

Communication

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

Chelsey A. Leisinger^{1,*}, Glen Adaniya², Melanie R. Freeman³, Erica J. Behnke⁴, Martha Aguirre⁵, Matthew D. VerMilyea⁶ and Mitchel C. Schiewe⁷¹ Ovation Fertility, Baton Rouge, LA 70817, USA² Ovation Fertility, Carmel, IN 46032, USA; gadaniya@ovationfertility.com³ Ovation Fertility, Nashville, TN 37203, USA; mfreeman@ovationfertility.com⁴ Ovation Fertility, Cincinnati, OH 45209, USA; ebehnke@ovationfertility.com⁵ Ovation Fertility, Las Vegas, NV 89117, USA; maguirre@ovationfertility.com⁶ Ovation Fertility, Austin, TX 78731, USA; mvermilyea@ovationfertility.com⁷ Ovation Fertility, Newport Beach, CA 92663, USA; mschiewe@ovationfertility.com

* Correspondence: cleisinger@ovationfertility.com



Citation: Leisinger, C.A.; Adaniya, G.; Freeman, M.R.; Behnke, E.J.; Aguirre, M.; VerMilyea, M.D.; Schiewe, M.C. Effect of Microfluidic Sperm Separation vs. Standard Sperm Washing Processes on Laboratory Outcomes and Clinical Pregnancy Rates in an Unselected Patient Population. *Reprod. Med.* **2021**, *2*, 125–130. <https://doi.org/10.3390/reprodmed2030013>

Academic Editor: Berthold Huppertz

Received: 19 May 2021

Accepted: 13 July 2021

Published: 26 July 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: A prospective, multicenter, randomized, sibling oocyte study was conducted with 86 couples to evaluate if a microfluidic sperm separation device improved ICSI sperm selection and subsequent cycle outcomes of fertilization, blastocyst utilization, ploidy, and clinical pregnancy rate when applied to a general patient population. Patients with at least 10 metaphase II oocytes were enrolled in the study and sibling oocyte groups were split in half. One half of the oocytes underwent ICSI with the control processed sperm and the other half were injected with sperm sorted by the ZyMöt microfluidic sperm separation device. Fertilization rate was recorded and resulting blastocysts were biopsied and evaluated for ploidy status with NGS. Euploid, non-mosaic embryos were randomly selected for single embryo transfer. A total of 787 oocytes were evaluated in the ZyMöt group and 777 in the control group. No statistical differences were observed between ZyMöt and control processing methods in any of the study outcomes evaluated. It is possible that the selection of normal, progressive sperm for ICSI, and the repair capacity of oocytes are sufficient to promote normal embryonic development in the general infertility population.

Keywords: sperm selection; microfluidic chip; ZyMöt; sperm wash; fertilization; blastocyst; euploid; sibling oocyte; pregnancy; unselected population

1. Introduction

The use of intracytoplasmic sperm injection (ICSI) has increased steadily since its successful application in 1992. In recent years, ICSI has been utilized in greater than half of the assisted reproductive cycles conducted each year, with many programs applying 100% ICSI cycles [1]. The selection of normal, highly progressive sperm for ICSI may result in improved fertilization and lower miscarriage rates [2]. Improving these parameters can have a significant effect on the outcome of a cycle for patients seeking fertility treatment. It has also been reported that total motile sperm count has a greater predictive value of high-quality embryo production, pregnancy results and the odds of miscarriage compared to any other WHO 2010 cut-off values [2].

Several methods are available for the isolation of motile sperm from a semen sample for ICSI. The most commonly employed sperm selection methods include density gradient centrifugation (DGC), direct swim-up, and swim-up wash. Each of these methods has their own limitations. The swim-up methods isolate a highly motile sperm population, but low numbers are often obtained [3,4]. Density gradient and swim-up wash methods employ centrifugation to form sperm pellets for the selective isolation of motile sperm

from debris and the removal of seminal plasma by washing, respectively [3]. However, the application of centrifugation has been shown to increase the production of reactive oxygen species (ROS) [5], which can lead to sperm plasma membrane damage and increased DNA damage [6–9]. Some reports indicate that increased DNA damage in human sperm has been associated with an increased incidence of childhood cancer [10,11]. In turn, there has been renewed interest in the investigation of alternative methods to isolate motile sperm that result in minimal ROS production and DNA damage.

Microfluidics is an emerging technology with applications in fertility, forensics, and diagnostics [12]. Sperm selection utilizing microfluidics has shown promise in selecting highly motile sperm with reduced ROS and DNA fragmentation for use in ART procedures [13,14]. Studies applying microfluidic sperm selection for ICSI have reported improved laboratory and clinical outcomes, such as the likelihood to produce an increased number of high-quality blastocysts, euploid blastocysts, and surplus high-quality blastocysts to freeze [15,16]. The present study was designed to evaluate the possible differences in fertilization, blastocyst utilization, ploidy, and clinical pregnancy rate when applying the ZyMöt microfluidic sperm separation device for sperm selection versus traditional processing methods to a general IVF patient population.

2. Materials and Methods

2.1. Participants

A prospective, randomized, multicenter, sibling oocyte study was carried out in 86 consenting couples with at least 10 metaphase II (MII) oocytes at retrieval, without regard to the DNA fragmentation status of the male partner. For male patients, only those with severe male factor (<1 million motile sperm/mL in the ejaculate) were excluded from the study. This study presented no additional risk to patients or gametes and was classified by the Research Ethics Committee as exempt from IRB approval 20 December 2019, study #OF191218B. Sperm to be used for ICSI underwent a split processing treatment: (1) a control processing procedure (DGC or swim-up wash procedure) and (2) treatment processing with an 850 µL ZyMöt microfluidic sperm separation device (DxNow, Inc., Gaithersburg, MD, USA).

2.2. Sperm Processing Methods

The control processing procedures were carried out according to standard protocols at each of four IVF centers. One center employed a swim-up wash procedure for 23 patients. Semen samples were centrifuged for 10 min in insemination media (IM), the supernatant was removed, the pellet resuspended in IM and centrifugation repeated. The supernatant was removed, and the pellet resuspended in 0.3 mL of IM. The sample was pipetted underneath 1–2 mL of IM in an organ culture dish and allowed to swim up for 15–60 min at 37 °C, 5% CO₂. After incubation, 1 mL of supernatant was removed and placed in a sterile culture tube until it was used for ICSI. Three centers applied DGC procedures for 63 patients. Protocols at the three centers included layering the semen sample over a gradient and centrifuging for 14–20 min. The pellet was then placed in sperm wash medium, centrifuged for 4–10 min, and resuspended in an appropriate volume for ICSI use.

An 850 µL ZyMöt device was used for treatment processing in each lab. Briefly, 850 µL of raw semen was added to the inlet and 750 µL of sperm wash was placed in the outlet port and overlaid on the device. The ZyMöt device was incubated at 37 °C for 30 min and 500 µL of medium with selected sperm was aspirated from the outlet port. All laboratories participating in the study received training from the manufacturer for the use of the ZyMöt device prior to the study.

2.3. Assessment of Laboratory Outcomes

Oocyte maturity was assessed after cumulus cells were removed by hyaluronidase. Patients with at least 10 MII oocytes were enrolled in the study. The MII oocytes of each patient were split equally into two groups for ICSI. Half of the oocytes underwent ICSI with the control processing procedure sperm and the other half underwent ICSI with sperm

sorted by the ZyMöt treatment processing group sperm. If the patient had an odd number of MII oocytes, the extra one was assigned to the ZyMöt treatment processing group.

Fertilization, embryo culture and biopsy were carried out using the standard protocols at each center. Fertilization was assessed on day 1 as zygotes possessing two pronuclei. All oocytes subjected to ICSI were evaluated for fertilization. All successfully fertilized zygotes were cultured to the blastocyst stage. Blastocyst formation was evaluated on days 5–7 using Gardner criteria [17]. Blastocysts of predominantly fair to good quality ($\geq 2\text{BB}$) underwent trophectoderm biopsy. Usable blastocysts that were produced and not subjected to biopsy were cryopreserved. Biopsy samples were sent to the same genetics laboratory (Ovation Fertility Genetics, Nashville, TN, USA) and evaluated for ploidy status using next-generation sequencing. Only euploid, non-mosaic embryos were randomly selected for single embryo transfer according to gender preference by patient and the best embryo quality grade irrespective of sperm treatment. Clinical pregnancy was defined as the presence of an intrauterine implantation sac with the detection of a fetal heartbeat on ultrasound.

2.4. Statistical Analysis

Statistical significance for all data analyses was set at $p < 0.05$. Fertilization, blastocyst utilization, ploidy, and clinical pregnancy rates within and between clinics were analyzed by Chi square or Fisher's exact test where appropriate, according to the variables being evaluated and the number of sample observations. Differences in fertilization, blastocyst utilization, and ploidy results between the treatment and control sperm processing methods were analyzed using a paired *t*-test. All statistical analyses were carried out using InStat (Graph Pad Software, San Diego, CA, USA).

3. Results

A total of 1564 MII oocytes from 86 female patients (mean age: maternal 34.7 ± 4.6 ; paternal 36.1 ± 5.4) were enrolled in the study. A total of 787 MII oocytes were allocated to the ZyMöt group and 777 were allocated to the control group. In the ZyMöt group, 296 blastocysts were produced and 283 of these blastocysts were analyzed for ploidy status. The control group produced 282 blastocysts and 265 of these blastocysts were analyzed for ploidy status.

Not surprisingly, some variations in laboratory outcomes were noted between centers enrolled in the study, yet there were no differences observed in any parameter evaluated when comparing treatment versus control methods within and between groups ($p > 0.05$). As a result, data were pooled for analysis. Combined laboratory results across centers did not exhibit any significant differences in embryo outcomes when comparing ZyMöt to control processing methods ($p > 0.05$) (Table 1).

Table 1. Comparison of embryo development outcomes in sibling oocytes subjected to ICSI using ejaculates split processed with ZyMöt microfluidic sperm separation treatment or control sperm processing procedures. No significant differences between treatments were observed, $p > 0.05$.

Laboratory Outcomes	ZyMöt		Control	
	Rate	%	Rate	%
Fertilization (2PN/MII)	604/787	77	592/777	76
Blastocyst (Blast/2PN)	296/604	49	282/592	48
Euploidy	165/283	58	151/265	57
Mosaicism	25/283	9	31/265	12
Clinical pregnancy	22/39	56	25/33	76

Interestingly, the rate of clinical pregnancies when transferring euploid embryos derived from the control group was 20% higher compared to ZyMöt, but this difference was not significant ($p = 0.0886$) (Table 1).

A higher blastocyst production was exhibited when employing a swim-up wash compared to DGC control processing ($p = 0.002$) (Table 2). No significant difference was observed in any of the other study outcomes evaluated when comparing the two control processing methods ($p > 0.05$) (Table 2).

Table 2. Comparison of ZyMöt treatment and control processing methods density gradient centrifugation (DGC) and swim-up wash in embryo development outcomes.

Laboratory Outcomes	ZyMöt		DGC		Swim-Up Wash	
	Rate	%	Rate	%	Rate	%
Fertilization (2PN/MII)	604/787	77	434/574	76	158/203	78
Blastocyst (Blast/2PN)	296/604 ^a	49 ^a	190/434 ^a	44 ^a	92/158 ^b	58 ^b
Euploidy	165/283	58	99/185	54	52/80	65
Mosaicism	25/283	9	20/185	11	11/80	14

^{a, b} Different superscripts denote significant difference within rows, $p < 0.05$.

4. Discussion

The present study is the first multicenter, prospective, randomized controlled sibling oocyte study to evaluate the effect of the ZyMöt microfluidic sperm sorting device on fertilization, blastocyst utilization, euploidy, and clinical pregnancy rates when compared to control sperm processing methods. The present study was applied to a general, unselected population of IVF patients with minimal exclusion criteria.

The present study observed no significant differences in blastocyst production, aneuploidy, or clinical pregnancy when comparing microfluidic sperm selection to control methods. Similarly, the only other sibling oocyte study available in the literature reported no difference in fertilization, blastocyst, or live birth rate comparing microfluidic sperm sorting to swim-up processing [18]. It is well established that DNA repair mechanisms exist within the oocyte and these mechanisms can repair a multitude of DNA anomalies that may be present due to damaged sperm [19–22]. Thus, the results of the present study and the data reported utilizing sibling oocytes may indicate that oocyte repair mechanisms are adequate in the study populations to offset DNA damage resulting from conventional sperm selection methods [18].

It has been repeatedly demonstrated that the application of a microfluidic device for sperm sorting results in sperm with increased motility and significantly reduced DNA fragmentation [13,14,23–26]. Considering these results, only a few studies have evaluated the effects of microfluidic sperm sorting on laboratory and cycle outcomes [4,15,16,18]. It is important to note that each of these studies employed the same microfluidic device as the present study [4,15,16,18]. Three of these studies describe the use of the Fertile Chip and in markets outside of the United States, ZyMöt devices were known under the “Fertile” brand [4,16,18]. In April 2020, Fertile became known worldwide as the ZyMöt device. The ZyMöt device is simple to use and requires significantly less technician training than conventional sperm processing methods. The device yields a highly motile population of sperm with minimal technician effort.

The results of other studies investigating the use of microfluidic sperm sorting to conventional methods are varied. One study conducted utilizing a microfluidic device found an increased total number of high-quality embryos produced in the microfluidic sperm sorted group compared to a conventional swim-up [16]. Like the present study, no differences in fertilization, clinical pregnancy, or live birth rate were observed [16]. Two studies have evaluated the effect of microfluidic sperm sorting on sperm DNA fragmentation and cycle outcome in patients with recurrent IVF failure [4,15]. One study evaluated the use of microfluidic sperm sorting in couples with disrupted sperm DNA and recurrent IVF failure and found that microfluidic sperm sorting decreased the sperm DNA fragmentation and increased the chances of obtaining a euploid conceptus [15]. A disadvantage of this study is the small number of patients enrolled, which led to a small number of embryos

and transfers for accurate statistical analysis [15]. Another study had a greater number of patients enrolled and found reduced sperm DNA fragmentation but no difference in fertilization or pregnancy rates in first-time IVF patients [4]. While microfluidic sperm sorting increased fertilization rates overall in that study, no effect on pregnancy rate was observed, and neither blastocyst nor euploid rates were examined [4].

Our study does have some limitations. There were minimal exclusion criteria for both the male and female partners to evaluate the application of microfluidic sperm sorting on a general unselected patient population. While our study population was quite broad, this did allow for a larger sample size than some studies that have evaluated microfluidic sperm sorting and laboratory outcomes. Another limitation was that the sperm DNA fragmentation status was unknown for the sperm used in this study. The improvement in euploidy status noted in previous studies when ZyMöt was applied to male factor patients with high degrees of sperm DNA fragmentation may have been lost in our overall study population.

In conclusion, the goal of the present study was to evaluate the application of a microfluidic sperm separation device in an unselected patient population. Our data suggest that the general application of sperm selection using the ZyMöt sperm separation device does not improve any of the outcome measures of fertilization, blastocyst utilization, ploidy, and clinical pregnancy rate. Further investigation is warranted to identify if there is truly a benefit in the application of microfluidic sperm sorting to couples with elevated DNA fragmentation and/or previously failed ICSI cycles.

Author Contributions: C.A.L., G.A., M.R.F., E.J.B., M.A., M.D.V. and M.C.S. contributed to study conception and design. C.A.L., G.A., M.R.F. and E.J.B. contributed to data acquisition and analysis. C.A.L. wrote the manuscript. G.A. and M.C.S. assisted in preparation of manuscript and figures for publication. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was approved by the Research Ethics Committee of Ovation Fertility, study # OF191218B. Date of approval 18 December 2019.

Informed Consent Statement: Patient consent was waived as this study presented no additional risk to patients or gametes and was classified by the Ovation Research Ethics Committee as exempt from IRB approval.

Acknowledgments: Discounted purchasing rates were provided by the ZyMöt distributor DxNow, Inc.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ICSI	Intracytoplasmic sperm injection
WHO	World Health Organization
DGC	Density gradient centrifugation
ROS	Reactive oxygen species
DNA	Deoxyribonucleic acid
ART	Assisted reproductive technology
IVF	In vitro fertilization
MII	Metaphase II
IRB	Institutional Review Board
IM	Insemination media

References

1. Centers for Disease Control and Prevention. Assisted Reproductive Technology National Summary Report 2016. Available online: <https://www.cdc.gov/art/pdf/2016-report/ART-2016-National-Summary-Report.pdf> (accessed on 8 September 2020).
2. Borges, E., Jr.; Setti, A.S.; Braga, D.P.A.F.; Figeira, C.S.; Iaconelli, A., Jr. Total motile sperm count has a superior predictive value over the WHO 2010 cut-off values for the outcomes of intracytoplasmic sperm injection cycles. *Andrology* **2016**, *4*, 880–886. [[CrossRef](#)] [[PubMed](#)]

3. Schuster, T.G.; Cho, B.; Keller, L.M.; Takayama, S.; Smith, G.D. Isolation of motile spermatozoa from semen samples using microfluidics. *Reprod. Biomed. Online* **2003**, *7*, 75–81. [[CrossRef](#)]
4. Yildiz, K.; Yuksel, S. Use of microfluidic sperm extraction chips as an alternative method in patients with recurrent in vitro fertilization failure. *J. Assist. Reprod. Genet.* **2019**, *36*, 1423–1429. [[CrossRef](#)] [[PubMed](#)]
5. Aitken, R.J.; Clarkson, J.S. Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques. *J. Androl.* **1988**, *9*, 367–376. [[CrossRef](#)] [[PubMed](#)]
6. Aitken, R.J.; Harkiss, D.; Buckingham, D.W. Analysis of lipidperoxidation mechanisms in human spermatozoa. *Mol. Reprod. Dev.* **1993**, *35*, 302–315. [[CrossRef](#)]
7. Altman, S.A.; Zastawny, T.H.; Randers-Eichhorn, L.; Cacciuttolo, M.A.; Akman, S.A.; Dizdaroglu, M.; Rao, G. Formation of DNA–protein cross-links in cultured mammalian cells upon treatment with iron ions. *Free Rad. Biol. Med.* **1995**, *19*, 897–902. [[CrossRef](#)]
8. Dizdaroglu, M. Oxidative damage to DNA in mammalian chromatin. *Mutat. Res.* **1992**, *275*, 331–342. [[CrossRef](#)]
9. Griveau, J.F.; Le Lannou, D. Effects of antioxidants on human sperm preparation techniques. *Int. J. Androl.* **1994**, *17*, 225–231. [[CrossRef](#)]
10. Ji, B.T.; Shu, X.O.; Linet, M.S.; Zheng, W.; Wacholder, S.; Gao, Y.T.; Ying, D.M.; Jin, F. Paternal cigarette smoking and the risk of childhood cancer among offspring of nonsmoking mothers. *J. Natl. Cancer Inst.* **1997**, *89*, 238–244. [[CrossRef](#)]
11. Sorahan, T.; Lancashire, R.J.; Hultén, M.A.; Stewart, A.M. Childhood cancer deaths and parental use of tobacco: Deaths from 1953 to 1955. *Br. J. Cancer* **1997**, *75*, 134–138. [[CrossRef](#)]
12. Jiang, L.; Korivi, N.S. Microfluidics: Technologies and applications. In *Nanolithography the Art of Fabricating Nanoelectric and Nanophotonic Devices and Systems*; Feldman, M., Ed.; Woodhead Publishing: Cambridge, UK, 2014; pp. 424–443.
13. Asghar, W.; Velasco, V.; Kingsley, J.L.; Shoukat, M.S.; Shafiee, H.; Anchan, R.M.; Mutter, G.L.; Tüzel, E.; Demirci, U. Selection of functional human sperm with higher DNA integrity and fewer reactive oxygen species. *Adv. Healthc. Mater.* **2014**, *3*, 1671–1679. [[CrossRef](#)]
14. Quinn, M.M.; Jalalian, L.; Ribeiro, S.; Ona, K.; Demirci, U.; Cedars, M.I.; Rosen, M.P. Microfluidic sorting selects sperm for clinical use with reduced DNA damage compared with density gradient centrifugation with swim-up in split semen samples. *Hum. Reprod.* **2018**, *33*, 1388–1393. [[CrossRef](#)]
15. Parrella, A.; Keating, D.; Cheung, S.; Xie, P.; Stewart, J.D.; Rosenwaks, Z.; Palermo, G.D. A treatment approach for couples with disrupted sperm DNA integrity and recurrent ART failure. *J. Assist. Reprod. Genet.* **2019**, *36*, 2057–2066. [[CrossRef](#)]
16. Yetkinel, S.; Kilicdag, E.B.; Aytac, P.C.; Haydardedeoglu, B.; Simsek, E.; Cok, T. Effects of the microfluidic chip technique in sperm selection for intracytoplasmic sperm injection for unexplained infertility: A prospective, randomized controlled trial. *J. Assist. Reprod. Genet.* **2018**, *36*, 403–409. [[CrossRef](#)]
17. Gardner, D.K.; Schoolcraft, W.B. In vitro culture of human blastocyst. In *Towards Reproductive Certainty: Fertility and Genetics and Beyond*; Jansen, R., Mortimer, D., Eds.; Parthenon Publishing: Carnforth, UK, 1999; pp. 378–388.
18. Kalyan, E.Y.; Celik, S.C.; Okan, O.; Akdeniz, G.; Karabulut, S.; Caliskan, E. Does a microfluidic chip for sperm sorting have a positive add-on effect on laboratory and clinical outcomes of intracytoplasmic sperm injection cycles: A sibling oocyte study. *Andrologia* **2019**, *51*, e134303. [[CrossRef](#)]
19. Ashwood-Smith, M.J.; Edwards, R.G. DNA repair by oocytes. *Mol. Hum. Reprod.* **1996**, *2*, 46–51. [[CrossRef](#)]
20. Ménézo, Y.; Dale, B.; Cohen, M. DNA damage and repair in human oocytes and embryos: A review. *Zygote* **2010**, *18*, 357–365. [[CrossRef](#)] [[PubMed](#)]
21. Sakkas, D.; Alvarez, J.G. Sperm DNA fragmentation: Mechanisms of origin, impact on reproductive outcome, and analysis. *Fertil. Steril.* **2010**, *36*, 1423–1429. [[CrossRef](#)] [[PubMed](#)]
22. Stringer, J.M.; Winship, A.; Zerafa, N.; Wakefield, M.; Hutt, K. Oocytes can efficiently repair DNA double-strand breaks to restore genetic integrity and protect offspring health. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 11513–11522. [[CrossRef](#)]
23. De Martin, H.; Cocuzza, M.S.; Tiseo, B.C.; Wood, G.J.A.; Miranda, E.P.; Monteleone, P.A.A.; Soares, J.M., Jr.; Serafini, P.C.; Srougi, M.; Baracta, E.C. Positive rheotaxis extended drop: A one-step procedure to select and recover sperm with mature chromatin for intracytoplasmic sperm injection. *J. Assist. Reprod. Genet.* **2017**, *34*, 1699–1708. [[CrossRef](#)]
24. Nosrati, R.; Vollmer, M.; Eamer, L.; San Gabriel, M.C.; Zeidan, K.; Zini, A.; Sinton, D. Rapid selection of sperm with high DNA integrity. *Lab. Chip* **2014**, *14*, 1142–1150. [[CrossRef](#)] [[PubMed](#)]
25. Schulte, R.T.; Chung, Y.K.; Ohl, D.; Takayama, S.; Smith, G. Microfluidic sperm sorting device provides a novel method for selecting motile sperm with higher DNA integrity. *Fertil. Steril.* **2007**, *88*, 76. [[CrossRef](#)]
26. Shirota, K.; Yotsumoto, F.; Itoh, H.; Obama, H.; Hidaka, N.; Nakajima, K.; Miyamoto, S. Separation efficiency of a microfluidic sperm sorter to minimize sperm DNA damage. *Fertil. Steril.* **2016**, *105*, 315–321. [[CrossRef](#)] [[PubMed](#)]