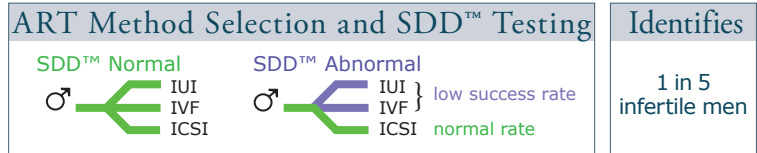




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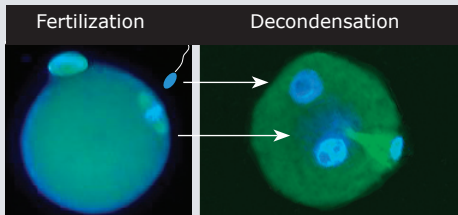
Sperm DNA Decondensation™ Test (SDD™)*

revised June 2007

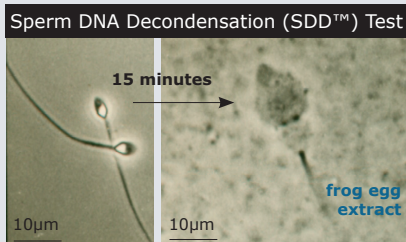


Science and Physiology

For human reproduction to occur successfully, sperm must be able to undergo decondensation normally after penetrating the egg. During decondensation, physical expansion of the sperm pronucleus occurs as protamines, DNA packaging proteins, are replaced with histones and the chromatin is reformatted.



Without a ready supply of human egg extract available, in the past there was no available method with which to measure the performance of human sperm decondensation. However, egg extract from the frog *Xenopus laevis* has been shown to simulate the human egg environment sufficiently to allow human sperm to undergo decondensation. This discovery led to the creation of the Sperm DNA Decondensation (SDD™) test in which the decondensation rate of 50-100 human sperm placed in frog egg extract can be measured¹. The SDD™ score is expressed as a percentage of sperm that have fully decondensed at 15 minutes relative to the normal control sperm.



An abnormal SDD™ score has been shown in a recent retrospective study to be associated with a highly reduced chance of successful IUI or IVF².

Indications for Testing

Suggested:

- **Initial diagnostic evaluation prior to first ART attempt**

Alternative:

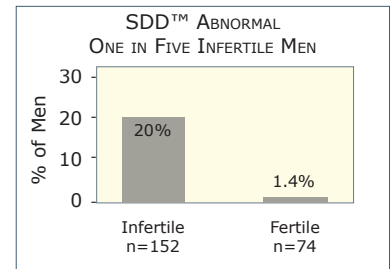
- Failed (non-ICSI) ART attempts (from fertilization to 1st trimester loss)
- Exposure to toxic agents or processes

Recent research has demonstrated, in approximately 1000 ART cycles, that ART selection can be guided by sperm DNA integrity testing using the SDFATM (SCSA®) test in conjunction with a standard semen analysis prior to ART treatment³. Recent research also demonstrates that SDD™ testing may provide similar guidance. Current data suggests that an abnormal SDD™ test identifies men less likely to succeed with IUI or IVF but with unchanged odds of success with ICSI. Patients with abnormal SDD™ scores may want to consider ICSI earlier in their treatment plans.

Clinical Evidence

SDD™ is abnormal in 20% of infertile men

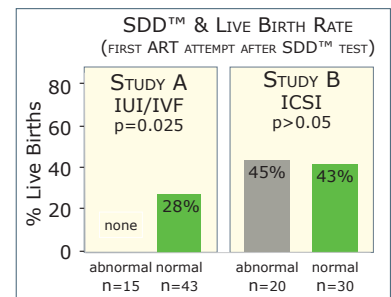
Four prospective, blinded studies⁴ demonstrated that the SDD™ test was abnormal (below 80%) in 20% of 152 infertile men (78 Male Factor, 74 Idiopathic, all with normal female partners) but in only 1.4% of 74 men of proven fertility (Graph 1)^{1,4-6}. In these studies, none of the SDD™ abnormal men had successful subsequent IUI, IVF or ICSI attempts. However, as with other sperm DNA tests, recent data suggest that current ICSI methods can overcome the abnormalities identified by the SDD™ test (see below).



GRAPH 1

SDD™ testing may differentiate between IUI/IVF and ICSI potential

In a retrospective study of 58 patients (Study A, Graph 2), the outcomes of the first IUI or IVF attempt subsequent to an SDD™ test were analyzed². In 43 patients with normal SDD™ scores, 28% (12) had successful IUI or IVF attempts. This was significantly different ($p=0.025$, Fisher's exact test) from 15 patients with abnormal SDD™ scores of whom 0% (0) had a successful first IUI or IVF attempt. Additionally, in a prospective, blinded study of 50 patients (Study B, Graph 2), the pregnancy rate post first trimester of males with SDD™ normal scores (43%, $n=30$) and SDD™ abnormal scores (45%, $n=20$) were not different ($p>0.05$) with 80% power to detect 38% difference⁷.



GRAPH 2

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* Formerly known as the Human Sperm Activation Assay (HSAA) Patent numbers: 5,358,847; 5,770,363 and 5,919,621

† SDD™ testing was performed on sperm from the same semen specimen used in the ART attempts. At the time of these studies, IUI, IVF, or ICSI methodology used did not include density gradients to isolate the "best" sperm for ART

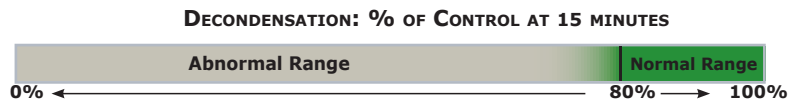
‡ Between the SDD™ normal and abnormal groups there were no significant differences in the average FSH level, female age, or male age

Test Methodology

Room temperature semen is permeabilized by lysolecithin and then is chemically reduced. The sperm are then exposed to egg extract from the frog *Xenopus laevis* for 15 minutes. 50-100 sperm are then visualized by phase contrast microscopy, scored for completion of DNA condensation, and expressed as a percentage of the DNA decondensation rate of the control sperm from fertile donors, which are processed in parallel.

Units and Ranges

The SDD™ score is the % of sperm undergoing full decondensation within 15 minutes, relative to the control sperm from fertile donors, ≥80% is within normal range, while <80% is abnormal.



Specimen Requirements

Contact Repromedix Client Services (1-800-667-8893 ext. 716) for shipping materials and instructions

- The test evaluates sperm. The test does not require fresh viable sperm and can be applied to sperm kept refrigerated for up to 9 days prior to shipment to Repromedix. Freezing sperm prior to shipping is NOT required
- Two cool packs must be frozen at least overnight, at between -10°C and -20°C
- When cool packs are received from Repromedix, place them in the freezer immediately
- Label the conical tube with patient name and date of collection
- Collect semen and allow to liquify at room temperature for 1 hour. Using the provided pipette, transfer more than 500,000 sperm into the 15 ml conical tube
- Store the sample in a 4°C refrigerator until ready for shipment (up to 9 days)

NOTE: DO NOT USE Test Yolk Buffer or cryoprotectants

For shipping:

- Wrap conical tube in plastic bubble wrap to avoid direct contact with cool packs
- Place wrapped semen tube between two frozen cool packs
- Pack in styrofoam box inside cardboard shipping carton
- Ship the specimen to Repromedix using the prepaid FedEx shipping mailer (ship Monday-Thursday ONLY)

NOTE: DO NOT FREEZE

NOTE: DO NOT SHIP with SDFATM specimens in dry ice shipping container

Follow Up or Treatments Based on the SDD™ Test Results

Normal Results (≥80%)

Neither the initial nor the follow up specimen are associated with reduced success rates for any ART method. It should be noted that other sperm tests such as the SDFATM test or the P34H test may identify additional sperm abnormalities not identified by the SDD™ test. For example, in a study of 227 patients tested for both SDD™ and SDFATM, 31% (45/147) of SDD™ normal patients were borderline or abnormal for SDFATM (Repromedix unpublished data).

Abnormal Results (<80%)

Based upon preliminary clinical studies, suggest the following may be considered

- ICSI treatment earlier or as the method of choice
- Inquiry regarding the possibility of exposures to substances such as environmental toxins, medications or drugs of abuse. Some evidence suggests that an abnormal SDD™ test may indicate exposure to reproductive toxicants ^{1,8,9}
- Referral to a urologist who may identify a varicocele. Preliminary, unpublished evidence suggests that patients with an abnormal SDD™ score and a varicocele may benefit from a varicocelectomy. Further studies are in progress to confirm this finding

Reproducibility

The reproducibility of SDD™ scores was evaluated in one unpublished retrospective study in which 2 fertile men each donated 25 semen specimens over a 3 year period. The size of the standard deviation in SDD™ score was 2 percentage points. However, sample to sample variation in infertile men is expected to be much higher. The SDD™ test may be repeated using a fresh specimen at 10 weeks after removal of toxic exposure/process or varicocele repair to verify improvement, or 10 weeks after an abnormal SDD™ test if no possible causal factors can be found.

	IUI	IVF	ICSI
SDD™ Normal	low	normal	normal
SDD™ Abnormal	low	low	normal

low (purple) | normal (green)

References

1. Brown DB and Nagamani M. Use of *Xenopus laevis* frog egg extract in diagnosing human male unexplained infertility. *Yale Journal of Biology and Medicine* **1992**; 65:29-38.
2. Leader B *et al.* Sperm DNA decondensation assay and selection of assisted reproductive technology method. Abstract (submitted to ASRM May **2007**)
3. Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J. and Giwercman A. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. *Human Reproduction* **2007**; 22:174-9
4. Brown DB *et al.* Some cases of human male infertility are explained by abnormal in vitro human sperm activation. *Fertility and Sterility* **1995**; 64(3): 612-622.
5. Brown DB, Maeker BJ, Meriano J and Casper RF. Utility of the human sperm activation assay in determining a sperm sample's efficacy for fertilization when used in ICSI pregnancy attempts. *Fertility and Sterility* **1998**; 70 (Suppl. 1) S144.
6. Diagnostic Systems Laboratories Validation study, (1995) Unpublished.
7. Merryman DC, Rivnay B, Honea KL and Brown D. Sperm DNA Decondensation (SDD™) and Sperm Penetration Assay (SPA) with Gradient Preparation Are Not Predictive of Pregnancy Outcome in In Vitro Fertilization (IVF) cycles with Intracytoplasmic Sperm Injection (ICSI). Abstract (submitted to ASRM May **2007**)
8. Sawyer DE and Brown DB. The use of an *in vitro* sperm activation assay to detect chemically induced damage of human sperm nuclei. *Reproductive Toxicology* **1995**; 9:351-357.
9. Sawyer DE and Brown DB. (2000) Diminished decondensation and DNA synthesis in activated sperm from rats treated with cyclophosphamide. *Toxicology Letters* **2000**; 114:1-26.