Stressful life events are associated with a poor in-vitro fertilization (IVF) outcome: a prospective study

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\textbf{BACKGROUND:} There is preliminary evidence to suggest an impact of stress on chances of achieving a pregnancy with in-vitro fertilization (IVF). The majority of the available research has focused on stress related to infertility and going through IVF-treatment, and it is still unclear whether non-fertility-related, naturally occurring stressors may influence IVF pregnancy chances. Our aim was to explore the association between IVF-outcome and negative, i.e. stressful, life-events during the previous 12 months.

\textbf{METHODS:} Prior to IVF, 809 women (mean age: 31.2 years) completed the List of Recent Events (LRE) and questionnaires measuring perceived stress and depressive symptoms.

\textbf{RESULTS:} Women who became pregnant reported fewer non-fertility-related negative life-events prior to IVF (Mean: 2.5; SD: 2.5) than women who did not obtain a pregnancy (Mean: 3.0; SD: 3.0) ($t(465.28) = 2.390, P = 0.017$). Logistic regression analyses revealed that the number of negative life-events remained a significant predictor of pregnancy (OR: 0.889; $P = 0.02$), when controlling for age, total number of life-events, perceived stress within the previous month, depressive symptoms, and relevant medical factors related to the patient or treatment procedure, including duration of infertility, number of oocytes retrieved and infertility etiology. Mediation analyses indicated that the association between negative life events and IVF pregnancy was partly mediated by the number of oocytes harvested during oocyte retrieval.

\textbf{CONCLUSION:} A large number of life-events perceived as having a negative impact on quality of life may indicate chronic stress, and the results of our study indicate that stress may reduce the chances of a successful outcome following IVF, possibly through psychobiological mechanisms affecting medical end-points such as oocyte retrieval outcome.

\textbf{Key words:} infertility / stress / negative life events / IVF / treatment outcome
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\textbf{Introduction}

Between 10 and 15\% of all couples experience fertility problems due to a variety of causes (Schmidt et al., 1995; Eugster and Vingerhoets, 1999; Juul et al., 1999), and infertility is increasing in the industrialized countries, possibly due to social and behavioural factors along with environmental exposures (Skakkebaek et al., 2006). Infertility is commonly defined as failure to conceive after at least 1 year of attempting to achieve a pregnancy with unprotected intercourse (Schmidt, 2006; Dansk Fertilitetstelsel, 2007). Couples suffering fertility problems frequently turn to artificial reproductive technology (ART), and it is assumed that ~50\% of all Danish couples experiencing infertility seek ART treatment (Schmidt et al., 1995). In Denmark, the number of initiated treatments with in-vitro fertilization (IVF) and intra cytoplasmatic sperm injection (ICSI) performed at public and private fertility clinics has increased by 83\%, from approximately 6000 per year to more than 11 000 per year, within the last 10 years (National Board of Health D, 2006; The Danish Fertility Society, 2009).

There is evidence to suggest that psychological factors may influence the chances of obtaining a pregnancy with IVF (Eugster and Vingerhoets, 1999; Klonoff-Cohen, 2005; Boivin and Schmidt, 2005). A number of studies have shown associations between distress, mostly anxiety and depression, and IVF-outcome, although a few studies have been unable to confirm this link (Milad et al., 1998; Lovely et al., 2003; de Klerk et al., 2008). Such findings are often reported as studies on effects of ‘stress’ on IVF-outcome (Harlow et al., 1996; Csemezzy et al., 2000; Lovely et al., 2003; Smeenk et al., 2005). However, the instruments used in these studies are generally measures of various types of distress which may be
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unrelated to external factors. The majority of the available studies therefore do not qualify as studies of stress.

The term ‘stress’ is, in fact, often confused with distress, which, although considered part of the same process, is a different phenomenon. The stress process is generally considered to consist of three aspects: (1) a stressor, i.e. an environmental demand, e.g. a life event or a series of life events, followed by (2) a set of appraisals and the perception of stress, which then may lead to (3) affective, behavioural and/or biological stress responses (i.e. distress) (Cohen et al., 1995). Perception of stress results when the individual finds the environmental demands taxing and/or threatening, and at the same time feels insufficiently able to cope with the demands due to lack of personal or environmental resources (Cohen et al., 1995).

According to the original conceptualization of a stressor, it is defined as ‘that which produces stress’ (Selye, 1976).

However, there is a challenge in assessing stressors in terms of life events, as the interpretation of and capacity to handle an experienced event both emotionally and practically is highly individual (Cohen et al., 1995).

For our purpose of examining the possible association between stress and reproductive outcomes, stress is defined as events or environmental demands (i.e. stressors), rated by the individual as having had a negative influence on her quality of life. These events will from here on be referred to as ‘negative life events’. The psychobiological effects of stress may potentially be long-lasting and persist even after the stressor has ceased to act (Selye, 1976).

Only very few studies of stress in the strict sense of the term and IVF-outcome have been conducted, focusing primarily on perceived marital stress and perceived stress caused by infertility and IVF-treatment. Infertility can indeed be very stressful for the affected couples (Berg et al., 1991; Eugster and Vingerhoets, 1999; Oddens et al., 1999; Webb and Daniluk, 1999; Peterson et al., 2003, 2006; Throsby and Gill, 2004; Schmidt et al., 2005a, b), and two large studies conducted with IVF-populations have found some support for infertility-related stress as a predictor of IVF-outcome (Klonoff-Cohen et al., 2001; Boivin and Schmidt, 2005). Another study, however, found the opposite result, i.e. higher infertility-related stress in the group that subsequently achieved a pregnancy (Cooper et al., 2007). One study measured IVF-treatment related stress (Klonoff-Cohen and Natarajan, 2004) and found this source of stress to be a predictor of the number of retrieved and fertilized oocytes. Women who prior to treatment had severe concerns about the medical aspects of the procedure, about missing work, or about finances, had up to 20% fewer oocytes retrieved and fertilized, which affected their pregnancy chances negatively. The third type of stressor studied in relation to IVF-treatment outcome, perceived marital stress, has also been associated with treatment outcome, in that the number of cycles-to-pregnancy was higher in women reporting high-marital stress compared with women reporting low-marital stress (Boivin and Schmidt, 2005). Preliminary evidence for an association between perceived marital stress and IVF outcome has also found partial support in two other studies (Stoleru et al., 1997; Verhaak et al., 2001). Finally, two studies (Facchinetti et al., 1997; Gallinelli et al., 2001) have provided evidence for an effect of experimentally induced stress on IVF-outcome using the Stroop Color Word Test (Stroop, 1935).

Taken together the available evidence is limited, yet suggestive of a stress effect on IVF-outcome. The majority of the available research has focused on perceived stress in relation to infertility and IVF-treatment, which may be confounded by the couples’ knowledge about their prognosis, and it is still unclear whether naturally occurring stressors, which are unrelated to infertility, may influence pregnancy chances following IVF. The aim of our study was therefore to investigate the possible association between negative life events and pregnancy chances in a sample of women in IVF-treatment at a large regional university hospital fertility clinic. We hypothesized that an increase in the number of negative life events would be associated with reduced chances of obtaining IVF-induced pregnancy. Stress may impact fertility possibly by interrupting menstrual cycles (Chrousos and Gold, 1992; Ferin, 1999) or through a poorer ovarian functional response to IVF-treatment (Klonoff-Cohen and Natarajan, 2004). We therefore planned to explore the possible role of the number of retrieved oocytes as a possible mediator of this association, if an association between negative life events and pregnancy chances was found.

Materials and Methods

Patients

A total of 837 women participated in this study from a cohort of approximately 1578 couples undergoing their first IVF-treatment cycle at the fertility clinic. The selection criteria were: (1) first IVF-cycle, (2) no previous attempts with IVF-treatment and (3) ability to read and understand Danish well enough to complete the questionnaires. Couples were excluded from the study in cases of PGD (Preimplantation Genetic Diagnosis) as the indication for these treatments in the clinic is usually genetic and not infertility. Another exclusion criterion was unplanned change of treatment type, e.g. from insemination to IVF due to too many follicles.

Procedure

As a part of a larger study, couples referred to IVF-treatment at the Fertility Clinic, University Hospital, Skejby between October 2001 and September 2006 received oral and written information about the study and an invitation to participate during their first treatment cycle. Women, who agreed to participate, received a baseline questionnaire package when presenting themselves at their first scheduled appointment with a fertility clinic nurse, taking place approximately on the 21th day of the cycle. At this meeting the patients were also instructed on how to administer hormonal down-regulation with a GnRH-agonist for 2 weeks followed by 8 days of stimulation with Follicle Stimulating Hormone (FSH). The baseline questionnaire was completed at home during this period and handed in by the patient approximately 2 weeks later when presenting herself for a follicle scan.

The physician registered medical and technical details in relation to the oocyte retrieval procedure and results of pregnancy tests were added when available. The study was accepted by the Regional Ethics Committee and approved by the Danish Data Protection Agency.

Measurements

Independent variables: stress measures

Negative life events. An adjusted version of The List of Recent Events (LRE) (Henderson et al., 1981) was used to measure the presence and psychological impact of major life events. The original version of the scale consists of an event list of 59 items describing various potentially stressful environmental events within the following categories: illness, injury and accident, bereavement, pregnancy/children, changes in...
relationships, living conditions, study/schooling, work/employment, finances and legal difficulties. For each event, the participants are to answer yes or no to the question 'Did you within the past 12 months experience [this event]?' For each event, the participants were asked to indicate whether the event had an impact on subjective well-being by answering the question: ‘Did this event have a negative impact on your life quality’ with yes or no. The LRE has been shown to adequately assess the presence of negative life events in the general population (Henderson et al., 1981), and has shown predictive strength in relation to physical health symptoms (Cohen et al., 1991). In the present study a shortened 37-item version of the list was used. We excluded events such as death of spouse, divorce, new partner, giving birth, since these events were irrelevant to the sample due to the hospital’s admission criteria for IVF-treatment. Two total scores are derived from the LRE: the total number of experienced events, which in the following is termed ‘number of events’, and total number of events ‘perceived as having had a negative impact on quality of life’, from here on termed ‘negative life events’. Thus from the total number of events, ‘negative life events’ refer to the (sum of) events evaluated by the person as having a negative influence on her quality of life.

Perceived stress. Perceived stress within the past month was assessed with the Perceived Stress Scale (PSS-10) (Cohen and Williamson, 1988), consisting of 10 items measuring the present level of self rated stress. The PSS-10 is a widely used measure that has been shown to be reliable and valid. The internal consistency of the PSS-10 was considered acceptable within this sample (Cronbach’s α = 0.85).

Independent variables: distress measures

Depressive symptoms. The Beck Depression Inventory (BDI-II) (Beck et al., 1996) was used to measure depressive symptoms. The BDI consists of 21 items measuring the severity of both somatic and cognitive-affective depressive symptoms. It is a widely used instrument for assessing depressive symptoms in various populations and has shown good reliability and validity. The internal consistency of the BDI in this sample was acceptable (α = 0.71).

Dependent variables

Pregnancy. A positive pregnancy test was defined by measurement of a serum HCG-level of >20 IU 2 weeks following embryo transfer. Pregnant patients were scanned routinely 5 weeks after embryo transfer. Clinical pregnancy was defined as a live intrauterine pregnancy detected by ultrasound. Where nothing else is specified, pregnancy and IVF-outcome refers to clinical pregnancy.

Additional measures

The number of oocytes retrieved at oocyte retrieval, the cause of infertility (male, tubal, endometriosis, ovulation factor, idiopathic) and the type of treatment (ICSI or IVF) were registered by the physician. In case of more than one cause of infertility, the most clinically significant cause was recorded as the primary cause. In the following, cause of infertility refers to the primary cause of infertility. Medical and treatment-related information and information on life style factors was extracted from the hospital register database and used for comparison between sample and non-participants in the cohort. BMI was calculated from self-reported weight and height data. Basal FSH was measured on cycle day 2–4.

Statistical analyses

The statistical analyses were conducted with the Statistics Package for the Social Sciences (SPSS) version 15.0.

In cases of multiple comparisons, Bonferroni adjustments were made in order to reduce the risk of type-I error by dividing the alpha level with the number of comparisons, resulting in accordingly adjusted p-levels (Tabachnick and Fidell, 2007). It has been argued that in large samples, normal distribution is not a prerequisite for the t-test (Lumley et al., 2002), thus in the analyses t-tests are chosen over non-parametric test in order to retain statistical power.

Univariate analyses were conducted, comparing patients who agreed to participate to the eligible non-participating patients in the cohort. Independent samples t-test was applied in order to explore differences between the two groups concerning female age, duration of infertility, total hormone dose before aspiration, number of previous births, number of previous provoked and spontaneous miscarriages, number of previous ectopic pregnancies. χ²-analysis was applied to assess differences in pregnancy outcomes, smoking habits and coffee intake.

Means and standard deviations were calculated for the independent variables (IVs) BDI, PSS-10 and LRE for women who subsequently did or did not achieve pregnancy following IVF-procedure. A series of independent samples t-test was carried out to assess differences between these two groups.

If differences in the number of negative life events between IVF-successful and non-successful women were found, the data were initially inspected for possible patient- or treatment-related confounders and mediators of this association; correlation analyses/linear regression techniques were applied to investigate associations with dependent variables (DVs): number of oocytes and negative life events, and logistic regression was used for detection of possible associations with pregnancy chances as a DV. Statistical information is provided for any significant results.

Next, a series of direct regression analyses were planned in order to examine the main hypothesis of an association between negative life events and IVF pregnancy chances, although controlling for relevant biological and medical factors relating to infertility and IVF-treatment procedure. Subsequently, to test the possible mediating role of number of oocytes for the association between negative life events and IVF pregnancy, we used the method described by Baron and Kenny (Baron and Kenny, 1986). Mediation is proposed to be established when (1) the IV significantly predicts the DV, (2) the IV significantly predicts the mediator, (3) the mediator predicts the DV when controlling for the IV and (4) when controlling for the mediator, the effect of the IV on the DV ceases (complete mediation) or is reduced (partial mediation) (Baron and Kenny, 1986). Finally, χ² analyses were conducted for each possible life event on the LRE in order to explore whether some types of events posed a higher risk for an adverse IVF-outcome than others.

Missing values

For all psychometric scales (excl. LRE), the proportion of missing values was computed, and missing values were substituted with the mean values for the remaining scale items. In cases where more than 50% of items in the respective scales were unanswered, the case was omitted from the analysis. Substituting missing values with the mean for the remaining scale items is recommended, provided that the internal reliability of the scales is high (i.e. α > 0.70) (Schafer and Graham, 2002).

Some variables from the hospital register contained a proportion of unsubstiuted missing data, and are primarily used for comparative analyses between participants and non-participants. N is reported for these analyses. In addition, exploratory analyses were conducted on the basis of hospital register data with FSH, BMI, coffee, smoking and duration of infertility. All other analyses are based on data collected as a part of this study.
Results

Study sample
Of the 837 couples, 18 cases had not completed questionnaires at baseline and were excluded from the data analyses. Another 10 cases were excluded for various reasons (puncture of cysts, spontaneous pregnancy, ovulation prior to oocyte retrieval, treatment cancellation or moving away). This resulted in a total of 809 couples in the final sample. Sociodemographic and reproductive characteristics of the sample can be seen in Table I.

Minor inconsistencies between numbers given in Table I and in the following comparative analyses between sample and non-participants are due to data being drawn from two different sources, i.e. study questionnaires and hospital register. Study questionnaire data are used to describe sample characteristics, as these data are more detailed.

Table I Socio-demographic and reproductive characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>Mean (SD) or %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD)</td>
<td>796</td>
<td>31.2 (3.9)</td>
<td>[21, 40]</td>
</tr>
<tr>
<td>Marriage/Living together</td>
<td>804</td>
<td>4–6 years (2 years)</td>
<td>[2, 8+]</td>
</tr>
<tr>
<td>Education (ISCED-based, UNESCO, 2009))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower secondary general (7 years)</td>
<td>4</td>
<td>0.5%</td>
<td></td>
</tr>
<tr>
<td>Lower secondary general (8–10 years)</td>
<td>46</td>
<td>5.7%</td>
<td></td>
</tr>
<tr>
<td>Upper secondary (11–13 years)</td>
<td>245</td>
<td>30.3%</td>
<td></td>
</tr>
<tr>
<td>Tertiary &lt; master degree (14–17 years)</td>
<td>366</td>
<td>45.2%</td>
<td></td>
</tr>
<tr>
<td>Tertiary master degree (≥18 years)</td>
<td>137</td>
<td>16.9%</td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>11</td>
<td>1.4%</td>
<td></td>
</tr>
<tr>
<td>Duration of infertility in months</td>
<td>668</td>
<td>31.5 (22.9)</td>
<td>[0–228]</td>
</tr>
<tr>
<td>Type of procedure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICSI long down-regulation</td>
<td>284</td>
<td>35.1%</td>
<td></td>
</tr>
<tr>
<td>IVF long down-regulation</td>
<td>385</td>
<td>47.6%</td>
<td></td>
</tr>
<tr>
<td>IVF/ICSI mild hormone stimulation</td>
<td>51</td>
<td>6.3%</td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>89</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>Cause of infertility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovulation factor</td>
<td>26</td>
<td>3.2%</td>
<td></td>
</tr>
<tr>
<td>Tubal</td>
<td>135</td>
<td>16.7%</td>
<td></td>
</tr>
<tr>
<td>Endometriosis</td>
<td>79</td>
<td>9.8%</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>351</td>
<td>43.4%</td>
<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>145</td>
<td>17.9%</td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>73</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>IVF-treatment outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive pregnancy test</td>
<td>280</td>
<td>34.6%</td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>217</td>
<td>26.8%</td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>14</td>
<td>1.7%</td>
<td></td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>776</td>
<td>6.93 (4.4)</td>
<td>[0–25]</td>
</tr>
<tr>
<td>Missing data</td>
<td>33</td>
<td>4%</td>
<td></td>
</tr>
</tbody>
</table>

No differences were found between patients who agreed to participate and the 522 eligible non-participating patients in the cohort (total n = 1359) with respect to age (n = 1359), duration of infertility (n = 1259) and daily amount of smoking (n = 1001), number of previous abortions (spontaneous or provoked), number of previous ectopic pregnancies and number of previous births (n = 729). Also, there was no statistically significant difference between the study group and the rest of the cohort as to pregnancy outcome from the first treatment cycle (n = 336) (data not shown).

The group of study participants represented a larger proportion of male factor infertility cases (46% male factor, 33% female factor and 21% idiopathic) compared with the non-participants (37% male factor, 31% female factor and 32% idiopathic) who, in return represented slightly more cases of idiopathic infertility (χ² (2) = 20.193, P = 0.0005) (n = 1359). In accordance, study participants represented a larger proportion of patients receiving ICSI treatment for male factor infertility (40% ICSI, 60% IVF) than the rest of the cohort (23% ICSI, 77% IVF) (χ²(1) = 39.220, P = 0.0005) (n = 1359). Study participants received a higher total hormone dose before aspiration (MEAN: 1650.60 IU, SD: 1592.36 IU) compared with the rest of the cohort sample (MEAN: 1252.03 IU, SD: 1795.29 IU) (t(1358) = −4.272, P = 0.0005) (n = 1358). Total hormone dose before aspiration showed a moderately skewed distribution. Among study participants there was a larger coffee intake compared with the non-participants in the cohort (χ²(3) = 9.082, P = 0.028) (n = 986). When correcting for multiple comparisons, using a Bonferroni adjustment of the alpha level (0.05/13 = 0.004), differences between participants and non-participants’ coffee habits ceased to be significant.

Comparing pregnant and non-pregnant women
Means and standard deviations for women who subsequently did or did not achieve pregnancy following the IVF-procedure on the IVs BDI, PSS-10 and LRE are shown in Table II. The BDI and negative life events showed moderately skewed distributions. A series of independent samples t-tests showed no significant differences between successful and unsuccessful IVF-patients for depressive symptoms, perceived stress and total number of life events (with an adjusted (0.05/3) alpha level of 0.02) (data not shown).

Initial testing of the main hypothesis showed that women who obtained a clinical pregnancy following the IVF-procedure reported a statistically significantly smaller proportion of negative life events than women who did not obtain pregnancy. These results are shown in Table II.

Correlations with reproductive outcomes
The number of oocytes retrieved during oocyte retrieval showed a small, but statistically significant negative correlation with number of negative life events (r = −0.10, n = 772, P < 0.01 (2-tailed)), and was also a statistically significant univariate predictor of pregnancy (OR = 1.072, n = 733, P = 0.0005). FSH is an indicator of potential ovarian response and the ovarian response is a predictor of IVF-outcome (Yih et al., 2005). Although FSH was a statistically significant univariate predictor of number of oocytes (β = −0.197, n = 495, P = 0.0005), we found no association between FSH and pregnancy, or between negative life events and FSH (no data shown).
We were therefore unable to establish FSH as a possible mediator, either of the association between negative life events and the number of oocytes or the association between negative life events and pregnancy. Similarly, although age was shown to be significantly associated with pregnancy (OR = 0.948, n = 783, P = 0.01), this variable was unrelated to negative life events (no data shown), and can thus likewise be excluded as a possible mediator. There were no significant differences in the number of oocytes retrieved as a function of BMI (data not shown). Smoking habits (n = 651), daily smoking habits (n = 521), and daily coffee intake (n = 518) were unrelated to clinical pregnancy (data not shown).

Effects of negative life events on pregnancy chances

To test whether negative life events predicted the likelihood of obtaining a positive IVF-treatment outcome, a series of logistic regression analyses were carried out with pregnancy versus no pregnancy as the DV and negative life events as the predictor, while controlling for total number of life events experienced, current perceived stress, depressive symptoms, and age.

Moderate to strong positive correlations were seen between the number of negative life events (LRE) and depressive symptoms (BDI), and between negative life events and perceived stress within the last month (PSS-10). Whether life events are negatively evaluated may be confounded by the person’s current level of perceived stress and depressive symptoms, and since the correlations were not sufficiently large to suggest problems related to multicollinearity (Tabachnick and Fidell, 2007), these two factors were included in the regression model.

The models provided an acceptable fit to the data as indicated by the omnibus test of model coefficients (Positive pregnancy test: χ²(5) = 17.03, P = 0.004, n = 764, Clinical pregnancy: χ²(5) = 16.35, P = 0.006, n = 764) explaining 2–3% of the variance in pregnancy rate (pseudo R²-statistics). The IV, negative life events, remained a statistically significant predictor of a positive pregnancy test following IVF, when controlling for depressive symptoms, perceived stress, age and total number of events experienced (OR: 0.909, P = 0.018). Concerning clinical pregnancy, there was a trend toward negative life events being a predictor IVF-outcome when controlling for the same variables (OR: 0.922, P = 0.063).

When using pregnancy as a DV, it is important to control for selected medical factors related to the IVF-patient and the treatment. We therefore conducted a second logistic regression analysis, additionally controlling for number of oocytes, duration of infertility in months and cause of infertility, categorized into female, male or idiopathic factor. Female factor was used as a simple contrast (reference group) in the analysis. The results are presented in Table III. Negative life events continued to be a predictor of clinical pregnancy chances when controlling for number of oocytes, cause of infertility and duration of infertility in the model (OR: 0.889, P = 0.02). The

<table>
<thead>
<tr>
<th>Table II</th>
<th>Differences between pregnant and non-pregnant women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measures</td>
<td>Pregnant Mean (SD)</td>
</tr>
<tr>
<td>Perceived stress</td>
<td>14.8 (6.0)</td>
</tr>
<tr>
<td>Depressive symptoms</td>
<td>7.2 (6.1)</td>
</tr>
<tr>
<td>Total number of life events</td>
<td>5.4 (3.0)</td>
</tr>
<tr>
<td>Negative life events</td>
<td>2.5 (2.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table III</th>
<th>Effects of stress measures on IVF outcome, when controlling for medical variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measures</td>
<td>Coefficient (B)</td>
</tr>
<tr>
<td>Negative life events</td>
<td>−0.118</td>
</tr>
<tr>
<td>Number of events</td>
<td>0.009</td>
</tr>
<tr>
<td>Depressive symptoms</td>
<td>0.005</td>
</tr>
<tr>
<td>Perceived stress</td>
<td>0.032</td>
</tr>
<tr>
<td>Age</td>
<td>−0.074</td>
</tr>
<tr>
<td>Number of oocytes</td>
<td>0.061</td>
</tr>
<tr>
<td>Female cause of infertility</td>
<td>1.586</td>
</tr>
<tr>
<td>Male cause of infertility</td>
<td>−0.269</td>
</tr>
<tr>
<td>Idiopathic infertility</td>
<td>−0.179</td>
</tr>
<tr>
<td>Duration of infertility</td>
<td>0.004</td>
</tr>
</tbody>
</table>

All variables used in the analysis are shown.
N = 588 (Missing cases: 221). Pseudo R²-statistics: Cox and Snell, R² = 0.048, Nagelkerke R² = 0.069.
LRE, events rated as having had negative impact on quality of life.
LRE, number of events.
results indicated that for every negative life event a patient had experienced within the past 12 months, there was approximately a 10% decrease in the chances of obtaining a clinical pregnancy.

Age and the number of oocytes retrieved were both significantly associated with chances of obtaining a clinical pregnancy, even when controlling for other medical factors related to the patient or the IVF-treatment. Duration of infertility in months and cause of infertility did not predict clinical pregnancy. As in the previous analyses, perceived recent stress and depressive symptoms did not emerge as significant independent predictors of the DV.

**Mediation analysis**

As a small correlation was observed between the number of negative life events and number of oocytes retrieved, we further examined whether the association between number of negative life events and pregnancy rates could possibly be mediated by number of oocytes harvested during oocyte retrieval.

A series of linear regression analyses showed that the number of negative life events significantly predicted both IVF-outcome and number of oocytes harvested during the oocyte retrieval procedure. Furthermore, when controlling for negative life events, the number of oocytes harvested predicted pregnancy. When controlling for oocytes harvested, the first association between negative life events and pregnancy ceased to be significant. The results of the mediation analysis are shown in Table IV. Applying the criteria for mediation (Baron and Kenny, 1986), the analysis showed the number of oocytes harvested during oocyte retrieval to be a partial mediator of the association between negative life events and pregnancy.

**Impact of specific types of life events**

Finally, analyses were conducted to explore whether failure to obtain a pregnancy was associated with specific life events. χ²-tests revealed a trend as to whether the woman reported having experienced violence or threats of violence within the past 12 months or not: 16 women reported experiences of violence or threats of violence and none of these women achieved a viable pregnancy following their first cycle of IVF. (χ²(1) = 6.130, P < 0.009 (Fishers Exact Test), total n = 795). Furthermore, a trend was seen with respect to whether couples had experienced prolonged or serious incidents of illness among close relatives. A larger proportion of the women who reported having had a parent with serious illness did not achieve IVF-pregnancy (21% pregnant) in the year following this event compared with those who did not report this type of event (29% pregnant) (χ²(1) = 3.691, P < 0.055, total n = 795) (Bonferroni adjusted p-level of 0.001 (0.05/37)).

**Discussion**

Our results showed that women who became pregnant following IVF-treatment reported fewer negative life events than women who did not obtain pregnancy. The data in this study indicate that the lower pregnancy rate associated with negative life events is partly mediated by a lower number of oocytes harvested. Our results thus extend previous findings linking the presence of infertility and relationship stress with impaired chances of achieving pregnancy with an IVF-procedure (Facchinetti et al., 1997; Stoleru et al., 1997; Gallinelli et al., 2001; Klonoff-Cohen et al., 2001; Verhaak et al., 2001; Boivin and Schmidt, 2005).

**Effects of negative life events on IVF-outcome**

Our results are in line with two of the three other studies exploring associations between environmental stressors and reduced chances of pregnancy following IVF-treatment. In a 12-month prospective study by Boivin and Schmidt (2005) of a cohort of 818 Danish couples in IVF-treatment, an association was found between baseline perceived infertility-related stress and pregnancy obtained within the following 12 months of treatment. Among couples reporting that fertility problems had disrupted their lives, were difficult to cope with, or had caused severe strain on their physical and mental health, fewer conceived within the subsequent 12 months of treatment when compared with couples not reporting such stressors. The authors also explored another potential environmental stressor, marital problems, and found that the number of cycles-to pregnancy was significantly higher in women reporting high perceived marital stress compared with women reporting low levels of this stressor. Women who reported that infertility had caused a crisis in their relationship, given rise to thoughts of divorce, or placed great strain on their sexual relationship thus had reduced chances of a successful IVF-outcome (Boivin and Schmidt, 2005). The preliminary evidence for an association between perceived marital stress and IVF-outcome has also found support in one other study (Stoleru et al., 1997). One study, however, has reported the opposite result (Cooper et al., 2007), but this study was potentially confounded by access to psychological counselling offered by the fertility clinic, which was not controlled for statistically. Together with our results, the limited available evidence is suggestive of an association between stress and fertility, with stress having a detrimental impact on chances of achieving a viable pregnancy through IVF-treatment.

Depression and perceived stress within the past month were not significant predictors of IVF-outcome in multivariate analysis. This is not an uncommon finding, and previous studies have shown low predictive quality of the particular measures used (BDI and PSS-10) concerning IVF-outcomes (Klonoff-Cohen et al., 2001; Smeenk et al., 2001). The BDI may be too exclusively designed for populations of
clinically depressed individuals, failing to capture ongoing mood shifts in psychologically healthy samples, and several studies have stated that IVF-patients are not clinically depressed (Greil, 1997; Eugster and Vingerhoets, 1999).

The PSS-10 measures current experiences of stress in that the timeframe is limited to feelings of stress during the past month, and we suggest that the fluctuations in current stress captured by the PSS-10 may not be potent enough to harm the reproductive system. Consistent with this, perceived stress did not emerge as a significant predictor of IVF-outcome. In contrast to the lack of effect of perceived current stress, we did find an effect of having experienced traumatic or negative life events within the past 12 months on pregnancy chances. Major life stressors may be viewed as a more chronic source of stress. The fact that the effect of negative life events on IVF-outcome was independent of effects of distress, i.e. depression and of perceived stress, warrants further discussion.

Negative life events may exert influence on the female reproductive system in two ways, either directly or indirectly (i.e. through life style behaviours). It is possible that the experience of negative life events may result in a subtle stress reaction, that may only be partially perceived (or even suppressed) by the individual. Although thus not necessarily being considered by the individual as a source of daily (perceived) stress, the negative life events or series of negative life events may over time lead to an increase in maladaptive coping strategies and life style behaviours (i.e. diet, drinking, smoking habits etc.) and thus indirectly impact pregnancy chances. The experience of highly stressful life events is generally known to be associated with behavioural health problems (i.e. maladaptive life style behaviours in terms of eating behaviours, alcohol and cigarette use) (Veenstra et al., 2007; Bacharach et al., 2008; Hooper et al., 2008; Ogden et al., 2009), and behavioural health problems are known to be associated with reduced fertilization chances (Klonoff-Cohen, 2005). Specifically, in one study the authors investigated female alcohol consumption and found this related to pregnancy chances (Klonoff-Cohen et al., 2003).

This possibility is however in need of further testing, as our study only used relatively simple measures of life-style behaviours and had no appropriate measure of alcohol consumption, which may be an important life-style variable in this context. BMI was unrelated to probability of a clinical pregnancy. However, as BMI was based upon self-reported measures, this may have given rise to bias.

A direct effect of negative life events on the female reproductive system may take place through release of stress hormones such as cortisol, which may cause estradiol inhibition indicated by impaired granulosa cell function and possibly compromised follicular maturation and lower number of oocytes to be harvested (Lancaster and Boivin, 2005). The more severe the stress, the more cycle disturbance is likely to be produced, with stress responses inhibiting the reproductive system through activation of the hypothalamic-pituitary-adrenal (HPA) axis (Chrousos and Gold, 1992).

In terms of psychological interventions, it seems premature to put forth suggestions, before direct and/or indirect pathways of a stress effect on pregnancy chances have been clarified. Should further research point to mediating effects of maladaptive coping strategies and life style behaviours (i.e. diet, drinking, smoking habits etc.) in the association between stress and IVF-outcome, cognitive-behavioural interventions could provide a useful tool for addressing these maladaptive ways of handling the psychological consequences of negative life events as well as for implementing alternative health behaviours. If the effect is unrelated to health behaviour, interventions targeted at conscious processing of psychological consequences of experiencing negative life events may alleviate psychobiological stress reactions and thereby possibly optimize pregnancy chances following IVF through a reduction of stress-related HPA-axis activity.

Mediating mechanisms

Stress (Facchinetti et al., 1997; Klonoff-Cohen et al., 2001; Gallinelli et al., 2001) and distress (Sanders and Bruce, 1999; Smeenk et al., 2001) have been found to be associated with a poorer ovarian functional response to IVF-treatment, and reduced fertilization, implantation and live birth rates. It has been suggested that stress may impact fertility by interrupting menstrual cycles (Demyttenaere et al., 1992) or by interacting with aspects of the IVF-treatment (Boivin and Takefman, 1995); our study has provided preliminary evidence for the latter.

The number of oocytes retrieved during oocyte retrieval is a critical characteristic of the ovarian response to IVF-treatment. The quality of the ovarian response is a potent predictor of IVF-outcome and has been shown to predict increased clinical pregnancy rates relatively independent of other factors such as maternal age (Yih et al., 2005). It has been reported that the biological response of the ovaries during IVF-treatment (e.g. number of follicles and number of oocytes) can be predicted by psychological variables (Lancaster and Boivin, 2005). This suggests a possible association between stress, number of oocytes, and pregnancy. Wishing to gain a more detailed knowledge about the mechanisms at work, we used mediation analysis to explore oocytes as a potential link in the stress-fertility association, and found that the number of oocytes retrieved was a partial mediator between negative life events and IVF-pregnancy.

Evidence for a link between stress and number of oocytes has been reported in a study by Klonoff-Cohen and colleagues, who prospectively studied infertility-related stress and IVF-treatment-related stress in 151 female IVF-patients (Klonoff-Cohen et al., 2001; Klonoff-Cohen and Natarajan, 2004). Although they did not find infertility-related stress to be a significant predictor of pregnancy, trends were found for this source of stress as a predictor of number of oocytes retrieved (Klonoff-Cohen et al., 2001). Concerning treatment-related stress, it appeared that women who prior to treatment had severe concerns about the medical aspects of the procedure, about missing work, or about finances, had up to 20% fewer oocytes retrieved and fertilized (Klonoff-Cohen and Natarajan, 2004).

Together with our findings, the results by Klonoff-Cohen et al. thus suggest a detrimental effect of stress on the number of oocytes that can be retrieved during procedure. Our results pointed to further consequences in terms of a reduction in pregnancy chances.

Impact of specific types of life events

Psychological stress refers to an individual’s psychological reaction when faced with challenges (stressors) exceeding his or her personal resources, i.e. coping abilities (Lazarus and Folkman, 1984). The total amount of psychological stress experienced is known to be influenced by the level of subjective control over the situation, which again is closely related to perceived coping possibilities. According to the theoretical and empirical works on stress, it is especially the prolonged
exposure to an environmental stressor and/or uncontrollable situations that lead to human psychological and psychobiological stress with potentially adverse consequences for health (Bloom and Lazerson, 1988). Trends were found in our study that long lasting illness within the nearest family and experiences with violence were more highly related to IVF-outcome than other stressors. Long lasting illness in the family and experienced violence are highly uncontrollable and therefore likely to produce a very high amount of psychological stress, which, without proper resolution, may also be of a long lasting character. Due to the relatively small number of participants having experienced such life events, these results must however be considered preliminary, and more studies are needed to confirm this finding.

Strengths and limitations

This is the first study to examine a possible association between IVF-outcome and stress following negative life experiences, even though taking into account procedural stress related to the immediate treatment situation. Investigating life events within the year before commencing treatment provides knowledge about stressors of potential damage to the reproductive system that take place at a very early point in time, compared with the time frame set in the existing studies on stress and IVF-outcome. This study also includes a sufficiently large sample to explore mechanisms while at the same time controlling for relevant demographic, clinical and treatment-related factors. Existing studies exploring psychological factors in IVF-treatment rarely include more than 100 participants, and most studies include less than 50 participants (e.g. Demyttenaere et al., 1991; Boivin and Takefman, 1995; Facchinetti et al., 1997; Csemiczky et al., 2000; Lovely et al., 2003), which limits the number of IVs in the statistical analyses and enhances the risk of type-2 error (Tabachnick and Fidell, 2007).

However, our study is not without limitations. We had no precise monitoring of non-response per se as questionnaires unfortunately were handed out at the clinic without complete registration of those who chose to participate and those who declined. Therefore a detailed non-responder analysis was not possible as some of the women may not have been offered participation. The problem was addressed by careful comparison between participants and non-participants in the cohort on the basis of hospital registry data on relevant variables. Comparisons of participants with non-participating women in the same cohort showed that participants included more cases of male factor infertility and ICSI treatment than non-participants, whereas the latter group consisted of more cases of idiopathic infertility. Treatment modality and infertility etiology were however unrelated to pregnancy in our study, and general differences for these two parameters are therefore less likely to affect the external validity of our findings concerning stress and IVF-outcome. In addition, differences were found concerning hormone doses, with study participants receiving a higher total hormone dose before aspiration compared with the rest of the cohort sample. This could have been due to a smaller proportion of patients in mild hormone stimulation protocol among participants, but, unfortunately, we have no registration of this particular treatment factor for the non-participating part of the cohort. However, the fact that participants completed the LRE prior to beginning hormone treatment, together with the similarity of pregnancy outcomes in the participant and non-participant groups, excludes hormone treatment as a possible confounder in the association between negative life events and IVF-outcome. Participants and non-participants were also generally similar with respect to age as well as life style factors, medical variables, and reproductive history, suggesting that our results can be considered relatively generalizable.

Use of variables from the hospital register meant acceptance of an appreciable proportion of missing values on some variables, thus results from these analyses must be interpreted with some caution, e.g. comparisons between participants and non-participants. In the analyses, we were unable to investigate any effects of alcohol consumption as we did not have an appropriate measure of this potential confounder. Future studies should include measures of alcohol consumption in order to assess this factor as a possible mediator of stress-infertility associations. Concerning the non-appearance of a statistically significant association of FSH, BMI and other life style habits with IVF-outcome, we cannot exclude lack of statistical power as a possible explanation.

Conclusion

Our results indicate that the number of negative life events influences IVF outcome, an effect that is partly mediated by number of oocytes harvested during treatment. A large number of negative life-events may indicate chronic stress, which may only be partially acknowledged by the individual, and which could reduce the chances of a successful IVF-outcome through a detrimental impact on the number of oocytes retrieved. Ovarian response is one of the most important predictors of pregnancy and may prove to be an important link in the association between psychological factors, e.g. stress, and pregnancy. More studies are needed, focusing specifically on stress and preferably including physiological measures, in order to properly clarify the mechanisms behind the stress-fertility association, both in terms of (1) indirect pathways of this association, i.e. health problem behaviour, and (2) direct effects in terms of psychoneuroendocrine mechanisms.

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References

Stressful life events and IVF outcome


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