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• *Original Contributions*

INDIVIDUALITY OF DNA DENATURATION PATTERNS IN HUMAN SPERM AS MEASURED BY THE SPERM CHROMATIN STRUCTURE ASSAY

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Abstract — Eight monthly semen samples from 45 men not known to be exposed to industrial toxicants were measured by the flow cytometric sperm chromatin structure assay (SCSA). This assay determines susceptibility of sperm DNA to in situ, acid-induced denaturation and is quantitated by the metachromatic shift of acridine orange fluorescence from green (native DNA) to red (denatured DNA). The observed green versus red fluorescence scattergram (cytogram) patterns were generally unique between donors and homogeneous within a donor over time. Within a donor, the cytogram patterns were the same whether intact sperm cells or detached nuclei were measured. For some individuals the cytogram patterns differed for some months and then returned to the original pattern. Intraclass correlations for mean and standard deviation of alpha t [α_t = red/(red + green) fluorescence] were higher (.67 to .90) than any classically measured semen variables, suggesting that SCSA results within an individual were more consistent than other measures. Furthermore, average within-donor CV of α_t parameters expressed as a percent of any given individual's means was around 10%, which is significantly lower than those derived from common semen measures. The SCSA is an objective, technically sound, biologically stable, sensitive, and feasible measure of semen quality.

Key Words: human semen quality; longitudinal study; flow cytometry; sperm chromatin structure assay; SCSA; DNA denaturation; acridine orange; intraclass correlation.

INTRODUCTION

There is growing concern about the reproductive consequences of exposing humans to the large, complex array of environmental and occupational chemicals. It is well known that semen quality may be dramatically altered by exposure to therapeutically administered chemicals (1,2) and some industrial chemicals (3–8). To detect toxic effects, it is of prime importance to develop more sensitive and practical methods by which putative alterations in fertility and germ cell quality may be investigated (9). The criteria established by Overstreet (10) for male reproductive risk assessment required that the tests be a) objective, b) technically sound, c) biologically stable, d) sensitive, and e) feasible. As detailed herein, all of these criteria appear likely to be met by our flow cytometric sperm chromatin structure assay (SCSA).

For studies on toxic exposure, sperm tests have received the most attention for monitoring human exposure to germ cell mutagens and male reproductive toxicants, because: 1) sperm can be easily obtained, 2) the assay is conducted on cells with paternal genome in the form involved in fertilization, 3) they may clearly demonstrate germ line damage, resulting from exposure to a toxic agent that has taken place in vivo, and 4) in contrast to many mutation assays and short term tests for carcinogenicity, evaluation of sperm abnormalities and changes in sperm count have a place in the conventional evaluation of compounds for effects on male fertility (11).

Considerable interest and research have been directed towards determining which semen quality tests are the best predictors of male fertility. Although there are many measures of semen quality employed to diagnose infertility and fertility reductions due to toxicant-

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induced damage, few studies (12,13) have been conducted to determine stability or variability of these semen parameters among the general population.

The current investigation was part of a recent NIOSH longitudinal study of semen quality of 45 men not known to be exposed to industrial toxicants (14). The effort here was directed specifically toward measurements of sperm chromatin structure by means of the SCSA (15–17) to determine the stability of sperm chromatin structure within a donor over time. Data from this assay, which measures the resistance of sperm DNA to in situ denaturation, have been shown to correlate toxic chemical exposure (17–19), drug exposure (20), and disease (21) with aberrations of sperm chromatin. In addition, limited studies on human (21,22) and bovine (22–24) semen have suggested a relationship between sperm chromatin structure and fertility.

The present study shows that sperm chromatin fluorescence staining patterns differed to varying degrees among many of the 45 donors. However, month to month similarities of staining patterns within individuals were evident. In some cases, varying patterns were seen for some of the months within a donor, possibly a result of some type of stress that affected spermatogenesis.

MATERIALS AND METHODS

Semen samples

Forty-five men responding to a newspaper advertisement in Cincinnati were paid to deliver one semen sample per month to the Andrology Laboratory at the National Institute for Occupational Safety and Health (NIOSH), Cincinnati. After an approximate 2-day abstinence period, samples were obtained by masturbation into a glass specimen jar and delivered, with protection from temperature extremes, to NIOSH within an hour of collection. Details of the recruitment procedure and semen evaluations by other criteria have been reported (14). Semen quality parameters measured at the NIOSH facility included sperm count, semen volume, percent motile sperm, sperm velocity, sperm morphology, and sperm viability, using light microscopy and a CellSoft semen analyzer. Flow cytometric measurements of semen began with samples collected on the second month of the 9-month study. Upon arrival of semen samples at the NIOSH laboratory, 100 μL aliquots of semen were diluted 10-fold with TNE buffer (0.15 M NaCl, 0.01 M Tris, 0.001 M EDTA, pH 7.4) containing 10% glycerol and frozen at − 100 °C in 1.5 mL microcentrifuge tubes (cat. #2075, West Coast Scientific, Hayward, CA). At the end of the 9 month collection period, all samples were sent on dry ice to South Dakota State University.

Sperm chromatin structure assay (SCSA)

Individual semen samples, stored at − 100 °C, were thawed in a 37 °C water bath and then immediately placed on crushed liquid ice. All succeeding steps were performed at 4 °C. Samples were diluted with TNE buffer to obtain a sperm concentration of $\leq 2 \times 10^6$ sperm/mL. A 200 μL aliquot was removed and admixed with 400 μL of a low pH detergent solution (0.15 M NaCl, 0.08 N HCl, 0.01% Triton X−100, pH 1.4). After 30 sec, 1.2 mL staining solution (6 μg/mL acridine orange [AO], chromatographically purified; Polysciences, Warrington, PA) in 0.2 M Na_2HPO_4, 1 mM disodium EDTA, 0.15 M NaCl, 0.1 M citric acid monohydrate, pH 6.0] was added, and the stained sample was placed into the flow cytometer sample chamber (16,25).

Semen samples used for instrument calibration were from a single human semen ejaculate, not a part of the Cincinnati samples, which was diluted in TNE buffer + 10% glycerol to a concentration of about 2×10^6 sperm/mL and aliquotted into several hundred 300 μL portions in 1 mL polycarbonate snapcap tubes and immediately frozen at − 100 °C. Calibration aliquots were thawed and measured at each start-up of the flow cytometer, before each donor's set of samples, and after every four samples within that set to ensure standardization and stability of the instrument from sample to sample and from day to day. Freezing and thawing mouse (18) or human sperm (in preparation) as described above have no effect on SCSA data relative to fresh samples. Semen calibration standards are advantageous over fluorescent beads since they are: a) more sensitive, especially for $SD\alpha_t$ values, and b) a biologic standard provides an internal control for potential experimental variables.

Fluorescence measuredments

Samples were measured on a Cytofluorograf 30 system equipped with a 100 mW argon ion laser operated at 35 mW and interfaced to a 2150 data handling system (Becton Dickinson Immunocytometry Systems, Braintree, MA). Green (515 to 530 nm) and red (>630nm) fluorescence values, corresponding to amounts of native and denatured DNA, respectively (26), were obtained from 5×10^3 cells per sample. Measurements began 3 min after AO staining with a flow rate of approximately 250 cells/sec. A single measurement was made for each sample, and all samples from an individual were measured in sequence, month 2 to 9. Duplicate measurements were made on one-fifth of the samples.

Sonication of samples

Each semen sample was diluted to a final volume of 0.5 mL with TNE buffer to obtain a sperm count of about 2×10^6 cells/mL and dispensed into a cryogenic vial with a sealed cap (Corning vial no. 25702, Corning, NY). A rubber stopper with proper sized hole was used

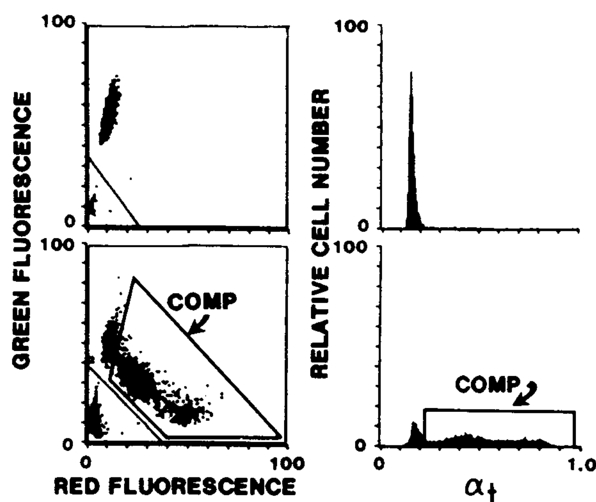

Fig. 1. Green versus red fluorescence cytograms and corresponding alpha t (α_t) frequency histograms of sperm from donor number 50 measured by the SCSA. The upper portion of the figure corresponds to a semen sample obtained one month prior to the sample corresponding to the lower portion. Increased red fluorescence (denatured DNA) with concomitant loss of green fluorescence (native DNA) is related to the extent of DNA denaturation. Cells outside the main population (COMPα_t) with abnormal chromatin are boxed off in the cytogram and corresponding frequency histogram.

to hold the sample vial on top of a Branson sonicator cup horn (VWR Scientific, Danbury, CT) so that it was situated just above the bottom of the cup horn cavity. Water (4 °C) was circulated in the cup horn to keep the sample cool. The sample was sonicated for 30 sec with a Branson 450 Sonifier (VWR Scientific) operating at a power setting of 3 and utilizing 70% of 1 sec pulses. Under these conditions somatic cells are totally disrupted and ≥ 95% sperm tails were removed from heads. This procedure utilizing sealed tubes provides protection from potential aerosolized infective agents in the samples.

Statistical analysis

Computer-generated means and standard deviations of green and red fluorescence and alpha t (α_t = red/total [red + green] fluorescence) (26) values were analyzed. The percentage of cells outside the main population of α_t (COMPα_t;16,17) was obtained by delineation of the main population of α_t and determining the percentage of cells inside and outside of it. This is accomplished by extrapolating the right hand slope of the α_t frequency histogram to the x axis. Data points to the left of this line are in the main population while those to the right are COMPα_t (see Figure 1). Fluorescent debris, found near the origin of the cytogram, was in almost all cases easily delineated and excluded from analysis.

The data were analyzed on a Compaq 386 PC computer using Statistical Analysis System (SAS;27) procedures. Similar to the companion study (14), the key statistics calculated, using the NESTED Procedure, included between-subject standard deviations (SD_B; variation from one donor to another); within-subject standard deviations (SD_W; variation of repeated samples within a donor); intraclass correlations (R_I; measurement of the size of between variance relative to total (between + within) variance, total coefficient of variation (CV_T), and within-subject coefficient of variation (CV_W).

The intraclass correlation (R_I) is a measure of repeatability, and a high correlation coefficient indicates that repeated measures from the same individual are similar. The coefficients of variation, CV_T and CV_W, help assess precision relative to the mean (14). CV_T provides the relative precision of a parameter value, measured once for a single subject, to represent the mean of the population over time. CV_W is an average of the 45 intradonor coefficients of variation for a given parameter and tells how precisely a single measurement of a parameter represents the true mean (over time) within a donor.

RESULTS

Data obtained from the sperm chromatin structure assay

Figure 1 shows representative data from the sperm chromatin structure assay of human sperm. The cytogram (scattergram) shows raw data with each point representing the coordinate of red and green fluorescence for an individual sperm. The dots in the lower left hand corner correspond to sample debris and are excluded from data analysis. The ability to differentiate debris from sperm signals is essential for accurate analysis. Although each sperm normally contains a uniform amount of DNA, an elongated distribution of green fluorescence is observed due to high refractive index and asymmetric shape of the sperm head, and the optical configuration of the flow cytometer (28). This optical artifact has no significant effect (16,17) on the α_t distribution as evidenced by its low coefficient of variation and the narrow frequency histogram. Cells outside the main population of the α_t distribution, (COMPα_t), are boxed off in Figure 1. COMPα_t corresponds to the percentage of cells with abnormal chromatin structure as measured by the SCSA. The extent of chromatin structure abnormality is represented by the mean and standard deviation of α_t ($\bar{X}_{\alpha_t}$ and SD_{α_t}, respectively; 16,17).

Effect of abstinence on SCSA values

The mean abstinence period prior to sample collection ranged from 2.64 ± 1.01 days (August) to 3.07 ±

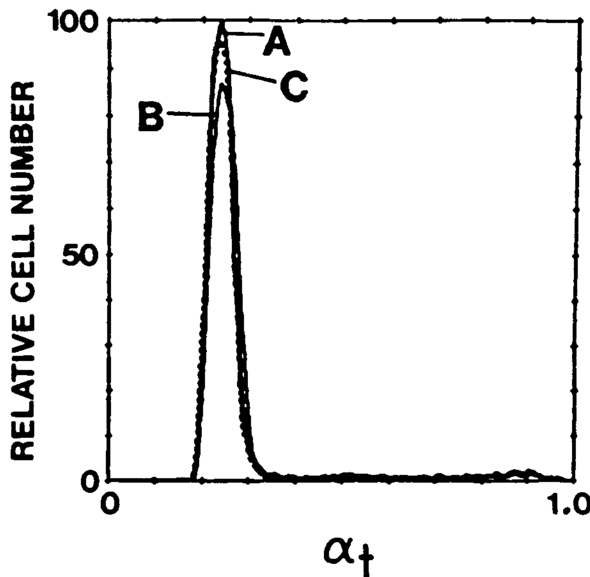

Fig. 2. α_t frequency histograms of three semen samples obtained from a single individual following: A) 7 days, B) 2 days, and C) 0.5 day abstinence.

1.73 days (December); the range of abstinence for all samples was 0.5 days to 11 days (14). Figure 2 shows that abstinence times from 0.5 to 7 days had no significant effect on α_t parameters measured within a single individual; COMPα_t values ranged from 10% to 11%.

Cytograms of eight monthly samples obtained from 15 men

Figure 3 displays cytograms of 8 successive monthly semen samples obtained from 15 of the 45 men; selection of donor cytograms was made on the basis of including a variety of the different patterns observed. Note the degree of pattern similarity within an individual over the months. Note also the similarities and differences of signal profiles between some of the donors. Subjective classifications can be made for cell staining distributions relative to the length of the cytogram pattern, width of main population, and abnormal patterns. Patterns formed by the distribution of cell fluorescence include narrow red profiles in main population (donors 2, 4, 50), wider red profiles (donors 20, 38), high green (donor 45), high green and wide red (donors 26, 47), normal green in the main population but wide in red fluorescence and a high level of denaturation (donor 33), high green and denatured DNA (donor 8), and highly irregular patterns (donor 42). When cells exit the main population, they generally follow an approximate 45 degree path as best illustrated in Figure 1. Cells with highly denatured DNA are represented by minimal green fluorescence and varying amounts of red fluorescence as illustrated by donors 8 and 26. The similarity in location of COMPα_t cells from month to month within a donor demonstrates the repeatability in degree of DNA denaturation of abnormal cells for an individual. Thus, the percentage of abnormal cells and the degree of their abnormality are alike from month to month within an individual donor.

Fluorescence patterns from several donors deserve mention. Note the unique patterns of donor 20 for which the upper portion of the main population is shifted to the right. For donor 26, the third cytogram down exhibits a sharp narrow line just to the left of the main population; this is due to bacteria in the sample. The patient was diagnosed as having prostatitis and treated with vibramycin; note the absence of this population in the next month's sample. Donor 50 received cortisone for a back problem during month 4 (cytogram 3); a notable change in staining pattern is evident which indicates a significant loss of semen quality in month 4 and a return to his normal pattern by month 5.

Effect of sonication on SCSA data

In other studies (16), some or all semen samples are sonicated to separate sperm heads from cytoplasmic components to ensure that any increased red fluorescence was not due to abnormally retained RNA in cytoplasmic droplets; these studies have shown no significant differences between intact, RNAsed and/or sonicated sperm samples. Thus, the assumption has been made that increased red fluorescence is due to denatured DNA. Due to laboratory safety considerations of possible HIV infected samples, sonication was not done in the first measurement of samples. After obtaining a sonifier that allowed sonication of samples in sealed tubes, previously measured samples from the second month that had been refrozen were rethawed and measured, and sonicated and measured.

From 38 samples analyzed the cytogram patterns were essentially identical before and after sonication including parameters such as shape, height, and width of the main population, percent of COMPα_t, and position of the COMPα_t cells as illustrated in Figure 4. The mean red fluorescence value of the 38 samples was reduced by $-4.3\% \pm 4.9\%$ following sonication, compared to the unsonicated samples. This is considered biologically insignificant.

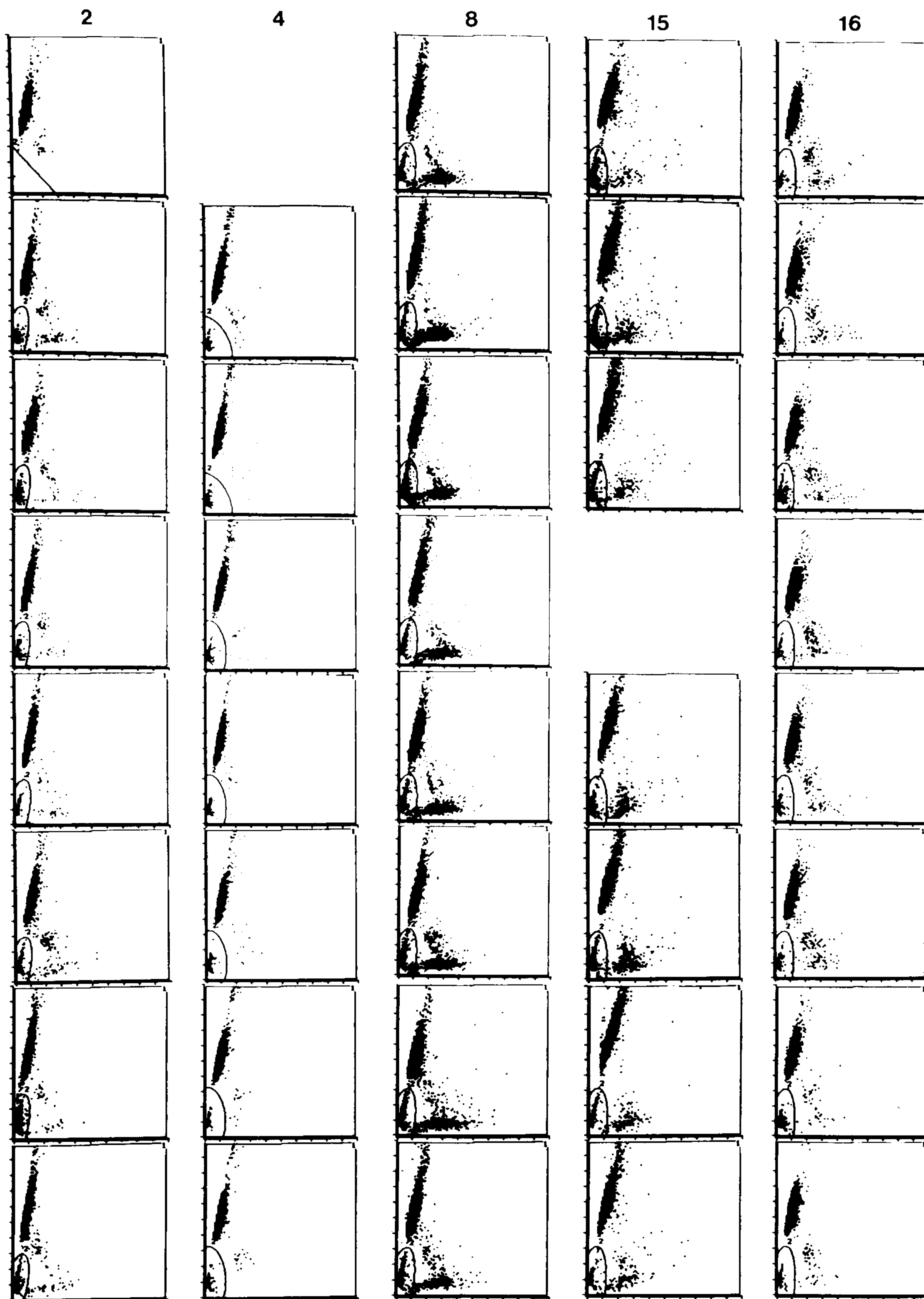

Fig. 3. Green versus red fluorescence cytograms of semen samples obtained on months 2 to 9 from a 9-month study for 15 of the 45 donors with identifying numbers ranging from 1 to 50. (Figure continues on 2 subsequent pages.)

(Fig. 3 continued.)

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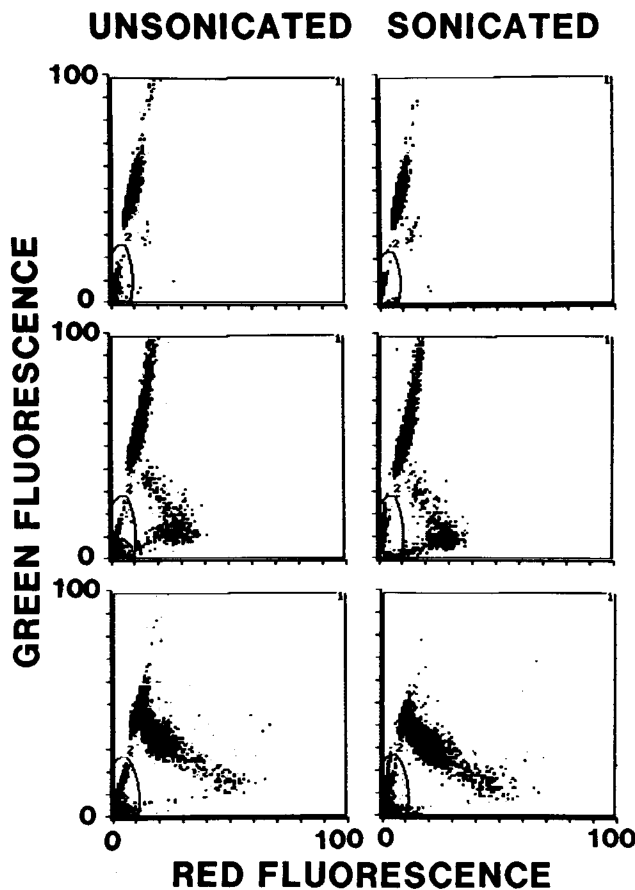

Fig. 4. Cytograms of semen samples from three donors measured by the SCSA prior to and after sonication.

Mean monthly α_t values

Figure 5 A–C shows the distribution and mean values for $\overline{X}\alpha_t$, $COMP\alpha_t$, and $SD\alpha_t$ for the 45 men during the 8-month collection period. The mean values are relatively constant over time, suggesting no significant seasonal effect, although not all months are represented.

Within- and between-donor variation

Figure 6 A–C plots the individual values for $\overline{X}\alpha_t$, $SD\alpha_t$, and $COMP\alpha_t$ obtained for each of 8 months. The values for individuals are more tightly clustered than the values between individuals. Table 1 shows values for intersubject and intrasubject variation in DNA denaturability. Standard deviations of FCM parameters between donors (SD_B) are larger than within a donor (SD_W), indicating that α_t parameters are much more highly repeatable within than between individuals. This is also evidenced by the high intraclass coefficients (R_I; ranging between 67 and 90). Note also that most CV_T (20% range) and CV_W (10% range) values are small, indicating low variability relative to the mean.

All of the data reported are based on a single measurement of each sample. However, semen samples from two different months for each of 36 men were independently measured twice. The repeatabilities between observations for $\overline{X}\alpha_t$, $SD\alpha_t$ and $COMP\alpha_t$ were 0.99, 0.99, and 0.97, respectively, and are in agreement with previous studies on bull semen (24).

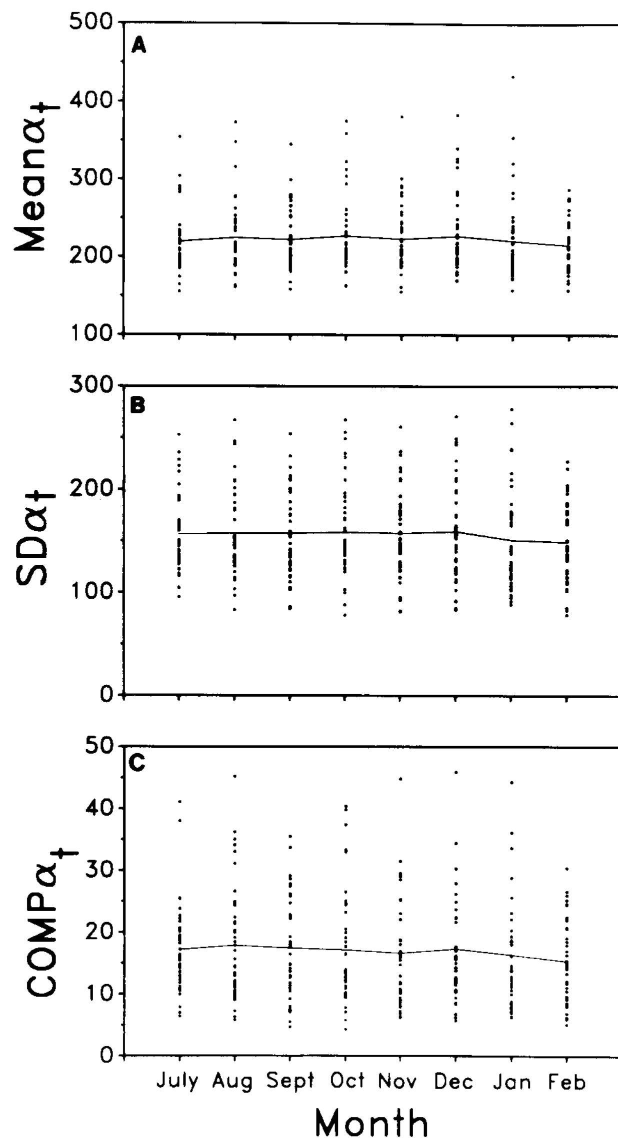

Fig. 5. α_t values by month. The points represent α_t distribution values of the semen samples for each month. The solid line represents the mean value for each month. A) $\overline{X}\alpha_t$, B) $SD\alpha_t$, C) $COMP\alpha_t$.

Correlation of SCSA DNA denaturation variables with conventional semen parameters

Table 2 shows low but significant negative correlations between most SCSA variables and sperm concentration, total sperm count, or percent abnormal sperm head morphology. Correlations between α_t parameters

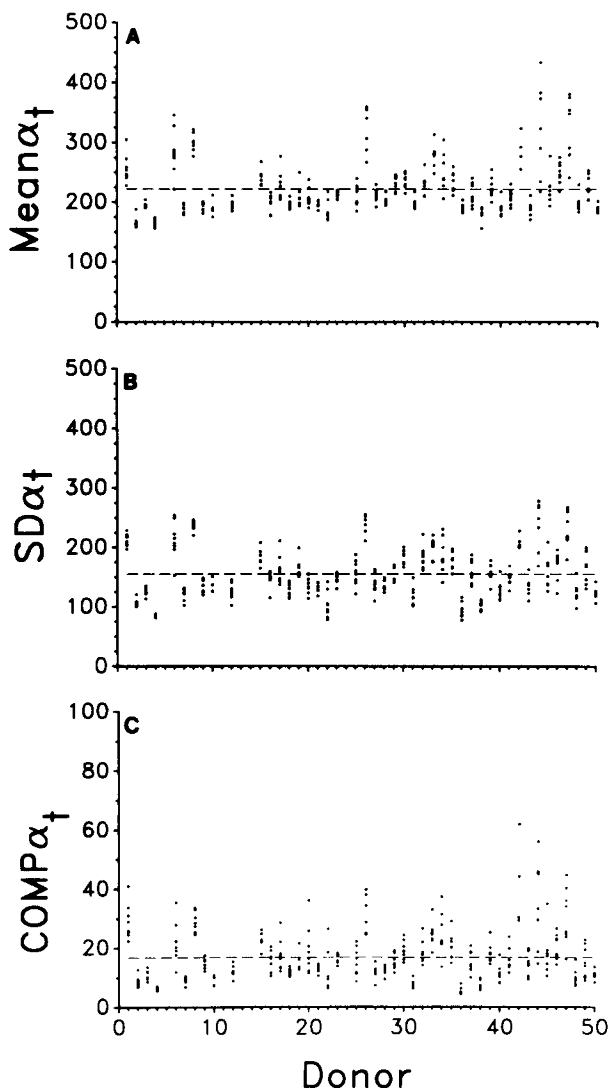

Fig. 6. α_t values by donor. The points represent α_t distribution values of the semen samples for each donor. The dashed line represents the mean value for all samples. A) $\overline{X}\alpha_t$, B) $SD\alpha_t$, C) $COMP\alpha_t$.

($SD\alpha_t$, $COMP\alpha_t$, and $\overline{X}\alpha_t$) and percent motile (range of $-.41$ to $-.43$, $P < 0.001$) and % hypoosmotically swollen sperm (range of $-.57$ to $-.63$, $P < 0.001$) were negative and significant. Very little correlation was found between α_t variables and percent viable (unstained) sperm (range of $-.22$ to $-.26$, $P < 0.001$).

DISCUSSION

The most interesting observation in this study was the consistency of unique AO staining patterns within individuals, suggesting that SCSA data can, in the absence of specific testicular stress, provide a baseline measurement of semen quality for individuals. Furthermore, if a baseline is established for an individual, deviations from that pattern may indicate the presence of testicular stress as has been observed in previous studies (18). The staining patterns illustrated by the cytograms are generated from green (native DNA) and red (denatured DNA) fluorescence of 5×10^3 acid-treated, AO-stained cells. The primary interest of this study is the measurement of low pH induced shift from green to red fluorescence which has been concluded to represent a shift from native DNA to denatured DNA. This and previous studies (16) provide evidence that the shift is not due to retention of cytoplasmic RNA or, if cytoplasmic RNA is present, that its contribution to red fluorescence is minimal relative to the increase of red fluorescence due to denatured DNA. Another cytogram pattern observed is one with abnormally increased intensity of green fluorescence (donor 45, Figure 3), likely due to increased native (nondenatured) DNA binding sites, perhaps resulting from abnormal exchange of histones for transition proteins and/or protamines (16). Other patterns include increased red fluorescence in the main population leading to a wider signal (donor 20), probably resulting from minor levels of increased DNA

Table 1. Descriptive statistics for flow cytometric measurements

FCM Parameter	Mean	SD_B	SD_W	R_I	CV_T	CV_W
$\overline{X}\alpha_t$	222.3	38.9	21.8	76	20	7
$SD\alpha_t$	155.3	38.7	18.0	82	28	10
$COMP\alpha_t$	16.8	7.2	5.0	67	52	23
Red $\overline{X}$	136.4	23.2	11.7	80	19	7
Red SD	88.9	19.8	11.6	75	26	11
Green $\overline{X}$	506.2	29.8	18.5	72	7	3
Green SD	153.1	32.2	11.6	89	22	7
Total $\overline{X}$	321.3	18.0	6.3	89	6	2
Total SD	76.4	13.1	4.5	90	19	5

SD_B = between-donor standard deviation.
SD_W = within-donor standard deviation.
R_I = intraclass correlation coefficient × 100.
CV_T = total coefficient of variation as a percentage of the overall mean.
CV_W = average within-donor coefficient of variation expressed as a percentage of any given individual's mean.

Table 2. Correlation between α_t parameters and other semen parameters

FCM Parameter	Conc.	Count	% Normal	% Motile	% Swollen	% Live
$SD\alpha_t$	$-.34^a$	$-.16^b$	$-.30^a$	$-.43^a$	$-.57^a$	$-.22^a$
$\bar{X}\alpha_t$	$-.26^a$	$-.10$	$-.29^a$	$-.42^a$	$-.60^a$	$-.25^a$
$COMP\alpha_t$	$-.29^a$	$-.16^b$	$-.36^a$	$-.41^a$	$-.63^a$	$-.26^a$
Red $\bar{X}$	$-.23^a$	$-.12^c$	$-.31^a$	$-.45^a$	$-.64^a$	$-.33^a$
Red SD	$-.24^a$	$-.11^c$	$-.32^a$	$-.43^a$	$-.64^a$	$-.31^a$
Green $\bar{X}$	.07	$-.16^b$	$-.03$	.04	$.13^c$	$-.01$
Green SD	$-.34^a$	$-.27^a$	$-.43^a$	$-.47^a$	$-.57^a$	$-.20^a$
Total $\bar{X}$	$-.08$	$-.24^a$	$-.24^a$	$-.27^a$	$-.32^a$	$-.24^a$
Total SD	$-.33^a$	$-.31^a$	$-.50^a$	$-.48^a$	$-.56^a$	$-.20^a$

$SD\alpha_t$ = standard deviation α_t. $\bar{X}\alpha_t$ = mean α_t. $COMP\alpha_t$ = cells outside main population α_t.
Red $\bar{X}$ and SD = mean and standard deviation of red fluorescence, respectively.
Green $\bar{X}$ and SD = mean and standard deviation of green fluorescence, respectively.
Total $\bar{X}$ and SD = mean and standard deviation of total (red + green) fluorescence, respectively.
$^aP < 0.001$, $^bP < 0.01$, $^cP < 0.05$.

denaturation relative to those samples exhibiting thin profiles.

Cytograms exhibiting a small percentage of $COMP\alpha_t$ that consistently appears in the same cytogram location from month to month indicate the same degree of deviation from normal. Speculation allows several possible explanations for this phenomenon. Perhaps a small percentage of stem cells carry a mutation that results in a consistent proportion of germ cells having altered genes for regulating chromatin structure. Wyrobek et al. (29) have suggested that a complex of about 25 genes controls mouse sperm head morphology. Studies in this laboratory have shown a high correlation between toxicant-induced sperm head morphologic abnormalities and altered sperm chromatin structure in the mouse (16, 18, 19). Also, an altered reproductive tract environment (gonadal or extragonadal) may affect developing germ cells in a manner that results in abnormal cytogram profiles.

The standard deviations of flow cytometric measurements were observed to repeat at least as well as the means of the measurements (Table 1). Previous studies on toxicant-altered mouse sperm (16, 18, 19) and bull semen quality (23,24) have shown $SD\alpha_t$ to be more highly correlated with toxicant dose and fertility potential, respectively, than the mean α_t values. $SD\alpha_t$ is strongly influenced by the extent of DNA denaturation, even if the population of cells exhibiting DNA denaturation is small, that is, low $COMP\alpha_t$. The observation that the degree of damage in a small percentage of cells may provide the best correlation to toxicant damage or fertility potential also suggests that the extent of damage to cells outside the main population is reflective of submeasurable damage in the main population.

The descriptive statistics show a higher degree of repeatability for flow cytometry measurements of chromatin structure (Table 1) than for semen parameters measured in the parallel study (14). From the previous measures of sperm count, semen volume, sperm velocity, percent mature sperm, percent normal morphology, percent viable sperm, and percent hypoosmotic swollen cells, the highest intraclass correlation was .62 for sperm count, indicating that a man with a relatively high sperm count would tend to continue to have a high sperm count, and a man with a relatively low sperm count would continue to have a low sperm count. However, the average coefficient of variation for within-subject sperm count was 44%, indicating that variability within a man is large relative to the mean, which, as stated by the authors (14), makes a precise measurement of sperm count difficult. In contrast to the above study (14), the range of intraclass correlations in the present study for the chromatin structure measures was .67 to .90 indicating a high repeatability of DNA susceptibility to in situ denaturation. The SCSA variable most useful in our studies (16) for correlations between chromatin measures, and fertility or toxicant damage was $SD\alpha_t$, which had an intraclass correlation in this study of 0.82 and a CV_w of 10%. Mean green fluorescence, reflecting native DNA stainability and degree of chromatin condensation, had an intraclass correlation coefficient of .72 and a CV_w of 3%. These values indicate a very high repeatability for an individual regarding sperm nuclear maturation. The low CV_w represents the relative precision of a single measurement of a parameter for representing the mean over time of the same individual (14).

SCSA data are independent measurements of semen quality that may or may not correlate well with other semen quality measurements. Previous reports (30) as well as unpublished observations of infertility clinic semen samples have shown that samples with good to excellent values for conventional semen quality tests (sperm count, morphology and motility) may exhibit poor chromatin quality. In this study, correlations between DNA denaturability and semen quality mea-

sures obtained in the companion study (14) ranged from poor to moderate. Sperm concentration, count, and morphology values show low but significant correlations with SCSA values (Table 2). Flow cytometric measurements correlated best with precent sperm swollen in hypoosmotic media (-0.57 to -0.63, $P < .001$), but did not correlate with percent unstained, live cells (-0.22 to 0.26, $P < .001$).

The SCSA should be suited to toxicology field studies and also analyses of infertility in an andrology clinic setting. Typically 50 to 100 μL of semen are placed into buffer and stored frozen until measured. Alternatively, raw semen can be frozen, shipped on dry ice to a flow cytometry facility, and diluted at time of measurement. Normal SCSA data obviously do not ensure donor fertility. However, our current opinion, based on thousands of measurements from animals, (bulls, stallions), and humans, is that poor quality sperm chromatin structure is highly indicative of male subfertility. Because the SCSA measures a characteristic of sperm that cannot presently be determined accurately by any other method, it should be of particular significance in identifying defective samples that appear normal by other semen quality criteria.

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