1° Workshop PREVIENI

Roma, 27 ottobre 2009

Integrità del DNA nemaspermico: un nuovo endpoint per lo studio della pressione ambientale sulla fertilità umana

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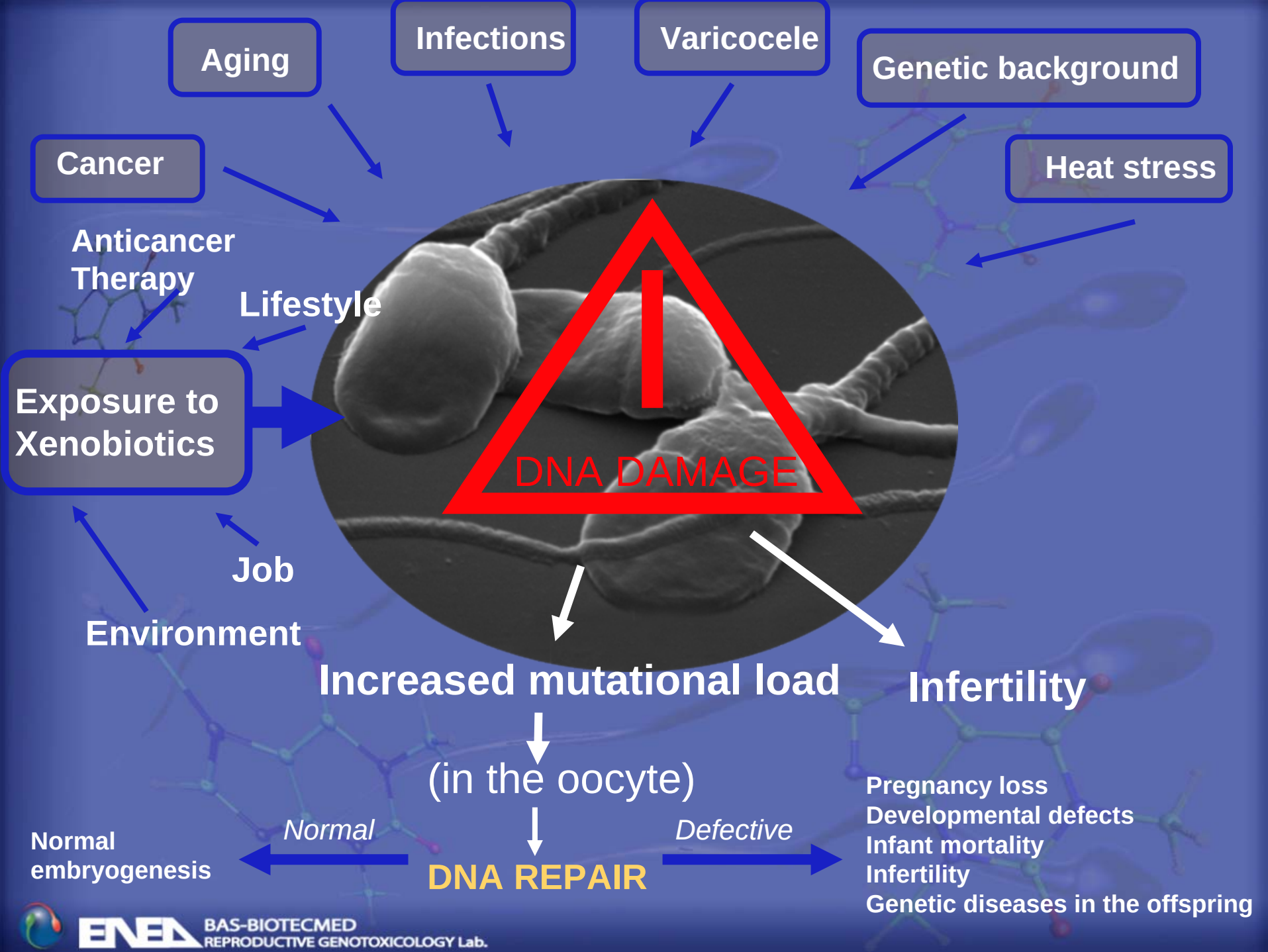
Semen quality is conventionally assessed by the visual scoring of sperm number and properties according to WHO guidelines

Need for new semen quality markers that may better:

- discriminate infertile from fertile men
- predict pregnancy outcome and the risk of adverse reproductive events (especially in ART)
- give information about sperm genomic integrity

Sperm DNA damage as a new candidate biomarker


Aging
Infections
Varicocele
Genetic background
Cancer
Heat stress
Anticancer Therapy
Lifestyle
Exposure to Xenobiotics
DNA DAMAGE
Job
Environment
Increased mutational load
Infertility
(in the oocyte)
Normal
Defective
Normal embryogenesis
DNA REPAIR
Pregnancy loss
Developmental defects
Infant mortality
Infertility
Genetic diseases in the offspring
ENEA
BAS-BIOTECMED
REPRODUCTIVE GENOTOXICOLOGY Lab.

Actual and potential DNA defects that may be transmitted by sperm

CHROMOSOME ABERRATIONS

Number
Aneuploidy
Polyploidy

Structure
Duplications/deletions
Insertions
Rearrangements

DNA CHEMICAL MODIFICATIONS

Premutational lesions
DNA adducts
Protamine adducts
SSB/DSB (non specific DNA breaks & fragmentation)

Epigenetic changes
Imprinting

DNA BASE MODIFICATIONS

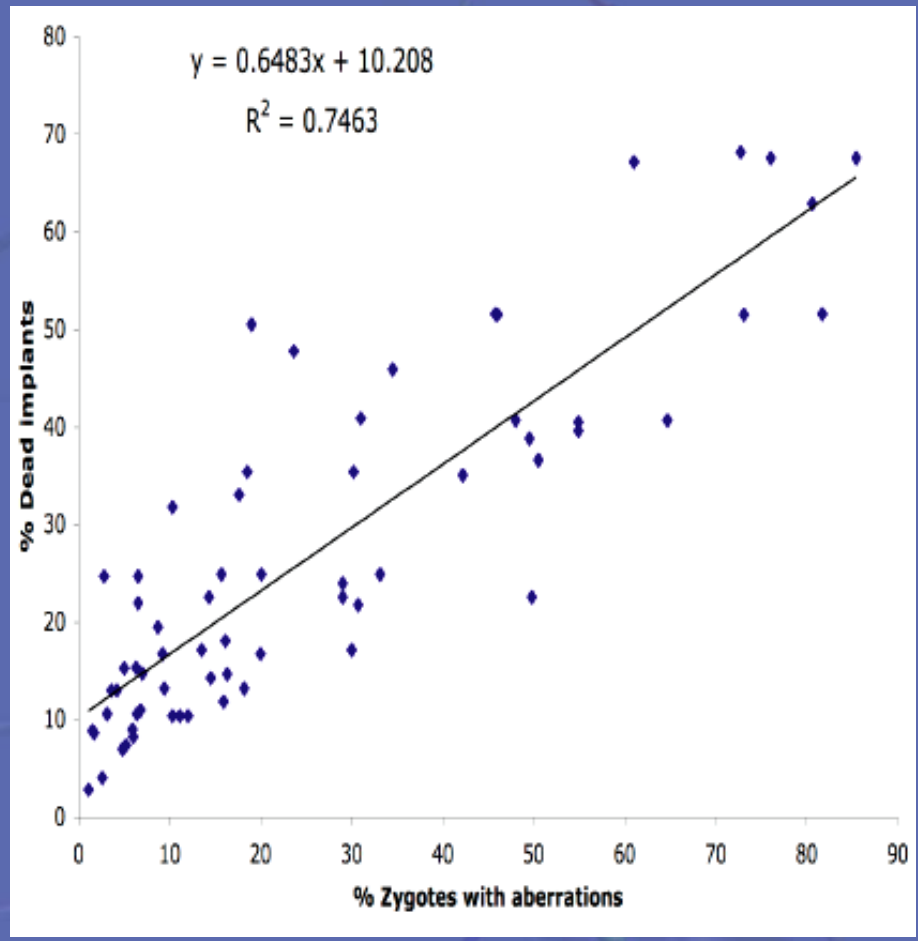
Changes in the number of trinucleotide repeats/ Expanded Simple Tandem Repeats (ESTR)

Gene mutations

A large body of evidence in rodents unequivocally shows that:

Paternal exposure to a variety of chemicals induces embryonic lethality and other abnormal reproductive outcomes

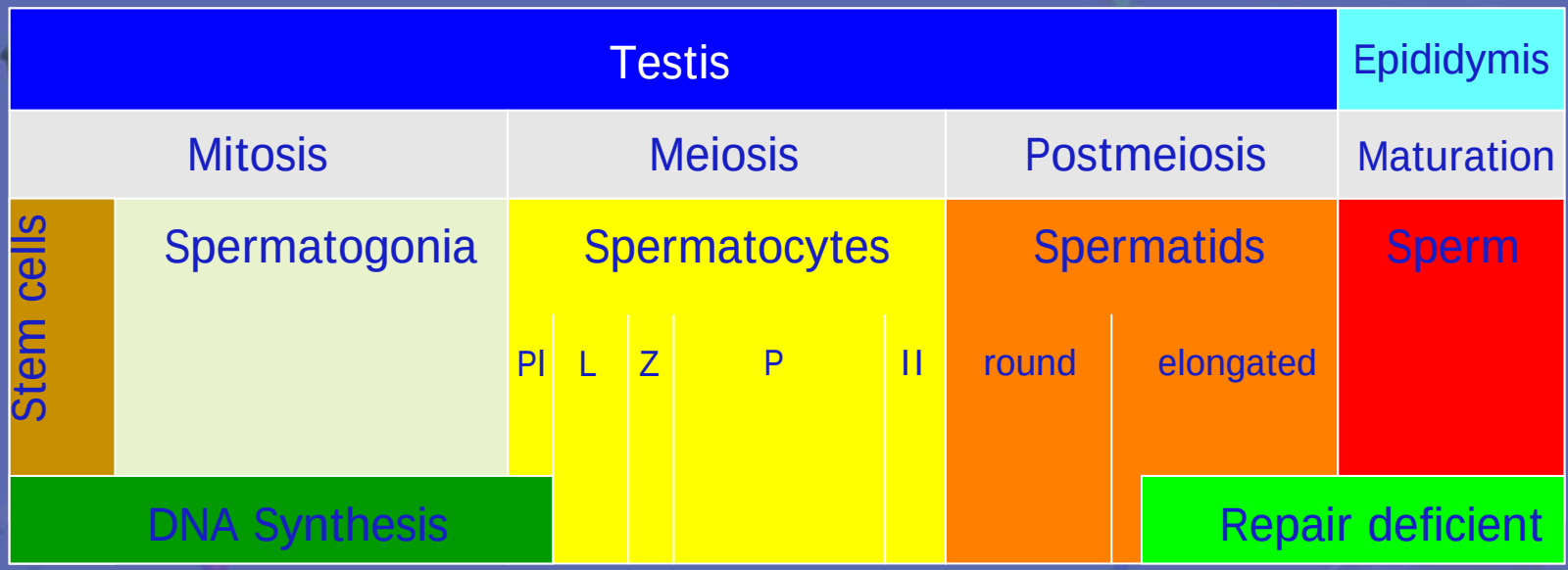
Chromosomal aberrations in zygotes are critical intermediate between paternal exposure and abnormal reproductive outcomes

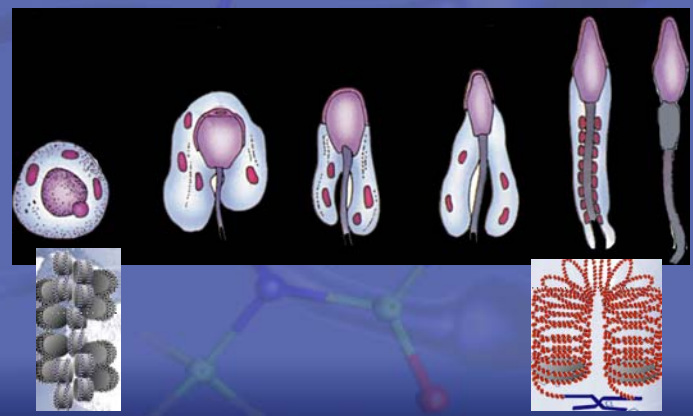


Marchetti & Wyrobek (2005) Birth Defect Res Part C 75:112-129

The ability to repair DNA damage is diminished during the last part of spermatogenesis

man	100 days	64	38	25	15	0
mouse	45 days	35	21	14	7	0





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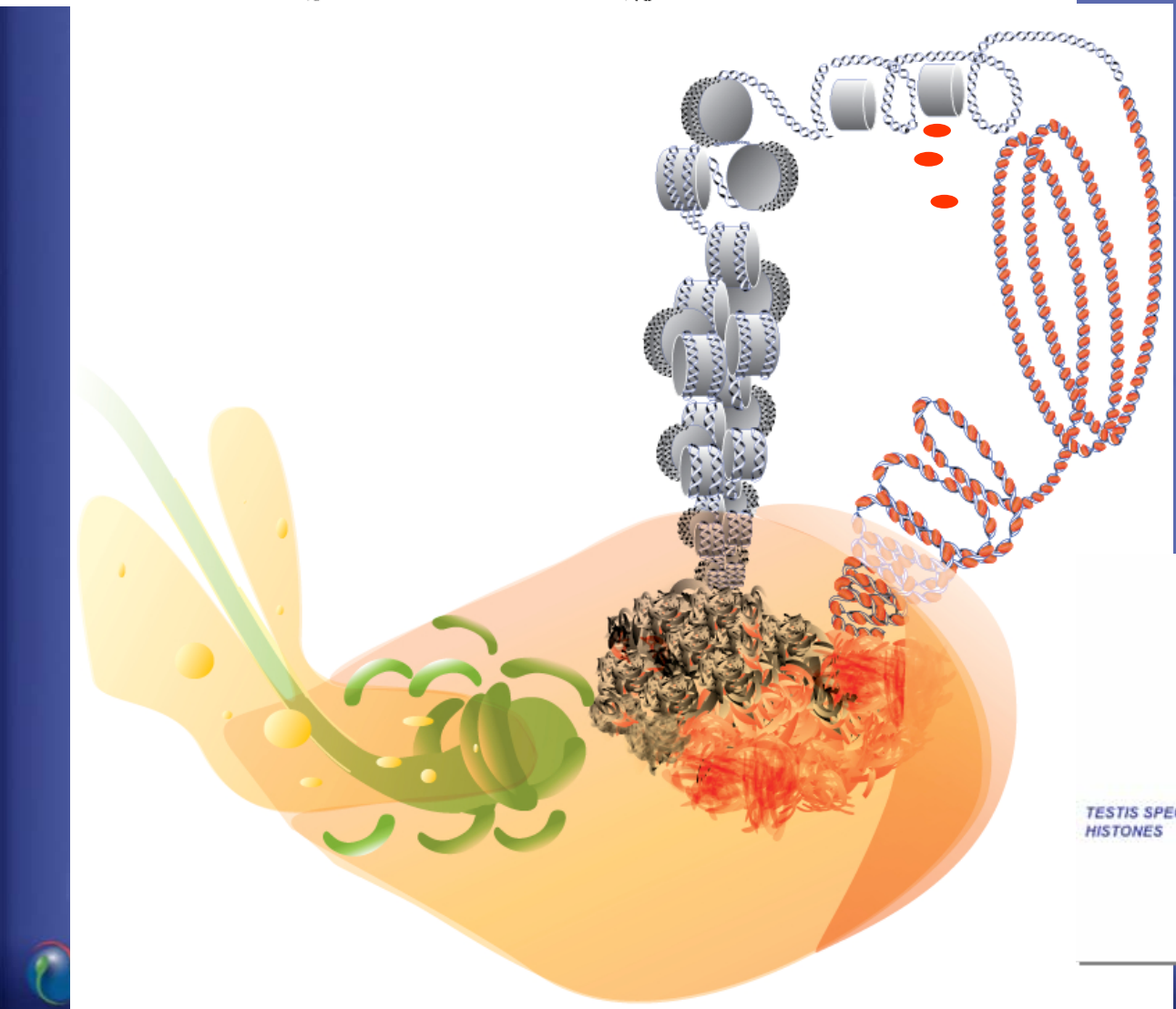
DNA Integrity Is Compromised in Protamine-Deficient Human Sperm

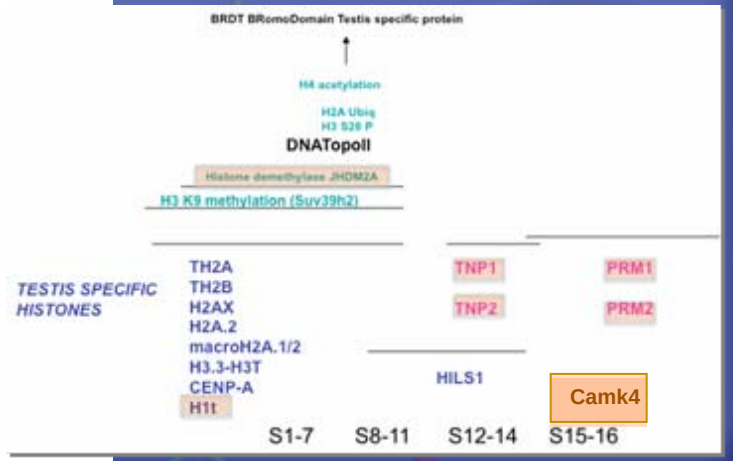
VINCENT W. AOKI,*†‡ SERGEY I. MOSKOVTSEV,§ JENNIFER WILLIS,§ LIHUA LIU,*‡
J. BRENDAN M. MULLEN,§ AND DOUGLAS T. CARRELL*†‡¶

BIOLOGY OF REPRODUCTION 69, 211–217 (2003)
Published online before print 5 March 2003.
DOI 10.1095/biolreprod.102.015115

Protamine 2 Deficiency Leads to Sperm DNA Damage and Embryo Death in Mice[1]

Chunghee Cho,[2] Haesook Jung-Ha,[3] William D. Willis,[3] Eugenia H. Goulding,[3] Paula Stein,[4] Zhe Xu,[4]
Richard M. Schultz,[4] Norman B. Hecht,[5] and Edward M. Eddy[3,6]





BIOLOGY OF REPRODUCTION 78, 761–772 (2008)
Published online before print 16 January 2008.
DOI 10.1095/biolreprod.107.065623

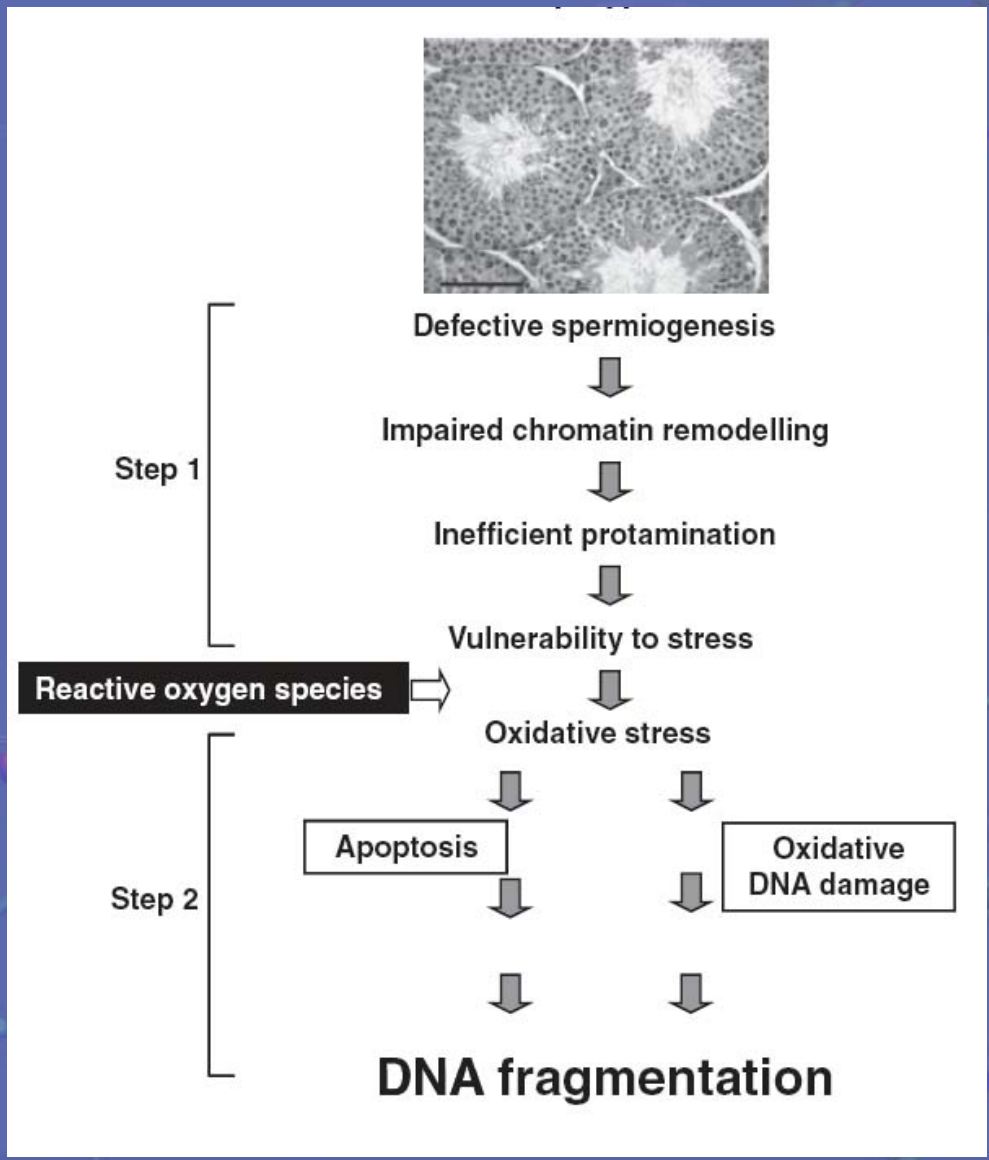
Long-Term Effects of Mouse Intracytoplasmic Sperm Injection with DNA-Fragmented Sperm on Health and Behavior of Adult Offspring[1]

Raúl Fernández-Gonzalez,[3] Pedro Nuno Moreira,[3] Miriam Pérez-Crespo,[3] Manuel Sánchez-Martín,[4] Miguel Angel Ramirez,[3] Eva Pericuesta,[3] Ainhoa Bilbao,[5] Pablo Bermejo-Alvarez,[3] Juan de Dios Hourcade,[3] Fernando Rodriguez de Fonseca,[5] and Alfonso Gutiérrez-Adán[2,3]

The results in our mice model suggest, first, that a risk is linked to the ICSI procedure itself, capable of producing alterations in the early embryo and long-term consequences, such as obesity and organomegaly, and, second, that a risk is linked to the use of ICSI with DFS, capable of producing genetic and epigenetic changes during preimplantation that may lead to altered fetal/placental development and, as a consequence, offspring with aberrant growth, behavior, early aging, and tumors. Because sperm with damaged DNA can fertilize oocytes during ICSI and no system exists to select spermatozoa with strand breaks, an evaluation of sperm DNA fragmentation level is crucial to prevent or reduce the risk of inducing genetic alterations in the offspring. Further studies in humans are needed to determine the threshold of sperm DNA fragmentation after which ICSI should not be recommended.

*

A two-step hypothesis of DNA damage in the male germline (Aitken et al., Int J Androl, 2008)



Age & sperm DNA damage

Assay	Cohort	Age range	Age Effect	Reference
SCSA	277 Danish farmers	18-55	↑	Spanò et al., 1998
SCSA	176 Swedish fishermen	32-63	↑	Rignell-Hydbom et al., 2005
SCSA	707 Europeans & Greenland Inuits	18-67	↑	Spanò et al., 2005
SCSA	102 US infertility patients	22-57	↑	Moskovtsev et al., 2005
SCSA	1125 US infertility patients	25-60	↑	Moskovtsev et al., 2005
SCSA	88 US men	20-80	↑	Wyrobek et al., 2006
COMET neut	60 UK infertility patients	29-47	↑	Morris et al., 2002
COMET neut	212 US infertility patients	20-54	↑	Hauser et al., 2003
COMET neut	26 US fertile and 40 infertile patients	20-57	↑	Singh et al., 2003
COMET neut	257 US infertile	22-54	↑	Trisini et al., 2004
COMET alk	71 Italian styrene workers	22-46	↑	Migliore et al., 2002
COMET alk	80 US non smokers	22-80	↑	Schmid et al., 2007
TUNEL	49 Greek fertile and 61 infertile	24-64	↑	Plastira et al., 2007
TUNEL	508 Brasilian infertile	22-59	↑	Vagnini et al., 2007
FISH structural	10 healthy non smokers 10 healthy non smokers	22-28 vs 65-80	↑	Sloter et al., 2007
TOTAL	3859			

The fraction of defective sperm (DFI) seems higher in infertile patients

Technique	Controls (%) N	Patients % N	Reference
SCSA	13.7±7.2 165	24.4 115	Evenson et al., Hum Reprod, 1999
SCSA	14.2 213	23.6 92	Boe-Hansen et al., Hum Reprod , 2006
SCSA	13±7.7 126	20.4±12.5 224	Erenpreiss et al., Asian J Androl, 2008
8-OH-2'-dG	$4.8/10^5$ 54	$10.0/10^5$ 60	Shen et al., J Androl, 1999

Generally, the fraction of DNA defective sperm is weakly negatively correlated with conventional WHO parameters

Technique	N	Conc	Morphology	Motility	Reference
FI M-TUNEL	262	N.S.	-0.44	-0.60	Henkel et al., RBMO, 2003
FI M-TUNEL	167			N.S.	Henkel et al., Fertil Steril, 2004
FI M-TUNEL	132	-.39	-.25	-.34	Borini et al., Human Reprod, 2006
FCM-TUNEL	1633	N.S.	N.S.	Neg corr	Cohen-Bacrie et al., FertilSteril, 2008

Sperm DNA integrity and natural conception

(modified from Evenson & Wixon, Fertil Steril 2008)

Technique	N	Results	O.R. (95% CI)	Reference
SCSA	165 US couples (The Georgetown study)	DFI increase if pregnancies in month 1-3, in month 4-12, and no pregnancies. No pregnancies for DFI>30% DFI>15% predicted 39% miscarriages	**6.54** (1.71-24.91)	Evenson et al., Hum Reprod 1999
SCSA	215 Danish first pregnancy planners	TTP inversely associated with DFI levels: starts decreasing >20% and sharply becomes negligible when >40%	**7.59** (2.54-22.67)	Spanò et al., Fertil Steril 2000
7-hydro-8-oxo-2'-deoxyguanosine (8-oxodG) levels in DNA	225 Danish first pregnancy planners	TTP inversely associated with 8-oxodG levels		Loft et al., Hum Reprod 2003

Human Reproduction Vol.22, No.1 pp. 174–179, 2007 doi:10.1093/humrep/del326
Advance Access publication August 18, 2006.

Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome

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[1]Fertility Clinic, Viborg Hospital (Skive), Skive, Denmark, [2]Fertility Centre, Scanian Andrology Centre, Malmö University Hospital, Malmö, [3]Division of Occupational and Environmental Medicine and Psychiatric Epidemiology, Lund University, Lund, Sweden and [4]Section of Toxicology and Biomedical Sciences, BIOTEC-MED, ENEA Casaccia Research Center, Rome, Italy

[5]To whom correspondence should be addressed at: Fertility Centre, Scanian Andrology Centre, Malmö University Hospital, Malmö, Sweden. E-mail: mona.bungum@med.lu.se

BACKGROUND: The sperm chromatin structure assay (SCSA) has been suggested as a predictor of fertility *in vivo* as well as *in vitro*. The available data however, have been based on limited numbers of treatments. We aimed to define the clinical role of SCSA in assisted reproduction. METHODS: A total of 998 cycles [387 intrauterine insemination (IUI), 388 IVF and 223 ICSI] from 637 couples were included. SCSA results were expressed as DNA fragmentation index (DFI) and high DNA stainable (HDS) cell fractions. Outcome parameters were biochemical pregnancy (BP), clinical pregnancy (CP) and delivery (D). **RESULTS: For IUI, the odds ratios (ORs) for BP, CP and D were significantly lower for couples with DFI >30% as compared with those with DFI ≤30%. No statistical difference between the outcomes of ICSI versus IVF in the group with DFI ≤30% was seen. In the DFI >30% group, the results of ICSI were significantly better than those of IVF. CONCLUSIONS: DFI can be used as an independent predictor of fertility in couples undergoing IUI. As a result, we propose that all infertile men should be tested with SCSA as a supplement to the standard semen analysis. When DFI exceeds 30%, ICSI should be the method of choice.**

TOBACCO smoke & sperm DNA damage:?

Assay	Controls	Smokers	Result	Reference
HPLC 8-OHdG (cotinine)	32 healthy	28 healthy	↑	Shen et al., 1997
SCSA (cotinine)	15 young	10 young	N.S.	Rubes et al., 1998
SCSA	35	35	DFI ↑	Potts et al., 1999
SCSA	45 (13 healthy + 32 infertile)	20 infertile	N.S.	Saleh et al., 2002
TUNEL	35	35	DFI ↑	Potts et al., 1999
TUNEL	69	28	N.S.	Sargerie et al., 2000
TUNEL	57 infertile	51 infertile	DFI ↑	Sepaniak et al., 2006
COMET alk	15 normo fertile	25 normo infertile	N.S.	Belcheva et al., 2004
COMET neut	183 infertile	67 infertile	N.S.	Trisini et al., 2004
FISH (cotinine)	15 young	10 young	Disomy Y↑ Disomy X, 18 N.S.	Rubes et al., 1998
FISH (cotinine)	28 healthy	17 healthy	Disomy X ↑ Disomy Y, 18 N.S.	Robbins et al., 1997
FISH	10 healthy	21 healthy	Disomy 13 ↑ Disomy 21, X, Y N.S.	Shi et al., 2001
Total	*489*	*302*		

Smoking affects male reproductive health after in utero exposure

Reduced sperm count in adult sons of heavy smoking mothers as shown by 4 papers based on some 4,000 men

Storgaard et al., Epidemiology 14: 278-86, 2003

Jensen et al., Am J Epidemiol 159: 49-58, 2004

Jensen et al., Hum Reprod 20: 2559-66, 2005

Ramlau-Hansen et al., Am J Epidemiol 165: 1372-9, 2007

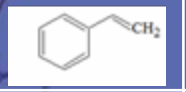
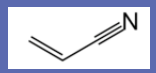
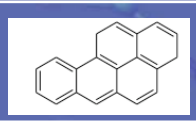
No evidence of alteration in sperm chromatin integrity (Storgaard et al., 2003)

AIR POLLUTION & sperm DNA damage: possible

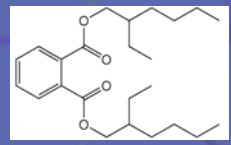
The Teplice program

Assay	Population	Result	Reference
SCSA	266 young Czech (111 Prachatice 151 Teplice)	DFI ↑ after high pollution only N.S. for WHO parameters (except morphology)	Selevan et al., 2000
SCSA	36 young Czech	In repeated measurements, association between exposure to period of high pollution (≥US air quality standard) and DFI N.S. for WHO parameters	Rubes et al., 2005
SCSA	35 young Czech	GSTM1 null men showed higher %DFI in response to exposure to intermittent air pollution	Rubes et al., 2007
FISH (X, Y, 8)	19 nonsmokers in winter (high) 16 nonsmokers in summer (low)	Association between high pollution and disomy YY Others disomy/ploidy N.S.	Robbins et al., 1999

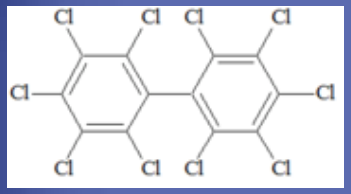
Occupational exposures reported to affect sperm DNA integrity in workers

Noxia	N	Country	Assay	Reference
Styrene 	111	Denmark Italy	SCSA Comet (alk) FISH	Kolstad et al., 1999 Migliore et al., 2002; 2006
Acrylonitrile 	30	China	Comet (alkaline) FISH	Xu et al., 2003
Lead	362	UK Belgium Italy	SCSA	Bonde et al., 2002
Non persistent pesticides	363	China USA Mexico	SCSA Comet (neutral) Comet (alkaline) TUNEL FISH	Sanchez-Peña et al., 2004 Meeker et al., 2004 Bian et al., 2004 Xia et al., 2005
PAH 	48	Taiwan	SCSA	Hsu et al., 2006

URINARY PHTHALATE METABOLITES AND SPERM DNA DAMAGE AS A POTENTIAL BIOMARKER OF SUSCEPTIBILITY

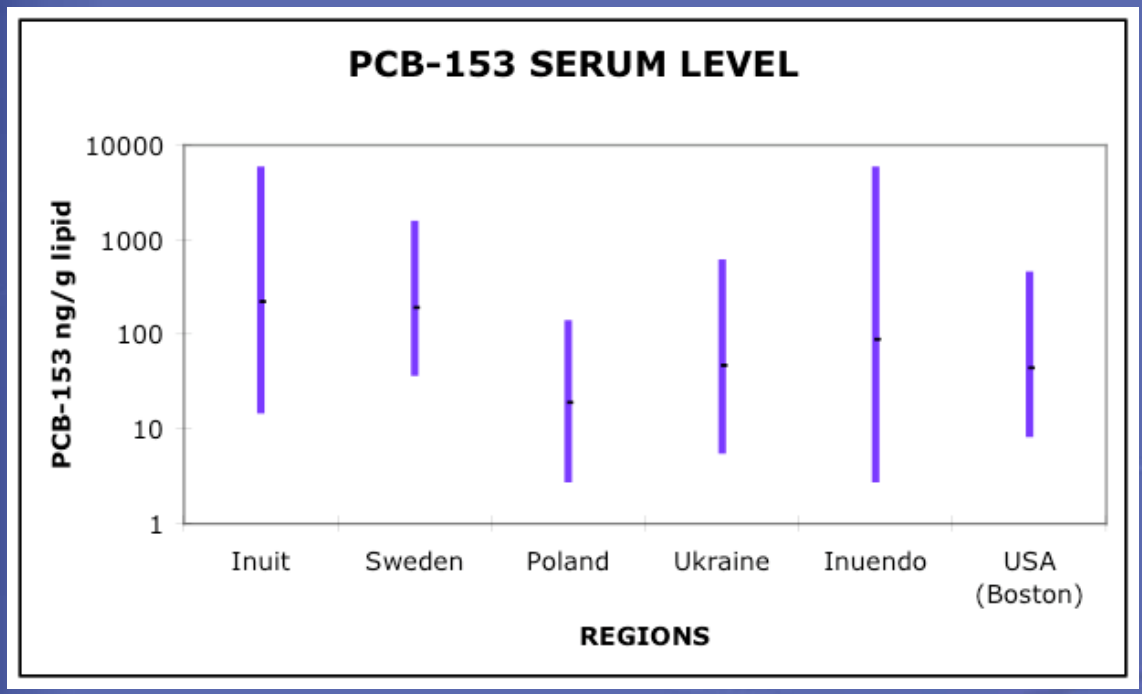


Phthalates	*Assay*	*Place*	*Cohort*	*Results*	*Reference*
MEP MMP MEHP MBP MBzP MOP MINP MCHP	COMET (neutral)	USA - Boston	168 infertile	Comet extent with MEP	Duty et al., Environ Health Perspect, 2003
MEHHP MEOHP DEHP	COMET (neutral)	USA - Boston	379 infertile	Comet extent/Tail Moment with	Hauser et al., Hum Reprod, 2007
MEP MEHP MBzP MBP phthalic acid	SCSA	Sweden	234 young conscripts from the general population	N.S.	Jönsson et al., Epidemiology, 2005
DEP DBP DEHP DMP DOP	SCSA	India - Lucknow	200 infertile 100 fertile	DFI correlated with DBP and DEHP mainly in urban and infertile populations	Pant et al., Toxicol Appl Pharmacol, 2008

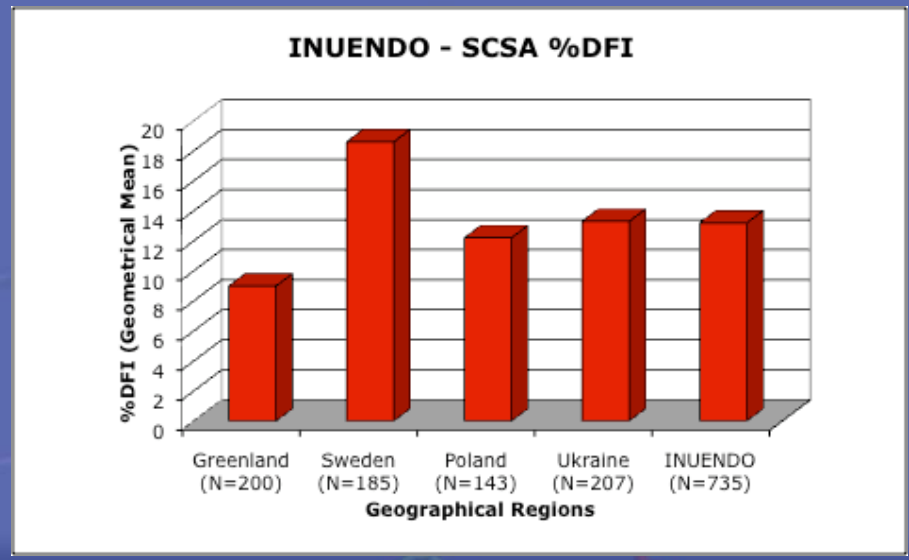


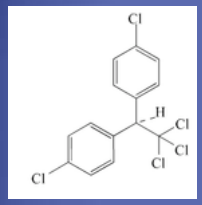
PolyChlorinated Biphenyls (PCBs) & sperm DNA damage: ?

Assay	Exposed	PCB-153 plasma conc (ng/g lipid) mean (min-max)	Result	Reference
COMET neut	212 infertile US men 36.1±5.2 yrs	44 (9-421) *57 congeners including PCB118, 138, 153*	N.S. Trend with PCB138	Hauser et al., 2003
SCSA	707 fertile men (193 Inuits, 178 Sweden, 141 Poland, 195 Ukraine) 33.7 (18-67.5) yrs	180 (3-5500)	DFI ↑ in Europeans	Spanò et al., 2005
TUNEL	652 fertile men (200 Inuits, 166 Sweden, 134 Poland, 152 Ukraine) 33.7 (18-67.5) yrs	180 (3-5500)	DFI ↑ in Europeans	Stronati et al., 2006
TOTAL	919			


PCB-153 SERUM LEVEL
PCB-153 ng/g lipid
10000
1000
100
10
1
Inuit
Sweden
Poland
Ukraine
Inuendo
USA (Boston)
REGIONS


INUENDO
INUENDO
ENEA

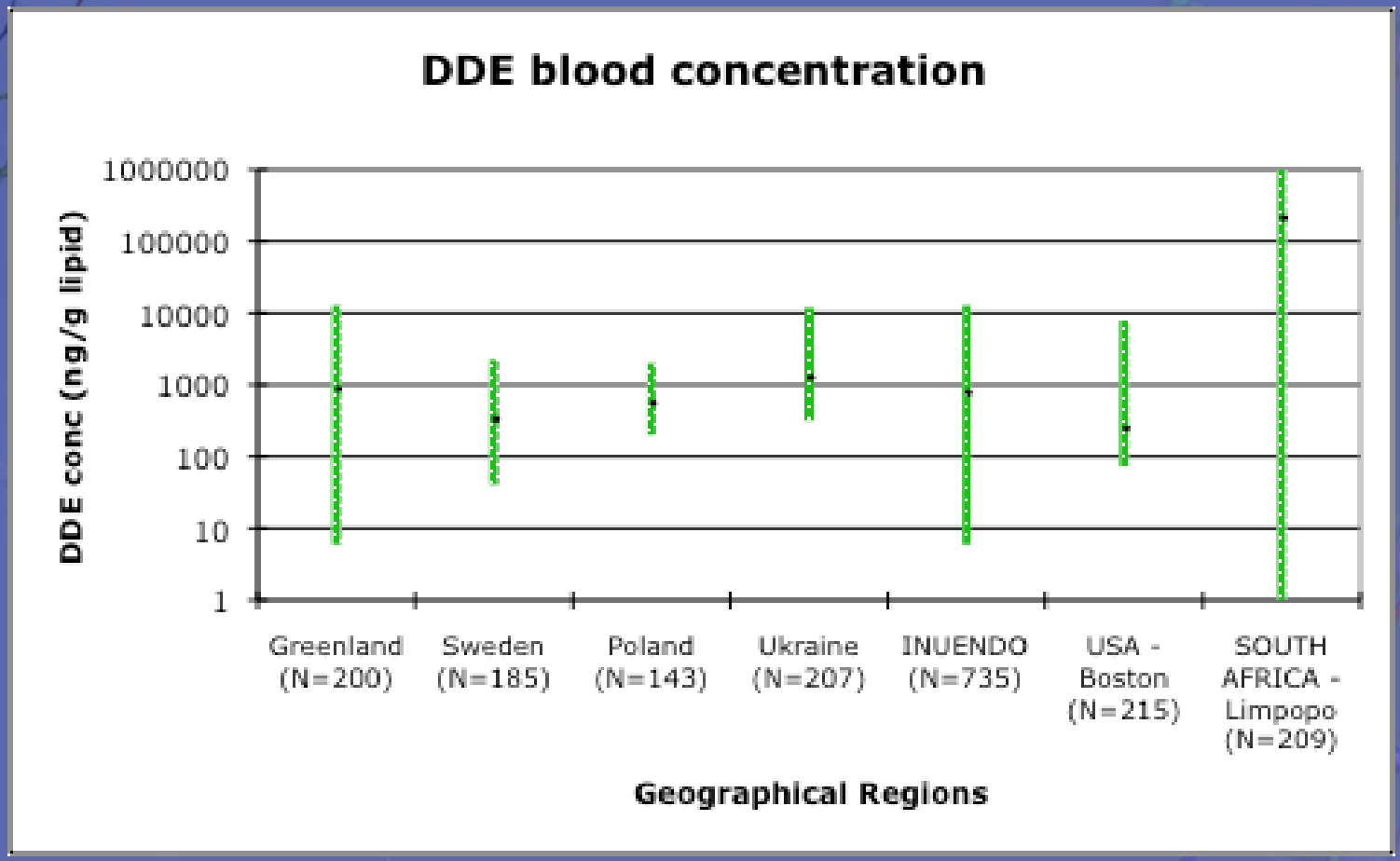

INUENDO - SCSA %DFI
%DFI (Geometrical Mean)
20
18
16
14
12
10
8
6
4
2
0
Greenland (N=200)
Sweden (N=185)
Poland (N=143)
Ukraine (N=207)
INUENDO (N=735)
Geographical Regions



DDT (Dichloro Diphenyl Trichloroethane) & sperm DNA damage: ?

Assay	Exposed	DDE plasma conc (ng/g lipid)	Result	Reference
COMET neut	212 infertile US men 36.1±5.2 yrs	254 (72.5-7776)	N.S.	Hauser et al., 2003
TUNEL	652 fertile men (200 Inuits, 166 Sweden, 134 Poland, 152 Ukraine) 33.7 (18-67.5) yrs	790 (6-13000)	DFI N.S.	Stronati et al., 2006
SCSA	707 fertile men (193 Inuits, 178 Sweden, 141 Poland, 195 Ukraine) 33.7 (18-67.5) yrs	790 (6-13000)	DFI N.S. AR (CAG)n in Europeans If CAG≤21 DFI 40% higher in the high DDE exposure group	Spanò et al., 2005 Giwercman et al., 2007
SCSA	202 South Africans from Limpopo Province (malaria) 22.9 (18-44) yrs	215,500 ± 210,600 (1-997,000)	DFI ↑ (r = 0.12) *(volume count motility viability morphology √)*	De Jager et al., Hum Reprod, 2009
TOTAL	1121			

DDE plasma concentrations detected worldwide



Environ Health Perspect 116: 269–277, 2008

Review

Fertility and Markers of Male Reproductive Function in Inuit and European Populations Spanning Large Contrasts in Blood Levels of Persistent Organochlorines

Jens Peter Bonde,[1] Gunnar Toft,[1] Lars Rylander,[2] Anna Rignell-Hydbom,[2] Aleksander Giwercman,[3] Marcello Spano,[4] Gian Carlo Manicardi,[5] Davide Bizzaro,[6] Jan K. Ludwicki,[7] Valentina Zvyezday,[8] Eva C. Bonefeld-Jørgensen,[9] Henning Sloth Pedersen,[10] Bo A.G. Jönsson,[2] Ane Marie Thulstrup,[1] and INUENDO[11,]*

The study did not provide direct evidence of hormone-like activity of PCB-153 and of p,p´-DDE Serum concentrations of these compounds were not consistently related to either endogenous or exogenous hormone activity in serum

Several links bewteen POP exposure and biomarkers of male reproductive function were identified:
1) An association between high CB-153 serum levels and low sperm counts was detected within a subgroup of men with short androgen receptor CAG repeat length
2) A relationship between increased CB-153 serum concentrations and decreased sperm motility was seen in all four studied regions, and indications of reduced neutral α-glucosidase activity in seminal plasma point to a post-testicular effect
3) Damage of sperm chromatin integrity was considerably less frequent in Greenlandic Inuits compared with that in European groups, and only in the latter was impairment of sperm chromatin integrity related to POPs
4) Fertility in terms of time taken to conceive was not related to POPs except in Inuits (a likely explanation of the latter was not identified)

CONCLUSIONS
POPs may interfere with male reproductive function without major impact on fertility. The data do not provide direct evidence for endocrine disruption, hence other mechanisms should also be considered.

CONCLUSIONS

Occupational and environmental exposures can affect the fraction of genetically abnormal sperm

For many compounds, the association is only suspected or suggested and needs further evaluation before conclusions can be drawn

Exposure assessment should be more accurate, especially dealing with compounds associated with personal lifestyles

Need of studies relating sperm DNA integrity in the adult with his past exposure during pregnancy

Health consequences in the offspring are presently unknown

Effects of chronic inflammatory pathologies and medical treatments together with the importance of dietary factors and nutriceuticals should be considered in future studies

Need of identifying vulnerable subpopulations, which is critical to our understanding of risk and the protection of public health

Need of research on the underlying mechanisms by which chemicals may alter human fertility