# Microfluidic Sperm Selection Enhances ICSI Outcomes by Selecting Spermatozoa with the Highest Chromatin Integrity

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## Abstract

**Study question:** Does selecting spermatozoa with superior chromatin integrity lead to higher implantation and clinical pregnancy rates with ICSI?
**Summary answer:** Microfluidic sperm selection (MFSS) provides spermatozoa with optimal chromatin integrity and yields higher implantation and clinical pregnancy rates with ICSI.
**What is known already:** Sperm preparation methods aim at providing specimen for insemination with the highest progressive motility independent of phenotypic and genomic integrity. Both single-strand (ss) and double-strand (ds) DNA nicks and breaks inhibit the ability of the male genome to support embryonic development. While different mechanisms are in place to prevent this phenomenon, they may be hindered by a defective epididymal function or a suboptimal or aged oocyte.
**Study design, size, duration:** From October 2016 to January 2019, consenting men (N=32) known to have higher DNA fragmentation in their ejaculate and prior ART failure had their ejaculates simultaneously processed by density gradient centrifugation (DGC) and MFSS. TUNEL was carried out on the raw specimens and on the differently selected aliquots. In men (N=13) treated by ICSI with their female partners, clinical outcomes were recorded. Semen parameters, chromatin integrity, embryo implantation, and pregnancy characteristics were compared.
**Participants/materials, setting, methods:** Fresh ejaculated specimens from consenting men were collected for standard semen analysis in accordance with WHO 2010 criteria. DGC and MFSS were used to isolate motile spermatozoa based on cell motility and fluid dynamics. Sperm chromatin fragmentation (SCF) was assessed by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) on at least 500 spermatozoa under a fluorescent microscope utilizing a threshold of ≥15%. ICSI was performed in the standard fashion.
**Main results and the role of chance:** A total of 32 men with an average age of 41±9 years had the following average semen parameters: concentration of 48.1±37 x $10^6$/mL, motility of 31.5±14.6, and 2.4±1% morphology. After DGC and MFSS, the sperm concentration was 33.8±25 and 11.6±12 x$10^6$/mL, with 59.4±33% and 97.6±9% motility, respectively (*P*<0.0001).
The morphology of the raw sperm sample improved from 2.4±1% to 4.0±1% after MFSS, while it remained at 2.6±1% after DGC. The average SCF decreased from 24% in raw samples to 15% following DGC and became 1.7% after MFSS processing (*P*<0.0001).
Couples (n=13) who underwent ICSI had an SCF in their raw sample of 30.3%, which reached 22% after DCG selection and was only 1.5% after MFSS (*P* < 0.0001). These couples (female age, 36.5±3 years; male age, 42±9 years) underwent 28 cycles with DGS sperm selection, achieving a fertilization rate of 67%. The implantation rate was only 3.4% (1/29) with a clinical pregnancy rate of 6.6% (1/15) that ended in pregnancy loss. Subsequently, these couples underwent ICSI cycles with MFSS and achieved a fertilization rate of 61%. The implantation rate rose to 31% (7/23) *(P<0.05)* with a clinical pregnancy rate of 54% (7/13) *(P<0.05)*. The pregnancy loss was 15.3% (2/13).
**Limitations, reasons for caution:** This study represents a preliminary experiment on a small number of subjects. If confirmed, MFSS yields a male gamete with the highest chromatin integrity, progressive motility, and improved morphology. MFSS should be used in couples afflicted by high levels of SCF in their raw ejaculate.
**Wider implications of the findings:** According to our study, SCF appears to be linked to the kinetic characteristics of the sperm cell. MFSS yielded the highest portion of progressive motility with the highest DNA integrity. This novel microfluidic system may serve to identify spermatozoa with the highest functional and genomic integrity.
**Study funding/competing interest(s):** None
**Trial registration number**: N/A

## Background

Sperm preparation methods aim at providing specimen for insemination with the highest progressive motility independent of phenotypic and genomic integrity. Both single-strand (ss) and double-strand (ds) DNA nicks and breaks inhibit the ability of the male genome to support embryonic development. While different mechanisms are in place to prevent this phenomenon, they may be hindered by a defective epididymal function or a suboptimal or aged oocyte.

## Methods

From October 2016 to January 2019, consenting men (n=32) known to have higher DNA fragmentation in their ejaculate and prior ART failure had their ejaculates simultaneously processed by density gradient centrifugation (DGC) and MFSS. TUNEL was carried out on the raw specimens and on the differently selected aliquots. SCF was measured by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) on at least 500 spermatozoa under a fluorescent microscope utilizing a threshold of ≥15%. ICSI was performed in the standard fashion.
In men (N=13) treated by ICSI with their female partners, clinical outcomes were recorded. Semen parameters, chromatin integrity, embryo implantation, and pregnancy characteristics were compared.

## Conclusions

According to our study, SCF appears to be linked to the kinetic characteristics of the sperm cell. MFSS yielded the highest portion of progressive motility with the highest DNA integrity. This novel microfluidic system may serve to identify spermatozoa with the highest functional and genomic integrity.

## Results

A total of 32 men with an average age of 41±9 years had the following average semen parameters: concentration of 48.1±37 x $10^6$/mL, motility of 31.5±14.6, and 2.4±1% morphology. After DGC and MFSS, the sperm concentration was 33.8±25 and 11.6±12 x$10^6$/mL, with 59.4±33% and 97.6±9% motility, respectively (*P*<0.0001). The morphology of the raw sperm sample improved from 2.4±1% to 4.0±1% after MFSS, while it remained at 2.6±1% after DGC **(Table 1)**.

**Table 1. Comparison of semen parameters between density gradient method and microfluidics**

| | | Selection | |
|---|---|---|---|
| **N=32** | **Raw** | **Density Gradient** | **Microfluidics** |
| **Male age (M±SD)** | 41±9 | 41±9 | 41±9 |
| **Concentration (x$10^6$ /mL)** | 48.1±37 | 33.8± 25 | 11.6± 12 |
| **Motility (%)** | 31.5±14.6 | 59.4± 33 | 97.6± 9 |
| **Morphology (%)** | 2.4±1 | 2.6±1 | 4.0± 1 |
| **DNA fragmentation (%)** | 24±9 | 15±7 | 1.7±1 |

The average SCF decreased from 24% in raw samples to 15% following DGC and became 1.7% after MFSS processing (*P*<0.0001) **(Figure 1)**. Couples (n=13) who underwent ICSI had an SCF in their raw sample of 30.3%, which reached 22% after DCG selection and was only 1.5% after MFSS (*P* < 0.0001). These couples (female age, 36.5±3 years; male age, 42±9 years) These couples female age, 36.5±3 years underwent 28 cycles with DGS sperm selection, achieving a fertilization rate of 67%.

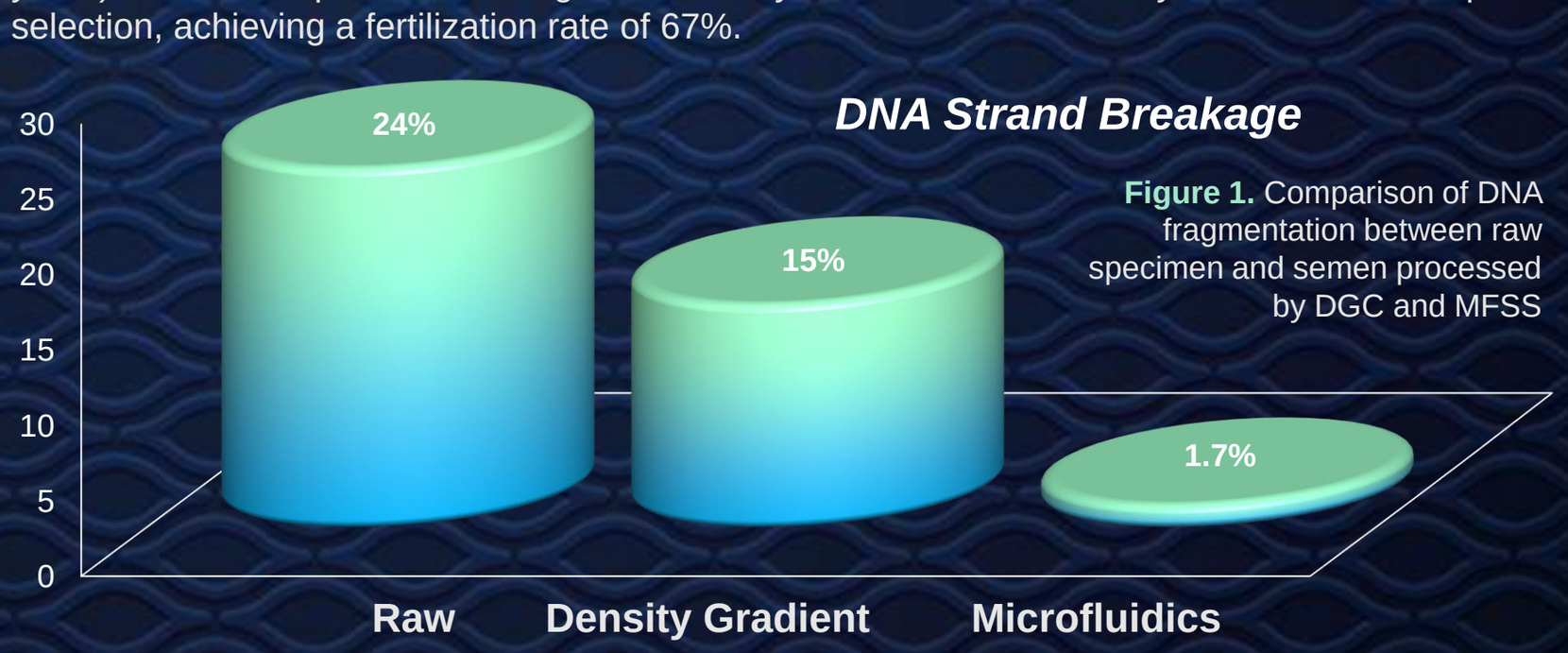


**Figure 1.** Comparison of DNA fragmentation between raw specimen and semen processed by DGC and MFSS

The implantation rate was only 3.4% (1/29), with a clinical pregnancy rate of 6.6% (1/15) that ended in pregnancy loss. Subsequently, these couples underwent ICSI cycles with MFSS and achieved a fertilization rate of 61%. The implantation rate rose to 31% (7/23) *(P*<0.05), with a clinical pregnancy rate of 54% (7/13) *(P*<0.05). Pregnancy loss was 15.3% (2/13) **(Figure 2)**.

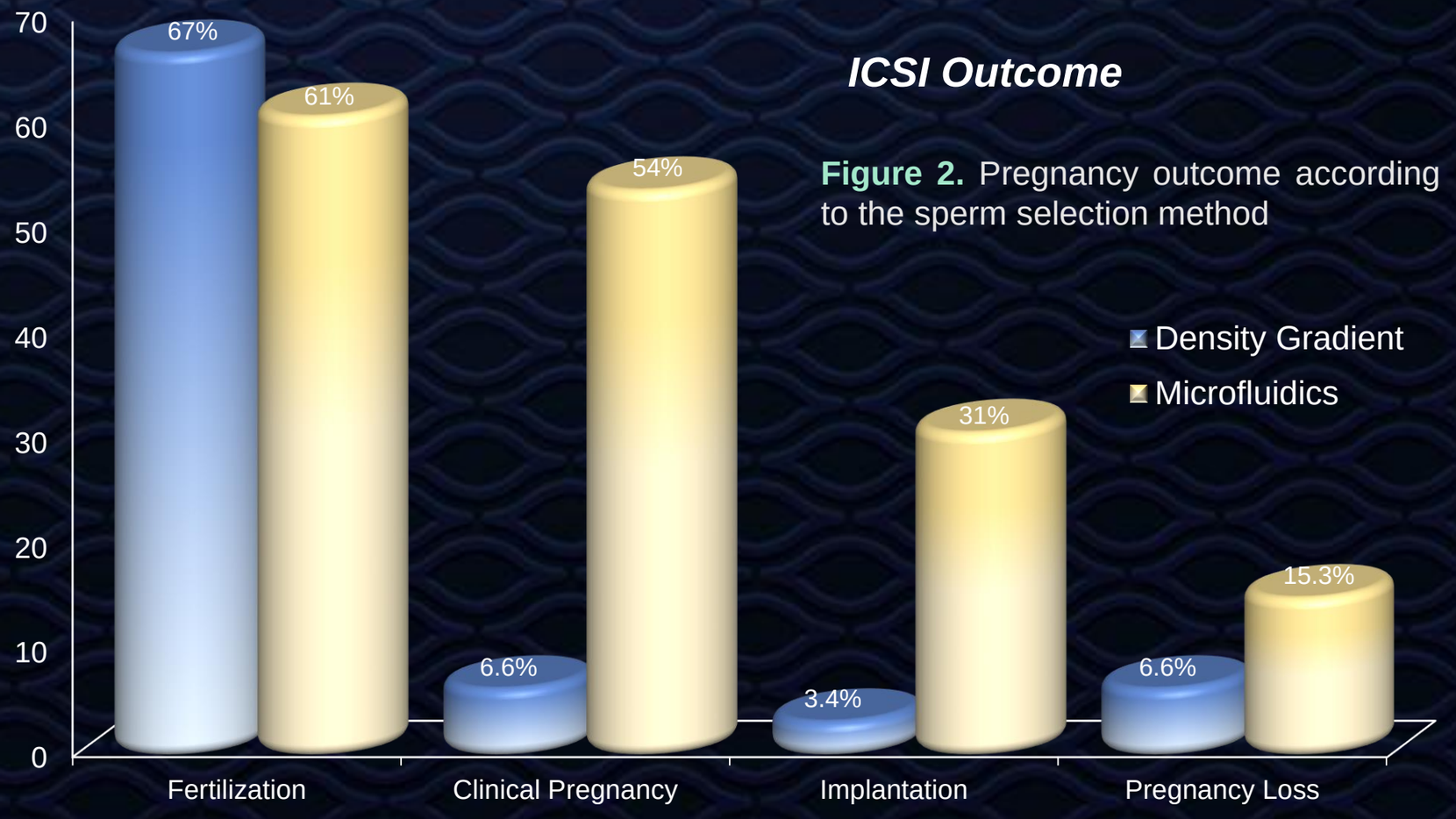


**Figure 2.** Pregnancy outcome according to the sperm selection method