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ORIGINAL ARTICLE

Semen Analysis

Decline of semen quality during IVF is not associated with subjective male stress

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The aim of the present study was to assess if semen quality declines during *in vitro* fertilization (IVF) and whether or not this phenomenon is triggered by chronic male stress. In order to test this hypothesis, we first investigated a retrospective cohort of 155 male IVF patients (testing cohort). Subsequently, we started a prospective cohort study in men undergoing their first IVF and assessed semen quality and subjective male chronic stress using a validated tool, i.e. the Fertility Problem Inventory (FPI) questionnaire. The association between stress and sperm quality decline measured 4–6 weeks before the start of IVF (T1) and at the day of oocyte retrieval (T2) was the primary outcome. Live birth rate, first trimester abortion and rate of poor responders were secondary outcomes. In the testing cohort, mean progressive motility, but not mean sperm density significantly declined. There were 78/154 (51%) men who showed a decline in semen density and 50/154 (32%) men who showed a decline in progressive motility. In the validation cohort, progressive motility declined, whereas, sperm density increased from T1 to T2. Of 78 men, 27 men had increased stress (FPI-score > 146). Sperm density and progressive motility were not significantly different in men with and without stress. However, in the presence of male stress, couples had a higher rate of poor responders, miscarriages and a lower rate of live births. Subjective stress is not associated with a decline in semen quality observed during IVF but may be associated with adverse pregnancy outcome.

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INTRODUCTION

It has been hypothesized that stress and stress-related factors have an influence on the autonomic, neuroendocrine and immune systems.¹ Reproduction is a central issue in most couples' lives. Thus, if a couple fails to conceive spontaneously, both partners are likely to experience frustration and disappointment, which could lead to stress. Supporting this assumption, the length of time to conceive has been demonstrated to be associated with stress in infertile couples.² In addition; procedure- and non-procedure-related stress may negatively affect the course and outcome of assisted reproduction techniques. For example, stressful life events are associated with poor *in vitro* fertilization (IVF) outcome³ and baseline psychological measures of stress, such as anxiety and depression, negatively influence the clinical pregnancy rate after IVF in women with tubal factor infertility.⁴ An association between anxiety and depression and IVF outcomes has been described in some,^{5–7} but not in other studies.⁸

Both the female and male partner of couples undergoing IVF are affected by stress, with women being more vulnerable to stress.⁹ For example, in a study of couples preparing for IVF, women consistently scored higher on multiple measures of psychological distress than their male partners.¹⁰ However, men also react to stress with a decline in reproductive parameters, as demonstrated by studies showing a negative impact of stress on semen quantity and quality.^{11,12} Various

types of stressors can affect semen quality, such as job stress,¹³ stress related to the recent loss of a family member,¹² stress resulting from war¹⁴ and even stress due to natural disasters, such as an earthquake.¹⁵ To date, only two studies have been published assessing the relationship between male psychological stress and semen quality among couples undergoing their first IVF (PubMed search 12 February 2013; search terms: stress, semen, sperm motility, sperm density, IVF, ART and outcome).^{16,17} In a study of 31 men undergoing IVF for the first time, sperm quantity and density significantly declined from before IVF to the day of oocyte retrieval. Semen parameter decline was associated with the perceived importance of producing an adequate semen specimen.¹⁶ Vellani *et al.*¹⁷ investigated 94 first-attempt IVF patients and 85 age-matched, random subjects and found increased levels of both state and trait anxiety to be associated with lower semen volume, sperm concentration and count, reduced sperm motility and increased sperm DNA fragmentation.

Based on these data, we hypothesized that semen quality declines during IVF and that male stress may trigger this phenomenon. In order to test this hypothesis, we investigated a retrospective cohort of male IVF patients (testing cohort) in order to confirm that there is a decline in semen quality during IVF at our institution. After confirmation of a semen quality decline, we started a prospective cohort study in men undergoing their first IVF and assessed semen quality and subjective

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stress using a validated tool, i.e. the Fertility Problem Inventory (FPI) questionnaire. This questionnaire has been designed and validated to reveal chronic stress, as indicated by high FPI scores.¹⁸ The objectives of the present study were: (i) to assess the influence of subjective stress measured at the start of IVF on the decline of semen quantity and quality in first-time male IVF patients compared to a baseline semen analysis and a semen analysis at the time of oocyte retrieval as the primary outcome; and (ii) to study the relationship between male subjective stress and the rate of poor responders, first trimester abortions and the live birth rate as secondary outcomes.

MATERIALS AND METHODS

This was a prospective and retrospective cohort study of men undergoing their first IVF at the IVF Unit of the Department of Reproductive Medicine and Clinical Endocrinology, Medical University of Vienna, Vienna, Austria, between March 2008 and June 2012. They had no previous ART treatment (intrauterine insemination or IVF/intracytoplasmic sperm injection therapy) in any other center before, and infertility duration in all cases was under 24 months.

We excluded all patients who had an existing psychological or psychiatric problem before or at the time of the IVF/intracytoplasmic sperm injection therapy. All men during that period undergoing their first IVF cycle who agreed to participate were eligible. The study protocol was approved by the Ethics Committee of the Medical University of Vienna. Written, informed consent was obtained from all participants before enrollment in the study. Each participant completed the FPI before the start of the first IVF cycle and provided two semen samples at the following times: 4–6 weeks prior to the first IVF cycle (T1) and at the time of oocyte retrieval (T2).

Sperm handling and assessment

Semen samples were collected by masturbation into a plastic container in a temperature-controlled setting. According to our study protocol, all the semen specimens were to be produced onsite. Men were asked to adhere to a 48–72 h abstinence period prior to semen sampling and whether they had kept to the abstinence time was checked on the day of collection. All the patients were confirmed. After collection, semen samples liquefied at room temperature for 30–45 min and were incubated at 37°C and analyzed within 1 h. Seminal fluid volume was measured to the nearest 0.1 ml with a 5 ml calibrated pipette. A microbiological exam was performed on all specimens. Undiluted semen (5 ml) was placed in a Makler chamber. The concentration of spermatozoa per milliliter was determined at a magnification of $\times 400$, and the total sperm count was calculated. Sperm concentration, quantity and progressive motility were assessed manually. The analysis was performed by trained laboratory workers according to the World Health Organization Laboratory Manual 1992.¹⁹ Although the new World Health Organization guidelines for semen analysis were published in 2010, we still used the criteria of 1992 in our study population in order to preserve the homogeneity in methods of our study.

IVF treatment

The antagonist protocol was used for all patients. The initial exams were performed on day three of the menstrual period, and included a transvaginal ultrasound examination to assess the antral follicle count and a blood sample for hormone analyses (thyroid stimulating hormone, follicle stimulating hormone, luteinizing hormone, estradiol and prolactin) using standard protocols at the Central Laboratory of the General Hospital of Vienna, Department of Laboratory Diagnostics, Medical University of Vienna, Vienna, Austria. The

protocol was initiated only in patients with normal concentrations of the above-mentioned hormones and normal-appearing ovaries without cysts or an antral follicle count of less than 15 in each ovary. The stimulation protocol was started on day 3 of the menstrual cycle, with a basal dosage of 200 IU of recombinant follicle stimulating hormone (Puregon, MSD Pharma). Monitoring was carried out by transvaginal sonography. When necessary, the follicle stimulating hormone dosage was adjusted according to the number and diameter of follicles. When adequate stimulation was achieved (three follicles of ≥ 18 mm in diameter), 10 000 IU of human chorionic gonadotropin (Pregnyl, MSD Pharma) were administered. Oocyte retrieval was performed 35 h after human chorionic gonadotropin injection. Conventional IVF, following standard techniques, was used.

A maximum of two embryos were transferred through a Wallace catheter between days 3 and 5 after oocyte retrieval. All patients received 10 mg of dydrogesterone (Duphaston, Solvay Pharma) orally, twice daily, and 200 mg of progesterone (Utrogestan, Meda Pharma) vaginally, three times daily, for luteal support. Biochemical pregnancy was defined as a positive urinary human chorionic gonadotropin test on day 14 after transfer. A clinical pregnancy was defined as a pregnancy verified by transvaginal sonography 5 weeks after embryo transfer.

During the whole IVF/intracytoplasmic sperm injection therapy, all the patients were offered the same counseling procedure for treatment. According to the therapy protocol, medical staff discusses lifestyle issues, like stress, depression and grieving during and after therapy and offered all the patients further psychological support from a clinical psychologist who specialized in issues associated with ART treatment. All the patients who participated in the study refused this option.

Patients were followed through the course of their pregnancies to evaluate the number of pregnancies, the number of miscarriages, extrauterine pregnancies, spontaneous abortions and the number of live births, including information about gestational week at delivery and delivery mode. Patients who delivered in another hospital were asked to provide a copy of the hospital delivery report after delivery.

FPI questionnaire

Perceived infertility-related stress was assessed using the German version of the FPI¹⁸ containing the following items: social concern; sexual concern; relationship concern; rejection of child-free lifestyle; need for parenthood; and global stress. All items were scored on a Likert scale, ranging from 1 (I do not agree) to 6 (I totally agree). For the purposes of this study, the primary endpoint, subjective stress, was defined according to Newton *et al.*¹⁸ as a FPI score $>84^{\text{th}}$ percentile in men, i.e. >146 .

Statistical analysis

Group differences for categorical variables were tested by Chi-square test and for continuous variables by Student's *t*-test. The FPI scores were compared with normative data¹⁸ using a one-sample *t*-test. Semen parameters were analyzed by repeated-measures ANOVA (analysis of variance) using time as a within-subjects factor and group as a between-factors grouping variable. Where appropriate, simple effect tests were conducted for significant main effects or interaction effect terms. We performed a multiple linear regression model to test whether the effect of stress on semen parameters was independent of potential confounders, such as age (<50 vs >50) and causative factor of the infertility (male factor vs female factor). A power calculation demonstrated that, with a sample size of 78, the study had a power of $>80\%$ to detect an absolute 40% difference in semen parameters at a significance level of 0.05, using a Mann-Whitney U test. A difference

of at least 40% was estimated to be clinically relevant. A $P < 0.05$ was considered statistically significant. Values are expressed as means \pm standard deviation unless indicated otherwise. We used the software Statistical Package for Social Sciences, version 11.0 for Windows (SPSS 11.0, SPSS Inc, Chicago, IL, USA) for statistical analyses.

RESULTS

Retrospective testing cohort

The retrospective testing cohort consisted of 155 men undergoing their first IVF. Mean semen density (50.0 ± 50.3 vs 43.8 ± 51.6 , $P = 0.2$) and mean progressive motility (10.1 ± 15.8 vs 5.0 ± 10.9 , $P = 0.001$) declined during IVF as evidenced by T1 and T2 comparison, but the difference was only statistically significant for mean progressive motility. Of 154 men, 78 (51%) showed a decline in semen density and 50/154 (32%) men showed a decline in progressive motility. A decline in semen density and progressive motility were seen both in male factor (56/110 and 29/110, respectively) and female factor (20/45 and 24/45, respectively) participants. The quantitative amount of semen density and progressive motility decline was evenly distributed among affected males. Specifically, a $<25\%$, 25% – 50% and $>50\%$ decline in semen density was observed in 22, 16 and 38 men, respectively. On the other hand, the quantitative amount of progressive motility decline was severe in most affected males. A $<25\%$, 25% – 50% and $>50\%$ decline in progressive motility density was observed in three, four and 46 men, respectively. In a multiple linear regression analysis, the presence of a male factor ($P = 0.02$), male smoking ($P = 0.04$) and endometriosis ($P = 0.002$), but not male body size ($P = 0.8$), female smoking ($P = 0.7$) and polycystic ovary syndrome ($P = 0.6$), independently influenced progressive motility.

Prospective validation cohort

Eighty-four men consented to participate in the prospective study and completed the FPI. The analysis was restricted to 78 men, for whom FPI scores, semen parameters and IVF outcomes were available. Participant characteristics of the men in the validation cohort are shown in **Table 1** and IVF in contrast to the testing cohort, mean semen density (33.5 ± 31.4 vs 39.8 ± 41.3 , $P = 0.05$) increased in the validation cohort and sperm volume (3.6 ± 1.9 vs 3.8 ± 1.8) was unchanged when T1 and T2 were compared. In accordance with the testing cohort, mean progressive motility (11.3 ± 18.2 vs 6.9 ± 11.6 , $P = 0.05$) declined during IVF.

FPI scores are depicted in **Table 2**. The mean FPI score (global stress) in the whole cohort was 134.2 ± 30.5 . Compared with a normative sample of the FPI,¹⁹ study participants had a significantly higher global stress score, as reflected by significantly higher scores in three of five subscales (**Table 2**). Twenty-seven of 78 men were categorized as having stress based on an FPI-score >146 . The presence of stress did not influence semen density, progressive motility and sperm volume (**Table 3**). However, in the presence of male stress, couples had a higher rate of poor responders, miscarriages and a lower rate of live births (**Table 4**).

Since male factor versus female factor infertility might induce different stress effects on semen quality, we replicated the analyses after grouping according to male and female factor infertility. The two groups did not differ regarding FPI scores, FPI grouping and IVF outcomes. Mean semen density and sperm volume at the two time points, T1 and T2, did not differ in the two groups. However, sperm motility was differently affected in the two groups, as can be seen in **Figure 1**. ANOVA demonstrated a significant group effect for progressive

Table 1: Patient characteristics of men and women undergoing their first IVF (validation cohort)

Patient sample validation cohort (n=78)	
Age (men, year)	33.5 \pm 6.1
Age (women, year)	29.7 \pm 4.6
Poor responders	6/78 (8%)
Pregnancy	28/78 (36%)
Live birth	21/78 (26%)
First trimester abortion	7/78 (9%)
Male/female factor infertility	57/21

IVF: *in vitro* fertilization

Table 2: Fertility problem inventory questionnaire scores

	Study group	Normative sample	t	P value
Social concern	22.3 \pm 8.5	22.1 \pm 9.3	0.32	NS
Sexual concern	16.2 \pm 7.5	14.6 \pm 5.9	2.13	0.05
Relationship concern	20.3 \pm 7.9	19.6 \pm 7.9	0.98	NS
Rejection of child-free lifestyle	33.0 \pm 7.9	26.4 \pm 7.7	7.70	0.001
Need for parenthood	42.4 \pm 8.6	33.9 \pm 10.0	8.82	0.001
Global stress	134.2 \pm 30.5	117.0 \pm 29.3	5.12	0.001

NS: not significant; s.d.: standard deviation. Data are presented as mean \pm s.d.

Table 3: Global fertility problem inventory stress score and sperm quality

	FPI score (mean \pm s.d.)	
	≤ 146 (n=50)	>146 (n=26)
Density T1 (10^6 ml ⁻¹)	33.4 \pm 31.6	23.2 \pm 25.1
Density T2 (10^6 ml ⁻¹)	41.2 \pm 39.1	28.7 \pm 30.1
Motility T1 (%)	10.9 \pm 18.7	11.2 \pm 19.3
Motility T2 (%)	6.7 \pm 11.0	3.1 \pm 6.2
Volume T1 (ml)	3.4 \pm 1.8	3.9 \pm 2.0
Volume T2 (ml)	3.7 \pm 2.0	4.0 \pm 1.5

s.d.: standard deviation; T1: 4–6 weeks before treatment; T2: at the time of egg retrieval

Table 4: Global fertility problem inventory stress scores of men and IVF outcome of female partners

	FPI score (%)	
	≤ 146 (n=51)	>146 (n=26)
Poor responder	3 (6)	3 (11)
Nonpregnant	29 (57)	15 (55)
Miscarriage	3 (6)	4 (14)
Live birth	16 (31)	5 (18)

IVF: *in vitro* fertilization

motility. Specifically, loss of progressive motility between T1 and T2 was significantly more pronounced in female factor compared to male factor study participants ($P = 0.02$; **Figure 1**).

DISCUSSION

In the present study, we found that sperm density did not change significantly during the course of first IVF in the testing and validation cohorts. However, functional sperm quality, as measured by progressive motility, was nearly halved in both cohorts with a pronounced and significant effect in the testing cohort. Our study hypothesis that the decline in semen quality in men undergoing their first IVF is influenced by male subjective stress was rejected. We found that FPI questionnaire scores did not influence semen density nor did they affect progressive motility or sperm volume. On the other hand, secondary outcomes such the rate of poor responders, miscarriages and live births were

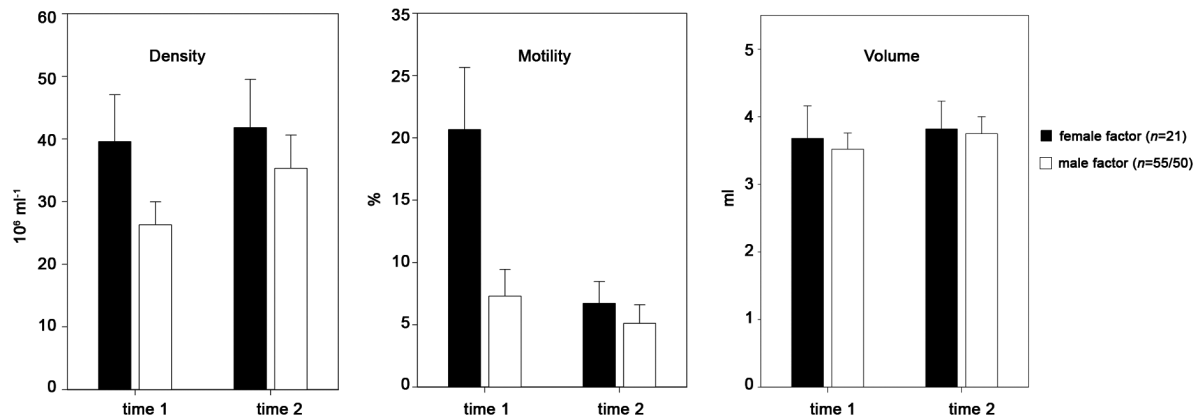


Figure 1 Semen quality (mean \pm standard error of the mean (s.e.m.)) as a function of male/female factor infertility. time 1: 4–6 weeks before treatment; time 2: at the time of egg retrieval.

negatively affected in the presence of male stress. Males with a male factor infertility had significantly lower sperm density and progressive motility compared to men with a female factor infertility. However, the decline in progressive motility during IVF was much more pronounced in males of couples with female factor infertility. This indicates that IVF couples with female factor infertility are more vulnerable to sperm quality decline. Multiple linear regression analysis indicated that male smoking and a history of endometriosis may play an etiologic role regarding this effect. It is also possible that a potential sperm quality decline does not become obvious in male factor infertility couples, since affected males have significantly lower sperm quality in the first place, as demonstrated in **Figure 1**.

Our results have to put in line with the data of Kentenich *et al.*²⁰ and Harrison *et al.*²¹ demonstrating a significant decline in sperm concentration, total sperm count and motility during IVF. According to the existing literature, not only stressful life events, but also socio-psycho-behavioral factors, such as occupational class, may be associated with decreased semen quality in fertile men.^{22–24} We can confirm in both the testing and the validation cohort that progressive motility seems to be markedly diminished during IVF. This is in accordance with the data reported by Vellani *et al.*¹⁷ who have reported increased levels of both state and trait anxiety to be associated with lower semen volume, sperm concentration and count, reduced sperm motility and increased sperm DNA fragmentation. Of note, semen density and semen volume did not deteriorate over time in our study between T1 and T2. Thus, we conclude that the functionally oriented measurement of progressive sperm motility may be a more stable and thus more reliable parameter of semen quality decline during IVF.

It is an interesting finding that the presence of male stress did not influence semen density, volume and progressive motility, but was indeed associated with a higher rate of poor responders, miscarriages and a lower rate of live births. If this is a true finding and not due to chance, male stress may adversely affect pregnancy outcome in first time IVF couples, but not via a reduction of semen density, volume or progressive motility. If the most obvious and direct male contribution, i.e. semen, is not the vehicle of the impact of male stress on pregnancy outcome, then more indirect interactions such as social behavior or female stress induced by the stressed male may be candidates for such an interaction.

Our study has strengths and weaknesses. For example, our study rejects the hypothesis that male stress reduces semen parameters by at least 40%. More subtle differences may be present, but would have been

missed by our study. In addition, the FPI consisting of items such as social concern, sexual concern, relationship concern and global stress has not been specifically developed for and may not be representative of stress among IVF couples. Also, the time points T1 (4–6 weeks before the start of IVF) and T2 (at the time of oocyte retrieval) were arbitrarily chosen. Therefore, our data do not rule out that stress measurements at other, more suitable time points, may identify an association between male stress and variations in sperm quality.

In addition, we have focused on chronic stress. The assessment of acute stress might be an additional valuable item when the relationship between sperm quality and acute physiological stress responses is being tested. Based on the physiology of sperm maturation, however, we feel that chronic stress is more likely to influence sperm quality measures compared to acute stress. This was the reason why we focused on the FPI questionnaire, an established tool for measuring chronic stress, with special emphasis on chronic stress as a result of fertility problems. Nevertheless, this study cannot comment on the potential influence of acute stress events on sperm quality.

In conclusion, data from the present study showed a decline of progressive motility, but not semen density in male partners during their first IVF in a retrospective and prospective setting. However, stress, as measured by the FPI questionnaire, was clearly not associated with sperm parameters. The way stress interacts with pregnancy-related parameters in IVF couples thus remains unclear. As a secondary finding, male stress did adversely affect pregnancy outcomes in first time IVF couples. Further research is needed to determine whether psychological interventions would be of any use in reducing the stress in males, and thus, may improve pregnancy outcome in this population.

AUTHOR CONTRIBUTIONS

KN has contributed to patient recruiting, interpretation of data, manuscript writing and editing. BRL contributed to data analyzing and statistical work. DET contributed to patient recruiting and performed data collection. MIS, KAW, JOH and LAH contributed to data analyzing, interpretation of data, mining of the data and manuscript editing. CBT has performed analyzing and interpretation of data, manuscript writing and editing and supervised the project. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interest.

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REFERENCES

- 1 Pike JL, Smith TL, Hauger RL, Nicassio PM, Patterson TL, *et al*. Chronic life stress alters sympathetic, neuroendocrine, and immune responsivity to an acute psychological stressor in humans. *Psychosom Med* 1997; 59: 447–57.
- 2 Greil AL. Infertility and psychological distress: a critical review of the literature. *Soc Sci Med* 1977; 45: 1679–704.
- 3 Ebbesen SM, Zachariae R, Mehlsen MY, Thomsen D, Højgaard A, *et al*. Stressful life events are associated with a poor *in vitro* fertilization (IVF) outcome: a prospective study. *Hum Reprod* 2009; 24: 2173–82.
- 4 Li XH, Ma YG, Geng LH, Qin L, Hu H, *et al*. Baseline psychological stress and ovarian norepinephrine levels negatively affect the outcome of *in vitro* fertilisation. *Gynecol Endocrinol* 2011; 27: 139–43.
- 5 Demyttenaere K, Nijs P, Evers-Kiebooms G, Koninckx PR. Coping, ineffectiveness of coping and the psychoendocrinological stress responses during *in vitro* fertilization. *J Psychosom Res* 1991; 35: 231–43.
- 6 Demyttenaere K, Nijs P, Evers-Kiebooms G, Koninckx PR. Coping and the ineffectiveness of coping influence the outcome of *in vitro* fertilization through stress responses. *Psychoneuroendocrinology* 1992; 17: 655–65.
- 7 Demyttenaere K, Bonte L, Gheldof M, Vervaeke M, Meuleman C, *et al*. Coping style and depression level influence outcome *in vitro* fertilization. *Fertil Steril* 1998; 69: 1026–33.
- 8 Boivin J, Takefman J. Stress level across stages of *in vitro* fertilization in subsequently pregnant and nonpregnant women. *Fertil Steril* 1995; 64: 802–10.
- 9 Smeenk JM, Verhaak CM, Vingerhoets AJ, Sweep CG, Merkus JM, *et al*. Stress and outcome success in IVF: the role of self-reports and endocrine variables. *Hum Reprod* 2005; 20: 991–6.
- 10 Boivin J, Shoog-Svanberg A, Andersson L, Hjelmstedt A, Bergh T, *et al*. Distress level in men undergoing intracytoplasmic sperm injection versus *in vitro* fertilization. *Hum Reprod* 2005; 14: 1403–6.
- 11 Gerhard I, Lenhard K, Eggert-Kruse W, Runnebaum B. Clinical data which influence semen parameters in infertile men. *Hum Reprod* 1992; 7: 830–7.
- 12 Fenster L, Katz DF, Wyrobek AJ, Pieper C, Rempel DM, *et al*. Effects of psychological stress on human semen quality. *J Androl* 1997; 18: 194–202.
- 13 Hjollund NH, Bonde JP, Henriksen TB, Giwercman A, Olsen J, Danish First Pregnancy Planner Study Team. The Danish First Pregnancy Planner Study Team. Job strain and male infertility. *Epidemiology* 2004; 15: 114–7.
- 14 Abu-Musa AA, Nassar AH, Hannoun AB, Usta IM. Effect of the Lebanese civil war on sperm parameters. *Fertil Steril* 2007; 88: 1579–82.
- 15 Fukuda M, Fukuda K, Shimizu T, Møller H. Decline in sex ratio at birth after Kobe earthquake. *Hum Reprod* 1998; 13: 2321–2.
- 16 Clarke RN, Klock SC, Geoghegan A, Travassos DE. Relationship between psychological stress and semen quality among *in vitro* fertilization patients. *Hum Reprod* 1999; 14: 753–8.
- 17 Vellani E, Colasante A, Mamazza L, Minasi MG, Greco E, *et al*. Association of state and trait anxiety to semen quality of *in vitro* fertilization patients: a controlled study. *Fertil Steril* 2013; 99: 1565–72.
- 18 Newton CR, Sherrard W, Glavac I. The Fertility Problem Inventory: measuring perceived infertility-related stress. *Fertil Steril* 1999; 72: 54–62.
- 19 World Health Organization. WHO laboratory manual for the examination of human semen and sperm–cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press; 1992: p4–29.
- 20 Kantenich H, Schmiady H, Radke E. The male IVF patient--psychosomatic considerations. *Hum Reprod* 1992; 1: 13–8.
- 21 Harrison KL, Callan VJ, Hennessey JF. Stress and semen quality in an *in vitro* fertilization program. *Fertil Steril* 1987; 48: 633–6.
- 22 An Y, Sun Z, Li L, Zhang Y, Ji H. Relationship between psychological stress and reproductive outcome in women undergoing *in vitro* fertilization treatment: psychological and neurohormonal assessment. *J Assist Reprod Genet* 2013; 30: 35–41.
- 23 Li Y, Li Y, Zhou N, Han X, Ma M, *et al*. Socio-psycho-behavioral factors associated with male semen quality in China: results from 1346 healthy men in Chongqing. *J Fam Plann Reprod Health Care* 2013; 39: 102–10.
- 24 Gollenberg AL, Liu F, Brazil C, Drobnis EZ, Guzick D, *et al*. Semen quality in fertile men in relation to psychosocial stress. *Fertil Steril* 2010; 93: 1104–11.

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