Maternal distress in late pregnancy alters obstetric outcomes and the expression of genes important for placental glucocorticoid signalling.

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Abstract

The experience of maternal distress in pregnancy is often linked with poorer obstetric outcomes for women as well as adverse outcomes for offspring. Alterations in placental glucocorticoid signalling and subsequent increased fetal exposure to cortisol have been suggested to underlie this relationship. In the current study, 121 pregnant women completed the Perceived Stress Scale, State Trait Anxiety Inventory and Edinburgh Postnatal Depression Scale in the third trimester of pregnancy. Placental samples were collected after delivery. Maternal history of psychiatric illness and miscarriage were significant predictors of poorer mental health in pregnancy. Higher anxiety was associated with an increase in women delivering via elective Caesarean Section, and an increase in bottle-feeding. Birth temperature was mildly reduced among infants of women with high levels of depressive symptomology. Babies of mothers who scored high in all stress (cumulative distress) measures had reduced 5-minute Apgar scores. High cumulative distress reduced the expression of placental HSD11B2 mRNA and increased the expression of placental NR3C1 mRNA. These data support a role for prenatal distress as a risk factor for altered obstetric outcomes. The alterations in placental gene expression support a role for altered placental glucocorticoid signalling in the relationship between maternal prenatal distress and adverse outcomes.

Key Words

Prenatal; Stress; Maternal; Pregnancy; Anxiety; Obstetric; Depression; Placenta

1. Introduction

There is now an extensive body of evidence showing that the *in utero* experience is a critical determinant of fetal outcome (Langley-Evans, 2006). One factor that has been extensively studied in this regard is the adverse effect of maternal prenatal distress on birth outcomes (Bussières et al., 2015). This is important, as understanding the relationship between prenatal distress and unfavourable births outcomes may allow for targeted maternal or fetal surveillance in high-risk pregnancies, or timely intervention to decrease the risk of an adverse outcome. The term 'prenatal distress' is often used to collectively refer to negative psychological wellbeing, and encompasses stress, anxiety and depression. The prevalence of prenatal distress is estimated to be 31%, 28% and 12% for stress, anxiety and depression respectively (McDonald et al., 2013). Thus, a significant proportion of women experience clinically significant levels of maternal distress during pregnancy, highlighting the need to study its impact on birth outcomes.

A number of epidemiological studies have shown that fetal exposure to maternal prenatal distress can alter fetal development and increase short and long term disease risk. Prenatal stress and anxiety increases the risk of preterm birth (PTB) and low birth weight (LBW) (Bussières et al., 2015; Ding et al., 2014; Khashan et al., 2009; Khashan et al., 2008). Prenatal depression has been found to increase the risk of operative deliveries (Hu et al., 2015), PTB and LBW (Grote et al., 2010). However, in a more recent meta-analysis, Accortt and colleagues reported significant variability in existing studies (Accortt et al., 2015), highlighting the need for further studies examining the association between prenatal depression and birth outcomes.

While the clinical outcomes have been the subject of intensive investigation, the molecular and biological parallels of these changes in human population is not well known.

Given the well-known effect of stress on glucocorticoid signalling, one proposed hypothesis is that alterations in placental glucocorticoid signalling, leads to overexposure of the fetus to maternal cortisol (Cottrell et al., 2013). This has been proposed as a key biological mechanism underpinning the programming effect of prenatal distress on poor outcomes (Cottrell et al., 2013). In particular, prenatal distress has been shown to alter the expression of three important genes in the placenta; 11-beta hydroxysteroid dehydrogenase type 2 (HSD11B2) (Jensen Pena et al., 2012; O'Donnell et al., 2012; Seth et al., 2015), the glucocorticoid receptor (NR3C1) (Palma-Gudiel et al., 2015) and FK506 binding protein (FKBP5) (Monk et al., 2016). Expression of these genes have been shown to correlate with infant birthweight (Green et al., 2015; Mulligan et al., 2012) and growth restriction (Zhao et al., 2014), suggesting that an altered placental glucocorticoid signalling may play a role in determining newborn outcome. Further alterations in the epigenetic status and expression of HSD11B2 (Appleton et al., 2015), NR3C1 (Sheinkopf et al., 2016), and FKBP5 (Paquette et al., 2014) have been shown to predictive of neurobehavioral problems in infancy.

The primary objective of this study was to evaluate the link between prenatal stress, depression and/or anxiety in late pregnancy on neonatal and obstetric outcomes in pregnant women receiving antenatal care at Cork University Hospital. The secondary goal was to examine whether prenatal stress, depression and/or anxiety in late pregnancy led to any changes in key genes involved in placental glucocorticoid signalling that have previously been linked to unfavourable birth outcomes and poorer neurodevelopment in infancy.

2. Materials and Methods

2.1 Participant recruitment

This study was carried out with full ethical approval from the Research Ethics Committee of Cork Teaching Hospital. Participants attending antenatal care at Cork University Maternity Hospital (CUMH), Cork, Ireland between July 2015 and September 2016 were invited to participate in this study. The inclusion criteria were; 1. 18 years of age or older, 2. English speaking, 3. having a current singleton pregnancy and 4. plans to give birth in in the maternity hospital. The participants were recruited when they were greater than 28 weeks' gestation to time of delivery. Participants were recruited in late pregnancy as this time corresponds to a period with a high prevalence of prenatal distress (Lee et al., 2007). Written informed consent was obtained from all women who agreed to take part and participants were asked to complete a combination of questionnaires to assess maternal distress (Khashan et al., 2014) and donate a small biopsy of their placenta following delivery. Detailed clinical and demographic data were collected from the medical notes of patients once the entire cohort had given birth. This data included information on maternal age, body mass index (BMI), previous obstetric complications, previous psychiatric history, medical conditions, current obstetric complications, birthweight, gender, gestational age, Apgar score, birth temperature, head circumference, neonatal resuscitation (if any), admissions to the neonatal intensive care unit (NICU) and mode of feeding on discharge from the hospital.

2.2 Questionnaires

This study used the 10-item Perceived Stress Scale (PSS). The PSS is a popular tool used to measure psychological stress and how individuals appraise stressful life events (Cohen et al., 1983). Higher Scores on the PSS are indicative of a higher level of perceived stress. In this study, a score of greater than or equal to 20 was used as the cut-off for the 'high stress' group.

Maternal anxiety was measured using the 6-item version of State Trait Anxiety Inventory (STAI). The 6 item STAI is a frequently used brief psychological measure of anxiety and the 6 item version which has been validated for use during pregnancy (Marteau and Bekker, 1992). As there are currently no recommended cut off scores for the STAI during pregnancy, women were deemed anxious if they scored in the top 25% of the cohort. Depressive symptoms were assessed using the Edinburgh Postnatal Depression Scale (EPDS). Consistent with previous studies, we used a score of 13 or greater to indicate a high probability of depression (Cohen et al., 1983; Rubertsson et al., 2011). These self-reported questionnaires were marked by a 4-point Likert Scale. In this study, the Cronbach's alpha of the PSS, STAI and EPDS were 0.867, 0.838 and 0.894 respectively.

2.3 Placental collection

Placental samples were collected within 3 hours of delivery. Placental weight was measured after the cord and membranes had been removed. 5 cross-sectional samples were randomly excised from each placenta to incorporate both the maternal and fetal sides. The samples were washed in dH_2O and immediately stored at -80 °C until further analysis.

2.4 RNA extraction, cDNA synthesis and PCR

RNA was extracted from placental samples using the Trizol method as per the manufacturer's instructions. Briefly, placental sample was homogenised in 3 ml of Trizol reagent and left on ice for 10 min. Samples were centrifuged at 12,000g for 5 min at 4°C and 1 ml of the supernatant was transferred into a new tube. 200µl of chloroform was added, mixed and left at room temperature for 3 min. The sample was centrifuged at 12,000g for 15 min at 4°C. The upper clear aqueous phase was removed and placed in a new 1.5ml tube, 500µl of isopropanol

was added and left at room temperature for 10 min. The samples were centrifuged at 12,000g for 10 min at 4°C. The supernatant was removed and the pellet was washed in 1 ml of 70% ethanol. The samples were again centrifuged, the supernatant was removed and the pellet was left to air dry and re-suspended in 70µl of RNase free H_2O. RNA concentration and quality was assessed using the Nanodrop 8000. 500 ng of placental RNA was reversed transcribed using the high capacity cDNA reverse transcription kit (Applied Biosystems) using the following parameters 25 °C for 10 min; 37 °C for 120 min; 85 °C for 5 min; and held at 4 °C until storage at 80 °C. Real time PCR was performed for HSD11B2, FKBP5, NC3R1 under the following cycling parameters; 50 °C for 2 min; 95 °C for 10 min; 40 repetitions of 95 °C for 15 s and annealing/elongating at 60 °C for 1 min. All samples were run in duplicate, cycle threshold values were recorded and analysis was performed using the $2^{-\Delta\Delta}$cycle threshold method (Livak and Schmittgen, 2002).

2.5 Data analysis

Data was collected and analysed on SPSS version 22 and Graphpad prism 5. Missing values were checked for and any questionnaire response with greater than 3 missing values were removed. Where medical records were unavailable, participants' questionnaire scores were also removed from the cohort. Normality was tested for using the Kolmogorov-Smirnov tests prior to beginning inferential statistics. Birthweight, birthweight centiles and placental weight ratios were normally distributed and were analysed using an unpaired student t-tests or one-way ANOVA with Tukeys post hoc. Placental weight, Apgar scores, birth temperature and head circumference displayed a non-normal distribution, therefore non parametric tests were used. For nominal data, binary logistic regression analysis with 95% confidence intervals was used. Multivariate data were evaluated with ordinal logistic regression analysis. Values of $p <$

0.05 were consider statistically significant. All analyses were adjusted for maternal age, body mass index (BMI) and social class.

In our analysis we grouped mode of delivery into 3 models. In model 1, mode of delivery was grouped as either spontaneous vaginal delivery (SVD) or operative. Model 2 included vaginal delivery versus caesarean section (CS) delivery. Model 3 grouped delivery as SVD, emergency CS (EMCS), elective CS (ELCS), vacuum (VD) or forceps delivery (FD). Additionally newborns were grouped into number of adverse birth outcomes based on six parameters; (a) admission to the NICU (b) newborn resuscitation received (c) delivered before 37 weeks' gestation (d) 5 minute Apgar score ≤ 7 (e) birth temperature $< 36.5°C$ and (f) birth centile ≤ 10 or ≥ 90.

3. Results:

3.1 Descriptive statistics

159 pregnant women were initially recruited into the study and completed the questionnaires. Participants were removed from the study if they were less than 28 weeks' gestation when they completed the questionnaires (13%), had incomplete survey information (3%) or if medical records were unavailable (7.5%) (Fig. 1). The final analysis included 121 participants. The mean age of the participants was 31.75 years (SD = 4.54, range 17 – 41). The average BMI was 26.33 (SD = 4.60, range 18.6 – 38.8). The mean gestational age at which the surveys were completed was 35.55 weeks' (SD 3.541, range 28 -41). The majority of the women in this study where multigravida (67.8%) or primigravida (26.4%). 2.5% of participants had delivered greater than 5 infants and 3.3% had greater than 7 previous deliveries. 23.1% of this population had a history of having one or more miscarriages (*n = 28*). Only 2 participants (1.7%) had a history of ectopic pregnancies and therefore no further analysis was conducted in relation to ectopic pregnancy history. 21.5% of the study population had a history of psychiatric illness (*n = 26*). Depression was the most common psychiatric illness in this population (9.9%, *n=12*), followed by anxiety (7.4%, *n=9*), postnatal depression (5%, *n=6*) and bipolar disorder (0.8%, n=1) (Table 1 & 2).

3.2 Perceived Stress Scale:

The mean PSS score in the entire study cohort was 16.36 ± 0.59 (Supplementary Fig.1 & Table 1). This number is comparable to other studies that have used the 10-item PSS to assess prenatal stress in the third trimester (Liou et al., 2013). High levels of perceived psychological stress was determined by a PSS score of greater than or equal to 20 (33.1%, *n = 40*). Participants with a score of less than or equal to 19 were deemed 'low stress' (66.9%, *n=81*). Women with high levels of stress during pregnancy were 2 times more likely to have a history of psychiatric

illness (OR 2.519; 95% CI=1.036–6.125). This relationship remained significant after controlling for maternal age, BMI and social class (aOR 2.591; 95% CI=1.033 – 6.499). When grouped based on type of psychiatric illness, maternal stress was not associated with depression, anxiety or PND individually (Table 3). Prenatal stress had no effect neonatal outcome (Fig. 2), mode of delivery, gestational age at delivery or infant feeding (Table 3). Additionally scoring on the PSS was related to neonatal resuscitations, admission to the NICU, birth centiles (Supplementary Table 2) or number of neonatal adversities (Supplementary Table 3).

3.3 State Trait Anxiety Inventory:

The mean score in the STAI was 6.17 ± 0.35 (Supplementary Fig.1 & Table 1). As there are currently no guidelines to indicate high anxiety in the perinatal period using the STAI, we defined highly anxious as the top quartile of scores in the cohort (27.3%, *n=33*). Previous depression, anxiety or PND alone was not associated with scoring in the STAI. However, when grouped by any psychiatric illness there was an association with scoring high in the STAI and having a history of psychiatric illness (OR 3.020; 95% CI=1.215–7.509). This relationship remained significant after including maternal age, BMI and social class into the model (aOR3.532; 95% CI=1.322-9.389) (Table 4). A history of miscarriage was associated with a 3 fold increased risk of scoring high in STAI (aOR 3.807; 95% CI=1.384-10.473) (Table 4), indicating previous pregnancy loss as a contributing factor to anxiety during pregnancy. Maternal anxiety was not related to the neonatal outcomes measured (Fig. 3). Women who scored in the high anxiety group were more than twice as likely to have an operative delivery as oppose to SVD (OR 2.443; 95% CI = 1.077-5.545). When analysed in model 3 this rose to a 4 fold increased risk of delivery via ELCS (OR 4.154; 95% CI = 0.359-2.489). The relationship remained significant after including maternal age, BMI and social class into the

model (aOR 5.411; 95% CI = 0.509-2.868) (Table 4). Similarly, participants with high anxiety were 3 times more likely to deliver before 39 weeks' gestation (aOR 3.235; 95% CI 1.182 – 8.855) (Table 4). Women who scored high on the STAI were more likely to bottle feed their infant prior to discharge from the hospital (aOR 2.710; 95% CI = 0.086-1.908) (Table 4).

3.4 Edinburgh Postnatal Depression Scale:

The mean EPDS was 9.07 ± 0.51 (Supplementary Fig.1 & Table 1). An EPDS score greater than or equal to 13 was used to mark participants as being 'highly likely depressed' (25.6%, *n=31*). Concordantly, participants who scored 12 or less were grouped in the 'not likely depressed' category (74.4%, *n = 90*). Participants who scored in the 'highly likely depressed' group were greater than 3 time more likely to have had a history of psychiatric illness (aOR 3.566; 95% CI=1.348-9.434). When stratified based on type of psychiatric illness (depression, stress or anxiety), women with a history of depression were 4 times more likely to be in the 'highly likely depressed' group after adjusting for maternal age, BMI and social class (aOR 4.451; 95% CI=1.198-16.538) (Table 5). Maternal history of miscarriage was associated with a three-fold increased risk of scoring high in the EPDS (aOR 3.878; 95% CI=1.420-10.590) (Table 5). Babies of women who were ranked in the 'highly likely depressed' group on the EPDS had a reduced temperature at birth ($P < 0.05$ *) (Fig. 4D). Maternal depressive symptomology did not affect mode of delivery, gestational age at delivery or infant feeding (Table 5). Additionally scoring on the EPDS was related to neonatal resuscitations, admission to the NICU, birth centiles (Supplementary Table 2) or number of neonatal adversities (Supplementary Table 3).

3.5 Cumulative group

As prenatal stress, anxiety and depression are highly correlated (Supplementary Fig. 2), a new exploratory group was created for participants who scored in the high category on all three questionnaires (14.9%, *n= 18*). Participants in this group were up to 3 times more likely to have a history of miscarriage (aOR = 3.903; 95% CI=1.224-12.446) (Table 6). The risk of having a history of psychiatric illness in this group rose to greater than 6 times (aOR = 6.229; 95% CI = 1.947-19.926). Similar to participants who ranked high in the EPDS, participants in this category were more likely to have a history of depression (aOR = 4.504; 95% CI=1.073-18.897) (Table 6). Infants born to women in the cumulative high stress group had a lower 5 minute Apgar score than other infants. Further, unlike babies from the low group, babies born to mothers in this group did not have a significant increase in their Apgar score from 1 to 5 minutes (Fig. 4E). Maternal cumulative distress had no effect on mode of delivery, gestational age at delivery or infant feeding (Table 6).

3.6 Placental gene expression:

To determine the relationship between prenatal distress and the expression of genes that play crucial roles in placental glucocorticoid signalling, we performed real time PCR on placental samples for three key genes; HSD11B2, FKBP1, NR3C1. Placentae were available for 16 of the high cumulative distress participants and 9 of the low scoring participants. Scoring in the high group was associated with a significant decrease in HSD11B2 ($p = 0.0296$) and an increase in NR3C1 ($p=0.048$) mRNA in the placenta (Fig. 6). Additionally placental NR3C1 was negatively correlated with placental weight ($r^2 = 0.159$; $p=0.025$) and birthweight centiles ($r^2 = 0.412$; $p=0.036$) (Supplementary Table 5). There was no effect of prenatal cumulative distress on the expression of FKBP5 ($p=0.101$) (Fig 6). Placental FKBP5 expression positively

correlated with maternal BMI ($r^2 = 0.185$; $p=0.025$) (Supplementary Table 6). Placental gene expression was not related to infant sex or obstetric outcomes (Supplementary Fig. 3)

4. Discussion:

The present study adds to the existing literature highlighting a negative role for prenatal distress in mediating adverse pregnancy and birth outcomes. Prenatal stress is commonly reported during pregnancy and varies in its definition. Psychological wellbeing encompasses a broad area of health that expands beyond diagnosable mental health disorders such as depression, anxiety and posttraumatic stress disorder. There are a wide range of screening tools used to identify women who may be at risk of stress, anxiety and depression that are commonly used to measure psychological status in pregnancy. The extensive tools available means prenatal stress is commonly defined and assessed in multiple ways, which has led to significant inconsistencies between studies. To characterise psychological distress in this study, we used the PSS, STAI and EPDS. A large meta-analysis demonstrated these questionnaires to have excellent reliability and to currently be the best available tools to measure stress, anxiety and depressive symptomology during pregnancy (Nast et al., 2013).

The current study does not support a strong association between maternal prenatal distress and neonatal outcomes. Although we identified a significant reduction in birth temperature among neonates from women with high depressive symptoms, and reduced new-born temperature is related to increased infant mortality (Soll, 2008), we recommend this result be interpreted with caution as infants of mothers who report depression had an average temperature of 36.43 °C, and infants are only considered clinically hypothermic when temperature drops below 36°C. We also found a reduction in the five minute Apgar score among babies from the high cumulative stress group. Further these babies did not display an increase in Apgar scores from 1 to 5 minutes. A low 5-minute Apgar score is associated with infant mortality (Thorngren-Jerneck and Herbst, 2001) and is predictive of poor neurological function later in life (Lie et al., 2010) (Moster et al., 2001; Seidman et al., 1991).

We found that prenatal anxiety was associated with an increased risk of women delivering via elective Caesarean Section and delivering before 39 weeks' gestation. The reduced gestation observed among these women is likely a reflection of the increased rates of elective CS in this highly anxious group, which are often scheduled for 39 weeks' gestation (Laye and Dellinger, 2006). In line with previous studies (Fairlie et al., 2009), we showed that women with high levels of anxiety in late pregnancy were more likely to bottle feed their infants prior to hospital discharge. This highlights a central role for anxiety in late pregnancy to mediate adverse obstetric outcomes, and may have implications for mode of feeding in the post-partum period.

Fetal over-exposure to maternal cortisol as a mediator of adverse fetal outcomes is well described in the literature (Reynolds, 2013). In the placenta, the fetus is protected from high levels of maternal cortisol by the actions of the enzyme HSD11B2. This enzyme breaks down cortisol into its inactive product cortisone and in doing so prevents fetal overexposure to maternal cortisol. Scientific enquires are now focusing on this enzyme to be a mediator of prenatal stress as depression in the perinatal period have been shown to reduce the expression of this enzyme in the placenta (Togher et al., 2014). Here we demonstrate a significant reduction in the expression of placental HSD11B2 mRNA in women who were in the high cumulative stress group. Additionally we find this group to have elevated levels of the NR3C1 receptor mRNA in the placenta and show a negative correlation between NR3C1 mRNA expression and birthweight. The association between these placental genes with infant neurodevelopment (Appleton et al., 2015), suggests dysregulation of these genes may lie at the core of the relationship between prenatal distress and adverse infant outcomes and highlights the potential to use these genes as potential biomarkers for altered neurodevelopment in infancy. This also suggests that molecular signatures of prenatal stress exposure may be present

in the placenta at birth which suggests that the placenta may be an accessible molecular barometer of the degree of stress exposure during pregnancy.

We identified a maternal history of psychiatric illness or miscarriage as significant predictors of poorer mental health in pregnancy. This is in line with previous studies where women with a history of early pregnancy loss have elevated levels of stress, anxiety and depression in subsequent pregnancies (McCarthy et al., 2015; Woods-Giscombé et al., 2010). Identifying these women and offering support measures to reduce the risk of mood disturbance in their current pregnancy may be beneficial in reducing neonatal and obstetric complications. Of interest prenatal yoga has been shown to enhance positive mood, reduce depressive and anxiety symptoms (Davis et al., 2015), and decrease salivary cortisol and alpha amylase levels (a marker of HPA activity) (Kusaka et al., 2016) in pregnancy. Additionally, a preliminary RCT showed significant improvement in anxiety and depressive symptoms in women receiving an antenatal mindfulness intervention (Woolhouse et al., 2014). Furthermore the 'Centering Pregnancy' trial which promotes *group* prenatal care has been shown to reduce rates of preterm birth and enhance breastfeeding initiation and is widely implemented across the United States (Ickovics et al., 2007). Examining the effectiveness of such strategies in a country specific manner will be crucial in progressing clinical practise and promoting interventions to manage maternal distress in pregnancy to ultimately improve maternal and birth outcomes in this high risk population.

Study limitations

The current study has several limitations. First the relatively limited sample size in this cohort may explain why we do not find an effect with prenatal distress and birth and placental weight. Additionally enrolling women after 28 weeks' gestation precludes our ability to measure any association with extremely PTB. Furthermore the earliest age at delivery in this cohort was 37

weeks' gestation which prevented us from investigating an association with pre-term delivery, which has a background incidence in our population of 4%. The limited ethnicity of our cohort did not allow us to examine any ethnic differences, which is an important factor when considering prenatal distress (Borders et al., 2015). Moreover, measuring maternal distress in late pregnancy only underscores the importance of other vulnerable periods during gestation. Finally, we were unable to assess or control for maternal antidepressant use or lifestyle factors such as exercise, diet, smoking or alcohol intake which may mediate the relationship between prenatal distress and adverse outcomes (Shapiro et al., 2013)

Conclusions

Despite these limitations the current study is important as it identifies prenatal anxiety in late pregnancy as a significant factor leading to increased opting for Caesarean Section delivery and increased bottle feeding of infants. Addtionally the changes in placental HSD11B2 and NR3C1 mRNA expression further supports the findings that prenatal distress can change the expression of genes playing a crucial role in placental glucocorticoid signalling in human populations. It also support the potential involvement of this mechanism in the programming effects of prenatal distress. Further studies conducted longitudinally throughout pregnancy and including both psychological and physiological measures of distress in larger and more ethnically diverse populations will help to clarify vulnerable individuals specifically at risk. Translating these results into prenatal clinics and establishing interventions to manage distress in pregnancy will be crucial in reducing morbidities in pregnancy and neonates.

Conflict of Interest:

The authors declare no conflict of interest.

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Figures

Figure 1: Flowchart of participants

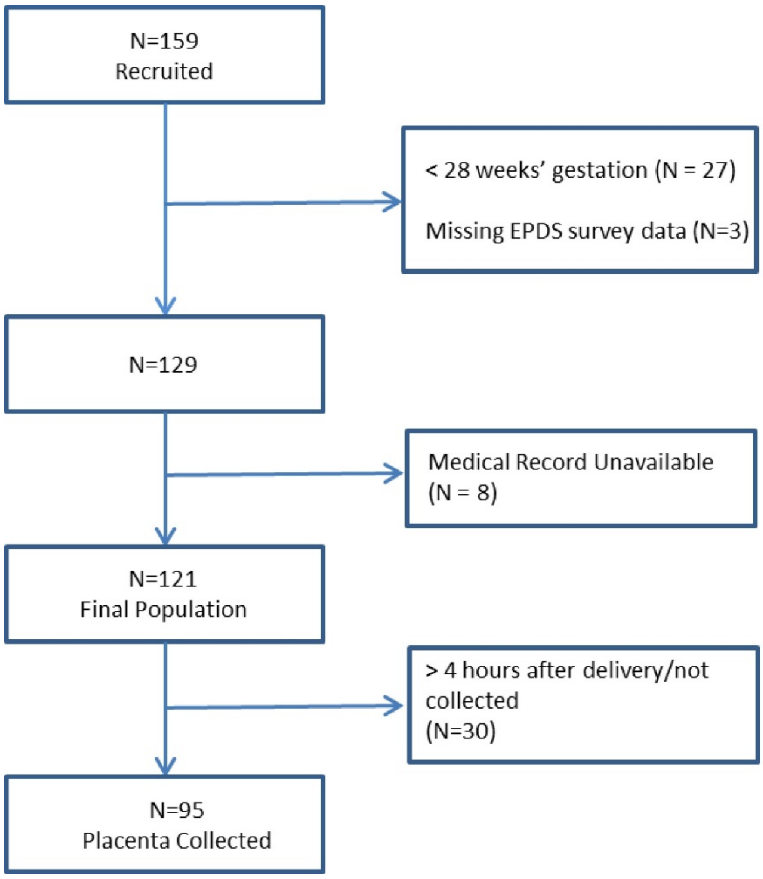


Figure 1: 159 questionnaires were completed with 21 (13.2%) removed from the dataset for being less than 28 weeks gestation and a further 5 (3.1%) removed for incomplete filling of the questionnaire. Medical records were unavailable for 12 (7.5%) of the participants. Placental samples were collected, frozen and biobanked for 100 of the remaining participants (82.6%).

Table 1: Descriptive statistics of categorical variables

Demographics	*N (%)*
Age (years)	
≤ 18	1 (0.8%)
19 – 24	8 (6.7%)
25 – 29	26 (21.8%)
30 – 35	48 (40.3%)
≥ 35	36 (30.3%)
*Missing	2 (1.7%)
BMI (kg/m²)	
Underweight (< 18.50)	0
Normal (18.5 – 24.99)	50 (41.7%)
Overweight (25 – 29.9)	44 (36.4%)
Obese (> 30.0)	26 (21.5%)
*Missing	1 (0.8%)
Social class	
1. Professional workers	14 (11.6%)
2. Managerial and technical	37 (30.6%)
3. Non-manual	8 (6.6%)
4. Skilled manual	28 (23.1%)
5. Semi-skilled	9 (7.4%)
6. Unskilled	8 (6.6%)
7. Unemployed	6 (5%)
8. Missing	11 (9.1%)

Medical History	
History of miscarriage	
Yes	28 (23.1%)
No	93 (76.9%)
History of ectopic pregnancy	
Yes	2 (1.7%)
No	119 (98.3%)
Psychiatric history	
History of any psychiatric illness	
Yes	26 (21.5%)
No	95 (78.5%)
History of depression	
Yes	12 (9.9%)
No	109 (90.1%)
History of anxiety	
Yes	9 (7.4%)
No	112 (92.6%)
History of postnatal depression	
Yes	6 (5%)
No	115 (95%)
Onset of delivery	
Spontaneous	51 (50.5%)
Induction	50 (49.5%)
*missing	20 (16.5%)
Rupture of membranes	

SROM	35 (34%)
PROM	3 (2.9%)
AROM	65 (63.1%)
Mode of delivery	
Spontaneous vaginal delivery	67 (55.8%)
(SVD)	4 (3.3%)
Forceps delivery (FD)	19 (15.8%)
Vacuum delivery (KVD)	10 (8.3%)
Emergency Casearan section	20 (16.7%)
(EMCS)	
Elective Casearan section (ELCS)	
Resuscitation at birth	
Yes	17 (14.4%)
No	101 (85.6%)
Admission to the Neonatal Unit	
Yes	5 (4.2%)
No	115 (95.8%)
Mode of Feeding on discharge	
Exclusive breast fed	56 (46.3%)
Mixed feeding	7 (5.8%)
Bottle fed	58 (47.9%)

Table 1: Descriptive statistics of categorical variables

Table 2: Descriptive statistics of continuous variables

Measure	*Mean (N)*	*SEM*	*SD*	*Range*
Maternal age	31.75	0.41	4.54	17 - 41
Maternal BMI	26.33	0.42	4.60	19 - 40
Birthweight	3654.67	40.66	445.49	2690 - 4800
Placental weight	558.33	10.84	106.29	300 - 900
Placental weight ratio	0.157	0.006	0.06	0.09 – 0.70
Birth Temperature	36.80	0.09	1.01	27 - 39
1 minute Apgar Score	8.54	0.10	1.19	3 - 10
5 minute Apgar Score	9.53	0.06	0.73	6 - 10
Gestational Age at delivery	39.58	0.09	1.04	37 - 41
Birthweight Centiles	47.58	2.46	26.87	1 - 98

Table 2: Descriptive statistics of continuous variables

Figure 2: Prenatal stress and neonatal outcomes

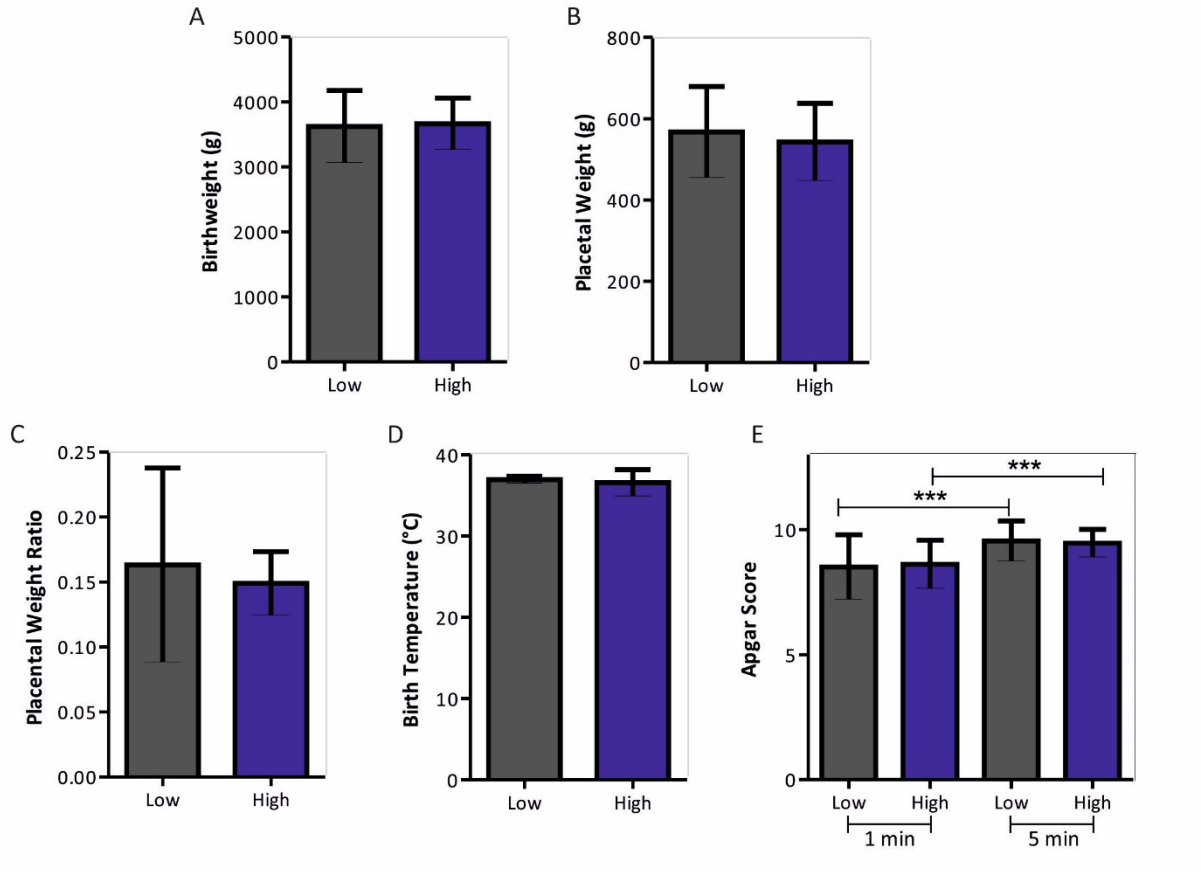


Figure 2: Graphical representation of birthweight (A), placental weight (B), placental weight ratio (C), birth temperature (D) and Apgar score (E) in neonates whose mothers were ranked in the high and low stress category. Unpaired students t-test between low and high on birth weight (A) and placental weight ratio (C) show no significant difference between groups $P > 0.05$. Mann Whitney test between low and high on placental weight (B) and birth temperature (D) show no significant difference between groups $P > 0.05$. Mann Whitney test show increased Apgar score from 1 to 5 minute (E) in low and high stress group ($P < 0.0001$ ***).

Table 3: Prenatal stress, maternal history and obstetric outcomes

Perceived Stress Scale			
		OR (CI; p-value)	aOR (CI; p-value)
Psychiatric history			
Psychiatric Illness		**2.519 (1.036 – 6.125 ; 0.042) ***	**2.591 (1.033 – 6.499 ; 0.042) ***
Depression		2.203 (0.663 – 7.338 ; 0.197)	2.648 (0.747 – 9.382 ; 0.131)
Anxiety		2.082 (0.696 – 10.867 – 0.149)	2.592 (0.622 – 10.796 ; 0.191)
Postnatal Depression		1.013 (0.178 – 5.780 ; 0.988)	0.837 (0.135 – 5.206 ; 0.849)
Miscarriage history			
Miscarriage		1.741 (0.730 – 4.155 ; 0.211)	2.278 (0.878 – 5.911 ; 0.091)
Mode of delivery			
Model 1	SVD	ref	ref
	Operative	1.023 (0.478 – 2.190 ; 0.954)	1.014 (0.466 – 2.204 ; 0.972)
Model 2	Vaginal	ref	ref
	CS	1.017 (0.423 – 2.441 ; 0.97)	1.178 (0.467 – 2.970 ; 0.729)
Model 3	SVD	ref	ref
	EMCS	0.511 (-2.302 – 0.961 ; 0.420)	0.503 (-2.345 – 0.969 ; 0.416)
	ELCS	1.364 (-0.720 – 1.340 ; 0.555)	1.706 (-0.556 – 1.625 ; 0.337)
	KVD	1.193 (-0.886 – 1.239 ; 0.745)	1.046 (-1.043 – 1.133 ; 0.935)
	FD	0.682 (-2.703 – 1.937 ; 0.746)	0.492 (-3.062 – 1.645 ; 0.555)
Gestational Age at delivery			
< 39 weeks gestation		0.673 (0.284 – 2.252 ; 0.673)	0.758 (0.266 – 2.157 ; 0.603)
Mode of Feeding			
Breastfed		ref	ref

Bottle-fed	1.111 (-0.674-0.885; 0.791)	1.059 (-0.737 – 0.852 ; 0.887)
Mixed	0.844 (-1.902-1.564; 0.848)	1.094 (-1.710 – 1.890 ; 0.922)

Table 3: Odds ratios assessing the relationship between prenatal stress, history of psychiatric illness, history of miscarriage, mode of delivery, gestational age at delivery and mode of feeding. Binary logistic regression analysis revealed participants with a history of psychiatric illness were more likely to score high on the PSS ($P < 0.05$ *). Adjusted for maternal age, BMI and social class.

Figure 3: Prenatal anxiety and neonatal outcomes

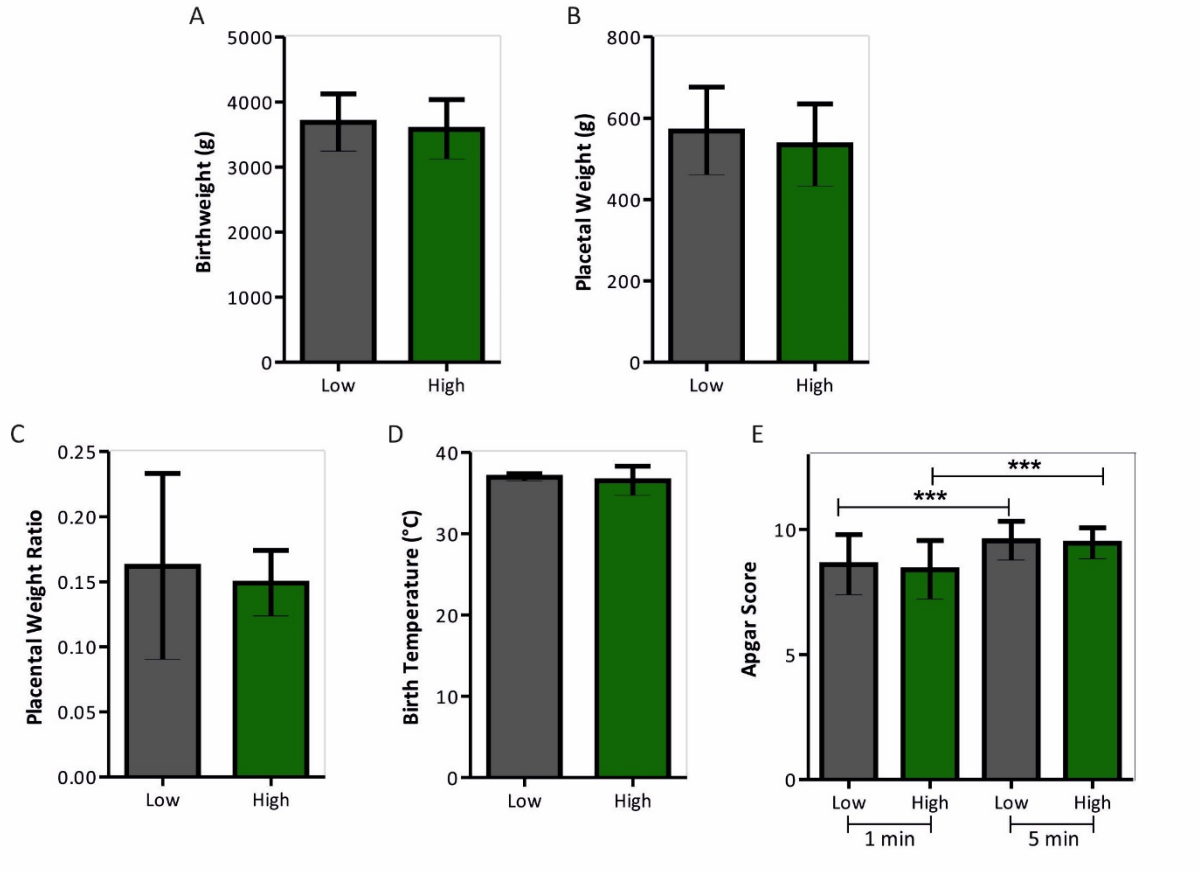


Figure 3: Graphical representation of birthweight (A), placental weight (B), placental weight ratio (C), birth temperature (D) and Apgar score (E) in neonates whose mothers were ranked in the high and low anxiety category. Unpaired students t-test between low and high on birth weight (A) and placental weight ratio (C) show no significant difference between groups $P > 0.05$. Mann Whitney test between low and high on placental weight (B) and and birth temperature (D) show no significant difference between groups $P > 0.05$. Mann Whitney test show increased Apgar score from 1 to 5 minute in low and high anxiety group ($P < 0.0001$ ***).

Table 4: Prenatal anxiety, maternal history and obstetric outcomes

State Trait Anxiety Inventory			
		OR (CI; p-value)	aOR (CI; p-value)
Psychiatric history			
Psychiatric Illness		**3.020 (1.215 – 7.509 ; 0.017) ***	**3.532 (1.322 – 9.389 ; 0.012) ***
Depression		2.066 (0.607 – 7.037 ; 0.246)	2.579 (0.699 – 9.515 ; 0.155)
Anxiety		1.367 (0.321 – 5.812 ; 0.672)	1.322 (0.275 – 6.358 ; 0.727)
Postnatal Depression		2.833 (0.542 – 14.806 ; 0.217)	2.509 (0.432 – 14.583 ; 0.306)
Miscarriage history			
Miscarriage		**3.163 (1.296 – 7.721 ; 0.011) ***	**3.807 (1.384 – 10.473 ; 0.010) ***
Mode of delivery			
Model 1	SVD	ref	ref
	Operative	**2.443 (1.077 – 5.545 ; 0.033) ***	2.264 (0.977 – 5.246 ; 0.057)
Model 2	Vaginal	ref	ref
	CS	2.222 (0.923 – 5.348 ; 0.075)	2.306 (0.895 – 5.937 ; 0.083)
Model 3	SVD	ref	ref
	EMCS	1.038 (-1.626 – 1.701 ; 0.965)	0.817 (-1.917 – 1.513 ; 0.817)
	ELCS	**4.154 (0.359 – 2.489 ; 0.009) ****	**5.411 (0.509 – 2.868 ; 0.005) ****
	KVD	2.423 (-0.227 – 1.997 ; 0.119)	2.235 (-0.345 – 1.954 ; 0.170)
	FD	1.385 (-2.017 – 2.668 ; 0.785)	1.013 (-2.424 – 2.451 ; 0.992)
Gestational Age at delivery			
< 39 weeks gestation		**3.182 (1.196 – 8.467 ; 0.020) ***	**3.235 (1.182 – 8.855 ; 0.022) ***
Mode of Feeding			
Breastfed		ref	ref

Bottle-fed	**2.964 (0.195 –1.978; 0.017)***	**2.710 (0.086 – 1.908 ; 0.032) ***
Mixed	3.917 (-0.293 – 3.023; 0.107)	5.189 (-0.148 – 3.441 ; 0.072)

Table 4: Odds ratios assessing the relationship between prenatal anxiety, history of psychiatric illness, history of miscarriage, mode of delivery, gestational age at delivery and mode of feeding. Binary logistic regression analysis revealed participants with a history of psychiatric illness or specifically depression and miscarriage were more likely to score high on the STAI ($P < 0.05$ *). High anxiety was associated with an increased risk of delivery by ELCS ($P < 0.01$ **). Prenatal anxiety related to increased risk of delivery before 39 weeks gestation ($P < 0.05$ *). Women in the high anxiety group were more likely to be bottle feeding on hospital discharge ($P < 0.05$ *). Adjusted for maternal age, BMI and social class.

Figure 4: Prenatal depressive symptoms and neonatal outcomes

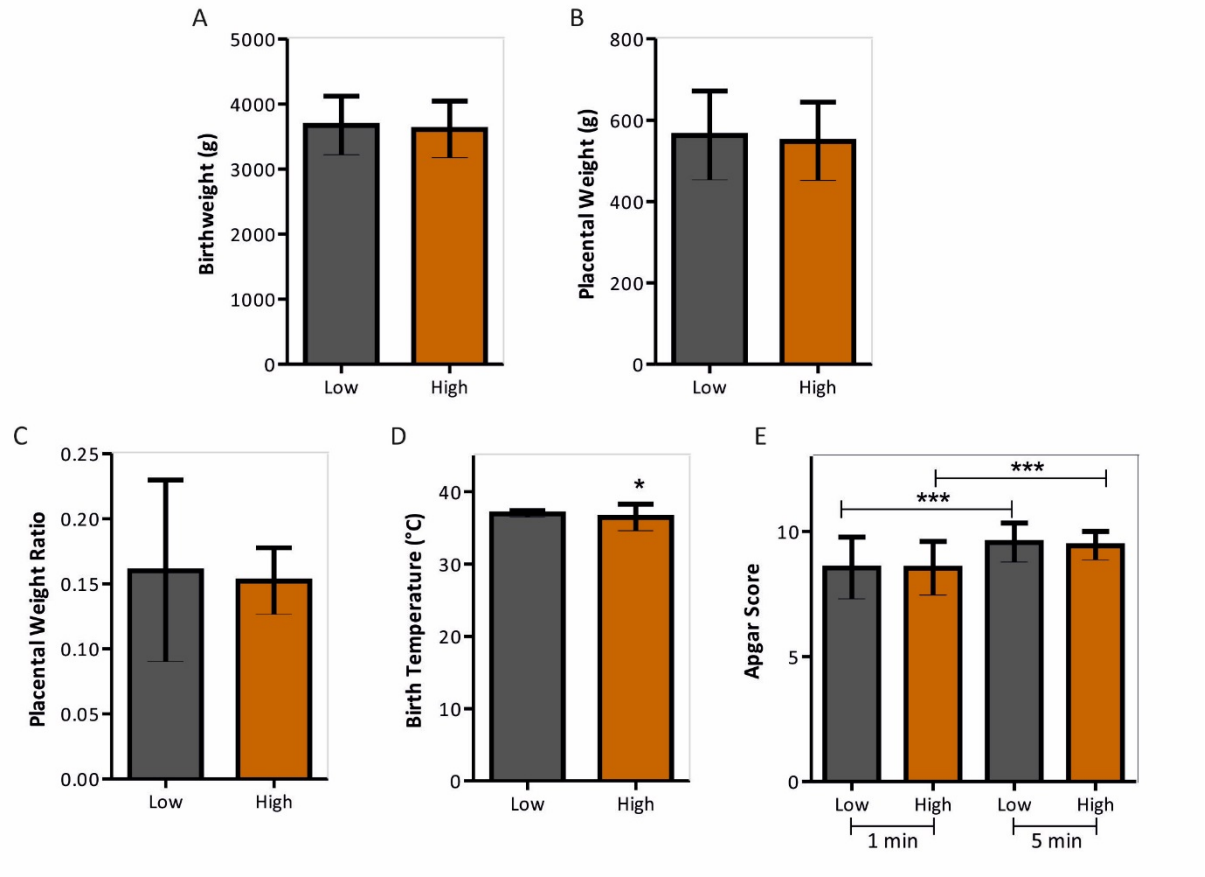


Figure 4: Graphical representation of birthweight (A), placental weight (B), placental weight ratio (C), birth temperature (D) and Apgar score (E) in neonates whose mothers were ranked in the high and low depressive category. Unpaired students t-test between low and high on birth weight (A) and placental weight ratio (C) show no significant difference between groups $P > 0.05$. Mann Whitney test between low and high on placental weight (B) show no significant difference between groups $P > 0.05$. Mann Whitney test show decrease birth temperature (E) among the high depressive symptoms group ($P < 0.05$ *). Mann Whitney test show increased Apgar score from 1 to 5 minute in low and high depressive symptom group ($P < 0.0001$ ***).

Table 5: Prenatal depressive symptoms, maternal history and obstetric outcomes

Edinburgh Postnatal Depression Scale			
		OR (CI; p-value)	aOR (CI; p-value)
Psychiatric history			
Psychiatric Illness		**3.429 (1.366 – 8.607 ; 0.009) ****	**3.566 (1.348 – 9.434 ; 0.010) ***
Depression		3.360 (0.995 – 11.341 ; 0.051)	**4.451 (1.198 – 16.539 ; 0.026) ***
Anxiety		2.519 (0.631 – 10.053 ; 0.191)	2.042 (0.475 – 8.776 ; 0.337)
Postnatal Depression		1.483 (0.258 – 8.522 ; 0.659)	1.111 (0.149 – 8.312 ; 0.918)
Miscarriage history			
Miscarriage		**3.611 (1.463 – 8.913 ; 0.005) ****	**3.878 (1.420 – 10.590 ; 0.008) ****
Mode of delivery			
Model 1	SVD	ref	ref
	Operative	1.739 (0.764 – 3.960 ; 0.187)	1.751 (0.743 – 4.128 ; 0.200)
Model 2	Vaginal	ref	ref
	CS	1.344 (0.538 – 3.361 ; 0.527)	1.618 (0.592 – 4.419 ; 0.348)
Model 3	SVD	ref	ref
	EMCS	0.946 (-1.713 – 1.603 ; 0.948)	0.950 (-1.755 – 1.652 ; 0.953)
	ELCS	2.038 (-0.379 – 1.804 ; 0.201)	2.456 (-0.275 – 2.072 ; 0.133)
	KVD	1.747 (-0.574 – 1.691 ; 0.334)	1.521 (-0.777 – 1.617 ; 0.492)
	FD	3.786 (-0.715 – 3.378 ; 0.202)	3.485 (-0.869 – 3.366 ; 0.248)
Gestational Age at delivery			
< 39 weeks gestation		2.154 (0.792 – 5.855 ; 0.133)	2.391 (0.841 – 6.796 ; 0.102)
Mode of Feeding			

Breastfed	ref	ref
Bottle-fed	1.520 (-0.433-1.272; 0.336)	1.301 (-1.621 – 2.147 ; 0.784)
Mixed	1.467 (-1.377-2.143; 0.670)	1.337 (-0.594 – 1.175 ; 0.520)

Table 5: Odds ratios assessing the relationship between prenatal depressive symptoms, history of psychiatric illness, history of miscarriage, mode of delivery, gestational age at delivery and mode of feeding. Binary logistic regression analysis revealed participants with a history of psychiatric illness or specifically depression and miscarriage were more likely to score high on the EPDS ($P < 0.05$ *, $P < 0.01$ **). Adjusted for maternal age, BMI and social class.

Figure 5: Prenatal cumulative distress and neonatal outcomes

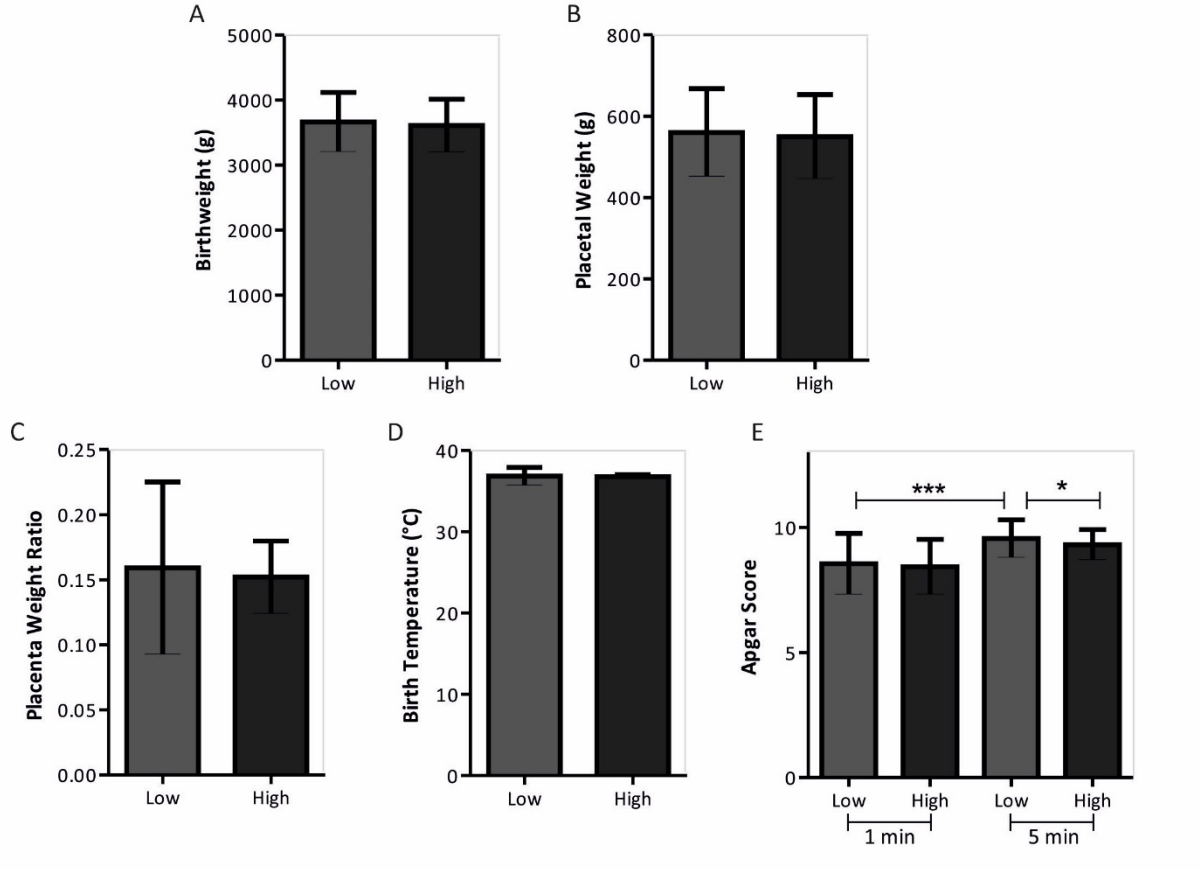


Figure 5: Graphical representation of birthweight (A), placental weight (B), placental weight ratio (C), birth temperature (D) and Apgar score (D) in neonates whose mothers were ranked in the high and low cumulative distress category. Unpaired students t-test between low and high on birth weight (A) and placental weight ratio (C) show no significant difference between groups $P > 0.05$. Mann Whitney test between low and high on placental weight (B) and birth temperature (D) show no significant difference between groups $P > 0.05$. Mann Whitney test show increased Apgar score from 1 to 5 minute in low cumulative distress group ($P < 0.0001$ ***) but not in high group ($P > 0.05$). Significant reduction in 5 minute Apgar score among high cumulative distress group (F; Mann Whitney test; $P < 0.05$ *).

Table 6: Prenatal cumulative distress, maternal history and obstetric outcomes

Cumulative distress			
		OR (CI; p-value)	aOR (CI; p-value)
Psychiatric history			
Psychiatric Illness		**5.059 (1.752 – 14.606 ; 0.003) ****	**6.229 (1.947 – 19.926 ; 0.002) ****
Depression		3.393 (0.902 – 12.763 ; 0.071)	**4.504 (1.073 – 18.897 ; 0.040) ***
Anxiety		3.233 (0.730 – 14.329 ; 0.122)	3.411 (0.649 – 17.916 ; 0.147)
Postnatal Depression		3.094 (0.523 – 18.302 ; 0.213)	3.136 (0.427 – 23.036 ; 0.261)
Miscarriage history			
Miscarriage		**3.320 (1.162 – 9.488 ; 0.025) ***	**3.903 (1.224 – 12.446 ; 0.021) ***
Mode of delivery			
Model 1	SVD	ref	ref
	Operative	2.193 (0.786 – 6.113 ; 0.133)	1.982 (0.684 – 5.739 ; 0.207)
Model 2	Vaginal	ref	ref
	CS	1.646 (0.558 – 4.852 ; 0.366)	1.758 (0.545 – 5.672 ; 0.345)
Model 3	SVD	ref	ref
	EMCS	0.952 (-2.258 – 2.161 ; 0.965)	0.704 (-2.647 – 1.945 ; 0.765)
	ELCS	2.857 (-0.230 – 2.329 ; 0.108)	**4.375 (0.005 – 2.947 ; 0.049) ***
	KVD	2.286 (-0.526 – 2.179 ; 0.231)	2.307 (-0.596 – 2.267 ; 0.252)
	FD	2.857 (-1.345 – 3.445 ; 0.390)	2.121 (-1.843 – 3.346 ; 0.570)
Gestational Age at delivery			
< 39 weeks gestation		1.557 (0.452 – 5.355 ; 0.483)	1.548 (0.431 – 5.558 ; 0.503)
Mode of Feeding			
Breastfed		ref	ref

Bottle-fed	1.638 (-0.535 – 1.522 ; 0.347)	1.409 (-0.730 – 1.415 ; 0.531)
Mixed	-	-

Table 6: Odds ratios assessing the relationship between prenatal cumulative distress, history of psychiatric illness, history of miscarriage, mode of delivery, gestational age at delivery and mode of feeding. Binary logistic regression analysis revealed participants with a history of psychiatric illness or specifically depression and miscarriage were more likely to be in high cumulative distress group ($P < 0.05$ *, $P < 0.01$ **). Prenatal cumulative distress was associated with increased delivery by ELCS (Ordinal regression; $P < 0.05$ *). Adjusted for maternal age, BMI and social class.

Figure 6: Placental gene expression

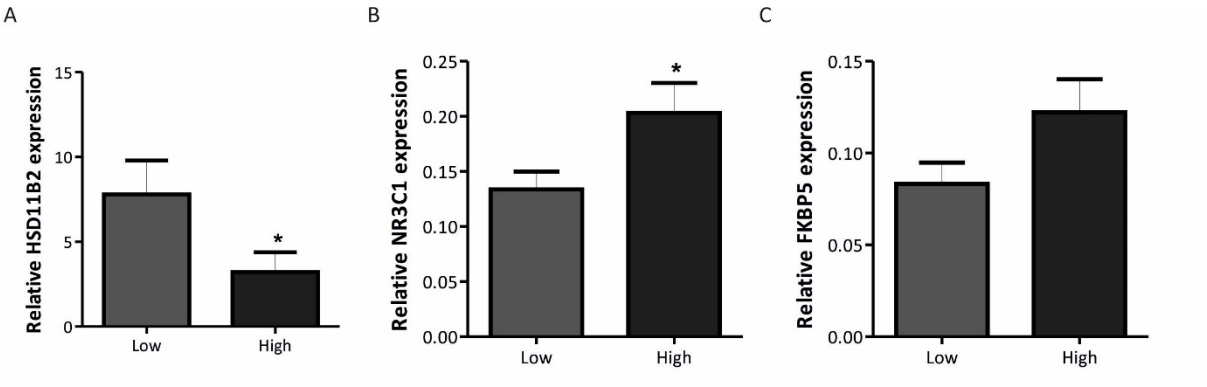
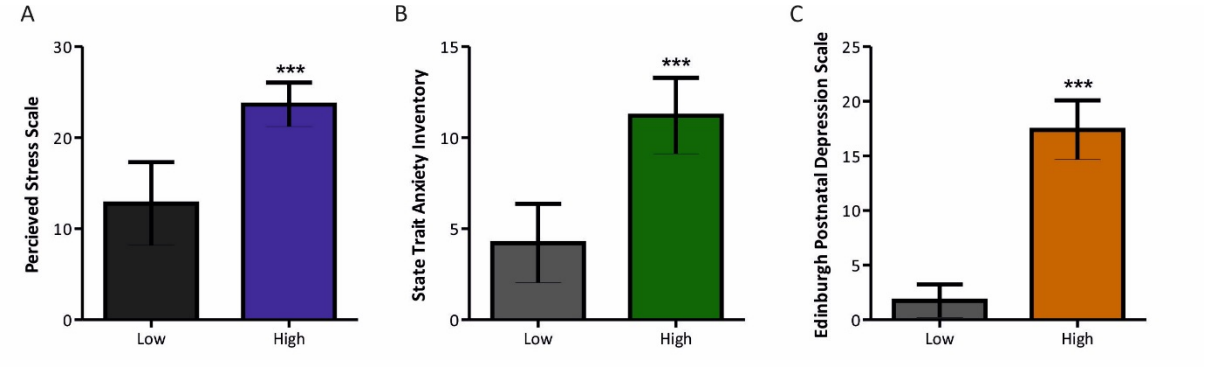


Figure 6: Real time PCR showing (A) HSD11B2, (B) NR3C1 and (C) FKBP5 expression in the term human placenta between participants in the low and high cumulative stress group using the 2-delta-Ct method (Low *n=9*, High *n=15*; Unpaired Student's t-test $P < 0.05$ *).

Supplementary Information

Supplementary *figure 1: Participants groupings based on Questionnaire Scorings*



Supplementary figure 1: Graphical representation of perceived stress scale (A), State Trait Anxiety Inventory (B) and Edinburgh Postnatal Depression Scale (C) scores between women ranked in the low and high categories. Unpaired student t-test show a significant difference between groups $P < 0.001$ ***, N=121.

Supplementary table 1: Global sample means of measured variables

Measure	*Population*	*Low*	*High*
	Mean ± SD (N)	Mean ± SD (N)	Mean ± SD (N)
Perceived Stress Scale	15.88 ± 6.5 (121)	12.75 ± 4.5 (81)	23.65 ± 2.4 (40)
State Trait Anxiety Inventory	6.17 ± 3.8 (121)	4.20 ± 2.1 (87)	11.21 ± 2.0 (34)
Edinburgh Postnatal Depression Scale	9.07 ± 5.6 (121)	6.52 ± 3.7 (90)	16.48 ± 2.9 (31)

Supplementary table 1: Global sample means of measured variables expressed as mean ± SD. High stress (PSS ≥ 20), low stress (PSS ≤ 19). High anxiety (STAI ≥ 9), low anxiety (STAI ≤ 8). High probability of depression (EPDS ≥ 13), low probability of depression (EPDS ≤ 12).

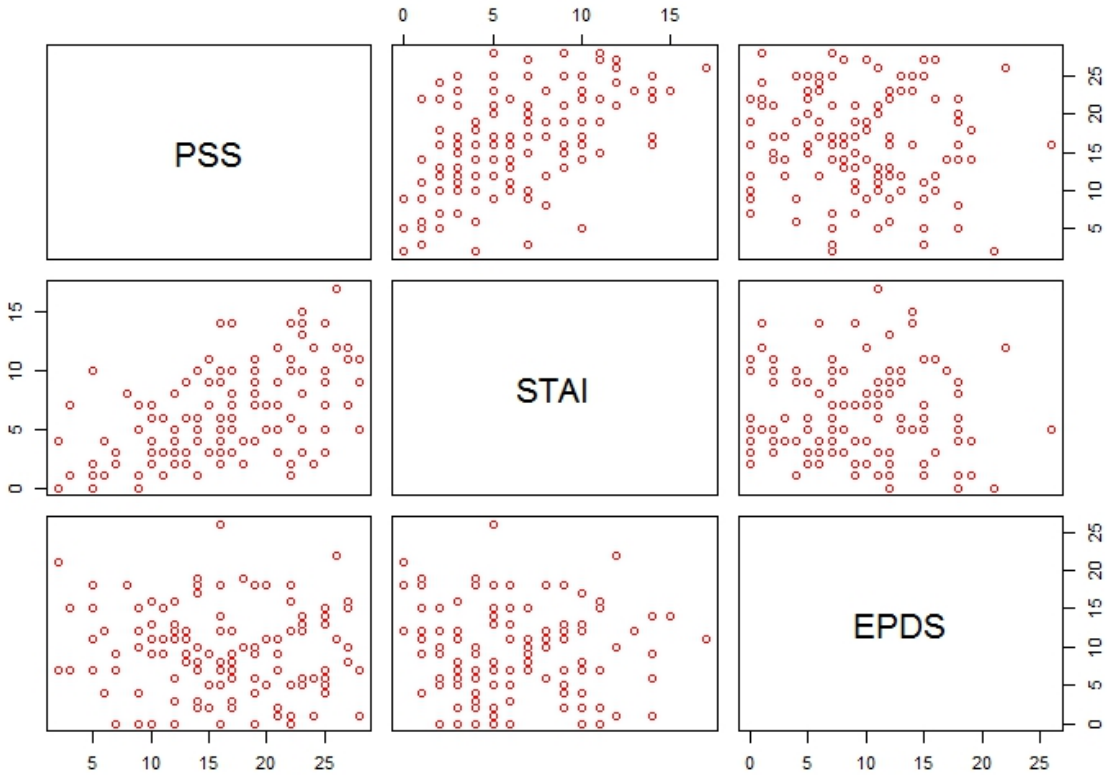
Supplementary table 2: Prenatal stress, anxiety and depression and other neonatal outcomes

Perceived Stress Scale		
	OR (CI ; *p*-value)	aOR (CI ; *p*-value)
Birth centiles	0.429 (0.115 – 1.606 ; 0.209)	0.395 (0.104 – 1.501 ; 0.173)
Neonatal Resuscitation	0.404 (0.109 – 1.501 ; 0.176)	0.460 (0.122 – 1.743 ; 0.253)
NICU Admission	1.405 (0.225 – 8.774 ; 0.716)	1.572 (0.240 – 10.312 ; 0.637)
Stat Trait Anxiety Inventory		
	OR (CI ; *p*-value)	aOR (CI ; *p*-value)
Birth centiles	1.279 (0.407 – 4.019 ; 0.673)	1.272 (0.396 – 4.080 ; 0.686)
Neonatal Resuscitation	1.142 (0.368 – 3.544 ; 0.818)	1.206 (0.375 – 3.873 ; 0.753)
NICU Admission	4.448 (0.708 – 27.954 ; 0.111)	1.109 (0.930 – 1.323 ; 0.250)
Edinburgh Postnatal Depression Scale		
	OR (CI ; *p*-value)	aOR (CI ; *p*-value)
Birth centiles	0.650 (0.172 – 2.456 ; 0.525)	0.602 (0.152 – 2.382 ; 0.469)
Neonatal Resuscitation	0.618 (0.164 – 2.324 ; 0.476)	0.654 (0.165 – 2.593 ; 0.546)
NICU Admission	2.071 (0.329 – 13.032 ; 0.438)	1.857 (0.277 – 12.460 ; 0.524)
Cumulative		
	OR (CI ; p-value)	aOR (CI ; p-value)
Birth centiles	0.363 (0.045 – 2.945 ; 0.342)	0.334 (0.039 – 2.855 ; 0.316)
Neonatal Resuscitation	0.764 (0.158 – 3.689 ; 0.738)	0.795 (0.158 – 4.003 ; 0.781)
NICU Admission	4.444 (0.685 – 28.828 ; 0.118)	1.007 (0.878 – 1.155 ; 0.919)

Supplementary table 2: Relationship between scoring in the PSS, STAI, and/or EPDS with infants in the lower 10th birth centile, neonatal resuscitations and NICU Admissions. Binary

logistic regression shows no significant difference between groups P>0.05. Adjusted for maternal age, BMI and social class.

Supplementary figure 2: Intercorrelations of questionnaire scores



Supplementary figure 2: Correlation matrices showing relationship between PSS, STAI and EPDS scores. Pearson correlation shows each questionnaire is significantly correlated. PSS and STAI ($r = 0.521$; $P < 0.001$ ***), PSS and EPDS ($r = 0.724$; $P < 0.001$ ***) and STAI and EPDS ($r = 0.678$; $P > 0.001$ ***). RStudio.

Supplementary table 3: Prenatal distress with number of newborn adversities

Perceived Stress Scale		
	OR (CI ; *p*-value)	aOR (CI ; *p*-value)
1 adverse outcome	0.669 (-1.245 – 0.442; 0.351)	0.648 (-1.302 - 0.435; 0.328)
2 adverse outcomes	0.341 (-3.276 – 1.122; 0.337)	0.335 (-3.310 – 1.124; 0.334)
3 adverse outcomes	0.852 (-2.607 – 2.287; 0.898)	0.878 (-2.628 – 2.468; 0.474)
Stat Trait Anxiety Inventory		
	OR (CI ; *p*-value)	aOR (CI ; *p*-value)
1 adverse outcome	0.914 (-0.974 – 0.794; 0.842)	0.860 (-1.076 – 0.773; 0.748)
2 adverse outcomes	1.325 (-1.492 – 2.055; 0.756)	1.440 (-1.445 – 2.174; 0.693)
3 adverse outcomes	1.325 (-2.174 – 2.736; 0.822)	0.809 (-2.769 – 2.346; 0.871)
Edinburgh Postnatal Depression Scale		
	OR (CI ; *p*-value)	aOR (CI ; *p*-value)
1 adverse outcome	0.914 (-0.974 – 0.794; 0.842)	0.914 (-1.019 – 0.839; 0.849)
2 adverse outcomes	--	--
3 adverse outcomes	1.325 (-2.174 – 2.736; 0.822)	1.090 (-2.517 – 2.690; 0.948)
Cumulative		
	OR (CI ; p-value)	aOR (CI ; p-value)
1 adverse outcome	1.025 (-1.056 – 1.105; 0.965)	0.986 (-1.148 – 1.120; 0.981)
2 adverse outcomes	--	--
3 adverse outcomes	2.818 (-1.449 – 3.521; 0.414)	1.610 (-2.143 – 3.094; 0.722)

Supplementary table 3: Odds ratios assessing the relationship between prenatal distress and number of newborn adversities. Newborns were grouped into number of adverse birth outcomes based on six parameters (a) Admission to the NICU (b) Newborn Resuscitation received (c) Delivered before 37 weeks gestation (d) 5 minute Apgar score ≤ 7 (e) Birth

Temperature < 36.5°C and (f) Birth Centile ≤ 10 or ≥ 90. Ordinal logistic regression analysis revealed no significant effect ($P > 0.05$). Adjusted for maternal age, BMI and social class.

Supplementary table 4: Linear regression analysis of placental HSD11B2 mRNA expression with maternal distress, demographics and neonatal outcomes

HSD11B2	*R2*	*β-coefficient*	*95% CI*	*p-value*
Birthweight	0.019	0.137	-0.783 – 1.547	0.505
Placental weight	0.037	0.119	-0.144 – 0.382	0.360
PWR	0.030	0.173	0.00 – 0.00	0.408
Birthweight centiles	0.012	0.109	-0.068 – 0.116	0.597
1 min Apgar	0.012	0.109	-0.002 – 0.003	0.597
5 min Apgar	0.011	0.103	-0.001 – 0.002	0.617
Birth Temperature	0.003	0.054	-0.001 – 0.001	0.794
Gravidity	0.000	-0.009	-0.009 – 0.009	0.964
Maternal Age	0.004	-0.064	-0.647 – 0.472	0.472
Maternal BMI	0.062	-0.248	-0.001 – 0.000	0.212
Social Class	0.009	-0.095	-0.954 – 0.594	0.636
PSS	*0.137*	*-0.370*	*-0.051 – 0.001*	*0.058*
STAI	0.070	-0.265	-0.025 – 0.005	0.181
EPDS	0.068	-0.260	-0.038 – 0.008	0.190

Supplementary table 4: Placental HSD11B2 expression and continuous variables. Linear regression analysis. $P > 0.05$.

Supplementary table 5: Linear regression analysis of placental NR3C1 mRNA expression with maternal distress, demographics and neonatal outcomes

NR3C1	*R2*	*β-coefficient*	*95% CI*	*p-value*
Birthweight	0.083	-0.288	-1961 – 325.796	0.153
Placental weight	***0.159***	***-0.398***	***-790.258 - -2.667***	***0.049***
PWR	0.086	-0.293	-0.867 – 0.146	0.155
Birthweight centiles	***0.170***	***-0.412***	***-180 - -6.380***	***0.036***
1 min Apgar	0.004	-0.064	-2.870 – 2.111	0.756
5 min Apgar	0.00	0.010	-1.714 – 1.794	0.962
Birth Temperature	0.038	-0.195	-1.267 – 0.455	0.340
Gravidity	0.003	-0.054	-9.854 – 7.583	0.791
Maternal Age	000	0.015	-547.008 – 588.035	0.941
Maternal BMI	0.023	0.151	-0.292 – 0.635	0.453
Social Class	0.005	0.074	-644.397 – 955.528	0.715
PSS	0.075	0.273	-8.437 – 45.959	0.168
STAI	0.003	0.052	-13.496 – 17.405	0.797
EPDS	0.007	0.084	-18.893 – 28.563	0.678

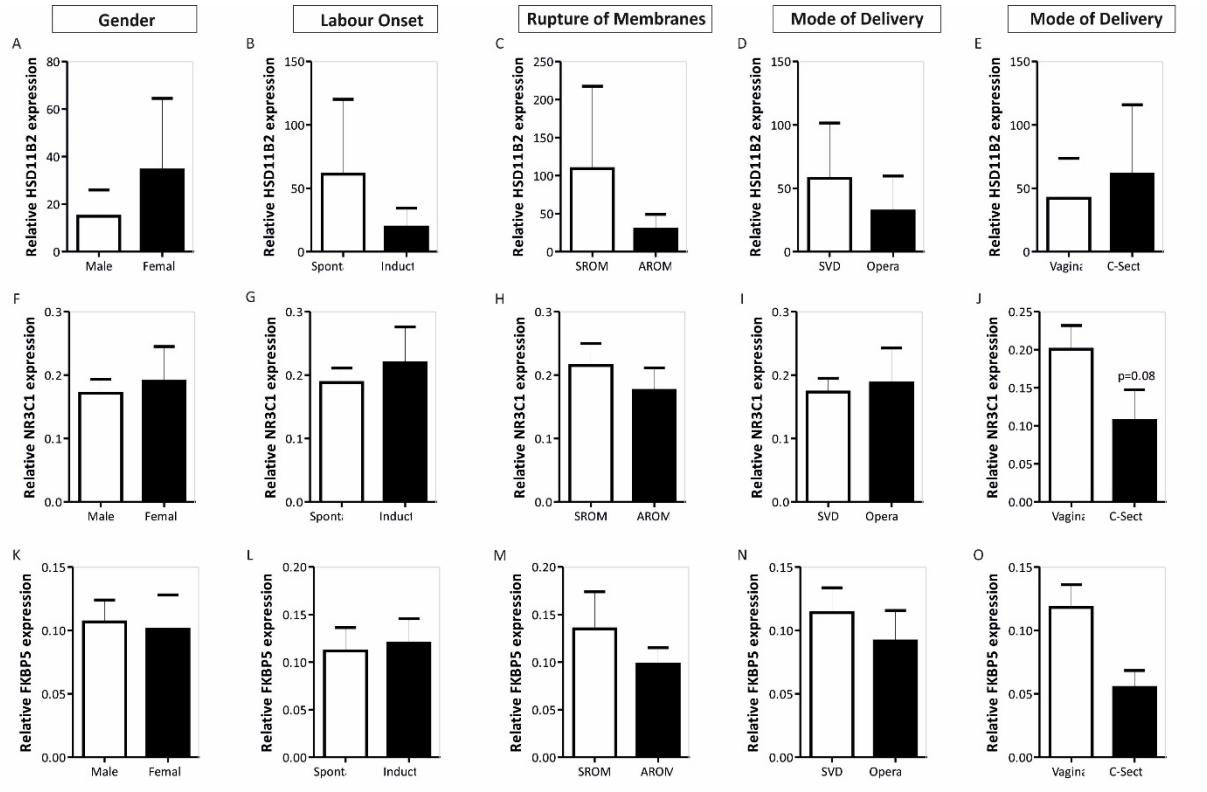
Supplementary table 5: Placental NR3C1 expression and continuous variables. Linear regression analysis. Placental weight and birthweight centiles associated with NR3C1 levels. $P < 0.05$ *.

Supplementary table 6: Linear regression analysis of placental FKBP51 mRNA expression with maternal distress, demographics and neonatal outcomes

FKBP51	*R2*	*β-coefficient*	*95% CI*	*p-value*
Birthweight	0.033	-0.183	-2955.102 – 1147.112	0.372
Placental weight	0.109	-0.330	-1010.482 – 106.235	0.107
PWR	0.020	-0.140	-0.959 – 0.487	0.506
Birthweight centiles	*0.150*	*-0.387*	*-308.452 – 0.616*	*0.051*
1 min Apgar	0.011	0.107	-3.244 – 5.473	0.603
5 min Apgar	0.002	0.05	-2.714 – 3.444	0.809
Birth Temperature	0.015	-0.122	-1.985 – 1.086	0.551
Gravidity	*0.012*	*-0.122*	*-19.559 – 11.174*	*0.579*
Maternal Age	*0.137*	*-0.370*	*-47.048 – 1.302*	*0.063*
Maternal BMI	***0.185***	***0.430***	***3.069 – 42.498***	***0.025***
Social Class	0.023	0.153	-862.086 – 1893.458	0.448
PSS	0.038	0.194	-25.554 – 72.702	0.332
STAI	0.008	0.088	-21.420 – 33.170	0.661
EPDS	0.045	0.212	-1.231 – 3.962	0.289

Supplementary table 6: Placental FKBP5 expression and continuous variables. Linear regression analysis. Maternal BMI positively associated with FKBP5 levels. $P < 0.05$ *.

Supplementary figure 3: Placental gene expression, gender and obstetric outcomes



Supplementary figure 3: Expression of placental HSD11B2, NR3C1 and FKBP5 in relation newborn sex and obstetric outcomes. No significant differences between groups. Unpaired Student's t-test. $P < 0.005$. Spontaneous (Spont), Induction (Induct), Spontaneous rupture of membranes (SROM), Artificial Rupture of membranes (AROM), Spontaneous vaginal delivery (SVD), Operative delivery (Opera) Vaginal delivery (Vagin) and C-Section delivery (C-Sect).

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Abstract

The experience of maternal distress in pregnancy is often linked with poorer obstetric outcomes for women as well as adverse outcomes for offspring. Alterations in placental glucocorticoid signalling and subsequent increased fetal exposure to cortisol have been suggested to underlie this relationship. In the current study, 121 pregnant women completed the Perceived Stress Scale, State Trait Anxiety Inventory and Edinburgh Postnatal Depression Scale in the third trimester of pregnancy. Placental samples were collected after delivery. Maternal history of psychiatric illness and miscarriage were significant predictors of poorer mental health in pregnancy. Higher anxiety was associated with an increase in women delivering via elective Caesarean Section, and an increase in bottle-feeding. Birth temperature was mildly reduced among infants of women with high levels of depressive symptomology. Babies of mothers who scored high in all stress (cumulative distress) measures had reduced 5-minute Apgar scores. High cumulative distress reduced the expression of placental HSD11B2 mRNA and increased the expression of placental NR3C1 mRNA. These data support a role for prenatal distress as a risk factor for altered obstetric outcomes. The alterations in placental gene expression support a role for altered placental glucocorticoid signalling in the relationship between maternal prenatal distress and adverse outcomes.