Effect of emotional stress on sperm quality

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Received September 24, 2007

\textit{Background & objectives:} Emotional stress plays a detrimental role on fertility. In this study male patients with idiopathic infertility were selected after evaluation of psychological stress to evaluate a positive effect of a stress therapy on their semen quality.

\textit{Methods:} A total of 20 patients with infertility were enrolled in the study and randomly divided in two groups. Ejaculates were examined by light and transmission electron microscopy (TEM). Meiotic segregation was also investigated by fluorescence \textit{in situ} hybridization (FISH). Ten patients were treated with Conveyer of Modulating Radiance (CRM) therapy and sperm characteristics and meiotic segregation were evaluated again three months at the end of treatment.

\textit{Results:} TEM data showed that, among sperm pathologies, necrosis and apoptosis were higher and the number of “healthy” sperm was significantly reduced in both groups of stressed men compared to reference values\textsuperscript{1}. The number of “healthy” sperm was significantly higher in the treated group after therapy, indicating a recovery of sperm quality, although no significant decrease in sperm pathologies was observed. FISH analysis showed that the mean frequencies of sex chromosomes disomies and diploidies significantly decreased after stress therapy.

\textit{Interpretation & conclusions:} The effects induced by stress also seem to include meiotic and structural alterations in sperm cells. The spermatogenic process was improved after a cycle of CRM therapy indicating that stress is an additional risk factor for idiopathic infertility.

\textbf{Key words} CRM therapy - emotional stress - FISH - sperm - TEM

It has been hypothesized that life stress alters the dynamic regulation of the autonomic, neuroendocrine, and immune systems\textsuperscript{2}. In many cultures social and family issues of reproduction are very important and it seems logical that a couple that fails to achieve the expected goal of reproduction would experience feelings of frustration and disappointment.

The literature regarding artificial insemination and the associated psychological, psychiatric and sexual disorders has mainly been carried out in the field of gynaecology\textsuperscript{3}, approaching the disorder from the point...
of view of the female partner. Very few studies have been reported on andrology. Lemyre et al\textsuperscript{6} described a Measure of Psychological Stress (MPS), a psychometric scale used for measuring styles of defence mechanisms. Chronic exposure to stress increases hypothalamic-pituitary-adrenal (HPA) axis activity and concomitantly reduces hypothalamic-pituitary-gonadal (HPG) axis activity. A study conducted on male rats showed that the sexual behaviour might be the most vulnerable aspect of male reproduction to acute and chronic stress due to the antagonistic relationship between testosterone and corticosteroids\textsuperscript{5}.

Most studies have rejected the theory of stress as the only factor in the aetiology of infertility; but there is growing evidence to show that stress is an additional risk factor for infertility. For example, it has been found that sperm quality decreases after a natural disaster, such as an earthquake\textsuperscript{6}. Emotional stress connected with work or, for example, a depressive reaction to infertility or its therapy, is one of the frequent causes of decreased semen quality\textsuperscript{7}. Stress interaction with the autonomic nerve functions may therefore interfere with both sperm numbers and semen volume and probably with sperm motility\textsuperscript{8}. Other studies confirm a negative influence of increased stress on the semen volume, on the percentage of normal morphological sperm shapes\textsuperscript{9} and on sperm concentration\textsuperscript{10}. Most studies investigating the association between psychological stress and semen quality lacked information on biochemical parameters. Only recently an increase in superoxide dismutase (SOD) activities\textsuperscript{11} and an increase in nitric oxide (NO) levels with a decrease in arginase activity in the L-arginine-NO pathway\textsuperscript{12} have been shown to be present in the seminal plasma of men in a condition of stress.

Recent advances in modern technologies have provided conveyer of modulating radiance (CRM) therapy. CRM therapy has been applied in clinical setting in many specialized areas for the treatment of illnesses and symptoms most frequently related to psychological stress. This therapy has been recognized by the Australian Clinical Trial Register (ACTR) and International Clinical Trials Registry Platform (ICTRP) of World Health Organization (WHO)\textsuperscript{13}.

We undertook this study to investigate the effect of CRM therapy on sperm quality from morphological and meiotic points of view in men with idiopathic infertility who were also stressed as evaluated by psychological test.

**Material & Methods**

**Patients selection**: A psychological test was performed at the beginning of the study to identify patients affected by stress. From January to December 2005. Twenty male patients (aged 29 to 37 yr) with idiopathic infertility were randomly selected at the Interdepartmental Centre for the Research and the Therapy of Male Infertility, Siena University, Italy, and their information was recorded in a database. Of the 20 men selected, 10 were allocated in the treated group (group I) and 10 as controls (group II). The presence of varicocele was excluded clinically and by Doppler sonography. In all selected patients, sexual development and medical histories were normal, patients did not have anatomical pathologies or hormonal imbalance, they were not carriers of genetic sperm defects and there was no consanguinity in their family histories. Microbiological investigations did not reveal any genitourinary infections. None of the patients had ever received hormone therapy. Only patients with an apparently normal 46, XY karyotype were included in this study. The presence of Y microdeletions was set up by PCR in patients with a number of sperm/ml lower than $15 \times 10^6$. For evaluation of hormonal profile, karyotype and of Y microdeletions 15 ml of blood were drawn from each patient. Patients were not smokers or drinkers and they had not been in contact with noxious substances. All patients have written and signed an informed consent to participate in the research. For this type of research any ethical approval is needed. Unfortunately at the end of the study only 20 men were able to furnish semen samples before and after treatment.

**Psychological test**: A standardized and validated self-reporting test for the measurement of psychological stress (MPS)\textsuperscript{4,14} was self-administered to the subjects in the treatment group, as well as in controls. The test is a questionnaire of 49 items for self-evaluation of answers, regarding stress conditions, and there is a system of elaboration of the results. Each item is based on clusters of stress condition: loss of self-control, irritability, psychological sensations, confusion, anxiety, depression, physical pain, hyperactivity and acceleration. Patient were expected to answer the questions about their psychological stress using 4 answers, according to the intensity of psychological stress condition (very much=4, much=3, little=2, none=1). The final score is expressed in total points (TP, $T = Z^* 10 + 50$) according to the summary of the results of each item and in per centile. The total points report normative data in the tables, in per centiles and T points ($T = Z^* 10 + 50$)\textsuperscript{14}.

In this study, total points, per centiles and the considered score were used. The considered score reported the subjective perception of stress.
The test was administered when patients were enrolled and it was repeated three months after the end of therapy in treated patients as well as in untreated patients.

**Semen analysis**

*Light and electron microscopy*: Semen samples were collected by masturbation after 4 days of sexual abstinence and examined after liquefaction for 30 min at 37°C. Volume, pH, concentration and motility were evaluated according to World Health Organization (WHO) guidelines. Semen analysis was repeated three-four months after the end of CRM therapy.

For electron microscopy, sperm samples were fixed in cold Karnovsky fixative and maintained at 4°C for 2 h. Fixed semen was washed in 0.1 mol/l cacodylate buffer (pH 7.2) for 12 h, postfixed in 1 per cent buffered osmium tetroxide for 1 h at 4°C, then dehydrated and embedded in Epon Araldite (Fluka, Germany). Ultra-thin sections were cut with a Supernova ultramicrotome (Reickert Jung, Vienna, Austria), mounted on copper grids, stained with uranyl acetate and lead citrate and then observed and photographed with a Philips CM10 transmission electron microscope (TEM; Philips Scientifics, Eindhoven, The Netherlands).

For each sample, 300 ultra-thin sperm sections were analysed. Major submicroscopic characteristics were recorded by a highly trained examiner who was blind to the experiment. TEM data were evaluated using the statistical mathematical formula by Baccetti et al. which calculates the number of spermatozoa free of structural defects (healthy) and the per centages of three main phenotypic sperm pathologies: immaturity, necrosis and apoptosis.

The lowest number of spermatozoa free of defects (healthy), assuring a normal fertility, is two million.

**Fluorescence in situ hybridization (FISH) analysis of sperm**: In order to evaluate aneuploidy frequency, FISH was performed according to Baccetti et al. on the sperm nuclei of patients. A mix of α-satellite DNA probes (CEP, Chromosome Enumeration Probes, Vysis, IL, USA) for chromosomes 18, X, and Y, directly labelled with different fluorochromes, was used. Sperm nuclei were scored according to published criteria. All samples were analyzed by an highly trained examiner.

Observation and scoring were performed using a Leitz Aristoplan Optical Microscope (Leica, Wetzlar, Germany), equipped with a fluorescence apparatus, with a triple bandpass filter for aqua, orange and green fluorochromes (Vysis) and a monochrome filter for 4’,6-diamidino-2-phenylindole (DAPI, Vysis).

**PCR analysis**: DNA was extracted from peripheral blood lymphocytes using the QIAamp DNA Blood kit (QIAGEN, Valencia, Calif).

PCR (Perkin Elmer Corp., Norwalk, CT) was performed according to EAA/EMQN best practice guidelines for molecular diagnosis of Y chromosomal microdeletions.

Control DNA was extracted from the blood of 10 male donors, aged 30-40 yr, with a documented history of fertility. DNA extracted from the blood of two fertile females was used as a negative control.

**Description of conveyer of modulating radiance CRM® and of neurological-psycho-physical optimization**: The Conveyer of Modulating Radiance (CRM) is an innovative medical device aimed at promoting the neuro-psycho-physical optimization (well-being and a reduction in the adaptive dysfunctional modifications in the nervous system induced by stress). It is a new medical instrument that uses the effects produced by a very low strength magnetic field on the central nervous system of the patient. The instrument used was authorized by the Italian Ministry of Health, Department of Technological Innovation in 2003 (DGFDM/III/P.36113), according to the 93/42/EEC Directive concerning medical devices. The instrument we used is registered under the trademark “Convogliatore di Radianza Modulante” CRM®. This radio-electric conveyer apparatus has radiated frequencies in the same range as the microwave (10.525 Ghz) but the radiated power is lower (below 10 mW). The effects produce an activation of the central nervous system that can optimize neuropsycomotor function and reduce the adaptive dysfunctional modification of the nervous system induced by stress.

The neurological-psycho-physical-optimization (NPPO) auricular therapy protocol was used to manage and optimize these modifications. The CRM probe was applied to seven specific points of the auricular pavilion, the same points that are also used in auricular therapy to treat neurovegetative symptoms and diseases. Eighteen sessions of NPPO with CRM therapy were administered to each patient after the first semen analysis and the MPS test.

The aim of CRM therapy was to optimize the responses of CNS against unknown alterations due to stress from continuous interaction with the environment.
Each therapeutic session lasted approximately three seconds. The protocol was painless, noninvasive, did not require the collaboration of the patient and was completely without side effects. Three months after the end of the CRM therapy, after a new, complete spermatogenic cycle, the MPS test and semen analysis were repeated.

**Statistical analysis:** Statistical analysis was performed using StatgraphicsPlus (vers.5.0, Rockville, MD).

Because the small sample size, to compare the differences in values in the examined variables of the groups (cases, controls, fertile controls), the Wilcoxon’s two-sided signed rank test was used for paired groups and the two sides Mann Whitney W test was utilized for independent groups.

**Results**

Stress status was evaluated in each patient by a psychological test. The final score was expressed in total points, and a considered score reference the subjective perception of stress was also reported. During pre-treatment evaluation total points and considered scores were similar in both the groups. Patients in group I received CRM therapy and during post therapy evaluation there was significant reduction in total points ($P<0.001$) and considered scores ($P<0.05$) in treated patients compared to controls (Table I). In group I, only one patient did not show important stress reduction. The mean of stress evaluation in group II (pre-study 101.4 vs post-study 102.3) was not reduced. There was little variation in stress evaluation values in all patients in group II.

PCR analysis was performed on peripheral blood lymphocytes of patients with a number of sperm/ml lower than $15 \times 10^6$ in order to exclude this well known genetic component for infertility. PCR did not reveal any microdeletions of the Y-chromosome.

The seminological features of the patients in both groups were analyzed by light and electron microscopy (Table II). In group I five patients had a normal sperm concentration and only one showed a progressive motility of >50 per cent, in group II, eight men showed a normal sperm concentration, but all of them had reduced progressive motility (a+b), lower than WHO parameters.

TEM analysis highlighted that two patients in group I had more than 2 million “healthy” sperm, the minimum number of well structured sperm required to be considered fertile. None of the patients in group II reached this value. The mean values of healthy sperm in groups I (2,300,949; $P=0.001$) and II (386,674; $P=0.00018$) were significantly lower (Table II) than reference values.

The main alterations in sperm pathologies were related to apoptosis and necrosis. Marginated chromatin and swollen and badly assembled mitochondria were the typical ultrastructural markers of apoptosis (Fig. 1), and reacted or absent acrosomes, nucleus with disrupted chromatin and broken plasma membrane (Fig. 2) were signs of necrosis.

The mathematical formula by Baccetti et al was used to calculate the per centage of these phenotypic sperm pathologies. Immaturity was not predominant in either group. Necrosis was significantly higher (group I 47.55% $P=0.004$; group II 46.09% $P=0.0017$) compared to reference value (21%). Finally, the presence of apoptosis in groups I and II (4.54%) was more than double that found in fertile controls, although it did not reach statistical significance (Table II).

In both groups, meiotic segregation, investigated by triple color FISH for chromosomes 18, X, and Y probes, was carried out on the sperm nuclei to evaluate aneuploidy frequency. A total score of 5096 sperm nuclei was found in group I, and 4967 were scored in group II.

The mean of frequencies of aneuploidy of chromosomes 18, X, and Y are summarized in Table III. In both groups, the mean frequencies of chromosome

<table>
<thead>
<tr>
<th>Cases</th>
<th>Pre CRM therapy evaluation</th>
<th>Post CRM therapy evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total P</td>
<td>Percentile</td>
</tr>
<tr>
<td>Group I</td>
<td>103.90±20.89</td>
<td>71.45±18.53</td>
</tr>
<tr>
<td>Group II</td>
<td>101.40±21.15</td>
<td>68.25±19.98</td>
</tr>
</tbody>
</table>

Values are mean ±SD of n=10.

Data were obtained before and after CRM therapy and compared using Wilcoxon’s rank test.

* $P<0.05$; ** $P<0.001$ compared to pre stress values
FIG. 2. TEM micrograph of necrotic spermatozoa from a stressed patient before treatment. It is characterized by absent acrosomes (aA) or acrosome with sparse content (sA), misshapen nuclei (aN) with marginated disrupted chromatin (mdCh). Axonemes, accessory fibers and fibrous sheaths are altered (aAX). Plasma membranes are broken (arrows). A longitudinal section of a sperm with normal nucleus (N) and acrosome (A) is also present. X 7,500.

FIG. 1. TEM micrograph of an apoptotic sperm from a stressed patient after treatment. It is characterized by misshapen acrosome (mA), altered nucleus (aN) with a vacuole (V). A large cytoplasmic residue (CR) embeds the axoneme (AX) and disorganized mitochondria (Mt). X 10,000.

Table II. Spermiogram and TEM data from semen samples of patients (group I) and controls (group II) before and after CRM therapy

<table>
<thead>
<tr>
<th>Cases</th>
<th>Sperm/ml X 10^6</th>
<th>Motility</th>
<th>Volume</th>
<th>Apoptosis per cent</th>
<th>Necrosis per cent</th>
<th>Immaturity per cent</th>
<th>“Healthy” sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Group I</td>
<td>58.55 ± 55.17 ±</td>
<td>26.2 ± 30.2 ±</td>
<td>3.72 ± 4.11 ±</td>
<td>8.21 ± 4.68 ±</td>
<td>47.55 ± 44.56 ±</td>
<td>57.65 ± 55.57 ±</td>
<td>2300949.6 ± 5542314.4 ±</td>
</tr>
<tr>
<td></td>
<td>63.03 ± 63.64 ±</td>
<td>17.05 ± 16.94 ±</td>
<td>1.14 ± 2.15 ±</td>
<td>8.27 ± 3.19 ±</td>
<td>18.11 ± 16.16 ±</td>
<td>18.24 ± 16.86 ±</td>
<td>5673363.5 ± 14723062.6</td>
</tr>
<tr>
<td>Group II</td>
<td>54.83 ± 36.04 ±</td>
<td>25.5 ± 21.2 ±</td>
<td>3.49 ± 3.33 ±</td>
<td>9.27 ± 10.08 ±</td>
<td>46.09 ± 43.80 ±</td>
<td>63.69 ± 68.20 ±</td>
<td>386674.2 ± 313594.3 ±</td>
</tr>
<tr>
<td></td>
<td>46.07 ± 29.01 ±</td>
<td>10.94 ± 7.36 ±</td>
<td>1.71 ± 0.89 ±</td>
<td>6.55 ± 6.32 ±</td>
<td>11.64 ± 12.32 ±</td>
<td>10.91 ± 13.10 ±</td>
<td>429767.8 ± 446034.8</td>
</tr>
</tbody>
</table>

Reference values °°, °°>20×10^6 °°>50 °°>2-6 4.80 ± 3.40° 21.00 ± 14.94° 55.10 ± 10.74° >2×10^6°
° Baccetti et al; °° WHO

Values are mean ± SD (n=10)
°P<0.05 compared to pre values.

All data were compared in each group before and after CRM therapy using Wilcoxon’s rank test; every value of groups I and II was compared with reference values using Mann Whitney W test
18 disomy were within the normal range; the means of frequency of diploidy and sex chromosome disomy were higher than reference values, but only diploidy reached statistical significance \((P=0.01)\) in group I. Three patients in group I showed all disomy and diploidy values within range; one man in group II showed FISH values within the normal range.

Patients in group I underwent Rinaldi-Fontani treatment (CRM therapy) and group II patients did not receive any much treatment. Both groups were re-examined three months after the end of the therapy.

The mean of progressive motility of sperm increased in group I, although it did not show significant recovery; in particular, it was noted that a patient reached a normal sperm concentration and motility compared to WHO parameters. Important improvement was observed in seminal parameters only in group I patients.

In order to quantify the effects of CRM therapy on sperm morphology, the sperm quality was analyzed by TEM after CRM therapy in both the groups and the data were compared with those obtained in the first examination.

In the treated group (group I), necrosis and immaturity did not show a significant decrease, whereas the per centage of apoptosis reached normal values (4.68%), however the mean per centage of the total number of “healthy” sperm was significantly higher, \((P<0.05)\), after treatment (Table II). In the control group (group II), no significant decrease was found in the per centage of sperm pathologies and the number of “healthy” sperm did not increase.

Regarding FISH data (Table III), in group I the mean frequency per centage of sex chromosome disomy and diploidy was significantly reduced after stress therapy treatment. Three patients recovered normal meiotic segregation. In group II the mean values of disomy and diploidy did not significantly decrease.

### Discussion

The use of electricity and magnetic fields in biomedical sciences, particularly in therapy of pathologies of the nervous system, is well known\(^{21,22}\). We evaluated CRM therapy as a new medical tool for stress management, applied to male infertility. The protocol is painless and non invasive, it does not require collaboration by the patient and there are no side effects. Moreover, this therapy is not pharmacological and it does not interfere with the concomitant use of other therapies.

The interaction done during the last two decades show that in a majority of cases, stress is the result and not the cause of infertility\(^{23}\). Although, various studies have demonstrated the importance of the mind-body connection and fertility, the psychosocial aspects of infertility have not been adequately addressed. Psychological factors such as depression, anxiety, and stress-induced changes in heart rate and cortisol level are predictive of a decreased probability of achieving a viable pregnancy\(^{24}\). A previous study showed a significant reduction in the general stress level and especially in correlated stress disorders such as loss of control and irritability, psycho-physical sensations, a sense of effort and confusion, depressive anxiety, pain and physical problems, hyperactivity when the CRM therapy was applied\(^{20}\).

In this study, we analyzed semen quality in a group of selected men showing a condition of psychological stress evaluated by the MPS test and an idiopathic infertility. Patients showed altered semen quality, particularly in progressive motility. Mental stress has

<table>
<thead>
<tr>
<th>Cases</th>
<th>Per cent diploidy</th>
<th>Per cent chromosome 18 disomy</th>
<th>Per cent sex chromosome disomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Group I</td>
<td>0.449 ± 0.30</td>
<td>&quot;0.366 ± 0.23&quot;</td>
<td>0.09 ± 0.03</td>
</tr>
<tr>
<td>Group II</td>
<td>0.446 ± 0.21*</td>
<td>0.350 ± 0.07</td>
<td>0.122 ± 0.07</td>
</tr>
<tr>
<td>Reference</td>
<td>0.28 ± 0.006</td>
<td></td>
<td>0.110 ± 0.003</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=10).

All data were compared in each group before and after CRM therapy using Wilcoxon’s rank test; every value of groups I and II was compared with reference values using Mann Whitney W test

\(P *=0.04\) \(** =0.01\) compared to pre values

\(*P= 0.03\) compared to reference values
already been shown to negatively influence sperm quality with an increase of superoxide dismutase. Among sperm pathologies, necrosis and apoptosis of sperm were higher than normal values. It has been demonstrated that the stress and glucocorticoid administration induce germ cell apoptosis in rat testes, mainly in spermatogonia. Before therapy, FISH analysis highlighted the presence of aneuploidy, particularly diploidy and sex chromosome disomy.

After a cycle of CRM therapy, a significant reduction was noted in points indicating the subjective perception of stress in the analysed subjects. We repeated all investigations on semen samples by light and electron microscopy; an improvement in sperm motility and a reduction in the per centage of apoptosis were observed, concomitant with a significant increase in “healthy” sperm and a significant decrease in aneuploidies. These results seem to suggest that CRM treatment optimizes psychophysical well-being, reducing the maladjusted responses to environmental stress and thus optimizing neuroendocrine responses, accompanied by a general improvement in spermatogenetic condition, as demonstrated with sophisticated tools such as TEM and FISH. Semen quality seems to improve in subjects when the spermatogonic process is not particularly compromised. Since CRM therapy probably has beneficial effects on the neuropsychophysical manifestations of stress, it could be particularly indicated in male infertility. These findings suggest the administration of CRM therapy to stressed men with idiopathic infertility. Stress reduction may improve sperm quality, and it may diminish the number of assisted reproduction treatment cycles required for pregnancy or even render more invasive techniques unnecessary. Further studies are needed on a larger population, also to verify the stability over time when using more than one cycle, although it could be very difficult to obtain and maintain a selected group, especially after therapy. Additional research is needed and evaluation of carefully designed psychological interventions must go hand-in-hand with improved recruitment strategies. In conclusion, our findings showed that stress may be an additional risk factor for idiopathic infertility in men and CRM therapy may be beneficial to improve the spermatogenic process.

References

13. Australian Clinical Trail Register (ACTR) and International Clinical Trials Registry Platform (ICTRP) of World Health Organization (WHO). 


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