

## Comparison of sperm DNA fragmentation tests

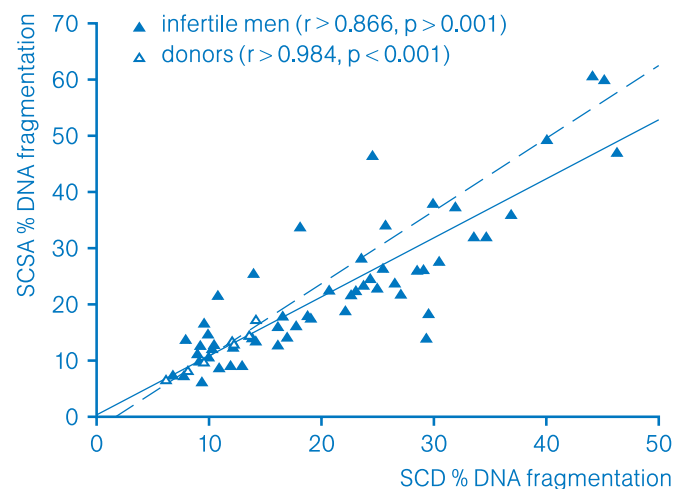
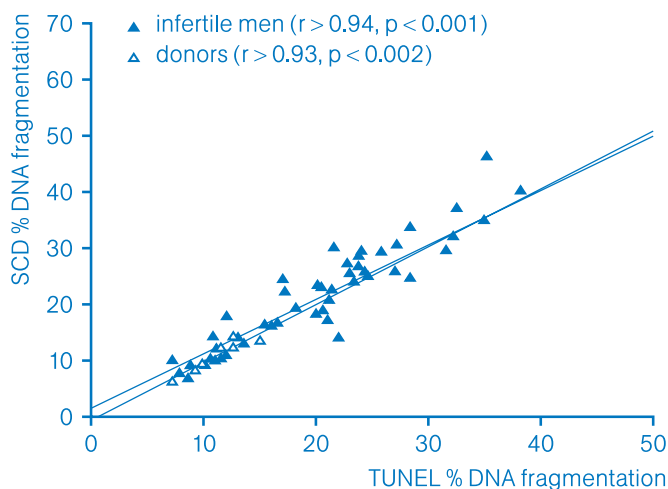
In order to evaluate sperm DNA fragmentation, Halotech has developed the **Halosperm®** kit: a fast and simple in vitro diagnostic kit based on the Sperm Chromatin Dispersion (SCD) technique first described by Fernández et al., 2003. Here is how our kit compares to other techniques available on the market:

### Underlying principles of the main sperm DNA fragmentation tests

ASSAY	PRINCIPLE	MEASURED PARAMETER	DETECTION METHOD
<b>TUNEL</b>	Addition of labeled dUDP nucleotides with terminal deoxynucleotidyl transferase to both SS and DS DNA breaks. Template-independent	% cells with labeled DNA	Fluorescence or bright field microscopy / Flow cytometry
<b>COMET</b>	Electrophoresis of sperm cells reveals fragmented DNA. Alkaline conditions denature DNA to reveal SS and DS DNA breaks. Neutral conditions reveal mostly DS breaks	% cells with migration tails containing fragmented DNA (also length of tail, % of DNA in tail)	Fluorescence microscopy
<b>SCSA</b>	Mild acid treatment denatures DNA with DS or SS breaks. Acridine orange binds DNA and fluoresces green with DS DNA (non-denatured) and red with SS DNA (denatured)	DNA Fragmentation Index (DFI): cells with red fluorescence divided by total number of cells (red +green). Expressed as %	Flow cytometry
<b>SCD</b>	Mild acid denaturation of DNA and lysis of protamines creates a chromatin decondensation halo around sperm head when DNA is intact and no halo when DNA is damaged	% cells with small or absent decondensation halo	Bright field microscopy

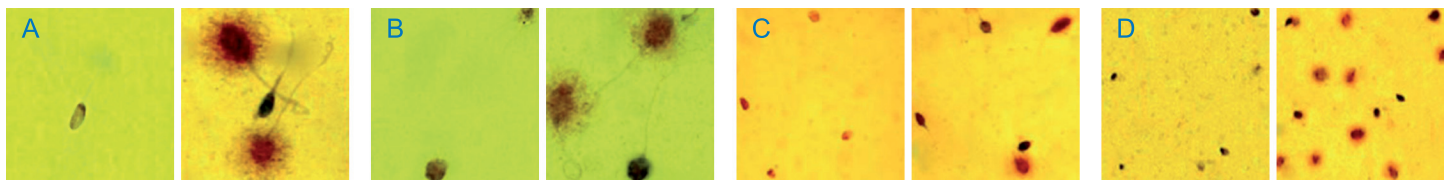
TUNEL, TdT (Terminal deoxynucleotidyl Transferase) –mediated dUDP nick-end labeling. SCSA, Sperm Chromatin Structure Assay. SCD, Sperm Chromatin Decondensation test, SS, Single-Stranded. DS, Double-Stranded. (Tarozzi et al., 2007, Zini and Sigman, 2009)–

### The results obtained with Halosperm® are comparable to those of TUNEL and SCSA



Sperm DNA fragmentation was analyzed in fertile donors (n=7, solid line) and infertile patients (n=60, dotted line). The correlations between the techniques (left, SCD and TUNEL, and right, SCD and SCSA) were analyzed using Student's t-test and are shown above each graph. (Adapted from: Chohan et al., 2006)

The same sperm cells are stained using TUNEL and Halosperm®



Sperm cells were embedded onto a slide and processed using the TUNEL assay followed by the SCD test. (A,B,C,D – left picture) Before Diff-quick staining, TUNEL positive sperms were stained brown. (A,B,C,D – right picture) After Diff-quick staining, TUNEL-positive sperms showed no halo and TUNEL-negative sperms showed a large halo. Pinhead sperm cells with no halo (Arrow) could not be observed with the TUNEL assay before Diff-quick staining. (Adapted from: Zhang et al., 2009).

Comparison of advantages and disadvantages for each technique

ASSAY	MAIN ADVANTAGES	MAIN DISADVANTAGES
TUNEL	<ol style="list-style-type: none"> <li>1. Can perform on few sperm</li> <li>2. For certain protocols use of fluorescence microscopy or flow cytometry may not be necessary</li> </ol>	<ol style="list-style-type: none"> <li>1. Thresholds not standardized</li> <li>2. Variable assay protocols</li> <li>3. Not specifically designed for human spermatozoa</li> <li>4. Labor intensive</li> </ol>
COMET	<ol style="list-style-type: none"> <li>1. Sensitive</li> <li>2. Can perform on few sperm</li> </ol>	<ol style="list-style-type: none"> <li>1. Requires complicated imaging software</li> <li>2. Variable assay protocols</li> <li>3. Unclear thresholds</li> </ol>
SCSA	<ol style="list-style-type: none"> <li>1. Many cells rapidly examined</li> <li>2. Most published data</li> </ol>	<ol style="list-style-type: none"> <li>1. Expensive equipment necessary</li> <li>2. Small variations in lab conditions affect results</li> <li>3. Calculations involve qualitative decisions</li> </ol>

(Adapted from Zini and Sigman, 2009)

List of competitive advantages offered by SCD technique - Halosperm® kit

1. Has been specifically designed for use with human spermatozoa and unlike some other techniques, there exists only one standard protocol that is highly reproducible. For instance, it was recently demonstrated that small variations in crucial steps of the TUNEL assay can greatly affect measures of sperm DNA fragmentation (Muratori et al., 2009).
2. Is one of the most economical techniques to measure sperm DNA fragmentation on the market.
3. Is the fastest technique available on the market. The turnaround time is under 1 hour, and multiple slides can be processed at a time.
4. Is the simplest and easiest technique available on the market. Only basic laboratory equipment is needed to perform the test, and slides can be analyzed with any bright field light microscope.
5. Can be used with few sperm cells, such as in cases of oligozoospermia.
6. Is a highly versatile tool that can also be used in research in conjunction with a number of other techniques, e.g. labeling for 8-oxoguanine to measure oxidative stress, FISH to measure chromosomal aberrations or immunocytochemistry to investigate protein status,...

References

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