A Proposed Method to Minimize Male Gamete Contribution to Aneuploidy in the Embryo Cohort

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Abstract

Study question: Can a new method to select spermatozoa enhance the genomic competence of the male gamete and yield euploid embryos in couples with all abnormal conceptuses?
Summary answer: Microfluidic selection yields spermatozoa with the highest progressive motility and genomic integrity, enabling the generation of chromosomally normal embryos despite a history of recurrent, total aneuploid conceptuses.
What is known already: The presence of a male factor can negatively impact embryo cleavage and chromosomal status. In addition, dysfunction of the male genital tract increases sperm chromatin fragmentation that, particularly in cases of double strand breaks, can lead to aneuploidy of the male gamete. Thus, in couples with a relatively young female partner, the recurrent appearance of aneuploid embryos may reflect the contribution of the male gamete.
Study design, size, duration: In a 19-month period, 7 couples with a history of high Sperm Chromatin Fragmentation (SCF) and embryo aneuploidy after several ART attempts underwent a successive cycle of ICSI where semen specimens were processed in a microfluidics chamber. Fertilization and clinical pregnancy rate were assessed and compared between different sperm preparation methods. SCF was assessed by TUNEL and sperm aneuploidy by FISH analysis. Chromosomal analysis was carried out by preimplantation genetic testing for aneuploidy (PGT-A) on conceptuses.
Participants/materials, setting, methods: Consenting men had their ejaculates screened by standard semen analysis according to WHO 2010 criteria. Ejaculate specimen were processed by density gradient and MFSS. SCF was measured by TUNEL utilizing a commercial kit (In Situ Cell Death Detection Kit, Roche). At least 500 spermatozoa were counted under fluorescent microscopy with an adopted threshold of 15%. FISH analysis was carried out on at least 1000 spermatozoa by 9 chromosome probes.
Main results and the role of chance: Seven couples (average maternal age, 38.3±6 years; average paternal age, 44.2±11 years) underwent 19 ICSI cycles. An average semen concentration of 11.5±16x106/mL, 18.5±16% motility, 2.0±0% normal morphology, and an SCF of 29.2±10% were found. The sperm aneuploidy rate was 4±2%. After selection by density gradient, the total motility of the sperm samples was 34.2±26%, resulting in a 60.4% fertilization rate. These cycles only generated 5 euploid embryos out of 23, which yielded two pregnancies, both resulting in miscarriage. Couples subsequently underwent 7 ICSI cycles in which the spermatozoa were processed by MFSS, which generated 98%±4 (P<0.0001) motility and an increased 4% morphology, while the SCF dropped to only 1.6±1 (P<0.0001). Although the fertilization rate was 67.1%, 7 euploid blastocysts out of 14 (50%) were obtained, yielding 5 out of 7 ongoing clinical pregnancies (71.4%; P<0.001).
Limitations, reasons for caution: This is a pilot study on a small number of subjects. However, this microfluidic method is capable of selecting spermatozoa with better morphology, higher chromatin integrity, and presumably lower sperm aneuploidy, which may likely yield euploid embryos in couples with subtle male factor infertility.
Wider implications of the findings: According to this study, the selection of a genomically competent male gamete may enhance the euploidy of the conceptus. Couples with a relatively young female partner and recurrent aneuploid embryos may benefit from MFSS selection to improve reproductive outcome due to an occult male factor.
Study funding/competing interest(s): None

Background

The presence of a male factor can negatively impact embryo cleavage and chromosomal status. In addition, dysfunction of the male genital tract increases sperm chromatin fragmentation that, particularly in cases of double strand breaks, can lead to aneuploidy of the male gamete. Thus, in couples with a relatively young female partner, the recurrent appearance of aneuploid embryos may reflect the contribution of the male gamete.

Methods

Consenting men had their ejaculates screened by standard semen analysis according to WHO 2010 criteria. Specimens were processed by density gradient and microfluidic sperm selection (MFSS) (**Figure 1**). SCF was measured by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) utilizing a commercial kit (In Situ Cell Death Detection Kit, Roche). At least 500 spermatozoa were counted under fluorescent microscopy, with an adopted threshold of 15%. Fertilization and clinical pregnancy rates were assessed and compared between the two preparation methods, and preimplantation genetic testing for aneuploidy (PGT-A) was performed on the resulting embryos.

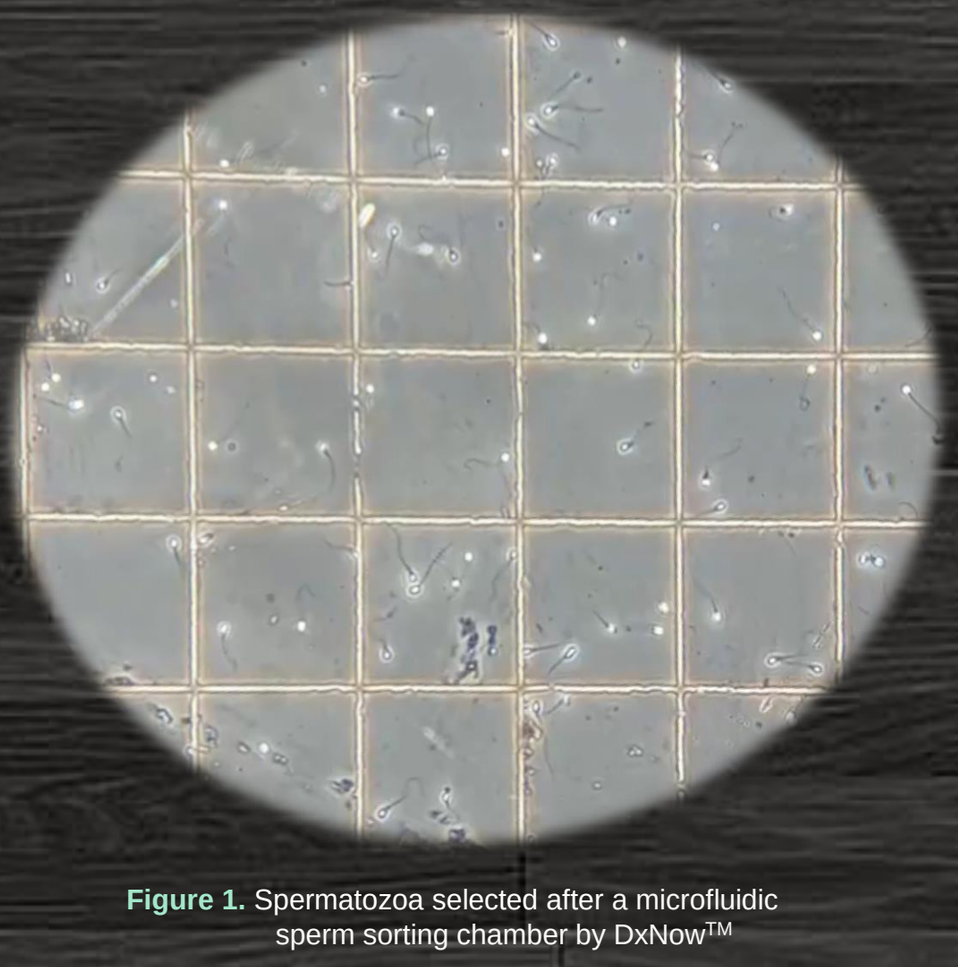

Figure 1. Spermatozoa selected after a microfluidic sperm sorting chamber by DxNow™

Results

Seven couples (average maternal age 38.3±6 yrs; average paternal age 44.2±11 yrs) underwent 19 ICSI cycles. An average semen concentration of 11.5±16x10^6/mL, 18.5±16% motility, and 2.0±0% normal morphology were found. When we compared the semen parameters of those samples processed by microfluidics to those processed by density gradient, we found that although concentration decreased, the progressive motility (P<0.0001) and normal morphology improved (**Figure 2**).

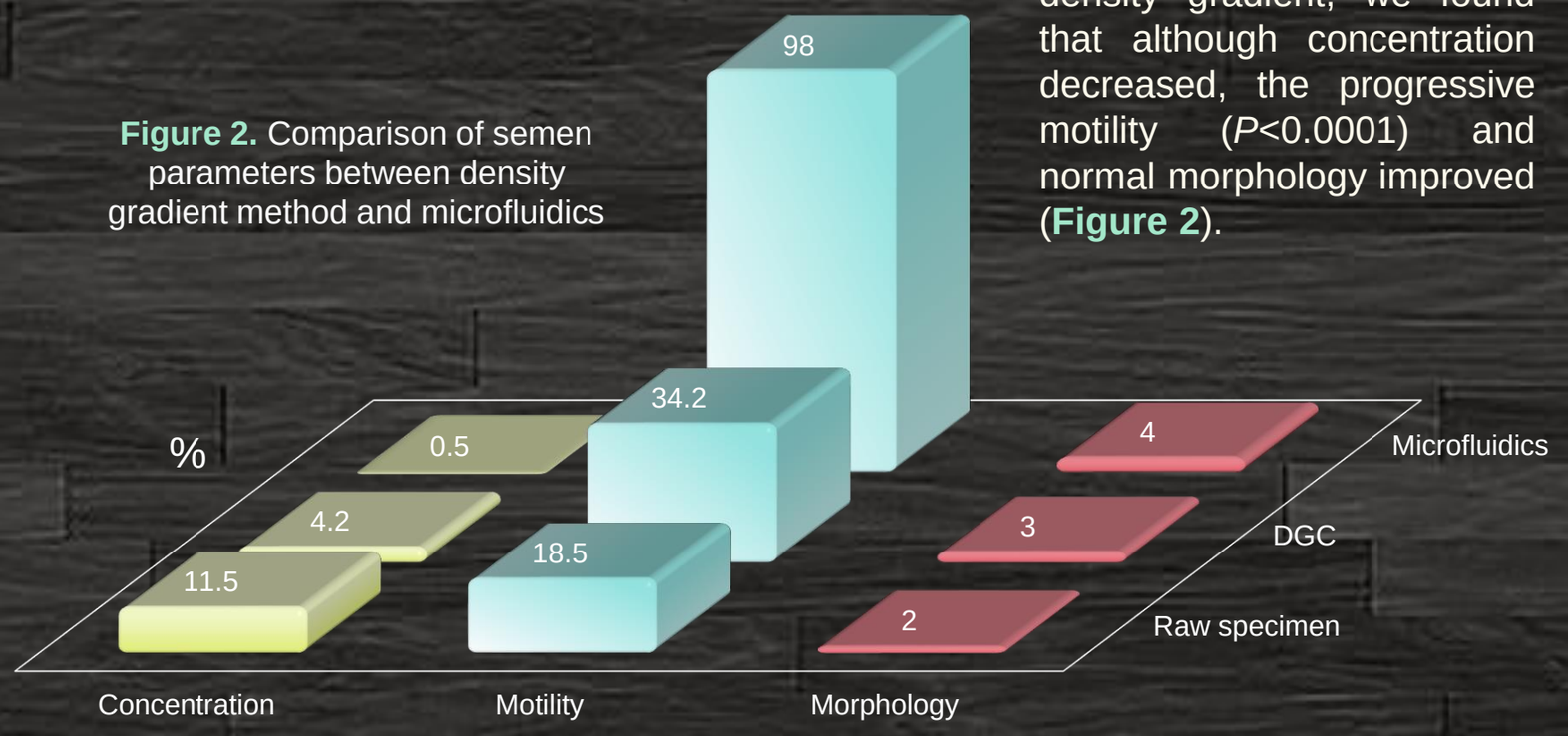

Figure 2. Comparison of semen parameters between density gradient method and microfluidics

When we assessed the SCF by TUNEL (**Figure 3**), we found that compared to 29.2% DNA fragmentation in the unprocessed specimens, spermatozoa processed by density gradient selection yielded 18% DNA fragmentation, which decreased to just 1.6% after microfluidics processing (**Figure 4**).

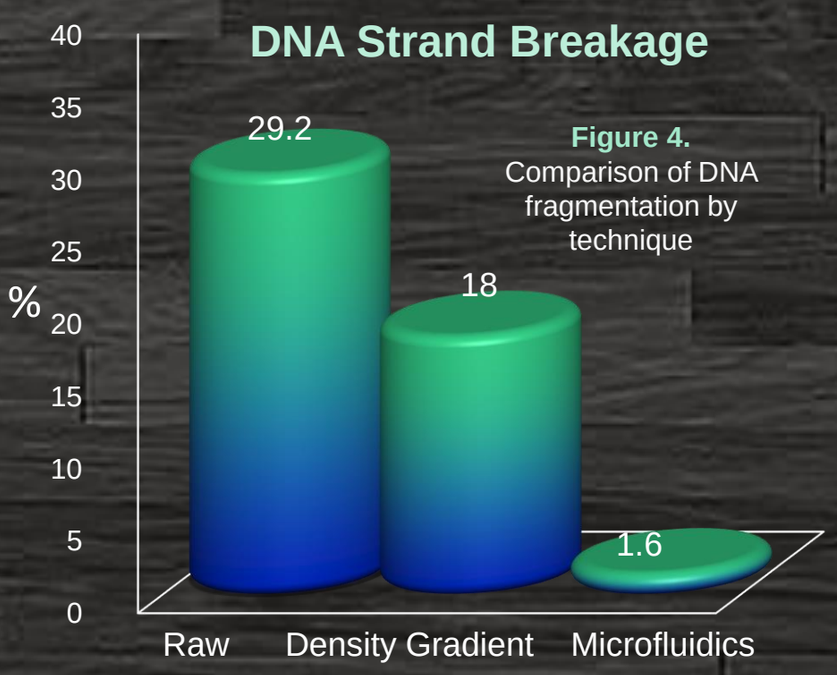

Figure 4. Comparison of DNA fragmentation by technique

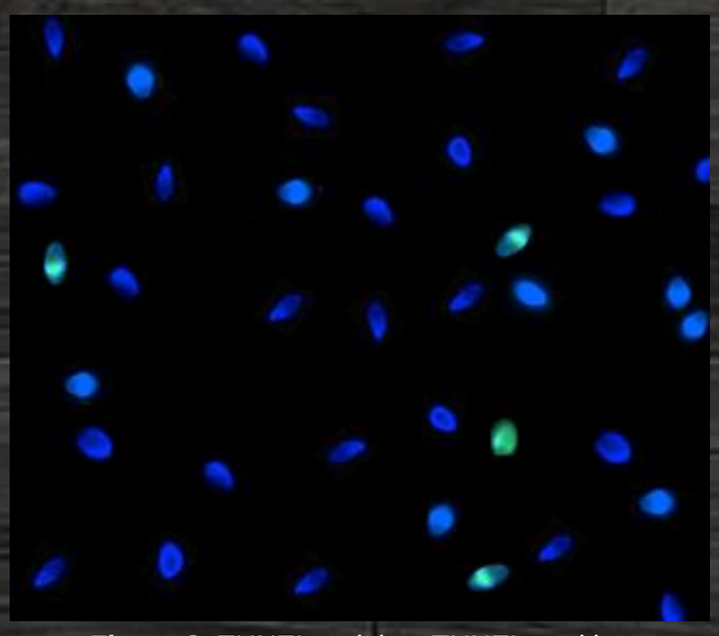

Figure 3. TUNEL staining. TUNEL positive spermatozoa (green) with DAPI counterstain (blue).

Couples subsequently underwent 7 ICSI cycles in which the spermatozoa were processed by density gradient or microfluidics. The cycles using density gradient sperm processing only generated 5 euploid embryos out of 23, which yielded two pregnancies, both resulting in miscarriage. Cycles utilizing microfluidics generated 7 euploid blastocysts out of 14 (50%), yielding 5 out of 7 ongoing clinical pregnancies (71.4%; P<0.001) (**Table 1**).

Table 1. Pregnancy outcomes for couples undergoing ICSI with DGC and MFSS specimens

	Selection	
	Density Gradient	Microfluidics
Couples	7	
Cycles	19	7
+bHCG	2	5
Clinical Pregnancy (+FHB) (%)	2 (10.5)	5 (71.4)
Pregnancy Loss	2	-

Conclusions

According to this study, the selection of a genomically competent male gamete may enhance the euploidy of the conceptus. Couples with a relatively young female partner and recurrent aneuploid embryos may benefit from MFSS selection to improve reproductive outcome due to an occult male factor.