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Sperm DNA fragmentation index and high DNA stainability do not influence pregnancy success following ICSI.

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Abstract

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 that underwent an ICSI cycle between 2009 - 2018

Setting: High-volume reproductive center.

Patients: Consecutive couples who underwent an ICSI cycle and had a semen analysis with subsequent DFI% and HDS% testing, evaluated by Sperm Chromatin Structure Assay (SCSA).

Interventions: Measurement of DFI% and HDS% prior to ICSI cycle.

Main Outcome Measures: To determine whether DFI% or HDS% of sperm was predictive of the number of ICSI cycles until the first clinical intrauterine pregnancy.

Results: A total of 550 couples who underwent 1050 ICSI cycles were analyzed. Of those, a total of 330 couples achieved pregnancy. As expected, in couples that achieved pregnancy, females were younger (33.7 ± 3.6 years vs 35.3 ± 3.4 years; $p < 0.001$) and underwent fewer cycles (2 [1-2] vs 2 [1-3]; $p = 0.001$). Importantly, the DFI% and HDS% were similar between couples who achieved pregnancy (DFI% = 12.9 [8-20]; HDS% = 9.3 [6.1-14.6]) and couples who did not (DFI% =12.2 [7.1-20.2]; HDS% = 9.1 [6.7-14]). A multivariable-adjusted analysis evaluating female age at the first cycle was negatively associated with pregnancy (OR = 0.827, 95% CI: 0.778 - 0.879; $p < 0.001$).

Conclusions: Neither DFI nor HDS at baseline influence 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.

Keywords: DNA fragmentation; high DNA stainability; ICSI; sperm

Capsule: Both DFI% and HDS% were not predictors of ISCI success, increase female age, and couples that required more ISCI attempts have lower chances of achieving pregnancy.

Introduction

Infertility has a prevalence of 8-12% worldwide amongst reproductive-age couples, with some regions reporting a prevalence as high as 30% (1). Approximately 40-50% of infertility cases are male factor related (2). This increase in infertility has led to the steady rise in the utilization of assisted reproductive 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 fully measure 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 increase 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% - 80% of infertile men(7). ROS are free radicals derived from oxygen which have a dual physiological and pathological effect. Low amounts of reactive oxygen species (ROS) are known to play a physiological role in sperm function; however, high levels of ROS induce oxidative stress and increase 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 (DFI) which measures abnormal chromatin structure, and high DNA stainable (HDS) which measures the amount of sperm in a semen sample having increased 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, 19). However, other studies suggest that there is no association between DFI and pregnancy rates, early abortion, oocyte fertilization, or the quality of embryos (20). 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 less than 30% per cycle(21), 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 cycles 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 & Methods

Study Design and Population

We conducted a retrospective study evaluating couples that 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 a 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, were missing data, had the cycle canceled for any reason, those using donor eggs or donor semen. Men with azoospermia or severe oligospermia due to Klinefelter syndrome and Y-chromosome microdeleteion and those who required surgical sperm retrieval were also excluded. In couples that 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, FL, USA.

Primary and Secondary Outcome

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 is then thawed in a 37°C water bath diluted with TNE buffer solution. The semen sample is subsequently treated with 400 µL of acid (pH = 1.20) for 30 seconds to denature DNA at the sites of strand breaks. The acridine orange (AO) staining solution is 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 are measure with ≥5000 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 by 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 (GnRH) antagonist short protocol; couples undergoing a frozen-thawed embryo transfer, a GnRH agonist suppression long protocol was utilized. Follicle stimulating hormone (Menopure) was used for ovarian stimulation with the dosage based on the patient's baseline FSH, female age, BMI, 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 prove and a 5 ¾ Pasteur pipette.

Statistical analysis

Statistical analysis was performed with SPSS version 24 software for Windows. Means and standard deviations (± SD) or medians and interquartile ranges were reported according to the data distribution, and comparison of continuous variables was performed using the U Mann-Whitney or Student T-test as required. Categorical variables were analyzed with a Chi-square test. Additionally, an 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 attempt. 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 utilized to assess differences in the number of cycles among DFI and HDS groups. A p-value < 0.05 was considered statistically significant.

Results

A total of 550 couples that underwent 1050 IVF/ICSI cycles were analyzed, of those, a total of 330 couples achieved pregnancy. In those couples that 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; $p < 0.001$) and underwent fewer cycle attempts (No pregnancy: 2 [1 – 3] years vs. Pregnancy: 2 [1 – 2]; $p = 0.001$). The DFI and HDS were similar between those who achieved pregnancy and those who did not ($p > 0.05$). The rest of the clinical and demographic variables are presented in Table 1. This cohort had a mean fertilization rate of 78%, blastocyst formation rate of 42% and embryo utilization rate of 35%.

After performing a univariable and a multivariable-adjusted risk analysis, it was shown that female age at the first cycle was negatively associated with pregnancy (OR = 0.827, 95% CI: 0.778 - 0.879; $p < 0.001$), and couples that underwent more number of ICSI attempts had lower probability of getting pregnant (OR = 0.597, 95% CI: 0.493 - 0.723; $p < 0.001$), neither the DFI% or HDS% were associated with an increase in chance of achieving pregnancy ($p > 0.05$) (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 2 cycles (95% CI: 1.84 - 2.16), with no significant difference in accordance to the categorized DFI ($p = 0.632$) or HDS ($p = 0.692$) (Table 3 and Figure 1). Furthermore, it was observed that in the first cycle - 156 couples achieved pregnancy (28.4%, 156/550). On the second cycle, an additional 122 couples achieved pregnancy (cumulative success = 50.5%, 278/550) and the third cycle an additional 42 achieved pregnancy (cumulative success = 58.2%, 320/550). The fourth cycle yielded an additional 8 pregnancies (cumulative success = 59.6%, 328/550), the fifth cycle yielded 1 additional (cumulative success = 59.8%, 329/550) and for the seventh, 1 additional couple achieved pregnancy (cumulative success = 60%, 330/550).

Discussion:

In our study, we evaluated 1050 of ICSI cycles in 550 couples. In couples that achieved pregnancy, the female was younger and underwent fewer cycle attempts. There were no significant associations between initial DFI and HDS category and those who achieved pregnancy. Overall the cumulative success for couples was 60% (330 couples that achieved pregnancy / 550 analyzed couples), on the first cycle success was 28.4% and cumulative success for 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 have limited potential to predict the number of attempts that a couple might undergo before pregnancy is achieved.

Our findings are comparable to previous studies. We found that females who achieved pregnancy were younger and these results are similar to Liang et al, who reported that female age is a predictor

for successful pregnancy after IVF(22). In 2018 Pacery concluded after reviewing 9 meta-analysis that evaluated DFI and pregnancy, that there remains little consensus about the use of DFI and whether it adds clinical value(23). Additionally, many studies have not found any difference between DFI and HDS to achieve pregnancy(12, 15, 16). However, Borges et al, 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 that other couples and underwent similar number of ICSI attempts as couples less percentage of initial DFI. Even though, DFI and HDS measurements have shown to be useful for varicocele and chemotherapy, these variables in context for ICSI appears to have limited role. In a clinical point of view, HDS has been less investigated than DFI. Jarre et al found that HDS above 15% was associated with a 5% increase in risk for early miscarriages[15] 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 US can cost $ 10,000 to $15,000 (25). Therefore, it is imperative to identify superfluous tests which may increase cost and preclude couples from attempting IVF or repeat IVF. Our study reports 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 utilization(26).

To our knowledge, our study evaluating 1050 cycles for ISCI is one of the largest series in current literature, as well as 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, 23, 27-29) 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 couple achieved additional pregnancies with ICSI treatment and considered only the first DFI and HDS result. 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, as the median number of ISCI attempts a couple undergo before achieving pregnancy was similar regardless the initial DFI or HDS. An increased female age and couples that required more ISCI 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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Table 1. Clinic and demographic characteristics of the couples that achieved and did not achieved pregnancy with ICSI failure.

Mean ± standard deviation, median [Interquartile range 25-75]; DFI = DNA Fragmentation Index; HDS = High DNA Stainable.

Table 2. Univariable and multivariable risk analysis for achieving pregnancy in 550 couples, comparing the clinic and demographic characteristics.

Table 3. Median number of ICSI attempts for the couples until achieving pregnancy in accordance with the initial DNA Fragmentation Index and High DNA Stainable category.

Figure 1. Kaplan-Meier Curves for pregnancy depending on the number of ICSI cycles, in accordance to A) Initial DNA Fragmentation Index category and B) Initial high DNA stainability category.

	Overall	No pregnancy	Pregnancy	p-value
Number of couples	550	220	330	
Number of cycles	1050	480	570	
Male age in years (at first cycle)	37.7 ± 6.5	37.9 ± 6.3	37.5 ± 6.7	0.491
Male prior fertility				
No (%)	344 (62.5%)	141 (64.1%)	203 (61.5%)	
Yes (%)	206 (37.5%)	79 (35.9%)	127 (38.5%)	0.541
Female age in years (at the first cycle)	34.3 ± 3.6	35.3 ± 3.4	33.7 ± 3.6	**< 0.001**
Female BMI in kg/m^2 (at the first cycle)	22.6 [20.4 - 25.8]	22.5 [20.2 - 25.6]	22.7 [20.5 – 26]	0.499
Male smoking history				
No (%)	467 (84.9%)	185 (84.1%)	282 (85.5%)	
Yes (%)	83 (15.1%)	35 (15.9%)	48 (14.5%)	0.662
Varicocele history				
No (%)	523 (95.1%)	213 (96.8%)	310 (93.9%)	
Yes (%)	27 (4.9%)	7 (3.2%)	20 (6.1%)	0.126
TMSC (10^6 motile sperm)	43.2 [9.9 - 101.5]	39.4 [9.9 - 95.3]	45.9 [9.9 - 107.6]	0.483
Sperm morphology (% normal forms)	3 [1 – 6]	3 [1 – 7]	1 [3 – 6]	0.473
DFI (%) (continues)	12.7 [7.8 – 20]	12.2 [7.1 - 20.2]	12.9 [8 – 20]	0.735
DFI (%) **categorized**				
DFI ≤ 15%	343 (62.4%)	139 (63.2%)	204 (61.8%)	
DFI 15.1-20%	72 (13.1%)	26 (11.8%)	46 (13.9%)	
DFI 20.1-25%	35 (6.4%)	12 (5.5%)	23 (7%)	
DFI 25.1-30%	42 (7.6%)	16 (7.3%)	26 (7.9%)	
DFI ≥ 30.1%	58 (10.5%)	27 (12.3%)	31 (9.4%)	0.723
HDS (%) (continues)	6.4 [9.2 - 14.4]	9.1 [6.7 – 14]	9.3 [6.1 - 14.6]	0.914
HDS (%) **categorized**				
HDS ≤ 7.5%	199 (36.2%)	81 (36.8%)	118 (35.8%)	
HDS 7.6-10%	113 (20.5%)	42 (19.1%)	71 (21.5%)	
HDS 10.1-15%	114 (20.7%)	48 (21.8%)	66 (20%)	
HDS ≥ 15.1%	124 (22.5%)	49 (22.3%)	75 (22.7%)	0.887
Median number of ICSI attempts or attempts until pregnancy (range)	2 [1 – 2] (1 – 10)	2 [1 – 3] (1 – 10)	2 [1 – 2] (1 – 7)	**0.001**

	Univariable			Multivariable		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Male age at first cycle (per year)	0.991	0.965 - 1.017	0.491	1.012	0.980 - 1.045	0.463
Male prior fertility						
No (%)	1			1		
Yes (%)	1.117	0.784 - 1.590	0.541	1.189	0.804 - 1.759	0.386
Female age at first cycle (per year)	0.881	0.837 - 0.927	**< 0.001**	0.827	0.778 - 0.879	**< 0.001**
Female BMI at first cycle (per kg/m^2)	1.011	0.974 - 1.050	0.554	1.008	0.968 -1.049	0.701
Male smoking history						
No (%)	1			1		
Yes (%)	0.900	0.560 - 1.445	0.662	0.979	0.586 - 1.637	0.937
Varicocele history						
No (%)	1			1		
Yes (%)	1.963	0.816 - 4.725	0.132	1.991	0.788 - 5.033	0.145
TMSC (10^6 motile sperm)	1.002	0.999 - 1.004	0.192	1.003	0.999 - 1.005	0.092
DFI (%) **categorized**						
DFI ≤ 15%	1			1		
DFI 15.1-20%	1.206	0.712 - 2.042	0.487	1.280	0.715 - 2.293	0.406
DFI 20.1-25%	1.306	0.629 - 2.711	0.474	1.518	0.676 - 3.408	0.312
DFI 25.1-30%	1.107	0.573 - 2.140	0.762	1.170	0.524 - 2.425	0.673
DFI ≥ 30.1%	0.782	0.447 - 1.368	0.390	0.751	0.389 - 1.451	0.394
HDS (%) **categorized**						
HDS ≤ 7.5%	1			1		
HDS 7.6-10%	1.160	0.722 - 1.866	0.539	1.376	0.815 - 2.322	0.233
HDS 10.1-15%	.944	0.592 - 1.506	0.808	1.189	0.706 - 2.005	0.515
HDS ≥ 15.1%	1.051	0.665 - 1.661	0.832	1.191	0.686 - 2.067	0.534
ICSI attempts (each additional attempt)	0.699	0.593 - 0.824	**< 0.001**	0.597	0.493 - 0.723	**< 0.001**

	Median number of ICSI attempts until pregnancy	95% CI	p-value
Overall	2	1.84 - 2.16	
DFI (%) categorized			
DFI ≤ 15%	2	1.81 - 2.19	
DFI 15.1-20%	2	1.53 - 2.47	
DFI 20.1-25%	2	1.51 - 2.49	
DFI 25.1-30%	2	1.28 - 2.72	
DFI ≥ 30.1%	2	1.27 - 2.73	0.632
HDS (%) categorized			
HDS ≤ 7.5%	2	1.72 - 2.28	
HDS 7.6-10%	2	1.68 - 2.32	
HDS 10.1-15%	2	1.64 - 2.36	
HDS ≥ 15.1%	2	1.61 - 2.39	0.692