200 Forest Street, 2nd Floor, Suite B
Marlborough, MA 01752 USA
tel: 800.667.8893 USA only
fax: 781.935.3068
CLIA#: 22D0884531

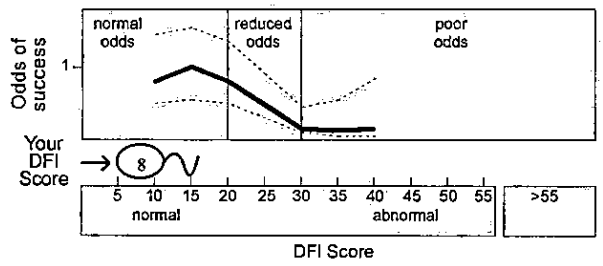
ReproSource® Fertility Diagnostics

The ReproSource®
Advanced Semen Report 2.0

Name:	Kramer, Ian	**Date of birth:**	02/19/1993
Clinician:	Richard O Burney MD	**Age:**	31
Specimen:	00556844	**Date of semen sample:**	04/09/2024

Your sperm DNA integrity test result
Sperm DNA Fragmentation Assay (SDFA)

Your DFI Score & odds of pregnancy with IUI



Your SDFA Score interpretation

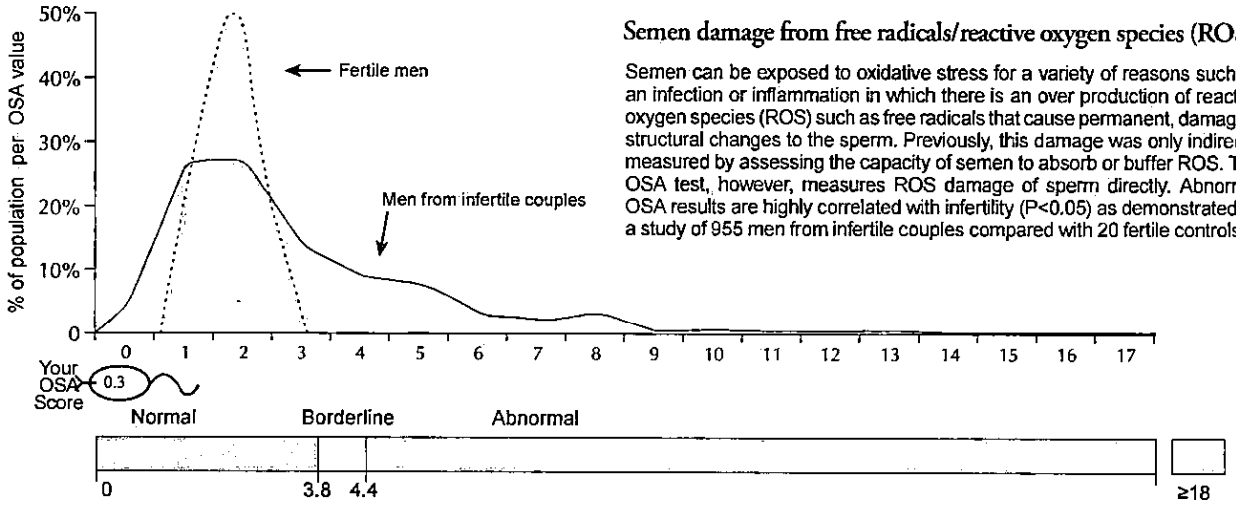
Your DFI result	Your predicted success rates			
	Natural	IUI	IVF	ICSI
normal	**normal**	**normal**	**normal**	**normal**

Comment: Your DNA Fragmentation Index (DFI) is normal

The DFI test results from the submitted semen specimen are associated with normal likelihood of success with IUI, IVF or ICSI.

Damage to your sperm from free radicals
Oxidative Stress Adduct (OSA) Test

A study of 955 men from infertile couples



Semen damage from free radicals/reactive oxygen species (ROS)

Semen can be exposed to oxidative stress for a variety of reasons such as an infection or inflammation in which there is an over production of reactive oxygen species (ROS) such as free radicals that cause permanent, damaging structural changes to the sperm. Previously, this damage was only indirectly measured by assessing the capacity of semen to absorb or buffer ROS. The OSA test, however, measures ROS damage of sperm directly. Abnormal OSA results are highly correlated with infertility ($P<0.05$) as demonstrated by a study of 955 men from infertile couples compared with 20 fertile controls.

Additional comments:

The ReproSource®

Advanced Semen Report 2.0

Patient Supplement

Patient: Kramer, Ian
Clinician: Richard O Burney MD
Date of Report: 04/17/2024

PRIVATE AND CONFIDENTIAL

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Fertility Diagnostics

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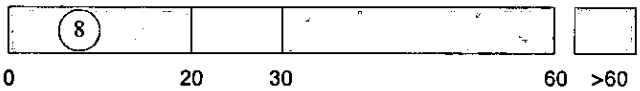
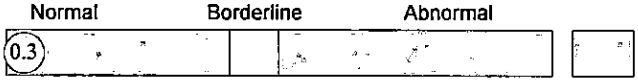
The ReproSource® Advanced Semen Report

ReproSource® Fertility Diagnostics

Patient:	Kramer, Ian	Clinician:	Richard O Burney MD
Gender: M Age: 31Y Date of birth:	02/19/1993	Phone:	1-205-996-3130 Fax: 1-205-996-3164
Specimen:	00556844	Address:	UAB Women and Infant Center
Reported:	04/17/2024		1700 6th Avenue S, Rm 9257A
Received:	04/12/2024 Time: 09:00		Birmingham, AL 35233
Collected:	04/09/2024 Time: 10:49		

Overview

DFI		Normal	DNA integrity
OSA		Normal	Damage from free radicals/ reactive oxygen species (ROS)
HDS		Normal	Levels of decondensed DNA (eg immature sperm)

Patient Results & Interpretation

Test Name	Normal Range	Unit	Result	Comment
DFI DNA Fragmentation Index	<20	%	8	Normal Predicted Success: Natural normal; IUI normal; IVF normal; ICSI normal Higher DFI scores correlate to lower success rates in natural or IUI attempts at pregnancy. Abnormal DFI results suggest the consideration of advancing directly to IVF or ICSI, treatments that lower the DFI score, and/or consultation with a urologist specializing in fertility.
Oxidative Stress Adduct	<3.8	uM	0.3	Normal The OSA test directly measures sperm damage from oxidative stress by quantifying the presence of "adducts," molecules in semen covalently modified by free radicals/reactive oxygen species. Men from 955 infertile couples demonstrated significantly higher results compared with 20 fertile controls (Fig 1, $p<0.05$). Low results have unclear clinical significance at this time.
HDS High DNA Stainability	<15	%	5	Normal The HDS Score provides supplementary information regarding the percent of cells with highly-staining DNA, and can be abnormal when high levels of immature sperm cells are present.

Additional comments:

References

1. Tirado E, Marquette M, Musto JD, Leader B The association of aging, oxidative stress and DNA integrity in human spermatozoa. *American Society of Andrology* 2010; Abstract. https://secureservercdn.net/198.71.233.187/h1k.f5b.myftpupload.com/wp-content/uploads/2019/08/2010_asa_program.pdf pg 64 #70
2. Evenson DP, Darzynkiewicz Z, Melamed MR. Relation of mammalian sperm chromatin heterogeneity to fertility. *Science*. 1980;210(4474):1131-1133. doi:10.1126/science.7444440
3. Bungum M, Humaidan P, Axmon A, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. *Hum Reprod*. 2007;22(1):174-179. doi:10.1093/humrep/del326
4. Evenson DP, Jost LK, Marshall D, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. *Hum Reprod*. 1999;14(4):1039-1049. doi:10.1093/humrep/14.4.1039
5. 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(1):43-50. doi:10.1016/s0015-0282(99)00462-8
6. Erenpreiss J, Bungum M, Spano M, Elzanaty S, Orbidans J, Giwercman A. Intra-individual variation in sperm chromatin structure assay parameters in men from infertile couples: clinical implications. *Hum Reprod*. 2006;21(8):2061-2064. doi:10.1093/humrep/del134

SDFA Overview & Supporting Data

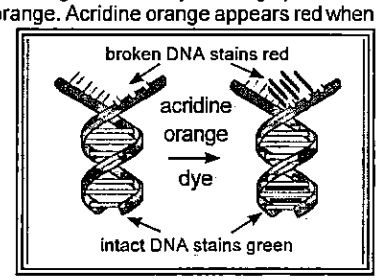
The SDFA measures the susceptibility of sperm dna to breakage or fragmentation

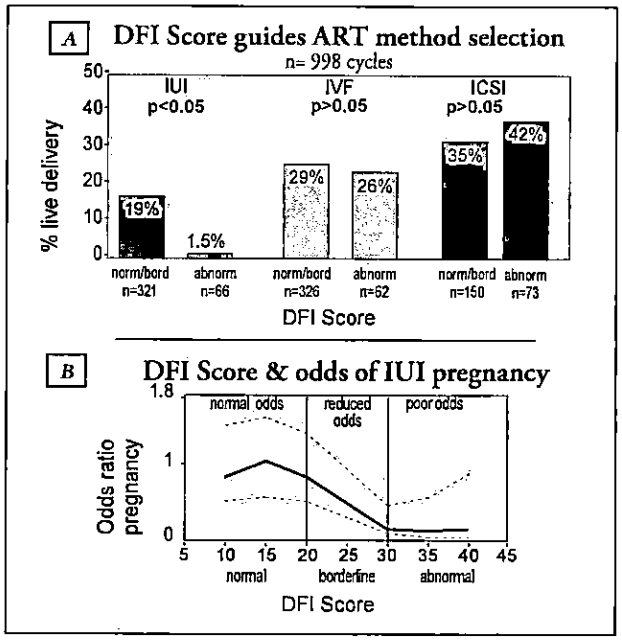
Clinical data: Clinical research indicates that abnormal DFI scores predict poor chance of success with IUI but improved ICSI success relative to conventional IVF than normally observed. Recent research with about 1000 cycles of ART (IUI, IVF, and ICSI) demonstrated that semen with abnormal DFI results had a 98.5% chance of failing IUI, but noted that live delivery rates with ICSI were nearly double those of conventional IVF [3].

Published frequency of abnormal DFI results				
All infertile couples	Male factor infertile couples	Unexplained infertile couples	Fertile couples	Refs
20% n = 998	33% n = 223	17% n = 387	3.5% n = 113	1-3

Reproducibility: Men with a semen DFI score of ≤10% are highly likely (94%, n=49) to have normal DFI scores when retested. However, the chance of having an abnormal DFI score upon retesting increases to 14% for DFI scores from 11 to 20 (n=133) and 27% for DFI scores from 21 to 30 (n=59) [6].

Science and physiology: The SDFA test evaluates the susceptibility of sperm DNA to fragmentation by staining sperm with a fluorescent dye, acridine orange. Acridine orange appears red when associated with broken DNA and green when associated with normal DNA. In the SDFA test, five thousand sperm are analyzed and amount of broken DNA is approximated by amount of red detected relative to red and green detected. This is reported as a % DNA fragmentation index (%DFI) [2,4,5].

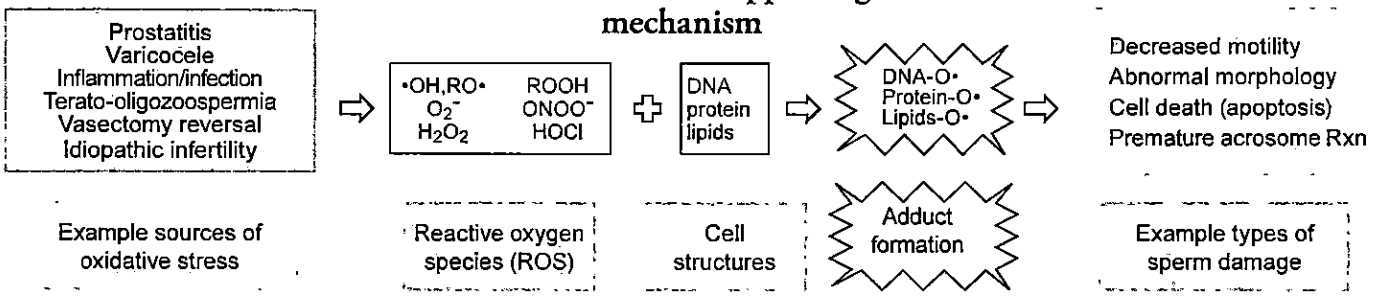




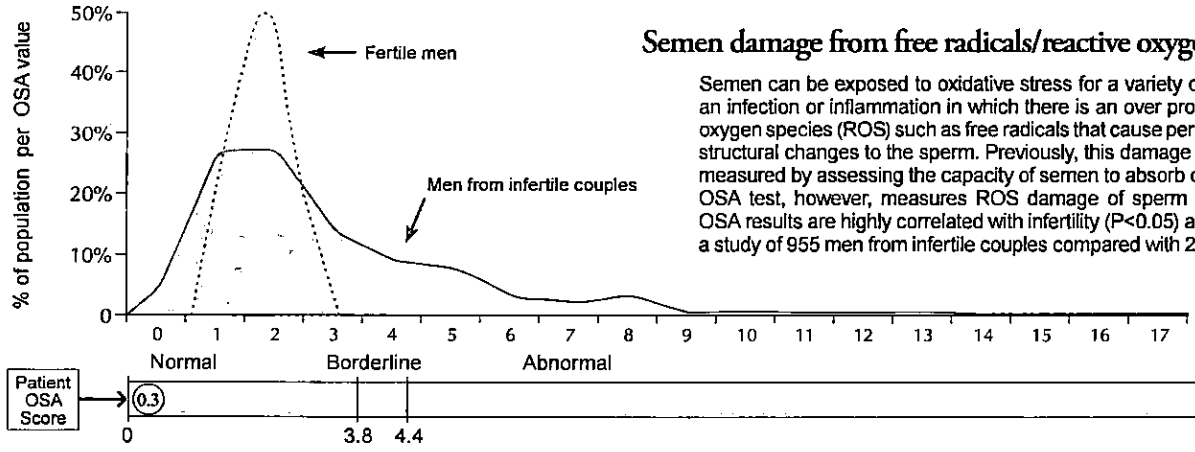
In fig a, live delivery rates by DFI category are shown for semen used in IUI, IVF, or ICSI. In fig.b, the odds ratio (solid line) and 95% confidence intervals (dashed lines) for pregnancy through IUI are shown by DFI score. Pregnancy became less likely with DFI scores between 20% and 30% and was unlikely when DFI scores exceeded 30%. 95% confidence intervals widened with DFI scores above 35% due to lower sample number. Figures adapted from Bungum et al. *Hum Rep* :174-9.

OSA overview and supporting data

mechanism



A study of 955 men from infertile couples



Semen damage from free radicals/reactive oxygen species (ROS)

Semen can be exposed to oxidative stress for a variety of reasons such as an infection or inflammation in which there is an over production of reactive oxygen species (ROS) such as free radicals that cause permanent, damaging structural changes to the sperm. Previously, this damage was only indirectly measured by assessing the capacity of semen to absorb or buffer ROS. The OSA test, however, measures ROS damage of sperm directly. Abnormal OSA results are highly correlated with infertility (P<0.05) as demonstrated by a study of 955 men from infertile couples compared with 20 fertile controls.

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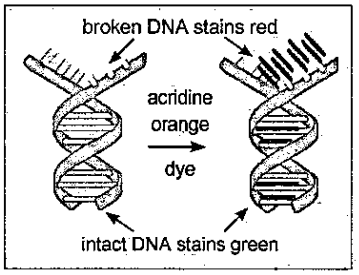
SDFA (Sperm DNA Fragmentation Assay)

An advanced semen testing approach

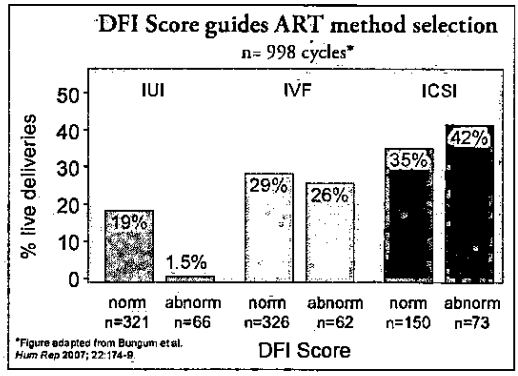


Science behind the test: the measurement of DNA damage

The SDFA test evaluates susceptibility of sperm DNA to breaking (fragmenting) when placed in acid and stained with a fluorescent dye, acridine orange. Acridine orange appears red when associated with broken DNA and green when associated with normal DNA. In the SDFA test, five thousand sperm are analyzed and the amount of broken DNA is approximated by the amount of red detected relative to red and green detected. This is reported as a percent DNA fragmentation index (% DFI), which reflects the proportion of sperm DNA susceptible to breakage in the tested semen sample [1,3].

Research supports guiding treatment with DFI Score

Clinical data: Clinical research indicates that abnormal DFI scores predict poor chance of success with IUI, but normal chance of success with IVF or ICSI [2, 4, 5]. Recent research, with about 1000 cycles of assisted reproduction (IUI, IVF, and ICSI), demonstrated that 98.5% of semen specimens with abnormal DFI results did not succeed with IUI. However, semen with abnormal DFI scores demonstrated normal success rates for IVF and ICSI, and interestingly, with ICSI success rates being almost double that of IVF with DFI-abnormal semen [2].



OSA (Oxidative Stress Adduct) Test

Directly measures sperm damage by free radicals

Male infertility is associated with oxidative stress/excess ROS

Prostatitis
Varicocele
Inflammation/infection
Terato-oligozoospermia
Vasectomy Reversal
Idiopathic Infertility

Example sources of oxidative stress

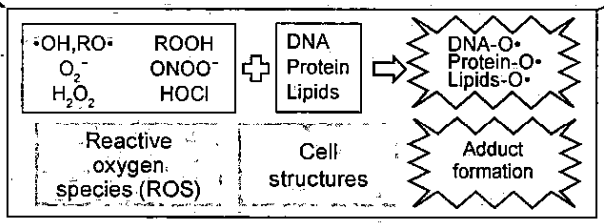
Many factors linked to male infertility, such as prostatitis or varicoceles, which are associated over production of reactive oxygen species (ROS) leading to oxidative stress. Many studies have confirmed that excess ROS damages sperm which is widely believed to be a leading cause of male infertility. A major issue hampering development of reliable fertility treatment protocols are reproducible tests that measure actual damage from oxidative stress in men.

Decreased Motility
Abnormal Morphology
Cell Death (Apoptosis)
Premature Acrosome Rxn

Example types of sperm damage

Adducts formation: evidence of semen damage by ROS

During oxidative stress there is an over production of ROS which includes free radicals and peroxides - highly reactive molecules that form permanent (covalent) bonds with many different molecules such as lipids, DNA, and proteins in the sperm, and the surrounding fluid in semen. The permanent modification of these molecules causes irreversible damage to sperm that are exposed to high levels of ROS. The damage manifests in many ways such as reduced ability of sperm to swim (motility), abnormal sperm shape (morphology), incorrectly timed acrosome reaction (required to penetrate the egg) and even sperm death.

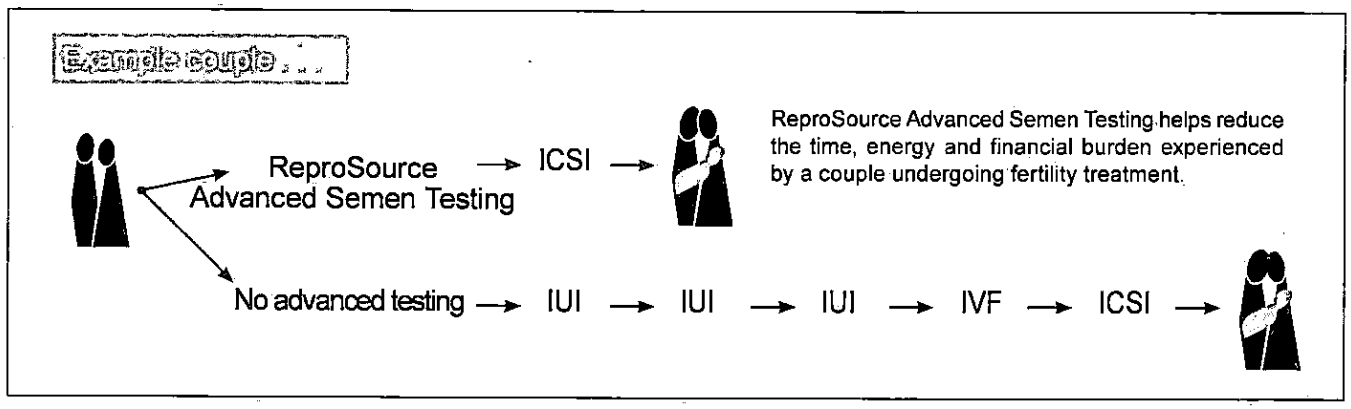


The OSA test: a direct measure of semen damage

Previous tests indirectly detected damage from ROS by measuring the ability of semen in a laboratory to absorb or buffer ROS. While this provides some insight into susceptibility of semen to damage by ROS, it does not answer the question of whether or not damage has actually occurred. The Oxidative Stress Adduct (OSA) test measures the levels of adducts formed from ROS and therefore directly assesses the level of damage caused by ROS. In a study of 955 men from infertile couples, the OSA test identified a significant portion with highly abnormal OSA scores (see pg 2).

More babies . . . sooner through proactive diagnostics

Reprosource testing helps select the optimal treatment first



Compared with IVF and ICSI, IUI is more natural, easier, and costs significantly less money. However, the true cost of an IUI cycle needs to account for the enormous drop out rate of couples who become discouraged after failing to have a child. Simply giving up is one of the biggest reasons for not having a child through advanced reproductive technology (ART). About 70% of all couples will not successfully have a child through IUI. ReproSource's Advanced Semen Report can help identify those couples ahead of time, thus avoiding the great physical, financial and emotional hardships associated with multiple IUI failures. Therefore, using the ReproSource Advanced Semen Report early in a couple's fertility can help reduce the time and cost involved in having a baby through fertility treatments.

Resources

American Society for Reproductive Medicine
www.asrm.org

European Society for Human Reproduction & Embryology
www.eshre.com

American Pregnancy Association
www.americanpregnancy.org

Resolve
www.resolve.org

Family Equality
www.familyequality.org

References

1. Evenson DP, Darzynkiewicz Z, Melamed MR. Relation of mammalian sperm chromatin heterogeneity to fertility. *Science.* 1980;210(4474):1131-1133. doi:10.1126/science.7444440
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6. Tirado E, Marquette M, Musto JD, Leader B The association of aging, oxidative stress and DNA integrity in human spermatozoa. *American Society of Andrology* **2010**; Abstract. Accessed March 9, 2022. https://secureservercdn.net/198.71.233.187/h1k.f5b.myftpupload.com/wp-content/uploads/2019/08/2010_asa_program.pdf pg 64 #70.

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