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Sperm DNA damage and semen quality
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· *Original Article* ·

Sperm DNA damage in men from infertile couples

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Abstract

Aim: To investigate the prevalence of high levels of sperm DNA damage among men from infertile couples with both normal and abnormal standard semen parameters. **Methods:** A total of 350 men from infertile couples were assessed. Standard semen analysis and sperm chromatin structure assay (SCSA) were carried out. **Results:** Ninety-seven men (28% of the whole study group) had a DNA fragmentation index (DFI) > 20%, and 43 men (12%) had a DFI > 30%. In the group of men with abnormal semen parameters (n = 224), 35% had a DFI > 20%, and 16% had a DFI > 30%, whereas these numbers were 15% and 5%, respectively, in the group of men with normal semen parameters (n = 126). Men with low sperm motility and abnormal morphology had significantly higher odds ratios (ORs) for having a DFI > 20% (4.0 for motility and 1.9 for morphology) and DFI > 30% (6.2 for motility and 2.8 for morphology) compared with men with normal sperm motility and morphology. **Conclusion:** In almost one-third of unselected men from infertile couples, the DFI exceeded the level of 20% above which, according to previous studies, the *in vivo* fertility is reduced. A significant proportion of men with otherwise normal semen parameters also had high sperm DNA damage levels. Thus, the SCSA test could add to explaining causes of infertility in cases where semen analysis has not shown any deviation from the norm. We also recommend running the SCSA test to choose the appropriate assisted reproductive technique (ART). *(Asian J Androl 2008 Sep; 10: 786–790)*

Keywords: infertility; sperm DNA damage; sperm chromatin structure assay; semen quality

1 Introduction

Sperm chromatin structure and DNA integrity are known to have a crucial influence on reproductive outcomes [1–7], including fertilization and embryonic transplantation rates in assisted reproductive technique (ART) procedures [6–9]. It has been suggested that, in contrast to standard semen parameters, which do not act as powerful discriminators between fertile and infertile men [10], sperm DNA damage assessment yields better prognostic value. It has been shown that fecundity starts to decrease when sperm DNA damage, expressed as the DNA fragmentation index (DFI) in sperm chromatin structure assay (SCSA), exceeds 20% [4, 5]. Above a threshold of 30%, chances for fertilization are close to zero, either by means of natural conception [4, 5] or intrauterine insemination (IUI) [6, 7]. This shows that the DFI

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possesses a high predictive value for male infertility *in vivo* (both natural and IUI fertilization). In addition, it has been suggested that in cases with a DFI > 30%, intracytoplasmic sperm injection (ICSI) should be the method of choice [7] because a high DFI is not associated with a decreased success rate in ICSI procedures, in contrast to IUI and *in vitro* fertilization (IVF) [6, 7, 11].

Infertile men are reported to have a higher fraction of sperm with chromatin defects and DNA breaks than fertile controls [12–14]. Sperm DNA damage assessment has been recommended as a complementary test in male infertility work-up by some authors [11, 15]. However, it is still unclear whether sperm DNA damage assessment should be introduced as a routine test in infertile men or only applied in selected cases. There are few studies addressing the issue of prevalence of high levels of sperm DNA damage among infertile men. Such prevalence was reported to be 17% when the 30% DFI threshold was used [12], and 58% using the 24% DFI threshold [13]. The latter threshold was chosen because all fertile men in that particular study showed a DFI below this level. In addition, there are conflicting data whether infertile men with normal standard semen parameters have increased levels of sperm DNA damage that could explain the infertility of the couple. Although some studies have shown increased sperm DNA damage (DFI > 24%) in 40% of these men [13, 14], other studies reported that infertile men with normal semen parameters infrequently (8%) have a DFI > 30% [12], or fail to show increased sperm DNA damage levels among infertile men with normal conventional semen parameters, as compared to fertile donors [16].

Therefore, the aim of this study was to investigate the prevalence of sperm DNA damage in a large group of infertile men with both normal and abnormal semen parameters in order to elucidate whether SCSA analysis can add to the information obtained by routine semen analysis when explaining the causes of infertility.

2 Materials and methods

2.1 Materials

Unless otherwise stated, all chemicals were obtained from Sigma Chemical (St. Louis, MO, USA).

2.2 Semen donors

This retrospective study involved 350 consecutive male patients from infertile couples referred to the Fertility Centre, Malmö University Hospital, Malmö, Sweden, for fertility counselling during the period 2001–2004. Infertility was defined as the inability to conceive after at least 1 year. No information regarding female partners was available for the study group.

2.3 Sperm quality measures

2.3.1 Standard semen parameter measurements

Samples were obtained by masturbation. Only one ejaculate from each patient was obtained. The patients were recommended 2–5 days of sexual abstinence, although in each case the actual abstinence period was noted. Samples were allowed to liquefy for 30 min. Standard semen parameters (volume, concentration and motility) were measured according to the World Health Organization (WHO) guidelines [17]. Sperm concentration was assessed using positive displacement pipettes and an improved Neubauer hemocytometer. Sperm motility was graded into four groups: rapid progressive motility, slow progressive motility, non-progressive motility, or immotile sperm. Sperm morphology was assessed after Papanicolaou staining following WHO guidelines for the staining procedure. A level of ≥ 5% was regarded as the threshold for normal morphology. Semen parameters were regarded as normal in men with sperm concentration ≥ 20 × 10^6/mL, progressive motility ≥ 50% and/or rapid progressive motility ≥ 25%, and a proportion of morphologically normal sperms ≥ 5%.

2.3.2 Sperm DNA damage assessment

Sperm DNA damage was evaluated by SCSA. The SCSA was applied following the procedure described earlier, using staining with acridine orange (AO) [18, 19]. An aliquot of unprocessed semen (13–70 µL) was diluted to a concentration of 1–4 × 10^6 sperm/mL with TNE buffer (0.01 mol/L Tris-HCl, 0.15 mol/L NaCl, and 1 mmol/L ethylenediaminetetraacetic acid [EDTA], pH 7.4). This cell suspension was treated with an acid detergent solution (pH 1.2) containing 0.1% Triton X-100, 0.15 mol/L NaCl, and 0.08 mol/L HCl for 30s, then stained with 6 mg/L purified AO (Polysciences, Warrington, PA, USA) in a phosphate–citrate buffer (pH 6.0). Under these experimental conditions, AO intercalated in double-stranded DNA emits green fluorescence, and AO associated with single-stranded DNA emits red fluorescence. Thus, sperm chromatin damage can be quantified by flow cytometry measurements of the metachromatic shift from green (native, double-stranded DNA) to red (relaxed,

single-stranded DNA) fluorescence. Measurements were stopped after measuring 10 000 spermatozoa using a FACSort flow cytometer (Becton Dickinson, San Jose, CA, USA). Adopting guidelines published by Evenson *et al.* [18], the extent of DNA denaturation was expressed in terms of the DFI, which is the ratio of red to total (red plus green) fluorescence intensity, using SCSAsoft 1.0 software (SCSA Diagnostics, Brookings, SD, USA). For the flow cytometer set-up and calibration, aliquots were used from a normal human ejaculate sample retrieved from the laboratory repository. All SCSA analyses were carried out by one person.

2.4 Data analysis

The prevalence of patients with a DFI > 20% and a DFI > 30% was calculated in the whole study group, and separately in patients with normal and abnormal standard semen parameters. The prevalence in groups was compared using Fisher's exact test. Odds ratios for having a high DFI (DFI > 20% and DFI > 30%) in men with abnormal vs normal sperm concentration (< 20 × 10^6/mL *vs.* ≥ 20 × 10^6/mL), sperm motility (< 50% progressive motility and < 25% rapid progressive motility *vs.* ≥ 50% progressive motility or ≥ 25% rapid progressive motility), and morphology (< 5% normal forms *vs.* ≥ 5% normal forms) were calculated using the logistic regression analysis model, including abstinence time and age as potential confounders. $P < 0.05$ were considered statistically significant. Statistical analysis was carried out using SPSS 15.0 software (SPSS, Chicago, IL, USA).

3 Results

Semen characteristics of the whole study group, and for study groups with normal (n = 126) and abnormal (n = 224) semen parameters, are given in Table 1. Men with normal semen parameters had a significantly lower DFI (13.0 ± 7.7) than men with abnormal semen parameters (20.4 ± 12.5, P < 0.001). Abstinence time, in contrast to the age of the patient, was found to be a significant confounder for the DFI (P = 0.015).

Ninety-seven men (28%) of the whole study group had a DFI > 20%, and 43 men (12%) had a DFI > 30%. There was a significantly larger proportion of men with high DFI levels in the group with abnormal semen parameters compared with the group with normal semen quality, 35% *vs.* 15% for DFI > 20% (P < 0.001), and 16% *vs.* 5% for DFI > 30% (P = 0.002).

Odds ratios (ORs) for high DFI levels in men with abnormal vs normal semen parameters are shown in Table 2.

Table 1. Descriptive statistics of all men from infertile couples who took part in this study, and for those with normal and abnormal semen parameters. Results expressed as means (SD). DFI, DNA fragmentation index.

Variables	Whole study group (n = 350)	Men with normal semen parameters (n = 126)	Men with abnormal semen parameters (n = 224)
Time of sexual abstinence (days)	4.5 ± 4.0	5.2 ± 5.8	4.1 ± 2.1
Semen volume (mL)	4.3 ± 1.9	4.2 ± 1.6	4.4 ± 2.1
Sperm concentration (× 10^6/mL)	61.2 ± 60.3	103.1 ± 64.0	42.1 ± 41.3
Progressive motility (%)	48.2 ± 19.7	64.4 ± 11.0	40.4 ± 17.4
Normal forms (%)	5.5 ± 4.2	9.7 ± 4.8	3.5 ± 3.0
DFI (%)	18.1 ± 12.0	13.0 ± 7.7	20.4 ± 12.5

Table 2. Odds ratios (ORs) for high DNA fragmentation index (DFI) in men with abnormal *vs.* normal sperm concentration (< 20 × 10^6/mL *vs.* ≥ 20 × 10^6/mL), sperm motility (< 50% progressive motility and < 25% rapid progressive motility *vs.* ≥ 50% progressive motility or ≥ 25% rapid progressive motility), and morphology (< 5% normal forms *vs.* ≥ 5% normal forms). CI, confidence interval.

Variables	DFI > 20 %			DFI > 30 %		
	OR	95% CI		OR	95% CI	
		Lower	Upper		Lower	Upper
Sperm concentration (× 10^6/mL)	1.5	0.8	2.7	0.6	0.3	1.3
Progressive motility (%)	4.0	2.2	7.3	6.2	2.5	15.1
Sperm morphology (%)	1.9	1.0	3.4	2.8	1.2	6.4

Men with both low sperm motility and abnormal sperm morphology had significantly higher ORs for showing high DFI levels compared with men with normal sperm motility and normal morphology.

4 Discussion

Our data indicate that almost one-third of men from infertile couples, regardless of their standard semen quality parameters, have sperm DNA damage at levels that have been shown to have a negative impact on fecundity (DFI > 20%) [4, 5]. Furthermore, a DFI above 30 % was found in 12 % of these men. Although association with couple fecundity has been found for standard sperm parameters, no clear cut-off levels for fertility or infertility have been established [10, 20]. However, a number of studies have shown that almost no conceptions *in vivo* occur when the DFI exceeds the level of 30 % [4–7]. Thus, in a significant proportion of males seeking clinical help for infertility, SCSA can help by explaining the reason for infertility.

Our data show that decreased sperm motility and morphology is a significant predictive factor for high sperm DNA damage. ORs for having high DNA damage were highest for diminished sperm motility, which is in line with previous data, showing that DNA damage has its strongest negative correlation with sperm motility among all semen parameters [14, 21–23]. Oxidative stress (reactive oxygen species [ROS] activity) damages cell membranes and inhibits sperm motility, and this same ROS activity likely causes sperm DNA fragmentation [3, 24]. However, even in men with normal sperm motility, as well as other standard semen parameters, DFI levels of more than 20% or 30% could be observed.

Apart from helping to explain the infertility problem of a couple, SCSA analysis was previously shown to help decision-making when an ART needs to be applied. It has been shown that IUI procedures are unsuccessful in men with DFI > 30% [6, 7]. Therefore, IVF or ICSI should be used, with the latter yielding higher success rates compared to IVF in these cases [6, 7, 11]. Sperm DNA damage assessment in infertile couples can be recommended to choose the most appropriate ART method, even if a man has a normal standard semen analysis. Although only 5% of these men, according to our data, will show a DFI > 30% it is probably still feasible to use SCSA to avoid IUI procedures with highly improbable chances of success. The limitation of this study is the lack of data for female partners from the infertile couples. It is possible that if we limited the group of men with normal semen parameters to those with partners without fertility problems, the proportions of men with DFI > 20% and DFI > 30% would be larger than 16% and 5%, respectively, as in our unselected group.

In conclusion, the proportion of men with a high DFI is greater among those with abnormal standard semen parameters compared with those with normal semen parameters, and an increased level of DFI is most often found in men with astheno- and teratozoospermia. However, in a significant proportion of men with otherwise normal semen parameters, the DFI exceeded the level of 20% above which, according to previous studies, *in vivo* fertility is reduced. Thus, the SCSA test could help to explain the causes of infertility in cases where semen analysis has not shown any deviation from the norm. Furthermore, because 16% of men with abnormal semen analysis, and 5% of men with normal semen analysis, showed a DFI > 30% (in the group of men from infertile couples unselected in regards to female factors), sperm DNA damage assessment is important for choosing the most appropriate ART method. IUI procedures should not be recommended when the DFI > 30%.

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