A Therapeutic Approach for Couples with Compromised Sperm DNA Integrity and a History of Aneuploid Embryos

Allison Petrini, Alessandra Parrella, Philip Xie, Zev Rosenwaks, and Gianpiero D. Palermo

Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, New York, USA

Abstract

Study question: Does selecting the highest progressively motile spermatozoa with optimal genomic integrity enhance the likelihood of generating euploid embryos?

Summary answer: Microfluidic sperm selection (MFSS) identified spermatozoa with the highest chromatin integrity, capable of generating euploid embryos in couples with a history of persistent implantation failure.

What is known already: Genomic impairment of the male gamete can hinder embryo cleavage and implantation. Dysfunction of the male genital tract increases both single-strand (ss) and double-strand (ds) DNA nicks and breaks that can inhibit the developmental competence of embryos.

In particular, ds DNA breaks present in the spermatozoa of fertile donors, at a concentration as high as 40%, may contribute to embryo aneuploidy with consequent implantation impairment.

Study design, size, duration: From October 2016 through January 2019, nine consenting couples with a history of embryo aneuploidy and/or recurring implantation failure and a male partner with high sperm chromatin fragmentation (SCF) in their ejaculate underwent a new ICSI cycle in which semen specimens were processed by density gradient centrifugation (DGC) and MFSS.

Participants/materials, setting, methods: SCF was measured by TUNEL on raw semen specimens as well as after DGC and MFSS. ICSI with pre-implantation genetic testing for aneuploidy (PGT-A) was carried out with spermatozoa selected by the two different methods. Fertilization and clinical pregnancy outcomes were assessed and compared between the two sperm selection methods. Embryo implantation and pregnancies were recorded after replacement of thawed euploid blastocysts.

Main results and the role of chance: A total of 9 men of average age 43±12 years had a mean sperm concentration of 55.1±47 x 10^6/mL, 30±14.7 motility, and 2.4±1% morphology. After DGC and MFSS, the sperm concentration was 36.2±48 and 11±17 x 10^6/mL, with 59±41% and 97±4% motility, respectively (P<0.0001).

The raw sample sperm morphology improved from 2.4±1% to 4.0±1% after MFSS, while after DGC it was 3±1%. The average SCF decreased from 31% in raw samples to 21% following DGC, and became 1.4% after MFSS processing (P<0.0001).

These couples (female partners, 37±5 years) underwent 12 cycles with DGC-selected spermatozoa and achieved a fertilization rate of 78% (75/96), which generated 30% (12/40) morphologically good-quality embryos. PGT-A did not show any euploid embryos for transfer. Subsequently, the couples underwent ICSI cycles with MFSS, showing a fertilization rate of 73% (76/104), with 41% (14/34) good-quality embryos.

In this group, 24% (8/33) of euploid embryos were identified and cryopreserved.

Four couples received a thawed single euploid blastocyst and all 4 became pregnant (P<0.0001), resulting in a clinical pregnancy rate per cycle of 44% (4/9) and 100% per transfer.

Limitations, reasons for caution: This study represents a preliminary experiment on a small number of subjects. While the oocyte contribution to aneuploidy cannot be discounted, MFSS was able to yield the highest progressive motile spermatozoa with optimal genomic integrity capable of enhancing the chances of generating euploid embryos.

Wider implications of the findings: The occasional presence of ds DNA in the male gamete has been considered responsible for contributing to embryo aneuploidy. MFSS of highly motile and genetically competent male gametes may enhance the chances of obtaining a euploid conceptus for transfer.

Background

Genomic impairment of the male gamete can hinder embryo cleavage and implantation. Dysfunction of the male genital tract increases both single-strand (ss) and double-strand (ds) DNA nicks and breaks that can inhibit the developmental competence of embryos. In particular, ds DNA breaks present in the spermatozoa of fertile donors, at a concentration as high as 40%, may contribute to embryo aneuploidy with consequent implantation impairment.

Methods

SCF was measured by TUNEL on raw semen specimens as well as after DGC and MFSS. ICSI with pre-implantation genetic testing for aneuploidy (PGT-A) was carried out with spermatozoa selected by the two different methods. Fertilization and clinical pregnancy outcomes were assessed and compared between the two selection methods. Embryo implantation and clinical pregnancies were recorded after replacement of thawed euploid blastocysts.

Results

A total of 9 men of average age 43±12 years underwent semen analysis **(Table 1)**. The average SCF decreased from 31% in raw samples to 21% following DGC, and became 1.4% after MFSS processing (P<0.0001) **(Figure 1)**.

Table 1. Comparison of semen parameters between DCG and MFSS

		Selection	
N=9	**Raw**	**Density Gradient**	**Microfluidics**
Male Age (M±SD)	43±12	43±12	43±12
Concentration (x10^6/mL)	55.1±47	36.2± 48	11.6± 12
Motility (%)	30±14.7	59± 41	97± 4
Morphology (%)	2.4±1	3±1	4.0±1

These couples (female partners, 37±5 years) underwent 12 cycles with DGC selected spermatozoa and achieved a fertilization rate of 78% (75/96), which generated 30% (12/40) morphologically good-quality embryos **(Table 2)**. PGT-A did not show any euploid embryos for transfer. Subsequently, the couples underwent ICSI cycles with MFSS, showing a fertilization rate of 73% (76/104), with 41% (14/34) good-quality embryos.

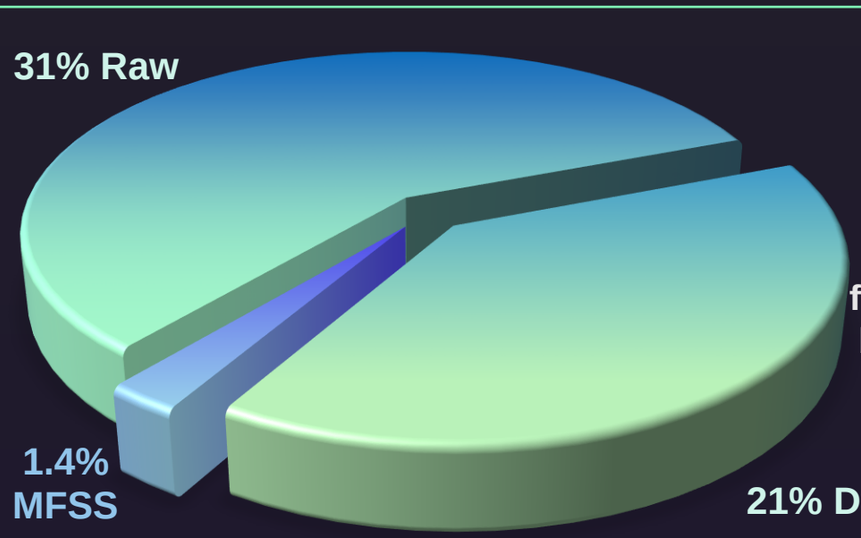

Figure 1. Comparison of DNA fragmentation by technique

Table 2. Cycle number, fertilization and PGT-A result after DGC and MFSS

	Selection	
Number of (%)	**Density Gradient**	**Microfluidics**
Cycles	12	9
Injected oocytes (M ± SD)	8±4	11.3±6
Fertilization rate (2PN) (%)	75/96 (78%)	76/104 (73%)
Embryos screened	40	34
Good Quality	12/40 (30%)	14/34 (41%)
Euploid	0	8/33 (24%)
Embryos transferred after PGT-A	2	4

In this group, 24% (8/33) of euploid embryos were identified and cryopreserved. Four couples received a thawed single euploid blastocyst and all 4 became pregnant (P<0.0001), resulting in a clinical pregnancy rate per cycle of 44% (4/9) and 100% per transfer **(Figure 2)**.

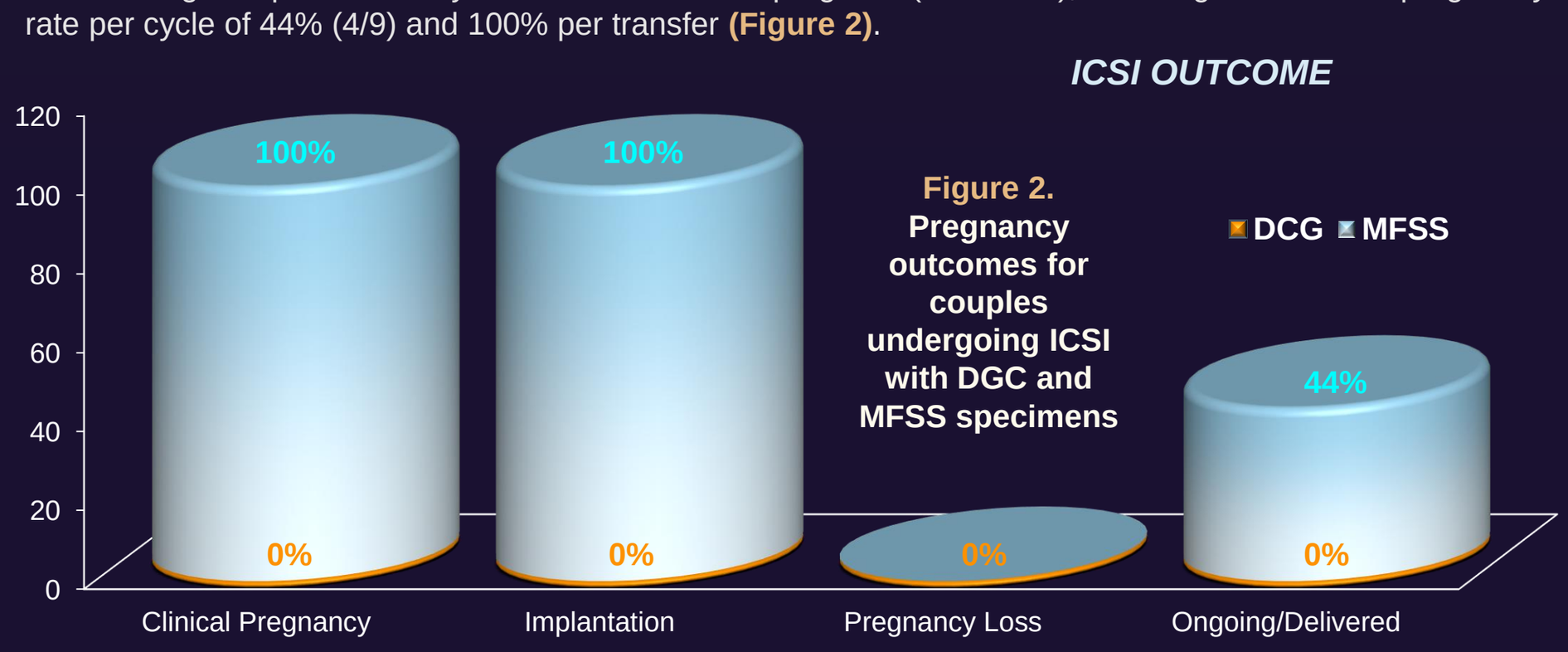

Figure 2. Pregnancy outcomes for couples undergoing ICSI with DGC and MFSS specimens

Conclusions

The occasional presence of ds DNA in the male gamete has been considered responsible for contributing to embryo aneuploidy. MFSS of highly motile and genetically competent male gametes may enhance the chances of obtaining a euploid conceptus for transfer.