Novel Techniques of Sperm Selection for Improving IVF

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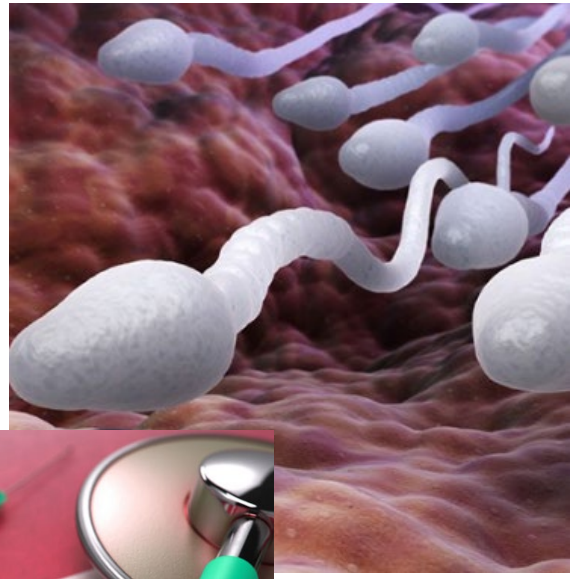
Selection of the best spermatozoa wye?

- Based on WHO, 10–15% of couples worldwide (more than 50 million) experience failure
- almost 50% of these infertility cases are attributed to male infertility
- assisted reproductive technologies (ARTs) such as intrauterine insemination (IUI), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI)
- However, the success rate has remained fixed merely at **30–40%** per cycle



*Pathway of sperm

with the oviduct and secretions such as glycoproteins and acidic mucopolysaccharides, which are produced by the secretory cells of the tubal epithelium

LCI revealed that 5%–15%of the sperm in the reservoir

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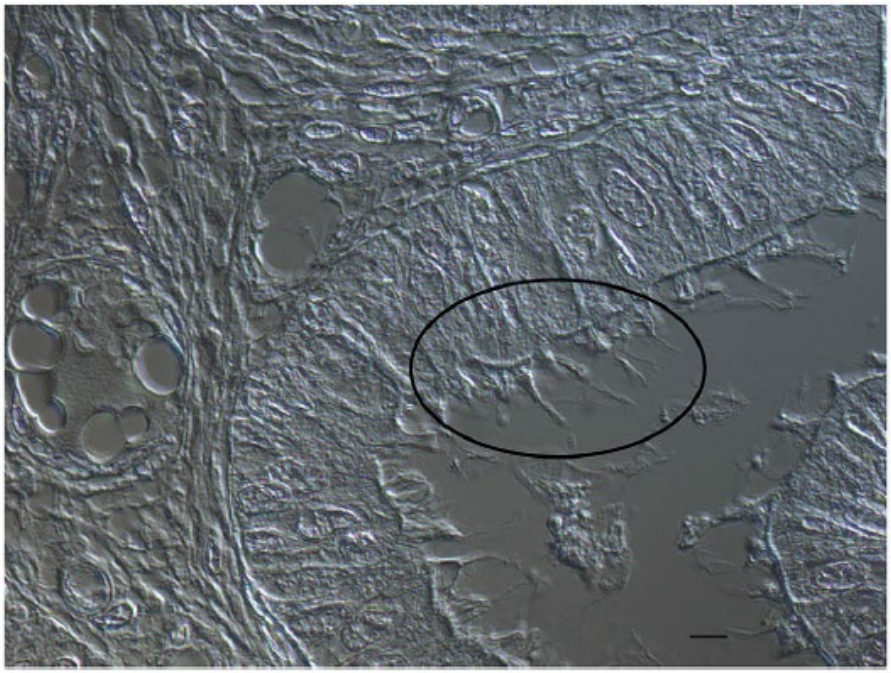

FIGURE 2 Formation of the sperm reservoir in the bovine oviduct as seen by differential interference contrast microscopy (DIC). Circles indicate bound sperm. Bar = 8 μm

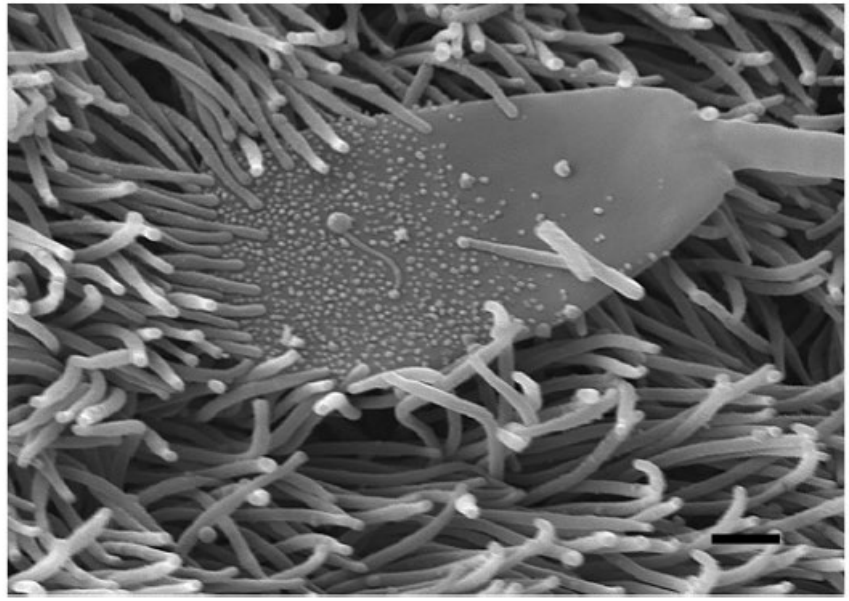

FIGURE 3 A bovine spermatozoon is bound to the cilia of the ampullar cells (scanning electron microscopy). Bar = 1 µm

“Therefore, simulating the environment inside the fallopian tube can help in selecting the right sperm”

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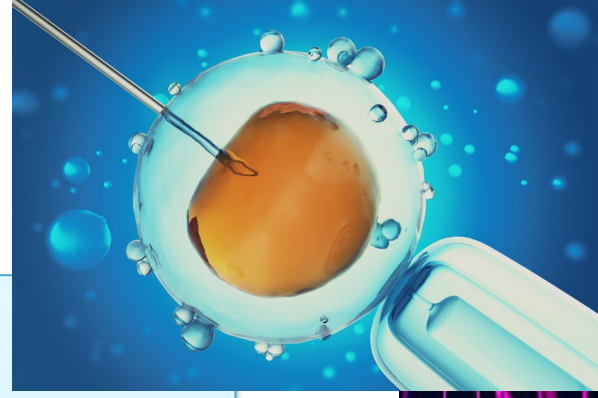
Sperm selection techniques:

NON- INVASIVE TESTS



1. Swim up
2. Density gradient
3. HOS test
4. Birefringence
5. Chemical inducer
6. LAISS laser-assisted
7. Sperm tail flexibility test (STFT)
8. Electrophoretic separation
9. Magnetic activated cell sorting (MACS)
10. Hyaluronic acid binding assay (HBA)
11. Spermatozoa-zona pellucida binding test (*ZPBA*)
12. Selection Based on Morphology–IMSI
13. Microfluidics
14. Horizontal Sperm Migration
15. The selection by motion
16. Machine learning

Classic methods

2021

cells

MDPI

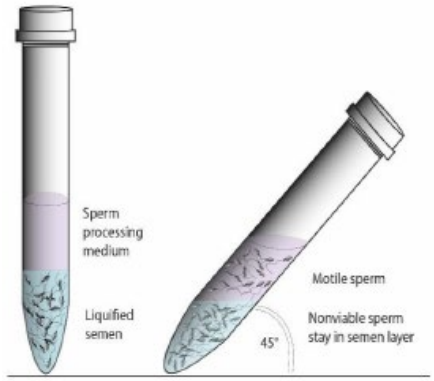
Review

Sperm Selection for ICSI: Do We Have a Winner?

Domenico Baldini [1,*], Daniele Ferri [1], Giorgio Maria Baldini [1], Dario Lot [1], Assunta Catino [2], Damiano Vizziello [3] and Giovanni Vizziello [1]

1. swim-up

- is a very soft technique, it produces a small load of ROS, but it recruits only mature activated sperm cells.
- SU method selects sperm cells with better motility, vitality and morphology than DGC.
  - the small portion of spermatic cells retrieved; only a maximum of 10% If samples are not centrifuged gently, many motile cells from pellets could potentially

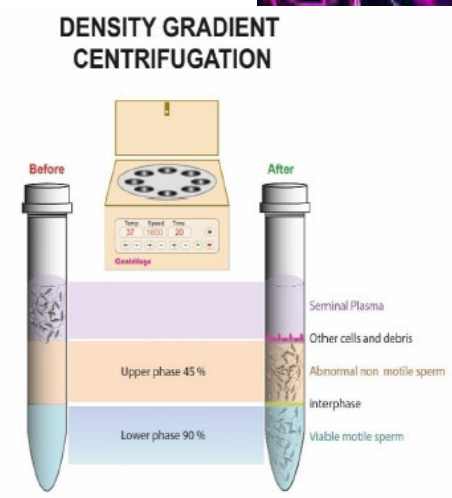


2. DGC

- is better than SU to select a higher percentage of capacitated sperm with hyper-activated motion

❖ using both methods in combination, better results

❖ Are not enough for DNA intact and ROS



So the question is:

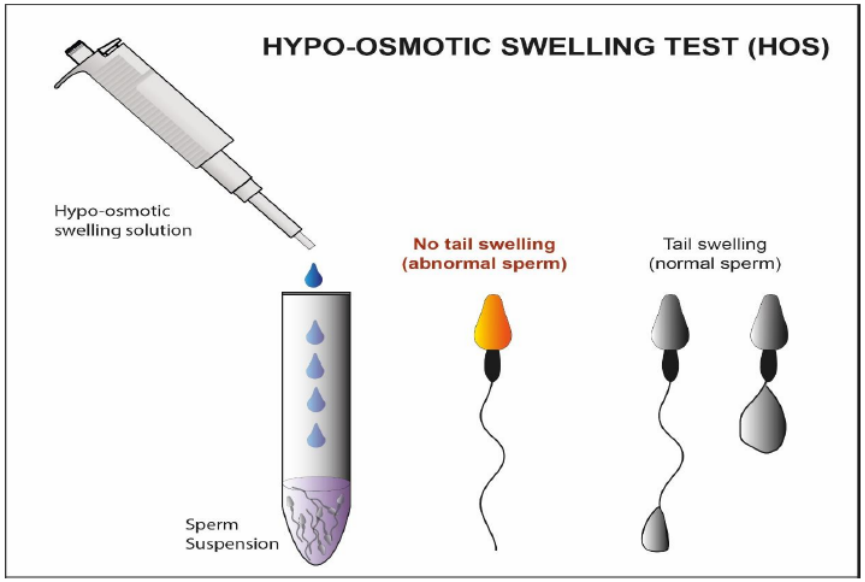
are there techniques that select spermatozoa with reduced level of chromatin or DNA damages?

HOW?

Advanced Methods

3. HOS test , Hypo-osmotic swelling test (HOST)

- an increased implantation rate when the HOST technique was used instead as selection method (45% vs. 11%).
- In a randomized study, They found significantly higher fertilization and pregnancy rates after ICSI performed with HOST selected sperm (Sallam et al).
- By comparing HOST and DGC, Charehjooy et al. found a significantly higher percentage of embryos that had good quality, implantation, and chemical pregnancy rates in couples undergoing ICSI with sperm selected by HOST.



4. Birefringence:

- In the mature sperm nucleus and acrosome, their is a strong intrinsic birefringence associated with nucleoprotein filaments / subacrosomal protein filaments, that are ordered in rods and longitudinally oriented.
- The localization of birefringence in the postacrosomal region was indicative of a spermatozoon that had undergone the acrosome reaction
- the selection of non-motile spermatozoa with birefringent heads resulted in an increase in clinical pregnancy and implantation rate (58% vs. 9% and 42% vs. 12%).
- higher implantation rates for those oocytes injected with reacted sperm (Gianaroli et al).

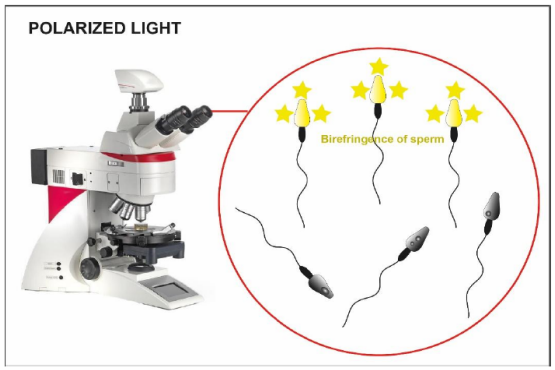
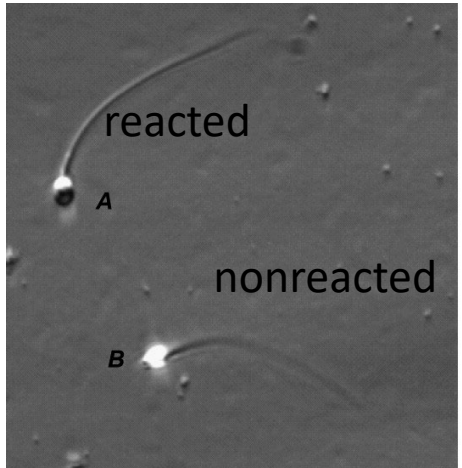
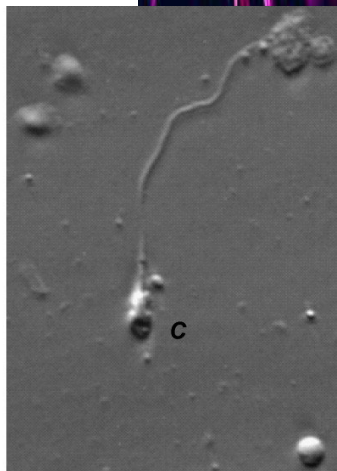
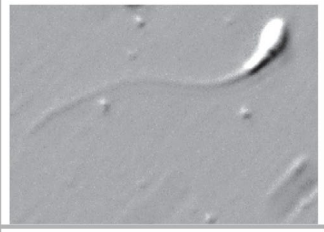
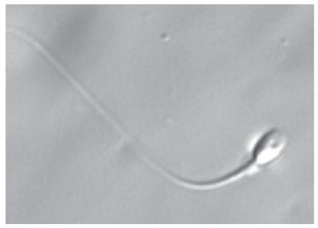
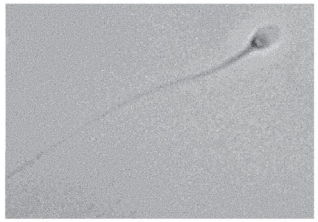


Figure 3. Microscope implemented with polarized light. The birefringence of the heads is clear in the viable sperm (yellow heads) compared to the not viable one where the birefringence is absent (dark heads).





Total birefringence		The whole sperm head shows a uniform birefringence.
Partial birefringence		The birefringence is localized in the post-acrosomal region.
No birefringence		The sperm head does not show any birefringence.

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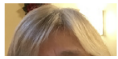
ARTICLE

Birefringence properties of human immotile spermatozoa and ICSI outcome



BIOGRAPHY

Maria Cristina Magli is an embryologist who has been coordinating the laboratories of the Italian Society for the Study of Reproductive Medicine since 1995. In ESHRE, she coordinated the Special

TABLE 3 PRESENCE OF HEAD BIREFRINGENCE IN EJACULATED AND TESTICULAR SPERM SAMPLES

	Ejaculated samples *n* = 83		Testicular samples *n* = 109	
	Birefringence	**No birefringence**	**Birefringence**	**No birefringence**
ICSI cycles	66 (79.5)[a]	17 (20.5)[b]	59 (54.1)[a]	50 (45.9)[b]
Age, years	34.8 ± 4.4	36.2 ± 3.9	34.7 ± 4.7	34.8 ± 4.1
Inseminated oocytes	432	94	443	370
Fertilized oocytes	283 (65.5)[c]	38 (40.4)[c]	276 (62.3)[d]	140 (37.8)[d]
Usable embryos	183 (64.7)	19 (50.0)	137 (49.6)[e]	53 (37.9)[e]
Transferred cycles	85	12	83	42
Clinical pregnancies (*n*, % per embryo transfer)	42 (49.4)[f]	2 (16.7)[f]	34 (41.0)[g]	5 (11.9)[g]
Implantation rate (%)	36.8	13.3	33.3[h]	10.2[h]
Miscarriages	5 (11.9)	0	4 (11.8)	1 (20.0)
Deliveries (*n*, cLBR per ICSI cycle %)	37 (56.1)[i]	2 (11.8)[i]	30 (50.8)[i]	4 (8.0)[i]

5. Chemical inducer

- Dimethylxanthines
- Papaverine
- Pentoxifylline

✓ Cause motility reactivation
✓ Comparing this technique with HOST, researchers have obtained a higher fertilization and pregnancy rate (32% vs. 16%)

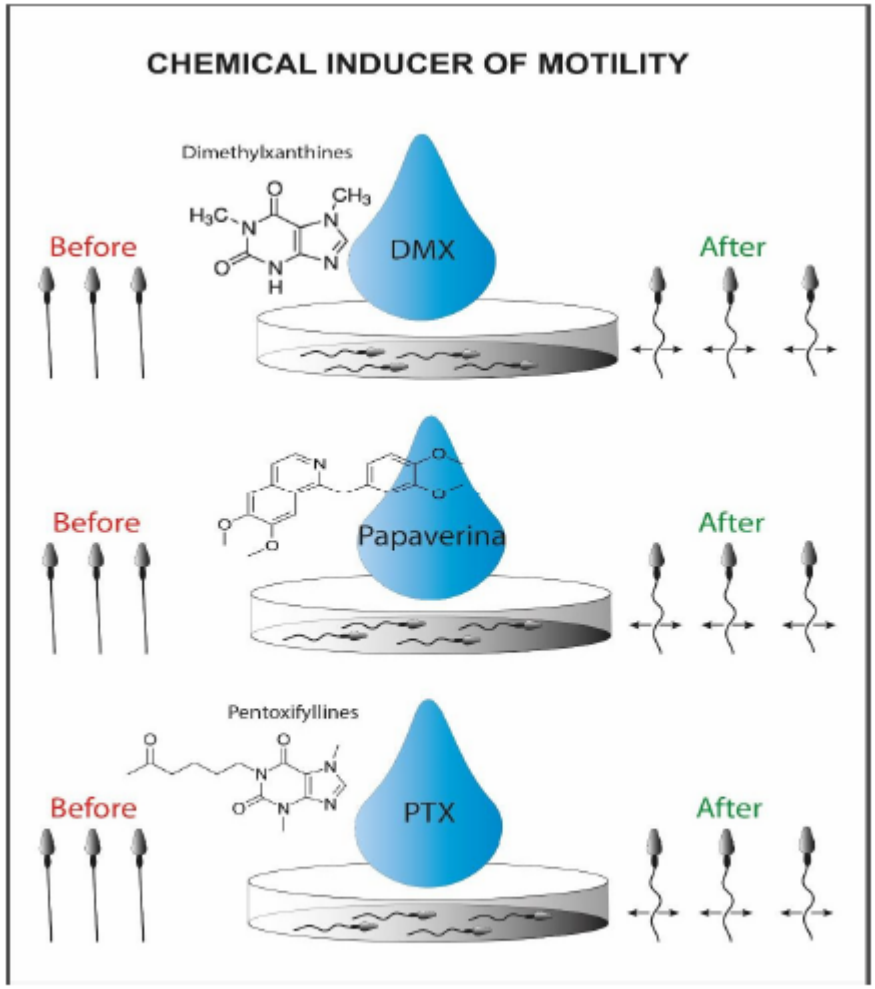


Figure 4. Schematic representation of chemical inducers of motility. On the left we can recognize the initial immotile sperms and on the right side the viable motile sperms activated by the chemical inducer

ATP/MgSO4 & myoinositol

- Myoinositol generates an increase in mitochondrial membrane potential, resulting in an intracellular increase of Ca2+.
- By incubating sperm cells frozen and then thawed from oligoasthenospermic patients with myoinositol, some research groups were able to recover a portion of sperm cells with significantly increased motility.
- The research group performed a series of ICSI by preincubating sperm cells the mitochondrial membrane potential which could lead to the occurrence of toxic effects

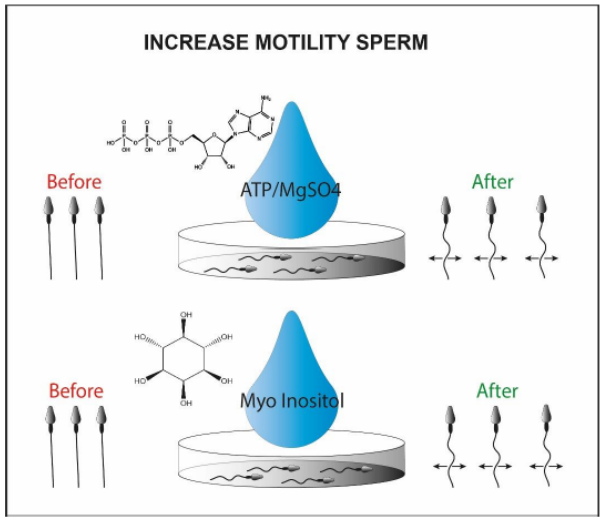


Figure 6. Sperm motility enhanced by ATP/MgSo4 or myo-inositol. Briefly in the picture we observe the immotile sperm before the treatment with the chemical inducer (left side) and the motile sperm cells after the chemical exposure (right side).

6. LAISS laser-assisted

- Laser irradiation causes the release in cytosol of second messengers, such as Ca2+ and ROS , and an increase in the synthesis of ATP,
- The sperm cell is then considered viable when its tail is coiled

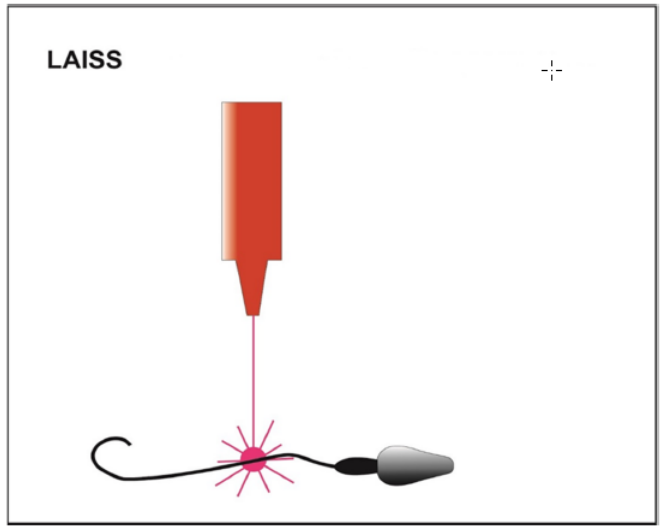
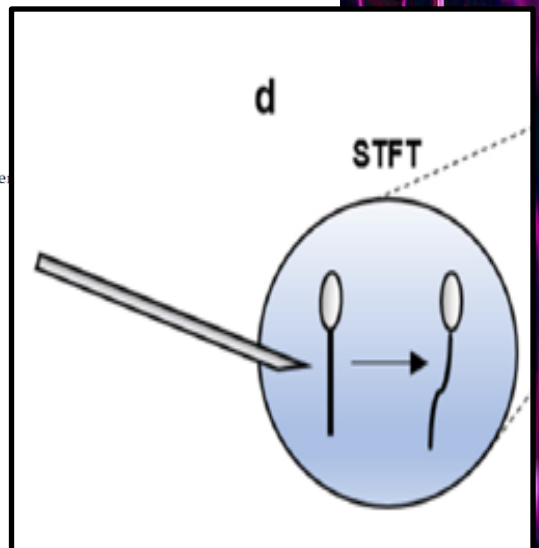


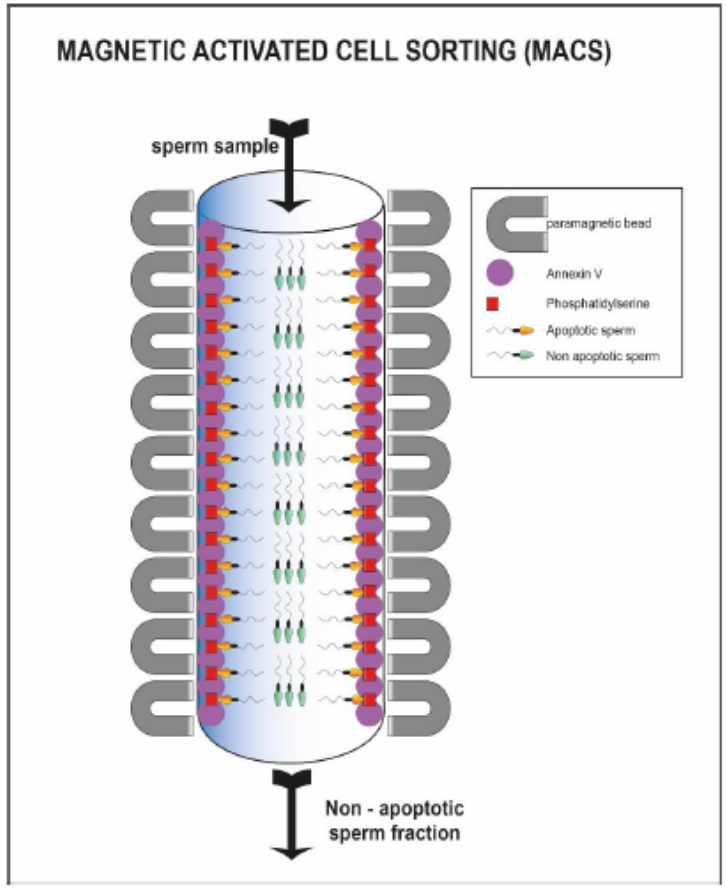
Figure 5. Schematic representation of LAISS (laser-assisted immotile sperm selection). The laser irradiation generates a slight movement of the tail in those viable sperms initially immotile.



7. Sperm tail flexibility test (STFT)

8. Magnetic activated cell sorting (MACS)

- It involves the use of magnetic microspheres conjugated to Annexin V (AV-MACS) that have a high affinity for phosphatidylserine.
- Use for cases of high nuclear fragmentation, idiopathic infertility, and patients with varicocele
- It was also shown that sperm selected by MACS before cryopreservation, had significantly higher sperm motility and cryosurvival rates and significantly higher levels of intact mitochondria after thawing (Grunewald et al.)



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MACS-DGC versus DGC Sperm Wash Procedure: Comparing Clinical Outcomes in Couples with Male Factor Infertility Undergoing ICSI: A Clinical Trial Study

Marziyeh Norozi-Hafshejani, M.Sc.[1,2], Marziyeh Tavalaee, Ph.D.[1*], Mohammad Hassan Najafi, M.D.[2], Farnaz Shapouri, Ph.D.[3], Maryam Arbabian, B.Sc.[1], Mohammad Hossein Nasr-Esfahani, Ph.D.[1,2*]

1. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Isfahan Fertility and Infertility Center, Isfahan, Iran
3. Memphasys Ltd, Sydney, Australia

- 206 infertile couples with teratozoospermia.
- 106 and 100 couples were considered for the study (DGC/MACS) and control group (DGC),
- top embryo quality on the day 3 (30.22 ± 3.59 vs. 17.96 ± 2.9, P=0.009), and implantation rate (18.12% vs. 10.42%, P=0.04) were higher in the study group

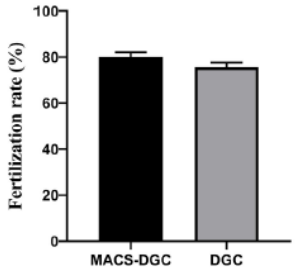
Table 2: Assessment of clinical parameters in this study

Study parameters	Study group n=106 MACS-DGC (%)	Control group n=100 DGC (%)
Cancelled cycle	13	8
Frozen cycle with no ET	2	2
Number fresh of ET cycle	54	57
Number frozen of ET cycle	37	33
Total ET cycle	91	90
Mean number of ET/transfer	1.75	1.81
Mean embryo score of transferred embryos	2.2	2.19
Number of clinical pregnancy	28/91 (30.76)	20/90 (22.22)
Implantation rate (sac/number ET)	(18.12)	(10.42)
Number of miscarriage	4	4
Number of singletons	19	15
Number of twins	5	1
Male/female	17/12	6/11
Sex ratio	1.42	0.54

DGC; Density gradient centrifugation, MACS; Magnetic-activated cell sorting, and ET; Embryo transfer.

Parameters	Study group 106 cases (MACS-DGC)	Control group 100 cases (DGC)	P-value
Male age (Y)	37.22 ± 00.64	37.58 ± 00.63	0.69
Female age (Y)	32.02 ± 00.55	31.55 ± 00.54	0.54
Sperm concentration (10^6/ml)	24.38 ± 01.52	25.04 ± 01.44	0.75
Sperm motility (%)	38.62 ± 01.36	35.71 ± 01.09	0.1
Abnormal sperm morphology (%)	98.31 ± 00.09	98.20 ± 00.07	0.34
Total injected oocytes	07.78 ± 00.53	07.92 ± 00.57	0.86
Previous ART	01.60 ± 00.09	00.90 ± 00.09	0.000

Data are presented as mean ± SD. DGC; Density gradient centrifugation, MACS; Magnetic-activated cell sorting, and ART; Assisted reproductive technology.



cells 2022 MDPI

Article

Effect of Sperm Selection by Magnetic-Activated Cell Sorting in D-IUI: A Randomized Control Trial

Cristina González-Ravina [1,2,3], Esther Santamaría-López [1,2,*], Alberto Pacheco [4,5], Julia Ramos [1], Francisco Carranza [1], Lucía Murria [2], Ana Ortiz-Vallecillo [2] and Manuel Fernández-Sánchez [1,2,3,6]

[1] IVI-RMA Seville, Avda. Américo Vespucio 19, 41092 Seville, Spain; cristina.gonzalez@ivirma.com (C.G.-R.); julia.ramos@ivirma.com (J.R.); francisco.carranza@ivirma.com (F.C.); manuel.fernandez@ivirma.com (M.F.-S.)

- Cryopreservation of sperm in donor intrauterine insemination (D-IUI) treatments increases sperm DNA f, so patients using these sperm samples can benefit from using this technique.
- study analyzed clinical outcomes of 181 D-IUI treatments.
- MACS was performed after density gradient centrifugation (DGC/MACS) in 90 thawed , whereas only DGC was performed in 91 thawed semen donor samples (CG).
- our results show significant differences in gestation, live birth, or miscarriage rates

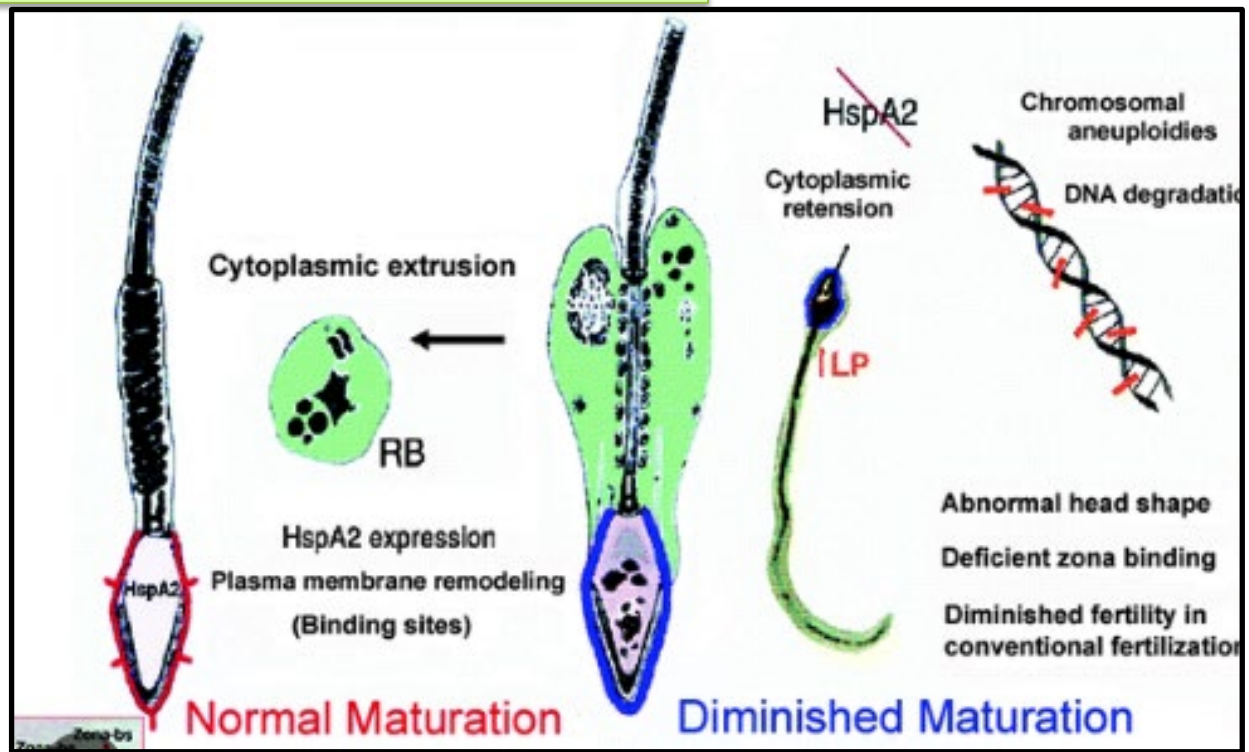
9. Hyaluronic acid binding assay (HBA)

Remodeling steps:

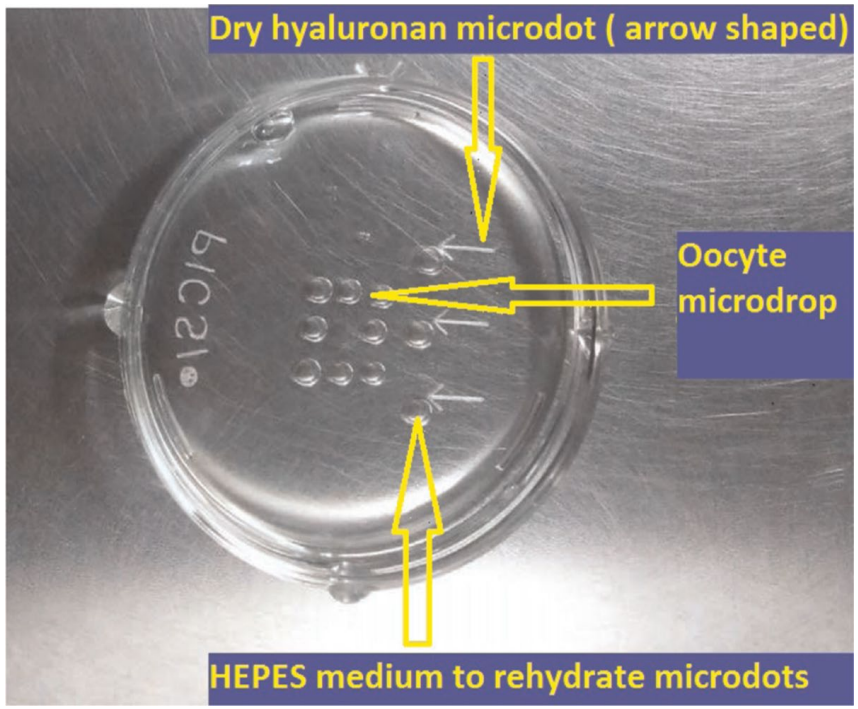
- Cytoplasmic extrusion
- Histone protamine exchange
- Shape properties changes
- Maturation of sperm plasma membrane

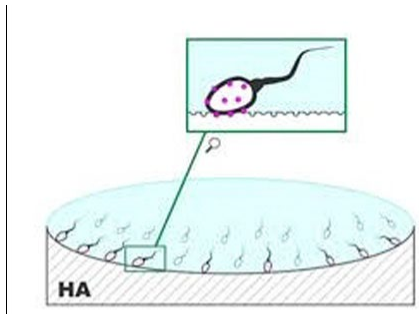
HspA2

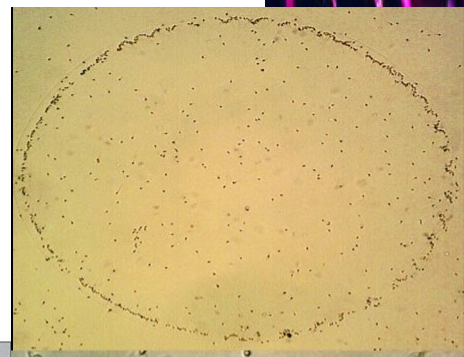
Formation of **Hyaluronan (HA)-binding and zona-binding** sites***(Huszar et al. 2007)***

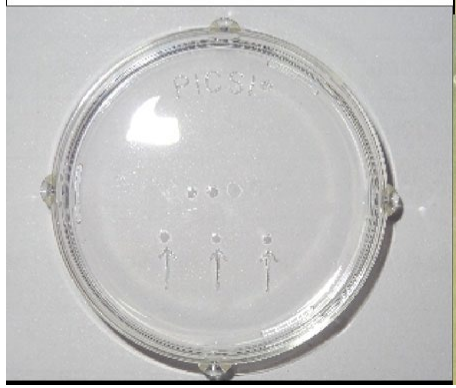


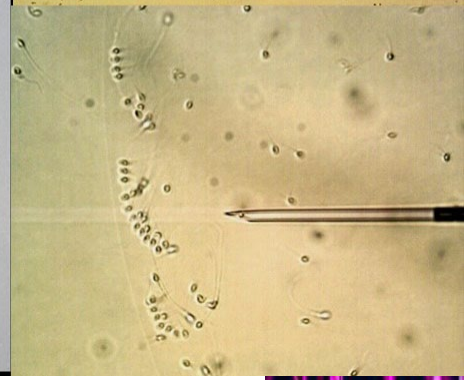
Selection of HA-bound spermatozoa (Physiological ICSI)

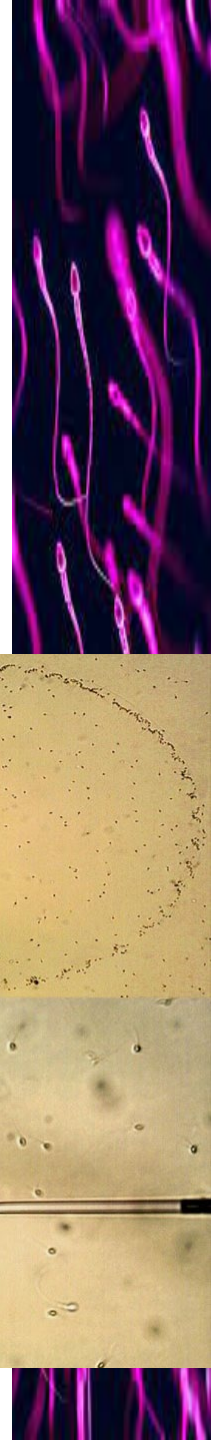


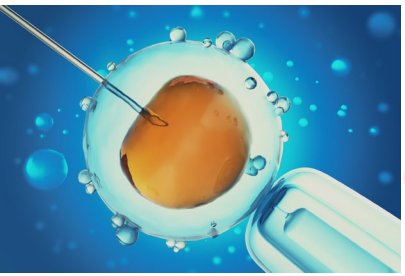












Outcome parameters: PICSI vs. ICSI

	Increased	decreased	similar to conventional insemination procedures
Fertilization rate	*Nasr-Esfahani 2008*		*Nijs 2009, Hong Ye 2006 (no predictive value), Sanchez2005, Janssens 2006, Worrilow 2006*
Embryo cleavage			*Janssens 2006, Worrilow 2006*
Blastocysts			*Worrilow 2006*
Pregnancy rate	*Worrilow 2006, 2009*		*Nijs 2009 (no predictive value), Nasr-Esfahani 2008*
Implantation rate			*Nasr-Esfahani 2008*
Miscarriage rate		*Worrilow 2006, Sanchez 2005*	
Delivery rate			*Nijs 2009 (no predictive value)*

Conclusions:

The clinical application/advantage has to be confirmed on higher Numbers of patients

10. Spermatozoa-zona pellucida binding test (*ZPBA*)

- **„natural biological selection“**
- Incubation of MI oocyte with SP for 2 h
- Remove with an ICSI pipette the SP that are bound to the ZP
- ZP-bound sperm are hyperactivated and have normal morphology and intact DNA.

- Jin et al. showed that ZP-bound sperm injection led to higher clinical pregnancy rate (60.5% vs 47.6%) and higher rates of high-quality embryos and useable embryos for transfer (66.1% vs 50.8% and 76.0% vs 66.3%), while they found no significant differences in fertilization and cleavage rates.

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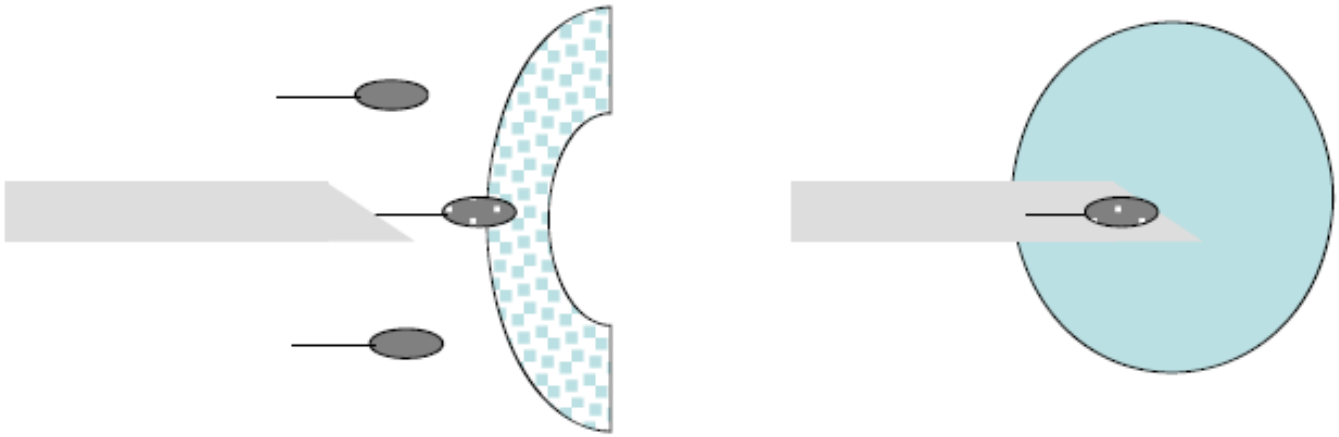
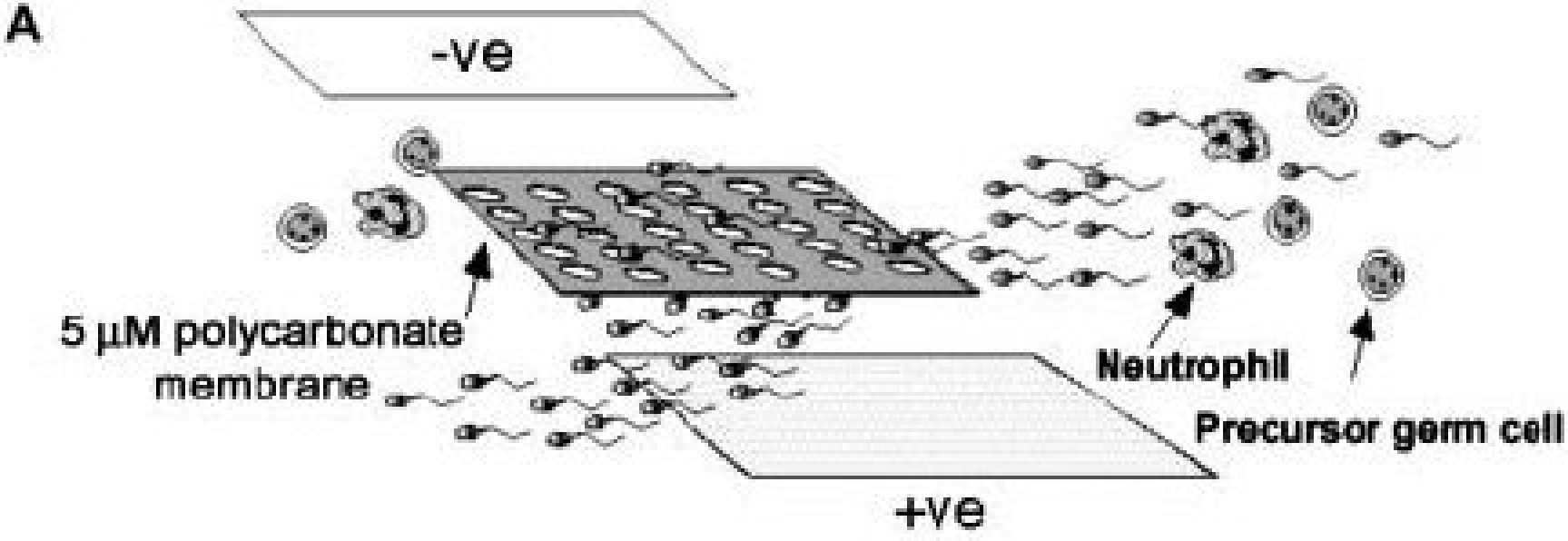


11. Electrophoretic separation

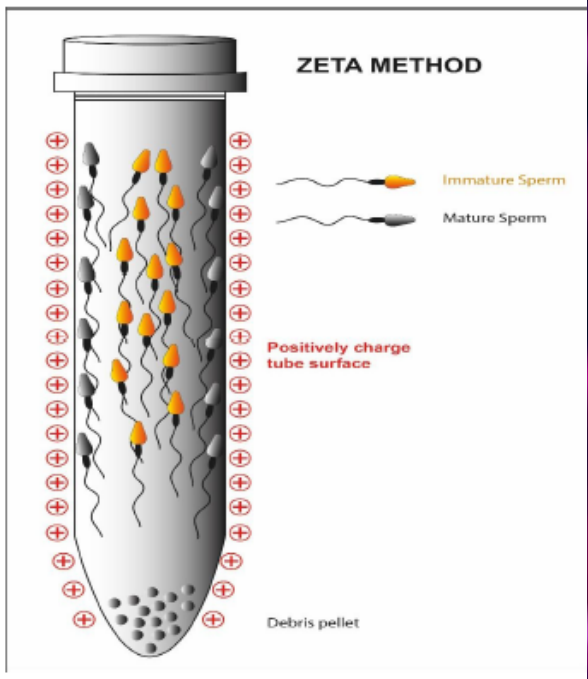
1. the highest quality spermatozoa contain glykocalyx, which is rich in salic acid residues, outside of the plasma membrane, negative electrical charge called “Zeta potential”.

2. spermatozoa can be separated from other contaminating electronegative cells (such as leukocytes and precursor germ cells) by virtue of their small cross sectional size



ZETA

- allow the collection of live spermatozoa with normal morphology and a high percentage of the integrity of the genetic material
- Nasr Esfahani et al. and Duarte et al. observed a significant increase in top quality embryos and pregnancy rate after ICSI by comparing DGC/Zeta
- Authors found that sex ratio male/female at birth was significantly lower in the DGC/Zeta group compared to DGC group.
- NO centrifugation: no generation of reactive oxygen species (Ainsworth et al., 2005)
- Nevertheless, the Zeta method leads to a
- low sperm recovery rate



12. Selection Based on Morphology–IMSI

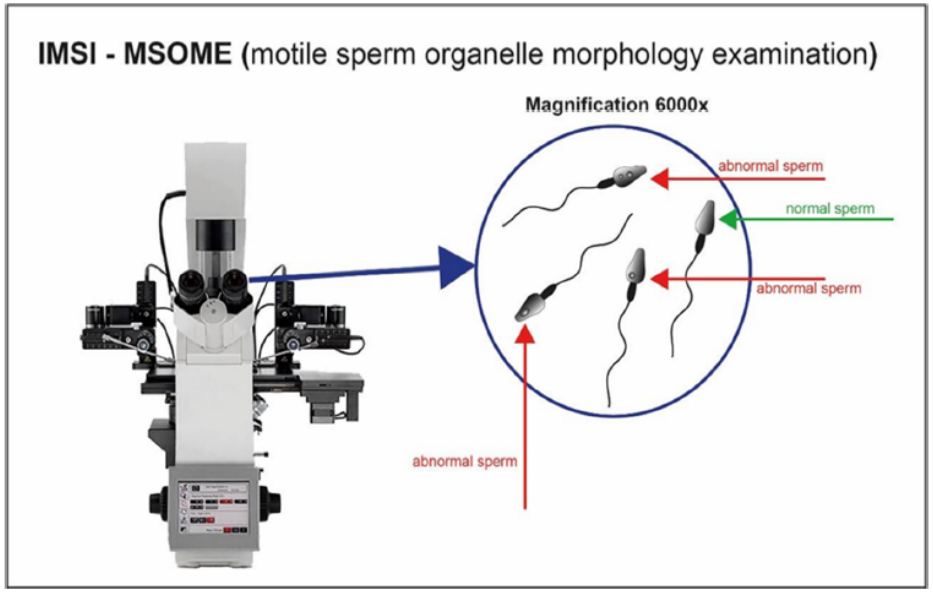


Figure 9. Sperm cell visualization with magnification system 6000×. Sperm cells with appropriate morphology are well selected through the high magnification.

IMSI

(Intracytoplasmic Morphologically Selected Sperm Injection)

(Bartoov et al., 2001, 2002, 2003)

MSOME

(Motile Sperm Organelle Morphology Examination)

Additional tool to

ICSI

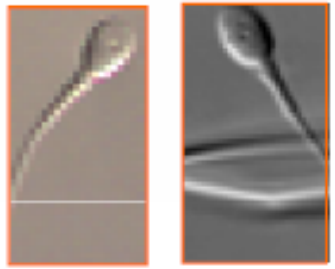
Spermatozoa class 1
(normal form, no vacuole)

..... (%)

(grade1)

Spermatozoa class 2
(normal form, max. 2 small vacuoles)

..... (%)



(grade 2)

„Normal Spermatozoa“

Spermatozoa class 3
(normal form, at least 1 large vacuole, > 2 small vacuoles)

..... (%)

(grade 3)

Spermatozoa class 4
(abnormal form, and vacuole(s))

..... (%)

(grade 4)

The IMSI Procedure Improves Laboratory and Clinical Outcomes Without Compromising the Aneuploidy Rate When Compared to the Classical ICSI Procedure

Daniel Luna[1], Roly Hilario[2], Julio Dueñas-Chacón[2], Rocío Romero[2], Patricia Zavala[2], Lucy Villegas[1] and Javier García-Ferreyra[1]

[1]FERTILAB Laboratory of Assisted Reproduction, Lima, Perú. [2]PROCREAR Fertility Center, Lima, Perú.

Libertas Academica
FREEDOM TO RESEARCH

2015

- 31 cycles of IMSI (*n* = 11) and ICSI (*n* = 20) procedures with preimplantation genetic diagnosis (PGD) performed between July 2011 and February 2015

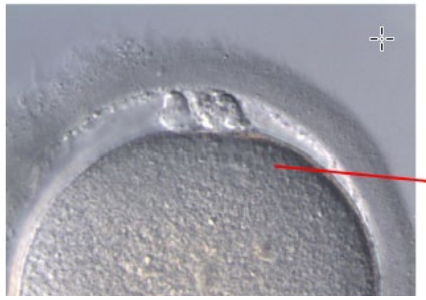
- The IMSI procedure significantly improves the embryo quality/development by increasing the implantation rates without affecting the chromosomal status of embryos.

Table 4. Clinical outcomes in the IMSI and ICSI groups.

	IMSI	ICSI
No. of cycles with embryo transfer	4	7
No. total of embryo transferred (Mean ± SD)	7 (1.75 ± 0.5)	11 (1.83 ± 0.41)
Cycles with at least one blastocyst at Day 5 (%)	55[a]	35
Cycles with embryos available to cryopreserve (%)	0	5
Pregnancy rate (%)	50	43
Implantation rate (%)	57[a]	27
Miscarriage rate (%)	0	33

Note: [a]Statistically significant difference between IMSI and ICSI groups ($P < 0.001$).

2015



Sperm Selection Method in ICSI

Author(s): Atsumi Yoshida

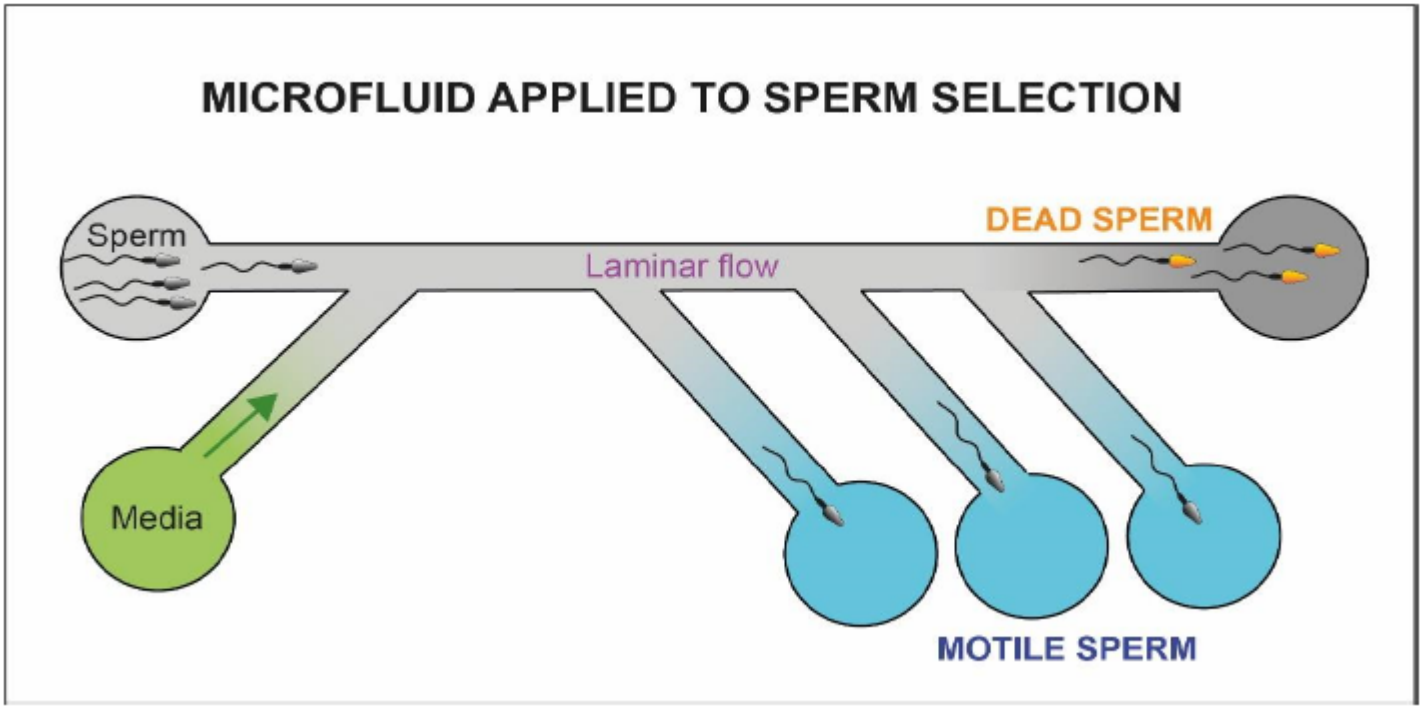
Source: Journal of Mammalian Ova Research, 32(1):19-28.

Table 1. Review of the literature

			No of cycles		Fertilization rate (%)			Implantation rate (%)			Pregnancy rate (%)			Abortion rate (%)			High-quality embryos		
			IMSI	ICSI	IMSI	ICSI	*p*	IMSI	ICSI	*p*	IMSI	ICSI	*p*	IMSI	ICSI	*p*	IMSI	ICSI	*p*
Bartoov [2] (2003)	Matching study	At least two previous consequent pregnancy failed ICSI cycle	50	50	64.5 ± 17.5	65.5 ± 21.5	NS	27.9 ± 26.4	9.5 ± 15.3	≤0.01	66	30	≤0.01	9	33	≤0.01	45.2 ± 28.2%	31.0 ± 19.5	≤0.01
Berkovitz [3] (2006)	Matching study	At least two previous consequent pregnancy failed ICSI cycle	80	80	67.4 ± 20.8	69.1 ± 22.6	NS	31.3 ± 36.3	9.4 ± 17.4	≤0.05	60	25	≤0.05	14	40	≤0.05	38.7 ± 31.6%	25.7 ± 28.3	≤0.05
Antinori [4] (2008)	Prospective randomized study	Severe oligoasthenoteratozoospermia	227	219	94.8	94.5	NS	17.3	11.3	0.007	39.2	26.5	0.004	16.9	24.1	NS	-	-	-
Knez [5] (2011)	Prospective randomized study	Poor semen quality with all arrested embryos following a prolonged 5-day culture in previous cycles	20	37	51.2	52.7	NS	17.1	6.8	NS	25	8.1	NS	-	-	-	-	-	-
Setti [6] (2011)	Prospective randomized study	Oligoasthenoteratozoospermia according to the 2010 WHO reference values	250	250	79.2	78.9	NS	23.8	25.4	NS	37.2	36.8	NS	18.4	17.9	NS	44.40%	37.30%	NS
Oliveira [7] (2011)	Prospective randomized study	Repeated implantation failure	100	100	64.5 ± 23.5	62 ± 26.5	NS	13.6	9.8	NS	26	19	NS	15.4	31.6	NS	1.4 ± 0.5 (mean ± SD)	1.5 ± 0.5 (mean ± SD)	NS
Balaban [8] (2011)	Prospective randomized study	Unselected infertile population	87	81	81.60 ± 10.65	80.97 ± 15.06	NS	28.9	19.5	NS	54	44.4	NS	-	-	-	-	-	-
Marci [9] (2013)	Prospective randomized study	Unselected infertile population	51	281	80	77.27	NS	16.67	16.83	NS	33.33	30.96	NS	5.26	17.78	NS	-	-	-
Leandri [10] (2013)	Prospective randomized study	First ART attempt for male infertility	116	139	56	63	<0.05	24	23	NS	31	33	NS	-	-		-	-	-

Revised list by Monte *et al.* [1]. Abbreviation: NS, not significant; IMSI, Intracytoplasmic Morphologically-selected Sperm Injection; ICSI, Intracytoplasmic Sperm Injection.

13. Microfluidics



- bypassed the need for semen sample centrifugation
- low sample consumption
- removing human error due to automation capability

scientific reports

2023

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OPEN

A novel microfluidic device with parallel channels for sperm separation using spermatozoa intrinsic behaviors

Ali Heydari[1], Mohammad Zabetian Targhi[1]✉, Iman Halvaei[2] & Reza Nosrati[3]

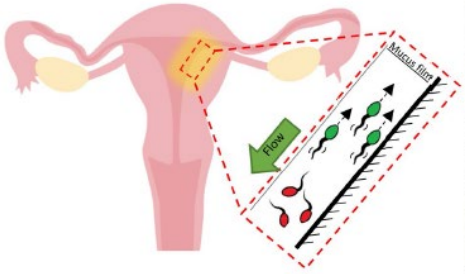
Isolating high-quality motile sperm cells is considered to be the main prerequisite for a successful

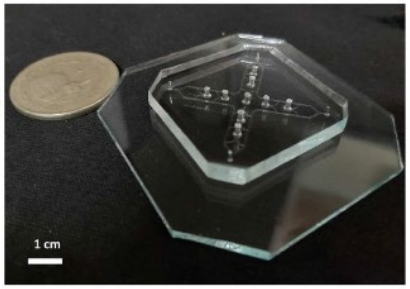
- Active microfluidic devices:

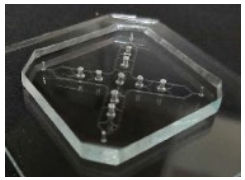
use external forces like optical traps, electrophoresis, and hydrostatic pressure.
they are invasive mechanism and include complicated experimental setups

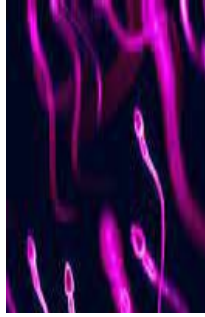
- Passive microfluidic systems:

the separation process is based on sperm's intrinsic behavior like boundary-following and response to external stimulants such as fluid flow, temperature, and chemical gradients. they separate sperm without damaging its morphology or DNA integrity by mimicking the in vivo environment



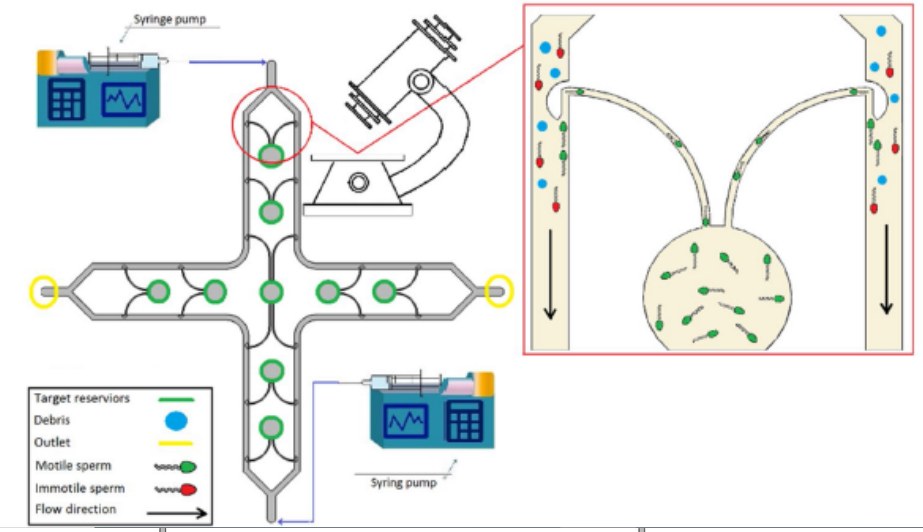




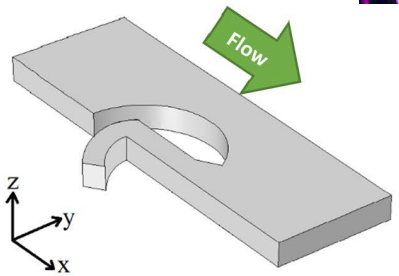


C

D

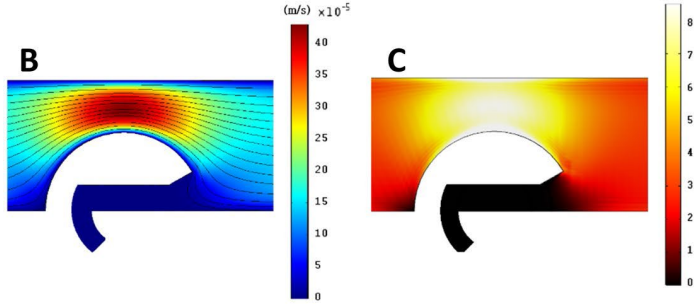

Syringe pump
Target reservoirs
Debris
Outlet
Motile sperm
Immotile sperm
Flow direction
Syring pump

A


Flow
z
y
x

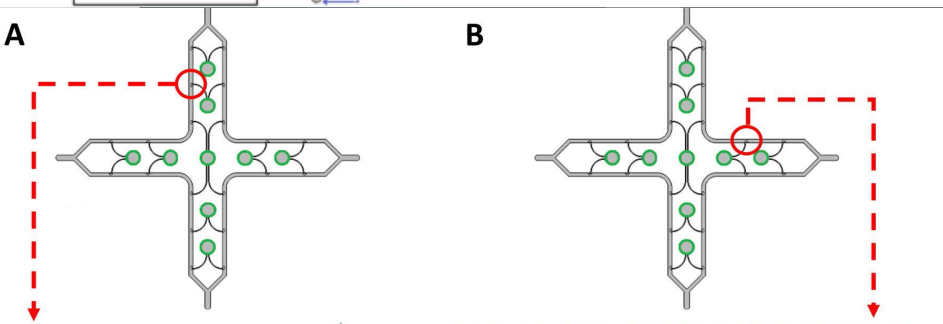
B

C

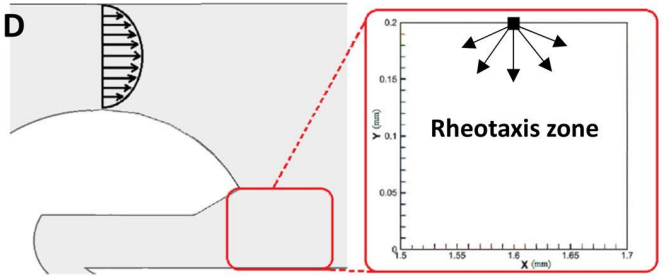

(m/s) ×10⁻⁵
40
35
30
25
20
15
10
5
0
(1/s)
8
7
6
5
4
3
2
1
0

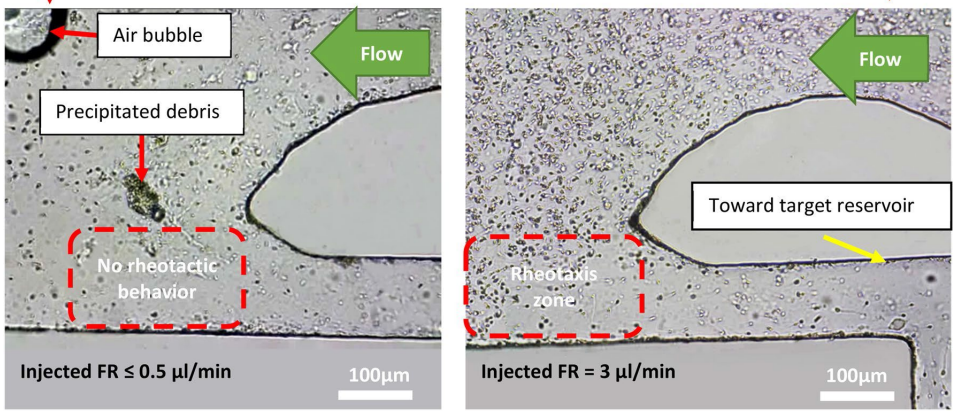
A

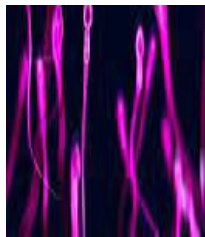
B

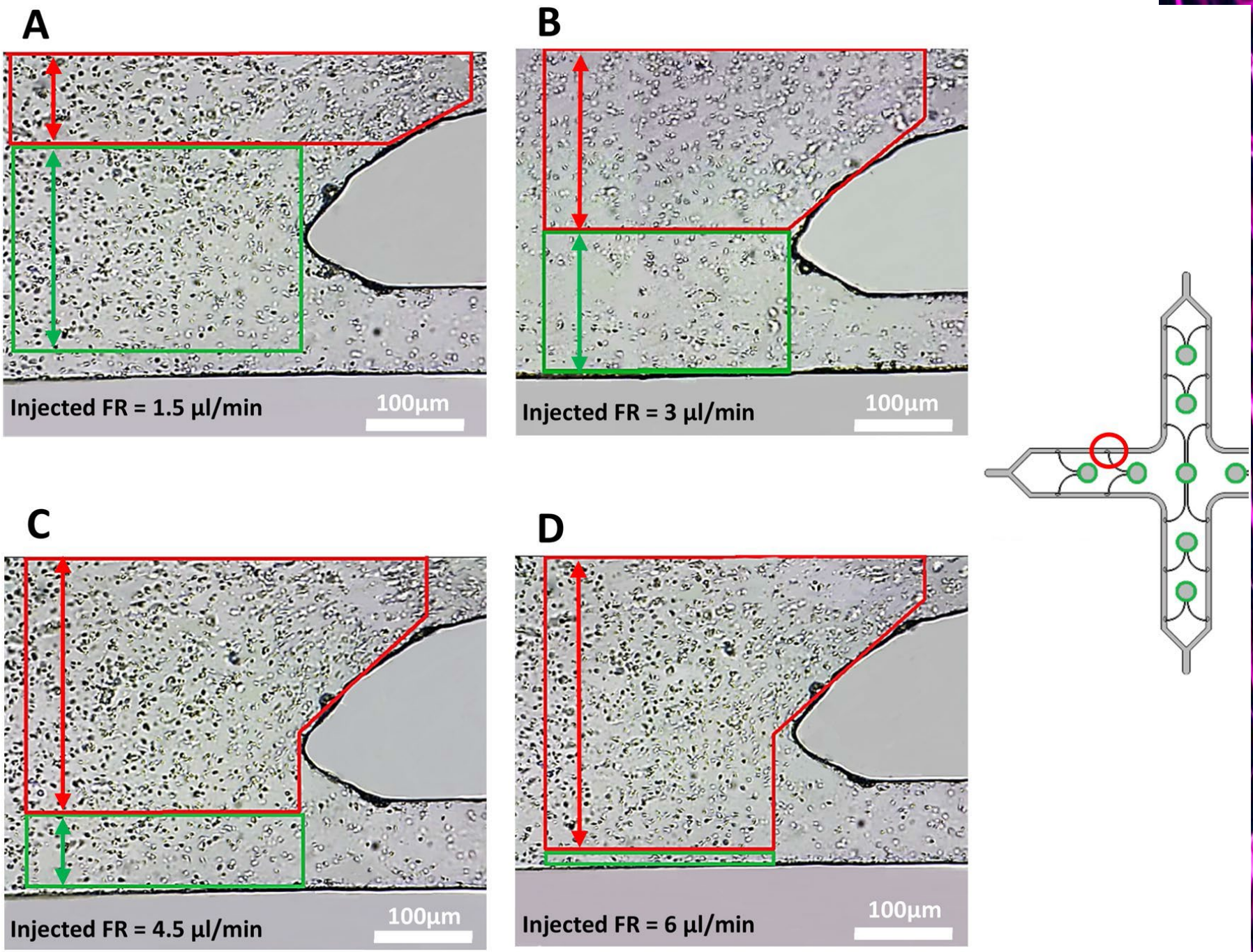


D


Rheotaxis zone
Y (mm)
X (mm)
0.2
0.15
0.1
0.05
0
1.5
1.55
1.6
1.65
1.7


Air bubble
Flow
Precipitated debris
No rheotactic behavior
Injected FR ≤ 0.5 µl/min
100µm
Flow
Toward target reservoir
Rheotaxis zone
Injected FR = 3 µl/min
100µm




A
Injected FR = 1.5 µl/min
100µm
B
Injected FR = 3 µl/min
100µm
C
Injected FR = 4.5 µl/min
100µm
D
Injected FR = 6 µl/min
100µm

PAPER

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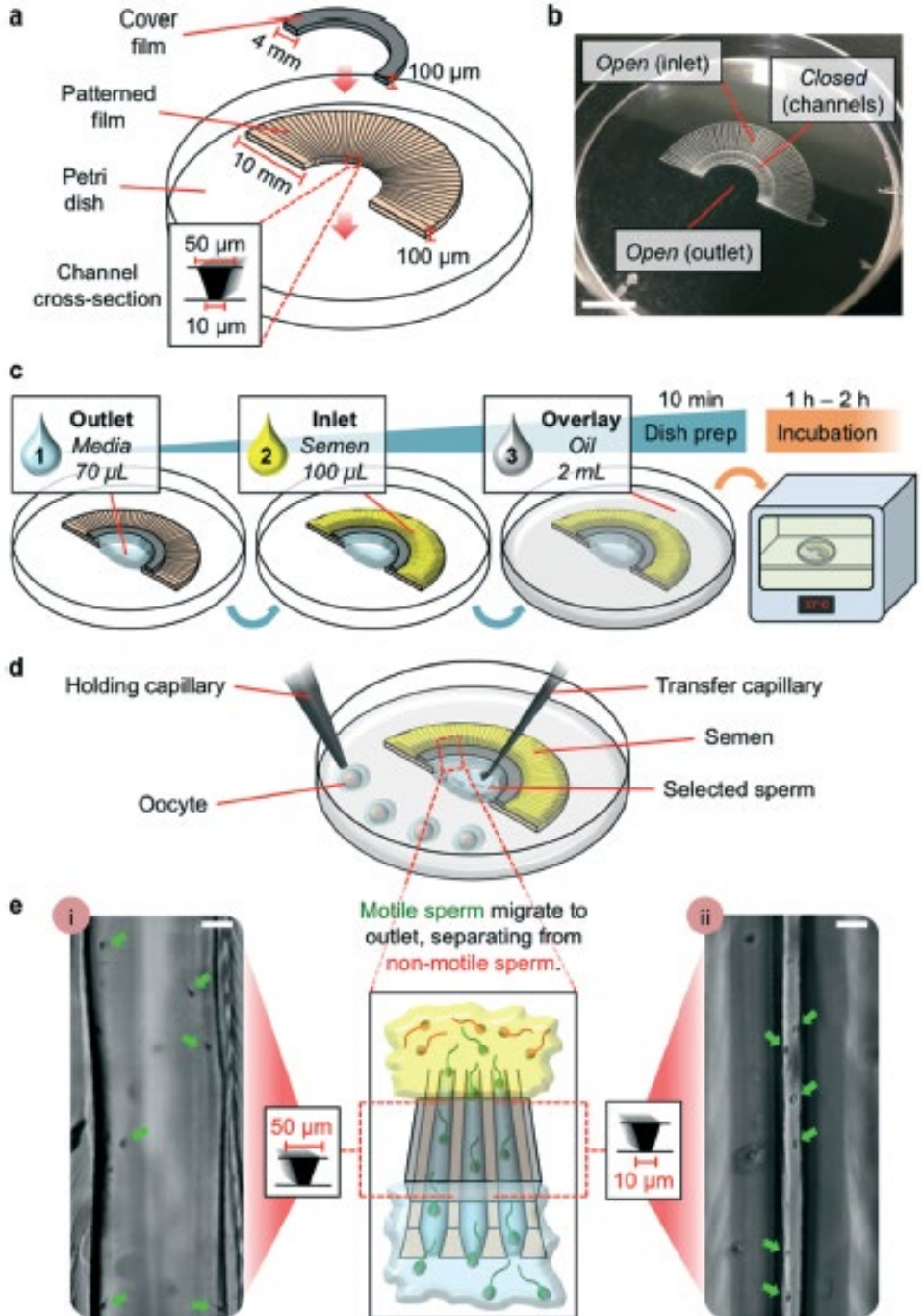
Cite this: *Lab Chip*, 2021, 21, 775

FertDish: microfluidic sperm selection-in-a-dish for intracytoplasmic sperm injection†

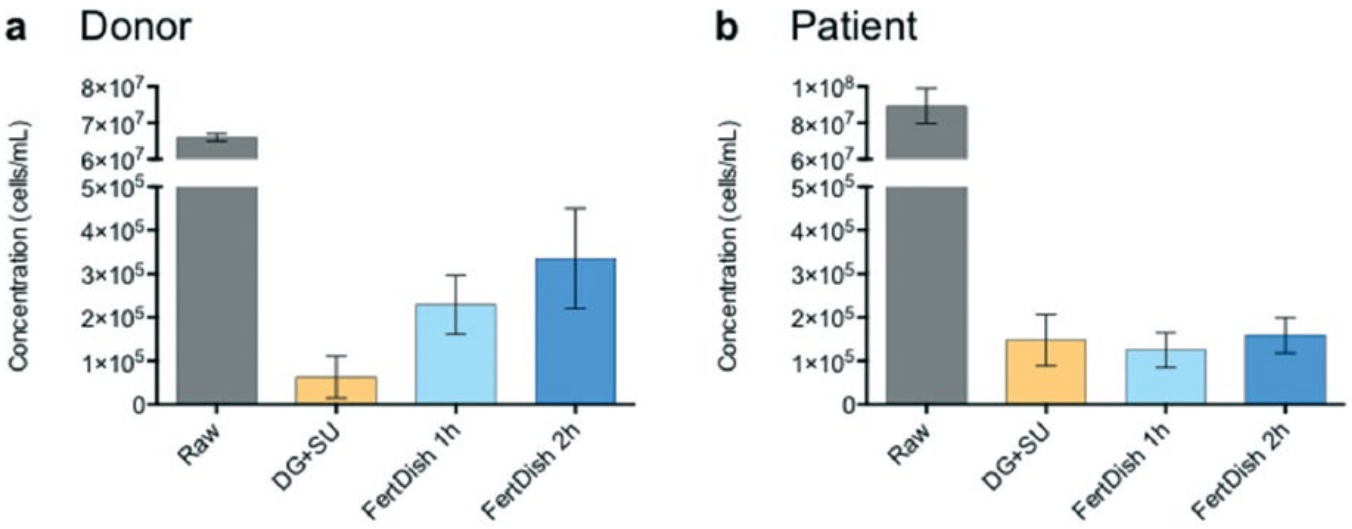
Sa Xiao, [a] Jason Riordon, [a] Mohammad Simchi, [a] Alexander Lagunov,[b] Thomas Hannam,[b] Keith Jarvi,[c] Reza Nosrati [d] and David Sinton *[a]

The selection of high quality sperm is critical for intracytoplasmic sperm injection (ICSI), a prevalent assisted reproduction technology. However, standard selection methods are time-consuming and fail to recover the most viable sperm, thereby limiting the ICSI success rate. Microfluidics enables rapid selection of viable

- The FertDish format mimics the clinician-familiar ICSI dish setup
- improvements in DNA fragmentation index of more than 91% (donor) and 74% (patient) versus raw semen
- The FertDish enables a high sperm recovery rate (>3.3 × 105 sperm per mL),
- loading the outlet with (PVP) media (70 µL),
- loading the inlet with raw semen (100 µL), and then overlaying the entire platform with paraffin oil (2 mL)



Sperm concentrations from donor and patient sample testing



a) Sperm concentrations from donor samples, including raw, DG + SU, and FertDish at 1 h and 2 h. b) Sperm concentrations from patient samples, including raw, DG + SU, and FertDish at 1 h and 2 h. N = 3 replicates for DG + SU and FertDish 1 h and 2 h. Error bars represent standard error of the mean in a and b.

reproductive medicine

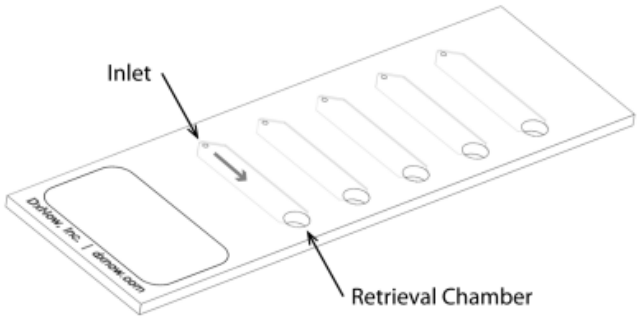
MDPI

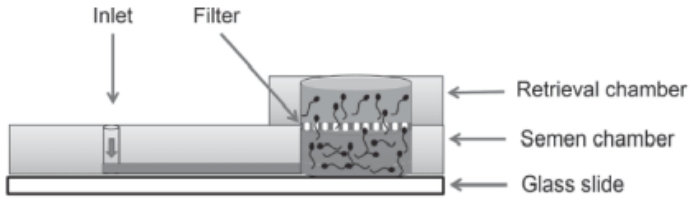
Communication

Effect of Microfluidic Sperm Separation Versus Standard Sperm Washing Processes on Laboratory Outcomes and Clinical Pregnancy Rates in an Unselected Patient Population

Chelsey A. Leisinger [1,*], Glen Adaniya [2], Melanie R. Freeman [3], Erica J. Behnke [4], Martha Aguirre [5], Matthew D. VerMilyea [6] and Mitchel C. Schiewe [7]

- the ZyMot device is a new, FDA-approved that helps embryologists select the highest-quality sperm
- prospective, multicenter, randomized, sibling oocyte study was conducted with 86 couples evaluate
- Patients with at least 10 metaphase II oocytes were enrolled in the study and sibling oocyte groups were split in half with ICSI and the other half were injected with sperm sorted by the ZyM¯ot
- No statistical differences were observed between ZyM¯ot and control processing methods in any of the study outcomes evaluated.





The ZyMōt Multi is available in two processing volumes, 850µL and

14. Horizontal Sperm Migration

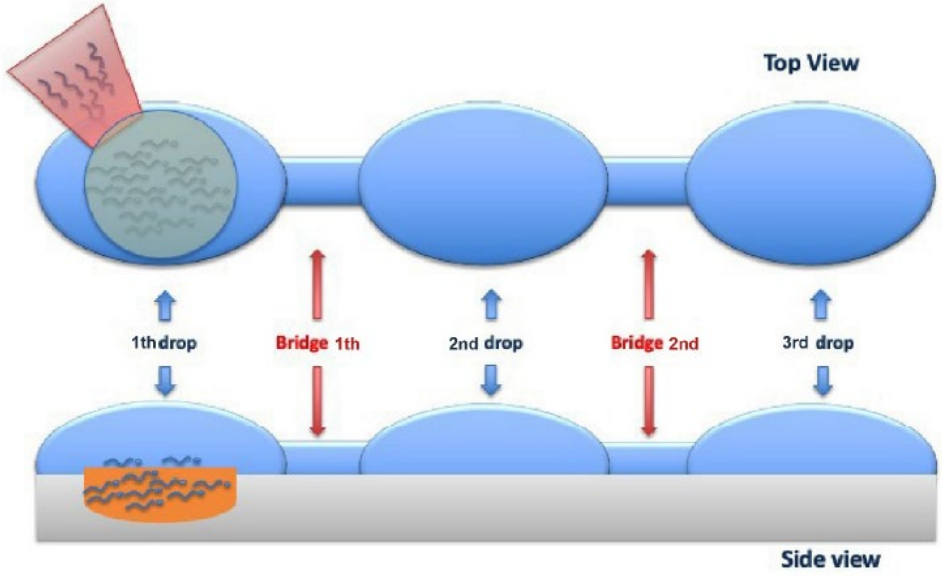


Figure 11. Top and Side viewing of the sperm cells horizontal migration from the first drop (where the cells are added) to the third drop (where the sperm cells are aspirated) through 2 bridges that link them.

- The technique allows the recovery of spermatozoa with high motility, normal morphology and minimal damage to the DNA, using a fast, safe, and economical procedure.
- segmentation and blastocyst rates are higher in the horizontal swim-up,
- lower quantity of harmful oxygen reactants
- clinical and ongoing pregnancy rates are numerically better in the horizontal swim-up than in conventional methods although there is no significant statistical difference.

Systems Biology in Reproductive Medicine

2020

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iaan20

Microfluidic sperm selection yields higher sperm quality compared to conventional method in ICSI program: A pilot study

Fatemeh Anbari, Mohammad Ali khalili, Abdul Munaf Sultan Ahamed, Esmat Mangoli, Ali Nabi, Fatemeh Dehghanpour & Mojdeh Sabour

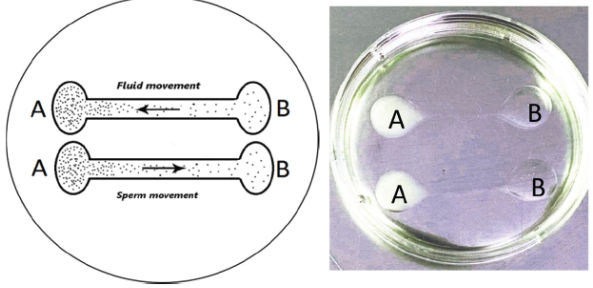


Figure 1. Design of microfluidic sperm sorting (MSS) technique. Droplets A and B (80 µL Ham's F10 medium supplemented with 5 mg/mL HSA) was loaded at the two ends of the dish at least2 cm apart and were connected by a narrow channel of medium created with a pipette tip. 60 µl of A droplet was replaced with 50 µl liquefied semen for 45 min. Due to the larger volume of the B droplet, the fluid flows from B to A droplet; while, the sperm cells moved toward B droplet. (A): droplet was semen input point. (B): droplet was sperm collection point.

- A total of 95 ICSI cases performed using sperm samples were prepared with our (MSS) Microfluidic Sperm Sorting or by Direct Swim Up (DSU; control)
- the rates of DNA fragmentation and immotile spermatozoa were significantly lower in MSS when compared to DSU group ($P < 0.001$).
- higher rates of high quality embryo formation ($P < 0.001$), implantation ($P = 0.04$) and pregnancy ($P = 0.05$)
- progressive motility of the sperm sample was higher after microfluid processing than after DSU.
- sperm concentration in MSS group was lower, which could be attributed to the spontaneous movement of sperm cells, not the centrifugation force

Original Article
2023 Mar 8. [Epub ahead of print]
https://doi.org/10.5468/ogs.22191
eISSN 2287-8580

2023

Obstetrics & Gynecology Science

Comparison between density gradient centrifugation method, an extended version of the horizontal swim up method and the combination of both for sperm selection

Malak Jamil, PhD[1,2], Hasnae Debbarh, PhD[1], Amal Kabit, MEd[2], Mohamed Ennaji, Lic[2], Loic Koumba, PhD[1], Ismail Kaarouch, PhD[2], Mohamed Zarqaoui, MD[2], Wassim Rhazi Senhaji, MD[2], El Mehdi Hissane, MD[2], Brahim Saadani, MD[2], Pierre Vanderzwalmen, PhD[3], Nourredine Louanjli, PhD[2], Rachida Cadi, PhD[1]

[1]Department of Biology, Laboratory of Molecular Genetic Physiopathology and Biotechnology, Ain Chock Faculty of Sciences, HASSAN II University Casablanca. [2]Labomac IVF Centers and Clinical Laboratory Medicine. Casablanca. Morocco. [3]IVF Centers Prof Zech. Bregenz. Austria

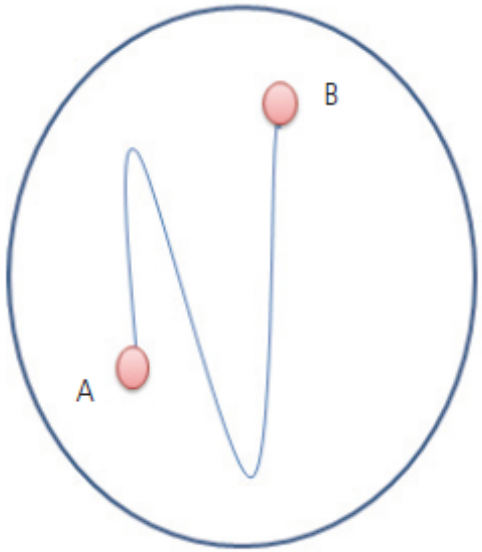


Fig. 2. Representation of the extended swim up by culture medium in a petri plate. (A) First drop where the sperm is added. (B) Second drop where sperm is migrating toward.

- normozoospermic semen samples
- the rates of DNA fragmentation and chromatin decondensation were significantly **lower** in extended horizontal SU samples than in DGC samples.
- The **lowest** rates of DNA fragmentation and chromatin decondensation corresponded to the samples treated with both methods (DGC/SU).
- **No significant difference** was found in the fertilization rate or day 3 embryos between sibling cultures.

Table 2. Comparison of the rate of DNA fragmentation/chromatin decondensation before and after density gradient migration, extended horizontal swim up, and the combination of both methods

	Before sperm treatment	After sperm treatment	*P*
Density gradient migration (n=97)			
Rate of DNA fragmentation	33.38±9.60	19.86±7.80	6.77×10^{-19}
Rate of chromatin decondensation	38.67±12.76	24.04±9.36	1.42×10^{-14}
Extended horizontal swim-up (n=97)			
Rate of DNA fragmentation	33.38±9.60	15.24±6.38	1.63×10^{-29}
Rate of chromatin decondensation	38.67±12.76	16.88±7.36	2.09×10^{-28}
Combination of both density gradient migration and extended horizontal swim-up (n=97)			
Rate of DNA fragmentation	33.38±9.60	6.02±6.26	3.38×10^{-46}
Rate of chromatin decondensation	38.67±1276	8.60±998	1.96×10^{-37}

Values are presented as mean±standard deviation.

Analysis of DNA fragmentation using TdT-mediated-dUTP nick-end labeling technique
Analysis of chromatin decondensation by the aniline blue technique

Table 4. Comparison of the rate of fertilization and day 3 embryos between culture A (ISCI with semen from density gradient migration) and culture B (ICSI with semen from the combination of density gradient migration and extended swim-up method)

	Culture A	Culture B	*P*
Fertilization rate (n=61)	74.11±27.91	71.72±31.05	NS
Rate of 8 cells embryos on day 3 (n=61)	68.76±32.44	60.03±36.74	NS

Values are presented as mean±standard deviation.
ICSI, intracytoplasmic sperm injection; NS, non significant.

15. The selection by motion

the selection of human spermatozoa with rotational motion might have the potential to improve ICSI outcome.

Accepted: 26 October 2017
DOI: 10.1111/and.12943

ORIGINAL ARTICLE

2017 WILEY ANDROLOGIA

Human sperm handling in intracytoplasmic sperm injection processes: In vitro studies on mouse oocyte activation, embryo development competence and sperm oxidation–reduction potential

S. Roychoudhury[1,2] | I. Maldonado-Rosas[3] | A. Agarwal[1] | S. C. Esteves[4] | R. Henkel[5] | R. Sharma[1]

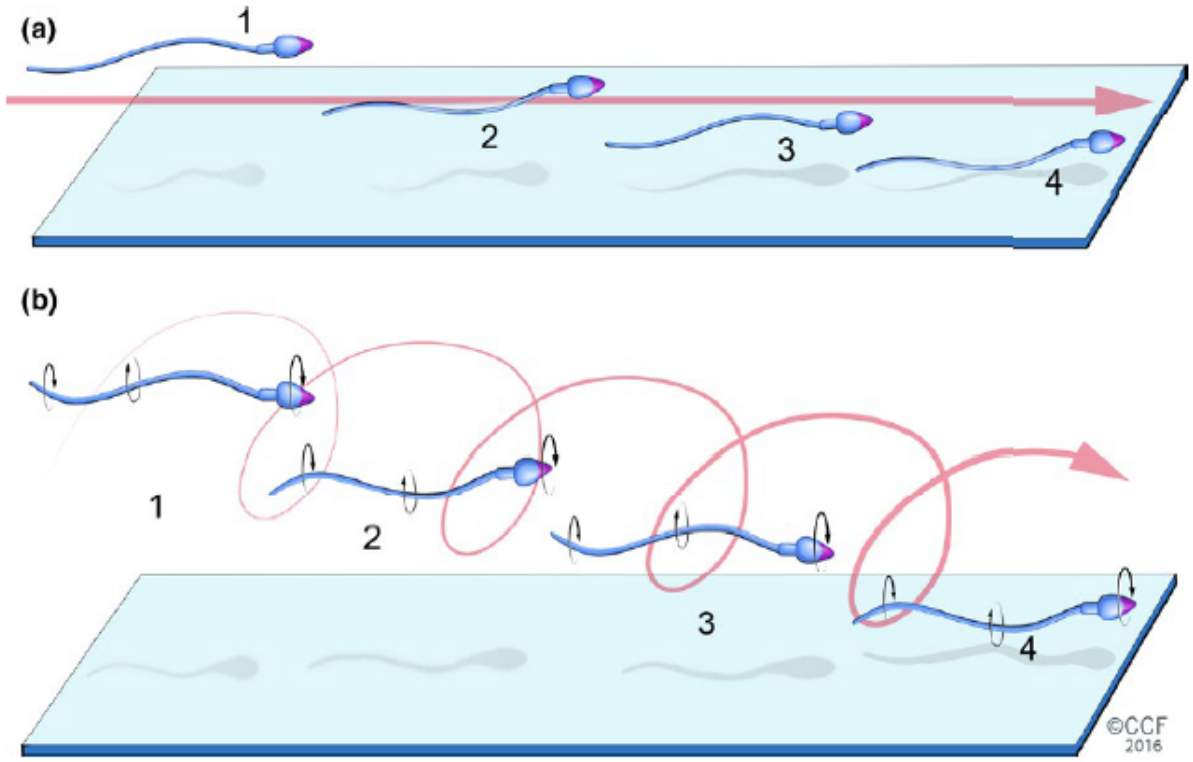


FIGURE 1 Linear and rotational motion of human spermatozoa. (a) Spermatozoa with linear motion change their position but not location by exhibiting two-dimensional movement in the bottom of a polyvinylpyrrolidone microdroplet, and (b) spermatozoa with rotational motion that change their position by gyrating over its own axis

REVIEWS

16. Machine learning

2021

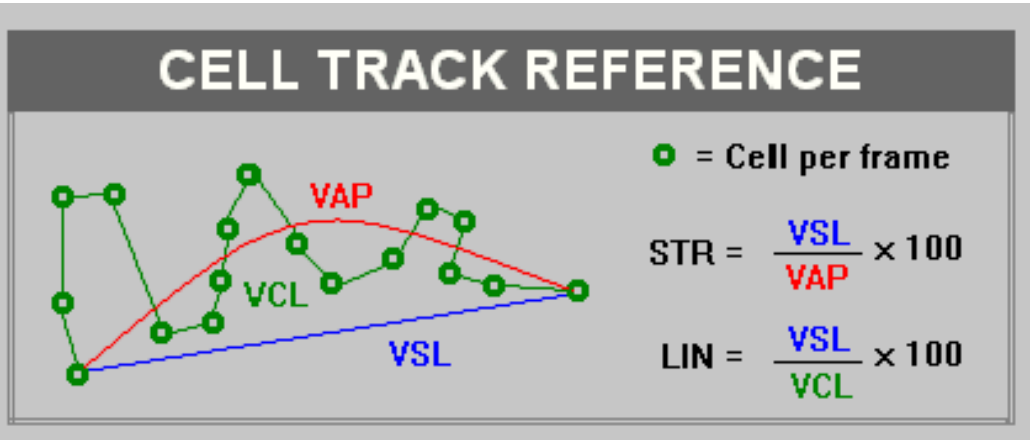
Machine learning for sperm selection

Jae Bem You[1,3], Christopher McCallum[1], Yihe Wang[1], Jason Riordon[1], Reza Nosrati[2] and David Sinton[1]

Abstract | Infertility rates and the number of couples seeking fertility care have increased worldwide over the past few decades. Over 2.5 million cycles of assisted reproductive technologies are being performed globally every year, but the success rate has remained at ~33%. Machine learning, an automated method of data analysis based on patterns and inference, is increasingly being deployed

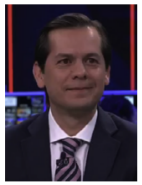
Current machine learning approaches

- FERTECH is image- processing software that classifies a 100 × magnified image of a sperm according to the WHO strict criteria.
- Later, an automated system called the Integrated Visual Optical System (IVOS) was developed that incorporates an optical setup and an image digitizing and analysis setup. This system enables automated analysis of an entire sperm- smeared slide
- using the support vector machine (SVM) classification algorithm, sperm classification as normal or abnormal was achieved based on sperm head morphology features obtained by extracting contour lines



Computer software (SiD) assisted real-time single sperm selection associated with fertilization and blastocyst formation

2022



BIOGRAPHY

Dr Chavez-Badiola graduated with honours from medical school in 1999. He is Medical Director and Founder of New Hope Fertility Mexico (2009), and Founder of IVF 2.0 LTD. His research interests include the meiotic spindle, the fertilization process and th applications of artificial intelligence in reproductive medicine.

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- 383 individual spermatozoa were retrospectively analysed from a dataset of 78 ICSI-assisted reproductive technology cycles.
- SiD software computes the progressive motility parameters, straight-line velocity (VSL) and linearity of the curvilinear path (LIN), of each sperm trajectory, along with a quantitative value, head movement pattern (HMP),
- LIN and HMP were found to be significantly different between successful and unsuccessful fertilization . These results suggest that the spermatozoa in the PF set had more linear trajectories, and larger variations related to head movement patterns.
- significantly higher SiD scores were found for those spermatozoa that achieved both successful fertilization (P = 0.004) and blastocyst formation. In the present study, three positive outcomes, i.e. SI, PF and PL, were related to higher means for all assessed parameters (VSL, LIN and HMP).
- higher velocity (VSL)improved blastocyst formation.

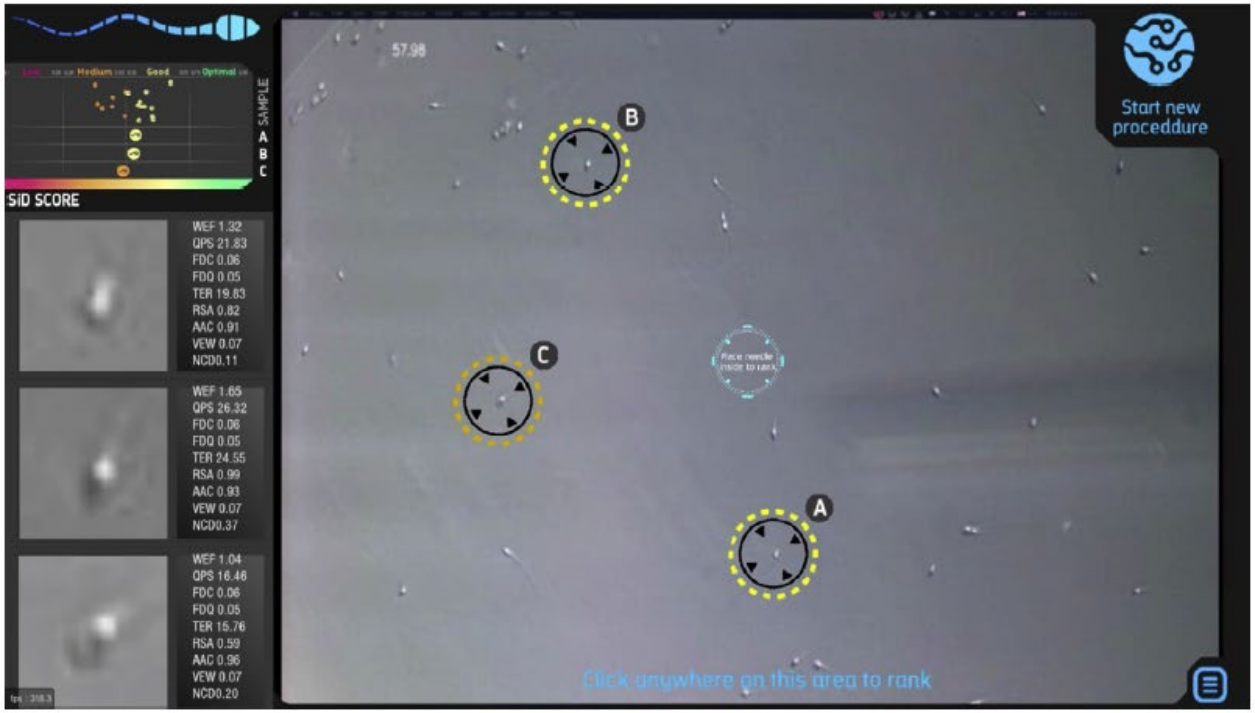

FIGURE 2 SiD's graphical user interface. Version SiD V1.0. The screen shows sperm selection and pick up shortly before intracytop injection.

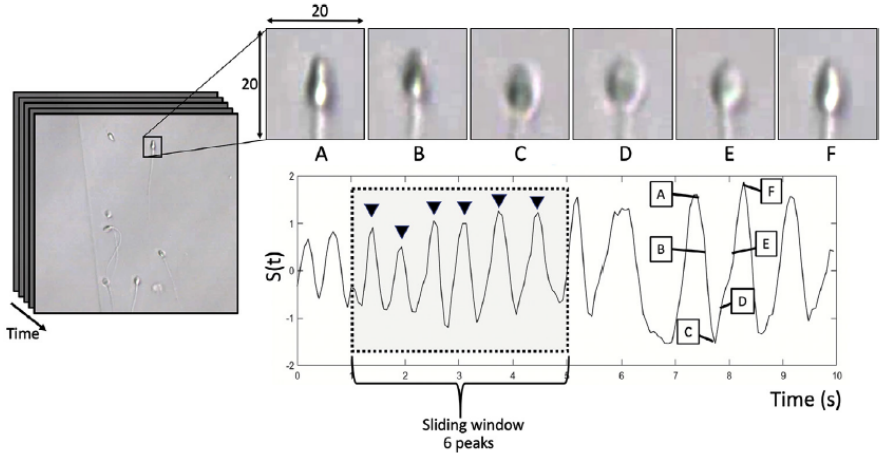

FIGURE 3 Sperm tracking and head movement pattern computation. Depiction of the sub-image generated around the centre of the detected sperm head and the correspondence between the sub-images of the sperm head across time and the mean intensity evolution signal S(t).

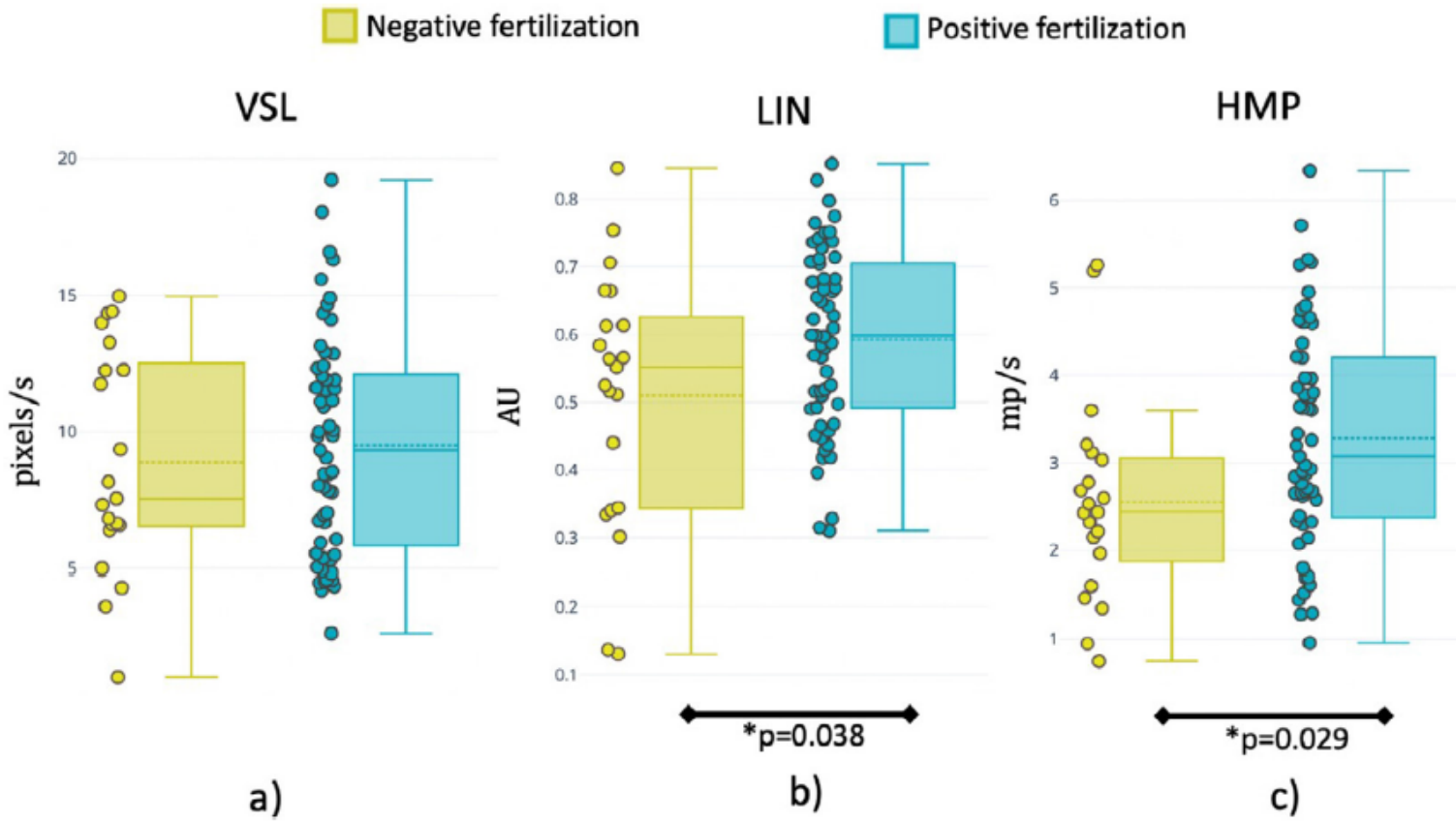

FIGURE 5 Motility parameters by fertilization outcome. Box plots showing the distribution of (a) straight-line velocity (VSL); (b) linearity of the curvilinear path (LIN); and (c) head movement pattern (HMP) values for the PF set, consisting of those selected and injected spermatozoa with a positive fertilization outcome (defined as the presence of two pronucei), and for the NF set, consisting of those spermatozoa with a negative fertilization result. *, significant difference between the means of the two sets using a Student's t-test.

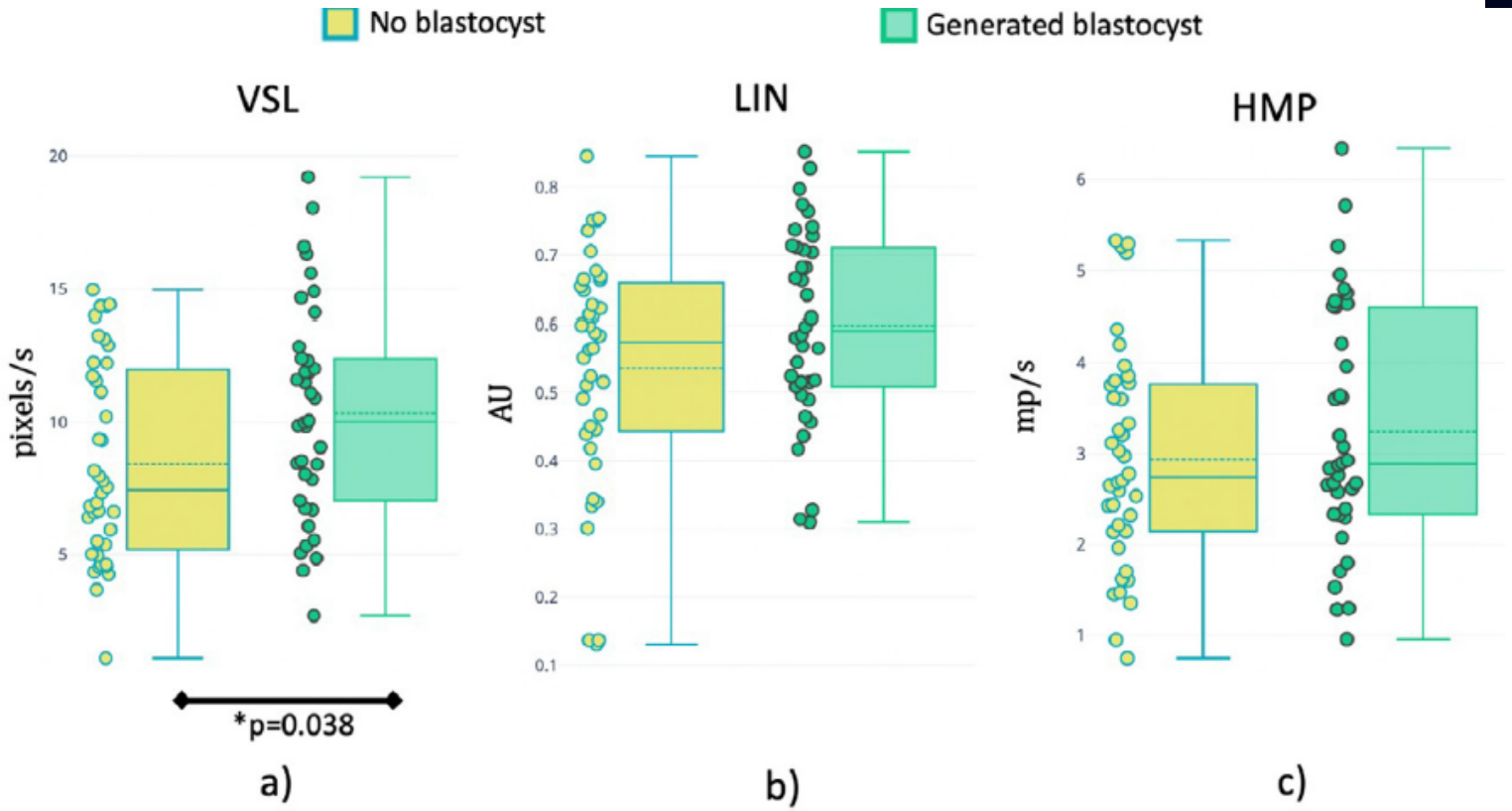

FIGURE 6 Motility parameters by blastocyst formation outcome. Box plots showing the distribution of (a) straight-line velocity (VSL); (b) linearity of the curvilinear path (LIN); and (c) head movement pattern (HMP) values for the PL set, consisting of the selected and injected spermatozoa that were associated with blastocyst generation, and the NL set, consisting of the injected spermatozoa that did not result in the generation of a blastocyst. *, significant differences between the distribution of the two sets using a Mann–Whitney U-test.

Thanks for your attention