Sperm deoxyribonucleic acid fragmentation index at the time of intracytoplasmic sperm injection and standard in vitro fertilization is correlated with lower fertilization but not with blastocyst genetic diagnosis

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Objective: To determine the effects of sperm deoxyribonucleic acid (DNA) fragmentation at the time of fertilization on in vitro fertilization (IVF) outcomes and genetic diagnosis using next generation sequencing.
Design: Prospective double-blinded study.
Setting: Private Clinic.
Patients: Couples (n = 150).
Intervention: In vitro fertilization with preimplantation genetic testing for aneuploidy and sperm DNA fragmentation assay, as in sperm chromatin structure assay the day of retrieval.
Main Outcome Measures: Laboratory outcomes are listed in the results section. Statistical analysis was performed using JMP, XYL-STAT, and STATA version 15.
Results: The sperm DNA fragmentation index (DFI) in the neat ejaculate did not predict fertilization rate, quality, blastulation, or genetic diagnosis. No statistically significant results were obtained comparing <15% with >15%, <20% with >20%, <30% with >30% except for DFI. No statistically significant differences in oocyte source age or male age were observed. No statistically significant differences comparing <15% with >15%, <20% with >20%, <30% with >30% DFI at the time of standard IVF or intracytoplasmic sperm injection (ICSI) were observed for % euploid, aneuploid, mosaic, blastulation, biopsied, or D5/total biopsied. The DFI of >15% had more good quality D3 embryos than the <15% group, as did the >20% group compared with the <20% group. The ICSI fertilization was significantly higher in all 3 lower percentage groups compared with the higher counterpart. Standard IVF had significantly more blastocysts/fertilized suitable for biopsy and more D5/total number biopsied than ICSI embryos despite no difference in DFI.
Conclusions: The DFI at fertilization is correlated with decreased fertilization for ICSI and IVF. (Fertil Steril Rep® 2023;4:183–9. ©2023 by American Society for Reproductive Medicine.)
Key Words: Sperm DNA fragmentation, Preimplantation genetic testing, fertilization, IVF outcomes, DFI

Previous studies have shown correlations with sperm deoxyribonucleic acid (DNA) fragmentation (SDF) and embryo development in the in vitro fertilization (IVF) laboratory (1–4). There have been conflicting reports about the possible effects of SDF on fertilization and whether intracytoplasmic sperm injection (ICSI) is an appropriate tool to counteract some of the deleterious effects (1–4). Early embryonic development before the maternal zygotic transition does not appear to be affected by SDF levels, but after the maternal zygotic transition, SDF is correlated with a higher incidence of embryo arrest, poor embryo quality, and slower development to the blastocyst stage (5–13). Consistently many studies, including several meta-analyses, have concluded that there is a strong correlation with failed implantation and pregnancy loss when the male partner has high SDF (4, 5, 7–9, 14, 15).

Preimplantation genetic testing for aneuploidy (PGT-A) is a popular tool used in assisted reproductive technology treatments currently, but very few studies have used this tool to determine if SDF is correlated with a higher incidence of embryo aneuploidy or mosaicism. The PGT-A diagnostic technology has improved dramatically in the past decade, and the advent of next generation sequencing (NGS) allows for the analysis of 1.1 million data points in the blastocyst's genome. Many studies have shown that NGS is a more powerful and accurate PGT-A tool than its predecessors, particularly in the detection of embryo mosaicism or segmental aneuploidies (16–24). Misdiagnosis by less powerful PGT-A platforms might explain why embryos diagnosed with euploid failed to implant or resulted in pregnancy loss. The use of these different biopsy methods, blastomere or trophectoderm, and different platforms in previous studies evaluating the relationship between SDF and embryo genetic diagnosis might explain conflicting results. The timing of SDF testing may also account for some conflicting results. Most studies used a previous diagnostic result and not the DNA fragmentation index (DFI) of the neat or processed sample used for IVF. The study design should be considered when comparing results because many are retrospective.

Garcia-Ferreyra et al., (25) in 2015, reported in a retrospective study of 32 donor oocyte cycles using blastomere biopsy and fluorescence in situ hybridization, but with a previously documented terminal deoxynucleotidyl transferase biotin-deoxyuridine triphosphate nick end labeling (TUNEL) result, found that men aged ≥50 years had significantly higher SDF, decreased blastocyst development, and higher aneuploidy rate (25). Kaarouch et al., (26) in 2015 attempted to look at aneuploidy and mosaicism by removing 2 blastomeres from the same embryo for fluorescence in situ hybridization. This was a prospective study with 39 couples that used a previously documented TUNEL result from diagnostic testing of the male partner. This study concluded that there was a significantly higher number of embryos with aneuploidy and mosaicism in men with high SDF. There was also a significantly lower implantation and pregnancy rate in men with high SDF with embryos diagnosed euploid (26). Gunnala et al., (27) in 2016, used a retrospective cohort study design with trophectoderm biopsy and array comparative genomic hybridization with a previously documented

TUNEL result. This study determined that there was no correlation between SDF and aneuploidy rate except when the female partner was aged ≤37 years (27). Gat et al., (28) in 2017, published a retrospective study using 134 couples over 177 cycles with trophectoderm biopsy and array comparative genomic hybridization with a previously documented sperm chromatin structure assay test result. This study did not report any differences in aneuploidy rate, blastocyst morphology, pregnancy rate, or loss (28).

Many studies have shown that SDF is correlated with poor IVF outcomes and a higher incidence of pregnancy loss without the use of PGT-A. The conflicting reports of PGT-A and SDF studies because of different biopsy methods and PGT-A platforms, and the timing of SDF data used to categorize the patient, reveals gaps in the literature and many research questions unanswered. The objective of our study was to determine the effects of SDF at the time of insemination on fertilization, embryo development, and blastocyst genetic diagnosis using NGS and pregnancy rates with the transfer of a euploid blastocyst.

MATERIALS AND METHODS

Institutional review board at Eastern Virginia Medical School approval was received (IRB# 17-11-EX-0222) before the beginning of the study. Patients who presented for IVF with PGT-A at Midwest Fertility Specialists (Carmel, IN) were consented to by the principal investigator (A.L.B.). Patients were not excluded for the use of donor oocytes or surgically derived sperm samples. The patients in the study were autologous couples using the male partner's ejaculated sperm over the course of a 6-month period in 2018. If they agreed to participate, the consent form along with the male lifestyle questionnaire (age, body mass index, smoking status, vitamins and supplement intake, abstinence period, area of residence, and heat exposure) was completed and witnessed before the oocyte retrieval. Patients were only excluded from the study (n = 16) if they later chose not to have embryo biopsy. The study included 166 couples, of which 150 completed the study. Of the 150 cycles, 133 had blastocysts for biopsy. On a per embryo basis, 480 blastocysts were analyzed.

Cycles included standard insemination, ICSI, or a combination of both methods. Oocytes were retrieved by transvaginal ultrasound, and cumulus-oocyte complexes were placed either in microdrops of G-IVF PLUS for standard insemination or in wells of G-IVF PLUS until later denudation for ICSI and placed in the incubator set at 7.3% carbon dioxide and 5% oxygen.

Sperm was collected by masturbation in a sterile collection cup. The semen sample was delivered to the IVF laboratory and placed on a warmer at 37 °C for liquefaction for 30–60 minutes. A basic semen analysis was performed measuring the sperm count (millions/mL), percent motile, volume, viscosity, and the presence of either white blood cells or sperm agglutination. A visual assessment of the morphology was documented as normal or abnormal by the same embryologist, the principal investigator, for all samples. Three vials with random numbers were selected and documented for each semen sample. An aliquot of the raw semen, approximately

0.5 mL, was placed in the first vial and flash-frozen in liquid nitrogen. The remaining semen was layered over a 2-layer gradient (Isolate, Irvine Scientific, Santa Ana, CA) for centrifugation at $3000 \times g$. for 15 minutes. The supernatant was removed, and the pellet was washed for an additional 5 minutes. The supernatant was removed. The pellet was resuspended with G-IVF PLUS to achieve a final concentration of approximately 2×10^6 motile sperm per mL. An aliquot of this sample was placed in the second vial labeled with a random number and flash-frozen in liquid nitrogen. The processed sperm sample was then placed in the incubator set at 7.3% carbon dioxide and 5% oxygen until the time of ICSI or insemination. The aliquot taken immediately postprocessing was used to evaluate the effectiveness of the sperm processing and to determine if sperm chromatin integrity changed from the time of processing to the time of insemination. The aliquots were analyzed using the sperm DNA fragmentation assay (SDFA) (acridine orange/flow cytometry SDFA) and the oxidative stress adduct (OSA) test, which directly measures sperm damage from oxidative stress by quantifying the presence of "adducts," molecules in semen covalently modified by free radicals/reactive oxygen species. The processing method of gradient and wash was effective for most patients, with an overall 40.2% decrease in DFI, a 27.3% improvement in OSA, and a 38.6% decrease in high-DNA stainability (HDS). However, 15.8% of men had an increase in DFI, and 20.3% had an increase in OSA from the neat semen sample to the sample used for IVF. The full data from the sperm study and the data from the lifestyle survey are presented in another body of literature.

Patients using surgically derived sperm samples from the epididymis or testes were also included in this study. However, only the remainder of the sperm sample after ICSI was frozen for analysis. These vials were also labeled with a random number. The processing of these samples before ICSI was a wash-only step in G-IVF PLUS (Vitrolife Sweden) for 5 minutes at $3000 \times g$. The processed sperm sample was then placed in the incubator set at 7.3% carbon dioxide and 5% oxygen until the time of ICSI. Samples were treated with pentoxifylline as needed right before ICSI.

Oocytes intended for ICSI were gently denuded using hyaluronidase (Hyal, Vitrolife, Sweden) and pipettes of decreasing size and were then incubated in G-IVF PLUS. The ICSI was performed using PVP (Irvine Scientific) and G-MOPS (Vitrolife) under warmed mineral oil (OvOil, Vitrolife) on a heated stage. The remaining sperm not used for ICSI in the tube was loaded into the third vial and flash-frozen in liquid nitrogen. Oocytes were cultured overnight in G-1 plus (Vitrolife) until approximately 18 hours postinjection. Zygotes with 2 pronucleus present were considered normally fertilized. The oocytes intended for standard insemination were cultured in microdrops of 35 μL of G-IVF PLUS covered in mineral oil (OvOil, Vitrolife). The processed sperm sample was added to each drop to achieve 200,000 motile sperm per mL. The remaining sperm not used for insemination in the tube was loaded into the third vial and flash-frozen in liquid nitrogen. The random numbers on the vial per each patient were written in a notebook and recorded in the data collection Excel file that is backed up on the server each night.

Samples were sent to ReproSource (Woburn, MA) for analysis in batches to further blind the data. All data from the study were double-blinded. After 84 completed cycles, the data were unblinded for the midstudy analysis. The remaining unblinding occurred after the study was closed to ensure no bias on any type of data collected.

Zygotes were cultured in G1 PLUS (Vitrolife) until day 3 of development. The embryos were assessed to determine cell number, percentage of fragmentation, and blastomere symmetry. A small tunnel was made in the zona pellucida using standard laser procedure. Embryos were cultured in corral dishes (CooperSurgical, Trumball, CT) in G2 PLUS covered in mineral oil (Vitrolife). On day 5 of development, embryos were graded and divided into blastocysts ready for trophectoderm biopsy, embryos that were developing but not yet suitable for biopsy, and arrested embryos were discarded. Embryos not yet suitable for biopsy were moved to fresh dishes of G2 (Vitrolife) and cultured to day 6. Trophectoderm biopsy was performed using standard laser procedure. The trophectoderm cells were analyzed using NGS by either Cooper Genomics (Detroit, MI) or Ovation Fertility (Nashville, TN). Embryos were vitrified using Cryotech (Cryotec, Shinjuku-ku, Japan) medium and device, and then moved to storage in a dewar until genetic results were obtained. Embryos were warmed per Cryotech procedure and a subsequent future frozen embryo transfer.

Sperm DFI and HDS were evaluated using the acridine orange with flow cytometry method (SDFA, ReproSource, Marlborough, MA). The OSA test (OSA, ReproSource) was used to quantify oxidative stress by measuring covalent reactions between reactive oxygen species and free radicals and the structures of sperm. The DFI of the neat, postgradient and sample used for insemination was calculated by analyzing 5000 sperm and determining the percentage that stained red, indicating DNA with double- or single-strand breaks, and the sperm that stained green, indicating healthy, undamaged DNA.

All cycle data were entered in the study spreadsheet by the principal investigator on completion of the cycle. The genetic diagnosis, gender, method of insemination, day of development, grade, ages of the oocyte, and sperm source were collected on a per blastocyst biopsied basis in an additional study tool. All data were evaluated by both the neat semen DFI and the DFI at the time of insemination.

All categorical data were analyzed using contingency tables and Pearson's χ^2 test in JMP software and considered statistically significant at $P = .05$. Patient level data for percentage fertilized by ICSI, total fertilized of retrieved oocytes, good embryos on day 3 (6 cell grade 3 or better) of the number fertilized, blastocyst formation of the number fertilized, biopsied of the number fertilized, and the number of embryos that arrested at day 3 of the number fertilized were compared between groups using Wilcoxon's rank sum test (JMP, SAS 2018, Cary, NC). Two proportion aggregate patient data in groups for percentage fertilized by ICSI, total fertilized of retrieved oocytes, good embryos on day 3 (6 cell grade 3 or better) of the number fertilized, blastocyst formation of the number fertilized, biopsied of the number fertilized, and the number of embryos that arrested at day 3

TABLE 1

Patient data grouped by sperm DNA fragmentation index (DFI) in the neat or insemination sample.

	< 15/ > 15 Neat	< 20/ > 20 Neat	< 30/ > 30 Neat	> 15/ > 15 Insem	< 20/ > 20 Insem	< 30/ > 30 Insem
Number (cycles)	75/51	73/58	101/30	106/44	117/33	129/21
Oocyte age	0.574	0.405	0.433	0.704	0.696	0.665
Male age	0.993	0.562	0.222	0.022	0.006	0.005
DFI	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
ICSI fertilization	0.286	0.27	0.022	<0.0001	<0.0001	<0.0001
Good day 3	0.175	0.796	0.490	0.004	0.009	0.119
D5 Bx/total	0.154	0.463	0.188	0.388	0.517	0.544
Euploid	0.160	0.461	0.661	0.135	0.146	0.630
Aneuploid	0.208	0.732	0.302	0.624	0.458	0.890
Mosaic	0.779	0.730	0.441	0.212	0.272	0.310
Blastulation	0.564	0.378	0.440	0.584	0.352	0.176
# Bx/total	0.598	0.439	0.784	0.937	0.386	0.040
Arrested day 3	0.957	0.583	0.402	0.208	0.261	0.141

Note: Aggregate patient data analyzed using 2×2 χ^2 analysis in XYSTAT. *P* values considered significant at *P*< .05.
Neat = raw ejaculate; Insem = sperm at the time of ICSI or IVF insemination; DFI = sperm DNA fragmentation index; D5 Bx/total = the number of blastocysts biopsied on day 5 divided by the total number biopsied; # Bx/total = the number biopsied divided by the number fertilized.

Broussard. Sperm DFI at fertilization and IVF outcomes. Fertil Steril Rep 2023.

of the number fertilized were analyzed in XYLSTAT (Excel, Microsoft, Redmond, WA) using χ^2 2×2 table analysis. Unadjusted and fully-adjusted multiple logistic regression models for DFI as a predictor of outcomes, taking into consideration the age of the man, age of the oocyte source, IVF/ICSI status, and autologous/OD status, were performed using STATA version 15 (StataCorp, College Station, TX). Two-sided statistical tests were evaluated at $\alpha = 0.05$.

RESULTS

In our cohort of 166 consented patients, 150 completed the study, with 133 having blastocysts biopsied. Couples were only excluded (n = 16) if they later chose not to have a biopsy performed on their blastocysts. A total of 480 blastocysts were biopsied, and data were analyzed. Data were categorized by the age of the oocyte source to include donor and autologous cycles. Some couples only had data on the sperm used for ICSI or IVF insemination because of low-sperm numbers.

Groups were compared using neat DFI and the DFI on the sample at the time of IVF insemination or ICSI. The parameters analyzed were ICSI fertilization (number fertilized/number injected), good day 3 embryos (embryos graded ≥ 6 cells with ≤ 5% fragmentation and no severe symmetry divided by the number fertilized), day 5 biopsied of the total number biopsied, number euploid, aneuploid, or mosaic of the total number biopsied, blastulation (number of any quality blastocysts divided by the number fertilized, the number biopsied of the total number fertilized, and the number of embryos that arrested on day 3 of the number fertilized). Mean oocyte age, male age, and DFI were also compared between groups. No statistically significant differences comparing all of the aforementioned parameters except for DFI (P<.0001) for the groups categorized by neat DFI: <15% to >15%, <20% to >20%, and <30% to >30%. No significant differences in oocyte source age or male age (P=.5743,.4048, and.4325, respectively) were observed. No statistical differences comparing <15% with >15%, <20%

with >20%, and <30% with >30% DFI at the time of standard IVF or ICSI were observed for % euploid, aneuploid, mosaic, blastulation, biopsied, or D5/total biopsied. The DFI of >15% had more good quality D3 embryos than the <15% group (P=.007), as did the >20% group compared with the <20% group (P=.004). The ICSI fertilization was significantly higher (P<.0001) in all 3 lower percentage groups compared with the higher counterpart. Cycles included standard insemination, ICSI, or a combination of both methods. Fertilization rates were analyzed for both methods. The fertilization method was recorded for all blastocysts that underwent biopsy. Standard IVF had significantly more blastocysts/fertilized suitable for biopsy (P<.0001) and more day 5 blastocysts of the total number biopsied than ICSI embryos (P=.0317) despite no difference in DFI (P=.1131) (Table 1 and Supplemental Table 1, available online).

All multiple logistic regression models included square and cubic polynomials for the DFI continuous variables. Results suggested no significant linear or nonlinear association between DFI, neat or at the time of insemination, and blastocyst quality or ploidy status, either before or after adjustment for age of the male, age of the oocyte source, ICSI status, and autologous/OD status. In both unadjusted and adjusted logistic regression models, a nonlinear positive association was observed between DFI in a neat sample and day 6 at biopsy. This association was not observed with the insemination sample.

The OSA, a measurement of oxidative stress in the sperm, and the HDS, a measurement of HDS that may be indicative of immature sperm and sperm with higher histone retention, were both analyzed for the sperm samples at the 3 different timepoints within the study. In a similar trend with DFI, the neat semen OSA and HDS were not significantly associated with % euploid, aneuploid, mosaic, blastulation, percentage biopsied, day 5/total biopsied, good quality day 3 embryos, or the number of embryos that arrested at day 3. However, the OSA score at the time of ICSI or IVF insemination was

TABLE 2

Pregnancy rates by sperm DNA fragmentation index of neat ejaculated sample.

	< 15%	> 15%	*P* value	< 20%	> 20%	*P* value	< 30%	> 30%	*P* value
Number of embryo transfers	48	67		65	50		87	28	
Pregnant (hCG >20)	64.6%	61.2%	.711	61.5%	64%	.787	62%	64.3%	.833
Fetal cardiac activity	56.3%	53.7%	.789	53.8%	56%	.818	54%	57%	.773
Pregnancy loss	16.1%	9.8%	.525	15%	18.8%	.671	27.8%	5.6%	.346

Note: Data analyzed by χ^2 analysis. No significant differences in pregnancy rate, fetal cardiac activity at 6 weeks gestation by transvaginal ultrasound, or pregnancy loss.
hCG = human chorionic gonadotropin.

Broussard. Sperm DFI at fertilization and IVF outcomes. Fertil Steril Rep 2023.

correlated with decreased total fertilization. An OSA score of 4.4, the high cut-off score correlated with male infertility, was correlated with lower total fertilization (*P*=.0027). The borderline score of 3.8 was negatively associated with total fertilization (*P*=.0446). The OSA, at 3.8 and 4.4, was positively correlated with DFI on both the neat semen sample and the sample used for insemination or ICSI (*P*<.0001). High-DNA stainability at the time of ICSI or IVF insemination was higher in groups with <10% HDS compared with moderate (10%–15%, *P*=.0283) and high (>15%, *P*=.0605, Table 2). The HDS was also positively correlated with DFI in the neat semen and at the time of insemination (*P*<.0001).

Frozen embryo transfers were performed using the best quality euploid blastocyst available. There was no difference in pregnancy rates, fetal cardiac activity at 6 weeks, or pregnancy loss comparing <15% to >15%, <20% to >20%, and <30% to >30% DFI on the neat semen sample or the sample used for ICSI or insemination (Table 3).

Post hoc power calculations were performed using STATA version 17 (StataCorp, College Station, TX). In the insemination sample, there were 44 with DFI of >15% and 106 with DFI of <15%; 33 with DFI of >20% and 117 with DFI of <20%; and 21 with DFI of >30% and 129 with DFI of <30%. Assuming a 2-sided χ^2 test for the difference between 2 proportions, $\alpha = 0.05$, and a baseline proportion of euploid embryos of 60%, we would have a power of 20%–40% to detect a difference of 10%–15% between exposure groups on proportion of euploid embryos. By contrast, assuming a 2-sided χ^2 test for the difference between 2 proportions, $\alpha = 0.05$, power = 0.80, and a baseline proportion of euploid embryos of 60%, we would be able to detect a difference of 0.23%–0.29%.

DISCUSSION

The mechanisms for how DNA fragmentation results in poor fertilization, embryo development, implantation, and live birth rates are unknown. In our study, we did not observe significant differences in embryo development. This included the number of good quality embryos on day 3, the number that were arrested at day 3, the number of blastocysts biopsied per the number fertilized, and the number biopsied on day 5 compared with day 6 with the exception of in the logistic regression model where we observed a positive correlation with day 6 embryos and neat DFI. In this model, the observed relationship was nonlinear and observed before and after adjustment for the age of the man, age of the oocyte source, ICSI status, and autologous/OD status. This observation of decreased morphokinetic development has been reported to be correlated with neat DFI by other studies. The factor of trophectoderm biopsy, which cannot be done until the blastocyst is well established and expanded, which may skew the perception of delayed development. Other developmental trends, such as the number of good quality embryos on day 3, the number that arrested at day 3, and the number of blastocysts biopsied per the number fertilized, have been reported in other studies but were not observed at a significant level in this study. Additional data analyses were performed to determine the possible lack of effects of SDF on developmental checkpoints. Data analyzed by only maternal age showed no differences in the quality of day 3 embryos, a slight trend toward higher blastulation rates in younger patients compared with advanced maternal age, and no difference in the number of embryos biopsied. There was, however, a significant difference in the percentage of euploid by maternal age. This is in agreement with the literature. Other developmental

TABLE 3

Pregnancy rates by DFI at the time of intracytoplasmic sperm injection or in vitro fertilization insemination.

	< 15%	> 15%	*P* value	< 20%	> 20%	*P* value	< 30%	> 30%	*P* value
Number of embryo transfers	91	33		97	25		109	13	
Pregnant (hCG >20)	58.3%	72.7%	.142	59.8%	72%	.262	60.6%	76.9%	.250
Fetal cardiac activity	50.5%	66.7%	.111	52.6%	64%	.306	53%	61.5%	.273
Pregnancy loss	11.3%	25%	.976	12%	16.6%	.110	24%	25%	.769

Note: Data analyzed by χ^2 analysis. No significant differences in pregnancy rate, fetal cardiac activity at 6 weeks gestation by transvaginal ultrasound, or pregnancy loss.
hCG = human chorionic gonadotropin.

Broussard. Sperm DFI at fertilization and IVF outcomes. Fertil Steril Rep 2023.

checkpoints, such as the number suitable for biopsy and the number that were biopsied on day 5 vs. day 6, are difficult to compare with other published studies in which fresh embryo transfer was the end point. To perform a trophectoderm biopsy, typically, a blastocyst needs to be further developed for safe biopsy. This means that more embryos are generally biopsied on day 6 for many patients, and very early blastocysts on day 5 may not be suitable for biopsy after another night in culture. Additionally, factors from the oocyte may also have masked some of the possible effects on the sperm. For many patients in the study, PGT-A was performed because of female factors, such as advanced maternal age, previous losses, or unexplained IVF failures. The patients in this study were not selected based on age, infertility diagnosis, or previous success or failures. It is possible that other effects of SDF were masked by female factors, such as good embryo development before the maternal zygotic transition. Additional future studies could investigate the role of SDF by controlling for female fertility by only using certain age groups, diagnosis, or by splitting cohorts of oocytes between sperm samples with different levels of SDF. Similar studies should also be performed across multiple centers at a larger scale. The patient population in this study, although a typical cross-section of patients undergoing IVF with PGT-A, may not be ideal for reaching adequate conclusions.

The one end point that was significant across different levels of SDF at the time of ICSI or insemination was fertilization. The mechanism of this correlation may be explained by the total health of the sperm. The SDF has been shown to be correlated with other abnormalities, such as abnormal histone retention, nuclear immaturity, and oxidative stress. Sperm with high fragmentation may also be aneuploid because of immaturity or the breaks in DNA resulting from errors during meiosis or mitosis. During fertilization, changes in the sperm, such as oxidative stress disrupting lipid membrane and acrosome function, can greatly disrupt the sperm's ability to fertilize the oocyte. In sperm with high-histone retention, which can be caused by oxidative stress, the sperm proteome can be greatly altered. This can have severe implications on the subsequent embryo and offspring (29–34). Studies are showing alterations in sperm gene expression are correlated with the same issues as SDF, such as fertilization, embryo development, implantation, and live birth rates (31, 34–41). The mechanism of SDF may be alterations of gene expression by fragments across key genes or the retention of histones across key developmental regions that the oocyte is unable to reconstruct at fertilization. Furthermore, breaks in the DNA strands of the sperm around the centrosome, the organizing center of the spindle, may affect fertilization. The centrosome is inherited from the sperm and is key in pronuclear formation after fertilization (42). The DNA breaks may interfere with this key step causing failed fertilization and subsequent development. A recent study showed that sperm factors involved in oocyte activation during the fertilization process were positively correlated with fertilization. These same factors had a negative correlation with SDF (43). Another study evaluated the male pronucleus and found that as sperm DNA damage increases, DNA methylation in the pronucleus increases, and histone acetylation decreases. They concluded that SDF interferes with

the active demethylation and insertion of histones into the male chromatin process that normally occurs in the oocyte after fertilization (44). This further shows that there are mechanisms related to SDF on an epigenetic level, and this may be caused by the fragments in the sperm DNA.

CONCLUSION

There have been numerous studies reporting failed intrauterine insemination (IUI) and overall failure to naturally conceive with couples who have high SDF. Our study highlighted the importance of SDF at the time of fertilization. Often in IUI cycles, the sperm is prepared by washing the semen sample to eliminate seminal plasma and concentrate the sperm cells, typically with no step like a gradient that would reduce SDF. Instead, SDF is likely increased because of the addition of the centrifugation step. Our results are in agreement as to how SDF might cause infertility by disrupting the step of fertilization, whether it is by ICSI, IVF insemination, or natural in vivo fertilization.

Much is still unknown about male infertility and the role the sperm and paternal DNA play in fertilization, embryo development, implantation, pregnancy, and offspring health. Future studies could potentially change how the infertile couple is treated. The findings from this study indicate that focusing on having the best processing methods to reduce DFI, HDS, and OSA will impact fertilization. There was no significant correlation between neat SDF and fertilization. This is promising for couples with diagnosed high DFI. With the proper sperm treatment to reduce SDF, it may be possible to eliminate the deleterious effects of SDF. This information has the potential to improve the success rates of fertility treatments and may even carry over to less invasive fertility treatments. Poor outcomes reported from IUI may be improved if a healthier sperm is available to fertilize the oocyte in vivo.

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