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Flow cytometric sperm chromatin structure assay as an independent descriptor of human semen quality[1]

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Semen quality is conventionally estimated according to the number, motility, and morphology of spermatozoa in the ejaculate. These parameters are pivotal in the laboratory assessment of male reproductive function and its possible impairment due to environmental and occupational exposure to toxic compounds.

However, it is widely acknowledged that the conventional measures of semen quality have several limitations in the detection of male reproductive effects and the characterization of male fertility status. More sensitive tests are needed to identify additional defects (not accessible under the light microscope) that make certain spermatozoa unable to fertilize.

Scientific curiosity has recently been aroused by the role of chromatin condensation and stability in relation to sperm fertilization potential. A variety of flow cytometric (FCM) methods, based on the uptake of DNA-specific fluorescent probes, is available with which to evaluate the process of sperm chromatin packaging and its derailment from normality (1).

In particular, the sperm chromatin structure assay (SCSA), which involves the use of acridine orange (AO) to monitor the susceptibility of sperm chromatin DNA (deoxyribonucleic acid) to acid-induced denaturation in situ (2), can offer several advantages. Anomalies in sperm chromatin structure can easily be detected by FCM measurement of the metachromatic shift of AO fluorescence from green (native, double-stranded DNA) to red (denatured, single-stranded DNA). Poor quality semen samples are characterized by the presence of sperm with an increased proportion of single-stranded DNA regions in their nuclei. Studies on humans and domestic animals have also suggested a relationship between poor sperm chromatin quality and impaired fertility (2). Furthermore, SCSA has been extensively applied in experimental reproductive toxicology studies, and sperm with chromatin defects have been induced in laboratory animals exposed to a variety of noxious agents (3, 4). The variability of the SCSA parameters among the human population, not biased by the presence of subfertile patients, has recently been described in a study carried out on 45 healthy American men, and the correlation between conventional andrological parameters and the SCSA data was found to be weak (5). In order to corroborate and extend these preliminary findings, semen samples from a cohort of healthy Danish men, not known to be exposed to industrial toxicants, underwent SCSA analysis, and the results have been compared with those derived from microscopic assessment.

Materials and methods

As part of a European concerted action on occupational hazards to male reproductive capability (6), 227 Danish

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men (age range 18—55 years) were enrolled in this study. Semen was collected by masturbation after 2—7 days of sexual abstinence. The samples were analyzed in a mobile laboratory (6), and the sperm concentration, morphology, and vitality were evaluated in accordance with the guidelines of the World Health Organization (7).

SCSA was applied according to the procedure of Evenson et al (5) on individual semen samples, stored at -80°C for several weeks before the FCM analysis. Thawed aliquots (0.2 ml) containing about 200 000 cells were subjected for 30 seconds to acidic denaturation at pH 1.4 and then stained with 6 µg/ml of chromatographically purified AO. The samples were analyzed by a Facstar Plus flow cytometer (Becton Dickinson, S. Josè, CA, USA). Altogether 5000 events were accumulated for each measurement and displayed as bivariate green versus red fluorescence cytogram patterns. Chromatin structure abnormalities, reflected by a shift from green (native, double-stranded DNA) to red (denatured, single-stranded DNA) fluorescence, were quantified in terms of αT (ratio between red and total fluorescence). αT was calculated for each sperm cell, and the distribution of the αT values was conveniently reported as ranging between channel 1 and 1024.

Relevant SCSA parameters included the mean (mean αT) and standard deviation (SD αT) of the αT frequency distribution together with the fraction of sperm with high values for red fluorescence intensity which accumulate in the histogram region defined as COMP αT (cells outside the main population). Correlation coefficients between the SCSA values and conventional measures of semen quality were computed using Spearman's nonparametric statistics (SAS Institute, Cary, NC, USA).

Results

Representative data from the FCM SCSA of a human semen sample are presented in figure 1. The bivariate cytogram pattern shows raw data with each point representing the coordinate of red and green fluorescence. Increased red fluorescence (denatured DNA) with concomitant loss of green fluorescence (native DNA) is related to the extent of DNA denaturation. SCSA parameters are then easily calculated on the corresponding αT frequency histogram. The mean αT and SD αT are expressed in arbitrary units (channel number) ranging between 1 and 1024, whereas the COMP αT is reported as the percentage of the whole sperm population. It has been observed that the signal profiles of the bivariate cytogram patterns can vary among persons in shape and position. Interestingly, poor quality sperm chromatin samples seem to be characterized by an increased green fluorescence intensity, perhaps resulting from an abnormal exchange of histones for protamines, or an increased COMP αT fraction, representing the cells with abundant denatured DNA regions, or both. The mean value of the COMP αT fraction was 15%.

The SCSA parameter variability is shown in table 1. Statistically significant (P<0.0001) negative and poor

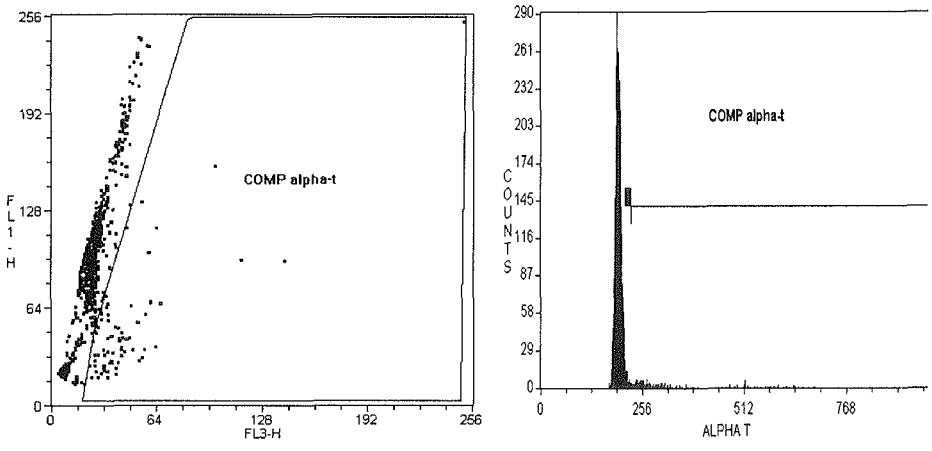

Figure 1. Representative green (FL1-H) versus red (FL3-H) fluorescence bivariate cytogram and the corresponding αT frequency histogram of sperm measured by the FCM SCSA. Cells with abnormal chromatin (COMP αT) are boxed off in the cytogram and in the corresponding αT histogram. (FCM = flow cytometric method, SCSA = sperm chromatin structure assay, αT= ratio between red and total fluorescence, XαT = mean of the αT frequency distribution,COMP αT = cells with abnormal chromatin)

Table 1. Descriptive statistics of the SCSA measurements. (SCSA = sperm chromatin structure assay, αT = ratio between red and total fluorescence, Mean αT = mean αT frequency distribution, SDαT = standard deviation of the αT frequency distribution, COMP αT = cells with abnormal chromatin)

SCSA parameter	Mean	SD	Range
Mean αT	224.9	23.2	194.5—381.6
SD αT	80.4	27.3	36.7—185.6
COMP αT	15.0	10.6	2.7—71.1

Table 2. Spearman's correlation coefficients between some SCSA and microscopy-derived parameters. (SCSA = sperm chromatin structure assay, αT = ratio between red and total fluorescence, Mean αT = mean αT frequency distribution, COMP αT = cells with abnormal chromatin)

Semen parameter	Mean αT	COMP αT
Sperm concentration	0.29	0.31
Morphologically normal forms (%)	0.38	0.38
Sperm vitality (%)	0.25	0.23

correlations were found between the SCSA variables and the sperm concentration, the percentage of sperm head abnormality, and the percentage of sperm viability (table 2), indicating that conventional measurements of semen quality may or may not correlate with the level of sperm chromatin abnormalities.

Discussion

SCSA parameters seem to be independent measurements of semen quality, as they show weak correlations with the conventional semen quality parameters of sperm concentration, morphology, and motility. Our results confirm and extend, because of the larger population under analysis, the poor correlation with conventional microscopic assessment reported previously (5). It is worth noting that SCSA results for a particular person were more consistent than those of any classically measured semen variable (5).

SCSA measurements reflect the heterogeneity of the nuclear chromatin conformotional changes of the different sperm cells in the ejaculate. It has been shown that chromatin anomalies can arise because of defective protamine deposition and the presence of residual endogenous DNA nicks (8). We can speculate that chromatin abnormalities influence sperm quality and male fertilizing ability, and the possibility that these spermatozoa can be more susceptible to the action of exogenous factors leading to further damage cannot be ruled out. SCSA seems able to detect chromatin abnormalities attributable to defective DNA-protamine packaging or to the presence of DNA nicks (9). Interestingly, associations between sperm chromatin defects and a decrease in fertilization rates have also been reported (10). Therefore, it seems reasonable that SCSA can be used in human reproductive toxicology studies and for the characterization of certain conditions of infertility since it rapidly identifies sperm with an increased chromatin susceptibility, regardless of the sperm morphology, motility, and number.

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