Selection of Spermatozoa with Higher Chromatin Integrity Through a Microfluidics Device

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ABSTRACT

Study question: We tested a simple method to enrich spermatozoa with higher progressive motility and superior chromatin status in men with normal and abnormal semen parameters.

Summary answer: A microfluidics device was able to isolate spermatozoa with higher progressive motility and the lowest incidence of chromatin fragmentation in oligo- and normo-spermic semen.

What is known already: Semen analysis is currently used as a method to screen for male factor infertility. Standard selection methods provide a cleaner and safer specimen for insemination with enhanced progressive motility albeit without any input on the genomic integrity of the spermatozoon. We learned that sperm DNA fragmentation is linked to motility and therefore a method that is capable of guaranteeing a richer proportion of the most progressive spermatozoa may also provide cells with the highest chromatin integrity.

Study design, size, duration: From October 2016 to January 2017, seminal samples of normozoo- (n=10) and asthenozoospermic men (n=10) were simultaneously processed by density gradient centrifugation (DGC) and by a new microfluidic sperm sorter (MFSS) chamber to allow selection of the most forwardly progressive motile sperm portion. Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) was carried out on the raw specimen and on the differently selected aliquots to assess sperm chromatin integrity (SCI).

Participants/materials, setting, methods: Consenting men had their ejaculates screened for infertility by standard semen analysis according to WHO 2010 criteria. Standard DGC, centrifugation and a MFSS chamber were used to isolate motile spermatozoa based on cell motility and fluid dynamics. SCI was assessed by TUNEL on at least 500 spermatozoa under fluorescent microscopy considering a ≥15% threshold.

Main results and the role of chance: The mean age for the men was 36.5±8 years. The average semen parameters for the normozoospermic men was: concentration 71.5±25 x 10^6/mL, motility of 44.8±4% and morphology of 2.9±1%. After DGC and MFSS the total motility was 86.4±5% and 98±1%, respectively (P<0.001) while the progressive motility was 84.7±5% and 97.6±2 %, respectively (P<0.001). The original sperm morphology of 2.9% became 4.0% after MFSS. In these men, the average SCI was 14.7 % in the raw ample; however, following DGC, the SCI decreased to 8.8% and after MFSS processing it reduced to 2.4% (P<0.001). For asthenozoospermic men, the average semen parameters were 54.4±45 x 10^6/mL concentration, 23.2±15% motility, and 2.1±1% morphology. Average motility improved to 45.1±34% following DGC and 97.7%±2 following MFSS processing (P<0.0001). Progressive motility from an initial 18.9%±13 became 41.9%±32 by DGC and 97.4%±2 by MFSS. Additionally, sperm morphology increased to 4.2±1% after MFSS. In asthenozoospermic men, a 23.1% SCF on the raw samples was reduced to 16.0% after DGC. However, following MFSS, SCF decreased to 1.8% (P<0.0001). In 2 couples with total embryo aneuploidy of preimplantation genetic screening (PGS), the MFSS was used to select spermatozoa with high chromatin integrity prior to ICSI. In one couple, after PGS, 1 blastocyst out of 3 was euploid.

Limitations, reasons for caution: This analysis is a pilot study on a small number of subjects. However, if confirmed, this microfluidic method, capable of selecting the portion of spermatozoa with the most progressive motility together with the highest level of chromatin integrity, may yield spermatozoa with superior embryo developmental competence for reproductive treatment.

Wider implications of the findings: SCI appears to be related to the kinetic characteristic of the human spermatozoa. MFSS yielded the highest progressively motile spermatozoa characterized by high DNA integrity. Couples with unexplained infertility and unable to achieve a pregnancy due to a concealed male factor may benefit from MFSS to improve their reproductive outcome.

BACKGROUND

DNA damage in spermatozoa is linked to infertility. It is associated with lower fertilization rate, poorer embryo development and reduced implantation rate. DNA fragmentation is strongly correlated with semen parameters such us motility and morphology characteristic. An elevate fraction of progressively motile spermatozoa is important to have a high DNA integrity.

METHODS

A total of 10 normozoo- men and 10 asthenozoospermic men were simultaneously processed by density gradient centrifugation and microfluidics sperm sorter device to select most progressively motile spermatozoa. SCF was assessed by Terminal Deoxynucleotidyl tansferase dUTP Nick End Labeling (TUNEL) (In Situ Cell Death Detection Kit, Roshe) on at least 500 spermatozoa under a fluorescent microscope utilizing a threshold of 15%.

Fig.1 Microfluidics Sperm Sorting chips Fertile and Fertile Plus developed from DxNow™

RESULTS

Semen characteristics for the normozoospermic men was: concentration 71.5±25 x 10^6/mL, motility of 44.8±4% and morphology of 2.9±1%. After DGC and MFSS the total motility was 86.4±5% and 98±1%, respectively (P<0.001) while the progressive motility was 84.7±5% and 97.6±2 %, respectively (P<0.001). The original sperm morphology of 2.9% became 4.0% after MFSS (Table 1)..

Table.1 Parameters of semen analysis of normozoospermoc men according to WHO 2010 criteria

Normozoospermic Men		Selection	
N=10	Raw	Density Gradient	Microfluidics
Volume (mL)	2.5±1	0.5±0	0.5±0
Concentration (x10^6)	71.5±25	46.4±18	19.8±10
Morphology (%NF)	2.9±1	3.0±1	4.0±1

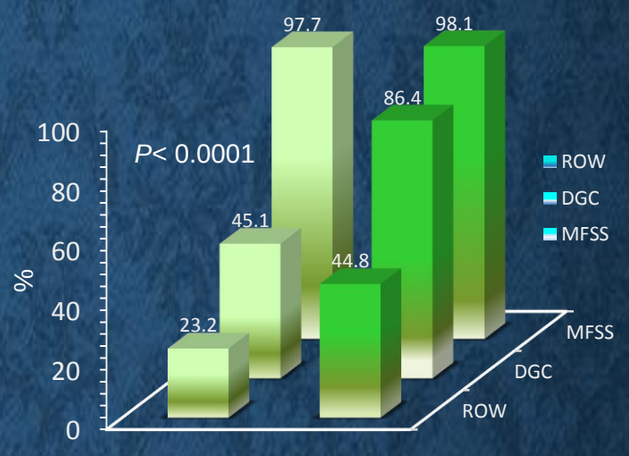

Fig.3 Comparison of motility between the RAW specimen and the semen processed by density gradient centrifugation and by a new microfluidic sperm sorter chamber

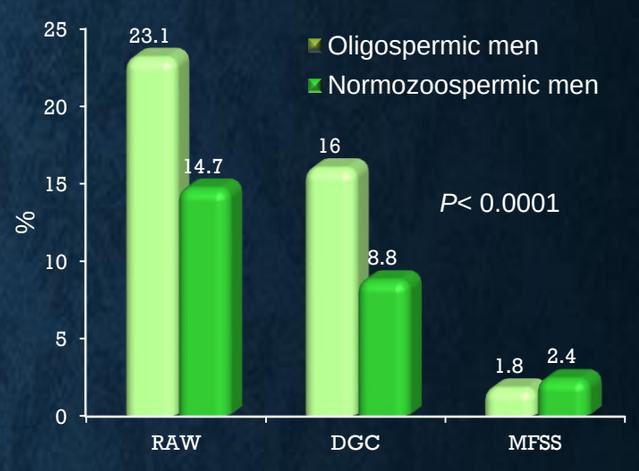

Fig.2 Comparison of DNA fragmentation between the ROW specimen and the semen processed by density gradient centrifugation and by a new microfluidic sperm sorter chamber

In these men, the average SCF was 14.7 % in the raw ample; however, following DGC, the SCF decreased to 8.8% and after MFSS processing it reduced to 2.4% (P<0.001).

Table.2 Parameters of semen analysis of asthenozoospermic men according to WHO 2010 criteria

Asthenozoospermic Men		Selection	
N=10	Raw	Density Gradient	Microfluidics
Volume (mL)	3.6±2	0.5±0	0.4±0
Concentration (x10^6)	54.4±45	36.3±31	11.8±15
Morphology (%NF)	2.1±1	2.1±1	0.2±0

In asthenozoospermic men, a 23.1% SCF on the raw samples was reduced to 16.0% after DGC. However, following MFSS, SCF decreased to 1.8% (P<0.0001). In 2 couples with total embryo aneuploidy of preimplantation genetic screening (PGS), the MFSS was used to select spermatozoa with high chromatin integrity prior to ICSI. In one couple, after PGS, 1 blastocyst out of 3 was euploid.

Fig.3 : Morphology of the sperm before and after MFSS.

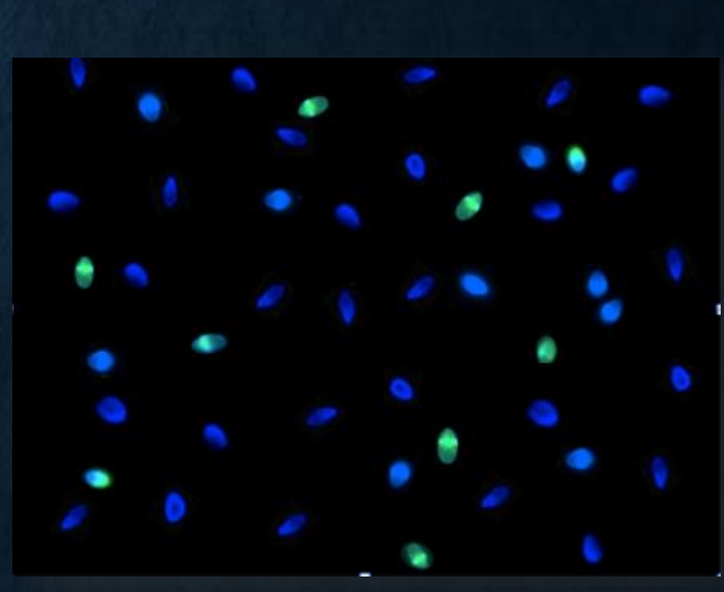

Fig.4: Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) immunoflourescent staining. TUNEL positive spermatozoa (in green) with the DAPI counterstain (in blu).

CONCLUSIONS

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