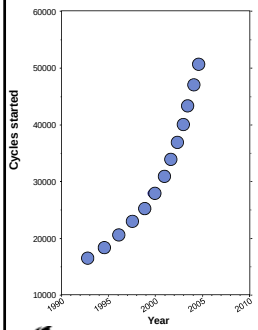
Where are we today and which questions remain? Clinical studies of male factor infertility

R. John Aitken

ARC Centre of Excellence for Biotechnology and Development, Discipline of Biological Sciences, School of Environmental and Life Sciences, The University of Newcastle, Newcastle, Australia.

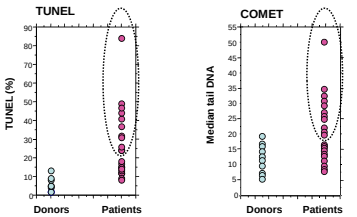
Contribution of ART to Australasian population

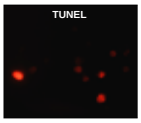
AIHW National Perinatal Statistics Unit

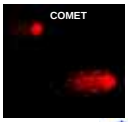


- One in every 35 babies produced by ART
- Major reason for referral is male factor
- Infertility affects 1 in 20 of the male population
- Most exhibit defective sperm function; largest single defined cause of human infertility

DNA damage in the male germ line







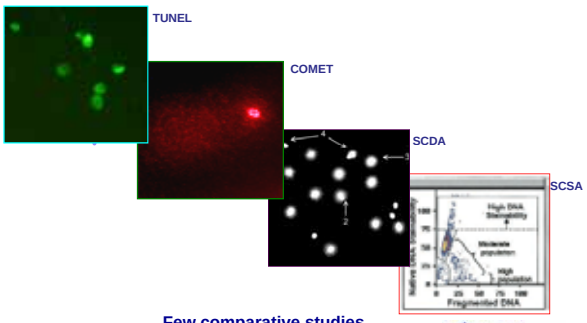
Reproductive consequences of DNA damage in the male germ line

- **Reduced pregnancy rates following natural or assisted conception** (Loft et al., 2003, Duran et al., 2002; Bungum et al., 2004).
- **Impaired fertilization** (Benchaib et al., 2003; Virro et al., 2004; Aitken 2004)
- **Disrupted preimplantation development** (Sakkas et al., 1998; Morris et al., 2002; Virro et al., 2004).
- **Increased rates of abortion** (Saleh et al., 2003; Carrell et al., 2003).
- **Increased rates of disease in children and young adults – eg cancer, complex neurological conditions** (Ji et al., 1997; Aitken and Krausz, 2001; Edwards and Ludwig, 2003; Aitken, 2004).

Where are we today and which questions remain?

- How should we measure DNA damage?
- How does the DNA damage originate?
- Clinically, how should we manage DNA damage in spermatozoa

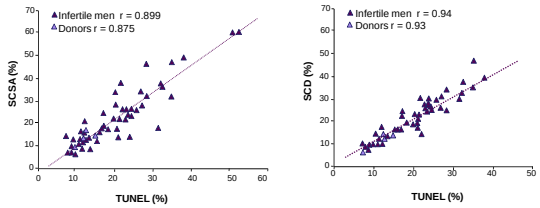
How should we measure DNA damage?



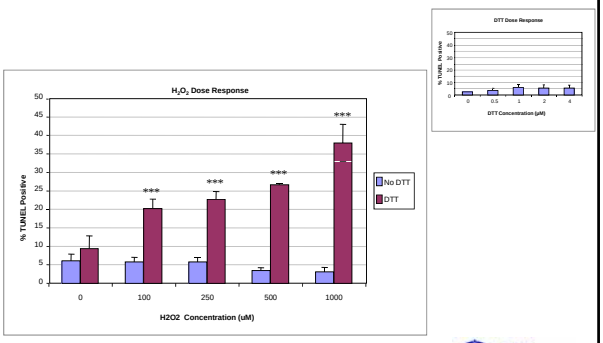
Few comparative studies

Relationships between DNA damage assays

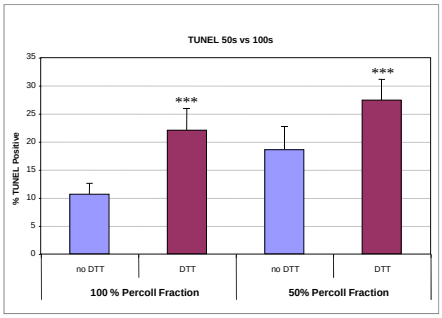
Chohun et al. J Androl 27, 53-59, 2006



DNA cross-linking of the TUNEL assay



Modified TUNEL assay and sperm fractions



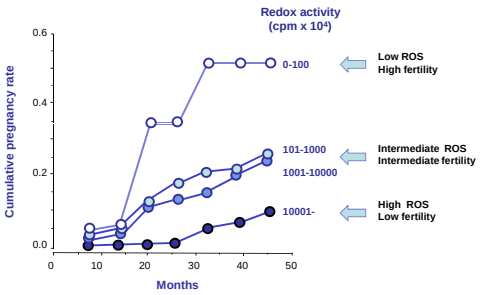
How does the DNA damage originate?

Oxidative stress

Abortive Apoptosis

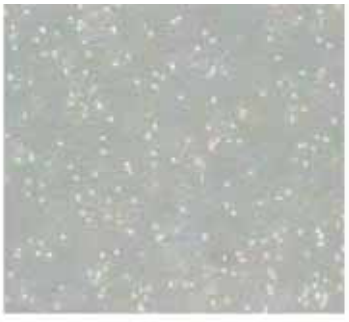
ROS and Spontaneous Pregnancy

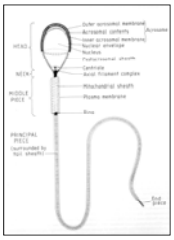
Spontaneous pregnancy in 139 couples characterized by normal female partner



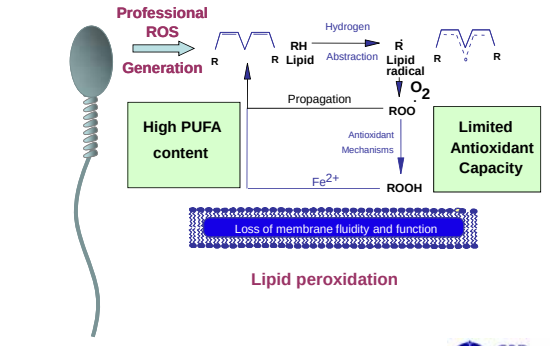
Aitken et al. (1991) Am J Obstet Gynecol

Human spermatozoa

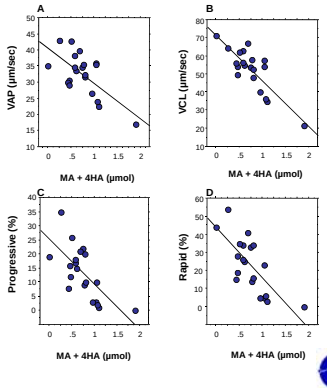




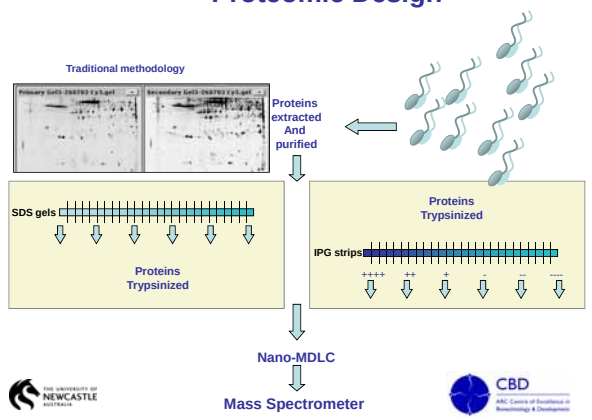
Susceptibility of Spermatozoa to Oxidative stress



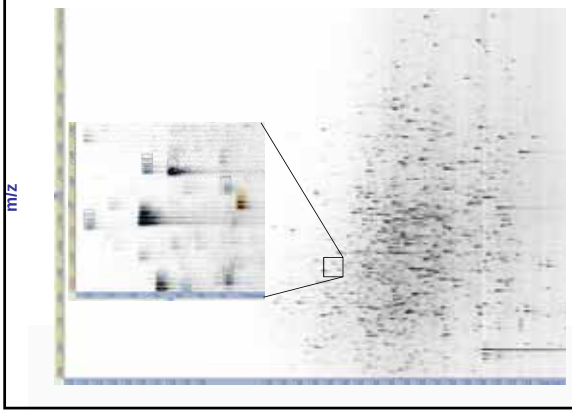
Lipid peroxidation and sperm movement



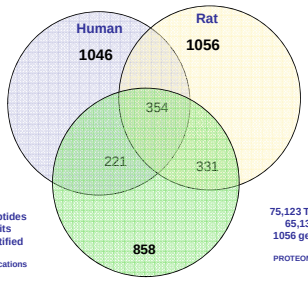
Proteomic Design



Example of one Virtual “2D Gel ”



Human vs Rat vs Mouse sperm proteomes



HUMAN
75,886 Total included Peptides
43,828 with 2 unique hits
1046 gene products identified

PROTEOMICS - Clinical Applications Volume 1, 2007, 524-532

RAT
75,123 Total included Peptides
65,133 with 2 unique hits
1056 gene products identified

PROTEOMICS. Volume 8, 2008, 2312-21

MOUSE
858 gene products identified

PROTEOMICS. Volume 8, 2008, 1720-30

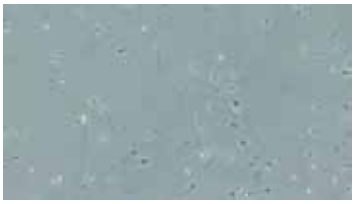
Receptors on the surface of human sperm

Tyrosine kinase/phosphatase receptors
Ephrin type-A receptor 8 precursor. Insulin receptor Isoform 1 of Granulocyte colony-stimulating factor receptor precursor. Isoform 1 of Prolactin receptor precursor. Isoform long of tyrosine-protein kinase transmembrane receptor ROR1 precursor. Protein tyrosine phosphatase, receptor type, c. Tyrosine-protein kinase receptor Tie-1 precursor
Seven–pass transmembrane receptors (GPCRs)
Cadherin EGF lag seven-pass G-type receptor 3 precursor. Isoform 1 of probable G-protein coupled receptor 116 precursor. G protein-coupled receptor 56 isoform B. Gastrin/Cholecystokinin type B receptor. Olfactory receptor 2AE1. P2Y purinoceptor 4
The Glutamate-gated Ion Channel (GIC) Family of Neurotransmitter Receptors
Glutamate [NMDA] receptor subunit epsilon 4 precursor Isoform 1 of Glutamate receptor, Ionotropic kainate 1 precursor
Progesterone receptor
Membrane-associated Progesterone receptor component 2 Adiponectin receptor protein 2
Transient receptor family
Transient receptor potential cation channel, subfamily M, member 1
Putative zona receptor glycoprotein
PH20, Basigin, ASA, Mannose-6-phosphate receptor

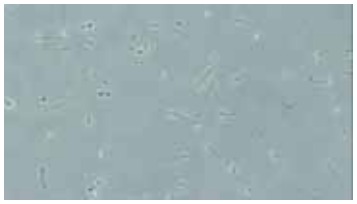
Baker et al., 2007

Apoptosis

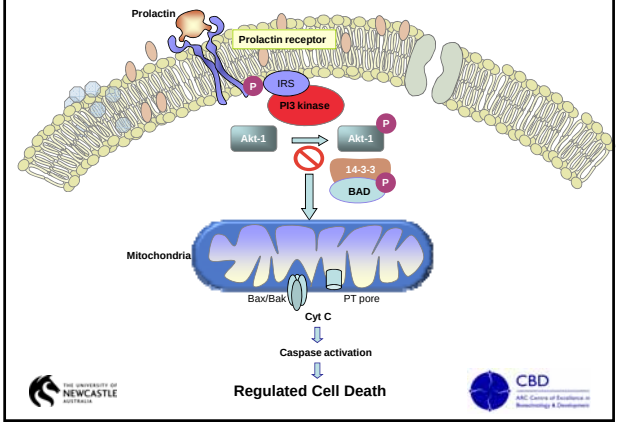
Control



Incubation 16h

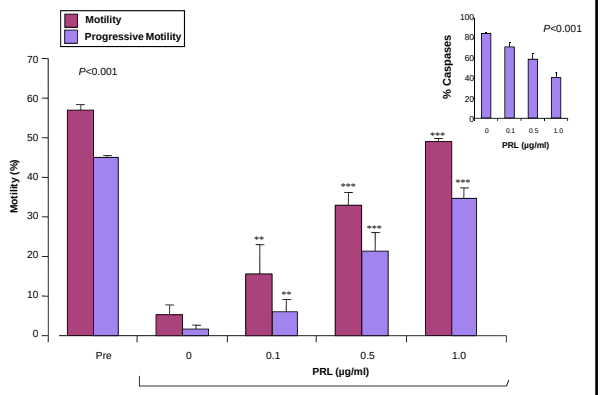


Apoptosis in spermatozoa



Regulated Cell Death

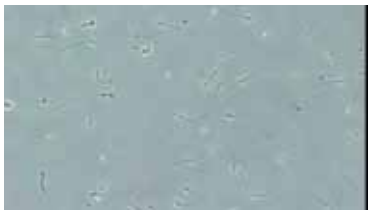
Effect prolactin on sperm motility



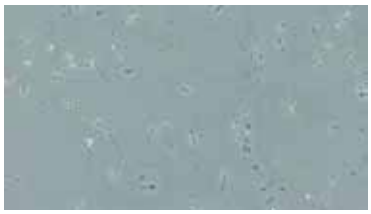
Ari Pujianto 2008

16 h incubation

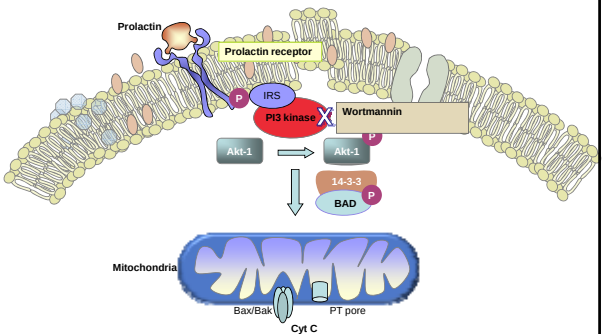
Control



Prolactin

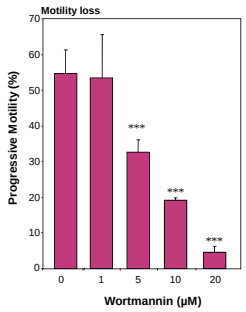


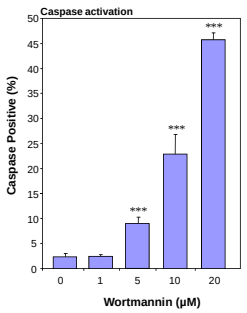
Apoptosis in spermatozoa



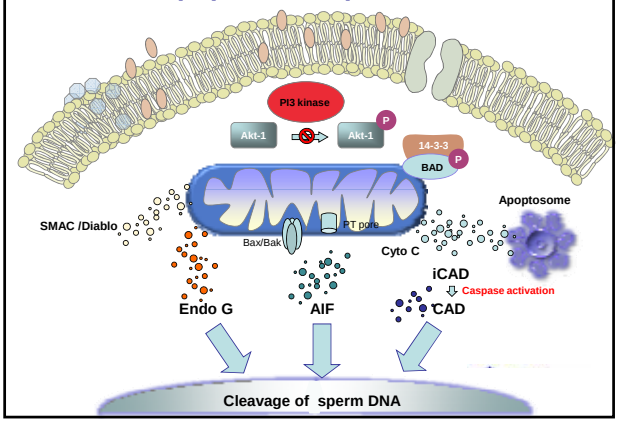
Regulated Cell Death

PI3 kinase inhibition

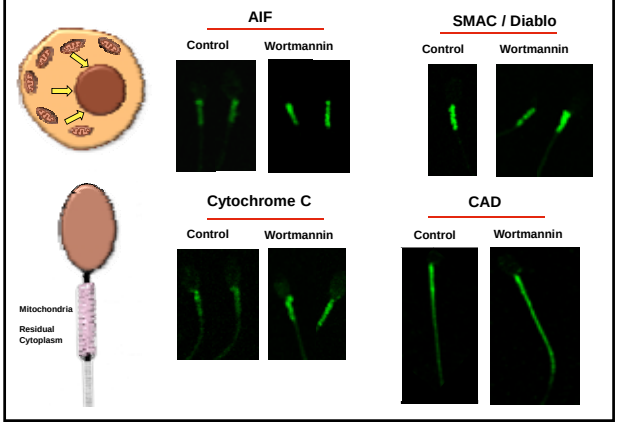




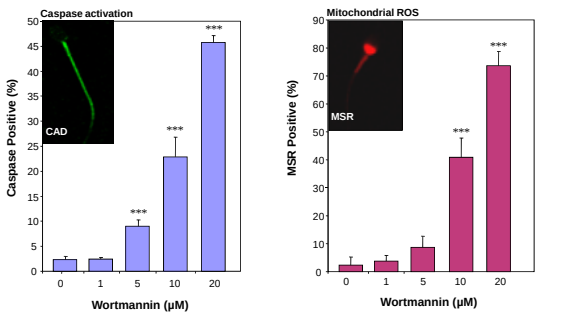
Apoptosis in spermatozoa



Effectors of apoptosis trapped in midpiece/tail

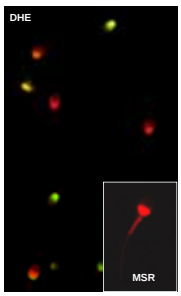


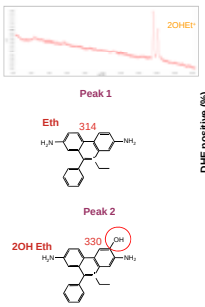
PI3 kinase and mitochondrial ROS

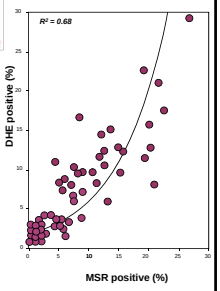


Superoxide generation by human sperm

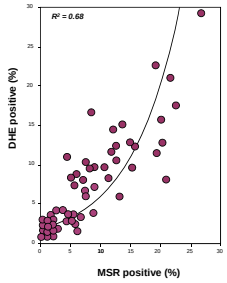
Superoxide anion generation

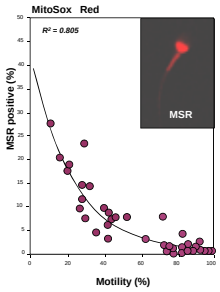




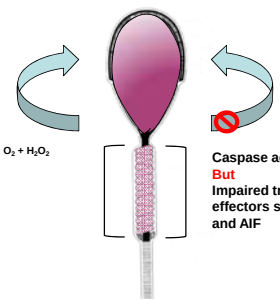


Mitochondrial ROS and sperm movement





Sperm can default to an apoptotic pathway

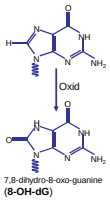


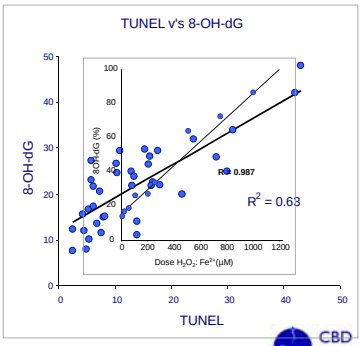
$2H^+ + 2O_2^{\cdot-} = O_2 + H_2O_2$

Caspase activation
But
Impaired translocation of effectors such as Endo G, CAD and AIF

Does mitochondrial ROS induce oxidative DNA damage in sperm?

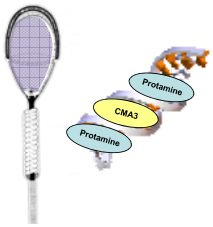
Oxidative Stress leads to DNA Damage

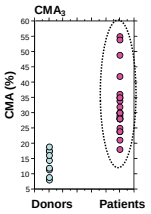


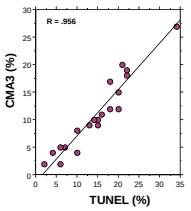


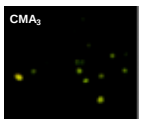
De Iuliis et al, in press

Chromatin protamination and DNA damage

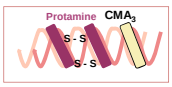




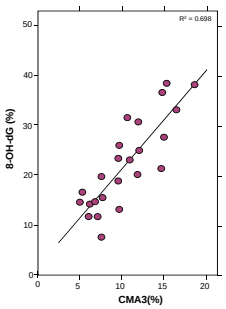




Oxidative DNA damage and chromatin remodelling



1. Poorly protaminated sperm chromatin is susceptible to DNA damage
2. Vulnerable DNA is attacked by ROS



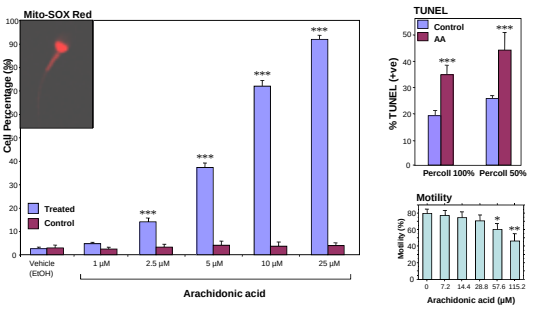
Two Step Hypothesis

Step 1: Poor chromatin remodeling creates a state of vulnerability to DNA damage

Step 2: DNA damage then induced by combination of internal and external factors

Polyunsaturated fats

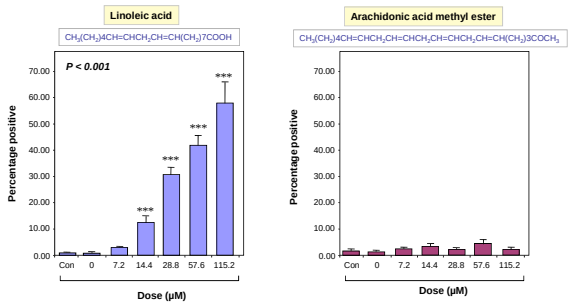
Arachidonic acid and mitochondrial ROS



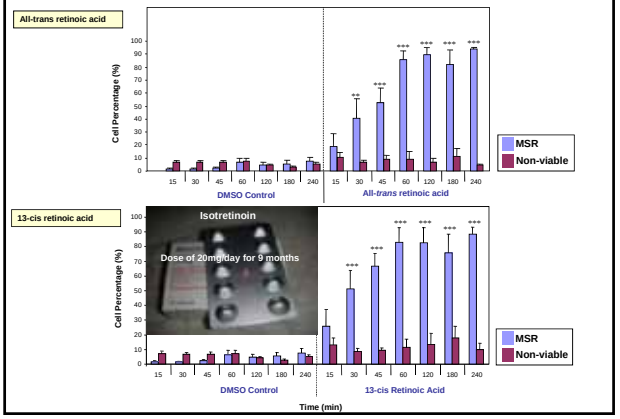
Recommended daily intake of PUFA 1-2 grams /day

Actual daily PUFA intake 20-50 grams /day

Amphipathic properties of PUFA are essential



Mitochondrial ROS induced by retinoids



Warning to male mobile phone users: chatting too long may cut sperm count

· US study based on 361 attending clinic
· Other scientists call for further investigations

Ian Sample in New Orleans
The Guardian, Tuesday October 24 2006

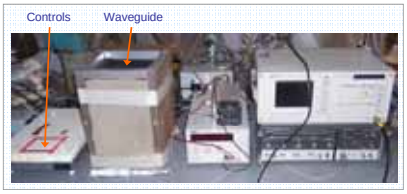
Non-biological parents to gain parental rights. Photograph: Derek Wilson/Corbis

Men who use mobile phones for long periods at a time may be at risk of damaging their sperm, according to research by American scientists.
Samples taken from men attending a fertility clinic revealed that their sperm declined steadily in number, quality and ability to swim as mobile phone usage increased. Where men used their mobiles for more than four hours a day, researchers found a 30% drop in sperm motility or movement and viability when compared with men who did not use a mobile phone.

guardian.co.uk

Electromagnetic Radiation

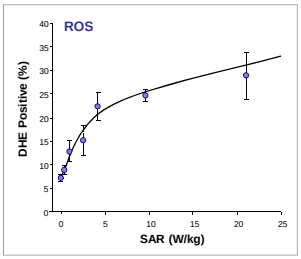
- **Radio frequency (RF-EMR)**
  - 1800 MHz
  - Specific absorption rates (SAR) 0.3 - 21 W/kg
  - 21°C
  - 16 hrs

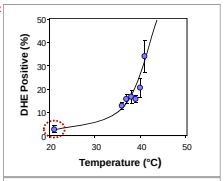


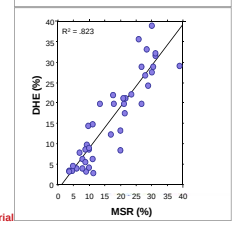
- Normal mobile phone power is ~0.5-1.5 W/kg
- At 21 W/kg the maximum temperature rise is 0.4°C

RF-EMR induces ROS

This is not a thermal effect

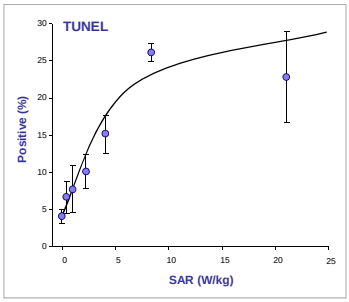


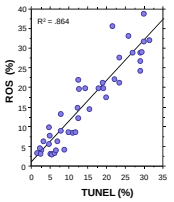


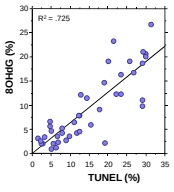


ROS are mitochondrial

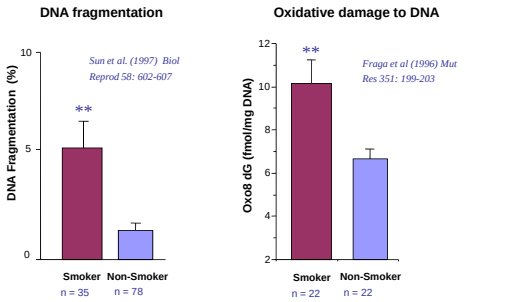
RF-EMR induces DNA fragmentation







Smoking and DNA damage to human sperm

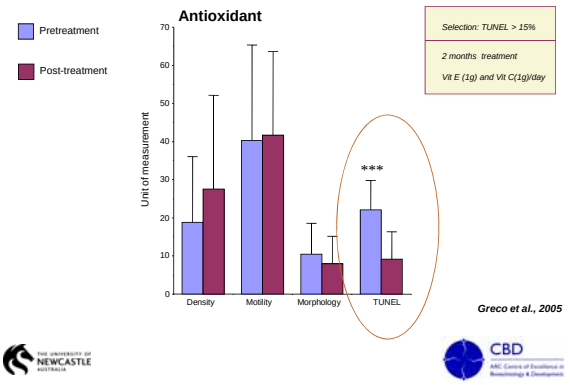


Factors capable of inducing DNA damage in the male germ line

Smoking	Hexachlorocyclohexane	Cryptorchidism
Alcohol	Trinitrotoluene	Testicular torsion
Chromic acid	Aflatoxin	Diabetes
Iron	Lindane	Hyperthyroidism
Lead	Adriamycin	Varicocele
Cadmium	Cisplatin	Infection
Uranium	Quinalphos	Physical exertion
Arsenic	Endosulfan	Hypogonadotrophism
Vanadate	Diethyl maleate	
Phthalate esters	Monensin	
Sulfur dioxide	Formaldehyde	
Sodium fluoride	Alloxan	
PCB/PCN	Streptozotocin	
Methoxychlor	Acrylamide	
Bisphenol A	Ozone	
Nonylphenol	Retinoids	
Cyclophosphamide	Catechol estrogens	

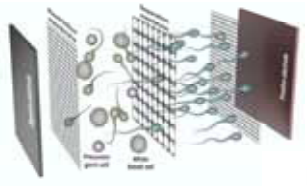
Aitken and Roman, 2008

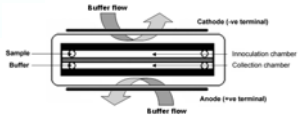
Impact of Vitamins E and C on DNA damage



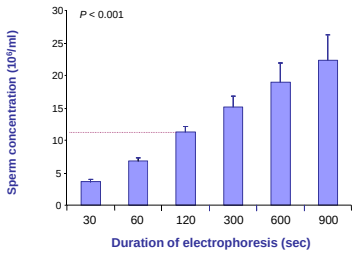
Greco et al., 2005

Spermsep CS10 System Schematics – Sperm Cell Separation



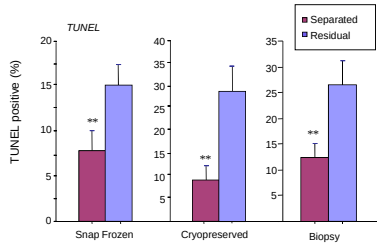


Electrophoretic sperm isolation

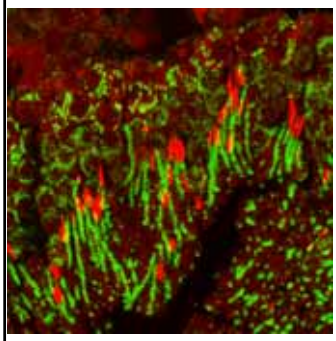


Electrophoretic isolation of spermatozoa

Reduction in DNA damage



2-step Hypothesis



Disordered spermiogenesis

⇩

Impaired chromatin remodelling

⇩

State of vulnerability

Mitochondrial Oxidative stress ⇩

Oxidative DNA base adduct formation

⇩

DNA fragmentation

Reproductive Science Group

University of Newcastle

Ari Pujianto, University of Newcastle

Sheena Lewis, Carmen McVicar: Queen's University , Belfast