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

TABLE 1

Distribution of semen parameters, and state and trait anxiety scores.

	First-attempt IVF patients (n = 94)				Controls (n = 85)				
Semen parameter	**Mean**	**SD**	**Minimum**	**Maximum**	**Mean**	**SD**	**Minimum**	**Maximum**	***P* value[a]**
Concentration, $\times 10^6$/mL	28.74	25.65	0.0	91.0	52.29	19.40	8.0	90.0	<.001
Volume, mL	3.14	1.59	0.6	8.0	3.11	0.86	1.2	5.3	
Total count, $\times 10^6$	83.72	88.87	0.0	491.0	164.31	79.22	13.5	354.2	<.001
Progressive motility, %	19.48	10.80	0.0	46.0	34.58	7.55	18.0	48.0	<.001
Total sperm motility, %	31.81	12.99	0.0	62.0	46.92	8.78	34.0	65.0	<.001
Normal morphology, %	8.56	5.91	0.0	21.0	11.98	6.14	1.0	29.0	<.001
DNA fragmentation, %	7.80	6.26	1.0	30.0	7.31	5.08	1.0	23.0	
State anxiety[b]	39.84	10.10	20.0	69.0	38.15	8.59	26.0	65.0	
Trait anxiety[b]	37.63	7.95	20.0	56.0	36.84	7.16	21.0	52.0	

Note: Abstinence time for all subjects was strictly controlled: minimum 3 days, maximum 5 days. Total count = Concentration × Volume. SD = standard deviation.
[a] Difference evaluated by a *t* test analysis (only statistically significant differences are shown).
[b] Evaluated by the State-Trait Anxiety Inventory (STAI) Y (Spielberger et al., 1986).

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three times for 3 minutes and air dried, counterstained with 6-diamino-2-phenylindole (DAPI), and stored at 4°C until the evaluation time. For each sample, negative controls were obtained by omitting the enzyme and fluorescein-conjugated dUTP. Approximately 200 sperm cells from each sample were scored blindly by two different operators.

Questionnaires

State-trait anxiety inventory Y. Acute and stable anxiety levels of the IVF patients and controls were assessed by administration of the Italian version of the State-Trait Anxiety Inventory (STAI) Y (17, 18). This questionnaire is divided in two parts: the first evaluates state anxiety by inquiring about the current emotional state. It is composed of 20 questions with responses on a 1–4 Likert scale, ranging from "not at all" (score 1) to "extremely" (score 4). Total scores range from 20 to 80, with higher scores indicating higher degrees of state anxiety. The second part assesses trait anxiety, asking the subjects to describe how they usually feel. It is composed of 20 questions with responses on a Likert 1–4 scale, ranging from "almost never" (score 1) to "almost always" (score 4). The STAI Y questionnaires were completed in approximately 20 minutes.

Work and social adjustment scale. Presence and seriousness of functional impairments caused by patients' medical experience or present in the controls were self-evaluated by the Work and Social Adjustment Scale (WSAS) (19, 20). This questionnaire rates the perceived disability in the areas of work, household activities, free time devoted to social activities, free time devoted to personal activities, and interpersonal relationships on a graphic 0–8 Likert scale, ranging from "not at all" (score 0) to "extremely" (score 8). The WSAS was completed in approximately 3 minutes.

Alcohol consumption and cigarette smoking. The men also were asked to report information on alcohol consumption and smoking, which represent a possible cause of decreased fertility and increased levels of seminal ROS along with sperm DNA fragmentation (21, 22). The ethanol consumption evaluation scale was as follows: 1 = no alcohol; 2 = 1–2 glasses of wine/beer a week; 3 = 1–2 glasses of wine/beer a day; and 4 = >2 glasses a day, including spirits. The smoking evaluation scale was as follows: 1 = no or occasional smoking; 2 = up to 10 cigarettes a day; 3 = 10–20 cigarettes a day; 4 = >20 cigarettes a day.

Statistical Analysis

A descriptive analysis of subjects' semen parameters, psychological factors, alcohol consumption, and cigarette smoking was performed. The patients' and control subjects' characteristics were then compared via a *t* test analysis. A linear regression analysis was employed to evaluate the relationship between single dependent variables (namely, semen volume, sperm concentration, total count, progressive and total motility, morphology, and DNA fragmentation) and the independent variables (i.e., state and trait anxiety, WSAS scores, and alcohol consumption/cigarette smoking). The association of anxiety to semen parameters was further investigated by analysis of variance (ANOVA) on split samples of subjects, resulting in either high scores ($\geq$47) or low scores ($\leq$30) of both state and trait anxiety. All analyses were performed using SPSS software version 15.0 (SPSS Inc.). $P<.05$ was considered statistically significant.

RESULTS

The descriptive statistics for the patients' and controls' semen parameters are reported in Table 1. Preliminary observations of the semen features of the patients' sample suggested that they represented a low fertility population, although their parameters did not reach the subfertile level according to World Health Organization (WHO) criteria (16). Differences with our random, control sample were therefore subjected to *t* test analysis and found to be statistically significant for sperm concentration, total count, progressive motility, total motility, and morphology. This confirmed that our patients' sample consisted of men with a particularly severe form of reproductive impairment. The semen parameters of our participants were then analyzed along with their psychometric characteristics and alcohol consumption/smoking behavior.

Anxiety and Functional Impairment

The state and trait anxiety of our patients and controls is reported in Table 1. Although the mean value of state anxiety of our sample was similar to the normative one for the Italian population (18), 26 (27.7%) of 94 subjects displayed a STAI score ≥ 47 (mean $\pm$ 1 SD) and were classified as high state anxious. This percentage is much higher than that observed in the general population (11.0%) (23), and is similar to that reported for patients before assisted reproduction (23.3%) (24). As for the controls, the mean value of state anxiety was also in line with the normative one for Italian population, even though the percentage of high anxious subjects (14 of 85, 16.5%) was slightly higher than that observed in the general population.

As for trait anxiety of both samples, it appeared to be distributed normally in terms of both the mean value (18) and the percentage of high anxious patients (17 of 94, 18.1%) or control subjects (12 of 85, 14.3%). When the WSAS scores were analyzed (Supplemental Table 1, available online), the mean value was not indicative of the self-perception of a particular functional impairment. In fact, values relative to the global score and the specific areas investigated were well below those reported in other investigations (20, 25) for both the patient and control samples.

Alcohol Consumption and Cigarette Smoking

Only a minority of the subjects of the two samples reported consuming alcohol regularly: 9.6% of patients and 9.4% of controls drank alcohol daily; 57.4% of patients and 53.0% of controls drank only occasionally. As shown in Supplemental Table 2 (available online), the mean values were representative of low ethanol abuse populations. A similar result was obtained for cigarette smoking (see Supplemental Table 2). Heavy cigarette smokers (>20/day) were found only among the patients and only in a small fraction (3.2%); the overall percentage of regular smokers (>10 cigarettes/day) was 16.0% for patients and 8.2% for controls.

Psychological Variables, Alcohol Consumption, Cigarette Smoking, and Semen Parameters

Linear regression analysis revealed an association of both state and trait anxiety, as well as other independent variables, to the patients' semen parameters. Specifically, state anxiety was associated with semen volume, sperm concentration, total sperm count, total motility, and DNA fragmentation. Trait anxiety was associated with semen volume, sperm concentration, total sperm count, and DNA fragmentation. Among other results, alcohol consumption was associated with sperm concentration, total sperm count, and progressive motility, but not with sperm DNA fragmentation; cigarette smoking was associated with normal sperm morphology; and age was associated with semen volume. The global results are reported in Table 2.

Linear regression analysis of the controls revealed that state and trait anxiety were associated with semen parameters, including sperm concentration, total sperm count, and motility in a similar manner to that observed for patients, but not the semen volume or sperm DNA fragmentation (Table 3). An association with the semen parameters of the controls was also found for alcohol consumption and cigarette smoking.

Association of State and Trait Anxiety to Semen Parameters

To investigate the specific association of anxiety with the semen parameters of IVF patients, we performed an analysis of variance (ANOVA) on split samples of subjects with either high or low scores (≥ 47 and ≤ 30, respectively) of both state and trait anxiety. The samples analyzed were composed as follows: 26 subjects with high state anxiety; 15 subjects with low state anxiety; 17 subjects with high trait anxiety; and 13 subjects with low trait anxiety. Among the patients with low or high scores in both state and trait anxiety, this analysis revealed a statistically significant difference in the mean values of sperm concentration and total count, total

TABLE 2

Interaction of psychological and other variables with semen parameters of IVF patients (n = 94).

Independent variable	Dependent variable	*P* value	β	R square
State anxiety	Semen volume	.045	−0.208	0.043
State anxiety	Concentration	.004	−0.295	0.087
State anxiety	Total count	<.001	−0.373	0.139
State anxiety	Total motility	.023	−0.234	0.055
State anxiety	DNA fragmentation	<.001	0.417	0.174
Trait anxiety	Semen volume	.023	−0.234	0.055
Trait anxiety	Concentration	.007	−0.278	0.077
Trait anxiety	Total count	<.001	−0.371	0.138
Trait anxiety	DNA fragmentation	<.001	0.376	0.141
Work (WSAS)	Concentration	.038	−0.215	0.046
HA (WSAS)	Concentration	.011	−0.261	0.068
HA (WSAS)	Total count	<.001	−0.405	0.164
HA (WSAS)	Progressive motility	.007	−0.275	0.076
HA (WSAS)	Total motility	.001	−0.351	0.123
PA (WSAS)	Concentration	.002	−0.313	0.098
PA (WSAS)	Total count	<.001	−0.412	0.169
PA (WSAS)	DNA fragmentation	.028	0.229	0.052
SA (WSAS)	Concentration	<.001	−0.390	0.152
SA (WSAS)	Total count	<.001	−0.490	0.240
SA (WSAS)	Total motility	.033	−0.220	0.048
IR (WSAS)	Concentration	.048	−0.205	0.042
IR (WSAS)	Total count	.008	−0.272	0.074
WSAS, total	Concentration	.001	−0.339	0.115
WSAS, total	Total count	<.001	−0.456	0.208
WSAS, total	Total motility	.012	−0.260	0.067
WSAS, total	DNA fragmentation	.039	0.215	0.046
Alcohol consumption	Concentration	.009	−0.269	0.073
Alcohol consumption	Total count	.001	−0.339	0.115
Alcohol consumption	Progressive motility	.020	−0.239	0.057
Cigarette smoking	Normal morphology	.015	−0.249	0.062
Age	Semen volume	.033	−0.220	0.048

Note: Only statistically significant interactions are shown ($P<.05$; linear regression analysis). β = expected change; HA = household activities; IR = interpersonal relationship; PA = personal activities; R square = explained variance; SA = social activities.

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TABLE 3

Interaction of psychological and other variables with semen parameters of control subjects (n = 85).

Independent variable	Dependent variable	*P* value	β	R square
State anxiety	Concentration	< .001	−0.429	0.184
State anxiety	Total count	< .001	−0.405	0.164
State anxiety	Total motility	.001	−0.359	0.129
Trait anxiety	Concentration	< .001	−0.436	0.190
Trait anxiety	Total count	< .001	−0.421	0.177
Trait anxiety	Total motility	.001	−0.331	0.110
Work (WSAS)	Concentration	.011	−0.275	0.076
Work (WSAS)	Total count	.004	−0.312	0.097
Work (WSAS)	Total motility	.001	−0.352	0.124
HA (WSAS)	Concentration	.013	−0.268	0.072
HA (WSAS)	Total count	.017	−0.257	0.066
HA (WSAS)	Progressive motility	.022	−0.248	0.062
HA (WSAS)	Total motility	.004	−0.309	0.095
PA (WSAS)	Progressive motility	.008	−0.288	0.083
PA (WSAS)	Total motility	.043	−0.220	0.049
SA (WSAS)	Concentration	.030	−0.236	0.056
SA (WSAS)	Total motility	.026	−0.241	0.058
IR (WSAS)	Concentration	.018	−0.256	0.065
IR (WSAS)	Progressive motility	.004	−0.313	0.098
IR (WSAS)	Total motility	.029	−0.237	0.056
WSAS, total	Concentration	.001	−0.343	0.118
WSAS, total	Total count	.009	−0.281	0.079
WSAS, total	Progressive motility	.002	−0.327	0.107
WSAS, total	Total motility	.000	−0.394	0.156
Alcohol consumption	Progressive motility	.012	−0.272	0.074
Cigarette smoking	Normal morphology	< .001	−0.425	0.180

Note: Only statistically significant interactions are shown (*P*< .05; linear regression analysis). β = expected change; HA = household activities; IR = interpersonal relationship; PA = personal activities; R square = explained variance; SA = social activities.

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TABLE 4

Association of state and trait anxiety to semen parameters.

Group	Independent variable	Dependent variable	*P* value
IVF patients[a]			
	State anxiety	Concentration	.004
		Total count	.003
		Total motility	.013
		DNA fragmentation	.002
	Trait anxiety	Concentration	.018
		Total count	.005
		Total motility	.003
		DNA fragmentation	.006
Controls[b]			
	State anxiety	Concentration	< .001
		Total count	.001
		Progressive motility	.040
		Total motility	.003
	Trait anxiety	Concentration	.001
		Total count	.001
		Total motility	.005

Note: Calculated by one-way ANOVA on split samples. Sample composition: High state anxiety (≥47), [a]N = 26, [b]N = 14; low state anxiety (≤30), [a]N = 15, [b]N = 13; high trait anxiety (≥47), [a]N = 17, [b]N = 12; low state anxiety (≤30), [a]N = 13, [b]N = 17. Only statistically significant differences are shown (*P*< .05).

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motility, and DNA fragmentation (Table 4). In summary, both state and trait anxiety displayed a strong association with the semen parameters of IVF patients.

An ANOVA was also performed on split samples of controls with the following composition: 14 subjects with high state anxiety; 13 subjects with low state anxiety; 12 subjects with high trait anxiety; and 17 subjects with low trait anxiety. Among the controls with low or high levels of state and trait anxiety, the analysis revealed a statistically significant difference in the mean values of sperm concentration, total count and motility, but not of sperm DNA fragmentation (Table 4). State anxiety was also found to be associated with progressive sperm motility. In summary, both state and trait anxiety revealed a slightly weaker association with semen parameters of the controls than with those of the IVF patients.

DISCUSSION

Even though the relationship between psychological stress and couple infertility is still not clear, an association of increased levels of psychological stress/anxiety with decreased semen quality in men has been hypothesized in several studies and is being supported by a growing number of observations (1–3, 9–12, 15, 26–28). Our study, aimed at analyzing the relationship of state and trait anxiety, a measure of the behavioral effects of acute and underlying stress, with semen parameters of a sample of men enrolled in an IVF program confirms and extends the previous data. Control data obtained from random subjects suggest that the overall results obtained from IVF patients may be extended to normally fertile men.

Subjects' Seminal and Psychological Characteristics

A preliminary analysis of semen parameters of our patients revealed that their mean sperm concentration and other main semen parameters were statistically significantly below the values observed in controls. Because the patients enrolled in our study were selected only for an absence of organic/iatrogenic infertility factors but no inquiry was performed on their general living environment, lifestyle habits, or other andrologic features, the major causes of their poor general semen quality may be represented by factors such as occupation, exposure to heat, chemical and/or physical agents, or organic factors including testicular volume, minor inflammation episodes, and/or non reported infections. However, the nature of those factors was not the object of this study.

Although they were characterized by levels of state and trait anxiety comparable to those found in the general Italian population, patients enrolled in our study displayed a peculiar distribution in their state anxiety scores. In fact, 27.7% of these men exhibited high levels of state anxiety, an incidence higher than observed in the general population (23, 29). This result was not surprising, as it was in agreement with the results reported, for instance, by Chen et al. (24) for patients before assisted reproduction treatment. It may thus be

concluded that state anxiety represents an emotional distress frequently observed in these patients. Because all men enrolled in our study were undergoing their first IVF attempt to ensure that their psychological conditions did not depend on the failure of previous attempts and they also reported the absence of other stressful conditions for at least 3 months before their examination, the high percentage of state anxious subjects confirms the view that needing assisted reproduction techniques induces per se a variation in psychological factors. Anxiety levels of the controls were also comparable to the normative ones for the Italian population, although the percentage of high state anxious subjects was slightly higher. In contrast to the observations for state anxiety, the percentage of patients and controls with high levels of trait anxiety is consistent with the reports for subjects who are not involved in assisted reproduction (17, 18).

As for the perception of impairment in everyday activities (WSAS), the levels displayed by patients and controls relative to the global scale as well as to specific areas were within the normal range (20, 25). For the patients' sample, this result may reflect the role of coping strategies; this is supported by the observation that, for instance, infertile men have better coping abilities and lower distress about their condition than their female partners (30). It may also be due to a reported tendency of infertile men to experience less sexual stress and anxiety than their partners, although they show similar anxiety symptoms related to their infertility status (31).

Association of State and Trait Anxiety to Semen Features

Differences in semen quality were observed with regard to both state and trait anxiety in the two samples. Regression analysis on the patients' sample showed that state anxiety had a strong association with semen parameters at both the macroscopic and cellular levels, including semen volume, sperm concentration, total count, and total motility as well as at the subcellular level for sperm DNA fragmentation. These results extend previous observations on the association of psychological stress and male fertility; for example, IVF patients experiencing distress over their medical status were characterized by poor sperm concentration and motility (3, 9), and stressed healthy volunteers showed low values of semen volume and sperm morphology (10–12). Results obtained with our controls also showed the association of state anxiety to sperm concentration, total count, and motility.

With the exclusion of total sperm motility, the semen parameters associated with state anxiety in IVF patients were also found to be associated with trait anxiety and at comparable levels. A similar effect was observed in the controls. To date, the number of reports describing the interaction of trait anxiety and human reproduction have been negligible, and no correlation has been shown thus far between such psychological feature and fertility (5, 32). Our results on trait anxiety represent, to our knowledge, the first data of this kind.

The association of state/trait anxiety to seminal parameters was confirmed by analysis of variance on split samples. In this analysis, high anxiety patients (STAI Y score ≥ 47) differed from low anxiety ones (STAI Y score ≤ 30) in sperm concentration, total count, total motility, and DNA fragmentation. This was observed in both state and trait anxiety. As for the controls, the same analysis confirmed the association of state and trait anxiety with sperm concentration, total count, and motility, and showed a specific association of state anxiety with progressive sperm motility. Neither psychological factor was associated with sperm DNA fragmentation. Besides the other results, the observation that state anxiety has a specific negative association with total and progressive sperm motility has to be taken into consideration as a possible acute stress factor of male infertility.

The results in IVF patients and controls show a similar association for state and trait anxiety with human semen quality, although it is more pronounced for IVF patients. Whether this difference depends on sample size or on a specific factor, or is related to the poorer quality of IVF patients' semen remains to be elucidated.

The association of state/trait anxiety to sperm concentration and total count calls into account the involvement of neuroendocrine factors that may produce alterations in the normal control of spermatogenesis. If this association may be easily explained for trait anxiety in light of its stability over time, it may also relate to state anxiety as a consequence of the diagnosis of couple infertility, as has been already suggested elsewhere (33), and the inclusion in an IVF program, which occurs several weeks before semen analysis. It is now well known that men experiencing stress/anxiety undergo a rise in activity of the hypothalamic-pituitary-adrenal axis, which inhibits the hypothalamic-pituitary-gonadal axis and results in a decrease in the levels of luteinizing hormone (LH) and testosterone (T) (34–36). The loss of conversion of androstenedione to T in Leydig cells is associated with lower average values of semen volume, sperm concentration, and motility (35).

On the other hand, the negative association of anxiety on male fertility observed at the cellular and subcellular levels, including DNA fragmentation, may involve mechanisms that control sperm cell viability by ensuring the quality of seminal plasma and including maintenance of the proper balance between generation of reactive oxygen species (ROS) and/or related molecules, and their scavenging by protective enzymes. High seminal plasma ROS levels resulting in oxidative stress are, in fact, considered one of the main reasons for testicular dysfunction and male infertility (36–38), as they are associated with sperm cell damage that affects sperm motility and DNA structure (39–41).

As suggested by Eskiocak et al. (15), seminal plasma ROS levels are increased under acute psychological stress and result in low sperm quality due to an enzymatic inability of the sperm cells to remove the ROS toxic product hydrogen peroxide. As of now, the relationship between psychological characteristics, seminal plasma ROS levels, and sperm quality in humans deserves further investigation; oxidative stress has to be considered as a possible link between anxiety and cellular/subcellular sperm features.

Functional Impairment and Semen Parameters

The use of the WSAS has been proven to be a valid measure of psychic disorders (20). Although the scores were well below the range indicating psychopathology (WSAS score ≥ 20), linear regression analysis showed that levels of reported functional impairment in our samples were associated with major semen parameters. This result was also verified for most of the WSAS subscales. Thus, it can be concluded that an increase in a subject's perceived disability is associated with poorer semen quality. The clinical relevance of this observation deserves further investigation.

Alcohol Consumption, Cigarette Smoking, and Semen Parameters

Alcohol consumption and cigarette smoking were evaluated in this study as indirect mechanisms possibly involved in the association of anxiety with reproductive human parameters. Another possible indirect mechanism, drug abuse, was not investigated.

The percentage of regular alcohol consumers and heavy cigarette smokers was very low in both the patient and control samples. Therefore, these behaviors cannot be taken into consideration to explain the general low sperm concentration of our group of patients. However, alcohol consumption was negatively associated with sperm concentration and total count as well as progressive motility in the IVF patients. In the controls, probably due to their higher sperm quality and the overall low level of substance abuse, the alcohol association was limited to sperm progressive motility.

These results confirm previous observations such as those of Muthusami and Chinnaswamy (42), who reported the detrimental effect of alcohol consumption on human semen quality in terms of semen volume, sperm count, motility, and normal morphology in association with a decrease in male sex steroids. The negative effect of alcohol on human semen parameters, including sperm DNA damage, was also reported by Varshini et al. (22).

As for cigarette smoking, it was found to affect sperm morphology both in IVF patients and in controls, confirming the observations of Zorn et al. (26) and Mitra et al. (43). Whether these effects act via seminal plasma ROS hyperproduction or other mechanisms it remains to be elucidated.

CONCLUSIONS

Taken together, our observations strongly suggest that trait and state anxiety may represent a significant factor involved in male infertility. This requires particular attention when infertility patients are considered. In fact, because the high scores in state anxiety of our patients could only be associated with their involvement in IVF procedures and not with their failure in previous attempts or with other stressful events, this further confirms that the diagnosis of couple infertility and the beginning of assisted reproduction programs represent additional psychological risk factors in male infertility. Therefore, besides the well-known organic infertility factors, social and psychological factors should be recognized as capable of interfering with sperm quality and pregnancy outcome; this in turn implies that a multidisciplinary approach should consider them as part of the management of infertile couples.

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SUPPLEMENTAL TABLE 1

Distribution of functional impairment scores.

Functional area	Mean	SD	Min	Max
IVF patients (n = 94)				
Work	2.17	1.99	0	7
Household activities	1.56	1.79	0	6
Free time devoted to social activities	1.76	2.13	0	8
Free time devoted to personal activities	1.53	2.05	0	8
Interpersonal relationships	2.70	2.29	0	8
All areas	9.72	8.36	0	30
Controls (n = 85)				
Work	2.08	1.24	0	5
Household activities	1.75	1.25	0	4
Free time devoted to social activities	1.91	1.53	0	7
Free time devoted to personal activities	1.80	1.26	0	5
Interpersonal relationships	1.88	1.21	0	6
All areas	9.42	4.47	0	23

Note: Evaluated by the Work and Social Adjustment Scale (WSAS) (Mundt et al., 2002). SD = standard deviation.

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SUPPLEMENTAL TABLE 2

Distribution of alcohol consumption and cigarette smoking.

Type of behavior	Mean	SD	Min	Max
IVF patients (n = 94)				
Alcohol consumption	1.82	0.66	1	4
Cigarette smoking	1.48	0.84	1	4
Controls (n = 85)				
Alcohol consumption	1.72	0.63	1	3
Cigarette smoking	1.29	0.64	0	3

Note: Alcohol and cigarette consumption were evaluated by respective evaluation scales (see *Materials and Methods* for details). SD = standard deviation.

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