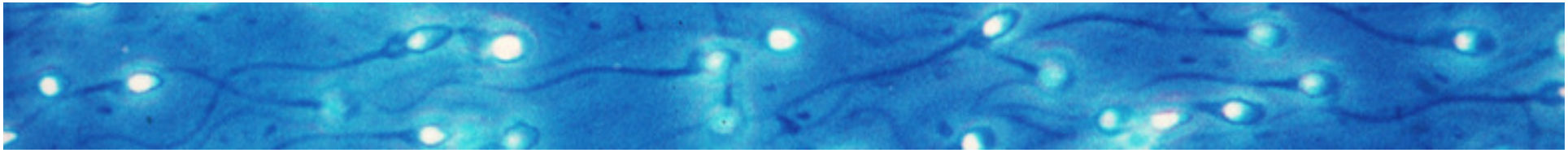

Why test for Oxidative Stress and DNA damage in sperm?

Sheena E. M. Lewis

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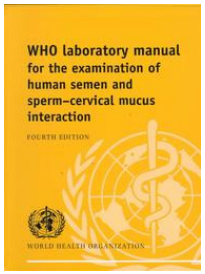
ESHRE Campus, Thessaloniki, 2009

Semen analysis

- **Essential for initial diagnosis**
- **Limited as prognostic tool for ART**
- **Only 20% of young Norwegian men achieve WHO values**
  Jorgensen et al, 2006
- **Only 46% of older men >45yrs (n=1174) meet all WHO values**
  Hellstrom et al, 2006
- **In infertility diagnosis-**
  Many men with 'below normal' values can be fertile
  Haugen et al, 2006
- **Men with 'normal' values can be infertile**
  Bonde et al, Lancet 1998



Clinical significance of semen profiles

No single parameter was diagnostic of infertility (n=1461)
Extensive overlap between fertile and infertile ranges
Morphology most powerful
Guzick et al, 2001

Morphology most powerful but volume and motility of limited value
Probability of pregnancy ↑ as concentration ↑ up to 40 x 10^6 /mL
then no further association (n=430)
extensive overlap between fertile and infertile ranges
Bonde, Skakkebaek et al, 1998

Concentration and motility were most powerful
Morphology poorest predictive power
- 50% of fertile men had abnormal morphology (n=719)
Nallella, Agarwal et al, 2006

243 fertile men had a mean of only 20% normal morphology
by WHO 1992 criteria
Chia et al, 1998

Regional and world- wide variation of semen parameters

- **Within USA, New York had highest concentrations (134 x 10^{6}/mL)**

  Iowa had lowest concentrations (48 x 10^{6}/mL)

  Thailand (52 x 10^{6} /mL)
- **In Japan, fertile men had lower semen quality, similar to Norway (20% < WHO)**
- **In Europe, Finland and Denmark's fertile men have markedly different semen profiles**

Fisch et al, 1996, Swan, 2006; Jorgensen et al, 2006; Iwamoto et al, 2006

Variability of semen parameters between and within individuals

- **Marked biological heterogeneity of semen between 243 fertile men**

  Chia et al, 1998

- **Even consecutive samples from same individuals** (twice a week for 120 weeks)

  WHO, 1990

  (673 samples from 7 men over 324 weeks)

  Mallidis et al,1991

Reference values have limited diagnostic value for infertility and are not predictive for ART

Intracytoplasmic sperm injection
ISCI- 1992

Success for men with poor semen quality

Only requirement is sperm viability

Natural barriers (poor motility or defective sperm zona binding) removed

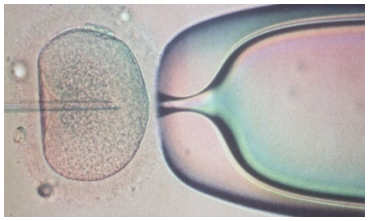
Usable with immature sperm

Pregnancy rates of 30-50%

The 'ISCI Escalation'-

almost twice as many cycles as IVF

-**reduction in andrological research**



ESHRE's European IVF Monitoring Consortium, 2008

Sperm DNA damage
Male Infertility

Environment

Endocrine disruptors

xenoestrogens

Anti-androgens

Toxic compounds

Occupation

Plastics and resins, solvents,
wood processing, metal industry,
Automobile, truck and aircraft mechanics
Sedentary or stressful job

Genetic Inheritance

CABVD
Robertsonian translocations
Y-chromosome deletions
Paternal Age

Lifestyle

diet
smoking
alcohol
recreational drugs
STDs
injury
infection

High levels of sperm DNA damage have some correlation with

- **Oligozoospermia**

  Irvine et al, 2000, Shayegon and Zini, 2002, Menezo et al, 2003, Schmid et al, 2003, O'Connell et al, 2003

- **Poor motility and morphology**

  O'Connell et al, 2003, Saleh et al, 2003

- **OAT**

  Gandini et al, 2000; Siddighi et al, 2004; Trisini et al, 2004; Huang et al, 2005, Appasamy et al, 2007

- **Cytoplasmic retention**

  Huszar et al, 2001, Aitken et al, 2006

- **mtDNA damage**

  O'Connell et al, 2003

DNA reproducibility compared to conventional parameters

- **DNA is more consistent than SA**

Schrader et al. 1988; Evenson et al. 1991; Zini et al. 2001; Loft et al. 2003

- **Sperm DNA has lower CV (20% cf >40%)**

Evenson et al, 1999,2000,2002, Zini et al, 2001; Loft et al, 2003
De Jonge et al, 2004

- **DNA has 'high monthly repeatability' within donors CV 10% cf 44% for conc, 78% for motility and 69% for morphology**

Evenson et al, 1991, Smit et al, 2007

Human Reproduction Vol.21, No.8 pp. 2061–2064, 2006
Advance Access publication May 9, 2006.
doi:10.1093/humrep/del134

Intra-individual variation in sperm chromatin structure assay parameters in men from infertile couples: clinical implications

J.Erenpreiss[1,2,5], M.Bungum[1,3], M.Spano[4], S.Elzanaty[1], J.Orbidans[2] and A.Giwercman[1]

- Retrospective study (n=282 consecutive patients)
- Attending for IUI, IVF or ICSI with 2-5 DNA tests
- Mean CV of DFI was 29%
- 37% (95% CI: 27%,49%) of patients with DFI>30% in 1st test had DFI<30% in 2nd test
- 27% (95%CI: 16%,40%) of patients with 21-30% DFI in 1st test had DFI>30% in 2nd test
- Intra- individual variation in DFI is significant
- Repeated DNA tests are necessary

Does Sperm DNA influence Fertility outcomes?

Nuclear DNA anomalies lead to:-

- **Failure of fertilization in IVF**

  Bianchi et al, 1993; Sun et al, 1997

- **Failure to implant in ICSI**

  Sakkas et al, 1996; Lopes et al, 1998

- **Increased time to conception**
- **Poor embryo development**

  Morris et al, 2002; Tomsu et al, 2002

- **Post-implantation loss and malformations**

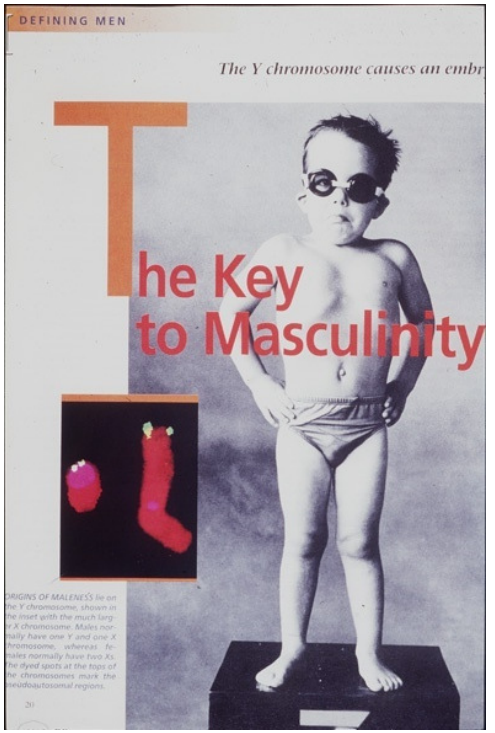
  Robaire et al, 1985

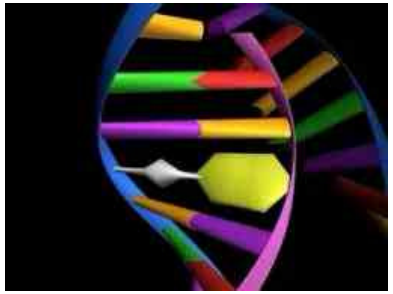
- **Increased miscarriage rate**

  Evenson et al, 1999; Carrell et al, 2003

- **Childhood cancers**

  Knight and Marrett, 1997





Sites and Causes of Sperm DNA Damage

Seminiferous tubules

Abortive apoptosis

Sakkas et al, 1999

Abnormal chromatin packaging

Manicardi et al., 1995, ;Carrell and Liu, 2001; Zhang et al, 2006

Epididymis

Incomplete repair of physiological nicks

Sakkas et al., 1999

Assault by senescent sperm and toxics

Hess, 1998; Moore, 1999

Aberrant SCF pathway

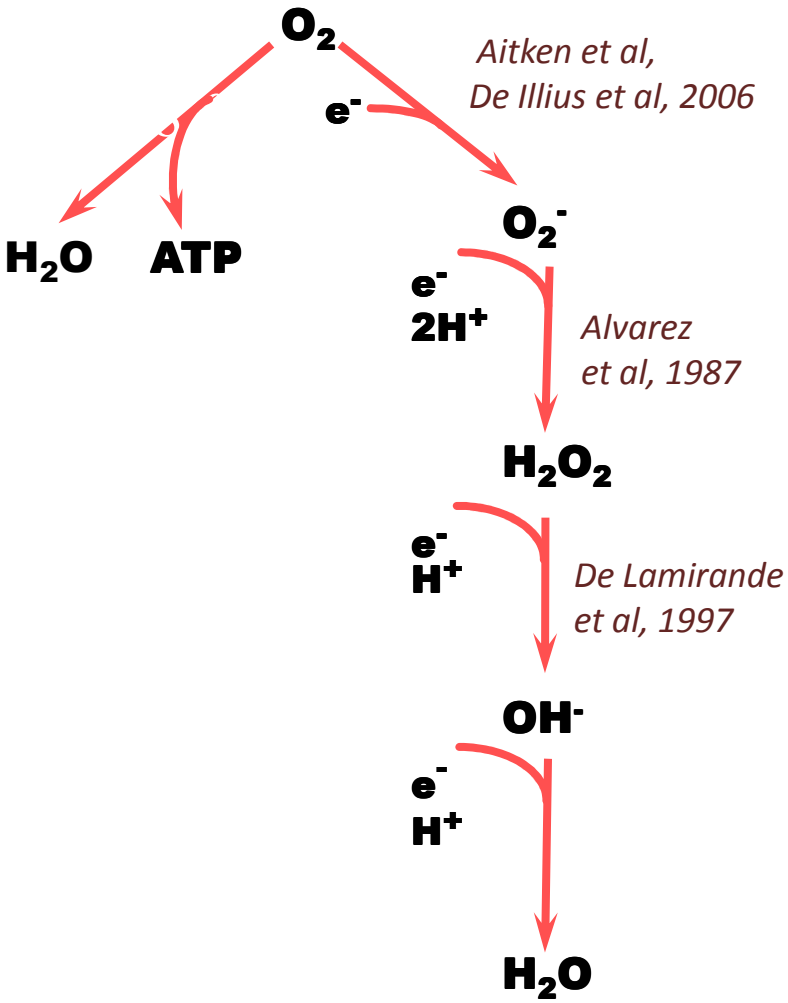
Shaman et al, 2007, Yamauchi et al, 2007

Post ejaculation

Clinical hazards imposed in ART labs

Oxidative Stress……..

Oxidative Stress and Fertility



Aitken et al, De Illius et al, 2006

Alvarez et al, 1987

De Lamirande et al, 1997

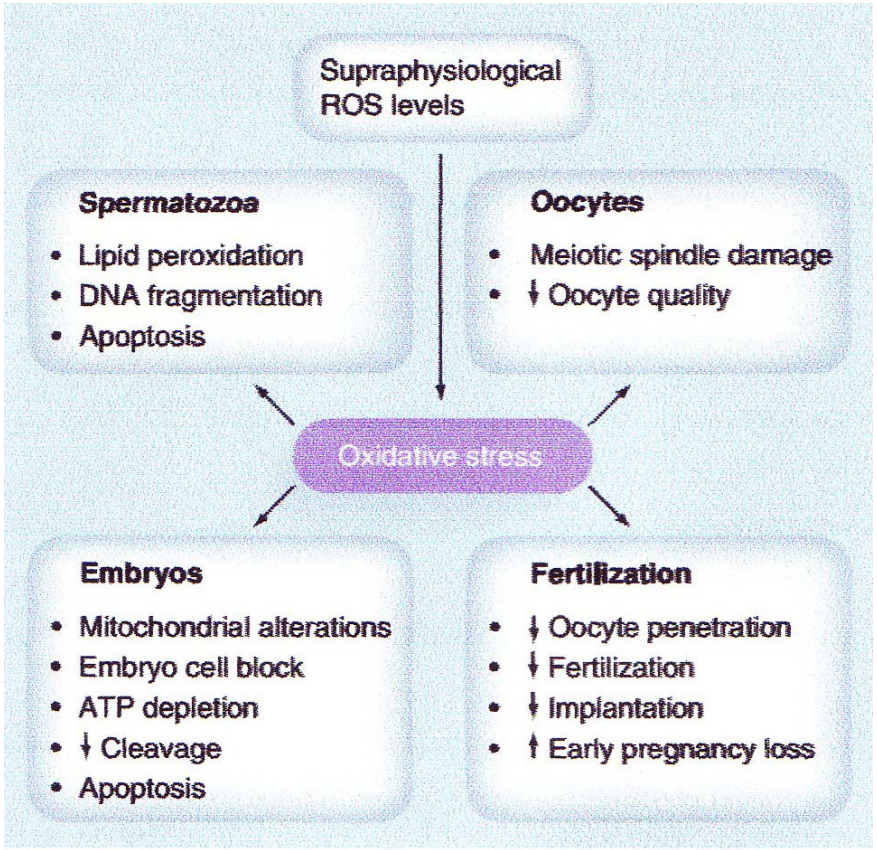
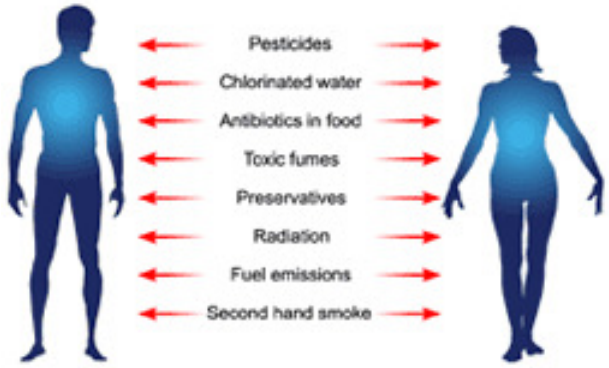
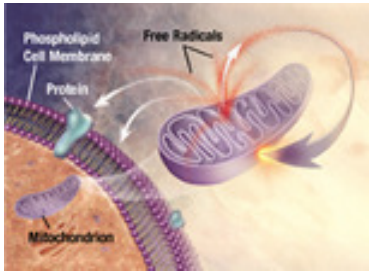


Figure 1. Effects of pathological levels of reactive oxygen species during IVF.
ROS: Reactive oxygen species.

Du Plessis et al, Expert Reviews, 2009

Oxidative Stress is a major cause of DNA damage





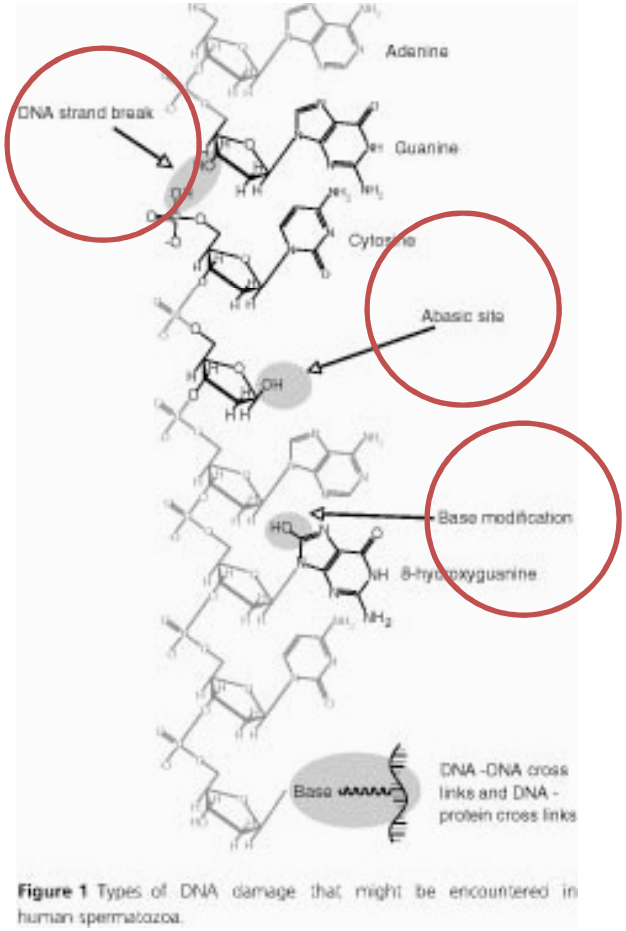
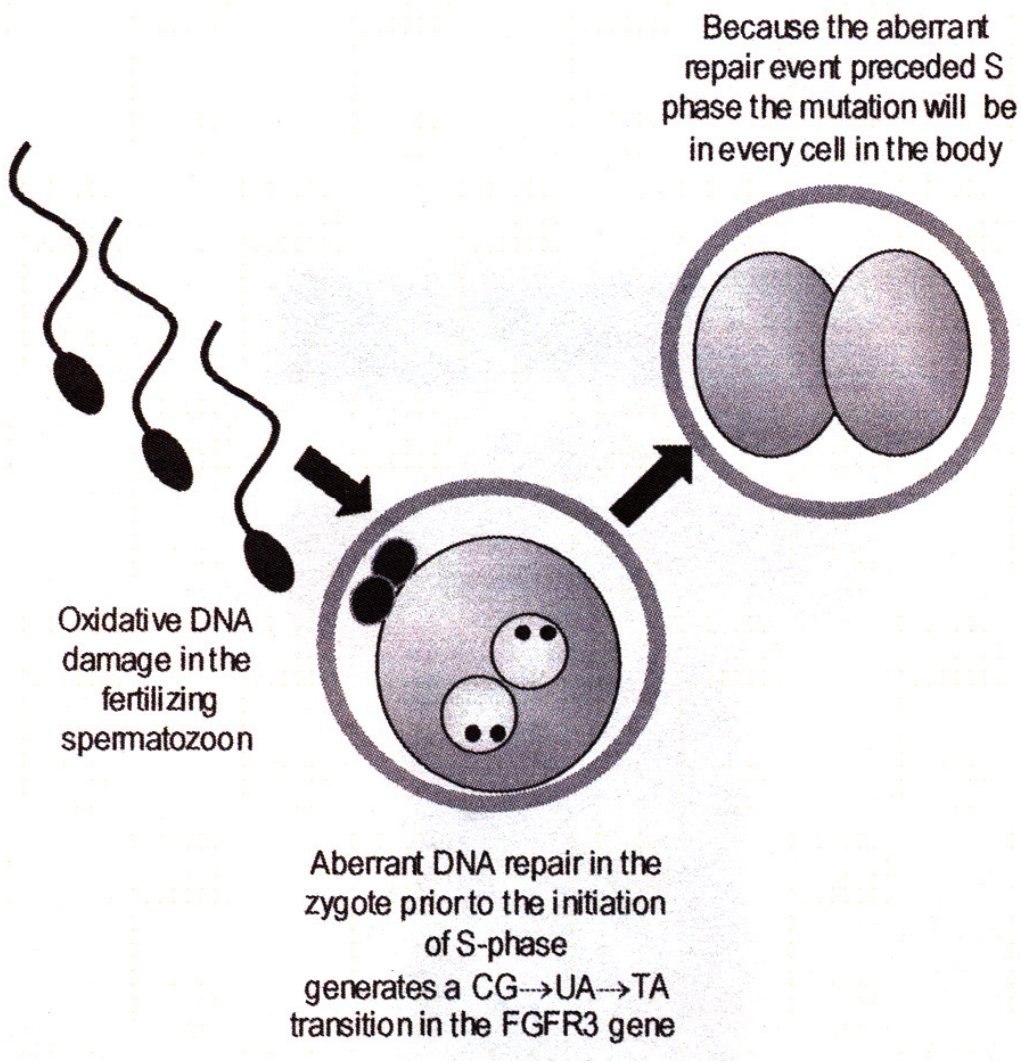


Figure 1 Types of DNA damage that might be encountered in human spermatozoa.

Sharma & Agarwal, 1996; Aitken & Krausz, 2001, Agarwal & Said, 2005; Lewis & Aitken, 2005, Peris et al, 2007;Lewis et al, 2008

Aitken and De Illius, 2009

Implications of sperm DNA



Aitken and de Iulius, 2007

Risk of Diseases in Offspring from Damaged Sperm DNA

- **Sperm DNA damage increases with ♂ Age**

  Singh et al, 2003; Wyrobek et al, 2006; Aitken and de Iulius, 2007

- **Oxidative damage increases with Age**
- **↑ ♂ age is associated with ↑ incidence of disease**

  -miscarriage *de Rochebrochard and Thonneau, 2002*

- **dominant genetic mutations-Achondroplasia and Apert Syndrome**

  Crow, 2000; Wyrobek et al, 2006

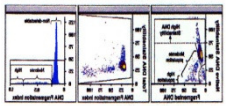
- **neurological Disorders -Schizophrenia , Autism and Bipolar Disease**

  Sipos et al, 2004; Frans et al, 2008

- **Birth defects- neural tube defects and even Downs Syndrome**

  McIntosh et al, 1995

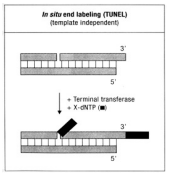
Methodologies to Evaluate Sperm DNA Damage



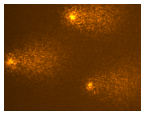
Strand breaks

- **Sperm Chromatin Structure Assay (SCSA)**

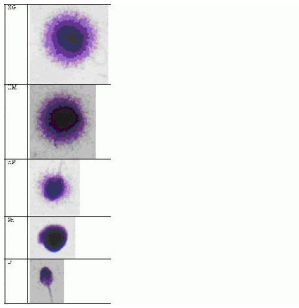
- **TUNEL**



- **Single-cell gel electrophoresis assay(Comet)**



- **Sperm Chromatin Dispersion Test (SCD)**



Chromatin packaging defects

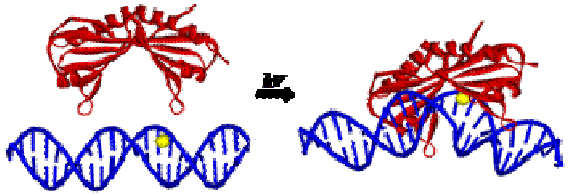
- **Acid Aniline blue**

- **Chromomycin A3**

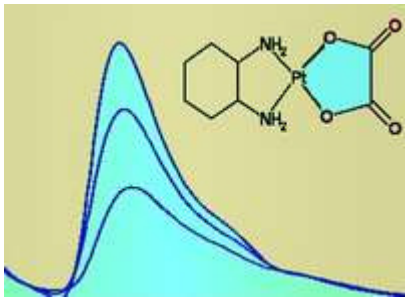
Novel tests- for biomarkers of OS in DNA

8-Hydroxy-2'-deoxyguanosine (8-OH2dG) - the most abundant DNA adduct

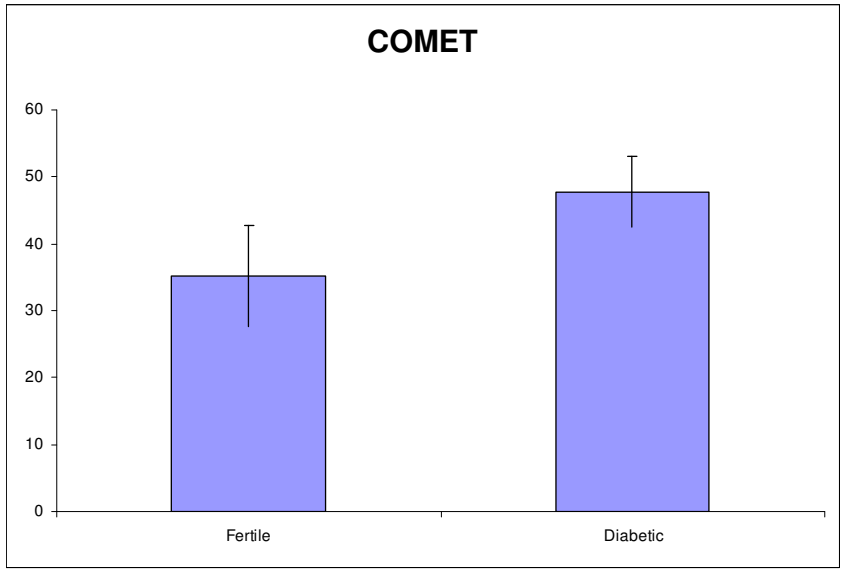
- In sperm, no repair and little antioxidant protection
- DNA exposed to ROS→ DNA adducts

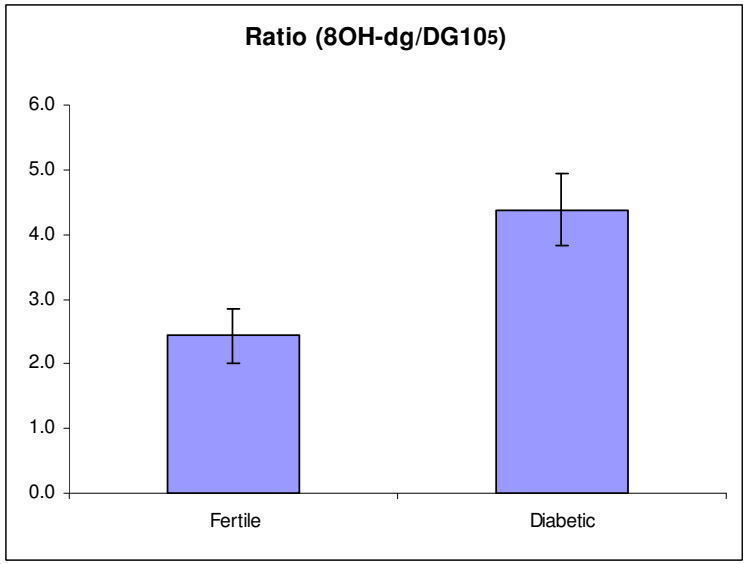


- Adducts are highly mutagenic
- 8-OH2dG can lead to a GC to TA transversion
- valuable biomarker of sperm health
- High Performance Liquid Chromatography



DNA damage caused by OS





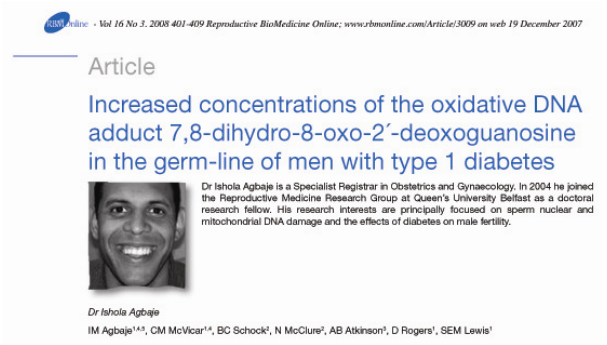
Strand breaks

oxidised bases

Subjects

Type 1 diabetics (n=27)

Non diabetics attending for investigation of infertility (n=29)


Vol 16 No 3. 2008 401-409 Reproductive BioMedicine Online; www.rbmonline.com/Article/3009 on web 19 December 2007

Article

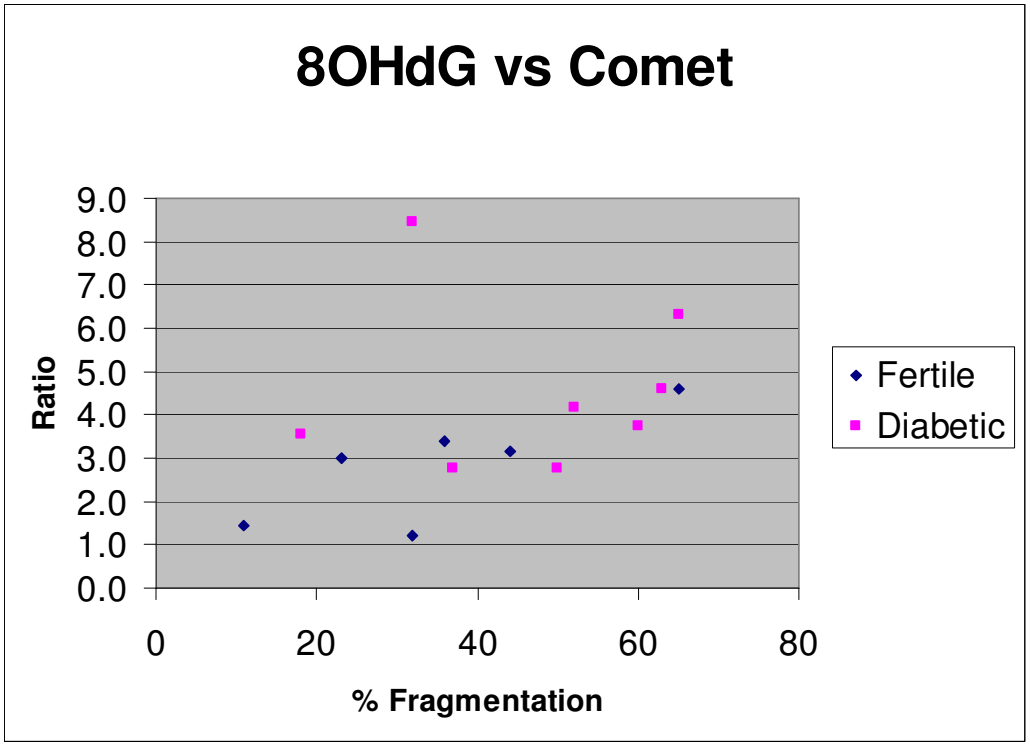
Increased concentrations of the oxidative DNA adduct 7,8-dihydro-8-oxo-2′-deoxoguanosine in the germ-line of men with type 1 diabetes

Dr Ishola Agbaje is a Specialist Registrar in Obstetrics and Gynaecology. In 2004 he joined the Reproductive Medicine Research Group at Queen's University Belfast as a doctoral research fellow. His research interests are principally focused on sperm nuclear and mitochondrial DNA damage and the effects of diabetes on male fertility.

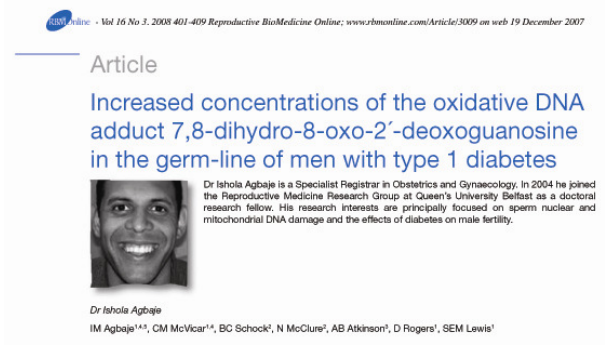
Dr Ishola Agbaje

IM Agbaje[1,4,5], CM McVicar[1,4], BC Schock[2], N McClure[2], AB Atkinson[3], D Rogers[1], SEM Lewis[1]

DNA damage caused by OS



R^2= 0.54



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Human Reproduction, Vol.24, No.9 pp. 2061–2070, 2009
Advanced Access publication on June 12, 2009 doi:10.1093/humrep/dep214

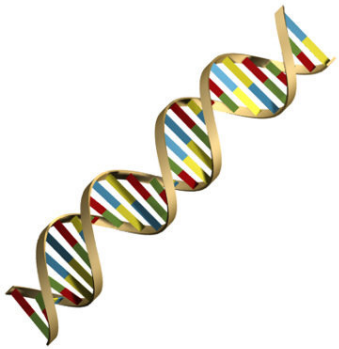
human reproduction ORIGINAL ARTICLE *Andrology*

Cryopreservation-induced human sperm DNA damage is predominantly mediated by oxidative stress rather than apoptosis

L.K. Thomson[1,4], S.D. Fleming[2], R.J. Aitken[3], G.N. De Iuliis[3], J.-A. Zieschang[1], and A.M. Clark[1]

Sperm DNA fragmentation and and 8OHdG	r	p
Native semen	0.756	<0.001
Post DCG	0.568	<0.001

Are sperm DNA tests useful as diagnostic or prognostic clinical tests?



For a test to be useful, it must have strong predictive capacity for pregnancy outcome and little overlap between fertile and infertile samples

IN VITRO FERTILIZATION

Do sperm DNA integrity tests predict pregnancy with in vitro fertilization?

John A. Collins, M.D.,[a] Kurt T. Barnhart, M.D.,[b] and Peter N. Schlegel, M.D.[c]

Journal of Andrology, Vol. 30, No. 3, May/June 2009

Are Tests of Sperm DNA Damage Clinically Useful? Pros and Cons

Review

ARMAND ZINI* AND MARK SIGMAN

(Diagnostic) Odds Ratios

An Odds Ratio gives us the chance of a pregnancy occurring if the test result is above our specified threshold

Odds ratios need to be > 2.0 to be useful
If CIs include 1.0, relationship is usually NS

Sensitivity- 1.00, if DNA damage above threshold prevents achievement of pregnancy in all cases

Specificity-1.00 , if all samples with DNA damage below threshold achieve pregnancy so their sum should approach 2.0

If Sensitivity plus Specificity >1.0, ORs are generally significant

TABLE 2

Diagnostic test properties: studies of the association between sperm DNA fragmentation and pregnancy.

Study	Treatment	Sens	Spec	Sens + Spec	Abnormal tests (%)	DOR	(95% CI)
Boe-Hanson et al., 2006 (46)	IVF	0.06	0.97	1.03	5	2.04	(0.38, 11.0)
	ICSI	0.36	0.57	0.94	38	0.76	(0.21, 2.73)
Borini et al., 2006 (52)	IVF	0.17	0.89	1.06	16	1.57	(0.38, 6.51)
	ICSI	0.71	0.75	1.46	60	6.55	(1.77, 24.3)
Bungum et al., 2004 (26)	IVF	0.17	0.85	1.02	16	1.16	(0.64, 2.12)
	ICSI	0.30	0.63	0.93	33	0.74	(0.42, 1.31)
Check et al., 2005 (47)	IVF	0.30	0.83	1.13	27	1.90	(0.61, 5.89)
Gandini et al., 2004 (48)	ICSI	0.38	0.44	0.83	45	0.52	(0.10, 2.74)
Host et al., 2000 (53)	IVF	0.34	0.80	1.14	30	1.91	(0.93, 3.91)
	ICSI	0.58	0.38	0.96	59	0.84	(0.29, 2.43)
Huang et al., 2005 (54)	IVF	0.22	0.83	1.04	19	1.30	(0.66, 2.56)
	ICSI	0.64	0.50	1.14	57	1.78	(0.76, 4.16)
Larson et al., 2000 (24)	IVF, ICSI	0.58	0.94	1.59	42	10.17	(1.77, 58.4)
Larson-Cook et al., 2003 (25)	IVF, ICSI	0.17	0.98	1.16	11	5.08	(1.24, 20.8)
Payne et al., 2005 (49)	IVF, ICSI	0.16	0.71	0.87	20	0.44	(0.15, 1.27)
Sell et al., 2004 (14)	IVF, ICSI	0.46	0.61	1.07	43	1.32	(0.43, 4.07)
Virro et al., 2004 (50)	IVF, ICSI	0.35	0.81	1.17	29	2.27	(1.30, 3.96)
Zini et al., 2005 (51)	ICSI	0.17	0.81	0.98	18	0.87	(0.24, 3.19)

Note: CI, confidence interval; DOR, diagnostic odds ratio; Sens, sensitivity; Spec, specificity.

Collins. Sperm DNA integrity tests. Fertil Steril 2008.

Sperm DNA Damage and IUI Outcomes

Author	Assay	n	Design	Threshold (%)	< Threshold Pregnancy (%)	> Threshold Pregnancy (%)	Pregnancy	OR	95%CI	P
Duran '02	TUNEL	154	prosp	4	NA	NA	13/154			
Muriel '06	SCD	100	prosp							
Bungum '07	SCSA	387	prosp	30	23.7	3.0	78/381	9.9	2.37,41.51	<0.001

Very useful test for IUI

Sperm DNA Damage and IVF Outcomes

Author	n	design	Female sel	Assay	Threshold (%)	< Preg (%)	> Preg (%)	Fert	Preg	OR	CI
Filatove ’99	176	-	none	Chromatin compaction	50	23	6	0	↓	6.33	1.82,22.08
Host ’00	175	Pro	none	TUNEL	4	NA	NA	↓	↓	1.92	0.92,4.04
Tomlinson ’01	140	-	none	ISNT	-	NA	NA	0	↓		
Tomsu ’02	40	Pro	<40	COMET	-	NA	NA	0	↓		
Morris ’02	20	Retro	<40	COMET	-	NA	NA	0	0		
Henkel ’03	208	Pro	None	TUNEL	37	34.7	18.7	0	0	2.24	1.09,4.58
Gandini ’04	12	Pro	None	SCSA	27	25	0	0	0		
Huang ’05	217	Retro	None	TUNEL	10	56.8	51.7	↓	0	1.30	0.66,2.56
Boe- Hansen ’06	139	Pro		SCSA	27	29	14.3		↓	2.43	0.28,20.83
Borini ’06	83	-	None	TUNEL	10	23.2	15.4	↓	↓	1.66	0.33,8.28
Bakos ‘07	45	-	None	TUNEL	-	NA	NA	↓	↓		
Benchaib ’07	84	pro	<40	SCSA	15	29	25	0	↓	0.46	0.11,2.00
Bungum ‘07	388	pro	<40	SCSA	30	33.7	29	0	↓	1.24	0.69,2.26
Frydman ‘07	117	pro	<40	TUNEL	35	57.8	23.5	0	↓	2.97	1.39,6.32
Lin ‘07	117	pro	<40	SCSA	27	51.3	54.4	0	↓	0.88	0.35,2.19

So is DNA damage a useful test for IVF?

- Combined odds ratio 1.67 for no pregnancy with high DNA damage (1.27-2.20) $p<0.01$
- Positive predictive value 74% but wrongly predicts failure in 26%

Collins et al, 2008; Zini et al, 2009

Sperm DNA Damage and ICSI Outcomes

Author	n	design	assay	Threshold (%)	< Preg (%)	> Preg (%)	Fert	Preg	OR	CI
Hammadeh ’96	61	Pro	A-Blue	29	18.5	35.3	0	↓	2.40	0.72,7.96
Host ’00	61	Pro	TUNEL	4	NA	NA	0	0	0.79	0.28,2.25
Virant-Klun ’02	183	Pro	AO	56	--	--	↓	0		
Morris ’02	40	Pro	COMET	-	NA	NA	0	0		
Henkel ’03	54	Retro	TUNEL	24	48	22.2	0	0	3.67	1.12,12.0
Gandini ’04	22	Pro	SCSA	30	44.4	55.6	0	0	0.36	0.06,2.08
Huang ’05	86	Retro	TUNEL	4	59.5	33.3	↓	0	1.80	0.76,4.27
Check ’05	104	-	SCSA	30	--	--	-	0	1.34	0.52,3.43
Zini ‘05	60	Pro	SCSA	30	51	55	0	0	0.87	0.23,3.22
Boe-Hansen ’06	47	Pro	SCSA	27	27.6	33.3		0	0.76	0.21,2.72
Borini ’06	50	-	TUNEL	10	45	10	0	↓	7.36	1.67,32.4
Muriel ’06	85	Pro	SCD	-	NA	NA	↓	0		
Benchaib ’07	218	pro	TUNEL	15	37.4	27.8	0	↓	1.55	0.70,3.41
Bungum ’07	223	Pro, consec	SCSA	30	37.3	47.9	0	0	0.65	0.37,1.14
Lin ’07	86	pro	SCSA	27	52.3	47.6	0	0	1.21	0.45,3.23
Bakos ‘07	68	-	TUNEL	35	NA	NA	0	↓		

Combined Odds ratio=1.20 (0.91,1.59)
p>0.05

so there is no clinical application as sperm DNA damage does not affect pregnancy rates after ICSI

- ISCI appears to bypass poor sperm DNA too

Zini et al, 2009

Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

Author	ART	n	Threshold	< Preg loss (%)	> Preg loss (%)	Preg loss (%)	Risk	OR	CI
Virro ’04	IVF and ICSI		30%	NA	NA				
Check ’05	ISCI	104		--	--	47	↑	2.27	0.45,1.59
Zini ’05	ISCI	60	30%	12	33	16	↑	3.67	0.46,29.42
Borini ’06	IVF	82	10%	15.8	50	6	↑	32.0	0.62,1663
Borini ’06	ICSI	50	10%	0	62.5	25	↑	108.0	1.73,6729
Benchaib ’07	IVF	84	30%	2.6	25	13	↑	10.0	0.87,114.8
Benchaib ‘07	ICSI	218	30%	2.8	8.3	13	↑	3.51	0.89,23.28
Lin ’07	ISCI	137	27%	11.8	40	12	↑	2.56	0.44,15.03
Lin ’07	IVF	86	27%	8.5	16.7	12	↑	5.00	0.97,25.77
Frydman ’07	ISCI	117	35%	10	36.8	19	↑	5.25	1.31,21.11
Bungum ’07	IVF	388	30%	24.4	19	22	0	0.73	0.23,233
Bungum ‘07	ICSI	223	30%	15.6	23.8	22	↑	1.69	0.63,4.49

So is DNA damage a useful test for predicting pregnancy loss?

- Combined odds ratio 2.48 (1.52-4.04) $p<0.0001$
- Positive predictive value of loss of 37% (high DNA damage) or 10% (low DNA damage) with sensitivity of 0.4
- However, 67% of couples with high DNA damage had normal offspring

Zini et al, 2009

Ito summarise the relationship between sperm DNA damage and pregnancy

in IUI: strong negative effect (OR=9.9)

in IVF : mild negative effect (OR=1.7)

in ISCI: no effect (OR=1.2)

Thus

↑Intervention from IUI to IVF to ICSI, the less impact sperm DNA damage has on early fertility check points

BUT in IVF and ICSI pregnancy loss: DNA damage has a moderate positive effect (OR=2.5)

ie an effect on fetal development

Systematic review and meta- analysis by Zini et al, 2008

Are we expecting too much from one test?

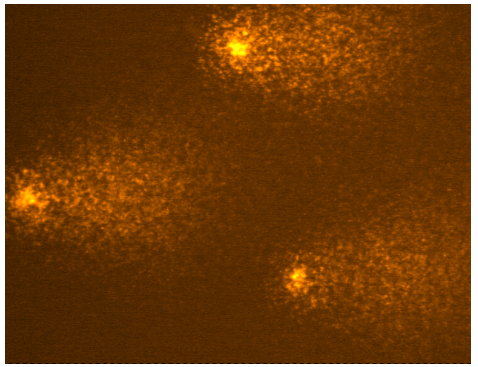
Other factors with important roles-

- **Sperm function**
- **Oocyte quality**
- **Embryo quality**
- **Uterine competence**
- **ORs are based on thresholds-**

  -how accurate are they?

Single Cell Gel Electrophoresis

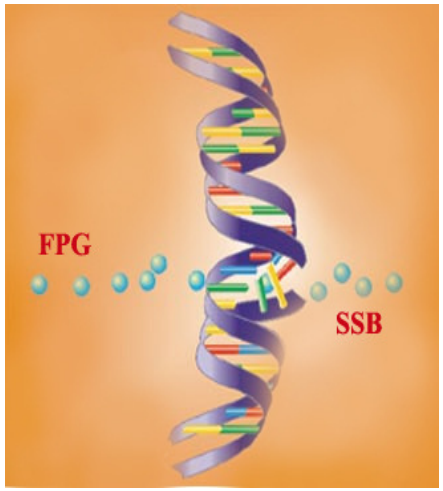
Comet assay



- more sensitive- detecting just 50 SSB/cell
- Inexpensive
- reproducible
- Requires low no of sperm(60,000/slide)
- Measure SSB + DSB and alkali labile sites

Another test for of DNA adducts

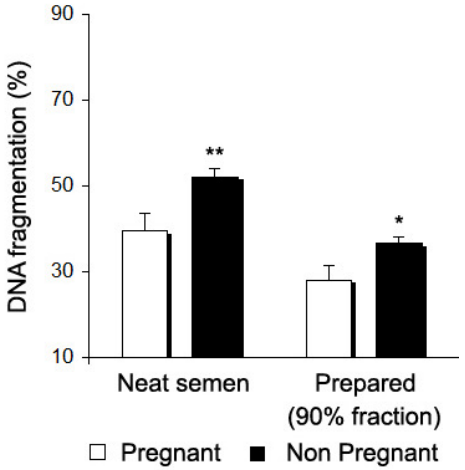
- **Formamidopyrimidine-DNA glycosylase; FPG**
- **converts 8OHdG to single strand breaks**
- **They can then be measured by Comet assay**

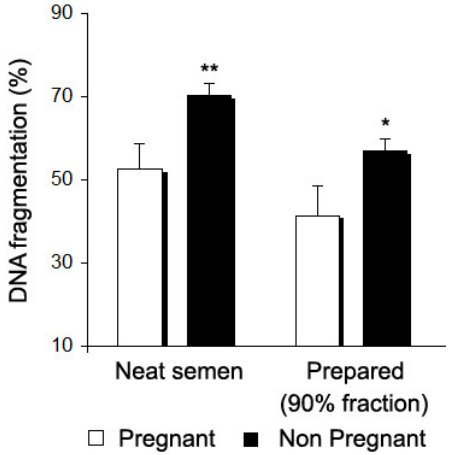


FPG extract kindly donated by Gunnar Brunborg, Institute of Public Health, Oslo, Norway

Relationship between sperm DNA fragmentation and pregnancy rates in IVF

Assay	Sample	n	ROC	CI	P
Comet	Native	146	0.649	0.57-0.79	0.013
	DCG	149	0.634	0.54-0.75	0.025
Comet + FPG	Native	64	0.698	0.60-0.91	0.024
	DCG	63	0.697	0.53-0.87	0.029

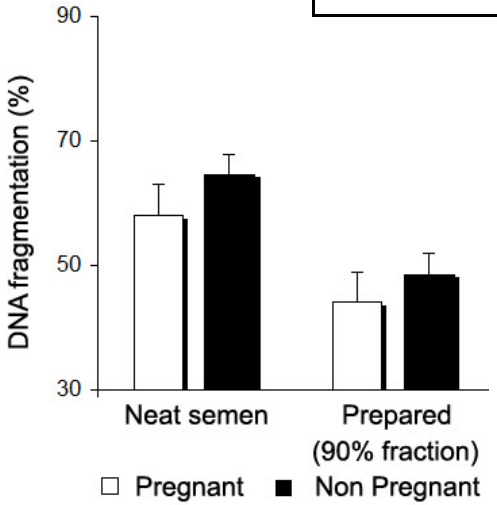


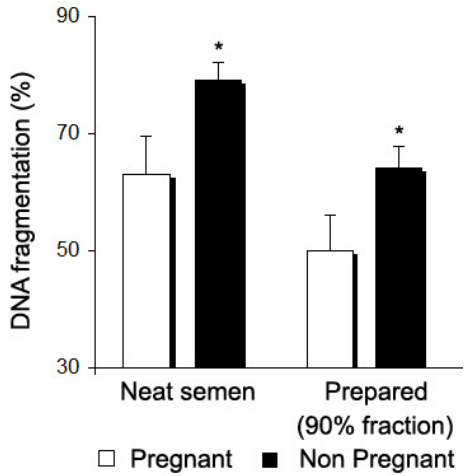


- **Native semen – 39.6 v 52.3 %**
- **DGC sperm – 28.0 v 36.5%**
- **Potential breaks constitute additional 12 – 20 %**
- **Adducts present in both native and DGC sperm**
- **No pregnancies when DNA damage > 48/62 %**

Relationship between sperm DNA fragmentation and pregnancy rates in ISCI

Assay	Sample	n	ROC	CI	P
Comet	Native	90	0.637	0.46-0.72	0.117
	DCG	89	0.553	0.43-0.69	0.271
Comet + FPG	Native	51	0.686	0.51-0.86	0.042
	DCG	51	0.702	0.53-0.87	0.027





- **No relationship between Comet and pregnancy**
- **Significant rel between Comet plus adducts and pregnancy**

Clinical significance of Comet using thresholds for native and DGC sperm in IVF & ISCI

Native	IVF			ICSI		
	<62%	>62%	OR (CI)	<62%	>62%	OR (CI)
Cycles started	114	35	--	43	47	--
Clinical pregnancies	25 (80.7%)	4 (36.4%)	3.54 (1.07-12.89)	16 (88.9%)	12 (54.6%)	1.73 (0.64-4.70)
Deliveries to date	17 (68.0%)	2 (50.0%)	5.46 (0.86-44.04)	8 (50.0%)	8 (66.7%)	1.40 (0.33-6.07)
Early pregnancy loss	3 (12.0%)	1 (25.0%)	2.44 (0.00-50.80)	5 (31.3%)	2 (16.7%)	2.27 (6.28-22.03)

DGC	IVF			ICSI		
	<48%	>48%	OR (CI)	<48%	>48%	OR (CI)
Cycles started	114	35	--	51	39	--
Clinical pregnancies	26 (74.3%)	3 (37.5%)	4.97 (1.06-32.03)	19 (86.4%)	9 (50.0%)	1.98 (0.71-5.62)
Deliveries to date	18 (69.2%)	1 (33.3%)	7.41 (0.80-177.8)	10 (45.5%)	6 (66.7%)	1.67 (0.40-7.40)
Early pregnancy loss	3 (11.5%)	1 (33.3%)	6.00 (0.0-328.36)	5 (26.3%)	2 (22.2%)	1.25 (0.14-12.40)

Strategies to Reduce Oxidative Stress

Antioxidant treatment

- **$ZnS0_4$/ folic acid and semen quality**

  Wong et al, 2002

- **Zn and Selenium and DNA quality**

  Menezo et al, 2007

- **Vit C and E and ICSI outcome**

  Rolf et al,, 1999; Greco et al, 2005

- **Menovit and IVF/ICSI outcome**

  Tremellen et al, 2007

Sperm DNA: organisation, protection and vulnerability – from basic science to clinical application

ESHRE Campus symposium

Stockholm, Sweden

21-22 May 2009

Organised by the ESHRE Special Interest Group “Andrology” in collaboration with the Karolinska Institutet (Centre for Andrology and Sexual Medicine, Department of Medicine, Huddinge, Stockholm, Sweden) with support from the Swedish Research Council (Vetenskaprådet).

Consensus document:

edited by Chris Barratt

Recommendations from Consensus Document

1. **Fundamental research is urgently required**
2. **Standardization of clinical assays**
3. **Animal Models**
4. **High quality clinical data is urgently required**
5. **Long term follow up of ART children**

Sperm DNA: organization, protection and vulnerability: from basic science to clinical applications
edited by Chris Barratt,
ESHRE Campus symposium, Stockholm, Sweden , 21-22 May 2009

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