Original Article

Improved sperm DNA fragmentation levels in infertile men following very short abstinence of 3–4 hours

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Background: Limited data exists on possible approaches to improve sperm DNA fragmentation index (DFI) when no identifiable cause is found. The effect of short abstinence on sperm parameters has been extensively studied, but rarely reported on the effect on DFI in infertile men. In this study, we aimed to determine whether a second ejaculate provided after very short abstinence demonstrates lower DFI rates in infertile men.

Methods: This prospective cohort study was conducted at Mount Sinai Hospital, Toronto, Canada, a tertiary university affiliated hospital. All men having DFI testing in addition to the standard semen analysis were identified via a prospectively collected database. Infertile men were instructed to provide two semen samples 3–4 hours apart (the first sample was given after 2–5 days of abstinence) to test the effect on DFI levels. Data analysis was performed for the comparison of the change in sperm parameters and DFI between samples and between men with DFI above and under 30%.

Results: A total of 52 men provided double ejaculates 3–4 hours apart. In the entire group, DFI decreased from 38.9%±21.4% to 35.1%±21.6% in the second sample (P<0.001). Semen volume was lower on the second sample (2.3±1.4 *vs.* 1.5±0.9 mL, P<0.001), while the remaining parameters did not change. Forty out of 52 patients (76.9%) had improved DFI (average of 6.0±4.0 percentage points). Change in DFI varied with 22/52 (42.3%) and 7/52 (13.5%) of patients found to have decreases in DFI >5% and >10% in the second ejaculate, respectively. For men with DFI of 30–40%, 64% (7/11) of DFIs reduced to the under 30% range. First DFI value was the only parameter associated with DFI decrease to under 30% in multivariate models [odds ratio (OR), 0.62; 95% confidence interval (CI): 0.39–0.98; P=0.04].

Conclusions: This study identified significant improvements in DFI in infertile men providing a second sample after 3–4 hours. Controlled trials are needed to determine if reproductive outcomes are improved using a second ejaculate for infertile men with high initial sperm DFI values.

Keywords: Abstinence; DNA fragmentation index (DFI); sperm DNA fragmentation (SDF); infertility; sperm

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Introduction

Infertility is estimated to impact nearly 15% of all couples worldwide, with male infertility accounting for approximately 40–50% of cases (1-3). Male factor as a cause of infertility is becoming more common with the increasing delay in paternal childbearing age (4) and age-related effect on sperm quality and reproductive outcomes (5). An additional factor is the possible global deterioration in sperm quality over the last few decades reported by several studies and meta-analyses (6-9), although some studies have shown contrasting results (10-12) and this subject is still in debate.

Sperm chromatin integrity is a promising biomarker in the evaluation of male infertility, as recently mentioned in the 6th edition of the World Health Organization (WHO) guidelines (13). Sperm DNA fragmentation (SDF) reflects the damage to sperm DNA integrity. SDF may result from several possible mechanisms, such as, varicocele, genitourinary tract infection, obesity, smoking, alcohol consumption and exposure to environmental factors that increase oxidative stress (14) with a common effect on breakage of sperm DNA.

The evaluation of DNA integrity by DNA fragmentation index (DFI) in the context of male infertility has gained popularity over the last decade and is now commonly used for semen quality assessment in infertile men with normal and abnormal sperm parameters, as well as in cases of recurrent miscarriages (15,16).

Several studies have shown that high DFI is associated with reduced fertilization rates, embryo quality, clinical pregnancy rates, ongoing pregnancy rates, and live birth rates (17-19). While other reports have demonstrated that increased DFI does not impair assisted reproductive technology (ART) outcomes (20,21), including cumulative live birth rates (22,23), there is now growing literature to support the association of high DFI with impaired in-vitro fertilization (IVF) pregnancy and miscarriage rates (18,24-28).

Over the last decade, several methods have been proposed to improve DNA integrity in attempt to improve spontaneous pregnancies and ART outcomes in men with high DFI. Though treating possible identifiable factors may contribute to a reduction in DFI levels (14), in most cases, the cause for elevated DFI is unknown, consequently, further investigation is required to establish a treatment approach for patients with idiopathic abnormal DFI levels, especially for those facing fertility treatments.

One possible mechanism thought to elevate DNA fragmentation is through exposure to reactive oxygen species (ROS) (29). Sperm are exposed to free radicals while being stored in the epididymis and seminal vesicles (30,31). Therefore, a possible approach to lower DNA damage is by providing a semen sample after short abstinence period to reduce the duration of exposure of sperm to the ROS in the epididymis and seminal vesicles (32,33). Few studies have investigated this approach with the evaluation of two consecutive samples given in 1 to 4 hours apart (23-36) using sperm chromatin dispersion test. Gosálvez *et al.* (34) and Dahan *et al.* (35) focused on normozoospermic or mostly normozoospermic men and showed an improvement in DFI (approximately 10%) after short abstinence. In the study by Kulkarni *et al.* (36), 17 men undergoing primary infertility treatment were evaluated for DFI change after short abstinence, with similar, though smaller, 3% improvement. A recent study by Tvrdá *et al.* (37) compared bacterial profile, as well as sperm parameters, membrane integrity, and DNA integrity of 51 men after 2 days and 2 hours abstinence periods and showed improved DNA integrity, among other parameters, after short abstinence. A meta-analysis by Barbagallo *et al.* (38) similarly showed that very short abstinence improves SDF in patients with abnormal sperm parameters.

Data however is still scarce on the effectiveness of this method in the infertile male population. Thus, establishing the basis for possible therapeutic modalities to improve

Highlight box

Key findings

- In infertile men providing double ejaculates 3–4 hours apart, DNA fragmentation index (DFI) improves in the second sample.
- Change in DFI varies, with 42.3% and 13.5% of patients found to have decreases in DFI >5% and >10% in the second ejaculate, respectively.
- For men with DFI of 30–40%, nearly two-thirds have a reduced DFI to the under 30% range.

What is known and what is new?

- Scarce data exists on the effectiveness of very short, 3–4 hours, abstinence, and its positive effect of DFI.
- This is the first study to focus on a population of men with male factor infertility and demonstrate that DFI improves in the second semen sample given shortly after the first sample.

What is the implication, and what should change now?

- Controlled trials are needed to determine if reproductive outcomes are improved using a second ejaculate for infertile men with high initial sperm DFI values.

sperm DFI in male factor infertility cases, remains a necessity.

In this study, we aimed to focus on a population of men with male factor infertility, referred to our male infertility clinic with abnormal semen analysis and investigate whether the DFI improves in the second semen sample given shortly (3–4 hours) after the first sample. We present this article in accordance with the STROBE reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-23-216/rc).

Methods

Study population and data collection

We performed a prospective cohort study at Mount Sinai Hospital, Toronto, Canada, a tertiary university affiliated hospital, between 2012 and 2016. We included men who presented for a fertility evaluation in our male infertility clinic for primary or secondary infertility (at least 12 months or more of regular unprotected sexual intercourse). Infertile men with abnormal sperm parameters who had a DFI measurement were included in this study. Exclusion criteria were previous semen analysis showing azoospermia, previous exposure to chemo-radiotherapy, testicular cancer, and surgical history of orchiectomy or Klinefelter syndrome. Patients were selected based upon their interest and willingness to participate, and the availability of laboratory services. These men were instructed to provide two consecutive semen samples for analysis, the first sample was given after an abstinence of 2–5 days and the second sample was provided within the next 3–4 hours. These two semen samples were assessed in the same andrology laboratory for standard semen analysis and DNA fragmentation evaluation by experienced andrology technicians.

Demographic data, general medical history, reproductive information, and risk factors for infertility were self-reported by men presenting for initial infertility assessment on a computer-based survey after informed consent was obtained. This data was later linked with semen analysis and DNA fragmentation data done at the Mount Sinai Hospital Andrology Laboratory, Toronto, Canada.

Semen analysis

The baseline sample was provided following 2–5 days of abstinence and the second 3–4 hours later. The two consecutive semen samples were provided by means of masturbation and delivered for assessment in the same laboratory (Mount Sinai Hospital, Toronto, Canada) and analyzed according to the 2010 WHO criteria (39). This evaluation included sample volume (mL), concentration (million/mL), motility (%), progressive motility (%), normal morphology (%), viability (%), and total motile count (TMC) (million). The classification of normal sperm parameters was according to the widely accepted WHO semen analysis reference range guidelines (39).

Macroscopic and microscopic evaluation of the semen was performed and an analysis of two specimens from each semen sample were used to determine the average value in the evaluation of the sperm concentration and motility using Makler chamber (Sefi Medical Instruments, Haifa, Israel) and computer-assisted sperm analysis (CASA). Morphology assessment was done using a microscopic high-power evaluation of at least 200 sperm for characteristics such as acrosome membranes intactness and shape of the sperm head, neck, midpiece, and tail.

Viability was measured by eosin-nigrosin assay, as previously described (40), and assessed by counting of at least 100 spermatozoa, followed by replicate counts of additional 100 sperm on each of the two slides with viable (sperm head unstained) and non-viable (sperm head stained) sperm count.

DNA fragmentation was tested on a frozen prepared semen sample using the sperm chromosome structure assay (SCSA), as previously described (41,42). Briefly, samples were treated for 30 seconds with 400 µL of a solution of 0.1% Triton X-100, 0.15 M NaCl, and 0.08 N HCl. After 30 seconds, 1.2 mL of staining buffer was admixed to the test tube, and the sample was analyzed by flow cytometry activated cell sorter (Caliburflow cytometer, Becton Dickinson, San Jose, CA, USA). A minimum of 5,000 cells were analyzed by a flow cytometry activated cell sorter scan interfaced with a data handler. The proportion of cells exhibiting an abnormal emission of red fluorescence, reflecting the percentage of sperm with denatured DNA, was recorded. Other assays such as sperm aneuploidy testing, sperm penetration assays, acrosome reaction or sperm epigenetics were not performed for the purpose of this study.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The collection of data and the analysis of the data in this database were

Table 1 Basic characteristics of the study population (n=52)

Parameters	Value
Age (years)	38.7±6.0 (38.1)
BMI (kg/m^2)	27.0±4.9 (26.5)
Smoker	10/52 (19.2)
Alcohol use	7/52 (13.5)
Marijuana use	5/52 (9.6)
Infertility (years)	3.9±3.9 (2.2)
Fathers to children	6/52 (11.5)
Chronic disease[†]	7/52 (13.5)
Medications use[‡]	10/51 (19.6)[§]

Data are presented as mean ± SD (median) or n/N (%). [†], the chronic disease parameter including cardiovascular disease or hypertension, inflammatory bowel disease, diabetes mellitus, major depressive disorder, and thyroid disease; [‡], medication used included levothyroxine for hypothyroidism, statins, selective serotonin reuptake inhibitors, and multi-vitamins and fertility supplements; [§], data on medication use was missing for 1 out of 52 patients. BMI, body mass index; SD, standard deviation.

approved by the Research Ethics Board of the Mount Sinai Hospital with reference numbers 05-0161-E (collection of data) and 07-0032-E (analysis of data) respectively. The date of the approval was October 18, 2005 and October 30, 2007. All participants have signed the Institutional Review Board (IRB)-approved informed consent form.

Statistical analysis

Quantitative variables were presented as either mean ± standard deviation (SD) or median. Differences in characteristics across samples were assessed using the Wilcoxon signed-rank test. Comparison of categorical parameters was done using the Pearson χ^2 test, as well as the Fisher's exact test, as indicated. Univariate and multivariate logistic regression models were used to test the association between demographics, parameters and the decrease in DFI between the semen samples. All hypothesis tests were two-tailed and P values <0.05 were considered statistically significant. Statistical analysis was performed in R Foundation for Statistical Computing, version 4.0.0 (The R Project for Statistical Computing, Vienna, Austria).

Results

During this study period, we referred 52 patients to the andrology laboratory to perform a trial of "double ejaculation", giving two semen samples 3–4 hours apart (the first after a 2–5-day abstinence period), in order to evaluate the levels of DFI in each sample.

The basic characteristics of the study population, including the baseline semen analysis results are presented in *Table 1*. The mean age was 38.7±6.0 (range, 27–59) years and the duration of infertility was 3.9±3.9 years.

A comparison between the first semen sample analysis and the second, given 3–4 hours apart, was done for the entire study population (*Table 2*). DFI significantly decreased in 3.8 percentage points (9.8%) in the second semen sample compared to the first DFI (mean ± SD, 35.1%±21.6% *vs.* 38.9%±21.4%, respectively, P<0.001). Forty patients (76.9%) had improved DFI in the second sample, 4 patients (7.7%) had the same DFI levels (change of less than 1%), and 8 (15.4%) had higher DFI after the very short abstinence. Among those with improved DFI, the average decrease in DFI was 6.0±4.0 percentage points (15.0% decrease).

This comparison also demonstrated lower volume in the consecutive semen sample (2.3±1.4 *vs.* 1.5±0.9 mL, respectively, P<0.001), while the remaining parameters were not significantly influenced. Overall, 24/52 (46.2%) men had initial TMC >5 million, with 6/24 (25.0%) declining to ≤5 million in the second sample.

Twenty-two out of 52 men (42.3%) and 7/52 (13.5%) were found to have an absolute decrease in DFI of more than 5 and 10 percentage points in their second ejaculate, respectively. For men with moderately increased DFI at the range of 30–40% in the first sample, 64% (7/11) of DFIs reduced to under 30% in the second. Further analysis evaluated the change in sperm parameters in men with a baseline DFI of 30% or less compared with men who had an initial high DFI of more than 30%. This comparison showed that the high DFI group (>30%) had a larger decrease in DFI percentage point in their second sample compared to those with DFI of 30% or less (–4.5±6.9 *vs.* –2.7±3.1 percentage points, respectively, P<0.001). The change in each sperm parameter between samples was similar in these groups, except for the change in viability, which improved in the second sample of the high DFI

Table 2 Semen analyses results and differences between the first and consecutive semen samples given 3–4 hours apart

Parameters	First sample	Second sample	Difference between samples	P value
Semen parameters				
DFI (%)	38.9±21.4	35.1±21.6	−3.8±5.7	<0.001*
Volume (mL)	2.3±1.4	1.5±0.9	−0.8±0.9	<0.001*
Concentration (million/mL)	44.6±52.7	38.1±49.5	−6.5±25.4	0.07
Motility (%)	18.9±12.5	17.9±10.7	−1.0±7.0	0.35
Progressive motility (%)	13.3±9.5	12.5±8.2	−0.9±5.9	0.41
Normal morphology (%)	10.2±8.0	10.1±8.1	−0.0±0.9	1.00
Viability (%)	53.5±21.9	52.9±20.0	0.0±10.2	0.80

Data are presented as mean ± SD. *, P<0.05. DFI, DNA fragmentation index; SD, standard deviation.

Table 3 Univariate and multivariate logistic regression models to predict second DFI of 30% or less in men with a DFI above 30% in their first semen analysis

Model	OR (95% CI)	N	P value
Univariable logistic regression			
Age	0.91 (0.74–1.07)	31	0.29
First DFI	0.64 (0.37–0.86)	31	0.03*
Father to children		31	0.99
0	Reference	26	
1	Not estimated	5	
Smoking		31	0.99
No	Reference	28	
Any smoking	Not estimated	3	
BMI	0.96 (0.75–1.17)	24	0.72
Multivariable logistic regression†			
Age	0.89 (0.60–1.30)	31	0.54
First DFI	0.62 (0.39–0.98)	31	0.04*

†, from the univariable model, patient's age and first DFI were entered into a multivariable model due to clinical importance or statistical significance; *, P<0.05. DFI, DNA fragmentation index; OR, odds ratio; CI, confidence interval; BMI, body mass index.

group (2.6±8.6 percentage points) while the DFI ≤30% group demonstrated a decrease (−3.9±11.3 percentage points) in the percent of viable sperm (P=0.03).

Univariate and multivariate logistic regression models were built to predict a second DFI ≤30% in men who had DFI >30% in their first semen analysis (*Table 3*). The univariate analysis included the parameters of age, first DFI, smoking, secondary infertility, and body mass index (BMI), with first DFI being the only statistically significant parameter (P=0.03). From the above, age (due to clinical importance) and first DFI (P=0.03 in the univariate model) were selected and entered into a multivariate model in which the parameter of first DFI [odds ratio (OR), 0.62; 95% confidence interval (CI): 0.39–0.98; P=0.04] was negatively predictive of DFI shift from above 30% to less in the second sample.

Univariate and multivariate logistic regression models for the prediction of a relative 15% decrease in DFI between the first and consecutive semen sample (*Table 4*) did not identify statistically significant independent predictive parameters, including baseline sperm parameters.

Discussion

In this study, we evaluated the effect of a short, 3–4-hour abstinence period, on sperm DFI of 52 infertile men. Semen parameters and DFI levels were compared between the first and second ejaculate for each patient in order to detect a change in DFI using this method. This study demonstrates a reduction in DFI in the population of interest—men with male factor infertility.

Our results demonstrate that this technique is associated with a decrease of 3.8 percentage points (9.8% relative decrease) in DFI and that 42% of patients will have at least a 5 percentage points absolute reduction in DFI. Moreover, the group of patients with baseline DFI higher than 30% will have a larger decrease in DFI compared to those with DFI of 30% or less (−4.5 *vs.* −2.7 percentage points decrease in DFI). While this absolute decrease in DFI, though

Table 4 Univariate and multivariate logistic regression models to predict at a relative 15% decrease or more in DFI in the second semen sample

Model	OR (95% CI)	N	P value
Univariable logistic regression			
Age	1.00 (0.91–1.10)	52	0.96
First DFI	0.97 (0.94–1.00)	52	0.07
Father to children	0.60 (0.08–3.37)	52	0.57
0	Referent	46	
1	Not estimated	6	
Smoking		52	0.99
No	Referent	44	
≤1 pack	0.44 (0.06–2.27)	7	0.35
2 packs or more	Not estimated	1	0.99
BMI	1.07 (0.94–1.24)	41	0.33
Multivariable logistic regression[†]			
Age	1.05 (0.94–1.17)	52	0.42
First DFI	0.97 (0.93–1.00)	52	0.06

[†], from the univariable model, patient's age and first DFI were entered into a multivariable model due to clinical importance. DFI, DNA fragmentation index; OR, odds ratio; CI, confidence interval; BMI, body mass index.

significant, is relatively small, our study also shows that almost two-thirds (64%) of patients with high DFI within the 30–40% range will have DFI value of less than 30% on the second semen test, which is widely used as a cutoff for abnormal DFI levels and for which reproductive outcomes were reported to be better compared to DFI >30% (14,43-46).

High levels of DFI in infertile men sets a challenge to physicians attempting to treat couples with male factor infertility, and thus, this is the population of interest with regard to therapeutic modalities to reduce DFI. Our study is unique by the inclusion of a population of only infertile men, referred to our male infertility clinic. We did not limit our study population to those with DFI above 30%, as the cutoff of DFI level associated with reproductive outcomes is still in debate. However, as many of the studies refer to this value, we performed a separate analysis to compare the repeat ejaculation technique by DFI above or below 30% and demonstrated a difference in the DFI drop in the second sample.

This study has several limitations, the main being the relatively small sample size and the lack of a control group and data on fertility outcomes with the second sample, which does not allow us to draw conclusions regarding its beneficial effect on intra-uterine insemination (IUI) or IVF outcomes. Moreover, while DFI only reflects DNA breakage it does not provide information on chromosomal numerical or structural errors, and therefore its decrease after short abstinence should be further studied and correlated with genetic testing and reproductive outcomes. Some patients were started on fertility supplements that include antioxidant and multi-vitamins prior to their infertility clinic visit or later and advised regarding lifestyle modification, before the semen samples were given. However, as each man served a self-control, this should not have caused any bias.

DFI assays were performed on frozen semen samples. As previously shown (47), DFI analysis done on fresh and frozen sperm samples provide identical results, and therefore the use of frozen semen samples for DFI analysis did not affect the DFI values.

Our findings are in agreement with the reports of several previous studies that investigated the change in SDF after very short abstinence (34-36). A study by Gosálvez *et al.* (34) evaluated 24 and 3 hours abstinence effect on DNA fragmentation in 21 normozoospermic infertile men and 12 sperm donors and showed lower baseline levels of SDF and improved results of sperm selection after short abstinence. A recent study, by Kulkarni *et al.* (36), evaluated the difference in DFI in 17 patients and showed a 3 percentage points (10.8%) decrease between tests. However, the only data reported was a comparison of means between samples and 88% of men provided the second sample after 1-hour and only one patient after a 3-hour abstinence. Dahan *et al.* (35) reported the results of 112 men who had their first semen analysis as part of an infertility work-up, and provided two semen samples, 3 hours apart. They showed a significant decrease in DFI in the second sample and reported that the factors predictive of a relative 30% or more decrease in DFI levels are age and use of antioxidant formulation, while the initial DFI was borderline statistically significant (P=0.06). Their important study is the largest so far to evaluate the effect of very short abstinence (3 hours) on DFI and provide multivariate regression model for factors predictive of the DFI change. However, the population in that study included patients on their first infertility work-up and first semen samples, with 60% of subjects (68/112) having normal sperm parameters according to the 2010 WHO guidelines in their initial semen analysis. Therefore, their results, and the results of the study by Gosálvez *et al.* (34), cannot be generalized to

infertile men with abnormal semen analysis. We, however, aimed to assess the effect of the very short abstinence on DFI levels among the infertile men with abnormal sperm parameters, thus we included only those with abnormal semen parameters. Our results showed similar improvement in DFI levels and additionally added data on the prediction of reduction of DFI levels from above 30% to under 30%.

Numerous studies have investigated the predictive value of DNA fragmentation levels on spontaneous abortion and ART outcomes—IVF and intracytoplasmic sperm injection (ICSI), with the use of the 30% cutoff value for abnormally high DFI (20,24,26).

Several studies and meta-analyses on DFI and ART outcomes concluded that though it is still debatable, data suggests that high SDF in couples undergoing IUI and IVF is associated with lower pregnancy rates and live birth rates as well as higher spontaneous abortion (20,22,24,26,43,48,49).

In our study, the regression models we created to predict a reduction in DFI to below the 30% cutoff, in those with initial DFI >30%, showed that the higher the initial DFI is, the lower the chances to reduce DFI to under 30%, as expected. However, we noticed that almost two-thirds (64%) of patients with a high DFI within the 30–40% range will have DFI value of less than 30% with the second assay.

Therefore, our results suggest that this technique, aimed to reduce DFI, should be further studied, especially its beneficial potential in cases of idiopathic slightly elevated DFI, as those within the 30–40% DFI range. Nevertheless, as we did not report on IUI or IVF outcomes, we cannot conclude regarding the association between 3 and 4 hours abstinence or even proven DFI decrease in this technique and fertility outcomes.

Several mechanisms on the link between short abstinence and improved SDF were hypothesized (38). It has been suggested that the main mechanism for SDF formation is the exposure of sperm to ROS (19), dead spermatozoa and leukocytes (50) during the transport of sperm from the testes to the epididymis and its storage there, resulting in sperm DNA breakage (30,31) The duration of transition through the epididymis varies and might be affected by the frequency of ejaculation, thus short abstinence might be associated with faster passage and decreased SDF. An additional theory focuses on the seminal plasma composition and its effect on SDF. Seminal plasma metabolites are secreted and accumulate with time and therefore short abstinence may result in an overall lower metabolites levels and subsequently lower fragmentation levels (51).

A previous study by Shen *et al.* (52) investigated the mitochondrial membrane potential (MMP), which correlates with sperm motility, in spermatozoa from samples given after several days *vs.* 1–3 hours of abstinence using proteomics technology. Their investigation demonstrated higher MMP after shorter abstinence, which represents another possible mechanism for improved sperm quality after short abstinence. Further research is required to identify these factors and enable prevention and treatment if needed.

Nevertheless, the technique of very short, 2–4 hours abstinence, to minimize the duration of exposure to potentially harmful ROS or other negative factors, presents encouraging results of reduction in DFI.

The association between very short abstinence and ART outcomes has been investigated recently in a systematic review and meta-analysis by Barbagallo *et al.* (53). The authors demonstrated significantly improved ART outcomes—implantation, clinical pregnancy, and live birth rates—following very short abstinence, compared to standard, 2 days or more, abstinence period in men with altered sperm parameters.

Conclusions

To conclude, this study demonstrates significant improvements in DFI in the second sample given within 3–4 hours from the first for men with infertility. Over 75% of patients had improved DFI. Patients with DFI above 30% demonstrate greater absolute decrease in DFI and the majority of those with moderately elevated DFI (30–40%) will revert to DFI under 30%. This data may encourage further controlled trials that are required to determine if the second ejaculate provides improved reproductive outcomes for men with initially high DFI values.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-23-216/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-23-216/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of the Mount Sinai Hospital with reference numbers 05-0161-E (collection of data) and 07-0032-E (analysis of data) respectively. The date of the approval was October 18, 2005 and October 30, 2007) and informed consent was obtained from all individual participants.

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