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Relationship between psychological stress and semen quality among in-vitro fertilization patients

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The purpose of this study was to determine the relationship between psychological stress and semen quality among men undergoing in-vitro fertilization (IVF). We assessed psychological variables, including self-reported stress, and sperm parameters in a group of 40 men undergoing IVF for the first time at a pre-IVF sampling period (T1) and at the time of egg retrieval (T2). Thirty-one patients completed the study. Results indicated that total and motile sperm concentration, total motile spermatozoa, and lateral head displacement decreased significantly from T1 to T2 in a high percentage of participants. In addition, the perceived importance of producing a semen specimen increased significantly ($P = 0.001$) from T1 to T2, and this change was significantly correlated ($P < 0.05$) with diminished semen quality at the time of oocyte retrieval. No decline in the semen quality or increase in perceived stress at egg retrieval was observed at T2 in male factor patients ($n = 7$). This study provides evidence for a significant decline in semen quality of male IVF patients at egg retrieval and demonstrates an inverse relationship between semen quality and specific aspects of psychological stress.

Key words: in-vitro fertilization/male infertility/semen/stress

Introduction

Infertile couples experience a wide range of physical and emotional stress during their attempts to conceive a child. The impact of this stress can be devastating, particularly to patients undergoing more advanced and involved procedures, such as in-vitro fertilization (IVF) (Baram *et al*., 1988; Newton *et al*., 1990). While the effects of psychological stress on female IVF patients have been well studied (Harlow *et al*., 1996; Milad *et al*., 1998), comparatively little is known about the impact of emotional stress on the male partner. Concern over the female partner undergoing egg retrieval, the importance of providing an adequate semen sample, and the uncertainty of fertilization results are but a few of the stresses commonly experienced by male IVF patients. It is not known whether the impact of stress is manifested in terms of altered semen quality at the time of the IVF procedure.

Previous studies have indicated that stress has a negative impact on various parameters associated with semen quality, including sperm concentration, motility and morphology (Moghissi and Wallach, 1983; Bents, 1985; Giblin *et al*., 1988). A decline in the semen quality of patients undergoing IVF has similarly been shown (Harrison *et al*., 1987; Kentenich *et al.*, 1992). Boivin *et al*. (Boivin *et al*., 1998) have recently demonstrated that men undergoing regular IVF and IVF with intracytoplasmic sperm injection (ICSI) exhibit similar levels of psychological distress. In these studies, however, the impact of psychological stress on semen changes observed in male IVF patients was not adequately measured.

The objectives of the present study were: (i) to identify changes in perceived stress and semen quality in first-time male IVF patients from a baseline analysis to the time of egg retrieval and; (ii) to study the relationship between psychological stress and semen quality in men undergoing IVF treatment. An additional purpose of the study was to determine whether environmental distractions associated with semen collection had a negative impact on semen quality.

Materials and methods

Patients and sampling periods

Forty of 118 (34%) male patients undergoing IVF for the first time agreed to participate in this study. First-time IVF patients only were included in the study to decrease the chance of habituation to the semen sample collection process that would occur among men undergoing IVF repeatedly. Of these, 31 completed the study, eight subjects chose not to continue after the first sampling period. One patient's cycle was cancelled before egg retrieval.

Each participant in the study signed a consent form approved by the hospital's Human Research Committee. Each patient provided a semen sample and completed a one-page questionnaire to assess anxiety levels at the following times: 4–6 weeks prior to the IVF cycle (baseline sample; T1) and at the time of oocyte retrieval (IVF sample; T2).

Sperm handling and assessment

Semen specimens were collected by masturbation in a temperature-controlled setting at the hospital. Men were asked to adhere to a 48–72 h abstinence period. Specimens were collected in sterile cups and allowed to liquefy at room temperature for 30–45 min, at which time the samples were processed.

Semen volume was measured to the nearest 0.1 ml with a calibrated pipette. Specimens were also assessed visually in terms of colour, viscosity, and debris, and any abnormalities were noted. Undiluted

Table I. Changes in sperm parameters from a baseline analysis (T1) to the time of oocyte retrieval (T2)

Sperm parameters	T1[a]	T2[a]	Change (%)	*P*-value
Total sperm concentration ($\times 10^6$/ml)	113.5 ± 19.7	68.9 ± 8.7	–39	0.034[b]
Motile sperm concentration ($\times 10^6$/ml)	76.9 ± 15.2	40.8 ± 7.4	–47	0.006[b]
Total motile spermatozoa ($\times 10^6$)	210.0 ± 46.8	111.7 ± 24.9	–48	0.002[b]
Lateral head displacement (μm)	2.2 ± 0.2	1.8 ± 0.2	–18	0.006[b]
Semen volume (ml)	2.9 ± 0.3	3.0 ± 0.3	+3	0.743
Normal forms (%)	52.9 ± 1.9	55.6 ± 2.4	+4.8	0.474

[a]Values are mean ± SEM.
[b]Significant differences from T1 to T2 using Wilcoxon's sign-rank test.

semen (5 μl) were placed in a Makler chamber and inserted into an automated semen analyser (Hamilton-Thorn, Danvers, MA, USA). Sperm concentration and quantity and quality of motility were assessed. Total motile spermatozoa (motile sperm concentration×volume) was calculated for each sample. Qualitative measures of sperm motility assessed included mean path, and progressive velocity, mean linear index, and mean lateral head displacement (LHD). In cases where the sperm concentration was $<10\times10^6$, a manual measurement was performed to assess semen quality. In these cases, 10 μl of diluted semen (1:20 with Ham's F-10 + 0.4% bovine serum albumin) was placed on a haemocytometer for determination of total sperm concentration, motile sperm concentration and forward progression. An additional aliquot of diluted semen was used to assess sperm morphology according to World Health Organization standards (World Health Organization, 1980).

Stress questionnaire

Psychological stress was measured in four ways. First, the Speilberger State Anxiety Inventory (Spielberger *et al.*, 1970) (STAI), which is widely used for assessing state or acute anxiety, was completed by all participants after collection of the semen specimen. The STAI asks the subject to describe how he feels 'right now' by responding to 20 questions with a 4-point response format ranging from 'not at all' (score 1) to 'extremely' (score 4). Total scores range from 20 to 80, with higher scores indicating greater anxiety. In addition to the STAI, there were two general appraisal questions assessing how stressful and how important it was for the subject to give a sample that day. These items also used the 4-point response format as above. Third, global indices of other types of life stresses such as stress with family members, friends, work, home and financial problems were assessed with single multiple-choice items. Last, nine items were also included to assess the degree to which the subject was distracted by his immediate environment, such as the presence of others outside the collection room and the hospital atmosphere. Five open-ended questions were included at the end of the questionnaire to determine whether the subject had lost any portion of the sample during the collection, when he had last ejaculated (date and time), how much alcohol he had consumed in the past week, whether he exercised regularly and whether he had taken any over-the-counter or prescription medications in the past month.

Statistical analysis

Changes in stress and semen parameters from T1 to T2 were assessed by the Wilcoxon sign-rank test (Glantz, 1981). Spearman's correlation for non-parametric data was used to test for correlations between changes in stress and semen parameters over the two sampling periods. In addition, a χ^2 analysis using the Yates' correction for continuity was used for individual comparisons of sperm-related changes over time. To determine whether environmental distractions associated with collection of the semen specimen were correlated with the level of stress or anxiety perceived by the patient, baseline (T1) measurements were analysed using Spearman's correlation test.

A second analysis was performed on patients grouped into either male factor or normozoospermic categories. For the purpose of these analyses, male factor patients were defined as those with fewer than 20×10^6 total motile spermatozoa in the ejaculate collected at the baseline sampling. The Wilcoxon sign-rank test was used for before-and-after comparisons, and Spearman's correlation test was used to determine significant correlations between stress and semen parameters.

Results

Sperm parameters

There was a significant decline in total sperm concentration (39% reduction), motile sperm concentration (47% reduction), and total motile spermatozoa (48% reduction) in semen specimens produced at the time of oocyte retrieval (T2) compared with baseline levels (T1) (Table I). Lateral head displacement (LHD) of the sperm head, a qualitative measure of sperm motility derived from the automated semen analysis, was significantly reduced from T1 to T2. No differences were observed in other quantitative and qualitative sperm parameters measured at the two sampling periods, including semen volume and sperm morphology.

A comparison of individual patients whose sperm parameters increased, decreased, or did not change from T1 to T2 is shown in Figure 1. For the purpose of these analyses, no change in a sperm parameter was defined as a deviation of 10% or less from T1 to T2. Total sperm concentration decreased in 61% of the patients from T1 to T2 (Figure 1A) and motile sperm concentration in 65% (Figure 1B). Total motile spermatozoa decreased significantly at T2 in 71% of the patients and increased or did not change in 29% ($P = 0.006$; Figure 1C). Lateral head displacement decreased in 58% of the patients and increased or did not change in 42% of participants (Figure 1D).

Stress parameters

Perceived importance of producing a semen specimen significantly increased from T1 to T2 ($P < 0.001$) (Table II). Ninety-four per cent of men indicated the highest response category for this question at the time of egg retrieval, compared with only 41% at baseline (T1). STAI scores and perceived stressfulness of providing a semen specimen did not change from T1 to T2, nor was there a difference in the perceived

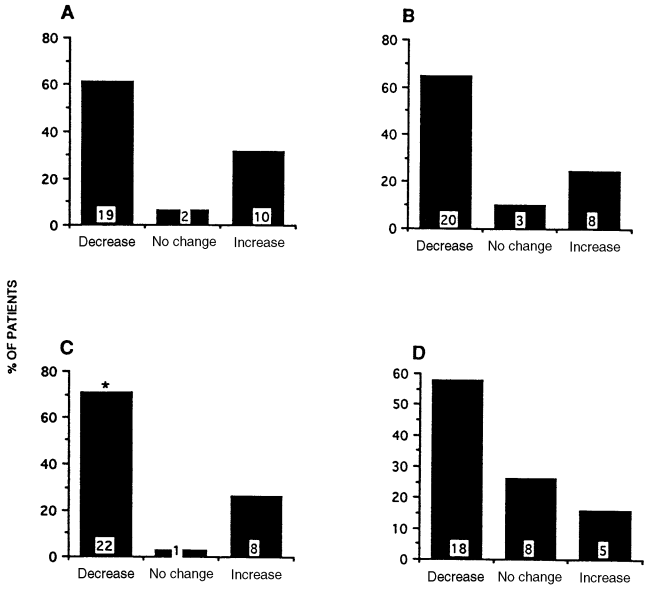

Figure 1. Individual comparison of changes in total sperm concentration (**A**), motile sperm concentration (**B**), total motile spermatozoa (**C**), and lateral head displacement (**D**) from T1 to T2 in male in-vitro fertilization patients [*significantly different (P = 0.006) from combined patients where there was either no change or an increase in the sperm parameter].

Table II. Changes in stress-related parameters from a baseline analysis (T1) to the time of oocyte retrieval (T2)

Stress parameters	T1[a]	T2[a]	*P*-value
Importance[c]	3.2 ± 0.2	3.8 ± 0.1	0.001[b]
Anxiety[c]	41.1 ± 1.6	42.2 ± 1.7	0.331
Stressfulness[c]	2.1 ± 0.2	2.1 ± 0.2	0.836
Environmental distractions	15.7 ± 0.9	15.3 ± 0.9	0.260

[a]Values are mean ± SEM.
[b]Significant difference from T1 to T2 according to Wilcoxon's sign-rank test.
[c]Increasing scale of 1–4.

level of distraction due to environmental factors over the sampling periods (Table II).

No significant differences between T1 and T2 were found for general stress related to family, friends, work, home, or finances. These were single items with a 4-point scale of severity. Means for these items ranged from a low of 1.2 for stress related to relationships with friends to 2.7 for work-related stress. There were no significant differences in scores from T1 to T2. The amount of alcohol consumed in the week prior to collection was assessed in categorical format. Results indicated that, at T1, 27% of men abstained, 49% had 1–3 drinks per week and 24% had 4–10 drinks. At T2, 35% abstained, 23% had 1–3 drinks per week and 35% had 4–10 drinks per week. Six per cent of the subjects did not answer the question. In terms of over-the-counter medication use, subjects were asked if they had used any in the prior week: at T1, 56% said they had, and at T2, 39% said they had.

Correlations between stress and sperm parameters

To address the question of a possible association between the psychological variables and specific sperm parameters, two separate analyses were performed. To determine whether there was a correlation between the psychological variables (state anxiety, perceived stressfulness, perceived importance) and sperm parameters, Spearman's correlation was applied to baseline samples (T1) only. Perceived importance of producing a semen sample was negatively correlated with both total sperm concentration (r = –0.36, $P < 0.02$) and total motile spermatozoa (r = –0.32, $P < 0.04$). STAI scores were negatively correlated with semen volume (r = –0.39, $P < 0.02$).

We were interested in whether changes in sperm parameters from T1 to T2 were associated with changes in psychological variables. These results are shown in Table III. Changes in perceived importance of producing a sample and STAI scores from T1 to T2 were significantly and inversely correlated with changes in several sperm parameters, including total sperm concentration, motile sperm concentration, and total motile spermatozoa. No correlations were found between perceived stressfulness and any of the sperm parameters measured.

Table III. Correlations between changes in sperm parameters and changes in stress parameters from T1 to T2

Stress parameter	Sperm parameter	Rho (r)	*P*-value
Importance	Total sperm concentration	–0.45	0.014[a]
	Motile sperm concentration	–0.43	0.019[a]
	Total motile sperm	–0.53	0.004[a]
	LHD	–0.02	0.909
Anxiety	Total sperm concentration	–0.37	0.043[a]
	Motile sperm concentration	–0.39	0.031[a]
	Total motile sperm	–0.43	0.019[a]
	LHD	–0.02	0.896
Stress	Total sperm concentration	–0.18	0.321
	Motile sperm concentration	–0.24	0.181
	Total motile sperm	–0.21	0.243
	LHD	–0.21	–0.243

[a]Significant correlation as analysed by Spearman's correlation test for non-parametric data.
LHD = lateral head displacement.

Male factor subsample

Seven patients (23%) from the study group were classified on the basis of their baseline semen analysis as having a male factor infertility problem. All of these men were aware of their diagnosis prior to the study. No decline in total and motile sperm concentration, total motile spermatozoa, or lateral displacement of the sperm head from T1 to T2 was observed in male factor subjects (Table IV). In fact, total motile spermatozoa increased or remained the same at T2 in five of the seven male factor patients. In comparison, the semen quality of non-male factor patients decreased significantly from the baseline analysis to the time of oocyte retrieval (n = 24; Table IV).

No differences in STAI scores or stress levels from T1 to T2 were observed in male factor patients. In terms of perceived importance non-male factor patients reported a significantly higher level of perceived importance of producing a specimen at T2 than at T1; no such increase was found in male factor patients (Table IV). It is important to note, however, that perceived importance of the baseline analysis (T1) was signi-

Table IV. Changes in sperm and stress parameters from T1 to T2 in male factor (MF; $n = 7$) and non-male factor (NMF; $n = 24$) patients

Sperm/stress parameter[c]	Patient type	T1[a]	T2[a]	*P*-value
Total sperm concentration ($\times 10^6$/ml)	MF	24.8 ± 4.8	50.6 ± 13.7	0.063
	NMF	139.3 ± 22.8	74.2 ± 10.4	0.003[b]
Motile sperm concentration ($\times 10^6$/ml)	MF	7.0 ± 2.4	15.7 ± 8.8	0.128
	NMF	97.4 ± 17.6	48.1 ± 8.7	0.002[b]
Importance	MF	3.6 ± 0.2	3.7 ± 0.3	0.706
	NMF	3.0 ± 0.2	3.9 ± 0.1	0.001[b]
Anxiety	MF	39.6 ± 2.7	38.7 ± 1.2	0.734
	NMF	41.5 ± 2.0	43.2 ± 2.1	0.235
Stressfulness	MF	2.1 ± 0.3	2.0 ± 0.3	0.706
	NMF	2.1 ± 0.2	2.1 ± 0.2	1.000

[a]Values are mean ± SEM.
[b]Significant differences from T1 to T2 according to Wilcoxon's sign-rate test.
[c]All on an increasing scale of 1–4.

Table V. Correlations between environmental distractions associated with semen collection and stress parameters at the baseline analysis (T1)

Stress parameter	Environmental distraction	Rho (*r*)	*P*-value
Anxiety	Presence of others	0.58	0.0003[a]
	Location of collection room	0.66	0.0001[a]
	Noise	0.41	0.009[a]
	Hospital atmosphere	0.50	0.002[a]
	Space limitations	0.32	0.046
	Absence of wife	0.23	0.146
Stress	Presence of others	0.55	0.0006[a]
	Location of collection room	0.60	0.0001[a]
	Noise	0.50	0.001[a]
	Hospital atmosphere	0.54	0.001[a]
	Space limitations	0.30	0.060
	Absence of wife	0.34	0.032[a]

[a]Significant correlations as analysed by Spearman's correlation test.

ficantly higher in male factor patients than in non-male factor patients (3.6 and 3.0 respectively; $P < 0.05$). The level of perceived importance measured at T2 did not differ between the normozoospermic and oligozoospermic groups (Table IV).

Environmental distractions

It was of interest to determine if environmental distractions, such as the physical layout of the semen collection facility added to the stress and anxiety experienced by our male IVF patients, and thus may have further impacted semen quality. The level of distractions caused by any of the variables measured did not increase at T2, nor were these parameters correlated with any changes in semen quality (data not shown). However, both STAI scores and perceived stressfulness were highly correlated with several distractions inherent to the sample collection process, including the presence of others in the waiting area, the location of the room, noise and the hospital environment (Table V). Environmental factors, however, were not associated with the perceived importance of producing a semen sample on a given day (data not shown).

Discussion

The results of this study show that the semen quality of men undergoing IVF treatment for the first time is diminished at the time of oocyte retrieval and provide preliminary evidence of a relationship between psychological state and semen quality. Total sperm concentration, total motile spermatozoa, and quantitative and qualitative sperm motility decreased significantly at the time of the egg retrieval compared with pre-IVF baseline values. Moreover, individual comparisons of semen quality over the two sampling periods indicated that this phenomenon occurred in a high percentage of the study participants. Others have reported a similar but less dramatic decline in semen quality associated with IVF. Harrison *et al.* (Harrison *et al.*, 1987) found sperm concentration, total sperm count, and motility to decrease slightly in 500 semen samples produced for IVF compared with those produced at the pre-IVF work-up. In a similarly designed study, Kentenich *et al.* (Kentenich *et al.*, 1992) reported that sperm concentration decreased significantly at the time of oocyte retrieval in 36% of male IVF patients compared with that obtained from an earlier examination. These studies did not limit their samples to first-time IVF patients nor did they specifically measure anxiety.

While it has generally been assumed that semen quality is affected by psychological stress, there have been few attempts at assessing either the level or type(s) of stress impacting on male IVF patients. Most studies assessing semen quality in IVF patients have failed to measure directly the stress involved in such procedures (Harrison *et al.*, 1987; Giblin *et al.*, 1988; Pellicer and Ruiz, 1989). Kentenich *et al.* (Kentenich *et al.*, 1992) used reported pleasantness and unpleasantness of specific events associated with IVF as experienced by the male partner, but did not attempt to correlate these feelings with changes in sperm parameters. In the present study, we evaluated specific psychological variables using cognitive appraisal and state anxiety immediately after subjects produced a semen specimen at a baseline sampling period and again after a specimen was produced at the time of egg retrieval. Using this strategy, we were able to assess changes in both psychological state and semen quality over the two sampling periods and to establish correlations between stress and semen parameters.

Men in the present study were found to have moderately high levels of anxiety both when providing a pretreatment semen sample and when providing a sample on the day of oocyte retrieval. Although levels of state anxiety did not significantly increase between the two sampling times, the

mean scores for the sample indicated that the procedures involved in providing a semen specimen were relatively stressful. The mean scores at T1 (41.1) and T2 (42.2) were higher than those obtained by Dziegielewski and Tyler (Dziegielewski and Tyler, 1989), who assessed anxiety and semen quality in men first presenting for an infertility evaluation. Other reported scores for a basis of comparison are 42.4 for men undergoing a surgical procedure (Speilberger *et al.*, 1970) and 43.6 for men who have been told they are HIV positive (Huggins *et al.*, 1991). Moreover, the average score for non-psychiatric male patients is 35.7 (Speilberger *et al.*, 1970). Therefore, although there was not a significant increase in reported anxiety state from the baseline assessment to the day of oocyte retrieval, the overall level of anxiety experienced by these men was clinically significant.

Two components contribute to the stressfulness of an event: the perceived importance of the event to the person (appraisal), and the person's belief regarding how well they could cope with the event (coping) (Lazarus and Folkman, 1984). In the present study, the perceived importance of producing a semen sample increased significantly from pretreatment to the day of oocyte retrieval, indicating that the men were aware of the increased importance of providing the T2 sample. Moreover, the heightened importance of providing a specimen at egg retrieval was significantly and negatively correlated with the semen quality of men involved in IVF treatment. To our knowledge, this is the first definitive evidence linking psychological appraisal and semen quality among male IVF patients.

It was interesting that male factor patients in the present study appeared to respond differently from normozoospermic patients. The perceived importance of providing a semen specimen by male factor patients did not increase, nor was there a decline in semen quality at the time of egg retrieval as was seen in the non-male factor patients. A possible explanation for this lies in the fact that the level of perceived importance in the male factor group was already elevated at T1 and remained high at T2. The male factor patients appeared to be keenly aware of the importance of their sample, even at the baseline sampling period as perceived importance remained high, semen parameters remained low. If perceived importance is associated with semen quality, then one would expect this relationship. The fact that some of the male factor patients exhibited a slight improvement in semen quality at T2 may reflect the degree of variability of semen samples within an individual patient (Cooper *et al.*, 1991). These results are consistent with the recent finding of Boivin *et al.* (Boivin *et al.*, 1998) that male factor patients undergoing IVF with ICSI report higher pre-IVF anxiety than normozoospermic men but that both groups' anxiety is equally high during the actual treatment. Boivin *et al.* (Boivin *et al.*, 1998) did not measure the correlation between psychological variables and subjects' semen parameters, however.

Environmental distractions associated with the sperm collection rooms, such as the presence of others, noise, and the hospital atmosphere were often a source of dissatisfaction to our patients. The level of this dissatisfaction, however, did not increase at the time of egg retrieval, nor were these factors significantly distracting or stress-invoking to be associated with detrimental changes in sperm parameters. Thus, modification of the physical layout of a semen collection facility should be focused on patient satisfaction, but may not be particularly relevant to the quality of the semen produced.

The mechanism by which psychological stress could affect semen quality is unclear. The spermatogenic cycle in the human male is approximately 70 days (the time required for an undifferentiated spermatogonium to develop and mature into a motile sperm cell; Frishman, 1995). Given the sampling interval (T1 to T2) of 30–45 days in the present study, it is unlikely that increasing stress experienced as oocyte retrieval approaches exerts a direct effect on sperm production *per se*. Rather, effects of stress may be indirect in nature via the hormonal component of spermatogenesis. There is evidence that such a phenomenon may be related to hormonal changes observed in the male during stressful events. Testicular biopsies obtained from prisoners awaiting sentencing, obviously under extreme stress, revealed complete spermatogenetic arrest in all cases (Steve, 1952). Milder forms of stress, such as that induced as a result of combat or surgery, have been shown to result in depressed testosterone concentrations in affected males (Kreuz *et al.*, 1972). This may be a result of activation of hormones from the hypothalamic–pituitary–adrenal axis, which are known to be elevated in response to stress (Guyton, 1989). McGrady (McGrady, 1984) noted that social stress in animals was related to diminishing testicular function via changes in luteinizing hormone (LH) and testosterone. Cui (1996) has demonstrated significantly lower semen volume and sperm concentration in a group of chronically stressed marmoset monkeys. These changes were attributed to lower concentrations of LH and testosterone (which were reduced in the stressed group). These changes appear to be mediated, according to Cui (Cui, 1996), by endogenous opioids in the hypothalamic–pituitary–adrenal axis. There is evidence for the role of opioids in blocking the inhibitory effects of stress on LH and testosterone by the administration of naloxone, an opioid agonist (Norman and Smith, 1992). Changes in LH and testosterone may further affect the sympathetic and parasympathetic systems in acute stress situations which directly affect testicular function and sperm quality.

The conclusions drawn from the present study are somewhat limited by the relatively small size of the sample. In addition, 23% of the participants dropped out of the study before completion. We believe that the drop-out rate was due to the sensitivity of the male IVF patients to issues regarding stress. Many of the participants seemed uncomfortable filling out the questionnaire, and it appeared that many of the men were attempting to minimize or mask any effects of stress related to the IVF procedure. This fact raises concerns regarding future subject recruitment and the necessity of obtaining more physiological indices to psychological stress. It would undoubtedly be beneficial to include certain hormonal measurements, such as urinary cortisol and plasma testosterone, in future studies involving stress and semen quality.

In conclusion, data from the present study showed a significant decline in the semen quality of IVF patients at the time of oocyte retrieval and provide evidence for a relationship between semen quality and specific aspects of psychological stress.

Further research is needed to determine whether either physical (frozen back-up semen samples) or psychological (relaxation training, guided imagery, or support groups) interventions would be helpful in reducing stress experienced by male IVF patients, with the potential benefit of minimizing stress-induced changes in semen quality.

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