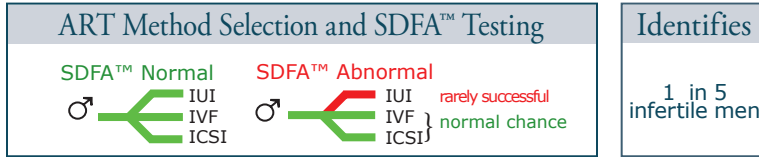




Repromedix
HELPING DOCTORS HELP COUPLES

Sperm DNA Fragmentation Assay (SDFA™)

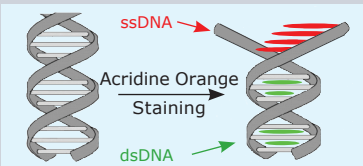
revised May 2007



Science and Physiology

The primary function of a sperm is to deliver male DNA to the egg, and therefore the quality of the DNA delivered is of clear importance to the developing embryo and pregnancy success. Standard semen analysis generally provides information about the quality of sperm as a delivery vessel (i.e., motility, concentration, morphology) but does not directly assess what is being delivered, namely the DNA. One way of assessing the quality or "integrity" of sperm DNA is by measuring the fragmented sperm DNA (DNA breaks). The quantity of breaks is reflected by the amount of DNA that exists as a single strand (ssDNA) rather than in the intact double strand form (dsDNA).

The fluorescent dye, acridine orange, emits red fluorescence when the dye binds to ssDNA via stacking and emits green fluorescence when inserted (intercalated) into dsDNA¹ (see figure below)



The SDFA™ test employs the same methodology as the SCSA® test in which sperm is treated with acid and then stained with acridine orange. 5000 sperm are then analyzed by flow cytometry, based on fluorescent intensity from which the ratio of red ssDNA to total DNA is calculated (see Test Methodology). This provides an objective biochemical assessment of the quality of sperm DNA^{1,2} the quintessential component of sperm. The amount of red fluorescing ssDNA in a sample correlates with the number of DNA breaks present making the SDFA™ an excellent measure of the burden of DNA damage³.

Indications for Testing

Suggested:

- Initial diagnostic evaluation prior to first ART attempt

Alternative:

- Failed natural conception or intrauterine insemination (IUI) attempt(s)
- Exposure to toxic agents or processes

The Sperm DNA Fragmentation assay (SDFA™), if used prior to the first ART attempt, can help couples and fertility specialists select the most appropriate ART treatment protocol. The SDFA™ test uses the same methodology as the SCSA® test and a blinded validation study at Repromedix showed a correlation of >0.96 between SDFA™ and SCSA®. Substantial recent research demonstrates that the SDFA™ test identifies those couples presenting with infertility who have a 15 fold reduced odds of IUI success, and therefore may want to consider IVF or ICSI earlier in their course of treatment⁴.

Clinical Evidence:

Approximately 1 in 5 men have abnormal SDFA™ results:

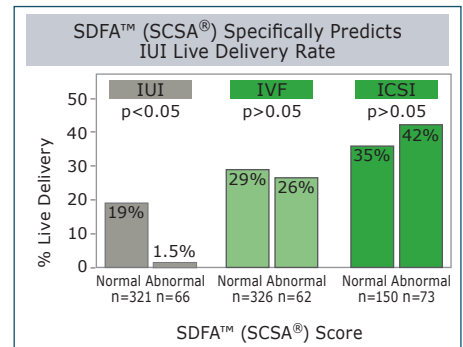
SDFA™ testing can benefit a significant number of couples although the percent of couples with abnormal SDFA™ scores depends upon the presence and type of male factor. A general approximation of the number of undifferentiated couples that would be helped with SDFA™ testing is 20%^{4,5}.

Prediction of natural conception:

In two studies totaling 380 couples attempting natural conception, increasing DFI values were correlated with low frequency of or failure to achieve pregnancy^{6,7}. Using a DFI cut-off value of >30%, all couples in one study either failed to achieve or achieved pregnancy only after 4 months⁶.

Selection of IUI versus IVF or ICSI:

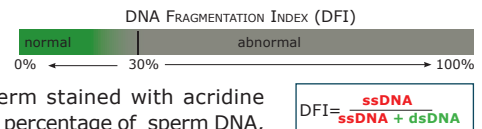
Previously published data suggested that an abnormal SDFA™ test predicted low success with IUI, IVF, or ICSI⁸⁻¹⁰. Although prediction of IUI failure appears to have been confirmed^{10,11}, several subsequent studies questioned this theory with respect to IVF and ICSI^{5,12,13}. Recently, in the most definitive research to date, a prospective analysis of approximately 1000 ART cycles (387:IUI, 388:IVF, 223:ICSI) from 637 patients, demonstrated that an abnormal SDFA™ test is highly predictive of IUI failure⁴. Live IUI delivery rates for patients with normal and abnormal SDFA™ scores were dramatically different (19% and 1.5% respectively). Live IVF and ICSI delivery rates were no different between SDFA™ abnormal or normal patients.* (See graph above)



Units and Ranges

DNA Fragmentation Index (DFI) is

generated from an analysis of 5000 sperm stained with acridine orange¹. The DFI is a ratio expressed as a percentage of sperm DNA, which is fragmented ssDNA (red fluorescence) divided by total sperm fluorescence, with both unfragmented dsDNA (green fluorescence) and fragmented ssDNA (red fluorescence)^{2,6}.



HDS Score is also generated by an analysis of 5000 sperm stained with acridine orange¹. It is a ratio expressed as a percentage of sperm DNA, intensely emitting green fluorescence, divided by the total sperm DNA fluorescence. HDS<15% is considered normal and HDS≥15% is considered abnormal^{2,6}.

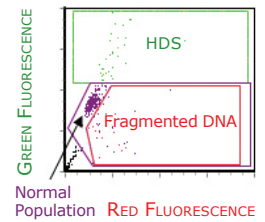
* Inclusion criteria: sperm count >10%/ml in raw semen; female partner age<40 years, BMI<30kg/m², baseline FSH<12 IU/l. Between the SDFA™ abnormal (DFI>30%) and normal (DFI≤30%), no significant differences were observed in: male/female age; female FSH and BMI; number of previous ART treatments; and sperm parameters. ART selection criteria: IUI, couples with unexplained infertility; IVF, mainly couples with female factor infertility; ICSI, total sperm count of <500,000 after gradient centrifugation.

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Test Methodology

The SDFATM test measures the sperm DNA fragmentation in 5,000 sperm per sample. Sperm are acid treated, stained using acridine orange (a cell permeant fluorescent dye), then analyzed by flow cytometry based on the fluorescent intensity ⁶ (SEE FIGURE TO RIGHT). Acridine orange emits green fluorescence when bound to normal dsDNA and red fluorescence when bound to damaged ssDNA. A positive and negative control are included in each test run. The measurement of sperm DNA fragmentation is reported as the DNA Fragmentation Index (see Units and Ranges). In addition, the prevalence of sperm intensely fluorescing green (High DNA Stainability or HDS) after acridine orange staining, is measured using flow cytometry.



Follow up and Treatments Based on SDFATM Results

Normal results (DFI <15%)

This indicates that the SDFATM test has not identified any sperm properties which correlate with reduced success for any ART method. It should be noted that other sperm tests such as the SDDTM test or the P34H test may identify additional sperm abnormalities not identified by the SDFATM test. For example, in a study of 227 patients tested for both SDDTM and SDFATM, 18% (22/124) of SDFATM normal patients were abnormal for SDDTM.

Borderline results (DFI 15% to <30%)

Although DFI <30 is most likely not associated with reduced IUI potential, patients with scores in the borderline range do have some risk of subsequent abnormal semen samples (see below).

Abnormal results (DFI >30%)

Abnormal SDFATM test results, based upon substantial clinical data, suggest the following may be considered:

- IVF or ICSI treatment earlier or as the methods of choice
- An abnormal SDFATM test may indicate exposure to reproductive toxicants such as medications, drugs of abuse and/or environmental toxins. Preliminary evidence suggests that the use of antioxidants in this setting may be beneficial ¹⁴, although definitive studies remain to be completed

	IUI	IVF	ICSI
SDFATM Normal	Green	Green	Green
SDFATM Abnormal	Red	Green	Green

rare Red normal Green

HDS Score

An abnormal HDS score indicates a high percentage of immature sperm present in patient semen which initial reports hypothesized may be due to impaired seminiferous tubules, Sertoli cell dysfunction, varicocele, illness, and/or too frequent ejaculation. However, analysis of the clinical significance of an abnormal HDS score has yielded conflicting results with some experts indicating an association with reduced fertility ^{5,8}, while other studies ⁹⁻¹², including the largest and most definitive study to date ⁴, show no association. Therefore at this time, no consensus exists regarding the use of HDS in clinical management.

Reproducibility

Standard semen analysis is known to show a high degree of variability in the same patient, with recent large studies reporting a coefficient of variation (CV) for concentration, motility, and morphology ranging from 20% to 54% ^{15,16}. The variation in the SDFATM test has been reported to be less than that of the standard semen analysis with initial studies suggesting a CV of <10% ⁶. However, recent research has suggested that the CV may be up to 35% ^{17,18}. No consensus exists regarding the exact CV, but the largest study to date suggests that men with a DFI<10 likely do not need repeat SDFATM testing. Men with a DFI>30 are likely to be abnormal again but if IUI is highly desired, repeat SDFATM testing may be worthwhile ¹⁸. If a reproductive toxicant or varicocele is thought to be a contributing factor and is removed, then repeat SDFATM testing is reasonable after 10 weeks or one complete spermatogenesis cycle. If a portion of sperm used for an IUI attempt is frozen, subsequent SDFATM testing may be performed on this specimen should the IUI cycle fail.

Specimen Requirements

Please contact the Repromedix Client Services Department (1-800-667-8893 ext. 716) for sample collection information, shipping material, and instructions. Semen samples for the SDFATM test must be collected and shipped according to Repromedix SDFATM specimen requirements.

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