

Stress and anxiety do not result in pregnancy wastage

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The association between stress and reproductive outcome is unclear. In-vitro fertilization (IVF) is psychologically stressful and has been shown to alter psychological markers such as cortisol, prolactin and progesterone. This study was designed to assess prospectively psychological and physiological markers of stress and to determine if they are related to pregnancy outcome. Forty patients were recruited from Northwestern Medical Faculty Foundation (Chicago, Illinois, USA) having obtained an initial positive β -human chorionic gonadotrophin (HCG) concentration 13 days after IVF with uterine embryo transfer. Patients underwent psychological and hormonal testing on three separate occasions (13, 20 and 27 days after embryo transfer) early in pregnancy. All subjects were followed to delivery. An adverse outcome was defined as a miscarriage before or after cardiac activity (including vanishing twin) or a loss before 20 weeks gestation. There was no difference in age, duration of infertility, diagnosis between patients experiencing an adverse pregnancy outcome ($n = 18$) and those that did not ($n = 22$). All patients were found to have high stress levels although this did not differentiate between groups of patients. There was no difference in hormonal markers of stress between patients. In conclusion, there is little association between psychological scores and physiological stress hormone concentrations. Also, it does not appear that high levels of anxiety and stress result in an adverse pregnancy outcome.

Key words: anxiety/infertility/in-vitro fertilization/miscarriage/stress

Introduction

Several studies have demonstrated that in-vitro fertilization (IVF) is psychologically stressful for women (Newton *et al.*, 1990; Litt *et al.*, 1992; Boivin and Takefman, 1995) but it is unclear whether the psychological stress adversely impacts on treatment outcome. Clinical experience tells us that many patients and clinicians agree with the statement, 'just relax and you'll get pregnant' with the implicit assumption that psychological stress or anxiety may prevent a woman from attaining and maintaining a pregnancy. From a physiological

perspective, it has been postulated that psychological stress alters levels of cortisol, prolactin and progesterone, which in turn have an adverse affect on pregnancy outcome. Recently several investigators have studied the proposed relationship between psychological distress and pregnancy outcome among IVF patients. Domar *et al.* (1990) reported decreases in anxiety and depression among a group of women who underwent a 10-session relaxation programme with a subsequent 34% pregnancy rate among the group attendees in a non-randomized, uncontrolled study. Boivin and Takefman (1995) concluded that psychological stress was related to IVF outcome in a group of women who kept daily ratings of stress throughout an IVF cycle.

While these studies suggest that there may be a relationship between psychological stress in women and IVF outcome, they are incomplete because they do not assess physiological markers of anxiety such as cortisol and prolactin. There are few studies that measure both the physiological and psychological aspects of anxiety during IVF and relate it to pregnancy outcome. One study (Demyttenaere *et al.*, 1991) found that anxiety and concentrations of prolactin and cortisol all increased significantly from baseline to the time of egg retrieval during IVF. Harlow *et al.* (1996) also found that levels of state anxiety and concentrations of prolactin and cortisol all increased during IVF but that there was no relationship between increased anxiety, hormone concentrations and pregnancy outcome. Other biochemical markers of stress have not been measured in relation to pregnancy outcome. Salivary α -amylase is known to be correlated with serum epinephrine concentrations (Chatterton *et al.*, 1996). Allopregnanolone is an active metabolite of progesterone and is a known modulator of γ -aminobutyric acid (GABA) receptors, resulting in anxiolytic effects. It has been suggested that allopregnanolone may enhance GABA-mediated inhibition during psychological stress (Majewska *et al.*, 1986; Harrison *et al.*, 1987; Peters *et al.*, 1988). Therefore further studies are needed to assess the intricate relationship between psychological anxiety, stress hormone concentrations and pregnancy outcome during IVF.

The purpose of the current study is to assess both psychological and physiological markers of stress and determine if they are related to pregnancy outcome.

Materials and methods

Subjects

The subjects in this study were recruited from the IVF programme at Northwestern Medical Faculty Foundation (Chicago, Illinois, USA). All patients underwent controlled ovarian stimulation using leuprolide

acetate for down-regulation (minimum of 10 days) followed by human menopausal gonadotrophins (HMG). Dosages were tailored for each patient but typically included follicle stimulating hormone (FSH) 150 IU with HMG 75 IU each day until the ovulatory dose of human chorionic gonadotrophin (HCG) was administered. When the lead follicle diameter of 20 mm was reached, 10 000 IU i.m. of HCG was injected, and therapy with leuprolide acetate and gonadotrophins was discontinued. Embryo transfer took place 72 h after oocyte retrieval. Luteal support was provided by progesterone in oil 25 mg i.m. every 12 h. Eleven days following uterine embryo transfer, patients underwent measurement of serum β -HCG concentration. If β -HCG was positive (≥ 3 IU/ml), patients were interviewed by telephone and enrolled. Patients returned 2 days later and underwent repeated measurement of serum β -HCG. At this time (day 13 after embryo transfer), additional blood samples were obtained, salivary samples were collected and the study questionnaire was completed. This series of tests was repeated weekly for two additional weeks. Follow-up ultrasound was performed and pregnancy outcome information obtained. In summary, 40 subjects were tested at 13 days, 27 at 20 days and 13 at 27 days after embryo transfer, and then followed through delivery. An adverse pregnancy outcome was defined as a loss before or immediately after fetal cardiac activity was observed, or a 'vanishing' embryo/fetus was identified before 20 weeks, including the loss of one embryo/fetus of a twin or triplet pregnancy. Those patients that were found to have a singleton, twin or triplet pregnancy who did not experience a loss were classified as 'no adverse outcome'. A biochemical pregnancy was defined as an initial serum HCG concentration ≥ 3 IU/l followed by subsequent serum HCG concentrations that eventually dropped, and a lack of confirmation of a clinical pregnancy by transvaginal ultrasound or histology. The study was approved by the Institutional Review Board of Northwestern University.

Anxiety measures

Two measures of anxiety were used to determine the levels of state anxiety during the study; the State-Trait Anxiety Inventory (STAI; Spielberger *et al.*, 1983) and the Pregnancy Anxiety Scale (PAS; Levin, 1991).

The STAI contains two, 20-item scales that assess anxiety, both current (state) and general (trait). Items are rated on a four-point scale with higher scores indicative of higher levels of anxiety. The scales are widely used and have been demonstrated to be reliable and valid (Spielberger *et al.*, 1983).

The PAS is a 10-item, multiple-choice questionnaire composed of questions specifically designed to assess anxiety related to pregnancy. An example of an item is, 'I read something that frightened me about being pregnant'. Responses were scored on a four-point scale, from 'strongly agree' to 'strongly disagree'. This measure was used to assess pregnancy-specific anxiety among previously infertile women and to determine if PAS scores were correlated with the STAI.

Perceptions of miscarriage

A question to assess the cognitive appraisal of risk of miscarriage was administered. Subjects were asked what they believed the 'average woman's' chances of a miscarriage would be on a scale of 0–100%. They were then asked what they believed their own chances for miscarriage would be on a scale of 0–100%. The difference between these two scores was used as an index of the woman's apprehension regarding miscarriage, with higher scores indicating the woman believed she had a higher than average chance of miscarrying.

Physiological measures

Amylase

Saliva was collected by use of an absorbent pad that was placed in the mouth for 1 min. The pad was then placed in the Salivette device

(Sarstedt Inc., Newton, NC, USA) and then kept in the freezer (-20°C) for between 0.5 and 16 h. After transport of the device to the laboratory, it was centrifuged to separate out the saliva. The saliva was then stored at -20°C for 1–2 months before assay. The amylase assay was conducted with materials supplied by Sigma (St Louis, MO, USA) as described previously (Chatterton *et al.*, 1996). The intra- and interassay coefficients of variation (CV) were 8.2 and 8.5%, respectively.

Cortisol

Saliva collected and stored as described above was analysed by a direct radioimmunoassay method as described previously (Chatterton *et al.*, 1996). The intra- and interassay CV were 12 and 14%, respectively.

Progesterone

Serum progesterone was assayed by a direct radioimmunoassay developed in this laboratory and described in detail previously (Chatterton *et al.*, 1991). The intra- and interassay CV were 8.5 and 10%, respectively.

Allopregnanolone

Serum allopregnanolone was assayed as described by Purdy *et al.* (1990a) with antiserum supplied by R.H. Purdy (LaJolla, CA, USA), [^3H]allopregnanolone supplied by New England Nuclear (Boston, MA, USA), and the pure reference material from Steraloids (Wilton, NH, USA). The intra-assay CV was 12% (all samples done in one assay).

Human chorionic gonadotrophin

Total serum β -HCG was analysed using a chemiluminescent immunoassay system (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA). The inter-assay CV was 8.5% (all done in different assays).

Prolactin

Prolactin (PRL) was assayed with materials obtained from the National Hormone and Pituitary Program, NIADDK. The reference and iodination materials were hPRL-RP-1 and hPRL-I-6, respectively. Iodination and radioimmunoassay were conducted according to the recommendation of the supplier. The intra-assay CV was 14% (all samples done in one assay).

Statistics

Statistical analyses were performed using SPSS Version 6.1.1 (SPSS Inc., Chicago, IL, USA). Comparisons between groups with respect to dichotomous variables were made using χ^2 tests. Fisher's exact test was used when the sample size was small. Pearson's multiple correlations were calculated among the variables. Comparisons with respect to continuous variables were undertaken using two-tailed *t*-tests and Mann-Whitney *U* analysis for data not normally distributed. *P* values < 0.05 were considered significant.

Results

All 40 subjects completed the first sampling period, 27 the second, and 13 the last period. Because of the relatively high attrition rate after the first sampling period, valid statistical comparisons were limited to the first and possibly to the second sampling period.

Effect of infertility diagnosis and age on outcome

There was no difference in duration of infertility, number of IVF cycles, nulliparity or mean age between patients experiencing and not experiencing an adverse pregnancy out-

Table I. Demographic information^a

	No adverse outcome (<i>n</i> = 22)	Adverse outcome (<i>n</i> = 18)
Age (years)	31.3 ± 7.58	35.2 ± 3.26
Years of infertility	2.9 ± 1.72	3.05 ± 1.67
No. of IVF cycles	1.54 ± 0.73	1.61 ± 0.6
Nulligravid (%)	59.1	66.6

There were no significant differences between groups.

^aValues are mean ± SD.

IVF, in-vitro fertilization.

Table II. Distribution of patients (%) by infertility diagnosis

Infertility diagnosis ^a	No adverse outcome (<i>n</i> = 22)	Adverse outcome (<i>n</i> = 18)
Male factor	32	11
Tubal factor	36	22
Endometriosis	5	11
Ovulation dysfunction	23	28
Uterine abnormality	9	22
Unexplained	9	22

^aSome patients were included in more than one category.

Table III. Distribution of pregnancy outcome among in-vitro fertilization (IVF) patients

Pregnancy outcome	No. of patients
<i>Adverse outcome</i>	
Biochemical pregnancy	6
Miscarriage (<6 weeks)	5
Miscarriage (10–20 weeks)	2
Vanishing triplet to twin	3
Vanishing triplet to singleton	2
Total	18
<i>No adverse outcome</i>	
Singleton	15
Twin	6
Triplet	1
Total	22

come (Table I). There was no difference in infertility diagnosis (Table II). The mean (± SD) number of embryos transferred did not differ statistically between patients experiencing an adverse outcome (4.9 ± 1.4) and those that did not (4.3 ± 1.1). Pregnancy outcome is summarized in Table III. The initial serum HCG measurement was made on day 11 following embryo transfer to confirm pregnancy. Day 11 serum HCG concentrations did not differ statistically between patients experiencing an adverse outcome (median: 23.6 IU/l, quartiles: 4.9–66.2) and those that did not (median: 55.5 IU/l, quartiles: 25.2–71.8). When patients presented 2 days later for the study (day 13 after embryo transfer) serum HCG concentrations differed significantly between those experiencing an adverse pregnancy outcome (median: 37.0 IU/l, quartiles: 9.22–160.0) and those that did not (median: 131.0 IU/l, quartiles: 80.7–212.5) ($P = 0.02$).

Relationship of psychological state to outcome and to physiological measures

Scores on the STAI were not significantly different between the adverse and non-adverse outcome groups (Table IV). Progesterone, cortisol and prolactin concentrations were also not significantly related to STAI scores (data not shown).

PAS scores also were not significantly related to outcome and had a low correlation ($r = 0.04$, $P > 0.05$) with the STAI scores, indicating little overlap in the constructs assessed by these measures. The PAS scores generally were less well correlated with the physiological measures.

A moderately high correlation was found between the subjects' estimation of the average chances of miscarriage and their own chances ($r = 0.64$, $P < 0.001$), indicating that the subjects had a reasonably positive attitude about their own pregnancies and did not greatly overestimate their chances of miscarriage.

Relationship of physiological measures to pregnancy outcome

The concentrations of serum progesterone were <50% as great in women with an adverse outcome as in those with continuing pregnancies at 13 days, despite all patients continuing to receive the same dose of supplemental progesterone (Table IV). Serum progesterone and allopregnanolone concentrations were highly correlated ($r = 0.83$, $P < 0.001$). Serum allopregnanolone concentrations were much higher than those reported in women during the menstrual cycle (Purdy *et al.*, 1990a). The ratio of allopregnanolone to progesterone during the menstrual cycle was 0.1, according to Purdy *et al.* (1990a), whereas the ratio was close to 1.0 in the pregnant women.

Serum prolactin concentrations (50.6 ± 4.0 ng/ml in all subjects), salivary cortisol concentrations (3.0 ± 0.2 ng/ml in all subjects) and salivary amylase (25.6 ± 3.7 U/ml in all subjects) did not differ between the outcome groups.

Discussion

Some studies have identified an inverse relationship between psychological stress and pregnancy outcome. Galletly *et al.* (1996) demonstrated that decreased stress, increased self-esteem and decreased depression were correlated with an improved fertility rate among obese, infertile women. Facchinetti *et al.* (1997) assessed the vulnerability to stress among women who subsequently became pregnant and those who did not. Although the sample size was small (16 pregnant, 33 not pregnant), those patients achieving pregnancy demonstrated less changes in systolic blood pressure, diastolic blood pressure and heart rate during stress testing in early pregnancy than those who did not achieve a pregnancy. Demyttenaere *et al.* (1994) found that women with subtle cyclic disturbances (luteal phase defects, endometriosis) have similar concentrations of cortisol compared with those with normal cycles, but did have an increase in serum prolactin concentrations. For these women there was also a stronger association between state anxiety and a negative IVF outcome.

Other studies have not confirmed the causative association between stress and infertility. Patients undergoing IVF certainly have a greater degree of anxiety than patients without infertility

Table IV. Psychological and physiological measures of stress and anxiety^a

	No adverse outcome	Adverse outcome	P-value	95% CI
Allopregnanolone ^b	63.63 ± 41.86 (n = 21)	40.48 ± 18.64 (n = 18)	0.011	(1.491, 44.80)
STAI ^b	42.15 ± 9.01 (n = 20)	45.0 ± 9.62 (n = 18)	NS	
Prolactin ^b	45.86 ± 26.2 (n = 21)	43.59 ± 27.73 (n = 18)	NS	
Progesterone ^b	77.94 ± 88.47 (n = 21)	36.81 ± 28.73 (n = 18)	0.011	(-3.06, 85.32)
Amylase ^b	23.4 ± 22.9 (n = 21)	15.95 ± 14.9 (n = 18)	0.097	(-5.294, 20.27)
Cortisol ^b	2.69 ± 1.24 (n = 20)	3.12 ± 1.66 (n = 17)	NS	

^aValues are mean ± SD.

^bPerformed 13 days after uterine embryo transfer.

STAI, State-Trait Anxiety Inventory (Spielberger *et al.*, 1983).

problems. Harlow *et al.* (1996) identified elevated concentrations of cortisol and prolactin, and higher levels of state anxiety in women undergoing IVF as compared with surgical control patients undergoing diagnostic laparoscopy. However, there was no difference in state or trait anxiety between the IVF patients who became pregnant versus those failing to achieve pregnancy. McMahon *et al.* (1997) found that IVF patients with trait anxiety scores comparable with controls had increased state anxiety even into the 30th week of gestation.

In the current study, there was no difference in self-reported anxiety between women that went on to experience an adverse pregnancy outcome and those that did not. In general, women not experiencing an adverse outcome had an apparently slightly lower level of state anxiety (as measured on the STAI) than did the adverse outcome group, but these differences were not significant. Women in both groups reported levels of anxiety consistent with those of IVF patients documented by others (Reading *et al.*, 1989; Harlow *et al.*, 1996), but higher than those of community norms (Knight *et al.*, 1983). Therefore, while these subjects are experiencing high levels of state anxiety, the anxiety does not appear to differentiate between those who will have an adverse pregnancy outcome and those who will not. Physiological markers of stress also were not correlated with pregnancy outcome. Salivary amylase, prolactin and cortisol concentrations were similar between groups.

We found plasma progesterone concentrations in the women with an adverse outcome were 50% lower than in women with continuing pregnancies as all received the same dose of supplemental progesterone. This difference may be due to much lower endogenous production of progesterone in women who miscarry. Alternatively, it may be that the administered progesterone was absorbed more slowly or metabolized more rapidly in the patients with low serum concentrations of progesterone.

Serum allopregnanolone concentration was also related to the outcome of pregnancy. Because of its high correlation with progesterone, the contribution of allopregnanolone cannot be considered an independently regulated factor. Nevertheless, the much higher values associated with uncomplicated, ongoing pregnancy may have biological significance for the pregnant woman since the analgesic and mood-altering effects of proges-

terone are exerted primarily through this metabolite (Purdy *et al.*, 1990b). The high values achieved in the women who maintained their pregnancies had no apparent deleterious effect, but the apparently lower anxiety reported on the STAI may be a result of the effects of this metabolite. In a similar fashion, lower concentrations of luteal phase allopregnanolone may contribute to anxiety, tension and depression in women with premenstrual syndrome (Rapkin *et al.*, 1997).

On the basis of our findings, it can be concluded that women with a positive serum β -HCG concentration following IVF often report very high levels of anxiety and stress. However, there is no correlation between these psychological scores and physiological stress hormone concentration. Finally, it does not appear that high levels of anxiety and stress result in an adverse pregnancy outcome.

Acknowledgements

This project was supported by a grant from the Kroch Center for Twin Studies (Chicago, IL, USA).

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Received on December 24, 1997; accepted on May 5, 1998