DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome

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Objective: To evaluate DNA fragmentation in morphologically normal sperm recovered from the same sample used for intracytoplasmic sperm injection (ICSI) and to correlate DNA damage with embryo quality and pregnancy outcome.
Design: Prospective study.
Setting: Academic center.
Patient(s): 36 infertile men participating in the ICSI program.
Intervention(s): Terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate-fluorescein nick end labeling (TUNEL) assay and morphologic assessment by phase contrast.
Main Outcome Measure(s): Simultaneous assessment of sperm morphology and DNA fragmentation by TUNEL assay was performed in the same cell, then the percentage of normal sperm with fragmented DNA (normal SFD) was correlated with embryo quality and pregnancy outcomes.
Result(s): A highly statistically significant negative correlation was found between the percentage of normal SFD and embryo quality. This association was confirmed for the transferred embryos and for the total embryo cohort. The receiver operating characteristics curve analysis demonstrated that the percentage of normal SFD and embryo quality were statistically significant predictors of pregnancy. When the percentage of normal SFD was $\leq$17.6 %, the likelihood of pregnancy was 3.5 times higher. No correlation was found between the percentage of total sperm with fragmented DNA (morphologically normal and abnormal) and ICSI outcomes.
Conclusion(s): The DNA fragmentation of morphologically normal sperm negatively impacts embryo quality and probability of pregnancy in ICSI cycles. (Fertil Steril® 2009;■:■–■. ©2009 by American Society for Reproductive Medicine.)

Key Words: Embryo quality, ICSI outcome, sperm DNA fragmentation, sperm morphology

Intracytoplasmic sperm injection (ICSI) has been recognized as a very efficient treatment modality for male factor infertility cases who present with poor sperm quality and/or failed conventional in vitro fertilization (IVF) cycles. The selection of the spermatozoon to be used for ICSI is based on the judgment of the embryologist, who chooses a motile spermatozoon with as good morphologic appearance as possible. However, the selected spermatozoa may have damaged DNA (1).

Many investigators (1–5) have shown "paternal effects" that can lead to a decrease in the success of assisted reproductive techniques (ART). A late paternal effect (2) has been mainly attributed to anomalies in the organization of the sperm chromatin (i.e., reduced chromatin condensation, chromosome anomalies, and increased DNA strand breaks or fragmentation). Several studies have suggested that paternal genomic alterations can compromise fertilization rate and embryo viability/quality and result in increased spontaneous miscarriage and minor or major birth defects (1, 3).

Sperm DNA fragmentation has been found to be associated with reduced natural conception, intrauterine insemination (IUI), and IVF outcome rates (6–13). For ICSI, contradictory results have been reported. While some reports did not find an effect (9, 14), others have shown a significant influence of sperm DNA fragmentation on fertilization and pregnancy rates (15–17). These discrepancies could be explained by the fact that during ICSI the sperm selection is based in the motility and the apparently normal morphology. However, the integrity of the DNA in the selected spermatozoon could be a major determinant of the overall success of this procedure.

It has been shown that sperm with fragmented DNA can fertilize eggs with the same efficiency as sperm without DNA fragmentation (18); however, if critical genes are damaged when the paternal genome is activated at day 3 (four to eight cell stage), embryo development failure is likely to occur. The inadvertent selection of spermatozoa with

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damaged DNA for ICSI may have untoward effects, compromising not only the normality of the embryos but also the resultant offspring. This highlights the need for strict monitoring and follow-up observation of the long-term health of children conceived by this technique. Nevertheless, there is sufficient evidence to suggest a negative effect of the use of spermatozoa with fragmented DNA (1). In addition, the negative consequences of using sperm with damaged DNA for short-term and long-term health have been recently demonstrated using animals models (19).

Based on the previous reports and our recent finding that motile spermatozoa with morphologically normal appearance can have damaged DNA (20), we further investigated the impact of DNA fragmentation in morphologically normal sperm on ICSI outcome, measured in terms of embryo quality and pregnancy potential. We focused our study on the identification of DNA fragmentation not only in the motile sperm (recovered by the swim-up technique) but also in the morphologically normal spermatozoa because these are the cells with a high probability of being selected by the embryologist at the time of oocyte injection for ICSI.

MATERIALS AND METHODS

Patients

This was a prospectively designed clinical study. The institutional review board of Eastern Virginia Medical School approved the study, and all participants gave written informed consent. Ejaculates from 36 men participating in the ICSI program were studied. All individuals had a normal physical examination, testes with normal volume, absence of varicocele, and negative semen cultures. A portion of the same processed semen sample used for ICSI was examined for each patient. The indications for ICSI included the diagnosis of male factor infertility based on clinical findings, the results of previous semen analyses showing poor sperm parameters, and/or a failure to achieve a pregnancy after ≥ 3 cycles of controlled ovarian hyperstimulation combined with intrauterine insemination therapy in cases with >5 million total motile spermatozoa (21). Couples with etiologic female factors, and women with fewer than four harvested mature oocytes were excluded from the study. Investigators assessing semen parameters and sperm DNA fragmentation were blinded to the ICSI results.

Sperm Preparation

Semen samples were collected by masturbation into sterile cups after 2 to 4 days of sexual abstinence. After liquefaction, semen characteristics and sperm parameters were assessed. Sperm concentration and progressive motility were assessed with an HTR-IVOS semen analyzer (version GS 771; Hamilton Thorne Research, Beverly, MA) and were manually monitored with fixed parameter settings (22). Motion parameters were examined after mixing the sperm suspension and loading a 5-μL aliquot into a Makler chamber (Mid-Atlantic Diagnostics Inc., Mt. Laurel, NJ). Sperm morphology was examined at $\times 1000$ magnification oil-immersion microscopy by strict criteria (23, 24) after staining with STAT III Andrology Stain (Mid-Atlantic Diagnostics, Inc.).

Motile spermatozoa were selected by the swim-up technique performed in human tubal fluid (HTF; Irvine Scientific, Santa Ana, CA) supplemented with 0.2% human serum albumin (HSA; Irvine Scientific). The spermatozoa were washed and incubated for 60 minutes at 37°C in fresh HTF-HSA. To retrieve the highly motile fraction, the volume from the top was removed. After the separation, part of the sperm sample was used for ICSI. The remainder portion of the purified sperm population with high motility was adjusted at a concentration of $5–10 \times 10^6$ spermatozoa/mL in HTF-HSA, and was stored at –196°C without cryoprotectant until examined for DNA fragmentation and morphologic features.

Samples were thawed in a 37°C water bath for 3 minutes immediately before assessment of DNA fragmentation and sperm shape, as previously reported elsewhere (20). An aliquot of approximately 25 μL was transferred to a multiwell slide (Cel-Line/Erie, Scientific Co, Portsmouth, NH) for examination of DNA fragmentation and morphologic normality. The DNA fragmentation and morphologic characteristics were evaluated simultaneously on the same sperm cell using immunofluorescence and phase contrast, respectively. Each sample was analyzed in duplicate droplets, and the results were averaged.

TUNEL Assay

Sperm DNA fragmentation was evaluated with terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate-fluorescein nick end labeling (TUNEL) assay with the In Situ Cell Death Detection Kit, Fluorescein (Roche Diagnostics GmbH, Mannheim, Germany). The assay uses fluorescein-dUTP to label single and double DNA strand breaks, according to the manufacturer's instructions, and the assay was performed as previously published elsewhere (8). Each sperm suspension was aliquoted in 25 μL on a multiwell slide, fixed with paraformaldehyde (final concentration 2%), permeabilized with 0.1% Triton X-100, and incubated in the dark at 37°C for 1 hour in the TUNEL reaction mixture containing 0.5 IU/μL of calf thymus terminal deoxynucleotidyl transferase and fluorescein-dUTP. Negative (omitting the enzyme terminal transferase) and positive (using deoxyribonuclease I, 1 IU/mL for 20 minutes at room temperature) controls were performed in each experiment. Mounting medium for fluorescence (Vectashield; Vector Laboratories, Burlingame, CA) was added before the evaluation to protect the fluorescence. A total of 200 cells were randomly analyzed per sample in a Nikon Eclipse E600 microscope (Nikon, Tokyo, Japan) at $\times 1000$ magnification oil-immersion objective. Each sperm cell was classified as DNA intact (no fluorescence) or DNA fragmented (green nuclear fluorescence).

In the negative controls, none of the cells showed fluo rescent staining, but in the positive controls (treated with deoxyribonuclease I), 100% of the cells showed DNA

fragmentation. The fluorescent staining of the spermatozoa persisted after several expositions under regular or fluorescence light during the counting procedure. The results were expressed as total sperm with fragmented DNA (total SFD) representing the Percentage of sperm DNA fragmentation/Total number of counted spermatozoa.

Simultaneous Examination of Normal Sperm Morphology and DNA Fragmentation

Immediately after TUNEL, and in the same droplets used for DNA fragmentation analysis, sperm morphology (without staining) was assessed as previously described elsewhere (20) in several randomly selected fields under phase-contrast microscopy (Nikon Eclipse E600) equipped with a SPOT-RT Slider digital camera (Diagnostic Instruments, Inc, Sterling, MI) using a ×1000 magnification oil-immersion objective. A total of 400 cells were evaluated in two droplets per patient. During this examination, every time a spermatozoon with normal morphology was found, the light was immediately switched to the fluorescence to determine DNA integrity. Spermatozoa were considered normal when the head had a normal shape, a symmetrical and oval head configuration, vacuoles occupying less than 20% of the head area, an acrosomal region comprising 40% to 70% of the head area, a symmetrical insertion of the tail, and absence of midpiece or neck defects (24, 25). The results were expressed as morphologically normal spermatozoa with fragmented DNA (normal SFD), representing the Percentage of sperm with normal morphologic characteristics having DNA fragmentation/Total number of morphologically normal sperm. A representative picture is shown in Figure 1.

ICSI and Embryo Quality Assessment

Ovarian stimulation protocols and embryo laboratory procedures used for IVF augmented with ICSI were accomplished using previously established protocols (26). At 16 to 20 hours after microinjection, oocytes were assessed for the presence of two pronuclei (normal diploid fertilization). Seventy-two hours after oocyte retrieval, embryos were classified according to cleavage and morphology score, and then were transferred to the uterus. Morphology grading followed the criteria established by Veeck (27) with modification as follows: grade 5, no fragmentation with equal-sized cells; grade 4, <20% fragmentation with equal-sized cells; grade 3, no fragmentation with unequal-sized cells; grade 2, >20% fragmentation with unequal-sized cells; and grade 1, >50% fragmentation. An individual embryo quality score was calculated by multiplying the number of blastomeres times the morphology grade. Two to three embryos were transferred on day 3 under abdominal ultrasound guidance.

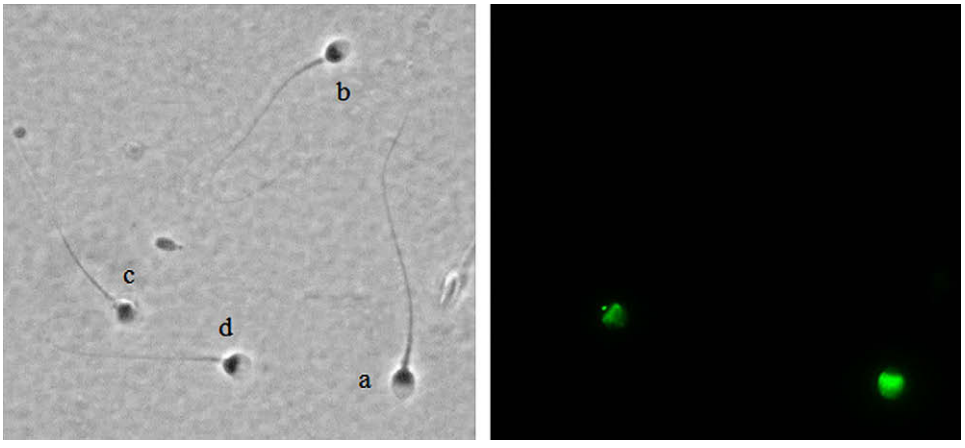
Two indexes were established to examine embryo quality (28). The first one, a mean embryo score (ES) for all embryos available for a given patient (total ES: total embryo score for the total cohort of embryos) was calculated as the Sum of scores of all available embryos/Total number of embryos of the cohort. The second one, a mean cumulative embryo score per transfer (transferred ES: embryo score for the transferred embryos) was calculated as the Sum of scores of transferred individual embryos/Number of embryos transferred in the cycle.

Statistical Analysis

Data are presented as mean ± standard deviation. The studied parameters were age of female and male participants, semen

FIGURE 1

Representative photomicrographs of the simultaneous assessment of normal sperm morphology and DNA fragmentation following swim up separation. *Left:* phase contrast. *Right:* TUNEL fluorescence. (*a*) Normal spermatozoon with DNA fragmentation; (*b*) normal spermatozoon without DNA fragmentation; (*c*) morphologically abnormal spermatozoa with DNA fragmentation; and (*d*) morphologically abnormal spermatozoa without DNA fragmentation.



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parameters of original (unprocessed) samples (sperm concentration, progressive motility, and normal [stained-strict] morphology), DNA fragmentation in total spermatozoa (total SFD) and DNA fragmentation in morphologically normal spermatozoa (normal SFD) obtained from the swim-up separated motile fractions, embryo quality (mean total ES and transferred ES), and clinical pregnancy (defined as the identification of gestational sacs by vaginal ultrasound at 7 weeks).

Various independent parameters of pregnant cycles were compared with those of nonpregnant cycles using the Mann-Whitney *U* test. Spearman's Rho was used for correlation analysis. Variables that were used in the correlation analysis were female and male age, all basic semen parameters evaluated (original sperm concentration, motility, and morphology), number of oocytes inseminated, number of oocytes fertilized (diploid), mean total ES, and mean transferred ES. Two forward stepwise multiple regression analyzes were performed to assess the determinants of mean transferred ES and mean total ES including the following parameters: male and female age, number of oocytes inseminated, number of oocytes fertilized, sperm morphology (phase-contrast analysis), total SFD, and normal SFD following the swim-up technique.

In addition, receiver operating characteristic (ROC) curves were constructed to assess the ability of normal SFD and mean transferred ES to predict pregnancy. A forward stepwise logistic regression analysis was performed to assess the value of selected parameters to predict pregnancy using likelihood ratios. The evaluated parameters were male and female age, number of oocytes inseminated and fertilized, post–swim-up sperm morphology (phase-contrast analysis), total SFD and normal SFD, and mean transferred ES and mean total ES. $P<.05$ was considered statistically significant.

RESULTS

Table 1 depicts the number of samples classified in different pathologic groups according to the semen analysis results in the 36 studied patients. A high proportion of samples showed isolated teratozoospermia (moderate and severe) while a smaller percentage of individuals had low sperm concentration and/or motility, with or without concomitant abnormal sperm morphology.

We compared couples who conceived after embryo transfer (n = 15) versus those who did not achieve a pregnancy (n = 21). There were no statistically significant differences in the number of oocytes retrieved and fertilized or the mean number of transferred embryos between the two groups. Moreover, there were no statistically significant differences in female or male age, basic sperm parameters results, or the total SFD when comparing the pregnant group with the nonpregnant group. However, the normal SFD was statistically significantly higher in the nonpregnant group. These results are shown in Table 2.

TABLE 1

Classification of basic semen analysis results in the study population.

Basic semen analysis	n	%
Isolated oligozoospermia ($<20 \times 10^6$ mL)	1	2.8
Isolated asthenozoopermia (<50 % progressive motility)	1	2.8
Isolated teratozoospermia		
Moderate (5% to 9 % normal forms)	15	41.7
Severe (≤4 normal morphology)	10	27.7
Oligoteratozoospermia	3	8.3
Asthenoteratozoopermia	4	11.1
Oligoasthenoteratozoospermia	2	5.6

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It is interesting that there was no correlation between normal SFD and the percentage of normal sperm morphology in the staining or phase-contrast analysis, nor with the sperm motility and concentration.

Determinants of Embryo Quality

Nonparametric analysis demonstrated a statistically significant negative correlation between normal SFD and mean total ES (r = –0.64, $P<.001$) as well as between normal SFD and mean transferred ES (r = –0.63, $P<.001$) (Fig. 2). The multiple regression analysis (r = 0.78, SE = 3.9, df = 29, $P<.001$) demonstrated that the normal SFD (B = –0.19, $P<.001$) and female age (B = –0.38, $P=.03$) were the main determinants of mean transferred ES among the evaluated parameters.

On the other hand, the determinants of mean total ES were normal SFD (B = –0.18, $P<.001$) and the number of oocytes fertilized (B = –0.44, $P<.03$) by multiple regression analysis (r = 0.73, SE = 3.9, df = 29, $P<.001$).

Predictors of Pregnancy

The ROC curve analysis demonstrated that normal SFD and mean transferred ES are useful predictors of pregnancy probability (Fig. 3). Table 3 summarizes the ROC curve analysis results. The area under the curve (0.7 and 0.75), *P* values (<.021 and <.002), and specificity (82.6 and 91.3) for normal SFD and mean transferred ES, respectively, showed a statistically significant predictive power for pregnancy.

Using ROC analysis we also determined the optimal cutoff values for pregnancy outcome prediction. According to these results, with a mean transferred ES >28, there was a 6.2 times increase in the probability of pregnancy, but with a mean transferred ES ≤28, there was a 0.5 times decrease in

TABLE 2

Comparison of female and male age, original semen parameters, and total sperm with fragmented DNA (SFD) and normal SFD among pregnant and nonpregnant groups.

Group	Pregnant (n = 15)	Nonpregnant (n = 21)
Female age	34.3 ± 4.5	34.2 ± 4.4
Male age	36.5 ± 5.2	36.4 ± 4.2
Oocytes retrieved	9.9 ± 4.5	11.3 ± 5.1
Fertilization rate (%)	83 ± 16	73 ± 20
Transferred embryos	2.4 ± 0.5	2.3 ± 0.5
Semen sperm concentration ($\times 10^6$/mL)	73.6 ± 55.7	75.1 ± 65.7
Semen sperm motility (%)	49.6 ± 17.7	55.4 ± 20.9
Semen sperm morphology (%)	5.2 ± 4.8	5.5 ± 5.5
Total SFD	15.6 ± 8.5	13.3 ± 12.3
Normal SFD	18.9 ± 20.0[a]	33.8 ± 19.4[a]

[a] $P=.03$.

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pregnancy. Additionally, if the normal SFD was ≤17.6%, the probability of pregnancy was 3.5 higher, but if the normal SFD was >17.6%, the pregnancy potential decreased by half.

A logistic regression analysis estimated a model to predict pregnancy (–2 log likelihood = 33.124, chi-square = 6.305 with 1 degree of freedom, $P=.012$). Only the mean transferred ES was included in this model (Exp B = 1.211, $P=.03$), probably because it was the single best independent predictor of conception. When the logistic regression analysis was performed excluding transferred ES from the list of parameters tested, the model estimated to predict pregnancy (–2 log likelihood = 34.321, chi-square = 5.109 with 1 degree of freedom, $P=.024$) included only normal SFD (Exp B = 0.958, $P=.04$) among the parameters analyzed.

DISCUSSION

The clinical introduction of ICSI has allowed many infertile men with severely affected sperm parameters the opportunity to become genetic fathers. However, ICSI is a more invasive technique than conventional IVF and bypasses the process of

FIGURE 2

Correlation between morphologically normal sperm with DNA fragmentation and embryo quality. (**A**) Scatterplot and linear regression between normal SFD (normal sperm with fragmented DNA) and mean total ES (embryo score for total embryos), r = –0.64, $P<.001$. (**B**) Scatterplot and linear regression between normal SFD (normal sperm with fragmented DNA) and mean transferred ES (embryo score for transferred embryos), r=–0.63, $P<.001$.

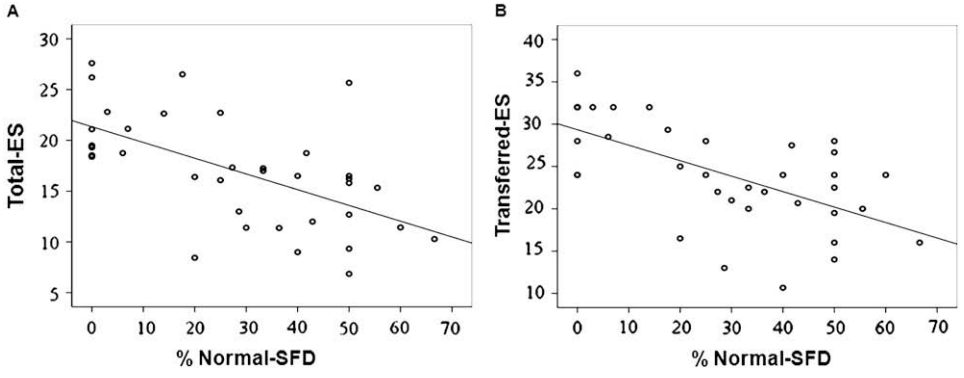

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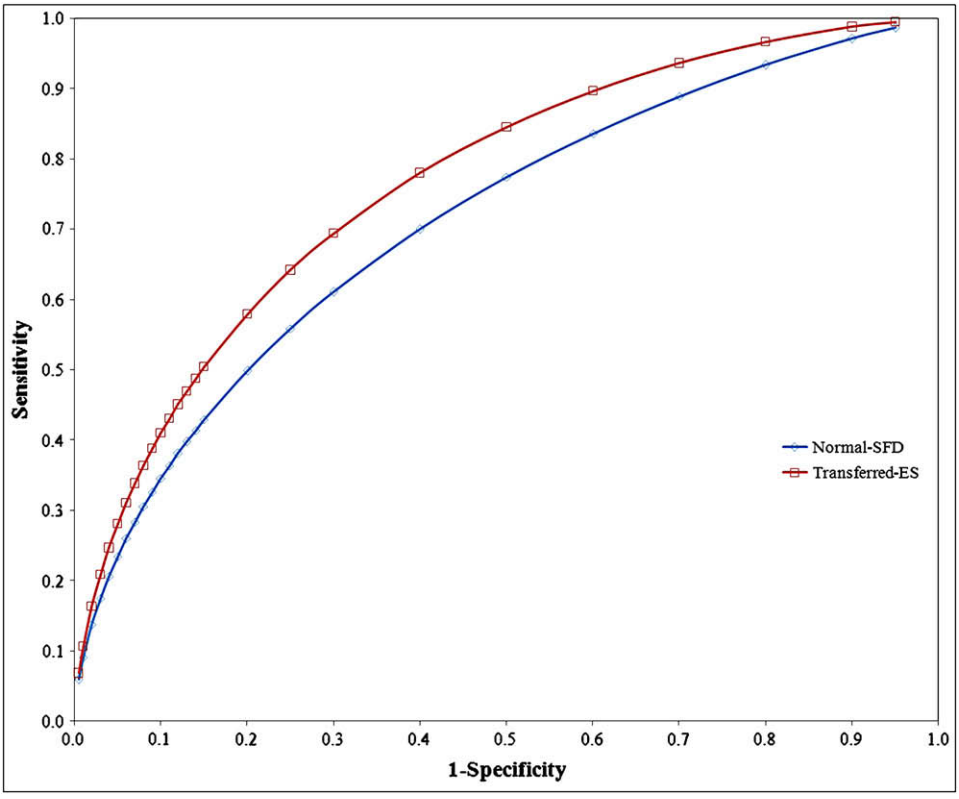
FIGURE 3

Receiver operating characteristics (ROC) curves for normal SFD (normal sperm with fragmented DNA) and mean transferred ES (embryo score for transferred embryos) to predict pregnancy. Quantitatively, the area under the curve is used to determine the accuracy of prediction. Area under the ROC curve for presence of normal SFD = 0.70 and transferred ES = 0.72.



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natural sperm selection. An increased risk of chromosomal abnormalities has been shown in ICSI offspring (29, 30). In addition, a significant increase in urogenital problems in male children born after ICSI was reported in a Swedish study (31). Others have also reported an association of major cardiovascular, urogenital, chromosomal, and musculoskeletal defects with the use of ICSI (32). Because selection for ICSI is based on sperm motility and normal morphology, and because sperm with damaged DNA cannot be recognized during the routine laboratory selection procedure, the inadvertent injection of spermatozoa with DNA damage into oocytes might be determinant of some of these problems.

Although some investigators have suggested the possibility that normal sperm may show DNA fragmentation (4), we have recently demonstrated, for the first time, that infertile men can present DNA fragmentation in the morphologically normal sperm population assessed by strict criteria. In addition, a recent report of unselected couples undergoing infertility treatment showed that 15.9 % of normal sperm selected by high magnification microscopy had DNA fragmentation, thus supporting our findings (33). This prompted us to conduct the present study to correlate the presence of DNA fragmentation in morphologically normal sperm and ICSI outcome.

The first major finding of our study was the demonstration of a statistically significant negative correlation between the percentage of morphologically normal sperm with fragmented DNA (normal SFD) and embryo quality measured as the mean embryo score of total embryos (total ES) after ICSI (Fig. 2). Importantly, the data also confirmed that this association was maintained when analyzing only the transferred embryos from a given patient, as normal SFD also had a statistically significant negative correlation with the mean transferred embryo score (transferred ES). These results support the fact that the presence of normal SFD has an important negative impact on the quality of embryos after ICSI. Contrary to other studies (2, 34), we did not find a statistically significant correlation between the total SFD and embryo quality, suggesting that analyzing the morphologically normal spermatozoa subpopulation enhances the ability to predict the impact of sperm DNA fragmentation on the embryo quality. This was supported by multiple regression analysis, which revealed that normal SFD, female age, and the

TABLE 3

Summary of the receiver operating characteristic (ROC) curve analysis: power of normal sperm with fragmented DNA (SFD) and mean transferred embryo score (ES) for pregnancy prediction.

Parameter	Area under the curve	95% Confidence interval	*P* value	Cutoff point	Sensitivity	Specificity	Positive likelihood ratio	Negative likelihood ratio	Positive predictive value	Negative predictive value
Normal FD	0.70	0.53–0.84	0.02	≤17.6%	61.5	82.6	3.5	0.5	66.7	79.2
Transferred ES	0.75	0.58–0.88	0.005	>28	53.9	91.3	6.2	0.5	77.8	77.8

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number of oocytes fertilized were the main determinants of embryo quality, superseding all other parameters in the prediction of mean total ES and transferred ES.

The second major finding was that the normal SFD and the mean transferred ES were statistically significant predictors of pregnancy when ROC curve analysis was performed (Fig. 3). Using optimal cutoff values and as estimated by the ROC curve analysis, both parameters showed high specificity and were accurate predictors of conception. In general, it is estimated that a test with a positive likelihood ratio (LR) >10 predicts outcome (in this case pregnancy) conclusively, whereas one with a LR 5 to 10 is a moderate and LR 2 to 5 a weak predictor of outcome (35). According to these criteria, we conclude that mean transferred ES is a moderate (LR: 6.2) and normal SFD a weaker (LR: 3.5) predictor of pregnancy in ICSI cycles. The normal SFD percentage predicts pregnancy indirectly through transferred ES, as indicated by their strong correlation and by the fact that in the logistic regression analysis only the mean transferred ES was included in the statistical model predictive of conception. In addition, when transferred ES was excluded from the list of parameters tested, the model estimated to predict pregnancy included only normal SFD. This finding supports our conclusion that normal SFD predicts pregnancy indirectly through transferred ES.

A number of studies have demonstrated an increased percentage of spermatozoa with damaged DNA in the ejaculate of infertile men as compared with healthy fertile donors (7, 16, 20). Although many studies have shown high levels of DNA fragmentation in infertile patients with abnormal sperm parameters (1, 4), it has also been reported that some patients with normal semen parameters and idiopathic infertility may present increased levels of DNA-damaged sperm (36).

In recent years, evaluation of sperm DNA fragmentation has been recognized as a predictor of the probability to conceive (4). The ability of natural conception declines as a function of the percentage of sperm with abnormal chromatin (6, 7). The negative impact of sperm DNA damage in pregnancy outcome has been shown for intrauterine insemination (IUI) (8, 9). In addition, a negative effect on fertilization (10), blastocyst development, and ongoing pregnancy (11) has been shown repeatedly in IVF.

With respect to ICSI, several studies have reported discrepancies on the impact of sperm DNA fragmentation. Some groups have showed a statistically significant negative association between the percentage of sperm with fragmented DNA and the ICSI fertilization rate (15), but others have reported no difference in the fertilization rate but a negative effect in the pregnancy rate (16) or embryo quality (17). Also, increased values of sperm DNA fragmentation have been proposed as a possible cause of low quality of postimplantation embryo and spontaneous abortion (2, 12, 34). On the other hand, some studies have failed to show any effect of sperm DNA fragmentation on ICSI outcomes (9, 14). These studies have evaluated DNA fragmentation in the total sperm

population (morphologically abnormal and normal spermatozoa), but during the ICSI procedure only the good-shaped sperm are selected for injection. This is why a DNA fragmentation analysis of the normal sperm subpopulation is a better predictor of embryo quality and pregnancy outcomes than an analysis the entire swim-up sample.

Notwithstanding these observations, spermatozoa with damaged DNA may retain the potential to fertilize and produce a viable embryo (2, 37). Although the oocyte has the capability to repair damaged paternal DNA, this ability is limited and depends on the grade of damaged DNA (38). Such damage can lead to a predisposition to mutations in the developing embryo with the potential to induce disease in the offspring (39).

The putative effect of sperm DNA damage on the health of future generations is not yet known. Some studies using animal models have indicated that sperm damaged by chemotherapeutic agents transmit heritable translocations, mutations and malformations to the next generation (40). The use of spermatozoa with damaged DNA through ICSI have indicated that genetic and epigenetic changes during pre-implantation may occur, leading to altered fetal development and, as consequence, offspring with aberrant growth, behavior, early aging and tumors (19). Functional bovine sperm with damaged DNA can normally fertilize the oocyte and no significant effect is observed during the first cleavages of the fertilized oocyte. However, a significantly negative effect in further embryo development can be observed as evidenced by embryo fragmentation, apoptosis and aberrant or no signs of mitotic spindle formation (38).

Previous studies have revealed a threshold of 15% to 20% of sperm with fragmented DNA (evaluated by TUNEL) over which pregnancy outcome is lower when ICSI is used (16, 34). Our results, however, showed that the percentage of total sperm with fragmented DNA (total SFD) is not associated with ICSI outcome. Instead, the proportion of morphologically normal sperm with fragmented DNA (normal SFD) was a good predictor of embryo quality and a weak predictor of pregnancy. Our results suggest that swim-up samples containing more than 17.6 % of morphologically normal sperm with fragmented DNA are associated with a higher possibility of generating poor quality embryos and are less likely to result in pregnancy.

In conclusion, although it is not possible to assess DNA integrity in the spermatozoa to be injected during ICSI, the evaluation of DNA fragmentation in morphologically normal sperm obtained from the motile fraction is apparently, albeit indirect, the closest way to reflect it. We have demonstrated that the DNA fragmentation in morphologically normal spermatozoa has a statistically significantly negative effect on embryo quality and pregnancy outcome in ICSI patients.

Even considering the relatively low number of samples analyzed in this study, we have demonstrated that the evaluation of sperm DNA fragmentation when assessing the morphologically normal cell population is useful in predicting embryo quality and it is also a predictor of pregnancy. We propose that this new way of evaluating spermatozoa could be used as a more reliable methodology in predicting ICSI outcomes.

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