

An investigation of the effect of maternal depression during pregnancy on maternal and infant hypothalamic-pituitary-adrenal axes

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*Dedicated to my grandparents Amma, Appaji, Aji, and Thatha,
and my parents, with love*

*“I almost wish I hadn’t gone down that rabbit-hole – and yet – and yet – it’s
rather curious, you know, this sort of life”*

- Alice in Wonderland

I. Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

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II. Summary

Pregnancy is a period of increased vulnerability for developing depression. Several maternal physiological systems undergo changes during pregnancy, thought to adapt the woman for gestation and lactation. The hypothalamic-pituitary-adrenal (HPA) axis also undergoes significant alterations during pregnancy. There is strong evidence in the literature for the role of the HPA axis in non-pregnant depression. Studies of the HPA axis in antenatal depression however, report diverse findings. An important consideration to make with any maternal illness during pregnancy is foetal health. The foetal programming hypothesis postulates that the foetus undergoes organisational adaptations to survive a stressful intrauterine environment. These adaptations endure into the postpartum period, and increase offspring vulnerability for physical and mental illness in later life. There is strong evidence to suggest that maternal antenatal depression may predispose the offspring to depression in adult life, an effect that may be mediated by the HPA axis. With the current literature in mind, the primary focus of this thesis is the HPA axis. The principal aims were to examine the HPA axis in antenatal depression, and investigate for any foetal programming effects of maternal depression during pregnancy on the infant HPA axis. The relationships between maternal early life adversity (ELA) and maternal and infant HPA axes were also explored.

Women were recruited into the study between 20 and 30 weeks gestation, and three groups were compared – women who were depressed during pregnancy (Depressed), euthymic pregnant women with a prior history of depression (History), and never-depressed pregnant women (Control). Mothers and their infants were then followed up at two and six months postpartum. Saliva samples were collected from mothers and infants at all time points in the study for cortisol quantification.

The maternal HPA axis during pregnancy was similar in all the three groups studied, with no apparent effect of antenatal depression on diurnal cortisol, total cortisol output, or the cortisol awakening response (CAR). There were no associations between maternal clinical measures during pregnancy and cortisol measures. These findings suggest that the physiological hypercortisolaemia of pregnancy may obscure any depression-driven HPA axis changes. One of the remarkable findings during

pregnancy was the higher rating scale scores observed in women in the History group compared to women the Control group. A prior history of depression is a known risk factor for antenatal depression. These findings would suggest that pregnancy may be a more challenging time for women with a history of depression who do not experience a full relapse, increasing their vulnerability. Although there were no effects of antenatal depression on maternal HPA axis during pregnancy, women with a history of ELA had higher evening cortisol levels compared to women with no history of ELA. This effect persisted at both time points postpartum. Maternal total cortisol output decreased significantly from pregnancy to two months postpartum in all three groups, indicating a return to non-pregnant HPA axis function. However, there were no differences in the cortisol awakening response between the time points in any groups, replicating current evidence that the cortisol awakening response is preserved during pregnancy.

Although there were no discernible HPA axis alterations seen in antenatal depression in this study, there is clear evidence for foetal programming of the HPA axis in infants born to women who were depressed during pregnancy. Infants in the Depressed group had higher baseline and post-stressor cortisol levels at six months. Of note, three women in the Depressed group experienced a relapse of depression at six months postpartum. Findings from this study suggest that maternal mood and cortisol measures during pregnancy work synergistically with maternal mood and cortisol measures at six months postpartum to influence infant baseline cortisol measures at this time point. Infant post-stressor cortisol levels at six months appear to only be influenced by concurrent maternal cortisol measures. There was no effect of trait depression or maternal early life adversity on infant HPA axis function.

The findings in this study highlight the need for further research into the foetal programming effects of maternal depression during pregnancy, in order to reduce illness burden in future generations. These findings also have clinical implications, emphasising the importance of increasing perinatal mental health services for early detection and management of maternal depression during pregnancy and postpartum.

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IV. Table of contents

CHAPTER 1: Introduction

1.1 Overview.....	2
1.2 Major depressive disorder.....	4
1.2.1 Epidemiology of major depressive disorder	6
1.2.2 Aetiology of major depressive disorder	6
1.2.3 Management of major depressive disorder.....	6
1.3 Perinatal depression.....	8
1.3.1 Antenatal depression.....	8
1.3.1.1 Epidemiology of antenatal depression	8
1.3.1.2 Aetiology of antenatal depression	9
1.3.1.3 Management of antenatal depression.....	10
1.3.1.4 Adverse obstetric and birth outcomes.....	11
1.3.2 Postpartum depression.....	12
1.4 The HPA axis in humans	14
1.4.1 The HPA axis in depression	18
1.5 The HPA axis in the perinatal period.....	20
1.5.1 The HPA axis during pregnancy.....	20
1.5.2 Parturition and the HPA axis	26
1.5.3 The HPA axis postpartum.....	26
1.5.4 Depressive symptoms during pregnancy and the HPA axis	28
1.5.5 Antenatal depression and the HPA axis	29
1.5.6 Postpartum depression and the HPA axis.....	33
1.5.7 Summary	33
1.6 The foetal and infant HPA axes.....	34
1.6.1 The foetal HPA axis	34
1.6.2 The infant HPA axis	35
1.7 Developmental programming.....	37
1.7.1 The foetal programming hypothesis.....	37
1.7.2 Animal studies.....	38
1.7.3 Depressive symptoms during pregnancy and the infant HPA axis.....	38
1.7.4 Antenatal depression and the infant HPA axis.....	39
1.7.5 Maternal HPA axis during pregnancy and the infant HPA axis	39
1.7.6 Later-life effects of prenatal stress in offspring	40
1.7.7 Sexual dimorphism in developmental programming.....	40
1.7.8 Mechanisms in foetal programming	42
1.7.9 Summary	44
1.8 Early life adversity.....	45
1.8.1 Childhood abuse	45
1.8.2 Early life adversity and depression	46
1.8.3 Early life adversity and the HPA axis	46
1.8.4 Early life adversity and the perinatal period	47

1.8.5	Early life adversity and the HPA axis in the perinatal period	47
1.8.6	Early life adversity and antenatal depression	48
1.8.7	Maternal early life adversity and intergenerational transmission of stress	49
1.8.8	Summary	49
1.9	Aims and structure of thesis	51
 CHAPTER 2: Methods		
2.1	Study design.....	54
2.2	Ethical considerations	54
2.3	Participants	54
2.3.1	Recruitment summary	54
2.3.2	Recruitment criteria.....	56
2.3.3	Method of selection.....	56
2.4	Clinical measures.....	59
2.4.1	Sociodemographic characteristics	59
2.4.2	Diagnostic clinical interview.....	60
2.4.3	Rating scales.....	60
2.5	Postpartum follow-up	61
2.5.1	After delivery	61
2.5.2	Two and six months postpartum	62
2.6	Biological measures.....	62
2.6.1	Saliva sample collection and storage	62
2.6.2	Cortisol analysis	63
2.6.3	Cortisol levels and cortisol awakening response parameters.....	64
2.7	Statistical analysis	66
 CHAPTER 3: Clinical aspects of mood states during pregnancy and postpartum		
3.1	Introduction	70
3.2	Methods	72
3.2.1	Recruitment	72
3.2.2	Rating scales.....	72
3.2.3	Statistical analysis	73
3.3	Results	74
3.3.1	Sociodemographic characteristics of sample.....	74
3.3.2	Pregnancy	76
3.3.2.1	Characteristics of sample during pregnancy	76
3.3.2.2	Medications during pregnancy	77
3.3.2.3	Clinical measures during pregnancy	79
3.3.3	Obstetric and labour outcomes	83
3.3.4	Postpartum	84
3.3.4.1	Characteristics of sample postpartum	84
3.3.4.2	Medications postpartum.....	86
3.3.4.3	Clinical measures postpartum.....	86

3.3.4.4	Trajectory of change in clinical measures from pregnancy to postpartum	90
3.3.5	Early life adversity	92
3.4	Discussion	94
3.4.1	Clinical aspects of mood states during pregnancy.....	94
3.4.2	Maternal mood states during pregnancy and clinical features postpartum.....	95
3.4.3	Trajectory of change in maternal clinical measures from pregnancy to postpartum	96
3.4.4	Distribution of maternal early life adversity	96
3.4.5	Obstetric and labour outcomes	97
3.4.6	Conclusions	97

CHAPTER 4: The HPA axis during pregnancy

4.1	Introduction	99
4.1.1	Study aims.....	102
4.1.2	Study hypotheses.....	102
4.2	Methods	103
4.2.1	Recruitment	103
4.2.2	Rating scales.....	103
4.2.3	Salivary cortisol	104
4.2.4	Cortisol measures	104
4.2.5	Statistical analysis	105
4.3	Results	106
4.3.1	Characteristics of sample	106
4.3.2	Maternal cortisol measures during pregnancy	106
4.3.2.1	Maternal cortisol levels.....	106
4.3.2.2	Maternal cortisol awakening response parameters during pregnancy	109
4.3.2.3	Maternal demographic variables and cortisol measures during pregnancy.	110
4.3.2.4	Maternal cortisol measures and clinical measures during pregnancy.....	110
4.3.2.5	Foetal sex and maternal cortisol measures during pregnancy	110
4.3.3	Maternal salivary cortisone during pregnancy	112
4.3.3.1	Antenatal depression and maternal cortisone levels during pregnancy	112
4.3.3.2	Maternal cortisone levels and clinical measures during pregnancy	112
4.3.3.3	Maternal cortisol and cortisone levels during pregnancy.....	114
4.3.3.4	Foetal sex and maternal cortisone levels during pregnancy.....	115
4.3.3.5	Maternal depression and cortisone/cortisol ratio during pregnancy	116
4.3.3.6	Foetal sex and maternal cortisone/cortisol ratio during pregnancy.....	116
4.3.4	Early life adversity and maternal cortisol measures during pregnancy	117
4.3.4.1	Early life adversity and maternal cortisol levels during pregnancy.....	117
4.3.4.2	Maternal early life adversity and maternal cortisol measures during pregnancy.....	119
4.3.4.3	Prediction of maternal cortisol levels with maternal early life adversity scores	121
4.3.4.4	Foetal sex as a moderator of the relationship between maternal early life adversity and cortisol measures during pregnancy.....	121
4.3.4.5	Maternal early life adversity and cortisone levels during pregnancy	123

4.4	Discussion	126
4.4.1	Effect of depression on maternal HPA axis during pregnancy.....	126
4.4.2	Effect of early life adversity on maternal HPA axis during pregnancy	128
4.4.3	Effect of foetal sex on maternal HPA axis during pregnancy	129
4.4.4	Conclusions	130

CHAPTER 5: The HPA axis during postpartum

5.1	Introduction	132
5.1.1	Study Aims	135
5.1.2	Study hypotheses.....	135
5.2	Methods	136
5.2.1	Recruitment	136
5.2.2	Salivary cortisol	136
5.2.3	Cortisol measures	137
5.2.4	Statistical analysis	137
5.3	Results	138
5.3.1	Characteristics of sample	138
5.3.2	Maternal cortisol measures postpartum	140
5.3.2.1	Maternal cortisol levels at two months postpartum	140
5.3.2.2	Maternal cortisol awakening response at two months postpartum	140
5.3.2.3	Maternal cortisone levels and cortisone/cortisol ratios at two months postpartum.....	140
5.3.2.4	Maternal cortisol levels at six months postpartum	143
5.3.2.5	Maternal cortisol awakening response parameters at six months postpartum	143
5.3.2.6	Maternal cortisone levels and cortisone/cortisol ratios at six months postpartum.....	143
5.3.3	Trajectory of maternal cortisol from pregnancy to postpartum.....	146
5.3.3.1	Maternal cortisol measures from pregnancy to two months postpartum in the whole sample	146
5.3.3.2	Maternal cortisol measures from pregnancy to two months postpartum within caseness groups.....	146
5.3.3.3	Association of maternal cortisol measures between pregnancy and two months postpartum	148
5.3.3.4	Maternal cortisol measures from pregnancy to six months postpartum in the whole sample	150
5.3.3.5	Maternal cortisol measures from pregnancy to six months postpartum within caseness groups	150
5.3.3.6	Association of maternal cortisol measures between pregnancy and six months postpartum.....	153
5.3.3.7	Maternal cortisol measures from two months to six months postpartum in the whole sample	154
5.3.3.8	Maternal cortisol measures from two months to six months postpartum within caseness groups	154
5.3.4	Maternal HPA axis associations at two months postpartum	157

5.3.4.1	Maternal demographic variables and cortisol measures at two months postpartum.....	157
5.3.4.2	Maternal clinical measures and cortisol parameters at two months postpartum.....	157
5.3.5	Maternal HPA axis associations at six months postpartum	160
5.3.5.1	Maternal demographic variables and cortisol measures at six months postpartum.....	160
5.3.5.2	Maternal clinical measures and cortisol parameters at six months postpartum	160
5.3.6	Maternal early life adversity and the HPA axis postpartum	162
5.3.6.1	Maternal early life adversity and cortisol measures at two months postpartum.....	162
5.3.6.2	Maternal early life adversity and cortisol measures at six months postpartum	163
5.4	Discussion	164
5.4.1	Trajectory of the HPA axis postpartum.....	164
5.4.2	Maternal HPA axis measures throughout the perinatal period	165
5.4.3	Effect of depression on postpartum HPA axis measures	166
5.4.4	Maternal early life adversity and postpartum HPA axis measures	167
5.4.5	Conclusions	167
 CHAPTER 6: The HPA axis in infants over the first six months of life		
6.1	Introduction	170
6.1.1	Study aims.....	173
6.1.2	Study hypotheses.....	173
6.2	Methods	174
6.2.1	Recruitment	174
6.2.2	Saliva samples	174
6.2.3	Cortisol analysis	174
6.2.4	Cortisol measures	175
6.2.5	Statistical analysis	175
6.3	Results	177
6.3.1	Characteristics of sample	177
6.3.2	Infant birth outcomes	178
6.3.3	Infant cortisol measures at two months.....	180
6.3.3.1	Infant cortisol stress response at two months	180
6.3.3.2	Infant cortisol measures at two months and maternal cortisol measures during pregnancy.....	182
6.3.3.3	Infant cortisol measures at two months and maternal cortisol measures at two months postpartum	185
6.3.3.4	Infant cortisol measures at two months and maternal rating scales	185
6.3.3.5	Maternal early life adversity and infant cortisol measures at two months..	185
6.3.4	Infant cortisol measures at six months	186
6.3.4.1	Infant cortisol stress response at six months	186
6.3.4.2	Infant cortisol measures at six months and maternal cortisol measures during pregnancy.....	188

6.3.4.3	Infant cortisol measures at six months and maternal cortisol measures at six months postpartum	192
6.3.4.4	Regression analysis of maternal cortisol and infant cortisol measures	194
6.3.4.5	Infant cortisol measures at six months and maternal rating scales.....	198
6.3.4.6	Maternal early life adversity and infant cortisol levels at six months	199
6.3.5	Trajectory of infant cortisol levels from two to six months after birth.....	200
6.4	Discussion	201
6.4.1	Infant birth outcomes	202
6.4.2	Trajectory of the infant HPA axis	203
6.4.3	Maternal antenatal depression and the infant HPA axis	203
6.4.4	Maternal mood postpartum and the infant HPA axis	205
6.4.5	Maternal early life adversity and the infant HPA axis.....	206
6.4.6	Conclusions	207
 CHAPTER 7: Discussion		
7.1	Overview.....	209
7.2	Pregnancy	209
7.3	The postpartum period	213
7.4	Trajectory of maternal HPA axis in the perinatal period	215
7.5	Developmental programming of the HPA axis in infants	216
7.6	Strengths and limitations of study	221
7.7	Future directions	223
7.8	Conclusions	225
 References		226
Appendix I: Study Information Flyer		252
Appendix II: Patient Information Sheet.....		253
Appendix III: Consent Form		257
Appendix IV: Confidential Recruitment Details		258
Appendix V: Participant Characteristics		259
Appendix VI: Saliva Sample Collection Instructions		260
Appendix VII: Perceived Stress Scale.....		261
Appendix VIII: Pittsburgh Sleep Quality Index.....		262
Appendix IX: Centre for Epidemiological Studies Depression Rating Scale.....		263
Appendix X: Childhood Trauma Questionnaire		264
Appendix XI: Hamilton Depression Rating Scale.....		265
Appendix XII: Mini International Neuropsychiatric Interview.....		269

V. List of tables

Table 1.1 DSM-5 diagnostic criteria for Major Depressive Disorder	4
Table 1.2 Studies demonstrating a positive association between clinical depression during pregnancy and the HPA axis	29
Table 1.3 Studies demonstrating no association between clinical depression during pregnancy and the HPA axis	30
Table 2.1 Coding of demographic variables	56
Table 3.1 Sociodemographic characteristics of sample during pregnancy	72
Table 3.2 Rating scale differences between groups during pregnancy	76
Table 3.3 Rating scale scores at two months and six months postpartum	82
Table 3.4 Repeated measures analysis of rating scale measures during pregnancy and at two months postpartum	85
Table 3.5 Distribution of early life adversity	86
Table 3.6 Distribution of early life adversity by caseness groups.....	87
Table 4.1 Maternal cortisol values during pregnancy	101
Table 4.2 Cortisol awakening response variables during pregnancy.....	103
Table 4.3 Differences in maternal cortisol measures during pregnancy by foetal sex.....	105
Table 4.4 Raw cortisone values at five time points from waking during pregnancy	107
Table 4.5 Effect of foetal sex on maternal cortisone levels during pregnancy.....	109
Table 4.6 Effect of foetal sex on maternal cortisone/cortisol ratios during pregnancy	110
Table 4.7 Early life adversity and cortisol levels during pregnancy	111
Table 5.1 Hamilton Depression Rating Scale score at pregnancy, two months, and six months postpartum	132
Table 5.2 Maternal cortisol values at five time points from waking at two months postpartum.....	135
Table 5.3 Maternal cortisol awakening response parameters at two months postpartum.....	135
Table 5.4 Maternal raw cortisol values at five time points from waking at six months postpartum.....	138
Table 5.5 Maternal cortisol awakening response parameters at six months postpartum....	138
Table 5.6 Trajectory of maternal cortisol measures from pregnancy to two months postpartum in the whole sample	140
Table 5.7 Correlation analysis of maternal cortisol measures at pregnancy and at two months postpartum	142
Table 5.8 Trajectory of maternal cortisol measures from pregnancy to six months postpartum.....	145
Table 5.9 Trajectory of maternal cortisol measures from pregnancy to six months postpartum within each group.....	145

Table 5.10 Correlation analysis of maternal cortisol measures at pregnancy and at six months postpartum	148
Table 5.11 Trajectory of maternal cortisol measures from two months to six months postpartum in the whole sample	150
Table 5.12 Trajectory of maternal cortisol measures from two months to six months postpartum within each group.....	150
Table 5.13 Correlation analysis of maternal demographics and cortisol measures at two months postpartum	154
Table 5.14 Correlation analysis of maternal demographics and cortisol measures at six months postpartum within the three groups.....	156
Table 5.15 Effect of early life adversity on cortisol levels at two months postpartum	157
Table 5.16 Effect of early life adversity on cortisol levels at six months postpartum	158
Table 6.1 Infant birth outcomes	173
Table 6.2 Mean differences in infant pre- and post-stressor cortisol levels at two months	174
Table 6.3 Infant cortisol stress responses at two months.....	175
Table 6.4 Correlation analysis of infant cortisol measures at two months and maternal pregnancy cortisol measures	178
Table 6.5 Mean differences in infant pre- and post-stressor cortisol levels at six months..	180
Table 6.6 Infant cortisol stress responses at six months	181
Table 6.7 Correlation analysis of infant cortisol measures at six months and maternal pregnancy cortisol measures	185
Table 6.8 Correlation analysis of infant cortisol measures at six months and maternal cortisol measures at six months postpartum.....	187
Table 6.9a Prediction of infant baseline cortisol at six months from maternal waking cortisol at pregnancy and at six months postpartum	189
Table 6.9b Prediction of infant baseline cortisol at six months from maternal total morning cortisol output at pregnancy and at six months postpartum	189
Table 6.9c Prediction of infant baseline cortisol at six months from maternal cortisol awakening response at pregnancy and at six months postpartum	190
Table 6.9d Prediction of infant post-stressor cortisol at six months from maternal waking cortisol at pregnancy and at six months postpartum	190
Table 6.9e Prediction of infant post-stressor cortisol at six months from maternal total cortisol output at pregnancy and at six months postpartum	191
Table 6.9f Prediction of infant post-stressor cortisol at six months from maternal cortisol awakening response at pregnancy and at six months postpartum	191
Table 6.10 Correlation analysis between infant cortisol at six months and maternal rating scales during pregnancy and six months postpartum.....	192
Table 6.11 Analysis of infant cortisol measures between two months and six months.....	194

VI. List of figures

Figure 1.1 Hormonal changes during pregnancy and the first 12 weeks postpartum	3
Figure 1.2 Diagrammatic representation of HPA axis activity	13
Figure 1.3 Diurnal variation of cortisol	14
Figure 1.4 The HPA axis during pregnancy	20
Figure 1.5 CRH and CRH-BP levels during gestation	21
Figure 2.1 Summary of study timeline	52
Figure 2.2 Cortisol awakening response parameters	62
Figure 2.3 Conceptual model of moderator interaction	64
Figure 2.4 Statistical model of moderator analysis	64
Figure 3.1 Recruitment outcome for each caseness group	73
Figure 3.2 Medication use during pregnancy	75
Figure 3.3 Mood and sleep rating scale scores during pregnancy	77
Figure 3.4 Childhood Trauma Questionnaire scores	78
Figure 3.5 Study participants at each time point	80
Figure 3.6 Mood and sleep rating scale scores at two months postpartum	83
Figure 3.7 Mood and sleep rating scale scores at six months postpartum	84
Figure 4.1 Diurnal variation of cortisol during pregnancy	102
Figure 4.2 Cortisol awakening response during pregnancy	102
Figure 4.3 Diurnal variation of cortisone during pregnancy	107
Figure 4.4 Correlations between maternal cortisol and cortisone levels during pregnancy	108
Figure 4.5 Early life adversity and maternal evening cortisol levels	112
Figure 4.6 Association between maternal early life adversity and evening cortisol levels during pregnancy	107
Figure 4.7 Moderation effect of foetal sex on the relationship between emotional abuse and maternal evening cortisol levels	116
Figure 4.8 Association between maternal cortisone/cortisol ratio at waking and early life adversity	118
Figure 4.9 Association between evening cortisone/cortisol ratio and early life adversity	119
Figure 5.1 Number of participants at each time point	133
Figure 5.2 Maternal diurnal variation of cortisol at two months postpartum	136
Figure 5.3 Maternal cortisol awakening response at two months postpartum	136
Figure 5.4 Maternal diurnal variation of cortisol at six months postpartum	139
Figure 5.5 Maternal cortisol awakening response at six months postpartum	139
Figure 5.6 Diurnal variation in maternal cortisol at pregnancy and two months postpartum in the whole sample	141

Figure 5.7 Diurnal variation in maternal cortisol at pregnancy and two months postpartum within each group	141
Figure 5.8 Whole sample correlation analysis between slope of regression line during pregnancy and slope at two months postpartum	143
Figure 5.9 Diurnal variation in maternal cortisol at pregnancy and six months postpartum in the whole sample	146
Figure 5.10 Diurnal variation of maternal cortisol levels at pregnancy and six months postpartum within each group.....	146
Figure 5.11 Diurnal variation in maternal cortisol at two months and six months postpartum in the whole sample	151
Figure 5.12 Diurnal variation in maternal cortisol at two months and six months postpartum within each group	151
Figure 5.13 Correlations between maternal age and cortisol measures at two months postpartum within the whole sample	153
Figure 5.14 Whole sample correlation analysis of maternal age and cortisol reactivity at six months postpartum	156
Figure 6.1 Number of infants at each study time point	171
Figure 6.2 Infant cortisol response to vaccination stressor at two months	175
Figure 6.3 Associations between infant post-vaccination cortisol at two months and maternal cortisol measures during pregnancy in the whole sample	177
Figure 6.4 Infant cortisol response to a vaccination stressor at six months	181
Figure 6.5 Association between infant baseline cortisol at six months and maternal cortisol measures during pregnancy	183
Figure 6.6 Association between infant cortisol reactivity at six months and maternal cortisol during pregnancy	184
Figure 6.4 Infant cortisol response to a vaccination stressor at six months	181

VII. List of abbreviations

11 β -HSD-2	11 beta-hydroxysteroid dehydrogenase-2
ACTH	Adrenocorticotrophic hormone
ALSPAC	Avon Longitudinal Study of Parents and Children
ANOVA	Analysis of variance
B1	Two-month time point (infant)
B2	Six-month time point (infant)
CAR	Cortisol awakening response
CBG	Corticosteroid-binding globulin
CES-D	Centre for Epidemiological Studies Depression rating scale
CRH	Corticotrophin-releasing hormone
CRH-BP	Corticotrophin-releasing factor-binding protein
CTQ	Childhood Trauma Questionnaire
CWIUH	Coombe Women and Infants University Hospital
DSM-5	Diagnostic and Statistical Manual of Mental Disorder, 5 th edition
EPDS	Edinburgh Postnatal Depression Scale
GR	Glucocorticoid receptor
HAM-D	Hamilton Depression rating scale
HPA	Hypothalamic-pituitary-adrenal
IL-1 β	Interleukin-1beta
IL-6	Interleukin-6
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LLE	Liquid-liquid extraction
LTC	Latent trait cortisol
MDD	Major depressive disorder
MINI	Mini International Neuropsychiatric Interview
MR	Mineralocorticoid receptor
MTBE	Methyl-tert-butyl ether

NMH	National Maternity Hospital
P	Pregnancy
PP1	Two months postpartum time point (mother)
PP2	Six months postpartum time point (mother)
PSQI	Pittsburgh Sleep Quality Index
PSS	Perceived Stress Scale
SCID	Structured Clinical Interview for DSM-5
TCD	Trinity College Dublin
TCIN	Trinity College Institute of Neuroscience
TNF- α	Tumour necrosis facror alpha

Chapter 1

Introduction

1.1 Overview

The perinatal period is comprised of the period of gestation and the following year postpartum (Scottish Intercollegiate Guidelines Network, 2012). During this time, a range of maternal systems undergo substantial alterations to provide for the needs of the foetus and to prepare for lactation. Pregnancy, with the physical, physiological, and psychological changes it brings, can be a challenging time in a woman's life. Human pregnancy has a gestational length of 37 to 42 weeks, calculated from the first day of the last menstrual period, and is divided into three trimesters. Implantation, embryonic growth, and placental formation take place during the first trimester. It is a period of rapid foetal growth, with many foetal organs developing during this time. The first trimester is also a period of high risk for miscarriage and developmental defects if exposed to teratogens. Growth continues in the second and third trimesters and is regulated by placental and maternal pituitary hormones (Newbern and Freemark, 2011).

Pregnancy sees an increase in several hormones, including progesterone and oestrogen, and the hypothalamic-pituitary-adrenal (HPA) axis hormones corticotrophin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), and cortisol [Figure 1]. After delivery, there is an immediate drop in progesterone and oestrogen, and the HPA axis hormones decrease slowly over several days [Figure 1]. These hormonal changes are thought to play a role in the development of perinatal mood disorders, which may lead to adverse foetal outcomes.

A summary of Major Depressive Disorder (MDD) will be presented in **Section 1.2**, and perinatal depression in **Section 1.3**. The HPA axis changes in MDD will be described in **Section 1.4**. The HPA axis in the perinatal period and in perinatal depression will be discussed in **Section 1.5**. The foetal and infant HPA axes will be detailed in **Section 1.6**. The evidence for foetal programming will be summarised in **Section 1.7**, and the implications of maternal early life adversity in the perinatal period in **Section 1.8**. The aims and structure of this thesis will be outlined in **Section 1.9**.

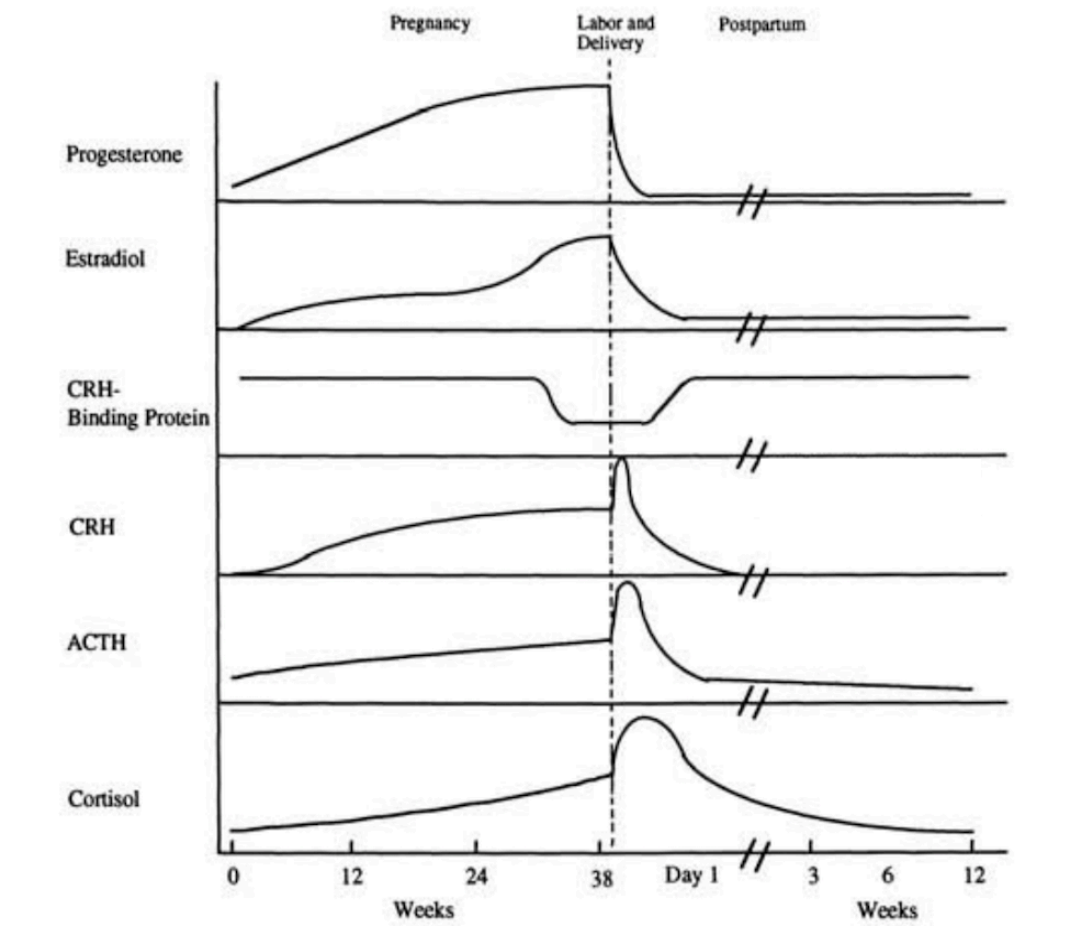


Figure 1.1 Hormonal changes during pregnancy and the first 12 weeks postpartum
CRH – Corticotrophin-releasing hormone, **ACTH** – Adrenocorticotrophic hormone
 (From Chrousos et al., 1998)

1.2 Major depressive disorder

MDD is a prevalent disorder, and is projected to be the second leading cause of global illness by 2030 (Mathers and Loncar, 2006). A complex interplay of genetic, developmental and environmental factors is implicated in the aetiology of MDD. The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (American Psychiatric Association, 2013) requires a two-week period of at least one core, and five other symptoms [described in Table 1.1] for a diagnosis of MDD to be made.

A categorical approach to depression such as the DSM-5 diagnostic criteria is important, particularly in clinical research where consistency is paramount. However, patients may sometimes present with subsyndromal depressive features that do not fulfil DSM-5 criteria for MDD. While a diagnosis of adjustment disorder or persistent depressive disorder may be helpful in these patients, a dimensional approach to depressive illness is often warranted in clinical practice. The DSM-5 describes certain specifiers for a major depressive episode, including anxious distress, mixed features, melancholic features, atypical features, psychotic features (which may be mood-congruent or mood-incongruent), catatonia, seasonal pattern, and finally, of interest in this study, peripartum onset.

Table 1.1 DSM-5 diagnostic criteria for Major Depressive Disorder

Two-week history of at least one core and five other symptoms	
Core symptoms	- Depressed mood - Markedly diminished interest or pleasure in activities
Other symptoms	- Significant unintentional change in weight or appetite - Insomnia or hypersomnia - Psychomotor agitation or retardation - Loss of energy - Feelings of worthlessness or inappropriate guilt - Indecisiveness or difficulty with concentration - Recurrent thoughts of death, suicidal ideation or plans
Other criteria	- Symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functionality - Symptoms not due to direct physiological effects of a substance or another medical condition - Occurrence of MDD not better explained by schizoaffective disorder or other psychotic disorders and there has never been a manic or hypomanic episode

1.2.1 Epidemiology of major depressive disorder

There is a female preponderance for depression, with female to male prevalence ratios of 2:1 (Kessler, 2003). With a lifetime prevalence of 16.5% for depression, there is a 20% prevalence rate in women, compared to 12% in men (Kessler et al., 1993, 2005; Rich-Edwards et al., 2006). Mean length of a depressive episode has been reported to be 16 weeks, with mean age of onset in the early to mid-twenties (Andrade et al., 2003; Kessler et al., 2003). Sociodemographic risk factors for developing depression include lower socioeconomic status and poverty, urban living, and unemployment (Andrade et al., 2003; Kessler et al., 2003).

1.2.2 Aetiology of major depressive disorder

MDD has a multifactorial aetiology, but the pathogenesis has not been fully elucidated. The clinical heterogeneity of MDD lends support to the hypothesis that different clinical subtypes of depression have different aetiologies. Genetic factors are known to play a strong role in the aetiology of depression, with a 30-40% influence on the development of depression (Sullivan et al., 2000). Environmental and developmental effects, such as childhood adversity, combined with a genetic loading, can increase the risk of developing depression (Kendler et al., 2002, 2006). Dysregulation of the HPA axis and glucocorticoid receptor (GR) function, epigenetic modifications of this system, and immune mechanisms have all been implicated in the pathogenesis of depression (Dantzer et al., 2008; Pariante and Lightman, 2008). The monoamine-deficiency hypothesis suggests depletion of the neurotransmitters serotonin, dopamine, or noradrenaline levels in the central nervous system as an aetiological basis for development of depression; other studies have reported altered GABAergic and glutamatergic neurotransmission (Hasler et al., 2007).

1.2.3 Management of major depressive disorder

Depression treatment may be categorised into biological, psychological, and social therapies. Biological treatment, including medication and electroconvulsive therapy, is the mainstay of treatment for moderate to severe depressive episodes. Mild depressive episodes are best treated with psychological therapies such as cognitive behavioural therapy (Cleare et al., 2015). Good practice indicates that a combination of treatment

modalities, involving examination of various psychosocial issues in the individual, is optimal. Complete remission is achieved in 70-90% of patients with depression, with 10-30% of patients remaining treatment-resistant (Keller, 2005; Sackeim, 2001).

Classes of antidepressant medication used in depression treatment include selective serotonin reuptake inhibitors, serotonin noradrenalin reuptake inhibitors, noradrenergic and specific serotonergic antidepressants, tricyclic antidepressants, and monoamine oxidase inhibitors. Although most antidepressants increase levels of serotonin, they are thought to have multi-modal function in targeting symptoms of depression, which in itself has multi-modal aetiology. Mood stabilisers and antipsychotics are often used adjunctive to antidepressants in treatment resistant depression. Physical therapies such as electroconvulsive therapy are an effective treatment option in severe depression (Cleare et al., 2015).

1.2.4 Impact of major depressive disorder

Depressive disorders impose considerable burden on patients. The disorder itself, in its relapsing and remitting nature, can lead to cognitive impairment, residual symptoms, and can impact on quality of life of patients. In addition to this, there is an increased risk of mortality with cardiovascular disease, cerebrovascular disease, and indeed, suicide. A Swedish population-based study found standardised mortality ratio (SMR) for suicide to be 20.9 in men and 27.0 in women, indicating that men and women with depression are 20.9 and 27 times, respectively, more likely to complete suicide than the general population (Osby et al., 2001). In addition to mortality burden, depression can lead to disability and economic burden with lost work days (Lépine and Briley, 2011).

1.3 Perinatal depression

1.3.1 Antenatal depression

Pregnancy is a period of heightened vulnerability for developing depression. The DSM-5 provides a 'peripartum onset' indication for major depression in the perinatal period, defined as the onset of depressive symptoms during pregnancy or in the first four weeks after delivery (American Psychiatric Association, 2013). The symptoms of depression remain the same as for MDD as described in Section 1.2 [Table 1.1]. Somatic symptoms of healthy pregnancy such as disrupted sleep, appetite, and energy levels are often considered to confound a diagnosis of MDD in the perinatal period, and may consequently be disregarded (Yonkers, Vigod, et al., 2011). However, Kelly and colleagues (2001) reported that women experiencing symptoms of depression or anxiety during pregnancy were more likely to report somatic symptoms (Kelly et al., 2001). Other studies have found that depressed women had more sleep disturbance than their non-depressed counterparts in the second and third trimesters of pregnancy, and that somatic symptoms, aside from appetite changes, are effective indicators of depression during pregnancy (Field et al., 2007; Nylen et al., 2013; Tomfohr et al., 2015). Somatic symptoms appear to be more enhanced in women experiencing depression during pregnancy; it would be prudent to include them in the diagnostic criteria, as recommended in the DSM-5.

1.3.1.1 Epidemiology of antenatal depression

Research on the prevalence rates of antenatal depression show varying rates worldwide, dependent on the method used to identify depression, socioeconomic status, and other methodological factors. A systematic review by Bennett and colleagues (2004) evaluating interview and self-report depression data from worldwide studies, found prevalence rates of 7.4% in the first trimester, 12.8% in the second trimester, and 12% in the third trimester. In another systematic review of studies using clinical diagnostic criteria for antenatal depression, Gavin and colleagues (2005) found a period prevalence of 18.4% during pregnancy, with 12.7% of these women having a major depressive episode. Fisher et al (2012) published a systematic review looking at studies from low- and middle-income countries, and found a 15.9% weighted mean prevalence of depression during pregnancy, increasing to 21.7% in studies using

diagnostic assessments. The Avon Longitudinal Study of Parents and Children (ALSPAC) in the UK found prevalence rates of 11.8% in the second trimester using the Edinburgh Postnatal Depression Scale (EPDS) as a screening tool (Evans et al., 2001). The EPDS is a commonly used screening tool for detecting possible depression in the perinatal period. However, it is important to note that it is not a diagnostic tool. Third trimester studies in the UK have found rates of 29% in a lower socioeconomic status population (Bolton et al., 1998), 9.8% (Johanson et al., 2000), and 13.5% (Evans et al., 2001).

A recent study of antenatal depression in Brazil found rates of 13.5% and 10.1% in the second and third trimester, respectively, using the Mini International Neuropsychiatric Interview (MINI), a clinically diagnostic tool (Castro E Couto et al., 2016). In the same study, much higher rates of 33.33% and 27.93% in the second and third trimester, respectively, were found when an EPDS score >11 was used to identify probable antenatal depression. Ashley et al (2016), in a US population study, reported similar rates of major depression in pregnant women compared to non-pregnant women, but higher rates of minor depression in pregnant women (16.6%) compared to non-pregnant women (11.4%). Our group conducted an anonymised multi-site Irish population survey of prevalence rates of antenatal depression using the EPDS (Jairaj et al., In preparation). Of the 5000 women who completed the EPDS questionnaire in obstetric services across Ireland, 15.8% scored above 12, indicating probable antenatal depression. In line with existing literature, prevalence rates of depression in that study increased with increasing gestational age.

1.3.1.2 Aetiology of antenatal depression

Study of the aetiology of antenatal depression, unlike that of MDD, is not extensive. Since pregnancy is an exceptional period of altered physiological systems, the aetiology of depressive illness during pregnancy is thought to be multifactorial. The HPA axis, as in MDD, is thought to play an important role in the aetiology of antenatal depression (Pariante, 2014). There are remarkable changes in the HPA axis during pregnancy, including an exponential rise in CRH and cortisol [described in detail in Section 1.5]. Levels of the steroid hormones oestrogen and progesterone are known to increase during pregnancy, and may contribute to the aetiology of antenatal

depression. However, O'Keane and colleagues (2011), in a study of hormonal changes in antenatal depression, found no differences in progesterone between depressed and non-depressed pregnant women.

The maternal immune system also undergoes substantial changes during normal pregnancy to prevent rejection of the foetal semi-allograft, and is thought to be another mechanism for the pathogenesis of antenatal depression. There is a mild elevation of both pro- and anti-inflammatory cytokines during healthy pregnancy (Curry et al., 2008; Kraus et al., 2010; Opsj n et al., 1993; Sharma et al., 2007; Vassiliadis et al., 1998). As inflammation is known to be associated with depression (Leonard and Maes, 2012), four studies have examined the role of cytokines in perinatal depressive symptoms. A positive correlation between IL-6 and IL-1  and self-rated depression scores, with no change in TNF-  levels was reported in some studies (Cassidy-Bushrow et al., 2012; Christian et al., 2009). However, no association was observed between depressed mood and IL-6 or TNF-  in one study using a clinician-rated depression scale (Blackmore et al., 2011).

A history of any psychopathology or ongoing psychosocial difficulties confer increased susceptibility for development of depression during pregnancy (Howard et al., 2014). Hormonal changes, in addition to physical, psychological and social changes, contribute to the increased vulnerability in this period (Bennett et al., 2004; Parry and Newton, 2001). Risk factors for developing depression during pregnancy include a past history of depression, life stress, poor social supports, unplanned pregnancy, domestic violence, lower socioeconomic status, maternal age, maternal race/ethnicity, lower educational attainment, lower income, unemployment, nulliparity, and a history of obstetric complications (Lancaster et al., 2010; O'Keane and Marsh, 2007).

1.3.1.3 Management of antenatal depression

An important aspect of the treatment of antenatal depression is the consideration that must be given to foetal health and the avoidance of any medication with teratogenic potential. Treatment of antenatal mood disorders is dependent on maternal treatment preferences, and may require a combination of psychotherapy and pharmacotherapy. Interpersonal therapy and CBT have shown some benefit in antenatal depression (Howard et al., 2014). Antidepressants are the mainstay of pharmacological treatment

of antenatal depression. However, due to ethical limitations for research on medication use during pregnancy, there have been no trials of antidepressant use for antenatal depression. Antidepressants are sometimes discontinued during pregnancy due to concerns about risks to the foetus. The evidence suggests increasing risk of relapse with antidepressant discontinuation during pregnancy, most evident in women with ≥ 4 previous episodes of depression, and in Black and Hispanic women (Cohen et al., 2006; Yonkers, Gotman, et al., 2011).

1.3.1.4 Adverse obstetric and birth outcomes

Antenatal depression may be associated with adverse obstetric and birth outcomes. Although there is a strong evidence base for poor infant birth outcomes in women diagnosed with depression during pregnancy, findings in the literature on obstetric outcomes in women diagnosed with depression vary. The authors of a systematic review of gestational diabetes and depression suggest that further studies are necessary to describe depression as a risk factor for developing gestational diabetes, as there is no consensus in study results to date (Ross et al., 2016).

However, studies examining antenatal depression and preeclampsia show a clear association between the two. One study examining the impact of depression and anxiety on preeclampsia risk included 109 women with pre-gestational depression or newly diagnosed depression in the first 20 weeks of gestation, and 32 women with similarly diagnosed anxiety (Qiu et al., 2009). A 2-fold increase in the risk of preeclampsia was observed in women diagnosed with a mood or anxiety disorder. Women with antenatal depression were found to have higher rates of preeclampsia and caesarean section deliveries in another study (Hu et al., 2015). Reduced placental perfusion due to genetic, behavioural, or environmental factors is thought to increase the risk of preeclampsia (Palmsten et al., 2012). In recent years, systemic inflammation and oxidative stress have also been implicated in the aetiology of preeclampsia (Nicholson et al., 2016). In view of the robust evidence for systemic inflammation in depression (Miller and Raison, 2016; Zunszain et al., 2012), it is possible that the increased risk of preeclampsia in depression is driven by inflammatory processes.

Evidence for adverse birth outcomes in women experiencing antenatal depression is more robust, with a meta-analysis report suggesting that antenatal depression was associated with increased risk of low birth weight (<2.5 kg) in developing countries, and in lower socioeconomic status populations in developed countries (Grote et al., 2010). There is also evidence for an increased risk of preterm birth (<37 weeks) in women diagnosed with depression during pregnancy (Eastwood et al., 2017; Grote et al., 2010). Antenatal depression therefore has implications for maternal obstetric and neonatal health.

1.3.2 Postpartum depression

MDD in the postpartum period has been well researched since the seminal work by Ian Brockington in the 1980s (Brockington, 1988; Brockington et al., 1988). Clinical features of MDD in the postnatal period are no different to the features of MDD outside the peripartum period. DSM-5 describes the postpartum period as the first four weeks after delivery - a contested time frame, as postpartum depression is often defined as the onset of depression in the first year after delivery (Pariante et al., 2014).

In a systematic review of the prevalence of perinatal depression, Gavin and colleagues (2005) estimated the point prevalence rate of postpartum depression to lie between 6.5% and 12.9% over the first year postpartum, with a period prevalence of 19.2% over the first three months postpartum. In another systematic review, postpartum depression prevalence was 19.8%, reducing to 16.1% when only studies utilising diagnostic assessments were included (Fisher et al., 2012). In fact, one study reported that about 38% of women experience depression over the first eight years of their child's life (Turney, 2012).

Despite its high prevalence rates, there is currently limited evidence for the aetiology of postpartum depression. Alterations in oestrogen and progesterone postpartum have been implicated in some studies (Bloch et al., 2000, 2003), while others lend support to the hypothesis that HPA axis perturbations postpartum [detailed in Sections 1.5.3 and 1.5.6] underlie the pathogenesis of postpartum depression (Magiakou, Mastorakos, Rabin, Dubbert, et al., 1996; Meltzer-Brody, 2011; Workman et al., 2012).

Management of postpartum depression is similar to non-perinatal depression management, with further considerations made for breastfeeding mothers, as some medications may pass in potentially unsafe amounts in breast milk.

1.4 The HPA axis in humans

The HPA axis plays a primary role in the neuroendocrine stress response system in humans [Figure 1.1]. In response to stress, the paraventricular nucleus (PVN) of the hypothalamus releases corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP). CRH, thought to drive the central HPA axis stress response with amplification by AVP (Gilles et al., 1982), enters pituitary portal blood and stimulates corticotroph cells of the anterior pituitary gland to produce adrenocorticotrophic hormone (ACTH). ACTH then acts on the adrenal cortex in humans to stimulate secretion of the glucocorticoid, cortisol (Dinan and Scott, 2005). The HPA axis is regulated by feedback inhibition with cortisol, which exerts direct negative feedback on the glucocorticoid receptors in the pituitary gland and the hypothalamus (Lightman and Conway-Campbell, 2010). Transient elevations in cortisol in response to an acute stressor are essential in maintaining homeostasis and adapting the individual to the environment. Cortisol has several effects on the body including neurogenesis, neuronal survival, neuronal excitability and memory acquisition, as well as metabolic and immune effects such as gluconeogenesis, peripheral insulin resistance and anti-inflammatory properties (Pariante and Lightman, 2008). Only 5% of cortisol in the blood is unbound and active, and 95% is bound to carrier proteins such as the corticosteroid binding globulin (CBG). CBG becomes saturated at 400-500 nMol/L of serum cortisol, indicating a higher percentage of free cortisol availability when plasma cortisol levels rise above this concentration. (Ballard, 1979; Lightman and Conway-Campbell, 2010). The adult reference range of serum cortisol in the morning, although variable between laboratories, is 100-600 nMol/L.

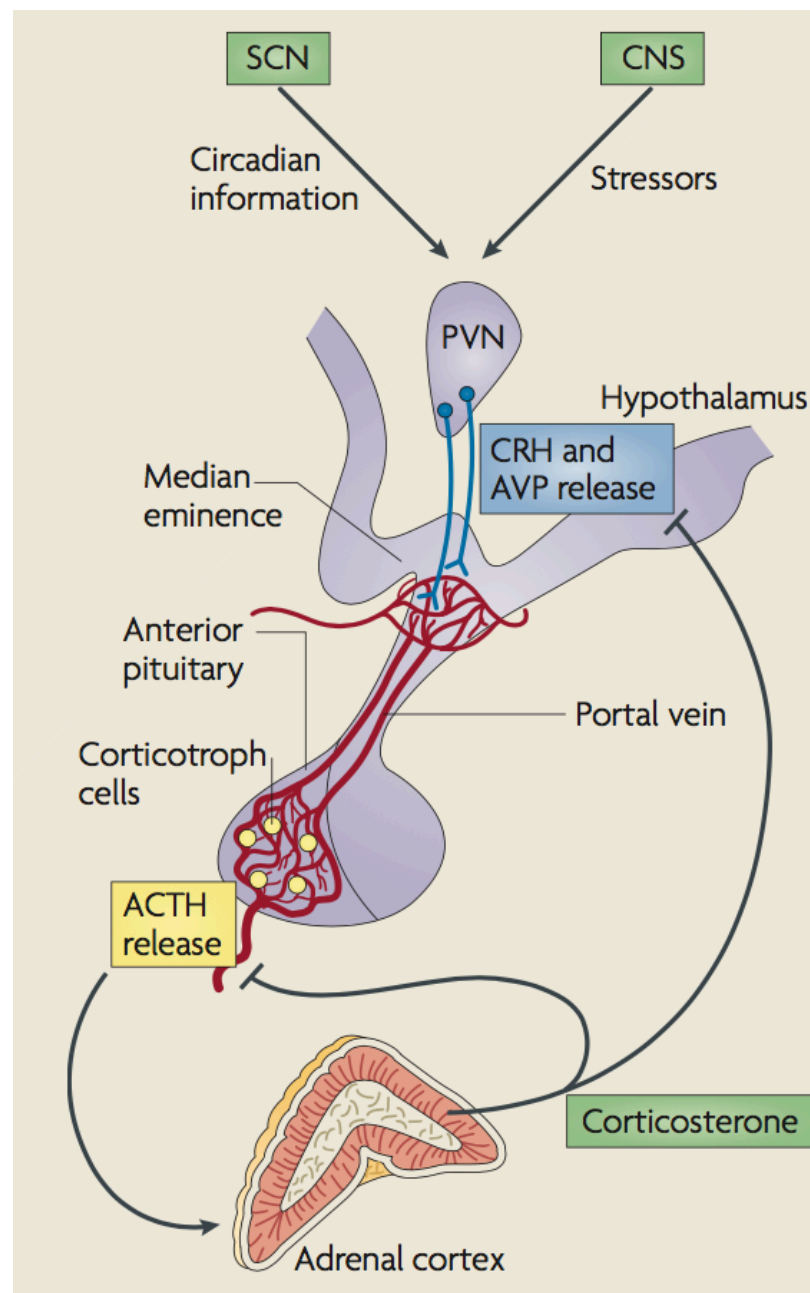


Figure 1.1 Diagrammatic representation of hypothalamic-pituitary-adrenal axis activity in humans. (Adapted from Lightman and Conway-Campbell, 2010)

SCN – suprachiasmatic nucleus of the hypothalamus, **CNS** – central nervous system, **PVN** – paraventricular nucleus of the hypothalamus, **CRH** – corticotrophin-releasing hormone, **AVP** – arginine vasopressin, **ACTH** – adrenocorticotrophic hormone.

Diurnal variation of cortisol

The circadian rhythm in humans and other mammals has evolved over time to align behaviours and physiological processes to the earth's geophysical rhythm. The circadian rhythm plays a role in preparing the body for daily recurring events such as awakening, by structuring the body's biochemistry to align with the rhythmic environment of the earth (Albrecht, 2011; Vaze and Sharma, 2013). In humans, cortisol follows a circadian rhythm with distinct diurnal secretion, regulated by the suprachiasmatic nucleus of the hypothalamus. Cortisol secretion is lowest around midnight, and begins to increase again towards the end of the sleep cycle, peaking around 30-45 minutes after awakening [Figure 1.2] (Pruessner et al., 1997). There is emerging evidence for an ultradian rhythm with cortisol within the diurnal rhythm, with an hourly release of cortisol pulses (Lightman and Conway-Campbell, 2010). The amplitude of these pulses are thought to influence the pattern of diurnal variation, and dysregulation of this intrinsic pulsatile activity of the HPA axis is thought to be associated with an increased incidence of depression (Pariante and Miller, 2001; Young et al., 2004).

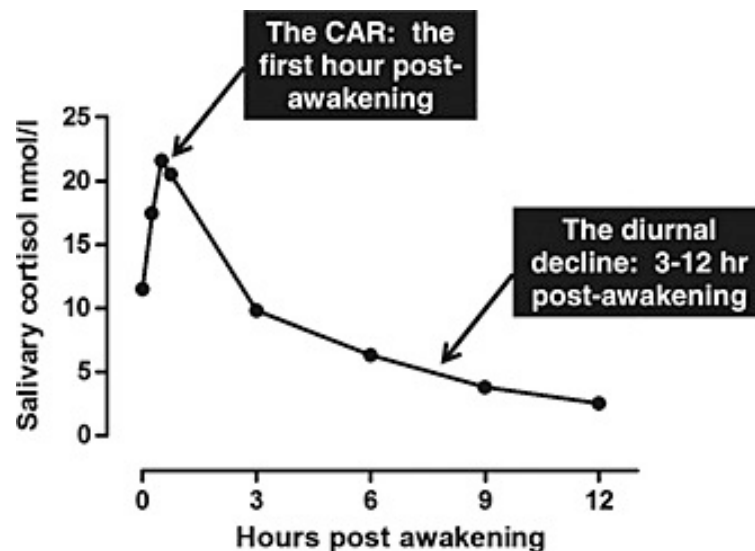


Figure 1.2 Diurnal variation in cortisol

The diurnal curve of cortisol, with the cortisol awakening response (CAR) in the first hour after awakening followed by a decline in levels over the course of the day. (From Smyth et al., 1997)

Cortisol awakening response

The cortisol awakening response (CAR) is a discrete part of the diurnal rhythm, defined by a rapid 50-160% increase in cortisol secretion over the first 45-60 minutes after awakening [Figure 1.2] (Clow et al., 2004). Initiated in response to awakening, it is thought to be a physiological response to mobilise the body from the resting state to a wakeful one (Pruessner et al., 1997; Wilhelm et al., 2007). There is a rapid attainment of consciousness upon awakening, followed by a period of 20-30 minutes where full alertness is reached. During this process, there is a rapid transition of activation in cortical and sub-cortical regions of the brain, which is thought to be associated with the initiation of the CAR (Clow et al., 2010). With awakening being comparable to a mild stressor, the CAR has been presented as an index of stress reactivity (Clow et al., 2010).

The hippocampus, with its high abundance of glucocorticoid and mineralocorticoid receptors (GR and MR), plays a key role in negative feedback of the HPA axis and regulation of the CAR (Fries et al., 2009). It is thought that marked activation of the hippocampus during sleep has an inhibitory effect on the HPA axis, and a switching off of the hippocampal effect on the HPA axis upon awakening leads to the rise in cortisol seen upon awakening (Clow et al., 2010). The CAR is also under the influence of the suprachiasmatic nucleus of the hypothalamus, which regulates the circadian rhythm and is sensitive to light (Clow et al., 2010).

The CAR was initially considered to be a stable individual trait marker (Fries et al., 2009; Pruessner et al., 1997). However, more recent evidence on the increased intra-individual state variability of CAR has brought this into question. Early time of awakening, negative experiences on the previous day, anticipation of the day ahead, increased levels of ambient light, jet lag, alcohol consumption, and ovulatory period of the menstrual cycle have all been found to be positively associated with the amplitude of the CAR (Law et al., 2013). A number of psychosocial and medical factors also influence the CAR in healthy individuals, but the findings on these are inconsistent. Stress, increasing age, and medical factors including cardiovascular disease, autoimmune disease, Alzheimer's disease have all been found to increase waking cortisol levels (Clow et al., 2010). Conversely, post-traumatic stress disorder is known to decrease waking cortisol levels (Fries et al., 2009).

1.4.1 The HPA axis in depression

A consistent biological finding in MDD is hyperactivity of the HPA axis (Holsboer, 2000). In an early study of the HPA axis in MDD, Sachar and colleagues (1970) found that cortisol levels correlated with measures of emotional arousal in depressed patients. Other studies followed, showing increased levels of CRH in cerebrospinal fluid and pituitary enlargement in MDD (Krishnan et al., 1991; Nemeroff et al., 1984). State-dependent adrenal hypertrophy was reported in one study, where the adrenal gland reverted to its normal size after resolution of depressive symptoms (Rubin et al., 1995).

The GR is implicated in the dysregulation of the HPA axis in depression. The GR, with its high affinity for exogenous glucocorticoids and lower affinity for endogenous glucocorticoids, is particularly important in regulating the HPA axis activity in the presence of high levels of endogenous glucocorticoids (Pariante and Lightman, 2008). Impairment of GR function, subsequently leading to disruption of cortisol-mediated negative feedback, is thought to explain HPA axis hyperactivity in depression (Anacker et al., 2011; O'Keane et al., 2012). In healthy individuals administered glucocorticoids, negative feedback to the HPA axis is executed through limbic GR, leading to a reduction in cortisol production. This HPA axis suppression in response to the administration of dexamethasone, an exogenous glucocorticoid, can be impaired in depressed patients (Arana et al., 1985). Evidence suggests that HPA axis dysregulation may be a result of early life experiences or adversity, which may have long-lasting effects on the HPA axis, predisposing the individual to depression in later life (Pariante and Lightman, 2008).

Salivary cortisol, despite the large intra- and inter-individual variability, can be a useful biomarker for assessing HPA axis function, particularly considering the ease of sample collection (Hellhammer et al., 2009). Variation in diurnal cortisol and the CAR in depressed individuals has been the subject of many studies, but the findings remain diverse. Increased waking cortisol and a flat diurnal curve in inpatients diagnosed with depression have been described in some studies, but these differences may not be as prominent in community samples, where patients would have lower illness severity (Dienes et al., 2013; Holsboer, 2000; Plotsky et al., 1998). A meta-analysis found small but significantly increased mean morning and evening cortisol values in studies of

depressed individuals compared to controls, with higher differences being reported in studies of higher depression severity (Knorr et al., 2010). The results of one study examining a community sample of 1,588 individuals indicated higher evening cortisol levels in patients with MDD than in controls (Vreeburg et al., 2009). Although there is some conflicting evidence for changes in CAR in depression, the evidence in general indicates that there is increased total morning cortisol output, as well as an increase in the CAR itself. Vreeburg and colleagues (2009) reported higher waking cortisol, total morning cortisol output, and CAR in depressed patients compared to controls. Other studies have variably reported increase in CAR (Bhagwagar et al., 2005) or blunted CAR in depressed patients (Jarcho et al., 2013). The finding of a blunted CAR in some depression studies may indicate lower depression severity, use of antidepressants, comorbid post-traumatic stress disorder, or atypical depressive symptoms.

These discrepancies in cortisol studies may be explained by the finding that free cortisol levels may not be a linear correlate of HPA axis functional activity (Hellhammer et al., 2009). Additionally, the high variability in salivary cortisol levels together with methodological differences in sampling may explain the paradoxical findings. Consistent methodology in collection of salivary cortisol with respect to timing and controlling for potential confounders may help to limit variability seen in cortisol studies.

The evidence presented above suggests a state effect of current depression on an individual's HPA axis. Further, it is postulated that a lifetime episode of depression may have a 'kindling' effect, predisposing an individual to recurrent episodes (Monroe and Harkness, 2005). It is therefore conceivable that a lifetime episode of depression may have a trait or 'scarring' effect on the HPA axis. The authors of three studies that have examined this hypothesis report higher cortisol levels in remitted MDD patients compared to those with no history of depression (Bhagwagar et al., 2003; Lok et al., 2012; Vreeburg et al., 2009), lending support to this hypothesis.

1.5 The HPA axis in the perinatal period

There are significant adaptations in the neuroendocrine stress system during the perinatal period [Figure 1.3]. These alterations are thought to adapt the maternal systems to gestation and lactation, and aid in foetal development.

1.5.1 The HPA axis during pregnancy

Placental CRH

The key instigator of changes to the HPA axis during pregnancy is the formation and growth of the placenta, which, uniquely in humans and higher primates, begins to produce placental CRH early in gestation (Mastorakos and Ilias, 2000; McLean and Smith, 1999; Robinson et al., 1989). CRH was first identified in sheep hypothalamus in 1981, and a year later, in the human placenta (Vale et al., 1981). Hypothalamic and placental CRH are identical in structure and both act on anterior pituitary corticotrophs (Smith and Thomson, 1991). There are two major forms of the CRH receptor – CRH-R₁ and CRH-R₂ (Chen et al., 1993). CRH-R₁ receptors can be found in large numbers in neocortical, cerebellar and sensory relay structures, whereas CRH-R₂ receptors are found in specific subcortical areas and peripheral tissues (Mastorakos and Ilias, 2003).

The placenta, along with the decidua and chorioamniotic membranes, produces CRH and stimulates the maternal HPA axis (Mastorakos and Ilias, 2003) [Figure 1.3]. CRH begins to be synthesised by placental syncytial cells around the 7th week of gestation (Riley and Challis, 1991) and circulating levels of maternal CRH begin to increase between the 8th and 10th weeks of gestation, rising exponentially during the last 6 weeks of pregnancy and peaking before delivery (Goland et al., 1988; Mastorakos and Ilias, 2000). Plasma CRH levels increase to 1,000 to 10,000 times higher than those of non-pregnant women (Thomson, 2013). The bioactivity of circulating CRH in pregnancy is influenced by corticotrophin-binding protein (CRH-BP), which binds to CRH with high affinity and renders it biologically inactive. CRH-BP is present in high concentrations through most of pregnancy, impeding the bioactivity of placental CRH until about 4-6 weeks before the onset of parturition, at which time CRH-BP levels begin to fall [Figure 1.4] (Linton et al., 1993; McLean et al., 1995). The rising CRH level

and the dropping CRH-BP level converge at a point about 3-5 weeks before the onset of spontaneous labour, with exponentially increased CRH levels coinciding with the gestational changes preparing the mother for parturition (McLean et al., 1995). There is no evidence for an association between these high CRH levels and depressive symptoms during the perinatal period (Zaconeta et al., 2015).

The placenta, myometrial smooth muscle, foetal membranes and foetal adrenal glands are all tissue targets of CRH during pregnancy (Grammatopoulos, 2008). Placental CRH plays a key role in the induction of labour, and will be discussed further in Section 1.5.1. High CRH levels in the second trimester are postulated to predict preterm birth, and women with low CRH levels in the second trimester were found to deliver post-term (Challis, 1998; Smith and Nicholson, 2007). McLean and colleagues (1995) propose that the length of gestation is determined at the start of pregnancy by a 'placental clock' which is controlled by the production of CRH (Challis, 1998; Smith, 1998). Furthermore, the placental trophoblast, in its juxtaposed position between maternal and foetal circulations, may be influenced by humoral factors from both mother and foetus (McLean et al., 1995).

Unlike hypothalamic CRH, placental CRH does not follow a circadian rhythm (Magiakou et al., 2000; Mastorakos and Ilias, 2003), and cortisol promotes, rather than inhibits, the secretion of placental CRH [Figure 1.3] (de Weerth and Buitelaar, 2005b; Glynn et al., 2013; Robinson et al., 1988). Since hypothalamic CRH does not cross the blood-brain barrier, circulating CRH during pregnancy is considered to be of placental origin (de Weerth and Buitelaar, 2005b; Riley and Challis, 1991).

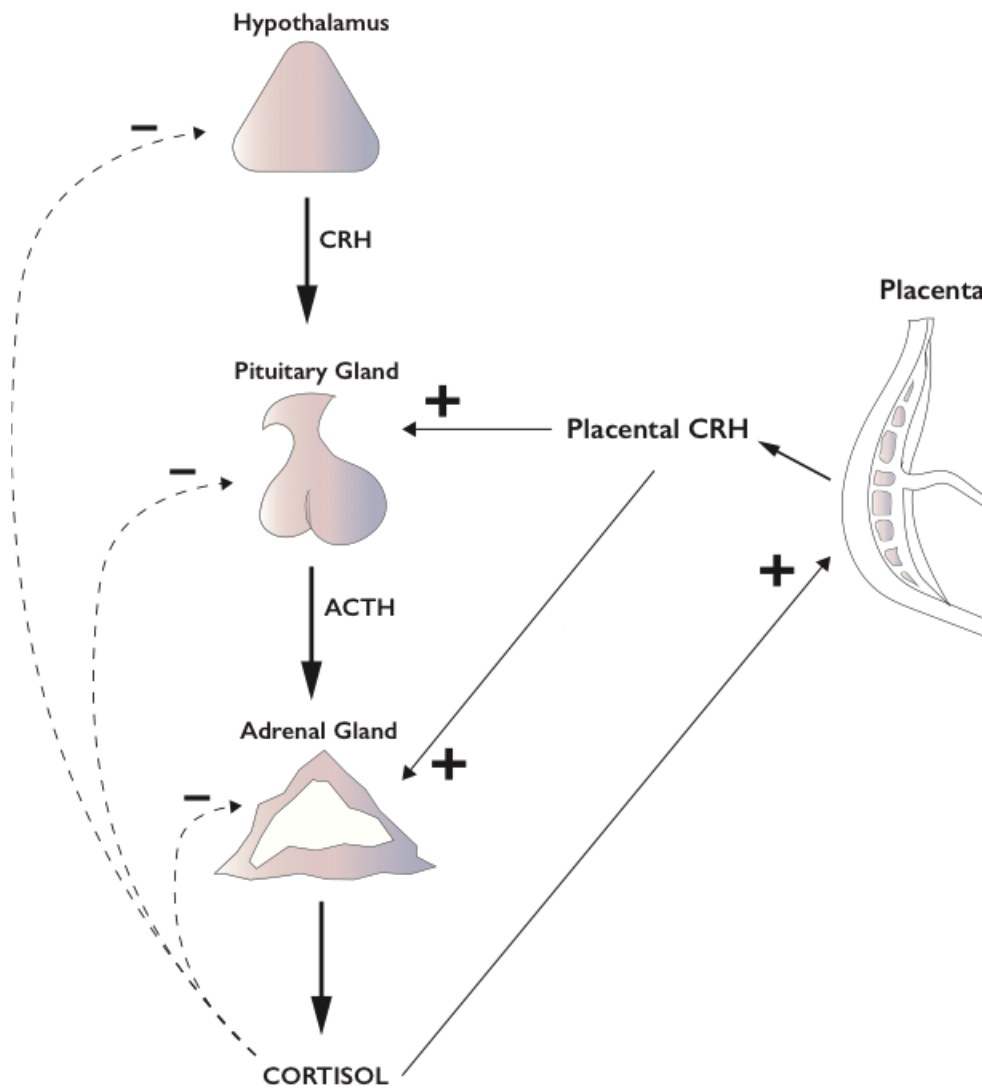


Figure 1.3 The HPA axis during pregnancy

Placental CRH stimulates the HPA axis during pregnancy, leading to a positive feed-forward loop with cortisol, which further stimulates placental CRH production.

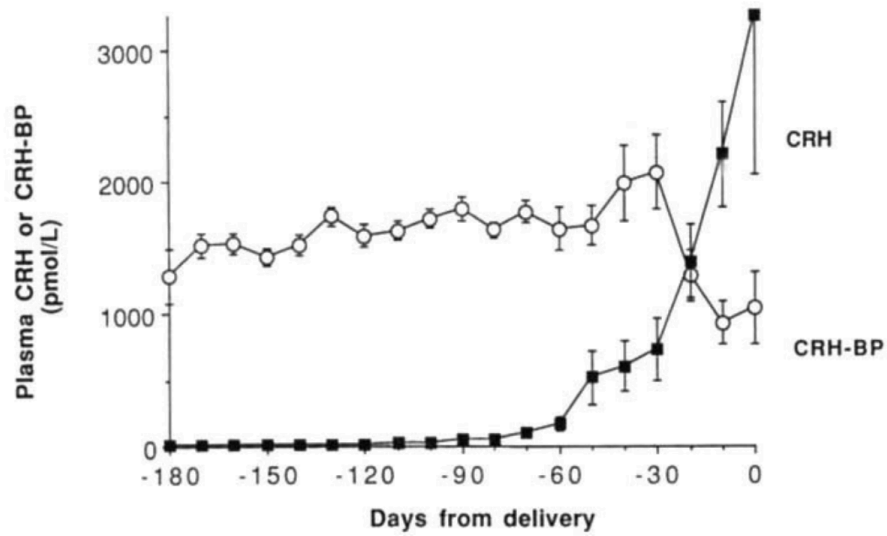


Figure 1.4 Corticotrophin-releasing hormone (CRH) and CRH-binding protein (CRH-BP) levels during gestation.

Levels of CRH-BP fall sharply 4-6 weeks before parturition, leading to higher levels of CRH, which then initiates parturition. (From McLean et al., 1995)

ACTH during pregnancy

The pituitary gland doubles in size during pregnancy, with a subsequent increase in circulating pituitary hormones as gestation progresses (Glynn et al., 2013). Placental CRH further stimulates the production of maternal ACTH (Mastorakos and Ilias, 2003). Despite the expectation for the exponential rise in circulating CRH to lead to similarly high levels of ACTH, the increase in ACTH remains within normal limits during pregnancy (Mastorakos and Ilias, 2003; Smith, 1998). The reduction in bioactivity of CRH by CRH-BP for most of pregnancy may explain this, or possibly the desensitisation of pituitary CRH receptors due to exposure to continuously high concentrations of CRH during pregnancy (Thomson, 2013). The circadian rhythm of ACTH is maintained through most of gestation (Magiakou, Mastorakos, Rabin, Margioris, et al., 1996; Mastorakos and Ilias, 2000).

Cortisol during pregnancy

There exists a state of mild but sustained physiological hypercortisolaemia during the first two trimesters of pregnancy (Mastorakos and Ilias, 2000). Cortisol levels begin to rise from about week 25 of gestation but, like ACTH, only show a modest rise initially (de Weerth and Buitelaar, 2005b). As pregnancy progresses, there is an oestrogen-stimulated increase in levels of cortisol-binding globulin (CBG), leading to a decrease in catabolism of cortisol by the liver and a subsequent increase in circulating cortisol levels (Duthie and Reynolds, 2013; Mastorakos and Ilias, 2003), up to twice that seen in non-pregnant populations (de Weerth and Buitelaar, 2005b; Smith, 1998). The adrenal glands become hypertrophied during pregnancy due to the increased production of cortisol from the adrenal zona fasciculata in response to circulating ACTH (Mastorakos and Ilias, 2000). Cortisol levels peak in the third trimester, and are similar to the levels seen in Cushing's syndrome at this stage of pregnancy (Mastorakos and Ilias, 2003).

Cortisol, like ACTH and unlike CRH, appears to maintain a diurnal rhythm during pregnancy (Allolio et al., 1990; Kivlighan et al., 2008; Nolten et al., 1980). The secretagogue AVP is thought to mediate the circadian rhythms for cortisol and ACTH (Magiakou, Mastorakos, Rabin, Margioris, et al., 1996). Findings on the HPA axis stress response in pregnant women are diverse. The cortisol awakening response is conserved during pregnancy, as evidenced by several studies. de Weerth and Buitelaar

(2005a) found a clear cortisol awakening response despite the presence of hypercortisolaemia in a study population of 119 pregnant women. Findings from another study suggested that, although the CAR is present during pregnancy, it is attenuated in the later stages of gestation, accompanied by an increase in baseline cortisol (Buss et al., 2009; Entringer et al., 2010). A higher cortisol response to awakening and a reduced dampening of the CAR was found to be associated with shorter gestational length in that study. The CAR in pregnancy may have an effect on birth outcomes, with one study indicating that maternal total cortisol output after awakening explained 19.8% of variance in birth weight, even after controlling for various confounding factors including gestational length (Bolten et al., 2011). Results from another study also indicated that a higher waking cortisol and blunted CAR was associated with shorter length of gestation (Entringer et al., 2011).

The cortisol stress response is maintained in the initial stages of pregnancy and is thought to be driven by AVP, since the maternal pituitary becomes desensitised to CRH after CRH receptor downregulation (McLean and Smith, 1999). Maternal physiological responses to stress have been shown to diminish during the latter stages of gestation in some studies, with reduced heart rate and blood pressure noted in response to stress (DiPietro et al., 2012; Entringer et al., 2010; Matthews and Rodin, 1992). Other studies have reported diminished psychological stress in later gestation, showing lower perceived stress in pregnant women in response to stressors including earthquakes and laboratory stress paradigms (Entringer et al., 2010; Glynn et al., 2001). Some researchers have found the HPA axis stress response to be similarly diminished in the latter stages of gestation. Several studies have been published indicating a blunting of the cortisol stress response in late gestation, but this has not been reported in early or mid-gestation. Kammerer and colleagues (2002) found no cortisol response to a cold stressor challenge in pregnant women in the third trimester, and reported a diminished CAR. Plasma cortisol response was absent in a study of exogenous CRH administration to third-trimester pregnant women (Schulte et al., 1990). Findings from a study by Entringer and colleagues (2010) indicated that women had higher baseline cortisol levels and a more attenuated CAR in the third trimester compared to the second, and a physical discomfort stressor elicited no cortisol response. Results from another study, however, suggested recent stressful life events to be associated with increased cortisol levels in late, but not early, gestation (Obel et al., 2005). Giesbrecht

and colleagues conducted two studies in this area, and found that psychological distress or periods of negative mood symptoms elicited a cortisol response throughout pregnancy (Giesbrecht et al., 2012, 2013). The findings are diverse, but most studies appear to indicate a dampening of the cortisol stress response in late gestation.

1.5.2 Parturition and the HPA axis

The process of labour commences during the final three weeks of gestation. Completion of foetal organ maturation, ripening of the cervix, and activation of myometrial contraction occur in a coordinated manner, and are thought to be subject to similar hormonal triggers (McLean and Smith, 1999). Placental CRH has a role in the induction of labour, activating multiple mechanisms that initiate parturition (Thomson, 2013). Not only does CRH stimulate increased production of prostaglandins from placental membranes, it also helps prepare the foetus for parturition. CRH stimulates foetal neurotransmitters involved in foetal stress responses and also stimulates the foetal adrenal glands to produce dehydroepiandrosterone-sulfate (DHEA-S), an essential precursor to oestrogen (Smith et al., 1998). Oestrogen is an important hormone for the initiation of contractions during parturition. When labour begins, the quiescent upper myometrium becomes sensitive to contractile signals from CRH-stimulated oxytocin and prostaglandins, and enters a highly contractile state as the lower section becomes relaxed, thus allowing for foetal expulsion (Challis et al., 2000; Smith and Nicholson, 2007).

1.5.3 The HPA axis postpartum

The HPA axis, following the significant alterations seen during pregnancy, undergoes abrupt changes in the early puerperium before gradually returning to its pre-pregnancy state in the weeks that follow. In healthy pregnancy, the delivery of the placenta leads to an acute withdrawal of placental CRH, thus dramatically reducing the levels of circulating CRH to the non-pregnant state within 48 hours (Mastorakos and Ilias, 2000; O'Keane, Lightman, Patrick, et al., 2011). A period of adrenal suppression, lasting weeks to months, follows on from treatment of conditions with endogenous high cortisol production such as Cushing's syndrome (Avgerinos et al., 1987; Fitzgerald et al., 1982; Gomez et al., 1993; Graber et al., 1965), and it is

hypothesised that similar perturbations of the HPA axis, with adrenal suppression, are seen postpartum following the physiological hypercortisolaemia of pregnancy (Kalantaridou et al., 2004). Cortisol levels peak during labour, and decrease after delivery (Kammerer et al., 2006). The adrenal hypertrophy of pregnancy persists postpartum, but unbound cortisol levels gradually return to normal over the first four days after delivery (Mastorakos and Ilias, 2003). One study showed no differences in cortisol levels between three, six, and 12 weeks postpartum, and a more recent study found that total plasma cortisol remained high at 2-3 months postpartum, while plasma free cortisol levels had returned to pre-pregnancy levels (Magiakou et al. 1996; Jung et al. 2011). It has been reported that, after a transient decrease, ACTH levels began to rise again between days three and six postpartum (O'Keane, Lightman, Patrick, et al., 2011). This is thought to indicate a re-establishment of hypothalamic CRH secretion following suppression by the hypercortisolaemia of pregnancy. Findings from another study suggested that ACTH returns to pre-pregnancy levels by day 5 postpartum (Okamoto et al., 1989).

The HPA axis appears to return to its pre-pregnancy function between five and 12 weeks postpartum. Plasma cortisol levels have been found in one study to be resistant to suppression by dexamethasone over the first two weeks postpartum, with resistance decreasing by about the third week and suppression returning to pre-pregnancy function by the fifth week (Owens et al., 1987). In keeping with the suppression of cortisol response to dexamethasone postpartum, women were found to have a blunted ACTH response to administration of ovine CRH at two weeks and six weeks postpartum in one study, with the ACTH response returning to pre-pregnancy levels by 12 weeks postpartum in healthy women (Magiakou et al. 1996).

Mechanisms underlying this mild suppression of the HPA axis postpartum are unclear, but one of the processes thought to be involved is the desensitisation of the anterior pituitary corticotrophs to CRH. Prolonged exposure of anterior pituitary corticotrophs to high levels of circulating CRH during pregnancy is thought to lead to a downregulation of pituitary CRH receptors, reducing their ability for maximal response (Glynn et al., 2013; Reisine and Hoffman, 1983; Thomson et al., 1990). Another plausible hypothesis for the mild adrenal suppression postpartum may be a downregulation of CRH production and hypothalamic CRH receptors from

overexposure to placental CRH during pregnancy (Gomez et al., 1993; Groer and Morgan, 2007).

1.5.4 Depressive symptoms during pregnancy and the HPA axis

A multitude of studies have examined the HPA axis in women experiencing depressive symptomatology measured using various depression rating scales during pregnancy. Predominantly, studies report an increase in HPA axis activity in women with high scores on depression rating scales, while some have found no association. In an early study, Lundy and colleagues (1999) found elevated third trimester cortisol levels in women with depressive symptoms. Later studies, five of which were from the same research group, found similar results. Higher first-pass morning urine cortisol levels were found in the second trimester in women with depressive symptoms in two studies (Field et al., 2001, 2004). Morning urine cortisol levels were also positively associated with depressive symptoms in the second and third trimesters in other studies (Diego et al., 2004; Field, Hernandez-Reif, Diego, Figueiredo, et al., 2006; Field, Hernandez-Reif, Diego, Schanberg, et al., 2006). Elevated plasma CRH levels in the early third trimester were associated with antenatal depressive symptoms in one study (Janet W. Rich-Edwards et al., 2008). In another, elevated evening cortisol levels were reported in women with high levels of antenatal depressive symptoms (Peer et al., 2013). One study investigated stable and momentary mood disturbance and cortisol levels during pregnancy, finding a 12.8% increase in cortisol per unit increase in mood disturbance on the Edinburgh Postnatal Depression Scale (EPDS) (Giesbrecht et al., 2012).

Findings from a further five studies indicate no association between depressive symptoms during pregnancy, measured using various depression rating scales, and HPA axis measures. Davis and colleagues (2007) found no association between maternal CES-D scores and afternoon salivary cortisol levels in the second or third trimesters. Results from another study indicated no association between cortisol levels and depression rating scale scores in early or late gestation (Pluess et al., 2010; Salacz et al., 2012; Shea et al., 2007a). In a study of maternal cortisol response to a film involving infant distress, no differences were evident in baseline cortisol levels between pregnant women with depressive symptoms compared to control pregnant

women (Murphy et al., 2015). However, women with depressive symptomatology had higher cortisol stress responses than the control group in this study. No association was evident between the HPA axis measures of ACTH, CRH and cortisol, and depressive symptoms during pregnancy in one study (Yim et al., 2009), while another found an inverse relationship between maternal CRH levels and CES-D scores during pregnancy (Meltzer-Brody et al., 2011).

1.5.5 Antenatal depression and the HPA axis

Despite the strong evidence base for the role of the HPA axis in major depressive disorder (MDD), few studies have examined clinically diagnosed depression during pregnancy in relation to the HPA axis. Of the eight studies that have reported on this, four found clinical depression during pregnancy to be associated with an increase in HPA axis measures [Table 1.2] (Field et al., 2007, 2009; O'Connor et al., 2014; O'Keane, Lightman, Marsh, et al., 2011), one found an association between comorbid depression and anxiety and cortisol (Evans et al., 2008), while three other studies found no association between clinical depression during pregnancy and HPA axis measures [Table 1.3] (Field et al., 2010; Hellgren et al., 2013; Parcells, 2010).

In a precursor to the present study, O'Keane and colleagues (O'Keane, Lightman, Marsh, et al., 2011) examined 27 depressed and 38 healthy control pregnant women and reported higher evening cortisol levels and higher CRH levels in depressed pregnant women compared to control pregnant women at 25 weeks gestation. In two earlier studies, clinical depression during pregnancy was associated with higher morning urine cortisol levels in Black women and in a mixed population (Field et al., 2007, 2009). O'Connor and colleagues (2014) examined cortisol values in depressed pregnant women compared to pregnant women with anxiety, and found depressed women to have lower waking cortisol levels but overall higher cortisol levels and a flattened diurnal curve compared to women with anxiety. There was no evidence for differences in CAR between the groups in this study.

No differences were observed in cortisol levels between clinically depressed pregnant women and healthy control pregnant women in one study, but higher mid-morning cortisol levels were evident in women with comorbid depression and anxiety (Evans et

al., 2008). Two studies found no differences in mid-morning urine cortisol or mid-morning salivary cortisol between clinically depressed pregnant women and healthy pregnant controls (Field et al., 2010; Parcels, 2010). In the latter study examining salivary cortisol, a positive association was reported between BDI-defined depression and cortisol levels (Parcells, 2010). Another study examined the CAR in depressed pregnant women, and reported no differences in waking cortisol levels or CAR compared to healthy controls (Hellgren et al., 2013). However, this study was conducted between 35 and 39 weeks gestation, where the notable physiological hypercortisolaemia may have rendered any waking cortisol differences undetectable.

Table 1.2 Studies demonstrating a positive association between clinical depression during pregnancy and the HPA axis

Study	Test Group	Comparison Group	Depression Measures	Sample Size	HPA Measures	Axis	Findings
Field et al, 2007	Depressed pregnant women (20-24 weeks)	Healthy control pregnant women	SCID, CES-D	83 Depressed 173 Healthy control	Morning urine cortisol levels		Higher cortisol levels in depressed women
Field et al, 2009	Depressed pregnant women (20 weeks, 32 weeks)	Healthy control pregnant women	SCID, CES-D	131 Depressed 205 Healthy control	First morning urine cortisol (only at 32 weeks)		Higher cortisol levels in depressed women
O'Keane et al, 2011	Depressed pregnant women (25 weeks, 36 weeks)	Healthy control pregnant women	SCID, HAM-D	27 Depressed 38 Healthy control	Salivary cortisol – diurnal samples Plasma CRH and ACTH		Evening cortisol and CRH levels higher in depressed women at 25 weeks, with no difference in ACTH levels
O'Connor et al, 2014	Depressed pregnant women (20 weeks, 32 weeks)	Pregnant women with anxiety	SCID, EPDS	<i>At 20 weeks:</i> 23 Depressed 24 Anxious <i>At 32 weeks:</i> 13 Depressed 23 Anxious	Salivary cortisol – diurnal samples		Lower waking cortisol levels in depressed women, higher cortisol levels across the day and flatter diurnal curve

SCID – Structured Clinical Interview for DSM-IV Disorders; **CES-D** – Centre for Epidemiological Studies Depression rating scale; **HAM-D** – Hamilton Depression rating scale, **EPDS** – Edinburgh Postnatal Depression Scale.

Table 1.3 Studies demonstrating no association between clinical depression during pregnancy and the HPA axis

Study	Test Group	Comparison Group	Depression Measures	Sample Size	HPA Measures	Axis	Findings
Evans et al, 2008	Depressed pregnant women (36 weeks)	Pregnant women - Healthy control - Anxiety disorder - Comorbid depression and anxiety	SCID, CES-D	16 Depressed 119 Healthy control 34 Anxiety disorder 9 Comorbid depression and anxiety	Mid-morning salivary cortisol		No difference in cortisol levels between depressed and control groups but higher cortisol levels in comorbid group
Field et al, 2010	Depressed pregnant women (20 weeks, 32 weeks)	Pregnant women - Healthy control - Anxiety disorder - Comorbid depression and anxiety	SCID, CES-D	181 Depressed 345 Healthy control 77 Anxiety disorder 308 Comorbid depression and anxiety	Mid-morning urine cortisol		No differences in cortisol levels between the groups at either time point
Parcells et al, 2010	Depressed pregnant women (26-28 weeks, 32-34 weeks)	Healthy control pregnant women	SCID, BDI-II	10 Depressed 46 Healthy control	Mid-morning salivary cortisol		No differences between depressed and healthy control groups but higher cortisol levels reported in women with BDI-defined depression
Hellgren et al, 2013	Depressed pregnant women (35-39 weeks)	Pregnant women - Healthy control - Prior history of depressive disorder	MINI, EPDS	38 Depressed 57 Healthy control 39 Previously depressed	Waking and morning salivary cortisol		No differences between groups

SCID – Structured Clinical Interview for DSM-IV Disorders; **CES-D** – Centre for Epidemiological Studies Depression rating scale; **BDI-II** – Beck Depression Inventory, **MINI** – Mini International Neuropsychiatric Interview, **EPDS** – Edinburgh Postnatal Depression Scale.

1.5.6 Postpartum depression and the HPA axis

Studies of the HPA axis in women with postpartum depression also report diverse results. Magiakou and colleagues (1996) found a sustained blunting of ACTH response to ovine CRH, even at 12 weeks postpartum, while non-depressed women had the return of a normal ACTH response by that stage postpartum. The authors found no differences in cortisol levels in depressed women postpartum compared to euthymic women (Magiakou, Mastorakos, Rabin, Dubbert, et al., 1996). No differences in ACTH or cortisol were reported in another study of women who were depressed postpartum compared to women who were euthymic, while a later study found that women with postpartum depression had HPA axis dynamics similar to women outside the perinatal period, with higher waking cortisol levels and a blunted response compared to non-depressed postpartum women (Jolley et al., 2007; Taylor et al., 2009).

1.5.7 Summary

To summarise, depressive symptomatology appears to increase HPA axis activity during pregnancy. However, findings on the relationship between antenatal depression and HPA axis function are paradoxical, with four studies finding HPA axis overactivity and four others finding no effect. As the aetiology of depression during pregnancy and postpartum depression remain unclear, further studies such as the current study including a diagnostic interview in addition to objective depression measures, are warranted to elucidate any relationship between perinatal depression and the HPA axis.

1.6 The foetal and infant HPA axes

1.6.1 The foetal HPA axis

The foetal HPA axis plays a critical role in the development and maturation of the foetus, and is also essential for immediate survival of the neonate in the postpartum environment. Consequently, adaptations in the foetal HPA axis in response to intrauterine stress, through foetal programming [See Section 1.7], may play a role in offspring mental and physical illness in later life. The structural components of the foetal HPA axis begin to develop during early gestation. Post-mortem studies of human foetuses have demonstrated the presence of ACTH in the foetal pituitary and cortisol in foetal adrenal tissue by 8-9 weeks gestation (Goto et al., 2006; Pavlova et al., 1968). CRH immunoreactivity was present in the foetal hypothalamus at 12 weeks gestation (Ackland et al., 1986).

Activity of the foetal HPA axis begins with maturation of the foetal pituitary glands (Mastorakos and Ilias, 2003; Perry et al., 1985; Wood, 2013). The foetal pituitary-adrenal axis, although immature, is functioning by the second trimester (McLean and Smith, 1999). CRH receptors have been found in the foetal pituitary, hypothalamus, and adrenal cortex from about 12 weeks gestation (Ackland et al., 1986; Jaffe et al., 1998). The foetal pituitary, in response to placental and foetal hypothalamic CRH, begins to produce ACTH, which is important for the development of the foetal adrenal cortex. The foetal adrenal gland primarily produces dehydroepiandrosterone-sulfate (DHEA-S), but begins *de novo* synthesis of cortisol around the 20th week of gestation (Partsch et al., 1991; Petraglia et al., 1987). One study examined live foetuses between 16-20 weeks gestation prior to termination of pregnancy. Higher concentrations of cortisol were found in the umbilical artery compared to the vein, indicating that the foetuses were producing cortisol by this gestational age (Partsch et al., 1991). Foetal cortisol plays an important role in foetal organ maturation – in particular, lung maturation. Post-mortem studies of preterm neonates from 24 weeks gestation showed the presence of GR and MR expression in the hippocampus (Noorlander et al., 2006).

Foetal stress responses are thought to be independent of maternal stress responses. Gitau and colleagues (2001) measured foetal cortisol levels in response to a foetal

blood sampling or intrauterine transfusion stressor and demonstrated that HPA axis responsivity begins by 20 weeks gestation, independent of the maternal stress response. In a later study, the same group found a rise in foetal ACTH and cortisol, but not CRH, in response to an intrauterine needling stressor, indicating that foetal plasma CRH is likely derived from another source such as the placenta (Gitau et al., 2004). CRH and ACTH were detected in foetal plasma in the second trimester in one study, and both were found to correlate with maternal CRH levels (Economides et al., 1987). Cortisol levels in foetally-derived amniotic fluid, a measure of foetal cortisol exposure, were found to correlate with maternal cortisol levels from week 17 of gestation, and a stronger correlation was observed in anxious mothers (Glover et al., 2009; Sarkar et al., 2007).

The foetus is thought to be protected from overexposure of cortisol *in utero* by the conversion of active cortisol to its inactive metabolite, cortisone, by placental 11β -hydroxysteroid dehydrogenase type 2 (11β -HSD-2) (Benediktsson et al., 1997). Although 11β -HSD-2 inactivates about 80% of maternal cortisol, 10-20% of active cortisol is thought to cross the placenta into the foetal circulation. One third of the variation in foetal cortisol is attributed to maternal cortisol, and it is proposed that the foetus is exposed to 5% of any elevations in maternal cortisol (Gitau et al., 1998, 2001). Levels of 11β -HSD-2 have been shown to increase throughout gestation (Schoof et al., 2001), before decreasing significantly between 38 and 40 weeks gestation, possibly to allow for any foetal organ maturation that may be mediated by cortisol (Murphy and Clifton, 2003).

1.6.2 The infant HPA axis

The neonatal HPA axis is critical in adapting the neonate to the extrauterine environment for survival. Although not fully mature at this stage, it is a functioning stress response system. There is no current consensus on the age of development of the cortisol circadian rhythm. While it is established that the circadian rhythm is not demonstrated in infants <1 month of age (Groschl et al., 2003), various reports suggest that the circadian rhythm appears by the age of two months, (De Weerth et al., 2003; Groschl et al., 2003), three months (Price et al., 1983; Spangler, 1991), two to three months (Antonini et al., 2000; Mantagos et al., 1998), six months (Lewis and Ramsay,

1995; Onishi et al., 1983), or nine months of age (Kiess et al., 1995). One study has demonstrated considerable intra-individual variability in the age of appearance of the circadian rhythm (De Weerth et al., 2003), which is thought to be associated with the establishment of a 24-hour sleep-wake cycle (De Weerth et al., 2003; Price et al., 1983; Santiago et al., 1996; Spangler, 1991). The infant CAR has been less extensively studied. Findings from one study suggested that infants under 16 months of age did not mount a CAR (Saridjan et al., 2010), and another study of infants aged 7-17 months also found no evidence for a CAR (Bright et al., 2012). However, Stalder and colleagues (2013), using more robust methodology, reported that infants began to demonstrate a CAR at two months of age.

Basal cortisol in infants also appears to have high intra-individual variability, with a decrease in basal levels reported from six weeks to five months, and a further decrease to one year of age (de Weerth and van Geert, 2002; Tollenaar et al., 2010). Accordingly, a systematic review of cortisol stress responses in infants found the response to be higher in younger infants, decreasing with age, notably after six months (Jansen et al., 2010; Lewis and Ramsay, 1995). This review also found that physical stressors, whether mild or painful, were better at eliciting a cortisol stress response compared to psychological stressors such as separation. This finding was replicated in another review, which found that vaccination stressor was superior in eliciting a mean increase in cortisol in infants up to six months of age (Gunnar et al., 2009). One study examined infant cortisol responses to a vaccination stressor at two and six months of age and reported both pre- and post-vaccination levels to decrease with age with no change in the cortisol stress response itself (Ramsay and Lewis, 1994). Although this reduction in cortisol levels was attributed to the increased ability for infants to self-soothe at six months, a further study by the same authors found no relationship between cortisol reactivity and infant behavioural regulation (Ramsay and Lewis, 2003).

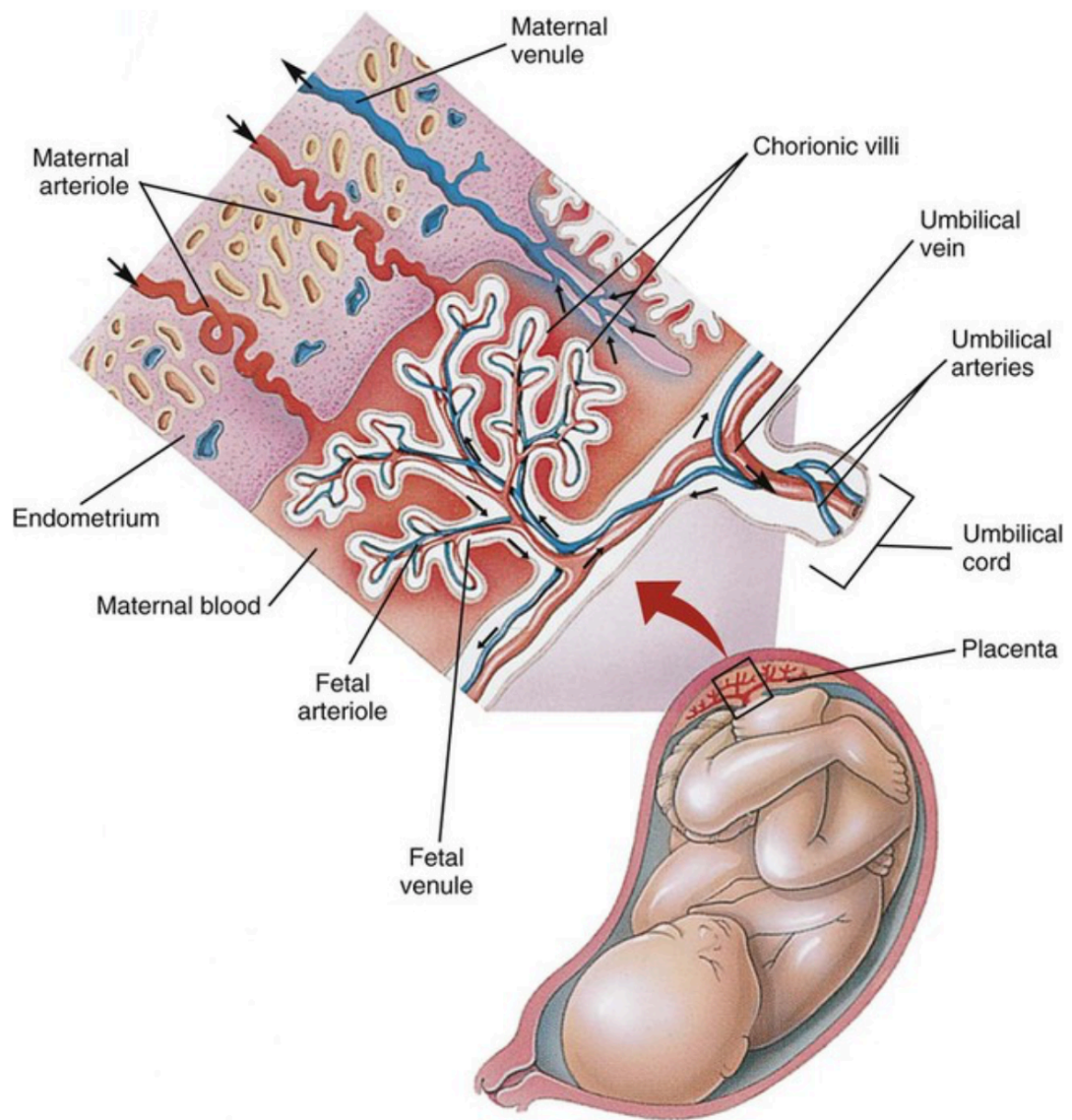


Figure 1.5 Diagrammatic representation of maternal-foetal circulation
 (From Thibodeau and Patton, 2010)

1.7 Developmental programming

1.7.1 The foetal programming hypothesis

In all pregnancies, the foetus receives intrauterine signals from the maternal environment, allowing it, through foetal plasticity, to adapt to the environment. These signals also inform the foetus about the likely nature of the postpartum environment. In the event of intrauterine perturbations due to poor nutrient supply or maternal physical or mental illness, intrauterine signals indicate to the foetus that the postpartum environment will be similarly perturbed. The foetus undergoes enduring physiological adaptations to survive the immediate intrauterine environment – termed foetal programming – and prepares for comparable environmental demands after birth. Foetal programming is thought to confer an evolutionary advantage to the foetus *in utero*, allowing it to preserve nutritional energy in order to overcome the immediate risks of the intrauterine environment to reach the end of gestation. However, evidence indicates that this transient benefit of foetal programming appears to come at a cost in later life, when it may confer an increased risk of physical and mental illness.

The foetal programming model was first proposed by David Barker in his seminal paper in 1995, where he provided an argument for intrauterine insults leading to poor foetal growth, which then predisposes the foetus to cardiometabolic disease in adulthood (Barker, 1995b). Barker's initial work in the 1980s demonstrated an association between foetal under-nutrition *in utero* and cardiovascular disease in later life (Barker et al., 1989; Barker and Osmond, 1986). He hypothesised that disproportionate foetal growth, secondary to under-nutrition in middle to late gestation, led to low birth weight, which was further associated with coronary artery disease, hypertension, stroke, and type 2 diabetes mellitus in adult life (Barker, 1995a, 1995b). The Dutch Hunger Winter study corroborated this finding, with women exposed to famine in middle to late gestation giving birth to low birth weight infants (Schulz, 2010). Conversely, maternal famine in early gestation led to normal birth weight infants, who went on to develop higher rates of adult obesity. It has now been established that foetal exposure to not only nutritional stress but also maternal psychosocial stress *in utero*, through overexposure of the foetus to excess glucocorticoids, can lead to long-lasting effects on the offspring and increase risk of

physical and mental illness in adult life (Meaney et al., 2007; Reynolds, 2013; Seckl, 1997, 2004). In particular, altered HPA axis activity in infants is thought to increase the infant's vulnerability to neuropsychiatric illness in adult life.

1.7.2 Animal studies

Studies in rats have shown that offspring exposed to prenatal stress exhibit downregulation of GR and MR leading to disrupted feedback regulation of the HPA axis, and increased secretion of glucocorticoids (Chung et al., 2005; Koehl et al., 1999; Levitt et al., 1996; Maccari et al., 2003; Welberg et al., 2000). Studies in nonhuman primates have similar findings, with exaggerated cortisol responses to stressors found in prenatally stressed offspring (Clarke et al., 1994; de Vries et al., 2007; Schneider et al., 2002).

1.7.3 Depressive symptoms during pregnancy and the infant HPA axis

Numerous studies have examined depressive symptoms during pregnancy and its effects on infant and neonatal HPA axis. Prenatal and postnatal depressive symptoms have been found to be associated with changes in infant cortisol stress responses (Feldman et al., 2009; Fernandes et al., 2015). An epigenetics study found a relationship between maternal EPDS scores in the second and third trimesters and increased methylation at the nerve growth factor inducible A (NGFI-A) binding site of the GR gene in infants (TF Oberlander, Weinberg, et al., 2008). This was further associated with increased cortisol stress response in three-month-old infants. A recent study investigated the effect of maternal depressive symptoms during pregnancy and infant cortisol responses to a vaccination stressor at two months in rural South India. The findings suggest that depressive symptoms during pregnancy are associated with increased cortisol stress reactivity in infants, and that this is a dose-dependent relationship, with higher maternal depression scores during pregnancy indicating higher infant cortisol reactivity to a stressor (Fernandes et al., 2014). Studies of neonatal urine cortisol levels have found higher levels in neonates born to women with high self-rated depression scores during pregnancy (Diego et al., 2004; Lundy et al., 1999). These findings indicate increased HPA axis responsivity in infants of mothers with high depression scores during pregnancy.

1.7.4 Antenatal depression and the infant HPA axis

Although there are a number of studies investigating the effects of depressive symptomatology during pregnancy on the infant HPA axis, only two have examined the programming effect of maternal clinical depression during pregnancy on the infant HPA axis. In one study of 911 pregnant women, Field et al (2010) compared infants of healthy control women to those born to women with Structural Clinical Interview for DSM-IV Disorders (SCID) diagnoses of depression alone, anxiety alone, and a diagnosis of comorbid depression and anxiety, using neonatal urinary cortisol 1-2 days postpartum as a measure. Cortisol was reported to be higher in neonates born to women with depression alone and those with comorbid depression and anxiety compared to infants born to women diagnosed with anxiety alone and control pregnant women (Field et al., 2010). Another study of six-month-old infants found that a maternal history of retrospectively diagnosed perinatal depression was associated with higher cortisol reactivity in the infants, while a lifetime history of maternal depression was associated with higher baseline and mean cortisol levels in infants (Brennan et al., 2008). These findings suggest that, as found in animal studies and studies of depressive symptoms during pregnancy, antenatal depression may programme the offspring HPA axis to have an exaggerated response to stress. The higher overall cortisol levels found in infants born to women with a lifetime history of depression may indicate a trait effect of depression on foetal programming of the infant HPA axis.

1.7.5 Maternal HPA axis during pregnancy and the infant HPA axis

Several studies have demonstrated an association between maternal cortisol measures during pregnancy and foetal and infant cortisol measures. Gitau and colleagues (2004) found a moderate correlation between maternal plasma cortisol levels during pregnancy and foetal plasma cortisol values. In another study examining maternal plasma cortisol and cord blood cortisol measures at delivery, maternal free plasma cortisol levels predicted 40% of foetal free plasma cortisol levels after taking into account confounders such as mode of delivery and estimated gestational age (Smith et al., 2011). A neonatal study found high maternal cortisol levels during pregnancy to be associated with higher neonatal cortisol responses to a heel-prick test conducted 24 hours after birth (Davis et al., 2011a). Amniotic fluid cortisol levels are considered to be

a good indication of foetal cortisol exposure; high amniotic fluid cortisol levels during pregnancy predicted higher baseline cortisol levels and blunted cortisol responsivity to a separation stressor in 17-month-old infants (T O'Connor et al., 2013). Mothers who experienced PTSD during pregnancy in response to the World Trade Centre collapse, and their infants, were found to have lower cortisol levels compared to mothers who did not develop PTSD during pregnancy in one study (Yehuda et al., 2005). These findings, suggesting that foetal exposure to excessive cortisol in utero may lead to altered HPA axis function in infancy, lend further support to the foetal programming hypothesis.

1.7.6 Later-life effects of prenatal stress in offspring

Studies of offspring mental health in relation to maternal stress during pregnancy involving natural disasters such as the Dutch Hunger Winter have found that offspring of mothers who experienced famine have increased rates of MDD compared to mothers who did not experience famine (Brown et al., 2000). Studies conducted in the South London Child Development Study have presented an argument for the association between antenatal depression and increased rates of offspring conduct problems and depression in adolescence (Hay et al., 2008, 2010; Pawlby et al., 2009). Postpartum depression was also examined in this cohort, and was found to mediate the relationship between antenatal depression and offspring depression. In a study of 4500 mother-offspring dyads participating in the Avon Longitudinal Study of Parents and Children (ALSPAC) in the UK, antenatal depression was reported to be an independent risk factor for offspring depression at 18 years (Pearson et al., 2013). Postpartum depression in this population was a risk factor for offspring depression in the event of low education levels in the mothers. These studies conducted in large cohorts provide strong evidence for the association between antenatal depression during pregnancy and later life depression in offspring, potentially mediated by altered HPA axis function.

1.7.7 Sexual dimorphism in developmental programming

Sexual dimorphism in adult mental illness is well delineated. Higher rates of depression and anxiety are seen in females, while the prevalence of ADHD, substance

use or antisocial personality disorders is higher in males (Kessler et al., 1993; Ramtekkar et al., 2010; Werling and Geschwind, 2013). There are also gender differences in foetal and neonatal outcomes, with males being more susceptible to preterm birth, low birth weight and intrauterine death compared to females. It remains unclear why this disparity exists between genders, but possible differences in foetal development, and subsequently, differing foetal programming effects have been put forward as an explanation.

Current evidence suggests that male and female foetuses adapt to intrauterine stress in different ways, and the placenta, in its juxtaposed position as the interface between the mother and the foetus, plays a key role in foetal development and programming (Clifton, 2010). The female placenta is thought to be more sensitive to stress signals, allowing the foetus to make adaptations to survive gestation to term. Results from one study examining maternal asthma and foetal growth suggest that, in women who suffer from chronic asthma, female foetuses are susceptible to poor growth while male foetuses had no change in their rate of development (Murphy et al., 2003). Conversely, in the event of acute asthma exacerbation in mothers, male foetuses showed compromised growth and were at an increased risk of preterm birth or intrauterine death (Murphy et al., 2005). In the same study, females with low birth weight had higher levels of circulating cortisol levels compared to male foetuses exposed to maternal asthma *in utero*. These findings suggest that female foetuses may respond to intrauterine stress in a different manner to males. Clifton (2010) puts forward the argument that male foetuses, in the presence of an intrauterine insult, continue to use their resources to grow normally, increasing their vulnerability for compromise in the event of another intrauterine stressor later in gestation. Females, conversely, conserve their resources in the presence of a stressful intrauterine environment and reduce growth, which allows them to survive gestation to reach the end of term, albeit with lower birth weight.

Emerging evidence suggests that foetal sex moderates the relationship between prenatal stress and infant outcomes (Clifton, 2010; Davis and Pfaff, 2014; Sandman et al., 2013). A cohort of the ALSPAC study were investigated for sexual dimorphism in the relationship between maternal depression during pregnancy and offspring depression in later life, and found female offspring to be more susceptible to

developing depression at 18 years compared to male offspring of women with antenatal MDD (Quarini et al., 2016). The Iowa Flood Study examined prenatal stress associated with a natural disaster, and its relationship with infant cortisol stress responses at 2½ years of age (Yong Ping et al., 2015). The authors reported an increase in cortisol response to a separation stressor in females only, with no effect in male infants. In another study, blunted cortisol stress responses were observed in male infants of women who had high scores on depression and anxiety rating scales during pregnancy, with no effect in female infants (Vedhara et al., 2012). In a recent study, Giesbrecht and colleagues (2016) reported differences in cortisol reactivity in 3-month-old male and female infants exposed to maternal distress during pregnancy. Low maternal CAR during pregnancy was associated with blunted reactivity in female infants and high cortisol stress responses in male infants, while high maternal CAR during pregnancy was associated with high cortisol stress responses in females and blunted responsivity in males. The evidence in this area appears to be limited and abstruse, indicating that the relationship between foetal sex and foetal programming warrants further exploration.

1.7.8 Mechanisms in foetal programming

The mechanisms underlying foetal programming remain unclear, although prenatal overexposure to cortisol with resulting physiological and epigenetic changes have been implicated. It is postulated that foetal overexposure to cortisol in utero leads to alteration of the 'set-point' of the foetal HPA axis, which then persists into the extrauterine environment and leads to perturbations in offspring HPA axis stress responses (Reynolds, 2013).

Placental 11β-HSD-2

In this regard, the placental enzyme 11β-HSD-2 has received much attention in the past decade. As outlined in Section 1.6.1, 11β-HSD-2 rapidly inactivates cortisol in the placenta, thus limiting foetal overexposure to cortisol. Animal studies have demonstrated reduced activity of 11β-HSD-2 with maternal stress, and raised cortisol and reduced hypothalamic GR expression in offspring of dams whose 11β-HSD-2 had been inactivated (Mairesse et al., 2007; Welberg et al., 2000). Human studies have found a negative association between maternal self-reported anxiety symptoms and

11 β -HSD-2 levels (Conradt et al., 2013; O'Donnell et al., 2012). Two studies to date have examined depressive symptoms during pregnancy using self-report measures and 11 β -HSD-2 levels with conflicting reports. One study reports a negative non-significant relationship between maternal depressive symptoms and 11 β -HSD-2 expression, while the other reports a positive non-significant relationship (O'Donnell et al., 2012; Reynolds et al., 2015). Only one study has examined this relationship using a pregnant population with clinically diagnosed depression, and found lower 11 β -HSD-2 expression in depressed women and those exposed to antidepressants during pregnancy (Seth et al., 2015). Additionally, the authors reported a negative association between depression and anxiety self-report measures and 11 β -HSD-2 activity. It remains unclear what role 11 β -HSD-2 plays in foetal programming.

Epigenetics

Epigenetics – stable alterations in gene expression in response to environmental stressors – has emerged as a potential mechanism for developmental programming in recent years. Environmental stressors in early life, when the epigenome undergoes profound developmental changes, are particularly associated with epigenetic modifications. The most studied epigenetic changes are those mediated through DNA methylation. There is evidence from both animal and human studies to suggest that early life stress leads to epigenetic modifications in the GR gene (NR3C1). In an influential study, Weaver and colleagues (2004) reported differences in DNA methylation at exon 1₇ GR promoter gene in the hippocampus of adult offspring exposed to high levels of maternal caregiving behaviours compared to offspring exposed to low levels of maternal caregiving. Offspring of high-care mothers also had less robust HPA axis stress responses and higher GR gene expression in the hippocampus compared to offspring of low-care mothers. Increased DNA methylation in the promoter region of the NR3C1 gene were observed in adult male mice that had experienced prenatal stress in another study, with resulting alterations in the HPA axis stress response (Mueller and Bale, 2008).

Oberlander and colleagues (2008) reported increased DNA methylation of the GR gene in infants born to mothers reporting high levels of depressive symptoms during the second and third trimesters of pregnancy. These infants also had higher cortisol stress responses at three months, which was postulated to be due to the increased GR

gene methylation. Similarly, prenatal depressive symptoms predicted increased *NR3C1* DNA methylation in male infants only at two months (Braithwaite et al., 2015). However, in a genome-wide study, no association between maternal psychiatric illness during pregnancy and infant DNA methylation was evident in any of the CpG sites studied, including in *Nr3C1* (Schroeder et al., 2012). The authors report that they did not examine the exact CpG sites that were previously studied, explaining the incongruence of their results from previous studies. One study examining the effects of severe maternal war stress in the Democratic Republic of Congo found increased cord blood *NR3C1* DNA methylation in neonates, which was further negatively correlated to infant birth weight, lending further support to the foetal programming hypothesis (Mulligan et al., 2012).

1.7.9 Summary

These findings provide robust evidence for maternal depression or depressive symptomatology during pregnancy leading to programming of offspring neurobiological systems and increasing the offspring's vulnerability to neuropsychiatric illness in later life. There is an increasing evidence base for the association between depressive symptomatology during pregnancy and altered HPA axis in infants, and the association between antenatal MDD and offspring depression in adult life has been elucidated in large-scale population studies. Although there is limited research to date on the effects of antenatal MDD on the infant HPA axis, combined evidence from the literature reviewed lends support to the potential for antenatal depression to adversely impact on infant HPA axis, predisposing the infant to depression in adult life.

1.8 Early life adversity

Significant progress has been made in the recognition of early life adversity (ELA) as a major public health concern. Although the role of childhood adversity in the development of later life psychopathology was first elucidated by Freud and other psychoanalysts (Freud, 1962), it is only in the past two decades that there has been increased interest in the long-term sequelae of childhood trauma. Childhood abuse has been shown to increase vulnerability for developing a range of psychiatric disorders including affective disorders, anxiety disorders, psychotic disorders, and substance use disorders in later life (Green et al., 2010; Keyes et al., 2012; Norman et al., 2012).

1.8.1 Childhood abuse

The World Health Organisation Consultation on Child Abuse Prevention (World Health Organisation, 1999) outlined the following definition for childhood abuse in 1999:

“Child abuse or maltreatment constitutes all forms of physical and/or emotional ill-treatment, sexual abuse, neglect or negligent treatment or commercial or other exploitation, resulting in actual or potential harm to the child’s health, survival, development or dignity in the context of a relationship of responsibility, trust or power.”

The recognised subtypes of childhood abuse are physical abuse, emotional abuse, sexual abuse, and neglect (Norman et al., 2012). The State of the Nation’s Children document on numbers of child welfare and abuse incidents reported to the Health Service Executive in Ireland found that rates of reporting have progressively increased annually from 23,268 in 2007 to 31,626 in 2011 (Department of Children and Youth Affairs, 2012). This increase may indicate an increase in reporting due to an increased awareness of the problem rather than an increase in actual occurrences of child abuse. Notably, aside from child welfare concerns that constituted 50% of reports made, child neglect was the most common subtype of abuse reported in Ireland, with a prevalence rate of 4.8 per 1,000 children. Emotional abuse was reported at 3.5 per 1,000 children, sexual abuse at 2.9, and physical abuse at 2.6 per 1,000 children. Overall 27.5 reports were made over child welfare or abuse per 1,000 children in 2011. Vitally, these

numbers indicate the numbers of reported abuse alone, and do not take into account the vast number of child abuse incidents that go unreported.

1.8.2 Early life adversity and depression

Several studies have now reported that all subtypes of ELA may be associated with an increased risk for the development of depressive episodes that are likely to be recurrent, persistent, and treatment-resistant (Lieberman et al., 2011; Nanni et al., 2012; Nemeroff, 2004; Norman et al., 2012). A meta-analysis conducted showed that sexual abuse in childhood imparted a higher risk of developing depression or anxiety in later life compared to physical abuse (Lindert et al., 2014). A dose-response relationship was found in some studies, with higher levels of emotional abuse being associated with greater increases in depressive symptoms in the presence of life stressors during adulthood (Shapero et al., 2014; Wise et al., 2001).

1.8.3 Early life adversity and the HPA axis

Studies of the HPA axis in children and adults with a history of trauma show diverse findings. In an early study of HPA axis responses to an ovine CRH challenge in sexually abused girls aged 7 to 15 years, the authors reported lower basal ACTH and ACTH response to ovine CRH, but similar cortisol levels and responses compared to control girls with no history of abuse (de Bellis et al., 1994). Conversely, one study of school-aged children with a history of maltreatment found higher afternoon cortisol levels in the maltreated group compared to the control group (Hart et al., 1996). Supporting these results, findings from another study indicated higher afternoon cortisol levels in children who experienced neglect during childhood (Bick et al., 2015).

Research on the HPA axis in adults with a history of childhood trauma have largely been conducted in depression studies, and have reported conflicting results. Suzuki and colleagues (2014) found blunted cortisol responses to an emotional stressor in depressed and non-depressed adults with a history of childhood trauma. Another study found increased CAR and total cortisol output in adults with a history of childhood trauma, regardless of their depression status (Lu et al., 2016). Hinkelmann and colleagues (2013) found low hair and salivary cortisol levels in adults with a history

of childhood abuse compared to those not exposed to childhood abuse, regardless of depression status. Heim et al (2008) reported increased ACTH levels to a stressor in women with a history of childhood abuse, whether they were depressed or not. Depressed women with a history of ELA in that study mounted higher ACTH and cortisol responses compared to non-depressed women with a history of ELA. An epigenetics study found childhood trauma, the severity of trauma, and the number of types of trauma to be associated with *NR3C1* methylation (Perroud et al., 2011). McGowan and colleagues (2009), reported increased *NR3C1* methylation and decreased levels of glucocorticoid receptor mRNA in post-mortem brains of suicide completers with a history of childhood abuse.

1.8.4 Early life adversity and the perinatal period

Childhood trauma, apart from increasing vulnerability to developing mental illness, can also have profound effects on the perinatal period. In a systematic review, Leeners and colleagues (2006) reported that pregnant women with a history of childhood trauma exhibited hypervigilant behaviour, were more stressed, more vulnerable to developing anxiety or depression, and had increased levels of suicidal ideation than women with no history of abuse. In women with a history of childhood sexual abuse, the different periods of pregnancy often triggered memories of the abuse, leading to avoidance of obstetric appointments and vaginal examinations, or a fear of the delivery process. Women with childhood trauma also reported more physical concerns, and were more likely to be abused during pregnancy, or have unscheduled contact with the antenatal clinic (Leeners et al., 2006). One study found women with a history of childhood trauma to have a 1.65-fold increased risk of stress-related sleep disturbance and a 2.11-fold increased risk of poor sleep quality (Gelaye et al., 2015), while another found that sleep quality mediated the negative association between childhood trauma and foetal heart rate (Gustafsson et al., 2016).

1.8.5 Early life adversity and the HPA axis in the perinatal period

Despite the disparity in results of studies examining the HPA axis in adults with a history of ELA, studies during pregnancy have consistently shown maternal ELA to be associated with increased levels of cortisol. In investigations of hair cortisol, a measure

of long-term cortisol levels, during pregnancy, three studies report a history of childhood trauma to be associated with higher hair cortisol levels (Buss, Stalder, et al., 2016; Schreier, Enlow, Ritz, Coull, et al., 2015; Schreier, Enlow, Ritz, Gennings, et al., 2015). However, the two studies by Schreier and colleagues appear to have been conducted on the same study population. Studies of the CAR in pregnant women have presented similar results. Bublitz and colleagues (2012) reported a larger CAR in women with a history of childhood sexual abuse compared to women with no history of abuse or those with a history of non-sexual abuse. A recent study reported that women with a history of physical abuse had higher total cortisol output during pregnancy, while women with a history of childhood sexual abuse exhibited an increased CAR compared to women with no history of abuse or low levels of childhood abuse (Buss, Entringer, et al., 2016). In contrast, Shea and colleagues (2007) found that higher childhood trauma scores were associated with lower waking cortisol levels.

There is limited published data on the possible effects of early life adversity (ELA) on the maternal HPA axis postpartum. One study of postpartum maternal cortisol response to a stressor involving infant distress has reported that mothers with a history of ELA have a lower cortisol stress response (Brand et al., 2010). In a study of the cortisol awakening response in postpartum women, those with a history of ELA had higher waking cortisol levels and a sustained increase in morning cortisol (Gonzalez et al., 2009).

1.8.6 Early life adversity and antenatal depression

Literature on childhood trauma and antenatal depression demonstrates that maternal childhood adversity is a strong predictor for developing depression during pregnancy. Childhood abuse, particularly physical and sexual, increased a woman's risk of developing depressive symptoms during pregnancy in one study, with stronger associations found with more recent abuse incidents (Benedict et al., 1999; Rich-Edwards et al., 2011). A history of childhood sexual abuse and emotional neglect was found to increase risk of mental disorders during pregnancy, including alcohol misuse and depressive symptoms, and predicted poorer maternal outcomes at one year postpartum (Lang et al., 2006). A history of childhood adversity was reported to be a strong predictor of MDD during pregnancy in a recent study (Plant et al., 2013). Two

systematic reviews have found maternal history of childhood abuse to be associated with depressive illness in the second and third trimesters of pregnancy (Alvarez-Segura et al., 2014; Choi and Sikkema, 2015).

1.8.7 Maternal early life adversity and intergenerational transmission of stress

Evidence to date on the effects of maternal ELA on pregnancy outcomes, offspring adversity during childhood, and subsequently, mental disorders, indicate that there may be a transgenerational effect of maternal ELA on offspring (Noll et al., 2008; Plant et al., 2013; Roberts et al., 2013; Wosu et al., 2015). However, only one study has investigated the relationship between maternal ELA and offspring HPA axis. Brand and colleagues (2010), in a study of maternal history of ELA and infant cortisol measures at six months, found lower baseline cortisol levels in infants born to mothers with a history of ELA. There were no observed differences in the cortisol response to a separation stressor. However, as physical stressors are superior to psychological stressors in eliciting an infant cortisol response, the use of a separation stressor may have been ineffective. Only one study to date has examined the relationship between maternal ELA and placental-foetal stress physiology, and reported maternal exposure to ELA to be associated with high placental CRH levels, with this association strengthening with increasing gestational age (Moog et al., 2016). The authors found that exposure to more than one trauma category led to even higher levels of placental CRH towards the end of gestation, with a 25% increase in placental CRH concentration being reported in women exposed to two categories of early life abuse. These results further support the hypothesis of intergenerational transmission of maternal ELA to the offspring.

1.8.8 Summary

It is clear from the evidence presented that ELA confers an increased risk for developing depression and other mental disorders in adult life, particularly during pregnancy. Whether this vulnerability is mediated by the HPA axis is unclear as there are conflicting reports on the association between ELA and the HPA axis in non-pregnant adults. However studies of maternal ELA and HPA axis during pregnancy report HPA axis hyperactivity in women with a history of ELA. With this in mind, it is

important to consider the risks of intergenerational transmission of ELA stress. Although there is strong evidence for the intergenerational transmission of ELA leading to poorer mental health outcomes in offspring, there is limited evidence to date for transmission of HPA axis changes.

1.10 Aims and structure of thesis

The HPA axis is the primary focus of this thesis. Since there are remarkable HPA axis alterations in the perinatal period, we sought to map its trajectory from pregnancy to postpartum. The literature review shows conflicting evidence for HPA axis dysfunction in antenatal depression. There is clear evidence in the literature for an association between prenatal stress and foetal programming of the infant's HPA axis, with limited evidence for the effects of antenatal depression. The principal aim of this thesis is to examine the HPA axis in antenatal depression, and describe any relationships with the infant HPA axis at two and six months that may be due to foetal programming effects. We also sought to evaluate the trajectory of the infant's HPA axis over the first six months of life.

The methodology of the study will be described in **Chapter 2**, including recruitment details, sample collection, laboratory analysis, and statistical analysis. Each of the data chapters (Chapters 3-6) will begin with a brief introduction, and include study aims and hypotheses, methodology, and results, ending with a brief discussion. **Chapter 3** will describe clinical data during the perinatal period, including the trajectory of change in clinical features during this time. The HPA axis during pregnancy and antenatal depression will be examined in **Chapter 4**, while HPA axis changes over the first six months postpartum in relation to perinatal depression will be described in **Chapter 5**. Infant HPA axis changes over the first six months after birth and any associations with maternal measures will be reported on in **Chapter 6**. A discussion of the findings in this thesis will comprise **Chapter 7**, with recommendations for future research.

1.9 Main study hypotheses

Mothers:

- Depressed women will demonstrate HPA axis dysregulation with higher waking cortisol levels, a blunted cortisol awakening response, and higher evening cortisol levels compared to women in the Control group during the perinatal period.
- Euthymic pregnant women with a history of depression will have higher waking cortisol levels compared to the Control group.
- Total cortisol output will decrease postpartum in the whole group, but dynamic cortisol responses (CAR) will remain the same in all groups.
- Maternal pregnancy and postpartum cortisol levels will have an association with maternal mood, stress, and sleep measures during pregnancy and in the postpartum period.
- Women with a history of ELA will have higher overall cortisol levels compared to women with no history of ELA throughout the perinatal period.

Infants:

- Infants born to women who were depressed during pregnancy will have higher baseline infant cortisol levels and higher vaccination stress responses.
- There will be an association between maternal clinical and cortisol measures during pregnancy and infant cortisol measures.
- There will be an association between infant cortisol measures and concurrent maternal clinical and cortisol measures.
- Maternal ELA will be associated with higher infant HPA axis measures.

Chapter 2

Methods

2.1 Study design

This thesis was conducted as part of a prospective longitudinal cohort study examining the HPA axis in maternal depression during pregnancy and the possible programming effects of antenatal depression on the infant's HPA axis and neurodevelopment. Women diagnosed with depression during pregnancy and women with a history of depression who were euthymic during pregnancy were compared to never-depressed pregnant women. Participants and their infants were followed up for six months after birth.

2.2 Ethical considerations

Ethical approval for conducting the study was obtained from the Research Ethics Committees in the National Maternity Hospital (NMH), Dublin; the Coombe Women and Infants University Hospital (CWIUH), Dublin; and The Rotunda Hospital, Dublin.

2.3 Participants

2.3.1 Recruitment summary

Women were recruited between 20 and 30 weeks gestation and, after a diagnostic clinical interview, completed self-report stress, sleep, and mood questionnaires [Figure 2.1]. Saliva samples at awakening (0 minutes), and 30 minutes, 60 minutes, 12 hours, and 12.5 hours after awakening were collected. Samples were quantified for cortisol levels, the main biological measure in this study, to enable assessment of cortisol diurnal variation and the cortisol awakening response. Delivery details, obstetric outcomes, and birth outcomes were collected after birth. At two and six months postpartum, self-rated stress, sleep, and mood scales, and clinician-rated HAM-D scales were completed, and women provided further saliva samples for cortisol analysis. To assess infant HPA axis activity, saliva samples were collected from infants pre- and post-vaccination, and the cortisol stress response was examined at two and six months postpartum.

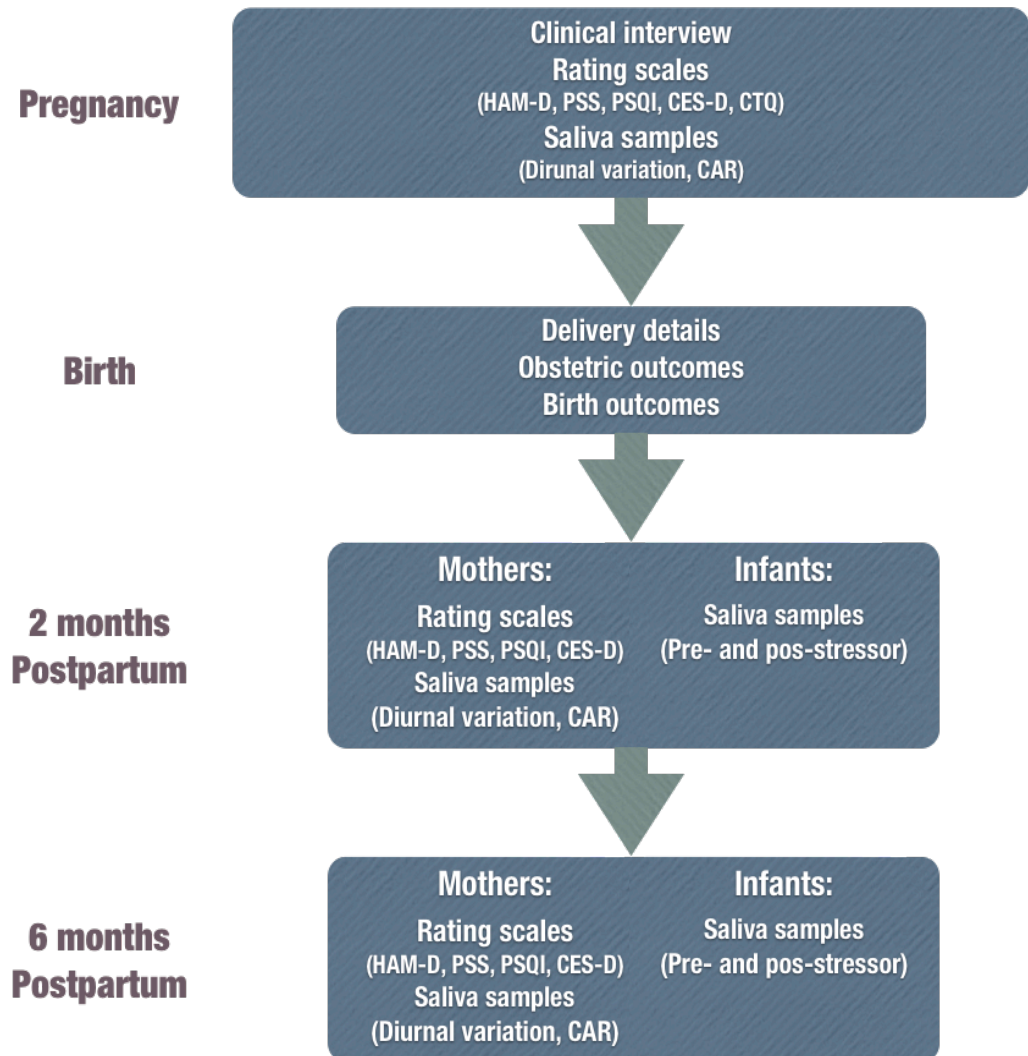


Figure 2.1 Summary of study timeline

Women were recruited during pregnancy, and mother-infant dyads were followed up at two and six months postpartum. Saliva samples for cortisol quantification were collected from mothers and infants. Maternal clinical rating scale measures were completed at all time points.

HAM-D – Hamilton Depression rating scale; **PSS** – Perceived Stress Scale, **PSQI** – Pittsburgh Sleep Quality Index; **CES-D** – Centre for Epidemiological Studies Depression rating scale; **CTQ** – Childhood Trauma Questionnaire; **CAR** – cortisol awakening response.

2.3.2 Recruitment criteria

Participants of all ages were recruited from maternity hospitals if they fulfilled inclusion criteria. Women were recruited into three groups – Depressed, History, and Control. Women who were depressed during pregnancy were recruited into the Depressed group, pregnant women with a prior history of depression who were euthymic during pregnancy were recruited into the History group, and never-depressed pregnant women were recruited into the Control group.

Inclusion criteria

Pregnant women with a DSM-5 diagnosis of MDD were considered for inclusion into the Depressed group. Women with previously diagnosed MDD were considered for inclusion into the History group if they were euthymic during pregnancy. The History group was included in the study to assess any potential trait effects of depression on the HPA axis. Women with no prior history of mental illness were eligible for inclusion into the Control group. Women between 20 and 30 weeks gestation were eligible for recruitment. This gestational age range was used as differences in HPA axis measures were evident between depressed and non-depressed women at around 25 weeks gestation in a prior study by O’Keane and colleagues (2011).

Exclusion criteria

Women with physical illness, chronic medical conditions, or significant obstetric complications during the index pregnancy were excluded from the study, as were women with non-singleton pregnancies. Women with current symptoms of, or a past history of, anxiety disorders, psychotic illness, or substance misuse were not eligible for participation. Women taking long-term medication, other than antidepressants or mood stabilisers, were excluded from the study. Women who were unable to communicate in English were not considered for the study due to ethical concerns with obtaining informed consent.

2.3.3 Method of selection

Participants were recruited from perinatal psychiatry clinics and antenatal clinic waiting rooms in the three maternity hospitals in Dublin.

Depressed group

I conducted two Perinatal Psychiatry clinics in two of the maternity hospitals in Dublin – NMH and CWIUH in order to recruit patients for this study. Pregnant women referred with low mood were triaged to my clinic, where I assessed them and considered their eligibility for participation in the study. Another avenue of recruitment into this group was from referrals from the Perinatal Psychiatrist or the Perinatal Advanced Nurse Practitioner in the hospitals. A brief description of the study was given to women between 20 and 30 weeks gestation who fulfilled criteria for MDD, after their clinical assessment. A brief information leaflet with researcher contact details [Appendix I] was also given to women during the appointment. If women were interested in participating at the time, a more detailed explanation and a Participant Information Sheet [Appendix II] were given. Patients signed the Consent Form [Appendix III] if they wanted to proceed with study participation. It was verbally reiterated that patients' participation in the study would not affect their care in the hospital.

Participants then completed four rating scales – the Perceived Stress Scale (PSS), the Pittsburgh Sleep Quality Index (PSQI), Centre for Epidemiological Studies Depression rating scale (CES-D), and the Childhood Trauma Questionnaire (CTQ). The clinician-rated Mini International Neuropsychiatric Interview (MINI) and the Hamilton Depression rating scale (HAM-D) were also completed at the time of recruitment. Participants were shown how to collect saliva samples and given a saliva collection pack, which included five sample collection tubes and written instructions for saliva collection [Appendix VI] [See Section 2.5]. A stamped and addressed padded envelope was also given to the patients, to post their saliva samples to the Trinity College Institute of Neuroscience (TCIN) laboratory for processing.

In most cases however, patients preferred to discuss the study with their partners and return on a different day for recruitment. In these instances, patients were given the option to attend either their maternity hospital or Trinity College Dublin (TCD) for the recruitment interview. The detailed Participant Information Sheet was emailed to patients prior to their recruitment interview. The study was discussed verbally and the diagnostic clinical interview completed, if it had not been completed previously.

Clinician- and participant-rated psychiatric rating scales were completed, and the procedure for saliva collection followed as described above. Of note, in this study, having the same researcher responsible for clinical care of the patient and the research evaluation increases the potential for observer bias, particularly in the clinical diagnosis of depression and in rating the HAM-D scale.

History group

Women with a lifetime history of clinical depression, diagnosed at any stage prior to the index pregnancy, were eligible for recruitment into this group. Women with a prior history of depression who were euthymic during the index pregnancy were either recruited from the Perinatal Psychiatry clinics or through referrals from the Perinatal Psychiatrist or the Advanced Nurse Practitioner in Perinatal Mental Health. A small number were recruited from antenatal clinic waiting rooms. Recruitment was either completed in the maternity hospitals or in TCD, following the procedure described above.

Control group

For recruitment of women with no history of mental illness, pregnant women between 20 and 30 weeks gestation were approached in the waiting room of antenatal clinics or ultrasound clinics and the study briefly discussed. Women recruited into the Control group were gestation-matched to women in the Depressed and History groups. Women were initially given a brief study leaflet with researcher contact details [Appendix I], and if interested in participating, a Participant Recruitment Form, including their contact details and any potential exclusion criteria [Appendix IV], was completed. Women were then contacted via phone or email a few days later to confirm participation in the study, and those who were contactable and interested in participating were given a date for the recruitment interview and emailed the detailed Participant Information Sheet [Appendix II]. Women attended either TCD or their maternity hospital for the clinical interview, and the procedure described above was followed.

2.4 Clinical measures

2.4.1 Sociodemographic characteristics

Maternal sociodemographic variables were collected at the time of recruitment interview [Appendix V]. Maternal age, BMI, gravidity, parity, pregnancy intent, and gestational week at the time of recruitment and at saliva sample collection were noted. Maternal ethnicity, education level, employment status, marital status, and levels of maternal social support were coded [Table 2.1]. Maternal smoking and alcohol use during the index pregnancy were coded Nil or Social use. Social use was categorised as any smoking during pregnancy or, for alcohol, ≥ 2 units of alcohol use per week. No provision was made for substance abuse as this was a criterion for exclusion. Information was obtained on domestic abuse during the index pregnancy. However, none of the participants in this study reported domestic abuse.

Table 2.1 Coding of demographic variables

Demographic Variables	Coding
Ethnicity	White African South Asian South East Asian Hispanic
Education	High School Some College University Degree Professional Qualification
Employment	Employed Unemployed
Marital Status	Single Co-habiting Married Divorced
Substance use (Smoking or alcohol)	Nil Social use
Social Support	Nil Good support

2.4.2 Diagnostic clinical interview

A clinical interview was conducted for each participant to assess eligibility and MDD diagnosis. Details about the patient's mental state during pregnancy, past psychiatric history, medical and obstetric history, medication use, substance use, family history of mental illness, personal history, and social history were collected during the interview. A diagnosis was made at the end of the interview, based on DSM-5 criteria. Modules A-C of the MINI were completed by the researcher during the interview process. The MINI is a brief, structured diagnostic interview schedule, particularly useful in clinical research for DSM-IV-TR and ICD-10 diagnosis of psychiatric disorders (Sheehan et al., 1998). Module A addresses Major Depressive Disorder, B Dysthymia, and C Suicidality [Appendix XII].

2.4.3 Rating scales

Participant-rated scales such as the PSS, PSQI, CES-D and CTQ, and the clinician-rated HAM-D scale, were completed at the time of recruitment. All the rating scales, except for the CTQ, were also completed at the two follow-up time points at two months and six months postpartum.

Perceived Stress Scale

The PSS is a 14-item self-report Likert scale that measures an individual's perception of stress over the preceding month (Cohen et al., 1983). The PSS is widely used in clinical research as a measure of the extent to which the current environment is appraised as stressful. [Appendix VII].

Pittsburgh Sleep Quality Index

The PSQI is 19-item self-report questionnaire that measures an individual's sleep quality over the previous month (Buysse et al., 1989). The PSQI evaluates seven components of sleep – sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of hypnotic medication, and daytime dysfunction. [Appendix VIII].

Centre for Epidemiological Studies Depression rating scale

The CES-D is a 20-item self-report depression questionnaire assessing depressed mood, low self-esteem, low energy, loss of appetite, and sleep disturbance (Radloff,

1977). Scores >16, although not diagnostic, are thought to indicate probable depression [Appendix IX]. The CES-D was used as an objective measure of mood in the present study over the EPDS, since it is more comprehensive and includes biological features of depression, which may be more markedly disturbed in depression during pregnancy.

Childhood Trauma Questionnaire

The CTQ is a 28-item self-report Likert scale that measures childhood maltreatment including emotional abuse and neglect, physical abuse and neglect, and sexual abuse (Bernstein et al., 2003). In addition to five questions for each domain of childhood adversity, there are three questions to address possible minimisation or denial of incidents of childhood adversity [Appendix X].

Hamilton Depression Rating Scale

The HAM-D is a 21-item clinician-rated scale of depression symptom severity (Hamilton, 1960). Scores are based on the first 17 items, while the final four items provide further information about the symptomatology. Items on the HAM-D evaluate various symptoms of depression including low mood, suicidal ideation, feelings of guilt, psychomotor activity, anxiety, and somatic symptoms. [Appendix XI].

2.5 Postpartum follow-up

2.5.1 After delivery

Consent was sought at the time of recruitment for the researcher to contact the mothers around the time of delivery. Mothers were given a courtesy phone call roughly a week after their estimated delivery date, to collect information on delivery and infant details. They were informed that a researcher would be in contact before the infant's two-month vaccination appointment to obtain details about the appointment date. Delivery details including method and onset of labour and labour complications were collected from maternal hospital medical records. Infant birth details including gestational age at birth, birth weight, head circumference, and Apgar scores at 1 minute and 5 minutes were collected from infant hospital medical records.

2.5.2 Two and six months postpartum

Mothers were contacted in advance of the infant's two-month and six-month vaccination to ascertain the date of the vaccination appointment. To facilitate infant saliva sample collection, either Niamh O'Leary, another researcher involved in the study, or I attended the appointments with the mothers.

Mother

Mothers collected their saliva samples at five time points over the course of the day (0, 30, 60 minutes, 12 hours, 12.5 hours after awakening) before the infant's vaccination appointment. At the appointment, mothers also completed the PSS, PSQI, and CES-D. HAM-D rating scales were also completed at the two time points.

Infant

Saliva samples were collected from the infants in the waiting room of the General Practitioner's clinic, before, and 30 minutes after the vaccination, to assess the cortisol stress response. Samples were collected at the two- and six-month vaccination appointments.

2.6 Biological measures

2.6.1 Saliva sample collection and storage

Saliva samples in the mother

Participants collected saliva samples at five time points over the course of one day at the time of recruitment during pregnancy. Women were given saliva collection packs at the time of the recruitment interview, which consisted of five labelled Salivette (Sarstedt) saliva tubes and an instruction sheet for saliva collection. Participants were advised to thoroughly rinse their mouths with water five minutes prior to sample collection, ensuring that they spit out all the water, and to place an absorbent swab provided in the saliva collection kit under the tongue for two minutes until the swab was saturated. They were asked to refrain from eating, drinking, or brushing their

teeth before the morning saliva sample collection. In order to improve compliance, participants were not asked to record waking times, times of saliva collection, or any events occurring around the time of sample collection. Swabs were then placed into an inner tube of the Salivette device, and the cap was placed on firmly. Saliva samples were collected at awakening (0 minutes), 30 minutes and 60 minutes after awakening, and then again at 12 hours and 12.5 hours after waking. Salivette tubes were stored in the participant's refrigerator for a day or two until they were posted out to TCIN in the self-addressed envelope provided. Once received, the samples were centrifuged for 10 minutes at 3,000 rbpm and room temperature. The inner tube was then discarded and the saliva divided into microtubes of 500µL aliquots and stored at -80°C.

Participants also provided saliva samples at the same five time points over the course of one day at two months and six months postpartum. Mothers collected their saliva samples the day before or the day after their infant's vaccination appointments. Samples were stored in the participant's fridge until collection by the researcher at the infant's vaccination appointment the following day, or until they were posted out to TCIN in the stamped and addressed envelope provided.

Saliva samples in the infant

Infant saliva samples were collected at the two- month and six-month vaccination appointments. Samples were collected in SalivaBio tubes (Salimetrics), designed for saliva sample collection in infants up to six months of age. These collection kits provide long absorbent swabs that enable the researcher to hold one end while leaving the other end in the infant's mouth for 1-2 minutes. This collection technique reduces choking hazard in infants. The swab was then placed in the saliva tube for transportation to TCIN, where they were centrifuged using the procedure described above.

2.6.2 Cortisol analysis

Saliva samples were then transported in three batches to Professor Brian Keevil's laboratory in the University Hospital of South Manchester for cortisol quantification. Cortisol levels were analysed using the Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) technique. LC-MS/MS has several advantages over

immunoassay techniques. In addition to having improved specificity and sensitivity, LC-MS/MS requires smaller sample volumes for analysis. This was of particular benefit in the present study as infant saliva samples were generally of small volume. In addition, LC-MS/MS can quantify several hormones simultaneously, producing results for cortisol and cortisone concentrations (Keevil, 2013; Rauh, 2010).

The laboratory protocol for cortisol and cortisone analysis was procured from Professor Keevil's laboratory. A Lower Limit of Quantification of 0.3 nMol/L was used for cortisol and cortisone levels in saliva. This was based on coefficients of variance of 9.4% and 4.9% for cortisol at 0.25 nMol/L and 0.5 nMol/L, respectively, and coefficients of variance of 14.1% and 5.2% at 0.25 nMol/L and 0.5 nMol/L for cortisone, respectively. Cortisol and cortisone were extracted by liquid-liquid extraction (LLE) to remove interfering substances and to minimise matrix effects. Samples were then extracted by mixing with methyl-tert-butyl ether (MTBE) following the addition of internal standard (Deuterated (D₄) cortisol and Deuterated (D₇) cortisone). Following extraction, the organic fraction was removed, evaporated and the residue reconstituted in 40% methanol. The reconstituted sample was then injected onto a C₁₈ reverse phase chromatography column connected to a tandem mass spectrometer to produce concentration levels for cortisol and cortisone.

2.6.3 Cortisol levels and cortisol awakening response parameters

Cortisol awakening response parameters of interest were calculated from the cortisol values obtained at 0 minutes (T₀), 30 minutes (T₃₀), and 60 minutes (T₆₀) after awakening. The Area Under the Curve (AUC) with respect to ground (AUC_g) and AUC with respect to increase (AUC_i) [Figure 2.2] were calculated using the following formulae provided by Fekedulegn and colleagues (Fekedulegn et al., 2007):

$$AUC_g = \left[\left\{ \frac{T_0 + T_{30}}{2} \right\} \times \text{time difference} \right] + \left[\left\{ \frac{T_{30} + T_{60}}{2} \right\} \times \text{time difference} \right]$$

$$AUC_i = AUC_g - [T_0 \times \text{total time between first and last measurement}]$$

The slope and intercept of a regression line fitted through the raw morning cortisol values were also calculated for statistical analysis in Microsoft Excel. The AUC_g and the intercept are measures of total cortisol production over the first hour after awakening, while the AUC_i and the slope of the regression line are indicators of the dynamic of cortisol change, or the cortisol awakening response (Khoury et al., 2015). Waking and evening cortisol levels are also used in analyses in this study.

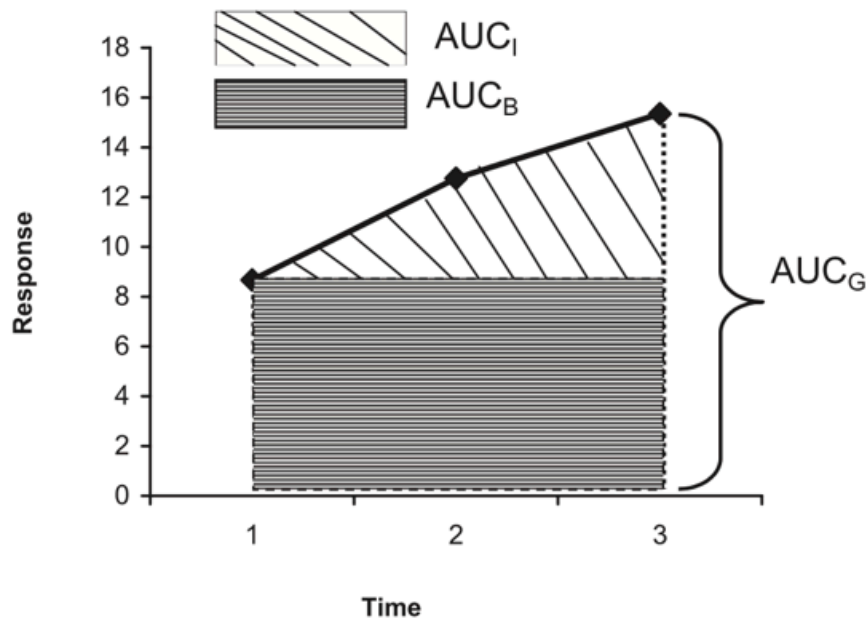


Figure 2.2 Cortisol awakening response parameters

The Area Under the Curve with respect to ground (AUC_g), indicating total cortisol output over the course of the first hour after awakening, and the Area Under the Curve with respect to increase (AUC_i), indicating the dynamic of cortisol increase are presented above. (From Fekedulegn et al, 2007)

2.7 Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics for Macintosh, Version 24 (IBM Corp, US). Continuous data is presented in means and standard deviations (SD), and categorical data in frequency and percentage (%). All data is reported to two decimal places, with the exception of p values, which are reported to three decimal places. Threshold for significance was set at 0.05 for all analyses. Data was assessed for normality with the Kolmogorov-Smirnov test. Raw cortisol data, which was not normally distributed, was log transformed prior to analysis to approximate a normal distribution and allow for general linear model analyses.

Non-parametric continuous data was assessed for group differences using the Mann-Whitney U test or the Kruskal-Wallis test, while normally distributed data was compared using the independent samples t-test or the one-way analysis of variance (ANOVA). Correlation analysis was conducted using Spearman's rank-order test in non-parametric data, and Pearson's correlation analysis in parametric data. General linear regression and hierarchical multiple regression were used in some association analyses. The Wilcoxon Signed-Rank test was used to assess repeated measures data in non-parametric samples. Categorical data was analysed using the Chi-square test for independence. Multiple comparisons were corrected for a false discovery rate using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995). All graphs were generated using GraphPad Prism, version 7.00 for Macintosh (GraphPad Software, La Jolla California, USA. www.graphpad.com).

Moderation analysis addresses the combined effect of two variables on an outcome, and was used to explore associations between cortisol measures and maternal and infant factors [Figure 2.3] (Field, 2013). Moderation is a regression-based model, and the analysis was conducted using a macro named PROCESS for SPSS and SAS, Version 2.16 (Andrew Hayes, Ohio State University, USA. www.processmacro.org). Conceptually, a moderator variable influences the relationship between a predictor and an outcome variable, without directly affecting the outcome variable [Figure 2.3]. The statistical model of moderation involves an interaction between the moderator and predictor variables [Figure 2.4] (Field, 2013). The PROCESS tool was used to assess the interaction term with simple slopes analysis. Simple slopes analysis produces the regression equation for the predictor and outcome variables at the mean, and 1SD

above and below the mean, for continuous moderator variables. In categorical moderator data, simple slopes analysis produces the regression equation for each categorical variable used in the analysis. Simple slopes data is then visualised on a graph, which allows interpretation of the slope and direction of the relationship between the predictor and outcome for each categorical moderator variable or the mean $\pm$ 1SD in continuous moderator data. Since moderation is a hierarchical linear regression model, it was ensured that the assumptions of linear regression were met. Only significant moderation interactions are reported in the thesis.

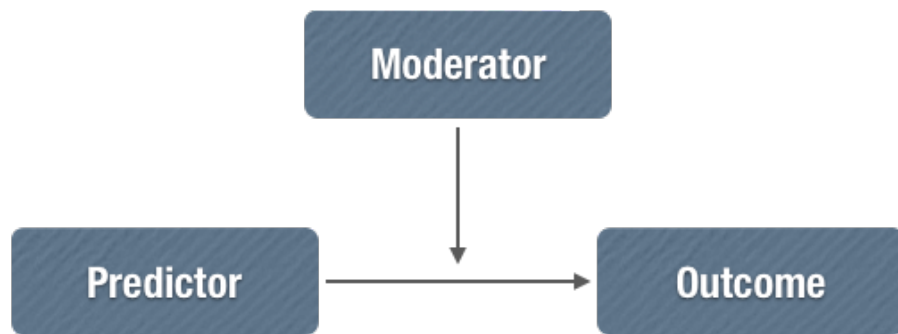


Figure 2.3 Conceptual model of moderator interaction

The moderator variable influences the relationship between the predictor and outcome variables. (Adapted from Field 2013)

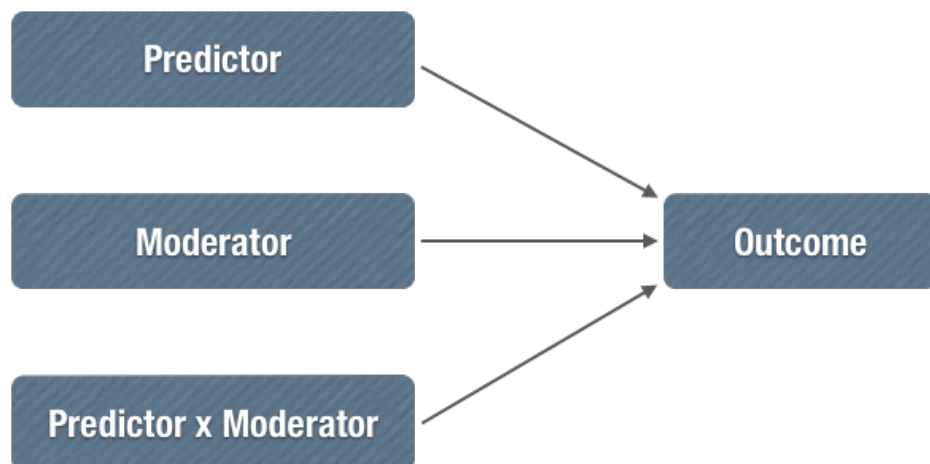


Figure 2.4 Statistical model of moderator analysis

The predictor and moderator variables must be included in the model, and the interaction term has to be significant for a moderation effect to be valid. (Adapted from Field 2013)

Chapter 3

Clinical aspects of mood states in pregnancy and postpartum

3.1 Introduction

Depression is a prevalent mental illness, and women are nearly twice as likely to report an episode of depression compared to men (Kessler et al., 1993). Women are most vulnerable to developing depression during their childbearing years, and pregnancy, with the notable physical, physiological, hormonal, and psychological changes it brings, confers a heightened risk (Bennett et al., 2004; Parry et al., 1995; Weissman et al., 1993). The prevalence of major depressive disorder (MDD) during pregnancy varies worldwide, and is generally higher in lower socioeconomic populations. A systematic review by Bennett and colleagues (2004) evaluating interview and self-report depression data from worldwide studies found prevalence rates of 7.4% in the first trimester, 12.8% in the second trimester, and 12% in the third trimester. It is often believed that the somatic symptoms of healthy pregnancy such as disrupted sleep, appetite, and energy levels may confound a diagnosis of MDD in the perinatal period (Yonkers, Vigod, et al., 2011). However, Kelly and colleagues (2001) reported that women with features of depression or anxiety were more likely to report somatic symptoms. In another study, Field and colleagues (2007) found that depressed women had more sleep disturbance than their non-depressed counterparts in the second and third trimesters of pregnancy. Findings in a recent Canadian study suggested that women reporting poorer sleep quality during pregnancy had higher levels of depressive symptoms (Tomfohr et al., 2015). Somatic symptoms appear to be more enhanced in women experiencing depression during pregnancy and consequently, it is prudent to include them in the diagnostic criteria for antenatal depression.

Current evidence for the association between antenatal depression and poor obstetric outcomes is conflicting. Women with antenatal depression were found to have higher rates of preeclampsia and caesarean section deliveries in one study (Hu et al., 2015). Another study of the impact of depression and anxiety on preeclampsia risk included 109 women with pre-gestational depression or depression diagnosed in the first 20 weeks of gestation, and 32 women with similarly diagnosed anxiety (Qiu et al., 2009). A 2-fold increase in the risk of preeclampsia was observed in women diagnosed with a mood or anxiety disorder. The authors of a systematic review of gestational diabetes and depression suggest that further studies are warranted to describe depression as a risk factor for developing gestational diabetes, as there is no consensus in study results to date (Ross et al., 2016).

An established theory in the aetiology of depression is that of the 'kindling' effect of depression, thought to increase the individual's vulnerability to recurrence (Monroe and Harkness, 2005). Consequently, a lifetime history of depression may augment the vulnerability for depression in the perinatal period, thus further increasing the risk of recurrence during this time (Lancaster et al., 2010). Apart from the conferral of an increased risk of recurrence, little is known about the effects of lifetime depression on clinical features during pregnancy. Further, a previous history of depression, or antenatal depression itself, can increase a woman's risk for postpartum depression (Silverman et al., 2017). About a third to half of postpartum depression is thought to begin during pregnancy (Gotlib et al., 1989; Wisner et al., 2013). In a systematic review of the prevalence of perinatal depression, Gavin and colleagues (2005) estimated the point prevalence rate of postpartum depression to lie between 6.5% and 12.9% over the first year postpartum, with a period prevalence of 19.2% over the first three months postpartum. In another systematic review, postpartum depression prevalence was 19.8%, reducing to 16.1% when only studies utilising diagnostic assessments were included (Fisher et al., 2012). Postpartum depression has been shown to be strikingly similar to non-postpartum depression, with somatic symptoms being a prominent feature (O'Hara and McCabe, 2013).

Early life adversity (ELA) is now recognised to be a risk factor for developing depression in non-pregnant populations (Heim et al., 2008; McCrory et al., 2012). A small number of studies examining the relationship between ELA and maternal depression during pregnancy have found no effects of ELA on antenatal depression (Dayan et al., 2010; Edwards et al., 2008; Gilson and Lancaster, 2008; Shea et al., 2007b). However, findings from the majority of studies examining this relationship suggest that there is a positive association between maternal history of ELA and depression during pregnancy (Benedict et al., 1999; Choi and Sikkema, 2015; Lang et al., 2006; Leigh and Milgrom, 2008; Plant et al., 2013).

3.2 Methods

3.2.1 Recruitment

All women in the study were recruited from three maternity hospitals – the National Maternity Hospital, Dublin; the Coombe Women and Infants University Hospital, Dublin; and The Rotunda Hospital, Dublin. Methods of recruitment varied depending on the individual clinical models in the hospitals but most depressed pregnant women and those with a history of depression were recruited from Perinatal Psychiatry clinics, or through referrals from members within the Perinatal Psychiatry service. For recruitment into the Control group, pregnant women were approached in the waiting room at their initial visit to the maternity hospitals and made aware of the study. A detailed explanation was given and, if interested, women were recruited into the study after obtaining informed written consent [See Section 2.3.2 for details on inclusion and exclusion criteria]. All study participants underwent a clinical interview, and were placed into the Depressed group if they fulfilled DSM-5 criteria for MDD, the History group if they were euthymic during pregnancy but had a prior history of depression, and the Control group if they had never been depressed. Women were recruited between 20 and 30 weeks gestation, and controls were gestation-matched to women in the Depressed and History groups.

3.2.2 Rating scales

The clinician-rated Hamilton Depression rating scale (HAM-D) was completed for each participant. Women also completed self-rated Perceived Stress Scale (PSS), Pittsburgh Sleep Quality Index (PSQI), the Centre for Epidemiological Studies Depression rating scale (CES-D), and the Childhood Trauma Questionnaire (CTQ). The CTQ is further categorised into five domains – Emotional Abuse, Physical Abuse, Sexual Abuse, Emotional Neglect, and Physical Neglect. CTQ scores were categorised into –ELA (no abuse) or +ELA (scores within the Low to Severe abuse classifications) within each abuse domain. Women with a history of ≥ 1 trauma subtype were analysed separately to women with no history of abuse.

3.2.3 Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics for Macintosh, Version 24 (IBM Corp, Armonk, NY). Data was assessed for normality using the Kolmogorov-Smirnov test. Data that followed a normal distribution was analysed using the independent-samples t-test or one-way analysis of variance. Non-normally distributed data was analysed using the non-parametric Mann-Whitney U test or the Kruskal-Wallis test. Univariate general linear model analysis for covariance was performed to control for covariates such as medication and obstetric complications. Chi-square test of independence was conducted to analyse distribution of categorical data across groups. Correlation analysis was conducted using Pearson's product-moment correlation for normally distributed data, and Spearman's rank-order analysis for non-parametric data. Wilcoxon Signed-Rank test was used to analyse repeated measures data.

3.3 Results

The sociodemographic characteristics of the sample will be described in **Section 3.3.1**. Clinical features of the sample during pregnancy will be discussed in **Section 3.3.2**, obstetric outcomes in **Section 3.3.3**, and postpartum clinical features in **Section 3.3.4**. Early life adversity and its effects on the clinical features during pregnancy and postpartum will be described in **Section 3.3.5**.

3.3.1 Sociodemographic characteristics of sample

The sociodemographic characteristics of the sample are presented in Table 3.1. Differences in mean age, gestation, gravidity and parity between Depressed, History and Control groups were analysed. The relationship among categorical demographic data across the three groups was explored. Age, gestation, gravidity and parity had similar means across the three groups with no significant differences.

Adjusted residuals in the Chi-square analysis were analysed to assess differences between individual groups. There were fewer than expected planned pregnancies (adjusted residual -3.5) and more than expected unplanned pregnancies in the Depressed group compared to the History and Control groups (adjusted residual 3.5). The Depressed group also had lower than expected levels of social support compared to the other two groups (adjusted residual -3.7), and more women than expected with no supports (adjusted residual 3.7). There were differences between groups in employment, with the History group having higher levels of unemployment (adjusted residual 2.9) and lower levels of employment (adjusted residual -2.9) than would be expected, and the Control group having more women than expected who were employed (adjusted residual 2.5) and fewer who were unemployed (adjusted residual -2.5). There were no differences in ethnicity, marital status, education, smoking and alcohol between the groups. Of note, the majority of the sample was Caucasian, married, and did not smoke or drink alcohol during pregnancy.

Table 3.1 Sociodemographic characteristics of sample during pregnancy

	Depressed (n=23)	History (n=31)	Control (n=46)	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
Age	31.60 (3.88)	32.29 (5.04)	33.91 (4.88)	$\chi^2=3.56$, p=0.169
Gestation	26.39 (4.78)	26.55 (2.99)	26.53 (3.92)	$\chi^2=1.47$, p=0.480
Gravidity	1.77 (0.87)	2.37 (1.10)	2.00 (1.12)	$\chi^2=4.88$, p=0.087
Parity	0.63 (0.85)	0.97 (0.76)	0.70 (0.76)	$\chi^2=3.65$, p=0.161
	n (%)	n (%)	n (%)	
Ethnicity				$\chi^2=6.89$, p=0.331
Caucasian	22 (95.7)	29 (93.5)	41 (89.1)	
African	1 (4.3)	0	1 (2.3)	
South Asian	0	0	3 (6.8)	
SE Asian	0	0	0	
Hispanic	0	2 (6.7)	1 (2.3)	
Pregnancy Intent				$\chi^2=12.44$, p=0.002*
Planned	12 (54.5)	27 (90.0)	40 (87.0)	
Unplanned	10 (45.5)	3 (10.3)	6 (13.6)	
Marital Status				$\chi^2=3.08$, p=0.545
Single	2 (8.7)	3 (10)	3 (6.5)	
Co-habiting	10 (43.5)	7 (23.3)	13 (28.3)	
Married	11 (47.8)	20 (66.7)	30 (65.2)	
Separated	0	0	0	
Education				$\chi^2=9.24$, p=0.161
High School	5 (22.7)	4 (13.3)	3 (6.5)	
Some College	1 (4.5)	5 (16.7)	6 (13.0)	
Undergraduate	15 (68.2)	16 (53.3)	25 (54.3)	
Postgraduate	1 (4.5)	5 (16.7)	12 (26.1)	
Employment				$\chi^2=9.12$, p=0.010*
Employed	18 (81.8)	19 (63.3)	42 (91.3)	
Unemployed	4 (18.2)	11 (36.7)	4 (8.7)	
Smoking				$\chi^2=2.37$, p=0.306
Non-smoker	20 (90.9)	27 (90.0)	45 (97.8)	
Smoker	2 (9.1)	3 (10.0)	1 (2.2)	
Alcohol				$\chi^2=5.97$, p=0.051
Nil in pregnancy	22 (100)	23 (76.7)	37 (80.4)	
>2 drinks/week	0	7 (23.3)	9 (19.6)	
Social Supports				$\chi^2=13.25$, p=0.001*
Nil	4 (17.4)	0	0	
Good Supports	19 (82.6)	30 (100)	46 (100)	

Note: Kruskal-Wallis test was used to analyse differences in continuous variables, and Chi-square test of independence used for categorical variables. Threshold for significance was set at 0.05.

3.3.2 Pregnancy

The clinical features of the sample during pregnancy, including mood profile, medication, substance use, and past psychiatric history will be described in this section. Self-rated and clinician-rated mood rating scales, as well as self-rated sleep and childhood trauma rating scales will be also be discussed in this section.

3.3.2.1 Characteristics of sample during pregnancy

In total, 100 participants were recruited into the study during pregnancy. The proportion of women recruited into each group is shown in Figure 3.1. All women in the **Depressed** group (n=23) had a diagnosis of MDD, as per DSM-5 criteria (American Psychiatric Association, 2013) at the time of recruitment. Twenty-two women in this group had a prior history of depression unrelated to the index pregnancy, and relapsed during the index pregnancy. Only one participant in the Depressed group experienced her first episode of depression during the index pregnancy. Women in the **History** group (n= 31) all had a lifetime history of MDD but were euthymic throughout the index pregnancy. Pregnant women who had never been diagnosed with depression or any mental illness were recruited into the **Control** group (n=46). The mean gestation at recruitment was 26 weeks for all groups [Table 3.1].

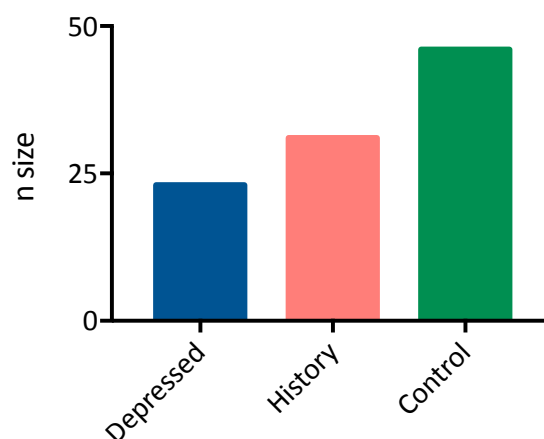


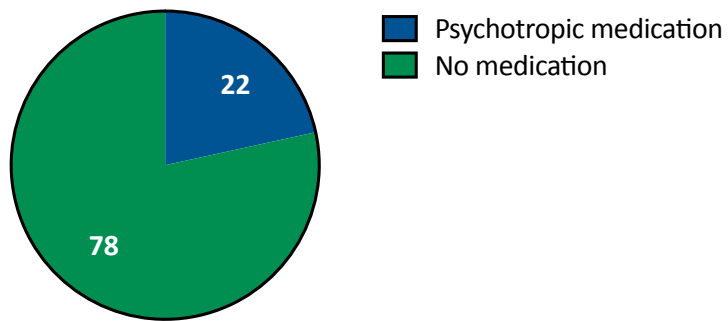
Figure 3.1 Recruitment outcome for each caseness group

Data is displayed as number of participants (n size) in each of the three groups – Depressed, History, and Control.

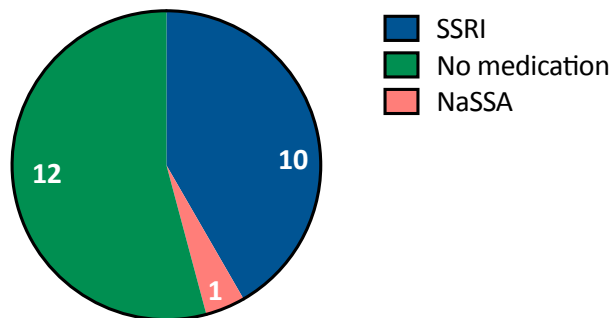
3.3.2.2 Medications during pregnancy

In this study, 22 women were taking psychotropic medication during pregnancy [Figure 3.2], 11 of whom were in the Depressed group, and a further 11 in the History group. Of these, 19 were prescribed selective serotonin reuptake inhibitors (SSRI); one was prescribed mirtazapine, a noradrenergic and specific serotonergic antidepressant (NaSSA); one was prescribed duloxetine, a serotonin-noradrenaline reuptake inhibitor (SNRI); and one was prescribed lamotrigine, a mood stabiliser.

a) Whole sample



b) Depressed



c) History

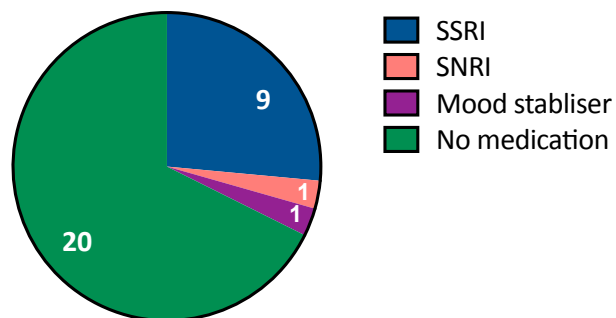


Figure 3.2 Medication use during pregnancy

- a) Distribution of psychotropic medication use within the whole pregnancy sample
- b) Distribution of psychotropic medication use within the Depressed group during pregnancy
- c) Distribution of psychotropic medication use within the History group during pregnancy

3.3.2.3 Clinical measures during pregnancy

Mean PSS, PSQI, CES-D, CTQ, and HAM-D scores between the Depressed, History, and Control groups were compared. Results of the scales are summarised in Table 3.2 and differences among the groups can be seen in Figure 3.3. The Depressed group had the highest scores in all the measures, and had significantly higher scores than the Control group in the PSS, PSQI, CES-D, and as would be anticipated, the HAM-D. The Depressed group had significantly higher scores than the History group in PSS, CES-D, and HAM-D scores also. The History group scored significantly higher than the Control group in PSS, CES-D, and HAM-D. However, none of the women in the History group fulfilled study criteria for MDD as described by DSM-5, indicating they had subsyndromal depressive features.

Overall, women in the Depressed group had higher scores compared to the History and Control groups in all the childhood trauma domains apart from Sexual Abuse and Physical Neglect [Table 3.2, Figure 3.4]. Pairwise comparisons showed that the Depressed group had significantly higher scores than the Control groups in the Emotional Abuse ($p<0.001$), Physical Abuse ($p=0.016$), and Emotional Neglect ($p<0.001$) categories. Within the Emotional Abuse domain, depressed women also had higher scores compared to those with a history of depression ($p=0.041$).

Table 3.2 Rating scale differences between groups during pregnancy

	Depressed (n=23)	History (n=31)	Control (n=46)	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
PSS	36.68 (5.18)	24.77 (7.32)	16.56 (7.61)	$\chi^2=50.99$, $p<0.001^*$
PSQI	10.20 (3.76)	7.53 (3.39)	5.66 (3.50)	$\chi^2=18.22$, $p<0.001^*$
CES-D	35.55 (12.28)	14.29 (8.64)	6.23 (5.69)	$\chi^2=51.95$, $p<0.001^*$
HAM-D	20.82 (5.01)	3.65 (4.39)	1.39 (1.55)	$\chi^2=57.50$, $p<0.001^*$
CTQEA	11.38 (5.09)	9.70 (5.53)	7.17 (3.42)	$\chi^2=18.39$, $p<0.001^*$
CTQPA	7.00 (3.89)	6.60 (3.20)	5.60 (2.11)	$\chi^2=7.92$, $p=0.019^*$
CTQSA	6.38 (3.76)	7.33 (5.90)	5.60 (2.57)	$\chi^2=1.54$, $p=0.463$
CTQEN	11.19 (4.74)	9.40 (4.33)	7.36 (2.97)	$\chi^2=15.25$, $p<0.001^*$
CTQPN	7.57 (3.20)	6.43 (1.91)	6.02 (1.62)	$\chi^2=4.99$, $p=0.082$

PSS – Perceived Stress Scale, **PSQI** – Pittsburgh Sleep Quality Index, **CES-D** – Centre for Epidemiological Studies Depression rating scale, **HAM-D** – Hamilton Depression Rating scale, **CTQ** – Childhood Trauma Questionnaire. **CTQEA** – CTQ Emotional Abuse, **CTQPA** – CTQ Physical Abuse, **CTQSA** – CTQ Sexual Abuse, **CTQEN** – CTQ Emotional Neglect, **CTQPN** – CTQ Physical Neglect.

Note: A Kruskal-Wallis test was conducted to compare differences between groups. Threshold for significance was set at 0.05.

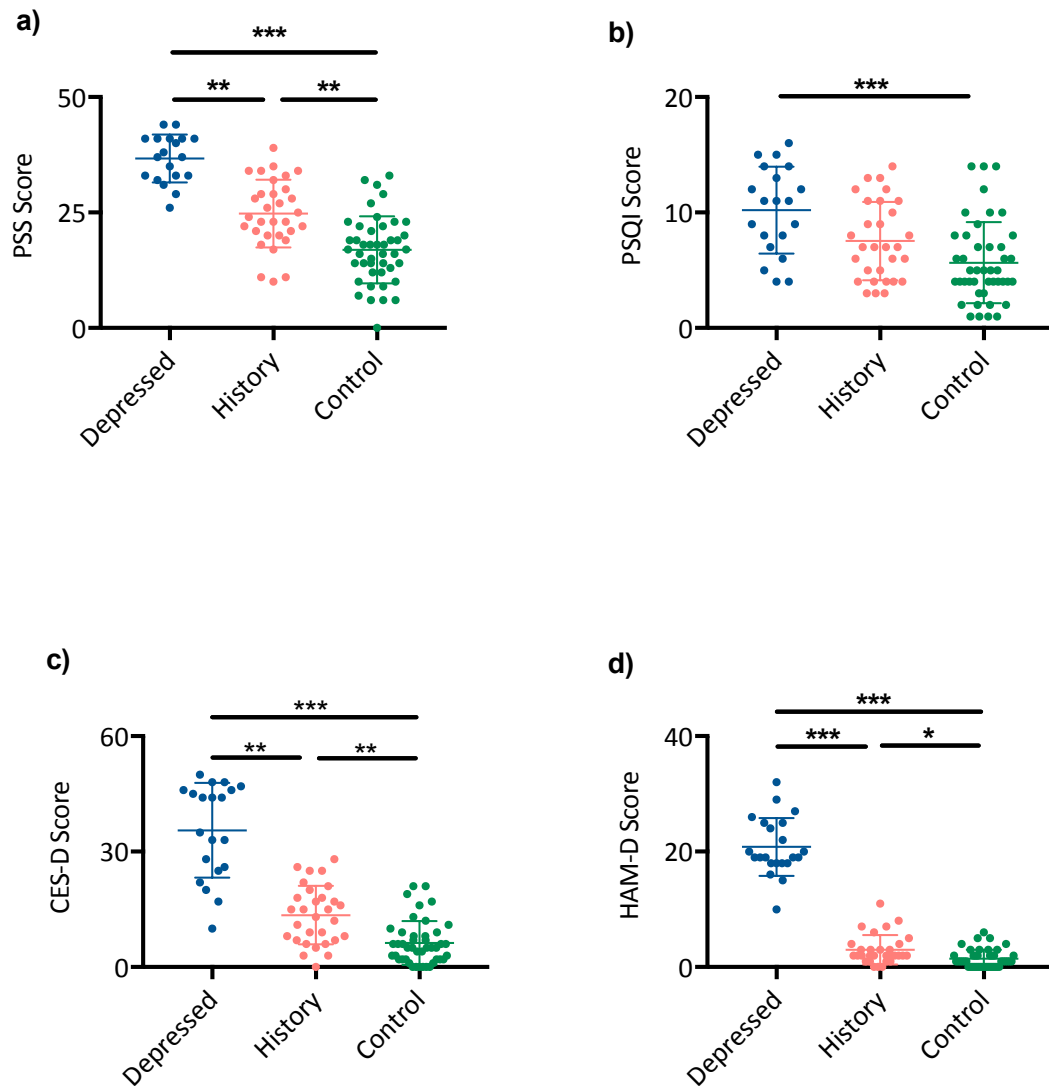


Figure 3.3 Mood and sleep rating scale scores during pregnancy

Perceived Stress Scale (PSS), Pittsburgh Sleep Quality Index (PSQI), Centre for Epidemiological Studies Depression rating scale (CES-D), and Hamilton Depression rating scale (HAM-D) scores during pregnancy across the groups. Means and SD for each group are depicted above.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

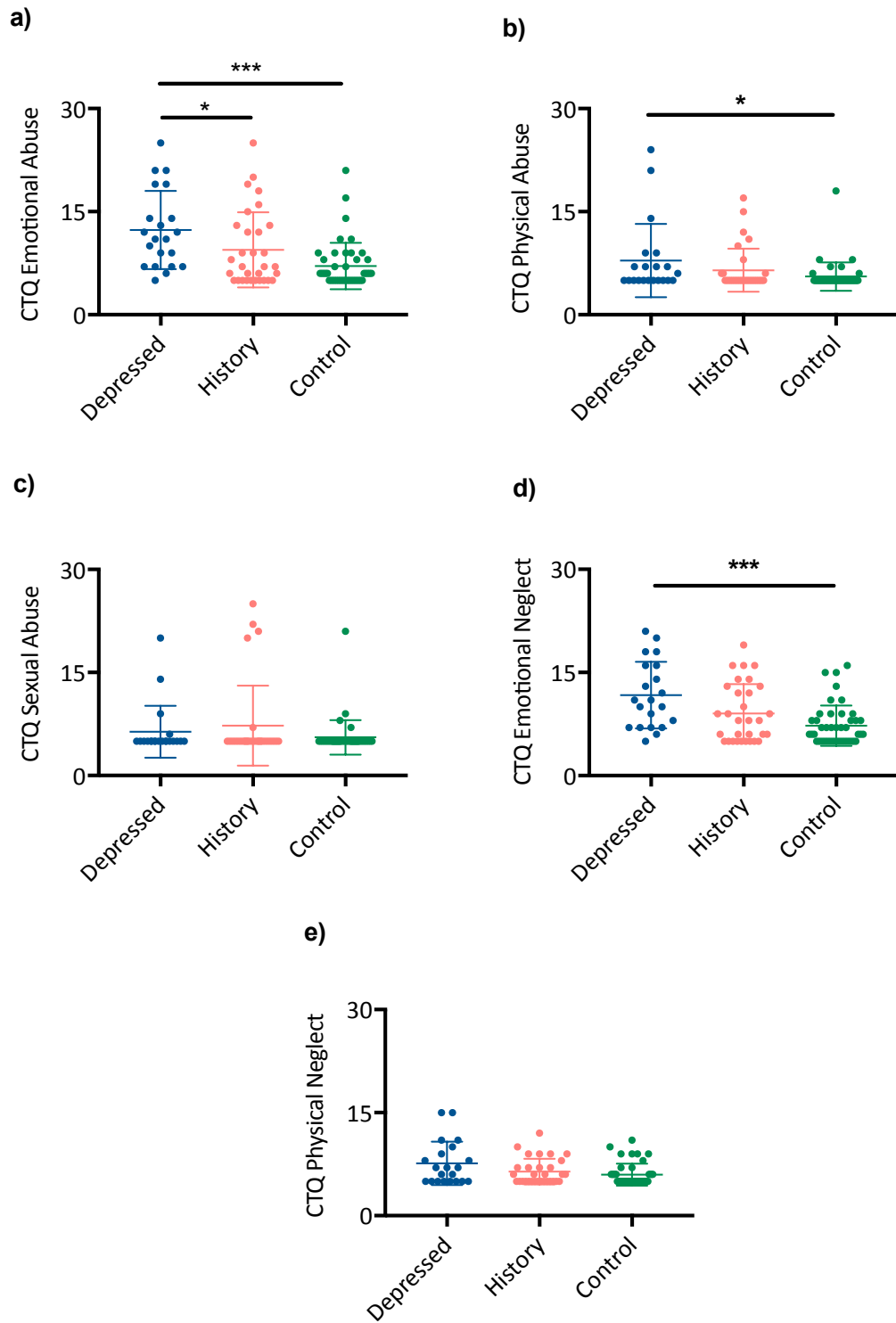


Figure 3.4 Childhood Trauma Questionnaire scores

a) CTQ Emotional Abuse scores; b) CTQ Physical Abuse scores; c) CTQ Sexual Abuse scores; d) CTQ Emotional Neglect scores; e) CTQ Physical Neglect scores.

* p<0.05, *** p<0.001

3.3.3 Obstetric and labour outcomes

Obstetric complications such as gestational diabetes and preeclampsia were exclusion criteria at the time of recruitment. However, some women in the study developed these complications after recruitment into the study. These factors were controlled for in the analyses. Two women, both in the Depressed group, developed preeclampsia during pregnancy. Two further women, one in the Depressed group and one in the Control group, developed gestational diabetes. In this sample, 16 women had induced labour, nine women had planned caesarean sections, while the rest had spontaneous labour onset. Five women had emergency caesarean sections, while the rest had vaginal deliveries. There were no significant differences in labour onset or method between the three groups.

3.3.4 Postpartum

In this section, the clinical features of the sample at two and six months postpartum, including mood profile, medication use, and measures of stress, sleep and self-report and clinician-rated mood will be described. This section also includes the trajectory of change in clinical features from pregnancy to postpartum.

3.3.4.1 Characteristics of sample postpartum

Study participants were followed up at two (PP₁) and six months (PP₂) postpartum. Due to the longitudinal nature of the study, not all participants had completed the study cycle at the time of writing this thesis. Notably, the number of women at each postpartum time point in this thesis indicates the number of women who have reached the particular time point in the study, and does not denote attrition rate. Six women dropped out of the study between pregnancy and two months post-partum, and one further participant dropped out between two months and six months postpartum. One participant was excluded after her infant was diagnosed with Apert's syndrome. Sixty-three women were analysed at the two months postpartum time point [Figure 3.5]. All participants were euthymic at two months. Forty-five participants were analysed at the six-month time point [Figure 3.5]. Three women were diagnosed with depression at six months, all belonging to the Depressed group.

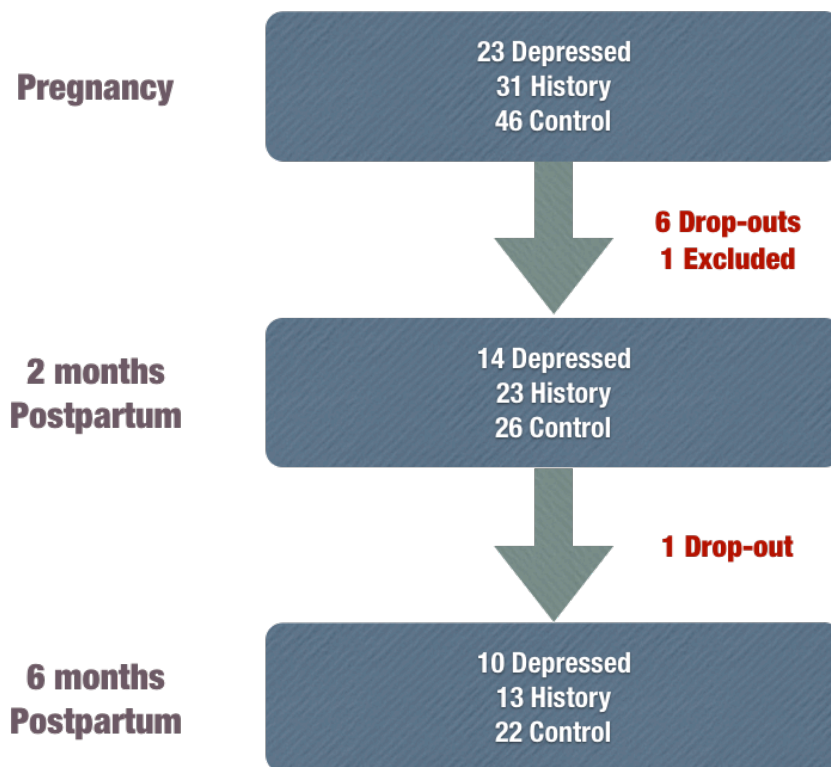


Figure 3.5 Study participants at each time point

3.3.4.2 Medications postpartum

At two months postpartum, 14 women remained on SSRIs, while two were prescribed lamotrigine, and one was prescribed duloxetine. At six months postpartum, nine women were taking SSRIs, and one each had been prescribed agomelatine, duloxetine, lamotrigine, and lithium.

3.3.4.3 Clinical measures postpartum

Results of all mood, stress, and sleep measures for PP₁ and PP₂ are summarised in Table 3.3, and individual differences between groups depicted in Figures 3.6 and 3.7. Women in the Depressed group were no longer clinically depressed at PP₁. However, the high HAM-D scores seen in the Depressed group during pregnancy persisted postpartum, although they were not as severe as the scores during pregnancy, and did not meet criteria for MDD. The Depressed group had significantly higher scores than the Control group in the PSS, PSQI, CES-D, and HAM-D, at both PP₁ and PP₂. The History group scored significantly higher than the Control group in the PSS at PP₁, and CES-D at both PP₁ and PP₂.

Table 3.3 Rating scale scores at two months (PP1) and six months (PP2) postpartum

	Depressed	History	Control	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
	(n=18)	(n=22)	(n=33)	
PP1 PSS	29.83 (10.71)	24.63 (8.85)	18.85 (7.00)	$\chi^2=12.95$, $p=0.002^*$
PP1 PSQI	10.67 (3.70)	7.53 (2.80)	6.91 (3.32)	$\chi^2=8.99$, $p=0.011^*$
PP1 CES-D	22.75 (15.08)	13.86 (11.99)	7.25 (6.31)	$\chi^2=14.08$, $p=0.001^*$
PP1 HAM-D	8.00 (7.16)	3.00 (2.53)	1.58 (1.47)	$\chi^2=16.19$, $p<0.001^*$
	(n=10)	(n=15)	(n=25)	
PP2 PSS	30.60 (11.48)	22.73 (9.46)	17.46 (8.56)	$\chi^2=10.77$, $p=0.005^*$
PP2 PSQI	10.40 (4.30)	6.47 (2.99)	5.84 (3.70)	$\chi^2=8.01$, $p=0.018^*$
PP2 CES-D	23.56 (14.60)	12.50 (8.59)	5.76 (7.23)	$\chi^2=16.85$, $p<0.001^*$
PP2 HAM-D	8.13 (8.14)	2.10 (1.91)	1.59 (1.12)	$\chi^2=6.95$, $p=0.031^*$

PSS – Perceived Stress Scale, **PSQI** – Pittsburgh Sleep Quality Index, **CES-D** – Centre for Epidemiological Studies Depression rating scale, **HAM-D** – Hamilton Depression rating scale.

Note: Kruskal-Wallis analysis was conducted to compare differences between means. Threshold for significance was set at 0.05.

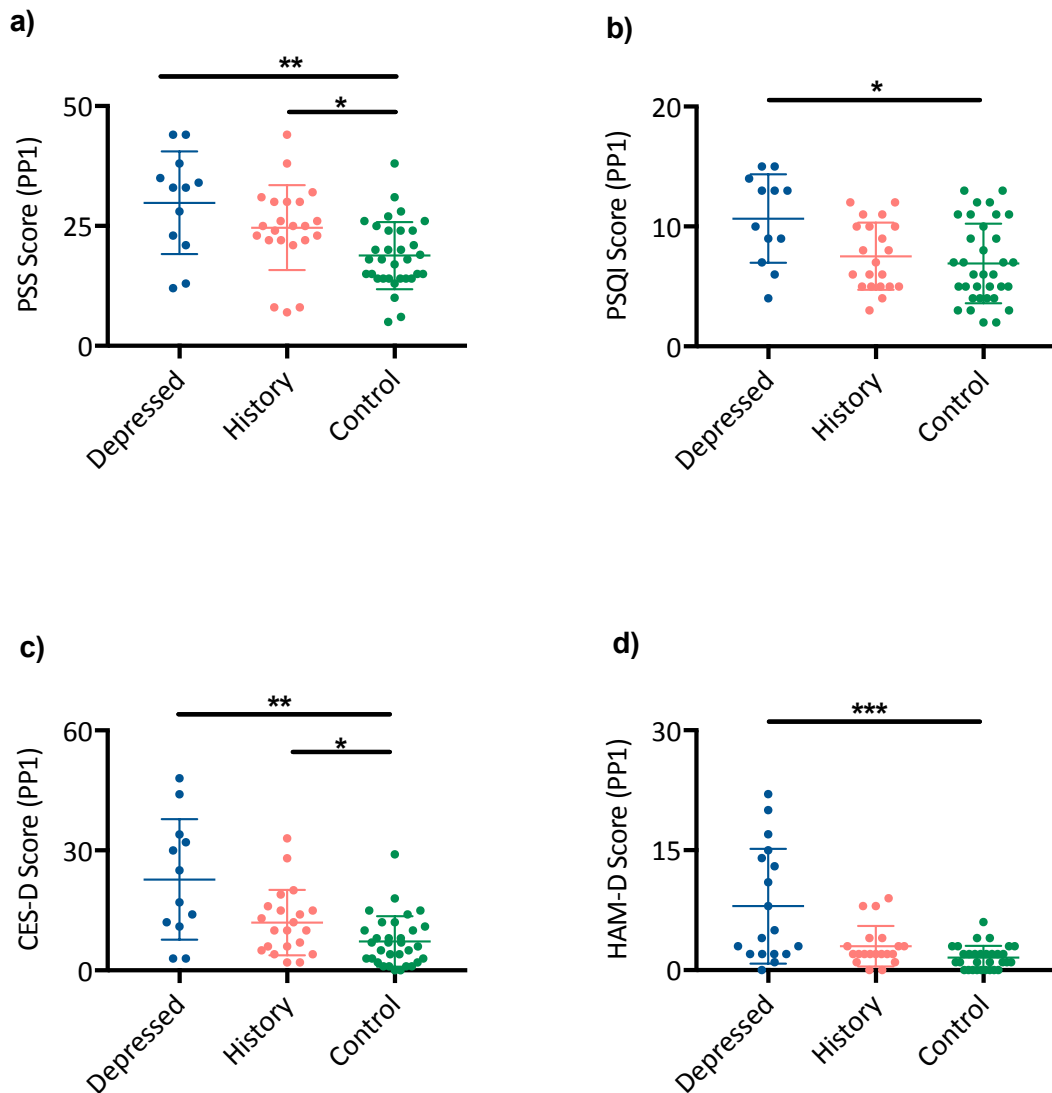


Figure 3.6 Mood and sleep rating scale scores at two months postpartum (PP1)

Perceived Stress Scale (PSS), Pittsburgh Sleep Quality Index (PSQI), Centre for Epidemiological Studies Depression rating scale (CES-D), and Hamilton Depression rating scale (HAM-D) scores at PP1. Means and SD for each group are depicted above.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

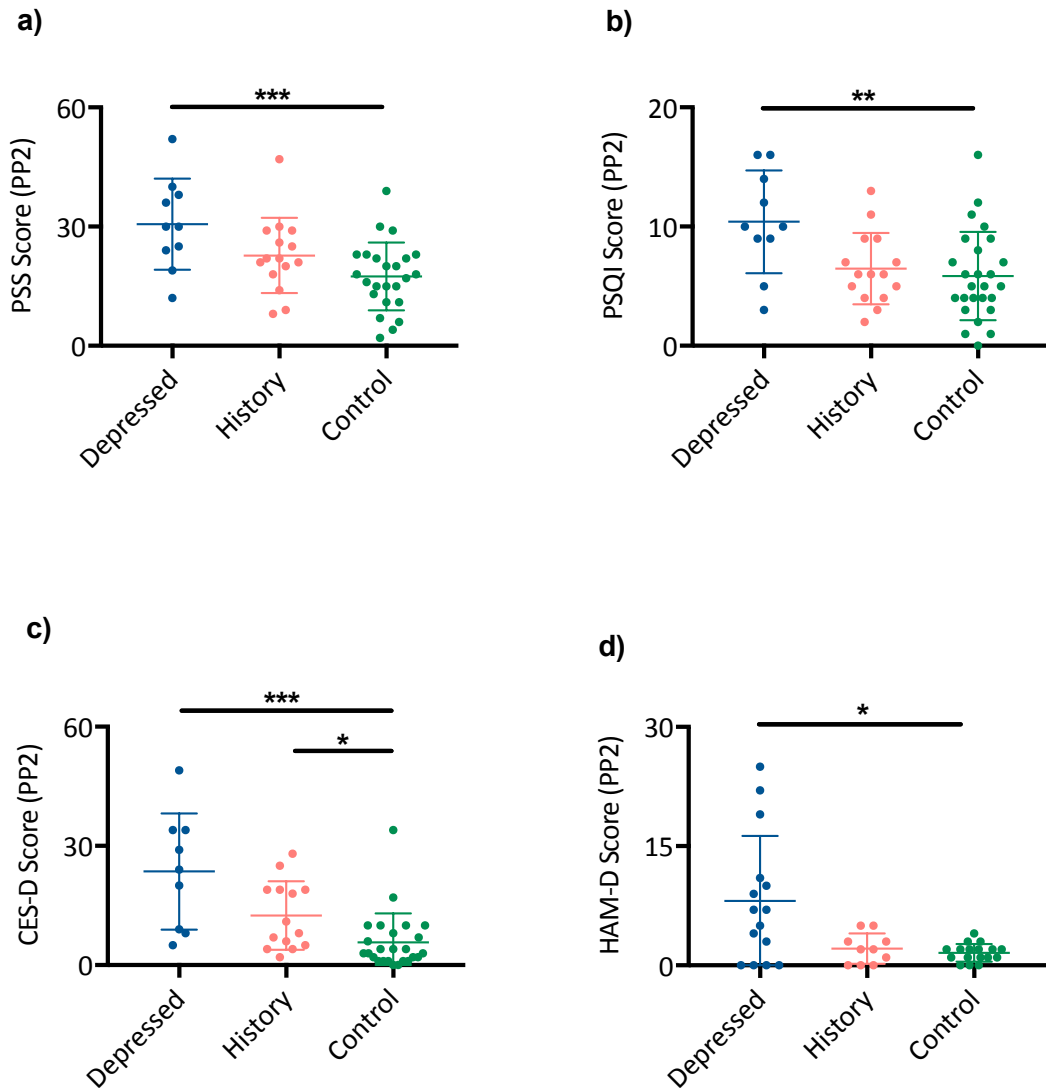


Figure 3.7 Mood and sleep rating scale scores at six months postpartum (PP2)

Perceived Stress Scale (PSS), Pittsburgh Sleep Quality Index (PSQI), Centre for Epidemiological Studies Depression rating scale (CES-D), and Hamilton Depression rating scale (HAM-D) scores at PP2. Means and SD for each group are depicted above.

* p<0.05, ** p<0.01, *** p<0.001

3.3.4.4 Trajectory of change in clinical measures from pregnancy to postpartum

Maternal PSS, PSQI, CES-D, and HAM-D scores were compared between the pregnancy and two month postpartum time points. In the whole sample analysis, HAM-D scores decreased significantly from pregnancy to PP1, while sleep quality scores significantly increased at PP1 [Table 3.4]. When the groups were analysed separately, there were no differences in rating scale scores between the two time points in the History and Control groups. Within the Depressed group, as would be anticipated as none of the women remained depressed at PP1, there was a significant reduction in HAM-D scores between pregnancy and PP1 [Table 3.4]. There were no differences between the two- and six-month time points in whole sample or individual group analyses.

Table 3.4 Repeated measures analysis of rating scale measures during pregnancy and at two months postpartum (PP1)

		Pregnancy	PP1	Statistic, p-value
		Mean (SD)	Mean (SD)	
Whole Sample		(n=95)	(n=69)	
	PSS	23.26 (10.39)	22.72 (9.25)	Z=-0.84, p=0.404
	PSQI	7.22 (3.90)	7.78 (3.48)	Z=-2.14, p=0.032*
	CES-D	15.03 (13.97)	12.28 (11.72)	Z=-1.20, p=0.230
	HAM-D	6.42 (8.55)	3.66 (4.76)	Z=-2.32, p=0.020*
Depressed		(n=20)	(n=18)	
	PSS	36.68 (5.18)	29.83 (10.70)	Z=-1.79, p=0.074
	PSQI	10.20 (3.76)	10.67 (3.70)	Z=-0.05, p=0.096
	CES-D	35.55 (12.28)	22.75 (15.08)	Z=-1.63, p=0.102
	HAM-D	20.81 (5.01)	8.00 (7.16)	Z=-3.58, p<0.001*
History		(n=31)	(n=22)	
	PSS	24.77 (7.32)	24.63 (8.84)	Z=-0.50, p=0.614
	PSQI	7.53 (3.39)	7.52 (2.80)	Z=-1.42, p=0.155
	CES-D	14.29 (8.64)	13.86 (11.99)	Z=-1.08, p=0.280
	HAM-D	3.64 (4.38)	3.00 (2.53)	Z=-0.03, p=0.977
Control		(n=45)	(n=34)	
	PSS	16.56 (7.61)	18.85 (7.00)	Z=-0.49, p=0.623
	PSQI	5.66 (3.50)	6.91 (3.32)	Z=-1.89, p=0.059
	CES-D	6.23 (5.69)	7.25 (6.31)	Z=-0.35, p=0.723
	HAM-D	1.39 (1.56)	1.58 (1.47)	Z=-1.92, p=0.045

PSS – Perceived Stress Scale, **PSQI** - Pittsburgh Sleep Quality Index, **CES-D** – Centre for Epidemiological Studies Depression rating scale, **HAM-D** – Hamilton Depression rating scale.

Note: A Wilcoxon Signed-Rank test was conducted within each group to analyse differences in clinical measures between pregnancy and two months postpartum. The Z statistic and p-value are presented in the table for each time point within each group. Threshold for significance was set at 0.05.

3.3.5 Early life adversity

Scores on the five domains of childhood trauma including Emotional Abuse, Physical Abuse, Sexual Abuse, Emotional Neglect, and Physical Neglect were split into two groups – No Trauma (-ELA) and Low to Severe Trauma (+ELA) as per the Childhood Trauma Questionnaire manual. The distribution of the sample across each domain is shown in Table 3.5. Analysis of the distribution of ELA domains across caseness groups showed group differences in the Emotional Abuse and Emotional Neglect categories [Table 3.6]. Adjusted residuals were obtained in the chi-square analysis to assess differences between groups in the variables with group differences. There were more women than expected with a history of emotional abuse or neglect (adjusted residual 3.0 and 3.1 respectively) and fewer than expected numbers with no history of emotional abuse or neglect (adjusted residual -3.0 and -3.1 respectively) in the Depressed group, with the opposite being found in the Control group.

Table 3.5 Distribution of early life adversity

	-ELA	+ELA
	n (%)	n (%)
CTQ Emotional Abuse	55 (59.1)	38 (40.9)
CTQ Physical Abuse	79 (85.9)	13 (14.1)
CTQ Sexual Abuse	80 (86.0)	13 (14.0)
CTQ Emotional Neglect	62 (68.1)	29 (31.9)
CTQ Physical Neglect	71 (76.3)	22 (23.7)
Total within sample	43 (46.2)	50 (53.7)

Note: This table presents the distribution of women with no history of early life adversity (-ELA) in each of the five domains on the Childhood Trauma Questionnaire (CTQ) compared to women with Low to Severe levels of early life adversity (+ELA) in each of the five domains.

Table 3.6 Distribution of early life adversity by caseness groups

CTQ Scores	Depressed n (%)	History n (%)	Control n (%)	Statistic, p-value
Emotional Abuse				$\chi^2=12.29$, p=0.002*
Nil	6 (30%)	16 (55.2%)	32 (76.2%)	
Low-Severe	14 (70%)	13 (44.8%)	10 (23.8%)	
Physical Abuse				$\chi^2=3.26$, p=0.196
Nil	16 (80%)	23 (79.3%)	39 (92.9%)	
Low-Severe	4 (20%)	6 (20.7%)	3 (7.1%)	
Sexual Abuse				$\chi^2=1.52$, p=0.468
Nil	16 (80%)	24 (82.8%)	38 (90.5%)	
Low-Severe	4 (20%)	5 (17.2%)	4 (9.5%)	
Emotional Neglect				$\chi^2=13.76$, p=0.001*
Nil	8 (40%)	18 (62.1%)	36 (85.7%)	
Low-Severe	12 (60%)	11 (37.9%)	6 (20%)	
Physical Neglect				$\chi^2=4.02$, p=0.134
Nil	12 (60%)	22 (75.9%)	35 (83.3%)	
Low-Severe	8 (40%)	7 (24.1%)	7 (16.7%)	

Note: A chi-square analysis was undertaken to assess the distribution of ELA domains across the caseness groups of pregnancy. Threshold for significance was set at 0.05.

3.4 Discussion

An interesting finding in this study was the effect of a prior history of depression on mood and stress measures during pregnancy and postpartum. The History group, comprised of euthymic pregnant women with a previous history of depression, had significantly higher perception of stress, as well as poorer subjective and objective mood scores compared to the Control group during pregnancy. Depressed pregnant women, as would be expected, also had higher scores on subjective and objective mood measures, poorer sleep quality, and higher perceived stress levels compared to the euthymic, never-depressed pregnant women in the Control group. These higher scores on mood, sleep, and stress measures in the Depressed group persisted postpartum, even at two months when the whole sample was clinically euthymic. Three women in the Depressed group experienced a relapse of depression at six months postpartum. Objective mood measures were lower at two months postpartum compared to the pregnancy time point both in the whole sample and in the Depressed group. Sleep scores were lower during pregnancy compared to the two months postpartum time point, indicating poorer sleep quality at two months postpartum. Overall, objective mood measures improved from pregnancy to two months postpartum, especially in the Depressed group. There were no differences in clinical measures between the two- and six-month time points.

3.4.1 Clinical aspects of mood states during pregnancy

As would be expected, depressed women had higher subjective and objective mood scores and higher levels of perceived stress during pregnancy compared to women who had never experienced depression. Women in the Depressed group also had significantly poorer sleep quality scores compared to women in the Control group, demonstrating that sleep disturbance, although generally present in healthy pregnancy, is further disrupted in the presence of antenatal depression. This has been reported in previous studies that have found that somatic symptoms, including sleep disruption, are more prominent in women experiencing clinical depression or depressive symptoms during pregnancy (Field et al., 2007; Kelly et al., 2001; Tomfohr et al., 2015). Women in the Depressed group also had significantly higher perceived stress and mood measures compared to women in the History group, but there were no differences in sleep scores between these groups.

Women in the History group had interesting differences in clinical features compared to the Control group during both pregnancy and postpartum. Since the women in the History group were not clinically depressed during pregnancy, it would be expected that they would have similar stress, sleep and mood measures compared to women in the Control group. However, in this study sample, women in the History group had significantly higher perceived stress and subjective and objective mood scores compared to the Control group, but lower than the Depressed group. These findings may be attributable to subsyndromal symptoms of depression in the History group. Additionally, some investigators propose that depression has a ‘scarring’ effect on an individual, which may then make them vulnerable to future recurrences (Monroe and Harkness, 2005). Although women in the History group did not relapse during pregnancy, it may be postulated that a prior episode of depression may have conferred an added effect that may have interacted with the heightened vulnerability resulting from pregnancy itself, leading to higher perception of stress, sleep disturbance, and lower subjective and objective mood. This indicates that, even without a clinical relapse of depression during pregnancy, women with a lifetime history of depression have more challenging pregnancies than women who have never experienced depression.

3.4.2 Maternal mood states during pregnancy and clinical features postpartum

Women who were depressed during pregnancy continued to have higher perceived stress and poorer sleep quality compared to the Control group at both two and six months postpartum. Interestingly, although all women in this sample were euthymic according to DSM-5 at two months postpartum, women in the Depressed group had higher subjective and objective mood scores compared to the Control group. This effect persisted at six months postpartum, at which point three women had been diagnosed with postpartum depression. However, this subgroup was not analysed separately as the sample size was too low to provide sufficient power for reliable statistical analyses. These findings suggest that women who experience depression during pregnancy may have either had partial resolution of symptoms or subsyndromal depressive symptoms by two months postpartum. Consequently, they remain vulnerable to clinical relapse during the postpartum period.

3.4.3 Trajectory of change in maternal clinical measures from pregnancy to postpartum

Within the whole sample, HAM-D scores decreased significantly between pregnancy and two months postpartum. This effect was only present in the Depressed group when the groups were analysed separately, as would be expected considering all participants were euthymic at two months postpartum. Sleep quality worsened at two months postpartum compared to the pregnancy time point in the whole sample, possibly due to the demands of infant feeding. There were no significant differences in mood, stress or sleep measures in the History and Control groups between pregnancy and two months postpartum. This would indicate that, although pregnancy can be associated with poorer sleep and increased stress, the demands of a young infant may be similarly stressful, with considerable sleep disturbance. In this instance, comparison to pre-pregnancy measures of stress, sleep, and mood would be useful, and should be a consideration for future studies.

3.4.4 Distribution of maternal early life adversity

In the current sample, 53.7% of women had experienced trauma in at least one trauma category. The commonest form of trauma in this sample was emotional abuse (40.9%), followed by emotional neglect (31.9%), and physical neglect (23.7%). This sample had a low prevalence of sexual abuse (14%). It is now well established in pregnant and non-pregnant samples that ELA can predispose the individual to depression and other mental illness in later life (Heim et al., 2008; McCrory et al., 2012). There is also clear evidence indicating an association between maternal childhood trauma and depression during pregnancy (Benedict et al., 1999; Choi and Sikkema, 2015; Lang et al., 2006; Leigh and Milgrom, 2008; Plant et al., 2013). In keeping with this, depressed women in the study had higher CTQ scores compared to controls in the Emotional Abuse and Emotional Neglect domains. However, since this study involved retrospective assessment of ELA, it is possible that depressed women recall higher subjectively negative childhood experiences, including abuse, making this a limitation of the study.

3.4.5 Obstetric and labour outcomes

Literature indicates that antenatal depression confers an increased vulnerability for preeclampsia, while the evidence is less clear for gestational diabetes (Hu et al., 2015; Qiu et al., 2009; Ross et al., 2016). Three women in the Depressed group developed either preeclampsia or gestational diabetes. However, due to the very small sample size, it is not possible to postulate on the effects of antenatal depression on obstetric complications. Additionally, there were no differences in labour onset or method between the groups, suggesting that, in this sample, antenatal depression had no effect on obstetric or labour outcomes.

3.4.6 Conclusions

The most striking finding in this study was the higher perception of stress and lower mood in euthymic pregnant women with a prior history of depression compared to pregnant women with no history of mental illness. This might be an indication of their increased vulnerability for relapse during pregnancy. There is clear evidence in the present study and in the general literature that women with a history of ELA are at increased risk of developing depression during pregnancy. Additionally, in women with antenatal depression, there may only be a partial resolution of symptoms by two months postpartum, which, along with the psychosocial stressors of the early postpartum period, can increase a woman's vulnerability for relapse. The perinatal period, therefore, can be a vulnerable period in a woman's life, particularly in the presence of prior depression or ELA. Therefore, early identification and monitoring of women with a history of depression or ELA during pregnancy, and adequate follow-up postpartum, may improve maternal and infant outcomes.

Chapter 4

**The hypothalamic-pituitary-adrenal axis
during pregnancy**

Relationship with mood and early life adversity

4.1 Introduction

Human pregnancy has a gestational length of 37 to 42 weeks, calculated from the first day of the last menstrual period. During this time, a range of maternal systems, including the hypothalamic-pituitary-adrenal (HPA) axis, undergo substantial alterations. The role of the HPA axis, the body's neuroendocrine stress system, has been explored in relation to clinical depression aetiology. However, there is a paucity of research into the HPA axis in clinical depression during pregnancy. Little is known also about the influence of early life adversity (ELA) and foetal sex on the HPA axis during pregnancy.

The primary function of the HPA axis is to modulate the body's response to stress. HPA axis activity begins with the release of corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular nucleus of the hypothalamus in response to stress. CRH and AVP stimulate the anterior pituitary to secrete adrenocorticotrophic hormone (ACTH) into the bloodstream. ACTH then stimulates the adrenal cortex to produce the glucocorticoid, cortisol (Dinan and Scott, 2005). Once released, cortisol binds to glucocorticoid and mineralocorticoid receptors in various parts of the body, where it mediates changes. Cortisol is also involved in feedback inhibition of the HPA axis through binding to receptors in the hypothalamus and anterior pituitary. In addition to its metabolic and immune functions, cortisol also has effects on neurogenesis, memory, and hippocampal structure within the brain (Pariante and Lightman, 2008).

A remarkable development during pregnancy is the production of CRH by the placenta, and to a smaller extent, the decidua and chorioamniotic membranes (Mastorakos and Ilias, 2003). Placental syncytial cells begin to produce CRH around the 7th week of gestation, with a subsequent increase in circulating CRH by the 10th week of gestation, rising exponentially until it reaches a peak before delivery (Goland et al., 1988; Mastorakos and Ilias, 2000; Riley and Challis, 1991). Consequently, the HPA axis, activated by circulating placental CRH, produces more cortisol. Unlike hypothalamic CRH, placental CRH is subject to a positive feed-forward mechanism by cortisol, further stimulating cortisol production (de Weerth and Buitelaar, 2005b; Robinson et al., 1988). This increase in cortisol levels is necessary for foetal organ maturation during the later stages of pregnancy (Liggins, 1994; Wadhwa, 2005).

The activity of 11β -HSD2, an enzyme that oxidises active cortisol into its inactive metabolite cortisone, is of interest in perinatal research. Placental 11β -HSD2 is thought to protect the foetus from overexposure of cortisol *in utero*, and disrupted regulation of this enzyme has been implicated in some studies of prenatal stress (Conradt et al., 2013; O'Donnell et al., 2012; Seckl, 1997; Seth et al., 2015; Togher et al., 2014). 11β -HSD2 activity can be measured by analysing the ratio of cortisone to cortisol, thereby determining the extent of conversion of cortisol into its inactive form.

There is a strong evidence base for the role of the HPA axis in major depressive disorder (MDD). However, few studies have examined antenatal depression in relation to the HPA axis. Of the eight studies that have reported on this, four have found depression during pregnancy to be associated with an increase in maternal cortisol levels (Field et al., 2007, 2009; O'Connor et al., 2014; O'Keane et al., 2011). Two of these studies by Field and colleagues (2007, 2009) reported higher morning cortisol levels in antenatal depression, while another by O'Keane and colleagues (2011) found higher evening cortisol levels. O'Connor and colleagues (2014) found lower waking cortisol levels and higher overall cortisol levels with a flattened diurnal curve in women with antenatal depression. Three other studies reported no effect of depression on the HPA axis during pregnancy (Field et al., 2010; Hellgren et al., 2013; Parcells, 2010), while one study found no difference in cortisol measures between depressed and non-depressed pregnant women but found higher cortisol levels in women with comorbid depression and anxiety (Evans et al., 2008). A trait effect of depression on the HPA axis, with higher cortisol levels in the non-depressed state in non-pregnant adults has been reported, and may be one of the mechanisms increasing vulnerability for recurrence in depression (Lok et al., 2012). Examining this effect in pregnant women, a recent study found no effect of a history of depression on the HPA axis during pregnancy (Hellgren et al., 2013).

There is good evidence that early life adversity (ELA) is associated with changes in the HPA axis in non-pregnant populations (Bremner et al., 2003; Cicchetti et al., 2010; Lemieux and Coe, 1995; Suzuki et al., 2014), predisposing an individual to developing depression and other mental illness in later life (Heim et al., 2008; McCrory et al., 2012). Current literature indicates that ELA continues to have an effect on HPA axis measures during pregnancy, with six studies showing an association between ELA and

higher cortisol levels during pregnancy (Bublitz and Stroud, 2012; Buss, Entringer, et al., 2016; Moog et al., 2016; Schreier, Enlow, Ritz, Coull, et al., 2015; Schreier, Enlow, Ritz, Gennings, et al., 2015). One study however, found a negative relationship between ELA and maternal waking cortisol levels (Shea et al., 2007a). These near-unanimous findings indicate that the impact of ELA on the HPA axis persists into pregnancy.

Sexual dimorphism in foetal health is a well-established concept, with differing rates of survival *in utero* for male and female fetuses. Indeed, there is also evidence of sexual dimorphism in mental illness in adulthood, with a higher prevalence of affective disorders in females and higher rates of developmental disorders such as autism spectrum disorders and ADHD in males (Kessler et al., 1993; Ramtekkar et al., 2010; Werling and Geschwind, 2013). Evidence in the foetal programming literature [Section 1.7] indicates that the maternal environment has an effect on the development of the foetus (Barker et al., 2002). Further emerging evidence suggests that in the male foetus, intrauterine resources are focussed on growth, even in a stressful maternal environment, increasing their susceptibility to preterm birth, low birth weight and intrauterine death compared to female fetuses. On the contrary, the focus in the female foetus appears to be on the allocation and conservation of resources and slowing the growth trajectory, especially when encountering a stressor in the maternal environment, thus increasing their chance of survival (Clifton, 2010; Hodyl et al., 2010). There is a reciprocal relationship between the foetus and the mother. While there is a large evidence base for the effects of maternal health on the foetus, the effects of the foetus on maternal health have been poorly studied. To date, two studies have investigated the associations between foetal sex and maternal cortisol levels with diverse results. In one of the studies, the authors found increased maternal cortisol levels up to the third trimester in women carrying male fetuses compared to those carrying females (DiPietro et al., 2011). A reversal after the third trimester, with women carrying females having higher cortisol levels. The findings of another study examining this relationship suggests that women carrying female fetuses have higher overall morning cortisol levels compared to males, with a more blunted cortisol stress response (Giesbrecht, Campbell, et al., 2015). There is still much left to be learned about this relationship, if indeed one exists.

4.1.1 Study aims

It is clear from the literature review that evidence on the impact of clinically diagnosed depression on maternal cortisol levels during pregnancy is equivocal. With this study, our first aim was to explore further the possible effects of state (current) as well as trait (past) depression on maternal HPA axis during pregnancy. Three groups of pregnant women were examined – Depressed (depressed during pregnancy), History (past history of depression but euthymic during pregnancy), and Control (healthy pregnant women with no history of mental illness). Early life adversity is known to increase the risk of depression and other mental illness in later life. It is also known to be associated with altered HPA axis function in adulthood. Our second aim was to examine the effect of ELA on maternal HPA axis during pregnancy. Finally, with some evidence for a possible effect of foetal sex on maternal physiological state during pregnancy, we sought to further explore this relationship.

4.1.2 Study hypotheses

- Depressed pregnant women will have higher waking cortisol levels and a blunted cortisol awakening response compared to women in the Control group.
- Depressed pregnant women will have higher evening cortisol levels compared to women in the Control group.
- Euthymic pregnant women with a history of depression will have higher waking cortisol levels compared to the Control group.
- Maternal cortisol levels will have an association with mood, stress, and sleep measures.
- Women with a history of ELA will have higher overall cortisol levels compared to women with no history of ELA.
- Women carrying male foetuses will have higher cortisol levels compared to women carrying females.

4.2 Methods

4.2.1 Recruitment

All women in the study were recruited from three maternity hospitals – the National Maternity Hospital, Dublin; the Coombe Women and Infants University Hospital, Dublin; and The Rotunda Hospital, Dublin. Methods of recruitment varied depending on the individual clinical models in the hospitals but depressed pregnant women and those with a history of depression were recruited from Perinatal Psychiatry clinics, or through referrals from staff members within the Perinatal Psychiatry service. To recruit into the Control group, pregnant women were approached in the waiting room at their initial visit to the maternity hospitals and made aware of the study. A detailed explanation of the study was given to all potential participants and, if interested, they were recruited into the study after obtaining informed written consent [See Section 2.3 for further details]. Pregnant women were recruited between 20-30 weeks gestation when possible, and healthy control pregnant women matched by gestational week were then recruited. All study participants underwent a clinical interview, and were assigned to the Depressed group if they fulfilled DSM-5 criteria for MDD, the History group if they were euthymic during pregnancy but had a prior history of depression, and the Control group if they had never been depressed.

4.2.2 Rating scales

The clinician-rated Hamilton Depression rating scale (HAM-D) was completed for each participant. Women also completed self-rated Perceived Stress Scale (PSS), Pittsburgh Sleep Quality Index (PSQI), the Centre for Epidemiological Studies Depression rating scale (CES-D), and the Childhood Trauma Questionnaire (CTQ). The CTQ is further categorised into five domains – Emotional Abuse, Physical Abuse, Sexual Abuse, Emotional Neglect, and Physical Neglect. Each trauma domain is classified into Nil (-ELA) or Low to Severe (+ELA) levels of trauma, as described by the CTQ Manual (Bernstein and Fink, 1998). Women with a history of ≥ 1 trauma subtype were analysed against women with no history of abuse.

4.2.3 Salivary cortisol

Women collected saliva samples using absorbent swabs in Salivette (Sarstedt) tubes at five time points over the course of one day – at waking (0 minutes), 30 minutes after waking, 60 minutes after waking, 12 hours after waking, and 12.5 hours after waking. Samples were then stored in the participant's refrigerator and posted out to Trinity College Institute of Neuroscience (TCIN) in pre-stamped envelopes. At arrival in TCIN, samples were centrifuged for 10 minutes at 3000 rpm at room temperature. Saliva was then divided into microtubes of 500µL aliquots and stored at -80°C. Saliva samples were transported to the University Hospital of South Manchester in three batches, where they were analysed for cortisol and cortisone levels by Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) in Professor Brian Keevil's laboratory. Due to its improved specificity and sensitivity, LC-MS/MS is considered to be a superior technique for cortisol quantification compared to immunoassay techniques [See Section 2.6.2] (Keevil, 2013). Cortisone and cortisol values were generated from the quantification analysis. A Lower Limit of Quantification of 0.3 nMol/L was used for cortisol and cortisone levels in saliva. This was based on coefficients of variance of 9.4% and 4.9% for cortisol at 0.25 nMol/L and 0.5 nMol/L, respectively, and coefficients of variance of 14.1% and 5.2% at 0.25 nMol/L and 0.5 nMol/L for cortisone, respectively.

4.2.4 Cortisol measures

Cortisol awakening response (CAR) parameters were calculated from the cortisol values obtained from the LC-MS/MS analysis. Using the three morning cortisol values, area under the curve (AUC) parameters were derived using formulae from Fekedulegn and colleagues (Fekedulegn et al., 2007). AUC with respect to ground (AUC_G), an estimate of total cortisol production over the morning, and AUC with respect to increase (AUC_I), a measure of the dynamic of the CAR reflecting cortisol sensitivity, were calculated. The intercept and slope of the regression line fitted through the raw morning cortisol values were also calculated, giving an indication of total cortisol output and dynamic of cortisol change, respectively.

4.2.5 Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics for Macintosh, Version 24 (REF). Data was assessed for normality using the Kolmogorov-Smirnov test. Raw cortisol values were log transformed to approximate a normal distribution. In order to account for values of zero, which does not have a logarithm, the constant 1 was added to all values prior to transformation. Outliers found after log transformation were removed from analyses to strengthen association analyses. Data that followed a normal distribution was analysed using the independent-samples t-test or one-way analysis of variance (ANOVA). Non-parametric data was analysed using the Mann-Whitney U test or the Kruskal-Wallis test. Univariate general linear model analysis for covariance was undertaken to control for covariates. Association analyses were conducted using Pearson's product-moment correlation for normally distributed data, or Spearman's rank-order analysis for non-parametric data. Multiple comparisons were corrected for a false discovery rate using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995). Linear regression modelling was undertaken for some analyses, and moderation analysis, a form of hierarchical regression modelling, was undertaken using the PROCESS tool for SPSS (Andrew Hayes, Ohio State University, USA. www.processmacro.org). These procedures are further explained in the relevant sections.

Hierarchical regression modelling was conducted to predict maternal cortisol outcome using various predictor variables. R^2 value, a measure of variation in the dependent variable explained by the independent variables, and the F statistic is shown in the relevant section, as well as the change in F with each model. In continuous variables, the unstandardised coefficient (B) indicates a change in the dependent variable for a one-unit change in the value of the independent variable. β represents the standardised coefficient, where the values of all the variables have been converted to the same scale for easier comparison. Threshold for significance was set at 0.05 for all analyses.

4.3 Results

In the Results section, characteristics of the sample will be described in **Section 4.3.1**, maternal cortisol measures during pregnancy in **Section 4.3.2**, and maternal cortisone measures during pregnancy in **Section 4.3.3**. Finally, ELA and the maternal HPA axis will be discussed **Section 4.3.4**.

4.3.1 Characteristics of sample during pregnancy

In total, 100 women were recruited into the study during pregnancy and categorised into the Depressed, History, and Control groups. Twenty-three women were clinically depressed during pregnancy, 31 were euthymic during pregnancy but had experienced a past episode of depression, and 46 were euthymic, never-depressed pregnant women. Saliva samples were provided by 16 women in the Depressed group, 25 women in the History group, and 40 women in the Control group. The clinical and demographic variables of the sample are described in Chapter 3.

4.3.2 Maternal cortisol measures during pregnancy

4.3.2.1 Maternal cortisol levels

Diurnal cortisol curves and the cortisol awakening response (CAR) for raw salivary cortisol measured at waking (0 minutes), 30 minutes after waking, 60 minutes after waking, 12 hours after waking (720 minutes), and 12.5 hours after waking (750 minutes) are portrayed in Figures 4.1 and 4.2 respectively. Raw cortisol values were log transformed in SPSS (Field, 2013), approximating a normal distribution to enable general linear model-based statistical analyses. The effect of caseness on maternal cortisol values during pregnancy was explored [Table 4.1]. There were no statistically significant differences between groups, and post-hoc analysis showed that the groups did not differ significantly from each other.

Table 4.1 Maternal cortisol values during pregnancy

	Depressed (n=16)	History (n=25)	Control (n=40)	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
T0	14.68 (13.62)	14.58 (7.73)	11.50 (5.00)	F=1.08, p=0.345
T30	15.30 (7.14)	15.83 (8.04)	12.89 (4.14)	F=1.34, p=0.269
T60	11.12 (6.52)	12.21 (4.79)	10.87 (4.01)	F=0.78, p=0.463
T720	2.58 (1.36)	2.54 (2.01)	2.44 (1.42)	F=0.13, p=0.883
T750	2.50 (1.41)	3.54 (4.31)	2.27 (1.30)	F=1.55, p=0.218

Note: Salivary cortisol was measured at waking (T0), 30 min from waking (T30), 60 min from waking (T60), 12 h from waking (T720), and 12.5 h from waking (T750). Raw cortisol values are presented in the table for the three groups. Differences in means were analysed using log transformed cortisol values in a one-way ANOVA, and the results presented in the last column. Threshold for significance was set at 0.05.

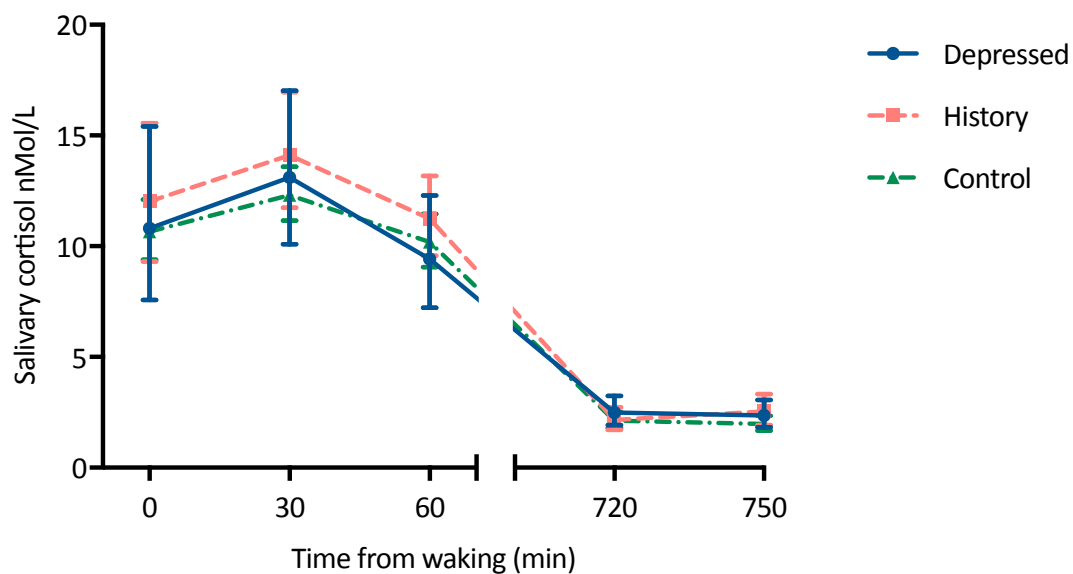


Figure 4.1 Diurnal variation of cortisol during pregnancy

Raw cortisol levels at the five time-points from waking (0 min) to 12.5 h (750 min) after waking in Depressed, History, and Control groups during pregnancy, with error bars. There were no statistically significant differences between groups at any of the time-points.

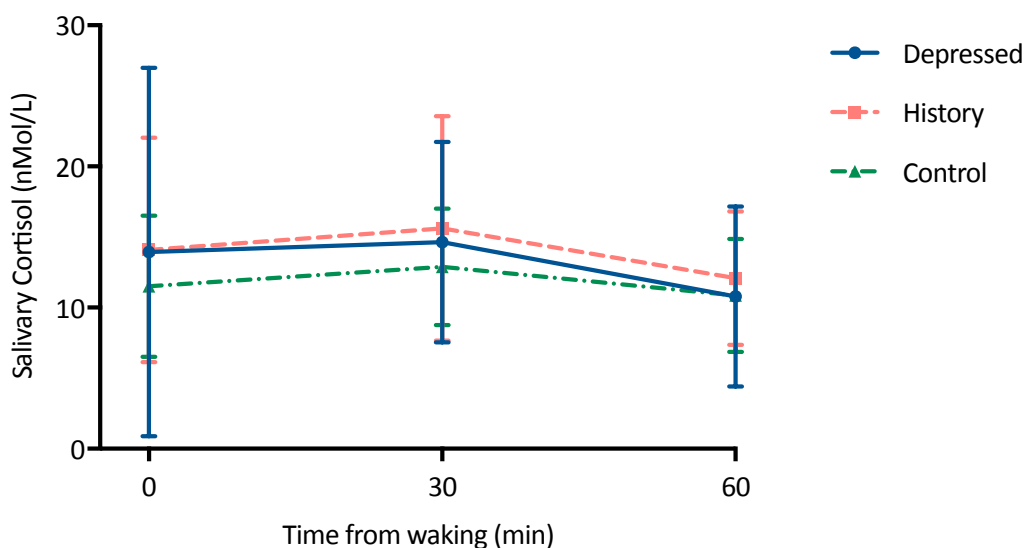


Figure 4.2 Cortisol awakening response during pregnancy

Raw cortisol levels at the three time-points from waking (0, 30, 60 min) in Depressed, History, and Control groups during pregnancy, shown with error bars. There were no statistically significant differences between groups.

4.3.2.2 Maternal cortisol awakening response parameters during pregnancy

The CAR was measured using the three morning cortisol levels (0, 30, and 60 minutes), and CAR parameters describing the AUC were derived from these [Table 4.2]. Measures of total cortisol output over the first hour after awakening (AUCg and intercept of regression line fitted through raw cortisol values) and measures of the dynamic of cortisol change in the first hour after awakening, i.e., the CAR (AUCi and slope of the regression line), are presented in the table. There were no significant differences between the groups. Post-hoc comparisons also failed to reach significance. Known confounders such as age, gestation, gravidity, parity, and medication use were controlled for in a univariate general linear model analysis of covariance, with no effect on results.

Table 4.2 Cortisol awakening response (CAR) variables during pregnancy

	Depressed (n=16)	History (n=25)	Control (n=40)	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
AUCg	788.29 (519.15)	808.31 (419.95)	695.49 (209.28)	F=0.04, p=0.958
AUCi	-92.51 (389.85)	33.73 (260.45)	56.76 (289.40)	F=1.28, p=0.283
Intercept	15.08 (12.36)	15.04 (8.36)	12.29 (4.80)	F=0.27, p=0.767
Slope	-0.06 (0.14)	-0.03 (0.11)	-0.02 (0.09)	F=0.24, p=0.786

AUCg – area under the curve with respect to ground, **AUCi** – area under the curve with respect to increase, **Intercept** – intercept of regression line fitted through raw cortisol values, **Slope** – slope of regression line fitted through raw cortisol values.

Note: CAR variables shown here are derived from raw cortisol values. CAR variables derived from log transformed cortisol values were used for statistical analysis. One-way ANOVA was conducted to examine differences between groups, and results are displayed in the last column. Threshold for statistical significance was set at 0.05.

4.3.2.3 Maternal demographic variables and cortisol measures during pregnancy

Correlation analyses were undertaken to assess the relationship of maternal age, gestational age, BMI, gravidity, and parity with maternal cortisol levels and CAR variables during pregnancy. There were no significant correlations observed.

4.3.2.4 Maternal cortisol measures and clinical measures during pregnancy

Correlation analyses between clinical measures including PSS, PSQI, CES-D, and HAM-D, and cortisol measures including maternal morning and evening cortisol levels and CAR parameters revealed no significant associations.

4.3.2.5 Foetal sex and maternal cortisol measures during pregnancy

Maternal cortisol measures were compared between women carrying male fetuses and those carrying females. No significant differences between male and female foetus groups were evident in whole sample analysis [Table 4.3]. Similarly, foetal sex did not appear to have an effect on maternal cortisol levels when the Depressed, History, and Control groups were analysed separately. Women carrying male and female fetuses were also analysed in separate groups for the effect of caseness on cortisol levels, with no significant differences evident.

Table 4.3 Differences in maternal cortisol measures during pregnancy by foetal sex

	Female Foetus (n=35)	Male Foetus (n=57)	Statistics, p-value
	Mean (SD)	Mean (SD)	
T0	12.37 (6.42)	13.53 (9.27)	t=-0.34, p=0.734
T750	2.73 (1.85)	2.70 (3.06)	t=0.51, p=0.607
AUCg	613.22 (391.93)	699.41 (428.32)	t=0.60, p=0.549
AUCi	19.18 (198.73)	4.45 (324.98)	t=0.47, p=0.640
Intercept	13.24 (6.30)	14.16 (8.61)	t=-0.04, p=0.967
SP2	-0.02 (0.83)	-0.34 (0.11)	t=-0.19, p=0.848

T0 – waking cortisol, **T750** – evening cortisol, **AUCg** – area under the curve with respect to ground, **AUCi** – area under the curve with respect to increase, **Intercept** – intercept of regression line fitted through raw cortisol values, **SP2** – slope of regression line fitted through raw cortisol values.

Note: The above raw cortisol values were log transformed and an independent-samples t-test conducted to assess differences between group means. Threshold for significance was set at 0.05.

4.3.3 Maternal salivary cortisone during pregnancy

4.3.3.1 Antenatal depression and maternal cortisone levels during pregnancy

Raw maternal cortisone levels were found to be normally distributed and were used for all analysis. The diurnal cortisone curve is shown in Figure 4.3. There were no significant differences in cortisone levels between groups [Table 4.4].

4.3.3.2 Maternal cortisone levels and clinical measures during pregnancy

Correlation analysis conducted to assess the relationship between cortisone and HAM-D, CES-D, PSS and PSQI scores during pregnancy showed no significant associations.

Table 4.4 Raw cortisone values at five time points from waking during pregnancy

	Depressed (n=16)	History (n=25)	Control (n=40)	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
Cortisone T0	39.82 (15.20)	43.58 (10.93)	40.89 (10.68)	F=0.57, p=0.570
Cortisone T30	47.80 (11.57)	52.62 (13.29)	51.92 (13.05)	F=0.68, p=0.507
Cortisone T60	40.05 (15.97)	44.48 (12.47)	45.50 (12.45)	F=0.98, p=0.379
Cortisone T720	13.35 (6.35)	13.73 (4.69)	14.91 (6.74)	F=0.49, p=0.618
Cortisone T750	13.11 (4.46)	16.08 (8.46)	14.30 (6.34)	F=1.06, p=0.351

Note: Salivary samples were obtained from women at waking (0 min), then at 30 min, 60 min, 12 h (720 min) and 12.5 h (750 min) after waking. Raw cortisone data was analysed for differences in means using a one-way ANOVA. Threshold for significance was set at 0.05.

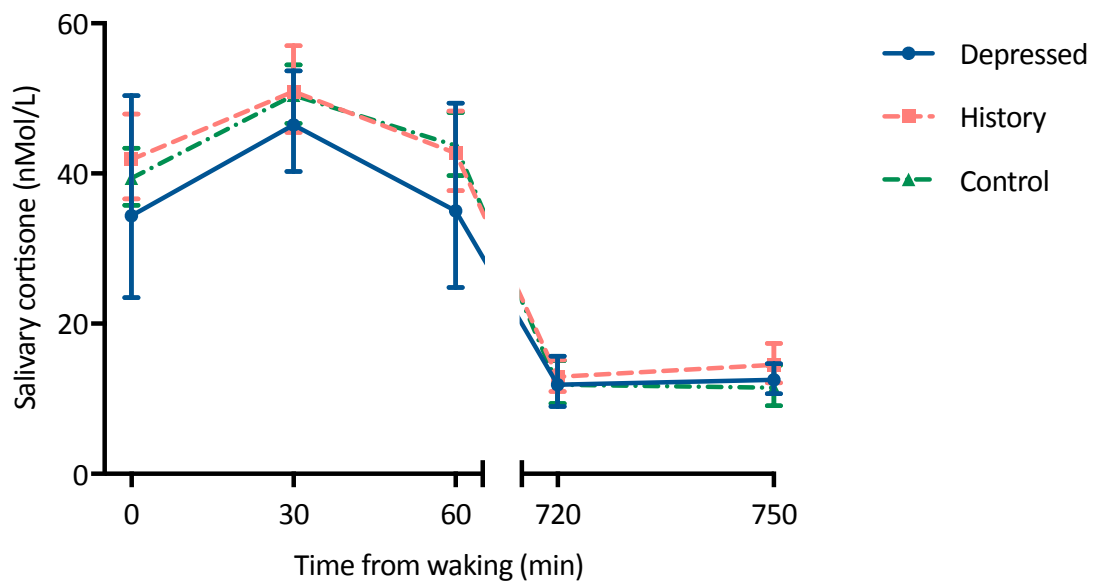


Figure 4.3 Diurnal variation of cortisone in pregnancy

Raw cortisone levels at the five time points from waking (0 min) to 12.5 h (750 min) after waking in the Depressed, History, and Control groups during pregnancy are presented here with error bars. There were no statistically significant differences between groups at any of the time points.

4.3.3.3 Maternal cortisol and cortisone levels during pregnancy

The relationship between maternal cortisol and cortisone at each of the five time points of saliva collection was analysed. Cortisol levels had significant, strong and positive correlations with cortisone at all the time points assessed [Figure 4.4].

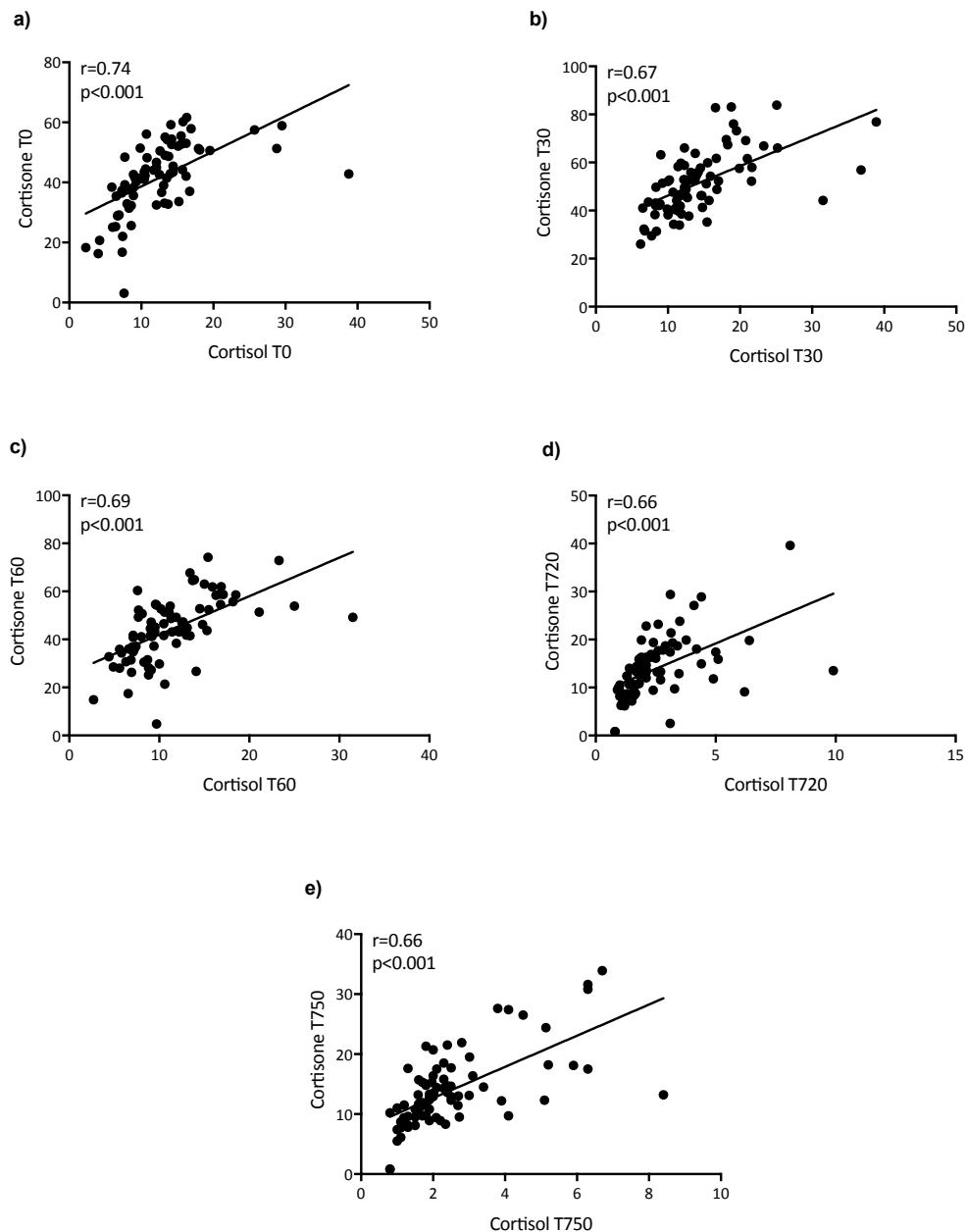


Figure 4.4 Correlations between maternal cortisol and cortisone levels during pregnancy. Spearman's rank-order correlation analysis was undertaken to assess this relationship. Threshold for significance was set at 0.05.

4.3.3.4 Foetal sex and maternal cortisone levels during pregnancy

Maternal cortisone levels in women carrying male foetuses and those carrying females were compared [Table 4.5]. Significantly higher cortisone levels were seen in women carrying male foetuses compared to those carrying female foetuses at 30 minutes after waking. There were no significant differences at the four other points of saliva collection.

Table 4.5 Effect of foetal sex on maternal cortisone levels during pregnancy

	Female (n=35)	Male (n=57)	Statistics, p-value
	Mean (SD)	Mean (SD)	
Cortisone T0	39.38 (10.46)	42.71 (12.38)	t=-1.19, p=0.236
Cortisone T30	47.88 (11.28)	53.92 (12.90)	t=-2.07, p=0.042*
Cortisone T60	42.28 (11.65)	45.53 (13.72)	t=-1.06, p=0.293
Cortisone T720	13.42 (4.93)	14.67 (6.64)	t=-0.87, p=0.386
Cortisone T750	14.77 (6.73)	14.53 (6.90)	t=0.15, p=0.877

Note: An independent-samples t-test was conducted to compare cortisone levels in women carrying male and female foetuses at waking (T0), 30 min after waking (T30), 60 min after waking (T60), 12 h after waking (T720) and 12.5 h after waking (T750). Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

4.3.3.5 Maternal depression and cortisone/cortisol ratio during pregnancy

There were no significant cortisone/cortisol ratio differences between Depressed, History, and Control groups during pregnancy. Controlling for foetal sex and gestational week in a univariate general linear model analysis of the values did not affect these results.

4.3.3.6 Foetal sex and maternal cortisone/cortisol ratio during pregnancy

Comparison of cortisone/cortisol ratios between women carrying female foetuses, and those carrying males showed a significant difference at 30 minutes after waking [Table 4.6]. Women carrying male foetuses had higher cortisone/cortisol ratios at this time-point compared to those carrying females.

Table 4.6 Effect of foetal sex on maternal cortisone/cortisol ratios during pregnancy

	Female (n=35)	Male (n=57)	Statistics, p-value
	Mean (SD)	Mean (SD)	
Ratio T0	3.49 (0.86)	3.79 (1.37)	t=-1.04, p=0.300
Ratio T30	3.59 (0.78)	4.09 (1.13)	t=-2.05, p=0.044*
Ratio T60	4.10 (0.81)	4.34 (1.51)	t=-0.94, p=0.351
Ratio T720	6.17 (2.15)	6.33 (2.38)	t=-0.31, p=0.761
Ratio T750	6.24 (2.23)	6.29 (2.47)	t=-0.09, p=0.932

Note: An independent-samples t-test was conducted to compare cortisone levels in women carrying male and female foetuses. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

4.3.4 Early life adversity and maternal cortisol measures during pregnancy

4.3.4.1 Early life adversity and maternal cortisol levels during pregnancy

Differences in maternal cortisol levels between groups with No ELA (-ELA) and groups with Low to Severe ELA (+ELA) were analysed at all five cortisol time points, and the significant results are shown in Table 4.7. Significantly higher evening cortisol levels were found in women with a history of emotional abuse, physical abuse, and emotional neglect [Table 4.7, Figure 4.5]. The group with a history of physical neglect also had a lower AUCi (-CTQPN: Mean=40.67 (248.82), +CTQPN: Mean=-75.58 (374.66); $Z=-2.07$, $p=0.039$). There was no effect of a history of sexual abuse on maternal cortisol levels. Women with a history of ≥ 1 trauma domain had higher evening cortisol levels, as well as higher waking cortisol levels [Table 4.7]. These effects persisted when maternal depression, HAM-D scores and medication use were controlled for in a univariate analysis.

Table 4.7 Early life adversity and cortisol levels (nMol/L) during pregnancy

		-ELA	+ELA	Statistics,
		Mean (SD)	Mean (SD)	p-value
T0	Emotional Abuse	12.19 (5.35)	14.77 (11.31)	$Z=0.77$, $p=0.441$
	Physical Abuse	12.26 (5.25)	18.81 (17.17)	$Z=1.06$, $p=0.291$
	Sexual Abuse	12.46 (6.23)	17.69 (15.49)	$Z=1.42$, $p=0.155$
	Emotional Neglect	12.30 (5.22)	15.17 (12.49)	$Z=0.42$, $p=0.673$
	Physical Neglect	12.28 (5.79)	15.64 (12.54)	$Z=0.98$, $p=0.325$
	Total Trauma Domains	12.30 (5.65)	13.98 (9.98)	$Z=2.95$, $p=0.003^*$
T750	Emotional Abuse	2.14 (1.18)	3.62 (3.92)	$Z=2.29$, $p=0.022^*$
	Physical Abuse	2.28 (1.34)	5.58 (5.99)	$Z=3.17$, $p=0.002^*$
	Sexual Abuse	2.43 (1.53)	4.46 (5.92)	$Z=0.99$, $p=0.318$
	Emotional Neglect	2.15 (1.07)	4.03 (4.38)	$Z=2.18$, $p=0.029^*$
	Physical Neglect	2.32 (1.42)	3.88 (4.63)	$Z=1.32$, $p=0.188$
	Total Trauma Domains	1.97 (0.86)	3.40 (3.51)	$Z=2.53$, $p=0.011^*$

Note: Waking (T0) and evening (T750) cortisol levels in groups with (+ELA) and without (-ELA) a history of different domains of early life adversity. A Mann-Whitney U test was conducted to compare differences, and findings were corrected for multiple comparisons. Threshold for significance was set at 0.05.

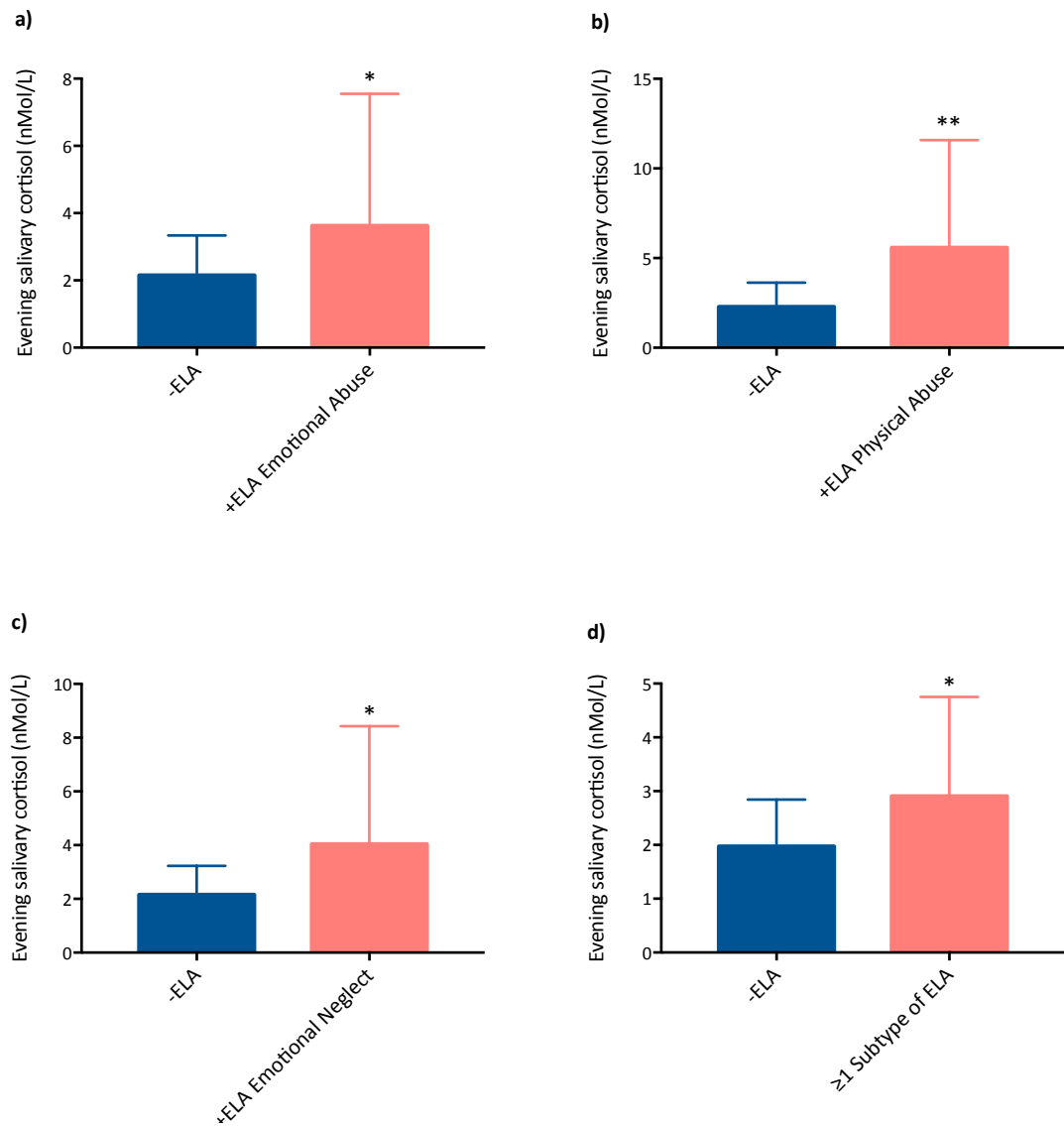


Figure 4.5 Early life adversity and maternal evening cortisol levels

A Mann-Whitney U test showed higher evening cortisol levels in women with a history of (+ELA) a) Emotional Abuse; b) Physical Abuse; c) Emotional Neglect; and d) ≥ 1 Subtype of ELA, compared to women who had experienced no early life adversity (-ELA).

* $p < 0.05$, ** $p < 0.01$

4.3.4.2 Maternal early life adversity and maternal cortisol measures during pregnancy

An association between maternal ELA and cortisol levels was evident in this sample. Maternal waking cortisol had a positive and significant correlation with physical neglect ($r=0.26$, $p=0.028$), while evening cortisol had moderate and positive correlation with physical abuse, emotional neglect, and total number of trauma domains ($r=0.30$, $p=0.007$) [Figure 4.6]. Maternal evening cortisol also had weak and positive correlations with emotional abuse and physical neglect [Figure 4.6]. The associations persisted after controlling for HAM-D scores.

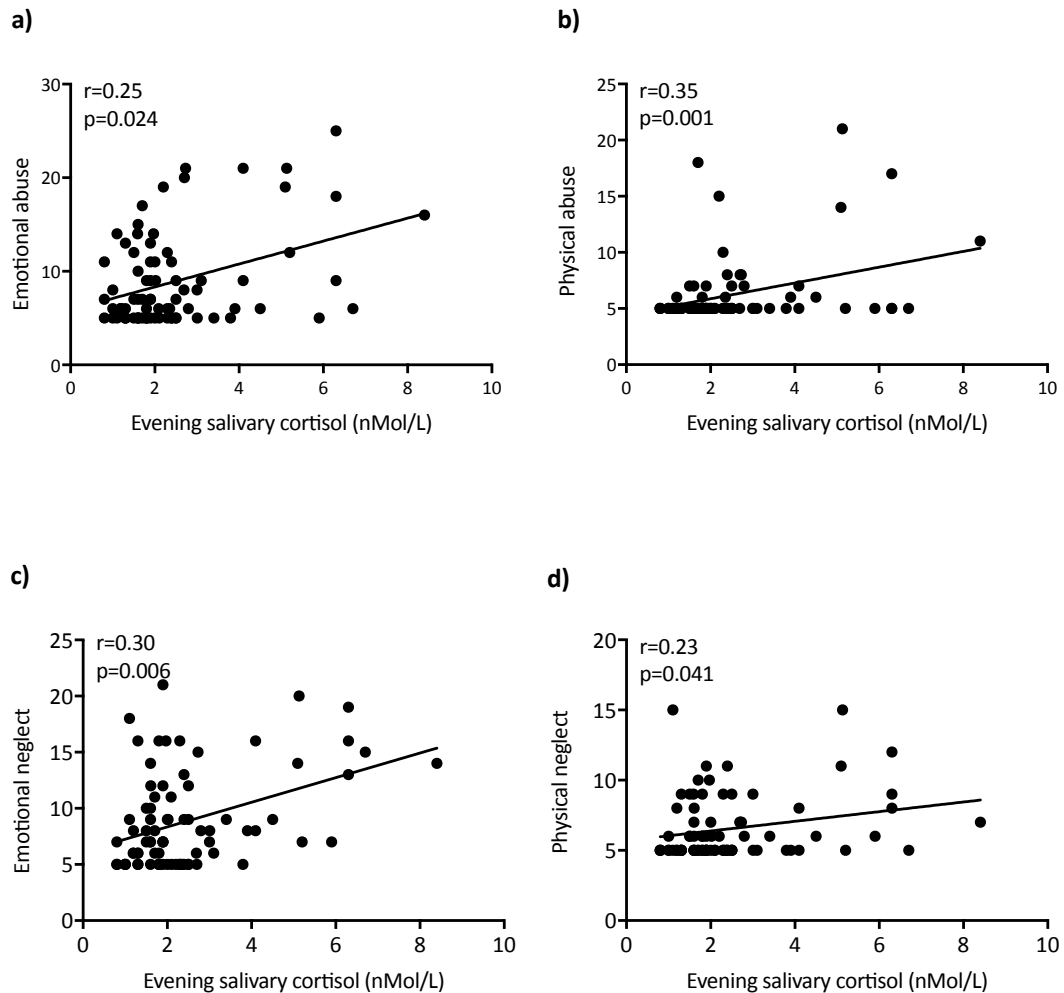


Figure 4.6 Association between maternal early life adversity and evening cortisol levels during pregnancy

Spearman's rank-order correlation analysis showed positive correlation between maternal evening cortisol and a) Emotional Abuse; b) Physical Abuse; c) Emotional Neglect; and d) Physical Neglect scores. Threshold for significance was set at 0.05.

4.3.4.3 Prediction of maternal cortisol levels with maternal early life adversity scores

Simple linear regression was performed to evaluate the predictive value of total ELA (classified as any level of trauma in ≥ 1 trauma domain) on maternal waking and evening cortisol levels. There was no significant predictive effect of ELA on maternal waking cortisol levels. However, simple linear regression demonstrated that ELA predicted 17% ($R^2=0.17$, $F=16.20$, $B=0.05$, $p<0.001$) of variance in maternal evening cortisol levels.

4.3.4.4 Foetal sex as a moderator of the relationship between maternal early life adversity and cortisol measures during pregnancy

Moderation analysis, a form of hierarchical linear regression, was conducted to assess the statistical significance of the interaction between CTQ scores and foetal sex on maternal mean morning and evening cortisol levels [See Section 2.7 for further explanation of moderation analysis]. A statistically significant moderator effect of foetal sex was found on the relationship between CTQ Emotional Abuse scores and maternal evening cortisol levels, with the interaction contributing to an additional 8% of total variance ($p=0.044$). Simple slopes analysis on these variables revealed a positive linear effect of foetal sex on the relationship between CTQ Emotional Abuse scores and maternal evening cortisol levels ($B=0.014$, $p=0.008$). Women carrying female foetuses had a non-significant negative linear relationship ($B=-0.002$, $p=0.695$) [Figure 4.7].

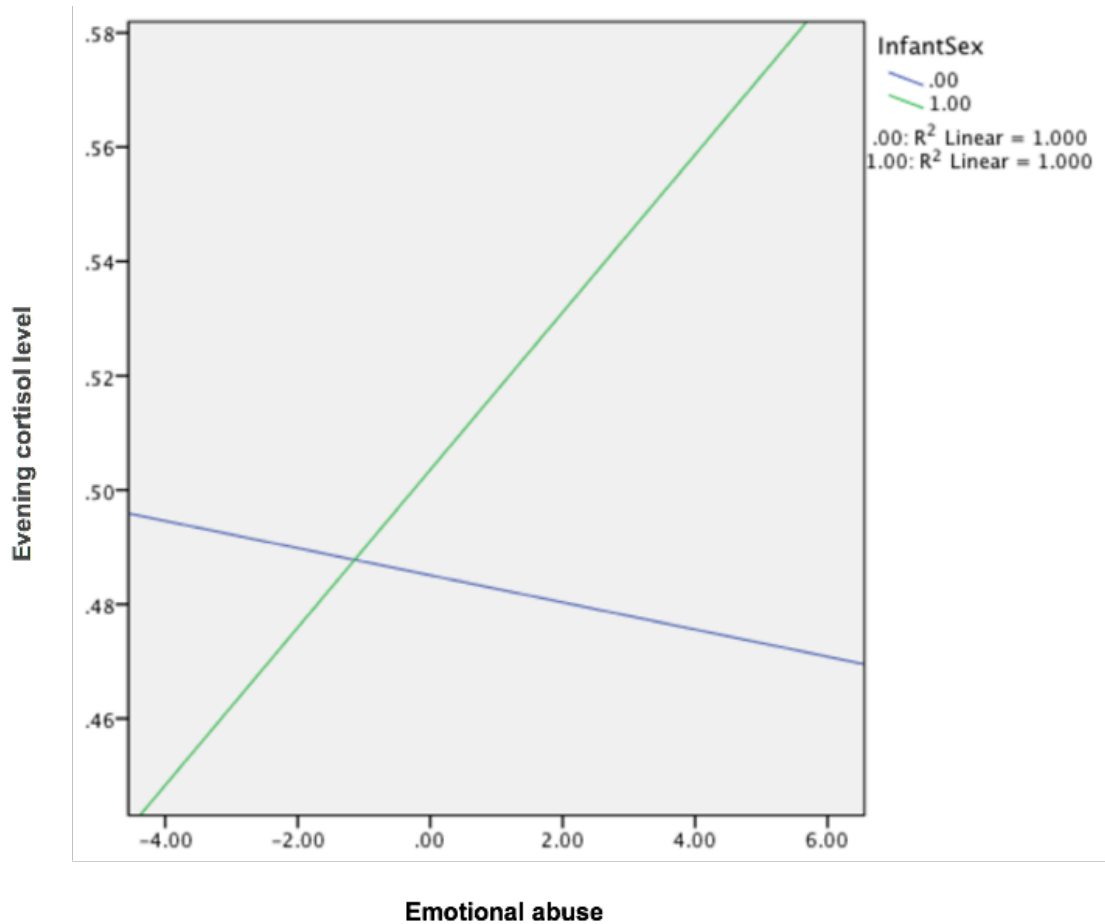


Figure 4.7 Moderation effect of foetal sex on the relationship between Emotional Abuse and maternal evening cortisol levels

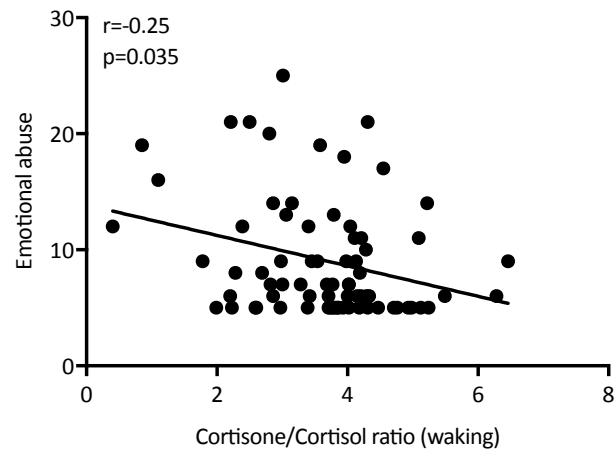
Male foetuses (InfantSex=1) had a significant positive linear moderation effect on the relationship between emotional abuse and maternal evening cortisol levels. Female foetuses (InfantSex=0) had a negative, non-significant moderation effect.

4.3.4.5 Maternal early life adversity and cortisone levels during pregnancy

Maternal cortisone levels across groups of women with no ELA and those with low to severe forms of ELA were analysed, with no significant differences observed. Maternal cortisone/cortisol ratios in groups of women with no ELA were compared to ratios in those with a history of any of the five subtypes of ELA. An effect of physical abuse was seen with the evening cortisone/cortisol ratio, with women in the +ELA Physical Abuse group having lower ratios (Mean=4.50, SD=2.18) compared to those in the -ELA Physical Abuse (Mean=6.53, SD=2.32, $t=2.71$, $p=0.008$).

Association analyses were conducted to assess the relationship between maternal cortisone and cortisone/cortisol ratios during pregnancy and maternal ELA subtypes. Since the data was not normally distributed even after log transformation, it was not possible to control for other variables such as HAM-D scores or clinical depression, which may influence this relationship. There were no significant correlations found between maternal cortisone levels and CTQ scores. Negative and significant correlations were found between the cortisone/cortisol ratio at waking and emotional abuse ($r=-0.25$, $p=0.035$), physical neglect ($r=-0.33$, $p=0.005$), global CTQ score ($r=-0.26$, $p=-0.029$), and ≥ 1 trauma subtype ($r=-0.26$, $p=-0.024$) [Figure 4.8]. A significant negative association was also found between ratio of cortisone/cortisol at 30 minutes after waking and emotional neglect ($r=-0.23$, $p=0.047$) and physical neglect ($r=-0.26$, $p=0.045$). Maternal evening cortisone/cortisol ratio negatively correlated with physical abuse ($r=-0.30$, $p=0.006$), global CTQ score ($r=-0.29$, $p=0.009$), and ≥ 1 trauma subtypes ($r=-0.23$, $p=0.038$) [Figure 4.9].

a)



b)

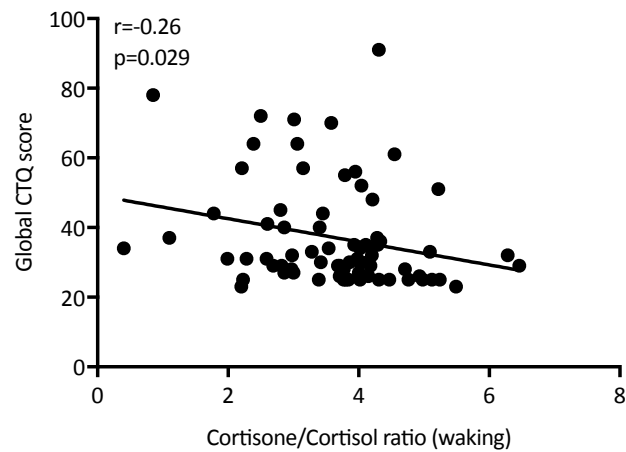
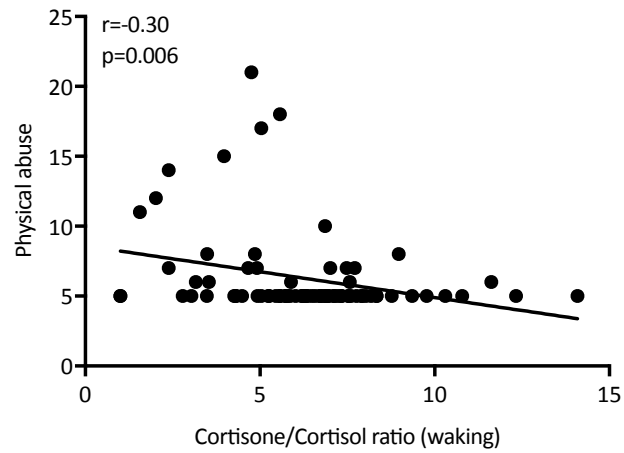


Figure 4.8 Association between maternal cortisone/cortisol ratio at waking and early life adversity

Spearman's rank-order analysis showed a negative correlation between waking cortisone/cortisol ratio and a) CTQ Emotional Abuse and b) CTQ Global Score. Threshold for significance was set at 0.05.

a)



b)

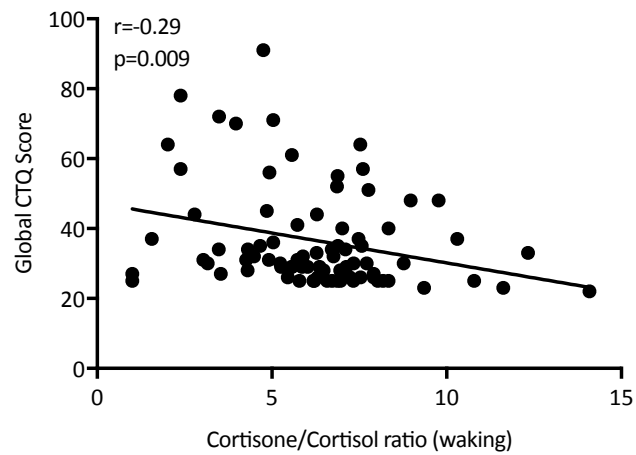


Figure 4.9 Association between evening cortisone/cortisol ratio and early life adversity

Spearman's rank-order analysis showed a negative correlation relationship between waking cortisone/cortisol ratio and a) CTQ Physical Abuse and b) CTQ Global Score. Threshold for significance was set at 0.05.

4.4 Discussion

The main positive finding in this study was the effect of early life adversity on maternal cortisol levels. Higher waking cortisol levels were found in women who had a history of abuse in ≥ 1 trauma domain. Higher evening cortisol levels were found in women with a history of abuse in ≥ 1 abuse domains – in particular, emotional abuse or neglect, or physical abuse. This relationship appeared to be moderated by male foetal sex, although foetal sex in itself had no effect on maternal cortisol measures during pregnancy. Women carrying male foetuses had higher peak cortisone levels, and accordingly, higher peak cortisone/cortisol ratios. Multiple negative correlations were also observed between maternal ELA domains and cortisone/cortisol ratios. There was no effect of state or trait depression, or clinical measures of depression, on the maternal HPA axis during pregnancy.

4.4.1 Effect of depression on maternal HPA axis during pregnancy

Although it is well established that the HPA axis plays a role in major depressive disorder, the relationship between the HPA axis and antenatal depression remains uncertain. Study findings to date are equivocal on whether the relationship between HPA axis and depression exists during pregnancy. There were no significant differences in cortisol measures between the groups in this study sample, disproving the study hypothesis. Although graphically, the Depressed and History groups appeared to have somewhat higher waking cortisol levels compared to the Control group, these differences were not statistically significant. Maternal CAR parameters followed this pattern, with no significant differences between the three groups. Known confounders such as age, BMI, gestation, parity, gravidity, and medication use were controlled for in the analysis, with no change in results, suggesting that depression had no effect on maternal cortisol levels during pregnancy in this sample. These findings contradict the results of the pilot study by O'Keane and colleagues (2011), which found higher evening cortisol levels in depressed women in the second trimester. Methodological differences may explain these findings. The study by O'Keane and colleagues used saliva samples collected over three consecutive days, in contrast to saliva samples collected over the course of one day in the present study. Cortisol averages in saliva samples collected over consecutive days may reduce the variability in cortisol levels, giving a more accurate cortisol concentration. However, in

order to increase compliance, samples were only collected over the course of one day in the current study.

Findings from the present study add to the evidence base of studies showing no associations between antenatal depression and the HPA axis (Evans et al., 2008; Field et al., 2010; Hellgren et al., 2013; Parcels, 2010). There is some evidence in non-pregnant samples for a trait, or scarring, effect of depression on the HPA axis (Lok et al., 2012). Only one other study investigated possible trait effects of the HPA axis in a pregnant sample and found no differences between groups (Hellgren et al., 2013). In the present study, since all the three groups had similar cortisol measures, we cannot comment on the study hypothesis of the presence of scarring effect in the HPA axis of women with a history of depression.

Maternal self-rated mood, stress, and sleep measures were examined for a relationship with cortisol measures. The positive relationship observed between maternal depression during pregnancy and the PSS, PSQI, CES-D, and HAM-D scores were reported in Chapter 3. However, there were no associations found between mood, stress or sleep measures during pregnancy and concurrent maternal cortisol measures, refuting the study hypothesis.

The exponential increase in cortisol in normal pregnancy, beginning from around gestational week 12 and peaking to about three-times normal levels in the third trimester is well recognised (Jung et al., 2011). These physiologically high levels of cortisol may obscure any increase in cortisol seen in antenatal depression, which may explain the similarity in cortisol levels across groups. In fact, even the hypercortisolaemia of Cushing's disease, although rare in pregnancy, is a challenging diagnosis to make, owing to the extent of physiological hypercortisolaemia present during healthy pregnancy (Lindsay and Nieman, 2005; Lopes et al., 2016). Additionally, there is a diminished physiological HPA axis stress response during pregnancy (de Weerth and Buitelaar, 2005b), particularly in the third trimester. Since the majority of the women in the current study were recruited at the end of the second trimester or early in the third trimester, it may be postulated that this physiological dampening of cortisol may also dampen any depression-driven cortisol responses. Further, there is evidence in the literature for dysfunction in the cortisol ultradian rhythm in

depression, driven by hypothalamic CRH (Lightman and Conway-Campbell, 2010; Pariante and Miller, 2001; Young et al., 2004). Similarly, there may be a disruption of the cortisol ultradian rhythm in antenatal depression, which may not be evident in the placental CRH-driven free cortisol measured in saliva.

Disrupted activity of the placental enzyme, 11β -HSD2, which is thought to protect the foetus from overexposure of cortisol *in utero* by oxidising active cortisol into its inactive metabolite cortisone, has been implicated in some studies of foetal programming by prenatal stress (Conradt et al., 2013; O'Donnell et al., 2012; Seckl, 1997; Seth et al., 2015). In the present study however, there were no significant differences between groups in cortisone levels and no differences in the ratio of cortisone/cortisol. Further, cortisone levels or the cortisone/cortisol ratio had no association with clinical measures. As would be expected, strong correlations were present between cortisone and cortisol levels during pregnancy.

4.4.2 Effect of early life adversity on maternal HPA axis during pregnancy

Few studies have examined the effect of ELA on the HPA axis during pregnancy. Of those that have, six studies have found that ELA is associated with higher cortisol levels during pregnancy (Bublitz and Stroud, 2012; Buss, Entringer, et al., 2016; Buss, Stalder, et al., 2016; Moog et al., 2016; Schreier, Enlow, Ritz, Coull, et al., 2015; Schreier, Enlow, Ritz, Gennings, et al., 2015), while one study found a negative relationship between ELA and waking cortisol levels (Shea et al., 2007a). In support of the majority of the study reports and the current study hypothesis, higher evening cortisol levels were found in women who had experienced either emotional trauma or physical abuse in the present study. Higher waking cortisol levels were found in women with a history of ≥ 1 trauma subtype. There were multiple negative correlations between maternal ELA domains and the cortisone/cortisol ratio, which reflect lower cortisone/cortisol ratios due to the high levels of cortisol evident in women with a history of ELA. Linear regression modelling in the current study showed that a history of ≥ 1 trauma subtype explained 17% of variance in maternal evening cortisol levels. These findings suggest that, although the driver of HPA axis dysregulation in depression may be masked during pregnancy, ELA-driven HPA axis hyperactivity appears to be amplified during pregnancy.

4.4.3 Effect of foetal sex on maternal HPA axis during pregnancy

Only two studies so far have examined the possible effects of foetal sex on maternal HPA axis during pregnancy. DiPietro and colleagues found women carrying male fetuses to have higher cortisol levels up to 30 weeks gestation, while women carrying females had higher cortisol levels after 30 weeks (DiPietro et al., 2011). Women carrying female fetuses had higher overall morning cortisol production in another study (Giesbrecht, Campbell, et al., 2015). With this emerging evidence in mind, women in this study were categorised into those carrying female fetuses and those carrying males, and their cortisol concentrations compared. There was no significant effect of foetal sex on maternal cortisol levels in this sample, even when the caseness groups were analysed separately, and when women carrying male and female fetuses were analysed separately. These findings contradict the study hypothesis. Notably, there was a male preponderance in this study sample, which may have skewed any effects of foetal sex on maternal cortisol measures. Women carrying male fetuses had significantly higher cortisone levels and a higher cortisone/cortisol ratio at 30 minutes after awakening. However, in the absence of a statistically significant difference in cortisol levels at this time point, it would not be prudent to speculate that this may be due to an increased conversion of peak cortisol into cortisone.

Foetal sex was found to have an influence on the relationship between ELA and maternal cortisol in this sample, with male fetuses exerting a positive linear effect and females exerting a non-significant negative linear effect. Women with a history of emotional abuse carrying male fetuses had higher mean evening cortisol levels compared to women with a history of emotional abuse carrying females. Although there were no overall differences in maternal cortisol production between women carrying male fetuses and those carrying females, these findings suggest sexually dimorphic effects of the foetus on maternal health. Male fetuses are thought to accelerate growth in utero, even in the presence of maternal stress (Clifton, 2010; Hodyl et al., 2010). It would appear that male fetuses in this sample, while focussing their resources on optimum growth in the presence of maternal ELA, may exert additional stress on the maternal HPA axis, explaining the positive linear effect on the relationship between maternal ELA and evening cortisol levels.

4.4.4 Conclusions

The hypotheses of antenatal depression and clinical measures during pregnancy having an effect on maternal cortisol levels were disproved in this study. Since there were no differences in cortisol levels between the three groups, we could not postulate about a trait effect of the HPA axis persisting during pregnancy. The reasons for the similarities in cortisol measures across the three groups are possibly complex, and may include the already physiologically high cortisol levels and the attenuation of the CAR in the third trimester of pregnancy. Maternal history of ELA was associated with higher evening cortisol levels, in accordance with our hypothesis. Additionally, this relationship was moderated by male foetal sex, despite foetal sex not having a direct effect on maternal cortisol levels during pregnancy, indicating sexually dimorphic foetal influence on maternal HPA axis. Although depression during pregnancy did not have an effect on maternal HPA axis during pregnancy, ELA appeared to continue to exert an activating effect on the maternal HPA axis.

Chapter 5

The trajectory of the hypothalamic-pituitary-adrenal axis postpartum

Relationship with mood and early life adversity

5.1 Introduction

The HPA axis, following the significant alterations seen during pregnancy, undergoes abrupt changes in the early puerperium before gradually returning to its pre-pregnancy state in the weeks that follow. In healthy pregnancy, the delivery of the placenta leads to an acute withdrawal of placental CRH, thus dramatically reducing the levels of circulating CRH to the non-pregnant state in the early puerperium (Mastorakos and Ilias, 2000; O'Keane, Lightman, Patrick, et al., 2011). A period of adrenal suppression lasting weeks to months follows on from treatment of conditions with endogenous high cortisol production such as Cushing's syndrome (Avgerinos et al., 1987; Fitzgerald et al., 1982; Gomez et al., 1993; Graber et al., 1965), and it is hypothesised that similar perturbations of the HPA axis, with adrenal suppression, are seen postpartum following the physiological hypercortisolaemia of pregnancy.

Cortisol levels peak during labour, and decrease after delivery (Kammerer et al., 2006; O'Keane, Lightman, Patrick, et al., 2011). Plasma cortisol levels were resistant to suppression by dexamethasone over the first two weeks postpartum in one study, with resistance decreasing by about the third week and suppression returning to pre-pregnancy function by the fifth week (Owens et al., 1987). In another study, no differences were reported in cortisol levels between three, six, and 12 weeks postpartum, and a more recent study found that total plasma cortisol remained high at 2-3 months postpartum, while plasma free cortisol levels had returned to pre-pregnancy levels (Jung et al., 2011; Magiakou, Mastorakos, Rabin, Dubbert, et al., 1996). The adrenal hypertrophy of pregnancy persists postpartum, but unbound cortisol levels gradually return to normal over the first four days postpartum (Magiakou, Mastorakos, Rabin, Dubbert, et al., 1996; Mastorakos and Ilias, 2003). It has been reported that, after a transient decrease, ACTH levels began to rise again between days three and six postpartum (O'Keane, Lightman, Patrick, et al., 2011). This was thought to indicate the re-establishment of hypothalamic CRH secretion following suppression by the hypercortisolaemia of pregnancy. Findings from another study also indicate that ACTH levels return to pre-pregnancy levels by day 5 postpartum (Okamoto et al., 1989). In accordance with the suppression of cortisol response to dexamethasone postpartum, one study found women to have a blunted ACTH response to administration of ovine CRH at two weeks and six weeks postpartum, with the

response returning to pre-pregnancy function by 12 weeks postpartum in healthy women (Magiakou, Mastorakos, Rabin, Dubbert, et al., 1996).

Mechanisms underlying this mild suppression of the HPA axis postpartum are probably numerous, but one of the processes thought to be involved is the desensitisation of the anterior pituitary corticotrophs to CRH. Prolonged exposure of these cells to high levels of circulating CRH during pregnancy is thought to lead to a downregulation of pituitary CRH receptors, reducing their ability for maximal response (Glynn et al., 2013; Reisine and Hoffman, 1983; Thomson et al., 1990). Another plausible contributory factor is the downregulation of CRH production and hypothalamic CRH receptors due to overexposure to placental CRH during pregnancy (Gomez et al., 1993; Groer and Morgan, 2007).

Studies of the HPA axis in women with postpartum depression report diverse results. Magiakou and colleagues (Magiakou, Mastorakos, Rabin, Dubbert, et al., 1996) found a sustained blunting of ACTH response to ovine CRH, even at 12 weeks postpartum, at which time non-depressed women, as discussed above, had the return of a normal ACTH response. The same study found no differences in cortisol levels between depressed women postpartum compared to euthymic women (Magiakou, Mastorakos, Rabin, Dubbert, et al., 1996). Another study also found no differences in ACTH or cortisol in women who were depressed at six or 12 weeks postpartum compared to women who were euthymic, while a later study reported that depressed women at 6-8 weeks postpartum had HPA axis dynamics similar to women outside the perinatal period, with higher waking cortisol levels and a blunted response compared to non-depressed postpartum women (Jolley et al., 2007; Taylor et al., 2009).

Although there is strong evidence for an association between maternal ELA and hyperactivity of the HPA axis during pregnancy (Bublitz et al., 2014; Buss, Entringer, et al., 2016; Moog et al., 2016), there is little published data on the possible effects of ELA on the maternal HPA axis postpartum. One study examined postpartum maternal cortisol responses to a laboratory stress paradigm involving infant distress, and found mothers with a history of ELA to have lower cortisol levels in response to this stressor compared to women with no history of ELA (Brand et al., 2010). In another study of the cortisol awakening response in the postpartum period, women with a history of

ELA had higher waking cortisol levels and a sustained increase in morning cortisol postpartum compared to controls (Gonzalez et al., 2009).

5.1.1 Study Aims

Despite the evidence that restoration of the HPA axis to non-pregnant levels following healthy pregnancies is preceded by a temporary period of mild HPA axis suppression, little is known about the changes of the HPA axis during the postpartum period in women who were depressed during pregnancy. In this study, we sought to measure maternal salivary cortisol over two time points postpartum – two months and six months, and to evaluate the trajectory of the HPA axis over this period, comparing the Depressed (depressed during pregnancy), History (past history of depression but euthymic during pregnancy), and Control (healthy pregnant women with no history of mental illness) groups. Since ELA has been shown to have an effect on cortisol levels in adulthood, and there is evidence for ELA-driven HPA axis changes during pregnancy, we examined maternal cortisol levels postpartum for persistence of ELA-driven changes.

5.1.2 Study hypotheses

- Total cortisol output will decrease postpartum, but dynamic cortisol responses (CAR) will remain the same in all groups.
- Higher cortisol levels will be seen in the Depressed group at the postpartum time points.
- There will be an association between maternal cortisol levels and sleep, stress, and mood measures at the corresponding time point.
- Women with a history of ELA will have higher cortisol measures compared to those with no ELA.

5.2 Methods

5.2.1 Recruitment

Women were recruited from three maternity hospitals – the National Maternity Hospital, Dublin; the Coombe Women and Infants University Hospital, Dublin; and The Rotunda Hospital, Dublin. Three groups of women were recruited into the study – women who were clinically depressed during pregnancy (Depressed group), those with a prior history of depression who were euthymic during pregnancy (History group), and never-depressed pregnant women (Control group). Mean gestational age at recruitment was 26 weeks. Clinical depression was diagnosed based on DSM-5 criteria for a major depressive episode (MDD), and a Mini International Neuropsychiatric Interview (MINI) completed. Hamilton Depression rating scales (HAM-D) were completed for all women as an objective measure of mood.

Women also completed self-rated scales including the Perceived Stress Scale (PSS), Pittsburgh Sleep Quality Index (PSQI), and the Centre for Epidemiological Studies Depression rating scale (CES-D) at two and six months postpartum. The Childhood Trauma Questionnaire (CTQ), completed during pregnancy, was also used in the analysis. The CTQ is subdivided into Emotional Abuse, Physical Abuse, Sexual Abuse, Emotional Neglect, and Physical Neglect categories. Women with a history of abuse in ≥ 1 trauma domain were compared to women with no history of abuse.

5.2.2 Salivary cortisol

Saliva samples were collected by women using absorbent swabs in Salivette (Sarstedt) tubes at five time points over the course of the day – at waking (0 minutes), 30 minutes after waking, 60 minutes after waking, 12 hours after waking (720 minutes), and 12.5 hours (750 minutes) after waking. Samples were then stored in the participant's refrigerator and either collected by researchers at the infant vaccination appointment, or posted out to Trinity College Institute of Neuroscience (TCIN) in pre-stamped and addressed envelopes. Upon arrival in TCIN, samples were centrifuged for 10 minutes at 3000 rpm at room temperature. Samples were then divided into microtubes of 500 μ L aliquots and stored at -80°C. Samples were then transported in batches to Professor Brian Keevil's laboratory in the University Hospital of South

Manchester for analysis. Cortisol and cortisone levels were analysed by Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS). Due to its improved specificity and sensitivity, LC-MS/MS is considered to be a superior technique for cortisol quantification compared to other immunoassay techniques [See Section 2.6.2].

5.2.3 Cortisol measures

In addition to waking and evening cortisol levels, total cortisol output and cortisol awakening response (CAR) parameters were calculated from the five cortisol values. Using the three morning cortisol values, area under the curve (AUC) parameters were derived (Fekedulegn et al., 2007). AUC with respect to ground (AUC_G), an estimate of total cortisol production over the morning, and AUC with respect to increase (AUC_I), a measure of the CAR, reflecting the dynamic of cortisol change, were calculated. A regression line was fitted through the morning cortisol values and the intercept (indicating total cortisol output) and slope (indicating CAR) of this line were also calculated.

5.2.4 Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics for Macintosh, Version 24 (REF). Data was assessed for normality using the Kolmogorov-Smirnov test. Despite log transformation, cortisol values remained non-normally distributed, and as such, raw values were used in most of the analyses in this chapter. Extreme high or low outliers after normality assessment were removed from the analysis. Data was analysed using the non-parametric tests Mann-Whitney U test or the Kruskal-Wallis test to compare means. Correlation analysis was conducted using Spearman's rank-order test. Findings were corrected for multiple comparisons using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995). Regression modelling was not undertaken since the non-normal distribution of data would lead to spurious results. Repeated measures analysis with the non-parametric Wilcoxon Signed-Rank test was conducted to analyse differences in cortisol between the pregnancy and postpartum time points.

5.3 Results

In the Results section, the characteristics of the sample will be discussed in **Section 5.3.1**. Maternal cortisol measures at two and six months postpartum will be described in **Section 5.3.2**. The trajectory of cortisol change from pregnancy to the postpartum period will be outlined in **Section 5.3.3**. Associations of maternal cortisol measures at two and six months postpartum will be discussed in **Section 5.3.4** and **Section 5.3.5** respectively. The effect of maternal ELA on cortisol measures postpartum will be discussed in **Section 5.3.6**.

5.3.1 Characteristics of sample

Study participants were followed up at two (PP₁) and six months (PP₂) postpartum. The number of women at each time point indicates the number of women who had reached this time point, and does not signify attrition rate. There were six drop-outs from pregnancy to PP₁, and one further drop-out from PP₁ to PP₂. One participant was excluded postpartum after her infant was diagnosed with Apert's syndrome. Sixty-three women were analysed at the two-month time point [Figure 5.1]. All participants were clinically euthymic at two months postpartum. Forty-five participants were analysed at the six-month time point [Figure 5.1]. Three women were diagnosed with depression at six months, all belonging to the Depressed group. HAM-D scores for the three time points are presented in Table 5.1. The Depressed group had significantly higher HAM-D scores compared to Controls during pregnancy ($p < 0.001$), at PP₁ ($p < 0.001$) and PP₂ ($p = 0.034$) [Table 5.1]. During pregnancy, the Depressed group also had significantly higher scores compared to the History group ($p < 0.001$), and the History group scored higher than the Control group ($p = 0.018$).

Table 5.1 Hamilton Depression rating scale scores at pregnancy, two months (PP1) and six months postpartum (PP2)

	Depressed		History		Control		Statistic, p-value
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	
Pregnancy	23	20.82 (5.01)	31	3.64 (4.39)	46	1.39 (1.56)	$\chi^2=54.50$, p<0.001*
PP1	14	8.00 (7.16)	22	3.00 (2.53)	24	1.58 (1.47)	$\chi^2=16.19$, p<0.001*
PP2	10	8.13 (8.14)	12	2.10 (1.91)	21	1.58 (1.12)	$\chi^2=6.95$, p=0.031*

Note: A Kruskal-Wallis test was conducted to analyse differences in means between the groups. Results were corrected for multiple comparisons. Significant threshold was set at 0.05.

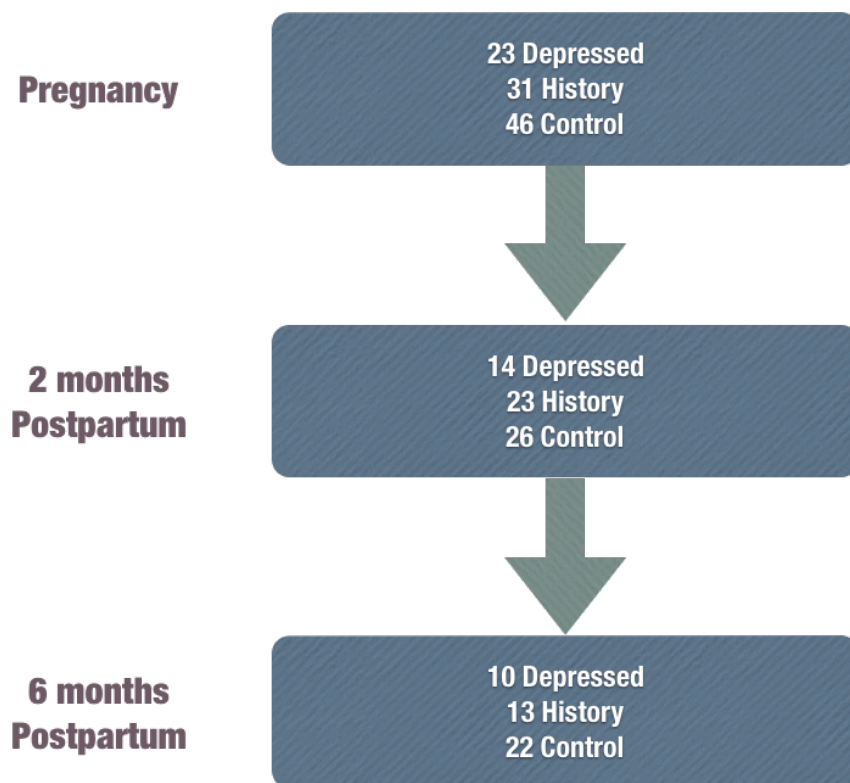


Figure 5.1 Number of participants at each time point.

5.3.2 Maternal cortisol measures postpartum

5.3.2.1 Maternal cortisol levels at two months postpartum

Maternal salivary cortisol levels measured at PP₁ are displayed in Table 5.2, and the diurnal variation of cortisol at this time point is presented in Figure 5.2. No significant differences in cortisol levels were found between the three groups at this postpartum stage. All participants in the study were euthymic at this time point. There was no effect of medication on cortisol values.

5.3.2.2 Maternal cortisol awakening response at two months postpartum

Cortisol parameters describing total cortisol production (AUC_G and intercept of regression line) and CAR (AUC_I and slope of the regression line) were derived from the three morning cortisol levels at PP₁ [Table 5.3, Figure 5.3]. There were no significant differences between the groups.

5.3.2.3 Maternal cortisone levels and cortisone/cortisol ratios at two months postpartum

There were no differences between the Depressed, History, and Control groups in either cortisone levels alone or the cortisone/cortisol ratio.

Table 5.2 Maternal cortisol values (nMol/L) at five time points from waking at two months postpartum (PP1)

	Depressed (n=13)	History (n=20)	Control (n=24)	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
PP1 T0	6.55 (2.64)	8.03 (5.00)	6.64 (3.60)	$\chi^2=0.90$, p=0.637
PP1 T30	7.90 (5.14)	8.29 (5.04)	9.99 (4.72)	$\chi^2=2.04$, p=0.361
PP1 T60	5.19 (5.08)	5.88 (3.67)	7.25 (4.18)	$\chi^2=3.85$, p=0.146
PP1 T720	2.30 (3.41)	0.88 (0.25)	2.88 (8.34)	$\chi^2=5.48$, p=0.065
PP1 T750	1.24 (0.61)	0.92 (0.45)	1.88 (2.85)	$\chi^2=3.91$, p=0.142

Note: Salivary cortisol was obtained from women at waking (T0), then at 30 min (T30), 60 min (T60), 12 h (T720) and 12.5 h (T750) after waking. Raw cortisol data was analysed using the Kruskal-Wallis test. Threshold for significance was set at 0.05.

Table 5.3 Maternal cortisol awakening response parameters at two months postpartum

	Depressed (n=13)	History (n=20)	Control (n=24)	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
AUCg	346.14 (261.62)	421.09 (264.07)	503.60 (212.24)	$\chi^2=1.75$, p=0.417
AUCi	32.24 (189.78)	35.36 (207.76)	106.01 (209.34)	$\chi^2=0.45$, p=0.800
Intercept	7.24 (2.92)	9.32 (5.54)	7.58 (3.45)	$\chi^2=1.09$, p=0.058
Slope	-0.02 (0.09)	-0.05 (0.08)	0.02 (0.08)	$\chi^2=4.79$, p=0.091

AUCg – area under the curve with respect to ground, **AUCi** – area under the curve with respect to increase, **Intercept** – intercept of regression line fitted through raw cortisol values, **Slope** – slope of regression line fitted through raw cortisol values.

Note: CAR variables shown here are derived from raw cortisol values. Kruskal-Wallis test was conducted analyse differences between groups. Threshold for significance was set at 0.05.

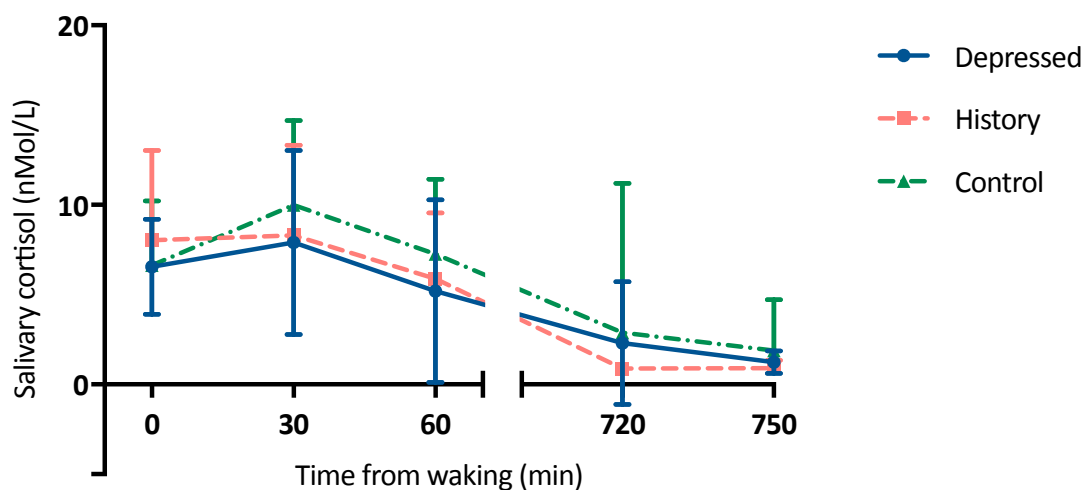


Figure 5.2 Maternal diurnal variation of cortisol at two months postpartum

Raw cortisol levels at the five time-points from waking (0 min) to 12.5h (750 min) after waking at two months postpartum in Depressed, History, and Control groups from pregnancy, with error bars. There were no statistically significant differences between groups at any of the time points.

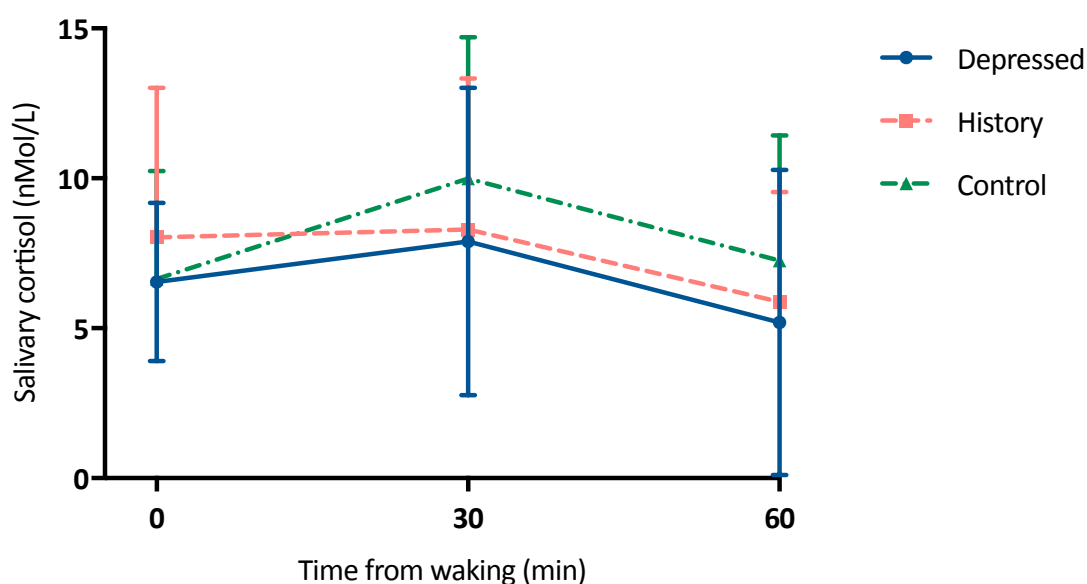


Figure 5.3 Maternal cortisol awakening response at two months postpartum

Raw cortisol levels at the three time points from waking (0, 30, 60 min) at two months postpartum in Depressed, History, and Control groups from pregnancy, shown with error bars. There were no statistically significant differences between groups.

5.3.2.4 Maternal cortisol levels at six months postpartum

Maternal raw cortisol levels measured at PP2 are displayed in Table 5.4, and the diurnal variation of cortisol at this time point is presented in Figure 5.4. Differences in cortisol between the three caseness groups were analysed. Three participants in the Depressed group had relapsed at this postpartum stage but were not analysed separately owing to the small sample size. There was a group effect at peak cortisol levels, with a significant difference between the History and Depressed groups ($p=0.030$) found after post-hoc analysis [Table 5.4]. This may be attributable to a type I error. There was no effect of medication on cortisol values.

5.3.2.5 Maternal cortisol awakening response parameters at six months postpartum

The parameters describing the measures of total cortisol output (AUC_G and intercept of regression line) and CAR (AUC_I and slope of regression line) were derived from the three morning cortisol values (0 min, 30 min, 60 min) [Table 5.5, Figure 5.5]. Significant group differences were found in the AUC_G, and pairwise comparisons showed that the Depressed group had significantly higher AUC_G compared to the History group ($p=0.039$).

5.3.2.6 Maternal cortisone levels and cortisone/cortisol ratios at six months postpartum

There were no differences in cortisone levels or cortisone/cortisol ratios between the Depressed, History, and Control groups at PP2.

Table 5.4 Maternal raw cortisol values (nMol/L) at five time points from waking at six months postpartum (PP2)

	Depressed (n=10)	History (n=13)	Control (n=22)	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
PP2 T0	11.58 (5.87)	7.74 (3.48)	8.38 (4.11)	$\chi^2=3.12$, p=0.210
PP2 T30	12.30 (3.52)	6.96 (3.95)	9.45 (3.34)	$\chi^2=7.54$, p=0.023*
PP2 T60	6.58 (1.53)	5.14 (3.69)	6.65 (3.43)	$\chi^2=1.91$, p=0.386
PP2 T720	1.76 (1.62)	0.95 (0.43)	1.20 (0.76)	$\chi^2=4.36$, p=0.113
PP2 T750	1.67 (1.22)	0.91 (0.21)	2.05 (4.48)	$\chi^2=2.84$, p=0.241

Note: Salivary cortisol was obtained from women at waking (T0), then at 30 min (T30), 60 min (T60), 12 h (T720) and 12.5 h (T750) after waking. Raw cortisol data was analysed using the Kruskal-Wallis test and corrected for multiple comparisons. Threshold for significance was set at 0.05.

Table 5.5 Maternal cortisol awakening response parameters at six months postpartum

	Depressed (n=10)	History (n=13)	Control (n=22)	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
AUCg	694.76 (352.28)	410.84 (214.57)	503.60 (212.24)	$\chi^2=7.16$, p=0.028*
AUCi	-105.55 (272.10)	-35.56 (282.57)	106.01 (209.34)	$\chi^2=0.67$, p=0.716
Intercept	12.46 (6.56)	8.94 (4.22)	7.58 (3.45)	$\chi^2=2.39$, p=0.302
Slope	-0.08 (0.11)	-0.05 (0.10)	0.02 (0.08)	$\chi^2=1.21$, p=0.546

AUCg – area under the curve with respect to ground, **AUCi** – area under the curve with respect to increase, **Intercept** – intercept of regression line fitted through raw cortisol values, **Slope** – slope of regression line fitted through raw cortisol values.

Note: CAR variables shown here are derived from raw cortisol values. Kruskal-Wallis test was conducted to look at differences in means between groups. Findings were corrected for multiple comparisons. Threshold for significance was set at 0.05.

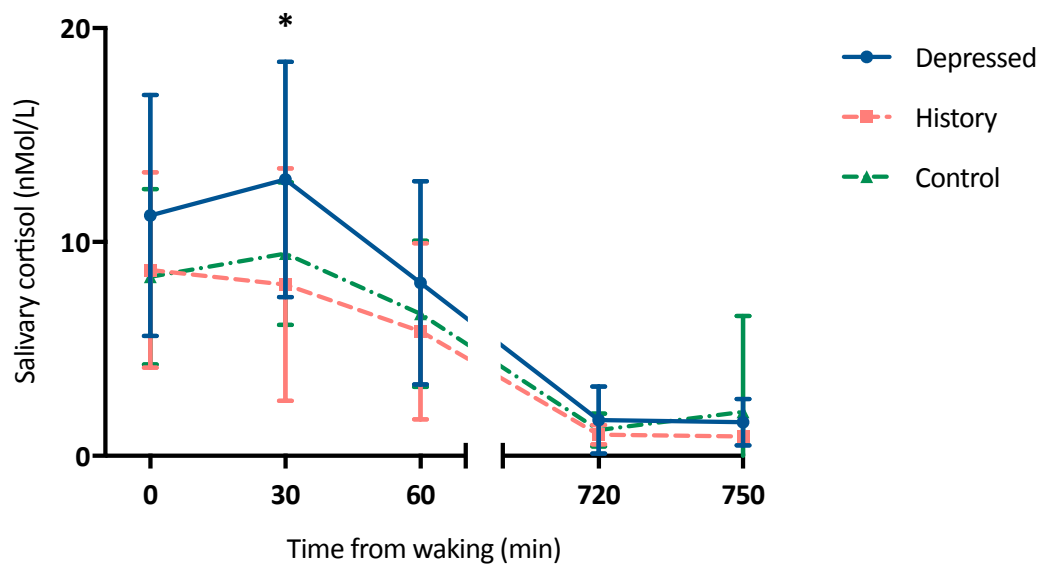


Figure 5.4 Maternal diurnal variation of cortisol at six months postpartum

Raw cortisol levels at the five time-points from waking (0 min) to 12.5 h (750 min) after waking in Depressed, History, and Control groups at six months postpartum, with error bars. Women in the Depressed group had significantly higher cortisol levels at T30 compared to the History group ($p=0.023$). * $p<0.05$

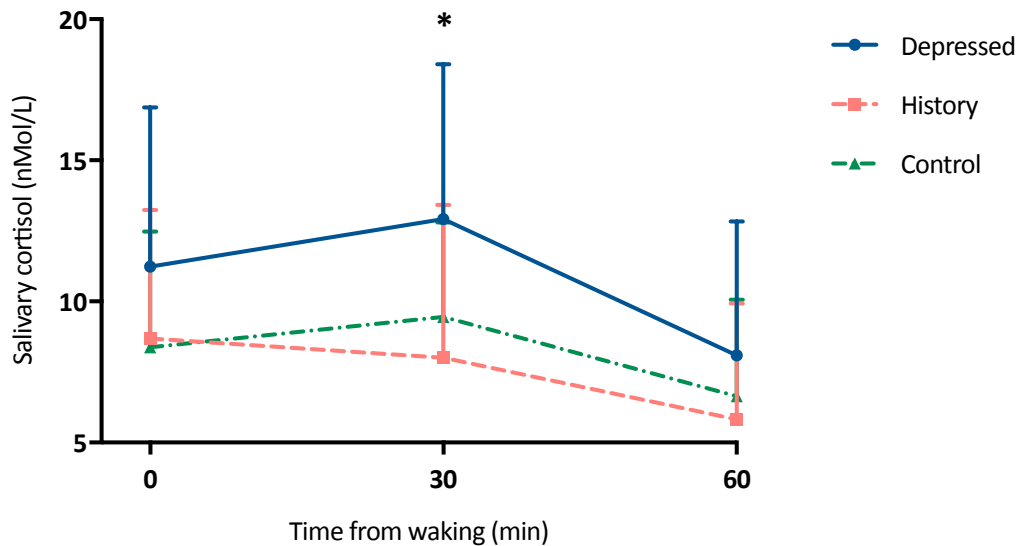


Figure 5.5 Maternal cortisol awakening response at six months postpartum

Raw cortisol levels at the three time-points from waking (0, 30, 60 min) in Depressed, History, and Control groups at six months postpartum, shown with error bars. Women in the Depressed group had significantly higher cortisol levels at T30 compared to the History group ($p=0.023$). * $p<0.05$

5.3.3 Trajectory of maternal cortisol from pregnancy to postpartum

5.3.3.1 Maternal cortisol measures from pregnancy to two months postpartum in the whole sample

The diurnal cortisol curves during pregnancy and PP1 are presented in Figure 5.6. The whole sample was analysed for differences in waking and evening cortisol levels, total cortisol production (AUCg, intercept of regression line) and CAR (AUCi and slope of regression line) between pregnancy and PP1. Waking cortisol levels, evening cortisol levels, and total cortisol production decreased significantly between the pregnancy time point and PP1, but there were no differences in the CAR measures [Table 5.6].

5.3.3.2 Maternal cortisol measures from pregnancy to two months postpartum within caseness groups

When each caseness group was analysed separately, similar to the whole group differences, there were significantly lower waking cortisol levels, evening cortisol levels, and total cortisol production within each of the three groups, with no significant difference in CAR [Figure 5.7].

Table 5.6 Trajectory of maternal cortisol measures from pregnancy to two months postpartum (PP1) in the whole sample

	Pregnancy (n=83)	PP1 (n=58)	Statistic, p-value
	Mean (SD)	Mean (SD)	
T0	13.10 (8.31)	7.04 (3.89)	Z=-4.90, p<0.001*
T750	2.71 (2.67)	1.19 (1.19)	Z=-5.03, p<0.001*
AUCg	752.13 (356.13)	430.94 (218.49)	Z=-4.76, p<0.001*
AUCi	15.49 (301.65)	45.38 (218.04)	Z=-0.21, p=0.834
Intercept	13.69 (7.91)	7.98 (4.13)	Z=-4.38, p<0.001*
Slope	-0.02 (0.11)	-0.02 (0.09)	Z=-0.25, p=0.805

Note: Waking (T0) and evening (T750) cortisol levels, measures of total cortisol production (AUCg and intercept of regression line), and measures of CAR (AUCi and slope of regression line) were analysed for differences at Pregnancy and PP2 with a Wilcoxon Signed-Rank Test. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

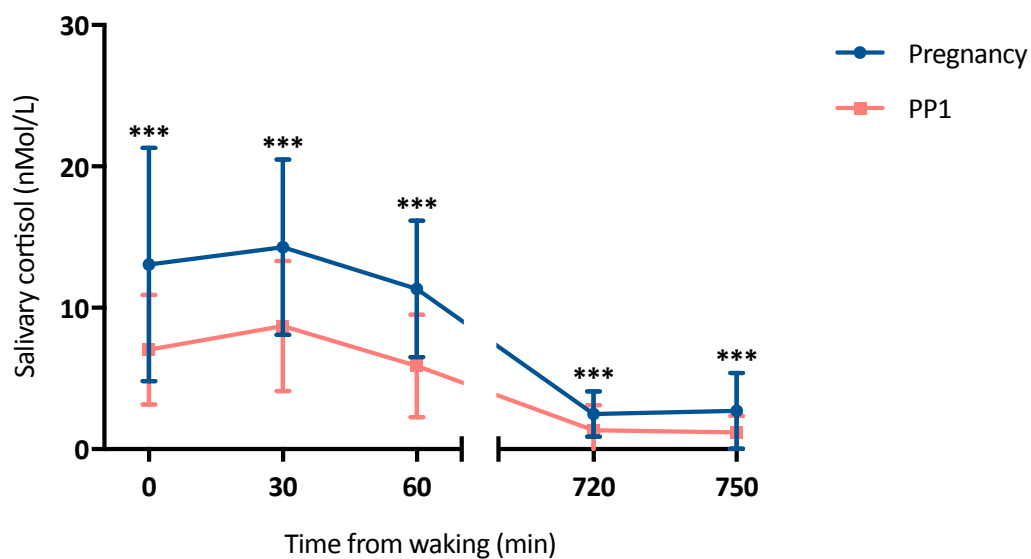


Figure 5.6 Diurnal variation in maternal cortisol at pregnancy and two months postpartum (PP1) in the whole sample

Maternal salivary cortisol was measured at five time points between 0 min and 12.5 h (750 min) after waking during pregnancy and at two months postpartum. Wilcoxon Signed-Rank test showed significant differences between the pregnancy and PP1 stages at all five cortisol time points.

*** $p < 0.001$

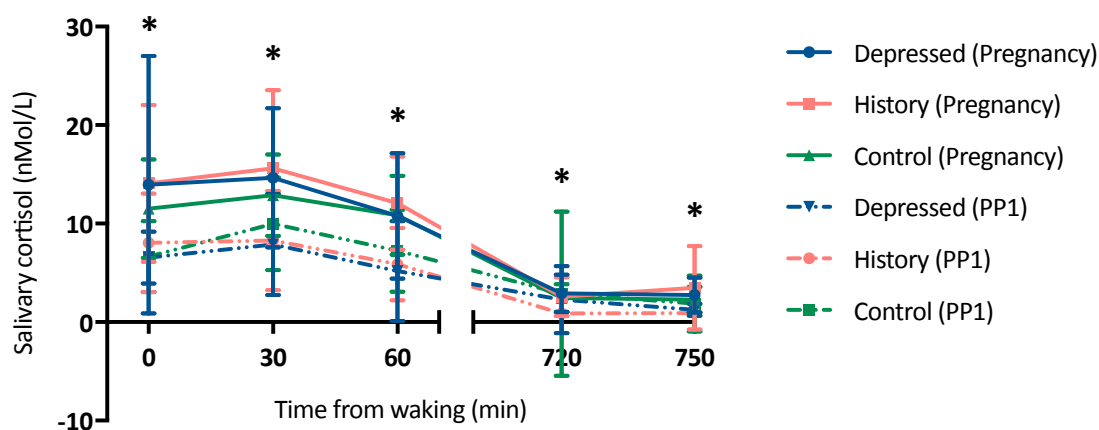


Figure 5.7 Diurnal variation in maternal cortisol at pregnancy and two months postpartum (PP1) within each group

Maternal salivary cortisol was measured at five time points between 0 min and 12.5 h (750 min) after awakening during pregnancy and at PP1. Wilcoxon Signed-Rank test showed significant differences between the pregnancy and PP1 stages in all levels of cortisol within each group, except for T720 in the Depressed group. * $p < 0.05$

5.3.3.3 Association of maternal cortisol measures between pregnancy and two months postpartum

The relationship between maternal cortisol levels and CAR parameters during pregnancy and those at PP1 was analysed in the whole sample [Table 5.7]. The slope of the regression line during pregnancy had significant positive correlations with AUCi ($r=0.302$, $p=0.033$) and slope of the regression line ($r=0.334$, $p=0.019$) at PP1 [Figure 5.8].

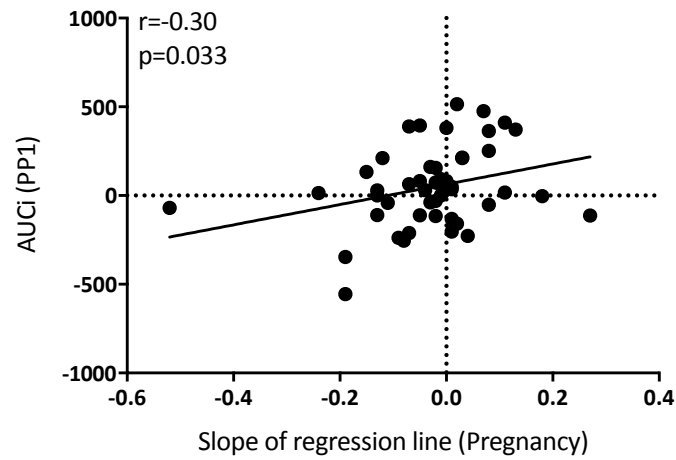
The analysis was then conducted separately in each group examining waking and evening cortisol, and one measure each of total cortisol output (AUCg) and CAR (AUCi) [Table 5.7]. Within the Depressed group, maternal waking cortisol at PP1 correlated strongly and positively with maternal pregnancy evening cortisol ($r=0.74$, $p=0.018$). Both maternal evening cortisol levels were strongly and positively correlated within the History group ($r=0.48$, $p=0.040$). There were no significant correlations within the Control group.

Table 5.7 Correlation analysis of maternal cortisol measures at pregnancy (P) and at two months postpartum (PP1)

		P T0	P T750	P AUCg	P AUCi
Whole Sample P: n=79 PP1: n=53	PP1 T0	0.08	0.13	-0.10	-0.18
	PP1 T750	-0.09	0.19	-0.17	-0.22
	PP1 AUCg	-0.12	0.12	0.01	0.02
	PP1 AUCi	-0.16	-0.02	0.02	0.19
Depressed P: n=16 PP1: n=10	PP1 T0	-0.03	0.74*	-0.14	-0.53
	PP1 T750	-0.27	0.05	-0.60	-0.42
	PP1 AUCg	-0.03	0.36	0.06	-0.16
	PP1 AUCi	-0.10	-0.29	0.13	0.34
History P: n=23 PP1: n=19	PP1 T0	0.12	-0.09	-0.08	-0.18
	PP1 T750	-0.05	0.48*	-0.22	-0.27
	PP1 AUCg	0.01	0.03	0.07	-0.02
	PP1 AUCi	-0.14	0.17	-0.16	0.11
Control P: n=40 PP1: n=24	PP1 T0	0.09	0.15	0.05	-0.03
	PP1 T750	-0.14	0.14	-0.30	-0.27
	PP1 AUCg	-0.11	0.17	0.27	0.29
	PP1 AUCi	-0.06	-0.04	0.15	0.26

Note: Spearman's rank-order correlation analysis was performed on maternal cortisol levels at pregnancy and at two months postpartum within each group. Spearman's rho correlation coefficient (r) is presented in the table. Results were corrected for multiple comparisons. * $p<0.05$

a)



b)

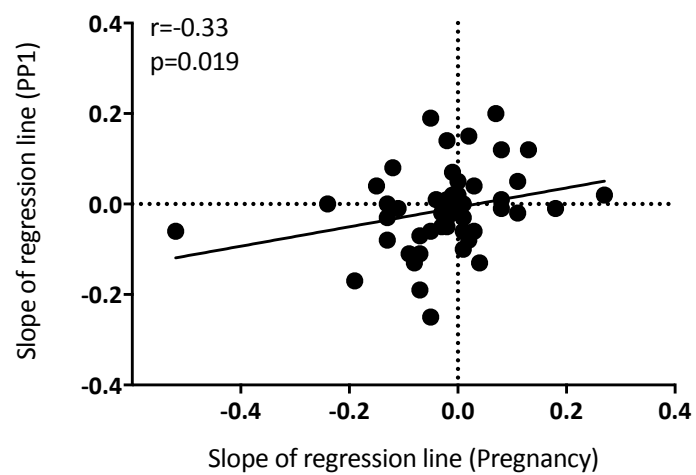


Figure 5.8 Whole sample correlation analysis between slope of regression line during pregnancy and cortisol measures at two months postpartum (PP1).

Correlation coefficients and p-values are shown in the figures. Slope of regression line during pregnancy was significantly moderately and positively correlated with AUCi and slope at PP1. Threshold for significance was set at 0.05.

5.3.3.4 Maternal cortisol measures from pregnancy to six months postpartum in the whole sample

Maternal diurnal cortisol measures at PP2 were compared to diurnal cortisol measures during pregnancy to analyse differences in waking and evening cortisol, total cortisol production, and CAR from pregnancy to PP2 [Figure 5.9, Table 5.8]. Waking and evening cortisol levels, as well as total cortisol production (AUCg, intercept) were significantly lower at PP2 compared to pregnancy. There were no significant differences in CAR measures between the two time points.

5.3.3.5 Maternal cortisol measures from pregnancy to six months postpartum within caseness groups

The analysis was repeated within each caseness group to examine the differences in cortisol measures between the two time points. The diurnal cortisol curve for each group is presented in Figure 5.10. There were no differences found in cortisol levels, total cortisol production or CAR within the Depressed group between pregnancy and PP2 [Table 5.9]. To reiterate, three women within this group were depressed at six months postpartum. The History and Control groups showed a significant reduction in cortisol levels and total cortisol production between pregnancy and PP2, with no change in CAR [Table 5.9].

Table 5.8 Trajectory of maternal cortisol measures from pregnancy to six months postpartum (PP2) in the whole sample

	Pregnancy (n=80)	PP2 (n=44)	Statistic, p-value
	Mean (SD)	Mean (SD)	
T0	13.10 (8.31)	8.93 (4.53)	Z=-3.11, p=0.002*
T750	2.71 (2.67)	1.12 (0.70)	Z=-3.97, p<0.001*
AUCg	752.13 (356.13)	468.08 (218.94)	Z=-3.68, p<0.001*
AUCi	15.49 (301.65)	-30.80 (214.05)	Z=-0.87, p=0.375
Intercept	13.69 (7.91)	9.37 (4.47)	Z=-2.97, p=0.003*
Slope	-0.02 (0.11)	-0.04 (0.08)	Z=-1.33, p=0.182

Note: Waking (T0) and evening (T750) cortisol levels, measures of total cortisol production (AUCg and intercept of regression line), and measures of CAR (AUCi and slope of regression line) were analysed for differences at Pregnancy and PP2 with a Wilcoxon Signed-Rank Test. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

Table 5.9 Trajectory of maternal cortisol measures from pregnancy to six months postpartum (PP2) within each group

		Pregnancy Mean (SD)	PP2 Mean (SD)	Statistic, p-value
		(n=17)	(n=9)	
Depressed	T0	14.68 (13.61)	11.58 (5.86)	Z=-0.17, p=0.886
	T750	2.50 (1.40)	1.67 (1.22)	Z=-1.01, p=0.310
	AUCg	788.28 (519.15)	589.21 (271.92)	Z=-0.85, p=0.389
	AUCi	-92.50 (389.85)	-105.54 (272.10)	Z=-0.17, p=0.866
	Intercept	15.07 (12.36)	12.47 (6.56)	Z=-0.11, p=0.917
	Slope	-0.06 (0.14)	-0.08 (0.11)	Z=-0.94, p=0.345
		(n=23)	(n=13)	
History	T0	14.57 (7.73)	7.74 (3.49)	Z=-2.54, p=0.012*
	T750	3.54 (4.30)	0.91 (0.21)	Z=-2.67, p=0.008*
	AUCg	808.07 (419.95)	359.02 (173.79)	Z=-2.67, p=0.008*
	AUCi	33.73 (260.45)	2.20 (248.22)	Z=-0.39, p=0.695
	Intercept	15.03 (8.36)	8.29 (3.31)	Z=-2.85, p=0.004*
	Slope	-0.03 (0.11)	-0.04 (0.09)	Z=-0.31, p=0.755
		(n=40)	(n=22)	
Control	T0	11.50 (5.00)	8.38 (4.11)	Z=-2.35, p=0.019*
	T750	2.28 (1.30)	1.00 (0.53)	Z=-3.16, p=0.002*
	AUCg	701.30 (203.55)	482.97 (197.09)	Z=-3.24, p=0.001*
	AUCi	46.84 (284.67)	-19.73 (164.94)	Z=-3.04, p=0.375
	Intercept	12.32 (4.80)	8.89 (3.84)	Z=-2.25, p=0.026*
	Slope	-0.02 (0.09)	-0.03 (0.07)	Z=-1.42, p=0.154

Note: Waking (T0) and evening (T750) cortisol levels, measures of total cortisol production (AUCg and intercept of regression line), and measures of CAR (AUCi and slope of regression line) were analysed for differences at Pregnancy and PP2 with a Wilcoxon Signed-Rank Test. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

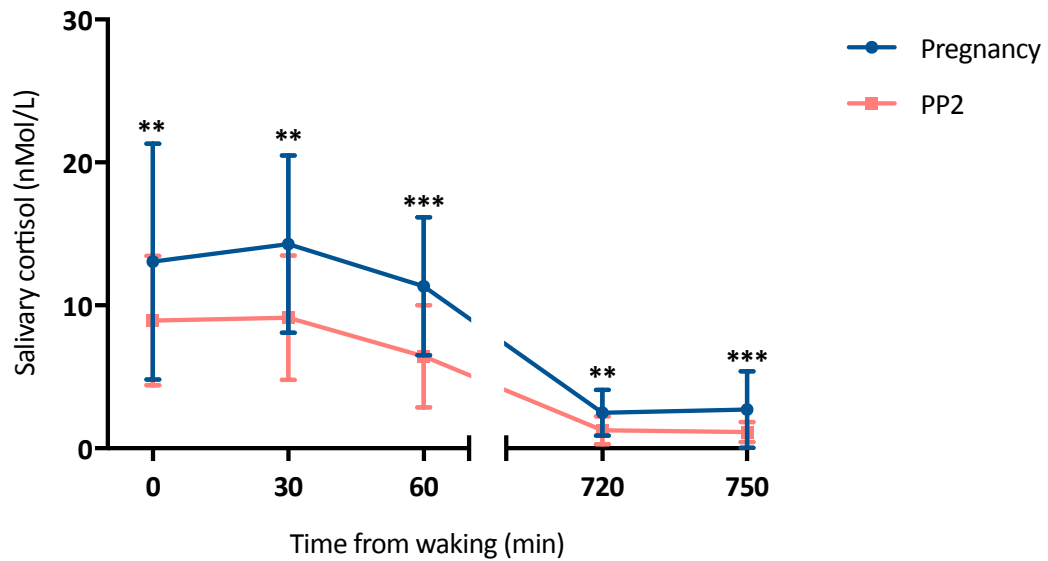


Figure 5.9 Diurnal variation in maternal cortisol at pregnancy and six months postpartum (PP2) in the whole sample

Maternal salivary cortisol was measured at five time points between 0 min and 12.5 h (750 min) after awakening during pregnancy and at PP2. Wilcoxon Signed-Rank test showed significant differences between the pregnancy and PP1 stages at all five cortisol time points. ** $p < 0.01$ *** $p < 0.001$

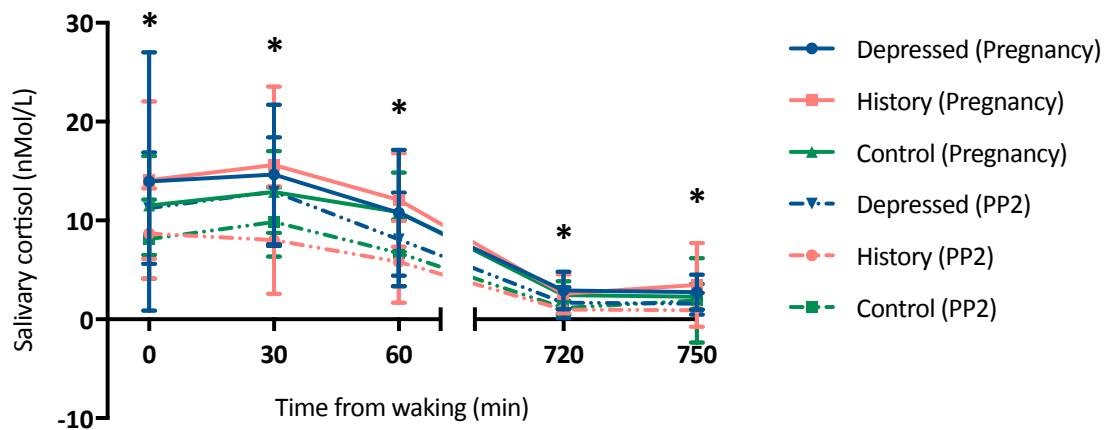


Figure 5.10 Diurnal variation of maternal cortisol levels at pregnancy and six months postpartum (PP2) within each group

Maternal salivary cortisol was measured at five time points between 0 min and 12.5 h (750 min) after awakening during pregnancy and PP2. Wilcoxon Signed-Rank test was conducted to analyse differences in cortisol. There were significant differences between groups at all five time points. * $p < 0.05$

5.3.3.6 Association of maternal cortisol measures between pregnancy and six months postpartum

The relationship between pregnancy and PP2 maternal cortisol waking cortisol levels, evening cortisol levels, total cortisol production and CAR was explored in the whole sample [Table 5.10]. Maternal waking cortisol at PP2 was positively correlated to maternal waking cortisol during pregnancy.

The analysis was repeated separately in each group. In the Depressed group, both maternal waking cortisol levels were strongly and positively associated [Table 5.10]. There were no significant correlations in the History group. Maternal evening cortisol levels were positively correlated in the Control group. [Table 5.10].

Table 5.10 Correlation analysis of maternal cortisol measures at pregnancy (P) and at six months postpartum (PP2)

		P T0	P T750	P AUCg	P AUCi
Whole Sample P: n=76 PP2: n=39	PP2 T0	0.33*	0.13	0.40*	0.01
	PP2 T750	-0.04	0.18	0.24	0.26
	PP2 AUCg	0.01	0.16	-0.02	-0.13
	PP2 AUCi	-0.32	0.02	-0.42	-0.16
Depressed P: n=16 PP2: n=7	PP2 T0	0.86*	-0.11	0.57	-0.04
	PP2 T750	0.15	-0.83	0.21	0.76
	PP2 AUCg	-0.29	0.41	-0.57	-0.32
	PP2 AUCi	-0.82	0.33	-0.68	0.21
History P: n=23 PP2: n=11	PP2 T0	0.40	0.25	0.10	-0.12
	PP2 T750	-0.74	-0.02	-0.39	-0.16
	PP2 AUCg	-0.03	0.28	-0.09	0.09
	PP2 AUCi	-0.56	-0.09	-0.27	0.07
Control P: n=40 PP2: n=21	PP2 T0	0.30	-0.05	0.48	0.13
	PP2 T750	0.07	0.55*	0.50	0.30
	PP2 AUCg	0.37	0.00	0.32	-0.22
	PP2 AUCi	0.01	0.14	-0.30	-0.47

Note: Spearman's rank-order correlation analysis was performed on maternal cortisol levels at pregnancy and at six months postpartum within each group. Spearman's rho correlation coefficient (r) is presented in the table. Results were corrected for multiple comparisons. *p<0.05 **p<0.01

5.3.3.7 Maternal cortisol measures from two months to six months postpartum in the whole sample

Maternal cortisol levels at the five time points over the course of the day at PP₁ were compared to maternal cortisol levels at the same five time points over the course of the day at PP₂ in the whole sample [Figure 5.11, Table 5.11]. The overall mean waking cortisol level was higher at PP₂ compared to PP₁, and there were no significant differences in the other measures.

5.3.3.8 Maternal cortisol measures from two months to six months postpartum within caseness groups

The analysis was repeated within each caseness group of pregnancy to examine the differences in cortisol values between PP₁ and PP₂. The diurnal variation of cortisol in each group is portrayed in Figure 5.12. There was a significant increase in waking cortisol and total cortisol production (AUC_G) from two months to six months postpartum within the Depressed group [Table 5.12]. In the Control group, there was a significantly higher intercept at PP₂ compared to PP₁. There were no differences in the History group between the two time points [Table 5.12].

Table 5.11 Trajectory of maternal cortisol measures from two months (PP1) to six months postpartum (PP2) in the whole sample

	PP1 (n=80)	PP2 (n=44)	Statistic, p-value
	Mean (SD)	Mean (SD)	
T0	7.04 (3.89)	8.93 (4.53)	Z=-2.41, p=0.016*
T750	1.19 (1.18)	1.12 (0.70)	Z=-0.06, p=0.950
AUCg	430.94 (218.49)	468.08 (218.94)	Z=-1.17, p=0.095
AUCi	45.37 (4.84)	-30.80 (214.05)	Z=-1.46, p=0.145
Intercept	7.98 (4.14)	9.37 (4.47)	Z=-1.73, p=0.084
Slope	-0.02 (0.09)	-0.04 (0.08)	Z=-0.13, p=0.898

Note: Waking (T0) and evening (T750) cortisol levels, measures of total cortisol production (AUCg and intercept of regression line), and measures of CAR (AUCi and slope of regression line) were analysed for differences at Pregnancy and PP2 with a Wilcoxon Signed-Rank Test. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

Table 5.12 Trajectory of maternal cortisol measures from two months (PP1) to six months postpartum (PP2) within each group

		PP1	PP2	Statistic, p-value
		Mean (SD)	Mean (SD)	
		(n=13)	(n=9)	
Depressed	T0	6.54 (2.64)	11.58 (5.86)	Z=-2.24, p=0.025*
	T750	1.24 (0.62)	1.67 (1.22)	Z=-0.81, p=0.416
	AUCg	399.39 (238.35)	589.21 (271.92)	Z=-2.19, p=0.028*
	AUCi	37.62 (204.50)	-105.54 (272.10)	Z=-1.01 p=0.314
	Intercept	7.24 (2.91)	12.47 (6.56)	Z=-1.68, p=0.093
	Slope	-0.02 (0.09)	-0.08 (0.11)	Z=-0.21, p=0.833
		(n=20)	(n=13)	
History	T0	8.04 (4.99)	7.74 (3.49)	Z=-0.18, p=0.859
	T750	0.92 (0.44)	0.91 (0.21)	Z=-0.37, p=0.715
	AUCg	421.09 (264.07)	359.02 (173.79)	Z=-0.80, p=0.422
	AUCi	35.35 (207.76)	2.20 (248.22)	Z=-0.18, p=0.861
	Intercept	9.32 (5.54)	8.29 (3.31)	Z=-0.87, p=0.382
	Slope	-0.05 (0.09)	-0.04 (0.09)	Z=-1.57, p=0.116
		(n=25)	(n=22)	
Control	T0	6.64 (3.59)	8.38 (4.11)	Z=-1.89, p=0.059
	T750	1.41 (1.74)	1.00 (0.53)	Z=-1.12, p=0.225
	AUCg	455.23 (168.90)	482.97 (197.09)	Z=-1.34, p=0.179
	AUCi	57.64 (239.90)	-19.73 (164.94)	Z=-1.46, p=0.145
	Intercept	7.33 (3.20)	8.89 (3.84)	Z=-2.17, p=0.030*
	Slope	0.01 (0.08)	-0.03 (0.07)	Z=-1.79, p=0.073

Note: Waking (T0) and evening (T750) cortisol levels, measures of total cortisol production (AUCg and intercept of regression line), and measures of CAR (AUCi and slope of regression line) were analysed for differences at Pregnancy and PP2 with a Wilcoxon Signed-Rank Test. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

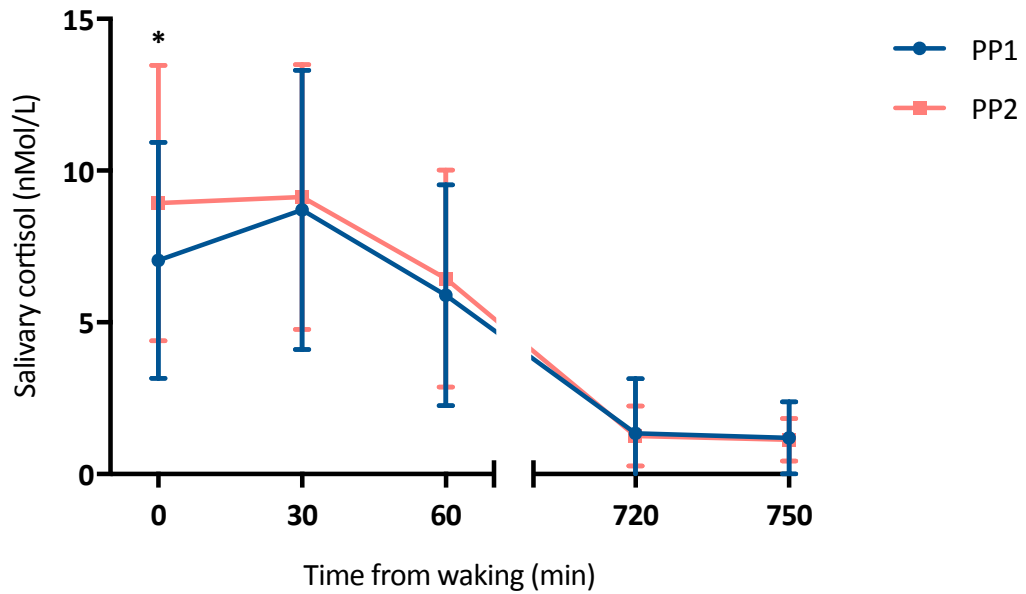


Figure 5.11 Diurnal variation in maternal cortisol at two months (PP1) and six months postpartum (PP2) in the whole sample

Maternal salivary cortisol was measured at five time points between 0 min and 12.5 h (750 min) after awakening at PP1 and at PP2. Wilcoxon Signed-Rank test showed significant differences between the pregnancy and PP1 stages at all five cortisol time points. * $p < 0.05$

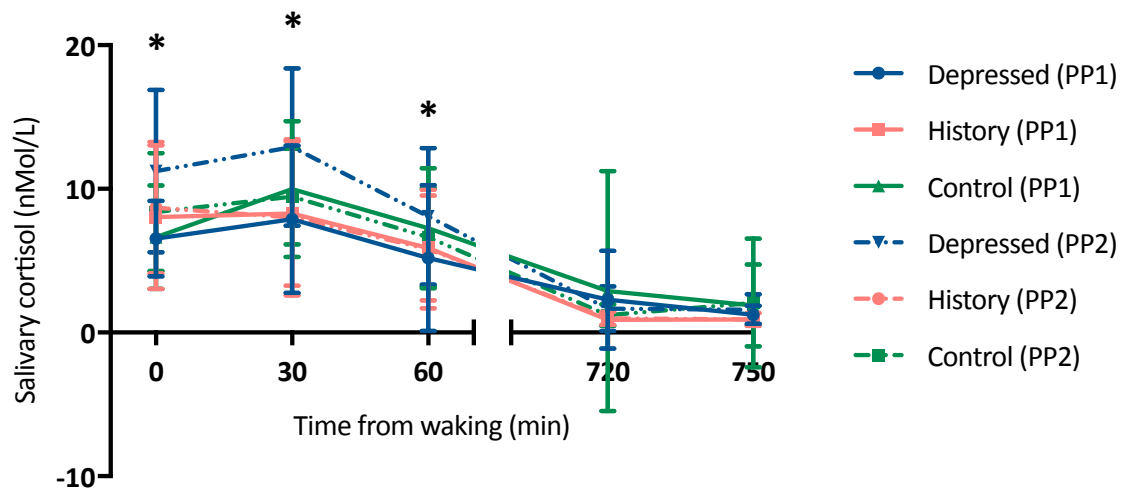


Figure 5.12 Diurnal variation of maternal cortisol levels at two months (PP1) and six months (PP2) postpartum within each group

Maternal salivary cortisol was measured at five time points between 0 min and 12.5 h (750 min) after awakening at PP1 and PP2. Wilcoxon Signed-Rank test showed significant differences between the PP1 and PP2 stages in all morning cortisol levels within the Depressed group only. * $p < 0.05$

5.3.4 Maternal HPA axis associations at two months postpartum

5.3.4.1 Maternal demographic variables and cortisol measures at two months postpartum

Correlation analyses were undertaken to assess the relationship between maternal age, gestational age, BMI, gravidity and parity, and maternal cortisol measures at two months postpartum. Maternal age had significant positive correlations with the slope of the regression line ($r=0.42$, $p=0.001$), a measure of the CAR. Maternal age had significant negative correlations with waking cortisol at this point postpartum ($r=-0.28$, $p=0.043$) [Figure 5.13].

When the groups were analysed separately, there were no significant correlations observed in the History and Control groups. Gravidity and parity were found to be strongly and positively associated with mean morning cortisol and intercept of the regression line within the Depressed group [Table 5.13]. Strong positive associations were found between maternal age and the slope of the regression line, as well as between parity and waking cortisol in the Depressed group. [Table 5.13].

5.3.4.2 Maternal clinical measures and cortisol parameters at two months postpartum

No significant associations between maternal PSS, PSQI, CES-D or HAM-D scores and cortisol measures were evident at PP1, either in the whole sample or individual group analyses.

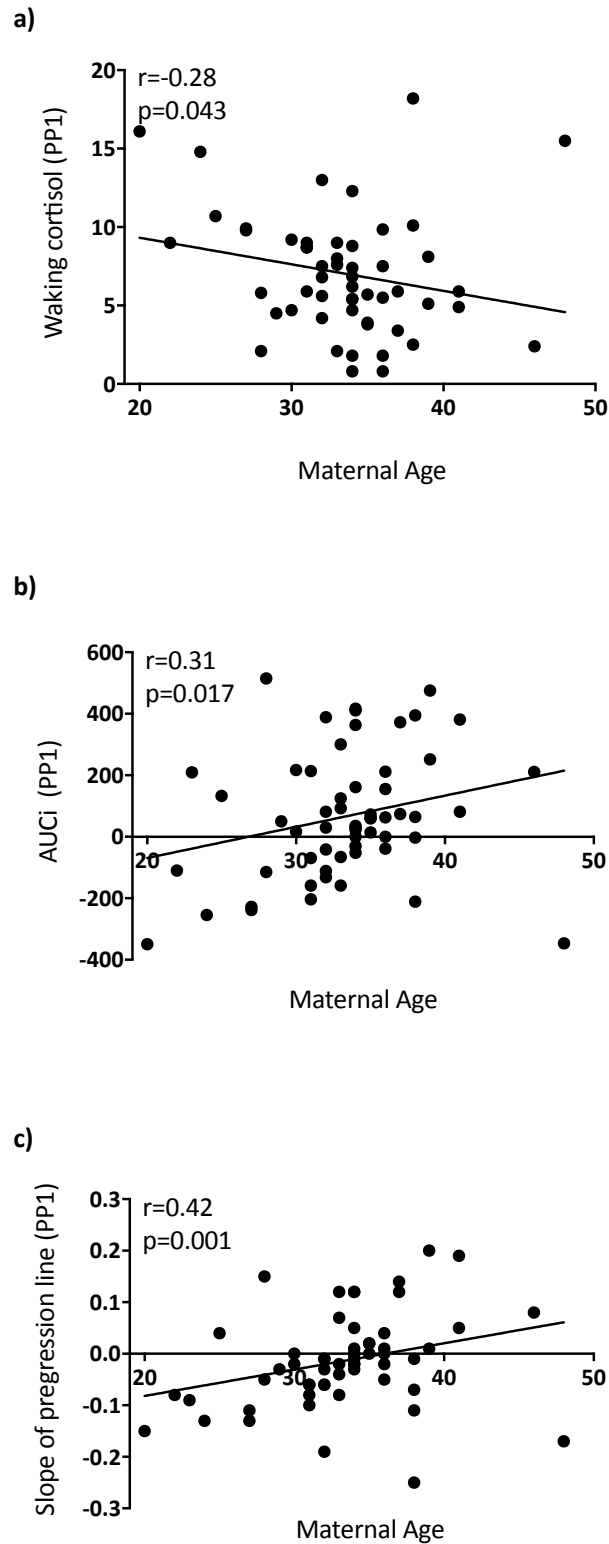


Figure 5.13 Correlations between maternal age and cortisol measures at two months postpartum (PP1) within the whole sample.

Spearman's rank-order correlation analysis was conducted to analyse association between maternal age and a) Waking cortisol, b) AUCi, and c) Slope of regression line at PP1. Threshold for significance was set at 0.05.

Table 5.13 Correlation analysis of maternal demographics and cortisol measures at two months postpartum (PP1)

		Age	Gravidity	Parity
Whole Sample	PP1 T0	-0.28*	0.58	0.64*
	PP1 T750	-0.05	-0.05	-0.01
	PP1 AUCg	0.03	0.12	0.22
	PP1 AUCi	0.31	0.23	0.19
	PP1 Intercept	-0.19	-0.20	0.04
	PP1 Slope	0.42**	0.21	0.14
Depressed	PP1 T0	-0.45	0.58	0.64*
	PP1 T750	-0.30	-0.14	-0.37
	PP1 AUCg	0.12	0.60*	0.53*
	PP1 AUCi	0.26	0.09	-0.05
	PP1 Intercept	-0.38	0.64	0.63*
	PP1 Slope	0.81**	0.09	-0.05
History	PP1 T0	-0.38	-0.25	-0.02
	PP1 T750	0.08	0.36	0.53
	PP1 AUCg	-0.28	0.05	0.27
	PP1 AUCi	0.29	0.43	0.38
	PP1 Intercept	-0.20	-0.07	0.06
	PP1 Slope	0.17	0.27	0.23
Control	PP1 T0	-0.05	-0.09	-0.14
	PP1 T750	-0.18	0.01	-0.15
	PP1 AUCg	0.21	-0.11	0.01
	PP1 AUCi	0.29	0.10	0.15
	PP1 Intercept	0.03	-0.23	-0.18
	PP1 Slope	0.28	0.16	0.12

Note: Spearman's correlation analysis was performed on maternal demographic and cortisol values within each group. Waking cortisol (T0), evening cortisol (T750), total cortisol output (AUCg, Intercept), and CAR (AUCi, slope) are displayed. Spearman's rho correlation coefficient (r) is presented in the table. Results were corrected for multiple comparisons. *p<0.05, **p<0.01

5.3.5 Maternal HPA axis associations at six months postpartum

5.3.5.1 Maternal demographic variables and cortisol measures at six months postpartum

Association analyses were undertaken to assess the relationship between maternal demographics of age, BMI, gravidity and parity, and maternal cortisol levels and CAR variables at six months postpartum in the whole sample [Figure 5.14]. Maternal age was found to have significant moderate positive correlations with AUCi ($r=0.32$, $p=0.036$), a measure of CAR. When the analysis was repeated within each caseness group, maternal age was significantly, and negatively, associated with waking cortisol level, and positively associated with AUCi within the History group [Table 5.14]. There were no significant correlations within the Depressed and Control groups.

5.3.5.2 Maternal clinical measures and cortisol parameters at six months postpartum

Correlation analyses were conducted to assess the association between maternal cortisol and CAR variables and PSS, PSQI, CES-D, and HAM-D scores at PP2. HAM-D scores were positively correlated with waking cortisol ($r=0.38$, $p=0.040$) and evening cortisol ($r=0.48$, $p=0.012$) at PP2. No significant associations with cortisol were observed in the other measures. When the groups were analysed separately, PSQI scores in the Depressed group were strongly positively correlated with waking cortisol ($r=0.76$, $p=0.046$) and Intercept ($r=0.90$, $p=0.015$) at PP2, indicating that women with poorer sleep quality had higher waking and total morning cortisol outputs. In the History group, CES-D scores were negatively correlated with AUCg ($r=-0.86$, $p=0.001$) at PP2, suggesting that women with lower perceived mood had lower morning cortisol production. There were no associations within the Control group.

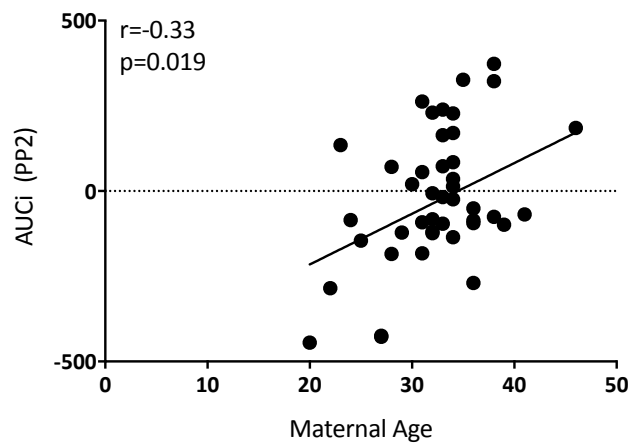


Figure 5.14 Whole sample correlation analysis of maternal age and cortisol responsivity at six months postpartum (PP2)

Spearman's rank-order analysis was conducted between maternal age and AUCi, a measure of CAR. Spearman's rho correlation coefficient and significance value are presented. Threshold for significance was set at 0.05.

Table 5.14 Correlation analysis of maternal demographics and cortisol measures at six months postpartum within the three groups

		Age	Gravidity	Parity
Depressed	PP2 T0	0.12	0.26	0.26
	PP2 T750	0.43	-0.20	-0.36
	PP2 AUCi	-0.38	-0.26	-0.26
	PP2 Intercept	-0.17	0.27	0.27
	PP2 Slope	-0.03	-0.22	-0.22
History	PP2 T0	-0.66	-0.08	0.18
	PP2 T750	0.13	-0.27	-0.22
	PP2 AUCi	0.84**	0.14	0.03
	PP2 Intercept	-0.10	-0.16	-0.11
	PP2 Slope	0.23	0.28	0.36
Control	PP2 T0	-0.09	-0.10	-0.20
	PP2 T750	0.33	0.39	0.29
	PP2 AUCi	0.09	0.09	-0.02
	PP2 Intercept	-0.11	-0.04	-0.21
	PP2 Slope	0.23	0.20	0.23

Note: Spearman's correlation analysis was performed on maternal demographic and cortisol values within each group. Waking cortisol (T0), evening cortisol (T750), total cortisol output (AUCg, Intercept), and CAR (AUCi, slope) are displayed. Spearman's rho correlation coefficient (r) is presented in the table. Results were corrected for multiple comparisons. *p<0.05, **p<0.01

5.3.6 Maternal early life adversity and the HPA axis postpartum

5.3.6.1 Maternal early life adversity and cortisol measures at two months postpartum

Differences were analysed in waking and evening cortisol values, measures of total cortisol output and the CAR between women with and without a history of childhood trauma (+ELA and -ELA respectively). Women with and without a history of each subtype of abuse were also compared. Women with a history of any abuse subtype during childhood had significantly higher evening cortisol levels compared to those who had no history of abuse [Table 5.15]. There were no significant differences in the separate domains of abuse measures.

Table 5.15 Effect of early life adversity (ELA) on cortisol levels (nMol/L) at two months postpartum (PP1)

		-ELA		+ELA		Statistics, p-value
		n	Mean (SD)	n	Mean (SD)	
PP1	Emotional Abuse	27	6.89 (3.56)	25	7.12 (6.84)	Z=0.14, p=0.891
T0	Physical Abuse	43	7.04 (3.97)	9	6.79 (3.84)	Z=0.13, p=0.905
	Sexual Abuse	46	7.10 (3.75)	6	6.23 (5.31)	Z=-0.86, p=0.408
	Emotional Neglect	36	7.17 (3.79)	16	6.61 (4.26)	Z=-0.56, p=0.579
	Physical Neglect	38	6.95 (3.76)	14	7.12 (4.43)	Z=0.11, p=0.910
	≥1 Trauma Domain	23	6.74 (3.35)	29	7.21 (4.35)	Z=0.06, p=0.956
PP1	Emotional Abuse	29	1.15 (1.47)	27	1.24 (0.81)	Z=1.92, p=0.055
T750	Physical Abuse	48	1.16 (1.21)	8	1.38 (1.10)	Z=0.78, p=0.845
	Sexual Abuse	48	1.18 (1.23)	8	1.23 (0.95)	Z=0.14, p=0.918
	Emotional Neglect	38	1.20 (1.34)	18	1.18 (0.80)	Z=0.40, p=0.686
	Physical Neglect	42	1.22 (1.33)	14	1.10 (0.62)	Z=0.29, p=0.765
	≥1 Trauma Domain	24	1.15 (1.61)	32	1.22 (0.76)	Z=2.35, p=0.019*

Note: A Mann-Whitney U test was conducted to assess differences in cortisol at waking (T0) and evening (T750). Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

5.3.6.2 Maternal early life adversity and cortisol measures at six months postpartum

Childhood emotional trauma was associated with higher waking and evening cortisol levels at six months postpartum, with significantly higher evening cortisol levels seen in women with a history of childhood emotional neglect [Table 5.16]. Women suffering from any abuse subtype during childhood also had significantly higher evening cortisol levels compared to those who had no history of abuse. Emotional neglect scores correlated positively with maternal evening cortisol at six months postpartum ($r=0.36$, $p=0.035$).

Table 5.16 Effect of early life adversity (ELA) on cortisol levels (nMol/L) at six months postpartum (PP2)

		-ELA		+ELA		Statistic, p-value
		n	Mean (SD)	n	Mean (SD)	
PP2	Emotional Abuse	22	8.49 (5.30)	18	9.53 (3.60)	Z=1.60, p=0.112
T0	Physical Abuse	35	8.95 (4.79)	5	8.98 (3.15)	Z=0.20, p=0.843
	Sexual Abuse	35	9.11 (4.68)	5	7.87 (4.13)	Z=-0.42, p=0.691
	Emotional Neglect	28	8.33 (5.15)	12	10.42 (2.50)	Z=2.08, p=0.036*
	Physical Neglect	30	8.62 (4.96)	10	9.96 (3.24)	Z=0.35, p=0.177
	≥1 Trauma Domain	19	8.85 (5.53)	21	9.06 (3.67)	Z=0.98, p=0.333
PP2	Emotional Abuse	22	1.28 (1.09)	16	1.21 (0.62)	Z=1.56, p=0.162
T750	Physical Abuse	33	1.26 (1.00)	5	1.25 (1.00)	Z=-0.69, p=0.557
	Sexual Abuse	34	1.25 (0.99)	4	1.36 (1.12)	Z=-0.34, p=0.766
	Emotional Neglect	27	0.98 (0.37)	11	1.96 (1.58)	Z=2.24, p=0.045*
	Physical Neglect	29	1.22 (1.16)	9	1.64 (2.95)	Z=0.21, p=0.866
	≥1 Trauma Domain	19	0.91 (0.35)	19	1.61 (1.28)	Z=2.42, p=0.030*

Note: A Mann-Whitney U-test was conducted to assess differences in cortisol, with waking (T0) and evening (T750) levels presented. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

5.4 Discussion

Maternal morning and evening cortisol levels had significantly decreased by two months postpartum, indicating a reduction in cortisol levels, as expected, from the hypercortisolaemia of pregnancy. There were no differences in maternal cortisol between the groups at two months postpartum. The reduction in morning and evening cortisol was maintained at six months postpartum in the whole sample, but not in the Depressed group. In fact, maternal waking cortisol and total cortisol production within the Depressed group was found to have significantly increased from two to six months postpartum. Of note, three women in the Depressed group had experienced a relapse of depression at six months postpartum. The Depressed group had higher peak cortisol and higher total morning cortisol output at six months postpartum, but the small sample size precluded further analyses to evaluate if these changes were driven by depression. Multiple associations were found between maternal cortisol measures during pregnancy and those at two and six months postpartum. Maternal age, gravidity, and parity had significant positive associations with maternal cortisol measures at two months postpartum, and only in the Depressed and History groups. Depression scores were also correlated with waking cortisol levels at six months postpartum, possibly indicating a return to non-pregnant HPA axis function, with changes that usually accompany the depressed state. Significantly, maternal history of ELA continued to exert an influence on maternal evening cortisol at both time points postpartum, although to a lesser extent than was evident during pregnancy.

5.4.1 Trajectory of the HPA axis postpartum

Although the HPA axis postpartum has been well studied in healthy pregnancies, little is known about the trajectory of the HPA axis postpartum in women who were depressed during pregnancy, or those with a history of depression who were euthymic during pregnancy. We sought to map the progression of the HPA axis in these women, comparing them to women who have never been depressed. A significant reduction was observed in waking and evening cortisol levels, as well as total cortisol output in the overall sample, and in each individual group, at two months postpartum. Although high cortisol levels would commonly be associated with a blunted CAR in the non-pregnant state, the dynamic of the CAR remained the same between pregnancy and postpartum time points, indicating that there was no alteration to the CAR even in the presence of

raised cortisol levels during pregnancy. In accordance with the study hypothesis, this replicates previous evidence that, despite an increase in overall cortisol levels, the CAR remains unaltered during pregnancy (de Weerth and Buitelaar, 2005a). The findings indicate that, by two months postpartum, the hypercortisolaemia of pregnancy had significantly decreased, corroborating the results from one study showing that cortisol returns to pre-pregnancy levels by the fifth week postpartum (Owens et al., 1987). Cortisol showed a modest decrease in one study by day 6 postpartum (O’Keane, Lightman, Patrick, et al., 2011), while findings from another study indicate that the return of cortisol to pre-pregnancy levels may take up to three months (Magiakou, Mastorakos, Rabin, Dubbert, et al., 1996).

Similarly, between pregnancy and six months postpartum, there was an overall reduction in waking and evening cortisol levels, and total cortisol production, with no differences in the CAR. When the caseness groups were analysed separately, the History and Control groups had lower cortisol levels and total cortisol production at six months postpartum, and there were no significant differences between pregnancy and six months postpartum in the Depressed group. This may be attributed to higher mean morning cortisol levels and higher total cortisol output observed within the Depressed group, where three women had experienced a relapse of depression. These findings will be discussed further in Section 5.4.3.

5.4.2 Maternal HPA axis measures throughout the perinatal period

An association between maternal cortisol measures during pregnancy and those in the postpartum period was evident in the whole sample, both at two months and six months postpartum. The associations were prevalent in the Depressed group at both time points. HPA axis associations between pregnancy and postpartum time points within the History and Control groups were less consistent. The stronger relationship between maternal pregnancy and postpartum cortisol measures in the Depressed group would suggest that women who are depressed during pregnancy, although not having significantly different cortisol levels to their non-depressed counterparts, have alterations in the HPA axis that are consistently present during pregnancy and postpartum. Importantly, these findings suggest that those who are depressed during pregnancy are vulnerable to developing depression in the postpartum period, in

accordance with the literature demonstrating increased risk for postpartum depression in women experiencing antenatal depression (O'Hara and Swain, 1996).

Maternal age, gravidity, and parity were associated with higher maternal cortisol levels postpartum, particularly in the Depressed group at two months postpartum. These findings indicate that lifetime depression may confer additional stress on the HPA axis in older mothers with more children. The absence of these associations at six months possibly indicates a dissipation of the difficulty in managing a newborn infant.

5.4.3 Effect of depression on postpartum HPA axis measures

There was no effect of caseness on maternal cortisol levels at two months postpartum, as would be expected since all participants were euthymic at this time point. However, as already mentioned, at six months postpartum there was a significant increase in peak cortisol and total morning cortisol output in the Depressed group, where three women had experienced a relapse. Further, maternal HAM-D scores were associated with waking and evening cortisol levels at this time point. It is well established that clinical depression leads to activation of the HPA axis in non-pregnant populations (Holsboer, 2000; Pariante and Lightman, 2008). However, this effect on the HPA axis was not apparent in the depressed cohort of the present study sample during pregnancy, possibly explained by the already physiologically high levels of cortisol during pregnancy. Upon return of the HPA axis to its non-pregnant state, the effect of depression on the HPA axis appears to have been re-established, leading to higher total cortisol levels. Studies of the HPA axis in postpartum depression report variable results, but those examining morning cortisol levels have reported that postpartum depression leads to higher waking cortisol levels and a blunted cortisol awakening response (Jolley et al., 2007; Taylor et al., 2009). However, in view of the small sample size of depressed women at six months postpartum in the current study, it can only be speculated, without being conclusive, that the HPA axis findings at this time point are an indication of the return to a non-pregnant HPA axis function, with changes usually seen in clinical depression. The absence of group differences in cortisone and the cortisone/cortisol ratio postpartum suggests that 11β -HSD2 does not play a role in the regulation of cortisol levels in postpartum depression. Despite existing literature indicating that antidepressants have a class-based effect on the HPA axis in non-pregnant adults,

medication use did not have an effect on cortisol findings in this study (Manthey et al., 2011; Vreeburg et al., 2009).

Women in the History group had lower, but not significantly so, evening cortisol measures compared to those in the Control group, both at two and six months postpartum. Although not significantly different, women in the History group demonstrated a trend for higher rates of childhood sexual abuse [See Chapter 3] compared to the Depressed and Control group. It may be speculated, in view of the finding of lower baseline cortisol levels in sexually abused girls and the enhanced negative feedback on the HPA axis seen in post-traumatic stress disorder (de Bellis et al., 1994; Shea et al., 2005), that the trend for lower cortisol levels in the History group in the present study may be attributable to childhood sexual abuse and hypoactive HPA axis responses to stress.

5.4.4 Maternal early life adversity and postpartum HPA axis measures

ELA continued to exert an influence on maternal cortisol levels at two months postpartum, with higher evening cortisol levels seen in women with a history of any subtype of trauma. The effect of any trauma subtype on maternal evening cortisol levels persisted at six months postpartum, with emotional neglect appearing to have a specific effect. Persistence of the relationship between ELA and maternal evening cortisol levels during pregnancy and postpartum indicates that the effect of ELA on cortisol measures is stable over time and resistant to the hypercortisolaemia of pregnancy, lending support to the study hypothesis. To my knowledge, this is the first study to have examined, and found a consistent association with, evening cortisol in the perinatal period and maternal ELA. Other studies have variously reported lower cortisol stress responses or higher waking cortisol levels and higher morning cortisol output in the postpartum period in women with a history of ELA (Brand et al., 2010; Gonzalez et al., 2009).

5.4.5 Conclusions

Overall, the findings from this study indicate that maternal total cortisol output declines from pregnancy to two months postpartum, with conservation of the CAR and diurnal cortisol rhythm throughout pregnancy. The absence of further changes in HPA axis measures from 2 to 6 months in the History and the Control groups suggests that the

HPA axis output has been restored to the non-pregnant state by 2 months postpartum. The HPA axis appears to be vulnerable to alterations from a number of factors in the postpartum period, including maternal depression and ELA, in addition to age, gravidity, and parity. With the precise aetiology of postpartum depression remaining largely unknown, we may postulate that these factors that influence the HPA axis postpartum may also increase a woman's vulnerability to developing postpartum depression. Consequently, it is important to recognise and address these vulnerabilities in the postpartum period, and offer increased support to women during this time.

Chapter 6

**The hypothalamic-pituitary-adrenal axis in
infants over the first six months of life**

6.1 Introduction

The foetal HPA axis plays a vital role in foetal development and maturation in the late stages of gestation, preparing it for birth, and is important for survival of the neonate postpartum. The structural components of the foetal HPA axis begin to develop during early gestation, and foetal HPA axis activity begins with maturation of the foetal pituitary gland (Mastorakos and Ilias, 2003; Perry et al., 1985). The foetal pituitary, in response to placental and foetal hypothalamic CRH, begins to produce ACTH, which is important for the development of the foetal adrenal cortex. The foetal adrenal gland primarily produces dehydroepiandrosterone-sulfate (DHEA-S), but begins *de novo* synthesis of cortisol around the 20th week of gestation (Partsch et al., 1991). Foetal stress responses are thought to be independent of maternal stress responses despite cortisol of maternal origin forming the majority of cortisol in foetal circulation (Gitau et al., 2001).

After birth, the neonatal HPA axis, through the secretion of adequate amounts of cortisol, is critical in adapting the neonate to the extrauterine environment. Although not fully mature postpartum, it is a functioning stress response system. Jansen and colleagues (2010) conducted a systematic review of studies examining infant cortisol stress responses to various stimuli at different ages up to 24 months and reported that infants had higher cortisol responses to stressors at younger ages, with the response decreasing with age, notably after six months. They found that physical stressors, whether mild or painful, were better at eliciting a stress response compared to psychological stressors such as separation. This finding was reiterated in a review which found that vaccination stressor elicited a higher mean increase in cortisol in infants up to six months of age, compared to psychological stressors (Gunnar et al., 2009). One study examined infant cortisol responses to a vaccination stressor at two and six months of age and found both pre- and post-vaccination levels to decrease with age, with no change in the cortisol stress response itself (Ramsay and Lewis, 1994). Although this reduction in cortisol levels was attributed to the increased ability for infants to self-soothe at six months, a further study by the same authors found no relationship between cortisol reactivity and infant behavioural regulation (Ramsay and Lewis, 2003).

The foetal programming hypothesis was first proposed by David Barker, whose early seminal studies demonstrated an association between foetal undernutrition *in utero* and cardiovascular disease later in life (Barker et al., 1989; Barker and Osmond, 1986). He later postulated that, in the presence of intrauterine stressors, the foetus becomes programmed to respond to stress in a certain way to overcome immediate risks, and carries these adaptations into the postpartum environment, leading to an increased risk of cardiometabolic disease (Barker, 1995b; Barker et al., 2002). Foetal programming is thought to confer an evolutionary advantage *in utero* by allowing the foetus to survive a stressful maternal environment to reach the end of gestation.

There is strong evidence in the literature for an association between antenatal depression and increased risk of preterm birth and low birth weight (<2.5kg) (Grote et al., 2010; Liu et al., 2016; O'Keane, Lightman, Marsh, et al., 2011; Staneva et al., 2015; Venkatesh et al., 2016). Low gestational age at birth, associated with poorer infant outcomes, may be considered to be an early effect of developmental programming (Boyle et al., 2012). The length of gestation is thought to be determined early in pregnancy by the 'placental clock', which is influenced by placental CRH (Challis, 1998; McLean et al., 1995; Smith, 1998). High levels of placental CRH are evident in antenatal depression, and high levels in the second trimester have been shown to increase the risk of preterm birth (Challis, 1998; O'Keane, Lightman, Marsh, et al., 2011; Smith and Nicholson, 2007). Additionally, there is clear evidence for an increased prevalence rate of preterm birth in males (Di Renzo et al., 2007; Verburg et al., 2016; Zeitlin et al., 2004). The mechanisms for this sexual dimorphism in foetal programming are unclear but sexually dimorphic differences in foetal development and the role of the placenta in obstetric complications may play role (Clifton, 2010).

Considering the known effects of depression on HPA axis output, the foetal programming hypothesis has been extended to investigate the effects of depression during pregnancy on offspring HPA axis. Most published studies to date have investigated the effects of depressive symptoms during pregnancy on offspring HPA axis and, of these, the majority are studies investigating young children or adolescents. Only two studies have examined the effect of clinically diagnosed maternal depression during pregnancy on infant HPA axis. In one large sample size study, Field et al (2010) compared infants of healthy control women to those born to women with Structured

Clinical Interview for DSM-IV-TR (SCID) diagnoses of depression alone, anxiety alone, or a diagnosis of comorbid depression and anxiety, using neonatal urinary cortisol 1-2 days postpartum as a measure. Cortisol was found to be higher in neonates born to women with depression alone and those with comorbid depression and anxiety (Field et al., 2010). Another study examined six-month-old infants and maternal depression diagnosed retrospectively using structured clinical interviews. A maternal history of retrospectively diagnosed perinatal depression was associated with higher cortisol reactivity in the infants and a lifetime history of maternal depression was associated with higher baseline and mean cortisol levels in infants (Brennan et al., 2008).

Other studies have examined the effect of psychiatric rating scale-defined depression, but not MDD, in infants up to six months of age. An epigenetics study found a relationship between maternal Edinburgh Postnatal Depression Scale (EPDS) scores during pregnancy and methylation of the GR gene in infants, which was further associated with an increased cortisol stress response at three months (TF Oberlander, Weinberg, et al., 2008). Other studies of neonatal urine cortisol levels have found higher levels in neonates born to women with high self-rated depression scores during pregnancy (Diego et al., 2004; Lundy et al., 1999).

Most studies on the effects of maternal *postpartum* depression on the offspring HPA axis have also been conducted on older children and adolescents. Feldman and colleagues (2009) investigated the effects of postpartum depressive symptoms on one-month-old infants and found that infants of mothers with high scores on depression rating scales had higher cortisol reactivity compared to those with mothers with lower depression scores (Feldman et al., 2009). Another study examining infant cortisol levels at birth, four, and 36 months, reported that infants with mothers later diagnosed with postpartum depression had higher cortisol levels at birth, but this effect did not persist at four or 36 months (Lucci et al., 2016).

There is a paucity of research into the intergenerational effects of maternal ELA on the offspring. Findings from the only study investigating this suggest that infants of mothers with a history of childhood trauma have lower baseline cortisol levels compared to those born to mothers with no history of trauma (Brand et al., 2010).

6.1.1 Study aims

Although there is some evidence for infant HPA axis programming consequent to maternal depressive symptoms during pregnancy, there is little data on the effects of operationally defined antenatal depression on infant HPA axis. The main aim of this study was to investigate whether a relationship exists between maternal antenatal depression and the infant HPA axis. Additionally, we sought to explore the relationship between maternal cortisol measures during the perinatal period and infant cortisol levels at two and six months. We then investigated the effects of maternal ELA on infant HPA axis. The trajectory of the infant HPA axis over the first six months of life was also outlined.

6.1.2 Study hypotheses

- Infants born to women who were depressed during pregnancy will have higher baseline infant cortisol levels and higher vaccination stress responses at two and six months after birth.
- There will be an association between maternal clinical and cortisol measures during pregnancy and infant cortisol measures at two and six months postpartum.
- There will be an association between infant cortisol measures and concurrent maternal clinical and cortisol measures at two and six months postpartum.
- Maternal ELA will be associated with higher infant HPA axis measures at two and six months.

6.2 Methods

6.2.1 Recruitment

All mothers in the study were recruited from three maternity hospitals– the National Maternity Hospital, Dublin; the Coombe Women and Infants University Hospital, Dublin; and The Rotunda Hospital, Dublin. All study participants underwent a diagnostic clinical interview, and were placed into the Depressed group if they fulfilled DSM-5 criteria for MDD, the History group if they were euthymic during pregnancy but had a prior history of depression, and the Control group if they had never been depressed. Infants of participants were categorised into the same groups as their mothers, and followed up at two months and six months of age. Information on infant gestational age at birth, birth weight, head circumference, and Apgar scores at 1 min and 5 min were collected.

6.2.2 Saliva samples

Saliva samples were collected from infants at two months and six months of age. Researchers attended the infant's vaccination appointment at the two time points, and collected saliva samples before the vaccination for the baseline value, and 30 min after the vaccination to assess the cortisol stress response to vaccination. The SalivaBio Infants' Swab kit (Salimetrics) was used for saliva collection. This kit included a long absorbent swab allowing the researcher to hold one end, thus reducing choking hazard, and a plastic tube for swab transportation. The swabs were then centrifuged for 10 minutes at 3000 rpm at room temperature, after which saliva was divided into microtubes of 100µL aliquots and stored at -80°C.

6.2.3 Cortisol analysis

Saliva samples were transported to the University Hospital of South Manchester, where they were analysed for cortisol and cortisone levels by Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) in Professor Brian Keevil's laboratory. LC-MS/MS is thought to have improved sensitivity and specificity for hormone concentrations than standard immunoassay methods [See Section 2.6.2] (Keevil, 2013; Rauh, 2010).

6.2.4 Cortisol measures

Baseline (pre-vaccination) and post-vaccination cortisol levels in infants are reported for two and six months. Mean infant cortisol and cortisol reactivity in response to a vaccination stressor are also described. In mothers, diurnal cortisol levels were measured. AUC_g and the intercept of the regression line fitted through raw cortisol values, both measures of total morning cortisol output are reported. AUC_i and the slope of the regression line fitted through raw cortisol values, which are both measures of the CAR, are also described.

6.2.5 Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics for Macintosh, Version 24 (REF). Data was assessed for normality using the Kolmogorov-Smirnov test. Raw cortisol values were log transformed to approximate a normal distribution. In order to account for values of zero, which does not have a logarithm, the constant 1 was added to all values prior to transformation. Extreme high values were removed from analysis. Outliers found after log transformation were also removed from analyses to strengthen association analyses. Data that followed a normal distribution was analysed using the independent-samples t-test or one-way analysis of variance, with Tukey's test used for post-hoc analysis. Non-normally distributed data was analysed using the non-parametric Mann-Whitney U test or the Kruskal-Wallis test. Univariate general linear model analysis for covariance was undertaken to control for covariates, including infant gestational age at birth, birth weight, and infant feeding prior to saliva sample collection. Correlation analysis was conducted using Pearson's product-moment correlation for normally distributed data, or Spearman's rank-order analysis for non-parametric data. Multiple comparisons were corrected for a false discovery rate using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995).

Hierarchical regression modelling was conducted to determine the predictive value of maternal cortisol measures during pregnancy and at six months postpartum on infant cortisol levels at six months. A table is presented for each model, and the variables

added to the previous model are shown in the Variables column. R^2 value, a measure of variation in the dependent variable explained by the independent variables, and R^2 change, representing the change in the R^2 value from the previous model, are also presented. The F statistic is shown, as well as the change in F with each model. In continuous variables, the unstandardised coefficient (B) indicates a change in the dependent variable for a one-unit change in the value of the independent variable. β represents the standardised coefficient, where the values of all the variables have been converted to the same scale for easier comparison. Threshold for significance was set at 0.05 for all analyses.

6.3 Results

In the Results section, the characteristics of the sample will be discussed in **Section 6.3.1**, birth outcomes in **Section 6.3.2**, infant cortisol measures at two months and their association with maternal measures in **Section 6.3.3**, infant cortisol measures at six months and their association with maternal measures in **Section 6.3.4**, and the trajectory of change in infant cortisol levels in **Section 6.3.5**.

6.3.1 Characteristics of sample

Six women dropped out of the study after pregnancy, and one was excluded as the infant was diagnosed with Apert's syndrome, a congenital disorder [Figure 6.1]. A total of 58 infant saliva samples were analysed for cortisol at two months, of whom 14 infants were born to women in the Depressed group, 18 to the History group, and 26 to the Control group. At the six month time point, 11 infants remained in the Depressed group, 12 in the History group, and 22 in the Control group [Figure 6.1]. The number of infants at each time point indicates the number of infants who have reached each time point, and does not denote attrition rate.

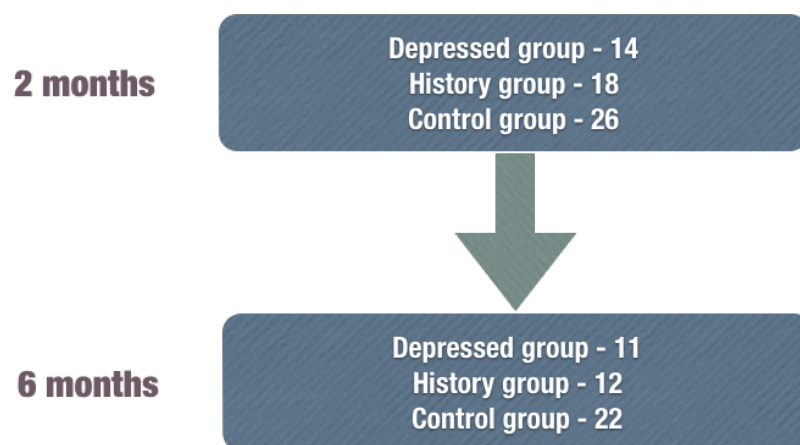


Figure 6.1 Number of infants at each study time point

6.3.2 Infant birth outcomes

Infant gestational age at birth, birth weight, head circumference, and Apgar scores at 1 min and 5 min were compared between the three caseness groups [Table 6.1]. Group differences were observed in gestational age at birth and infant birth weight in the whole sample analysis. Infants in the Depressed group had lower gestational age at birth compared to both the Control group ($p=0.002$) and the History group ($p=0.012$). Infants in the Depressed group also had lower birth weight than those in the History group ($p=0.040$). However, birth weight for gestational age was in the 99th percentile for all infants in the study.

Male and female infants were then analysed separately [Table 6.1]. Male infants in the Depressed group had significantly lower gestational age at birth compared to male infants in History ($p=0.039$) or Control ($p<0.001$) groups, while there were no differences among groups in female infants.

Association analyses revealed no significant correlations between maternal cortisol measures during pregnancy and infant birth outcomes. There were no observed associations between birth outcomes and infant cortisol measures at two or six months.

Table 6.1 Infant birth outcomes

		Depressed Mean (SD) (n=22)	History Mean (SD) (n=28)	Control Mean (SD) (n=45)	Statistic, p-value
Whole sample	Gestational age at birth	38.87 (1.44)	39.59 (1.26)	40.20 (2.26)	F=3.48, p=0.035*
	Birth weight	3.40 (0.45)	3.86 (5.42)	3.57 (0.62)	F=3.48, p=0.036*
	Head circumference	34.08 (1.22)	35.18 (0.89)	33.80 (6.38)	F=0.38, p=0.689
	Apgar score				
	1 min	8.60 (0.83)	8.54 (1.05)	8.81 (0.90)	F=0.60, p=0.551
	5 min	8.80 (0.77)	8.95 (0.38)	9.06 (0.25)	F=1.78, p=0.177
Females	Gestational age at birth	39.36 (1.14)	39.58 (1.26)	39.32 (3.50)	F=0.04, p=0.964
	Birth weight	3.39 (0.29)	3.89 (0.77)	3.37 (0.84)	F=1.30, p=0.292
	Head circumference	35.55 (0.97)	35.67 (1.27)	34.78 (1.25)	F=1.04, p=0.377
	Apgar score				
	1 min	8.57 (1.13)	8.67 (0.51)	8.58 (1.44)	F=0.01, p=0.988
	5 min	8.57 (1.13)	9.00 (0.00)	9.08 (0.29)	F=1.54, p=0.236
Males	Gestational age at birth	38.38 (1.61)	39.69 (1.34)	40.63 (1.11)	F=11.60, p<0.001*
	Birth weight	3.44 (0.62)	3.87 (0.45)	3.67 (0.45)	F=1.95, p=0.156
	Head circumference	33.62 (1.45)	34.96 (0.67)	32.93 (8.84)	F=0.25, p=0.784
	Apgar score				
	1 min	8.57 (0.53)	8.42 (1.28)	8.94 (0.23)	F=1.78, p=0.184
	5 min	9.00 (0.00)	8.92 (0.47)	9.05 (0.23)	F=0.59, p=0.558

Note: Mean infant gestational age at birth (weeks), birth weight (kg), head circumference (cm), and Apgar scores at 1 min and 5 min were compared across the caseness groups of pregnancy in the whole group and for males and females with a one-way ANOVA. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

6.3.3 Infant cortisol measures at two months

6.3.3.1 Infant cortisol stress response at two months

Pre- and post-vaccination infant cortisol values at two months (B1) were compared and there were significantly higher post-vaccination values in the whole sample, indicating a cortisol stress response to the vaccination [Table 6.2]. When groups were analysed separately, this result was found to be present in the Depressed group but not significantly so in the History and Control groups, although there was a trend for increased post-stressor cortisol levels in infants in the Control group.

Infant cortisol measures were analysed for differences across the maternal caseness groups [Table 6.3]. Figure 6.2 depicts infant cortisol values pre- and post-vaccination stressor. There were no differences among the three groups in any of the variables.

Table 6.2 Mean differences in infant pre- and post-stressor cortisol levels at two months (B1)

	B1 T0	B1 T30	Statistic, p-value
	Mean (SD)	Mean (SD)	
Whole sample	5.00 (5.44)	7.87 (4.42)	t=-3.03, p=0.004*
Depressed	3.16 (2.98)	9.05 (4.62)	t=-2.76, p=0.024*
History	7.04 (7.75)	6.93 (2.57)	t=-0.74, p=0.476
Control	4.60 (4.15)	7.90 (5.10)	t=-1.96, p=0.064

Note: Infant cortisol levels measured pre- (T0) and post-vaccination (T30) stressor were compared using a paired-samples t-test. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

Table 6.3 Infant cortisol stress response at two months (B1)

	Depressed (n=14)	History (n=18)	Control (n=26)	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
B1 T0	3.16 (2.98)	7.04 (7.75)	4.60 (4.15)	F=1.38, p=0.261
B1 T30	9.05 (4.62)	6.93 (2.57)	7.90 (5.10)	F=0.49, p=0.614
B1 Mean	4.82 (3.26)	6.77 (5.00)	6.00 (3.23)	F=1.22, p=0.308
B1 RT	2.65 (5.93)	-2.01 (8.14)	2.70 (6.90)	F=1.30, p=0.285

Note: Infant cortisol levels measured pre- (T0) and post-vaccination (T30) stressor, mean infant cortisol and cortisol reactivity (RT) were log-transformed and their means compared across the caseness groups of pregnancy with a one-way ANOVA. Threshold for significance was set at 0.05.

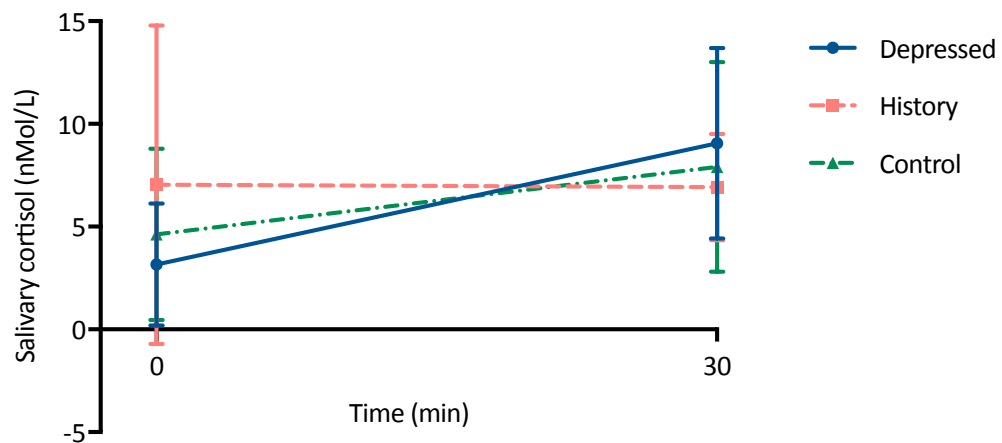


Figure 6.2 Infant cortisol response to a vaccination stressor

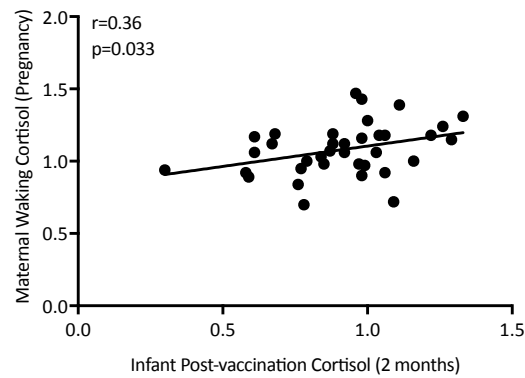
Infant cortisol levels pre- (0 min) and post-vaccination (30 min) within each caseness group of pregnancy are presented in the graph. There were no significant differences between groups.

6.3.3.2 Infant cortisol measures at two months and maternal cortisol measures during pregnancy

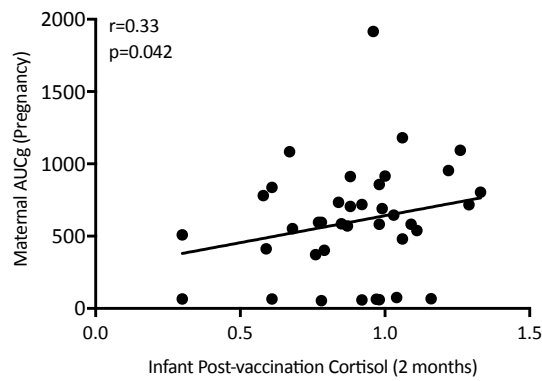
Correlation analysis was conducted in the whole sample between infant cortisol measures at B1 and maternal cortisol measures during pregnancy [Table 6.4]. Infant B1 post-stressor cortisol levels were significantly positively correlated with maternal cortisol measures during pregnancy in the whole sample, including waking cortisol ($r=0.36$, $p=0.033$), total cortisol output, and intercept ($r=0.39$, $p=0.018$) [Figure 6.3].

Infants within each maternal caseness group of pregnancy were then analysed separately [Table 6.4]. In the Depressed group, mean B1 infant cortisol was very strongly positively correlated with maternal waking cortisol during pregnancy, as well as measures of total morning cortisol secretion (AUCg and intercept), and CAR (AUCi). In the Control group, infant post-vaccination cortisol levels were strongly correlated with maternal waking cortisol. There were no significant correlations within the History group.

a)



b)



c)

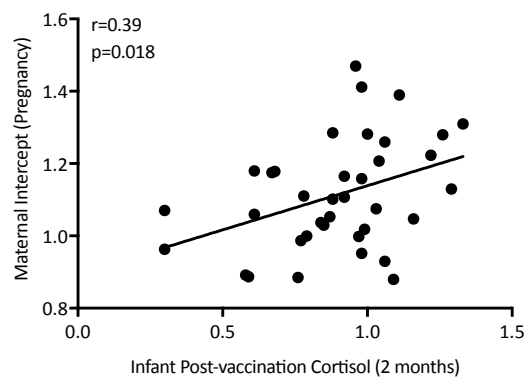


Figure 6.3 Associations between infant post-vaccination cortisol at two months and maternal cortisol measures during pregnancy in the whole sample

Pearson's correlation analysis was conducted between infant cortisol at two months and maternal pregnancy cortisol values. The significant relationships are presented, with Pearson's correlation coefficient (r) and p -values reported. Threshold for significance was set at 0.05.

Table 6.4 Correlation analysis of infant cortisol measures at two months (B1) and maternal pregnancy cortisol measures (P)

		B1 T0	B1 T30	B1 Mean	B1 RT
Whole Sample P: n=76 B1: n=39	P T0	-0.13	0.36*	0.13	0.28
	P T750	-0.16	-0.10	-0.18	0.09
	P AUCg	-0.17	0.33*	0.06	0.32
	P AUCi	0.17	-0.18	0.07	0.01
	P Int	-0.12	0.39*	0.25	0.22
	P Slope	-0.10	-0.15	-0.22	-0.05
Depressed P: n=16 B1: n=7	P T0	0.08	0.34	0.88*	0.62
	P T750	-0.29	0.14	-0.58	-0.39
	P AUCg	0.32	0.54	0.94**	0.68
	P AUCi	0.52	0.04	0.83*	0.61
	P Int	0.13	0.31	0.89*	0.62
	P Slope	-0.08	0.16	-0.73	-0.40
History P: n=23 B1: n=11	P T0	-0.22	0.03	-0.27	0.45
	P T750	-0.27	-0.54	-0.24	0.47
	P AUCg	-0.42	0.20	-0.55	0.52
	P AUCi	0.02	0.10	-0.07	-0.01
	P Int	-0.01	0.07	-0.07	0.48
	P Slope	-0.21	0.41	-0.23	-0.13
Control P: n=40 B1: n=21	P T0	-0.23	0.58**	0.14	0.01
	P T750	0.07	0.13	0.06	-0.11
	P AUCg	-0.09	0.38	0.11	-0.16
	P AUCi	0.25	-0.39	-0.01	-0.11
	P Int	-0.22	0.53	0.17	-0.02
	P Slope	-0.02	-0.34	-0.19	0.01

AUCg – area under the curve with respect to ground, **AUCi** – area under the curve with respect to increase, **Intercept** – intercept of regression line, **Slope** – slope of regression line fitted through raw cortisol values.

Note: Infant cortisol levels pre-vaccination (B1 T0) and post-vaccination (B1 T30), mean infant cortisol and reactivity (RT) at six months were correlated with maternal cortisol at waking (T0), and evening cortisol levels (T750), as well as cortisol awakening parameters during pregnancy (P). Pearson's correlation coefficients (r) for the variables are displayed in the table above. Results were corrected for multiple comparisons. *p<0.05 **p<0.01

6.3.3.3 Infant cortisol measures at two months and maternal cortisol measures at two months postpartum

Association analyses conducted between infant cortisol measures at two months and concurrent maternal cortisol measures found no significant associations.

6.3.3.4 Infant cortisol measures at two months and maternal rating scales

Association analyses were conducted between infant cortisol measures and maternal pregnancy clinical measures including the Perceived Stress Scale (PSS), Pittsburgh Sleep Quality Index (PSQI), Centre for Epidemiological Studies Depression rating scale (CES-D) and the Hamilton Depression rating scale (HAM-D). There were no significant relationships in this analysis. The analysis was repeated for infant cortisol values at B1 and concurrent maternal clinical measures. Post-stressor infant cortisol showed a moderate positive correlation with maternal PSQI score at two months postpartum ($r=0.36$, $p=0.027$).

6.3.3.5 Maternal early life adversity and infant cortisol measures at two months

Infant cortisol measures were analysed for differences at two months between two groups – infants of mothers with a history of ELA and infants of mothers with no history of ELA. There were no significant differences between the groups.

Association analyses were performed to assess the relationship between maternal Childhood Trauma Questionnaire (CTQ) scores and infant cortisol measures at two months. CTQ scores were analysed within each trauma domain of Emotional Abuse, Physical Abuse, Sexual Abuse, Emotional Neglect, and Physical Neglect. There were no significant associations in this analysis.

6.3.4 Infant cortisol measures at six months

6.3.4.1 Infant cortisol stress response at six months

Pre- and post-vaccination cortisol values were analysed for differences at six months (B2). Significantly higher post-vaccination cortisol levels were observed when the whole sample was analysed, indicating a cortisol stress response [Table 6.5]. When the analysis was repeated in each group, higher post-vaccination cortisol levels were only found in the Control group. There were no differences between pre- and post-vaccination cortisol levels in the Depressed and History groups.

Infant cortisol measures at B2 were analysed for differences across the caseness groups [Table 6.6, Figure 6.4]. Analysis conducted on log-transformed values showed significant groups differences in infant baseline cortisol, post-vaccination cortisol, and mean cortisol. Post-hoc analysis found that infants in the Depressed group had significantly higher baseline cortisol levels compared to the Control group ($p=0.010$); higher post-stressor cortisol levels compared to the Control ($p=0.022$) and History ($p=0.001$) groups; and higher mean cortisol compared to the Control ($p<0.001$) and History ($p=0.001$) groups.

Table 6.5 Mean differences in infant pre- and post-stressor cortisol levels at six months (B2)

	B2 T0	B2 T30	Statistic, p-value
	Mean (SD)	Mean (SD)	
Whole sample	4.96 (9.15)	6.65 (6.00)	$t=-3.55, p=0.001^*$
Depressed	11.52 (16.34)	12.13 (8.58)	$t=-1.37, p=0.201$
History	3.40 (3.15)	3.46 (2.12)	$t=-0.67, p=0.516$
Control	3.10 (4.28)	5.50 (3.76)	$t=-4.77, p<0.001^*$

Note: Infant cortisol levels measured pre- (T0) and post-vaccination (T30) stressor were compared using a paired-samples t-test. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

Table 6.6 Infant cortisol stress response at six months (B2)

	Depressed (n=11)	History (n=12)	Control (n=20)	Statistic, p-value
	Mean (SD)	Mean (SD)	Mean (SD)	
B2 T0	11.52 (16.34)	3.40 (3.15)	3.10 (4.28)	F=4.92, p=0.012*
B2 T30	12.13 (8.58)	3.46 (2.12)	5.50 (3.76)	F=7.53, p=0.002*
B2 Mean	11.84 (10.36)	3.50 (2.06)	4.70 (3.74)	F=11.90, p<0.001*
B2 RT	0.61 (15.88)	-0.23 (3.64)	2.70 (6.17)	F=1.21, p=0.315

Note: Infant salivary cortisol levels obtained pre- (T0) and 30 min after a vaccination stressor (T30), mean infant cortisol and cortisol reactivity (RT) are presented in the table. A one-way ANOVA was conducted on log-transformed values to analyse differences in means between the groups. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

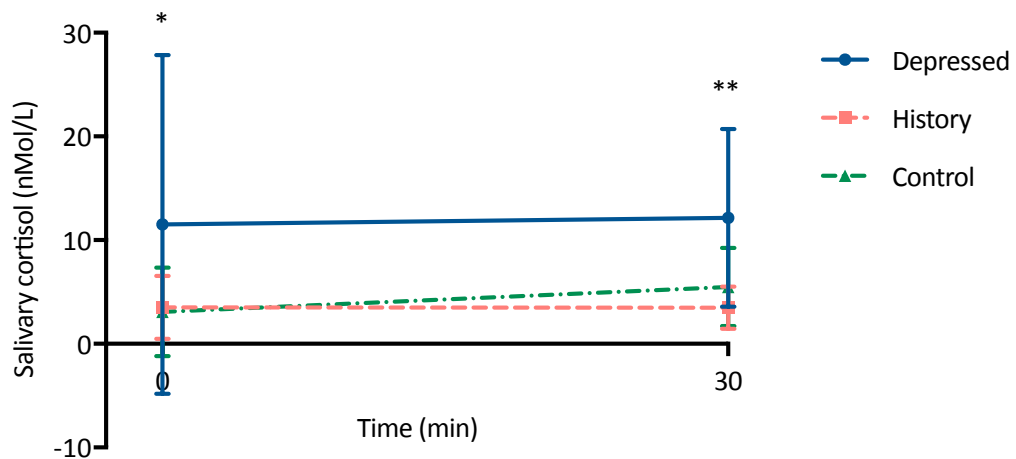


Figure 6.4 Infant cortisol response to a vaccination stressor at six months

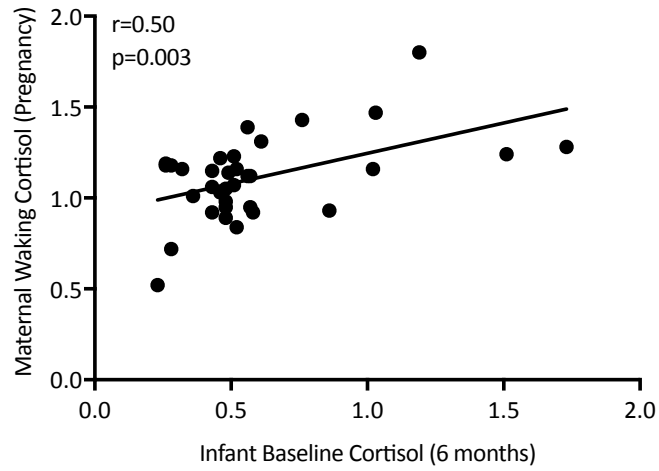
Infant cortisol stress response pre- (0 min) and post-vaccination (30 min) within each caseness group of pregnancy is presented. *p<0.05 **p<0.01

6.3.4.2 Infant cortisol measures at six months and maternal cortisol measures during pregnancy

Correlation analysis was conducted between infant cortisol measures at six months and maternal pregnancy cortisol measures [Table 6.7]. Infant baseline cortisol was significantly and positively associated with maternal pregnancy waking cortisol, as well as the intercept of the regression line [Figure 6.5]. Infant cortisol reactivity had a significant and negative relationship with maternal waking cortisol and intercept [Figure 6.6].

Infants in each caseness group were then analysed separately [Table 6.7]. At six months, only infants born to mothers who were depressed during pregnancy had very strong positive correlations with maternal cortisol levels during pregnancy. Infant baseline cortisol was very strongly associated with maternal intercept of regression line during pregnancy in the Depressed group.

a)



b)

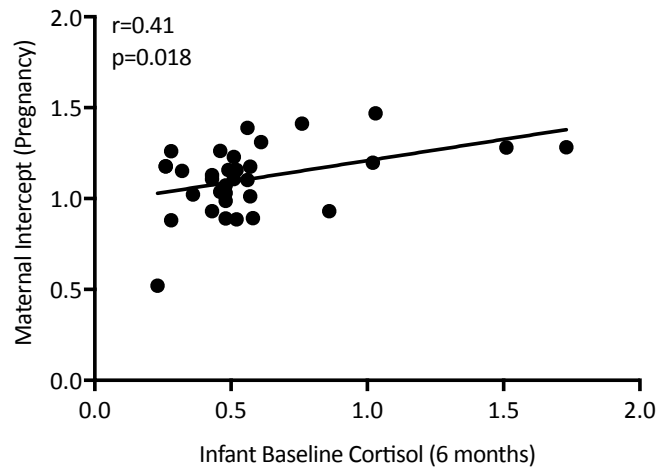
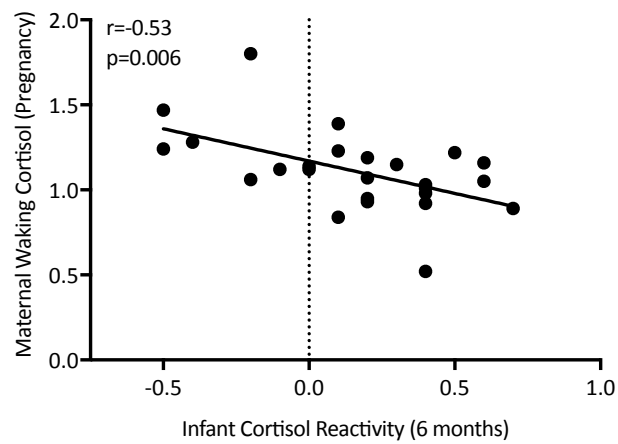


Figure 6.5 Association between infant baseline cortisol at six months and maternal cortisol measures during pregnancy

Pearson's correlation analysis of infant baseline cortisol at six months with a) maternal waking cortisol and b) maternal intercept of regression line fitted through raw cortisol values of pregnancy. Threshold for significance was set at 0.05.

a)



b)

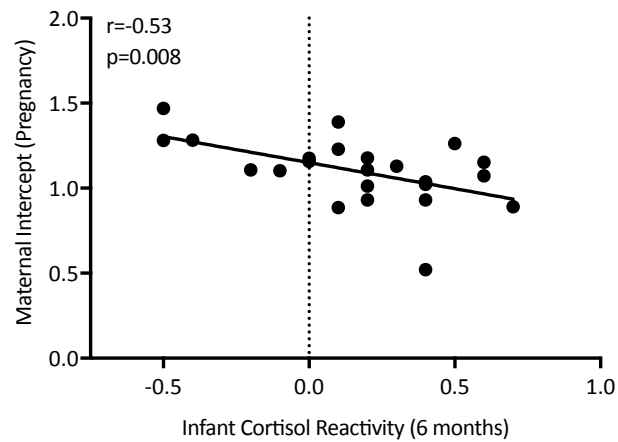


Figure 6.6 Association between infant cortisol reactivity at six months and maternal cortisol during pregnancy

Pearson's correlation analysis of infant cortisol reactivity at six months with a) maternal waking cortisol and b) maternal intercept of regression line fitted through raw cortisol values of pregnancy. Threshold for significance was set at 0.05.

Table 6.7 Correlation analysis of infant cortisol measures at six months (B2) and maternal pregnancy cortisol measures (P)

		B2 T0	B2 T30	B2 Mean	B2 RT
Whole Sample M1: n=76 B2: n=39	P T0	0.50*	0.08	0.36	-0.53*
	P T750	0.09	-0.03	-0.02	-0.20
	P AUCg	0.07	0.11	0.12	-0.12
	P AUCi	-0.03	0.02	0.17	0.06
	P Int	0.41*	-0.03	0.24	-0.53**
	P Slope	-0.11	0.04	0.07	0.05
Depressed M1: n=16 B2: n=7	P T0	0.71	0.34	0.67	-0.67
	P T750	-0.03	-0.17	-0.23	-0.14
	P AUCg	0.66	0.74	0.89	-0.64
	P AUCi	0.26	0.17	0.27	-0.34
	P Int	0.90*	0.68	0.95	-0.80
	P Slope	-0.78	-0.46	-0.83	0.63
History M1: n=23 B2: n=11	P T0	0.56	0.43	0.67	-0.49
	P T750	-0.05	-0.50	-0.41	-0.68
	P AUCg	-0.27	0.25	-0.34	0.77
	P AUCi	-0.50	-0.18	-0.50	-0.66
	P Int	0.59	0.46	0.73	-0.48
	P Slope	-0.27	-0.29	-0.20	-0.21
Control M1: n=40 B2: n=21	P T0	0.18	-0.19	-0.35	-0.25
	P T750	0.27	0.15	0.04	0.06
	P AUCg	0.15	0.04	0.12	0.01
	P AUCi	-0.01	0.15	0.29	0.15
	P Int	0.21	-0.17	-0.29	-0.29
	P Slope	0.25	-0.18	0.26	-0.09

AUCg – area under the curve with respect to ground, **AUCi** – area under the curve with respect to increase, **RT** – reactivity, **Intercept** – intercept of regression line fitted through raw cortisol values, **Slope** – slope of regression line fitted through raw cortisol values.

Note: Infant cortisol levels pre-vaccination (B2 T0) and post-vaccination (B2 T30), mean infant cortisol and reactivity at six months were analysed with maternal cortisol at waking (T0), and evening cortisol levels (T750), as well as cortisol awakening parameters during pregnancy (P). Pearson's correlation coefficients (r) for the analyses are displayed in the table above. Results were corrected for multiple comparisons. *p<0.05 **p<0.01

6.3.4.3 Infant cortisol measures at six months and maternal cortisol measures at six months postpartum

Correlation analysis conducted between infant cortisol at six months and concurrent maternal cortisol at six months postpartum revealed multiple significant correlations in the whole sample [Table 6.8]. Maternal waking cortisol was positively correlated with infant baseline and post-stressor cortisol. Maternal CAR was negatively correlated with infant baseline cortisol and mean cortisol.

When the groups were analysed separately, all the significant values were within the Depressed group [Table 6.8]. Results indicate that higher maternal waking cortisol and higher total morning cortisol output (intercept) at six months postpartum were associated with higher infant baseline cortisol levels in the Depressed group. Maternal waking cortisol was highly correlated with mean infant cortisol. There were no significant associations in the History and Control groups.

Table 6.8 Correlation analysis of infant cortisol measures at six months (B2) and maternal cortisol measures at six months postpartum (PP2)

		B2 T0	B2 T30	B2 Mean	B2 RT
Whole Sample M1: n=76 B2: n=39	PP2 T0	0.51**	0.40*	0.48	-0.37
	PP2 T750	0.11	0.20	0.08	-0.18
	PP2 AUCg	0.00	0.08	-0.11	0.01
	PP2 AUCi	-0.53**	-0.30	-0.59**	0.36
	PP2 Int	0.43	0.34	0.46	-0.34
	PP2 Slope	-0.39	-0.36	-0.60**	0.19
Depressed M1: n=16 B2: n=7	PP2 T0	0.85**	0.72	0.87*	-0.76
	PP2 T750	0.03	0.63	0.47	0.04
	PP2 AUCg	-0.20	0.10	-0.13	0.44
	PP2 AUCi	-0.67	-0.31	-0.56	0.80
	PP2 Int	0.97**	0.69	0.97	-0.99
	PP2 Slope	-0.89	-0.69	-0.94	0.94
History M1: n=23 B2: n=11	PP2 T0	0.62	0.10	0.08	-0.79
	PP2 T750	0.11	-0.19	-0.46	-0.42
	PP2 AUCg	0.22	-0.12	-0.75	-0.36
	PP2 AUCi	-0.51	-0.05	-0.78	0.24
	PP2 Int	0.28	0.11	-0.07	-0.77
	PP2 Slope	-0.29	-0.28	-0.78	0.29
Control M1: n=40 B2: n=21	PP2 T0	-0.20	0.27	0.12	0.25
	PP2 T750	0.44	0.27	-0.37	-0.56
	PP2 AUCg	-0.07	0.00	-0.12	-0.02
	PP2 AUCi	0.18	-0.41	-0.43	-0.42
	PP2 Int	-0.10	0.20	0.07	0.14
	PP2 Slope	-0.01	-0.38	-0.47	-0.31

AUCg – area under the curve with respect to ground, **AUCi** – area under the curve with respect to increase, **RT** – reactivity, **Intercept** – intercept of regression line, **Slope** – slope of regression line fitted through raw cortisol values.

Note: Infant cortisol levels pre-vaccination (B2 T0) and post-vaccination (B2 T30), mean infant cortisol and reactivity at six months were correlated with maternal cortisol at waking (T0), and evening cortisol levels (T750), as well as cortisol awakening parameters at six months postpartum (PP2). Pearson's correlation coefficients (r) are displayed in the table above. Results were corrected for multiple comparisons. *p<0.05 **p<0.01

6.3.4.4 Regression analysis of maternal cortisol and infant cortisol measures

Cortisol levels exhibit a high level of variance in general, as cortisol is sensitive to a number of factors. Therefore, it was of interest in this study to examine the effect of maternal factors on variance in infant cortisol measures. Hierarchical multiple regression was conducted to determine the predictive value of maternal cortisol measures during pregnancy and at six months postpartum on variance in infant cortisol levels at six months [See Section 6.2.6 for full description]. Regression modelling in this section describes two models [Table 6.9a – Table 6.9f]. For each model, the variables added to the previous model are shown in the Variables column. The first model (Model 1) contains a measure of maternal cortisol during pregnancy as a variable (waking cortisol level, intercept or slope of regression line), and Model 2 includes maternal cortisol measures both during pregnancy and at six months postpartum. R^2 value, R^2 change, the F statistic, the change in F, the unstandardised coefficient (B), and the standardised coefficient (β) are all presented in the table.

Maternal waking cortisol during pregnancy and at PP2 predicted 25% and 13% of variance respectively in infant baseline cortisol level at B2, together accounting for 38% of variance in baseline infant cortisol at six months. [Table 6.9a]. Maternal intercept during pregnancy and at six months, a measure of total morning cortisol secretion, predicted 17% and 11% of variance respectively in infant baseline cortisol levels at B2, together accounting for 28% of the variance in infant baseline cortisol at 6 months [Table 6.8b]. Maternal CAR (slope) only at PP2 and not during pregnancy, predicted 15% of variance in infant B2 baseline cortisol. [Table 6.9c].

None of the pregnancy cortisol measures were associated with infant post-vaccination cortisol levels. Maternal waking cortisol at PP2 explained 15% of variance in infant B2 post-stressor cortisol levels [Table 6.9d]. Similarly, total cortisol secretion in the first hour after awakening (intercept) and the CAR (slope) at PP2 each predicted 13% of variance in infant post-vaccination cortisol levels at B2 [Table 6.9e and Table 6.9f].

Table 6.9a Prediction of infant baseline cortisol at six months (B2 T0) from maternal waking cortisol (T0) at pregnancy (P) and six months postpartum (PP2)

Variables	B2 T0			
	Model 1		Model 2	
	B	β	B	β
Constant	-0.27		-0.60	
P T0	0.79*	0.50*	0.60*	0.37*
PP2 T0			0.57*	0.39*
R^2	0.25		0.38	
F	10.40		9.54	
R^2 change	0.25		0.13	
F change	10.40**		6.78*	

R^2 = measure of variation in the dependent variable explained by the independent variable(s); R^2 change = the change in the R^2 value from the previous model; F = F statistic, F change = change in F with each model; B = unstandardised coefficient; β = standardised coefficient.

Note: Hierarchical regression modelling conducted to predict infant cortisol values from maternal pregnancy and postpartum cortisol measures is presented above. * $p < 0.05$

Table 6.9b Prediction of infant baseline cortisol at six months (B2 T0) from maternal total morning cortisol output (Intercept) at pregnancy (P) and six months postpartum (PP2)

Variables	B2 T0			
	Model 1		Model 2	
	B	β	B	B
Constant	-0.27		-0.58	
P Intercept	0.79*	0.41*	0.61	0.32
PP2 Intercept			0.51*	0.35*
R^2	0.17		0.28	
F	6.20		5.84	
R^2 change	0.17		0.11	
F change	6.20*		4.74*	

R^2 = measure of variation in the dependent variable explained by the independent variable(s); R^2 change = the change in the R^2 value from the previous model; F = F statistic, F change = change in F with each model; B = unstandardised coefficient; β = standardised coefficient.

Note: Hierarchical regression modelling conducted to predict infant cortisol values from maternal pregnancy and postpartum cortisol measures is presented above. * $p < 0.05$

Table 6.9c Prediction of infant baseline cortisol at six months (B2 T0) from maternal cortisol awakening response (Slope) at pregnancy (P) and six months postpartum (PP2)

Variables	B2 T0			
	Model 1		Model 2	
	B	β	B	β
Constant	0.59		0.52	
P Slope	-12.56	-0.11	-7.09	-0.06
PP2 Slope			-32.13	-0.38*
R ²	0.01		0.15	
F	0.36		2.73	
R ² change	0.01		0.14	
F change	0.36		5.07*	

R² = measure of variation in the dependent variable explained by the independent variable(s); R² change = the change in the R² value from the previous model; F = F statistic, F change = change in F with each model; B = unstandardised coefficient; β = standardised coefficient.

Note: Hierarchical regression modelling conducted to predict infant cortisol values from maternal pregnancy and postpartum cortisol measures is presented above. *p<0.05

Table 6.9d Prediction of infant post-stressor cortisol at six months (B2 T30) from maternal waking cortisol (T0) at pregnancy (P) and six months postpartum (PP2)

Variables	B2 T30			
	Model 1		Model 2	
	B	β	B	β
Constant	0.65		0.33	
P T0	0.12	0.08	-0.06	-0.04
PP2 T0			0.55*	0.23*
R ²	0.01		0.16	
F	0.24		3.09	
R ² change	0.01		0.15	
F change	0.24		5.91*	

R² = measure of variation in the dependent variable explained by the independent variable(s); R² change = the change in the R² value from the previous model; F = F statistic, F change = change in F with each model; B = unstandardised coefficient; β = standardised coefficient.

Note: Hierarchical regression modelling conducted to predict infant cortisol values from maternal pregnancy and postpartum cortisol measures is presented above. *p<0.05

Table 6.9e Prediction of infant post-stressor cortisol at six months (B2 T30) from maternal total morning cortisol output (Intercept) at pregnancy (P) and six months postpartum (PP2)

Variables	B2 T30			
	Model 1		Model 2	
	B	β	B	β
Constant	0.84		0.54	
P Intercept	-0.06	-0.03	-0.23	-0.13
PP2 Intercept			0.50*	0.38*
R ²	0.00		0.13	
F	0.03		2.47	
R ² change	0.00		0.13	
F change	0.03		4.90*	

R² = measure of variation in the dependent variable explained by the independent variable(s); R² change = the change in the R² value from the previous model; F = F statistic, F change = change in F with each model; B = unstandardised coefficient; β = standardised coefficient.

Note: Hierarchical regression modelling conducted to predict infant cortisol values from maternal pregnancy and postpartum cortisol measures is presented above. *p<0.05

Table 6.9f Prediction of infant post-stressor cortisol at six months (B2 T30) from maternal cortisol awakening response (Slope) at pregnancy (P) and six months postpartum (PP2)

Variables	B2 T30			
	Model 1		Model 2	
	B	β	B	β
Constant	0.78		0.72	
P Slope	-0.45	-0.04	0.30	0.00
PP2 Slope			-28.21*	-0.36*
R ²	0.00		0.13	
F	0.06		2.44	
R ² change	0.00		0.13	
F change	0.06		4.82*	

R² = measure of variation in the dependent variable explained by the independent variable(s); R² change = the change in the R² value from the previous model; F = F statistic, F change = change in F with each model; B = unstandardised coefficient; β = standardised coefficient.

Note: Hierarchical regression modelling conducted to predict infant cortisol values from maternal pregnancy and postpartum cortisol measures is presented above. *p<0.05

6.3.4.5 Infant cortisol measures at six months and maternal rating scales

Association analyses between infant cortisol at six months and maternal clinical measures during pregnancy showed several significant associations in the whole sample analysis as shown in Table 6.10. There were no significant associations when the groups were analysed separately. The analysis was repeated to examine the association between infant cortisol at six months and maternal clinical measures at six months postpartum with multiple significant associations observed [Table 6.10].

Table 6.10 Correlation analysis between infant cortisol at six months (B2) and maternal rating scales during pregnancy and six months postpartum (PP2)

		B2 T0	B2 T30	B2 Mean	B2 RT
Pregnancy	PSS	0.26	0.30	0.64**	-0.15
	PSQI	0.39*	0.30	0.63**	-0.16
	CES-D	0.39*	0.31	0.59**	-0.14
	HAM-D	0.42**	0.47**	0.44*	-0.10
PP2	PSS	0.01	0.15	0.14	0.11
	PSQI	0.15	0.45**	0.42*	0.23
	CES-D	0.15	0.36	0.38	0.15
	HAM-D	0.26	0.45**	0.47**	0.13

PSS – Perceived Stress Scale; **PSQI** – Pittsburgh Sleep Quality Index; **CES-D** – Centre for Epidemiological Studies Depression rating scale; **HAM-D** – Hamilton Depression rating scale.

Note: Spearman’s correlation analysis was conducted between infant pre- (T0) and post-vaccination (T30) cortisol, mean cortisol, and cortisol reactivity (RT) at B2 and maternal rating scales at pregnancy and PP2. Spearman’s rho correlation coefficient (r) is presented in the table. Results were corrected for multiple comparisons. *p<0.05, **p<0.01

6.3.4.6 Maternal early life adversity and infant cortisol levels at six months

Infant cortisol measures were analysed for differences at six months between two groups – infants of mothers with a history of early life adversity (ELA) and those of mothers with no history of ELA. No significant differences were observed between the groups even after controlling for depression scores.

Correlation analysis was conducted between infant cortisol measures and maternal CTQ scores within each of the five domains of abuse. Maternal CTQ Sexual Abuse scores correlated significantly and positively with infant post-vaccination cortisol levels ($r=0.31$, $p=0.045$).

6.3.5 Trajectory of infant cortisol levels from two to six months after birth

Infants' pre- and post-stressor cortisol, mean cortisol and cortisol reactivity were analysed for differences between two months and six months [Table 6.11]. Infant post-vaccination cortisol was lower at six months in the sample as a whole. Infants were then analysed in separate groups [Table 6.11]. In the Depressed group, mean cortisol was significantly higher at six months compared to two months. Within the History and Control groups, infants had significantly lower post-stressor cortisol levels at six months than at two months. Infants born to Control mothers also had a significant reduction mean cortisol from the two-month to the six-month time point. There were no significant differences in cortisol reactivity between the two time points.

Table 6.11 Analysis of infant cortisol measures between two months (B1) and six months (B2)

		B1	B2	Statistic, p-value
		Mean (SD)	Mean (SD)	
Whole Sample B1: n=34 B2: n=34	T0	4.43 (4.91)	5.23 (9.98)	F=0.05, p=0.833
	T30	8.63 (4.45)	6.99 (7.05)	F=5.78, p=0.023*
	Mean	5.91 (3.92)	5.85 (6.90)	F=0.18, p=0.677
	RT	2.24 (7.04)	0.83 (8.52)	F=1.20, p=0.284
Depressed B1: n=9 B2: n=9	T0	2.51 (2.37)	12.24 (17.90)	F=4.00, p=0.080
	T30	8.78 (4.90)	13.82 (10.22)	F=0.32, p=0.594
	Mean	4.78 (2.77)	12.22 (11.49)	F=20.62, p=0.006*
	RT	4.33 (6.53)	-0.04 (1.16)	F=0.57, p=0.484
History B1: n=12 B2: n=12	T0	6.51 (7.29)	3.28 (3.27)	F=2.29, p=0.161
	T30	8.17 (2.67)	2.20 (1.55)	F=28.91, p=0.003*
	Mean	6.94 (5.34)	3.50 (2.06)	F=2.12, p=0.205
	RT	-1.15 (7.94)	-0.23 (3.62)	F=2.10, p=0.207
Control B1: n=15 B2: n=15	T0	4.04 (3.25)	2.26 (0.53)	F=3.77, p=0.074
	T30	8.75 (5.02)	5.72 (3.93)	F=6.29, p=0.025*
	Mean	5.79 (3.30)	4.25 (3.67)	F=23.00, p=0.001*
	RT	3.63 (6.00)	2.13 (2.13)	F=0.05, p=0.833

Note: Mean (SD) of raw infant cortisol values measures at baseline (T0) and 30 min post-stressor (T30), mean infant cortisol and cortisol reactivity (RT) are presented in the table. Data was log transformed and means compared between two and six months of age with repeated measures ANOVA. Results were corrected for multiple comparisons. Threshold for significance was set at 0.05.

6.4 Discussion

Birth outcomes varied by group and infant sex in this sample, with male infants in the Depressed group having lower gestational age at birth compared to female infants and infants in the History and Control groups. Infants in the Depressed group also had lower birth weight compared to the other groups. This was accounted for by the lower gestational age as all infants in the study were in the 99th percentile in birth weight for gestational age. There were no differences in cortisol stress responses among infants at two months of age across the three maternal caseness groups. However, there were some associations between infant cortisol measures at two months and maternal cortisol measures during pregnancy in the whole sample and in the Depressed and Control groups. The strong positive relationship between maternal cortisol measures and depression scores during pregnancy, and infant HPA axis measures at six months was one of the most striking findings in this study. At the six-month time point, infants born to women who were depressed during pregnancy had a different cortisol profile compared to infants in the other two groups, with higher pre- and post-stressor cortisol levels and higher overall mean cortisol levels. There were also notable correlations between infant cortisol measures at six months and maternal clinical and cortisol measures during pregnancy in the whole sample, and in the Depressed group only. Infant cortisol measures at six months were also correlated with concurrent maternal clinical and cortisol measures. Maternal cortisol measures, both during pregnancy and at six months postpartum, were found to predict infant cortisol variance at six months. Interestingly, despite the effect of ELA on maternal HPA axis during pregnancy and postpartum, there was no apparent effect of maternal ELA on infant HPA axis at two or six months of age.

Infants in the History and Control groups appeared to have higher post-vaccination cortisol levels at two months compared to six months in the overall sample, with no difference in cortisol reactivity. Mean infant cortisol levels were lower in the Control group at six months compared to two months. However, in infants born to women in the Depressed group, mean infant cortisol levels increased significantly at six months. In this context it is worth noting that three women in the Depressed group experienced postpartum depression at six months postpartum.

6.4.1 Infant birth outcomes

There is a strong evidence base for an association between antenatal depression, low birth weight and shortened gestational length (Grote et al., 2010; Liu et al., 2016; O'Keane, Lightman, Marsh, et al., 2011; Staneva et al., 2015; Venkatesh et al., 2016). Accordingly, infants in the Depressed group in the present study had lower gestational age at birth and birth weight than the History and Control groups. All infants were in the 99th percentile in birth weight for gestational age, indicating that the low birth weight was consistent with a shortened gestational length. Although infants in the Depressed group had significantly lower gestational age at birth than infants in the History and Control groups, they did not meet criteria for preterm birth or low birth weight. Interestingly, and in line with current evidence indicating a male preponderance for preterm birth, the shortened gestational length in the Depressed group was only observed in male infants (Di Renzo et al., 2007; Verburg et al., 2016; Zeitlin et al., 2004). Gestational age at birth in female infants in the Depressed group did not differ significantly from female infants in the History or Control groups. Male infants in the Depressed group however, had a significantly lower gestational age at birth compared to male infants in the History and Control groups, indicating that the group difference in the whole sample may be attributed to the male infants alone. These sexually dimorphic differences in infant birth outcomes, and only in the Depressed group, indicate that male and female foetuses do indeed respond to intrauterine stress in different ways. There are suggestions in the literature that male foetuses, in the presence of an intrauterine insult, continue to focus their resources on growth and development, thus increasing their susceptibility for preterm birth (Clifton, 2010; Murphy et al., 2005). Females are thought to conserve resources for future growth, slowing their growth trajectory in the presence of intrauterine stressors. It is likely in the current study that, in the presence of maternal depression during pregnancy, the male foetus continued growth and development at an accelerated rate, increasing vulnerability to a shortened gestational length compared to infants in the History and Control groups.

These findings lend support to the foetal programming hypothesis (Barker, 1995b). One plausible explanation for lower gestational age at birth in infants of mothers with antenatal depression may be increased placental CRH production in these women (Challis, 1998; O'Keane, Lightman, Marsh, et al., 2011). Evidence suggests that raised

placental CRH during the second trimester of pregnancy resets the ‘placental clock’ and increases the risk of preterm delivery (Challis, 1998; O’Keane, Lightman, Marsh, et al., 2011; Smith and Nicholson, 2007). Although there were no associations between maternal cortisol measures during pregnancy and infant birth outcomes in this study, it is possible that CRH levels, if measured, may have had an association with infant birth outcomes. The negative effect of antenatal depression on infant gestational age, rather than birth weight, suggests an accelerated gestation rather than compromised foetal growth in utero, consistent with the placental clock hypothesis of preterm birth.

6.4.2 Trajectory of the infant HPA axis

The trajectory of development of the infant HPA axis over the first six months showed that, in the overall sample, infants had lower post-stressor cortisol levels at six months compared to two months, replicating other studies examining the development of the cortisol stress response in infants (Jansen et al., 2010; Ramsay and Lewis, 1994). In accordance with the literature, there were no differences in cortisol reactivity between the two time points. These findings indicate a process of maturation of the developing HPA axis in the overall sample. On individual group analysis, infants in the History and Control groups had lower post-stressor cortisol levels at six months but infants in the Depressed group had significantly higher mean cortisol levels. These higher infant cortisol levels in the Depressed group may reflect maternal mood and cortisol levels at the six-month time point, since these maternal measures appear to be associated with infant cortisol measures in this sample. These findings indicate that the infant HPA axis at two months may still be in the initial stages of development with higher cortisol stress responses, and appears to have matured further at six months.

6.4.3 Maternal antenatal depression and the infant HPA axis

No differences were observed in infant cortisol measures at two months across the maternal caseness groups. There was, however, an association between infant cortisol response to stress at this time point and maternal cortisol measures during pregnancy in the overall sample, and in the Depressed and Control groups. Infant post-stressor cortisol levels were positively associated with maternal waking cortisol and measures of total morning cortisol output during pregnancy, indicating that high maternal

cortisol levels during pregnancy are associated with high cortisol stress responses in infants at two months, regardless of maternal mood status during pregnancy. The relationship between maternal cortisol measures during pregnancy and infant cortisol measures at two months was present mainly in the Depressed group. In the Depressed group, mean infant cortisol was positively associated with maternal waking and morning cortisol measures, and CAR during pregnancy. In the Control group, infant post-stressor cortisol values were positively associated with maternal waking cortisol during pregnancy. This suggests that, regardless of maternal mood status, there is a relationship between maternal cortisol measures during pregnancy and infant cortisol measures at two months postpartum. However, since the infant HPA axis is still immature at two months, these associations may not have any clinical significance.

At six months postpartum, there was a significant group effect in infant pre- and post-vaccination cortisol levels and mean cortisol. In line with the study hypothesis, infants born to mothers who were depressed during pregnancy had higher overall cortisol measures compared to those born to women in the Control and History groups. Although pre- and post-vaccination cortisol values were raised in infants in the Depressed group, there was no change in cortisol reactivity. Additionally, there were positive associations with baseline infant cortisol at six months and maternal pregnancy waking cortisol and total morning cortisol output in the whole sample analysis. When the groups were analysed separately, correlations were observed only in the Depressed group. Infant baseline cortisol was positively correlated to maternal total cortisol output measures during pregnancy. Regression modelling revealed that maternal pregnancy cortisol measures explained 17-25% of variance in baseline infant cortisol measures at six months. In addition, infant cortisol levels at six months also exhibited a strong relationship with maternal sleep, stress, and mood scores during pregnancy. These findings support the study hypothesis that maternal cortisol and clinical measures during pregnancy will influence infant cortisol measures.

These findings suggest that maternal cortisol measures and clinical measures during pregnancy have an impact on infant cortisol measures over the first six months of life. These findings replicate the results of studies examining the effects of clinically diagnosed antenatal depression on infant HPA axis, finding higher cortisol measures and stress responses in infants of women with antenatal depression (Brennan et al.,

2008; Field et al., 2010). In contrast to state depression, there was no effect of a maternal history of depression on infant cortisol levels at two or six months after birth.

6.4.4 Maternal mood postpartum and the infant HPA axis

All study participants were euthymic at two months postpartum. Perhaps for this reason, there was no evidence of a relationship between maternal and infant cortisol levels at this time point. However, infant post-stressor cortisol levels at this time point were positively associated with maternal sleep scores, suggesting a possible relationship between maternal stress and infant HPA axis. This association may also reflect poor sleep in a stressed infant with subsequent sleep disturbance in the mother.

Three women in the Depressed group experienced a relapse of depressive illness at six months postpartum. At this time point, there was a group effect in infant cortisol with infants in the Depressed group having higher cortisol levels compared to the History or Control groups. In accordance with the study hypothesis, there were strong associations between infant and maternal cortisol measures in the whole sample at this time point. On separate group analysis, these associations were only evident in the Depressed group, indicating an effect of maternal mood state on infant cortisol levels at this time point. Infants of mothers with high morning cortisol production had high mean cortisol levels in the Depressed group at this time point. Regression modelling showed that 11-15% of variance in pre- and post-stressor infant cortisol measures at six months was predicted by maternal cortisol measures at six months postpartum. Infant cortisol measures were also positively associated with maternal sleep and mood measures at six months postpartum, indicating that infants of mothers with poor sleep and low mood had higher cortisol levels.

These results suggest that maternal cortisol output strikingly influences infant cortisol output at six months postpartum, but only in the Depressed group. This appears to be related to the maternal mood state at this time point, demonstrated by the relationship between maternal clinical measures and infant cortisol measures at the six-month time point. Some studies on neonates and nine-month-old infants have found maternal postpartum depression to have an effect on infant cortisol levels

(Feldman et al., 2009; Lucci et al., 2016). However, the small sample size of depressed women at six months postpartum in the present study would preclude any speculation about the effects of postpartum depression on infant HPA axis at six months. However, the evidence for a relationship between maternal cortisol and clinical measures and infant cortisol measures at six months, regardless of maternal mood status, is strong. Although there is an association between maternal pregnancy cortisol measures and infant cortisol measures at two months, there are no observed differences between infant groups at this time point. This would indicate that a combined effect of maternal pregnancy and postpartum cortisol measures, as observed at the six-month time point, may be necessary to influence infant cortisol measures at the concurrent time point postpartum.

Additionally, infants in the History group demonstrated no cortisol stress response to a vaccination stressor at both two and six months of age. Although not significant, women in the History group had lower cortisol levels compared to those in the Control group, and demonstrated a trend for higher rates of childhood sexual abuse on the CTQ [See Chapters 3 and 5]. These findings suggest that HPA axis hypoactivity seen in women in the History group with a history of childhood sexual abuse may be associated with a blunted cortisol stress response to vaccination in infants.

6.4.5 Maternal early life adversity and the infant HPA axis

Although maternal ELA had an apparent influence on maternal evening cortisol levels during pregnancy and postpartum, there were no effects of maternal ELA on infant cortisol levels at two or six months of age, and no associations between maternal evening cortisol levels and infant cortisol measures. These findings refute the study hypothesis of a transgenerational effect of maternal ELA on infant HPA axis. Only one other study has examined the possible transgenerational effects of maternal ELA on offspring HPA axis and found lower baseline cortisol levels in infants born to mothers with a history of ELA compared to infants whose mothers had no history of ELA (Brand et al., 2010). These findings were not replicated in the current study. This may be explained by methodological disparities in the studies, as the effects of maternal ELA on offspring HPA axis was a secondary aim in the present study. Further, there was an overlap between depression and ELA in this sample, with a higher incidence of

maternal ELA in women in the Depressed group. It would appear that maternal state depression and cortisol levels may have a stronger effect on infant HPA axis measures, obscuring any possible effects of ELA.

6.4.6 Conclusions

The present study provides robust evidence for foetal programming in maternal depression during pregnancy, mediated through the HPA axis. Antenatal depression appears to have a negative effect on infant birth outcomes, only in male infants. In keeping with other studies demonstrating the reduction in stress responses as the infant HPA axis matures, there was a reduction in infant cortisol stress responses between two and six months in the History and Control groups. There was an increase in cortisol levels from two to six months in the Depressed group, likely explained by the influence of maternal mood status and cortisol measures. Maternal cortisol measures during pregnancy appear to have a strong effect on infant baseline cortisol levels at six months, while maternal cortisol values at six months postpartum appear to have a strong effect on infant post-stressor cortisol responses at this time point. These results indicate that synergistic actions of maternal HPA axis measures during the perinatal period influence infant HPA axis measures, which may have an effect on mental and physical health in later life. The clear indication that state depression during pregnancy influences infant HPA axis activity is compelling reason to actively manage depression during pregnancy.

Chapter 7

Discussion

7.1 Overview

A longitudinal prospective study of maternal depression during pregnancy, its clinical features, and the accompanying changes in the maternal and infant HPA axis forms the core of this thesis. The relationship between HPA axis dysregulation and clinical depression is well accepted. However, less is known about the relationship between antenatal depression and the HPA axis during pregnancy. With this, and the possible foetal programming effects of maternal HPA axis during pregnancy in mind, we sought to study the HPA axis in mothers and infants during the perinatal period. A summary of the main findings, their relationship with the current literature and their possible clinical implications will be discussed in this section. Strengths and limitations of the study and suggestions for future research will also be explored.

7.2 Pregnancy

The HPA axis and depression during pregnancy

One hundred pregnant women, 23 of whom were depressed, were recruited into this study at a mean gestational age of 26 weeks. Women who were depressed during pregnancy had no significant differences in their cortisol measures, cortisone levels, or cortisone/cortisol ratios during pregnancy compared to never-depressed pregnant women, in line with four studies that found no relationship between antenatal depression and the HPA axis (Evans et al., 2008; Field et al., 2010; Hellgren et al., 2013; Parcels, 2010). The present study adds to the evenly divided evidence base for the effect of antenatal depression on maternal HPA axis, giving further support to studies showing no effect. The reasons for discrepancies in these studies may relate to different methodologies, varied clinical definitions of depression, differences in gestational age at sample collection, and in particular, different participant samples used for cortisol quantification. In fact, varying results were found in different studies conducted by the same group (Field et al., 2007, 2009, 2010). Diverse study methodology is likely to be a principal factor, but the notable changes in the HPA axis during pregnancy probably obscure any possible functional differences between depressed and control study populations. One major issue in the studies is the gestational age at sample collection for analysis of cortisol measures. There is evidence to indicate that the maternal HPA axis becomes less responsive to stress as gestation

progresses and cortisol levels rise, particularly in late gestation (Entringer et al., 2010; Kammerer et al., 2002; Schulte et al., 1990). Most studies investigating the association between antenatal depression and HPA axis changes have variously examined women at 20, 32 or 36 weeks gestation. Only one other study, by O’Keane and colleagues (2011), reported on HPA axis measures around 25 weeks gestation, and found higher evening cortisol levels in women diagnosed with depression during pregnancy. Most studies examined morning cortisol levels; the only other study to examine diurnal levels found higher cortisol levels throughout the day and a flatter diurnal curve (O’Connor et al., 2014).

Clinical aspects of depression during pregnancy

Although there were no observed HPA axis alterations in women who were depressed during pregnancy in this sample, the clinical features of depression were clearly evident, and were not obscured by the somatic changes of the healthy pregnant state. Depressed pregnant women had a higher perception of stress, poorer sleep quality, and lower subjective and objective mood compared to women in the Control group. Notably, these features were not associated with cortisol measures during pregnancy. Somatic features of pregnancy, such as disturbances in sleep, energy, or appetite, were previously considered to confound a diagnosis of depression and were often excluded from diagnostic criteria (Yonkers, Vigod, et al., 2011). However, my findings support recent evidence indicating that somatic features are more prominent in depressed, compared to non-depressed, pregnant women (Field et al., 2007; Kelly et al., 2001; Nylén et al., 2013; Tomfohr et al., 2015). Accordingly, DSM-5 criteria for peripartum depression is no different to the criteria for MDD (American Psychiatric Association, 2013).

In line with current literature on the risk factors for depression during pregnancy, depressed pregnant women were more likely to have had unplanned pregnancies and poorer social supports than the rest of the sample (Lancaster et al., 2010). In fact, there were four participants with poor social supports in the study sample, and all belonged to the Depressed group. Literature suggests that lower socioeconomic status is a risk factor for domestic violence during pregnancy (James et al., 2013). In this study sample, the absence of any reported domestic violence may be a reflection of the higher socioeconomic status of the population.

There is a clear link between preeclampsia and antenatal depression in the literature, but the evidence for an association between gestational diabetes and antenatal depression is tenuous (Hu et al., 2015; Qiu et al., 2009; Ross et al., 2016). Four women in the study developed obstetric complications. Although three of them were depressed during pregnancy, the small number precludes speculation about the association between antenatal depression and obstetric complications in this sample.

An interesting section of this study sample was the History group, comprised of euthymic pregnant women with a lifetime history of depression. This group was included in the study to investigate possible trait effects of depression on the HPA axis during pregnancy. A prior history of depression is known to increase the risk of developing depression during pregnancy (Lancaster et al., 2010). In keeping with this, 22 women out of 23 in the Depressed group in this study had a past history of depression. Recurrence after an initial episode of MDD is a consistent finding (Bhagwagar and Cowen, 2008) and may possibly result from a scarring effect on the HPA axis from a prior episode of depression (Lok et al., 2012). None of the women in the History group in this sample experienced a relapse of depression during pregnancy. They would, therefore, be expected to have similar stress and mood measures to women in the Control group. However, there were striking clinical differences between the History group and the Depressed and Control groups. Although self-report symptoms of depression may be higher in women during pregnancy compared to the postpartum period (Evans et al., 2001), women in the History group had higher stress and mood measures compared to the Control group, although not as high as the Depressed group. HPA axis measures in the History group, as would be expected given the findings in the Depressed group, were not significantly different to measures in the Control group. There is clear evidence in this study that a prior history of depression is a risk factor for depressive relapse during pregnancy. Additionally, women with a prior history of depression who do not relapse during pregnancy appear to experience elevated stress and lower mood, indicating that pregnancy is a more challenging period for these women compared to women with no history of mental illness.

Early life adversity and the HPA axis during pregnancy

Maternal early life adversity had an interesting effect on maternal cortisol measures, even after accounting for depression. The distribution of women with and without a history of ELA was reasonably even in this sample, with 54% having a history of ELA. Women in the Depressed group had higher ELA scores than women in the Control group, particularly in the emotional trauma domains, in keeping with the well-accepted association between ELA and antenatal depression (Benedict et al., 1999; Lang et al., 2006; Rich-Edwards et al., 2011). Women with a history of ELA had higher waking and evening cortisol levels during pregnancy compared to women with no history of ELA in the present study. A history of ELA predicted 17% of variance in maternal evening cortisol levels during pregnancy, after controlling for depression. These findings are in line with the literature demonstrating higher HPA axis measures in pregnant women with a history of ELA compared to pregnant women with no history of ELA (Bublitz and Stroud, 2012; Buss, Entringer, et al., 2016; Buss, Stalder, et al., 2016; Schreier, Enlow, Ritz, Coull, et al., 2015; Schreier, Enlow, Ritz, Gennings, et al., 2015). Only one study investigated the effects of both antenatal depressive symptoms and ELA on maternal CAR and found a history of ELA to be associated with lower waking cortisol levels after controlling for confounders such as medication use (Shea et al., 2007b). However, depression scores, which may have confounded the results of the study, were not accounted for in the analysis. In the present study however, since all analyses of ELA controlled for depression, it may be postulated that ELA has an effect on maternal HPA axis during pregnancy irrespective of maternal mood status.

Sexually dimorphic effects of the foetus on maternal HPA axis

There is a reciprocal relationship between the pregnant woman and her foetus *in utero*. A strong evidence base exists for the effects of maternal health status on foetal health, and forms the basis of the developmental programming hypothesis (Barker, 1995b). The effects of the foetus on maternal physiological systems, however, are poorly studied. Literature on the possible effects of foetal sex on maternal HPA axis is limited and studies report paradoxical results. Higher first and second trimester cortisol levels in women carrying male foetuses were reported in one study while another found higher morning cortisol levels and blunted CAR in women carrying females (DiPietro et al., 2011; Giesbrecht, Campbell, et al., 2015). The current study

found no sexually dimorphic effects of foetal sex on maternal cortisol levels during pregnancy. Cortisone levels and subsequently, cortisone/cortisol ratios were higher 30 minutes after awakening in women carrying male foetuses. However, with no observed differences in cortisol levels the significance of these results is unclear. A higher prevalence of male infants in this study may have skewed results of both the cortisol and cortisone measures.

Although foetal sex did not directly influence maternal cortisol levels in this study, male foetal sex moderated the relationship between ELA and maternal evening cortisol levels with a positive linear effect. In addition to the evolving literature for an effect of foetal sex on maternal HPA axis during pregnancy, there is emerging evidence that male foetuses have a different intrauterine growth pattern compared to females (Clifton, 2010; DiPietro et al., 2011; Giesbrecht, Campbell, et al., 2015). Male foetuses are thought to focus their resources on growth even in the presence of intrauterine stress, thus increasing their susceptibility for poor birth outcomes (Clifton, 2010). This would suggest that male foetuses, in their efforts to maximise growth, may be increasing stress on maternal physiological systems and enhancing the effect of ELA on maternal HPA axis during pregnancy. In the present study, female foetuses had a non-significant negative linear effect on the relationship between maternal ELA and evening cortisol levels, suggesting that the large proportion of male foetuses in this study sample may have driven the overall effect of higher evening cortisol levels during pregnancy. The effects of various domains of ELA on the HPA axis appear to be more enhanced during pregnancy compared to the postpartum period. This finding may be explained by the positive linear effect of male foetuses on the relationship between maternal ELA and HPA axis during pregnancy.

7.3 The postpartum period

There were no differences between groups in cortisol measures at two months postpartum, not a surprising finding as all women were euthymic at this time point. However, women in the Depressed group, although euthymic, had higher clinical scores in all domains measured, with higher perceived stress, poorer sleep quality, and lower subjective and objective mood, compared to women in the Control group. This may indicate residual symptoms of a partially resolved depressive episode. Although

they had lower scores than women in the Depressed group, women in the History group had higher scores on clinical measures compared to the Control group at two months postpartum. They had higher levels of perceived stress and lower subjective mood, suggesting ongoing difficulty in the postpartum period compared to controls. Interestingly, maternal age, gravidity, and parity were positively associated with cortisol measures at two months only in the Depressed and History groups, indicating that a history of depression is associated with HPA axis changes in the early postpartum period in older mothers with more children, suggesting enduring vulnerability in this period.

Additionally, the History group had lower evening cortisol levels and lower total morning cortisol output postpartum compared to the Control group, although these findings were not statistically significant. The History group in this sample also had a history of ELA. Hinkelmann et al (2013) found lower hair and salivary cortisol levels in adults with a history of ELA, regardless of their depression status. It is possible that women with a history of depression *and* ELA exhibit hypocortisolemia in the postpartum period.

Three women in the Depressed group experienced a relapse of depression at six months postpartum, and this group continued to have higher scores than women in the Control group on all clinical measures. Higher total morning cortisol output was observed in the Depressed group and maternal clinical measures were associated with cortisol measures at six months. We could speculate that by six months postpartum in the present study, the HPA axis has returned to its non-pregnant state, and the higher cortisol levels observed in the Depressed group reflect HPA axis changes that are normally observed in clinical depression (Vreeburg et al., 2009). However, in view of the small sample size of depressed women postpartum, these findings are not conclusive. Studies of the HPA axis in postpartum depression have all been conducted within the first three months postpartum. Some have reported no differences in cortisol levels between depressed and euthymic women postpartum, while one study found a blunted CAR in women with postpartum depression (Jolley et al., 2007; Magiakou, Mastorakos, Rabin, Dubbert, et al., 1996; Taylor et al., 2009). These diverse findings indicate the need for further study into the role of the HPA axis in postpartum depression in a larger population.

Maternal early life adversity and the HPA axis postpartum

The effect of ELA on maternal evening cortisol levels persisted postpartum in the present study. Few studies have examined the HPA axis postpartum in women with a history of ELA. One reported a lower cortisol response to a stress paradigm involving infant distress in postpartum women with a history of ELA compared to women with no history of ELA (Brand et al., 2010). Another study examined the CAR in postpartum women with a history of ELA and found higher waking cortisol levels and a sustained increase in morning cortisol output (Gonzalez et al., 2009). There was no effect of ELA on maternal morning cortisol measures postpartum in the current study. An effect of maternal depression on morning cortisol measures at six months postpartum may predominate any effects of ELA on these measures. However, there was a consistent effect of ELA on maternal evening cortisol levels during pregnancy and postpartum, even after controlling for depression. This would suggest that the effect of ELA on the maternal HPA axis is independent of depression dynamics in the HPA axis.

7.4 Trajectory of maternal HPA axis in the perinatal period

There were striking reductions in cortisol measures in all groups from pregnancy to two months and six months postpartum. There were no significant differences in cortisol measures between the two time points in the History and Control groups, indicating a return of the HPA axis to the non-pregnant state by two months postpartum. The Depressed group however, had higher cortisol output at six months postpartum compared to two months, possibly explained by a relapse of depression in three women in this group at six months. These findings may reflect the restoration of the HPA axis from the hypercortisolaemia of pregnancy by two months postpartum, regardless of mood status during pregnancy. Free cortisol levels are thought to return to normal over the first four days after delivery (Mastorakos and Ilias, 2003; O'Keane, Lightman, Patrick, et al., 2011). One study found no differences in cortisol levels between three, six, and 12 weeks postpartum (Magiakou, Mastorakos, Rabin, Dubbert, et al., 1996). My findings corroborate a recent study which found that free cortisol levels had returned to pre-pregnancy levels by 2-3 months postpartum (Jung et al., 2011). There was no change in the cortisol awakening response (CAR) between pregnancy and either postpartum time points in this study, in line with current evidence that the CAR is preserved during pregnancy (de Weerth and Buitelaar,

2005a). Clinical depression scores decreased from pregnancy to the two month postpartum time point in the Depressed group only, reflecting that these women were euthymic at this time point, although with only partial resolution of symptoms, as suggested by the clinical features discussed above. Sleep measures worsened from pregnancy to two months postpartum, possibly due to the demands of infant feeding.

Interestingly, there were multiple associations between cortisol measures during pregnancy and those at the postpartum time points. These associations between pregnancy and postpartum cortisol measures were evident in all the groups at six months postpartum. This demonstrates that, although the HPA axis is in overdrive during pregnancy, individual secretory patterns remain constant through the pregnant and the non-pregnant states. The associations between pregnancy and postpartum cortisol measures were strongest in the Depressed group, suggesting that any differences that may exist in the HPA axis between depressed and non-depressed pregnant women are concealed within the hypercortisolaemic state of pregnancy. It is conceivable that HPA axis dynamics in antenatal depression are predominantly driven by hypothalamic CRH, and may not be evident in the placental CRH-driven salivary free cortisol measured. There is evidence that the ultradian rhythm of cortisol pulsatile activity, driven by the hypothalamus, may be disrupted in depression (Lightman and Conway-Campbell, 2010; Pariante and Miller, 2001; Young et al., 2004). Accordingly, any changes in the ultradian rhythm during pregnancy may be obscured by the hypercortisolaemia of placental CRH origin. Since placental CRH also stimulates ACTH output in addition to cortisol, and hypothalamic CRH does not cross the blood-brain barrier, there are unfortunately no circulating HPA axis markers that could be measured to further investigate this hypothesis.

7.5 Developmental programming of the HPA axis in infants

Birth outcomes and sexual dimorphism

There is clear evidence in this study for the developmental programming effects of antenatal depression on the infant HPA axis. Infants born to women who were depressed during pregnancy had poorer birth outcomes, with lower gestational age at birth and lower birth weight, although there were no apparent associations between

these factors and maternal cortisol measures during pregnancy. Although infants in the Depressed group had lower birth weight compared to the other two groups, all infants in the study were in the 99th percentile in birth weight for gestational age. This would indicate that the lower birth weight is consistent with a shortened gestational length. Shortened gestational length is a well-established adverse outcome associated with maternal depression during pregnancy, while the evidence for an association between antenatal depression and low birth weight is less robust (Grote et al., 2010; Liu et al., 2016; Staneva et al., 2015; Venkatesh et al., 2016).

The lower gestational age in infants in the Depressed group may be mediated by placental CRH. The length of gestation is thought to be determined early in pregnancy by a 'placental clock', controlled by placental CRH (Challis, 1998; McLean et al., 1995; Smith, 1998). High placental CRH levels found in antenatal depression are hypothesised to be driven by a cortisol feed-forward loop (O'Keane, Lightman, Marsh, et al., 2011). Although there were no observed differences in maternal cortisol measures in depressed pregnant women compared to euthymic pregnant women in the present study, there is clear association between maternal cortisol measures during pregnancy and infant cortisol measures only in the Depressed group. This would suggest that there were HPA axis dynamics specific to the Depressed group, possibly leading to foetal overexposure of cortisol *in utero*, which may not have been detected by the free cortisol measures alone. This potential foetal overexposure to intrauterine cortisol may have shortened the length of gestation in infants of mothers who were depressed during pregnancy. Although we did not measure CRH in the present study, high levels of CRH were reported in the second trimester in women experiencing antenatal depression in other studies (O'Keane, Lightman, Marsh, et al., 2011; J. W. Rich-Edwards et al., 2008). Infants born to mothers with antenatal depression, with their lower gestational age at birth, have poorer health outcomes in childhood with higher rates of hospital visits and increased risk of long-term illness (Boyle et al., 2012).

Notably, in support of the literature demonstrating sexual dimorphism in infant birth outcomes, only male infants in the Depressed group had low gestational age at birth compared to male infants in History and Control groups. Female infants in the Depressed group did not have significantly differ from the rest of the sample in gestational age at birth. A retrospective Australian population study spanning 30 years

found clear male predominance for preterm birth, and other studies have reported similar findings (Di Renzo et al., 2007; Verburg et al., 2016; Zeitlin et al., 2004). In another large-scale study, male-male twins had the highest rate of preterm births compared to female-female twins and male-female twins (Tan et al., 2004).

There are several proposed mechanisms for this sexual dimorphism in infant birth outcomes. It is now well accepted that excess exposure to cortisol *in utero* impairs foetal growth, and along with sex-specific placental 11 β -HSD-2 expression, it is proposed as a mechanism underlying preterm birth (Challis et al., 2013; Seckl and Holmes, 2007). The placenta, in its juxtaposed position between the mother and the foetus, plays a key role in foetal development and programming (Clifton, 2010). The female placenta has been shown to be more sensitive to stress signals, allowing the foetus to make adaptations to survive gestation to term. Female foetuses, in the presence of intrauterine stress, slow their growth trajectory and conserve their resources for future growth, increasing their chances of survival (Murphy et al., 2003). Male foetuses are thought to grow at an accelerated rate compared to females in general. Contrary to females, they do not make any adaptations to their growth trajectory even in the presence of intrauterine insults, making them more susceptible to preterm birth (Clifton and Murphy, 2004; Murphy et al., 2005). Other proposed mechanisms for the sexual dimorphism in preterm birth include increased susceptibility to intrauterine infection in males, sex-specific gene expression in the placenta, and higher cytokine expression in female placentas compared to male, at least in the presence of maternal asthma (Gabory et al., 2013; Scott et al., 2009). Sexual dimorphism in foetal development warrants further research, as enhanced understanding of the mechanisms involved may help reduce offspring illness, which in itself has a sexually dimorphic distribution.

Regulation of the infant HPA axis

Current evidence suggests that the infant HPA axis is only beginning to be endogenously regulated at two months, although some studies suggest that the circadian rhythm does not appear until nine months (De Weerth et al., 2003; Kiess et al., 1995). In the present study, mean cortisol levels and cortisol stress responses in infants in the History and Control groups were higher at two months compared to measures at six months. This is in accordance with the literature showing high infant

cortisol responses in early infancy, with an overall decrease with age up to the six-month time point (Jansen et al., 2010). Infants in the Depressed group, however, had higher pre- and post-stressor cortisol levels at six months compared to two months. Regression modelling indicated that these high infant cortisol measures at six months were predicted by a synergistic effect of maternal cortisol measures during pregnancy and cortisol measures at the concurrent time point postpartum. There were no differences in cortisol reactivity between two and six months in the present sample, in line with current evidence showing that although there is an overall decrease in cortisol levels, cortisol reactivity remains stable in infants (Ramsay and Lewis, 1994).

Maternal depression and the infant HPA axis

A number of studies have examined the effects of depressive symptoms during pregnancy on the infant HPA axis and found higher cortisol reactivity in infants born to mothers with high depressive symptomatology (Fernandes et al., 2014; Field et al., 2004; Lundy et al., 1999; T Oberlander et al., 2008). One study that examined the effect of clinical depression during pregnancy found higher cortisol levels in neonates 1-2 days postpartum (Field et al., 2010). Another study used retrospective depression diagnoses and reported higher cortisol reactivity in six-month-old infants born to mothers with retrospectively diagnosed perinatal depression (Brennan et al., 2008). In the current study, infants in the Depressed group had higher pre- and post-vaccination cortisol levels, and higher mean cortisol levels compared to the rest of the sample. Additionally, there were several correlations between infant cortisol levels and maternal cortisol and clinical measures during pregnancy and postpartum, mainly evident in infants born to women in the Depressed group. These findings add to the evidence base that a relationship exists between maternal cortisol levels during pregnancy and infant cortisol levels (Davis et al., 2011b; TG O'Connor et al., 2013; Yehuda et al., 2005).

In the current study, maternal cortisol measures during pregnancy and at six months postpartum predicted 17-25% and 11-15% of variance respectively in infant baseline cortisol levels at six months. Maternal cortisol measures at six months postpartum predicted 13-15% of variance in the post-stressor cortisol levels in infants at six months. These findings suggest that, although maternal cortisol measures were not significantly different across groups during pregnancy, there may be underlying HPA

axis dynamics, not evident in salivary cortisol measures, that modify baseline HPA axis function in infants. The infant post-stressor cortisol level at six months appears to only be influenced by maternal cortisol measures at the corresponding time point. This suggests that baseline cortisol secretion is influenced by the maternal HPA axis during pregnancy and the cortisol stress response is influenced by concurrent maternal stress and HPA axis activity. The small sample size of depressed women postpartum would preclude any speculation about the effect of postpartum depression on the infant HPA axis. These are novel findings and the first to demonstrate a synergistic effect of maternal cortisol measures during pregnancy *and* postpartum on infant cortisol measures.

An interesting finding in this study was the lack of cortisol response to a vaccination stressor in infants born to women with a history of depression, at both two and six months of age. This outcome may be a reflection of maternal hypocortisolemia, as discussed in Section 7.3, in this group postpartum. However, Suzuki et al (2014) found blunted cortisol stress responses in adults with a history of childhood trauma. It may therefore be speculated that infants born to women with a history of depression and ELA do not mount a cortisol stress response in the first six months of life.

Maternal early life adversity and the infant HPA axis

Contrary to findings in the only other study examining the intergenerational transmission of maternal ELA stress and infant HPA axis, there was no effect of maternal history of ELA on infant cortisol levels at either two or six months in this study (Brand et al., 2010). There were no associations evident between maternal evening cortisol measures and infant measures, in spite of the consistent finding of elevated evening cortisol levels in women with a history of ELA across the perinatal period. Perinatal maternal depression and maternal morning cortisol measures, however, appear to be associated with infant HPA axis function. Further exploration into HPA axis activity during pregnancy in antenatal depression and in ELA is warranted to extricate the HPA axis dynamics of each.

7.6 Strengths and limitations of study

This study has numerous strengths, including the prospective longitudinal study design, diagnosis of depression made with a clinical interview, and evaluation of the HPA axis at different time points in mothers and infants. Most studies of perinatal depression and the HPA axis offer a cross-sectional observation of prenatal depressive symptoms. This is the first study of the HPA axis in mother-infant dyads, to my knowledge, that has examined clinical depression longitudinally from pregnancy to six months postpartum. This study design allowed us to outline the trajectory of the HPA axis during the perinatal period in depressed and healthy women, and in infants over the first six months after birth. Additionally, several potential maternal and infant confounding factors were taken into account in this study, thus reducing bias.

The small sample size is one of the main limitations of this study. The Depressed group, owing to its small size of 23 participants, may not be adequately powered to show significant differences in cortisol levels. There were only three women with postpartum depression in this study. Consequently, we could only speculate about any effects on the HPA axis at this point.

Additionally, this is a rather homogenous sample – the majority of participants were Caucasian, educated, married, and did not smoke or drink alcohol, even socially, during pregnancy. Participants were recruited from three maternity hospitals in Dublin, two of which are located in socially deprived areas and areas with high migrant populations. Social deprivation and migration are known risk factors for depressive illness. Despite this, most women in this study were educated, employed, in stable relationships, and Irish. This may be a reflection on the demographic of women agreeing to participate in a time-intensive longitudinal study. A larger, more diverse, sample size would have increased the representativeness of this study sample, but with negative effects on the power of the study.

Observer bias is a limitation of the study. Having the same person responsible for evaluation for recruitment of pregnant women into the study, and for their treatment will have introduced bias into the study. This would be of particular significance when diagnosing clinical depression and scoring the HAM-D rating scale.

Medication use was another limitation of the study. All participants received treatment as usual, and 22 women in the Depressed and History groups took psychotropic medication including antidepressants and mood stabilisers during pregnancy. Although there was no effect of medication on findings in the present study, altering medication dose and bioavailability during the perinatal course of this study must be considered, in addition to the effects of antidepressants on the maternal HPA axis. Antidepressants have been found to have a class-driven effect on the HPA axis, with patients taking tricyclic antidepressants having a blunted CAR, and those taking selective serotonin reuptake inhibitors having higher evening cortisol levels (Manthey et al., 2011; Vreeburg et al., 2009). Although medication use did not have an effect on maternal and infant cortisol findings in covariance analyses in this study, it is possible that SSRIs may have had developmental programming effects on the infant HPA axis. Studies have found lower cord blood cortisol levels, and lower basal evening cortisol levels in infants exposed to prenatal SSRIs (Davidson et al., 2009; TF Oberlander, Grunau, et al., 2008). Ideally, women taking part in this study would have been medication-free but this would have made an already-challenging recruitment process more arduous.

There were some limitations with saliva sample collection methodology. In order to improve compliance, participants were not instructed to record times of saliva sample collection, and were relied on to collect their samples at the required time points. While some participants admitted to missed sampling times and repeated a full day of sampling, others may not have mentioned any discrepancies in sampling times. Inaccurate sampling times are known to vastly affect CAR measurement, and some studies even use electronic devices to remind participants to record times (Stalder et al., 2016). Additionally, participants were not required to record any food or drink intake during the period of saliva sample collection in the morning. Food and caffeinated drinks can affect salivary cortisol concentration, and may therefore have affected the results (Stalder et al., 2016). However, researchers collected infant saliva samples at the appropriate times, and confounders such as infant feeding between sample collection times were recorded, ensuring more accurate cortisol measurement.

Finally, there were several missing data points, as participants often did not return all saliva samples. Some participants, particularly women who were depressed during

pregnancy, did not return any saliva samples, resulting in missing data sets. This is to be expected in a clinical study of depression where participants are likely to have low levels of motivation and poor cognitive function consequent to depression.

7.7 Future directions

In view of the large variability in results of studies examining antenatal depression and the HPA axis, multi-centre studies with consistent methodology would be ideal in assessing this relationship. This may not be feasible however, and may not be fruitful. It may be more pragmatic to move on from this question to that of the effects of maternal depression during pregnancy on offspring HPA axis function. The strong association between maternal and infant HPA axis function indicates that cortisol measures used in this study are adequate to explore this relationship further.

Foetal programming is clearly a multifactorial process. The strong evidence for foetal HPA axis programming in antenatal depression in the absence of overt maternal HPA axis dysfunction during pregnancy in this study indicates that larger studies investigating depressed pregnant women and infant HPA axis function would be useful to establish this relationship and explore other possible mechanisms involved. The placenta, as the interface between maternal and foetal circulation, is emerging as a driver of developmental programming changes. Placental dysfunction, possibly mediated by epigenetic changes, can influence maternal mood and subsequently, foetal development (Janssen et al., 2016). Placental sampling may be a useful addition to future studies to establish the role of the placenta in foetal programming. Extension of the present study to investigate the offspring HPA axis and epigenetic changes in childhood may provide valuable information on potential biomarkers for foetal programming effects of antenatal depression.

Cortisol analysis in current research only measures state cortisol, which is subject to considerable inter- and intra-individual variability. Additionally, during pregnancy, the physiologically raised cortisol values may confound any underlying HPA axis dynamics, especially in antenatal depression. Although cortisol output and the cortisol awakening response are useful indicators of state-specific changes, an indicator of stable cortisol changes may be a more valuable measure in perinatal research, as they

may be driving the foetal programming effects of antenatal depression. Latent state-trait cortisol modelling uses correlations among different cortisol samples collected over a period of at least three consecutive days to identify a latent trait cortisol factor, which is a stable measure of HPA axis function (Stroud et al., 2016). A latent state cortisol factor is also derived, giving an indication of immediate changes in HPA axis function. Latent trait cortisol may be useful in perinatal research, as it gives an indication of stable cortisol measures that are unaffected by the state-specific HPA axis changes during pregnancy (Giesbrecht, Bryce, et al., 2015).

From a clinical perspective, it is evident that maternal mental health during pregnancy has an impact on offspring mental health. It would be judicious, therefore, to take into consideration the mother-infant dyad, rather than the mother alone, in perinatal mental health management. In addition, there is growing evidence for the role of paternal depression in infant outcomes. A large population study has demonstrated an increased risk of preterm birth in the presence of paternal antenatal depression (Liu et al., 2016). An association between paternal perinatal depression and infant emotional and conduct problems in childhood has been reported in some studies (Ramchandani et al., 2005; Sweeney and MacBeth, 2016). Further, paternal antenatal depression can increase the risk of paternal postnatal depression, and this has been shown to lead to increased relationship difficulties which may then impact on infant outcomes (Underwood et al., 2017). Therefore, future studies of antenatal depression should include the father to further elucidate the combined effects of parental depression on infant outcomes.

The findings in this study have implications not only for future research but also for service development in the field of perinatal mental health. Improving the detection and management of perinatal depression would be a clear recommendation based on the findings in this thesis. My findings also indicate that the mother-infant dyad, rather than the mother alone, should be the focus of specialist perinatal intervention in women experiencing perinatal depression. Further research into foetal programming effects of antenatal depression can help reduce disease burden of future generations.

7.8 Conclusions

Although depression during pregnancy did not have an effect on maternal HPA axis function during pregnancy in this study, there is clear evidence for HPA axis dysregulation in infants born to mothers who were depressed during pregnancy. Antenatal depression was associated with adverse infant birth outcomes including lower gestational age at birth and lower birth weight. Maternal depression during pregnancy was also associated with higher pre- and post-stressor cortisol levels in infants at six months, and maternal cortisol measures during pregnancy predicted infant baseline cortisol levels at six months. This would indicate that maternal HPA axis during pregnancy does indeed have an effect on infant HPA axis measures, despite the absence of overt HPA axis dysfunction in antenatal depression. Additionally, maternal cortisol measures at six months postpartum predicted post-stressor cortisol levels in six-month-old infants. These are novel findings, suggesting that maternal cortisol levels during pregnancy and postpartum work in a synergistic manner to influence infant cortisol levels at six months. This is the first longitudinal perinatal study to report a relationship between ELA and maternal evening cortisol levels during pregnancy, persisting into the postpartum period. Maternal ELA, however, did not have an effect on the infant HPA axis. The novel finding of moderation of the relationship between maternal ELA and pregnancy HPA axis measures by male foetal sex adds to the emerging evidence base for sexually dimorphic foetal effects on maternal physiology during pregnancy. Antenatal depression, therefore, can have significant impact on infant HPA axis and birth outcomes. My results indicate that early recognition and appropriate management of depression in the perinatal period could improve health outcomes of future generations.

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Appendix I: Study Information Flyer



Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin



National
Maternity
Hospital



Coombe
Women's Hospital



Call for pregnant women to participate in clinical study

We need your help...

A study of cortisol in women and their babies



Cortisol is a hormone that rises to very high levels in healthy pregnancy. Cortisol has also been shown to increase in depression. In this study we are measuring cortisol to see if there are any differences in levels between pregnant women who are depressed and those that aren't depressed. We also want to see how your baby's cortisol levels change in the first year after birth.

You are suitable if...

- You are depressed
- You are **not** depressed

Your role in the study...

- Assessment with Dr Chai Jairaj during your pregnancy
- Providing saliva samples for measurement of cortisol levels and a blood sample during your pregnancy

Your baby's role in the study...

- Saliva samples will be collected from your baby at 8 weeks, 6 months, and one year after birth
- Baby's development and play activity will be observed with a 5-minute video, to be analysed by experienced Psychologists

Benefits...

- **€30 travel reimbursement** for taking part in the study!
- FREE developmental and behavioural assessments for your baby in the first year
- Regular contact with Psychiatrist if required and referrals to the service as necessary
- Assisting in medical research by helping us to better understand the development of depression and to improve the treatment and prevention of depression for future generations

If you wish to participate in this study, please contact in confidence...

Dr Chai Jairaj (Research Registrar in Psychiatry): cjairaj@tcd.ie 086 370 1387

Appendix II: Patient Information Sheet



INFORMATION FOR PARTICIPANTS:

Depression during pregnancy: a study of stress hormone systems in women and their babies

Thank you for taking the time to read this Information Sheet. We would be most grateful if you would consider taking part in our research project.

This study is being organized by **Professor Veronica O'Keane**, Consultant Psychiatrist from Trinity College Dublin, **Dr Jennifer Donnelly**, Consultant Obstetrician in the Rotunda Hospital, **Professor Eleanor Molloy**, Consultant Neonatologist in Coombe Women and Infants University Hospital & **Professor Fionnuala McAuliffe**, Consultant Obstetrician in the National Maternity Hospital. This project has approval from the Research Ethics Committee in both the Coombe Women and Infants University Hospital and the National Maternity Hospital.

What is the Research Project about?

Basically, we are looking at the effects of depression during pregnancy on the woman's body and on the developing baby. Feeling stressed is a normal human experience but clinical depression is a more profound and prolonged sadness, hopelessness and loss of enjoyment of almost everything, with accompanying sleep, appetite and energy changes. Depression during pregnancy (antenatal depression) can often be neglected because some symptoms of depression such as poor sleep or appetite and low energy levels may be attributed to normal pregnancy. It is a treatable condition but if left untreated, can persist into the postnatal period. About half of what we call postnatal depression actually begins during pregnancy. If you have any concerns about your mental health, please inform the researchers, your Midwife or your Obstetrician.

We want to study three main issues. First, we want to see if depression during pregnancy is having an effect on the hormone cortisol in pregnant women, which is measured in saliva. Cortisol is a hormone that is released in high quantities during the later stages of pregnancy and is important for fetal development. We would also like to take one blood sample to measure possible immune changes in pregnancy. The cortisol/immune systems can affect the way genes work; this process is called epigenetics and this will be measured also in blood and saliva samples.

Second, we want to see if depression during pregnancy leads to any changes in the baby's cortisol/epigenetic systems after birth. This will be done by collecting baby saliva samples at their vaccinations at 2 months, 6 months and 12 months.

Third, we are looking at your baby's developmental trajectory and interaction at 2 months, 6 months, and 12 months.

Should we have any concerns about this, we will contact your GP, with your consent.

Why am I being asked to participate in this research project?

For our research study, we are looking for pregnant women who are clinically depressed and also those who feel well. You will not directly benefit from participation in this study but the findings from the study will help us to understand what is happening in depression during pregnancy and open up new pathways to treat depression during pregnancy.

What would my role be in this Research Project?

Dr Chai Jairaj will talk you through the study, and explain what is involved. Basically, we would like to measure your cortisol/immune function around the six-month (25 weeks) time-point during pregnancy.

What is specifically involved for me?

We would like to:

1. Talk to you about your mental health and fill in some questionnaires, which will take about 40 min.
2. Take some saliva samples to measure cortisol. We will give you instructions about taking the saliva samples.
3. Take a blood sample (10mL, collected separately to usual antenatal bloods) to measure immune and epigenetic markers. We will collect the blood and saliva at the 6-month time point in pregnancy. This may leave transient minor bruising or pain in the area of blood collection.

What is specifically involved for my baby?

Dr Jairaj will be in contact with you following the birth of your baby. We will access your hospital chart to get details about your baby's health soon after birth – birth weight, head circumference and Apgar score.

1. To measure baby cortisol secretion we will take saliva samples before and 30 minutes after your baby's vaccination injections. Baby saliva is collected by placing a swab under your baby's tongue for two minutes. Either Chai Jairaj or Niamh O'Leary, our baby research worker will meet you at your baby's vaccination appointment at two months, six months and 12 months to take this sample.
2. We may also take a saliva sample from you and your baby at six months to look for any differences between mother and baby in a specific gene (epigenetics).
3. We would like to look at baby development and interaction at each time point. At seven weeks we will look at baby development using the Neonatal Behavioral

Assessment Scale (NBAS) which shows us about baby reflexes and emerging interaction skills. At six and 12 months we will look at your baby's emerging thinking, language and motor skills using the Bayley Scale of Infant Development (BSID). We will also take a three-minute video of you interacting or playing with your baby at each time point. Your details will be kept entirely confidential and this video will only be used for the purpose of the study. If there are any concerns, a report of the video session or infant assessments may be shared with medical practitioners with parental consent.

What are the possible risks, discomforts, side effects or inconveniences?

Should the assessment reveal that you are depressed you will be offered the option of referral to a specialist perinatal psychiatry service in the hospital or your local psychiatrist to discuss possible treatment options.

What measures will be taken to ensure confidentiality?

You will be given a study number and all your information will be stored using this number, rendering it unidentifiable. The key to your data will be held by Prof Veronica O'Keane in a locked facility in Trinity College Dublin. All the saliva and blood samples that we collect from you will be coded and may be stored in Trinity College Dublin for a period of 10 years. We may contact you in the next 10 years to invite you to participate in future research that has obtained appropriate Research Ethics Committee approval. Please note that, in the event of child protection concerns, it is mandatory throughout the HSE to report any concerns to the Rotunda Hospital.

Is there likely to be a benefit to me or will there be potential benefits to improve future medical care?

There is a wide variation in cortisol levels in healthy people, and the same is true during pregnancy. Your cortisol levels are unlikely to tell us anything about how stressed you are as an individual as we do not know enough about pregnancy, depression and cortisol levels to be able to judge your level as normal or not. This is one question that we hope to answer with this study.

If we show that depression in women during pregnancy is changing the developing baby's cortisol system, we want to show that these effects may be reversed in the first year of life.

At the completion of the project, will I be informed of the research outcome?

Yes, we will ask you whether you want to know the results from our project and we will send you on copies of our reports and publications when they are ready.

More Information?

You may wish to discuss participation in this research project with your family and/or with your doctor. Please feel free to ask for further information before deciding if you will take part.

Consent

Participation in this study is voluntary and you may, of course, withdraw from the study at any point and this will not change the care that you receive in any way.

My rights as a Participant

1. I am informed that no information regarding my medical history will be divulged and the results of any tests involving myself will not be published so as to reveal my identity.
2. The detail of the procedure proposed has also been explained to me, including the anticipated length of time it will take, the frequency with which the procedure will be performed and an indication of any discomfort which may be expected.
3. Although I understand that the purpose of this research project is to improve the quality of medical care, it has also been explained that my involvement may not be of any benefit to myself.
4. I have been given the opportunity to have a member of my family or a friend present while the project was explained to me.
5. I understand that this research project has been approved by the Research Ethics Committee of the National Maternity Hospital.
6. I have received a copy of this document for my records.

Name and phone number of emergency contact:

Dr Chai Jairaj 086 370 1387

Appendix III: Consent Form



THE
UNIVERSITY
OF DUBLIN



Consent Form for REDEEM study

Depression during pregnancy: a study of stress hormone systems in women and their babies

Principal Investigators: Professor Fionnuala McAuliffe, Professor Eleanor Molloy, Professor Veronica O'Keane

I have read the Information Leaflet and it has been explained to me by

_____.

I understand the Information Sheet and have had time to consider my participation in the study. I have this Information Sheet to keep and I understand the purpose, extent and possible effects of my involvement. I have been asked whether I would like to have a family member or friend present while this project was being explained to me.

I understand that it is my decision to take part in the study and that if I decide to withdraw from the study at any time without explanation, this will not affect my access to the best available treatment and care from the National Maternity Hospital/Coombe Mother & Infant's University Hospital.

Signed _____

Date _____

Print name _____

Witnessed by _____

Date _____

Print name _____

If you have any concerns or questions regarding the study, please contact: Dr Chai Jairaj 086 370 1387

Appendix IV: Confidential Recruitment Details



TRINITY COLLEGE DUBLIN
COLAISTE NA TRÍONÓIDE

THE
UNIVERSITY
OF DUBLIN



National
Maternity
Hospital



THE
ROTUNDA
HOSPITAL
DUBLIN

CONFIDENTIAL Recruitment Details

Date: _____ MRN: _____

Name: _____

Mobile: _____

DOB: _____ BMI: _____

Address: _____

Email: _____

How far along are you in your pregnancy? _____

When is your estimated delivery date? _____

Do you have any major medical problems? _____

Have you ever been diagnosed with (please tick):

- | | | |
|---|--|--|
| ☐ Anxiety | ☐ Depression | ☐ Other mental disorder |
| ☐ Bipolar disorder | ☐ Schizophrenia | |

Please list all your current medications: _____

Appendix V: Participant Characteristics

Date:
Study #:

PERINATAL DEPRESSION STUDY

NAME: _____

DOB: _____ TEL: _____

ADDRESS: _____
(urban/rural)

ETHNICITY: _____ MARITAL STATUS: _____

PREG INTENT: _____ BMI: _____

GESTATION: _____ EDD: _____

GP DETAILS: _____

- ☐ Informed that GP will be sent study information
- ☐ Would like to be sent study publications

EMPLOYMENT
STATUS: _____

EDUCATION: _____

PAST PSYCH HX: _____

MED HX: _____

OBSTETRIC HX: _____

MEDICATIONS: _____

SUBSTANCE
MISUSE/SMOKING: _____

SOCIAL SUPPORT: _____

DOMESTIC
VIOLENCE: _____

EARLY CHILDHOOD
EXPREIENCES: _____

NEGATIVE LIFE
EVENTS: _____

Appendix VI: Saliva Sample Collection Instructions

Participant Step-by-Step Instructions Saliva Sample Collection

- The purpose of the saliva collection is to measure the changes in stress hormones throughout the day.
- The saliva will be collected by placing the swab provided under your tongue for 1-2 minutes after which you will place the swab in the tube provided. Please see detailed instructions below.
- The tubes will be labelled with time points corresponding to each collection after waking.
- You will have **5 collections** in total. You should put them in the plastic bag provided and place the samples in the refrigerator.
- Please **post the tubes to Dr Chai Jairaj in Trinity College Dublin in the pre-stamped and addressed envelope provided.**

The tubes will be labelled for the specific times below. Please collect saliva at each of these consecutive times after waking for **one** day.

1. 0 minutes (on waking)
2. 30 minutes (after waking)
3. 60 minutes (after waking)
4. 12 hours (after waking, in the pm)
5. 12.5 hours (after waking, in the pm)

For example, if you wake up at 7.00 am, you will be collecting samples at:

1. 7.00 am (approximately)
2. 7.30 am
3. 8.00 am
4. 7.00 pm
5. 7.30 pm

How to take samples:

Take the tube marked with the appropriate time point, remove the blue cap on the tube and then remove the swab in the smaller tube. Place the swab under your tongue for 1-2 minutes. Then return the saturated swab into the smaller tube and close it firmly with the blue top. Please ensure that the saturated swab is placed in the smaller tube that clicks into the larger tube. Place all samples in the refrigerator until you post them.

Please do not brush your teeth prior to collecting the morning samples. Please rinse your mouth out with water 5 min before taking a sample (Please make sure that you only collect saliva and not water from rinsing your mouth out!) If you forget to take a sample, just move on to the next time point.

If you have any questions, please contact Dr Chai Jairaj at 086 370 1387

Appendix VII: Perceived Stress Scale

PSS

Name: _____

Age: _____

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate *how often* you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is, don't try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate.

In the last month...	0 Never	1 Almost Never	2 Some- times	3 Fairly Often	4 Very Often
1. How often have you been upset because of something that happened unexpectedly?					
2. How often have you felt that you were unable to control the important things in your life?					
3. How often have you felt nervous and "stressed"?					
4. How often have you dealt successfully with irritating life hassles?					
5. How often have you felt that you were effectively coping with important changes that were occurring in your life?					
6. How often have you felt confident about your ability to handle your personal problems?					
7. How often have you felt that things were going your way?					
8. How often have you found that you could not cope with all the things that you had to do?					
9. How often have you been able to control irritations in your life?					
10. How often have you felt that you were on top of things?					
11. How often have you been angered because of things that happened that were outside of your control?					
12. How often have you found yourself thinking about things that you have to accomplish?					
13. How often have you been able to control the way you spend your time?					
14. How often have you felt difficulties were piling up so high that you could not overcome them?					

Appendix VIII: Pittsburgh Sleep Quality Index

Pittsburgh Sleep Quality Index (PSQI)

Name: _____

Age: _____

Gender: _____ Male _____ Female

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. When have you usually gotten up in the morning? _____
4. How many hours of actual sleep do you get at night? (This may be different than the number of hours you spend in bed) _____

5. During the past month, how often have you had trouble sleeping because you...	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason:				
6. During the past month, how often have you taken medicine (prescribed or "over the counter" to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)
9. During the past month, how would you rate your sleep quality overall?				

Appendix IX: Centre for Epidemiological Studies Depression Rating Scale

Center for Epidemiological Studies Depression Scale (CES-D), NIMH

Name: _____

Age: _____

Gender: _____ Male _____ Female

	During the Past Week			
	Rarely or none of the time (less than 1 day)	Some or a little of the time (1-2 days)	Occasionally or a moderate amount of time (3-4 days)	Most or all of the time (5-7 days)
1. I was bothered by things that usually don't bother me.				
2. I did not feel like eating; my appetite was poor.				
3. I felt that I could not shake off the blues even with help from my family or friends.				
4. I felt I was just as good as other people.				
5. I had trouble keeping my mind on what I was doing.				
6. I felt depressed.				
7. I felt that everything I did was an effort.				
8. I felt hopeful about the future.				
9. I thought my life had been a failure.				
10. I felt fearful.				
11. My sleep was restless.				
12. I was happy.				
13. I talked less than usual.				
14. I felt lonely.				
15. People were unfriendly.				
16. I enjoyed my life.				
17. I had crying spells.				
18. I felt sad.				
19. I felt that people dislike me.				
20. I could not get "going."				

Appendix X: Childhood Trauma Questionnaire

CTQ

Name: _____

Age: _____

Gender: _____ Male _____ Female

When I was growing up...	Never True	Rarely True	Sometimes True	Often True	Very Often True
1. I didn't have enough to eat.					
2. I knew that there was someone to take care of me and protect me					
3. People in my family called me things like "stupid," "lazy," or "ugly."					
4. My parents were too drunk or high to take care of the family.					
5. There was someone in my family who helped me feel that I was important or special.					
6. I had to wear dirty clothes.					
7. I felt loved.					
8. I thought that my parents wished I had never been born.					
9. I got hit so hard by someone in my family that I had to see a doctor or go to the hospital.					
10. There was nothing I wanted to change about my family.					
11. People in my family hit me so hard that it left me with bruises or marks.					
12. I was punished with a belt, a board, a cord, or some other hard object.					
13. People in my family looked out for each other.					
14. People in my family said hurtful or insulting things to me.					
15. I believe that I was physically abused.					
16. I had the perfect childhood.					
17. I got hit or beaten so badly that it was noticed by someone like a teacher, neighbor, or doctor.					
18. I felt that someone in my family hated me.					
19. People in my family felt close to each other.					
20. Someone tried to touch me in a sexual way, or tried to make me touch them.					
21. Someone threatened to hurt me or tell lies about me unless I did something sexual with them.					
22. I had the best family in the world.					
23. Someone tried to make me do sexual things or watch sexual things.					
24. Someone molested me.					
25. I believe that I was emotionally abused.					
26. There was someone to take me to the doctor if I needed it.					
27. I believe that I was sexually abused.					
28. My family was a source of strength and support.					

Appendix XI: Hamilton Depression Rating Scale

THE HAMILTON RATING SCALE FOR DEPRESSION

(to be administered by a health care professional)

Patient's Name _____

Date of Assessment _____

To rate the severity of depression in patients who are already diagnosed as depressed, administer this questionnaire. The higher the score, the more severe the depression.

For each item, write the correct number on the line next to the item. (Only one response per item)

- _____ **1. DEPRESSED MOOD** (Sadness, hopeless, helpless, worthless)
0= Absent
1= These feeling states indicated only on questioning
2= These feeling states spontaneously reported verbally
3= Communicates feeling states non-verbally—i.e., through facial expression, posture, voice, and tendency to weep
4= Patient reports VIRTUALLY ONLY these feeling states in his spontaneous verbal and non-verbal communication
- _____ **2. FEELINGS OF GUILT**
0= Absent
1= Self reproach, feels he has let people down
2= Ideas of guilt or rumination over past errors or sinful deeds
3= Present illness is a punishment. Delusions of guilt
4= Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations
- _____ **3. SUICIDE**
0= Absent
1= Feels life is not worth living
2= Wishes he were dead or any thoughts of possible death to self
3= Suicidal ideas or gesture
4= Attempts at suicide (any serious attempt rates 4)
- _____ **4. INSOMNIA EARLY**
0= No difficulty falling asleep
1= Complains of occasional difficulty falling asleep—i.e., more than 1/2 hour
2= Complains of nightly difficulty falling asleep
- _____ **5. INSOMNIA MIDDLE**
0= No difficulty
1= Patient complains of being restless and disturbed during the night
2= Waking during the night—any getting out of bed rates 2 (except for purposes of voiding)

Adapted from Hedlung and Vieweg, The Hamilton rating scale for depression, *Journal of Operational Psychiatry*, 1979;10(2):149-165.

6. INSOMNIA LATE

- _____ **0=** No difficulty
1= Waking in early hours of the morning but goes back to sleep
2= Unable to fall asleep again if he gets out of bed

7. WORK AND ACTIVITIES

- _____ **0=** No difficulty
1= Thoughts and feelings of incapacity, fatigue or weakness related to activities; work or hobbies
2= Loss of interest in activity; hobbies or work—either directly reported by patient, or indirect in listlessness, indecision and vacillation (feels he has to push self to work or activities)
3= Decrease in actual time spent in activities or decrease in productivity
4= Stopped working because of present illness

8. RETARDATION: PSYCHOMOTOR (Slowness of thought and speech; impaired ability to concentrate; decreased motor activity)

- _____ **0=** Normal speech and thought
1= Slight retardation at interview
2= Obvious retardation at interview
3= Interview difficult
4= Complete stupor

9. AGITATION

- _____ **0=** None
1= Fidgetiness
2= Playing with hands, hair, etc.
3= Moving about, can't sit still
4= Hand wringing, nail biting, hair-pulling, biting of lips

10. ANXIETY (PSYCHOLOGICAL)

- _____ **0=** No difficulty
1= Subjective tension and irritability
2= Worrying about minor matters
3= Apprehensive attitude apparent in face or speech
4= Fears expressed without questioning

11. ANXIETY SOMATIC: Physiological concomitants of anxiety, (i.e., effects of autonomic overactivity, "butterflies," indigestion, stomach cramps, belching, diarrhea, palpitations, hyperventilation, paresthesia, sweating, flushing, tremor, headache, urinary frequency). Avoid asking about possible medication side effects (i.e., dry mouth, constipation)

- _____ **0=** Absent
1= Mild
2= Moderate
3= Severe
4= Incapacitating
-

12. SOMATIC SYMPTOMS (GASTROINTESTINAL)

_____ **0=** None

1= Loss of appetite but eating without encouragement from others. Food intake about normal

2= Difficulty eating without urging from others. Marked reduction of appetite and food intake

13. SOMATIC SYMPTOMS GENERAL

_____ **0=** None

1= Heaviness in limbs, back or head. Backaches, headache, muscle aches. Loss of energy and fatigability

2= Any clear-cut symptom rates 2

14. GENITAL SYMPTOMS (Symptoms such as: loss of libido; impaired sexual performance; menstrual disturbances)

_____ **0=** Absent

1= Mild

2= Severe

15. HYPOCHONDRIASIS

_____ **0=** Not present

1= Self-absorption (bodily)

2= Preoccupation with health

3= Frequent complaints, requests for help, etc.

4= Hypochondriacal delusions

16. LOSS OF WEIGHT

_____ **A.** When rating by history:

0= No weight loss

1= Probably weight loss associated with present illness

2= Definite (according to patient) weight loss

3= Not assessed

17. INSIGHT

_____ **0=** Acknowledges being depressed and ill

1= Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.

2= Denies being ill at all

18. DIURNAL VARIATION

_____ **A.** Note whether symptoms are worse in morning or evening. If NO diurnal variation, mark none

0= No variation

1= Worse in A.M.

2= Worse in P.M.

_____ **B.** When present, mark the severity of the variation. Mark "None" if NO variation

0= None

1= Mild

2= Severe

19. DEPERSONALIZATION AND DEREALIZATION (Such as: Feelings of unreality;
Nihilistic ideas)

- _____ **0=** Absent
 1= Mild
 2= Moderate
 3= Severe
 4= Incapacitating

20. PARANOID SYMPTOMS

- _____ **0=** None
 1= Suspicious
 2= Ideas of reference
 3= Delusions of reference and persecution

21. OBSESSIONAL AND COMPULSIVE SYMPTOMS

- _____ **0=** Absent
 1= Mild
 2= Severe

Total Score _____

Presented as a service by

GlaxoWellcome

Glaxo Wellcome Inc.
Research Triangle Park, NC 27709
Web site: www.glaxowellcome.com

Appendix XII: Mini International Neuropsychiatric Interview

Patient Name:	_____	Patient Number:	_____
Date of Birth:	_____	Time Interview Began:	_____
Interviewer's Name:	_____	Time Interview Ended:	_____
Date of Interview:	_____	Total Time:	_____

	MODULES	TIME FRAME	MEETS CRITERIA	DSM-IV	ICD-10
A	MAJOR DEPRESSIVE EPISODE	Current (2 weeks)	†	296.20-296.26 Single	F32.x
		Recurrent	†	296.30-296.36 Recurrent	F33.x
	MDE WITH MELANCHOLIC FEATURES	Current (2 weeks)	†	296.20-296.26 Single	F32.x
	Optional			296.30-296.36 Recurrent	F33.x
B	DYSTHYMIA	Current (Past 2 years)	†	300.4	F34.1
C	SUICIDALITY	Current (Past Month)	†		
		Risk: † Low † Medium † High			
D	MANIC EPISODE	Current	†	296.00-296.06	F30.x-F31.9
		Past	†		
	HYPOMANIC EPISODE	Current	†	296.80-296.89	F31.8-F31.9/F34.0
		Past	†		
E	PANIC DISORDER	Current (Past Month)	†	300.01/300.21	F40.01-F41.0
		Lifetime	†		
F	AGORAPHOBIA	Current	†	300.22	F40.00
G	SOCIAL PHOBIA (Social Anxiety Disorder)	Current (Past Month)	†	300.23	F40.1
H	OBSESSIVE-COMPULSIVE DISORDER	Current (Past Month)	†	300.3	F42.8
I	POSTTRAUMATIC STRESS DISORDER	Current (Past Month)	†	309.81	F43.1
J	ALCOHOL DEPENDENCE	Past 12 Months	†	303.9	F10.2x
		Past 12 Months	†	305.00	F10.1
K	SUBSTANCE DEPENDENCE (Non-alcohol)	Past 12 Months	†	304.00-.90/305.20-.90	F11.1-F19.1
		Past 12 Months	†	304.00-.90/305.20-.90	F11.1-F19.1
L	PSYCHOTIC DISORDERS	Lifetime	†	295.10-295.90/297.1/ 297.3/293.81/293.82/ 293.89/298.8/298.9	F20.xx-F29
		Current	†		
	MOOD DISORDER WITH PSYCHOTIC FEATURES	Lifetime	†	296.24/296.34/296.44	F32.3/F33.3/ F30.2/F31.2/F31.5 F31.8/F31.9/F39
		Current	†	296.24/296.34/296.44	
M	ANOREXIA NERVOSA	Current (Past 3 Months)	†	307.1	F50.0
N	BULIMIA NERVOSA	Current (Past 3 Months)	†	307.51	F50.2
		ANOREXIA NERVOSA, BINGE EATING/PURGING TYPE	†	307.1	F50.0

O	GENERALIZED ANXIETY DISORDER	Current (Past 6 Months)	†	300.02	F41.1
P	ANTISOCIAL PERSONALITY DISORDER Optional	Lifetime	†	301.7	F60.2

Which problem troubles you the most? Indicate your response by checking the appropriate check box(es). _____ ↑

A. MAJOR DEPRESSIVE EPISODE

(MEANS : GO TO THE DIAGNOSTIC BOXES, CIRCLE **NO** IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

A1	Have you been consistently depressed or down, most of the day, nearly every day, for the past two weeks?	NO	YES
A2	In the past two weeks, have you been much less interested in most things or much less able to enjoy the things you used to enjoy most of the time?	NO	YES
	IS A1 OR A2 CODED YES?	NO	YES

A3 Over the past two weeks, when you felt depressed or uninterested:

- | | | |
|---|----|-------|
| a Was your appetite decreased or increased nearly every day? Did your weight decrease or increase without trying intentionally (i.e., by $\pm 5\%$ of body weight or ± 8 lbs. or ± 3.5 kgs., for a 160 lb./70 kg. person in a month)?
<small>IF YES TO EITHER, CODE YES.</small> | NO | YES * |
| b Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)? | NO | YES |
| c Did you talk or move more slowly than normal or were you fidgety, restless or having trouble sitting still almost every day? | NO | YES * |
| d Did you feel tired or without energy almost every day? | NO | YES |
| e Did you feel worthless or guilty almost every day? | NO | YES |
| f Did you have difficulty concentrating or making decisions almost every day? | NO | YES |
| g Did you repeatedly consider hurting yourself, feel suicidal, or wish that you were dead? | NO | YES |

ARE **5** OR MORE ANSWERS (**A1-A3**) CODED **YES**?

NO **YES ***

***MAJOR DEPRESSIVE
EPISODE, CURRENT***

IF PATIENT HAS CURRENT MAJOR DEPRESSIVE EPISODE CONTINUE TO A4,
OTHERWISE MOVE TO MODULE B:

- | | | |
|--|----|-----|
| A4 a During your lifetime, did you have other episodes of two weeks or more when you felt depressed or uninterested in most things, and had most of the problems we just talked about? | NO | YES |
|--|----|-----|

- | | | |
|---|--|--|
| b In between 2 episodes of depression, did you ever have an interval of at least 2 months, without any depression and any loss of interest? | | |
|---|--|--|

NO **YES**

***MAJOR DEPRESSIVE
EPISODE, RECURRENT***

* If patient has Major Depressive Episode, Current, use this information in coding the corresponding questions on page 5 (A6d, A6e).

MAJOR DEPRESSIVE EPISODE WITH MELANCHOLIC FEATURES (optional)

(MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE NO, AND MOVE TO THE NEXT MODULE)

IF THE PATIENT CODES POSITIVE FOR A CURRENT MAJOR DEPRESSIVE EPISODE (**A3 = YES**), EXPLORE THE FOLLOWING:

A5	a	During the most severe period of the current depressive episode, did you lose almost completely your ability to enjoy nearly everything?	NO	YES
	b	During the most severe period of the current depressive episode, did you lose your ability to respond to things that previously gave you pleasure, or cheered you up? IF NO: When something good happens does it fail to make you feel better, even temporarily?	NO	YES
		IS EITHER A5a OR A5b CODED YES ?	NO	YES

A6 Over the past two week period, when you felt depressed and uninterested:

a	Did you feel depressed in a way that is different from the kind of feeling you experience when someone close to you dies?	NO	YES
b	Did you feel regularly worse in the morning, almost every day?	NO	YES
c	Did you wake up at least 2 hours before the usual time of awakening and have difficulty getting back to sleep, almost every day?	NO	YES
d	IS A3c CODED YES (PSYCHOMOTOR RETARDATION OR AGITATION)?	NO	YES
e	IS A3a CODED YES FOR ANOREXIA OR WEIGHT LOSS?	NO	YES
f	Did you feel excessive guilt or guilt out of proportion to the reality of the situation?	NO	YES

ARE **3** OR MORE **A6** ANSWERS CODED **YES**?

NO	YES
Major Depressive Episode with Melancholic Features Current	

B. DYSTHYMIA

(MEANS : GO TO THE DIAGNOSTIC BOX, CIRCLE **NO**, AND MOVE TO THE NEXT MODULE)

IF PATIENT'S SYMPTOMS CURRENTLY MEET CRITERIA FOR MAJOR DEPRESSIVE EPISODE, DO NOT EXPLORE THIS MODULE.

B1	Have you felt sad, low or depressed most of the time for the last two years?	NO	YES
B2	Was this period interrupted by your feeling OK for two months or more?	NO	YES
B3	During this period of feeling depressed most of the time:		
a	Did your appetite change significantly?	NO	YES
b	Did you have trouble sleeping or sleep excessively?	NO	YES
c	Did you feel tired or without energy?	NO	YES
d	Did you lose your self-confidence?	NO	YES
e	Did you have trouble concentrating or making decisions?	NO	YES
f	Did you feel hopeless?	NO	YES
	ARE 2 OR MORE B3 ANSWERS CODED YES ?	NO	YES

B4Did the symptoms of depression cause you significant distress or impair
your ability to function at work, socially, or in some other important way?

NO	YES
<i>DYSTHYMIA</i>	
<i>CURRENT</i>	

C. SUICIDALITY

In the past month did you:

				Points
C1	Suffer any accident?	NO	YES	0
	IF NO TO C1, SKIP TO C2; IF YES, ASK C1a,:			
C1a	Plan or intend to hurt yourself in that accident either passively or actively?	NO	YES	0
	IF NO TO C1a, SKIP TO C2; IF YES, ASK C1b,:			
C1b	Did you intend to die as a result of this accident?	NO	YES	0
C2	Think that you would be better off dead or wish you were dead?	NO	YES	1
C3	Want to harm yourself or to hurt or to injure yourself?	NO	YES	2
C4	Think about suicide?	NO	YES	6

IF YES, ASK ABOUT THE INTENSITY AND FREQUENCY OF THE SUICIDAL IDEATION:

Frequency

Intensity

Occasionally	Mild
Often	Moderate
Very often	Severe

Can you control these impulses
and state that you will not act
on them while in this program?

Only score 8 points if response is NO. NO YES 8

C5	Have a suicide plan?	NO	YES	8
C6	Take any active steps to prepare to injure yourself or to prepare for a suicide attempt in which you expected or intended to die?	NO	YES	9
C7	Deliberately injure yourself without intending to kill yourself?	NO	YES	4
C8	Attempt suicide?	NO	YES	10
	Hoped to be rescued / survive			
	Expected / intended to die			

In your lifetime:

C9	Did you ever make a suicide attempt?	NO	YES	4
----	--------------------------------------	----	-----	---

IS AT LEAST **1** OF THE ABOVE (EXCEPT C1) CODED **YES**?

IF YES, ADD THE TOTAL NUMBER OF POINTS FOR THE ANSWERS (C1-C9)
CHECKED 'YES' AND SPECIFY THE LEVEL OF SUICIDE RISK AS
INDICATED IN THE DIAGNOSTIC BOX:

MAKE ANY ADDITIONAL COMMENTS ABOUT YOUR ASSESSMENT
OF THIS PATIENT'S CURRENT AND NEAR FUTURE SUICIDE RISK IN
THE SPACE BELOW:

NO YES

***SUICIDE RISK
CURRENT***

1-8 points Low
9-16 points Moderate
≥ 17 points High