Sperm DNA Fragmentation

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Learning Points

SDF:

- What is it
- Why does it happen
- How do we measure it
- How is it related to reproductive outcomes
- What can we do about it

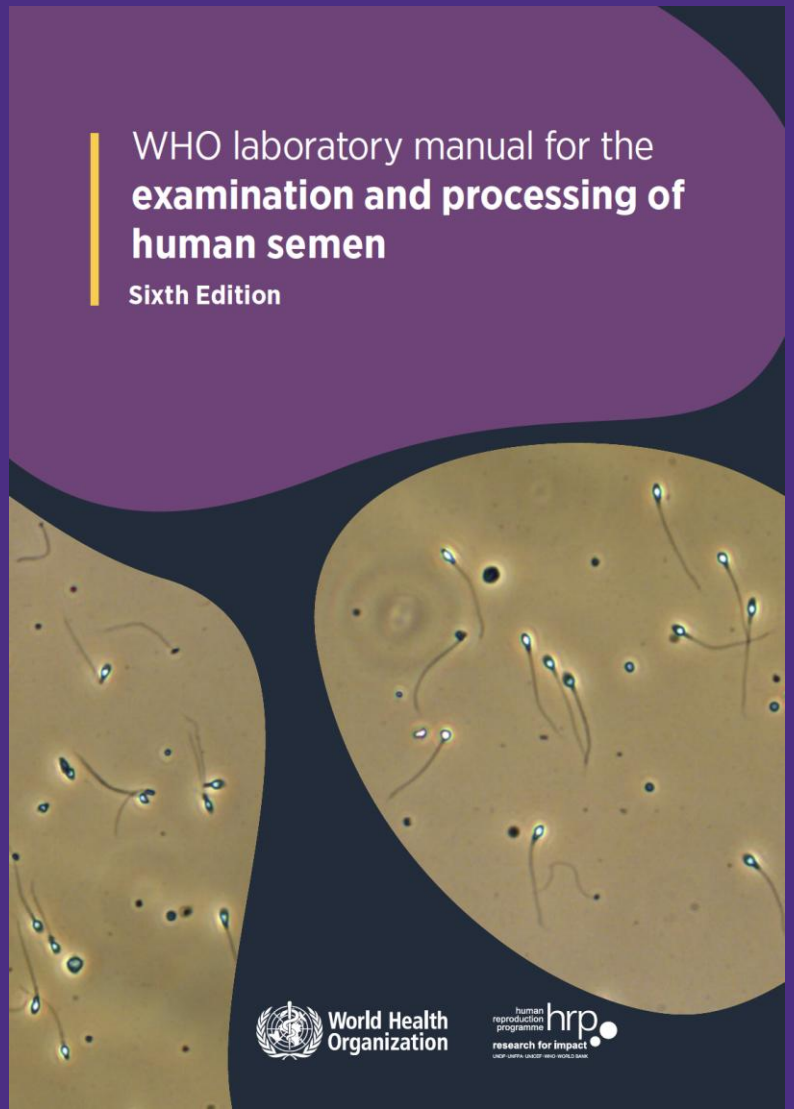
Not a complete review of individual studies or meta-analyses (see references in Handout). Rather, a guide to understanding.

History

DNA fragmentation in human sperm

- Narendra Pal Singh et al., 1989 Comet
- Donald Evenson 1980 (bull), 1990s SCSA
- Sun et al., 1997 TUNEL
- Fernandez et al., 2005 SCD

WHO 6th ed.



“Since sDF is only partially related to semen quality… it could represent an important addition in the work-up of male infertility, becoming one of the most discussed and promising biomarkers in basic and clinical andrology .”

“Sperm DNA is re-packaged by protamines to decrease nuclear size and protect genetic material”

Is that entirely true?

1. Artificial induction of oxidative damage shows that DNA is the MOST SENSITIVE site of damage, before lipid peroxidation or protein modification.

2. Spermatozoa with damaged DNA often have normal motility and morphology.

3. Damaged DNA does NOT prevent fertilization

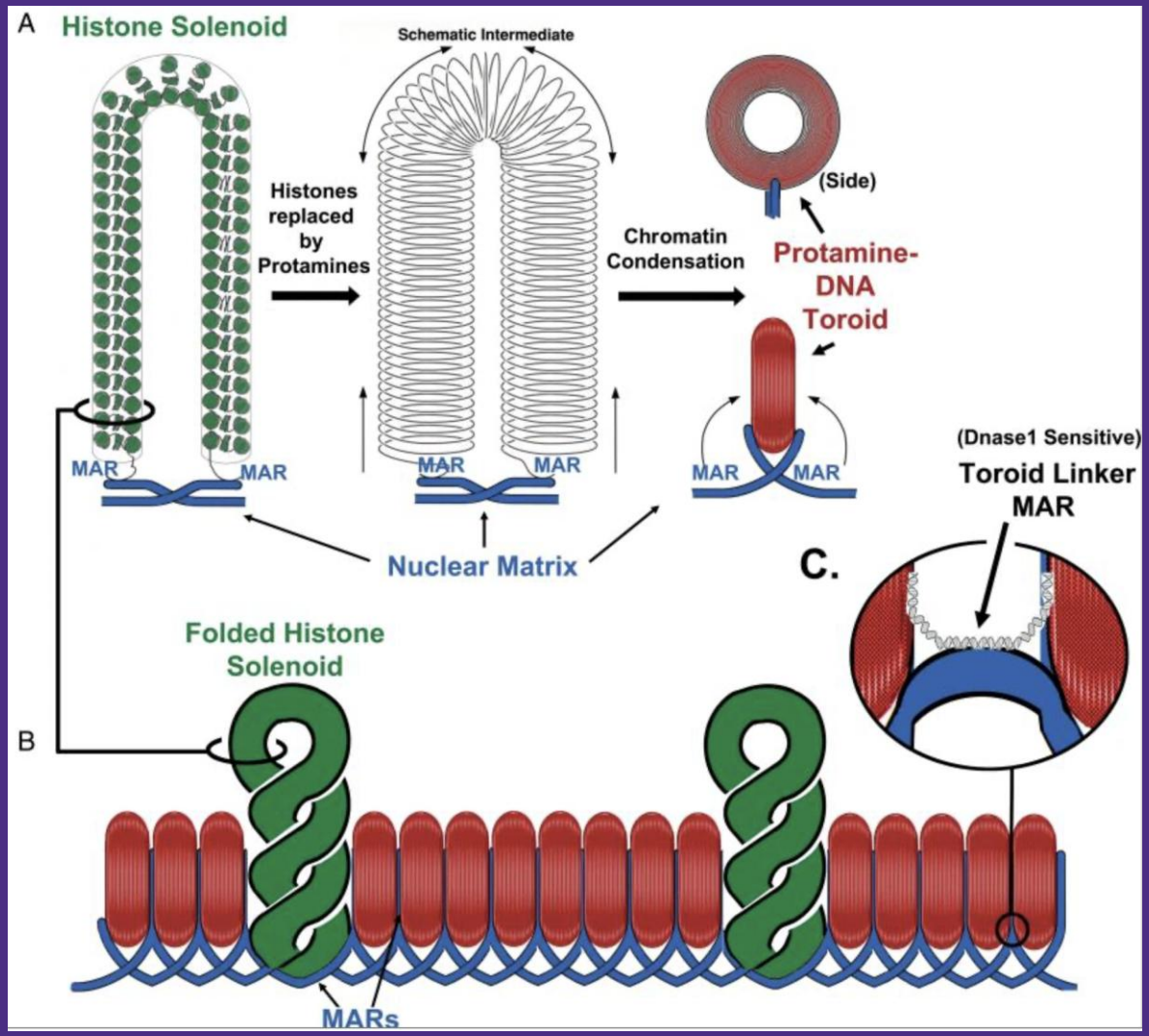
Consequences of Sperm DNA Fragmentation

- Mature sperm do not possess repair mechanisms.
- Damaged sperm DNA does not preclude fertilization. Paternal genome crucial at d4-5.
- Fertilized eggs may not correctly repair damaged sperm DNA, especially with DSB or double-double breaks.
- Unrepaired paternal DNA may result in post-fertilization embryonic failure and, potentially, childhood diseases.

Aitken et al 1998 Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biol Reprod 59: 1037-46

Aitken 1999 The Amoroso Lecture. The human spermatozoon – A cell in crisis? Reproduction 115:1-7

Condensed sperm DNA has nuclease sensitive sites between protamine-DNA toroids, and uncondensed DNA in histone solenoids.



Up to 15% of human sperm DNA remains in histone solenoids. These may contain DNA critical for embryo development and may be sensitive to DNA damage.

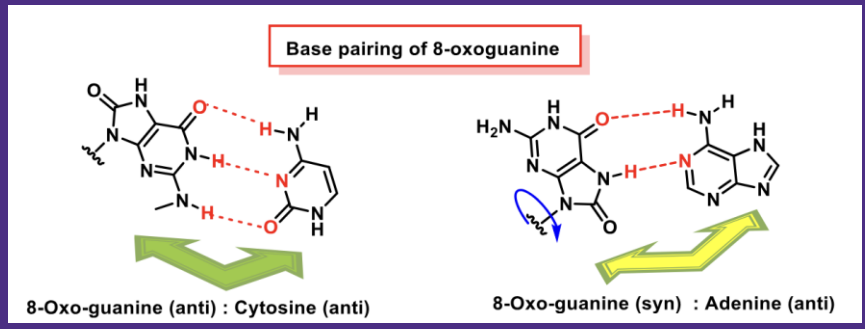
Figure from Ward 2010
Mol Hum Reprod 16:30

Types of sperm DNA damage

Base modifications
Abasic sites

Single Strand Breaks (SSB)

Double Strand Breaks (DSB)
Double Double Breaks



Chemical Insights into Oxidative and Nitrative Modifications of DNA
Celia María Curieses Andrés, José Manuel Pérez de la Lastra, Celia Andrés Juan, Francisco J. Plou and Eduardo Pérez-Lebeña
Int. J. Mol. Sci.* **2023, *24*(20), 15240

How do we measure Sperm DNA Damage?

Abasic and modified bases cause SSB under acidic or alkaline conditions.

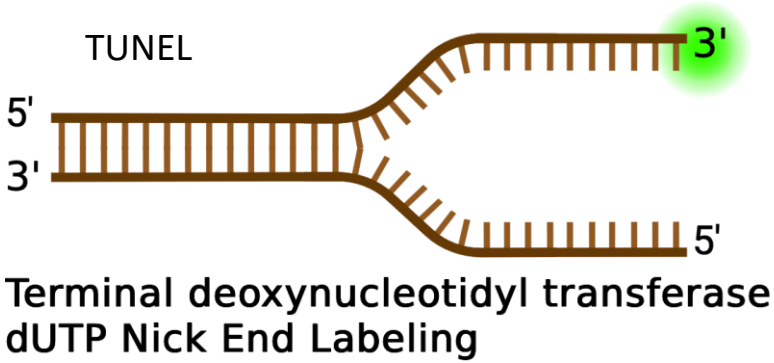
Break Type	TUNEL	SCSA (acid)	SCD (acid)	Neutral Comet	Alkaline Comet/Diffusion
SSB	++	+++	-	+	+++
DSB	-	-	++	+++	+
Double DSB	-	-	-	+++	+
Apoptosis	+	+	-	+	+++

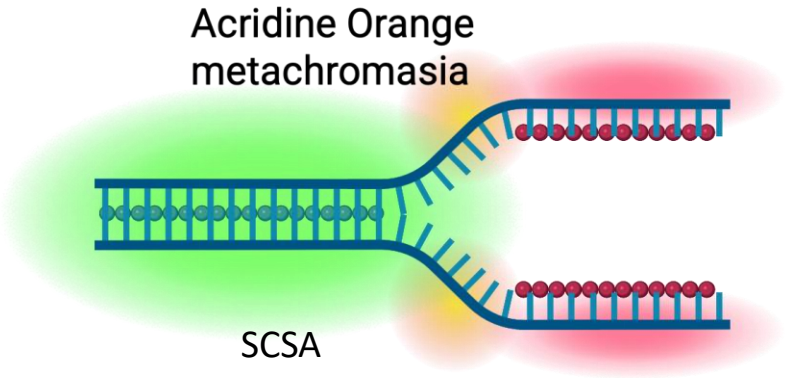
Modified bases such as 8-OH-dG may be measured by HPLC or ELISA

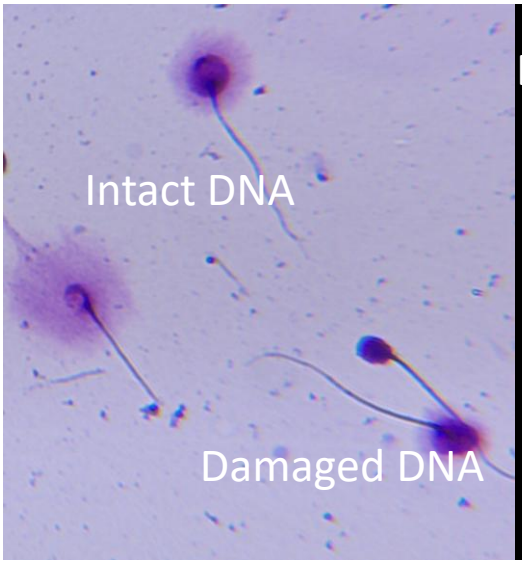
New methods provide sequence data: NGS to identify abasic sites; NGS with immunoppt; SSB-seq; BLESS (Breaks Labeling, Enrichment on Streptavidin and next-generation Sequencing) and similar for DSB; SSiNGLe (single-strand break mapping at nucleotide genome level) .

What Sperm DNA Fragmentation Methods Measure

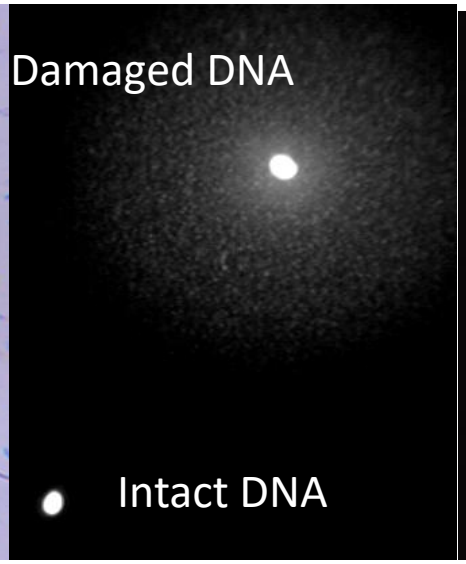
Also: see Handout



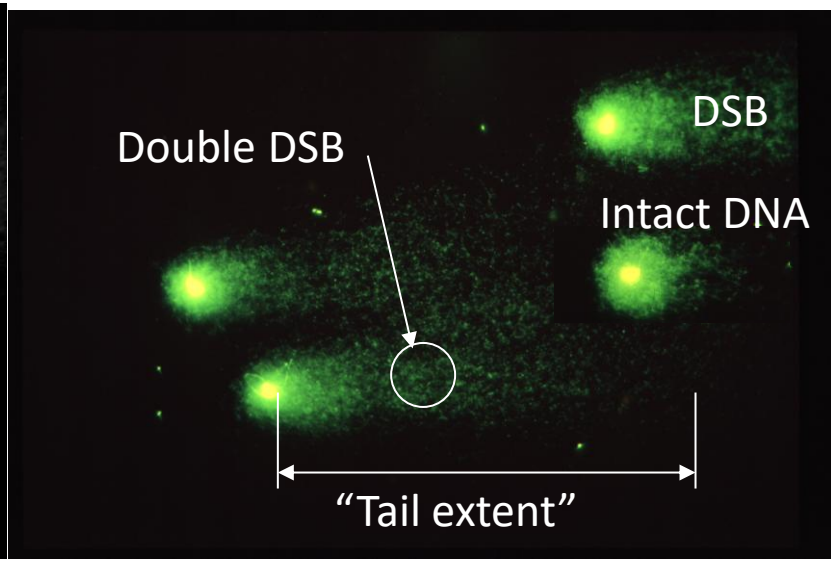




SCD "Halo"



Alkaline Diffusion



Neutral Comet
Comet

DNA Repair

Common throughout your body

Single-strand breaks (SSBs) are usually caused by oxidative stress, and are repaired by nucleotide excision, base excision, and mismatch repair. However, unresolved repair often leads to programmed cell death.

Oocytes are really good at DNA repair but may not be able to repair all kinds of damage, especially DSB and Double DSB.

Spermatozoa have NO enzymes to repair damaged DNA!

Where does Sperm DNA Fragmentation occur?

First site: Spermatogenesis

DNA breaks are a normal part of life, growth and reproduction. Replication and meiosis involve separation of DNA strands, allowing errors such as breaks, modifications and loss of bases. These are normally repaired. About 5-10,000 occur each day.

Spermatocytes are especially susceptible to toxicants and heat. Up to 20% or more undergo, or start, programmed cell death (apoptosis).

"apoptosis" (ἀπόπτωσις) Greek: "dropping off" or "falling off". "…we propose that the stress should be on the penultimate syllable, the second half of the word being pronounced like "ptosis" (with the "p" silent)…" From: Kerr, Wyllie, Currie 1972. "Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics". *British Journal of Cancer*. **26** (4): 239–257.

Other Tests for Apoptosis

DNA "ladder" of digested nuclear DNA: typical of somatic cells, not of sperm.

Apoptic bodies: Remnants of apoptotically degenerated cells; common in semen (eg, M540 bodies); may contain DNA and ROS-generating enzymes.

Apoptosis pathway: Caspases, BCL/BAX, FAS/FasLigand, p53

Externalized Phosphotidyl Serine: binds to Annexin V. Typical for somatic cells. Possible marker of aborted apoptosis in sperm. Sperm also externalize PS during capacitation.

Where does Sperm DNA Fragmentation occur?

Second site: Post-Spermiation (Male reproductive tract - semen)

DNA condensation continues in the epididymis, so DNA remains vulnerable.

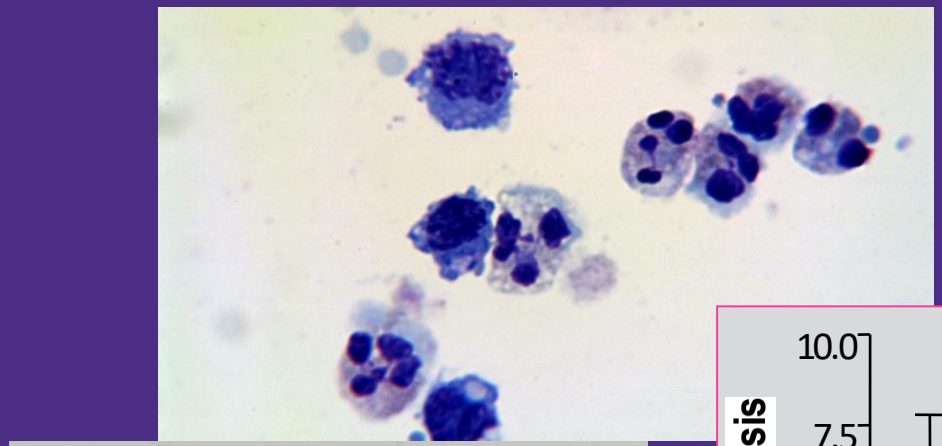
Apoptosis initiated in testis is “on hold” in some sperm.

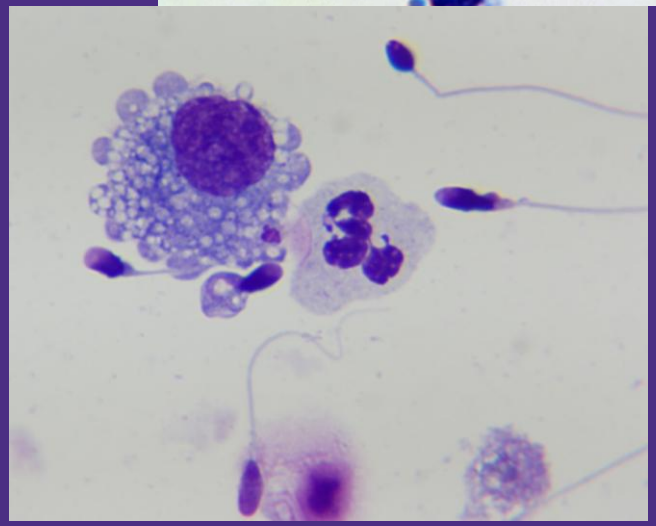
Inflammation is fairly common in prostate, allowing immune cells into semen. Infection, less common, brings in more immune cells.

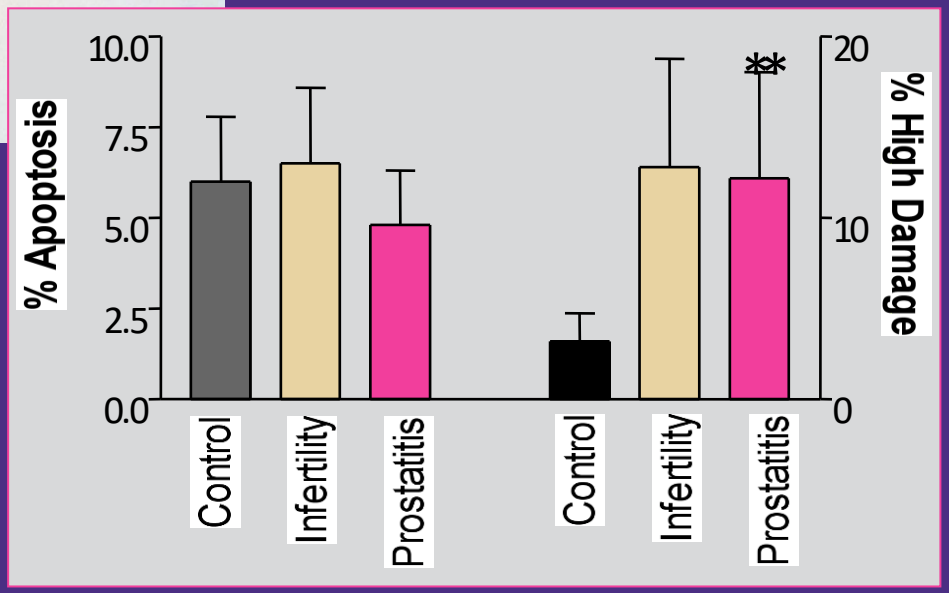
Reactive oxygen/nitrogen species induce DNA damage. Probably 100,000 per day in each person…

Oxidative stress is induced when ROS>antioxidants.

Leukocyte-induced sperm DNA damage







Muller et al 2009

Where does Sperm DNA Fragmentation occur?

Third site: During and after sperm separation from semen; cryopreservation

IN THE LAB! IN THE OR!

Aspiration, centrifugation, exposure to VOCs, UV, heat, shear, loss of antioxidants, extrinsic chemicals (culture media), etc.

Cryopreservation induces osmotic, physical, chemical and temperature stresses during both freezing and thawing.

In the female reproductive tract?

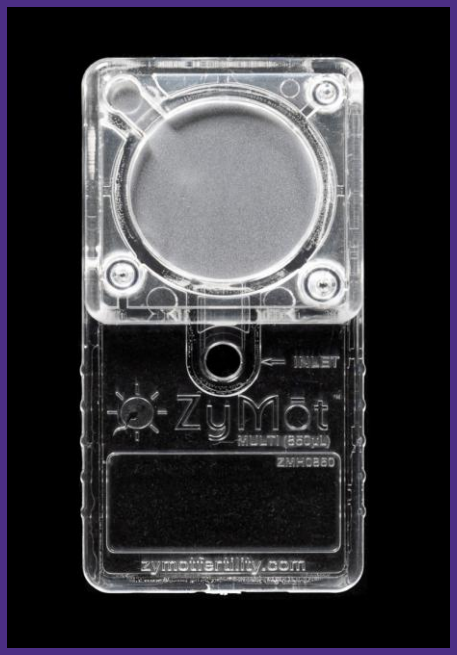
Sperm Separation and Purification Methods

How many centrifugations? What RCF?

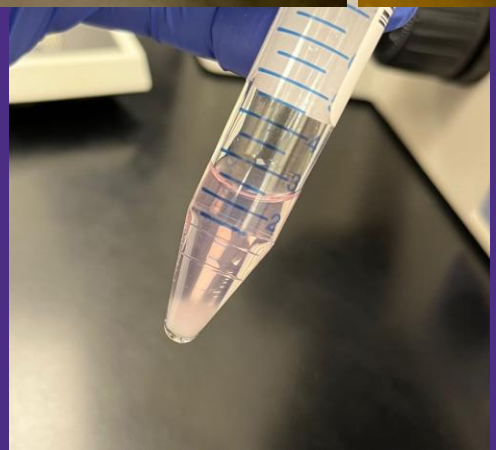
Density Gradients

40/80%

25/45/65/90%



Channels, Pores



Direct Swim Out

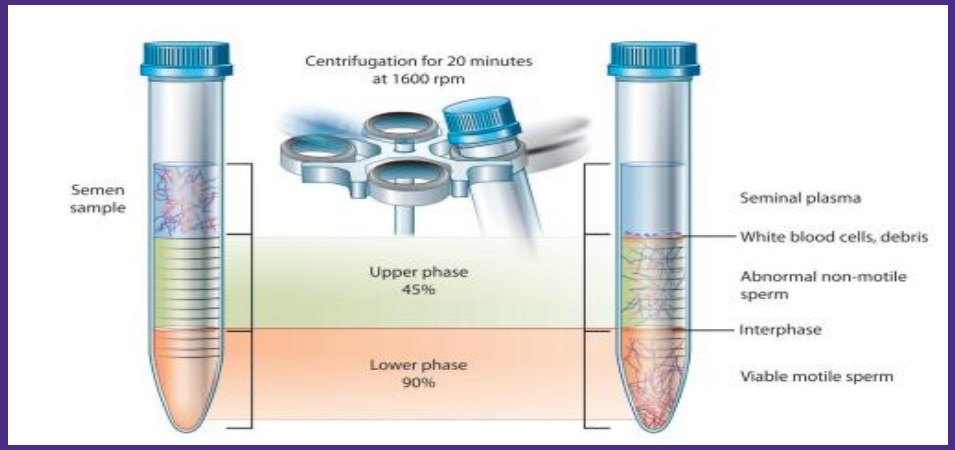
Centrifugation: A Necessary Evil?

At what force (xg) should we centrifuge sperm?

(Show of hands)

A. ≤ 300 RCF
B. 400-1,000 RCF
C. > 1,000 RCF

Don't confuse speed (RPM) with force (RCF, xg)!



Always use an aerosol cap on buckets!

Photo courtesy of Beydola T, Sharma RK, Agarwal A. 2013. "Sperm Preparation And Selection Techniques." In *Male Infertility Practice*, Chapter 29: pp. 244-251. Jaynee Brothers Medical Pubishers. New Delhi.

Does DNA Fragmentation Increase with Centrifugal Force?

Preliminary experiment: Student is repeating with more samples

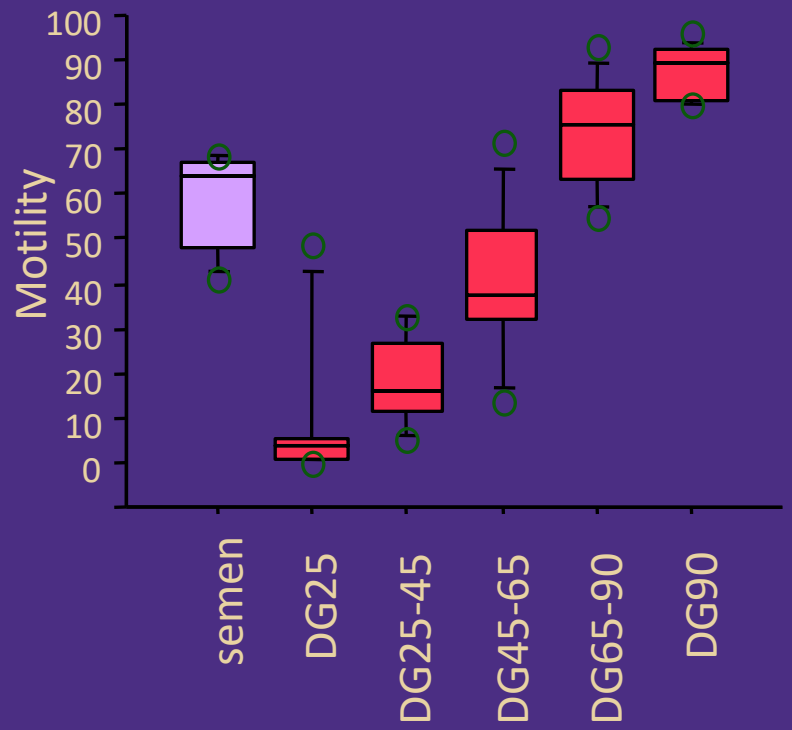
Observation: IVF Lab centrifuge made a hard sperm pellet. It was improperly set to 800xg (no digital readout of RCF). IVF case had good fertilization but poor blast survival.

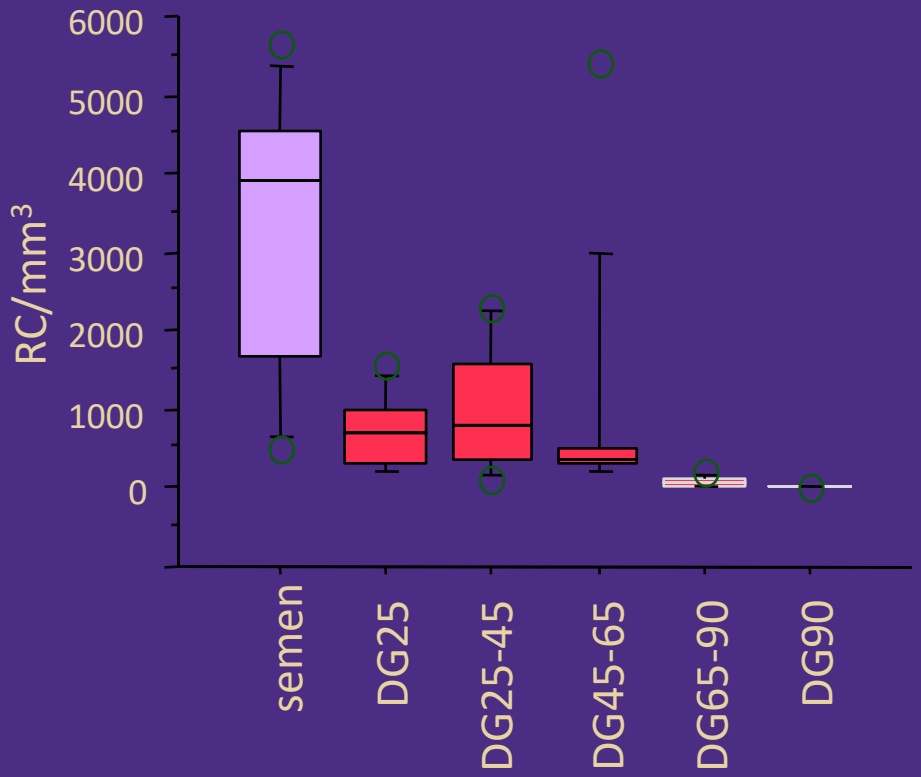
I ran DNA Fragmentation tests on patient's semen and hard pelleted sperm. Semen DFI: 9; Pellet DFI: 43 (Alkaline DNA Diffusion)

Experiment: Centrifuged semen on MultiStep Gradients at different RCF for 30 min, washed 1x 7 min at 250xg. Semen DFI = 8% Alkaline damage.

RCF	% Pr Motile	% Rap Lin	DFI
250xg	95	44	
600xg	96	45	
800xg	95	38	

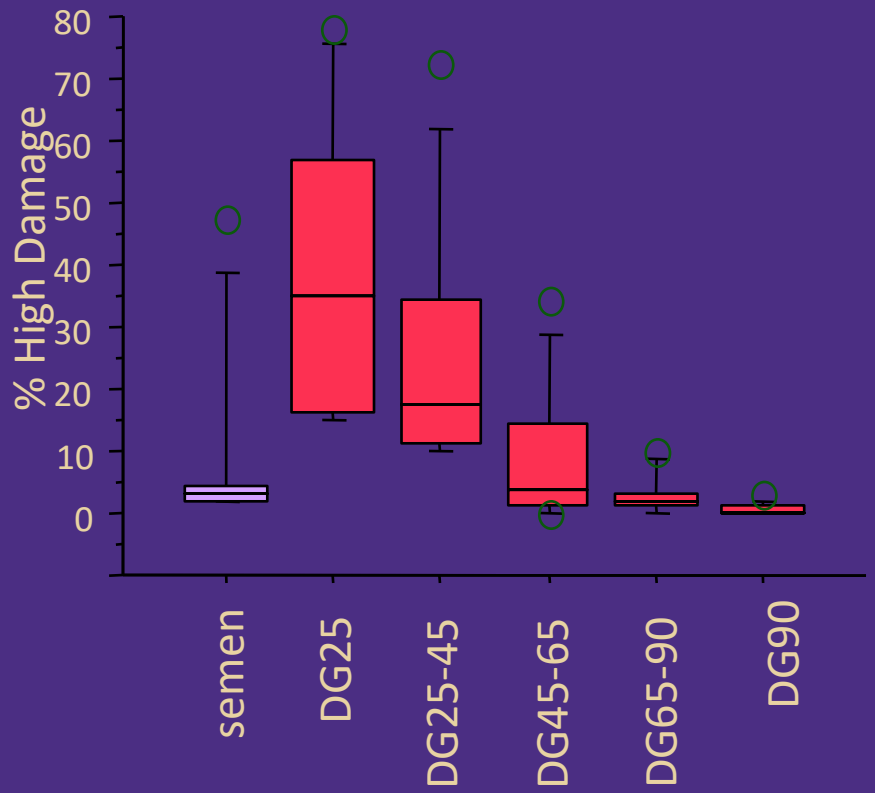
Purification of Sperm by Multi-Step Density Gradient

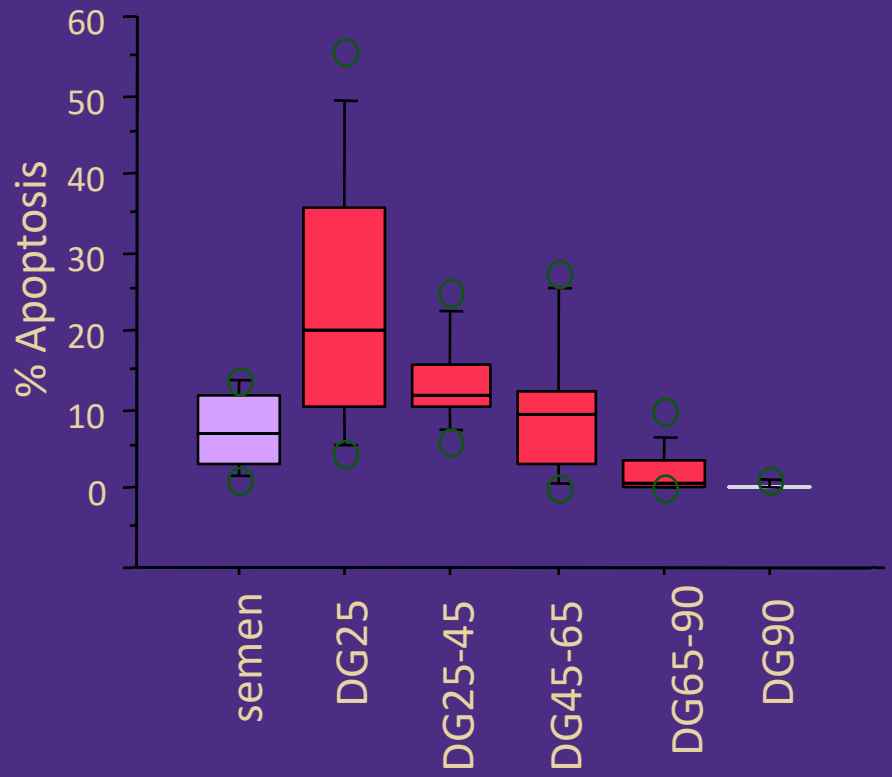




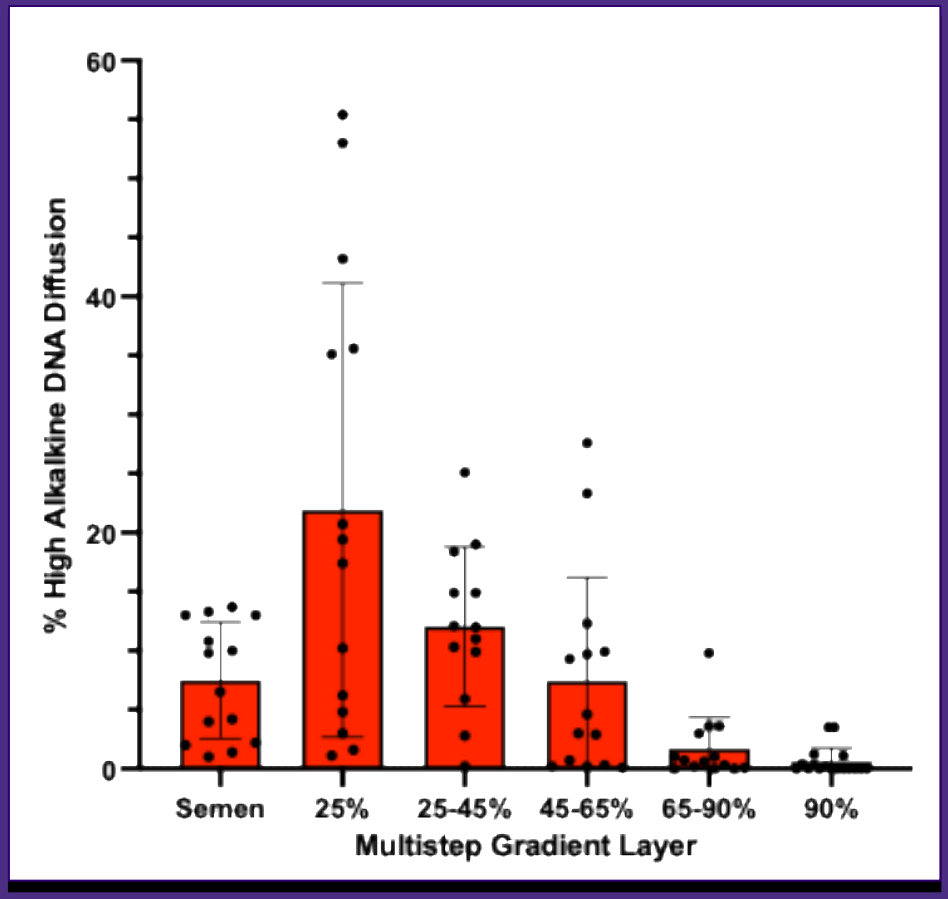
Dec 2007

Selection of Sperm with Low DNA Damage and Low Apoptosis





Alkaline Sperm DNA Damage in MSDG



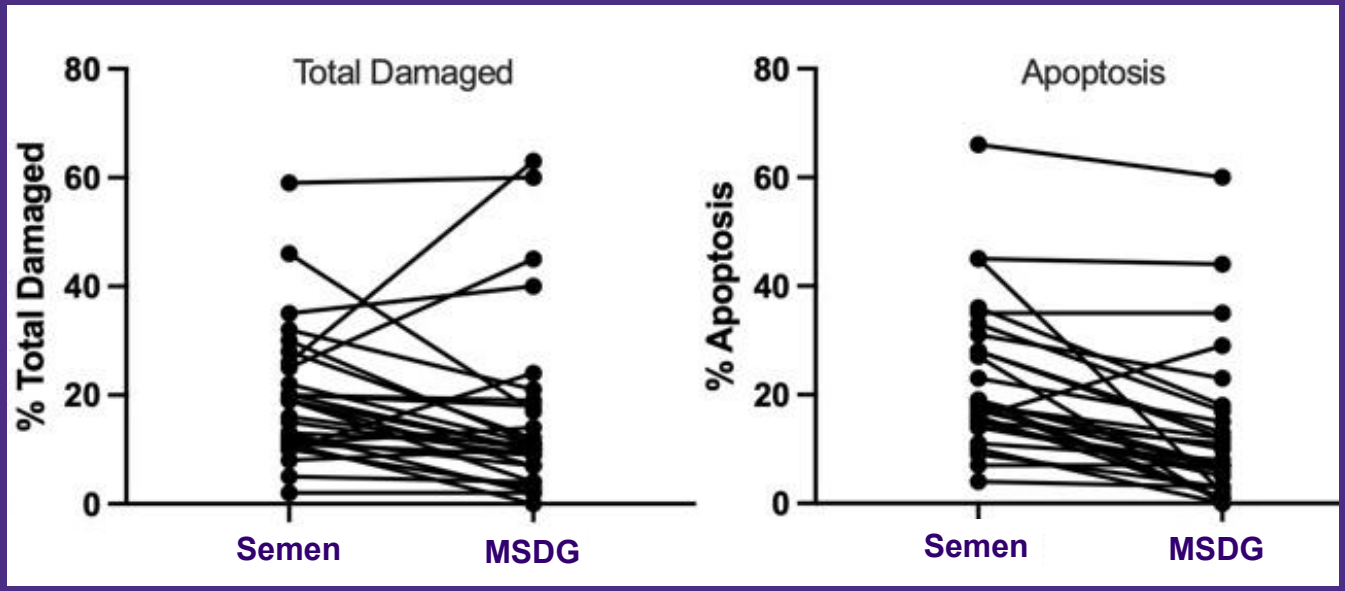
Optimizing Sperm Selection for DNA Integrity

28 Male patients with history of IVF failure and/or recurrent pregnancy loss

8 couples had IVF without, then with MSDG sperm prep:

Fert	72%	80%
Blast	28%	54%
Clin Preg	0%	25%

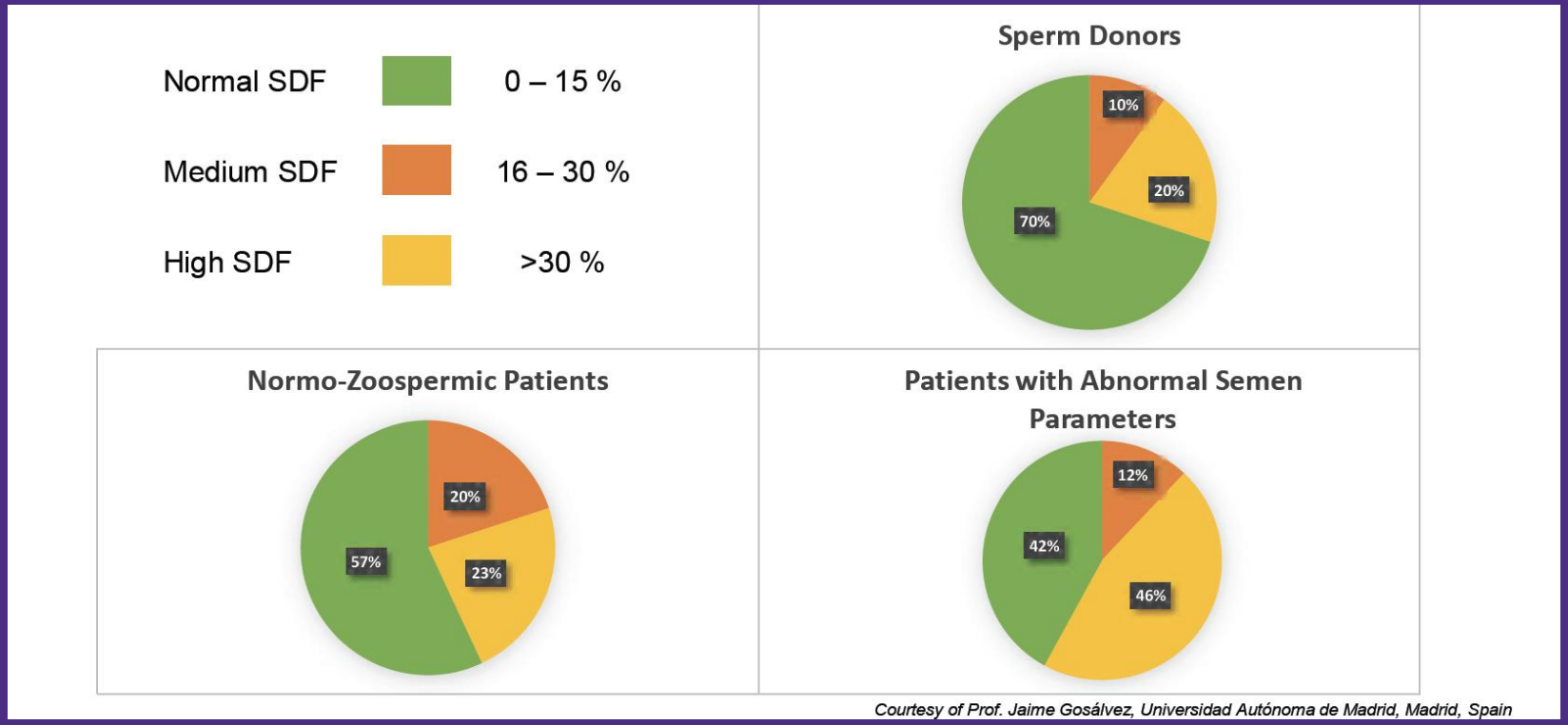
Unselected sperm vs. Multistep Density Gradient



Lindell, Muller, Nicholson et al. *in prep*

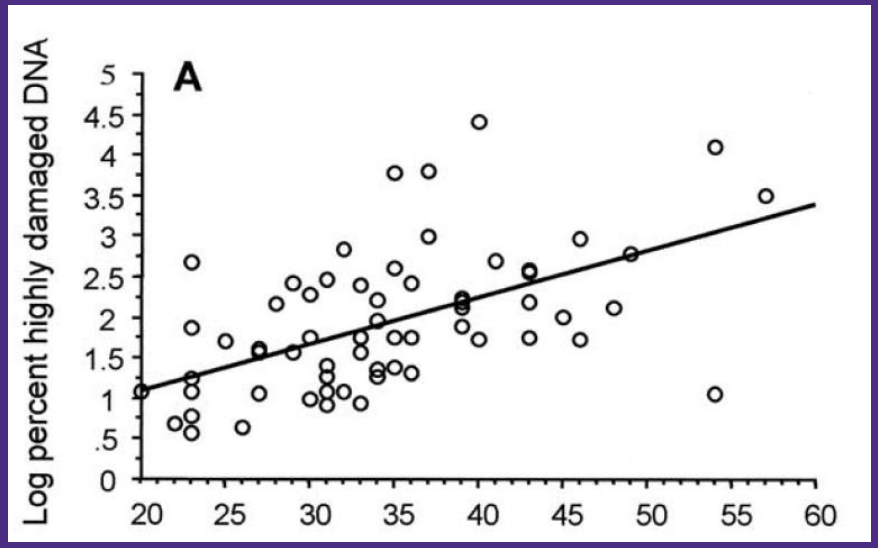
Wilcoxon signed rank test: p=0.030 p=0.0001

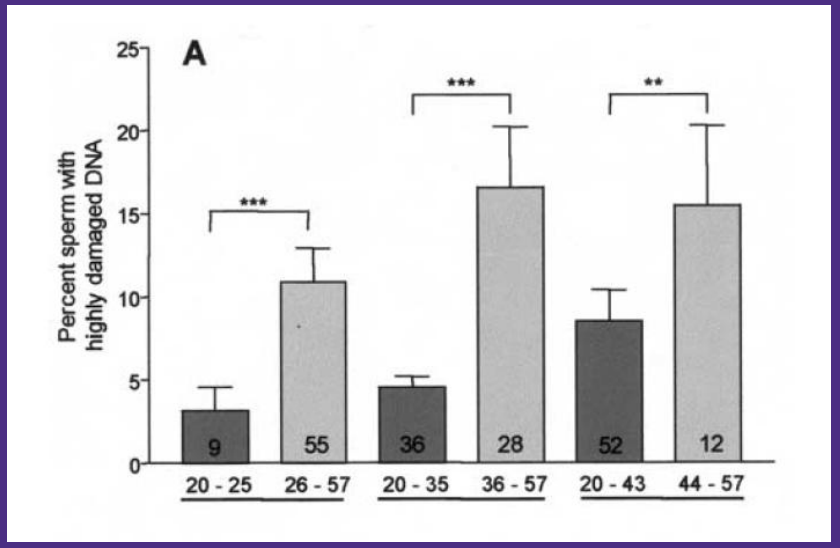
Prevalence of SDF (assessed by SCD)



Courtesy of Prof. Jaime Gosálvez, Universidad Autónoma de Madrid, Madrid, Spain

Sperm DNA Fragmentation increases with Age





Singh, Muller, Berger 2003

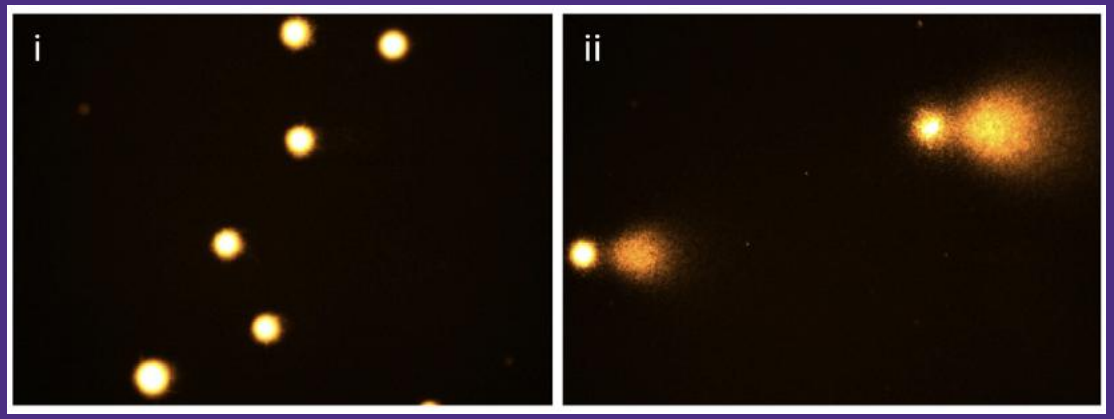
Is Sperm DNA predictive of recurrent pregnancy loss?

Drakeley et al. 2026 U Liverpool and Examen Ltd, Belfast RBMO

About 50% of RPL is of undetermined etiology, although female age, genetics, lipids and uterine factors account for most diagnoses.

100 men from couples with RPL, 81 sperm donor samples.

Neutral and Alkaline Comets performed at a commercial lab

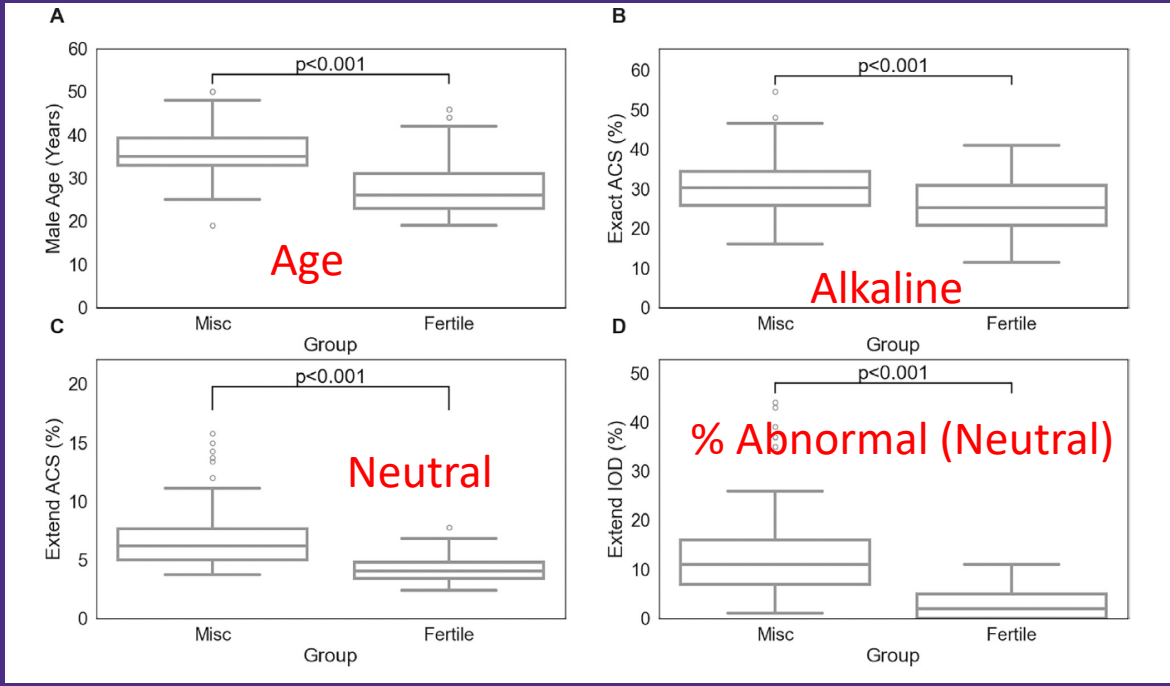


Normal Abnormal "Extend" (Neutral) Comet

Data:

- Amount of DNA in "tail"
- % Abnormal sperm

RPL and Comet (Drakeley et al. 2026)



Considerable overlap between groups even though statistically different.

<u>% Abn Neutral Comet</u>
Specificity = .78
Sensitivity = .84
Pos Pred Val = .79
corr for prevalence = 0.012

LR+ = 3.8
A man with an abnormal Neutral Comet is almost 4 times as likely to be in a couple with RPL than a man with a normal result.
Caveat: These calculations are based on selected fertile and RPL populations.

Sperm DNA Fragmentation

TAKE-HOME MESSAGES

Different types of DNA errors cause different types of breaks.

Different tests measure different aspects of DNA errors.

All tests have advantages and limitations.

Tests must be evaluated for validity, especially PPV and LR+.

DNA fragmentation is just one of many (hundreds?) of reasons for male infertility... (To say nothing of female infertility)…

Always remember:

KEEP SPERM HAPPY!!

Thank you!

Questions?

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