

Oxidative DNA damage in human sperm influences time to pregnancy

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BACKGROUND: Oxidative stress and related DNA damage in human sperm may be important for fecundity and pregnancy outcome. **METHODS:** We studied the level of oxidative DNA damage in terms of 7-hydro-8-oxo-2'-deoxyguanosine (8-oxodG) in sperm DNA among 225 first-pregnancy planners. Over the six menstrual cycle follow-up time, after cessation of contraception, 135 pregnancies were conceived. **RESULTS:** The likelihood of pregnancy occurring in a single menstrual cycle was inversely associated with the 8-oxodG level ($P < 0.01$). The odds ratio of pregnancy in each of the first three or all six follow-up menstrual cycles was 0.42 (0.23–0.78; 95% CI) and 0.61 (0.36–0.91) per unit increase in the log 8-oxodG/100 000 dG ratio after adjustment for potential confounders, (including sperm concentration) respectively. The intra-individual coefficient of variation of 8-oxodG in 2–6 monthly repeated sperm samples from 116 men was 19% for the 8-oxodG/dG ratio, whereas the inter-individual coefficient of variation was 49%. The 8-oxodG level was not significantly associated with smoking, consumption of alcohol or caffeine, exposure to welding fumes or the plasma levels of sex hormones. **CONCLUSIONS:** The data suggest that oxidative damage to sperm DNA influences fecundity and the level of damage is relatively constant within an individual and not influenced by smoking.

Key words: 8-oxodeoxyguanosine/male fecundity/oxidative DNA damage/smoking/time to pregnancy

Introduction

The postulated secular, geographical and inter-individual variation in semen quality has been partly attributed to environmental factors, including estrogens, pesticides, phthalates, polychlorinated biphenyls (PCBs), air pollution and tobacco smoke acting in pre- and perinatal life or directly on sperm after puberty (Sharpe *et al.*, 1993; Vine *et al.*, 1994; Fisch and Goluboff, 1996; Vine, 1996). Besides potential hormone disrupting effects, most of the agents have the capacity to induce oxidative stress, which could damage sperm DNA. The need for study of oxidative stress in sperm and male infertility has recently been emphasised (Sharma *et al.*, 1996).

In human sperm DNA, substantial oxidative modification in terms of the oxidized deoxynucleoside, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), at the level of 2–4 per 100 000 deoxyguanosines (dG) has been demonstrated (Fraga *et al.*, 1991; 1996; Shen *et al.*, 2000). The level of 8-oxodG in sperm

DNA has been reported to be increased in smokers and the level correlated with the intake and seminal plasma concentration of vitamin C, the most important antioxidant in sperm (Fraga *et al.*, 1991; 1996; Shen *et al.*, 1997). If not repaired, 8-oxodG modifications in DNA are mutagenic and may cause embryonic loss, malformations or childhood cancers (Fraga *et al.*, 1991). Moreover, this modification could be a marker of oxidative stress in sperm which could also have negative effects on sperm function (Ni *et al.*, 1997; Chen *et al.*, 1997a;b; Shen *et al.*, 2000). Accordingly, the level of oxidative modifications in seminal DNA may be a valuable biomarker of environmental factors affecting sperm.

In a population of 266 healthy men from a cohort of 430 first pregnancy-planning couples, we studied oxidative modification in terms of 8-oxodG levels in DNA from up to six repeated semen samples and the relationship with life-style factors and semen quality in terms of volume, concentration and motility as well as with apparent male fecundity in terms of probability

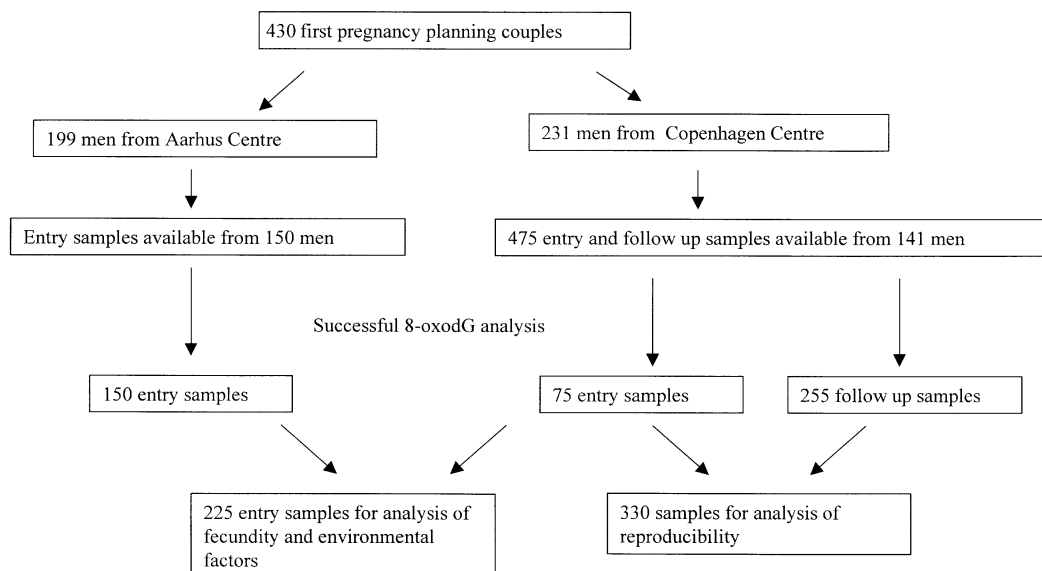


Figure 1. Diagram of available semen samples and successful analysis of 8-oxodG from a study of first pregnancy-planners.

Table I. Characteristics of female partners of 225 men with successful analysis of sperm samples for 8-oxodG

	Median	Interquartile range
Age (years)	25	24–27
Body weight (kg)	64	57–70
Height (cm)	169	165–173
Cycle length (days)	28	24–32
Consumption of:		
Cigarettes (no/day)	0	0–1
Alcohol (drinks/week)	3	1–6
Caffeine (mg/week)	256	123–450

of pregnancy during six menstrual cycles follow-up after cessation of contraception.

Materials and methods

The present material was part of a two-centre study of fecundity and measures of semen quality in 430 first pregnancy-planning couples described in detail elsewhere (Bonde *et al.*, 1998a;b). In brief, the study population was recruited by contacting, by means of a postal information brochure, 52 255 members of the trade unions for metal workers, office workers, nurses and daycare workers between 20–35 years of age, who had no children and who were co-habiting with a person of the opposite sex and the same age range. Eligible couples were those planning to discontinue contraception to achieve a pregnancy and had no previous reproductive history in either partner. The protocol was approved by the local ethics committee and the subjects granted informed consent in accordance with the Helsinki declaration. The subjects of the present study consisted of 141 and 150 men recruited from the Copenhagen and Jutland areas respectively.

Information regarding body weight, height, smoking, consumption of coffee, tea and alcohol and occupational exposures was obtained from a questionnaire. During follow-up over a period of six menstrual

cycles, after discontinuation of contraception, occurrence of pregnancy was verified by a physician or commercial pregnancy tests.

Semen samples were collected by masturbation. The subjects were asked to keep 2–3 days of abstinence prior to sample collection and report the actual abstinence period. The first sample was collected at entry and stored at -80°C . Subsequent samples were collected every month from cessation of contraception for at least 3 months and up to 6 months if conception had not occurred. Thus, each subject collected 3–6 samples. Except for the entry sample the samples were stored at -20°C for <3 months. The samples from the Copenhagen area and Jutland were stored for <12 and <30 months respectively at -80°C until analysis. The semen quality was determined in the entry sample in terms of volume, concentration, total number (volume \times concentration), motility and morphology. Number of sperm cells were counted in a Bürker-Türk or Makler chamber. The residual semen, after securing material for other analyses, was used for analysis of 8-oxodG in DNA. The repeated samples were available for 8-oxodG analysis from the Copenhagen area subjects only.

A total of 475 repeated semen samples from 141 individuals from the Copenhagen area was available for analysis of 8-oxodG in DNA (Figure 1). Of these samples, 65 were run in batches with insufficient analytical quality control and thus excluded, and sufficient DNA could not be extracted from 80 samples. A total of 330 samples from 116 subjects were successfully analysed. Seventy-five entry samples from the Copenhagen area and all the 150 available entry samples from Jutland were successfully analysed. Probably due to a correlation between seminal volume and availability of sufficient material for 8-oxodG analysis there were significant differences between sperm cell concentration in samples with successful 8-oxodG analysis ($58; 30\text{--}95 \times 10^6/\text{ml}$; median; interquartile range; $n = 225$) and in those without successful analysis ($42; 17\text{--}78 \times 10^6/\text{ml}$; $n = 193$) in the whole population ($P = 0.00048$; Mann–Whitney U -test).

The personal characteristics of the female partners of the 225 male subjects with successful 8-oxodG analysis are summarized in Table I. Of these women 19 (8.4%) reported being told by a doctor that they had one of the following diseases: salpingitis, ovarian cyst, gonorrhoea, perforated appendicitis or other diseases that may affect fecundity, whereas 63 (28%) were reported to be smokers.

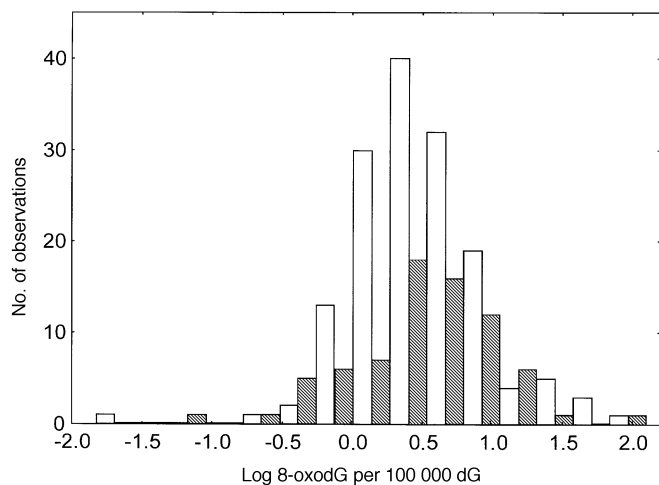


Figure 2. Frequency histogram of oxidative DNA damage in terms of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG per 10^5 dG) in DNA from sperm samples from 225 men, of whom 74 were smokers (hatched bars) and 151 were non-smokers (open bars).

Analysis of 8-oxodG in sperm DNA

The levels of 8-oxodG and dG in nuclear sperm DNA were determined by HPLC with electrochemical and UV detection as previously described (Loft *et al.*, 1999). In principle, DNA is extracted from nuclei isolated from the sample and digested to the deoxynucleosides by means of nuclease P1 and alkaline phosphatase. The level of 8-oxodG is reported per 10^5 unoxidized dG. The assay coefficient of variation was 6.9% as assessed from 41 samples analysed in triplicate.

In separate experiments calf thymus DNA (Sigma, St Louis, MO, USA) was enzymatically digested and analysed on the HPLC apparatus before and after being subjected to the isolation procedure used for sperm DNA. Similarly, dG was analysed before and after being subjected to enzymatic digestion. No generation of 8-oxodG was found under these circumstances (data not shown). The 8-oxodG levels were stable under the storage conditions at either -20°C or -80°C during the period.

Data analysis

The 8-oxodG to dG ratio in DNA from the entry sample was used for data analysis. For each variable the fit to the normal distribution before and after log transformation of the data was tested by probit analysis and the Kolmogorov–Smirnov test.

The relationship between the recorded host factors and the log 8-oxodG/dG ratio was investigated in bivariate analysis. The *t*-test or Mann–Whitney *U*-test were used for the effect of binominal variables according to the distribution. Pearson product moment or Spearman rank correlations coefficients were calculated for continuous variables. The effect of all recorded host factors was investigated by stepwise multiple regression analysis with forwards variable selection as well as backwards variable exclusion. The sperm concentration was log transformed before analysis.

The relationship between the explanatory variables, including log 8-oxodG level, and the time to pregnancy was investigated by a discrete time survival analysis, where the time scale was the number of menstrual cycles since entering the study. This analysis was carried out as a logistic regression on the total number of observed cycles with the outcome pregnant/not pregnant and with cycle number as well as the variables of interest as explanatory variables, as previously described (Bonde *et al.*, 1998a). For the 225 couples with available 8-

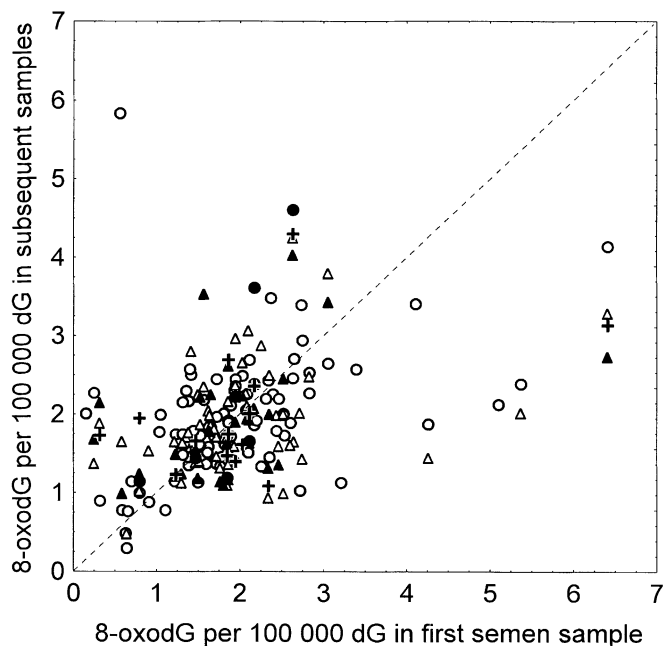


Figure 3. Repeatability of oxidative DNA damage in terms of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG per 10^5 dG) in DNA from 1–6 sperm samples collected once a month from 116 men. The level of 8-oxodG in up to five follow-up sperm samples is plotted on the level in the initial sample. (open circle = 2.; open triangle = 3.; closed triangle = 4.; + = 5.; closed circle = 6. sample).

oxodG level in the entry sperm sample, a total of 886 menstrual cycles (in which sexual intercourse was reported between cycle days 11–20) were included in the analysis. For a separate analysis of the likelihood of pregnancy within the first three menstrual cycles, 570 cycles were available for analysis. Odds ratios were calculated to describe the relationship between menstrual cycle outcome (pregnant/non-pregnant) and the level of 8-oxodG as well as sperm density and smoking status. The odds ratios were adjusted for menstrual cycle number as well as other potential confounders, including laboratory, occupation, age of woman, presence/absence of female urogenital disorders, woman's body-mass index (BMI), length of menstrual cycle, oral contraception as last method of contraception and woman's or man's smoking status.

Results

Figure 2 shows the distribution of the 8-oxodG to dG ratio in sperm DNA among the male subjects fitted to a log-normal distribution according to probit analysis and Kolmogorov–Smirnov tests. The inter-individual variation in the 8-oxodG level was substantial, i.e. the coefficient of variation was 49%, whereas the intra-individual variation of the 8-oxodG to dG ratio in repeated samples from the same man was much less, i.e. the coefficient of variation was 19.6% (Figure 3). Moreover, there was no systematic change within a subject from the first to the last sample.

During the follow-up period of the first three menstrual cycles 81 women (36%) conceived whereas a total of 135 (60%) conceived during the full six-cycle follow-up period. The likelihood of pregnancy during the six-menstrual cycle

Table II. Odds ratios for the occurrence of pregnancy during a single menstrual cycle in relation to oxidative DNA damage and sperm concentration in 225 first pregnancy-planning couples followed for six menstrual cycles

	Pregnancy within three cycles follow up			Pregnancy within six cycles follow up		
	No. pregnant	No. cycles	Odds ratio (95% CI)	No. pregnant	No. cycles	Odds ratio (95% CI)
8-oxodG level						
10 ⁻⁵ per dG increase [†]	81	570	0.46 (0.28–0.78)	135	886	0.66 (0.44–0.99)
Adjusted*			0.45 (0.25–0.79)			0.59 (0.38–0.92)
<1.23 8-oxodG/10 ⁵ dG	22	129	1.0	30	196	1.0
1.23–1.6 8-oxodG/10 ⁵ dG	26	149	0.98 (0.52–1.84)	40	216	1.25 (0.74–2.10)
Adjusted*			0.93 (0.48–1.36)			0.98 (0.56–1.71)
>1.6–2.2 8-oxodG/10 ⁵ dG	21	155	0.75 (0.39–1.45)	36	244	0.93 (0.55–1.59)
Adjusted*			0.64 (0.31–1.36)			0.77 (0.44–1.34)
>2.2 8-oxodG/10 ⁵ dG	12	137	0.46 (0.21–0.97)	29	230	0.67 (0.36–1.23)
Adjusted*			0.44 (0.20–0.97)			0.64 (0.35–1.17)
Sperm concentration						
1×10 ⁶ /ml increase ^{†*}	81	570	1.26 (0.93–1.71)	135	886	1.18 (0.96–1.45)
Smoking (men)						
Non-smoker	62	377	1.0	95	571	1.0
Smoker	19	193	0.55 (0.32–0.94)	40	315	0.71 (0.48–1.07)
Adjusted*			0.66 (0.37–1.19)			0.97 (0.62–1.53)

[†]Log transformed before analysis, odds ratio relates to transformed value.

*Adjusted for menstrual cycle number, laboratory location, occupation of index person, age of women, presence/absence of female urogenital disorders, women's body-mass index, length of menstrual cycle, oral contraception as last method of contraception and women's or men's smoking status.

Table III. Relationship between anthropometric variables, life style factors and semen quality with the level of 8-oxodG in seminal DNA in 225 healthy men

	Mean ± SD or median (range)	Correlation with 8-oxodG level
Body weight (kg)	81 ± 11	Rs = 0.03
Body height (cm)	182 ± 6	Rs = -0.01
Age (years)	28 ± 3	Rs = -0.01
Consumption of		
Cigarettes (no/day)	0 (0–40)	Rs = 0.02
Caffeine (mg/day)	414 (0–2500)	Rs = -0.09
Alcohol (drinks/week)	7 (0–42)	Rs = 0.04
Welding during last 3 months (h/day)	0 (0–7.5)	Rs = -0.08
Seminal characteristics		
Volume (ml)	3.21 (0.5–9.0)	Rs = 0.02
Sperm concentration (×10 ⁶ /ml)	58 (3.3–408)	Rs = -0.14*
Total number of sperm	230 (3.6–1148)	Rs = -0.13*
Immotile sperm (%)	38 (19–70)	Rs = 0.03
Morphology (% normal)	41 ± 10	Rs = -0.11
Days of sexual abstinence (<i>n</i> = 200)	4.19 (1–30)	Rs = 0.13

**P* < 0.05; Rs = Spearman correlation coefficients.

follow-up was significantly inversely associated with the 8-oxodG level (Table II). The association was strongest in the first three cycles. The relationship between the likelihood of pregnancy and the 8-oxodG level was independent of smoking and other potential confounders (Table II) as well as of sperm concentration. With further adjustment for log sperm concentration in the logistic regression model the odds ratio of pregnancy in the first three and all six follow-up menstrual cycles was 0.42 (0.23–0.78; 95% CI) and 0.61 (0.39–0.96) per unit increment in the log 8-oxodG/100 000 dG ratio respectively.

The likelihood of pregnancy was also associated with the sperm concentration, morphology and total number of sperm after three and six cycles of follow-up as previously shown for the whole population (Bonde *et al.*, 1998a), although the associations were not statistically significant in the present material (Table II). This was probably due to selection of men with surplus sperm material for analysis of 8-oxodG. An apparently negative effect of the men's smoking status on the likelihood of pregnancy was partly related to confounding by women's smoking status and was not significant after adjustment for that.

Table IV. Comparison of semen quality and 8-oxodG level between smokers and non-smokers among 225 men

	Non-smokers (n = 151)	Smokers (n = 74)	95% CI for difference (% of non-smoker mean)
Seminal volume (ml)	3.19 ± 1.58	3.26 ± 1.54	-16.2-11.2
Sperm concentration (×10 ⁶ /ml)*	60 (1.1-262)	57 (4.5-142)	-6.0-6.6
Number of sperm*	156 (0.6-666)	149 (3.6-445)	-5.9-5.4
Immotile sperm (%)	36.5 ± 11.3	37.0 ± 16.0	-11.3-8.6
Morphology (% normal)	41.7 ± 9.6	40.2 ± 9.4	-2.6-10.1
8-oxodG per 100 000 dG*	1.83 ± 1.04	1.79 ± 0.91	-23.8-32.4

*Log transformed before analysis and estimation of CI for difference; values are mean ± SD or median (range).

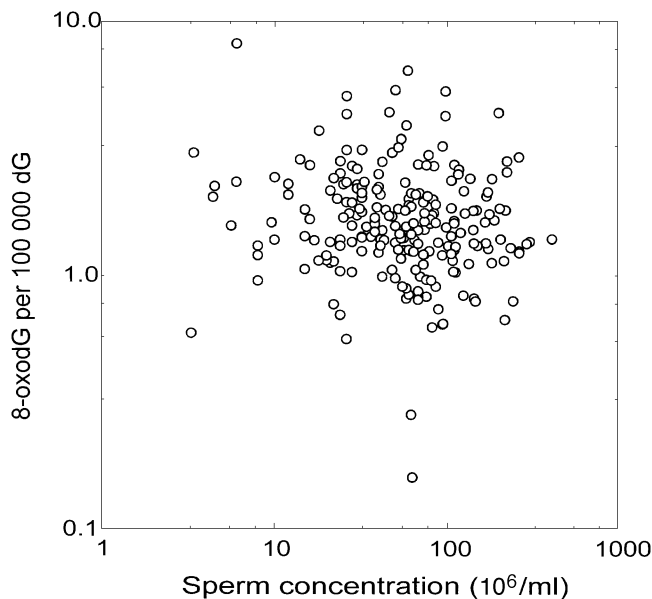


Figure 4. Relationship between the oxidative damage in terms of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG per 10⁵ dG) in sperm DNA and the sperm concentration in samples from 225 men.

None of the recorded anthropometric variables, life-style factors or occupational exposures were significant predictors of the 8-oxodG level in seminal DNA, either in bivariate analysis or in multiple regression analysis (Tables III and IV). There were no differences in the levels of 8-oxodG between the subjects from the different trade unions (data not shown). Similarly, the level of 8-oxodG among 80 men reporting daily welding was 1.67 ± 0.79 per 10^5 dG and 1.90 ± 1.09 8-oxodG per 10^5 dG among 145 men not reporting welding ($P = 0.11$; t -test on log transformed values). Similarly, there was no difference in sperm quality measures between smokers and non-smokers among the men (Table IV).

The 8-oxodG to dG ratio showed a significant negative correlation with the sperm density ($R = 0.14$) and the total number of sperm (Table III; Figure 4) whereas there was no significant correlation with the semen volume, morphology or fraction of immobile sperm (Table III). In multiple linear regression, with log transformed values where necessary, none

of the recorded variables were predictors of the 8-oxodG level (data not shown). Similarly, there was no significant correlation between the 8-oxodG to dG ratio and plasma/serum hormone levels, including testosterone, LH, FSH, estradiol and inhibin ($n = 197$; data not shown).

Discussion

The present data corroborate that oxidative modification in terms of 8-oxodG is present in sperm DNA to an extent corresponding to other cells in the body. Moreover, the data indicate a decreasing likelihood of pregnancy with increasing levels of 8-oxodG, suggesting that oxidative DNA damage is an independent predictor of fecundity. The 8-oxodG level was weakly inversely correlated with the total sperm count and density and relatively constant in an individual, whereas none of the recorded life-style or occupational factors were significantly associated with the level of 8-oxodG or independent predictors of fecundity. Contrary to other reports (Fraga *et al.*, 1996; Shen *et al.*, 1997), the smokers in the present data did not have increased levels of 8-oxodG in sperm DNA.

Previously, the level of 8-oxodG has been shown to be elevated in semen from infertile patients compared with healthy subjects (Kodama *et al.*, 1997; Shen *et al.*, 1999; 2000). The present data show that among men without prior knowledge of their fecundity the level of 8-oxodG correlate with their probability of conceiving a child. Moreover, this relationship was independent of the sperm concentration and other potential confounders, including smoking. Interestingly, the strongest predictive value for the 8-oxodG level was related to the first three menstrual cycles after cessation of contraception. This attenuation of the predictive value occurred despite that the level of 8-oxodG appeared to be relatively constant within an individual for at least 6 months.

An inverse relationship between the level of 8-oxodG in DNA and sperm density or number has been reported previously (Kodama *et al.*, 1997; Ni *et al.*, 1997; Shen *et al.*, 2000). In view of the reported declining sperm count in the last decades (Carlsen *et al.*, 1992; Sharpe *et al.*, 1993; Vine *et al.*, 1994; Auger *et al.*, 1995; Fisch and Goluboff, 1996; Fisch *et al.*, 1996; Vine, 1996) a possible association between sperm count and oxidative DNA damage in semen appears interesting. 8-OxodG is a mutagenic DNA modification which could cause

embryonic loss, malformations or childhood cancers (Fraga *et al.*, 1991). Moreover, 8-oxodG is a marker of oxidative stress which *per se* has negative effects on sperm function (Ni *et al.*, 1997; Chen *et al.*, 1997a;b; Shen *et al.*, 2000). However, the data should be interpreted with caution. A major problem regarding the measurement of 8-oxodG in DNA relates to artefactual oxidation of dG during the analysis (Collins *et al.*, 1997; Helbock *et al.*, 1998; Loft *et al.* 1999; ESCODD, 2002). Among a number of other factors low amounts of DNA are prone to give high values of the 8-oxodG to dG, possibly partly due to contamination of the HPLC system with dG (Loft *et al.*, 1999). Although our method includes maximum precautions against this problem and a fixed number of sperm cells was analysed when possible, it cannot be excluded that the DNA yield is a potential confounder. In agreement with the present data some reports have not found significant associations between 8-oxodG in DNA and motility in sperm or donor age (Fraga *et al.*, 1991; 1996), whereas reports have found inverse correlations with cells with normal morphology and motility in samples from infertile men (Kodama *et al.*, 1997; Shen *et al.*, 1999; 2000).

In two other cross-sectional studies, one with 41 men from California and Argentina, and one with 60 men from China, 50–60% higher levels of 8-oxodG in sperm DNA was found in smokers as compared with non-smokers (Fraga *et al.*, 1996; Shen *et al.*, 1997). In contrast, in the present population of 225 men, the 81 smokers did not have increased levels and the 95% confidence interval (CI) exclude differences of such magnitude. The 8-oxodG level in sperm DNA was ~ 1.8 per 10^5 dG in our material, i.e. well within the range of the levels in American men with average levels of 2.1 per 10^5 dG in subjects with unknown smoking status and 1.3 and 2.0 per 10^5 dG in non-smokers and smokers (Fraga *et al.*, 1991; 1996). In the study from China the reported average levels were 3.9 and 6.2 per 10^5 dG in non-smokers and smokers respectively (Shen *et al.*, 1997). Thus, our data and the lack of effect of smoking on 8-oxodG level are not likely to be a result of artefacts, as these were controlled for in our assay development. However, there are several potential differences between the study populations. In our samples the sperm count was independent of smoking and much lower than in the American samples, which had a total count of 279 and 358×10^6 per sample on average in smokers and non-smokers respectively (Fraga *et al.*, 1996). In the Chinese study the total sperm number was more similar to our data and not significantly different between smokers and non-smokers. In another American study the sperm count was significantly lower in samples with low vitamin C concentration, i.e. 167×10^6 per sample as compared with 294×10^6 in samples with high vitamin C (Fraga *et al.*, 1991). Similarly, the 8-oxodG level in sperm DNA was highly dependent on the intake and seminal plasma concentration of vitamin C as shown by a significant correlation among 24 men and a depletion and replenishment study in eight men (Fraga *et al.*, 1991; Jacob *et al.*, 1991). Similarly, in a small study of infertile men a combination of vitamin C, α -tocopherol and glutathione for 2 months decreased the levels of 8-oxodG in sperm to the levels of fertile men (Kodama *et al.*, 1997). Smokers have decreased plasma levels of vitamin C

(Lykkesfeldt *et al.*, 1997) due to both increased useage and lower intake as compared with non-smokers (Lykkesfeldt *et al.*, 1996; Zondervan *et al.*, 1996). In the study on 8-oxodG in sperm from American men the intake of vitamin C assessed by questionnaire in a subset of men was 26% lower in smokers than in non-smokers although the level of vitamin C in seminal plasma was not significantly different between the groups (Fraga *et al.*, 1996). Nevertheless, the level of α -tocopherol was significantly lower in seminal plasma from smoking as compared with non-smoking American men (Fraga *et al.*, 1996). Unfortunately, it was not possible to assess the intake of antioxidant vitamins or the levels in seminal plasma in the present study. In general, the intake and plasma concentration of vitamin C are low in Danish men (Lykkesfeldt *et al.*, 1996; 1997). Accordingly, the discrepancy between American, Chinese and Danish men with respect to the apparent effect of smoking on the 8-oxodG level in seminal DNA is as yet unexplained but could partly be related to differences in genetic background, sperm counts and diet, including vitamin C intake. It is also possible that the necessary selection of samples with surplus semen material affected our results.

Many studies have attempted to assess the effect of tobacco smoking on semen quality, yielding variable results. A meta-analysis showed that sperm concentration and volume may be decreased by 13–18% on average in smokers (Vine *et al.*, 1994). A more recent large study including around 500 smokers and 500 non-smokers found no effect of smoking on standard semen parameters (Trummer *et al.*, 2002). Moreover, chromatin structure was not affected in smokers (Sergerie *et al.*, 2000). On the other hand, DNA adducts from polyaromatic hydrocarbons present in cigarette smoke have been reported to be elevated in sperm from smokers (Zenzes *et al.*, 1999) and substances in seminal plasma from smokers seem to decrease the viability of sperm (Zavos *et al.*, 1998). In line with the described apparent relationship with oxidative DNA modification, ascorbic acid may be important for standard measures of sperm quality. Thus, supplementation with vitamin C 200 or 1000 mg per day increased sperm concentration and viability in heavy smokers (Dawson *et al.*, 1992). However, in the present relatively small population there were no significant differences in sperm quality between smokers and non-smokers or in fecundity after controlling for women's smoking status, which is an important determinant of fecundability (Jensen *et al.*, 1998). As for the level of 8-oxodG in sperm DNA, factors other than smoking may be more important for sperm quality in Danish men.

There was no effect of exposure to welding on the level of 8-oxodG in sperm in the present material. Nevertheless, welding fumes, particularly from stainless steel welding, may contain oxidants such as hexavalent chromium, possibly inducing oxidative stress and genotoxicity. Actually, stainless steel welding was associated with an increased risk of spontaneous abortion in spouses in the full cohort of the present study, suggesting genotoxic effects in the sperm (Hjollund *et al.*, 2000).

A number of markers of DNA damage and chromatin integrity have been assessed as predictors of pregnancy outcome. In the present study, and other reports in particular,

the sperm chromatin structure assay has been valuable (Spano *et al.*, 1998; Evenson *et al.*, 1999; 2002; Larson *et al.*, 2000). The level of 8-oxodG in sperm DNA may be added to these assays, although the analysis is far from trivial, due to the risk of artificial oxidation of the DNA during analysis (ESCODD, 2002)

In the present study selection bias may have occurred during the recruitment of subjects, due to the necessary selection of samples with surplus semen material as well as the required successful analysis of the samples for 8-oxodG. Indeed, the association between the sperm concentration and the likelihood of pregnancy was not significant in the subgroup of the present study. It is possible that the 8-oxodG level is not a significant predictor of the likelihood of pregnancy in subjects with a low sperm count, which will be the dominant predictor. Similarly, it cannot be excluded that selection affected the apparent lack of association between the life-style and occupational factors and the level of 8-oxodG in this study.

In conclusion, the present study confirmed the substantial presence of oxidative modification in terms of 8-oxodG in seminal DNA and showed that the level is relatively constant in an individual. Moreover, the data indicate that a high level of oxidative DNA damage independently predicts decreased male fecundity. The 8-oxodG level was weakly inversely correlated with the sperm count and density but not with other measures of semen quality. None of the recorded occupational and life-style factors, including smoking, were significant independent predictors of the level. Accordingly, 8-oxodG is an interesting biomarker of oxidative damage in seminal DNA rewarding further study and development.

Acknowledgements

Christopher Krumbach and Anette Haumann are thanked for expert technical assistance. This work was supported by the Danish Strategic Environmental Research Programme, Aarhus University Research Foundation, the Danish Medical Research Council and the Danish Health Insurance Foundation.

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Submitted on June 26, 2002; accepted on January 14, 2003