Sperm DNA Damage and Semen Quality Impairment After Treatment With Selective Serotonin Reuptake Inhibitors Detected Using Semen Analysis and Sperm Chromatin Structure Assay

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Purpose: Semen parameters and sperm DNA were evaluated in patients receiving selective serotonin reuptake inhibitors.
Materials and Methods: Semen samples were obtained from 74 fertile, depressed men treated with selective serotonin reuptake inhibitors (group 1) and 44 healthy fertile volunteers who served as a control (group 2). Two semen analyses and physical examinations were completed in all participants. Sperm chromatin structure assay was used to detect sperm DNA fragmentation.
Results: The mean ± SE total sperm count in patients receiving selective serotonin reuptake inhibitors and in normal subjects was 61.2 ± 11.4 million and 186.2 ± 31.4 million, respectively ($p = 0.001$). Patients in group 1 had a mean motility of 48.2% ± 4.6%, which was significantly lower than the mean of 66.2% ± 4.4% in normal controls ($p = 0.01$). Normal sperm morphology was detected in 14 patients (18.9%) and 23 controls (52.3%) ($p = 0.001$). Patients treated with selective serotonin reuptake inhibitors had a mean of 8.1% ± 5.4% normal forms per ejaculate. A significant increase in the amount of denatured single strand DNA in total cellular DNA was found in patients treated with selective serotonin reuptake inhibitors compared with that in controls (43.2% ± 11.4% vs 21.4% ± 10.6%, $p = 0.01$). Each semen analysis parameter significantly correlated with treatment duration.
Conclusions: Selective serotonin reuptake inhibitors can impair semen quality and damage sperm DNA integrity. Further studies are needed to replicate our findings.

Key Words: testis, spermatozoa, DNA damage, serotonin uptake inhibitors, depression

Selective serotonin reuptake inhibitors have become the most commonly prescribed drugs for depression. Although these drugs are safe, adverse events occur in a certain proportion of patients. More than a third of patients receiving SSRIs discontinue treatment due to sexual side effects. About 5% of the adult population in the United Kingdom receive an antidepressant with similar prescribing rates in the United States.[1]

Approximately half of the cases of infertility are due to male factors. In many cases the underlying cause of male infertility is not discovered and, therefore, the condition is considered idiopathic. The gene transcription factor AP-1 is an important target for antidepressant drugs. This protein recognizes a specific DNA sequence (TGACTCA) in the promoter region of several genes.[2] It has been shown that there are consensus binding sites for the AP-2 transcription factor family in regulatory regions of the genes in the serotonergic system.[3] Citalopram is an SSRI. Chronic administration of citalopram significantly decreases the DNA binding activity of AP-2.[5] Serotonin (5-HT) can also cause strand cleavage in DNA through an oxidative mechanism in the presence of copper ions (Cu++).[4] Two molar equivalents of Cu++ can be reduced to Cu+ by serotonin. This is done through a reaction involving 2 electron oxidation of the phenolic ring to a quinone methide.

At our fertility clinic several patients have presented who have shown improved semen parameters after discontinuing SSRI. Tanrikut and Schlegel reported on 2 patients referred for evaluation of male infertility who were receiving SSRIs, including citalopram, sertraline and venlafaxine.[5] They had oligo-asthenospermia and were recommended to stop any medications. Repeat semen analyses performed 1 to 2 months after discontinuing antidepressants showed significant improvements in semen quality.

SCSA is a flow cytometric technique. The susceptibility of sperm nuclear DNA to denaturation can be measured by SCSA.[6] The presence of DNA strand breaks increases the susceptibility of sperms to acid denaturation.[3] Therefore, one can quantify the degree of chromatin fragmentation by SCSA. SCSA uses the metachromatic properties of acridine orange. Results are usually shown as green fluorescence (double strand or native DNA in sperm chromatin) vs red fluorescence (single strand or heat denatured DNA in sperm chromatin) bivariate cytogram patterns. SCSA is particularly suitable for epidemiological studies. Differentiation of diploid spermatogonia into mature haploid sperm involves a continuum of dramatic biochemical events and morphological alterations. One or more of these biochemical events and morphological alterations can be disturbed by physiological stress and/or exposure to environmental agents, such as various chemical agents and drugs. During the last decade SCSA has been used extensively in groups of men exposed to

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pesticides, lead, styrene, solvents, polychlorinated biphenyls and air pollution.[7] SCSA has an advantage of low within-individual variability, and intra-assay and interassay variation comparison with those of traditional semen analysis.[8] To our knowledge this is the first extensive study of semen parameters and sperm DNA in patients on SSRIs.

MATERIALS AND METHODS

Study Design

From March 2006 to April 2007, 74 outpatients 20 to 50 years old with depression who were being treated with an SSRI were enrolled in this study. Included patients were required to meet Diagnostic and Statistical Manual of Mental Disorders, 4th edition (text revision) criteria for a current major depressive disorder and have a score of 17 or greater on the 17-item Hamilton Rating Scale for Depression.[9] Participants were recruited through local advertisements for a study of fertility capacity while being treated with citalopram, escitalopram, fluoxetine, paroxetine or sertraline for depression. Table 1 lists dosing characteristics in the study population. The 44 age matched, normal, healthy, normozoospermic (according to WHO criteria) volunteers without a history of infertility were recruited as control group. To obtain controls a female obstetric nurse approached each pregnant woman attending the prenatal clinic and explained the purpose of the study. If the woman agreed to the study, she then sought the consent of her husband to participate. Only couples who had never attended the infertility clinic were recruited and this information was confirmed separately with each spouse.

All patients before the start of antidepressive therapy and controls had fathered at least 1 child. None of the patients with depression had attempted to initiate pregnancy after the initiation of SSRIs. All participants provided written informed consent before entering the study, which was done in accordance with the Declaration of Helsinki. The human ethics committee approved the study protocol. No remuneration was offered.

Inclusion and Exclusion Criteria

Patients were included in the study if they were diagnosed with a primary major depression disorder, were younger than 50 years and had been receiving SSRIs 6 months or greater for depression. Participants in each group with a history of epididymo-orchitis, venereal disease, alcohol consumption, tobacco or illicit drug use, any toxin exposure or concomitant medical problems known to be associated with decreased fertility, such as varicocele, were excluded from study.

Evaluations

All patients and controls underwent physical examination, including genital and rectal examinations, as performed by the author (MRS). These men completed a detailed paternity questionnaire, including information on marriage and cohabitation, paternity, health problems, drug use, chemical exposure, other information that may affect fertility and demographic information. Testicular volume was measured with scrotal ultrasound by a sonologist. To rule out depression all controls underwent a routine clinical psychiatric interview.

TABLE 1. *Antidepressant dosing in overall study population*

Medication	Mean ± SD Dose (mg/day) (range)
Citalopram	32.4 ± 9.76 (20–40)
Escitalopram	13.24 ± 4.52 (10–20)
Fluoxetine	28.41 ± 16.04 (10–60)
Paroxetine	25.14 ± 12.16 (20–45)
Sertraline	72.4 ± 35.28 (50–200)

All participants provided semen samples following an abstinence period of not less than 2 but not more than 5 days. When there was no more than a 20% difference in semen parameters, semen analysis was performed at least twice. When values differed by more than 20%, a third test was done. Each sample was tested at least twice for sperm density, motility and morphology. The total number of sperm in the ejaculate was calculated as the sperm concentration times specimen volume. All semen analyses were done in accordance with established 1999 WHO criteria except sperm morphology, which was assessed using the Kruger parameters. Normal WHO values included a sperm concentration of 20×10^6 spermatozoa per ml or greater and 50% or greater motility with forward progression. Using the Kruger strict criteria males with greater than 14% normal forms were considered normal.

Sperm morphology was evaluated by examining stained smears of 5 to 10 μl semen. Briefly, slides were fixed with Diff-Quick™ fixative for 15 seconds, stained with Diff-Quick solution I for 10 seconds and finally stained with Diff-Quick solution II for 5 seconds. The slides were dried at room temperature. The criteria for normal spermatozoa were a smooth oval configuration of the head with a well-defined acrosome occupying 40% to 70% of the sperm head, absent neck, mid piece and tail defects, and no cytoplasmic droplets more than half the size of the sperm head with a sperm head length of 5 to 6 μm and a diameter of 2.5 to 3.5 μm. Sperm DNA damage was assessed by SCSA.

SCSA

This test was done by laboratory personnel at our institution. SCSA was performed in 5,000 sperm from each of the 74 patients and 44 controls. Briefly, sperm samples were diluted to a concentration of 2×10^6 cells per ml with TNE, composed of 0.15 mol/l NaCl, 0.1 mol/l tris and 1 mmol/l ethylenediaminetetraacetic acid (pH 7.4). They were the stained by adding 1.2 ml acridine orange staining solution. The extent of DNA denaturation was determined by measuring the metachromatic shift from green fluorescence (acridine orange intercalated into double strand nucleic acid) to red fluorescence (acridine orange associated with single strand DNA). Cells were then immediately analyzed in a Coulter® Epics® XL™ Flow Cytometer. Red fluorescence from single strand DNA was measured using a 675 nm detector and green fluorescence from double strand DNA was measured using a 525 nm detector. The most important parameter of SCSA is the percent DFI, which represents the population of cells with DNA damage. For the flow cytometer setup and calibrations a reference semen sample retrieved from the laboratory repository was used. This reference sample was not characterized as fertile or infertile but was used for quality control.

Results are shown in terms of the equation, αt = [red/(red + green) fluorescence], expressed as a percent, and as (COMP)

αt, where COMP represents cells outside the main population. COMP αt was calculated as the percent of cells in the population showing an αt value of greater than 26%. DFI was generated using ListView software (Phoenix Flow Systems, San Diego, California).

Statistical Analysis

Results are expressed as the mean ± SE. Differences in sociodemographic variables between groups were determined with the paired t test and 1-way ANOVA for dichotomous and normally distributed continuous variables, respectively. Pairwise comparisons of the percent of denatured DNA measured by SCSA of sperm from patients and volunteers were analyzed with the Mann-Whitney U test. Data not normally distributed were analyzed by Kruskal-Wallis ANOVA on ranks. The Spearman's rank correlation coefficient rs was used to assess associations between semen analysis variables and treatment duration. Because many semen variables were not normally distributed, we used square root or squared transformations. When several semen samples were obtained, the mean value of each parameter was used. Average values of semen parameters in the 2 groups are expressed as the mean ± SE. Differences in sperm density, motility and morphology were evaluated for significance using the nonparametric Wilcoxon signed rank test. Spearman rank correlation coefficients were calculated to determine associations between SCSA results and semen parameters with $p < 0.05$ considered statistically significant. Statistical analysis was performed using the computer statistical package SPSS® 10.0 and SAS® 6.4.

RESULTS

Patient Disposition and Demographics

The mean age of patients and controls was 34.6 (range 27 to 49) and 34.4 years (range 27 to 49), respectively. The mean history of depression was 3.3 ± 0.7 years. Of the patients 13 (17.6%), 8 (10.8%), 26 (35.1%), 11 (14.9%) and 16 (21.6%) had received citalopram, escitalopram, fluoxetine, paroxetine and sertraline, respectively.

Semen Analysis and Testicular Volume

Mean testicular volume measured by ultrasonography was 24.7 ± 2.7 and 25.4 ± 3.1 ml in patients treated with SSRIs and controls, respectively ($p = 0.1$). Mean seminal volume in patients and controls did not differ significantly (2.6 ± 1.3 and 3.2 ± 1.1 ml, respectively, $p = 0.08$). Based on WHO criteria 45 patients (60.8%) and 4 controls (9.1%) had an abnormal sperm concentration ($p = 0.001$). Table 2 shows semen analysis and SCSA test results. Mean sperm density in the patient group was significantly lower than that in the control group (18.6 ± 4.3 × 10^6/ml vs 61.2 ± 10.3 × 10^6/ml, $p = 0.001$). Patients treated with SSRIs had higher abnormal sperm motility than controls (49 or 66.2% vs 3 or 6.8%, $p = 0.001$). Using the normal morphological sperm ratio of 14% according to the Kruger criteria 14 (18.9%) and 23 participants (52.3%) in groups 1 and 2, respectively, had normal sperm morphology ($p = 0.001$). The normal morphological level was 8.1% ± 5.4% and 19.8% ± 7.8% in patients and controls, respectively ($p = 0.001$, table 2).

SCSA

A significant increase in the amount of denatured single strand DNA in total cellular DNA was found in patients treated with SSRIs compared with controls (43.2% ± 11.4% vs 21.4% ± 10.6%, Mann-Whitney U test $p = 0.01$, table 2). There was a negative association between sperm motility and DNA damage. Sperm motility was recorded as the percent of total and rapid progressive motility. Each assessment of motility significantly correlated with SCSA data ($r = -0.214$, $p = 0.014$ and $r = -0.216$, $p = 0.018$, respectively).

Different SSRI Types

The type of SSRI used had no impact on semen parameter data. Furthermore, the type of SSRI did not correlate with SCSA results.

Treatment Duration

We observed a positive correlation between the duration of SSRI therapy and abnormal semen parameters. When stratifying patients according to duration (6 to 12, 12 to 24 and more than 24 months), significant differences were detected for each semen analysis parameter (each Kruskal-Wallis test $p = 0.01$, table 2). Based on Pearson correlations each semen analysis parameter significantly correlated negatively with treatment duration, including sperm concentration ($r = -0.34$, $p = 0.037$), percent of motile sperm ($r = -0.35$, $p = 0.026$), percent of normal morphology ($r = -0.032$, $p = 0.026$) and total motile sperm ($r = -0.41$, $p = 0.035$). Treatment duration was positively associated with sperm DNA damage ($r = 0.44$, $p = 0.027$).

DISCUSSION

To our knowledge we report the first extensive study to show detrimental effects of SSRIs on semen parameters and

TABLE 2. *Routine semen parameters and SCSA results in participants*

	No.	Mean ± SE Total Sperm Count (×10^6)	Mean ± SE % Motility	Mean ± SE % Normal Forms	Mean ± SE % DFI
Normal controls	44	184.1 ± 31.4	66.4 ± 4.1	19.8 ± 7.8	21.4
Pts	74	61.4 ± 11.2	48.6 ± 4.4	8.1 ± 5.4	43.2
p Value		0.001	0.01	0.02	0.01
Treatment duration (mos):					
6–12	27	23.2	51.2	25.7	31.4
13–24	22	18.1	44.6	20.3	39.7
p Value		0.03	0.02	0.02	0.03
24*	24	14.6	35.7	10.8	45.6

* Vs 6 to 12 and 13 to 24 months $p = 0.01$.

sperm characteristics. Moreover, of some concern is the finding that these patients also had sperm DNA damage. Therefore, patients treated with SSRIs are at risk for impaired fertility.

SSRIs came onto the market almost 20 years ago. They may induce retarded ejaculation and anorgasmia. This known side effect of SSRIs has served as the rationale for their use for premature ejaculation. In this study at 12 months after treatment with SSRIs about two-thirds of the patients had decreased semen quality. In an early study 3 weeks of treatment with 200 mg desmethylimipramine daily was associated with a significant decrease in sperm viability in 5 depressed patients.[10] In another study compared to 37% of the samples in 15 age matched controls all sperm samples were abnormal in 11 men treated with 75 mg clomipramine for 3 months.[11] The rate of sperm DNA damage assessed by SCSA was high in patients.

Based on the literature there is a higher probability of pregnancy if the maximum proportion of cells revealing evidence of DNA damage (DFI) does not exceed approximately 30%.[6] The reported DFI in healthy controls in this study was 21.4% ± 10%. Sperm DNA fragmentation can be evaluated in various ways. We used one of the most sensitive assays (SCSA) to detect any sperm DNA abnormalities. In SCSA the sperm chromatin is exposed to acid denaturation, which exposes single strand DNA to acridine orange binding as an aggregate and differentiates them from unfragmented double strand DNA.[12] SCSA is a useful clinical test because of its high repeatability and its ability to measure an aspect of fertility that differs from what can be offered by traditional semen analysis.[3] Using SCSA sperm DNA damage was significantly higher in patients on SSRIs than in normal controls. Neither sperm motility nor morphology was a statistically significant predictor of the proportion of sperm cells with DNA abnormalities as assessed by SCSA, confirming sperm DNA integrity as an independent parameter for sperm quality.

Alkylating agents and aromatic amines are known to preferentially mutate guanine bases at the 39-end of a run of guanines.[13] It has been shown that the GG*GC*CC sequence is a hot spot for other DNA damaging agents, including Cu-H_2O_2 induced reactive oxygen species.[14] Nudell et al reported increased mutations in testicular DNA in infertile men with azoospermia using sequence analysis of a polymorphic marker.[15] Serotonin is capable of causing DNA cleavage in the presence of copper ions through the generation of reactive oxygen species, such as the hydroxyl radical.[16] DNA damage by hydroxyl radicals is well established. As mentioned, serotonin can bind to DNA and copper ions. Therefore, since copper is an essential component of chromatin, the formation of a ternary complex of serotonin-Cu++ DNA is possible. This is the probable mechanism of DNA cleavage with 5-HT. The adverse effect of SSRIs on emission and ejaculation is well-known. Therefore, it is possible that SSRIs could also negatively influence sperm transport. Patients with spinal cord injury have impaired sperm transport and they also tend to have abnormal semen parameters and sperm DNA integrity. This might occur in patients on SSRIs.

It has been previously reported that the 5-HT and gonadotropin releasing hormone systems are in close proximity, particularly in the olfactory bulbs and in the preoptic-anterior hypothalamic area.[17] Serotonin in the medial basal hypothalamus inhibits LH secretion. Endocrinological etiologies of male infertility or subfertility include hypopituitarism, resulting in deficient follicle stimulating hormone or LH. While to our knowledge no mechanism has been identified to explain potential SSRI induced changes in semen quality, neuroendocrine factors may have a role. For example, serotonin increases prolactin by inhibiting dopamine and by stimulating the activity of prolactin releasing factors.[18] An increase in serum prolactin can impair fertility through 3 mechanisms, including at the hypothalamic level by increasing dopamine, which suppresses gonadotropin releasing hormone release, at the pituitary level by suppressing follicle stimulating hormone and LH release, and at the gonadal level by suppressing gonadal hormone production.[19] SSRIs are known to augment the 5-HT receptor mediated stimulation of prolactin secretion.[20] In the current study sperm concentration, percent motile sperm, percent normal morphology, total motile sperm and SCSA results correlated negatively with treatment duration. Therefore, the duration of treatment may be crucial, which could be clarified by further studies of the long-term effects of SSRIs in humans.

Our study is not without limitation. In this study none of the patients underwent hormonal evaluation. We did not include endocrine parameters. Indeed, impaired semen quality and damaged sperm DNA integrity might be due to endocrine dysfunction of the reproductive axis in depression. The association between depression and hypothalamus-pituitary-adrenal axis dysfunction is well-known but to our knowledge the association between depression and hypothalamus-pituitary-testis axis dysfunction has yet to be determined. We hope that these data will help physicians inform patients about the likely effects of SSRIs on fertility capacity and provide a basis for further investigations in the future.

CONCLUSIONS

Patients receiving SSRIs had statistically significant impairment in all semen parameters and increased sperm DNA damage. Whether the cessation of SSRI treatment reverses these adverse effect on spermatogenesis remains unclear. Further studies are needed to elucidate the impact of the observed impairment of semen quality on fertility.

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Abbreviations and Acronyms

5-HT	=	5-hydroxytryptamine
AP	=	activator protein
DFI	=	DNA fragmentation index
LH	=	luteinizing hormone
SCSA	=	sperm chromatin structure assay
SSRI	=	selective serotonin reuptake inhibitor

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