Association of state and trait anxiety to semen quality of in vitro fertilization patients: a controlled study

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Objective: To investigate the relationship between semen quality and state/trait anxiety in patients enrolled in an in vitro fertilization (IVF) program and in control subjects.
Design: Cross-sectional study.
Setting: Centre for Reproductive Medicine and Biology, European Hospital, Rome.
Patient(s): Ninety-four first-attempt IVF patients and 85 age-matched, random subjects recruited in the period July 2006 through March 2008.
Intervention(s): None.
Main Outcome Measure(s): Behavioral features of stress, including state and trait anxiety, self-perceived impact of physical disturbance on everyday activities, ethanol consumption, cigarette smoking, and semen parameters such as semen volume, sperm concentration, total count, motility, morphology, and DNA fragmentation.
Result(s): Increased levels of both state and trait anxiety were associated with lower semen volume, sperm concentration and count, reduced sperm motility, and increased sperm DNA fragmentation of IVF patients, thus influencing seminal parameters at the macroscopic and cellular/subcellular levels. Similar results were obtained in the controls.
Conclusion(s): Our data confirm previous observations with state anxiety and show that trait anxiety also is negatively associated with male fertility. (Fertil Steril® 2013;99:1565–72. ©2013 by American Society for Reproductive Medicine.)
Key Words: IVF, semen quality, sperm DNA fragmentation, sperm motility, state anxiety, trait anxiety

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Psychological stress is considered as one of the causes of idiopathic infertility in both men and women (1, 2). Together with physical stresses, psychological stress strongly affects infertile couples undergoing in vitro fertilization (IVF) procedures (3, 4). However, although the impact of psychological stress has been well analyzed in women (5–7), questions remain with respect to the association of this type of stress with male reproductive parameters. This problem ought to be addressed, as male reproductive impairments represent the cause of or a contributing factor to couple infertility in approximately 50% of cases (8, 9).

To date, studies performed in male IVF patients have examined the relationship between semen quality and stress/anxiety, personality factors, and coping strategies. Several of these studies have reported an association between higher stress levels and lower semen quality. The parameters

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associated with psychological stress include semen volume, sperm concentration, motility, and morphology (3, 9–12). However, the results that have been obtained by the various studies are difficult to compare because different psychological aspects were investigated. In addition, these results usually included state anxiety, a transient condition relative to a subject's momentary feelings and emotional state; in IVF patients, state anxiety may strongly depend on the acute emotional distress of the medical experience.

In contrast, trait anxiety, the temperament aspect of anxiety, reflects stable personality variables that induce each individual to react to possibly dangerous situations with high-level emotional responses, independent of external situations. The question of an association of trait anxiety with male semen parameters remains open. To test the hypothesis that trait anxiety may have a measurable association with major reproductive male parameters, we have compared the relationship between state and trait anxiety and semen features in men undergoing treatment in an IVF program as compared with random control subjects. In addition to standard semen parameters, our analysis included sperm DNA fragmentation, a molecular alteration that results in decreased male fertility (13), which is induced by high levels of reactive oxygen species (ROS) in the seminal plasma (14), possibly associated with higher levels of psychological stress (15).

All patients analyzed were at their first IVF attempt. We excluded those who had experienced previous unsuccessful cycles because this may potentially have influenced their psychological responses and increased their level of state anxiety.

MATERIALS AND METHODS

Patients

Male first-attempt IVF patients of the Centre for Reproductive Medicine and Biology, European Hospital in Rome were recruited during the period of July 2006 to March 2008. Initially, 293 patients were interviewed and received the questionnaires. Of these, 222 returned the questionnaires, and 15 were excluded because of incomplete responses. From the remaining sample of 207 patients, we further excluded 34 who presented with a diagnosis of varicocele; 53 with a diagnosis of urogenital tract infection, orchitis, or retractile testes; 18 who had undergone urogenital surgery of any kind, including scleroembolization or tying of sperm ducts; and 8 who had a genetic condition (Klinefelter syndrome) or chronic disease (diabetes mellitus). The final sample consisted of 94 men who reported no stress factors in the previous 3 months, with the exclusion of the knowledge of their medical condition. Although 8 of these men reported having had children with previous spouses, 86 were characterized by primary infertility. No inquires were directed to the men's partners. The patients' ages ranged from 29 to 49 years (mean: 38.91 $\pm$ 4.54 standard deviation [SD]).

The controls were recruited randomly during the same period from among the male participants in "Health Care Day" at the center. Among the 124 men who were interviewed and received the questionnaires, 39 were excluded for the same criteria as described for the patient sample, including an absence of stress factors in the previous 3 months. The final control sample consisted of 85 men, whose ages ranged from 31 to 48 years (mean: 37.71 $\pm$ 3.76 SD).

The study was approved by the European Hospital ethics committee and was conducted according to the guidelines of the World Medical Association Declaration of Helsinki. All participants gave written informed consent.

Semen Assessment

The week before semen collection, each patient and control participant was asked to observe a strict 3- to 5-day sexual abstinence period before his next visit. Semen specimens were then collected by masturbation at the clinic and were allowed to liquefy at room temperature for 30 minutes. Specimens were handled according to the guidelines previously described elsewhere (16) with minor modifications. The parameters evaluated included semen volume, sperm concentration, morphology, and motility. A terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) test was performed to assess the fraction of cells with fragmented DNA.

The semen volume was measured in a calibrated pipette with an accuracy of 0.1 mL. During this phase, other features of each sample were evaluated, including color, viscosity, and presence of debris. The measurement of sperm concentration was performed on undiluted semen by placing a 10-μL aliquot in a Makler chamber and counting under an inverted microscope (Nikon Italia) at $\times$200 magnification. The total sperm count (TC) relative to each patient was calculated by multiplying the sperm concentration by the semen volume.

For the assessment of sperm motility, 10-μL semen samples were streaked on a glass slide, and, where possible, at least 100 sperm cells were scored under $\times$200 magnification to determine the percentage of [a] progressive motile, [b] nonprogressive motile, [c] and immotile cells. The total percentage of motile sperm cells was calculated by addition: a + b. When fewer than 100 sperm cells were recovered, they were classified as described previously, and the relative percentages were calculated accordingly.

The percentage of morphologically normal sperm cells was evaluated on 10-μL samples, streaked on a glass side, air-dried, and subjected to Papanicolaou staining. Sperm cells were analyzed under $\times$1,000 magnification for the shape and size of the head, presence of vacuoles, shape and size of the neck region, presence of cytosolic residues, length of the flagellum, and presence of one or two flagella (16).

For the TUNEL assay, sperm cells from each sample were washed in HEPES-buffered medium, pelleted at 1,700 rpm for 10 minutes, and resuspended in fresh HEPES-buffered medium. Sperm cells were streaked on a glass slide, air-dried, fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4, for 15 minutes at room temperature, and air-dried again. Cells were then permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate for 2 minutes at 4°C, washed twice, and labeled by use of a commercial kit and according to the manufacturer's procedure (Roche Applied Science). After incubation in the presence of the enzyme and fluorescein-conjugated dUTP for 1 hour at 37°C in the dark, the cells were washed in PBS