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EFFECT OF EMOTIONAL STRESS ON SPERM QUALITY

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Effetti dello stress psicologico sulla qualità dello sperma

Background e obiettivi:
Lo stress emozionale gioca un ruolo dannoso sulla fertilità. In questo studio pazienti di sesso maschile con infertilità idiopatica sono stati selezionati dopo valutazione per la presenza di stress psicologico, per determinare l'effetto positivo di una terapia per lo stress sulla qualità del loro sperma.

Metodo
In questo studio sono stati arruolati un totale di 20 pazienti con infertilità e suddivisi in modo random in due gruppi. L'eiaculato è stato esaminato con Microscopia Elettronica (TEM). La suddivisione meiotica è stata indagata con Tecniche di Ibridazione Fluorescente in situ (FISH). Dieci pazienti sono stati trattati con terapia con Convogliatore di Radianza Modulante (CRM Terapia); le caratteristiche dello sperma e la suddivisione meiotica è stata di nuovo valutata dopo tre mesi dalla fine del trattamento.

Risultati
I dati della TEM mostrano che, tra le patologie spermatiche, la necrosi e l'apoptosi sono superiori e il numero dei liquidi spermatici "sani" sono significativamente ridotti in entrambi i gruppi di uomini stressati comparati con i valori di riferimento.
Il numero dei liquidi spermatici "sani" era significativamente superiore nel gruppo dei trattati dopo la terapia, indicando un recupero della qualità dello sperma.
L'analisi alla FISH mostra che le minime frequenze delle disomie e della diploidie del cromosoma sessuale si riducono significativamente dopo terapia per lo stress.

Interpretazioni e conclusioni
Gli effetti indotti dallo stress sembrano anche includere la meiosi e le alterazioni strutturali nelle cellule spermatiche.
Il processo della spermatogenesi è migliorato dopo un ciclo di CRM Terapia indicando che lo stress è un fattore di rischio aggiuntivo per infertilità idiopatiche.

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Effect of emotional stress on sperm quality

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***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.**

***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 *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.**

***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[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.**

***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.**

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[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[3], approaching the disorder from the point

of view of the female partner. Very few studies have been reported on andrology. Lemyre *et al*[4] 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[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[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[7]. Stress interaction with the autonomic nerve functions may therefore interfere with both sperm numbers and semen volume and probably with sperm motility[8]. Other studies confirm a negative influence of increased stress on the semen volume, on the per centage of normal morphological sperm shapes[9] and on sperm concentration[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[11] and an increase in nitric oxide (NO) levels with a decrease in arginase activity in the L-arginine-NO pathway[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) [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 were 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 $15x10^6$. 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 (MSP)[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 ($T=Z^* 10+50$)[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, *p*H, concentration and motility were evaluated according to World Health Organization (WHO) guidelines[15]. 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 (*p*H 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*[16] 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[1].

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*[17]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[17,18]. 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 pratice guidelines for molecular diagnosis of Y chromosomal microdeletions[19].

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[20] 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[4,14].

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 $15x10^6$ 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[15].

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[1].

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*[16] 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 I. Stress evaluation with MPS test in patients (group I) and controls (group II)

Cases	Pre CRM therapy evaluation			Post CRM therapy evaluation		
	Total P	Percentile	Considered score	Total P	Percentile	Considered score
Group I	103.90±20.89	71.45±18.53	3.55±1.39	85.90±9.75**	52.70±15.39**	2.50±0.67*
Group II	101.40±21.15	68.25±19.98	3.86±1.74	102.30±19.68	71.30±20.42	3.62±1.35

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;**<0.001 compared to pre stress values

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

Cases	Sperm/ml X 10^6		Motility		Volume		Apoptosis per cent		Necrosis per cent		Immaturity per cent		"Healthy" sperm	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Group I	58.55 ± 63.03	55.17 ± 63.64	26.2 ± 17.05	30.2 ± 16.94	3.72 ± 1.14	4.11 ± 2.15	8.21 ± 8.27	4.68 ± 3.19	47.55 ± 18.11	44.56 ± 16.16	57.65 ± 18.24	55.57 ± 16.86	2300949.6 ± 5673363.5	5542314.4 ± 14723062.6*
Group II	54.83 ± 46.07	36.04 ± 29.01	25.5 ± 10.94	21.2 ± 7.36	3.49 ± 1.71	3.33 ± 0.89	9.27 ± 6.55	10.08 ± 6.32	46.09 ± 11.64	43.80 ± 12.32	63.69 ± 10.91	68.20 ± 13.10	386674.2 ± 429767.8	313594.3 ± 446034.8
Reference values °°,°,°°>20×10^6			°°>50		°°2-6		4.80 ± 3.40°		21.00 ± 14.94°		55.10 ± 10.74°		>$2\times10^{6°}$	

° Baccetti *et al*[1]; °° WHO[14]

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

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.

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.

Table III. FISH data in sperm nuclei of semen samples of stressed men (group I) and stressed controls (group II) before and after CRM treatment.

Cases	Per cent diploidy		Per cent chromosome 18 disomy		Per cent sex chromosome disomy	
	Pre	Post	Pre	Post	Pre	Post
Group I	0.449 ± 0.30	**0.366 ± 0.23	0.09 ± 0.03	0.10 ± 0.01	0.362 ± 0.25	*0.298 ± 0.15
Group II	0.446 ± 0.21+	0.350 ± 0.07	0.122 ± 0.07	0.101 ± 0.03	0.330 ± 0.14	0.284 ± 0.08
Reference values	0.28 ± 0.006		0.110 ± 0.003		0.230 ± 0.004	

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

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

already been shown to negatively influence sperm quality with an increase of superoxide dismutase[11]. 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[25]. 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 spermatogenic process is not particularly compromised. Since CRM therapy probably has beneficial effects on the neuropsycophysical manifestations of stress[13], 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[26]. 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.

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Commentary

Emotional stress & male infertility

Infertility imposes a major psychological burden on patients. It affects an estimated 10-15 per cent of couples, and in roughly half of these cases the defect can be traced to the male[1]. A large portion of these men is infertile because of abnormalities in sperm parameters. Paradoxically, the use of assisted reproductive technology such as intracytoplasmic sperm injection (ICSI) has increased the need for identifying and understanding the basis of sperm abnormalities of unknown origin. The explosive growth in the use of assisted reproduction techniques (ART) focuses our attention on the fact that spermatogenic defects may be transmitted by ICSI more readily than by *in vivo* fertilization[2]. Therefore, it is of utmost importance to identify sperm abnormalities before directing the algorithm of infertility management towards ART.

Despite the current exciting phase of andrology research, which has been endowed with significant developments, many of the aetiological factors for the lack of fecundity remain unidentified. Hence, the term "unexplained infertility" has become one of the established diagnoses. The diagnosis of unexplained infertility may represent misfortune as a result of laws of chance or a limitation of knowledge of reproductive physiology. Ideally, the diagnosis would specifically identify couples with real but subtle defects in reproductive function that are not detected by available methods. In practice, however, unexplained infertility is a diagnosis of exclusion that is made when a couple is involuntarily infertile and no abnormalities are revealed by a standard infertility evaluation. Emotional stress could be one of these factors that are consistently overlooked but yet play a significant role in the aetiology of infertility.

There is wealth of data describing the impact of emotional stress on the female's reproductive health[3,4]. Psychological stress may reduce the female reproductive performance in various ways including the autonomic nervous system, the endocrine and immune systems. There is, however, a lack of clear consensus as to the definition and measurement of 'psychological stress', bringing into question the nature and strength of any putative association[5]. Therefore, the evidence appears to be limited and not consistent across studies. Although reported studies support an association between increased levels of psychological stress and impaired reproductive performance, there is lack of consensus in defining and measuring stress levels. In turn, the level of precision in determining a cause-effect relationship is low.

The relationship between emotional stress and male fecundity is similarly controversial. While initial reports about the impact of distress on male fertility were rather anecdotal, in the last few decades more systematic research has been conducted. The correlation between sperm quality and distress has been computed in several studies. Given the wide range of values of sperm parameters, it is not too surprising that these cross-sectional studies led to contradictory results[6]. Whether infertility is a chronic stressor for couples suffering from infertility or stress may interfere with spermatogenesis and fertility rate remains to be identified, it is possible that both hypotheses are interchangeably related and constitute the components of a vicious circle.

Much research has focused on the change of sperm quality in men exposed to stress. A recent study has assessed the effect of the Lebanese civil war on sperm parameters[7]. It was reported that the sperm concentration was significantly lower during the war compared with the postwar period. However, the percentage of abnormal sperm morphology increased in the postwar period. The significant decline in sperm concentration could be attributed to the increased stress level during the war[7]. Supporting evidence is provided

in a study by Fukuda *et al* that reported decreased sperm quality subsequent to a natural disaster such as an earthquake[8]. On the other hand, controlled studies have also revealed that basic sperm parameters declined during the course of ART treatment. These studies were based on the rationale that medical examinations and involvement in the treatment of the spouse are stressful for the majority of patients. Therefore, a decline in sperm quality was hypothesized when comparing the last pre-treatment semen analysis with the specimen from the treatment cycle several weeks later[6]. The impact of stress goes beyond affecting the couple's ability to bear children to affect their actual marital relation. Stress associated with ART treatments can be both a causative factor for infertility and a cause for tense marital relationships. A positive impact of successful ART cycles on marital relations was reported[9].

Despite the overwhelming evidence associating stress with male infertility, the relationship appears to be still far from being established. Contradictory reports overshadow positive findings and cast serious doubts whether such a relationship really exists. A study conducted in Denmark found no associations between any semen characteristic or sexual hormones and any job strain variable[10]. Interestingly, personality attitudes, psychopathological symptoms and biological parameters also appear to play a role in male infertility. A negative correlation was found between seminal parameters and 'extraversion', 'anxiety' and 'psychoticism'[11].

Multiple factors could act as modulators for the suboptimal sperm quality found in the presence of emotional stress. Antioxidant enzymes of the seminal plasma, superoxide dismutase (SOD) and catalase were monitored in normal healthy medical students during academic examination period. Results indicated that during stress period, stress scores and SOD activities increased significantly compared to the non-stress period. Spermatozoa concentrations, motility index and percentage of rapid progressive motility decreased under stress. This could be attributed at least in part to redox imbalance[12]. The "readthrough" variant of acetylcholinesterase (AChE-R) provides another pathway for stress-induced infertility. AChE-R is involved in the cellular stress response in a variety of mammalian tissues. Transgenic mice overexpressing AChE-R displayed reduced sperm counts, decreased seminal gland weight, and impaired sperm motility compared with age-matched nontransgenic controls. Sperm head AChE-R staining was also conspicuously reduced in samples from human couples for whom the cause of infertility could not be determined, similar to the pattern found in transgenic mice[13]. These findings indicate that AChE-R is involved in impaired sperm quality, which suggests that it is a molecular marker for stress-related infertility.

The study by Colledel *et al*[14] provides additional insight regarding the impact of psychological stress on the male reproductive function. The data clearly showed that sperm quality using transmission electron microscopy (TEM) was declined in a group of men identified as stressed. Most importantly, the study shows that the employment of Conveyer of Modulating Radiance (CRM) was associated with a significant improvement in sperm quality and decrease in sex chromosomes disomies and diploidies as evidenced by fluorescence *in situ* hybridization (FISH) analysis. The data presented in the study show an overall improvement in sperm parameters following the administration of CRM therapy. However, this does not consistently appear in all cases, thus, it appears that the beneficial effects of CRM will depend on individual factors. In turn, more research is still needed to identify those cases that would benefit the most from a similar approach. Nevertheless, the above mentioned study offers an alternative approach to male infertility that is based on identifying and treating the emotional stress factor as well as other conventional parameters. Such a holistic approach may be of added value in management of men with suboptimal sperm quality.

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