Zymōt

Sperm separation device

Brussels IVF

De Munck Neelke

Disclaimer

Consultant for Cooper

Brussels IVF

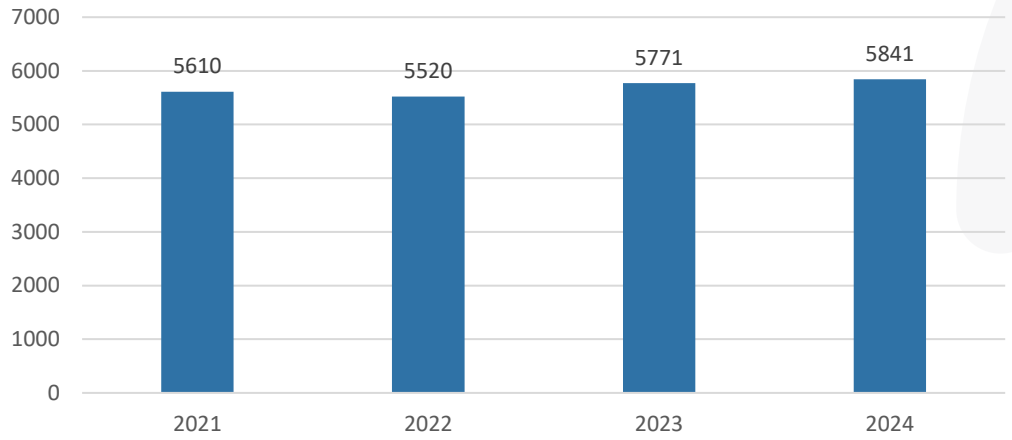
https://www.brusselsivf.be/

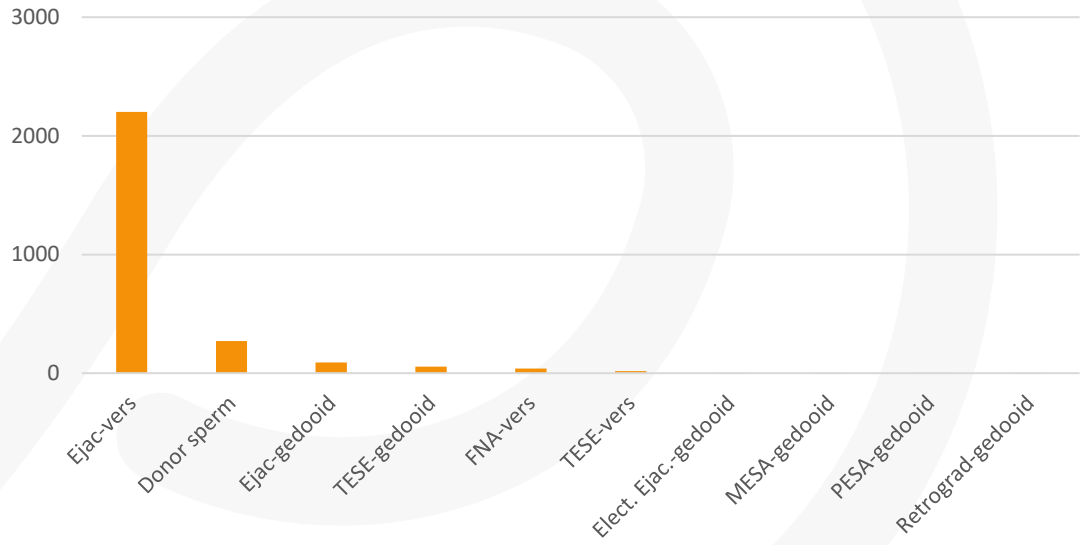
Brussels, Jette

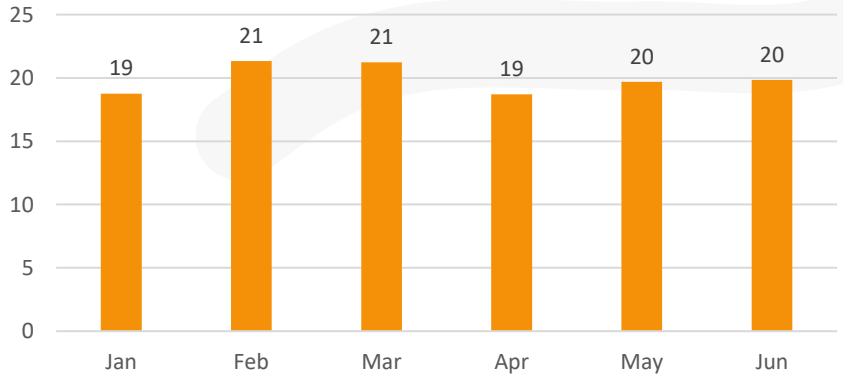
University hospital

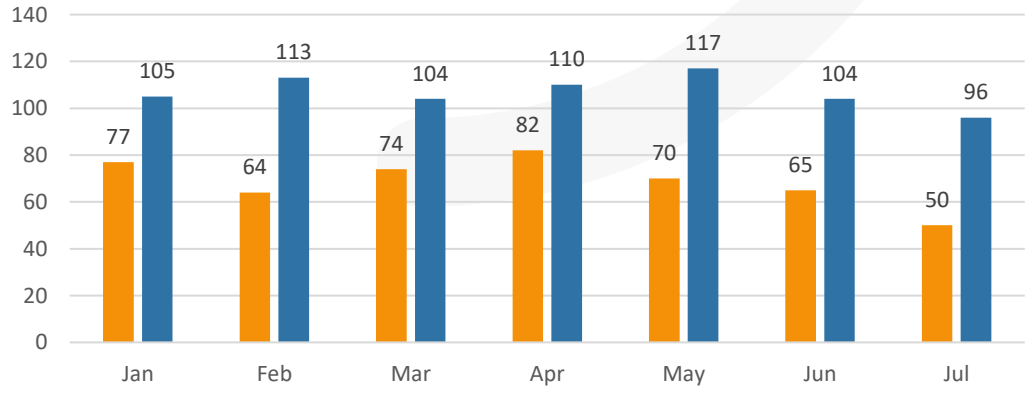
Brussels IVF

OPU, sperm samples, Nr of transfers, inseminations



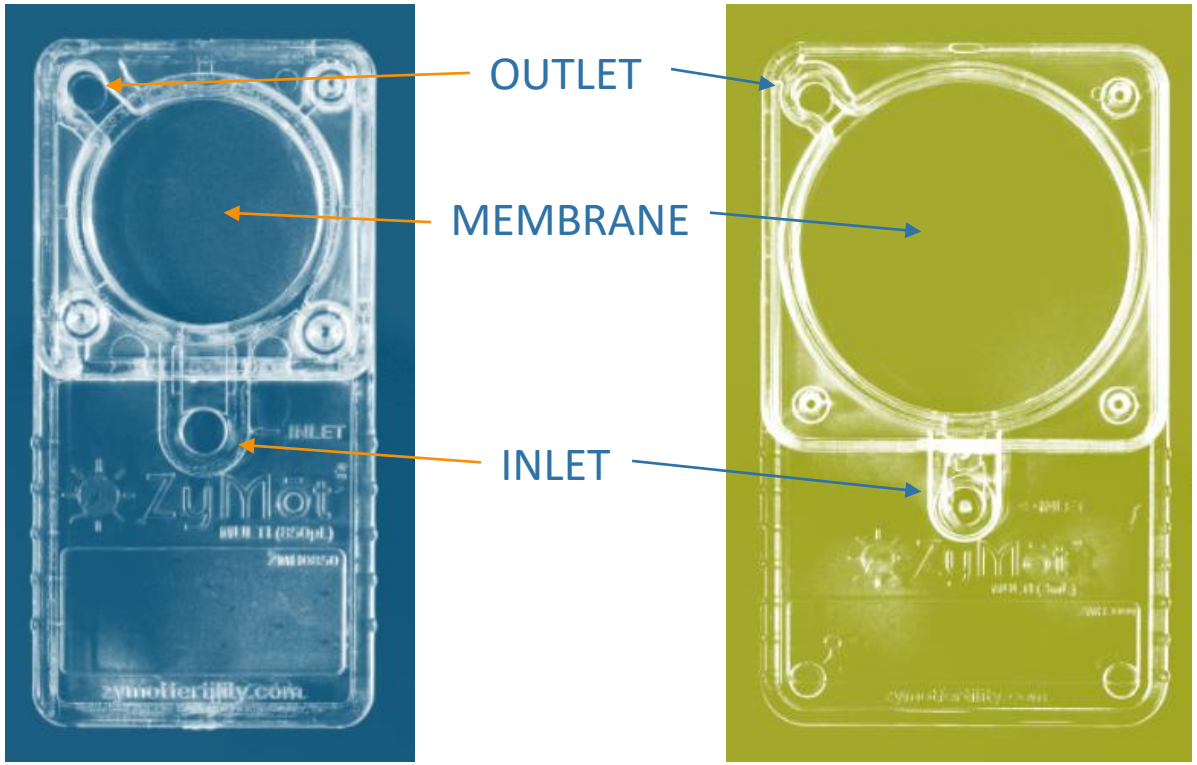






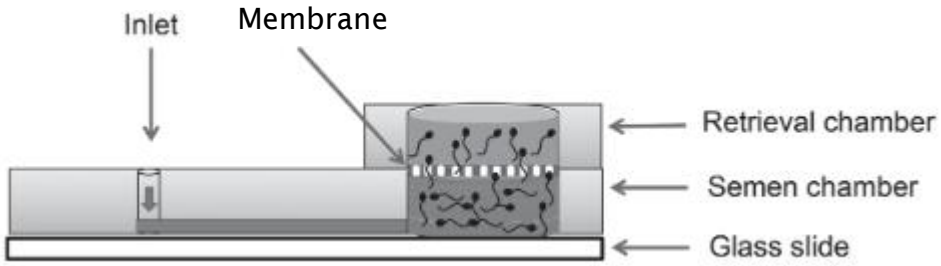
Zymōt

The device



850 µl

3 mL



850 µl device

- IN: 850 µl
- OUT: 500 µl

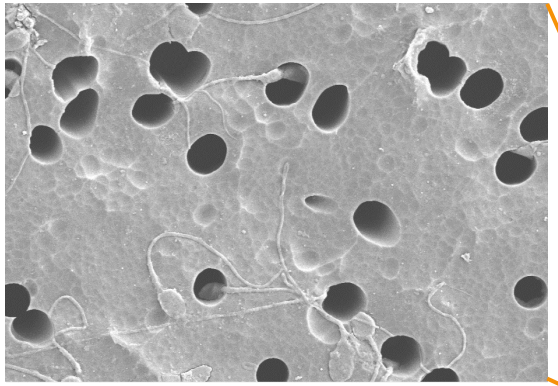
3 mL device

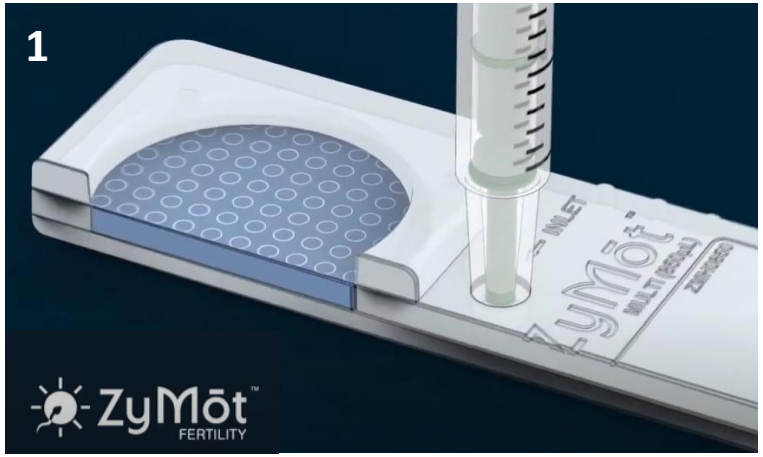
- IN: 3 mL
- OUT: 1-1.5 mL

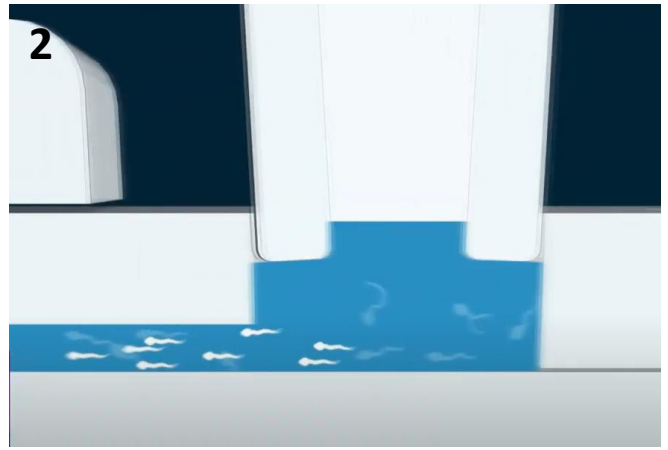
Zymōt

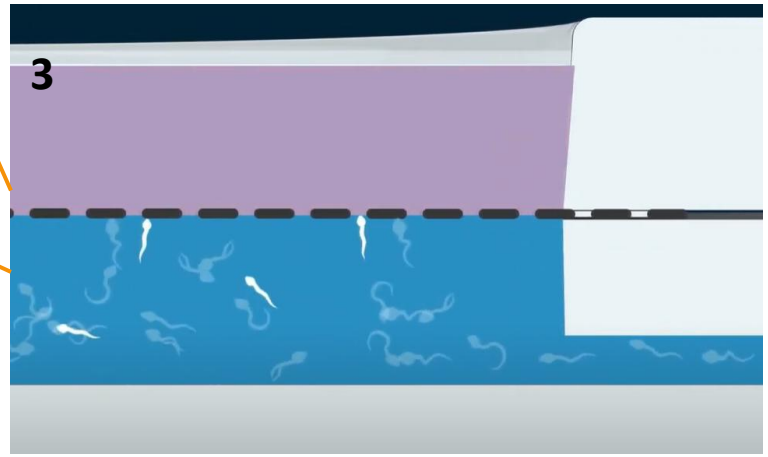
The device

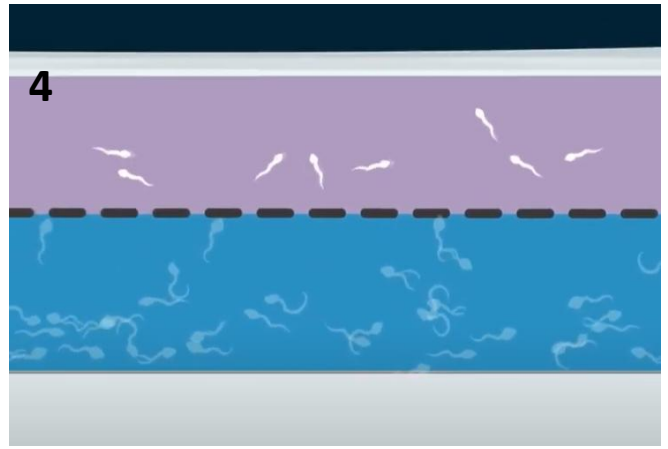
1. Apply sperm
2. Sperm sorting (30')
3. Aspirate selected sperm











Zymōt

What is known already?

- Centrifugation or DGC
- DNA fragmentation/genomic integrity
- ROS/cellular stress
- Fertilization/blastulation
- Embryo euploidy
- Outcome
- IUI

Zymōt

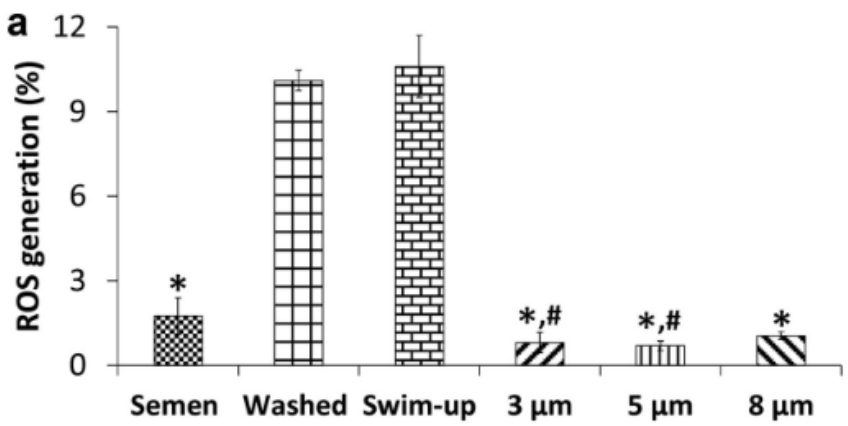
What is known already?

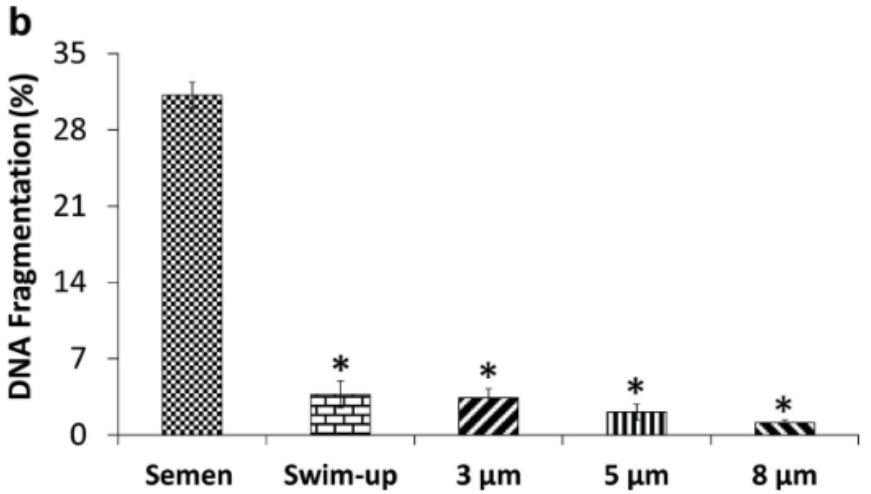
Materials Views
www.MaterialsViews.com

ADVANCED HEALTHCARE MATERIALS
www.advhealthmat.de

Selection of Functional Human Sperm with Higher DNA Integrity and Fewer Reactive Oxygen Species

*Waseem Asghar, Vanessa Velasco, James L. Kingsley, Muhammad S. Shoukat, Hadi Shafiee, Raymond M. Anchan, George L. Mutter, Erkan Tüzel, and Utkan Demirci**





- Reduced ROS
- Reduced DNA fragmentation

Zymōt

What is known already?

Abstract citation ID: deac107.078
P-082 Microfluidic-based device selects sperm with less DNA damage and higher motility, what else?

M.L. Pardiñas Garcia[1], J.M. De los Santos[2], T. Viloria[2], D. Ortega-Jaen[1], A. Martin[1], M.J. De los Santos[2]

[1]IVI Foundation-IIS La Fe, Research and Innovation, Valencia, Spain
[2]IVIRMA Valencia, IVF Laboratory, Valencia, Spain

Study question: Does the microfluidic-based sperm selection device ZyMōt improve sperm parameters and other laboratory key performance indicator (KPI) values compared to the conventional swim-up method?

Summary answer: The microfluidic-based sperm selection device ZyMōt selects sperm with lower DNA fragmentation and higher motility than conventional swim-up method in intracytoplasmic sperm injection (ICSI) cycles.

What is known already: Elevated levels of sperm DNA fragmentation (SDF) in semen samples have been associated with poor embryo development and low pregnancy rates. SDF refers to breaks in the sperm's genetic material, mainly due to defects during spermatogenesis and other factors such as reactive oxygen species that are favored by centrifugation. Conventional sperm selection methods, by integrating centrifugation into their protocols, have become ineffective in selecting sperm with low SDF. In order to solve this problem and improve reproductive outcomes, microfluidic-based devices such as ZyMōt have been designed to avoid centrifugation and select spermatozoa with low SDF.

Study design, size, duration: Prospective, experimental, single-center study conducted from June to December 2021. A total of 14 couples with ≥ 10 retrieved oocytes were recruited for an intra-patient comparison. Semen sample was split and processed for ICSI by the conventional swim-up method or by the microfluidic-based device ZyMōt. Each fraction was used to fertilize half of the total number of oocytes retrieved. SDF index, semen parameters, useful blastocyst rate, fertilization rate and morphokinetic variables were observed.

Participants/materials, setting, methods: Oocytes retrieved were from own (n = 96) and donation cycles (n = 93). From each patient, the cohort of oocytes was divided into two groups: 1) inseminated with spermatozoa selected by swim-up and 2) inseminated with spermatozoa selected by ZyMōt 850 µL device. Embryo evaluation and development were then followed by time-lapse monitoring using EmbryoScope. Sperm chromatin dispersion (SCD) assay was used to measure SDF, analyzed by ImageJ. Each treatment followed routine protocol established in the clinical practice.

Main results and the role of chance: SDF index was significantly lower in ZyMōt group in comparison with swim-up group (10% vs 20%), indicating a better selection by the ZyMōt of sperm with less DNA breaks. Additionally, ZyMōt group also presented a significantly greater number of spermatozoa with progressive motility (96.9% vs 95.4%). In contrast, useful blastocyst rate showed a slightly, but not significantly, increment in ZyMōt group compared to swim-up group (53.2% vs 46.6%). No significant differences in fertilization rate or sperm recovery rate were observed between groups. Regarding morphokinetic parameters, timing variables from first cell division to blastocyst stage (t2-tB) showed no significant correlation with ZyMōt group contrasted with swim-up. Blastocysts were evaluated and a value was assigned with respect to their quality (A to D). There was a higher number of embryos with A grade in ZyMōt group and a higher number of embryos with D grade in swim-up group. The annotated variables were assessed using paired *t*-test and P value <0.05 was considered statistically significant.

Limitations, reasons for caution: The impact on reproductive outcomes may vary depending on whether the breakage is single- or double-stranded, however, SCD is not able to distinguish between them. These, together with the oocyte's ability to repair sperm damage, lead us to the explanation for the non-significance on embryo quality.

Zymōt

What is known already?

Human Reproduction, Vol.37, No.7, pp. 1406–1413, 2022
Advance Access Publication on May 6, 2022 https://doi.org/10.1093/humrep/deac099

human reproduction ORIGINAL ARTICLE *Andrology*

Microfluidic preparation of spermatozoa for ICSI produces similar embryo quality to density-gradient centrifugation: a pragmatic, randomized controlled trial

Molly M. Quinn [1,*], Salustiano Ribeiro[2], Flor Juarez-Hernandez[2], Rhodel K. Simbulan[2], Liza Jalalian[2], Marcelle I. Cedars[2], and Mitchell P. Rosen[2]

Table II Embryo data following ICSI with sperm prepared by microfluidic separation or density-gradient centrifugation.

	*Control (n = 140)	Microfluidic processing (n = 157)	P-value[a]	Absolute difference 95% CI[d]
Fertilization rate 2PN/MII %	79.4 (19.4)	75.2 (17.8)	0.055	4.2 (−0.1, 8.4)
High-quality D3 embryo rate[b] (high quality/2PN) %	66.0 (25.8)	68.0 (30.3)	**0.541**	**−2.0 (−8.5, 4.5)**
High-quality D3 embryos #	6.9 (5.6)	7.2 (5.6)	0.687	−0.3 (−1.5, 1.0)
High-quality blastocyst rate[c] (high quality/2PN) %	37.4 (25.4)	37.4 (26.2)	0.985	−0.6 (−6, 5.9)
High-quality blastocyst #	4.1± 3.7	4.1± 3.8	0.940	−0.0 (−0.9, 0.8)

Data are mean (SD).
[a]Student's *t* test.
[b]6c or greater <10% fragmentation, symmetry: even/slightly uneven.
[c]Expansion grade 3 or greater, A/B for inner cell mass and trophectoderm.
[d]95% CI for difference in means.
*Density-gradient centrifugation.
2PN, two pronuclei, MII, metaphase II.

Zymōt

What is known already?

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Table III Embryo transfer outcome data.

	*Control	Microfluidic processing	P-value[a]	Absolute difference 95% CI
Clinical pregnancy rate (per ET)	78/136 = 57.4%	82/159 = 51.6%	0.321	5.8 [−5.6, 17.2]
Ongoing pregnancy rate (per ET)	60/136 = 44.1%	70/159 = 44.0%	0.987	0.1 [−11.3, 11.5]
Clinical pregnancy rate (per patient analysed in ITT**)	78/140 = 55.7%	82/157 = 52.2%	0.445	3.5 [−7.8, 14.8]
Ongoing pregnancy rate (per patient analysed in ITT)	60/140 = 42.9%	70/157 = 44.6%	0.764	−1.7 [−13.0, 9.6]

[a]Chi-square.
*Density-gradient centrifugation.
**Intention to treat analysis.
ET, embryo transfer.

STUDY QUESTION: Does processing of spermatozoa for IVF with ICSI by a microfluidic sperm separation device improve embryo quality compared with density-gradient centrifugation?

SUMMARY ANSWER: Patients randomized to microfluidic sperm preparation had similar cleavage- and blastocyst-stage embryo quality and clinical and ongoing pregnancy rates to those who underwent standard sperm processing for IVF with ICSI.

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What is known already?

P-65 6:30 AM Tuesday, October 19, 2021

DOES MICROFLUIDIC SPERM SORTING IMPROVE EMBRYO DEVELOPMENT AND EUPLOIDY RATES IN PATIENTS UNDERGOING ICSI? Alex Robles, M.D.,[1] Evan Akiva Reshef, MD,[1] Robert W. Prosser, MSc,[1] Eric J. Forman, M.D.,[2] Zev Williams, M.D., PhD.[1] [1]Columbia University Fertility Center, New York, NY; [2]Columbia University Fertility Center.

OBJECTIVE: To determine if the use of the ZyMot microfluidics sperm sorting device improves embryo development and euploidy rates compared to conventional density gradient centrifugation in patients undergoing intracyoplasmic sperm injection (ICSI) with preimplantation genetic testing for aneuploidies (PGT-A).

MATERIALS AND METHODS: This was a retrospective cohort study comparing the outcomes of ICSI/PGT-A cycles that used the ZyMot device for sperm processing versus previous cycles that used density gradient centrifugation in the same cohort. As such, patients served as their own controls. Data was collected from one fertility center in New York City between April 2019 and February 2021. The primary outcome was blastocyst rate. Secondary outcomes included the average number of fertilized embryos per cycle as well as euploidy rates. The decision to use ZyMot was based on disappointing blastulation rates in the previous cycle.

RESULTS: 86 patients were identified who had a prior ICSI/PGT-A cycle using density gradient for sperm preparation followed by an ICSI/PGT-A cycle using the ZyMot microfluidics device for sperm preparation. A 1:1 comparison was performed that demonstrated a statistically significant difference in the average number of blastocysts as well as blastocyst rates obtained in the Zymot cycles vs the density gradient cycles (3 vs. 2, P=0.014) and (40.2% vs. 29.2%, P=0.02) respectively. There was also a statistically significant difference in the euploidy rate in the ZyMot cycles compared to the density gradient cycles (43% vs. 33%, P=0.016). The mean age of the female patients was 37.7.

	ZyMot Cycle	Density Gradient Cycle	P value
Total # of Cycles	86	86	NS
Mean # of Eggs Retrieved	12.6	12.2	NS
Mean # of MII Oocytes	9.8 (77.4%)	9 (74%)	NS
Mean # of 2PN Embryos	7.3 (74.8%)	6.8 (75.5%)	NS
Mean # of Blastocysts	3 (40.2%)	2 (29.2%)	P = 0.014
Total # of Euploid Embryos	103 (43%)	55 (33%)	P= 0.016

CONCLUSIONS: In patients with a previous ICSI/PGT-A cycle using density gradient for sperm selection, a subsequent ICSI cycle using the ZyMot microfluidics device yielded improved blastulation rates and higher euploidy rates. These results were true despite a similar number of mature eggs , and normally-fertilized embryos per cycle. Larger prospective studies are needed to validate the findings.

IMPACT STATEMENT: The Zymot microfluidics device may improve blastulation and euploidy rates in patients who previously had unsuccessful ICSI/PGT-A cycles that used conventional density gradient centrifugation for sperm preparation.

Zymōt

What is known already?

Comparison of microfluid sperm sorting chip and density gradient methods for use in intrauterine insemination cycles

Funda Gode, M.D.,[a,b] Taylan Bodur, M.D.,[b] Fatma Gunturkun, Ph.D.,[c] Ali Sami Gurbuz, M.D.,[d] Burcu Tamer, M.Sc.,[b] Ibrahim Pala, M.Sc.,[b] and Ahmet Zeki Isik, M.D.[b,e]

TABLE 2

Sperm parameters of the study groups before and after sperm preparation.

Characteristic		Microchip	Gradient	SMD	*P* value
Baseline	Sperm concentration (10^6/mL)	49.74 ± 34.13	52.66 ± 34.60	0.09	.51
	Sperm motility (%)	52.50 ± 15.46	55.30 ± 16.53	0.17	.17
	TPMSC	35.96 ± 37.69	70.66 ± 61.65	0.67	.00[a]
After preparation	Sperm concentration (10^6/mL)	16.79 ± 13.21	34.20 ± 27.91	0.79	.00[a]
	Sperm motility (%)	96.34 ± 7.29	84.42 ± 10.87	1.27	.00[a]
	TPMSC	22.85 ± 21.12	18.85 ± 15.08	0.22	.09

Note: Student's *t*-test. Data were presented as mean ± standard deviation. TPMSC = total motile sperm count ($\times 10^6$).
[a] $P < .05$.

Gode. Microchip and density gradient methods. Fertil Steril 2019.

	Microchip	Gradient	P value
Pregnancy rate (%)	18.4	15.15	>0.5
Clinical pregnancy rate (%)	15.03	12.87	>0.5
Ongoing pregnancy rate (%)	15.03	9.09	>0.5

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Brussels IVF experience

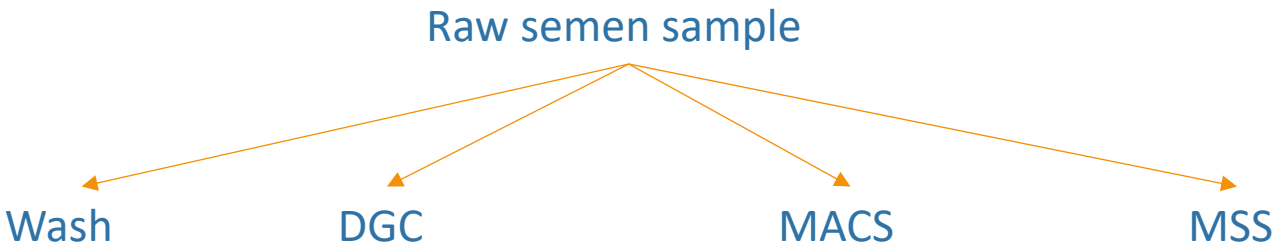
Journal of Assisted Reproduction and Genetics
https://doi.org/10.1007/s10815-024-03168-9

ASSISTED REPRODUCTION TECHNOLOGIES

Optimized sperm selection: a highly efficient device for the isolation of progressive motile sperm with low DNA fragmentation index

Ileana Mateizel[1] · Annalisa Racca[2] · Eleni Aligianni[3] · Elisa Distasi[4] · Yoni Baert[5,6] · Ingrid Segers[1] · Danijel Jankovic[1] · Celine Schoemans[1] · Koen Wouters[1] · Herman Tournaye[1] · Neelke De Munck[1]

Received: 7 December 2023 / Accepted: 3 June 2024



Analysis

- Motility
- Morphology
- Acrosome index
- DNA fragmentation
- Vitality

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Brussels IVF experience

Table 1 Patient and unprocessed semen sample characteristics

No. of samples	52
Age (years)	35.9 ± 6.8
Days of abstinence	4.1 ± 2.4
Volume (ml)	4.6 ± 1.8
Concentration ($\times 10^6$)	58.9 ± 31.2
Progressive motility (%)	58.3 ± 12.3

Results are expressed as mean ± SD

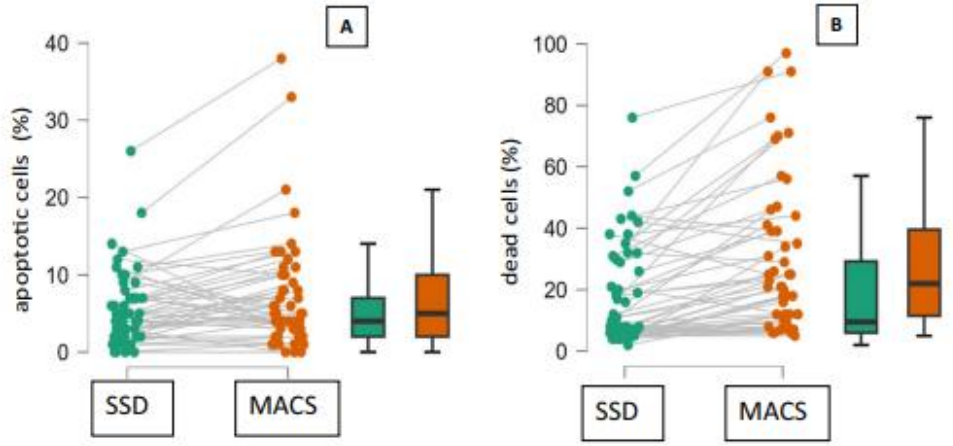

Table 2 Descriptive analysis of the effect of four different preparation techniques on concentration, progressive motility, normal morphology, AI, and DFI

	SW	DGC	MACS	SSD
Concentration ($\times 10^6$)	61.7 ± 35.4 (17.5–193.0)	13.0 ± 11.6 (0.8–68)	8.4 ± 9.2 (0.61–49.6)	15.1 ± 14.2 (1.5–69.0)
Progressive motility (%)	54.3 ± 10.6 (23–86)	74.3 ± 11.8 (38–90)	77.2 ± 12.5 (37–92)	88.6 ± 4.2 (73–96)
Normal morphology (%)	3.3 ± 2.9 (0–13)	4.1 ± 3.1 (0–13)	4.2 ± 3.7 (0–18)	5.1 ± 3.9 (0–16)
AI (%)	8.5 ± 4.9 (1–20)	9.7 ± 6 (1–30)	8.7 ± 4.9 (0–19)	10.8 ± 6.8 (1–30)
DFI (%)	6.2 ± 4.6 (0.8–26.1)	2.7 ± 3.2 (0.2–14)	2.1 ± 4.3 (0.9–20.8)	0.2 ± 0.4 (0–2.3)

Results are expressed as mean ± SD (minimum–maximum)

SW sperm wash, *DGC* density gradient centrifugation, *MACS* magnetic activated cell sorting, *SSD* sperm separation device, *AI* acrosome index, *DFI* DNA fragmentation index

Zymōt

Brussels IVF experience

Embryo quality after sperm selection using microfluidic technology or density gradient centrifugation: a sibling oocyte study

Wouters K.[1], Mateizel I.[1], Schoemans C.[1], Segers I.[1], Van Asbroeck J.[1], Jankovic D.[1], Krunic M.[1], Tournaye H.[1], De Munck N.[1]

Alpha congress 2024

Sibling oocyte study

Primary endpoint: utilisation rate

- Sample size: 253 in each arm

Inclusions:

- Ejaculated sperm
- ≥ 6MII

	DGC	Zymot	P value
Female age (y)	34.5 ± 4.4		
Partner age (y)	37.5 ± 5.5		
Sperm concentration (mil/ml)	2.9 ± 1.2	3.5 ± 5.0	0.16
Progressive motility (%A+B)	74.0 ±17.2	89.9 ± 10.4	<0.01

	DGC	Zymot	P value chi2
No of COCs = 658			
No of MII= 532	265	267	
Fertilization/MII (%)			
2PN	208 (78.5)	209 (78.3)	0.96
1PN	11 (4.2)	7 (2.6)	0.46
3PN	8 (3.0)	6 (2.2)	0.77
Deg	15 (5.7)	13 (4.9)	0.83
Not fertilized	22	22	
Day 3 EQ/2PN (%)			
Excellent+Good	159 (76.4)/2PN	161 (77.0)/2PN	0.97
Day 5 EQ/2PN (%)			
Excellent+Good	86 **(41.3)/2PN**	76 **(36.4)/2PN**	**0.30**
Utilized Embryos			
Nr Es transferred	10	10	
Nr Es cryo	94	97	
d5	*61*	*62*	
d6	*26*	*30*	
d7	*7*	*5*	
Utilization rate (%)			
Per no of MII	**39.2**	**40.1**	**0.91**
Per no of BV	**50.0**	**51.2**	**0.88**

Conclusion: Since fertilisation, embryo quality and utilisation rate are similar in both groups, the use of Zymot 850µl®device offers the advantage of eliminating the negative effects and time-consuming nature of centrifugation. Another advantage is that scheduling of semen samples can be planned more efficiently.

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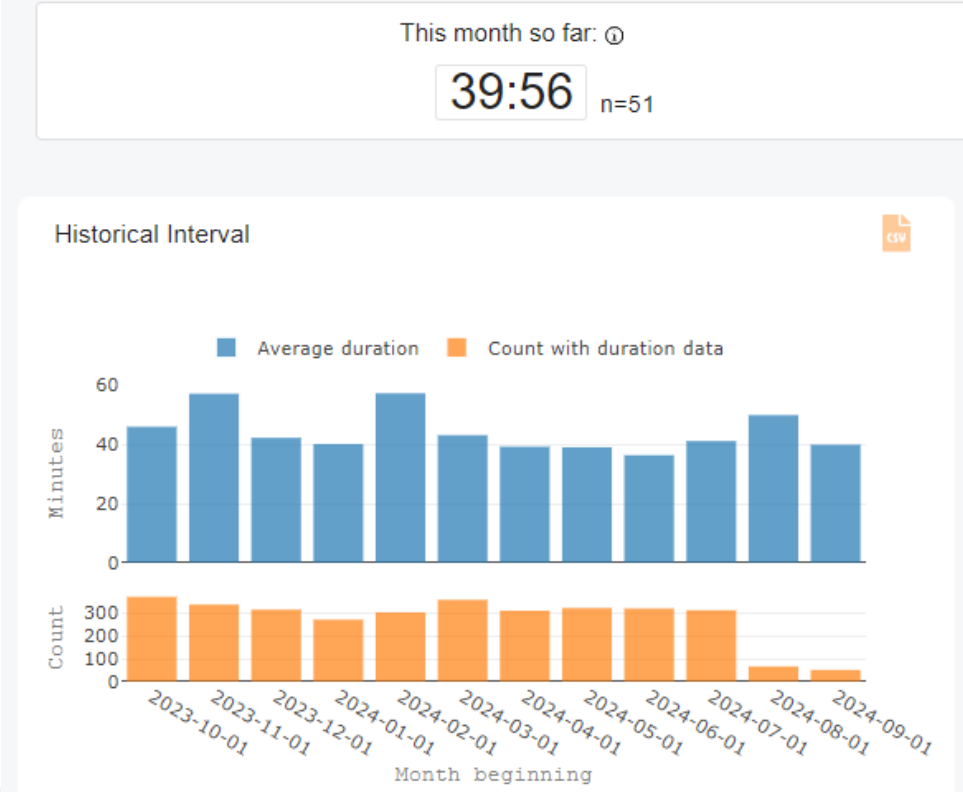
What do we win?

Monitoring work load, timings of procedures

DGC



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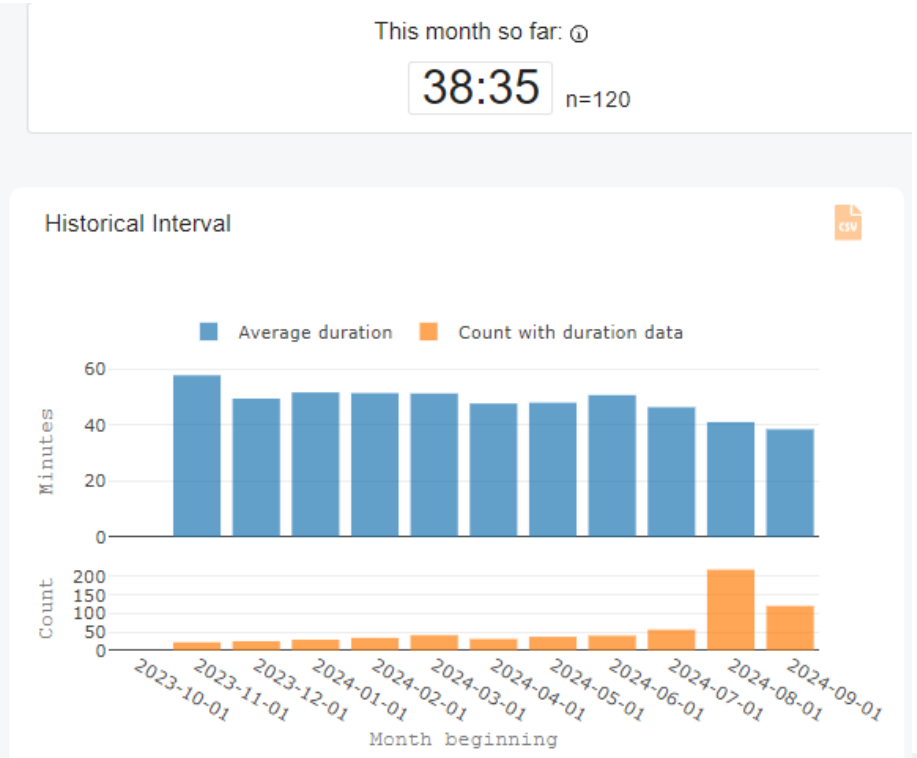
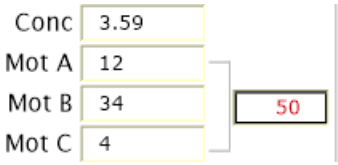
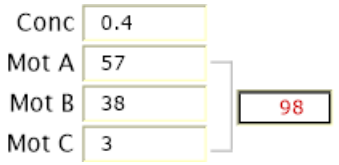
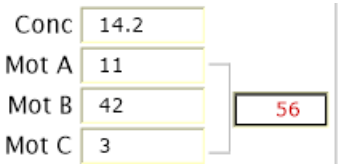
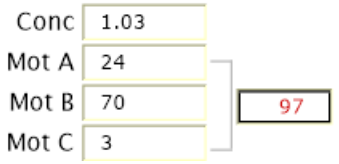
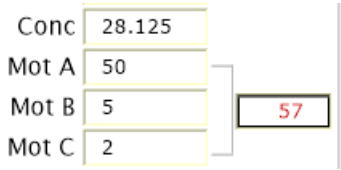
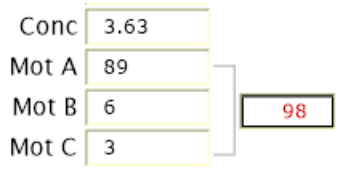
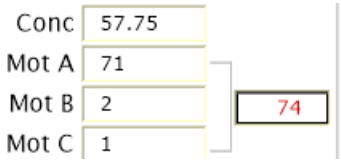
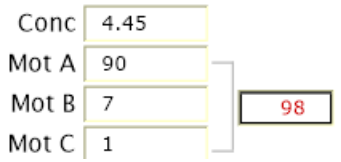


Zymōt

Recovery

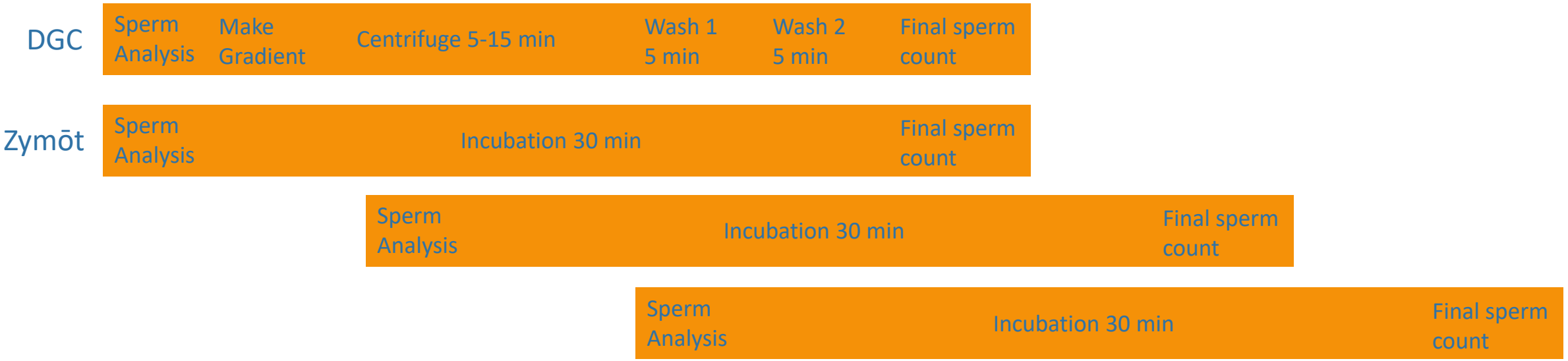
	Raw sample	After Zymot
Sample 1		
Sample 2		
Sample 3		
Sample 4		

Zymōt

How to start in the lab?

Benefits?

- Improved motility
- Improved DFI
- Improved sperm preparation time: flexibility



Zymōt

How to start in the lab?

Which patients?

- > 1 million/ml
- >10% A+B
- Frozen samples
- IUI

How to integrate this?

- Arrange schedule for sperm sample preparation
- Lab set-up:
  - Division in time and space!
  - Incubator
  - Protocol
  - Training*
  - Evaluate!

VALIDATE!

Thank you!

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