Austin Journal of Clinical Pathology

Open Access

Austin Publishing Group

Research Article

Can the Sperm Chromatin Dispersion Test and Terminal Uridine Nick-End Labeling by Flow Cytometry Technique be used Interchangeably to Measure Sperm DNA Damage in Routine Laboratory Practice?

Grèze C[1]*, Guttmann A[2,3], Pons-Rejraji H[1,4], Vasson MP[5,6], Lornage J[7], Janny L[1,4], Ouchchane L[2,3] and Brugnon F[1,4]

[1]Assistance Médicale à la Procréation, Hôpitaux Universitaires, France

[2]Department of Public Health, Clermont-Ferrand University, France

[3]Institut Pascal, Université Clermont Auvergne, France

[4]Imagerie Moléculaire et Stratégies Théranostiques, Université Clermont Auvergne, France

[5]Unité de Nutrition Humaine, Université ClermontAuvergne, France

[6]Unité de Nutrition, Centre de Lutte contre le Cancer Jean-Perrin, France

[7]Service de Médecine de la Reproduction, CHU de Lyon, Bron, France

***Corresponding author:** Grèze C*, Hôpitaux Universitaires de Strasbourg, Laboratoires de Biologie de la Reproduction et de Diagnostic préimplantatoire - Site du CMCO - 1 place de l'hôpital - BP 426 – 67091, Strasbourg Cedex, France

Received: July 13, 2018; **Accepted:** July 26, 2018; **Published:** August 02, 2018

Abstract

Numerous tests have been proposed to evaluate sperm DNA integrity. To assess the Sperm Chromatin Dispersion (SCD) test in an andrology laboratory, twenty-five men attending Clermont-Ferrand (France) University Hospital's Center for Reproductive Medicine were recruited. Sperm DNA damage was measured in the same semen samples using the SCD test and the flow cytometry TUNEL assay (TUNEL/FCM) after density gradient centrifugation.

SCD test reliability between readings, readers or slides was clearly established with very high agreement between measurements (ICC at 0.97, 0.95 and 0.98 respectively). Despite very good agreement between the SCD test and TUNEL/FCM (ICC at 0.94), the SCD test tended to slightly but significantly underestimate DNA damage compared with TUNEL ($p = 0.0127$). This systematic difference between the two techniques was -3.39 ± 1.45% (mean ± SE).

Andrology laboratories using the SCD test to measure sperm DNA damage need to know that it appears to give slightly under estimated measurements compared to TUNEL/FCM. However, this systematic under estimation is very small in amplitude, and the both techniques give almost perfectly congruent test results in our study. Our study underlines that each laboratory should validate its method to assess sperm DNA damage before implementing it in routine andrology lab practice.

Keywords: Andrology laboratory; DNA damage; Flow cytometry; Spermatozoa; Sperm chromatin dispersion test; TUNEL

Abbreviations

ART: Assisted Reproductive Techniques; DGC: Density Gradient Centrifugation; FCM: Flow Cytometry; ICC: Intraclass Correlation Coefficient; SCD: Sperm Chromatin Dispersion; TdT: Terminal deoxynucleotidyl Transferase; TUNEL: Terminal Deoxynucleotidyl Transferase Dutp Nick End Labeling

Introduction

Sperm DNA damage is an important semen quality parameter and a potential predictive biomarker of fertility [1-3]. Accurate determination of sperm DNA damage has important implications for assisted reproductive technology practice, but the lack of standardization is a bottleneck to routine use of sperm DNA integrity tests [4,5].

Numerous tests have been proposed to evaluate sperm DNA integrity. Sperm DNA fragmentation can be measured by Terminal Uridine Nick-End Labeling (TUNEL), which remains a reference technique for direct measurement of DNA strand, breaks [6-8]. The use of Flow Cyto Metry (FCM) to detect sperm with DNA fragmentation by TUNEL is considered a more reliable technique than a slide-based analysis as it allows quick and easy automated measurement of a large number of spermatozoa. Moreover, it is clearly demonstrated that FCM is a sensitive, objective and precise method for detecting DNA fragmentation in spermatozoa [6,9,10]. However, it requires expensive instrumentation and is not easy to apply in routine analysis. Sperm Chromatin Dispersion (SCD) is an assay that measures the susceptibility of sperm to DNA denaturation [11]. After acid denaturation and nuclear protein removal, sperm without DNA fragmentation forms a halo whose diameter decreases with degree of DNA damage. SCD thus looks to be quick, easy and well-adapted to routine lab assessment of human sperm DNA fragmentation, but halo readings need an evaluation of intra and inter-observer reliability to validate their reproducibility.

Here we assessed the reliability of the SCD test coupled to bright-field microscopy and ran the very first comparison of the SCD test against the TUNEL assay with FCM for each sperm sample. The aim of the study was to determine whether the SCD test and TUNEL/FCM can be used interchangeably to measure DNA damage in routine andrology lab practice.

Citation: Grèze C, Guttmann A, Pons-Rejraji H, Vasson MP, Lornage J, Janny L, et al. Can the Sperm Chromatin Dispersion Test and Terminal Uridine Nick-End Labeling by Flow Cytometry Technique be used Interchangeably to Measure Sperm DNA Damage in Routine Laboratory Practice?. Austin J Clin Pathol. 2018; 5(1): 1056.

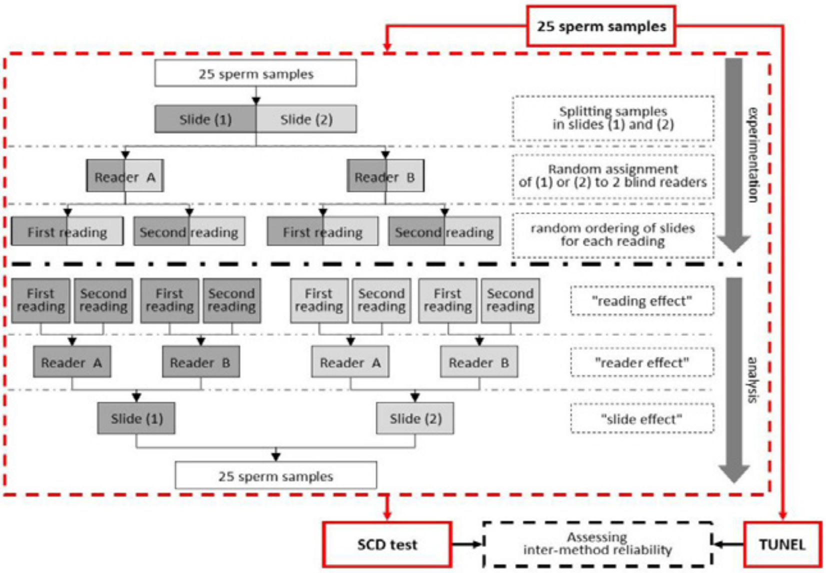

Figure 1: Study design: DNA damage was measured in sperm samples by both the SCD test and the TUNEL assay. For SCD test: The intra-observer, inter-observer and inter-slide reliabilities were successively assessed. Afterwards, the inter-method reliability between the SCD test and the TUNEL assay was performed.

Materials and Methods

Study design and procedures

The decision was made to evaluate the sperm DNA damage not on neat semen but after density gradient centrifugation. This should allow getting results within the potential available population of spermatozoa intended to be used in Assisted Reproductive Techniques (ART). After density gradient centrifugation, sperm DNA damage was measured in sperm samples by both the SCD test and the TUNEL assay. Since the SCD test is a non-automated and subjective method, inter-slide reliability for readings of the same sperm sample and the intra- and inter-observer reliabilities of the same slide were assessed. Afterwards, we assessed the inter-method reliability between the SCD test and TUNEL/FCM for the measurements of sperm DNA damage (Figure 1).

Ethics statement

This study was performed on 25 men attending Clermont-Ferrand (France) University Hospital's Center for Reproductive Medicine for fertility issues. Written informed consent was obtained from each man before the inclusion of any sperm sample in the GERMETHEQUE bio bank, authorized by the ethical committee (CPP Sud-Est VI, DC 2008-558). The study was registered at https://clinicaltrials.gov (NCT 02252510). This work complies with the Code of Ethics of the World Medical Association.

Sperm collection and preparation

All 25 patients attended the Center because of fertility issues, and no sperm donor was included. One semen sample was collected for each of them.

The andrology laboratory has implemented a quality management system based on the International Standard ISO 15189.

Semen samples were collected by masturbation into sterile containers after a period of 2-3 days of sexual abstinence. After semen liquefaction for 30 min at 37°C, basic semen analysis was performed according to World Health Organization guidelines (WHO, 2010), except for sperm morphology assessment, which was done according to David morphological classification.

To isolate sperm cell populations, a two-step discontinuous Sperm Filter® (Cryo Bio System, Rambouillet, France) gradient (90-45%) diluted in Sperm Preparation Medium® (Origio, Limonest, France) was applied on the sperm samples. The purified sperm population was recovered from the 95% layer, washed in Sperm Prep Medium® (750g, 8 min) and suspended in a suitable volume of phosphate buffer saline (PBS, Sigma-Aldrich, Lyon, France) supplemented with 1% (v/v) Bovine Serum Albumin (Sigma-Aldrich, Lyon, France) according to the manufacturer's protocol for SCD tests (Halotech DNA, Spain) to reach a final concentration of 5 to 10.106 spermatozoa.mL-1.

Sperm DNA damage

SCD test (Halosperm® kit): The SCD test was performed using the Halosperm® kit based on the manufacturer's protocol (Halotech DNA, Spain). The Eppendorf tubes of low-melting point agarose provided in the kit were placed in a water bath at 90–100°C for 5 min. At the same time, the pre-coated slides were placed on a tray at 4°C for 5 min. From this point, the protocol was applied twice in a row for each prepared sperm sample in order to get two slides in the end (50 slides for 25 sperm samples). The melted agarose was quickly added with 60µL of each sperm sample and mixed. 60µL were thus pipetted twice in a row for each sperm sample and put in two different Eppendorf tubes. The chilled pre-coated slides were pipetted with 20µL of the cellular suspensions, immediately covered (22×22 mm cover slip), then held at 4°C for 5 min. Once the gel formed with the spermatozoa embedded inside, the cover slips were gently removed and the denaturation solution provided in the kit was applied for 7 min at room temperature. The slides were then placed in the lysing solution for 25 min, and washed with distilled water for 5 min at room temperature. After dehydration by successive

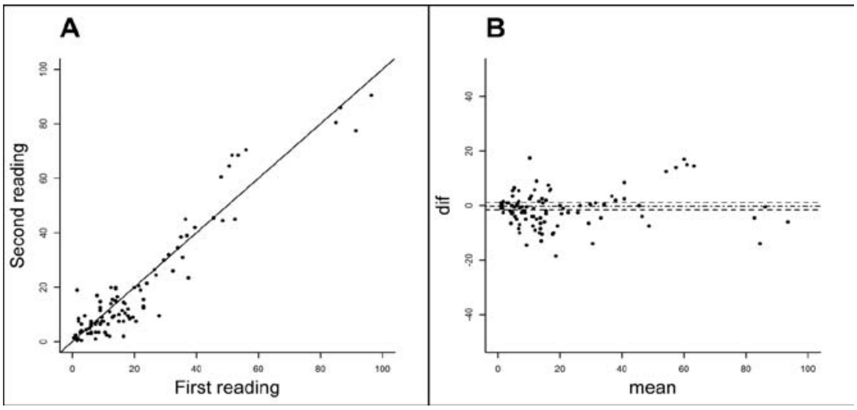

Figure 2: "Reading effect" for SCD test: Scatter plot (A) of second *vs.* first readings by the same reader and Bland-Altman plot (B) where the difference (dif: second minus first reading) is plotted against the mean (mean: arithmetic mean of two readings of each slide), the mean difference is shown as a dash-dot line and its 95% confidence limits are shown as two dashed lines.

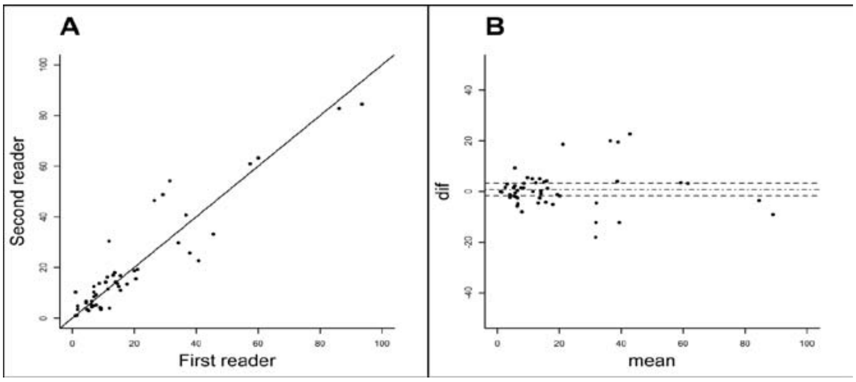

Figure 3: "Reader effect" for SCD test: Scatter plot (A) of second reader *vs* first reader of the same slide and Bland-Altman plot (B) where the difference (dif: second reader minus first reader) is plotted against the mean (mean: arithmetic mean of two readers for each slide), the mean difference is shown as a dash-dot line and its 95% confidence limits are shown as two dashed lines.

increasing concentrations of ethanol (70%, 90% and 95%), the slides were dried and readied for bright-field microscopy by staining for 15 min with Wright staining solution (Merck 1.01383.0500, Darmstadt, Germany) and PBS (1:1, Merck 1.07294.1000, Darmstadt, Germany). These staining solutions are not provided in the kit, but are used by Fernández [12]. The slides were mounted using Eukitt® mounting medium (O. Kindler GmbH & Co, Germany), a colorless medium with crystal-clear optics, which does not change color nor structure of mounted material (according to the technical data sheet). The slides were then stored in the dark at room temperature. This approach thus made it possible to take different readings at different times.

Positive controls were performed for each measurement. After incubation in permeabilization solution (0.1% sodium citrate, 0.1% Triton X-100) for 30 min at room temperature, spermatozoa were treated by DNAse I (Roche Diagnostics GmbH, Germany) at a final concentration of 3 IU.mL-1 at 37°C for 30 min and washed in PBS/ BSA 1% (v/v) before measuring DNA damage by a SCD test as detailed above.

As described previously [12], the observed spermatozoa were scored in five patterns. A total 200 spermatozoa were scored per slide and per observer.

As the aim of the study was to assess the reliability of the SCD test, the study design (see below) was planned such that each sperm sample (once migrated) was split by preparing two slides. Each slide was read independently by two different blinded readers in random order. The coding of the slides had been done by a third person. Each reader ignored the value of the measure obtained by the other reader and each reader performed a double reading, also in random order. Slides had been re-coded before any reassessment by the same observer.

TUNEL assay: The TUNEL assay was performed with flow cytometry as previously described before [13]. to select the population of spermatozoa and to discard the debris and round cells. DNA fragmentation was detected with the "in situ cell death detection kit" according to the manufacturer's protocol (Roche, Meylan, France). Briefly, 1.5×106 washed spermatozoa were fixed with 2% para formaldehyde for 30 min at room temperature. The spermatozoa were then rinsed and incubated for 3 min in permeabilization solution containing 0.1% Triton X-100 (v/v) in 0.1% citrate (w/v) on ice. After washing, the spermatozoa were labeled with 50µL labeling solution containing dUTP and 50µL Terminal Deoxynucleotidyl Transferase (TdT). After counterstaining with 2mg.mL-1 Propidium Iodide (PI), measurement was performed by flow cytometry.

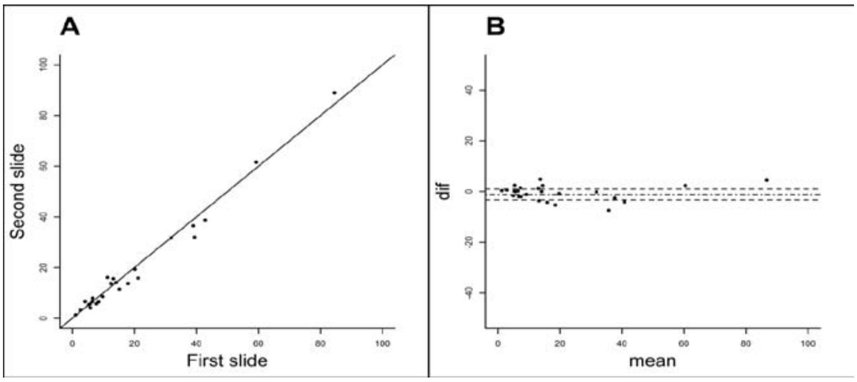

Figure 4: "Slide effect" for SCD test: Scatter plot (A) of second slide *vs* first slide of the same sperm sample and Bland-Altman plot (B) where the difference (dif: second slide minus first slide) is plotted against the mean (mean: arithmetic mean of two slides for each sperm sample), the mean difference is shown as a dash-dot line and its 95% confidence limits are shown as two dashed lines.

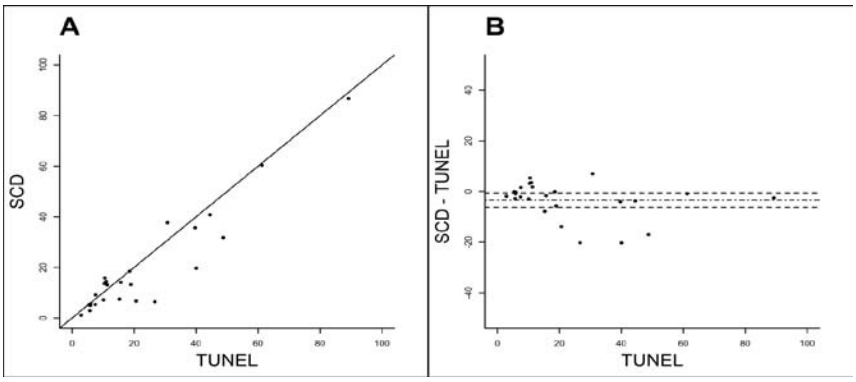

Figure 5: Inter-method reliability between the SCD test and TUNEL: Scatter plot (A) of the SCD *vs* TUNEL and Bland–Altman plot (B) where the difference (SCD minus TUNEL) is plotted against the TUNEL, the mean difference is shown as a dash-dot line and its 95% confidence limits are shown as two dashed lines.

For each sample, we ran a negative control by omitting the TdT enzyme and a positive control by incubating the spermatozoa with 3IU DNase I for 15 min at 37°C in Tris-HCl buffer before labeling. Flow cytometry was performed on an Epics XL cytometer (Beckman-Coulter, USA). A minimum of 20,000 spermatozoa were examined for each assay. Spermatozoa obtained in the plots of CMF were gated by using Side-Angle Light Scatter (SSC) and Forward-Angle Light Scatter (FSC). This was done to put out of the gate, debris and other cells than spermatozoa.

An argon laser delivered a 488 nm excitation wavelength. Green fluorescence (TUNEL-positive cells) was detected with FL1 (using a 525-nm band-pass filter) and red fluorescence (PI-positive cells) with FL3 (using a 620-nm band-pass filter). Both fluorescence signals were recorded after logarithmic amplification. Rate of labeled cells was calculated by the flow cytometer software.

Statistical analyses: All analyses are based on the same sperm samples from 25 patients. The first focus of the study was the reliability of SCD test in measuring sperm DNA damage. We tested for the following potential factor effects: the effect of preparing several slides from the same sperm sample (referred to as "slide effect"), the effect of involving several readers for the same slide (referred to as "reader effect"), and the effect of one reader reading the same slide several times (referred to as "reading effect"). Thus, regarding the SCD test, each sperm sample was split into two slides, each slide was read by two readers (the same pair of trained observers for the whole study), and each reader read each slide twice. The reliability of SCD was assessed using a hierarchical frame following the same scheme for each factor. First, the factor effect was tested through a discordance test using a paired Student t-test or a non-parametric signed-rank test if differences showed non-normal distribution (assessed by a Shapiro-Wilk test). When the tests found no significant discordance on a factor, the concordance between the two modalities of this factor was estimated using the Intra Class Correlation Coefficient (ICC) [14]. In cases of non-discordant values and very good to almost perfect concordance (ICC at 0.8 or more), the two available values were lumped together by computing their mean. Once a factor was assessed, analyses moved on to focus on the next factor, following the same scheme. Analyses followed a hierarchical schedule, first testing the "reading effect", then the "reader effect" and finally the "slide effect", according to the average differences which were expected to sort in ascending order from difference between readings, then between readers, and lastly between slides. Scatter plots and Bland–Altman plots were graphed for each factor analysis (see (Figures 2 to 4)).

If, as expected, the quantifications of DNA damage measured by SCD were sufficiently reliable and reproducible, inter-method reliability between SCD and TUNEL was assessed following the same

Table 1: Patients and semen characteristics.

Variable	N	Mean	Median	Std Dev	Minimum	Maximum	Lower Quartile	Upper Quartile
Age (yrs)	25	38.60	38.0	6.06	30.0	55.0	35.0	40.0
Sperm concentration (million/mL)	25	110.04	80.0	102.24	15.0	432.0	41.0	150.0
Total sperm ouput (million)	25	395.39	243.6	379.85	57.5	1530.0	161.5	567.3
Progressive motility (%)	25	39.40	40.0	14.33	13.0	70.0	30.0	49.0
Total motility (%)	25	47.92	50.0	14.33	23.0	82.0	37.0	55.0
Initial vitality (%)	25	74.56	74.0	9.47	53.0	88.0	67.0	82.0
Initial normal sperm morphology (%)	25	15.70	16.0	8.48	1.0	31.0	11.0	21.0
Normal sperm morphology after sperm preparation (%)	25	24.39	25.0	11.56	2.0	43.0	18.0	34.0

experimental design in these same 25 patients.

Finally, we assessed the relationship between sperm parameters and DNA damage (through both SCD test and TUNEL assay) by performing non-parametric Spearman correlation coefficient tests.

All statistical analyses were performed using SAS v9.4 for windows (SAS Institute Inc., Cary, NC) with a double-sided type I error set at 0.05.

Results

Patients and semen characteristics

Main results are presented in (Table 1).

Intra-method (SCD) and inter-method (SCD versus TUNEL) reliability analyses

The key results on the reliability analyses are reported in (Table 2).

Reliability assessment for SCD test measurement of DNA damage

The reliability of the SCD test in measuring sperm DNA damage was assessed by analyzing the “reading”, “reader” and “slide” effects. For each of the 25 sperm samples, two slides were prepared and each slide was read twice by two readers. The observers read the slides in random order and independently.

“Reading” effect was assessed within 200 readings. Mean difference between the first and second reading of the same slide by the same reader was -0.20 ± 0.70% (mean ± SE) and was not significantly different from 0 (p = 0.3975). ICC was 0.97, reflecting an almost perfect agreement of SCD test measures between readings (see the scatter plot and Bland–Altman plot in (Figure 2)).

“Reader” effect was assessed within 100 readings, pooling both readings by the same reader by their mean since there was almost perfect reading-to-reading agreement. Mean difference between readers of the same slide was 0.82 ± 1.25% (mean ± SE) and was not significantly different from 0 (p = 0.8213). ICC was 0.95, reflecting a very good agreement of SCD test measures between readers (see the scatter plot and Bland –Altman plot in (Figure 3)).

“Slide” effect was assessed within 50 readings, pooling measures from both readers of the same slide by their mean since there was very good reader-to-reader agreement. Mean difference between slides of the same sperm sample was -1.14 ± 1.08% (mean ± SE) and was again not significantly different from 0 (p = 0.5195). ICC was 0.98, reflecting an almost perfect agreement of SCD test measures between two slides from the same sperm sample (see the scatter plot and Bland–Altman plot in (Figure 4)).

SCD test measurements from both sides of the same sperm sample were lumped together by their mean since there was almost perfect slide-to-slide agreement, leading to 25 measurements of DNA damage from SCD tests.

Inter-method reliability between the SCD test and TUNEL

The inter-method reliability for measuring sperm DNA damage was assessed within 50 readings since each sperm sample (n=25) was first split in two to perform each DNA damage measurement. As shown in (Figure 5), DNA damage exhibited distribution with quite a wide range of values, for both TUNEL/FCM and SCD. The mean ± SE value of DNA damage was 22.6 ± 4.2% for TUNEL/FCM and 19.2 ± 4.0% for SCD. DNA damage ranged from 3 to 89.2% for TUNEL/FCM and from 1.2 to 86.8% for SCD. The median (and inter quartile) limits were 15.3% ([7.6 – 30.7]) for TUNEL/FCM and 12.5%

Table 2: Assessment of reliability of DNA damage (in percentages): Reliability analysis within SCD test and inter - method reliability between the SCD test and the TUNEL assay.

Reliability analysis	Effect	N	Mean difference (SE)	p-value*	ICC**
Within SCD	reading	200	-0.205 (0.70)	0.3975	0.9663
	reader	100	0.816 (1.25)	0.8213	0.9468
	slide	50	-1.142 (1.08)	0.5195	0.9825
Between SCD and TUNEL	technique	50	-3.392 (1.45)	0.0127	0.9383

*p-value of the signed-rank test.
**ICC = Intra Class Correlation Coefficient.

Table 3: Correlations between sperm DNA damage and sperm characteristics.

Sperm characteristics (N = 25)	SCD		TUNEL	
	SCC*	p-value	SCC*	p-value
Total sperm output	0.12615	0.5479	-0.0039	0.9854
Sperm concentration	0.06193	0.7687	-0.0573	0.7855
Progressive motility	-0.448	0.0247	-0.4749	0.0164
Total motility	-0.6031	0.0014	-0.6493	0.0004
Sperm morphology after sperm preparation	-0.3133	0.1272	-0.353	0.0835
Initial sperm morphology	-0.4204	0.0364	-0.5732	0.0027
Initial sperm vitality	-0.431	0.0315	-0.4379	0.0286

*SCC=Spearman Correlation Coefficient

([6.4 – 20.1]) for SCD. Mean difference between methods of the same sperm sample was -3.39 ± .45% (mean ± SE) and turned out to be significantly different from 0 ($p = 0.0127$). Nevertheless, as shown by scatter plot and Bland–Altman plot (Figure 5), DNA damage measurements were very close to each other. Compared to TUNEL, SCD tends to underestimate DNA-damage with a systematic offset of about 3.4%.

Inter-method ICC was 0.94, meaning that despite a systematic offset of -3.39%, the results from these two methods can be considered very highly concordant.

Correlation between sperm DNA damage and standard semen parameters

Significant negative correlations between sperm DNA damage (using both SCD test and TUNEL assay results) and sperm characteristics were found for progressive motility, total motility, vitality, and initial morphology. No significant correlation was observed between sperm DNA damage and total sperm output, sperm concentration, or sperm morphology after sperm preparation.

The results on the correlation analyses are reported in (Table 3).

See Supplementary Information (Supplementary (Tables 1 and 2)) for the exhaustive data sets.

Discussion

Sperm DNA damage can result from defective chromatin packaging [12], abortive apoptosis [15,16]. Or oxidative stress [17]. A high level of sperm DNA damage negatively influences live birth rate [18]. Since sperm DNA integrity is an important component of fertility, andrology labs need an accurate method for measuring sperm DNA integrity. Sperm Chromatin Dispersion (SCD) test and the TUNEL assay are two available methods to measure sperm DNA damages. The TUNEL assay with flow cytometry detection is considered as the reference method for detecting DNA breaks [19]. But does not lend itself to easy routine practice. Here we clearly showed that in our laboratory the SCD test with bright microscopy is highly reliable, accurate and does not require andrology labs to invest in expensive new instrumentation.

Since the SCD test is a non-automated method, we first analyzed the potential subjectivity in measurements by a blinded experiment with two different readers. Our results clearly showed high reliability between readings of the same reader, between readers of the same slide, and even between slides of the same sperm sample. Our results are in accordance with a previous study [12]. Showing very low within-reader and between-reader variability in 6 readings of 4 different readers. However, it is important to note that the readers have to be trained to perform the measurements. Indeed, it was not always easy to distinguish the difference between class 2 and 3 spermatozoa described by Fernández [20]. Using the SCD test. Mounted slides with known SCD test results should be kept as a reference to enable regular training of expert readers and ensure high inter-reader reliability. Furthermore, each andrology lab needs to optimize its staining conditions as it is crucial to easily distinguish the halo from the core. The optimal staining, i.e. the time required to get an easily distinct halo of dispersed DNA loop, has to be found in each lab. The halo must not be too dim, which would risk making the outer edge hard to see, nor too intense, which would risk making the borderline between halo and core difficult to determine. Using a microscope eyepiece reticle could help distinguish the different classes of observed spermatozoa.

Our analysis showing high reliability between two slides from the same sperm sample brings novel findings and underlines that the SCD test is a robust measure despite the fact that this technique is non-automated.

The inter-method analysis performed here revealed high agreement between results from the SCD test and the flow-cytometry TUNEL assay. This is the first study to thoroughly compare these two methods on the basis of measurements performed in the same sperm samples. Another study reported a high correlation between these two techniques [21]. In frozen sperm samples, but failed to perform a reliability analysis. Another study [22]. Showed a higher level of sperm DNA damage when measurements were performed with SCD test compared to the TUNEL assay with detection by Fluorescence Microscopy (FM). This is not in accordance with our results but limitations of FM are well-known. Indeed a few hundred cells are observed by this method with the risk of fluorescence bleaching during analysis and relying on human eye. With the detection by FCM, the gating of the population of interest in the dot plots warrants special care. In this study we followed previous gating protocols as published by Grizard [23]. Some authors working on TUNEL/FCM technique excluded semen samples with considerable leukocyte spermia [10,24]. The selection by Density Gradient Centrifugation (DGC) removed the major part of round cells, leukocytes and debris, which made the TUNEL/FCM technique easier to carry out and more reliable. The sperm selection methods such as DGC are known to improve general sperm parameters and to reduce sperm DNA fragmentation [25]. However, the improvement of sperm DNA integrity may not be as important as the improvement of sperm motility [26-28]. As expected, in our study, sperm DNA damage in sperm suspensions after DGC, assessed with both TUNEL/FCM and SCD techniques exhibited distribution with a wide range of values. Working on prepared samples gave supplementary work for this study, but we thought it was important to do that way, keeping in mind that if the results finally led to the choice of SCD test as a routine technique, the procedure would directly be validated on prepared samples. A further clinical study would possibly be conducted in a second time on intra-uterine insemination and *in-vitro* fertilization cycles. This perspective could be helpful to define a cut-off value, predictive of clinical pregnancies and live birth rates after ART.

Despite high reliability between SCD test and flow-cytometry TUNEL assay results from the same sperm sample, a systematic higher proportion of sperm DNA damage was observed by TUNEL. This systematic offset may be explained by lower detection sensitivity (eye-dependent), fewer cells analyzed, and differences in the principles underpinning these two methods. Indeed, the SCD test measures the susceptibility of DNA to acid denaturation [11] while the TUNEL assay measures DNA fragmentation by incorporation of modified nucleotides (dUTP) at the site of DNA damage [29,30]. This systematic difference confirms the lack of correlation showed by previous study [31]. Reporting differences in quantified DNA damage, and implies that only results measured by either the SCD test or the TUNEL should be cross-compared.

In our setting, the rapid solidification of the agarose for the SCD test could be a drawback when testing numerous sperm samples in parallel. We determined that a maximum of 3 tests could be performed together. As the Halosperm® kit is not compatible for fixed samples; we performed the test on only fresh sperm samples, though simultaneous tests would become possible if the samples were thawed at the same time. Our fresh-only protocol ensured that the study was not affected by the potential effect of cryopreservation on DNA damage [32]. Flow cytometry TUNEL is able to measure a higher number of samples, but the assay is also time-consuming and requires an expensive flow cytometer. We used positive controls to validate our assays but it enhanced the time of measurements. A good option could be to use positive controls frozen in advance. The SCD test is a quick and easy technique to implement in routine practice (1.5 hours), and in contrast to flow-cytometry TUNEL, it can also be adapted to low-spermatozoa-count sperm samples.

When taking into account all cost parameters and the feasibility in routine andrology lab settings, the SCD test emerges as a more suitable option than the flow-cytometry TUNEL assay although the cost of the test is higher than TUNEL assay. This is mainly explained by the expensive investment for acquisition and maintenance of flow cytometer.

Both the SCD test and the TUNEL assay pointed to statistically significant negative correlations between sperm DNA damage and sperm motility and morphology. This is consistent with previous studies [33-35] and confirms that male infertility is associated with poor sperm DNA integrity. The lack of negative correlation with sperm concentration may be explained by the high initial sperm concentration required to perform our numerous reliability analyses.

Conclusion

The SCD test offers a practicable and reliable option. Our study showed that despite a systematic offset of 3.39%, results from the SCD test and from TUNEL/FCM can be considered almost perfectly concordant. Andrology labs need to look carefully at which technique to use to evaluate sperm DNA damage. Before implementing SCD test, it is necessary to validate it by each andrology laboratory, as performed in this study. External quality controls exist for the standard sperm parameters (sperm output, motility and morphology) but none were found for sperm DNA damages tests. This is a real weakness for the standardization of these tests and reduces the scope of clinical studies proposing clinical cut-off values.

References

1. Evenson D, Wixon R. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. Reprod Biomed Online. 2006; 12: 466-472.
2. Spanò M, Bonde JP, Hjøllund HI, Kolstad HA, Cordelli E, Leter G. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. Fertil Steril. 2000; 73: 43-50.
3. Zhao J, Zhang Q, Wang Y, Li Y. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. Fertil Steril. 2014; 102: 998-1005.
4. Beshay VE, Bukulmez O. Sperm DNA damage: how relevant is it clinically?. [Miscellaneous Article]. Curr Opin Obstet Gynecol. 2012; 24: 172-179.
5. The clinical utility of sperm DNA integrity testing: a guideline. Fertil Steril. 2013; 99: 673-677.
6. Erenpreiss J, Jepson K, Giwercman A, Tsarev I, Erenpreisa J, Spano M. Toluidine blue cytometry test for sperm DNA conformation: comparison with the flow cytometric sperm chromatin structure and TUNEL assays. Hum Reprod Oxf Engl. 2004; 19: 2277-2282.
7. Bujan L, Walschaerts M, Brugnon F, Daudin M, Berthaut I, Auger J, et al. Impact of lymphoma treatments on spermatogenesis and sperm deoxyribonucleic acid: a multicenter prospective study from the CECOS network. Fertil Steril. 2014 Sep; 102: 667-674.
8. Domínguez-Fandos D, Camejo MI, Ballescà JL, Oliva R. Human sperm DNA fragmentation: correlation of TUNEL results as assessed by flow cytometry and optical microscopy. Cytom Part J Int Soc Anal Cytol. 2007; 71: 1011-1018.
9. Perreault SD, Aitken RJ, Baker HWG, Evenson DP, Huszar G, Irvine DS, et al. Integrating new tests of sperm genetic integrity into semen analysis: breakout group discussion. Adv Exp Med Biol. 2003; 518: 253-268.
10. Sergerie M, Laforest G, Bujan L, Bissonnette F, Bleau G. Sperm DNA fragmentation: threshold value in male fertility. Hum Reprod Oxf Engl. 2005; 20: 3446-3451.
11. Fernández JL, Muriel L, Rivero MT, Goyanes V, Vazquez R, Alvarez JG. The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. J Androl. 2003; 24: 59-66.
12. Fernández JL, Muriel L, Goyanes V, Segrelles E, Gosálvez J, Enciso M, et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertil Steril. 2005; 84: 833-842.
13. Brugnon F, Ouchchane L, Pons-Rejraji H, Artonne C, Farigoule M, Janny L. Density gradient centrifugation prior to cryopreservation and hypotaurine supplementation improve post-thaw quality of sperm from infertile men with oligoasthenoteratozoospermia. Hum Reprod Oxf Engl. 2013; 28: 2045-2057.
14. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychol Bull. 1979; 86: 420-428.
15. Brugnon F, Ouchchane L, Verheyen G, Communal Y, Van der Elst J, Tournaye H, et al. Fluorescence microscopy and flow cytometry in measuring activated caspases in human spermatozoa. Int J Androl. 2009; 32: 265-273.
16. Sakkas D, Seli E, Bizzaro D, Tarozzi N, Manicardi GC. Abnormal spermatozoa in the ejaculate: abortive apoptosis and faulty nuclear remodelling during spermatogenesis. Reprod Biomed Online. 2003; 7: 428-432.
17. Aitken RJ, Jones KT, Robertson SA. Reactive Oxygen Species and Sperm Function—in Sickness and in health. J Androl. 2012; 33:1096-1106.
18. Osman A, Alsomait H, Seshadri S, El-Toukhy T, Khalaf Y. The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis. Reprod Biomed Online. 2015; 30: 120-127.
19. Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril. 2010; 93: 1027-1036.
20. Fernández JL, Muriel L, Goyanes V, Segrelles E, Gosálvez J, Enciso M, et al. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertil Steril. 2005; 84: 833-842.
21. Ribas-Maynou J, García-Peiró A, Fernández-Encinas A, Abad C, Amengual MJ, Prada E, et al. Comprehensive analysis of sperm DNA fragmentation by five different assays: TUNEL assay, SCSA, SCD test and alkaline and neutral Comet assay. Andrology. 2013; 1: 715-722.
22. Feijó CM, Esteves SC. Diagnostic accuracy of sperm chromatin dispersion test to evaluate sperm deoxyribonucleic acid damage in men with unexplained infertility. Fertil Steril. 2014; 101: 58-63.
23. Grizard G, Ouchchane L, Roddier H, Artonne C, Sion B, Vasson M-P, et al. *In vitro* alachlor effects on reactive oxygen species generation, motility patterns and apoptosis markers in human spermatozoa. Reprod Toxicol Elmsford N. 2007; 23: 55-62.
24. Marchiani S, Tamburrino L, Olivito B, Betti L, Azzari C, Forti G, et al. Characterization and sorting of flow cytometric populations in human semen. Andrology. 2014; 2: 394-401.

25. Amiri I, Ghorbani M, Heshmati S. Comparison of the DNA Fragmentation and the Sperm Parameters after Processing by the Density Gradient and the Swim up Methods. J Clin Diagn Res JCDR. 2012; 6: 1451-1453.

26. Ebner T, Shebl O, Moser M, Mayer RB, Arzt W, Tews G. Easy sperm processing technique allowing exclusive accumulation and later usage of DNA-strand break-free spermatozoa. Reprod Biomed Online. 2011; 22: 37-43.

27. Stevanato J, Bertolla RP, Barradas V, Spaine DM, Cedenho AP, Ortiz V. Semen processing by density gradient centrifugation does not improve sperm apoptotic deoxyribonucleic acid fragmentation rates. Fertil Steril. 2008; 90: 889-890.

28. Zini A, Mak V, Phang D, Jarvi K. Potential adverse effect of semen processing on human sperm deoxyribonucleic acid integrity. Fertil Steril. 1999; 72: 496-499.

29. Tesarik J, Mendoza-Tesarik R, Mendoza C. Sperm nuclear DNA damage: update on the mechanism, diagnosis and treatment. Reprod Biomed Online. 2006; 12: 715-721.

30. Zini A, Albert O, Robaire B. Assessing sperm chromatin and DNA damage: clinical importance and development of standards. Andrology. 2014; 2: 322-325.

31. Zhang L, Qiu Y, Wang K, Wang Q, Tao G, Wang L. Measurement of sperm DNA fragmentation using bright-field microscopy: comparison between sperm chromatin dispersion test and terminal uridine nick-end labeling assay. Fertil Steril. 2010; 94: 1027-1032.

32. Zribi N, Feki Chakroun N, El Euch H, Gargouri J, Bahloul A, Ammar Keskes L. Effects of cryopreservation on human sperm deoxyribonucleic acid integrity. Fertil Steril. 2010; 93: 159-166.

33. Benchaib M, Lornage J, Mazoyer C, Lejeune H, Salle B, François Guerin J. Sperm deoxyribonucleic acid fragmentation as a prognostic indicator of assisted reproductive technology outcome. Fertil Steril. 2007; 87: 93-100.

34. Velez de la Calle JF, Muller A, Walschaerts M, Clavere JL, Jimenez C, Wittemer C, et al. Sperm deoxyribonucleic acid fragmentation as assessed by the sperm chromatin dispersion test in assisted reproductive technology programs: results of a large prospective multicenter study. Fertil Steril. 2008; 90: 1792-1799.

35. Shen HM, Dai J, Chia S-E, Lim A, Ong C-N. Detection of apoptotic alterations in sperm in subfertile patients and their correlations with sperm quality. Hum Reprod Oxf Engl. 2002; 17: 1266-1273.

Austin J Clin Pathol - Volume 5 Issue 1 - 2018
ISSN : 2381-9170 | www.austinpublishinggroup.com

Citation: Grèze C, Guttmann A, Pons-Rejraji H, Vasson MP, Lornage J, Janny L, et al. Can the Sperm Chromatin Dispersion Test and Terminal Uridine Nick-End Labeling by Flow Cytometry Technique be used Interchangeably to Measure Sperm DNA Damage in Routine Laboratory Practice?. Austin J Clin Pathol. 2018; 5(1): 1056.