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Stress and stress-related hormones during in-vitro fertilization treatment

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Whether stress and infertility are linked as cause or consequence is unclear, and there is no consensus on the most appropriate methods for measuring stress in infertile women. To address this question, we measured changes in biochemical and questionnaire-based assessments of stress in infertile women. Median baseline, follicular phase and pre-operative serum prolactin (229, 311 and 457 mIU/l) cortisol (278, 369 and 496 nmol/l) and state anxiety score (38, 40 and 49) respectively all increased during stimulated in-vitro fertilization (IVF) treatment. There was no such increase in a control group having similar laparoscopic surgery unrelated to infertility, or in women having unstimulated IVF without laparoscopy, suggesting that anxiety levels are greatest in stimulated IVF, increase as a result of the treatment, and are adequately reflected by state anxiety scores. Baseline serum prolactin in unstimulated IVF (384 mIU/l) was significantly higher than control (177 mIU/l), although this was not reflected in serum cortisol or state anxiety score. Trait anxiety was constant within and between groups, suggesting that stress is not contributing greatly to the infertility. Women who achieved a pregnancy had similar state anxiety scores to those who failed, suggesting that the degree of anxiety observed during IVF treatment is unlikely to influence the chance of pregnancy. There was a trend towards lower trait anxiety in women who became pregnant, but the numbers were small.

Key words: anxiety/cortisol/IVF/prolactin/stress

Introduction

It is well recognized that stress and infertility are linked. However, the causal or consequential nature of this link remains unclear. In a review, Edelmann and Connolly (1986) were unable to confirm that there were psychological causes of infertility. They concluded that infertility clearly has psychological consequences for some couples, although the underlying mechanisms are poorly understood. Golombok (1992) felt that the impact of fertility on psychological function was complex and subject to a variety of factors.

Serum prolactin, a known stress marker (Pepperell, 1981), is commonly elevated in infertile women (Robyn *et al.*, 1981). Hyperprolactinaemia affects gonadotrophin secretion (Moult *et al.*, 1982; Sauder *et al.*, 1984) or may directly affect the ovary (McNatty *et al.*, 1974; McNeilly *et al.*, 1982). Glucocorticoid secretion, the classic adaptive response mechanism to stress (Selye, 1939), may also affect fertility (Peyser *et al.*, 1973; Moberg, 1987) by actions on gonadotrophin secretion (Suter and Schwartz, 1985) or directly on the ovary (Schoonmaker and Erickson, 1983; Harlow *et al.*, 1987). Beneficial effects of glucocorticoid treatment on follicular development and clinical pregnancy rate have been reported (Kemeter and Feichtinger, 1986; Polak de Fried *et al.*, 1993). However, it is not clear whether the effects on follicular development are due to a reduction in endogenous glucocorticoids or to a direct effect of the synthetic glucocorticoid on the ovary. Furthermore, not all studies have been able to demonstrate these effects (Lee *et al.*, 1994).

In addition to hormonal markers, behavioural features of stress can be measured by a variety of questionnaires (see Harrison, 1990), of which the State-Trait Anxiety Inventory (STAI; Spielberger, 1983) is particularly suitable, being a relatively 'pure' assessment of anxiety. Anxiety is regarded as the main psychological problem faced by infertile couples (Golombok, 1992). Reading *et al.* (1989) observed high state ('of the moment') anxiety in women at 6 weeks gestation after assisted conception methods compared with natural conception. In another study, it was found that infertile women had higher state anxiety than fertile women, and that trait (i.e. relating to underlying emotional background) anxiety was higher in women with luteal phase insufficiency compared with those whose infertility was due to other causes (Pesch *et al.*, 1989). However, as highlighted in their review, Edelman and Connolly (1986) pointed out that most studies relating infertility to stress were without adequate controls and the measured stress could not readily be dissociated from the effects of infertility treatment itself.

Therefore, to investigate further the links between stress and infertility, we have measured the changes in serum prolactin, serum and urinary cortisol, and STAI scores during the treatment cycle of women undergoing in-vitro fertilization (IVF) compared with a control group of women having similar gynaecological surgery unrelated to infertility. The first part of the study examined whether there was a relationship between hormonal markers and STAI scores. Having established this link, the second part of the study followed the stress levels in a larger group of women undergoing IVF treatment to see whether there was a relationship between stress and treatment outcome.

Materials and methods

Subjects

Women attending the gynaecology and reproductive medicine clinics at St Michael's Hospital, Bristol, UK, were recruited for the study, which was approved by the Ethical Committee of the local Health Authority. In part 1 of the study involving hormonal measurements (see below) three groups were studied. The control group included women undergoing laparoscopy for sterilization (n = 24). The second group (unstimulated IVF) included women having IVF during an unstimulated cycle, unperturbed by gonadotrophin treatment, and with oocyte recovery undertaken transvaginally with ultrasound guidance (n = 25). The third group (stimulated IVF) comprised women having diagnostic laparoscopy combined with oocyte collection for IVF during gonadotrophin-stimulated cycles (n = 26). Part 2, comprising just a questionnaire study, was on women having diagnostic laparoscopy following gonadotrophin stimulation of their ovaries, after pituitary desensitization with a gonadotrophin-releasing hormone agonist (n = 95). In all cases, only a single cycle of gonadotrophin treatment was included in the study.

Study design

In part 1, all three groups completed a STAI questionnaire (Spielberger, 1983), collected 24-h urine samples and had a peripheral blood sample collected on three separate occasions (except the control group which only had two samples collected): (i) baseline sample at initial consultation; (ii) a follicular phase sample during the early follicular phase of the treatment cycle, between days 2 and 4, in the two IVF groups and (iii) a pre-operative sample on the day prior to surgery in the control group, or on the day the dominant follicle reached 15 mm diameter in the unstimulated IVF group, or on the day of human chorionic gonadotrophin injection in the stimulated IVF group. All blood samples were collected between 0800 and 1200 h.

The median interval from consultation to surgery in the three groups was 9.0 (range 3–15), 6.0 (3–13) and 6.0 (3–26) weeks respectively, and was not significantly different between groups (Mann–Whitney U-test).

Urine sample volume was noted, and an aliquot of urine and the corresponding blood sample were centrifuged immediately. The serum and sediment-free urine were stored at –20°C until required for assay.

In part 2, to assess whether anxiety affects the chance of pregnancy, a larger group of women having stimulated IVF were given only STAI questionnaires on the three occasions listed above. An additional luteal phase questionnaire was given 5 days after embryo transfer to those women who achieved successful fertilization.

Hormone assays

Cortisol was extracted from urine samples with dichloromethane and reconstituted in cortisol-free serum prior to assay. Serum and extracted urine samples were assayed using DELFIA fluoroimmunoassay kits, donated by Wallac Oy (Turku, Finland). The range of the assay was 30–1600 nmol/l. Interassay precision, expressed as the coefficient of variation of two pools of serum, was 5.6% (mean 568.6 nmol/l) and 6.7% (mean 929.8 nmol/l). This assay had a significant cross-reaction with cortisone (37%).

Serum prolactin was measured using DELFIA fluoroimmunoassay kits (Wallac UK Ltd, Milton Keynes, Bucks, UK). The range of the assay was 9–9000 mIU/l. Interassay precision of two serum pools was 8.2% (mean 236 mIU/l) and 10.6% (mean 955 mIU/l).

Statistical analysis

Hormone data and anxiety questionnaire scores were compared by one-sample Wilcoxon signed rank test, Mann–Whitney U-test or Mood median test as appropriate.

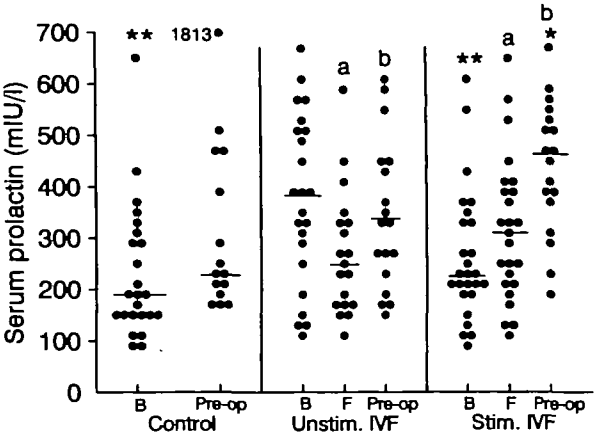

Figure 1. Serum prolactin concentrations in control, unstimulated (Unstim.) and gonadotrophin-stimulated (Stim.) IVF cycles. Individual values are shown, with median values represented by a horizontal bar. Samples were collected at consultation (baseline: B), during the follicular phase of treatment (F) and 1–2 days pre-operatively (Pre-op). $^{*}P < 0.05$ stimulated versus control and unstimulated IVF cycles; $^{**}P < 0.01$ control and stimulated versus unstimulated IVF cycles (Mann–Whitney U-test). $^{a}P < 0.05$ follicular phase versus baseline; $^{b}P < 0.05$ pre-operative versus follicular phase (Wilcoxon one-sample).

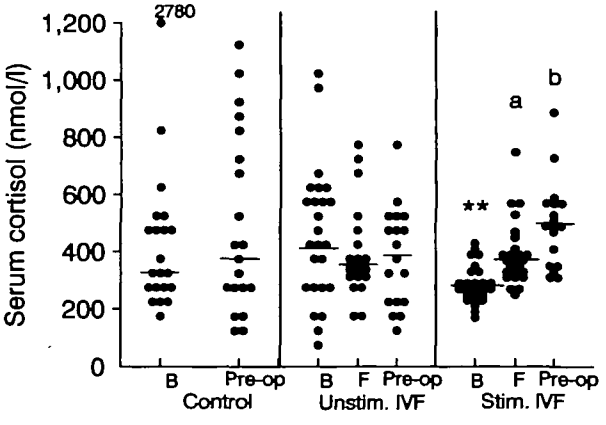

Figure 2. Serum cortisol concentrations in control, unstimulated and gonadotrophin-stimulated IVF cycles. Individual values are shown, with median values represented by a horizontal bar. Samples were collected at consultation, during the follicular phase of treatment and 1–2 days pre-operatively. For abbreviations, see Figure 1. $^{**}P < 0.01$ stimulated versus unstimulated IVF cycles (Mann–Whitney). $^{a}P < 0.05$ follicular phase versus baseline; $^{b}P < 0.05$ pre-operative versus follicular phase (Wilcoxon one-sample).

Results

Serum hormone concentrations

Serum prolactin concentrations are presented in Figure 1. In the control group, median concentrations were 186 mIU/l in the baseline sample, and rose to 238 mIU/l in the pre-operative sample, although this difference was not significant. By comparison, the median baseline concentration was significantly higher ($P < 0.01$) in the unstimulated cycle IVF group (384 mIU/l). Within the unstimulated IVF group, the concentration was significantly lower ($P < 0.05$) in the follicular phase sample (247 mIU/l), and increased in the pre-operative sample (338 mIU/l). In the stimulated IVF group, the median baseline

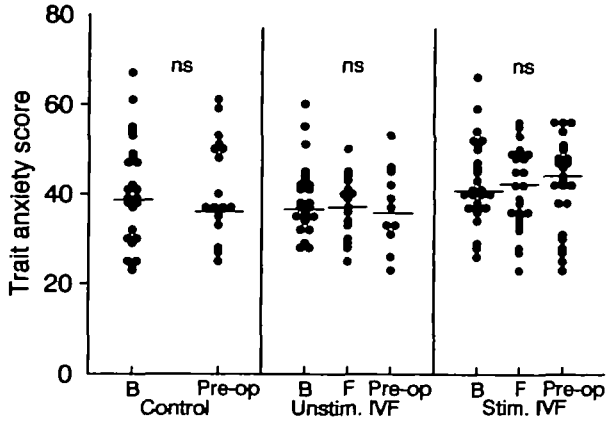

Figure 3. Trait anxiety scores in control, unstimulated and gonadotrophin-stimulated IVF cycles. Individual values are shown, with median values represented by a horizontal bar. Samples were collected at consultation, during the follicular phase of treatment and 1–2 days pre-operatively. For abbreviations, see Figure 1. ns = not significant between and within groups.

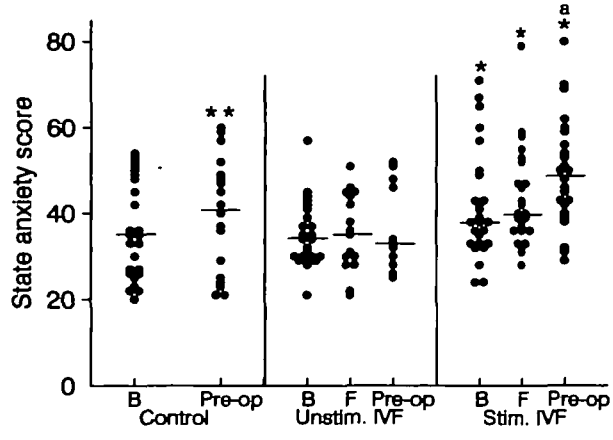

Figure 4. State anxiety scores in control, unstimulated and gonadotrophin-stimulated IVF cycles. Individual values are shown, with median values represented by a horizontal bar. Samples were collected at consultation, during the follicular phase of treatment and 1–2 days pre-operatively. $*P < 0.05$ stimulated versus unstimulated IVF cycles; $**P < 0.05$ control versus stimulated IVF cycles (Mann–Whitney U-test). $^{a}P < 0.01$ pre-operative versus baseline or follicular phase (Wilcoxon one-sample).

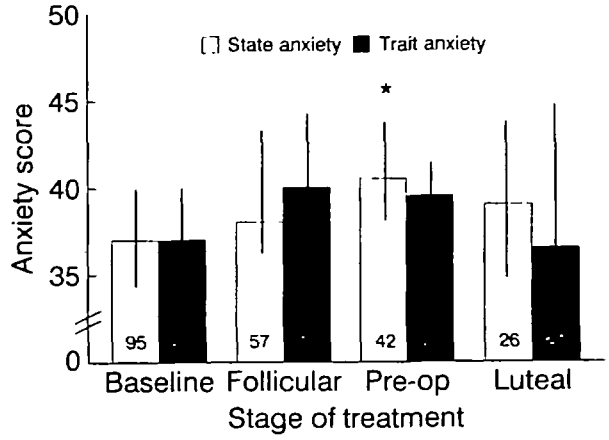

Figure 5. State and trait anxiety in 95 cycles of stimulated in-vitro fertilization treatment. The values represent median ± 95% confidence interval. Values at the base of each bar are the numbers of observations. $*P < 0.05$ pre-operative (Pre-op) versus baseline.

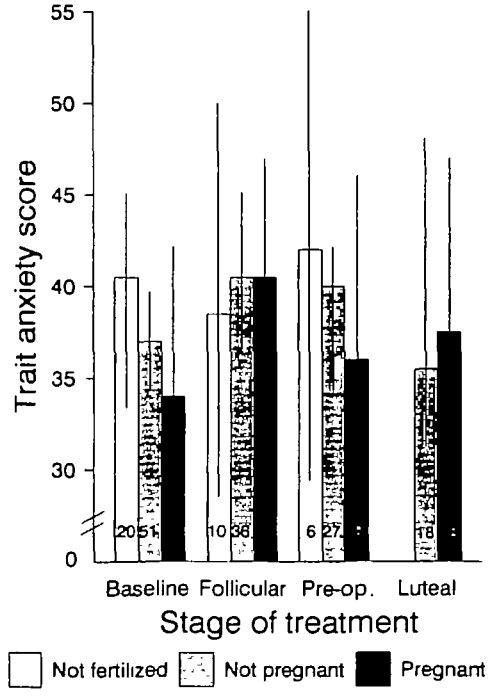

Figure 6. Trait anxiety scores according to pregnancy outcome. The values represent median ± 95% confidence interval. Values at the base of each bar are the numbers of observations. Luteal phase samples were not collected in cycles which were cancelled or where the oocytes failed to fertilize. Pre-op = pre-operative.

concentration (210 mIU/l) was similar to that in the control group, but significantly lower ($P < 0.01$) than in the unstimulated IVF group, and within the stimulated IVF group the concentration increased significantly ($P < 0.05$) in both the follicular phase (320 mIU/l) and pre-operative (460 mIU/l) samples.

Serum cortisol concentrations are shown in Figure 2. In the control group, median concentrations did not increase significantly in the pre-operative sample, and were no different from those in the unstimulated cycle IVF group. In the stimulated IVF group, the median baseline concentration (278 nmol/l) was significantly lower ($P < 0.01$) than in the unstimulated IVF group, and increased both in the follicular phase (369 nmol/l) and pre-operative samples (496 nmol/l).

Median 24 h urinary cortisol concentrations ranged between 115 and 165 nmol/24 h, and were not significantly different between or within groups.

State and trait anxiety scores

Median state and trait anxiety scores for part 1 of the study are shown in Figures 3 and 4. Trait anxiety was similar between and within groups. Median values were between 37 and 43.5 and were not significantly different (Figure 3).

State anxiety was significantly higher ($P < 0.05$) in the stimulated compared with the unstimulated group at all three time-points (38 versus 34, 40 versus 35 and 49 versus 33) (Figure 4). State anxiety also increased significantly

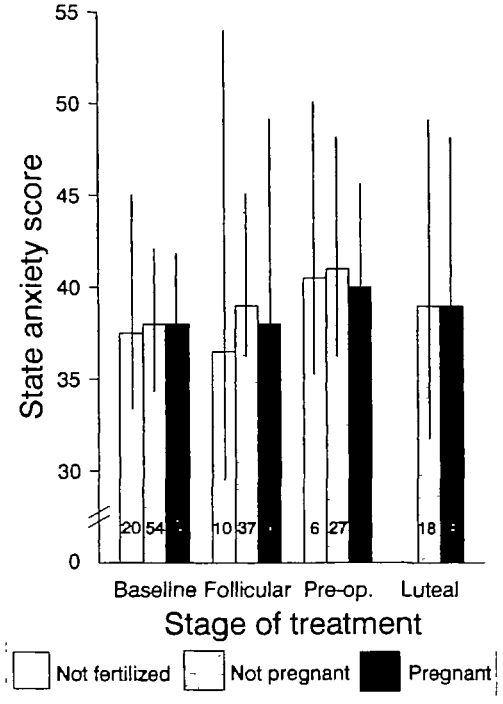

Figure 7. State anxiety scores according to pregnancy outcome. The values represent median ± 95% confidence interval. Values at the base of each bar are the numbers of observations. Luteal phase samples were not collected in cycles which were cancelled or where the oocytes failed to fertilize. Pre-op = pre-operative.

($P < 0.01$) during treatment in the stimulated IVF group (38 versus 40 versus 49).

State and trait anxiety scores for all women in part 2 of the study are shown in Figure 5. There was a high drop-out rate during the study, reflected in the reduction in numbers at the later stages of treatment. To account for this, comparisons within categories were made using the Wilcoxon one-sample test for paired samples. Median trait anxiety was lowest at consultation (baseline) and during the luteal phase (37 and 36.5 respectively) and highest during the follicular phase and pre-operatively (40 and 39 respectively), although these differences were not significant. State anxiety was also lowest at the baseline point (37) and was significantly higher ($P < 0.05$) at the pre-operative point (40.5). The score fell slightly in the luteal phase (39), although this was not significant.

Trait anxiety scores according to outcome are shown in Figure 6. Median baseline (37 and 34) and pre-operative (40 and 36) trait anxiety appeared to be higher in women who failed to become pregnant compared with those who became pregnant, but these differences were not significant, and numbers were few. Trait anxiety did not differ during treatment in any of the groups (Mood median test).

State anxiety scores are shown in Figure 7. Although there appeared to be a rise in state anxiety levels up to the pre-operative time-point, this was not sustained in the luteal phase and the changes were not significantly different (Mood median test). During the follicular and pre-operative phases, state anxiety appeared to be higher in women who failed to become pregnant compared with those who achieved a pregnancy (39 versus 38 and 41 versus 40 respectively), but again these differences were not significant.

Discussion

Our results show for the first time in a controlled study that women undergoing gonadotrophin-stimulated IVF treatment have higher state anxiety and serum cortisol and prolactin concentrations than women undergoing comparable laparoscopic surgery for reasons not associated with infertility treatment. Women having non-laparoscopic IVF during an unstimulated cycle were intermediate. We have also shown that trait anxiety is not higher in infertile women compared with controls, suggesting that anxiety *per se* is not contributing to their infertility.

O'Moore *et al.* (1983) demonstrated a higher state anxiety in infertile women compared with fertile controls which has been attributed to a reduction in coping mechanisms in infertile women (Pesch *et al.*, 1989). However, state anxiety was not measured during IVF treatment cycles. In the only study relating state anxiety to the stage of treatment, highest levels were found at the time of oocyte retrieval and embryo transfer (Johnston *et al.*, 1987), although there was no control group or biochemical measurement of stress.

Higher prolactin and urinary cortisol concentrations have been demonstrated in infertile women compared with controls (O'Moore *et al.*, 1983), and stress and state anxiety were also higher in women conceiving after IVF compared with natural conception (Reading *et al.*, 1989). We have shown that state anxiety, serum cortisol and serum prolactin increase during gonadotrophin-stimulated IVF treatment. This extends previous studies (Harper *et al.*, 1985).

Serum prolactin concentrations rose during unstimulated cycle IVF treatment, in contrast to the findings of McNeilly and Chard (1974), who found no consistent changes in cycling women. Lenton *et al.* (1982) demonstrated a slight rise in serum prolactin during the follicular phase in non-conception cycles. In the latter study, prolactin concentrations did rise consistently in the follicular phase of conception cycles in women treated with clomiphene citrate, which was attributed to the raised oestrogen concentrations (Lenton *et al.*, 1982). In our study, prolactin concentrations also rose significantly during the follicular phase of gonadotrophin-stimulated cycles. This rise in serum prolactin during gonadotrophin stimulation is similar to the transient oestrogen-mediated hyperprolactinaemia reported by others (Healy and Burger, 1983; Kauppila *et al.*, 1988; Hoffman *et al.*, 1989). However, in the light of the increases in serum cortisol and state anxiety observed in this group, it seems likely that stress may also be a causative factor in the prolactin rise. Harper *et al.* (1985) showed a positive correlation between state anxiety and serum prolactin in women attending an infertility clinic, which supports this argument. Furthermore, although raised follicular phase prolactin was correlated with peak oestradiol concentrations during gonadotrophin stimulation, Hoffman *et al.* (1989) found a high incidence of hyperprolactinaemia in basal (cycle day 3) samples, which they argued might be a consequence of stress.

Urinary cortisol did not appear to be a sensitive marker of stress in our study, and the concentrations did not show the same differences as those seen in serum cortisol, either during treatment or between groups. O'Moore *et al.* (1983) also failed to find a difference in 24 h urinary cortisol concentrations between controls and women with unexplained infertility.

The 35% rise in serum cortisol during the follicular phase of stimulated cycles is similar to the rise observed during the normal menstrual cycle (Gennazani *et al.*, 1975). It is therefore possible that the rise in cortisol observed in the present study is oestrogen-mediated, although the 80% increase between baseline and pre-operative samples suggests that stress may also be involved. We do not know whether free cortisol is affected, but since cortisol-binding globulin increases in response to gonadotrophin treatment (Andersen *et al.*, 1992), it is difficult to predict the effect on free cortisol. Whilst the rise in serum cortisol during gonadotrophin-stimulated IVF may be due to an increase in stress during treatment, it is not known whether this rise in cortisol has any effect on ovarian function. Fateh *et al.* (1989) demonstrated higher cortisol concentrations in follicles from which the oocytes failed to fertilize, suggesting a possible inhibitory effect of cortisol on fertilization. This may be mediated by effects on steroidogenesis, as indicated by the observations of Michael *et al.* (1993a), who showed inhibitory effects of cortisol on luteinizing hormone-stimulated granulosa cell pregnenolone production *in vitro*. However, the concentration of intrafollicular cortisol may be regulated by ovarian 11β-hydroxysteroid dehydrogenase, which inactivates cortisol to cortisone, and was found to be absent in women who became pregnant during gonadotrophin-stimulated IVF (Michael *et al.*, 1993b). Furthermore, baseline cortisol concentrations were higher, and there were blunted cortisol responses to provoked stress in infertile women compared with fertile controls (Lindheim *et al.*, 1995).

Trait anxiety is indicative of underlying stress and, if raised in women with infertility, would suggest a causative effect. Women with unexplained infertility (O'Moore *et al.*, 1983; Harrison *et al.*, 1986) and luteal phase insufficiency (Pesch *et al.*, 1989) had higher trait anxiety than fertile controls. In contrast, we found no increase in trait anxiety in infertile women, nor did Reading *et al.* (1989) in women conceiving after IVF or gamete intra-Fallopian transfer (GIFT), nor Modell *et al.* (1990) in women with polycystic ovarian disease. There were insufficient subjects in the present study to compare the results according to diagnostic classification. There was a trend towards lower trait anxiety in women who became pregnant, but the numbers were too small to be statistically conclusive. Further studies with a larger group of women are required to identify the interactions between stress, cause of infertility and pregnancy outcome.

If stress were reducing fertility during IVF treatment, one would expect state anxiety to be higher in women who failed to become pregnant. This was not the case in the present study and, although the number of pregnancies was too small to draw firm conclusions, our results support the notion that the amount of stress experienced during gonadotrophin-stimulated IVF treatment is insufficient to reduce the overall chance of conception. This does not rule out, however, the possibility that conception during unstimulated IVF, in which only a single follicle develops, could be jeopardized by the level of stress and associated hormonal changes.

In conclusion, we have demonstrated an increase in state anxiety in women undergoing gonadotrophin-stimulated IVF treatment, in parallel with increases in serum prolactin and cortisol, which indicate that state anxiety may reflect underlying changes in biochemical markers of stress. There is no evidence that stress predisposes women to infertility, since trait anxiety levels were similar in all groups. Our results suggest that despite increases during treatment, the amount of stress experienced by women having gonadotrophin-stimulated IVF does not significantly reduce their chance of achieving a pregnancy.

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