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Sperm chromatin structure assay (SCSA®) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles

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Objective: To determine the relationship between sperm chromatin structure assay (SCSA) parameters (DNA fragmentation index [DFI] and high DNA stainability [HDS]), and conventional IVF and IVF/intracytoplasmic sperm injection (ICSI) outcomes.

Design: Retrospective review and prospective study.

Setting: Private IVF clinic.

Patient(s): Two hundred forty-nine couples undergoing first IVF and/or ICSI cycle.

Intervention(s): IVF, ICSI, blastocyst culture.

Main Outcome Measure(s): DFI, HDS, conventional semen parameters, IVF, ICSI.

Result(s): IVF and ICSI fertilization rates were not statistically different between high- and low-DFI groups. More men with ≥15% HDS had lower (<25% and <50%) IVF fertilization rates. High DNA stainability was not related to ICSI fertilization rates. High DNA stainability did not affect blastocyst rates or pregnancy outcomes. Men with ≥30% DFI were at risk for low blastocyst rates (<30%) and no ongoing pregnancies. Men with ≥30% DFI had more male factors. World Health Organization thresholds were not predictive of ongoing pregnancy.

Conclusion(s): The relationship between HDS and poor IVF fertilization rates provides preliminary evidence that ICSI may be indicated in men with ≥15% HDS. Men with high levels of DNA fragmentation (≥30% DFI) were at greater risk for low blastocyst rates and failure to initiate an ongoing pregnancy. The SCSA provides valuable prognostic information to physicians counseling couples before IVF and/or ICSI cycles. (Fertil Steril® 2004;81:1289–95. ©2004 by American Society for Reproductive Medicine.)

Key Words: Sperm chromatin structure assay, SCSA, DNA fragmentation, DFI, HDS, IVF, ICSI, blastocyst rate, pregnancy, spontaneous abortion

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Male factor as the etiology of infertility has been shown to have a significant, negative effect on the number of embryos that develop to the blastocyst stage (1, 2). Therefore, sperm quality should be scrutinized to determine the role it plays in poor blastocyst development and/or the failure to initiate a term pregnancy. Male factor infertility correlates significantly with blastocyst numbers (1, 2) and quality (3).

Furthermore, blastocyst formation favors those zygotes resulting from IVF over intracytoplasmic sperm injection (ICSI) (4, 5). Although technical aspects may play a role in influencing ICSI embryo development, the low incidence of blastocyst development likely stems in part from the relatively high incidence of fragmented DNA and chromosomal abnormalities within the sperm population selected for ICSI (4). These data indicate that irreparable abnormalities in the paternal genome affect blastocyst development even when the sperm is injected directly into the oocyte via ICSI (1).

The embryonic genome is activated on day 3, and its transcriptional products supersede the regulatory control provided by maternal messages stored in the oocyte. Blastocyst culture

maintains the embryo in vitro during and after activation of the embryonic genome, allowing scientists to witness the earliest expression of an "errant paternal genome" (6). Embryo abnormalities seen in vitro can be more directly related to male factors because the results can be assessed without the interference of confounding female factors (e.g., uterine and endocrine abnormalities) that may lead to embryo wastage or miscarriage post-transfer (7). In vitro assessment of the embryos allows the development of noninvasive selection criteria against abnormal, nonviable embryos and may lessen the chance that a child will be conceived from sperm with an abnormal genome (6). Furthermore, blastocyst culture aids scientific endeavors designed to study the role that the paternal genome plays in blastocyst development, implantation, and clinical pregnancies.

Gaining a better understanding of the impact of male factors in abnormal blastocyst development is critically important to clinical practice as only 20% of human embryos implant after transfer to the uterus (8). Transfer of embryos at the blastocyst stage provides the possibility of selecting better-quality embryos with a higher implantation potential (5). Even more critical may be evidence indicating that the most significant phenotypic manifestations of paternal genome abnormalities occur during postimplantation development (9). Therefore, genetic abnormalities in the paternal genome may be a significant source of miscarriages, 50% of which are currently unexplained (7).

The role of paternal genome abnormalities in miscarriage is underestimated because the assays used in clinical practice measure major numerical or structural abnormalities but do not measure minor but potentially global chromosomal damage (DNA nicks, double strand breaks; 9). Sperm from infertile men have an elevated rate of these "minor" DNA abnormalities (10–17), which may lead to the observed abnormal blastocyst development, failed implantation, and spontaneous miscarriages in conceptions using these sperm. If evaluated in men before IVF and/or ICSI, sperm DNA abnormalities would likely identify the cause of infertility in a large percentage of patients (18). Therefore, evaluating the paternal genome may be a significant upgrade to the diagnostic and prognostic information provided by the conventional male factor infertility examination.

Over the past decade, several techniques have emerged as potential protocols for evaluating chromatin/DNA integrity. The sperm chromatin structure assay (SCSA) measures the percentage of sperm with a high susceptibility to low pH-induced DNA denaturation and is expressed as the DNA fragmentation index (%DFI). DNA fragmentation index is a highly accurate, repeatable measure of DNA quality that is proportionate to the level of DNA strand breaks in sperm (19, 20). In addition to quantifying DNA strand breaks through DFI, the SCSA simultaneously identifies the percentage of sperm with immature nuclear development. The nuclear chromatin (DNA and protein) structure of these immature sperm is abnormal, with a characteristically high level of DNA stainability (%HDS). In a study of semen samples collected monthly for 8 consecutive months, the SCSA parameters were more repeatable than the conventional semen parameters of sperm count, motility, and morphology (21). Other studies have shown that the DFI can be an excellent indicator of environmental pollution (22), smoking (23, 24), and exposure to industrial toxicants (25, 26). Additional factors that affect human SCSA data include febrile illness (27), age (24), and cancer (28–30).

In a *Science* article published in 1980, the SCSA showed a clear difference in DNA quality between proven fertile and infertile men (no conceptions over 1 year) and bulls (11). In a subsequent comprehensive male factor fertility study of couples attempting to conceive without assisted reproductive techniques, the SCSA distinguished between highly fertile, moderately fertile, and sub-/infertile men (12). In this study, pregnancy rates were significantly lower when men had a high percentage of immature sperm ($\geq$15% HDS). Furthermore, no couple achieved a pregnancy when the DFI was $\geq$30% during the month of conception. Similarly, three other studies including a total of 146 patients reported no sustained pregnancies after IUI/IVF/ICSI when the DFI was $>$27% in the raw ejaculate (13, 17, 18, 31).

To our knowledge, the research reported here is the first to compare SCSA parameters (DFI and HDS) to IVF/ICSI fertilization, blastocyst development, spontaneous abortions, and chemical and ongoing pregnancies.

MATERIALS AND METHODS

Patients

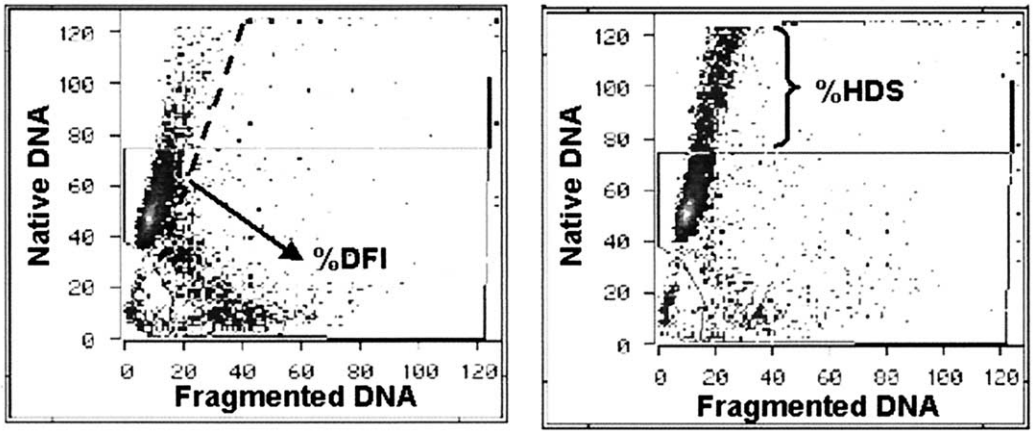
Two hundred forty-nine couples commencing their first IVF cycle had SCSA testing done on the male partner. Data from the first 63 couples were looked at retrospectively. After this, every couple planning a cycle had SCSA testing done before their egg retrieval. South Dakota State University's Institutional Review Board approved this research study. Approval was not sought in the private IVF clinic because of the lack of such a review board. Patients were grouped according to their SCSA data. Men with $<$30% DFI were classified as having low levels of DNA fragmentation, and men with $\geq$30% DFI were classified as having high levels of DNA fragmentation. A second, independent grouping was based on HDS. Men with $<$15% HDS were said to have low levels of immature sperm, and men with $\geq$15% HDS were said to have high levels of immature sperm.

SCSA Protocols

The SCSA protocol has been described elsewhere by Evenson et al. (12, 32). Each semen sample was frozen in LN_2 without cryoprotectant and transported in a LN_2 dry shipper via Federal Express to SCSA Diagnostics (Brookings, SD) for SCSA testing. From each semen sample, two

FIGURE 1

Native (green fluorescence) versus fragmented DNA (red fluorescence) cytograms showing the two SCSA parameters of interest. *Left*: semen sample with a high percentage of sperm with high levels of DNA fragmentation (%DFI). *Right*: semen sample with a high percentage of sperm with HDS (%HDS).



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separate measurements of 5,000 sperm cells each were evaluated by flow cytometry. High DNA stainability was calculated based on the percentage of sperm with high levels of green fluorescence (*y*-axis, Fig. 1). Native (green fluorescence, *y*-axis) versus fragmented DNA (red fluorescence, *x*-axis) cytograms (Fig. 1) were used to determine the DFI of the entire sperm population. The percentage of sperm with high levels of DNA fragmentation (%DFI) was quantified based on the increased ratio of red/[red + green] fluorescence.

Assisted Reproductive Techniques

Stimulation Protocol and Oocyte Retrieval

All women were suppressed with 0.1 cc of Lupron SC (Abbott Laboratories Ltd., Montreal, Quebec, Canada) starting on day 21. Suppression ranged from 13 to 20 days for IVF scheduling purposes. When E_2 levels were below 100 pmol/L, ovarian stimulation was started with pure FSH [either Gonal-F (Serono Canada Inc., Oakville, Ontario) or Puregon (Organon Canada Ltd., Scarburough, Ontario)] in 96% of patients. The remaining 4% of patients had either pure LH (Lhadi, Serono Canada, Ltd.) or Humegon (Organon Canada Ltd.) added to their protocol. The mean number of 75 unit ampoules injected was 26.5 per cycle, and the mean E_2 level was 4,537 pmol/L at the time of hCG [Profasi (Serono Canada Inc.) 10,000 units] administration. At least two lead follicles were 18 mm at the time of hCG administration as determined by transvaginal ultrasound. Egg retrieval was performed 35 hours after hCG injection. Egg retrieval was performed under neurolept anesthesia using Demerol, Propofol (Abbott Laboratories Ltd.), and Versed.

Seventy-five percent of patients had both conventional IVF and ICSI performed, splitting the number of eggs equally for both procedures. Eighteen percent had all of their eggs injected for male factor reasons, and 7% had only conventional IVF performed. After egg retrieval, oocytes were rinsed of follicular fluid in HTF-Hepes + 10% HSA (human serum albumin; Bayer Corp. Canada, Toronto, Ontario). Oocytes were transferred to organ culture dishes (Falcon, BD Biosciences, Franklin Lakes, NJ) that contained 800 μL of pre-equilibrated P1 + 10% substitute serum supplement (SSS; Irvine Scientific Canada, Mississauga, Ontario). Oocytes were incubated for 4–6 hours (37°C; 5% CO_2, 5% O_2, and 90% N_2) before insemination (IVF) or injection (ICSI).

Fertilization Assessment, Embryo Culture, and Grading

Oocytes were examined for evidence of fertilization 16–18 hours after insemination or injection. Normally fertilized ova (2PN; 2PB) were transferred to organ culture dishes containing 90 μL of pre-equilibrated P1 + 10% SSS. Zygotes were incubated for 48 hours at 37°C in 5% CO_2, 5% O_2, and 90% N_2. On day 3, embryos were transferred to organ culture dishes containing 90 μL of pre-equilibrated blastocyst medium + 10% SSS (Irvine). Embryos were incubated for an additional 48 hours until day 5.

Blastocyst Grading and Transfer

On the morning of day 5, embryos were examined for further cleavage to the blastocyst stage. Blastocyst development was characterized according to Gardner and Schoolcraft's blastocyst grading system (33). Any blastocyst that scored less than 3 and that had more than 60 cells was graded

TABLE 1

Female and male factors within DNA fragmentation index (DFI) and high DNA stainability (HDS) groups.

				Conventional semen parameters			
	Years of infertility	Female age	No. of mature eggs	Volume (mL)	Count (million)	Motility (% motile)	Morphology (WHO)
DFI							
Group 1, DFI <30%, n = 178	4.7 ± 3.0	34.1 ± 3.8	8.7 ± 4.1	3.2 ± 1.4	107.6 ± 90.2	49.1 ± 17.0	53.4 ± 16.5
Group 2, DFI ≥30%, n = 71	4.2 ± 2.6	33.9 ± 4.4	8.5 ± 4.1	3.4 ± 1.3	54.1[a] ± 66.0	35.2[b] ± 15.6	41.0[c] ± 17.0
HDS							
Group 1, HDS <15%, n = 207	4.5 ± 2.9	34.2 ± 4.0	8.5 ± 4.1	3.2 ± 1.3	104.6 ± 88.5	47.5 ± 17.2	51.7 ± 16.7
Group 2, HDS ≥15%, n = 42	4.4 ± 2.7	33.1 ± 3.8	9.1 ± 4.1	3.6 ± 1.7	32.3[b] ± 47.4	33.8[b] ± 16.4	40.6[a] ± 18.9

Note: Values represent mean ± SD for DFI and HDS groups. Values within column and within sperm chromatin structure assay variable (DFI or HDS) with different superscripts are significantly different. WHO = World Health Organization.

[a] $P<.01$.

[b] $P<.0001$.

[c] $P<.001$.

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as an early blastocyst. Immediately before transfer, the blastocysts were transferred to HTF-Hepes + 30% SSS. Blastocysts were loaded in 10–20 μL of HTF-Hepes + 30% SSS in the tip of an Edwards-Wallace embryo replacement catheter (Cooper Surgical, Shelton, CT). Only day 5 blastocyst data were used in this manuscript. All embryos were graded by one embryologist who was blinded to the %DFI and %HDS for the sperm sample used in that couple's cycle.

Couples had up to but not greater than two blastocysts transferred on the fifth day after egg retrieval. Embryo transfer (ET) was performed under ultrasound guidance using an Edwards-Wallace catheter (Cooper Surgical). Patients were requested to remain supine for 30 minutes after transfer and then have limited activity for the next 48 hours. Serum βhCG levels were drawn 12 days after ET. All patients were supported with P vaginal suppositories (200 mg) 24 hours after egg retrieval. If the βhCG level was greater than 200 on day 12, patients started hCG support (2,500 units) IM every 3 days until the twelfth week of gestation. Ultrasound was performed between 6 and 6.5 weeks to confirm fetal viability. Women with a positive βhCG without a pregnancy at the time of ultrasound or that miscarried before the twelfth week were identified as having a spontaneous abortion.

Statistical Analysis

Each semen sample was measured twice in tandem by the SCSA. Statistical analysis was completed using the mean DFI and HDS of the replicate runs. Pearson's χ^2 test was used to measure the relationship between DFI groups (≥30% DFI vs. <30% DFI) and HDS groups (≥15% HDS vs. <15% HDS) and the presence of male factors and IVF/ICSI outcomes [low fertilization rate (<25% and <50%), low blastocyst rate (<30%), spontaneous abortion, and chemical and ongoing pregnancy]. No biologically meaningful linear relationships (regression analysis) were identified between IVF/ICSI outcomes and conventional semen parameters (volume, count, motility, or morphology) or SCSA parameters (DFI or HDS). Analysis of variance and regression analysis were used to determine the relationships between SCSA parameters and conventional semen parameters as well as female age, years of infertility, and total number of mature eggs.

RESULTS

SCSA Groups

The 249 IVF and/or ICSI patients included in the study were divided based on their SCSA-defined DNA fragmentation levels, low %DFI (<30% DFI, n = 178) and high %DFI (≥30% DFI, n = 71). Men were also divided based on their SCSA-defined DNA stainability, low HDS (<15% HDS, n = 207) and high HDS (≥15% HDS, n = 42). Female age, years of infertility, and average number of mature eggs collected from the female partners did not vary significantly between the DFI or HDS groups (Table 1).

Conventional Semen Parameters and DFI

Men with ≥30% DFI were 6.9 times (3.8–12.62, confidence bounds) more likely to have one or more abnormal conventional semen parameter(s) and therefore be identified as "male factor." The means of sperm count ($P=.001$), motility ($P<.0001$), and World Health Organization (WHO) morphology ($P=.0002$) were significantly lower in men with ≥30% DFI (Table 1). Individually, motility ($r^2 = 0.17$, $P<.0001$), count ($r^2 = 0.12$, $P<.0001$), and WHO morphology ($r^2 = 0.13$, $P<.0001$) were significant predictors of DFI. In multiple regression analysis, motility and count significantly predicted 21% ($P<.01$) of the variation in DFI.

TABLE 2

Percentage of couples with low fertilization rates (<25% and <50%) in DNA fragmentation index (DFI) and high DNA stainability (HDS) groups.

	Fertilization rate <25%			Fertilization rate <50%		
	IVF	ICSI	IVF and ICSI	IVF	ICSI	IVF and ICSI
DFI						
Group 1, DFI <30%	9 (n = 13/144)	4 (n = 4/114)	2 (n = 4/178)	28 (n = 40/144)	12 (n = 14/114)	18 (n = 32/178)
Group 2, DFI ≥30%	24 (n = 8/34)	7 (n = 4/60)	7 (n = 5/70)	32 (n = 11/34)	23 (n = 14/60)	21 (n = 15/70)
HDS						
Group 1, HDS <15%	8 (n = 13/157)	5 (n = 7/138)	3 (n = 6/206)	25 (n = 40/157)	16 (n = 22/138)	17 (n = 36/206)
Group 2, HDS ≥15%	38[a] (n = 8/21)	3 (n = 1/36)	7 (n = 3/42)	52[b] (n = 11/21)	17 (n = 6/36)	26 (n = 11/42)

Note: Values within a column and within sperm chromatin structure assay variable (DFI or HDS) with different superscripts are significantly different. Values are percents.

[a] $P<.001$.

[b] $P<.01$.

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Conventional Semen Parameters and HDS

Men with ≥15% HDS were 4.8 times (2.4–9.3, confidence bounds) more likely to have one or more abnormal conventional semen parameter(s) and therefore be identified as male factor. The means of sperm count ($P<.0001$), motility ($P<.0001$), and WHO morphology ($P=.004$) were significantly lower in men with ≥15% HDS (Table 1). Individually, motility ($r^2 = 0.09$, $P<.0001$), count ($r^2 = 0.11$, $P<.0001$), and WHO morphology ($r^2 = 0.09$, $P<.0001$) were significant predictors of HDS. In multiple regression analysis, motility and count significantly predicted 15% ($P<.01$) of the variation in HDS.

Fertilization Rates and SCSA Parameters

Although trends were evident, fertilization rates were not statistically different between the high- and low-DFI groups (Table 2). However, the percentage of couples with low (<25%, $P=.001$) and moderately low (<50%, $P=.01$) IVF fertilization rates was significantly higher in couples including men with ≥15% HDS (Table 2). There was no relationship between ICSI fertilization rates and HDS. After ICSI, men with >15% HDS were at an equal risk (0.392–2.8, confidence bounds) of having <50% fertilization rates as those men with <15% HDS. Combined, IVF and ICSI fertilization rates were not significantly related to HDS.

Blastocyst Development and SCSA Parameters

Overall, men with ≥30% DFI had a greater chance of having poor (<30%) blastocyst rates ($P=.003$; Fig. 2). When IVF and ICSI data were divided, there was still a trend for low blastocyst rates in IVF ($P=.07$) and ICSI ($P=.03$) in men with ≥30% DFI. There was no significant relationship between ≥15% HDS and poor blastocyst rates. Furthermore, no WHO threshold for semen volume, sperm count, motility, or morphology was predictive of poor blastocyst rates.

Pregnancy Outcomes

Men with high levels of DNA fragmentation (≥30% DFI) had a lower chance of initiating a chemical pregnancy than

FIGURE 2

Percentage of couples with a low blastocyst rate (<30%) between DFI groups. No significant differences were found between HDS groups and blastocyst rate.

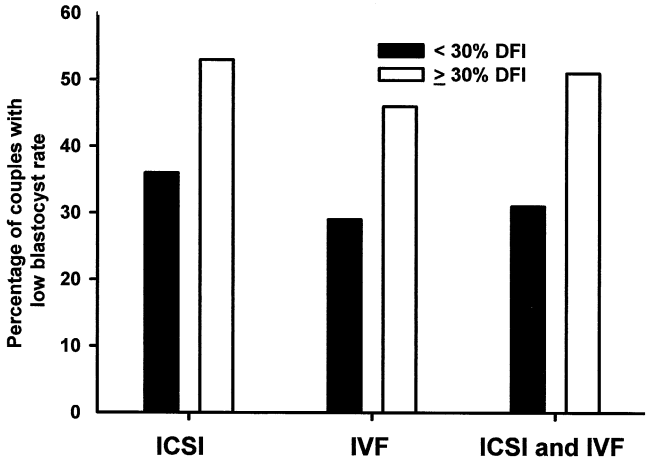

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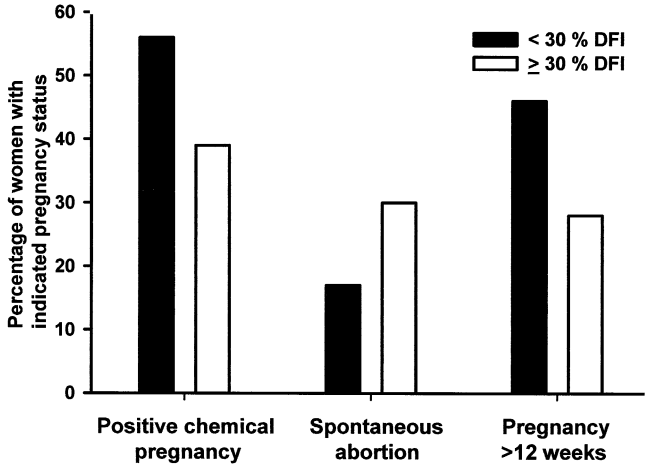
FIGURE 3

Female partners of men within group 2 (≥30% DFI) had a lower rate of chemical pregnancy ($P=.02$) and a higher rate of spontaneous abortion ($P=.11$) and a significant decrease in pregnancies ongoing at 12 weeks of gestation ($P<.01$). HDS groups and the WHO thresholds for normal semen analysis (volume, density, motility, and morphology) were not significantly related to these measures of pregnancy outcome.



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men with <30% DFI ($P=.02$; Fig. 3). This trend was compounded by the increase in spontaneous abortions in men with ≥30% DFI ($P=.11$; Fig. 3). The cumulative effect of these trends lead to the significant decrease in pregnancy rates found in men with high levels of DNA (28%) fragmentation versus those men with <30% DFI (47%, $P<.01$; Fig. 3). There was no relationship between HDS and chemical pregnancies, spontaneous abortions, or ongoing pregnancies. Similarly, no WHO thresholds for normal conventional semen parameters were significantly predictive of pregnancy outcomes.

DISCUSSION

Most fertility clinics evaluate semen samples simply by conventional analysis, which does not ensure the absence of a male factor problem (31, 34). Although men with abnormal semen parameters were at a significantly greater risk of having high levels of DNA fragmentation, the best multiple regression model, including count and motility, predicted only 21% and 15% percent of the variation in DFI and HDS, respectively. When all conventional semen parameters were normal, 18% of the men still had ≥30% DFI and fell into the high-risk category for poor blastocyst development and failure to initiate an ongoing pregnancy. Equally important, 39% of the men with abnormal semen parameters did not have high levels of DNA fragmentation. These men were effectively treated with IVF/ICSI and fell into the group of men (<30% DFI) that had a 47% chance of initiating a term pregnancy.

Ahmadi and Ng (35) have previously reported in a mouse model system that spermatozoa with defective DNA can fertilize an oocyte and produce high-quality early-stage embryos, but then, as the extent of the DNA damage increases, the likelihood of a successful term pregnancy decreases. Similarly, this and previous studies show that high levels of DNA fragmentation were not significantly associated with a decrease in fertilization rates (13, 14, 17, 34, 36). Instead, the deleterious consequences of fragmented paternal DNA became evident when the embryonic genome was activated. Specifically, high levels of sperm DNA fragmentation (≥30% DFI) were manifest in a significant decrease in blastocyst and ongoing pregnancy rates with a trend toward a lower rate of chemical pregnancies and a higher rate of spontaneous abortions.

The relationship between a high percentage of immature sperm (≥15% HDS) and low IVF fertilization rates supports the finding from an in vivo fertility study that showed that HDS was related to a decrease in pregnancies (12). Of clinical significance, ICSI fertilization rates were not related to HDS. Therefore, ICSI apparently compensated for the sperm functional abnormalities associated with HDS and eliminated any significant influence that a high percentage of immature sperm had on fertilization rates. It is possible that ICSI is bypassing a natural selection barrier, which ensures that fertilization will not occur when the chromatin is not properly organized. Yet immature sperm (HDS population) do not have an increase in the level of DNA fragmentation (DFI population). Therefore, the negative effect that immature sperm has on pregnancy rates is likely due to intact DNA being complexed with precursor protamines (23) or an altered ratio of protamine forms (37).

Depending on the couple's history and number of reproductive treatment cycles, the SCSA test may give couples a long-awaited answer to the cause of their infertility. Sperm donation may be a viable alternative for some of these couples, especially when poor blastocyst quality and repeated failed cycles are thought to be secondary to sperm DNA fragmentation. In our program, 12 couples elected for treatment with donor sperm after discussion about their abnormal SCSA results (≥30% DFI). These 12 couples had experienced poor blastocyst development (no high-grade blastocysts available for transfer) and unsuccessful pregnancy outcomes. Nine of these 12 couples conceived within three cycles of donor insemination, ending years of infertility. The positive results with donor sperm indicate that the damaged paternal genome of the male partners' sperm contributed to poor preimplantation embryo development in the couples' previous cycles. These results are supported by other studies showing that poor embryo quality (2), decreased implantation rates, and increased spontaneous abortion rates associated with severe male factors (38) improve

when the women were inseminated with donor sperm (39, 40). Therefore, insight into the DNA integrity of the sperm offer a direct explanation for poor embryo development and pregnancy rates in our patients and lead to more successful treatments.

The SCSA test is a valuable noninvasive assay with adequate sensitivity and repeatability to identify men at increased risk of poor blastocyst rates and negative pregnancy outcomes in a clinical setting. Men with ≥30% DFI are at an increased risk of having poor blastocyst development even though their fertilization rates are not significantly lower than men with <30% DFI. Therefore, culturing zygotes to the blastocyst stage may allow the selection of blastocysts after the activation of the embryonic genome and offer these couples an increased likelihood of pregnancy.

Performing the SCSA test on a semen sample before undergoing IVF or ICSI can offer couples insight into their risk of these negative outcomes that is not provided by standard semen analysis alone. Physicians may advise couples that have ≥30% DFI that their SCSA test score indicates they have a significantly lower chance of achieving an ongoing pregnancy due to the quality of the paternal genome. At this juncture, we believe that the SCSA makes a solid contribution to the semen analysis profile and may be an effective diagnostic and prognostic tool to evaluate male factor infertility.

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