Pregnancy-specific anxiety, ART conception and infant temperament at 4 months post-partum

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STUDY QUESTION: Is anxiety focused on the pregnancy outcome, known to be particularly salient in women conceiving through assisted reproductive technology (ART), related to difficult infant temperament?

SUMMARY ANSWER: While trait anxiety predicts infant temperament, pregnancy-focused anxiety is not associated with more difficult infant temperament.

WHAT IS KNOWN ALREADY: A large body of research has provided convincing evidence that fetal exposure to maternal anxiety and stress in pregnancy has adverse consequences for child neurodevelopmental, behavioural and cognitive development, and that pregnancy-specific anxiety (concerns related to the pregnancy outcome and birth) may be of particular significance. Women conceiving through ART are of particular interest in this regard. Research over more than 20 years has consistently demonstrated that while they do not differ from spontaneously conceiving (SC) women with respect to general (state and trait) anxiety, they typically report higher pregnancy-specific anxiety. While research suggests normal behavioural and developmental outcomes for children conceived through ART, there is some evidence of more unsettled infant behaviour during the first post-natal year.

STUDY DESIGN, SIZE, DURATION: The longitudinal cohort design followed 562 nulliparous women over a 7-month period, during the third trimester of pregnancy and at 4 months after birth.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Approximately equal numbers of nulliparous women conceiving through ART (n = 250) and spontaneously (SC: n = 262) were recruited through ART clinics and nearby hospitals in Melbourne and Sydney, Australia. Participants completed three anxiety measures (state, trait, pregnancy specific) at time 1 in the third trimester of pregnancy and a measure of infant temperament at time 2, 4 months after birth. At time 1, relevant socio-demographic, pregnancy (maternal age, smoking, alcohol, medications, medical complications) information was recorded and at time 2, information regarding childbirth (gestation, infant birthweight, mode of delivery) and post-natal (concurrent mood) variables was recorded and controlled for in analyses.

MAIN RESULTS AND THE ROLE OF CHANCE: In the third trimester of pregnancy, women conceiving through ART reported lower state and trait anxiety, but higher pregnancy-focused anxiety than their SC counterparts (all Ps < 0.05). Hierarchical regression analyses including mode of conception, all anxiety variables and relevant covariates indicated that while trait anxiety in pregnancy predicted more difficult infant temperament (P < 0.001), pregnancy specific and state anxiety did not. Mode of conception predicted infant temperament; with ART women reporting less difficult infant temperament (P < 0.001) than their SC counterparts.

LIMITATIONS, REASONS FOR CAUTION: The major limitations in the study are the reliance on a self-report measure of infant temperament and the fact that the study did not assess quality of caregiving which may moderate the effect of pregnancy anxiety on infant temperament.

WIDER IMPLICATIONS OF THE FINDINGS: This study is the first to our knowledge to prospectively examine the impact of gestational stress (pregnancy anxiety) on infant temperament in women conceiving through ART. Findings confirm existing research indicating that trait anxiety in pregnancy is associated with difficult infant temperament and suggest that pregnancy-specific anxiety (measured in the third trimester) is not implicated. These findings are reassuring for women conceiving through ART whose pregnancies may be characterized by particularly intense concerns about the wellbeing of a long sought after baby.

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Introduction

A substantial body of research indicates that maternal exposure to psychosocial and/or biological stress during gestation is associated with adverse neurodevelopmental, temperamental and behavioural outcomes in offspring (see Sandman and Davis, 2012 for a recent review). While mechanisms are not yet fully understood, fetal programming in utero is proposed, with maternal glucocorticoids and/or compromised placental blood flow believed to impact on the developing fetal hypothalamic–pituitary–adrenal axis and central nervous system. Offspring vary in vulnerability with most not affected, suggesting a gene–environment interaction model is most likely whereby changes to the prenatal environment interact with genetic factors in defining the phenotype at birth (Glover, 2011).

The phenotype of interest in the current study is infant temperament, an infant’s characteristic ways of responding emotionally and behaviourally, stable across contexts. Three broad factors have been consistently identified by researchers: surgency (approach to novelty), negative affectivity/soothability and effortful control or persistence (Rothbart and Hwang, 2002). A ‘difficultness’ construct conceptualized as negative mood, withdrawal from novel situations and low adaptability has been of particular interest given the challenges such temperaments present to parents and also evidence suggesting dysregulated temperaments may be a precursor to emotional and behavioural disorders in later childhood (Putnam et al., 2002). While temperament is believed to have physiological and/or genetic origins, it is acknowledged that caregiving behaviour may have an influence and, for parent report measures, parent perceptions also need to be considered.

Pregnancy and perinatal events have also been linked to unsettled temperament in the first post-natal year (Hughes et al., 2002). The fetal programming hypothesis proposes that the uterine environment, particularly exposure to elevated maternal cortisol, may predispose infants to difficult temperament. Prospective studies using laboratory measures of infant temperament have shown self-reported anxiety symptoms in the third trimester of pregnancy to be associated with heightened negative reactivity to novel stimuli in 4-month-old infants (Davis et al., 2004), and anxiety in the second trimester to greater fearfulness in a laboratory task in 14–18-month-old infants (Bergman et al., 2007). A clinical diagnosis of anxiety disorder in pregnancy has been associated with heightened cry reactivity in 4-month-old infants (Werner et al., 2007), while no main effect of pregnancy anxiety diagnosis was found in another study (Grant et al., 2010). Studies using maternal reports of infant temperament have linked high scores on questionnaire measures of general anxiety in the third trimester of pregnancy with parent ratings of more difficult temperament in infancy (Austin et al., 2005).

There is growing evidence that assessment of general anxiety may underestimate specific and intense anxiety and fears related to pregnancy (Huizink et al., 2004; Blair et al., 2011; Buss et al., 2011) and that pregnancy-specific anxiety may be a better predictor of child outcomes (Buss et al., 2011). Pregnancy-specific anxiety in early pregnancy has been linked to infant attention regulation at 3 and 8 months (Huizink et al., 2002); and, in a further follow-up of this sample, to restless disruptive temperament at 27 months (Gutteling et al., 2005). More recent evidence indicates pregnancy specific, but not state anxiety, assessed between 13 and 17 weeks gestation is associated with negative affectivity in offspring (Blair et al., 2011).

Given concern about the adverse impact of gestational stress in general, and pregnancy-focused anxiety more specifically, it seems surprising that (to our knowledge) prospective studies have not yet been conducted in pregnant women with elevated medical risk. Women conceiving through assisted reproductive technology (ART) are of particular interest in this regard. Qualitative studies suggest that from the moment of conception their pregnancy experience is emotionally intense, with feelings of elation at achieving a pregnancy juxtaposed with deep concerns about the survival and wellbeing of the unborn baby (McMahon et al., 1999). Quantitative studies over two decades have consistently shown that compared with those conceiving spontaneously, women conceiving through ART report comparable symptoms of depression, state and trait anxiety, but higher anxiety focused on the outcome of the pregnancy (Hammarberg et al., 2008). Reviews indicate, however, that children conceived through ART, show comparable neurodevelopmental (Middelburg et al., 2008) and behavioural outcomes (Golombok, 2003) compared with spontaneously conceived children.

In contrast, some studies focused on the first post-natal year suggest infants conceived through ART may have more difficult and/or dysregulated temperaments compared with those conceived spontaneously (Hammarberg et al., 2009). Interestingly, however, the mothers in this study also indicated that they subjectively experienced their infants as less ‘difficult’ than the average baby. A series of studies confirming relatively high admission rates of women conceiving through ART to residential programmes which assist parents with unsettled infant behaviour (Hammarberg et al., 2009; Fisher et al., 2012) is indirectly suggestive of early infant temperament difficulties.

In this paper, we report on prospective associations between anxiety in pregnancy and infant temperament at 4 months in a large sample of pregnant women where approximately half conceived using ART. We first aimed to explore the differential impact of various types of anxiety. Given a possible genetic contribution, we expected that maternal trait anxiety would be associated with more difficult infant temperament. We also expected to replicate previous findings linking higher state anxiety in the third trimester of pregnancy with more difficult temperament. We further predicted that
Pregnancy-focused anxiety may explain additional variance since this measure taps into anxiety that may not be captured by other measures.

Our second aim was to examine whether factors indicative of obstetrically higher risk pregnancies (e.g. older maternal age, ART conception) would moderate any association between anxiety and infant temperament. Given equivocal empirical findings to date, we also compared temperament in infants conceived through ART and those conceived spontaneously. In testing hypotheses, we controlled for a wide range of demographic, pregnancy, perinatal and post-natal variables associated with pregnancy after ART conception, which might influence child temperament.

Materials and Methods

Participants and recruitment

Women in this study were part of a large prospective study (Parental Age and Transition to Parenthood Australia, PATPA) that examined the impact of maternal age and mode of conception on psychological adjustment during the transition to parenthood. After receiving approval from all relevant institutional ethics committees nulliparous women in the third trimester of pregnancy were approached to participate at seven ART clinics in two large Australian cities (ART group) and also at private and public hospital antenatal clinics and clinics in the geographic vicinity of the ART clinics [spontaneously conceiving (SC) group]. Inclusion criteria were as follows: nulliparous and able to speak English sufficiently well to complete study materials. Given the larger study focus on maternal age and mode of conception, we recruited consecutive cohorts of approximately equal numbers of women conceiving through ART and spontaneously equally distributed across three age-groups: 20–30 years; 31–36 years; and 37 years or older.

Measures

Predictor variables: prenatal anxiety

General anxiety: trait (time 1) and state (time 1 and time 2). The Spielberger State-Trait Anxiety Inventory (STAI: Spielberger et al., 1983) was administered in the third trimester of pregnancy and the state scale re-administered at the post-natal follow-up. The STAI comprises 20 items that evaluate general (trait) and current (state) feelings of tension, anxiety and nervousness. Internal consistency was high (Cronbach’s alphas: A-Trait 0.90; A-State 0.91 pregnancy, 0.82 post-natal, respectively). Scores of >40 have been shown to indicate clinically significant anxiety in pregnant women (Grant et al., 2008).

Pregnancy-focused anxiety (time 1). The five-item ‘Anxiety concerning Health and Defects in the Child’ scale from the Baby Schema questionnaire (Gloger-Tippelt, 1983) was used to assess pregnancy-focused anxiety (PFA). The scale has good reliability (Cronbach’s alpha = 0.89 in the current study) and has been shown in previous reports to have high face validity and to discriminate between women conceiving through ART and those conceiving spontaneously (McManus et al., 1997, 2011). Items referring explicitly to concerns about the unborn baby, e.g. ‘I keep worrying about whether my child will be physically or mentally handicapped after birth’; ‘Sometimes I am afraid I will lose this child’ are rated on a six-point scale ranging (strongly disagree) to (strongly agree); higher scores indicate more anxiety.

Dependent variable

Short temperament scale for infants (time 2). This 30-item questionnaire (Sanson et al., 1985) assesses temperament in infants aged 4–8 months and has been extensively validated in Australian infants. Items are behaviourally specific, refer to how typically an infant responds in a particular way, and are rated by the parent on a Likert scale 1 (almost never) to 6 (almost always). A composite difficulty score (EDS) is derived by averaging scores from the approach (infant’s response to novel places, people, e.g. ‘For the first few minutes in a new place or situation the baby is fretful’); co-operation (infant behaviour during caregiving, e.g. ‘The baby continues to fret during nappy change despite efforts to distract him/her’); and irritability (negative affectivity and crying, e.g. ‘The baby continues to cry despite several minutes of soothing’) factors (18 items, Cronbach’s alpha = 0.79 in the current study). Higher scores indicate a more difficult temperament. This composite score has been validated in relation to observed behaviour (Allen and Prior, 1995) and was the dependent variable in the current study.

Control variables

Study design variables and demographics. We considered gestational weeks when mothers completed the anxiety assessments in pregnancy, child age when mothers completed the temperament ratings, maternal age at pregnancy assessment, educational level of the mother dichotomized as tertiary (university/post-school education versus no tertiary education) and marital status (married/de facto versus single), collected at time 1.

Biomedical variables in pregnancy. We developed a composite dichotomous variable to represent presence of gestational complications (one or more of hypertension, diabetes, bleeding during pregnancy: yes/no, time 1), and also considered use of prescription and/or non-prescription medications (yes/no, time 1). Because numbers reporting smoking and drinking alcohol during pregnancy were very low, these variables were also coded dichotomously (no smoking versus smoking; non-drinkers versus having at least one drink per week). Since there is some evidence that depression in pregnancy may also influence the developing fetus (Davis et al., 2004), we also assessed depression symptoms in pregnancy using the 10-item Edinburgh Post-natal Depression Scale (EPDS) (Cox et al., 1987), validated for use during pregnancy (Murray and Cox, 1990). The scale was repeated at 4 months post-partum and demonstrated good reliability (Cronbach’s alphas 0.86; 0.87 for the pregnancy and post-natal assessments, respectively).

Perinatal variables. Information regarding perinatal and post-natal variables was collected at the post-natal contact 4 months after birth. The following variables all coded dichotomously (yes/no) were considered: neonatal intensive care admission, elective Caesarean section and intervention during childbirth related to fetal distress (instrumental vaginal delivery, unplanned Caesarean section). We also generated a ratio variable for birthweight (grams) over gestational age (weeks) to index intrauterine growth restriction.

Post-natal variables. Post-natal variables were sex, breastfeeding for at least 2 months (yes/no) and concurrent mood at 4 months post-partum (depression, state anxiety symptoms). Questionnaires (EPDS and A-State) described above and used in pregnancy were repeated concurrently with child temperament ratings.

Procedure

In the third trimester of pregnancy and at 4 months post-partum mothers participated in a structured telephone interview (demographic data and
pregnancy health at the pregnancy interview; birth outcomes, infant sex and breastfeeding at the post-natal interview) and also completed questionnaires (maternal anxiety and depression pregnancy and post-natal, infant temperament post-natal). The questionnaire was delivered online for the majority, while a small number completed paper and pencil and returned by post.

Statistical analysis

Analyses were conducted using SPSS Version 19 (IBM corporation, Sydney). Data were inspected for missing values. Less than 5% of questionnaire items were missing; values were imputed using mean substitution when fewer than 10% of items were missing, with the exception of pregnancy-focused anxiety (cronbach’s alpha = 0.89) where values were imputed in cases where one of the five items was missing. The dependent variable, infant temperament (EDS difficulty score) was normally distributed. Preliminary univariate analyses (t-tests comparing means, χ² analyses for categorical variables) provided descriptive statistics on key study variables according to mode of conception (see Table I). Correlational analyses for categorical variables) provided descriptive statistics on key study variables according to mode of conception (see Table I). Correlational analyses (Pearson’s correlation) were used to identify variables (demographic, biomedical, perinatal and post-natal variables) correlated with the dependent variable for inclusion in multivariate analyses to test the study hypotheses. Hierarchical linear regression analyses were conducted to test study hypotheses by examining the relative contributions of the three pregnancy anxiety variables first and then entering the control variables. To test whether age and mode of conception moderated relations between pregnancy anxiety and temperament, relevant interaction effects were also tested. For all analyses, probability values \( P \leq 0.05 \) were considered significant.

Results

Sample details and attrition analyses

Six hundred and nineteen women gave consent and also completed pregnancy assessments; 27 were excluded as they received infertility treatment that did not involve oocyte retrieval and embryo transfer. Participation rates were 53.8 and 46.2% for women conceiving with ART and spontaneously, respectively. In total, 545 eligible women (92%) completed all post-natal assessments, 94% of ART and 91% of SC participants. Women who did not complete the post-natal follow-up were younger and less likely to have tertiary education (P-values <0.001). They did not differ on the key predictor variables in pregnancy: state, trait or pregnancy-focused anxiety. Given potential genetic contributions to infant temperament we excluded nine women because the mother was not genetically related to the child (used donor eggs or embryos to conceive), and also excluded 24 women because of multiple pregnancies, as twin pregnancies differ physiologically in many ways from singleton pregnancies. The sample for analyses thus comprised 512 women (250 ART; 262 SC). See Table I for details of the sample and descriptive statistics for the study variables, presented according to mode of conception and for the sample overall. In summary, as typical in volunteer samples, participants were socio-economically advantaged: most were partnered (97%) had a tertiary education (68%) were in professional occupations (77%).

Preliminary analyses

Relations among anxiety measures

State and trait anxiety were strongly correlated \( (r = 0.76) \), while pregnancy-focused anxiety was only modestly associated with state \( (r = 0.30) \) and trait anxiety \( (r = 0.29) \), respectively, all \( P \)-values <0.001.

Relations between potential covariates and dependent variable (EDS)

The following variables were significantly correlated with EDS and were therefore included in multivariate analyses: marital status (single), maternal education (non-tertiary); use of non-prescription medications in pregnancy; smoking in pregnancy; depression symptoms (pregnancy and 4 months post-partum); state anxiety at 4 months post-partum, all \( P \)-values <0.05. All other correlations were non-significant (see Table II).

Mode of conception comparisons

χ² analyses indicated that women conceiving through ART were more likely than those conceiving spontaneously to report gestational complications in pregnancy, elective Caesarean section and babies admitted to neonatal intensive care. T-Tests indicated that they were older and reported lower depression scores in pregnancy. With regard to pregnancy anxiety variables, women conceiving through ART reported significantly lower scores for state and trait anxiety, but significantly higher scores for pregnancy-focused anxiety. Finally, women conceiving through ART reported significantly lower scores for the dependent variable EDS: difficult temperament score, all \( P \)-values <0.05 (see Table I).

Multivariate analyses

In order to test our study hypotheses, we built a hierarchical regression model with the following order of entry: Step 1: predictor variables (state and trait anxiety in pregnancy, pregnancy-focused anxiety); Step 2: pregnancy, perinatal and demographic covariates that showed marginal \( (P < 0.10) \) or significant associations with the dependent variable at the univariate level, as well as maternal age and mode of conception. In Step 3, we included the interaction product terms for both maternal age and mode of conception and the three anxiety measures (see Table II).

Step 1 showed that while state and trait anxiety were correlated with the EDS score at a univariate level, pregnancy-focused anxiety was not. Only trait anxiety significantly predicted EDS in multivariate analyses that included all anxiety measures, with higher maternal trait anxiety associated with more difficult infant temperament. In Step 2, the following variables were significantly correlated with higher infant EDS score (more difficult infant temperament): spontaneous conception, tertiary education, use of non-prescription medicines in pregnancy and higher state anxiety at 4 months post-natal. At Step 3, none of the interaction effects were significant. Trait anxiety remained significant in all models. The final model (including interaction effects) accounted for 21% of the variance in EDS (difficult temperament score).

Discussion

This study sought to examine associations between various measures of anxiety in the third trimester of pregnancy and maternal reports of child temperament difficulty 4 months after birth. In a novel contribution, we examined these relations in women conceiving spontaneously and with ART. The study focused in particular on the potential contribution of pregnancy-focused anxiety, known to be salient in women
conceiving with ART. Findings indicated that pregnancy-focused anxiety assessed in the third trimester of pregnancy was not associated with more difficult infant temperament. While state anxiety was associated with infant temperament at a univariate level, when all three of the anxiety measures were considered together, only trait anxiety predicted difficult temperament. Further, trait anxiety remained significant after controlling for post-natal mood and pregnancy health, perinatal and demographic variables.

Mode of conception predicted infant temperament after controlling for all other variables, with mothers conceiving through ART reporting

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sample (n = 512)</th>
<th>SC (n = 262)</th>
<th>ART (n = 250)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic</td>
<td>M (SD)</td>
<td>M (SD)</td>
<td>M (SD)</td>
</tr>
<tr>
<td>Maternal age (years)**</td>
<td>33.65 (4.37)</td>
<td>32.20 (4.46)</td>
<td>35.10 (4.28)</td>
</tr>
<tr>
<td>Gestational age (weeks) pregnancy assessment</td>
<td>31.68 (2.42)</td>
<td>31.59 (2.39)</td>
<td>31.78 (2.46)</td>
</tr>
<tr>
<td>Baby age (weeks) post-natal assessment</td>
<td>18.84 (2.26)</td>
<td>18.78 (2.27)</td>
<td>18.90 (2.26)</td>
</tr>
<tr>
<td>Have tertiary education</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Partnered</td>
<td>355 (68.10)</td>
<td>183 (68.82)</td>
<td>172 (66.44)</td>
</tr>
<tr>
<td>Professional occupation</td>
<td>496 (96.87)</td>
<td>255 (97.32)</td>
<td>241 (96.40)</td>
</tr>
<tr>
<td>Bio-medical variables pregnancy**</td>
<td>389 (76.80)</td>
<td>194 (73.81)</td>
<td>195 (78.60)</td>
</tr>
<tr>
<td>Had gestational complications*</td>
<td>160 (31.25)</td>
<td>72 (27.48)</td>
<td>88 (35.20)</td>
</tr>
<tr>
<td>Smoked cigarettes</td>
<td>14 (27.34)</td>
<td>10 (3.81)</td>
<td>4 (1.61)</td>
</tr>
<tr>
<td>Used prescription medications</td>
<td>85 (16.60)</td>
<td>44 (16.79)</td>
<td>41 (16.41)</td>
</tr>
<tr>
<td>Used non-prescription medications</td>
<td>240 (46.87)</td>
<td>113 (43.13)</td>
<td>127 (50.10)</td>
</tr>
<tr>
<td>Mood variables (pregnancy)</td>
<td>M (SD)</td>
<td>M (SD)</td>
<td>M (SD)</td>
</tr>
<tr>
<td>A-state score**</td>
<td>31.50 (8.50)</td>
<td>32.52 (9.62)</td>
<td>30.49 (7.39)</td>
</tr>
<tr>
<td>A-trait score*</td>
<td>33.67 (8.28)</td>
<td>34.51 (8.55)</td>
<td>32.83 (8.02)</td>
</tr>
<tr>
<td>Pregnancy-focused anxiety score*</td>
<td>18.59 (6.21)</td>
<td>17.91 (6.08)</td>
<td>19.27 (6.35)</td>
</tr>
<tr>
<td>Depression symptoms (EPDS score)**</td>
<td>5.12 (4.28)</td>
<td>5.73 (4.54)</td>
<td>4.51 (4.03)</td>
</tr>
<tr>
<td>Perinatal variables</td>
<td>39.40 (1.66)</td>
<td>39.70 (1.52)</td>
<td>39.20 (1.80)</td>
</tr>
<tr>
<td>Birthweight (g)**</td>
<td>3380 (504)</td>
<td>3443 (449)</td>
<td>3317 (559)</td>
</tr>
<tr>
<td>Neonatal intensive care*</td>
<td>92 (18.00)</td>
<td>37 (14.12)</td>
<td>55 (22.00)</td>
</tr>
<tr>
<td>Elective Caesarean section*</td>
<td>69 (13.47)</td>
<td>25 (9.54)</td>
<td>44 (17.60)</td>
</tr>
<tr>
<td>Emergency Caesarean section</td>
<td>141 (27.54)</td>
<td>73 (27.6)</td>
<td>68 (27.21)</td>
</tr>
<tr>
<td>Instrumental delivery</td>
<td>112 (21.87)</td>
<td>65 (24.81)</td>
<td>47 (18.81)</td>
</tr>
<tr>
<td>Post-natal variables (4 months)</td>
<td>M (SD)</td>
<td>M (SD)</td>
<td>M (SD)</td>
</tr>
<tr>
<td>Depression symptoms (EPDS score)</td>
<td>5.15 (4.14)</td>
<td>5.40 (4.20)</td>
<td>4.91 (4.08)</td>
</tr>
<tr>
<td>Anxiety symptoms (A-state score)</td>
<td>30.93 (8.46)</td>
<td>31.06 (8.42)</td>
<td>30.80 (8.50)</td>
</tr>
<tr>
<td>Child temperament (difficulty EDSb score)**</td>
<td>2.38 (0.55)</td>
<td>2.46 (0.54)</td>
<td>2.30 (0.56)</td>
</tr>
<tr>
<td>Activity</td>
<td>3.99 (0.81)</td>
<td>4.01 (0.81)</td>
<td>3.97 (0.82)</td>
</tr>
<tr>
<td>Approach*</td>
<td>1.97 (0.66)</td>
<td>2.04 (0.68)</td>
<td>1.91 (0.64)</td>
</tr>
<tr>
<td>Co-operation</td>
<td>2.30 (0.62)</td>
<td>2.35 (0.66)</td>
<td>2.25 (0.59)</td>
</tr>
<tr>
<td>Irritability**</td>
<td>2.86 (0.87)</td>
<td>2.98 (0.87)</td>
<td>2.74 (0.86)</td>
</tr>
<tr>
<td>Rhythmicity</td>
<td>2.68 (0.83)</td>
<td>2.69 (0.83)</td>
<td>2.67 (0.83)</td>
</tr>
<tr>
<td>Child sex (male)</td>
<td>270 (52.70)</td>
<td>139 (53.72)</td>
<td>131 (52.43)</td>
</tr>
<tr>
<td>Breastfed at least 2 months</td>
<td>432 (84.37)</td>
<td>223 (85.11)</td>
<td>209 (83.60)</td>
</tr>
</tbody>
</table>

*No participants reported drinking alcohol, three did not answer the question.
**EPDS, Edinburgh post-natal depression scale; EDS, temperament difficulty score comprised of approach, co-operation, irritability, higher scores indicate more difficulty; mean in community sample Australian infants = 2.50.
ART and SC groups differ P < 0.05.
**ART and SC groups differ P < 0.01.
Bold values indicate modes of conception differences.
### Table II: Summary statistics for hierarchical regression analysis predicting difficult temperament score (EDS).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Zero-order correlation with (EDS)</th>
<th>Model I (Base model with only anxiety measures) (n = 509)</th>
<th>Model II (Base model + covariates) (n = 501)</th>
<th>Model II (Base model + covariates + interactions) (n = 501)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R²Δ = 0.067***</td>
<td>R²Δ = 0.13***</td>
<td>R²Δ = 0.011</td>
</tr>
<tr>
<td>Anxiety variables</td>
<td></td>
<td>B</td>
<td>S² (%)</td>
<td>B</td>
</tr>
<tr>
<td>A-Trait score (third trimester, trait anxiety)</td>
<td>0.251***</td>
<td>0.230***</td>
<td>2.2</td>
<td>0.138*</td>
</tr>
<tr>
<td>A-State score (third trimester, state anxiety)</td>
<td>0.209***</td>
<td>0.051</td>
<td>0.23</td>
<td>0.032</td>
</tr>
<tr>
<td>Pregnancy-focused anxiety score</td>
<td>0.031</td>
<td>-0.051</td>
<td>0.11</td>
<td>0.044</td>
</tr>
<tr>
<td>Pregnancy-focused anxiety score (third trimester, PFA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covariates a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age (at pregnancy assessment)</td>
<td>-0.039</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode of conception (0 = spontaneous conception, 1 = ART)</td>
<td>-0.142***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status (0 = has partner, 1 = single)</td>
<td>0.118*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiary education (0 = no, 1 = yes)</td>
<td>-0.199***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of non-prescription med in pregnancy (0 = no, 1 = yes)</td>
<td>0.100*</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Smoking during pregnancy (0 = no, 1 = yes)</td>
<td>-0.086*</td>
<td></td>
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<tr>
<td>Edinburgh depression scale score (3rd trimester)</td>
<td>0.176***</td>
<td></td>
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<tr>
<td>Edinburgh post-natal depression scale score (4 months post-natal)</td>
<td>0.278***</td>
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<tr>
<td>Spielberger state anxiety score (4 months post-natal)</td>
<td>0.322***</td>
<td></td>
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<td>Interactions b</td>
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<tr>
<td>Overall model significance</td>
<td>R² = 0.067***</td>
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*Only significant covariates entered into model. Sample size varies due to missing data.

Interactions could be specified as follows: maternal age × trait anxiety; maternal age × state anxiety; maternal age × pregnancy-specific anxiety; mode of conception × trait anxiety; mode of conception × state anxiety; mode of conception × pregnancy-specific anxiety.

**P < 0.01.

***P < 0.001.
infants with a less difficult temperament. Since pregnancy-focused anxiety may be particularly salient in older women and those conceiv-
ing through ART, we postulated that mode of conception and mater-
nal age might moderate associations with infant temperament, but no 
significant interactions were found.

Findings are consistent with previous research linking maternal trait 
anxiety in the third trimester of pregnancy with infant temperament 
traits involving heightened negative affectivity (Davis et al., 2004; 
Austin et al., 2005; Bergman et al., 2008). Links between maternal 
trait anxiety and difficult temperament suggest a possible genetic 
effect (Jorm et al., 2000). This interpretation is further supported by 
our finding that situational anxiety measures (state anxiety, pregnancy-
focused anxiety) were not predictive of infant temperament when trait 
anxiety was included in the model. While biased maternal reporting 
with more trait-anxious mothers reporting more negativity in their 
infants is also possible (Vaughn et al., 1981), the fact that we con-
trolled for concurrent state anxiety and depression symptoms makes 
this interpretation less likely. In terms of the fetal programming 
model, a significant predictive effect for trait anxiety could potentially 
be attributable to genetic sensitization (Oniszczenko and Dragan, 
2005) and/or the infant being exposed to anxiety for a longer time 
during gestation. The intensity and duration of antenatal stress as 
well as inadequate maternal coping mechanisms have been identified 
as important in other research (Van den Bergh et al., 2005). Trait 
anxiety measures assess general ways of responding and are presumed 
to be stable and enduring while state and pregnancy-focused anxiety 
reflects only current feelings.

Consistent with previous research (Huizink et al., 2004; Buss et al., 
2011) pregnancy-focused anxiety in this large sample was only mod-
estly correlated with state and trait anxiety indicating that the 
measure was tapping into a separate anxiety dimension. However, 
null findings for pregnancy-focused anxiety contrast with some previ-
ous reports (Huizink et al., 2002; Gutteling et al., 2005; Blair et al., 
2011). Comparisons across studies are constrained, however, due 
to between-study differences in timing of assessment of anxiety and 
child temperament and unique features of our sample (a large 
number of older mothers and mothers conceiving through ART). 
Effects of gestational stress have been reported at various stages in 
pregnancy and different mechanisms are likely to operate at different 
stages. Longitudinal studies suggest pregnancy anxiety is most intense 
in the first and third trimesters (Figueirido and Conde, 2011). Al-
though recent studies have reported a significant post-natal impact 
of pregnancy-specific anxiety assessed between 13 and 17 weeks, 
Buss et al. (2011) reported overall stability for pregnancy-focused 
anxiety and a review of previous studies reported the strongest post-
natal effects for pregnancy-focused anxiety at 27–28 weeks (Van den 
Bergh et al., 2005), not markedly different from the timing of assess-
ment in the current study (30–32 weeks). One study of women con-
ceiving through ART using a retrospective design identified gestational 
stress between 31 and 40 weeks as having the most significant asso-
ciation with various child behavioural outcomes in middle childhood 
(Rice et al., 2010).

Studies of women pregnant after using ART have reported anxiety 
is highest in the first trimester (Hjelmstedt et al., 2003), but also that 
this anxiety decreases as the pregnancy progresses, a trend also noted 
in studies of SC women (Buss et al., 2011). Our measure of 
pregnancy-specific anxiety focused explicitly on concerns about the 
survival and wellbeing of the unborn infant and these concerns may at-
tenuate somewhat by the third trimester, when a live birth is more 
likely. It seems reasonable to speculate, however, that the fetuses of 
mothers conceiving through ART may have been exposed to higher 
pregnancy-specific anxiety earlier in the pregnancy. A longitudinal 
design with measures at different points in pregnancy is needed to 
more fully explore the impact of pregnancy-specific anxiety in 
women conceiving through ART. Although the measure in the 
current study differed from those used in previous studies, the 
measure has previously been shown to discriminate ART and SC 
women and there was a similar focus on fears about the pregnancy 
outcome.

Findings that women conceiving through ART reported less difficult 
infant temperament were somewhat unexpected, although recent 
findings have also shown mothers who conceive through ART more 
likely to report a general impression of their infants as less difficult, 
despite ratings of more dysregulated sleeping and feeding on a tem-
perament measure (Hammarberg et al., 2009). These researchers 
have suggested this discrepancy (greater objective difficulty ratings jux-
tapoed with a lower subjective difficulty rating) may reflect the fact 
that women conceiving through ART are more tolerant regarding 
their child’s temperament in the context of feeling fortunate to 
finally have a child through ART. However, the general impression 
index used in that study was a single question and more subjective 
compared with the ‘difficulty’ composite in the current study, which 
was based on ratings of typical infant behaviour from three scales: ir-
ritability, co-operation (with caretaking activities) and approach (be-
haevioural response in novel situations). In addition, the present 
study is the first to control for a large number of pregnancy and peri-
natal variables which may influence infant temperament and which 
differentiate women conceiving through ART from their SC counterparts 
(birthweight ratio to gestational age, Caesarean section, neonatal in-
tensive care). Further research using observational measures of 
infant temperament is needed, however, to determine the extent to 
which the findings reflect maternal perceptions (and possible positive 
reporting) versus actual infant behaviour.

The multivariate model tested in the current study explained 21% of 
the variance in child temperamental difficulty. Among the confounding 
variables tested, results indicated that having a higher (tertiary) educa-
tion, being single and use of non-prescription medications (common, 
almost 50%) were associated in multivariate analyses with more diffi-
cult child temperament. The finding regarding higher education is un-
expected, but may possibly be related to unrealistic expectations of 
motherhood and/or difficulties adjusting to the lifestyle changes a 
needy baby requires. Findings regarding non-prescription medications 
in pregnancy require further investigation. Also contrary to expect-
ation, smoking was negatively associated with the difficulty score, 
however, there were very few women in the study who smoked. Post-
natal maternal state anxiety was also positively correlated with the 
difficulty score while post-natal depression symptoms were not signifi-
cant when concurrent anxiety was included in the model.

Strengths and limitations

The study had several strengths including the large sample (with equal 
numbers of women conceiving spontaneously and through ART), low 
attrition rates, minimal exclusion criteria and assessment of a large
number of potentially confounding variables. Several limitations need to be acknowledged. The sample was socio-economically low-risk, which, while typical of women conceiving through ART (Hammarberg et al., 2008) limits generalizability. The validity of parent reports of infant temperament has been the subject of extensive debate and research (Lancaster et al., 1989; Rothbart and Hwang, 2002) and, as discussed above, observational measures may be particularly important in the unique assisted conception context. Nonetheless, there is considerable evidence from reviews (Rothbart and Hwang, 2002) that parent report measures have objective validity, and the EDS difficulty score has been previously validated against observed behaviour (Allen and Prior, 1995).

Finally, the current study did not include assessments of caregiving. It is also possible that the link between trait anxiety and child temperament was mediated or moderated by quality of maternal caregiving. Several recent studies have shown that the quality of the post-natal environment can attenuate or exacerbate the effects of prenatal exposure to anxiety or stress (Bergman et al., 2008; Kaplan et al., 2008; Grant et al., 2010) and this represents an exciting direction for future research and intervention.

Conclusions and clinical implications
This study confirms earlier work associating trait anxiety in pregnancy with parent reports of difficult temperament and suggests that pregnancy-specific anxiety (at least as measured in the third trimester) is not associated with difficult temperament in the child. Pregnancy after ART conception is psychologically complex and challenging, with mixed emotions of elation and an understandable fear of pregnancy loss most likely based on a particular reproductive history. Compounding this, ‘worrying about worrying’ and any potential adverse impact that this worry may have on the developing child may be a further psychological burden, and lead to unnecessary maternal guilt (Oates, 2002). The current study findings suggest clinicians could reassure women conceiving after ART that their concerns about the pregnancy outcome are unlikely to adversely impact on their child.

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Authors’ roles
C.A.M. (lead investigator) was involved in designing the study, overall responsibility for the NSW study site and wrote the first draft of the manuscript; J.B. contributed particularly to data analysis, critical discussion and interpretation as well as first draft; F.L.G., K.H., K.W., D.S. and J.F contributed to study design, and critical discussion and draft manuscript. J.F. was responsible for the Victorian study site.

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Conflict of interest
The authors declare there are no conflicts of interest, actual or potential, related to the submitted manuscript.

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