

Sperm DNA fragmentation index and high DNA stainability do not influence pregnancy success after intracytoplasmic sperm injection

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Objective: To evaluate the ability of sperm DNA fragmentation index (DFI) and high DNA stainability (HDS) to influence the chance of achieving pregnancy in couples undergoing intracytoplasmic sperm injection (ICSI) cycles.

Design: A retrospective study evaluating couples who underwent an ICSI cycle between 2009 and 2018.

Setting: Reproductive center.

Patient(s): Consecutive couples who underwent an ICSI cycle and had a semen analysis with subsequent DFI and HDS testing, evaluated using Sperm Chromatin Structure Assay.

Intervention(s): Measurement of DFI and HDS prior to ICSI cycle.

Main Outcome Measure(s): To determine whether DFI or HDS of sperm was predictive of the number of ICSI cycles until the first clinical intrauterine pregnancy.

Result(s): A total of 550 couples who underwent 1,050 ICSI cycles were analyzed. Of those, a total of 330 couples achieved pregnancy. As expected, in couples who achieved pregnancy, females were younger and underwent fewer cycles. Importantly, the DFI and HDS were similar between couples who achieved pregnancy (DFI% 12.9; HDS% 9.3) and couples who did not (DFI% 12.2; HDS% 9.1). A multivariable-adjusted analysis evaluating female age at the first cycle was associated negatively with pregnancy.

Conclusion(s): Neither DFI nor HDS at baseline influenced the chances of a couple to achieve pregnancy after ICSI. Increased female age and couples who underwent more ICSI cycles were associated with lower chances of achieving pregnancy. (Fertil Steril Rep® 2020;1: 233-8. ©2020 by American Society for Reproductive Medicine.)

Key Words: DNA fragmentation, high DNA stainability, ICSI, sperm

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Infertility has a prevalence of 8% to 12% worldwide among reproductive-age couples, with some regions reporting a prevalence as high as 30% (1). Approximately 40% to 50% of infertility cases are male factor related (2). This increase in infertility has led to the steady increase in the use of assisted reproduc-

tive techniques (ART) worldwide in the last four decades (3). When assessing male reproductive potential, traditionally, evaluation has relied on conventional semen analysis (4). However, traditional semen analyses lack the capacity to measure fully sperm dysfunction at the cellular and molecular level,

and reference parameters cannot predict success or failure in the context of ART (5). Thus, with the increased use of in vitro fertilization and intracytoplasmic sperm injection (IVF/ICSI) (6), it is imperative to have tools that predict ART success based on male fertility potential to set expectations for couples.

Reactive oxidative species (ROS) in sperm are elevated in 30% to 80% of men with infertility (7). ROS are free radicals derived from oxygen that have a dual physiological and pathological effect. Low amounts of ROS are known to play a physiological role in sperm function; however, high levels of ROS induce oxidative stress and increase

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sperm DNA fragmentation impairing testicular spermatogenesis and steroidogenesis, epididymal sperm maturation, and fertilization (8, 9). Additionally, defective spermatogenesis affects sperm DNA integrity (10). Therefore, it has been suggested that sperm DNA damage might play a role in predicting the success of IVF/ICSI (11–16). This has led to the creation of tools that assess sperm DNA quality such as the sperm chromatin structure assay (SCSA). SCSA yields two main sperm variables: DNA fragmentation index (DFI), which measures abnormal chromatin structure, and high DNA stainability (HDS), which measures the amount of sperm in a semen sample having increased the amount of retained histones due to the lack of full protamination (11, 13). These parameters have been used to characterize sperm chromatin (protein) defects (13). Several studies have investigated the role of the DFI and HDS in IVF/ICSI. Some studies suggest that increases in the DFI affect pregnancy rate (17). Elevated HDS has been shown to predict pregnancy failure (18) and is associated with miscarriage (15). However, other studies suggest that there is no association between DFI and pregnancy rates, early abortion, oocyte fertilization, or quality of embryos (19). Furthermore, it has been suggested that current methods for sperm DNA fragmentation analysis have a low capacity to predict IVF/ICSI outcomes (12). Given that ICSI success has remained <30% per cycle (20) and the paucity of evidence regarding the role of HDS and DFI on ICSI, we analyzed the effect of DFI and HDS at baseline and their capacity to predict the number of ICSI cycle attempts that a couple must undergo until pregnancy is achieved. We hypothesized that the DFI and HDS at baseline will affect the number of ICSI cycles until first pregnancy is achieved and might provide an additional parameter in setting expectations for couples before starting ICSI.

MATERIALS AND METHODS

Study Design and Population

We conducted a retrospective study evaluating couples who underwent an ICSI cycle between January 2009 and December 2018 at a high-volume reproductive center in Miami, Florida. All couples who underwent an ICSI cycle during this time had semen analysis and SCSA testing done for the male counterpart. Using the Society for Assisted Reproductive Technology database, all couples who underwent an ICSI cycle during this time were identified and the number of cycles they underwent was recorded as well as the outcomes for each cycle. Couples were excluded if they previously underwent ICSI, they were missing data, they had the cycle canceled for any reason, and those using donor eggs or donor semen. Men with azoospermia or severe oligospermia due to Klinefelter syndrome and Y-chromosome microdeletion and those who required surgical sperm retrieval also were excluded. In couples who achieved pregnancy, the number of ICSI cycles were considered until the first clinical intrauterine pregnancy; subsequent pregnancies were not considered in this analysis. This study was submitted to and approved by the institutional review board of the University of Miami, Miller School of Medicine, Miami, Florida.

Primary and Secondary Outcomes

The primary outcome of our study was to determine whether DFI of sperm was predictive of the number of ICSI cycles until success, defined as the first clinical intrauterine pregnancy. The secondary outcome was whether HDS could predict the number of ICSI cycles until success.

SCSA Analysis

SCSA diagnostics required a minimum of 0.20 mL with a concentration of ≥ 0.5 million/mL. The frozen semen was then thawed in a 37°C water bath diluted with TNE (Tris and EDTA) buffer solution. The semen sample was treated subsequently with 400 μ L of acid (pH1.20) for 30 seconds to denature DNA at the sites of strand breaks. The acridine orange staining solution was added to stain the sites of single-strand DNA breaks red and the double-strand DNA breaks green. Using flow cytometry twice, the sperm cells were measured with $\geq 5,000$ sperm each time. The DFI was calculated using measurements of the red fluorescence, divided by the total amount of red and green fluorescence together. The HDS was calculated based on the sperm population in the semen sample that has an abnormally high level of DNA staining resulting from a lack of full protamination, indicating an increased amount of retained histones, which are indicative of the amount of sperm chromatin defects. As part of a standardized clinical practice, the semen sample that was collected for SCSA analysis was obtained in an office visit prior the appointment in which the sample for ICSI was collected.

Oocyte Retrieval

Couples undergoing a fresh embryo transfer received a gonadotropin-releasing hormone antagonist short protocol; for couples undergoing a frozen-thawed embryo transfer, a gonadotropin-releasing hormone agonist suppression long protocol was used. Follicle-stimulating hormone (menopur) was used for ovarian stimulation with the dosage based on the patient's baseline follicle-stimulating hormone, female age, body mass index, and ovarian volume. A transvaginal ultrasound was used to monitor ovarian response to determine the size and count of follicles. Oocyte retrieval was done using a transvaginal ultrasound probe and a 53/4 Pasteur pipette.

Statistical Analysis

Statistical analysis was performed with SPSS version 24 software for Windows (IBM). Means \pm standard deviations or medians and interquartile ranges were reported according to the data distribution, and comparison of continuous variables was performed using the Mann-Whitney *U* or Student *t* test as required. Categorical variables were analyzed with a chi-square test. Additionally, a univariable and multivariable risk analysis was performed to determinate the association (odds ratio [OR]) between pregnancy and the clinical characteristics of the couple, initial DFI and HDS category, and the number of cycles attempted. The median number of cycles until achieving first clinical pregnancy and their 95%

confidence interval (95% CI) were obtained through a Kaplan-Meier analysis, and a log-rank test was used to assess differences in the number of cycles among DFI and HDS-groups. $P < .05$ was considered statistically significant.

RESULTS

A total of 550 couples who underwent 1,050 IVF/ICSI cycles were analyzed; of those, a total of 330 couples achieved pregnancy. In those couples who achieved pregnancy, females were younger at the start of the cycles (no pregnancy 35.3 ± 3.4 years vs. pregnancy 33.7 ± 3.6 years; $P < .001$) and underwent fewer cycle attempts (no pregnancy 2 [1–3] years vs. pregnancy 2 [1–2]; $P = .001$). The DFI and HDS were similar between those who achieved pregnancy and those who did not ($P > .05$). The rest of the clinical and demographic variables are presented in Table 1. This cohort had a mean fertilization rate of 78%, a blastocyst formation rate of 42%, and an embryo use rate of 35%.

After performing a univariable and a multivariable-adjusted risk analysis, it was shown that female age at the first cycle was associated negatively with pregnancy (OR 0.827; 95% CI 0.778–0.879; $P < .001$), and couples who underwent more ICSI attempts had lower probability of getting pregnant

(OR 0.597; 95% CI 0.493–0.723; $P < .001$); neither the DFI or HDS were associated with an increase in chance of achieving pregnancy ($P > .05$) (Table 2). We then performed a Kaplan-Meier analysis, which showed that the median number of ICSI cycle attempts until half of the couples achieved pregnancy was two cycles (95% CI 1.84–2.16), with no significant difference in accordance with the categorized DFI ($P = .632$) or HDS ($P = .692$) (Table 3 and Fig. 1). Furthermore, it was observed that in the first cycle that 156 couples achieved pregnancy (28.4%; 156/550). In the second cycle, an additional 122 couples achieved pregnancy (cumulative success 50.5%; 278/550) and in the third cycle an additional 42 achieved pregnancy (cumulative success 58.2%; 320/550). The fourth cycle yielded an additional eight pregnancies (cumulative success 59.6%; 328/550), the fifth cycle yielded one additional pregnancy (cumulative success 59.8%; 329/550), and in the seventh cycle one additional couple achieved pregnancy (cumulative success 60%; 330/550).

DISCUSSION

In our study, we evaluated 1,050 ICSI cycles in 550 couples. In couples who achieved pregnancy, the female was younger and underwent fewer cycle attempts. There were no

TABLE 1

Clinic and demographic characteristics of the couples who achieved and did not achieve pregnancy with intracytoplasmic sperm injection failure.

Characteristic	Overall	No pregnancy	Pregnancy	P value
No. of couples	550	220	330	
No. of cycles	1,050	480	570	
Male age (y) at first cycle	37.7 ± 6.5	37.9 ± 6.3	37.5 ± 6.7	.491
Male prior fertility (%)				
No	344 (62.5)	141 (64.1)	203 (61.5)	
Yes	206 (37.5)	79 (35.9)	127 (38.5)	.541
Female age (y) at the first cycle	34.3 ± 3.6	35.3 ± 3.4	33.7 ± 3.6	< .001
Female BMI (kg/m ²) at the first cycle	$22.6 (20.4-25.8)$	$22.5 (20.2-25.6)$	$22.7 (20.5-26)$.499
Male smoking history (%)				
No	467 (84.9)	185 (84.1)	282 (85.5)	
Yes	83 (15.1)	35 (15.9)	48 (14.5)	.662
Varicocele history (%)				
No	523 (95.1)	213 (96.8)	310 (93.9)	
Yes	27 (4.9)	7 (3.2)	20 (6.1)	.126
TMSC (10 ⁶ motile sperm)	$43.2 (9.9-101.5)$	$39.4 (9.9-95.3)$	$45.9 (9.9-107.6)$.483
Sperm morphology (% normal forms)	3 (1–6)	3 (1–7)	1 (3–6)	.473
DFI (%) (continuous)	$12.7 (7.8-20)$	$12.2 (7.1-20.2)$	$12.9 (8-20)$.735
DFI (%) categorized				
≤ 15	343 (62.4)	139 (63.2)	204 (61.8)	
15.1–20	72 (13.1)	26 (11.8)	46 (13.9)	
20.1–25	35 (6.4)	12 (5.5)	23 (7)	
25.1–30	42 (7.6)	16 (7.3)	26 (7.9)	
≥ 30.1	58 (10.5)	27 (12.3)	31 (9.4)	.723
HDS (%) (continuous)	$6.4 (9.2-14.4)$	$9.1 (6.7-14)$	$9.3 (6.1-14.6)$.914
HDS (%) categorized				
≤ 7.5	199 (36.2)	81 (36.8)	118 (35.8)	
7.6–10	113 (20.5)	42 (19.1)	71 (21.5)	
10.1–11	114 (20.7)	48 (21.8)	66 (20)	
≥ 15.1	124 (22.5)	49 (22.3)	75 (22.7)	.887
ICSI attempts or attempts until pregnancy, median (range)	2 (1–2) (1–10)	2 (1–3) (1–10)	2 (1–2) (1–7)	.001

Note: Data presented as mean \pm standard deviation and median (interquartile range 25–75), unless stated otherwise. BMI = body mass index; DFI = DNA fragmentation index; HDS = high DNA stainability; ICSI = intracytoplasmic sperm injection; TMSC = total motile sperm count.

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TABLE 2

Univariable and multivariable risk analysis for achieving pregnancy in 550 couples, comparing the clinic and demographic characteristics.

Characteristic	Univariable			Multivariable		
	OR	95% CI	P value	OR	95% CI	P value
Male age (y) at first cycle	0.991	0.965–1.017	.491	1.012	0.980–1.045	.463
Male prior fertility (%)						
No	1			1		
Yes	1.117	0.784–1.590	.541	1.189	0.804–1.759	.386
Female age (y) at first cycle	0.881	0.837–0.927	<.001	0.827	0.778–0.879	<.001
Female BMI (kg/m ²) at first cycle	1.011	0.974–1.050	.554	1.008	0.968–1.049	.701
Male smoking history (%)						
No	1			1		
Yes	0.900	0.560–1.445	.662	0.979	0.586–1.637	.937
Varicocele history (%)						
No	1			1		
Yes	1.963	0.816–4.725	.132	1.991	0.788–5.033	.145
TMSC (10 ⁶ motile sperm)	1.002	0.999–1.004	.192	1.003	0.999–1.005	.092
DFI (%) categorized						
≤ 15	1			1		
15.1–20	1.206	0.712–2.042	.487	1.280	0.715–2.293	.406
20.1–25	1.306	0.629–2.711	.474	1.518	0.676–3.408	.312
25.1–30	1.107	0.573–2.140	.762	1.170	0.524–2.425	.673
≥ 30.1	0.782	0.447–1.368	.390	0.751	0.389–1.451	.394
HDS (%) categorized						
≤ 7.5	1			1		
7.6–10	1.160	0.722–1.866	.539	1.376	0.815–2.322	.233
10.1–15	.944	0.592–1.506	.808	1.189	0.706–2.005	.515
≥ 15.1	1.051	0.665–1.661	.832	1.191	0.686–2.067	.534
ICSI attempts (each additional attempt)	0.699	0.593–0.824	<.001	0.597	0.493–0.723	<.001

Note: BMI = body mass index; CI = confidence interval; DFI = DNA fragmentation index; HDS = high DNA stainability; ICSI = intracytoplasmic sperm injection; OR = odds ratio; TMSC = total motile sperm count.

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significant associations between initial DFI and HDS category and those who achieved pregnancy. Overall, the cumulative success for couples was 60% (330 couples who achieved pregnancy/550 analyzed couples); on the first cycle success was 28.4% and cumulative success for the second cycle was 50.5%. The Kaplan-Meier analysis further corroborates this

observation, showing the median number of cycle attempts until pregnancy was ~2 cycles. This finding was similar for all DFI- and HDS-stratified groups, suggesting that an initial evaluation of DFI and HDS has limited potential to predict the number of attempts that a couple might undergo before pregnancy is achieved.

Our findings are comparable with previous studies. We found that females who achieved pregnancy were younger and these results are similar to the results of Liang et al. (21), who reported that female age is a predictor for successful pregnancy after IVF. In 2018 Pacey et al. (22) concluded, after reviewing nine meta-analyses that evaluated DFI and pregnancy, that there remains little consensus about the use of DFI and whether it adds clinical value. Additionally, many studies have not found any difference between DFI and HDS to achieve pregnancy (12, 15, 16). However, Borges et al. (23), in a study of 445 cycles analyzed for DFI, found that DFI corresponded with negative outcomes for pregnancy, with a cut-off value for DFI at ≥30%. In our study, couples with a DFI >30% had similar outcomes to other couples and underwent similar number of ICSI attempts as couples less percentage of initial DFI. Although DFI and HDS measurements have shown to be useful for varicocele and chemotherapy, these variables in context for ICSI appear to have a limited role. From a clinical point of view, HDS has been less investigated than DFI. Jerre et al. (15) found that HDS

TABLE 3

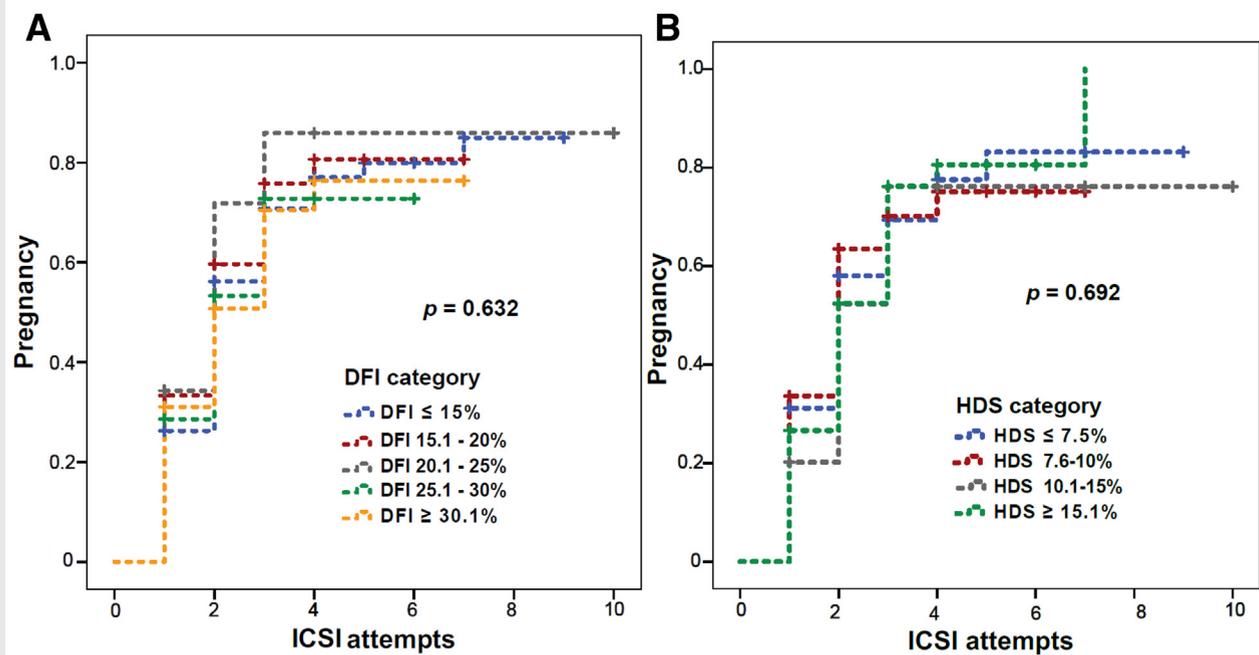
Median number of intracytoplasmic sperm injection attempts for the couples until achieving pregnancy in accordance with the initial DNA fragmentation index and high DNA stainable category.

Characteristic	ICSI attempts until pregnancy, median	95% CI	P value
Overall	2	1.84–2.16	
DFI (%) categorized			
≤ 15	2	1.81–2.19	
15.1–20	2	1.53–2.47	
20.1–25	2	1.51–2.49	
25.1–30	2	1.28–2.72	
≥ 30.1	2	1.27–2.73	.632
HDS (%) categorized			
≤ 7.5	2	1.72–2.28	
7.6–10	2	1.68–2.32	
10.1–15	2	1.64–2.36	
≥ 15.1	2	1.61–2.39	.692

Note: CI = confidence interval; DFI = DNA fragmentation index; HDS = high DNA stainability; ICSI = intracytoplasmic sperm injection.

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FIGURE 1



Kaplan-Meier curves for pregnancy depending on the number of intracytoplasmic sperm injection (ICSI) cycles, in accordance with (A) initial DNA fragmentation index (DFI%) category ($P = .632$) and (B) initial high DNA stainability (HDS) category ($P = .692$).

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>15% was associated with a 5% increase in risk for early miscarriages, but there is conflicting data in other studies (13, 16).

Infertility and its associated treatment have a large economic burden (24). One IVF cycle in the United States can cost \$10,000 to \$15,000 (25). Therefore, it is imperative to identify superfluous tests that may increase cost and preclude couples from attempting IVF or repeat IVF. Our study reports that DFI and HDS values had no statistical significance in the number of cycles for IVF required to achieve pregnancy. With the high price of these tests, the dearth of value added may not justify their use (26).

To our knowledge, our study evaluating 1,050 cycles for ICSI is one of the largest series in the current literature, and is the largest series investigating the association between DFI as well as HDS and pregnancy success. Other strengths include that all ICSI treatments were performed in the same IVF center, and all DFI/HDS testing was done in the same commercial laboratory using SCSA, a commercially available standardized assay. Additionally, we presented demographic characteristics in both male and female partners that can affect pregnancy. Multiple studies have questioned the use of DFI and HDS as a predictor for pregnancy outcomes (13, 15, 16, 19, 22, 23, 27), however, few studies have evaluated the number of ICSI cycles needed to achieve pregnancy, which is valuable information that can be used to predict the cost-effect or financial investment once starting ICSI treatment and set couples expectations. Limitations of our study include its retrospective nature with a moderate sample size, no inclusion of the interval between cycles, no data on embryo quality, only analysis of the first

pregnancy of couples regardless if couples achieved additional pregnancies with ICSI treatment, and consideration of only the first DFI and HDS results. Additionally, another limitation is that sperm DFI can change over time due to a multitude of reasons, and this may be the case in couples who underwent multiple rounds of ICSI. Further research is needed regarding DFI and HDS in IVF cycles and their association with embryo quality, incidence of miscarriage, recurrent IVF failure, and further pregnancy outcomes.

CONCLUSION

Our study showed that both DFI and HDS have limited utility as predictors of pregnancy achievement because the median number of ICSI attempts a couple undergo before achieving pregnancy was similar regardless the initial DFI or HDS. An increased female age and couples who required more ICSI attempts had lower chances of achieving pregnancy. Further studies are needed to confirm the true value of sperm DFI and HDS testing.

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