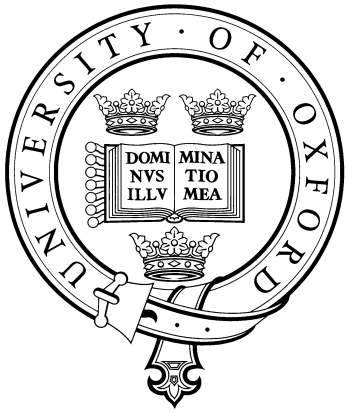
Does Prenatal Maternal Depression Predict Foetal and Infant Development? A Study of Mothers and Infants in Rural South India



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To José Estevam Remedios Fernandes and Christine Ethel Fernandes

Abstract

Introduction

Prenatal maternal depression is associated with an increased risk of psychopathology in childhood. The understanding of the mechanisms underlying this association is limited. Further, despite high rates of prenatal depression in the developing world, no research investigating this issue exists from these settings.

Objectives

The primary objectives of this thesis are to study the association between prenatal maternal depression and the following early offspring outcomes in a non-smoking, non-alcohol consuming prenatal sample from rural, South India:

i. Foetal stress responsivity, measured through foetal heart rate (FHR)
ii. Infant stress responsivity, measured through infant cortisol response to immunisation
iii. Infant temperament.

Methods

194 pregnant women from Solur, India were assessed for depression. The first 67 mothers with elevated symptoms of prenatal depression and the first 66 controls underwent FHR monitoring to study foetal stress responsivity. 58 mother-infant dyads returned at 1.5-3 months post birth. Infant salivary cortisol was measured before and after immunisation. Information on infant temperament and maternal postnatal depression (PND) was also collected.

Results

Twenty nine mothers (14.9%) met a diagnosis of major depression during pregnancy while 67 (34.5%) had elevated symptoms of prenatal depression.

Whilst there were no linear association between prenatal depression and foetal responsivity, a curvilinear (U shaped) association existed with the foetuses of mothers with very high and very low levels of prenatal depression having elevated stress responses compared to those with moderate levels of prenatal depression.

Prenatal depression predicted infant cortisol responsivity independent of PND (B=13.08, p=0.02).The relationship between infant cortisol responsivity and prenatal depression was also U shaped.

There was no association between prenatal depression and infant temperament.

Conclusions

This is the first study from the developing world investigating the relationship between prenatal depression and offspring outcomes. It provides evidence suggestive of the programming influence of prenatal depression on the developing offspring.

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LIST OF ABBREVIATIONS USED IN THIS THESIS

∑AUC	Summation of the area under the curve
11BHSD2	11 beta hyrdoxy steroid dehydrogenase 2
ACTH	Adrenocorticotrophic hormone
ANOVA	Analysis of variance
AUC	Area under the curve
AVP	Arginine vasopressin
BBV	Beat to beat variability in foetal heart rate
BE	Beta endorphin
BMI	Body mass index
bpm	Beats per minute
BSID	Bayley Scales of Infant Development
CIDI	Composite International Diagnostic Interview
CNS	Central nervous system
CRH	Corticotrophin releasing hormone
DoHaD	Developmental origins of health and disease
DPT Vaccine	Diphtheria, pertussis and tetanus vaccine
DSM-IV TR	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision
ECL	Electrochemiluminescence
EPDS	The Edinburgh Postnatal Depression Scale
FHR	Foetal heart rate
FM	Foetal movements
g/dL	Grams per decilitre
GBP (£)	Great Britain Pound
GIMP	GNU Image Manipulation Program
HPA axis	Hypothalamic-Pituitary-Adrenal Axis
IBQ	Infant Behaviour Questionnaire
ICD-10	The International Statistical Classification of Diseases and Related Health Problems, 10th Revision
INR	Indian Rupee
K10	The Kessler 10 Scale of Psychological Distress
LAMICs	Low and middle income countries
LBW	Low Birth weight
min.	Minute
MINIPlus	The Mini International Neuropsychiatric Interview
mL	Millilitre
mm	Millimetre
nmol/l	Nano mol per litre
NA	Noradrenaline
NIS	National Immunisation Schedule of India
NST	Non stress test
pCRH	Placental corticotrophin releasing hormone
PND	Postnatal depression
PPD	Postpartum depression
RCT	Randomised Controlled Trial
SCID	Structured Clinical Interview for DSM Disorders
SD	Standard Deviation
SMBP	The Solur Mother and Baby Project
SSQ	Social support questionnaire
WHO	The World Health Organisation
µL	Microliter
χ^2	Chi-square

CHAPTER 1
INTRODUCTION

1 INTRODUCTION

'The history of man for the nine months preceding his birth would, probably, be far more interesting, and contain events of greater moment than all the three score and ten years that follow it.'

Samuel Taylor Coleridge

Depression is one of the most commonly encountered psychiatric conditions (Desai and Jann 2000). According to WHO estimates, depression is projected to be one of the leading causes of disability globally in 2020, second only to ischemic heart disease (Murray and Lopez 1996). Although the lifetime prevalence of depression in the general population is estimated at 6-17% (Blazer, Kessler et al. 1994; Kessler, Barker et al. 2003), it is well established that women report higher rates of the disorder during their childbearing years (21.3%) compared to men (12.7%) (Kessler, Zhao et al. 1997; Kessler 2003).

Depression is an important cause of morbidity, disability and mortality among women. It is the single leading cause of years lived with disability (Moussavi, Chatterji et al. 2007). Depression has been shown to have the potential to interfere with the subject's ability to function in a personal and societal context, and to fulfil social roles including those relating to motherhood and childcare. Not only is it associated with higher health-care costs than any other disorder in the primary health care setting (Sartorium, Ustun et al. 1996), but depression bears broader social costs in the contexts of the family and the society. In addition, it often occurs as a co-morbid condition with other psychiatric disorders, particularly substance abuse.

The period surrounding pregnancy and childbirth is of special importance in the context of depression. This is not only because women are more likely to develop symptoms during this time (Kumar and Robson 1984; O'Hara, Neunaber et al. 1984), but because young offspring have been shown to be sensitive to the effects of maternal depression. Depression during pregnancy and in the postnatal period is a well-established risk factor for disturbances in offspring development. Previous research has focussed on the effects of postnatal maternal depression on child development and has reported associations with delayed cognitive and language skills and, a higher incidence of behavioural and sleep problems (Murray and Cooper 1997; Beck 1998). Recently, evidence from animal and human literature suggests that the association between maternal mental health and disturbances in offspring development may begin even before birth (Talge, Neal et al. 2007).

Foetal and infant life are periods of considerable developmental plasticity during which the growth and differentiation of various organ systems is coupled with the early origins of motor, sensory and learning behaviour (Hepper 2006). As the developing organism draws on stimuli from its external environment to drive this process, it is evolutionarily conditioned to be sensitive to environmental cues. The combination of ontogenic sensitivity and developmental plasticity make foetal and infant life periods of increased developmental vulnerability to teratogenic external influences. In recent years, there has been increased interest in the potential negative effect of maternal psychological stress during pregnancy (particularly prenatal maternal depression and anxiety) on foetal and long-term outcomes.

1.1 PRENATAL DEPRESSION AND OFFSPRING DEVELOPMENT

A considerable body of evidence, drawing from animal and human literature, has reported an association between prenatal maternal depression and offspring development. Prenatal maternal stress has been associated with short attention spans, poor motor development and increased distress to novelty in non-human primates (Schneider 1992 (a); Schneider 1992 (b); Schneider and Coe 1993; Schneider, Roughton et al. 1999). Increased aggression, anxiety and fearfulness have been noted in the offspring of pregnant rodents exposed to laboratory induced stress (Chapillon, Patin et al. 2002). Similar associations have been noted in prospective studies in humans. In particular, prenatal maternal depression and anxiety have been associated with more difficult temperament, poorer cognitive and language skills, and an increase in behavioural and emotional problems during childhood (Glover and O'Connor 2002; Van den Bergh 2004; Austin, Hadzi-Pavlovic et al. 2005; Talge, Neal et al. 2007). It has also been found to predict preterm delivery and low birth weight (Lundy, Jones et al. 1999; Field, Diego et al. 2004). More recently, the effects of prenatal depression on disturbances in foetal growth and neurodevelopment have also been reported (Allister 2001; Dieter, Field et al. 2001; DiPietro, Costigan et al. 2003; DiPietro, Kivlighan et al. 2010).

The association between prenatal depression and aberrant offspring development has largely been attributed to the 'programming effects' of maternal depression on the developing neural system. One of the key neuronal substrates for these programming effects is the Hypothalamic Pituitary-Adrenal (HPA) axis of the developing offspring (Talge, Neal et al. 2007). The HPA axis initiates and regulates the organism's response to stress (Fig. 1) and is extremely sensitive to changes in the intra-uterine environment during early development. In prenatally stressed mothers, these changes in the intra-uterine milieu include an increased level of cortisol and placental corticotrophin releasing hormone (pCRH) in the amniotic fluid,

decreased utero-placental blood flow following maternal sympathetic activation in response to prenatal stress and the teratogenic consequences of high risk behaviours seen in depressed women particularly poor nutrition, prenatal smoking, alcohol consumption and drug abuse (Talge, Neal et al. 2007).

Studies investigating stress responses in the offspring of prenatally depressed mothers have provided evidence suggestive of the programming influence of prenatal depression on the offspring's HPA axis. The offspring of prenatally stressed rodents have been found to display higher adrenocorticotropic hormone (ACTH) and cortisol at baseline and elevated responses in the same following exposure to laboratory induced stressors (Takahashi, Kalin et al. 1988; Takahashi and Kalin 1991; Takahashi, Haglin et al. 1992; Henry and Kabbaj 1994; Egliston, McMahon et al. 2007). Human inquiries report higher baseline cortisol levels during infancy and at 10 years in the offspring of prenatally depressed and anxious mothers (Lundy, Jones et al. 1999; Field, Diego et al. 2004; O'Connor, Ben-Shlomo et al. 2005). Further, maternal peripartum depression has been found to predict infant cortisol reactivity to stress (Brennan, Pargas et al. 2008). Prenatal depression has also been found to influence indirect measures of the foetal stress responsivity, namely foetal cardiac habituation to induced maternal stress or vibroacoustic stimuli (Allister 2001; DiPietro, Costigan et al. 2003; Sandman, Glynn et al. 2003).

Although the influence of prenatal maternal depression on the development of the offspring has been well documented, the mechanisms governing this association are as yet not fully understood. The Barker hypothesis and the foetal programming hypotheses both provide a pioneering insight into these effects.

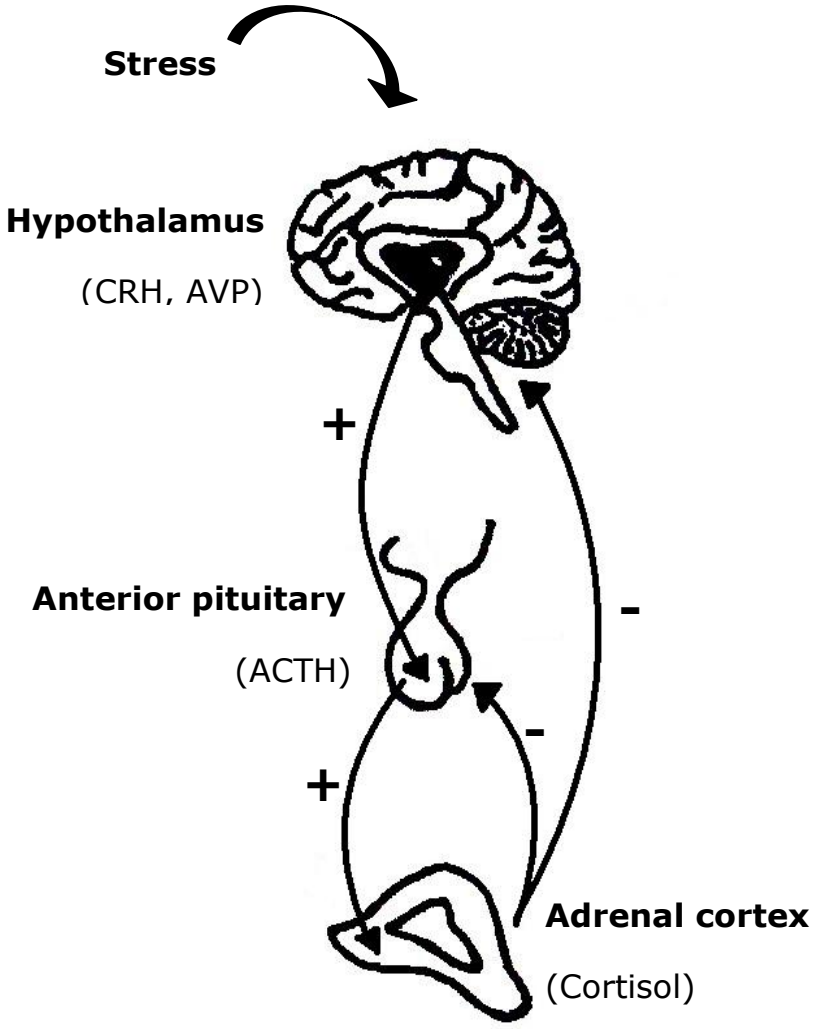

Fig. 1 Schematic representation of the Hypothalamic-pituitary-adrenal (UCLA 22/06/2011) (HPA) axis.
Note: CRH = corticotrophin releasing hormone, AVP = arginine vasopressin, ACTH = adrenocorticotropin releasing hormone.

The Barker Hypothesis

The Barker hypothesis stems from David Barker's findings of an association between low birth weight and later risk of cardiovascular disease in the UK in 1986 (Barker, Osmond et al. 1989). The theory contends that the risk for adult disease has antecedents in early life. It suggests that early environmental experiences (including those during intra-uterine life) such

as low levels of nutrition, result in metabolic adaptations that increase the risk for disorders such as diabetes mellitus, hypercholesterolemia and cardiovascular disease in later life (Barker 2001). This phenomenon, also referred to as the 'thrifty phenotype hypothesis', explains the developmental origins of health as a consequence of early adaptations to environmental cues that alter the developmental trajectory in order to aid survival (Barker 2001; Hales and Barker 2001). This theory therefore also explains the developmental origins of disease as the consequence of adaptation to early environmental cues that are not in congruence with the later environment.

The Foetal Programming Hypothesis

The foetal programming hypothesis (Gluckman, Hanson et al. 2005; DiPietro, Novak et al. 2006) further explains the developmental origins of health and disease (DoHaD) as a consequence of neuronal plasticity during intra-uterine life. During certain 'windows of sensitivity', the developing embryo or foetus adapts itself to the intra-uterine milieu in preparation for its survival and optimal functioning after birth. This is termed a 'predictive adaptive response' and has been hypothesised to confer the individual with a developmental advantage from an evolutionary perspective (Glover 2011). However, altered intra-uterine environments, as seen in conditions of poor prenatal nutrition, maternal alcohol consumption and cigarette smoking during pregnancy, and maternal psychological stress, could result in programming effects on foetal organ systems with potentially negative consequences. The foetal programming hypothesis contends that it is this altered 'programming' of the foetus that leads to an increased risk for subsequent health problems during childhood and later life.

1.2 PRENATAL DEPRESSION AND ANXIETY: QUESTIONS OF COMORBIDITY

There has been much debate about the categorisation of depression and anxiety as separate diagnostic classes in official nosologies (Mineka, Watson et al. 1998). While some researchers assert these disorders as single entities, others propose a more unitary concept arguing that high rates of comorbidity and similar emotional and temporal features suggest a single underlying dimension of psychological distress (Hodges 1990; Brady and Kendall 1992; Feldman 1993; Mineka, Watson et al. 1998). Nearly 50% of individuals with major depression have been found to suffer from comorbidity; with anxiety, substance abuse and eating disorders occurring most frequently (Sanderson, Beck et al. 1990; Kendler, Gallagher et al. 1996). In an epidemiological survey of 20,291 individuals in the USA almost half (47.2%) of those with a major depressive disorder met criteria for a comorbid anxiety disorder (Rieger, Pirke et al. 2004). Comorbidity has also been associated with increased levels of psychopathology and poorer psychosocial functioning than either depression or anxiety alone (Kendler, Gallagher et al. 1996; Mineka, Watson et al. 1998).

High levels of comorbidity have been reported between maternal anxiety and depression in the postnatal period. Postnatal depression scores at 14 and 30 weeks post birth have been reported to be strongly correlated with anxiety scores at the same points in time ($r_{14\ weeks}$= 0.48, $p<0.001$ and $r_{30\ weeks}$=0.82, $p<0.001$) (Stuart, Couser et al. 1998). Concurrent maternal depression and anxiety has also been associated with more adverse developmental outcomes in children than depression only. Comorbid depression and anxiety postnatally has been associated with lower levels of emotional availability in mothers and an increased risk of insecure infant attachment at 14 months as compared to groups with depression only and controls (Carter, Garrity-Rokous et al. 2001).

Although the comorbidity between depression and anxiety has been well established, more research has been done on the effect of prenatal anxiety (rather than prenatal depression) on child outcomes (Van den Bergh, Mulder et al. 2005). Prenatal anxiety has been associated with lower orientation scores in neonates, increased reports of infant crying and difficult infant temperament at 7 months and lower mean mental and motor scores in infants at 12 months (Van den Bergh 1990; Brouwers, van Baar et al. 2001) . The effect of prenatal anxiety on child development has been reported to be somewhat stronger than that of prenatal depression (Van den Bergh, Mulder et al. 2005). Prenatal anxiety has been found to predict difficult infant temperament at 4-6 months (Austin, Hadzi-Pavlovic et al. 2005) and behavioural and emotional problems in children at 4 years (O'Connor, Heron et al. 2002) independent of prenatal depression and postnatal maternal mood. However, a number of inquiries focussing on prenatal depression rather than prenatal anxiety have found the former to be associated with less optimal neonatal behaviour, increased infant crying and more difficult infant temperament. A summary of these studies is presented in Chapter 2.4.

This thesis focuses on the investigation of prenatal maternal depression rather than maternal anxiety in the context of foetal and infant development in rural South India. This is because previous inquiries from the Indian sub-continent have focussed on the former and therefore prevalence estimates for the prenatal depression rather than anxiety exist from these settings. Further, screening measures for prenatal depression, particularly the Edinburgh Postnatal Depression Scale, have been previously used in Indian populations (Chandran, Tharyan et al. 2002; Patel, Rodrigues et al. 2002; Rahman, Iqbal et al. 2003) while the use of questionnaire measures for prenatal anxiety has not been documented. Nevertheless, because of the high degree of overlap between symptoms of depression and anxiety, a screening measure for

general psychological distress (the Kessler 10 Scale) including items for both depression and anxiety, was included in the study protocol.

1.3 RATIONALE FOR THIS THESIS

There are few prospective inquiries that trace the programming effects of prenatal depression on the offspring from prenatal to postnatal life. Some studies have sought to understand the mechanisms by which prenatal maternal depression influences the offspring by measuring these effects as early as possible, even before birth. Based on the findings of these reports, a number of mechanisms have been hypothesised, however, evidence is limited. In addition, a number of methodological considerations limit the interpretation of these findings. These include small sample sizes, difficulties in the non-invasive, indirect assessment of foetuses, inadequate control for confounding influences (particularly maternal smoking and alcohol consumption during pregnancy) and the use of only psychological or biological measures in assessing offspring outcomes rather than a combination of both. Of particular importance is the fact that all studies investigating these effects have been carried out in the western world. This is despite higher rates of prenatal depression (20-45%) being reported from the developing world (Da-Silva, Moraes-Santos et al. 1998; Rahman, Bunn et al. 2007) in comparison to the developed world (7-12%) (Bennett, Einarson et al. 2004). Further 90% of the world's children aged under 5 years (2.9 billion) and 80% of the world's pregnancies (168.8 million) occur in the developing world (United Nations Report 2009). The generalisabilty of findings from western populations to developing world populations may be limited due to differences in the patterns of psychological stress, environmental stressors (such as poverty and food security) and parenting encountered in these settings.

1.4 AIMS OF THE THESIS

The objectives of this thesis are three-fold. First, this thesis aims to contribute to the understanding of the mechanisms governing the association between prenatal depression and offspring development by investigating the effect of prenatal maternal depression on foetal and infant stress responsivity. This is done by assessing foetal cardiac responses to a stressor, i.e. vibroacoustic stimulation, and infant cortisol responsivity to immunisation. Second, this thesis seeks to address the dearth of literature from the developing world by investigating, for the first time, the influence of prenatal maternal depression on the aforementioned offspring outcomes, and infant temperament, in a study population from rural, South India. Third, this thesis attempts to control for the influences of maternal smoking and alcohol consumption during pregnancy (which have confounded previous studies) by carrying out the inquiry in an entirely non-smoking, non-alcohol consuming prenatal population.

1.5 STRUCTURE OF THE THESIS

This thesis is structured at two levels, a phase-driven design and a hypothesis-oriented approach (Fig 2). The following chapter (Chapter 2) outlines previous work in the field using a hypothesis-driven approach and includes sub-sections on prenatal depression effects on foetal responsivity, infant cortisol responsivity, infant temperament and birth outcome. Chapter 3 states the aims and objectives of the thesis. Chapter 4 describes the materials and methods in a phase-driven template. The methodology is described based on the time points of assessment in the prospective design i.e. a prenatal phase (at ≥28 week gestation), a birth phase and a postnatal phase (1.5-3 months post-birth). A brief overview of the statistical analyses performed in the thesis is described in this chapter. The findings of the thesis are

reported in Chapter 5 in a hypothesis-oriented approach. Each of the six sub-sections reports the findings from the specific hypothesis investigated (for example, the association between prenatal maternal depression and foetal stress responsivity) and consists of its own introduction, details on data processing and specific statistical analyses conducted, the findings and a brief discussion of the same. These individual findings are discussed in context with each other and the broader goals of the thesis in the final discussion in Chapter 6. The conclusions of the thesis are then presented in Chapter 7.

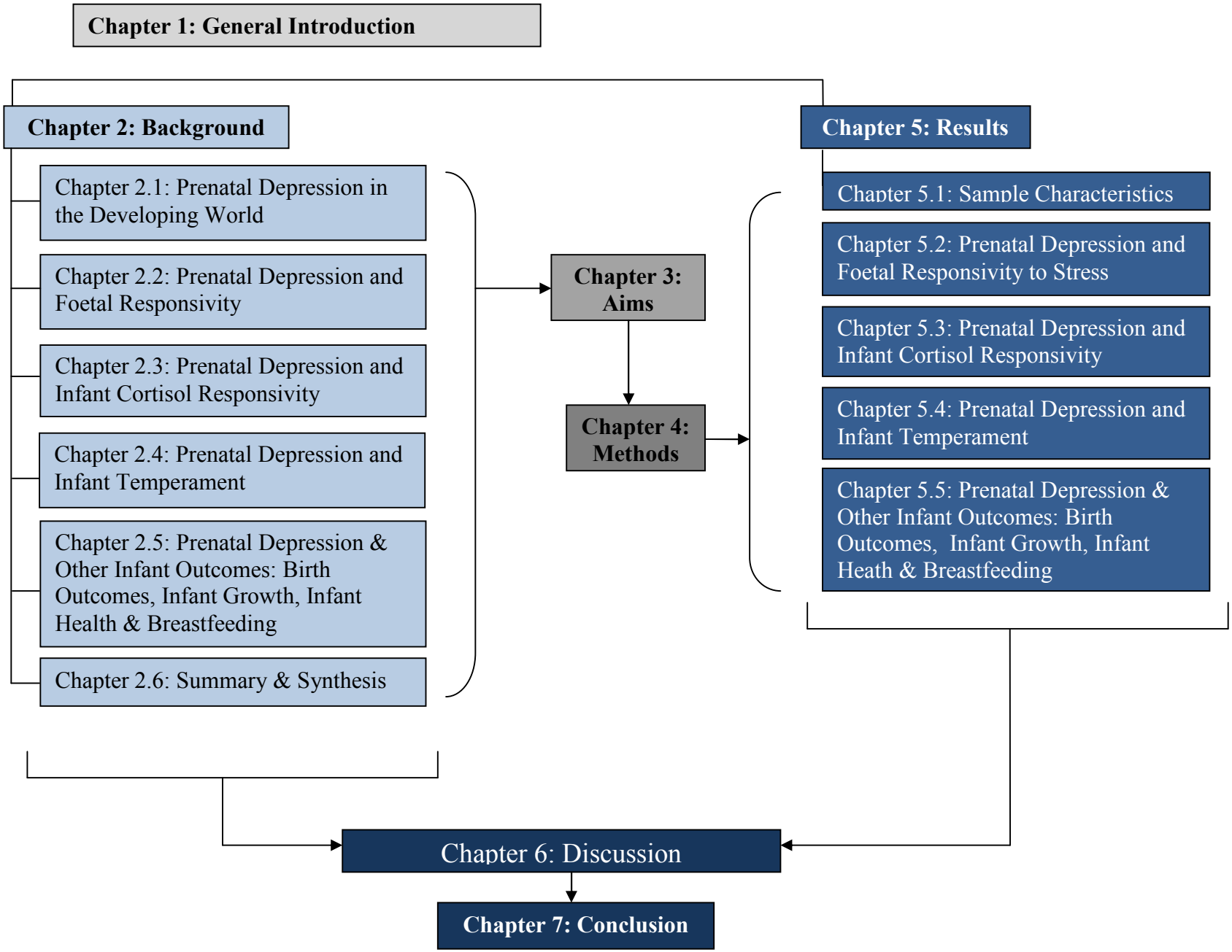

Fig. 2 Schematic representation of structure of the thesis.

CHAPTER 2
BACKGROUND

2.1 PRENATAL DEPRESSION IN THE INDIAN SUBCONTINENT

2.1.1 INTRODUCTION

Depression is the most common psychiatric condition occurring in women of childbearing age. The disorder significantly impairs the functioning of the individual and affects a substantial number of women across the world. Inquiries from western literature have reported that 6.5% to 12.9% of women develop symptoms of postnatal depression (PND) within 4 weeks of giving birth (Gavin, Gaynes et al. 2005). Data from low and middle income countries (LAMICs)[a] however report higher prevalence rates of PND ranging from 28-36% in Pakistan (Husain, Creed et al. 2000), 22% in Bangladesh (Gausia, Fisher et al. 2009) and 7.2-25% in China (Wang, Jiang et al. 2003). Rates of 13-37.1% in sub-Saharan Africa (Lawrie, Hofmeyr et al. 1998; Tesfaye, Hanlon et al. 2010), 10.2-50% in Chile (Florenzano, Botto et al. 2002; Jadresic, Nguyen et al. 2007) and 11.4-56% in Brazil (Surkan, Kawachi et al. 2008) have also been reported.

There are few epidemiological investigations into the prevalence of PND in India. A study of 359 women from Tamil Nadu in South India reported a prevalence of 11% while another study from the coastal state of Goa found 28% of women to develop PND (Chandran, Tharyan et al. 2002; Patel, Rodrigues et al. 2002).

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[a]The categorisation of countries into low income (<$1,005), middle income ($ 1,006-$12,275)and high income ($12,276) are based on their gross national income (GNI) per capita using criteria established by the World Bank (http://go.worldbank.org/K2CKM78CC0).

Recent attention to the developmental origins of child health and disease has increased interest in prenatal maternal depression and its effect on child development. Further, prenatal depressive symptoms have been known to predate postnatal depression in approximately two-thirds of women (O'Hara, Neunaber et al. 1984; Chandran, Tharyan et al. 2002; Patel, Rodrigues et al. 2002; Rahman, Iqbal et al. 2003). Most investigations into the prevalence of prenatal depression have however emanated largely from the western world. Third trimester estimates of prenatal depression range from 5% in France (Verdoux, Sutter et al. 2002) and 15.1% in England (Evans, Heron et al. 2001) to 17.4% in Sweden (Josefsson, Berg et al. 2001) and 19.6% in the United States (O'Heron 1991; Bennett, Einarson et al. 2004). The number of inquiries from the developing world, however, are limited. A study from Hong Kong reported a prevalence of 8.7% (Chung, Lau et al. 2001) while 37.9% of Brazilian women from a low socio-economic group were reported to suffer from prenatal depression (Da-Silva, Moraes-Santos et al. 1998).

2.1.2 Prevalence of Prenatal Depression in the Indian Sub-Continent

There are few reliable inquiries on which to determine an overall prevalence rate of prenatal depression for the Indian sub-continent. In a study of 359 women of low socio-economic status from a rural development block in the South Indian town of Vellore, 58 met criteria for an ICD-10 diagnosis of depression during their third trimester of pregnancy yielding a prevalence rate of 16.2% (Chandran, Tharyan et al. 2002). In another study of mothers from Goa, India, 42.06% of the sample scored high for symptoms of prenatal depression on screening questionnaires (Patel, Rodrigues et al. 2002). In a rural community based sample in Pakistan, 25% of women in the third trimester of pregnancy met criteria for an ICD-10 diagnosis of prenatal depression (Rahman 2007). In a rural community in Bangladesh, 33% of

pregnant women scored high for symptoms of prenatal depression on screening questionnaires while 14% admitted to contemplating suicide during their pregnancy (Gausia, Fisher et al. 2009). A recent report from Lahore, Pakistan found 42.7% of urban Pakistani women to have prenatal depression (Imran and Haider 2010). A comparison of the prevalence rates of prenatal depression between Indian sub-continent and the western world are presented in Fig 3 below.

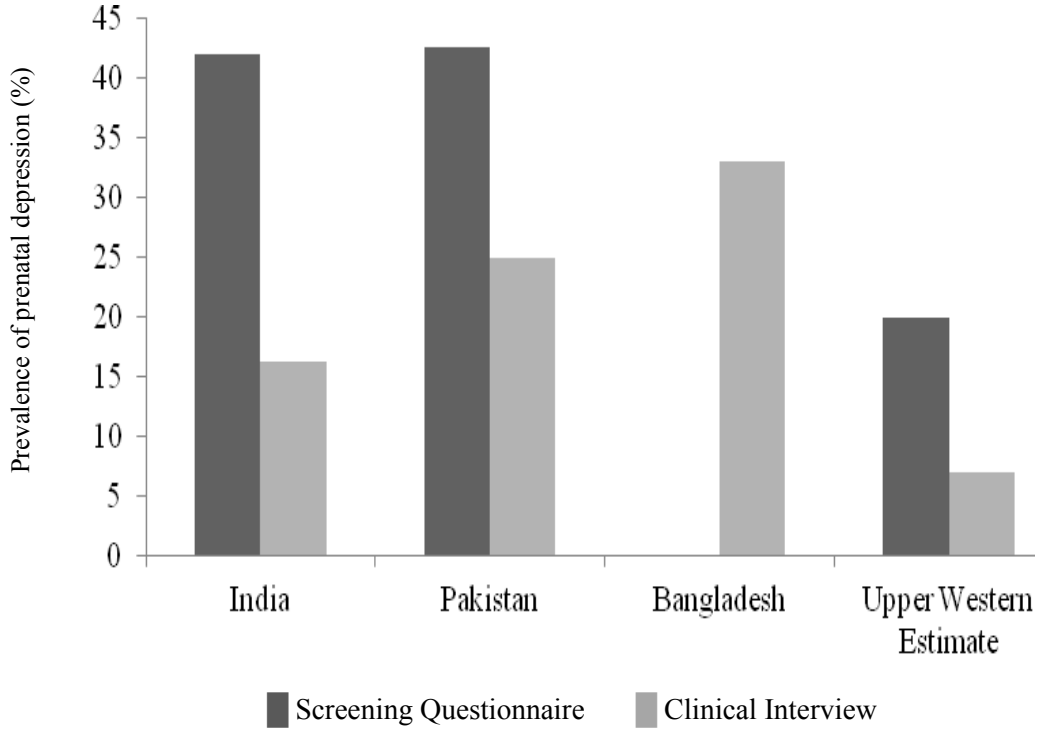


Fig. 3 Comparison of the prevalence of prenatal depression between the Indian Sub-continent and the Western World.

Note: Supporting references for prevalence rates: India (Chandran, Tharyan et al. 2002; Patel, Rodrigues et al. 2002), Pakistan (Rahman 2007; Imran and Haider 2010), Bangladesh (Gausia, Fisher et al. 2009), Western World (Bennett, Einarson et al. 2004). Clinical interview data for the prevalence of prenatal depression in Bangladesh was not available.

2.1.3 RISK FACTORS FOR THE DEVELOPMENT OF PRENATAL DEPRESSION AMONG INDIAN WOMEN

The higher prevalence of prenatal depression among women in low and middle income countries may reflect increased exposure of the prenatal population in these settings to multiple depression-related risk factors. While some of these risk factors for prenatal depression exist in both the developing and western world, high levels of poverty, low levels of education, and increased exposure to conflicts, violence and diseases are specific to developing world populations. However, exposure to certain socio-cultural stressors such as the bias against the birth of a female child is more specific to the Indian Sub-continent, the Middle East and East Asia.

The important risk factors for the development of prenatal depression in the Indian Subcontinent are summarised in Table 1. Financial hardship as reflected through poverty, hunger due to insufficient money to buy food and low levels of maternal and paternal education are well established risk factors for prenatal depression in both the western world and the developing world.

Social adversity, particularly physical abuse by the intimate partner has emerged as one of the most significant predictors of perinatal depression in both developing and western populations. In a sample from rural Bangladesh, 6% of women were repeatedly physically abused by their husbands during their current pregnancy (Gausia, Fisher et al. 2009). Spousal violence and marital discord were important predictors of suicidal ideation among pregnant women in this sample. Higher rates are reported from India where 14-46% of women report physical abuse during pregnancy, the violence being directed to the pregnant woman's

abdomen in a large proportion of cases (Jejeebhoy 1998; Purwar, Jeyaseelan et al. 1999; Varma, Chandra et al. 2007). The explanations for this phenomenon appear to be rooted in the cultural traditions of these patriarchal societies where male dominance and spousal abuse are tolerated. Low levels of education and the lack of financial independence further make women vulnerable to domestic violence in these settings. In India, a common tradition of '*pathibhakti'* (devotion to husbands) constrains women from reporting spousal abuse, seeking help or returning to their parental homes (Chandran, Tharyan et al. 2002). This is further complicated by the practise of living in a joint family with the husband's parents and brothers, their wives and children. In such cases verbal and physical abuse by the mother-in-law and sisters-in-law is not uncommon and an important risk factor for prenatal depression (Patel, Rodrigues et al. 2002; Gausia, Fisher et al. 2009; Imran and Haider 2010).

2.1.4 PRENATAL DEPRESSION IN THE INDIAN SUBCONTINENT: VARYING CONTEXTS

There are a number of contexts in which prenatal depression differs between developing world populations and western populations. As summarised above, nearly one in four women from the developing world suffer from prenatal depression as compared to one in ten of their western counterparts. However not only is a higher prevalence of prenatal depression reported from LAMICs, the rate of recovery during the postnatal period has been reported to be lower than those seen in western populations (Rahman 2007).

The socio-economic stressors operating in the developing world differ from those in western settings. High levels of poverty and economic stress and simultaneous exposure to multiple risk factors such as conflict, violence, migration and infectious diseases, particularly HIV

Risk Factor	Supporting Reference
Socio-economic Factors	
Poverty[b]	Patel et al 2002, 2006; Rahman et al 2003; Rahman and Creed 2007
Maternal Employment	Patel et al 2002
Low levels of maternal and paternal education[b]	Patel et al 2002; Rahman and Creed 2007; Gausia et al 2009
Having 5 or more children	Rahman and Creed 2007
Socio-cultural Factors	
Problems with in-laws	Rahman and Creed 2007; Gausia et al 2009; Patel et al 2002; Imran and Haider 2010
Family's preference for a male child	Gausia et al 2009; Patel et al 2002; Dhillon et al 2010
Given birth to a female child previously	Patel et al 2002
Poor relationship with parents	Imran and Haider 2010
Lack of help at home	Patel et al 2002; Gausia et al 2009
Social Support Factors	
Inadequate social support[b]	Patel et al 2002; Dhillon et al 2010
Marital discord[b]	Patel et al 2002 ;Rahman et al 2003; Imran and Haider 2010
Domestic violence[b]	Patel et al 2002; Gausia et al 2009; Imran and Haider 2010
Alcoholism in a family member	Patel 2002
Miscellaneous Factors	
Previous history of mental illness[b]	Imran and Haider 2010; Gausia et al 2009; Dhillon et al 2010
Unplanned pregnancy[b]	Patel et al 2002; Gausia et al 2009; Dhillon et al 2010

[b] These risk factors are also reported in western populations.

Table 1 Risk factors for the development of prenatal depression in the Indian Sub-Continent.

AIDS, make women from LAMICs more vulnerable to depression that those from the high income countries (Wachs, Black et al. 2009; Lund, Breen et al. 2010). Lower levels of education and financial independence, poorer nutrition, limited control over their reproductive health and the stigma associated with mental illness in the developing world places women at higher risk of developing depression in these settings (Wachs, Black et al. 2009).

Certain socio-cultural factors specific to the Indian subcontinent contribute to increasing the risk of prenatal depression in Indian women. An important example is the preference for a son – this tradition is deeply rooted in South East Asian societies and has historical connections with family economics, the dowry system and the performance of funeral rites. The pressure to give birth to a male child is an important independent risk factor for prenatal depression in the Indian subcontinent (Patel, Rodrigues et al. 2002; Gausia, Fisher et al. 2009). An Indian woman who has given birth to a female child is 1.6 times more likely to be depressed in her subsequent pregnancy than a woman who has given birth to a male child (Patel, Rodrigues et al. 2002). Unsurprisingly, the birth of a male child is a protective factor against the development of postnatal depression in India (Patel, Rodrigues et al. 2002). No such association between maternal depression and sex of the child has been reported from the western world (Robertson, Grace et al. 2004). Another example is that of the joint family system according to which Indian mothers reside in their husband's house along with their spouse's extended family. Strained family relationships, particularly with the mother-in-law, assume greater importance as a risk factor for the development of depression (Chandran, Tharyan et al. 2002). This is compounded by the fact that the pregnant woman may be unable to carry out household chores as before because of the physical symptoms of pregnancy including morning sickness, fatigue and body ache. The lack of physical help in household

tasks and parental support further increases the woman's risk for depression (Chandran, Tharyan et al. 2002). No analogous association exists from the western world.

Thus not only are higher rates of socio-economic adversity and spousal abuse reported from the developing world, cultural-specific stressors such as the bias against a female child and abuse by the mother-in-law are also prevalent in Indian societies. These influences may multiply the risk for depression in women, particularly during pregnancy when the woman is unable to perform physical tasks as before and when the family's preference for the sex of the child becomes apparent.

2.1.5 CAVEATS IN THE INVESTIGATION OF PRENATAL DEPRESSION IN INDIA

The limited inquiries investigating prenatal depression in the Indian subcontinent have yielded impressive results given the methodological limitation of working with culturally heterogeneous, rural samples spanned across 1.7 million square miles and speaking more than 20 official languages. However, there are gaps in our understanding of prenatal depression in the context of the Indian subcontinent. The lack of screening measures designed for prenatal populations in the developing world and the use of western questionnaires in rural, developing world settings raise questions about the reliability of these instruments in measuring prenatal depression in a different socio-cultural context. The limited use of structured clinical interview schedules and the difficulties encountered in the translation of culture-specific items into vernacular languages further compromise the reliability of findings.

There are two important caveats in the investigation of prenatal depression specific to Indian inquiries. First, samples are drawn from a specific city, village or district and are not

necessarily representative of the general population of a country as culturally and ethnically diverse as India. Evidence of such diversity is found not only between states but within different districts of the larger states themselves. Studies involving large samples drawn from different parts of the country and representing all major population sub-groups need to be undertaken. Second, the stigma associated with mental illness in India prevents women with depression from reporting symptoms and seeking help. This would contribute to an under-estimation of the prevalence of prenatal depression in the country. These two issues are difficult to address even in studies employing large sample sizes and of a methodologically sound design.

It is particularly important to undertake such studies in developing world settings where high rates of social adversity are encountered and where effective mothering is an important predictor of the child's well-being and survival in the first year of life.

2.1.6 Prenatal Depression from the Perspective of the Developing World

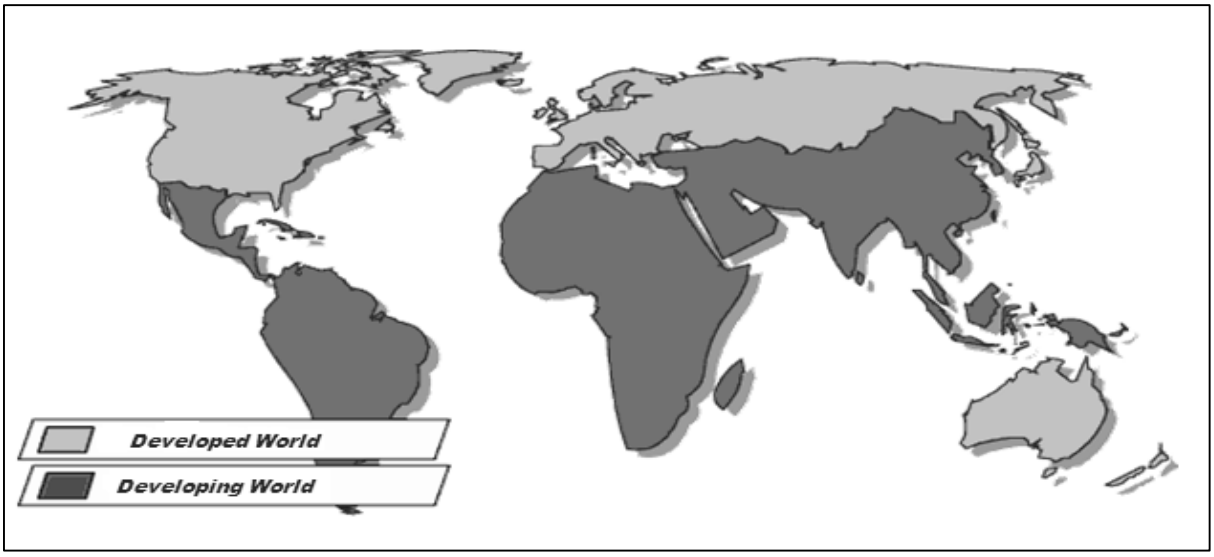
More than 90% of the world's population of children live in developing countries (Parsons, Young et al. 2011). Not only does the developing world report higher rates of prenatal depression, but a significantly larger proportion of the world's pregnancies occur here compared to the western world. These result in more than ten times the number of foetuses being exposed to the effect of maternal depression during intra-uterine life in the developing world compared to western settings (Fig. 4).

India is home to 447 million of the word's 3.2 billion children (UNICEF 2005-2009; United Nations Report 2009), 126 million of which are under 5 years of age. An epidemiological

survey conducted in 2005 reported 12.5% of the children in the city of Bangalore to suffer from psychiatric problems (Srinath 2005). The study found 1.8% of children ages below 4 years and 0.2% of children between the ages of 4 and 16 years to be diagnosed with behavioural and emotional disorders.

2.1.7 CONCLUSIONS

The estimates of prenatal depression from the developing world are higher than those in the western world. High rates of prenatal depression (11-46%) are reported from India (Chandran, Tharyan et al. 2002; Patel, Rodrigues et al. 2002). The effect of prenatal depression on child outcome has not been addressed in the developing world in general and particularly in the Indian subcontinent. This is despite the fact that the majority of the world's children live in the developing world, the greater proportion of the world's pregnancies occur in LAMICs and higher rates of social adversity are reported from these settings.



	Developed World	Developing World
Prevalence of Prenatal Depression (Patel, Rodrigues et al. 2002; Rahman, Iqbal et al. 2003; Bennett, Einarson et al. 2004)	10-20%	25-40%
Number of global pregnancies annually (%) (United Nations Report 2009)	42.2 million (20%)	168.8 million (80%)
Number of children (%) (United Nations Report 2009)	0.3 billion (9.4%)	2.9 billion (90.6%)
Lower estimate of number of foetuses exposed to the effects of prenatal depression annually (%)	4.2 million (9%)	42.2 million (91%)

Fig. 4 Comparison of the prevalence of prenatal depression, number of births and number of foetuses exposed to the effects of prenatal depression between the developed and developing world (United Nations Report; World Fertility Data 2008 (United Nations Report 2009). Image taken from the BBC (BBC 2011).

2.2 PRENATAL DEPRESSION AND FOETAL CARDIAC RESPONSIVITY

2.2.1 INTRODUCTION

Recent literature has provided evidence of an association between prenatal maternal depression and aberrant foetal responses to stimulation. Due to the methodological limitations encountered in studying foetal behaviour using non-invasive techniques, inquiries have relied on assessments of foetal heart rate, foetal movement and sleep as indirect measures of foetal responsivity. The use of foetal heart rate responses to a stressor has emerged as a useful paradigm to study intra-uterine foetal stress responses.

In the following sub-section, a brief overview of the embryology and neurophysiology of the foetal heart is presented. This is followed by a discussion of the findings of studies reporting associations between prenatal maternal depression and foetal cardiac responsivity, and the mechanisms underlying these effects.

2.2.2 EMBRYOLOGY OF THE FOETAL HEART

The embryonic[c] heart develops from the splanchnic mesenchyme in the third week of gestational life. The process begins with the development of two endocardial tubes in the cardiogenic region of the trilaminar germ disc located at the cranial end of the embryo (Fig. 5). These tubes undergo considerable folding and fuse together into a single heart tube by the 21st-22nd day of gestation. The primitive heart thus begins to beat by the 22nd day of gestation (Hsu 2004).

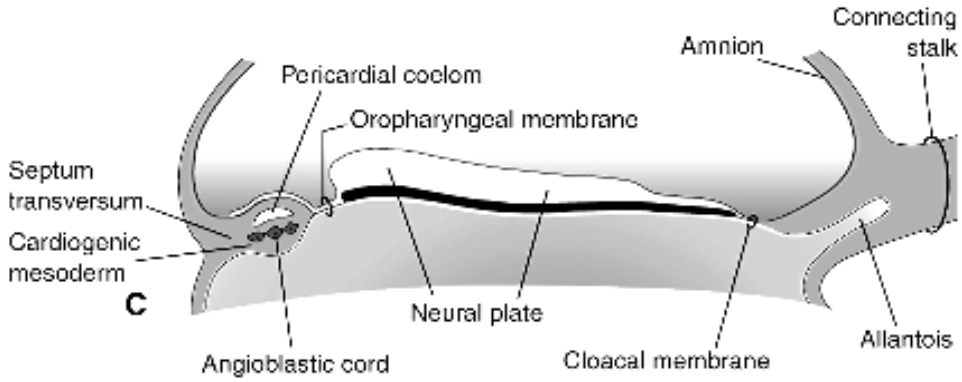


Fig. 5 Development of heart tube from the cardiogenic mesoderm (week 3 of gestation) (Columbia.edu 2006)

A series of constrictions divide the heart tube into a number of primitive chambers consisting of the sinus venosus, primitive atria, ventricle and bulbus cordis. The sinus venosus receives

[c]The germinal period is described as from conception till 2 weeks of gestational life. The embryonic period is described as from the 2nd to the 8th week of gestational life. The foetal period is described as from the 8th week of gestational life till birth (Hepper 2006).

blood from the vitelline veins (from the yolk sac), the umbilical veins (from the placenta) and the common cardinal veins (that return blood to the heart from the body of the embryo).

The vitelline veins develop into the portal circulation and the common cardinal veins develop into the caval system in adult life, however the umbilical veins are obliterated after birth. The bulbus cordis continues caudally as the truncus arteriosus and the conus cordis. The aortic sac, aortic arches and subsequently the aorta arise from the former while the pulmonary arteries arise from the latter.

The bulbus cordis and the primitive ventricle grow faster than other structures and form the bulboventricular loop. The two primitive atria fuse to form a single common atrium between the 22^{nd} – 28^{th} day of gestation giving rise to the two-chambered foetal heart (Fig. 6). This is followed by the development of two septae in the common atrium, the first referred to as the septum primum and the second, which develops a few weeks later, called the septum secondum. The septum secondum persists as the inter-atrial septum in adult life. During foetal life however, an opening in this septum called the foramen ovale shunts oxygenated blood from the right atrium to the left atrium thus by-passing the non-functional foetal lungs. The pressure of the baby's first breath causes the foramen ovale to close immediately after birth in most cases.

By day 28 the ventricular septum begins to grow upwards from the ventricular floor dividing the common ventricle into two. Around the same time, the endocardial cushions appear. These give rise to most of the cardiac skeleton that divide the heart into the atrial and ventricular sides (the atrio-ventricular septum).

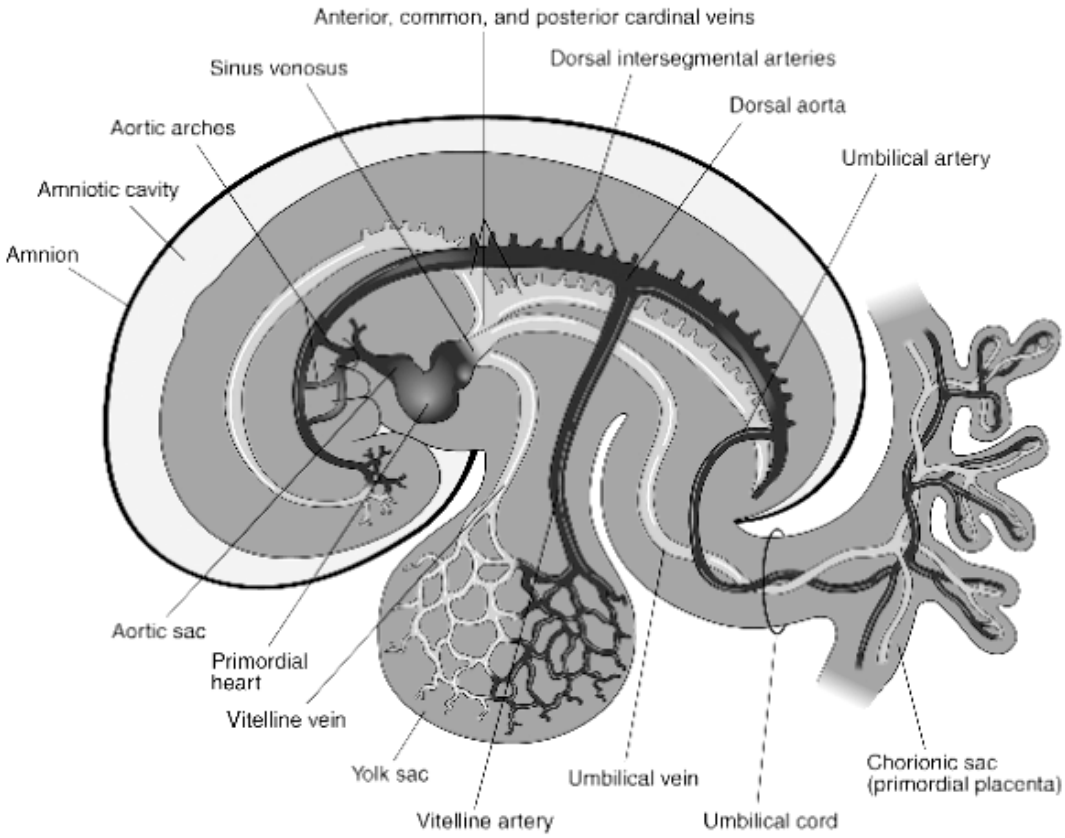


Fig. 6 Development of two chambered embryonic heart [Week 4 of gestation; (Columbia.edu 2006)].

The final large morphological change to take place in the foetal heart is the partition of the outflow tract. The truncus arteriosus develops into the aorta and the conus cordis develops into the pulmonary arteries early in the eight week of foetal life. Thus by week 8 the development of the four chambered embryonic heart is complete (Fig. 7).

2.2.3 FOETAL CIRCULATION

The foetal circulation differs considerably from adult circulation in that blood by-passes entry into the lungs (Fig. 7). This is because the foetal heart largely receives oxygenated blood (via the umbilical veins) and the foetal lungs are non-functional during intra-uterine life.

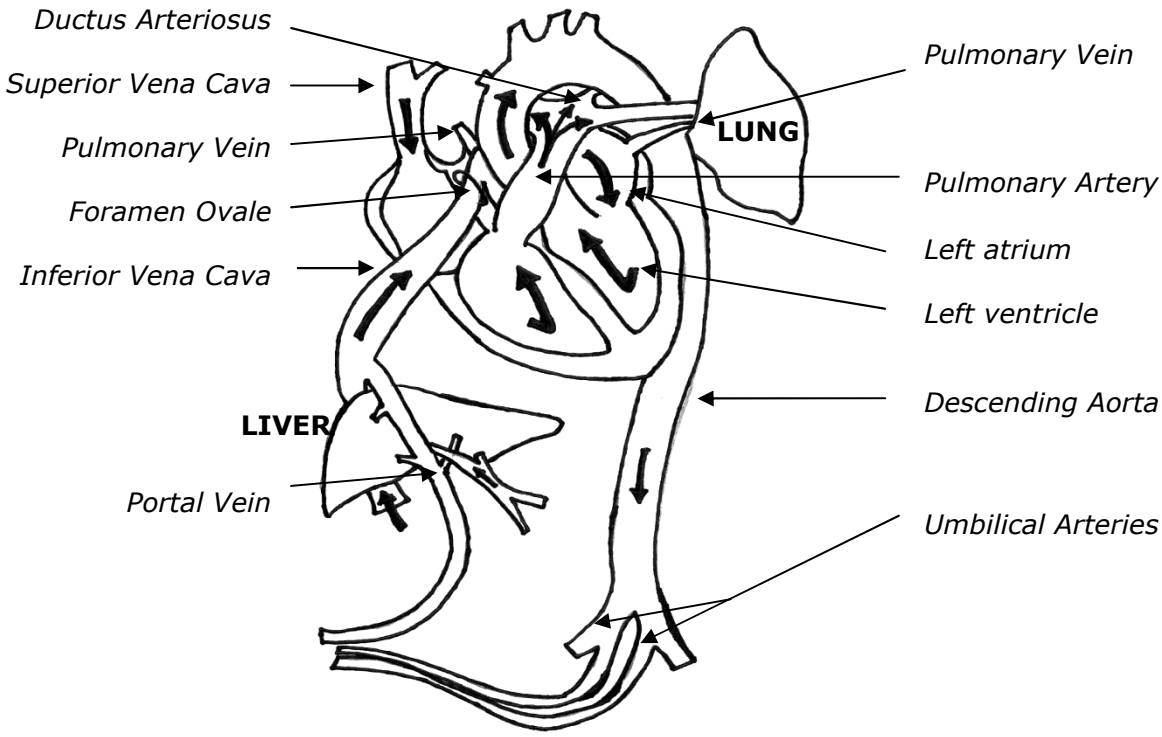


Fig. 7 Foetal circulation and schematic depiction of four chambered embryonic heart.

Oxygenated blood from the placenta (~80% saturated) and a small amount of deoxygenated blood from foetal extremities are delivered to the right atrium of the foetal heart through the inferior vena cava. Most of this blood passes through the formen ovale into the left atrium and then to the left ventricle. A small proportion of blood flows by way of the right ventricle into the pulmonary trunk. This blood is returned by the pulmonary veins into the left atrium and

mixes with the oxygenated blood received from the right atrium reducing oxygen saturation to ∼58%. From the left ventricle, blood enters the ascending aorta and foetal circulation (Richtsmeier 1999).

2.2.4 FACTORS CONTROLLING FOETAL HEART RATE (FHR)

The foetal heart has three intrinsic centres of pacemaker activity. The fastest pacemaker is the sinoatrial node, located in the wall of the right atrium. The next fastest centres of pacemaker activity are the atrioventricular node and the ventricle (Nageotte and Gilstrap 2009). The foetal heart is similar to the adult heart in that it is supplied by both the sympathetic and parasympathetic components of the autonomic nervous system. The precise period of embryonic development when nervous innervation of the foetal heart begins is not known.

The Sympathetic Nervous System

The sympathetic nerves originate in the lateral horn of the spinal cord and innervate the musculature of the heart. The sympathetic nervous system has two important influences on FHR:

1. The exertion of a constant, tonic stimulus maintaining foetal heart rate at a high level under normal conditions.
2. The provision of a reserve mechanism to improve cardiac performance, myocardial contractility and cardiac output during intermittent stressful conditions.

Normally, there is a tonic sympathetic influence on the foetal heart however this may increase as much as two-fold during foetal hypoxia (Nageotte and Gilstrap 2009).

The Parasympathetic Nervous System

The parasympathetic nervous system consists of the vagus nerve (the 10th cranial nerve originating in the medulla oblongata) and innervates the sinoatrial and atrioventricular nodes. The vagal innervation of the foetal heart has two important functions:

1. A tonic influence whereby vagal stimulation decreases the intrinsically high foetal heart rate maintained through the influence of the sympathetic nervous system.
2. An oscillatory stimulus which is responsible for beat to beat variability in FHR.

The influence of vagal tone increases as the gestational age of the foetus progresses. An increase in vagal influence on FHR is also observed in conditions of acute foetal hypoxia (Nageotte and Gilstrap 2009).

Chemoreceptors and Baroreceptors

Central and peripheral chemoreceptors are present in the medulla oblongata, and the arch of aorta and carotid sinus, respectively. In the adult, they are known to respond to an increase in carbon-dioxide and a decrease in oxygen saturation in blood by causing a reflex increase in heart rate and blood pressure. Their role in the foetus is, however, poorly understood (Nageotte and Gilstrap 2009).

Baroreceptors are sensitive to changes in arterial blood pressure and are located in the arch of aorta and the carotid sinus. They function via the vagus and glossopharyngeal nerve to decrease heart rate in response to elevated blood pressure (Nageotte and Gilstrap 2009).

The functioning of chemoreceptors and baroreceptors represent protective attempts by the body to maintain homeostasis in conditions of destabilisation.

Central Nervous System

The medulla oblongata contains the vasomotor centres that are responsible for the integration of the numerous central and peripheral inputs that influence foetal heart rate. The processing of these varied inputs result in the net foetal heart rate and variability measured during prenatal assessments (Nageotte and Gilstrap 2009).

Hormonal Regulation of Foetal Heart Rate

Adrenaline and noradrenaline produced by the foetal adrenals in response to stress increases FHR, cardiac output and blood pressure. Umbilical blood flow and the patency of the ductus arteriosus is maintained by various prostaglandins and arachidonic acid metabolites. Angiotensin II, nitric oxide, atrial natriuretic hormone, neuropeptide Y, thyrotropin-releasing hormone and cortisol also participate in the regulation of the foetal heart (Nageotte and Gilstrap 2009).

2.2.5 MEASUREMENT OF FOETAL HEART RATE

The measurement of foetal heart rate through external devices is one of the most common methods of foetal surveillance. Since their introduction 35 years ago (Nageotte and Gilstrap 2009), these methods have been widely employed in clinical and research settings to detect foetal compromise resulting from foetal hypoxia, acidosis and cardiac dysfunction. Decisions based on assessments of foetal heart rate contribute to the clinical management and treatment of women during late gestation and parturition. Foetal heart rate is measured either in isolation (as seen in low-resource settings) or in combination with other techniques (such as ultrasound foetal examination and tocography) to assess foetal well-being. External

measurements of foetal heart rate are non-invasive, easy to use and economical. They facilitate continuous monitoring of the foetus without any adverse effects.

The measurement of foetal heart rate by means of an ultrasound transducer placed on the mother's abdomen during the maternal resting state is termed a Non Stress Test (NST). The ultrasound transducer works on the principle of the Doppler effect. High frequency sound waves produced by the transducer are reflected back by the mechanical action of the foetal heart and converted into electrical signals by the FHR Monitor.

Foetuses are classified as healthy if the prenatal NST is 'reactive'. A reactive NST is one in which at least two accelerations in FHR lasting a minimum of 15 seconds and rising a minimum of 15 beats/min above the established baseline FHR occur during 20 minutes of assessment. Most healthy, term foetuses demonstrate reactive NSTs (Tucker, Miller et al. 2009). Approximately 12% and 6% of third trimester foetuses do not meet criteria for reactive NSTs at 30 and 40 minutes post-assessment respectively (Harman 2009). Non-reactive NSTs are seen in conditions of foetal compromise, central nervous system abnormalities and maternal drug ingestion and warrant urgent medical attention. Falsely reassuring NSTs occur at the rate of 4-5 per 1000 (Nageotte and Gilstrap 2009).

Components of Foetal Heart Rate:

The following are the components of the foetal heart are measured during continuous FHR monitoring:

I. Baseline FHR

The normal baseline FHR is between 110-160 beats per minute (bpm) (Tucker, Miller et al. 2009). Rates <110 bpm and >160 bpm are termed bradycardia and tachycardia

respectively. Bradycardia is seen in cases of severe foetal hypoxia and compromises in utero-placental blood flow. Tachycardia is observed during maternal and foetal infection and foetal exposure to maternally consumed drugs such as cocaine and methamphetamine.

II. Beat to Beat Variability (BBV)

Baseline BBV are fluctuations in the baseline FHR of 2 cycles per minute (6-25 bpm) or greater (Tucker, Miller et al. 2009). Absent or minimal BBV are seen in conditions of progressive foetal hypoxia, acidemia and blunted autonomic regulation of FHR.

III. Accelerations

An acceleration in FHR is defined as an abrupt increase in FHR at least 15bpm above baseline FHR lasting for at least 15 seconds from the onset to return to baseline (Tucker, Miller et al. 2009). Accelerations indicate adequate foetal oxygenation and normal autonomic regulation of FHR. Accelerations in FHR occur due to the release of catecholamines and autonomic stimulation. They commonly precede or occur along with foetal movement (FM) (Tucker, Miller et al. 2009). Concordance between FHR accelerations and FM are an indicator of foetal central nervous system (CNS) maturity (DiPietro, Costigan et al. 2002b).

IV. Decelerations

Decelerations are decreases in FHR at least 15 bpm below baseline FHR. They are categorised as early, late and variable decelerations depending upon their time of onset (Tucker, Miller et al. 2009). Early and late decelerations are gradual in onset and associated with uterine contractions. Variable decelerations begin abruptly and are

unrelated to uterine contractions. Early decelerations have no relationship to foetal oxygenation. Late and variable decelerations however represent a protective foetal response to transient ischemia.

Habituation in Foetal Heart Rate Responses to Potentially Stressful Stimuli:

Habituation is the progressive decrease in response by an organism when it is repeatedly stimulated. It is a basic form of learning and represents an intact and fully functioning central nervous system. Habituation measured through FHR responses has been investigated since the 1980s as a marker of foetal CNS integrity. In a study of 40 term foetuses near term, Leader et al found highly significant differences in foetal heart rate habituation patterns to vibroacoustic stimuli between high risk and normal pregnancies ($p<0.01$) (Leader, Baillie et al. 1982; Leo R. Leader 1982). Foetal movement habituation has been shown to predict postnatal behaviour at 18 and 36 months of age (Leader and Dore 1979).

2.2.6 PRENATAL DEPRESSION: ASSOCIATIONS WITH FOETAL CARDIAC RESPONSES

The assessment of foetal heart rate is a useful measure to study the effects of prenatal depression on foetal development and cardiac responsivity using non-invasive techniques. Although previous reports provide evidence suggestive of the programming influence of prenatal maternal depression on postnatal offspring outcomes, such as temperament, behaviour, cognitive performance and language skills (Talge, Neal et al. 2007), there is limited substantiation of these influences on the foetus. This is attributed to the methodological challenges of intra-uterine assessments. These include the limited availability of non-invasive techniques to assess foetal responsivity, the restricted ability to collect

biological samples (such as plasma and saliva) from foetuses, the inability to assess foetal behavioural responses to laboratory induced stressful tasks (such as those employed during postnatal assessments) and the ethical constraints of exposing foetuses to stressors. Nevertheless, two techniques have emerged as useful measures to assess foetal responses non-invasively – the use of real time ultrasound and the measurement of foetal heart rate responses to a stressor.

The use of real time ultrasound allows the assessment of foetal behavioural states by measuring foetal movement (FM), eye opening, sleep and breathing patterns. However, the technique is expensive and requires specialised training. The duration of assessments is long (1-2 hours) and findings depend on the expertise and experience of the sonologist. The technique is thus not suitable for research in low-income settings.

The use of FHR monitoring at baseline and in response to an external stressor (such as a vibroacoustic stimulus) has emerged as a useful paradigm to assess foetal responsivity in-utero. The technique utilises a FHR monitor, commonly available in most prenatal clinics, and does not require a high degree of specialised training. FHR traces are digitalised and analysed by computer software and not subject to rater biases. Further, biological variables constructed from FHR data afford a quantification of the foetal response to the stressors. In this paradigm, foetuses are either stimulated directly using a vibroacoustic stimulus or indirectly by inducing maternal cognitive stimulation.

Summary of Previous Literature

There have been six studies that have investigated the association between prenatal psychological distress and foetal cardiac responsivity to stress in humans. In addition two studies have investigated the association of maternal neuroendocrinological markers of

psychopathology with foetal cardiac responsivity to a vibroacoustic stimlus. The findings of these studies are summarised in Table 2.

In the only study to have investigated the association between prenatal depression and foetal cardiac responses to vibroacoustic stimulation, Allister and colleagues reported elevated FHR in the foetuses of depressed mothers compared to controls (Allister 2001). This association remained significant during baseline, stimulation and post-stimulation periods. As compared to controls, the foetuses of depressed mothers display a 3.5 fold decrease in FHR in the time taken to return to baseline during the recovery period.

Monk and colleagues have investigated the influence of prenatal anxiety on foetal cardiac responses to maternal cognitive stimulation (Monk, Fifer et al. 2000; Monk, Myers et al. 2003). In the first study, the authors reported greater increases in FHR during the stimulation period in the foetuses of anxious mothers compared to controls (Monk, Fifer et al. 2000). Compared to the control group which showed a gradual decrease in FHR to baseline levels during the recovery period, this group first displayed a gradual increase in FHR post-stimulation followed by a decrease in heart rate to baseline levels. No differences between the two groups were detected during the baseline period. The results from the second study are similar in that elevated increases in FHR were noted in the high anxiety group of mothers (Monk, Myers et al. 2003). However, the findings differed from the earlier study in that the foetuses of anxious mothers showed greater decreases in FHR in the post-stimulation period compared to controls. The reason for this inverse response is unclear.

The influence of prenatal stress on foetal cardiac responsivity has been studied by DiPietro and colleagues. Concordance between FHR and FM in an index of neurological maturation

and the authors report associations between high levels of prenatal stress and reduced FHR-FM coupling (DiPietro, Hodgson et al. 1996a). In later studies, elevated levels of perceived stress during pregnancy were associated with decreased variability in FHR and smaller decreases in FHR following maternal cognitive stimulation (DiPietro, Hodgson et al. 1996b; DiPietro, Costigan et al. 2003).

Two studies have investigated the association of maternal neuroendocrinological markers of psychopathology namely ACTH, beta endorphin (BE) and CRH (Sandman, Wadhwa et al. 1999; Sandman, Glynn et al. 2003). Although these inquiries did not include a measure of prenatal depression or anxiety, the authors report the foetuses of mothers with high levels of BE relative to ACTH show delayed habituation to vibroacoustic stimulation (Sandman, Glynn et al. 2003). High levels of BE are associated with a number of psychiatric disorders including depression, anxiety, schizophrenia and obsessive compulsive disorders (Kline, Li et al. 1977; Darko, Risch et al. 1992). In an earlier study the authors report low levels of dishabituation in the foetuses of mothers with elevated levels of plasma CRH (Sandman, Wadhwa et al. 1999). Elevated levels of CRH are associated with symptoms of major depression (Arborelius, Owens et al. 1999).

The results of these studies suggest that prenatal depression may negatively influence foetal responses to a stressor. However not all previous work is consistent with this. A recent inquiry from the United States has reported moderate levels of prenatal stress to be associated with accelerated neurological maturation in foetuses as measured through concordance in physiological measures of foetal behavioural states (DiPietro, Kivlighan et al. 2010). A similar association has been reported in animal literature. Exposure to mild stress during intra-uterine life was associated with better neuronal differentiation, enhanced spatial learning and

improved exploratory behaviour in rodents (Meek, Burda et al. 2000; Fujioka, Fujioka et al. 2001; Fujioka, Fujioka et al. 2006; DiPietro, Kivlighan et al. 2010). Sandman and colleagues have found evidence that the relationship between maternal endocrine dysregulation and FHR responses is non-linear (Sandman, Glynn et al. 2003). The authors report that a cubic solution explained this finding better than a linear model. Moderate levels of prenatal maternal stress have also been found to be associated with better BSID scores in children at 24 months of age (DiPietro, Novak et al. 2006).

Thus while some studies have provided evidence of a linear relationship between prenatal depression and fetal responsivity, others suggest the possibility of a non-linear relationship whereby moderate exposure to prenatal stress is associated with lower stress responsivity in foetuses and improved cognitive performance in children.

Limitations of Previous Work

Although the results of previous inquiries are interesting, there are a number of methodological limitations to consider when interpreting these findings. First, in 3 of the 8 studies, foetuses were stimulated indirectly through maternal cognitive stimulation. The extent to which the findings of these studies are a consequence of the individual mother's mood, temperament and stress response to the challenging task is not known. Second, in all previous work mean values of FHR during and after exposure to the stressor were considered. Measures of total foetal response to stimulation and change in FHR between these periods were not considered. The latter afford a better quantification of fetal stress responsivity than FHR values averaged across periods of stimulation and non-stimulation. Third, these studies have included sub-groups of women who smoked and consumed alcohol during their pregnancy. The extent to which the teratogenic effects of cigarette smoke and alcohol

confound the findings is not known. Finally, all the studies were conducted on western world populations. Different socio-environmental stressors such as poverty, poor maternal nutrition and low levels of social support may exist in these settings and place women at higher risk for developing prenatal depression. The findings of studies on western populations cannot be generalised to developing world populations without replication in these settings.

Study	Location & Sample Size	Details of Foetal Assessment	Details of Variables		Summary of Results
			Maternal	Foetal	
Prenatal Depression and FHR Responses					
Allister at al (2001)	Rhode island, USA Sample size: 20	Gestational Age: 34.1 weeks (SD 1.20 weeks) Duration of assessment: 20 minutes	Prenatal depression (BDI)	FHR response to VAS	Foetuses of depressed mothers had elevated FHR at baseline, stimulation and post-stimulation. They showed a 3.5 times delay in return to baseline during the recovery period.
Prenatal Anxiety and FHR Responses					
Monk et al (2000)	New York, USA Sample size: 17	Gestational Age: 37 weeks (SD 1 week) Duration of assessment: 13 minutes	Prenatal anxiety (STAI)	FHR response to maternal cognitive stimulation (mental arithmetic or Stroop task)	Foetuses of anxious mothers had significantly greater increases during the stressor period. As compared to controls, they showed a different pattern of return to baseline during the recovery period.

Monk et al (2003)	New York, USA Sample size: 32	Gestational Age: 36 weeks (SD 1 week) Duration of assessment: 15 minutes	Prenatal anxiety (STAI)	FHR response to maternal cognitive stimulation (Stroop task)	Foetuses of anxious mothers had greater FHR increases to the Stroop task and greater decreases in FHR during the recovery period.
Prenatal Stress and FHR Responses					
DiPietro et al (1996a)	Baltimore, USA Sample size: 31	Gestational Age: 20, 24, 28, 32, 36 and 38-39 weeks Duration of assessment: 50 minutes	Prenatal stress (Hassles and uplifts scale)	FHR – FM coupling	High prenatal stress was associated with less FHR-FM coupling.
DiPietro et al (1996b)	Baltimore, USA Sample size: 34	Gestational Age: 20, 24, 28, 32, 36 and 38-39 weeks Duration of assessment: 50 minutes	Prenatal stress (Hassles and uplifts scale)	FHR response to VAS	High prenatal stress was associated with less FHR variability.

DiPietro et al (2003)	Baltimore, USA Sample size: 137	Gestational Age: 24 and 36 weeks Duration of assessment: 100 minutes (subsampled to 7.3 minutes)	Maternal stress during the Stroop task (maternal & observer rated)	FHR response to maternal cognitive stimulation (Stroop task)	Foetuses of mothers who perceived the Stroop as stressful showed less decline in FHR and BBV during the recovery period.
Endocrinological Markers of Prenatal Psychopathology and FHR Responses					
Sandman et al (2003)	California, USA Sample size: 135	Gestational Age: 32.5 weeks (SD 1.49 weeks) Duration of assessment: Not mentioned	Prenatal maternal ACTH and beta endorphin (BE)	FHR habituation to a series of 10 VAS	Foetuses of mothers with high levels of BE relative to ACTH showed delayed habituation.
Sandman et al (1999)	California, USA Sample size: 33	Gestational Age: 32.1 weeks (SD 0.8 weeks) Duration of assessment: 45 minutes	Prenatal maternal CRH	FHR dis-habituation following exposure to 41 VAS	Foetuses of mothers with elevated CRH exhibited re-emergence of their habituated FHR response (dishabituation) more slowly.

BDI: Beck Depression Inventory (Beck et al 1977); STAI*: State - Trait Anxiety Inventory (Spielberger et al., 1970)*

Table 2 Summary of studies investigating the association of prenatal maternal psychological distress and foetal responsivity to a stressor.

2.2.7 PRENATAL DEPRESSION AND FOETAL RESPONSIVITY: UNDERLYING MECHANISMS

A number of hypotheses have been proposed to explain the association between prenatal maternal depression and aberrant foetal responses to a stressor however the precise mechanisms underlying the association is not known. There are gaps in the knowledge on the role of genetics in the heritability of stress responses. Further, literature explaining the influence of maternal smoking, alcohol consumption and substance abuse during pregnancy on foetal responses is limited. Nevertheless, previous literature has provided some evidence of the mechanisms underlying the association between prenatal depression and foetal stress responses as described below.

I. The HPA Axis

The HPA axis has emerged to be the primary neurological substrate for the programming effects of maternal depression on the foetus (Talge, Neal et al. 2007). Evidence from animal literature has provided evidence in support of the programming effects of laboratory induced prenatal maternal stress on postnatal offspring development. In both rodent and non-human primate models, exposure to gestational stress has been associated with long term behavioural outcomes (Schneider 1992 (b); Weinstock 1997; Schneider, Moore et al. 2002), impaired cognitive functioning (Chapillon, Patin et al. 2002) and a down-regulation of glucocorticoid receptors in the hippocampus and hypothalamus (Barbazanges, Piazza et al. 1996). Human inquiries have reported associations between severe prenatal stress and lower levels of baseline cortisol in infants (Yehuda, Engel et al. 2005). The correlation between maternal and amniotic fluid cortisol has been found to be higher in anxious mothers as compared to controls (Glover, Bergman et al. 2009).

The effect of prenatal maternal depression on reformatting the developing foetus' HPA axis has been attributed to the influence of elevated levels of maternal cortisol in the intra-uterine environment. Prenatal depression is known to stimulate the maternal HPA axis resulting in an elevation of maternal cortisol (Wadhwa, Culhane et al. 2001b; Wadhwa, Sandman et al. 2001c) which crosses the placenta to enter the fetal circulation. Gitau et al suggest that although maternal cortisol may contribute to about 40% of the variance in fetal cortisol concentrations (Gitau, Cameron et al. 1998), most is metabolized by the placental enzyme 11-beta hyrdoxy steroid dehydrogenase Type II (11BHSD2) into inactive cortisone (Wadhwa 2005; Weinstock 2007) so that the amount reaching the foetus is small (20%). Recent reports however suggest that prenatal stress down regulates the activity of 11BHSD2 (Mairesse, Lesage et al. 2007) and may increase placental permeability to cortisol (Glover, Bergman et al. 2009). Further, the release of increased placental corticotrophin releasing hormone (CRH) in response to prenatal depression and anxiety elevates both maternal cortisol and the concentration of cortisol in the intra-uterine environment (Glover, Bergman et al. 2009). Researchers suggest that areas of the foetal brain with high densities of CRH receptors, such as the hippocampus, are particularly sensitive to the elevated CRH and cortisol (Sandman, Wadhwa et al. 1999). Evidence from animal models report excessive glucocorticoid exposure is neurotoxic to hippocampal pyramidal cells (Sapolsky 1985a; Sapolsky, Krey et al. 1985b; Smith, Makino et al. 1995). As the functions of the hippocampus include learning, memory and emotional processing, it is possible that the association between prenatal depression and disturbances in foetal responsivity is due to the former's influence on these areas of the foetal brain.

II. Role of the Placenta

The placenta regulates foetal exposure to maternal factors and assists in preparing the foetus for optimal functioning in the extra-uterine environment. It serves two important functions in regulating foetal exposure to cortisol. First, it prevents the foetus from exposure to excess maternal cortisol through the action of the barrier enzyme, 11BHSD2, situated strategically at the placental interface with the maternal circulation (O'Donnell, O'Connor et al. 2009). The concentration of cortisol in foetal circulation is thus 13 times lower than that in maternal circulation (Gitau, Fisk et al. 2001). Reduced activity of 11BHSD2 has been reported in mothers with high levels of prenatal stress (O'Donnell, O'Connor et al. 2009). Second, the placenta itself secretes CRH beginning from the 7th week of gestation (Grino, Chrousos et al. 1987; Jones and Challis 1989). The production of placental CRH (unlike hypothalamic CRH) is not down-regulated by elevated levels of maternal glucocorticoids. Instead a positive feedback system exists, elevating maternal and foetal cortisol further in depressed women (Sandman, Wadhwa et al. 1999).

III. Activation of the Maternal Sympathetic-Adrenal System

The activation of the maternal sympathetic-adrenal system in response to prenatal depression and anxiety results in an increase in maternal levels of adrenaline and noradrenaline (Mulder, Robles de Medina et al. 2002). The placenta appears to be impermeable to catecholamines (Giannakoulopoulos, Teixeira et al. 1999); however their effect on the maternal cardiovascular system could impact on the foetus. Maternal sympathetic activation induces vasoconstriction in uterine arties resulting in a decrease in utero-placental blood flood (Copper, Goldenberg et al. 1996). This could limit the amount of oxygen and nutrients supplied to the foetus thereby compromising healthy foetal neurodevelopment. Sympathetic activation also promotes myometrial contractility increasing uterine vasoconstriction further.

Finally, it is possible that the effects of prenatal programming may be explained not only by the individual functioning of regulatory systems such as the HPA axis and sympathetic-adrenal system, but by the complex interactions between them (O'Donnell, O'Connor et al. 2009).

IV. Other Biological Processes

It is important to note that other biological processes may contribute to the association between prenatal depression and foetal responsivity. Despite limited scientific understanding of these mechanisms, evidence exists to support three plausible hypotheses.

First, previous literature suggest that the heritability of HPA axis functioning may play an important role in the prenatal programming of foetal stress responses. A study of 29 monozygotic twins found genetic factors to account for 40-45% of the variance in salivary cortisol (Young, Aggen et al. 2000). A meta-analytic review of twin studies estimated a heritability of 62% in baseline cortisol levels in a combined sample of 399 twin pairs (Bartels, Van den Berg et al. 2003). Polymorphisms in the glucocorticoid receptor gene have also been associated with altered cortisol metabolism (Rosmond, Chagnon et al. 2000a; Rosmond, Chagnon et al. 2000b). Second, depression is associated with high risk behaviours in mothers including smoking, alcohol consumption, the use of recreational drugs and weight loss (Goldenberg 1991). These may exert negative consequences on foetal development. Further, because depressed mothers display less health seeking behaviour than non-depressed mothers, they are at risk for receiving inadequate prenatal care (Miller 1992). Finally, prenatal depression has been associated with poor maternal nutrition (Allister 2001). Poor maternal nutrition is associated with intra uterine growth retardation and poor foetal development (Godfrey, Robinson et al. 1996). Further, a prenatal diet low in protein has been associated

with decreased functioning of the placental enzyme, 11BHSD2 (Bertram, Trowern et al. 2001).

2.2.8 CONCLUSIONS

Previous literature has provided evidence of an association between prenatal stress and altered foetal cardiac responses to a stressor. While some studies have reported negative consequences of prenatal depression on foetal stress responsivity, others have presented the possibility of a non-linear association. These studies suggest that exposure to moderate levels of prenatal stress may be associated with improved foetal responsivity to stress. Although the findings of these studies are of relatively small magnitude, they represent a foray into the investigation of the influences of prenatal depression during very early life. The patterns in foetal responsivity may also serve as markers for behavioural characteristics seen during later life.

A number of hypotheses have been proposed to explain these relationships. Although the role of the HPA axis and the activation of the maternal sympathetic-adrenal system appear to be well established, there is limited understanding of the mechanisms governing the association of prenatal depression with foetal responsivity. Importantly, the role of genetics and harmful maternal behaviours, including prenatal smoking and alcohol consumption, cannot be ignored.

All studies investigating the association of prenatal depression on foetal responsivity to a stressor have emanated from the western world, in particular from the USA. There are no investigations into the same from the developing world despite high levels of prenatal depression and different socio-economic stressors being reported from these settings. Further,

previous studies have not quantified foetal response to stimulation and compared FHR across various periods of assessment. It is possible that this may afford a better indication of foetal HPA axis functioning in the context of prenatal depression.

2.3 PRENATAL DEPRESSION AND INFANT CORTISOL RESPONSIVITY

2.3.1 INTRODUCTION

The Hypothalamic-Pituitary-Adrenal (HPA) axis has emerged as a key candidate for the programming influences of prenatal maternal depression on offspring development. Evidence from animal and human literature points to disturbances in glucocorticoids linked to the HPA axis (cortisol in humans and corticosterone in rats) in the offspring of prenatally stressed mothers. The programming influences of prenatal maternal depression on glucocorticoid production in offspring, particularly in response to stressful conditions, have also been linked to negative reactivity, behavioural outcomes and impairments in cognition (Brown, Varghese et al. 2004; Davis, Glynn et al. 2007; Talge, Neal et al. 2007).

The following sub-sections present an overview of the HPA axis, the influence of prenatal depression on the same and the mechanisms underlying these effects.

2.3.2 THE HPA AXIS: STRUCTURE AND FUNCTION

The HPA axis is a central regulatory network linking the central nervous system (CNS) with the endocrine system. Although it plays a crucial role in the body's stress response mechanism, it is also important for supporting normal physiological functioning. The neurocircuitary of the HPA axis consists of three endocrine structures – the hypothalamus, the pituitary and the adrenal gland.

The hypothalamus is a specialised structure located above the brain stem in the base of the brain. It serves as the integrating centre for interoceptive and exteroceptive stimuli (stimuli that originate within and outside the CNS respectively). The hypothalamus contains neurosecretary neurons which synthesise and release peptides and catecholamines in response to these stimuli.

The pituitary gland is a pea-shaped gland situated just below the hypothalamus and connected to it by a specialised capillary plexus termed the hypophyseal portal circulatory system. The gland consists of two parts – the anterior pituitary and the posterior pituitary, each of which secretes specialised hormones upon stimulation by the hypothalamus.

The adrenal glands are situated above the upper poles of the right and left kidneys. They consist of an inner medulla (secreting catecholamines) and an outer cortex (secreting steroid hormones) in response to stimulatory signals from the pituitary gland.

The HPA Axis and the Stress Response Mechanism

In conditions of stress, the hypothalamus produces corticotrophin releasing hormone (CRH) and/or arginine vasopressin. These are delivered by means of the hypophyseal portal circulatory system to the anterior pituitary and stimulate the release of adrenocorticotrophic hormone (ACTH) into the bloodstream. ACTH travels through the bloodstream and stimulates the adrenal glands resulting in a release of cortisol from the adrenal cortex and adrenaline and noradrenaline from the adrenal medulla. Cortisol enters the bloodstream and travels to multiple organs exerting its effects on metabolism, vascular reactivity, the immune system and the body's response to stress. In addition, elevated plasma levels of cortisol exert a negative feedback effect on the hypothalamus and pituitary, decreasing the secretion of CRH and ACTH respectively and thereby ultimately decreasing its own production. The initial rise in cortisol is necessary for the body's response to stress and the subsequent dampening in its secretion is necessary for the body to return to a state of normal homeostatic balance once the stressful condition has passed (Kudielka and Kirschbaum 2005; Field, Diego et al. 2008a). Fig. 1.1 on page 6 illustrates the HPA axis and the negative feedback loop initiated by cortisol secretion.

The regulatory effect of cortisol on the HPA axis ensures that the body does not overreact in response to a stressor (Munck, Guyre et al. 1984). This constant adjustment in the concentration of a hormone around a target level has been termed allostatic load (Adinoff, Iranmanesh et al. 1998). Chronic overactivation of the stress response system results in a high allostatic load (McEwen and Stellar 1993; McEwen 1998a) and a constant elevated state of anxiety and fear (McEwen 1994). A high allostatic load may also result in a number of negative health consequences in the long-term such as depression, anxiety, schizophrenia (Field, Diego et al. 2008c), hypertension, diabetes and cardiovascular disease (McEwen

1998b). Conversely, chronic HPA hypoactivity has been associated with an increase in autoimmune disorders such as lupus erythematosis, multiple sclerosis and rheumatoid arthritis (Kudielka and Kirschbaum 2005).

The HPA axis demonstrates a circadian rhythm with levels of cortisol varying at different points of time during the day. Thus, in addition to short-term fluctuations throughout the day, cortisol levels in humans decrease during late evening, reaching their lowest point during the middle of the night, begin to increase several hours before awakening peaking around 0800 – 0900 hours in the morning (Fig. 8).

The HPA Axis in Pregnancy

Pregnancy is regarded as a state of hypercortisolaemia. Third trimester levels of maternal ATCH and cortisol are 2 and 3 times respectively higher than the levels during early pregnancy or in the non-pregnant state (Sandman, Glynn et al. 2006). From the 8^{th} week of gestation onwards the placenta begins to secrete increasing amounts of CRH into the foetal and maternal circulation so that levels of maternal CRH at 31 weeks of gestation are more than 20 times that during early pregnancy (Fig 9; (Sandman, Glynn et al. 2006)). The rising levels of unbound placental CRH in the maternal bloodstream establish a positive cascade with the maternal HPA axis (unlike the negative feedback effect of hypothalamic CRH). This results in an elevation of ACTH and cortisol in the maternal blood.

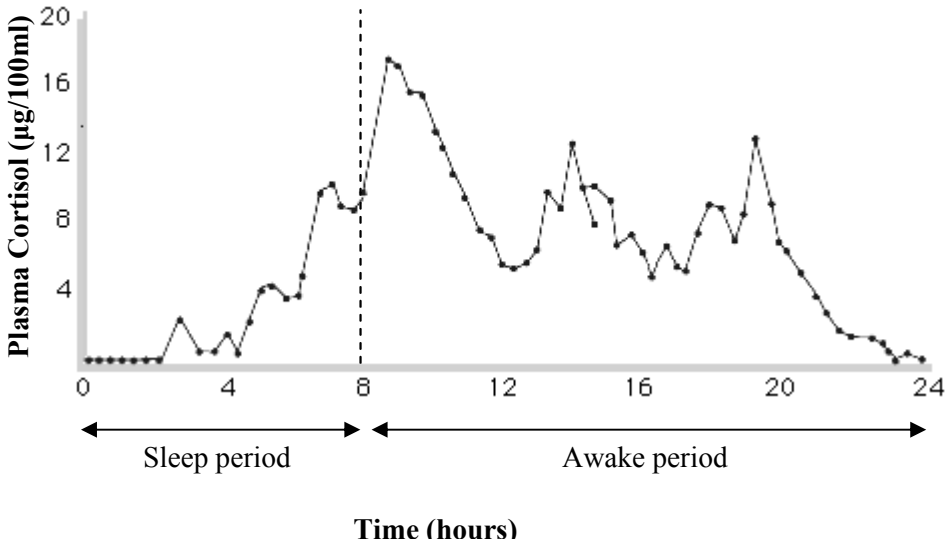


Fig. 8 Diurnal rhythm in cortisol levels (Weitzman, Fukushima et al. 1971).

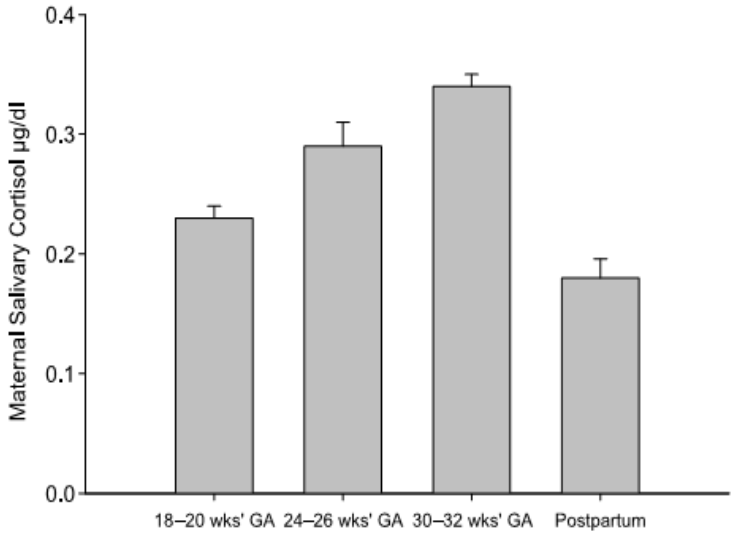


Fig. 9 Rise in maternal cortisol over the course of pregnancy. Comparisons between consecutive time points are statistically significant [Taken from (Davis, Glynn et al. 2007)].

However, despite the elevated levels of maternal cortisol during pregnancy, mothers do not suffer from symptoms of glucocorticoid excess. This is because high prenatal levels of oestrogen stimulate the production of corticosteroid binding globulin (CBG). CBG is a plasma protein that binds approximately 75% of cortisol in the circulation. As more CBG is produced during pregnancy, more cortisol is bound and the fraction of free (unbound) cortisol decreases. ACTH secretion increases in response to low levels of free cortisol and stimulates further cortisol production until the level of free cortisol returns to normal (Mitrovic 2003).

The physiological rise in cortisol levels during pregnancy is necessary for normal foetal growth, foetal lung maturation and the preparation of the foetus for postnatal life (McEwen 2000). Endogenous glucocorticoids regulate the production of prostaglandins in the placenta upregulating oxytocin expression and interfering with placental progesterone signalling. Further, cortisol regulates its own concentration within the placenta and foetal membranes by affecting the expression and the activity of 11BHSD2 (Norwitz and Lye 2009).

Foetal levels of cortisol are derived from the transplacental transport of maternal cortisol. Maternal cortisol has been found to account for about 40% of the variance in foetal cortisol levels in 13-35 week old foetuses (Gitau, Cameron et al. 1998; Field, Diego et al. 2008c). In addition, the foetal adrenal secretes cortisol in to foetal circulation (Hennessy, Coghlan et al. 1982). Further, enzymes in the foetal membranes and foetal lung reduce inactive cortisone to active cortisol resulting in an increase in foetal cortisol levels (Pearson Murphy 1981).

2.3.3 CORTISOL: AN INTRODUCTION

Cortisol is the end product of the HPA axis. It is the major glucocorticoid in humans. Approximately 75% of cortisol in circulation is bound to CBG, 15% is bound to albumin and about 10% remains unbound or free. It is this unbound fraction of cortisol that is biologically active via glucocorticoid receptors and controls the transcription of genes in many cells.

Cortisol exerts effects on many organ systems under conditions of normal physiological functioning and stress. During normal conditions, cortisol regulates the body's metabolism, exerting anabolic effects on the liver and catabolic effects on muscle, adipose tissue and lymphoid tissue (Mitrovic 2003). It facilitates the action of other hormones such as glucagon and catecholamines on the regulation of metabolism and blood pressure. It modulates the electrical activity of hippocampal and limbic neurons, decreases rapid eye movement sleep and increases the amount of time spent awake.

Under conditions of stress, cortisol levels rise dramatically enabling the body to cope with the stressor and maintain homeostasis in adverse conditions. Surges in cortisol in response to stress protect vital metabolic functions at the expense of others (e.g. the mobilisation of energy stores from the liver and adipose tissue to organs of importance such as the brain and the heart). Cortisol also inhibits the immune system and suppresses local inflammatory responses exerting an important 'brake' on the potentially damaging influences of unregulated inflammatory responses (Strachan and Walker 2002).

2.3.3.1 *DISORDERS OF CORTISOL SECRETION*

The regulation of cortisol production is vital for the maintenance of homeostasis under normal and stressful conditions. Dysregulation in cortisol is of clinical importance and is manifested through medical conditions such as Cushing's syndrome and Addison's disease (Table 3).

Cushing's syndrome is characterised by symptoms of cortisol excess due to excessive activation of glucocorticoid receptors (Strachan and Walker 2002). These include centripetal obesity, hypertension, muscle weakness and easy bruising. Hyperglycaemia, osteoporosis, menstrual abnormalities and an increased propensity to developing infections are also associated with Cushing's syndrome. The causes of Cushing's syndrome are attributed to pituitary tumours (Cushing's disease), ectopic ACTH-secreting tumours in the lung and pancreas (bronchial carcinoid and pancreatic carcinoma), adrenal tumours, chronic glucocorticoid therapy, alcoholism and major depressive illness (Pseudo-Cushing's Syndrome).

Addison's disease is a potentially fatal condition resulting from inadequate secretion of cortisol and/or aldosterone (Strachan and Walker 2002). Patients may present with chronic features (such as weight loss, malaise and anorexia) or acute circulatory shock including severe electrolyte disturbances, hypotension and hypoglycaemia. Cortisol levels are very low in these patients and treatment is by way of glucocorticoid and mineralocorticoid replacement therapy.

Plasma Cortisol (Reference range: 150-550 nmol/l (at 0800 hours); <200 nmol/L (at 2200 hours)[a]		
↑ **Cortisol**	**Primary**	Cushing's syndrome
	Secondary	Ectopic tumours, Cushing's disease, Pseudo-Cushing's syndrome
↓ **Cortisol**	**Primary**	Addison's disease, Nelson's syndrome
	Secondary	Pituitary tumours, Sheehan's syndrome

Table 3 Clinical conditions associated with Hyper- and hypocortisolaemia.
Reference range for cortisol is taken from Davidson's Principles and Practises of Medicine, 19th edition pg. 1218.

2.3.3.2 MEASUREMENT OF CORTISOL

Cortisol is easily measured in peripheral blood, saliva and urine. The dynamics of cortisol secretion facilitate a variety of potential paradigms to measure cortisol. A central concern in research is the selection of the most appropriate measure and design for the hypothesis and study population.

Initially, researchers focussed on measures of cortisol at baseline to assess the functioning of the HPA axis. However, results were inconsistent and there was limited agreement as to whether morning, evening or night-time cortisol was the best measure (Egliston, McMahon et al. 2007). Further, a single measure of cortisol did not provide information about the circadian rhythm or HPA axis functioning in response to stress. Another popular technique is the use of serial cortisol measurements throughout the day. Although this affords a better measurement

of the diurnal rhythm of cortisol secretion (Egliston, McMahon et al. 2007), disruptions in the circadian rhythm of cortisol secretion are but one manifestation of HPA axis dysregulation. A third paradigm gaining in popularity is the measurement in the change in cortisol from pre-stress to post-stress levels. This method is a more appropriate marker for HPA axis reactivity to stress. Some researchers have raised concerns about the validity of a single pre- and post-stress measure of cortisol in measuring peak stress reactivity and affording an adequate assessment of the recovery phase of the stress response (Austin, Hadzi-Pavlovic et al. 2005; Egliston, McMahon et al. 2007) . The use of multiple pre and post-stressor samples has been suggested to be superior to single measurements (Egliston, McMahon et al. 2007). Recently, the use of saliva to measure cortisol has increased in popularity because of the ease, relative cost-effectiveness and ability of participants to collect their own samples. Further, salivary cortisol levels accurately reflect the unbound, biologically active fraction of cortisol found in plasma (Kirschbaum and Hellhammer 1994).

2.3.4 CORTISOL AND DEPRESSION

Approximately 50% of patients with endogenous depression have elevated levels of cortisol (Reus, Joseph et al. 1985). Compared to controls, depressed patients have been found to demonstrate higher levels of plasma cortisol, increased and more frequent bursts of cortisol secretion, a loss of circadian pattern in cortisol secretion and a failure to suppress cortisol production following the administration of dexamethasone, an exogenous glucocorticoid, in the dexamethasone suppression test (DST) (Reus, Joseph et al. 1985). Higher baseline cortisol levels and HPA axis activation in the evening has been associated with the higher cortisol responses to stress reported in patients with a major depressive disorder (MDD) (Young, Haskett et al. 1994; Young, Lopez et al. 2000). Among patients with MDD, DST non-

suppression has been associated with a higher relapse rate than if DST results normalise (Targum 1984).

Nevertheless, people with major depression rarely manifest symptoms of cortisol excess unlike patients with Cushing's syndrome. This has been attributed to the decreased number of glucocorticoid receptors in depressed patients compared to patients with Cushing's syndrome (Huizenga, Herder et al. 2000). It is possible that depressed patients however do manifest sub-clinical symptoms of hypocortisolaemia such as subtle changes in the differential white blood cell count (Brown, Varghese et al. 2004).

Elevations in cortisol associated with MDD have also been reported to have a range of systemic effects. These include a decrease in hippocampal volume and impairments in declarative memory, cognitive impairment and deficits in working memory, truncal obesity, osteoporosis, hypertension, diabetes and peptic ulcers (Brown, Varghese et al. 2004). These symptoms are similar to features of the metabolic syndrome seen in patients with Cushing's disease. However, it is not known whether these symptoms are due to cortisol excess per say or whether they are the consequences of changes in sleep, appetite and activity levels seen in patients with MDD (Brown, Varghese et al. 2004).

2.3.5 CORTISOL: ASSOCIATIONS WITH PRENATAL STRESS AND OFFSPRING DEVELOPMENT

Maternal Cortisol: Association with Prenatal Depression

Prenatal depression has been associated with elevated levels of maternal cortisol beyond the normal physiological increase seen in pregnancy. In a sample of 63 third trimester women, elevated levels of cortisol and NA were reported in depressed mothers as compared to controls (Lundy, Jones et al. 1999). Maternal cortisol was found to be significantly correlated with prenatal depression and anxiety scores in another sample (Diego, Field et al. 2005). In a case control study of 140 women in the second trimester of pregnancy, elevated levels of cortisol were reported in the group of prenatally depressed mothers as compared to controls (Field, Diego et al. 2004). Similar findings have been reported in other studies (Field 1995; Aaron Jones, Field et al. 1998).

Prenatal Maternal Cortisol: Association with Offspring Development

Elevated levels of prenatal cortisol in mothers have been associated with adverse outcomes in offspring. High levels of maternal cortisol predicted decreased foetal growth (Diego, Field et al. 2005; Field, Diego et al. 2006), the failure of foetal habituation to vibroacoustic stimulation (Sandman, Wadhwa et al. 1999) and premature births (Field, Diego et al. 2004). Lower birth weights, increased irritability and elevated levels of urinary cortisol have been reported in the neonates of mothers with increased prenatal cortisol as compared to controls (Field, Diego et al. 2004; Field, Diego et al. 2006). In one study, increased crying and fussing were observed in the infants of high cortisol mothers at 1-3, 5, 7 and 18-20 weeks post birth (de Weerth, van Hees et al. 2003). Further, infants of depressed mothers with high neonatal

cortisol have been reported to have delays in motor and cognitive development at 3 years of age (Graham, Heim et al. 1999; Field, Diego et al. 2008c).

2.3.6 PRENATAL DEPRESSION: ASSOCIATION WITH INFANT CORTISOL

Evidence from animal literature has shown exposure to laboratory induced stress during gestation to be associated with aberrations in stress responses during postnatal life. Higher basal levels of ACTH and a greater, more prolonged elevation of the same in response to tail shock stressors have been reported in the offspring of prenatally stressed rodents as compared to controls (Takahashi, Kalin et al. 1988; Takahashi and Kalin 1991; Takahashi, Haglin et al. 1992) . The pups of prenatally stressed rodents have been reported to demonstrate a delay in return to baseline levels following cessation of the stimulus.

The limited inquiries investigating this association in human have reported findings similar to those described in animal literature. In a Dutch sample, deBruijn and colleagues reported prenatal depression to be associated with raised cortisol at baseline, during exposure to a stressful task and post-stressor in 103 toddlers (deBruijn, Bakel et al. 2009). Findings from a US based sample of 189 mother-infant dyads have shown maternal peripartum depression to predict infant cortisol reactivity at 6 months (Brennan, Pargas et al. 2008). Prenatal maternal stress was found to predict children's cortisol reaction to the first day of school in a sample of 29 five year olds (Gutteling, Weerth et al. 2005). One study of 45 preterm infants however found exposure to exogenous steroids during intra-uterine life to be associated with a dampened cortisol response to immunisation at 4 and 12 months of age (Glover, Miles et al. 2005). Nevertheless, the findings of these studies support an independent effect of prenatal glucocorticoids on postnatal offspring cortisol responses to stress.

Further, the neonates and infants of prenatally depressed mothers have been found to have higher levels of cortisol than controls (Lundy, Jones et al. 1999; Field, Diego et al. 2004). In a follow-up sample of the ALSPAC cohort, awakening and afternoon salivary cortisol levels at 10 years of age was found to be associated with prenatal anxiety ($r=0.25-0.32$, $p < .05$) (O'Connor, Ben-Shlomo et al. 2005). Thus pre-existing literature provides substantial evidence in support of the programming influence of prenatal maternal depression on the offspring's HPA axis with the children of prenatally depressed mothers displaying elevated baseline levels of cortisol and disturbances in cortisol response to stress as compared to the children of non-depressed mothers.

Many human inquiries have focussed on serial measurements of cortisol in children as an outcome measure (Lundy, Jones et al. 1999; Field, Diego et al. 2004; Gutteling, Weerth et al. 2005; O'Connor, Ben-Shlomo et al. 2005). A measurement of cortisol responsivity to a stressor however would afford a better assessment of the programming effect of prenatal maternal depression on the child's HPA axis. Some studies have employed the use of a laboratory based paradigm involving infant exposure to a series of stressful tasks such as arm restraint, maternal separation and frustrating play tasks (Brennan, Pargas et al. 2008; deBruijn, Bakel et al. 2009). The use of such paradigms provide an insight into the programming influences of prenatal depression on the child's stress response mechanism, however they are limited in that they cannot be administered to very young infants, are culture-specific, laboratory based and necessitate a resource intensive design involving specialised training of researchers. The use of infant cortisol response to a common stressor during infancy, such as immunisation, represents an easy, affordable, universally applicable alternative. However, the only inquiry to have employed a pre/post-immunisation paradigm was conducted on a sample of preterm infants exposed to exogenous glucocorticoids during

foetal life (Glover, Miles et al. 2005). The use of this paradigm in normal, healthy infants has not been explored.

Further, it is possible that previous reports have been confounded by maternal smoking and alcohol consumption during pregnancy. Smoking has been associated with high levels of prenatal stress and higher overall levels of evening cortisol in women (Obel, Hedegaard et al. 2005). Chronic alcohol consumption has also been associated with elevations in cortisol (Adinoff, Iranmanesh et al. 1998). However, the influence of prenatal smoking and alcohol consumption on the maternal and foetal HPA axis is not known.

Finally, all previous reports into the influence of prenatal maternal depression on infant cortisol responsivity to stress has emanated from western populations. There are no inquiries investigating the association in the developing world.

2.3.7 UNDERLYING MECHANISMS

Although the mechanisms underlying the influence of prenatal maternal depression on disturbances in offspring cortisol responsivity to stress remain to be determined, a number of hypotheses have been put forth to explain these effects.

I. Direct Effect of Maternal Cortisol on the Foetus

Maternal cortisol crosses the placenta and contributes directly to foetal cortisol levels. Gitau and colleagues suggest that maternal cortisol may account for about 40% of the variance in foetal cortisol (Gitau, Cameron et al. 1998). This is despite the regulatory action of the placental barrier and the enzyme 11BHSD2 which metabolises active cortisol into inactive

cortisone and prevents it from entering the amniotic sac (see 2.2.7 page 45-46). In addition, placental CRH stimulates the production of cortisol in both the mother and the foetus further contributing to an increase in cortisol in the intra-uterine environment.

Exposure to elevated levels of cortisol during intra-uterine life exerts programming influences on neural structures rich in glucocorticoid receptors (Sandman, Glynn et al. 2003; Austin, Hadzi-Pavlovic et al. 2005). These include the hippocampus and parahippocampal regions of the foetal brain. Findings from animal literature report prolonged prenatal exposure to glucocorticoids to be associated with dendritic atrophy, decreased neurogenesis and neuronal death in these structures (Egliston, McMahon et al. 2007). Thus exposure to elevated levels of maternal cortisol during intra-uterine life can negatively impact foetal growth and development (Kapoor, Dunn et al. 2006; Weinstock 2007) and result in a reformatting of the foetal HPA axis.

II. Effects on Utero-placental Blood Flow

Prenatal depression is associated with elevated levels of cortisol and NA (Lundy, Jones et al. 1999). These hormones influence uterine vasoconstriction (Kandel, Schwartz et al. 2000; Girod and Brotman 2004; Field and Diego 2008d). NA exerts direct vasoconstrictive effects on uterine arteries resulting in a decrease in utero-placental blood flow and placental hypoxia (Steele, Warren et al. 1993; Yousif, Chandrashekar et al. 2003). This limits the supply of nutrients and oxygen to the foetus.

Elevated maternal cortisol decreases the density of uterine artery adrenoreceptors potentiating the vasoconstrictive effect of NA on the uterine arteries (Xiao, Huang et al. 2003). The stressors of foetal hypoxia and nutritional deprivation, together with the increased incidence

of preeclampsia (Knuist, Bonsel et al. 1998) associated with uterine artery vasoconstriction, could be responsible for aberrations in foetal development in general and foetal neurodevelopment (including the foetal HPA axis) in particular.

III. Heritability of HPA Axis Activity

There is the possibility that disturbances in HPA axis functioning may be genetically determined. A study of monozygotic twins found 40-45% of the variance in salivary cortisol to be contributed to by genetic factors (Young, Aggen et al. 2000). A review of twin studies reported a heritability of 62% in baseline cortisol levels (Bartels, Van den Berg et al. 2003). Dysregulation in cortisol metabolism has been associated with polymorphisms in the GR gene (Rosmond, Chagnon et al. 2000a). However, it is important to note that 40-60% of the variance in HPA axis functioning is contributed to by non-genetic factors. The prenatal environment appears to be an important candidate among these factors.

IV. Other Factors

High risk behaviours and poor maternal nutrition are important factors to consider in the association between prenatal maternal depression and infant cortisol responsivity to stress. First, increased levels of alcohol consumption, smoking and recreational drug are reported in prenatally depressed mothers as compared to non-depressed mothers (Goldenberg 1991). The contribution of the neurotoxic effects of alcohol and smoking on foetal development is not known. Second, low levels of nutrition are reported in prenatally depressed mothers (Goldenberg 1991). A low protein diet has been associated with decreased 11BHSD2 activity (Bertram, Trowern et al. 2001) and may contribute to increased foetal exposure to maternal cortisol in depressed mothers.

2.3.8 CONCLUSIONS

The regulation of the body's response to stress by the HPA axis is carried out through cortisol. Elevated levels of cortisol are present during pregnancy and this is needed for healthy foetal growth and maturation. However, depression is associated with increases in cortisol and the levels of cortisol seen in prenatally depressed mothers are higher than the normal physiological elevations in cortisol encountered during pregnancy. There is a possibility that increased levels of cortisol may exert programming influences on the foetal HPA axis. These effects may be reflected as elevated baseline levels of cortisol and elevated cortisol responses to stress in infants and children exposed to prenatal maternal depression during intra-uterine life compared to the infants and children of non-depressed mothers. The mechanisms underlying this association are not clearly defined; however evidence exists to support the direct effect of maternal cortisol on the foetus and on utero-placental blood flow.

Importantly, all studies investigating the association between prenatal depression and infant cortisol responsivity to stress have been carried out in the western world. No inquiries exist from the developing world. Further, the effect of prenatal depression on cortisol response to immunisation in healthy, term infants has not been explored.

2.4 PRENATAL DEPRESSION: ASSOCIATIONS WITH INFANT TEMPERAMENT

2.4.1 INTRODUCTION

A recent body of evidence has emphasised the importance of prenatal maternal mood and the intra-uterine environment, to the development of infant[d] temperament. In this sub-section, the findings of studies reporting an association between prenatal depression and infant temperament are reviewed and discussed.

2.4.2 THE CONCEPT OF TEMPERAMENT

The premise of the concept of temperament lies in observations that children, even from a very young age, show considerable individual variability in reactions to their environment. These early individual differences have become a major focus of scientific attention because of their associations with behaviour, personality and neurocognitive development in later life.

Rothbart and Derryberry have defined temperament as "individual differences in emotional, motor and attentional reactivity measured by latency, intensity and recovery of response, and self-regulation processes such as effortful control that modulate reactivity" [(Rothbart 2007)

[d]Infancy is considered as the period between birth and the child's first birthday.

p.207)]. Broadly speaking, temperament refers to individual differences in reactivity and self-regulation presumed to be determined by the individual's genetic endowment and influenced across time by environmental exposure and maturity. Findings from twin studies suggest that genetics accounts for approximately 20% to 60% of the variability in temperament within a population, while the remaining 40-80% is attributable to environmental factors (Saudino 2005). A study of 59 candidate genes found significant associations between temperament traits and certain serotonin, dopamine, noradrenaline and GABA genes (Comings, Gade-Andavolu et al. 2000). These genes however accounted for only 1.5-3.5% of the variance in the temperamental characteristics of the sample (Comings, Gade-Andavolu et al. 2000).

An important hallmark of Rothbart's theory is the continuity of temperamental characteristics over time (Komsi, Räikkönen et al. 2006). A number of studies have provided evidence in support of this developmental continuity. In a US based cohort, significant associations were reported in maternally rated characteristics of child temperament from 3 months to 7 years (Rothbart, Ahadi et al. 2000). Temperamental continuity was also reported in a Finnish sample of 231 children from 6 months to 5.5 years (Komsi, Räikkönen et al. 2006). Caspi and colleagues have shown behavioural characteristics in early childhood to be associated with adjustment problems in early and late adolescence and early adulthood (Caspi, Moffitt et al. 1996). In this study, toddlers classified as 'under-controlled' at three years were, at 21 years of age, 2.9 times more likely to be diagnosed with anti-social personality disorder, 2.2 times more likely to be diagnosed with alcohol dependence and 16.8 times more likely to attempt suicide. Inhibited three year olds were 2.2 times more likely to suffer from depression, 2.9 times more likely to have been arrested and 6.5 times more likely to have attempted suicide at 21 years compared to controls.

Assessments of temperament in childhood have therefore emerged as a promising psychobiological marker of psychological and/or psychiatric morbidity in later life. Although there is limited consensus on the method of choice to assess infant temperament, to date three techniques exist to measure temperamental characteristics:

i. Parent-report questionnaires
ii. Home observations
iii. Laboratory Observations

The most commonly used method to assess infant temperament is through parent-report questionnaires, particularly maternally reported questionnaires (Rothbart 1986; Rothbart 1990). Although such measures are subject to reporter and recall biases in parental perceptions of their child's behaviour, a high degree of convergence has been reported between maternal reports and observer-rated reports of infant temperament at 3, 6 and 9 months of age (r=0.24-0.50) (Rothbart 1986). Further, parental reports sample across the spectrum of the caregiver's experience with the child and tap in a wide range of infant behaviours observed across a large time-frame and made under normal, everyday circumstances (Rothbart 1990). In contrast, home and laboratory observations, while inherently unbiased by parental perceptions, expectations and mood, sample a limited range of the child's behaviour across a short time span and to a pre-determined range of situations. In addition, the ease and low cost of administering parent-report questionnaires adds to the popularity of this technique (Rothbart 1990).

2.4.3 PRENATAL DEPRESSION: ASSOCIATIONS WITH INFANT TEMPERAMENT

Although it is assumed that temperament is genetically determined (Austin, Hadzi-Pavlovic et al. 2005), a number of inquiries in the past two decades have emphasised the role of the prenatal environment on the development of infant temperament. Prenatal maternal mood, particularly maternal depression and anxiety, have been reported to influence infant temperament. A summary of the important studies investigating this association is presented in Table 4.

Of the 16 studies summarised below, 15 reported associations between prenatal maternal mood, and infant behaviour and temperament. Of the 15 studies; 8, 5 and 2 inquiries reported associations between and infant temperament and measures of prenatal depression, anxiety and stress respectively. Compared with controls, higher levels of withdrawal and depressive symptoms have been reported in the neonates of prenatally depressed mothers (Lundy, Jones et al. 1999; Field, Diego et al. 2001; Field, Diego et al. 2004). Prenatal maternal depression has been associated with less optimal neonatal performance on the Brazelton scale, particularly for habituation, orientation, motor, range of state and autonomic stability clusters (Lundy, Jones et al. 1999; Brouwers, van Baar et al. 2001; Field, Diego et al. 2001; Field, Diego et al. 2004; Diego, Field et al. 2005; Field, Diego et al. 2008a). Prenatal depression has been associated with increased reports of fussiness, crying and difficult temperament during infancy independent of postnatal maternal mood (Werner, Myers et al. 2007; McGrath, Records et al. 2008). In a sample of 247 two month olds, prenatal depression was associated with negative infant reactivity independent of postnatal maternal mood (Davis, Glynn et al. 2007).

Prenatal anxiety has been associated with lower orientation scores, poor attentional regulation, increased crying and more difficult temperament during infancy (Van den Bergh 1990; Brouwers, van Baar et al. 2001; Huizink, Robles De Medina et al. 2002). One study from Australia has found prenatal anxiety to predict difficult infant temperament independent of prenatal depression (Austin, Hadzi-Pavlovic et al. 2005). However, not all previous literature is consistent with this. A study of 267 American born infants found prenatal anxiety to be associated with less optimal behaviour in female babies at 7-10 days post birth (Farber, Vaughn et al. 1981). No association was however detected during subsequent postnatal assessments at 3 and 6 months and no such relationship was observed in male offspring (Farber, Vaughn et al. 1981).

Some studies, however, report a lack of association between prenatal maternal depression and infant temperament, particularly after controlling for the confounding influences of maternal postnatal mood, socio-economic adversity and marital discord. In a USA based sample of 314 infants assessed at 4 months, Kaplan and colleagues did not find maternal prenatal psychiatric status to predict any of the dimensions of temperament in a sample of 314 four month olds (Kaplan, Evans et al. 2008).

The results of studies investigating associations between prenatal maternal depression (and anxiety) and infant temperament are not entirely consistent across population groups and are subject to a large number of confounding influences. There is large variability in the measures used to assess maternal mood and child outcomes (see 2.4.4). The numbers of participants assessed in these studies also differ widely (range: 51-1562 mother-infant dyads). Nevertheless, evidence from western populations does exist to support an association between prenatal maternal depression and difficult infant temperament.

2.4.4 LIMITATIONS OF PREVIOUS WORK

While a number of inquiries have prospectively investigated associations between prenatal maternal depression (and anxiety) and infant temperament, the heterogeneity in methodologies constrains the comparability of the results between different studies and limits the generalisability of findings to other samples. The main limitations of these studies are described as follows:

1. Heterogeneity of Measures:

i. Heterogeneity of Measures used to Assess Prenatal Maternal Mood:

Twenty different self-report questionnaires were employed in the 16 studies summarised in the Table 4 to assess prenatal maternal mood. These scales assessed varying aspects of maternal mood ranging from prenatal depression (n=3 scales), prenatal anxiety (n=3) and prenatal stress (n=12) to maternal behavioural inhibition/activation (n=1). Three of these questionnaires (the PRAQ, MSI and PESI) were developed by the authors for the study and have not been used by other research groups. Only two studies employed a structured clinical diagnostic interview schedule to obtain a clinical psychiatric diagnosis of maternal mood disorder during pregnancy.

ii. Heterogeneity of Measures used to Assess Infant Temperament:

A number of measures are employed to assess infant temperament. In the sixteen studies summarised below, seven different maternal-report questionnaires and four different observer-rated techniques were used to assess infant temperament. Only four studies used a combination of both observer and

maternal ratings of infant temperament, while nine studies relied solely on observer–rated measures and three studies relied only on maternal reports.

These brief observations emphasise the limitation in comparability of the findings of these studies as different measures are used to assess both exposure and outcome variables. The absence of a clinical interview schedule for maternal mood disorders raises questions about the reliability of relying solely on maternal self-report measures of depressed mood.

2. Wide Range of Postnatal Age at Assessment:

Assessments of infant temperament are not confined to a particular age but in general span any period during the first year of life. This ranges from a few hours after birth to 12 months after birth. The potential of measures to detect subtle differences in behaviour in very young infants is questionable given the limited repertoire of neonatal behaviours.

3. Lack of Adequate Control for Confounding Influences:

The effect of prenatal depression on infant temperament is moderated by a number of potential confounding influences, the most important of which are:

i. Maternal smoking, alcohol consumption and substance abuse during pregnancy
ii. Maternal psychotropic medication during pregnancy
iii. Postnatal depression
iv. Socio-economic adversity
v. Marital discord and intimate partner violence

It is important that inquiries are designed to control for these influences, as some reports have found the association between prenatal depression and infant temperament to be completely mediated by these factors (Servili, Medhin et al. 2010). Only four of the sixteen studies summarised below reported controlling for prenatal smoking, alcohol consumption and postnatal maternal mood.

4. Need for Multiple Postnatal Assessments of Infant Temperament

Many studies were confined to one prenatal and one postnatal assessment to investigate the association between prenatal depression and infant temperament. In studies where the prenatal assessment was carried out in very young infants, multiple postnatal assessments during the first year may provide more information about the effect of prenatal depression on the developmental trajectory of infant temperament. In the table below, only five studies included more than one assessment of the infant in the postnatal period.

5. Attrition:

A substantial number of studies experienced high attrition rates (three studies reported attrition in excess of 50%). It is possible that this may represent selective attrition of women at highest risk for perinatal psychopathology.

2.4.5 UNDERLYING MECHANISMS

The current understanding of the mechanisms underlying the association between difficult infant temperament and, prenatal depression and anxiety is limited. Nevertheless a number of hypotheses based on biological and psychosocial processes have been proposed as follows:

I. Direct Effects of Maternal Cortisol on Offspring Neuroendocrinology

Elevated levels of cortisol are reported in prenatally depressed mothers compared to controls (Field, Diego et al. 2004). Maternal cortisol crosses the placenta and contributes directly to foetal cortisol levels. Despite about 80% of maternal cortisol attempting to cross the placenta being inactivated by the placental enzyme 11 beta hyrdoxy steroid dehydrogenase 2 (11BHSD2), it has been reported that maternal cortisol contributes to up to 40% of the variance in foetal cortisol levels (Gitau, Cameron et al. 1998; Wadhwa 2005; Weinstock 2008). Prenatal depression has been found to decrease the activity of 11BHSD2 increasing placental permeability to cortisol (O'Donnell, O'Connor et al. 2009). It has been suggested that exposure to elevated levels of maternal cortisol exerts potential programming influences on areas of the foetal brain rich in glucocorticoid receptors such as the hippocampus (Sandman, Glynn et al. 2003). This may contribute to the high levels of cortisol and noradrenaline (NA) and low levels of dopamine and serotonin reported in the neonates of prenatally depressed mothers (Lundy, Jones et al. 1999; Field, Diego et al. 2004). High levels of cortisol and NE are associated with high sympathetic arousal while low levels of dopamine and serotonin are related to depression-like behaviours observed in the neonates of depressed mothers (Field, Diego et al. 2004).

II. Activation of the Maternal Sympathetic-Adrenal System

Prenatal depression induces the activation of the maternal sympathetic-adrenal system. This is associated with a constriction in uterine arteries and a subsequent decrease in utero-placental blood flow limiting the supply of oxygen and nutrients to the foetus (Copper, Goldenberg et al. 1996). It is possible that this may contribute to disturbances in foetal neurodevelopment observed as problems in infant temperament during later life.

III. Effect on Postnatal Depression and Parenting

Parental sensitivity is known to play an important role in the development of self-regulatory behaviours in infancy (Crockenberg and Leerkes 2004). Inadequate stimulation and arousal modulation, low levels of emotional availability and withdrawn or intrusive mother-infant interactions are reported in depressed mothers compared to controls (Field 1995; Field, Diego et al. 2001). As symptoms of prenatal depression are known to predate postnatal depression (PND) (Patel, Rodrigues et al. 2002; Austin, Hadzi-Pavlovic et al. 2005), it is possible that the association between difficult infant temperament and prenatal depression is mediated entirely by its continuation into the postnatal period and the subsequent impact of PND on mothering. This association is complicated by a bi-directional relationship in which sympathetically aroused infants may distress depressed mothers further, elevating maternal depression and deteriorating mother-infant interaction (Lundy, Jones et al. 1999).

IV. Heritability of Temperament Characteristics

Associations between temperament traits and a number of serotonin, dopamine, NE and GABA genes have been reported (Comings, Gade-Andavolu et al. 2000). Twin studies suggest a heritability of 20% to 60% in temperament characteristics within a population

(Saudino 2005) suggesting that genetic factors could contribute to the findings of an association between prenatal depression and infant temperament.

V. Other Confounding Influences

The influence of prenatal smoking, alcohol consumption and recreational drug use may complicate the relationship between prenatal depression and infant temperament (Huizink, Robles De Medina et al. 2002). In addition, the role of shared environmental stressors such as poverty, conflict and violence, may contribute independently to prenatal depression, postnatal depression, mothering and infant temperament (Kiernan and Huerta 2008).

2.4.6 CONCLUSION

Findings from pre-existing literature may be generalised to suggest an association between prenatal maternal depression and difficult infant temperament, independent of the influence of postnatal maternal mood. However, evidence in support of this association is mixed as three of the sixteen studies did not report an independent effect of prenatal depression on infant temperament after controlling for prenatal anxiety, postnatal maternal mood and socio-economic confounders. The variance in these findings may be attributed to issues pertaining to the heterogeneity of measures, the wide range of sample sizes and the timing of postnatal assessments. The results must therefore be interpreted with caution and in the context of the study population.

Importantly, all inquiries were carried out in high income countries (Fig 10). No reports of the association between prenatal depression and infant temperament exist from low and middle income countries (LAMICs) where high rates of prenatal depression, social adversity and malnutrition exist (Wachs, Black et al. 2009). Further, 80% of global pregnancies occur in the

developing world compared to 20% in the western world (United Nations Report 2009). Compared to their western counterparts, ten times the number of children from the developing world are at risk for disturbances in development due to exposure to prenatal depression during intra-uterine life (Fig. 4 Chapter 2.1).

There is limited literature on the association between lifetime maternal depression and child development in the developing world. This work has focussed mainly on the cognitive and motor aspects of infant development in the context of postnatal depression. Compared to controls, the infant of postnatally depressed mothers were found to score lower on the Bayley Scales of Infant Development (BSID) at 6 and 12 months of age in one sample from Bangladesh and another from Goa, India (Patel, DeSouza et al. 2003; Black, Baqui et al. 2007). Information on the effect of prenatal maternal depression on child development is limited to one inquiry from South Africa (Ramchandani, Richter et al. 2010). In this study of 953 children from a socio-economically deprived region of Soweto-Johannesburg, prenatal maternal stressors, particularly partner conflict and family adversity, were associated with a 2.5 fold increase in the risk of childhood behavioural problems at 2 years of age (Ramchandani, Richter et al. 2010). However, there are no inquiries into the association between prenatal depression and infant temperament from LAMICs.

Compared to the western world, high estimates of prenatal depression and associated risk factors, increased undernutrition and anaemia during pregnancy, and exposure to multiple environmental adversities such as poverty, conflicts, migration and infectious diseases, are reported from the developing world (Wachs, Black et al. 2009). Further, different cultural

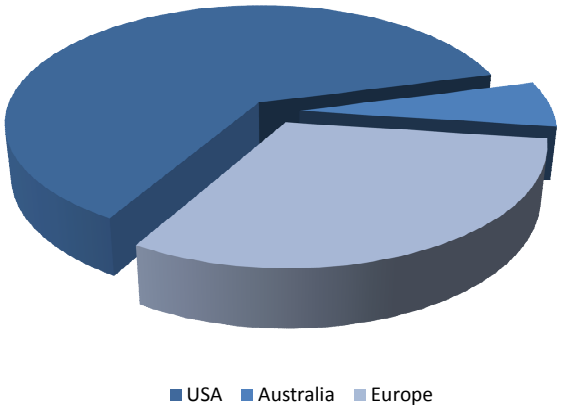


Fig. 10 Graph showing distribution of prospective, longitudinal studies investigating the associations between prenatal depression and infant temperament (≤12 months). There are no studies from the developing world.

practises relating to pregnancy, child birth and child care operate in these settings (Rodrigues, Patel et al. 2003). The findings of an association between prenatal depression and infant temperament in western populations therefore cannot be generalised to developing world populations without replication in these settings. Further, it is possible that due to the variations in the environments between high income countries and LAMICs, different patterns of association may exist between prenatal depression and infant temperament in the latter.

Study	Location & Sample Size (n)	Measures Used		Age of Assessment of Infant	Summary of Results
		Prenatal Maternal Depression	Infant Temperament		
Tiffany Field et al; Infant Behaviour & Development (2001)	USA n=120	CES-D, BIS/BAS, STAI, POMS	NBAS	3-5 days	Prenatal maternal depression is associated with lower neonatal scores on habituation, orientation, motor, range of state and autonomic stability clusters and higher scores on withdrawal and depressive symptoms and excitability. NBAS scores are lower in the groups of neonates with 'withdrawn' compared to 'intrusive' depressed mothers.
Miguel A. Diego et al; Infant Behaviour & Development (2005)	USA n=252	CES-D	NBAS	14 days	Prenatal depression is associated with more stress behaviour, increased neonatal fussing/crying and less optimal NBAS scores.
Tiffany Field et al; Infant Behaviour & Development (2008)	USA n=200	SCID	NBAS	< 1 month	Prenatal maternal dysthymia is associated with lower neonatal orientation and motor scores and more depressive symptoms in neonates.
Tiffany Field et al; Infant Behaviour & Development (2004)	USA n=140	CES-D, STAI, POMS	NBAS	2 days	Prenatal depression is associated with lower neonatal NBAS scores on habituation, orientation, motor, range of state and autonomic stability clusters.

Ellen A. Farber et al; Early Human Dev. (1981)	USA n=267	IPAT Anxiety Scale	NBAS, Mother-infant inter-action during feeding at home	7-10 days, 3 & 6 months	Prenatal maternal anxiety is associated with less optimal neonatal behaviour in female offspring. No associations were detected in male offspring and in older infants.
Brenda L. Lundy et al; Infant Behaviour & Development (1999)	USA n=63	CES-D, DIS, MSI, STAI	NBAS	7 days	Prenatal maternal depression is associated with less optimal neonatal scores on orientation, excitability and withdrawal clusters of the NBAS and more abnormal neonatal reflexes.
Elysia Poggi Davis et al; J. Am. Acad. Of Child and Adolesec. Psychiatry (2007)	USA n=247	CES-D, STAI, PSS	IBQ – fear subscale	2 months	Average prenatal depression is associated with negative infant reactivity independent of postnatal maternal mood.
Lauren A. Kaplan et al; Early Human Dev. (2008)	USA n=314	STAI, CES-D, SCID	IBQ, 10 minute free-play session	4 months	Maternal prenatal psychiatric status did not predict any of the dimensions of infant temperament.
Jacqueline M. McGrath et al; Infant Behaviour & Development (2008)	USA n=139	CES-D, PDPI-R	The Child-bearing Health Questionnaire	2 months and 6 months	Prenatal depression is associated with increased maternal reports of difficult infant temperament independent of maternal abuse, prenatal anxiety and postnatal depression at 2 and 6 months post-birth.

Elizabeth A. Werner et al; Dev. Psychobiology (2007)	USA n=52	SCID	Kagan's battery, Infant Behaviour Questionnaire	4 months	Prenatal (independent of postnatal) psychiatric diagnosis is associated with a 4 fold greater odds of the infant having a higher cry reactivity classification .
Anja C. Huizink et al; J. Am. Acad. Of Child and Adolesec. Psychiatry (2002)	The Netherlands n=230	PRAQ, PSS. EPL	BSID, Infant Charac-teristics Questio-nnaire	3 months & 8 months	Pregnancy specific anxiety accounted for 3.3% of the variance in infant attention regulation at 3 months. Higher perceived stress during pregnancy is associated with more difficult infant behaviour at 3 and 8 months independent of postnatal depression.
Evelien P. M. Brouwers et al; Infant Behaviour & Development (2001)	The Netherlands n=131	STAI	BNBAS, BSID	3 weeks, 12 months	Prenatal anxiety is associated with lower orientation scores on the NBAS in the neonate and lower mean mental and motor scores on the BSID at 12 months.
Bea R. Van den Bergh et al; Journal of Pre and Perinatal Psychology and Health (1990) 5:2	Belgium n=70	STAI, PAS, PSC, LES, Coping List	Prechtl scales, QNB, BSID, CMQ	7 days, 2.5 months, 7 months	Prenatal maternal anxiety was associated with reports of increased crying and more frequent change of state in neonates. Prenatal anxiety was also associated with difficult infant temperament at 2.5 and 7 months.
Eva Mohler et al; Early Human Dev. (2006)	Germany n=102	PESI, LEBI, SCL-90-R	Kagan's battery	4 months	Increased maternal emotional stress during pregnancy is associated with lower affective reactivity to novel stimuli in infants.

Margerete Rieger et al; Annals of the New York Academy of Sciences (2004)	Germany n=87	TICS, PDQ, PSS, LES	NBAS	3-5 days	Chronic pregnancy stress is associated with lower neonatal scores for orientation and state regulation. High pregnancy stress is associated with lower neonatal scores for robustness, alertness and endurance.
Marie Paul Austin et al; Early Human Dev. (2005) 81: 183-190	Australia n=1562	STAI, EPDS & life event stress	Short infant temper-ament question-nnaire	4-6 months	Prenatal anxiety is associated with an increased risk of difficult infant temperament (OR 1.96) independent of pre- and postnatal depression. No independent effect of prenatal depression on infant temperament.

Table 4 Summary of prospective, longitudinal studies investigating the associations between prenatal depression and infant temperament.

List of abbreviations of measures used:

STAI: State - Trait Anxiety Inventory (Spielberger et al., 1970)
EPDS: Edinburgh Postnatal Depression Scale (Cox et al., 1987)
CES-D: Centres for Epidemiologic Studies Depressed Mood Scale (Radloff et al., 1977)
PDPI-R: The Predictors of Postpartum Depression Inventory – Revised (Beck et al., 2002, 2006)
SCID: Structured Clinical Interview for DSM IV (First et al., 1997)
PRAQ: The Pregnancy Related Anxiety Questionnaire (Huizink 2000)
PSS: The Perceived Stress Scale (Cohen et al., 1983)
EPL: The Everyday Problems List (Vingerhoets et al., 1989)
DIS: Diagnostic Interview Schedule (Robins et al., 1981)
MSI: Maternal Stress Interview (Field et al.,1988)
QNB: Questionnaire on Neonatal Behaviour (Broussard et al, 1979)
PESI: Prenatal Emotional Stress Index (Mohler et al., 2006)
LEBI: The Leipzig Event and Stressors Inventory (Richter and Guthke, 1996)
SCL-90-R: The Symptom Check List-90-Revised (Derogatis et al., 1973)
POMS: Profile of Mood States (McNair et al., 1997)
TICS: The Trier Inventory for the Assessment of Chronic Stress (Schulz et al., 1998.)
LES: Life Experience Scale (Sarason et al., 1978)
PAS: Pregnancy Anxiety Scale (Shaefer et al., 1960)
PSC: Pregnancy Symptom Checklist
Fagley et al., 1982; Kumar et al., 1984)
IPAT: Institute for Personality and Ability Testing Scale (Cattell and Scheider, 1963)
BIS/BAS: Behavioural Inhibition Scale/Behavioural Activation Scale (Carver and White, 1994)
BSID: The Bayley Scales of Infant Development (Bayley, 2006)
BNBAS: The Brazelton Neonatal Behaviour Assessment Schedule (Brazelton et al., 1984)
CMQ: Carey & McDevitt Questionnaire (Carey and McDevitt, 1978
PDQ: Prenatal Distress Questionnaire (Yali etal., 1999)

2.5 Prenatal Depression: Associations with Birth Outcomes, Infant Growth & Breastfeeding

2.5.1 Introduction

Maternal mental health is an important predictor of healthy infant growth and development. Maternal depression has been associated with lower weights and smaller head circumferences at birth and an increased risk for premature births (Wadhwa, Sandman et al. 1993; Field, Diego et al. 2006). Depressed mothers have been known to display impairments in mother-infant interaction, decreased health seeking behaviour, poorer parenting and reduced breastfeeding compared to non-depressed mothers (Avan, Richter et al. 2010).

Infants are particularly vulnerable to the effects of maternal depression because of their dependency on caregivers for their nutritional and health needs. Maternal depression is associated with a reduced ability of the mother to fulfil these needs of the infant. Prenatal depression has been associated with an increased risk of infant diarrhoea (OR 1.84, 95% CI 1.12-3.03) (Ross, Hanlon et al. 2011) and hospital admissions (OR 2.0, 95% CI 1.1-4.3) (Mandl, Tronick et al. 1999).

The following sub-section discusses the associations of prenatal depression with birth outcome, infant physical growth and breastfeeding.

2.5.2 PRENATAL DEPRESSION: ASSOCIATIONS WITH BIRTH OUTCOME

Prenatal depression has been associated with a range of adverse obstetric outcomes for both mother and infant. Depression during pregnancy has been found to predict an increased risk of puerperal pathologies including pre-eclampsia, placental abnormalities and an increase in the rate of operative deliveries (RR 2.28 95% CI: 1.15 to 4.53) (Kurki, Hiilesmaa et al. 2000; Chung, Lau et al. 2001; Jablensky, Morgan et al. 2005). Prenatal depression has also been associated a higher incidence of preterm deliveries (Steer, Scholl et al. 1992; Sandman, Wadhwa et al. 1997; Orr, James et al. 2002; Dayan, Creveuil et al. 2006). Importantly, prenatal depression has been found to predict low birth weight in neonates (Steer, Scholl et al. 1992; Paarlberg, Vingerhoets et al. 1995). In a study of 143 infants in Rawalpindi, Pakistan, Rahman and colleagues have reported the birth weight of infants exposed to prenatal depression during intra-uterine life to be significantly lower (2910 grams) than controls (3022 grams; $z = 2.09$, $p < 0.05$) (Rahman 2007). A unit increase in depression scores was found to reduce birth weight in infants by 9.1 grams (95% CI: -16.0 to – 2.3) (Hoffman and Hatch 2000) in a US based sample of pregnant women of lower occupational status.

A number of hypotheses have been proposed to explain the higher incidence of adverse obstetric outcome associated with prenatal depression. First, the effect of elevated maternal cortisol in prenatally depressed mothers has been implicated in the higher incidence of low birth weight and preterm births. Higher rates of foetal growth restriction, decreased placental size and pre-eclampsia have been reported in animals exposed to glucocorticoids during

gestation (Field, Diego et al. 2006). Second, increased uterine artery constriction and decreased utero-placental blood flow have been reported in depressed mothers. This occurs because of influence of prenatal depression on (i) the activation of the maternal sympathoadrenal system and (ii) the maternal HPA axis (resulting in raised levels of pCRH). This limits the supply of oxygen and nutrients to the foetus resulting in a higher incidence of low birth weight and preterm birth (Kloet 2003; Field, Diego et al. 2006). Third, there are a number of environmental factors that are associated with a higher risk for both prenatal depression and adverse birth outcomes. These include poor maternal nutrition during pregnancy, poverty, infectious diseases and environmental adversity such as disasters, conflict and migration (Wachs, Black et al. 2009).

2.5.3 POSTNATAL MATERNAL DEPRESSION AND INFANT PHYSICAL GROWTH

UNICEF estimates that over 200 million children in the under 5 age group are undernourished (Patel, Rahman et al. 2004) with more than half of child deaths occurring globally attributable to malnutrition (Rahman, Iqbal et al. 2004). Further in what is referred to as the 'Asian enigma', a higher prevalence of undernutrition is reported from South East Asia (50%) compared to Africa (30%) notwithstanding similarities in the levels of poverty, government policies, child mortality and agricultural produce (Ramalingaswami, Jonsson et al. 1996). This has been attributed to intra-uterine malnourishment, low birth weight and inequality between men and women in South East Asia.

Infants of depressed mothers show significantly more growth retardation than the infants of non-depressed mothers (Patel, Rahman et al. 2004). This is of significance in low and middle income countries (LAMICs) where maternal competence in childcare is an important

predictor of child wellbeing and survival in the first year of life. Poverty, overcrowding and poor sanitation also contribute to increasing environment adversity and decreasing child survival in these countries.

Table 5 describes the four studies from the Indian subcontinent investigating the association between maternal depression and infant growth. In a study of 171 babies from Goa, India postnatal maternal depression (PND) was associated with the infant being below the 5th centile for age-adjusted weight (RR 2.3, 95% CI: 1.1-4.7) and length (RR 2.9, 95% CI: 1.3-6.8) at 6 months (Patel, DeSouza et al. 2003; Patel, Rahman et al. 2004). This association remained significant after controlling for the confounding influences of birth weight, breastfeeding and the physical health of the infant. In a clinic based, case control study from Vellore, south India, PND predicted a seven fold increase in risk for infant undernutrition (OR 7.4, 95% CI: 1.6-38.5) at six to twelve months after controlling potential confounders (Anoop, Saravanan et al. 2004). Similarly, PND was associated with infant stunting (OR 3.5, 95% CI 1.58-8.6) and under nutrition (OR 3.2, 95% CI 1.1-9.9) in a sample of six month olds from Pakistan (Rahman, Iqbal et al. 2004) independent of maternal socio-economic status, infant sex, birth weight, maternal education and family structure. A study from rural Bangladesh however, found no association between maternal depression after birth and infant physical growth at six months of age (Black, Baqui et al. 2009). Nevertheless, by twelve months the infants of depressed mothers experienced linear growth faltering significant and chronic enough to cause stunting.

Study	Location & Sample Size (n)	Infant Age at Assess-ment	Child Outcome Assessed	Association of Child Outcome with Maternal Depression
Patel et al 2003	Goa, India (urban) n=171	6 months	Underweight	RR 2.3 (95% CI: 1.1-4.7)
			Stunting	RR 2.9 (95% CI: 1.3-6.8)
Anoop at al 2004	Vellore, Tamil Nadu, India (semi-urban) n=144	6-12 months	Malnutirtion	OR 3.1 (95% CI: 0.9-9.7)
Rahman et al 2004	Rawalpindi, Pakistan (rural) n=172	6 months	Underweight	OR 3.5 (95% CI: 1.5-8.6)
			Stunting	OR 3.2 (95% CI: 1.1-9.9)
		12 months	Underweight	OR 3.0 (95% CI: 1.5-6.0)
			Stunting	OR 2.8 (95% CI: 1.3-6.1)
Black et al 2009	Matlab, Bangladesh (rural) n=221	6 months	Underweight	r = -0.05, p>0.05
			Stunting	r = -0.01, p>0.05
		12 months	Underweight	r = -0.10, p>0.05
			Stunting	r = -0.13, p<0.05

Table 5 Studies from the Indian subcontinent investigating the association between maternal mental health and infant growth.

Similar associations between maternal depression and infant growth have been reported from other developing countries. In a case-control study from Nigeria, compared to controls, infants of mothers with depression were found to have poorer growth at three (weight OR 3.41, 95% CI: 1.30-8.52; length OR 3.28, 95% CI: 1.03-10.47) and six months post birth (weight OR 4.21, 95% CI: 1.36-13.20; length OR 3.34, 95% CI: 1.18-9.52) (Adewuya, Ola et al. 2008). Jamaican mothers with undernourished children were found to have higher depression scores than mothers of well-nourished children (Baker-Henningham, Powell et al. 2003), while a study from a socio-economically deprived area in São Paulo, Brazil found pre-schoolers with severe protein energy malnutrition to be 2.8 times more likely to have a depressed mothers than controls (95% CI: 1.2-6.9)(Miranda, Turecki et al. 1996). This association remained significant after adjusting for birth weight, maternal education and family income. Another Brazilian study found PND to predict short stature in children independent of parenting efficacy (Surkan, Kawachi et al. 2008). The study however did not find an association between maternal depression and the child being underweight. Similarly, a study of peri-urban African mothers and infants from Khayelitsha, near Cape Town, found no clear associations between PND and infant growth (Tomlinson, Cooper et al. 2006).

Thus, the findings of previous literature linking postnatal maternal depression to infant growth are mixed. While some studies have reported maternal depression to predict infant undernutrition, others have not found evidence of such an association. Further, there appears to be variability in findings from studies conducted in different parts of the developing world.

Prenatal Maternal Depression and Infant Growth

There are no reports of the effect of prenatal maternal depression on infant growth in the developing world. All reports from LAMICs that have explored the association between

maternal depression and child growth have focussed solely on postnatal depression. Limited evidence from the western world has reported associations between prenatal depression and poor motor development in infants exposed to the prenatal maternal depression during intra uterine life (Huizink, Robles De Medina et al. 2002; Huizink, Medina et al. 2003; Field, Diego et al. 2006). However, these studies investigate the effect of prenatal depression on infant motor performance and not on infant anthropometric indices of physical growth such as weight and length.

Intra-uterine growth and birth weight are well established predictors of later growth and development. Prenatal depression is known to impact foetal growth and has been associated with low birth weight and the birth of babies that are small for gestational age (Field, Diego et al. 2006). It is important that its effect on postnatal growth be explored since low birth weight babies are at a disadvantaged start for postnatal physical growth. In addition, the symptoms of prenatal depression are known to predate PND. PND has been reported to impair breastfeeding and the mother's ability to care for her child (Cooper, Murray et al. 1993; Murray and Cooper 1997).

Mechanisms linking perinatal maternal depression and infant growth retardation:

Three mechanisms have been proposed to explain the links between perinatal maternal depression and poor infant growth (Fig. 11).

First, prenatal depression has been associated with decreased health seeking behaviour and a higher incidence of risk taking behaviours including smoking and unhealthy eating in mothers (Patel, Rahman et al. 2004). This influences foetal growth and, consequently, the new-borns of prenatally depressed mothers have been shown to demonstrate lower weights,

lengths and smaller gestational ages at birth (Copper, Goldenberg et al. 1996) placing them at higher risk for under nutrition and poor health outcomes postnatally.

Second, maternal depression impacts the emotional quality of parenting. Depressed mothers have been found to be less sensitive to the needs of their infants (Murray, Fiori-Cowley et al. 1996; Murray, Hipwell et al. 1996) . They also report increased difficulties with breastfeeding which in turn contribute to impaired infant growth (Henderson, Evans et al. 2003).

Finally, it is possible that the higher incidence of psychosocial adversity (including poverty and poor levels of social support) seen in depressed mothers, may be causal to both perinatal depression and impaired child growth and development.

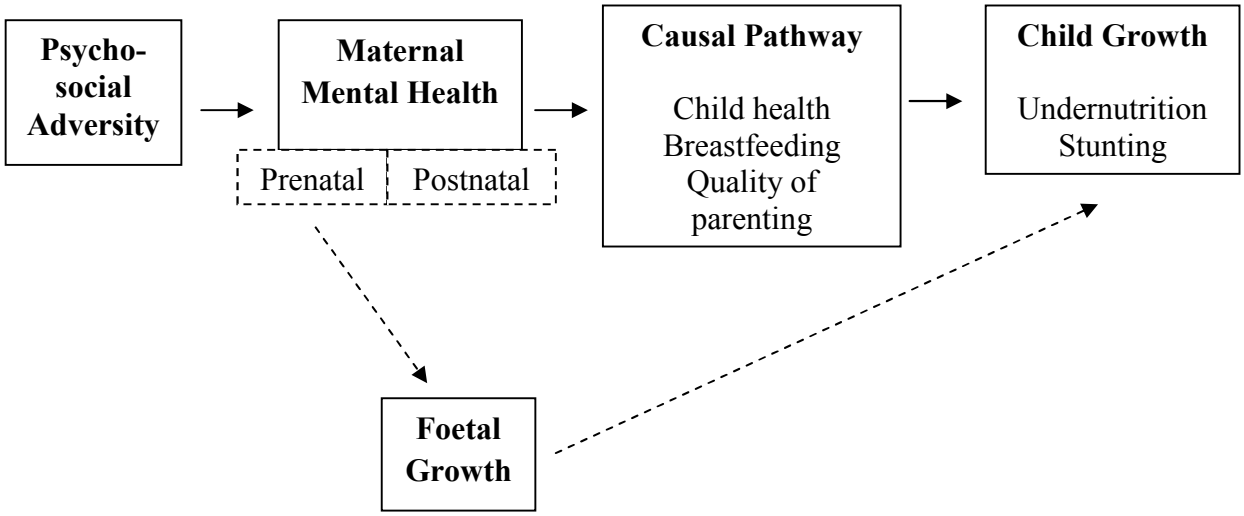


Fig. 11 Conceptual framework for the effect of perinatal maternal depression on child growth (Harpham, Huttly et al. 2005).

2.5.4 PERINATAL MATERNAL DEPRESSION AND BREASTFEEDING:

"For babies everywhere the benefits of breastfeeding are undisputed. But for babies in developing nations, breastfeeding is imperative"

The Right Reverend Simon Barrington-Ward

The progress of Nations 1997, UNICEF report

Breastfeeding is an important part of mothering and is promoted by obstetricians and midwives globally. The World Health organisation (WHO) strongly recommends exclusive breastfeeding for the first six months of life, with early initiation within one hour of birth (Barrington-Ward 2007). The WHO estimates that 50.9% of Indian infants between 2 and 3 months are exclusively breastfed. Of these infants, 25.4% received their first feed within an hour of birth (Barrington-Ward 2007).

The nutritive and immunological properties of breast milk contribute to the large number of health benefits associated with breastfeeding (Barrington-Ward 2007). These include enhanced cognitive development in infancy; a lower incidence of respiratory tract infections, gastrointestinal illness, asthma and atopy in childhood and a protective effect against obesity and chronic diseases (cardiovascular disease, atherosclerosis, inflammatory bowel disease) in adult life (Allen and Hector 2005). Breast milk can provide all the infant's calorific requirements up to six months of age and one half to one third between 6-12 months and 12-24 months respectively (WHO, 2010). Breastfeeding has also been reported to lower the risk of the development of breast and ovarian cancer in the mother and promotes recovery after childbirth (Allen and Hector 2005).

Maternal depression has been associated with reduced duration of breastfeeding and dissatisfaction with breastfeeding (Henderson, Evans et al. 2003). Perinatal psychosocial

stress has been reported to predict delayed initiation of breastfeeding in an Ethiopian sample (RR 2.8, 95% CI: 1.3-6.2) (Hanlon, Medhin et al. 2009). A report from Pakistan has shown that up to 40% of women who stop breastfeeding in the first three months after delivery suffer from depression (Taj and Sikander 2003).

PND is also associated with decreased and less efficacious breastfeeding. In a study of mothers and infants from Goa, India, depressed mothers were more likely to report infant difficulties with breastfeeding and sucking (RR 2.4, 95% CI: 1.5-3.9 and RR 1.7, 95% CI: 1.1-2.9) (Patel, Rodrigues et al. 2002). In a large Australian cohort, PND was associated with early cessation of breastfeeding (adjusted hazard ratio 1.25, 95% CI 1.03–1.52) (Henderson, Evans et al. 2003). In the study, mothers with early onset depression breastfed their infants for a median duration of 6.5 months compared to 7 months for those with late onset depression and 9.7 months for controls. A study of 226 Barbadian mothers reported breastfeeding attitudes to be associated with maternal mood at 6 months (Galler, Harrison et al. 2006).

While the association between PND and breastfeeding is well established, there are no inquiries investigating its association with prenatal depression. This is important because the influence of PND on breastfeeding problems may be contributed to by low levels of maternal-foetal attachment developed prenatally. Maternal-foetal attachment is known to predict postnatal mother-infant interactions (Müller 1996; Siddiqui and Hägglöf 2000), and compared to controls, lower levels are displayed by depressed mothers (Lindgren 2001). Further symptoms of prenatal depression are known to predate PND in about two-thirds of mothers (Chandran, Tharyan et al. 2002; Patel, Rodrigues et al. 2002).

2.5.5 CONCLUSIONS

A substantial body of literature, from the western world and the developing world, has provided evidence in support of the influence of prenatal maternal depression on adverse birth outcomes, particularly a higher incidence of low birth weight, preterm births and puerperal pathologies.

There are however no inquiries into the association between prenatal depression, and infant physical growth and breastfeeding, from the developing world despite high rates of prenatal depression and infant mortality being reported from these settings. Although inquiries linking maternal depression in the postnatal period with infant undernutrition do exist from the developing world, findings are mixed and some studies have not found evidence of an association.

In conclusion, a significant gap in the understanding of the association between prenatal maternal depression and infant growth exists. Further, the influence of prenatal depression on breastfeeding is not known.

2.6 SUMMARY AND SYNTHESIS

The association between postnatal maternal depression and problems in child development is well established (Murray and Cooper 1997). A recent body of literature has found prenatal depression to be an important risk factor for disturbances in child development. Evidence exists to suggest that these influences may begin even before birth.

High rates of prenatal depression are reported from low and middle income countries (LAMICs). India reports a prevalence rate of 16.2% of a diagnosis of major depression during pregnancy and 25% of elevated symptoms of prenatal depression (Chandran, Tharyan et al. 2002; Patel, Rodrigues et al. 2002). This is in contrast to rates of 10% of a diagnosis of prenatal depression and 20% of elevated depressive symptoms reported from the western world (Bennett, Einarson et al. 2004; Gavin, Gaynes et al. 2005). Women in the developing world are exposed to multiple risks factors for depression because of the high rates of socio-economic adversity, poverty, malnutrition, infectious diseases and violence reported from these settings (Wachs, Black et al. 2009; Lund, Breen et al. 2010). More than 90% of the world's population of children live in developing countries, 13.9% of which reside in India (United Nations Report 2009). Over 80% of global pregnancies occur in LAMICs (United Nations Report 2009). Despite this, there are no inquiries into the influence of prenatal depression on foetal and infant development from these settings. All previous inquiries have emanated from North America, Europe and Australia and it is possible that these findings may not be generalisable to rural populations in developing countries.

One of the key neuronal substrates for the effects of prenatal depression on disturbances in child development is the offspring's hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis regulates the organism's response to stress. It has been proposed to be particularly sensitive to the influence of maternal depression and the associated elevations in maternal cortisol because of the high density of glucocorticoid receptors in the hippocampal and parahippocampal areas of the foetal brain (Sandman, Wadhwa et al. 1999). Research has therefore attempted to investigate disturbances in offspring stress responsivity as a possible marker for the potential programming influences of prenatal depression on offspring development.

The previous sections have provided an overview of the literature investigating the association of prenatal maternal depression with foetal and infant development, in particular (i) foetal cardiac responsivity to a stressor, (ii) infant cortisol responsivity to stress and (iii) infant temperament.

Despite the methodological difficulties encountered in assessing foetuses using non-invasive techniques, six inquiries from the western world have studied the association between prenatal maternal stress and foetal stress responsivity by measuring changes in foetal heart rate (FHR). Although only one has investigated this in the context of prenatal depression, the findings suggest an association between elevations in foetal heart rate (FHR) during foetal exposure to a stressor and prenatal depression, anxiety and/or stress (Monk, Fifer et al. 2000; Allister 2001; Monk, Myers et al. 2003). No associations between prenatal depression and foetal responsivity were detected during baseline FHR monitoring (Monk, Fifer et al. 2000; Monk, Myers et al. 2003). Further, in two different reports, Sandman and colleagues argue that the relationship between neuroendocrinological markers of prenatal psychopathology and foetal stress responsivity may not be linear, but non-linear (cubic) in nature (Sandman, Wadhwa et

al. 1999; Sandman, Glynn et al. 2003). Despite the limited number of inquiries into patterns of foetal responsivity in the context of prenatal depression and anxiety, the findings provide evidence suggesting that the potential programming influence of prenatal depression on offspring development may be observed even during foetal life. The inquiries are however limited in that many of them employ indirect techniques of foetal stimulation using maternal cognitive responses to stressful tasks (Monk, Fifer et al. 2000; DiPietro, Costigan et al. 2003; Monk, Myers et al. 2003). The studies also use mean FHR to study foetal responsivity rather than quantifying the magnitude of foetal response to stimulation. In addition, all inquiries have been carried out in western populations and in samples where prenatal smoking and alcohol consumption may confound the findings.

Investigations into the influence of prenatal maternal depression on postnatal responsivity to stressors have relied on measures of infant cortisol and temperament. Broadly speaking, prenatal maternal depression has been found to predict elevated infant cortisol responses to stressful tasks in different samples at 0.5, 3, 5 and 10 years of age (Gutteling, Weerth et al. 2005; O'Connor, Ben-Shlomo et al. 2005; Brennan, Pargas et al. 2008; deBruijn, Bakel et al. 2009). This effect has been reported to be independent of the influence of postnatal maternal mood. In addition, compared to controls, disturbances in baseline, awakening and afternoon cortisol has been reported in the children of prenatally depressed mothers (Lundy, Jones et al. 1999; Field, Diego et al. 2004; Glover, Miles et al. 2005). Although the mechanisms underlying this association are as yet not well understood, these findings suggest the possibility of a programming influence of maternal depression (and elevated maternal cortisol) on the developing offspring's HPA axis. However, all previous inquiries have been carried out on populations from high income countries and no evidence from LAMICs exists. It is possible that prenatal smoking and alcohol consumption may confound the findings and

no inquiries have been carried out in samples where these behaviours are not reported. In addition, inquiries have relied on infant exposure to laboratory based stress-provoking tasks and investigations into cortisol responses to a routine stressor such as immunisation have not been studied in healthy, term infants.

Evidence linking prenatal depression to difficult infant temperament is mixed and while some studies have found prenatal depression to predict disturbances in neonatal behaviour and infant temperament (Field, Diego et al. 2001; Davis, Glynn et al. 2007; McGrath, Records et al. 2008) others have not reported evidence of such an association (Austin, Hadzi-Pavlovic et al. 2005; Kaplan, Evans et al. 2008). The heterogeneity of measures used to assess maternal mood and infant outcomes, and the wide range in sample size and the infant's age at assessment, limits comparability of these findings. Further, no evidence into the association between prenatal depression and infant temperament exists from the developing world and all previous inquiries have been carried out in western populations.

Prenatal depression has also been linked to other infant outcomes. These include a higher incidence of low birth weight and preterm deliveries (Wadhwa, Sandman et al. 1993; Field, Diego et al. 2006). However, although infant undernutrition and problems with breastfeeding have been associated with postnatal depression, the association with prenatal depression has not been studied (Cooper, Murray et al. 1993; Patel, Rodrigues et al. 2002; Rahman, Iqbal et al. 2004). This is important in the context of the developing world where high rates of infant mortality are reported and where mothering plays a crucial role in infant survival in the first year of life (Patel, Rahman et al. 2004).

The mechanisms underlying the association between prenatal depression and offspring development are poorly understood and require further investigation. However, a number of potential mechanisms have been hypothesised namely:

i. Foetal exposure to elevated levels of maternal cortisol, in the foetuses of depressed mothers compared to controls, because of:
    (a) Increased levels of maternal cortisol (Lundy, Jones et al. 1999)
    (b) Increased secretion of placental corticotrophin releasing hormone (Sandman, Wadhwa et al. 1999)
    (c) Down regulation of the placental enzyme 11 beta hyrdoxy steroid dehydrogenase 2 and increased placental permeability to cortisol (Glover, Bergman et al. 2009).
ii. Activation of the sympathetic nervous system in prenatally depressed mothers resulting in decreased utero-placental blood flow (Copper, Goldenberg et al. 1996).
iii. Heritability in HPA axis functioning (Young, Aggen et al. 2000).
iv. High risk behaviours such as increased prenatal smoking, alcohol consumption, recreational drug use and low levels of nutrition and health seeking behaviours in depressed mothers (Goldenberg 1991).
v. Continuation of prenatal depression into the postnatal period and its subsequent influence on maternal-infant interaction and mothering (Murray and Cooper 1997; Patel, Rodrigues et al. 2002).

Conclusion

In conclusion, evidence from previous literature suggests that prenatal depression may be associated with disturbances in foetal stress responsivity, infant cortisol responsivity and

infant temperament. The inquiries are however limited in number and evidence in support of the possibility of a programming effect of prenatal depression is mixed. Interestingly, DiPietro and colleagues also suggest the possibility that moderate levels of prenatal stress may be associated with positive outcomes in offspring such as accelerated neurological maturation in foetuses and improved cognitive performance in infants (DiPietro, Novak et al. 2006; DiPietro, Kivlighan et al. 2010). Further, all previous inquiries have been carried out in western populations and no inquiries studying these effects exist from the developing world despite high rates of prenatal depression being reported from these settings. In addition, previous research has not been carried out in samples where the confounding influences of prenatal smoking and alcohol consumption are not reported.

Further research into the relationship of prenatal depression with foetal and infant responsivity to stressors is needed before these processes and the mechanisms underlying them can be clearly understood. Importantly, future research in this field must, in addition to high income countries, also be directed towards the needs of families in the developing world.

CHAPTER 3
AIMS & OBJECTIVES

3 AIMS AND OBJECTIVES

Aim

The aim of this thesis is to investigate the association between maternal prenatal depression and foetal and infant responsivity to stress in a non-smoking, non-alcohol consuming prenatal population in rural South India.

3.1 PRIMARY OBJECTIVES:

The primary objectives of this thesis are to examine any association between prenatal depression during the third trimester of pregnancy, in a non-smoking non-alcohol consuming population in rural south India, and:

i. Foetal Responsivity as measured through foetal cardiac responses to vibroacoustic stimulation
ii. Infant cortisol responsivity to immunisation at 57.7 days (SD: 23.2 days) post birth
iii. Infant Temperament.

Primary Hypotheses:

i. Prenatal maternal depression in the third trimester of pregnancy is associated with increased foetal response to stress measured through the total magnitude of foetal heart rate (FHR) responses to vibroacoustic stimulation. Compared to controls, foetuses of depressed mothers show higher FHR during the post-stimulation period and a greater increase in FHR from baseline to post-stimulation levels. Further,

compared to controls, the foetuses of depressed mothers show delayed habituation to vibroacoustic stimulation.

ii. Compared to the infants of non-depressed mothers, the infants of prenatally depressed mothers have a greater cortisol response to immunisation at 35.56 weeks post-birth.

iii. Prenatal maternal depression in associated with increased maternal reports of difficult infant temperament at 35.56 weeks post-birth.

3.2 SECONDARY OBJECTIVES:

The secondary objectives of this thesis are:

i. To determine the prevalence of prenatal maternal depression in the third trimester of pregnancy and its relationship to socio-demographic variables particularly socio-economic adversity and social support

ii. To examine any association between prenatal depression and adverse birth outcomes

iii. To examine whether the physical growth of the infant, and the duration and uptake of breastfeeding, are associated with prenatal depression.

Secondary Hypotheses:

i. Compared to rates in high income countries, prenatal maternal depression is more common in rural South Indian women and is associated with a number of socio-demographic variables including low levels of education and family income, and poor social support.

ii. Prenatal maternal depression during the third trimester of pregnancy is associated with low birth weight and an increased incidence of preterm deliveries.

iii. Compared to the infants of controls, infants of prenatally depressed mothers have lower weights and lengths for age. Prenatal depression is associated with lower uptake of breastfeeding and reduced duration of breastfeeding in those mothers who breastfeed their infants.

Chapter 4
Methods

4 METHODS

This longitudinal cohort study was carried out under the title of the Solur Mother and Baby Project (SMBP) in the village of Solur in rural South India. Participants were assessed prospectively in three phases – a prenatal assessment in the third trimester of pregnancy, extraction of data relating to birth outcomes at birth and a postnatal assessment at 1.5 to 3 months after birth. The following chapter describes the research design and methodology of the study.

Study Location and Design

Study Location

The study was carried out in the village of Solur in rural South India (See Appendix VC). Solur is located in the Magadi *taluk* (sub-district) of the Ramanagara district of the south Indian state of Karnataka, approximately 60 miles south of the state capital of Bangalore (Fig. 12). It measures 17.35 square kilometres in area and has been inhabited for more than 200 years. The roughly 4700 inhabitants live on an average annual income of INR 10,000-15,000 (GBP 125-187.5). The village is a largely agronomical society although a small proportion of

Fig. 12 Map of India showing the location of Solur.

the populace is involved in manual labour in surrounding factories, sericulture (the rearing of silk worms) and the running of small local businesses. The village has an average literacy rate of 80% and a male to female ratio of 712.16 females per 1000 males. Approximately 60% of the inhabitants belong to the Hindu religion while 25% are Muslims and 15% are Christians. The village has 750 children below the age of 18 years. Solur houses basic amenities such as a primary health centre, an *anganwadi* (local crèche), a primary school and a post-office. In addition, the village also has a charitable mission hospital for obstetrics and gynaecology run by a religious congregation of Catholic nuns.

Karnataka is the eighth most populous state in the country with a total population of 52.73 million, 66 % of which live in villages (The Registrar General & Census Commissioner 2010-11). The official and most widely spoken language is Kannada. Ramnagara is one of the 30 districts of Karnataka state of which Magadi is a taluk. According to the 2001 census data of India, Magadi has a total population of 25,000 with 3,119 children below the age of 6 years. The male to female ratio is 961.2, with a literacy rate of 67% and 58% among males and females respectively. The population consists largely of Kannada speaking people though some may speak Hindi, Tamil, Tulu or Urdu as well.

The study was based in Snehalaya Hospital (*sneha = love, laya = the abode of*), a charitable mission hospital located in the village of Solur. Snehalaya is a 40 bedded hospital offering heavily subsidised healthcare to the economically disadvantaged. It caters to the obstetric, gynaecological, paediatric and general health care needs of Solur and the surrounding villages (an overall rural populace of approximately 90,000). It houses an operation theatre, delivery room, incubators for neonatal care, an outpatient clinic and prenatal, postnatal and general wards. Snehalaya Hospital is run by the religious congregation of the Sisters of Charity of

Capitanio and Gerosa. It is staffed by one senior doctor, two junior doctors (medical interns), six senior nurses and approximately 80 other non-specialised staff. The hospital runs an out-patient unit 5 days a week and conducts between 80-150 prenatal assessments per clinic. It also runs a mobile clinic twice a week during which 6 members of staff travel to distant villages to administer healthcare. An orphanage, a school of nursing, a women's empowerment unit and a nursery are attached to the hospital.

Collaborations

The study was run in collaboration with the following institutions:

i. Snehalaya Hospital, Solur, Magadi Taluk , Ramnagara District, Karnataka, India
ii. St. John's Research Institute, St. John's National Academy of Health Sciences, Bangalore, India

Study Design

The study was longitudinal and observational in design. Participants meeting inclusion criteria were recruited from the prenatal outpatient clinic of Snehalaya Hospital in their third trimester of pregnancy and invited to participate in the study. Assessments were carried out prospectively in three phases – a prenatal assessment, a birth phase and a postnatal assessment (Fig. 13). A summary of the exposure and outcome variables assessed in the study are summarised in Table 6. A brief overview of the study methodology is described below. The details of the prenatal assessment, the birth phase and the postnatal assessment are described in Sections 2.1, 2.2 and 2.3 respectively.

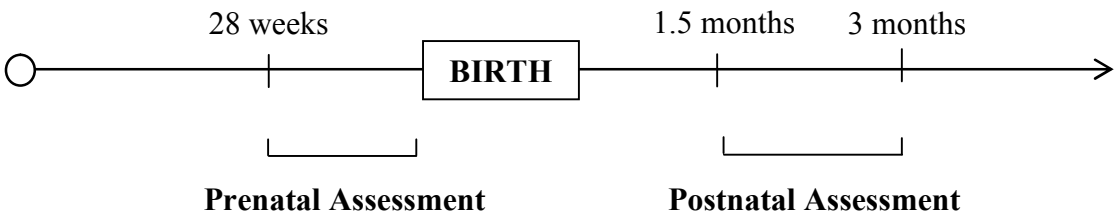


Fig. 13 Schematic representation of the three-phase, prospective study design.

Recruitment: Women attending the prenatal clinic at Snehalaya Hospital and meeting the following inclusion criteria were approached to participate in the study:

i. They were in their third trimester of pregnancy with singleton foetuses with no congenital anomalies - as detected by ultrasound investigation.
ii. They spoke one of the study languages i.e. Kannada (the local language) or English.
iii. They had no prior history of a severe psychiatric disorder namely schizophrenia or other psychotic illness, and had not suffered from major depression prior to the onset of their current pregnancy.
iv. They were not on any prescribed psychotropic medication since the onset of pregnancy.

The aims, methodology and longitudinal nature of the study were explained to potential participants and they were invited to participate. A patient information sheet, translated into Kannada, was given to all women who displayed an interest in the study (See Appendix IA for English and Kannada versions of the patient information sheet). Written, informed consent was obtained from those who agreed to participate in the study. Consent was obtained in the language of choice as expressed by the participant (See Appendix IB for English and Kannada versions of the informed consent sheet).

Construct		Measure	Prenatal Phase	Birth Phase	Postnatal Phase
Exposure Variable					
Prenatal Maternal Depression		EPDS	+		
		K10	+		
		MiniPlus	+		
Outcome Variables					
Primary Outcome Variables	**Foetal Stress Responsivity**	Foetal cardiac responses to VAS	+		
	Infant Stress Responsivity	Infant cortisol response to immunisation			+
	Infant Temperament	Infant behaviour questionnaire			+
Secondary Outcome Variables	**Birth Outcome**	Extraction of birth outcome date from hospital records		+	
	Infant Physical Growth	Infant weight, length & head circumference			+
	Breastfeeding	Breastfeeding questionnaire			+
Confounding Variables					
Postnatal Maternal Depression		EPDS			+
		K10			+
		MiniPlus			+
Social Variables		Socio-demographic data	+		
		SSQ	+		
Medical & Obstetric Variables		Prenatal health data sheet	+		
		Maternal nutrition (weight, height, body mass index)	+		
		Postnatal data sheet			+

VAS=Vibroacoustic stimulation; SSQ=Social support questionnaire

Table 6 Summary of study design.

Prenatal Assessment: The prenatal assessment was carried out in the third trimester of pregnancy. Mothers were assessed for prenatal depression and information on socio-demographic status, social support and maternal health was obtained. Foetuses were assessed for responses to vibroacoustic stimulation, and mothers were invited to return for follow-up at 1.5-3 months after birth.

Birth Phase: Data pertaining to birth outcome, such as birth weight, gestational age at birth, type of delivery and complications relating to birth, was extracted from hospital records within 24 hours of the baby being born. Participating mothers who delivered their babies at Snehalaya Hospital were reminded to return for postnatal follow-up at this stage.

Postnatal Assessment: Mothers and infants returned at 1.5-3 months after birth. Mothers were assessed for postnatal depression. A small amount of infant saliva was collected before and after infants received their routine dose of the diphtheria-pertussis-tetanus (DPT) combined vaccine and assayed for cortisol at a later date. Mothers reported on the temperament of their infants and infant anthropometry (weight, length and head circumference) was measured.

The 1.5-3 months postnatal period coincides with the universal immunisation programme of the national immunisation schedule (NIS) of India. Infants are given their first, second and third dose of DPT vaccine at 1.5, 2.5 and 3.5 months of age. Synchronising the postnatal assessment with the NIS allowed us the opportunity to collect infant saliva before and after immunisation. In this model, immunisation represents the unpleasant, pain-evoking stressor in a pre-stress/post-stress paradigm to study infant cortisol responsivity to stress. All children are

routinely exposed to this stressor. It was also hoped that coinciding the postnatal assessment with the immunisation of the infant would help to reduce attrition in the sample.

At both pre- and postnatal assessments, mothers identified as clinically depressed were referred to the senior doctor of Snehalaya Hospital (who was the local collaborator for this study) for further assessment. Participants found to require referral to a tertiary psychiatric clinic by the senior doctor were referred to the Department of Psychiatry at St. John's Medical College Hospital, Bangalore (the local collaborating institution). Arrangements were made for the provision of subsidised healthcare at St. John's Medical College Hospital for these participants of the study.

Power and Sample Size Estimation

There was limited data on which to base the estimation of power for this study, particularly because of the lack of literature from the prenatal developing world population, the heterogeneity in similar studies carried out in the western world and the large variability in the methods used to assess maternal depression and infant outcomes. It was therefore decided to establish a design which could detect a difference substantial enough to be considered clinically significant. A moderately large effect size of 0.6 or greater was therefore selected for this study. In support of this, findings from a study by Bergman and colleagues (Bergman, Sarkar et al. 2007) show a correlation of -0.39 between prenatal stress and child developmental outcomes (namely cognition and fearfulness) corresponding to an effect size of 0.6 (standardised difference).

To detect an effect size of 0.6, the sample size of 66 depressed participants and 66 controls was computed for the prenatal assessment. This calculation allows for a postnatal attrition rate

of 32%, which is the rate commonly encountered in longitudinal studies of a similar design (O'Connor, Heron et al. 2002), while still providing power in excess of 80% at the significance level of 5% (one-tailed). The follow-up sample would therefore be required to consist of 50 depressed and 50 non-depressed mother-infant dyads. All power calculations for this study were carried out using the GPOWER software (Buchner, Erdfelder et al. 1997).

Using the lowest reported prevalence of maternal depression in India [19%, (Chandran, Tharyan et al. 2002)], sample size calculations showed that 347 participants would have to be screened for prenatal depression in order to obtain 66 women who scored positive for depressive symptoms.

4.1 THE PRENATAL ASSESSMENT

The design and methodology employed during the prenatal assessment of the study are described in this sub-section. All aspects of data collection during the prenatal assessment, including the recording of foetal responses, were carried out by the author of this thesis. The processing of FHR data was carried out by two researchers blind to the prenatal mood status of the participants.

4.1.1 PARTICIPANTS

Participants were women in their third trimester of pregnancy (≥28 weeks gestational age) who attended the outpatient clinic at Snehalaya Hospital, Solur for their prenatal check-ups.

4.1.2 STUDY DESIGN

Participants were assessed for symptoms of prenatal depression using two screening questionnaires – the Edinburgh Postnatal Depression Scale [EPDS (Cox, Holden et al. 1987)] and the Kessler 10 Scale of Psychological Distress [K10 (Kendler, Gallagher et al. 1996; Kessler, Barker et al. 2003)]. Although designed as a self-report measure, the low levels of literacy in the sample and the unfamiliarity of the rural population with self-report questionnaires necessitated an interviewer administered design. Participants scoring above the threshold for depression on either the EPDS (≥13) or the K10 (≥4) were categorised as

depressed while those scoring below the cut-offs on both scales were classified as controls. A structured clinical interview, the Mini International Neuropsychiatric Interview Plus, version 5.0.0 [MINIPlus (Sheehan, Lecrubier et al. 1997)], was then administered to all participants in order to obtain an ICD-10 diagnosis of a major depressive disorder (WHO 1994).

The first 66 participants to score high for symptoms of prenatal depression on either the EPDS or the K10 and the first 66 controls then completed a social support questionnaire [SSQ (Ramthal, Thomas et al. 2011)] designed for use in the rural South Indian population. Socio-demographic details, data on maternal health during pregnancy, marital discord and the woman's preference for the gender of her unborn child were collected using a semi-structured interview adapted from Vikram Patel's study of 270 mothers in Goa, India (Patel, Rodrigues et al. 2002). Maternal height (in centimetres) and weight (in kilograms) were measured using a standard tape measure and an adult weighing scale respectively. Data on the most recent maternal haemoglobin value (measured in grams per decilitre, g/dL) during the current pregnancy were extracted from hospital records. Further details on the socio-demographic and maternal data sheets can be found in Appendix II A.

The sub-group of 66 depressed participants and controls then participated in the foetal heart rate (FHR) monitoring protocol to assess foetal responsivity. FHR was measured at baseline, during exposure to repeated vibroacoustic stimulation and post-stimulation over a period of 30 minutes. Further details on the FHR monitoring protocol are described in the section that follows.

4.1.3 MEASURES

4.1.3.1 PSYCHOMETRIC QUESTIONNAIRES

The Edinburgh Postnatal Depression Scale

The EPDS is a self-report screening instrument for pre- and postnatal depression (See Appendix IIB). It consists of 10 items based on a one week recall. Each item is scored from 0-3, with some items being reverse scored, yielding a total score of 0-30. Although designed to screen a broad population for postnatal depression (Cox, Holden et al. 1987), the EPDS has also been found to be a reliable screening instrument for prenatal depression. Its advantage lies in its ability to filter out the biological symptoms of pregnancy, such as fatigue, body ache and a change in sleep and appetite patterns, when screening women for prenatal depression.

In a validation study on 100 UK-based pregnant women, Murray and Cox reported the EPDS to have a sensitivity of 64%, a specificity of 90% and a positive predictive value of 50% at a cut-off score of 13 and above in screening for major and minor prenatal depression (Murray and Cox 1990). This cut-off has also been employed in a study of pre- and postnatal depression among low income Brazilian women (Da-Silva, Moraes-Santos et al. 1998). In the current study, a cut-off score of 13 and above was used to assess prenatal depression in concordance with previous reports from the Indian subcontinent (Chandran, Tharyan et al. 2002; Patel, Rodrigues et al. 2002).

However, questions have been raised about the appropriateness of the use of the EPDS in countries other than the UK because of the presence of certain culture-specific items that present difficulties in translation and comprehension in culturally diverse populations. This is reflected in reports of different threshold scores in different populations, e.g. ≥10 in Chile and

the United Arab Emirates (Bashiri and Spielvogel 1999), ≥11 in Lithuania (Bunevicius, Kusminskas et al. 2009) and ≥14 in Malta (Felice, Saliba et al. 2006).

Nevertheless, the EPDS is one of the most popular screening measures for perinatal depression in both the western world and the developing world. It is non-intrusive and well accepted by participants, easy to administer, quick to complete and has been validated in a variety of population groups across the world (Lee, Yip et al. 1998; Lawrie, Hofmeyr et al. 1998 ; Adouard, Glangeaud-Freudenthal et al. 2005; Benjamin, Chandramohan et al. 2005; Santos, MatijasevichI et al. 2007). Further, it has been widely used in assessing maternal depression in the Indian subcontinent (Chandran, Tharyan et al. 2002; Patel, Rodrigues et al. 2002).

The Kessler 10 Scale of Psychological Distress

The K10 is a self-report screening measure of psychological distress (See Appendix IIC). It was developed based on extensive psychometric analyses in large general population samples using modern item response theory models (Kessler 2003). It has been used in the World Health Organisation's Mental Health Initiative and in national health surveys in the US, Canada and Australia (Furukawa, Kessler et al. 2003; Kessler 2003).

The K10 consists of 10 self-report items scored 0-4 yielding a total score of 0-40. Participants rate the psychological distress experienced by them on a 5-point Likert scale using a 4-week recall period.

The K10 has been validated for screening for mental illness in the general population in the developing world. A survey of 598 participants from Goa, India found the K10 to be a reliable

screening instrument for psychological distress in the general population at a cut-off score of ≥7 (Patel, Araya et al. 2008). A report from the Australian National Survey of Mental Health and Wellbeing found the K10 to have a sensitivity of 90% and a specificity of 72% at a cut-off score of ≥5 in screening for current DSM-IV disorders (Andrews and Slade 2001). Only one inquiry from the developing world has validated the K10 in screening for perinatal maternal depression (Baggaley, Ganaba et al. 2007). This study, conducted in Burkina Faso, West Africa, validated the measure against an interview with a local psychiatrist and found the K10 to be an acceptable screening tool for postnatal depression at a cut-off score of ≥4. A cut-off score of ≥4 on the K10 was thus adopted to screen mothers for prenatal depression in the Solur Mother and Baby Project.

Since its development in 2003, the K10 has been gaining popularity as a screening instrument for psychological distress in research settings in the western and developing world. It has items to assess symptoms of both depression and anxiety and has been translated into many languages. However, the K10 was not specifically designed to screen for perinatal depression and thus includes the biological symptoms of depression which are also features of normal pregnancy. Nevertheless, its ease in administration and scoring makes it conducive for use by overburdened healthcare workers in low-resource settings. Further, its items are not culture-specific, are applicable in a wide range of settings, and lack meaningful gender and education biases (Baillie 2005).

The Mini International Neuropsychiatric Interview Plus

The MINIPlus was the reference standard diagnostic interview used in this study (Appendix IID). It has been validated against the SCID for a DSM-III diagnosis and the Composite International Diagnostic Interview (CIDI) for an ICD-10 diagnosis (Sheehan, Lecrubier et al.

1997). The MINIPlus contains 26 modules to assess the major axis I psychiatric disorders in DSM-IV TR and ICD-10 and is reported to have good test-retest and inter-rater reliability.

The MINIPlus was selected over other gold standards for use in this study because it has been widely used in research in India, covers a broad range of disorders and is easy to administer (Fernandes, Srinivasan et al. 2011).

The Social Support Questionnaire

The social support questionnaire used in this study was adapted from the Assessment of Parental Wellbeing and Behaviours (Ramthal, Thomas et al. 2011). The Assessment of Parental Wellbeing and Behaviours was designed to assess levels of social support in a socio-economically disadvantaged South Indian population and was readily available in the local language, Kannada. The questionnaire was developed following an intensive procedure of translating, adapting and piloting and consists of 6 informational items (pertaining to the participant's access to help on a routine basis) and 6 items on emotional support and positive appraisal. It was validated on 532 primary caregivers of school going children of poor socioeconomic status in Bangalore, India and has an internal consistency of 0.88. The social support questionnaire adapted from the same for use in this study consists of the 12 self-report items of the Assessment of Parental Wellbeing and Behaviours and 3 additional items on intimate partner violence including alcoholism in the family. The items are scored 0-3 yielding a total score of 0-45. Items are reverse-scored so that higher scores represent lower levels of social support (Appendix IIE).

Translation Protocol

The EPDS, K10 and MINIPlus were not available in the local language (Kannada) and were translated to the same using a standard translation-back translation protocol as per the WHO World Mental Health Initiative Interview Translation Guidelines (Harkness, Pennell et al. 2008). The forward and back translations of all three measures were undertaken independently by two bilingual health professionals proficient in English and Kannada. They were instructed to pay careful attention to the conceptual equivalent of phrases as per the WHO guidelines due to the difficulties encountered in the translation of certain idiomatic expressions from English into the local language. This was emphasised to ensure a clear, concise, conceptually accurate translation that was easily comprehended by a rural population with low levels of literacy.

All translations were revised by both the translators, by the senior collaborator at Snehalaya Hospital (a qualified medical professional, bilingual in Kannada and English) and by the author of this thesis. The final Kannada versions were obtained following a consensus on the context, comprehensibility and technical and conceptual equivalence of the translations. Pre-testing was carried out prior to the commencement of the study on ten women from a rural background at the prenatal clinic at Snehalaya Hospital.

4.1.3.2 THE FOETAL HEART RATE (FHR) MONITORING PROTOCOL

Apparatus

1. **The FHR Monitor**

FHR was measured using the Philips Avalon FM20 FHR Monitor which provides continuous monitoring of FHR (Fig. 14). The FHR monitor consists of an ultrasound

Fig. 14 Photograph of the Philips Avalon FM20 FHR Monitor.

transducer, an electronic circuit that converts raw data into a visual display, a touchscreen visual display unit that displays the FHR signal and a marker that prints the trace out onto heat-sensitive graph paper. The transducer produces ultrasound waves at a frequency of 1 MHz (SD 100Hz) every 100µs.

The FHR monitor works on the principle of the Doppler Effect. When the ultrasound transducer is placed on the maternal abdomen, the device generates high-frequency (range) ultrasound waves that are transmitted into the tissues (Tucker, Miller et al. 2009). These waves are reflected back to the transducer at different frequencies when they strike a moving interface such as the foetal heart. The change in frequencies is used to calculate the rate of motion of the foetal heart. The Doppler signal is affected by changes in the position of the transducer or the foetus.

This technique is extremely popular and is utilised in prenatal monitoring in clinical settings worldwide. However, an important limitation is the loss of contact between the transducer and the maternal abdominal wall resulting in signal loss (Tucker, Miller et al. 2009). This may occur because of foetal movement, maternal movement or the slipping of the traducer after being positioned. Further, maternal heart rate may be counted if the transducer is placed over major maternal vessels, such as the aorta, giving a spuriously low FHR reading. Nevertheless, the technique is non-invasive, easy to apply, and may be used in conjunction with other measures such as tocometry (measurement of intra-uterine pressure), ultrasound monitoring of the foetus and maternal heart rate monitoring. There are no adverse effects on either the mother or the foetus. The technique can also be used to measure FHR as early as 26 weeks of gestation (Tucker, Miller et al. 2009).

2. The Vibroacoustic Stimulus

The vibroacoustic stimulus (VAS) used to stimulate foetuses was an electronic artificial larynx (EAL). The device produces a broad-band noise at a fundamental frequency of 65 Hz ±7%, with sound pressure levels measured at 1 m in air averaged at 68 dB. It has been well documented to elicit cardioacceleratory responses in healthy foetuses (Visser, Mulder et al. 1989; Kisilevsky, Muir et al. 1992) and is commonly used in non-stress tests (D'Elia, Pighetti et al. 2005). Although exposure to the VAS has been associated with transient changes in FHR and foetal movement, there is no evidence that vibroacoustic stimulation is associated with harmful neurological or auditory effects in foetuses and in children exposed in utero (Nyman, S et al. 1991; Arulkumaran, Skurr et al. 1991). Further, inquiries investigating the effect of maternal hormones and prenatal maternal stress on the foetus have employed vibroacoustic stimulation as a method to study foetal responses (Sandman, Wadhwa et al. 1999; Allister 2001; Sandman, Glynn et al. 2003).

Procedure

Women participating in the assessment of foetal responsivity were asked to lie supine in a hospital bed in a quiet, comfortably lit room. The position of the foetal heart and head was determined by auscultatory and palpatory methods respectively. The ultrasound transducer was placed at the point of localisation of the foetal heart in order to obtain the maximum signal strength. Participants were reminded to inform the researcher of any distress caused to themselves or their foetus during any part of the procedure and were assured that the procedure would be abandoned upon their request.

The FHR was recorded at baseline while the mother was resting in the bed for a period of 10 minutes, i.e. from minute 00:00 to minute 09:59. Following this, the EAL was placed on the maternal abdomen in close proximity to the foetal head and one vibroacoustic stimulus lasting 3 seconds in duration was applied to the maternal abdomen at the beginning of every minute from minute 10:00 to minute 19:00. Thus, the foetus was exposed to a series of 10 consecutive stimuli each 57 seconds apart. FHR recording was recorded continuously during this period of stimulation. Vibroacoustic stimulation was stopped at minute 19:03 of the protocol and post-stimulation FHR was measured from this point until minute 30:00. A total of 30 minutes of continuous FHR recording was thus obtained (Fig. 15).

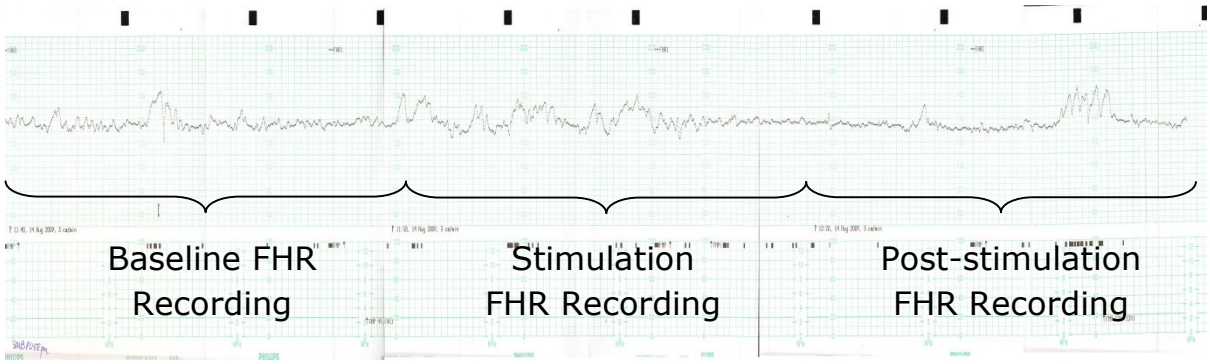


Fig. 15 Graph obtained from an FHR assessment as per the FHR monitoring protocol of the study showing the three periods of continuous FHR recording.
Note: This graph has been compressed to fit the dimensions of this page.

4.1.3.3 ASSESSING FOR POTENTIAL CONFOUNDING INFLUENCES

Data pertaining to the participant's age and education, education and occupation of the partner, family income, and the number of persons living in the same house as the participant was collected through an interview. Information on maternal health during pregnancy and obstetric data including parity, previous history of abortions, intra-uterine deaths and pregnancy-related complications was extracted from hospital records and verified through an interview with the mother.

Data on maternal nutritional status was obtained by measuring the participant's height and weight and calculating her body mass index (BMI) in kg/m^2. This was supplemented by information on the participant's most recent haemoglobin test value during the current pregnancy measured in g/dL and extracted from hospital records.

Data on the level of social support experienced by the participant was obtained from the Social Support Questionnaire. Information on the participant's and the family's preference for the sex of the foetus to be male, and on a history of alcoholism in a family member was collected through an interview with the participants.

4.1.4 PROCESSING OF FHR TRACES

Raw FHR data was transported to the UK and all FHR traces were processed at the Department of Psychiatry, University of Oxford, UK. The traces were processed by two researchers (Mary Renton and Juliet Zani) who underwent rigorous training in the processing protocol and were blind to the prenatal depression status of the mother. Three software programs – the GNU Image Manipulation Program v.2.4 (GIMP; © 2003-2011 The GIMP Development Team), MATLAB r2009b (©MathWorks) and Microsoft Excel 2009 (© Microsoft) - were used to process the data using a four-step process as described below.

I. Graphical Processing of FHR Traces

FHR traces were scanned into the computer in parts and consecutive parts were merged to recreate a panorama of the FHR trace using the GNU image manipulation program (see Fig. 15 for an example of a panoramic image recreated in GIMP). The green gridlines and artefacts were filtered out in order to obtain a black signal on a white background. Fig. 16 depicts the processed image for the trace in Fig. 15.

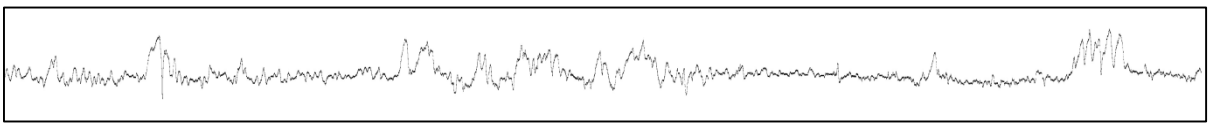


Fig. 16 Graphically processed FHR trace showing the FHR signal (in black) against a white background. Note: This graph has been compressed to fit the dimensions of this page.

II. Data Extraction using ProcessFHR and SumAUC

The black and white trace was then exported to the program MATLABr2009b. A novel code, *ProcessFHR*, designed in collaboration with the Department of Medical Electronics, BMS College of Engineering, Bangalore, India and the Department of Robotics, University of Oxford, UK was used to extract FHR data from the processed signal for every second of assessment.

The area under the curve (AUC) for every FHR response to vibroacoustic stimulation was calculated using another novel code, *SumAUC*, designed in collaboration with the Department of Mathematics, University of Oxford. This code uses a system of polynomial approximation utilising the least squares method to fit the data. The areas under the curve for specified epochs of time were calculated using an open-source software system, Chebfun, in conjunction with *SumAUC* (Trefethen, Hale et al. 2009). The specified epochs of time coincided with each minute of stimulation e.g. Minute 10:00 to 10:59, 11:00 to 11:59 until 19:00 to 19:59.

III. Export of Data to Excel

The data extracted using *ProcessFHR* and *SumAUC* were saved in .csv format and exported to Excel where mean FHR and BBV were calculated for the periods of baseline, stimulation and post-stimulation recording. The AUCs for the FHR responses to each of the 10 stimuli were calculated for each foetus. These were then added together to yield a summary variable of total foetal responsivity for each foetus. This variable was termed the *summation of the area under the curve* (∑AUC). The data so obtained for every foetus was merged into a single dataset for the prenatal sample and exported to the Statistical Package for Social Sciences v.15.0 (©IBM) for statistical analysis.

IV. Manual Interpretation of FHR Traces

Manual interpretation of the FHR traces to determine habituation was conducted independently by the two researchers. Habituation was defined as the failure of a foetus that has previously responded to vibroacoustic stimulation to respond by means of an acceleration in FHR (for the definition of an acceleration in FHR see page 34 in Section 2.2) to the 9th and 10th stimuli.

The process of determining whether the FHR response met criteria for an acceleration in FHR is described as follows. First, the minimum dimensions of a rectangle necessary to completely fit under the curve of the FHR response for it to be labelled acceleration was constructed on a piece of transparent paper. This was calculated to be a square of breath 7.5 mm (corresponding to 15 seconds) and length 7.5 mm (corresponding to 15 beats on the scaled paper). Second, baseline FHR was marked on the FHR trace (Fig. 17) after calculating the same in Excel. Third, the square was placed under every FHR response during the stimulation period to determine if it met criteria for an acceleration in FHR. Finally, the number of accelerations were counted and habituation was subsequently determined. The inter-rater reliability using this technique for coding habituation was 90%.

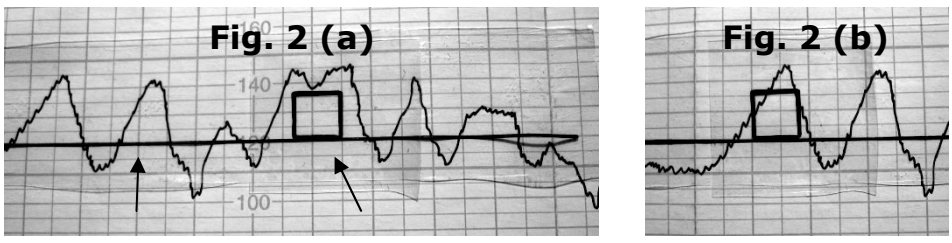


Fig. 17 Photographs of FHR responses meeting criteria for an acceleration in FHR (a) and not meeting criteria for an acceleration in FHR (b). The straight black line represents the mean baseline FHR.

No standardised technical criteria to define habituation exist to date (Thompson and Spencer 1966; Austin, Hadzi-Pavlovic et al. 2005). However, the threshold for foetal habituation used in this study was selected on the premise that previous investigations of habituation in foetuses have defined habituation as the failure of the foetus to increase FHR by ≥10 bpm for 5 consecutive stimuli when presented with up to 50 stimuli (Leader, Baillie et al. 1982; Austin, Hadzi-Pavlovic et al. 2005). The failure of the foetus to consecutively respond to more than 10% of the total number of stimuli was thus chosen to represent habituation. As the FHR protocol in this study was limited to foetal exposure to 10 stimuli, a slightly higher ratio of non-responsivity to the total number of trials was selected to denote habituation in this study compared to previous reports, i.e. 2 out of 10 stimuli (20%) in this study compared to 5 out of 50 stimuli (10%) in previous inquiries.

It is unlikely that failure of foetuses to respond to the 9th or 10th VAS trial in this study could be attributed to neuronal fatigue. This is because the total number of stimuli the foetuses were presented with was too small to cause nervous fatigue (Thompson and Spencer 1966). Further, given the short duration of exposure (10 minutes), the limited number of stimuli (n=10) and the use of the same VAS for all stimulation trails, it is unlikely that foetal response to the 9th and 10th stimuli could be attributed to dishabituation (recovery from habituation) (Thompson and Spencer 1966).

4.1.5 Labelling and Storage of Data

Data for each participant collected at the prenatal assessment, including the FHR trace, was added to the participant's file. Each file and FHR trace was tagged with a unique three digit number and stored in a locked cupboard. Data pertaining to the participant's name, address,

study number and hospital number was not stored with the prenatal data, but filed separately and stored in a separate locked cabinet.

4.1.6 INVITATION TO POSTNATAL FOLLOW-UP

Participants were requested to return for follow-up 1.5-3 months after giving birth, when the immunisation of their infants were scheduled. Each participant was given a study card containing her study number, the date of the follow-up assessment and the researcher's name and contact details (see Appendix IC). The study number was tagged onto the participant's hospital file for easy identification at the time of birth and postnatal follow-up. Mothers were thanked for their participation in the study and reimbursed for their travel and meal expenses for the day (INR 100 or GBP 1.25).

4.2 EXTRACTION OF BIRTH DATA FROM HOSPITAL RECORDS

Data pertaining to birth weight, preterm births, type of delivery and complications during labour, delivery and immediately post-partum were extracted from hospital records by the author of this thesis.

Birth weight

Birth weight is measured within 30 minutes of birth for all deliveries conducted at Snehalaya Hospital. It is recorded in kilograms and rounded off to the nearest two decimal places.

Infants who weigh less than 2500 grams at birth, regardless of gestational age, are considered to be of low birth weight (Iams, Romero et al. 2009).

Preterm Births

Births before 259 days (37 weeks) from the last menstrual period, or 245 days after conception, are defined as preterm births (Iams, Romero et al. 2009).

Type of Delivery

Details on the type of delivery were extracted from hospital records. This included normal vaginal delivery, assisted delivery through forceps or vacuum extraction of the baby and operative delivery (lower segment caesarean section) under spinal anaesthesia.

Complications

Information on the following puerperal complications was extracted from hospital records:

i. **Complications During Labour:** This consisted of complications during the first stage of labour i.e. from the onset of uterine contractions to the complete dilation of the cervix. Foetal malpresentations, failure of induction, meconium stained liquor, foetal distress and the non-progression of labour are included in this group.

ii. **Complications During Delivery:** This included complications during the second stage of labour i.e. from the dilatation of the cervix to the birth of the baby and included the presence of perineal, vaginal and cervical tears and the umbilical cord around the baby's neck.

iii. **Immediate Post-partum Complications:** This comprised of complications following the birth of the baby and included postpartum haemorrhage, adherent placenta and haematomas.

Reminder of the Postnatal Follow-up

Participating mothers who delivered their babies at Snehalaya Hospital were visited in the postnatal ward and reminded to return for follow-up when their infants were between 1.5 to 3 months old.

All participants were sent a letter to their home address one month before their babies turned 1.5 months of age. Those who had telephones or mobile phones were also contacted through the same to remind them to return for the postnatal assessment.

4.3 THE POSTNATAL ASSESSMENT

This sub-section describes the design and methodology of the postnatal assessment. All postnatal data, including the collection, handling and transport of saliva samples, was carried out by the author of this thesis.

4.3.1 PARTICIPANTS

Mothers who had completed the prenatal assessment and had given birth to a healthy baby with no history of birth hypoxia, cerebral palsy or severe medical and/or surgical morbidity were followed up at the immunisation clinic of Snehalaya Hospital between 1.5 to 3 months after birth. Their infants were also assessed at this stage. Mother-infant dyads were excluded from postnatal follow-up if mothers had started psychotropic medicine.

4.3.2 STUDY DESIGN

The postnatal assessment was conducted when the infants were between 1.5 to 3 months of age and scheduled to receive their first or second dose of the DPT vaccine as per the National immunisation Schedule of India. Further, 3 months is an important qualitative turning point in behavioural development characterised by the disappearance of endogenous (reflexive) smiling and infant fussiness and the emergence of exogenous (social) smiles and the recognition of objects (Spitz 1965; Emde, Gaensbauer et al. 1976). At this age, seeking & maintaining social contact becomes important.

All mother-infant dyads returning for follow-up were assessed in Snehalaya Hospital between 0900 hours and 1200 hours. Mothers were assessed for postnatal depression using the EPDS, the K10 and the MINIPlus (further details on these measures are available in Section 4.1 pp. 116-119). Mothers also completed a questionnaire on the breastfeeding of their infants. Information on maternal health, infant health, infant immunisation status and maternal satisfaction with the gender of her baby was collected using a semi-structured interview (See Appendix IIF).

Mothers reported on the temperament of their infants using the Infant Behaviour Questionnaire (Rothbart 1981). Although the IBQ was designed to be a self-report questionnaire, the unfamiliarity of the rural populace with Likert scales and the low levels of literacy among the sample in this study, necessitated an interviewer administered design.

At some point during the interview with the mother, a baseline saliva sample was collected from the infant. This was conducted employing the standard procedure using sorbettes when the infant was in the quiet, alert phase of activity. Mothers then proceeded to the immunisation clinic where infants received their first or second dose of the DPT vaccine depending on the age of the infant. Twenty minutes later a second saliva sample was collected from the infants. A baseline saliva sample was collected from mothers at the time of collection of the first infant sample using the passive drool technique. Saliva samples were assayed for cortisol at a later date.

The weight, length and head circumference of the infants was then measured. Mothers were thanked for their participation in the study and were reimbursed for their travel and meal expenses.

4.3.3 QUESTIONNAIRE MEASURES

The Infant Behaviour Questionnaire

The Infant Behaviour Questionnaire (IBQ) is a caregiver report instrument designed to assess infant temperament (See Appendix IIG). Caregivers report on items relating to specific aspects of their infant's behaviour in the previous week. When asked how often the infant displays a particular behaviour during the past week, the caregiver reports on a 7 point scale ranging from 'never' to 'always'.

The IBQ was designed to assess six dimensions of temperament in infants (Table 7). It was developed following rigorous item analysis of 463 questionnaires administered to the parents of 3, 6, 9 and 12 month olds (Rothbart 1981; Rothbart 1986). The mean item scale correlation for the IBQ ranges from 0.41 to 0.77 and internal consistency ranges from 0.67 to 0.84 with a median of 0.79 (Rothbart 1986). The IBQ has been shown to have good convergence with observer-rated home assessments of infant temperament and other temperament questionnaires such as Carey and McDevitt's Revised Infant Temperament Questionnaire, and Bates Infant Characteristics Questionnaire (Goldsmith and Rothbart 1991). The IBQ has been reported to have good stability from 3 to 6 months, 6 to 9 months and 3 to 9 months of age (Rothbart 1986). Further, the IBQ has been shown to be an effective instrument for assessing temperament even in very young infants less than 3 months of age and has been reported to have good stability from 2 weeks to 2 months, and 2 months to 12 months of age (Worobey and Blajda 1989).

The six dimensions of infant temperament assessed by the IBQ are presented in Table 7 [taken from (Rothbart 1981)]. The dimensions with positive loadings i.e. smiling and laughter, duration of orienting and soothability have been averaged to yield a score of mean

infant positive reactivity called the positive reactivity composite (Miceli, Whitman et al. 1998). The dimensions with negative loadings namely activity level, distress and latency to approach novel and sudden stimuli, and distress to limitations have been averaged to yield a negative reactivity composite reflecting the infant's mean negative reactivity (Miceli, Whitman et al. 1998). A global reactivity score, representing the infant's overall negative reactivity relative to its positive reactivity has also been constructed by subtracting the positive reactivity composite from the negative reactivity composite (Miceli, Whitman et al. 1998).

Dimension	Definition
Activity Level	Child's gross motor activity including squirming and movement of arms and legs
Smiling and Laughter	Smiling or laughter
Distress and Latency to Approach Novel and Sudden Stimuli	The child's distress and/or extended latency in approaching stimuli which are novel and/or intense
Duration of Orienting	The child's extended attention towards a single object, by means of vocalisation, viewing and/or interaction, when no sudden change in stimulation occurs
Distress to Limitations	Distress shown by the child (including crying and fussing) in a variety of frustrating situations such as waiting for food and being confined in a position or place
Soothability	A reduction in the child's fussing and/or crying when soothed by the caretaker

Table 7 Definitions of the six dimensions of infant temperament measured by the IBQ [taken from Table1; M. K. Rothbart (1981) Child Development 52:2, p. 573].

The IBQ is commonly used in the assessment of infant behaviour in a variety of research settings in the Western world and is one of the most popular questionnaire measures of infant temperament (Davis, Glynn et al. 2007; Werner, Myers et al. 2007; Kaplan, Evans et al. 2008). However, concerns have been raised about the reliability of caregiver reports of infant temperament. Potential sources of bias could be attributed to the influence of the characteristics, mood and temperament of the informant; recall biases; acquiescence biases; rater biases; biases in the comprehension of the questionnaire and biases in parental interpretation of infant behaviours (Rothbart 1990). Although these influences are important to consider they may be minimised by meticulous item construction, careful questionnaire administration and asking the caregiver about relatively recent events (Rothbart 1990). However, in spite of the potential sources of error in caregiver reports they have several advantages over home and laboratory based observer-rated assessments. These include the tapping into parental knowledge of child behaviours which is both broad and deep and made under normal circumstances unlike observer reports, which are limited to a short time frame and a specific set of situations (Rothbart 1990). Questionnaires are easy to administer and quick to complete. Further, they require no specialised training or facilities for coding as do video-taped assessments. Finally, the good correspondence of caregiver report measures of infant temperament and observer-rated reports of the same provides adequate evidence of the reliability of maternal reports.

There are no questionnaire measures designed to assess infant temperament in the developing world and we did not identify any studies assessing infant temperament in the Indian subcontinent. Further, we did not find any evidence of the validation of the IBQ in India or its translation into Kannada despite contacting the author (Mary K. Rothbart), the local collaborating institution and well-known researchers in the field from the Indian subcontinent.

Nevertheless, the IBQ was selected as the measure of infant temperament because it has been widely used in similar studies on western populations and is one of the only questionnaires validated for use in very young infants (less than 3 months of age). As it was not available in the local language, Kannada, it was translated into the same using the protocol described on page 120 of Section 4.1. The scale was piloted on a sample of 10 mothers with 1.5-3 month old infants attending the immunisation clinic at Snehalaya Hospital.

The Breastfeeding Questionnaire

The breastfeeding questionnaire was adapted from the breastfeeding data sheet compiled by Vikram Patel and colleagues in a study of 270 mothers and infants from Goa, India (Patel, Rodrigues et al. 2002). The questionnaire consists of 9 items on breastfeeding and 2 items on the general health of the baby (Appendix IIH). The items are not scored on a Likert scale but rather yield categorical information on the nature, type and duration of breastfeeding, problems associated with breastfeeding and the initiation of breastfeeding. The questionnaire was translated into Kannada using the protocol described on page 120 and piloted on a sample of 10 mothers along with the translated version of the IBQ.

4.3.4 SALIVA: COLLECTION, HANDLING AND ASSAY

The use of saliva as a diagnostic fluid is rapidly gaining in popularity. A wide variety of hormones and metabolites including cortisol, oestrogen, oestradiol, progesterone, testosterone, amylase and secretary immunoglobulins can be measured in saliva. Not only can saliva be collected with ease, but the non-invasiveness of the collection procedure increases acceptability by participants and avoids changes in hormone levels precipitated by the collection procedure itself e.g. rises in cortisol provoked by venous sampling (Salimetrics

2010). Further, the presence of binding proteins complicates attempts to measure steroid hormones such as cortisol. Saliva contains only unbound cortisol and therefore is believed to be a direct measure of the biologically active, free fraction of the hormone in the serum (Gozansky, Lynn et al. 2005).

The cortisol response to an invasive procedure such as immunisation takes 10-20 minutes to develop and a post-stress sample 20 minutes after such an exposure captures the peak response (Glover, Miles et al. 2005; Egliston, McMahon et al. 2007). The use of cortisol responses to a stressor using a pre-stress/post-stress paradigm is a common method of studying the response of the HPA axis to stress (Glover, Miles et al. 2005; Gutteling, Weerth et al. 2005; Brennan, Pargas et al. 2008).

4.3.4.1 Method of Saliva Collection in Infants and Mothers

Saliva was collected in infants at least 30 minutes after being breast-fed using the recommended technique with sorbettes (Salimetrics 2011). Sorbettes consist of hydrocellulose micro-sponges shaped as an arrow-head attached to a plastic shaft [Fig. 18(a)]. Two sorbettes were placed under the infant's tongue and left in place for 90 seconds until the micro-cellulose sponges begin to puff up. This has been reported to allow the sponge to absorb 200-300 μL of pooled saliva. Infants were not prevented from sucking on the sorbettes if they began to do so. Once collected, the sorbettes were immediately placed in sorbette storage tubes labelled with the mother-infant dyad's study number. The tubes were temporarily stored in an ice-box at four degrees Celsius for less than 30 minutes before being centrifuged [Fig. 18(b)].

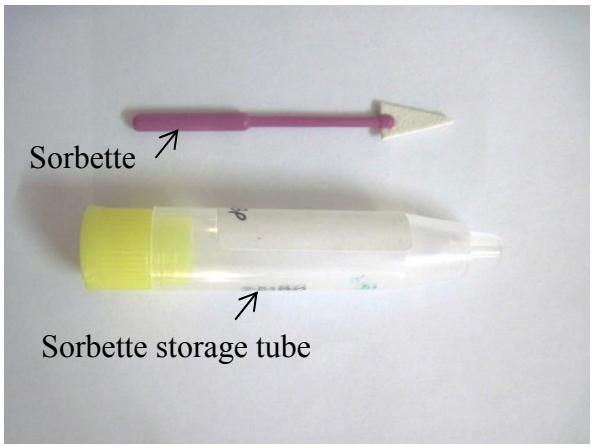


Fig. 18 (a)

Fig. 18 (b)

Fig. 18 Photographs of the sorbette collection device (a) and temporary storage of samples in an ice-box at 4^0C (b).

Two saliva samples were collected from infants – one at baseline and the other 20 minutes after the infant was immunised. Mothers were instructed to refrain from breastfeeding their infants after immunisation.

Saliva was collected from mothers using the passive drool technique which is the recommended technique for adults (Salimetrics 2011). Mothers were asked to rinse their mouths with water 10 minutes prior to collection and instructed to drool into a saliva collection tube until 3 mL of saliva was collected. They were instructed to focus on collecting the watery portion of saliva rather than foam and were offered the option of using a straw. No salivary stimulants were given to mothers and mothers were requested to refrain from consuming food and drink for at least 45 minutes before the assessment.

The time of collection of all samples was noted. All samples were stored in sorbette storage tubes labelled with a special identification number corresponding to the mother's study number.

4.3.4.2 Timing of Saliva Collection in Infants and Mothers

All infant and maternal saliva samples were collected between 0900 hours and 1200 hours. This period was chosen because it coincides with the circadian rhythm of cortisol secretion in adults when cortisol production is the highest. Furthermore, this was the period during which the immunisation clinic at Snehalaya Hospital operated and mothers usually brought their babies for immunisation during this time. Although the timing for the collection of infant saliva could not be further standardised within this period, it is important to note that, in this study, emphasis was placed on the infant's cortisol response to immunisation (the difference between baseline and post-immunisation levels) and not on the infant's diurnal pattern of cortisol secretion. It is also important to note that although it is generally established that infants develop the circadian rhythm of cortisol secretion seen in adults by 2 to 3 months of age (Egliston, McMahon et al. 2007), some infants in this study would be too young for this rhythm to be observed yet.

4.3.4.3 Saliva Processing, Storage and Transport

All saliva samples were collected in sorbette storage tubes and temporarily stored in an ice-box [Fig. 18 (b)]. They were then brought to room temperature and centrifuged at 3000 revolutions per minute (rpm) for 15 minutes to extract saliva from the sorbettes (Fig. 19). Following this, the sorbettes were discarded from the sorbette storage tube. The sorbette storage tubes with the extracted saliva were transported in the ice-box to a freezer in Snehalaya Hospital where they were stored at -20^{0}C under continuous temperature monitoring (Fig. 20).

The assay of saliva for cortisol was conducted at a laboratory in the city of Bangalore, 60 miles away from Solur. All saliva samples were transported by road in a vaccine carrier filled with dry ice under continuous temperature monitoring. The temperature was not allowed to drop below -20^{0}C at any point.

Fig. 19 Extraction of saliva following centrifuging.

Fig. 20 Storage of saliva samples in a freezer (open view) below -20^{0}C.

4.3.4.4 Salivary Cortisol Assays

Salivary cortisol assays were conducted at the Analytical Physiology Laboratory, Department of Physiology, St. John's Medical College Hospital, Bangalore in January 2010. The assay of cortisol levels in saliva was conducted using the Elecsys 2010 Immunoassay System (^{TM}Roche Diagnostics, Fig. 21). This is an automated multi-channel analyser for in-vitro qualitative determinations. The Elecsys 2010 works on the principle of Electrochemiluminescence (ECL). ECL is a unique and highly sensitive luminescence (light) detection system that amplifies the desired signal and filters out undesired noise and signals. It has the sensitivity of detecting 1 part in 1.3 billion with only 2 standard deviations of variation. Cortisol in saliva was measured using the protocol provided by the manufacturer of

the Salimetrics™ cortisol kit (See Appendix IIIA). All procedures were automated and carried out by the Elecsys 2010.

Fig. 21 Photograph of the Elecsys 2010 Immunoassay System.

4.3.5 ANTHROPOMETRIC MEASUREMENT OF THE INFANT

Infant weight, length and head circumference was measured as described below.

Infant weight was measured in grams up to 3 decimal places using an electronic weighing scale (Fig. 22). Items of clothing were removed from the baby and care was taken to ensure that the entire body of the infant was in the weighing pan.

Infant length was measured using an infantometer (Fig. 23). The baby was placed supine on the infantometer with its head held firmly against the head board by the mother. The legs were straightened, keeping the feet at right angles to the legs with the toes pointing upwards. A free foot board was brought in firm contact with the soles of the infant's heels and the length of

the baby was measured from the scale attached to the infantometer. All items of clothing and footwear interfering with the measurement of infant length were removed from the baby.

The head circumference of the baby was measured using a standard measuring tape calibrated in millimetres. The tape was passed around the infants head and the maximum circumference from the occipital protuberance to the supraorbital ridges was measured.

Fig.22 Photograph of the electronic infant weighing scale.

Fig.23 Photograph of the infantometer.

4.3.6 Assessment of Potential Confounding Influence

Information on postnatal maternal depression was collected using the EPDS, the K10 and the MINIPlus. Data on obstetric complications, maternal health, infant health, immunisation status and maternal (and family) satisfaction with the sex of the infant was collected using a semi-structured interview (See Appendix IIF). Postnatal maternal salivary cortisol data at baseline was collected to assess for its confounding influences on infant cortisol. This was selected as a proxy measure to control for genetic influences on HPA axis functioning.

4.3.7 CONCLUSION OF THE ASSESSMENT

Mothers were thanked for their participation as well as for the participation of their infants. They were advised to contact the author of this thesis or the senior collaborator at Snehalaya Hospital for details on the results of the study. Mothers were also reimbursed for their travel and meal expenses.

4.4 STATISTICAL ANALYSES

Statistical analyses for all sections and sub-sections of this thesis have been performed in SPSS unless otherwise stated. A brief outline of the statistical tests performed in this thesis is mentioned below. Details on specific analyses performed for each hypothesis is presented at the beginning of the results section for each respective hypothesis.

Exposure Variable: The exposure variable was prenatal depression defined as a score of ≥12 on the EPDS or ≥4 on the K10. To further explore the association of prenatal depression with foetal and infant outcomes, associations with the group of high scorers who met criteria for a current clinical diagnosis of major depression (clinical cases) were also examined[e].

Primary Outcomes: The primary outcome variables relating to the three primary hypotheses were (i) FHR responses to stress, (ii) infant cortisol responsivity and (iii) infant temperament.

Secondary Outcomes: The secondary outcome variables included those relating to birth outcomes, infant physical growth, infant health and breastfeeding. The continuity of stress responsivity from intra-uterine to extra-uterine life was also studied.

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[e] In the two group comparisons, mothers scoring high for symptoms of prenatal depression are referred to as *high scorers* and non-depressed mothers are referred to as *controls*. In the three group comparisons, mothers scoring high for symptoms of prenatal depression and meeting criteria for a current clinical diagnosis of major depression are referred to as *cases*. Mothers with elevated symptoms of prenatal depression without a clinical diagnosis are referred to as *high scorers without MDD* and non-depressed mothers are referred to as *controls* in the three group comparison.

4.4.1 STATISTICAL ANALYSES

An exploratory data analysis was conducted for each outcome variable. Correlations between exposure and outcome variables were tested for each hypothesis. The following statistical tests were then performed to study associations between prenatal depression and the outcome variable in question:

1. **Primary Analysis:** Offspring outcomes were compared between the group of mothers with elevated symptoms of prenatal depression (high scorers) and controls. This two-group comparison was performed using parametric (independent sample t tests, χ^2 tests) and non-parametric tests (Mann Whitney tests) for normally and non-normally distributed outcomes as appropriate.
2. **Secondary Analysis:** The association between clinically diagnosed prenatal depression and the outcome variables was tested by a three-group comparison between controls, high scorers and cases using an analysis of variance (ANOVA).
3. **Regression Analysis:** For postnatal outcome variables, hierarchical linear regression analyses were conducted to determine whether prenatal depression predicted infant outcomes independent of postnatal depression and other confounding influences.

A brief description of the analyses performed for each hypothesis is described below:

Chapter 5.1 Sample Characteristics

An exploratory data analysis was performed to determine the socio-demographic characteristics of the sample. The prevalence rate of prenatal depression was calculated and attrition rate encountered in the study was determined. The association between maternal

socio-demographic characteristics and prenatal depression was studied using steps 1 and 2 above for the prenatal sample as well as the attrition sample.

Chapter 5.2 Prenatal Depression: Associations with Foetal Stress Responsivity

The association between prenatal depression and foetal responsivity to stress was tested using Steps 1 and 2 above. Further, foetal stress responsivity variables were plotted against prenatal depression (EPDS) quintiles. Curve estimation techniques were then used to determine whether a linear or a quadratic model fit the association best.

Chapter 5.3 Prenatal Depression: Effects on Infant Cortisol Response

The effect of prenatal depression on infant cortisol responsivity was tested using two-group and three-group comparisons as described in Steps 1 and 2 above. The effect of postnatal depression on infant cortisol response was also tested. A regression analysis was performed to determine whether prenatal depression predicted infant cortisol independent of postnatal depression and maternal cortisol. Infant and maternal cortisol quintiles were plotted against EPDS quintiles to test whether a pattern of association existed between prenatal depression and cortisol. The resulting curves were analysed using curve estimation techniques.

Chapter 5.4 Prenatal Depression: Associations with Infant Temperament

A factor analysis for six dimensions of infant temperament measured on the IBQ was conducted. The association between prenatal depression and infant temperament was tested using Steps 1 and 2. Infant temperament was plotted against EPDS quintiles and curve estimation techniques were used to determine the pattern of association. A supplementary analysis was conducted to determine whether postnatal depression, birth weight and sex of the

infant, infant age, weight percentile, breastfeeding status and health predicted infant temperament.

An ancillary analysis using continuous correlations was conducted to determine whether associations between foetal responsivity, infant cortisol responsivity and infant temperament existed in the sample.

Chapter 5.5 Prenatal Depression: Associations with Birth Outcome, Postnatal Infant Health and Breastfeeding

All birth outcome variables (except birth weight) were dichotomised and associations with prenatal depression tested using two-group and three-group comparisons as described in Steps 1 and 2.

Infant physical growth (weight, length and head circumference), infant health and breastfeeding were compared between the infants of prenatally depressed mothers and controls using Steps 1 and 2. The potential confounding influence of postnatal depression, infant sex, birth weight and current infant health status on these outcomes was tested using parametric and non-parametric tests for normally and non-normally distributed infant outcomes respectively. Regression analyses were conducted to determine if an independent effect of prenatal depression on these infant outcomes existed.

4.5 ETHICS

The protocol for this study was approved by the Institutional Ethics Review Board, St. John's Medical College Hospital, Bangalore, India (IERB Study Reference Number 63/2009) and the Oxford Tropical Research Ethics Committee, University of Oxford, UK (OXTREC Reference Number 27-09).

Details of the study protocol approved by the ethical review boards of the University of Oxford and St. John's Medical College Hospital are presented in Appendix VB.

CHAPTER 5
RESULTS

5 RESULTS

The findings of this thesis are presented in the following sections as described below. Each section begins with a brief introduction into the respective hypothesis followed by a description of the specific statistical tests employed in the analysis. The section then reports the findings of this thesis pertaining to the hypothesis in question. This is concluded by a brief discussion of these results in the context of pre-existing literature and the mechanisms underlying the findings.

The overall consideration of the findings, and the strengths and limitations of the study as a whole is discussed in Chapter 6.

The various sections of this chapter are:

5.1 Sample Characteristics

This section describes the characteristics of the sample and reports the prevalence rate of prenatal depression in rural South India. The findings pertaining to the associations of prenatal depression with socio-demographic variables, obstetric variables and social support are also presented. The rate of attrition encountered in this study is reported.

5.2 Prenatal Depression: Associations with Foetal Responsivity

This section describes the findings of the association between prenatal depression and foetal cardiac responsivity to vibroacoustic stimulation.

5.3 Prenatal Depression: Associations with Infant Cortisol Responsivity

This section describes the findings related to the association between prenatal depression and cortisol response to immunisation in infants.

5.4 Prenatal Depression: Associations with Infant Temperament

The findings of the relationship between prenatal depression and the six dimensions of infant temperament are reported in this section. This is followed by the description of the findings of a supplementary analysis investigating the association between foetal and infant responsivity.

5.5 Prenatal Depression: Associations with Other Infant Outcomes

The findings of this thesis pertaining to the relationship between prenatal depression and, birth outcomes, infant physical growth, infant health and breastfeeding are reported in this section.

In Sections 5.2-5.5, the association between foetal and infant outcomes, and prenatal depression, is examined as follows:

i. **Primary Comparisons:** Foetal and infant outcomes are compared between the offspring of mothers who scored above the threshold for symptoms of prenatal depression on either the EPDS or the K10 (high scorers) and the offspring of non-depressed mothers (controls).
ii. **Secondary Comparisons:** The group of high scorers was split into those who scored high for symptoms of prenatal depression and met a diagnosis for a major depressive disorder (MDD) during pregnancy (cases) and mothers who had elevated symptoms of

prenatal depression but did not meet a diagnosis of MDD (high scorers without MDD). Offspring outcomes in these 2 groups were compared against controls.

5.1: SAMPLE CHARACTERISTICS

5.1.1 INTRODUCTION

One hundred and ninety six women meeting inclusion criteria were recruited from the prenatal clinic of Snehalaya Hospital. Of these, 194 women consented to participate in the study. The first 67 participants to score above the threshold for prenatal depression on either screening questionnaire (≥13 on the EPDS or ≥4 on the K10), and the first 66 participants to score below this threshold (controls), underwent foetal heart rate (FHR) monitoring in accordance with the study protocol[f]. The characteristics of this sample of 133 mothers from rural South India are presented in this section. Further information was collected from the participants at the time of delivery and at 1.5-3 months post birth.

5.1.2 STATISTICAL ANALYSES

The following statistical analyses were conducted:

I. Sample Characteristics

The sample characteristics for the prenatal sample (n=133) were determined for socio-demographic, obstetric and social support variables.

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[f] Participants who scored below the threshold for elevated symptoms of prenatal depression on the screening questionnaires (EPDS or K10) were labelled *controls* (n=66). Those scoring above this threshold (n=67) were labelled '*high scorers*' (of this, a sub-group of 28 participants who scored high for elevated symptoms of prenatal depression on screening questionnaires and met a diagnosis for a major depressive episode (MDD) during pregnancy were labelled '*cases'*). Unless specifically stated otherwise, where the group is described as depressed, this refers to the group of *high scorers* (n=67).

II. Prevalence Rate of Prenatal Depression

Two prevalence rates of prenatal depression were calculated using the initial sample of 194 as the denominator:

(i) The prevalence of a major depression disorder (MDD) during pregnancy: This was calculated as the percentage of women found to meet a clinical diagnosis of prenatal MDD.

(ii) The prevalence of symptoms of prenatal depression: This was calculated as the percentage of women found to score high for symptoms of prenatal depression on screening questionnaires.

III. Prenatal Depression and Sample Characteristics

Two sets of analyses were conducted to determine the association between prenatal maternal depression and maternal socio-demographic, medical and obstetric characteristics.

(i) **Primary comparisons:** Associations between prenatal maternal depression and sample characteristics were compared between high scorers for symptoms of prenatal depression and controls using parametric (Independent sample t test) and non-parametric tests (Mann-Whitney tests) for normally and non-normally distributed variables respectively.

(ii) **Secondary comparisons:** The group of mothers who scored high for symptoms of prenatal depression on screening questionnaires was split into two sub-groups: those with a diagnosis of prenatal MDD (cases) and those without (high scorers without MDD). Sample characteristics in these two groups were compared against controls using the analysis of variance (ANOVA) and Chi-Square tests.

IV. Characteristics of Attrition Sample

Attrition rates were calculated and sample characteristics were compared between the attrition sample and the follow-up sample using independent sample t tests and Chi-Square tests.

V. Prevalence of Postnatal Depression

The prevalence of depression in the postnatal follow-up sample was calculated. The continuity of depression from the prenatal to the postnatal period was examined.

5.1.3 RESULTS

5.1.3.1 SAMPLE CHARACTERISTICS

Socio-demographic Characteristics

The socio-demographic characteristics of the 133 women who participated in the prenatal assessment are presented in Table 8. The mean age of the sample was 21.5 (SD: 2.6) years. The majority of participants were literate (97.0%), married (97.7%) and housewives (84.2%). The average family income was GBP 59.28 (63.79) per month and 22 participants (16.5%) reported having gone hungry for a day at least once in the past 6 months because of insufficient money to buy food. Twenty eight participants (21.1%) reported living with a person who abused alcohol frequently.

Variable	N	Descriptive Characteristics	
Maternal Variables			
Participant's Age	133	Mean (SD):	21.5 (2.6) years
		Range:	18 – 31 years
Participant's Educational Qualification	133	None:	3.0% (n=4)
		Primary education (upto grade 5):	20.3% (n=27)
		Secondary education (grade 5-10):	50.4% (n=67)
		High school degree (grades 11 & 12):	23.3% (n=31)
		Higher education (studying further than a high school degree):	3.0% (n=4)
Participant's Occupation	133	Housewife	84.2% (n=112)
		Light work (tailor, teacher, software)	7.5% (n=10)
		Hard physical labour (agriculture, manual labour)	8.3% (n=11)
Paternal Variables			
Partner's Age	130	Mean:	28.6 (4.4) years
		Range:	20 - 50 years
Partner's Educational Qualification	130	Illiterate:	4.6% (n=6)
		Primary education (up to grade 5):	25.4% (n=33)
		Secondary education (grade 5-10):	40.8% (n=53)
		High school degree (grades 11 & 12):	15.4% (n=20)
		Higher education (studying further than a high school degree):	13.8% (n=18)
Partner's Occupation	130	Daily wage worker:	43.8% (n=57)
		Semi-skilled job:	38.5% (n=50)
		Skilled/professional:	17.7% (n=23)
Marital Variables			
Marital Status	133	Married:	97.7% (n=130)
		Unmarried:	0.8% (n=1)
		Widowed:	1.5% (n=2)
Number of Years of Marriage	131	Mean:	2.63 (1.50) years
		Range:	0.6 - 10 years
Consanguinity	133	None	66.7% (n=88)
		3rd degree consanguinity	9.8% (n=13)
		2nd degree consanguinity	23.5% (n=31)
Demographic and Socio-economic Variables			

Religion	133	Hindu	96.2% (n=128)
		Muslim	2.3% (n=3)
		Christian	1.5% (n=2)
Number of people living in the same house as the participant	133	Mean (SD):	4.66 (2.06) members
		Range:	2–12 members
Type of Family	133	Nuclear	21.8% (n=29)
		Joint	31.6% (n=42)
		Three generation	46.6% (n=62)
Monthly income in INR	117	Mean(SD):	INR 4446.15 (4784.63) GBP 59.28 (63.79)
		Range:	INR 0-45,000 (GBP 0-600)
Is this enough to meet the family's daily needs?	133	Yes	53.4% (n=71)
		No	46.6% (n=62)
Is the family in debt?	131	No	84.2% (n=112)
		Yes	14.3% (n=19)
Has the participant ever gone hungry for a day in the past 6 months because of lack of money to buy food?	133	No	83.5% (n=111)
		Yes	16.5% (n=22)
Family h/o alcoholism	133	No	78.9% (n= 105)
		Yes	21.06% (n= 28)
		Of which n=12 had alcoholic spouses	

INR: Indian rupees; h/o: history of

Table 8 Socio-demographic characteristics of the sample.

Obstetric and Medical Characteristics

The obstetric and medical characteristics of the sample are reported in Table 9. Participants were assessed at a mean gestational age of 35.56 weeks. The majority had planned pregnancies (n=128) and were expecting their first baby (n=82). Fifty five women (41.4%) experienced illness during their pregnancy. This included anaemia, pregnancy induced hypertension and fever. Of these, twelve (21.0%) experienced a severe illness during their pregnancy such as eclampsia and severe anaemia necessitating blood transfusions.

The biological characteristics of the sample are presented in Table 10. The mean haemoglobin level of participants as measured during the third trimester of pregnancy was 10.31 g/L. The mean body mass index of the sample was 21.54 kg/m^2 which is within the normal range of 18.5-24.9 kg/m^2.

Medical/Obstetric Characteristic	N	Descriptive Characteristics
Illness During Pregnancy	133	No: 58.6% (n=78) Yes: 41.4% (n=55)
Obstetric Score	133	Primiparous: 61.7% (n= 82) Multiparous: 38.3% (n= 51)
Planned Pregnancy	133	Planned: 96.2% (n=128) Unplanned: 3.8% (n=5)
Conception	133	Spontaneous: 99.2% (n=132) Assisted: 0.8% (n= 1)
Past h/o Infant Death	133	No: 94.7% (n=126) Yes: 5.3% (n=7)
Medical Illness Other Than That Associated with Pregnancy	133	No: 94.7% (n=126) Yes: 5.3% (n=7)
H/o Illness in the Family in the Past 6 Months	133	No: 74.4% (n= 99) Yes: 25.6% (n= 34)
H/o Mental Illness in the Family	133	No: 98.5% (n=131) Yes: 1.5% (n=2)
No. of Previous Abortions	133	None: 91.0% (n=119) At least one: 9.0% (n=14)
No. of Previous IUDs	133	None: 98.5% (n=131) At least once: 1.5% (n=2)

h/o: history of IUDs = Intra-uterine deaths

Table 9 Obstetric and prenatal medical characteristics of the sample.

Maternal Biological Measure	N	Mean (SD)	Range
Haemoglobin (g/L)	127	10.31 (1.17)	7.60-13.60
Weight (kilograms)	129	54.42 (8.74)	40.00-86.00
BMI (kilograms/metre^2)	118	21.54 (2.98)	15.79-31.64
Gestation age at assessment (weeks)	133	35.56 (3.04)	28.00-40.71

Table 10 Prenatal biological characteristics of sample.

Gender Related Variables

The sample characteristics for gender related variables are presented in Table 11. Twenty seven mothers (20%) had daughters from previous pregnancies. While nineteen participants expressed a preference for their foetus to be male, twenty four mothers reported that their family members expressed preferences for a son. The bias against daughters and the preference for male children reflects a deeply rooted tradition of gender bias prevalent in the Indian sub-continent.

Social Support

The mean social support score of the sample on Social Support Index was 15.04 (SD: 9.07). Social support score is reverse coded and women scoring higher have lower levels of social support. Twenty seven participants (20.4%) reported verbal abuse by their partners during their pregnancy, while seven participants (5.3%) reported physical abuse.

Gender Related Characteristic	N	Descriptive Characteristics
Previous Female Children	133	No previous females: 79.7% (n=106) Previous female children: 20.3% (n=27)
Previous Male Children	133	None: 90.9% (n=121) At least one: 9.0% (n=12)
Participant's Preference for the Gender of the Foetus	130	No preference: 70.7% (n=94) Female: 12.8% (n=17) Male: 16.5% (n=19)
Family of the Participant's Preference for the Gender of the Foetus	77	No preference: 53.2% (n=41) Female: 15.6% (n=12) Male: 31.2% (n=24)

Table 11 Gender related sample characteristics.

5.1.3.2 PREVALENCE OF PRENATAL DEPRESSION

Of the 194 participants screened for prenatal depression, twenty nine (14.9%) met clinical criteria for a major depressive episode during their current pregnancy. Sixty seven (34.5%) scored high for elevated symptoms of prenatal depression based on screening questionnaires.

Across the sample (n=133), the mean EPDS and K10 scores were 8.07 (SD: 6.07) and 6.77 (SD: 7.26) respectively. The distribution of EPDS and K10 scores in the sample are presented in Figures 24 and 25 respectively. EPDS and K10 scores were strongly correlated with each other ($r=0.89$, $p<0.01$).

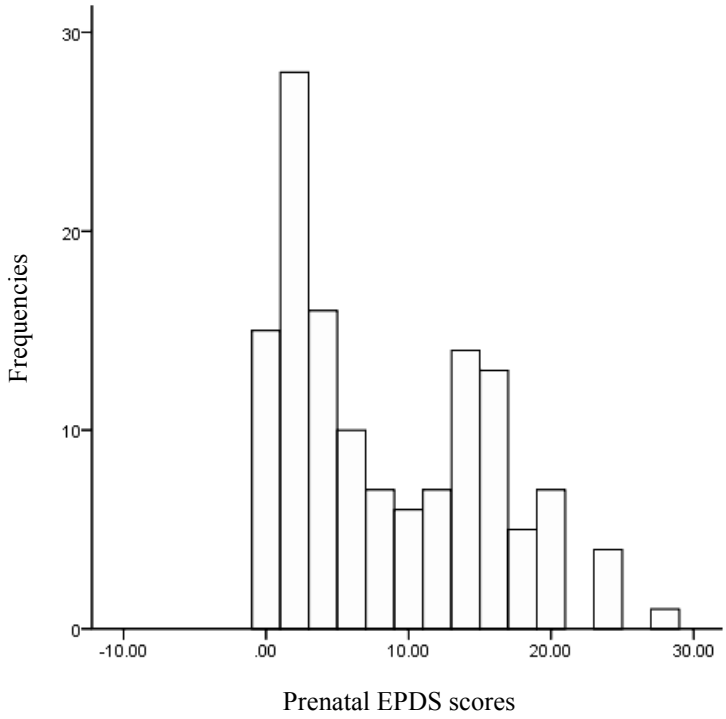

Fig. 24 Distribution of EPDS scores in the sample.

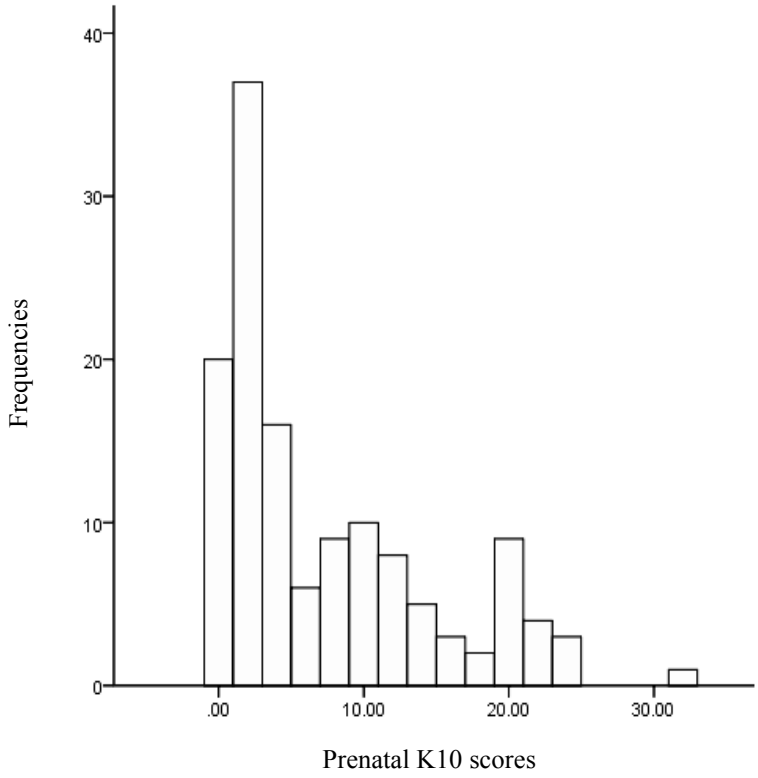

Fig. 25 Distribution of K10 scores in the sample.

The distribution of EPDS and K10 scores between high scorers and controls (n=133) are presented in Table 12 [a] and [b]. Both scores were significantly higher in cases as compared to high scorers without MDD (t=-5.39, p<0.01; t=-5.34, p<0.01 for the EPDS and K10 respectively). Although the mean EPDS and K10 scores for controls was low, none of the participants classified as controls on the screening questionnaires met a diagnosis of major depression during the clinical interview.

	EPDS		**K10**	
	Controls	**High Scorers**	**Controls**	**High Scorers**
N	66	67	66	67
Mean (SD)	2.30 (2.26)	13.74 (5.10)	1.12 (1.01)	12.32 (6.41)
Range	0-10	4- 27	0-3	4-32

Table 12[a] Primary Comparisons: EPDS and K10 scores in controls and high scorers for symptoms of prenatal depression.

	EPDS			**K10**		
	Controls	**High Scorers without MDD**	**Clinical Cases**	**Controls**	**High Scorers without MDD**	**Clinical Cases**
N	66	38	29	66	38	29
Mean	2.30	11.32	16.93	1.12	9.32	16.28
(SD)	(2.26)	(4.67)	(3.77)	(1.01)	(4.86)	(6.11)
Range	0-10	4-24	13-27	0-3	4-21	6-32

Table 12[b] Secondary Comparisons: EPDS and K10 scores in controls, high scorers without MDD and cases of prenatal MDD.

The range of the EPDS scores for the depressed group was 4-27. Although the lower limit of this range is below the EPDS cut-off of 13, a score of ≥4 on the K10 was also used to classify women as high scorers for prenatal depression and this may account for the wide range of EPDS scores in the depressed group. In addition, 43 participants scored above the threshold on both the EPDS and K10, while 24 participants (35%) scored above the threshold on the K10 but not on the EPDS.

The validity of the EPDS and K10 as screening instruments for prenatal depression in rural south India was also assessed. The gold standard used for comparison in this sample (n=194) was an ICD-10 diagnosis of MDD assessed on the MINIPlus. A receiver-operating characteristic analyses showed both scales to be good screening instruments for prenatal depression in rural South India at a cut-off of ≥13 on the EPDS (sensitivity = 100%, specificity = 84.90%, area under the curve (AUC) = 0.95) and ≥6 on the K10 (sensitivity = 100%, specificity = 81.30%, AUC = 0.95) (Fernandes, Srinivasan et al. 2011). The receiver operating characteristic curves for the EPDS and the K10 are presented in Appendix IVA.

5.1.3.3 PRENATAL DEPRESSION: ASSOCIATION WITH SAMPLE CHARACTERISTICS

Prenatal Depression and Socio-Demographic Characteristics

Prenatal depression was associated with maternal reports of hunger in the past 6 months (Table 13). Single mothers (unmarried or widowed) had elevated depression scores compared to married participants. Prenatal depression was not associated with maternal age, education, employment, religion, type of family or income.

Socio-demographic Variable	Sample Characteristics		Inter-Group Comparisons	
	Controls N = 66	High Scorers N = 67	Test statistic	p value
Maternal				
Age	Mean: 21.5 years (SD: 2.6)	Mean: 21.9 years (SD: 2.85)	t = 0.51	0.48
Education: Less than a High School Degree	n=45 (68.18%)	n=53 (79.10%)	χ^2=2.15	0.56
Occupational Status: Employed	n=10 (15.15%)	n=11 (16.42%)	χ^2=0.06	0.97
Paternal				
Age	Mean: 28.6 years (SD: 4.9)	Mean: 28.9 years (SD: 4.4)	t=0.01	0.97
Education: Less than a High School Degree	n=46 (69.70%)	n=46 (68.66%)	χ^2=3.10	0.54
Occupation: Daily Wage Worker	n=25 (37.88%)	n=32 (47.76%)	χ^2=1.57	0.46
Marital				
Single Mother	n=0 (0%)	n=3 (4.48%)	χ^2=2.93	0.23
Years of Marriage	Mean: 2.70 (SD: 2.38)	Mean: 2.85 (SD: 2.51)	t=0.71	0.40
1st or 2nd Degree Consanguinity	n=22 (33.33%)	n=22 (32.84%)	χ^2=0.99	0.61

Demography				
Religion: Hinduism	n=63 (95.45%)	n=65 (97.01%)	χ^2=0.30	0.82
Number of Family Members Co-habiting	Mean: 4.40 (SD: 2.00)	Mean: 4.88 (SD: 2.11)	t=0.09	0.76
Joint/3 Generation Family	n=48 (72.73%)	n=56 (83.58%)	χ^2=3.50	0.17
Monthly Income in GBP	Mean: £56.95 (SD: £74.65)	Mean: £56.03 (SD: £41.91)	t=0.05	0.83
Insufficient Finances to meet Daily Needs	n=32 (48.48%)	n=30 (44.78%)	χ^2=0.35	0.56
Presence of Debt	n=6 (9.10%)	n=13 (19.40%)	χ^2=2.43	0.12
Experience of Hunger	n=6 (9.10%)	n=16 (23.88%)	**χ^2=4.92***	**0.03**

Table 13 Associations between prenatal depression and socio-demographic characteristics.

Prenatal Depression: Associations with Obstetric and Health related Variables

The association of prenatal depression with obstetric and biological variables are presented in Table 14. Prenatal depression was associated with recent illness in a family member. It was not associated with pregnancy related illness, previous abortions and intra-uterine deaths and maternal biological characteristics namely weight, BMI (at assessment) and haemoglobin (the most recent third trimester level reported in the hospital records).

	Sample Characteristics		Inter-group Comparisons	
			Controls/High Scorers	
	Controls N = 66	High Scorers N=67	Test Statistic	p value
Obstetric and Medical Characteristics				
Illness during Pregnancy	n=22 (33.3%)	n=33 (49.3%)	$\chi^2 = 2.95$	0.09
Primiparity	n=42 (63.6%)	n=40 (59.7%)	$\chi^2 = 0.47$	0.49
Number of Previous Abortions	Mean: 0.12 (SD: 0.38)	Mean: 0.12 (SD: 0.37)	U= 2199.50	0.93
Number of Previous Intra-Uterine Deaths	Mean: 0.02 (SD: 0.12)	Mean: 0.01 (SD: 0.12)	U= 2208.50	0.97
Unplanned Pregnancy	n=2 (3.0%)	n=3 (4.5%)	$\chi^2 = 0.16$	0.69
Assisted Conception	n=0 (0%)	n=1 (1.5%)	$\chi^2 = 0.96$	0.33
Medical Illness other than that associated with Pregnancy	n=2 (3.0%)	n=5 (7.5%)	$\chi^2 = 1.22$	0.27
Illness in the Family during the Past 6 Months	n=10 (15.2%)	n=24 (35.8%)	**$\chi^2 = 6.92$**	**<0.01**
Mental Illness in the Family	n=0 (0%)	n=2 (3.0%)	$\chi^2 = 1.94$	0.16
Biological Characteristics				
Haemoglobin (g/dL)	Mean: 10.18 (SD: 1.14)	Mean: 10.33 (SD: 1.21)	t = -0.70	0.49
Weight (kilograms)	Mean: 55.26 (SD: 9.26)	Mean: 54.19 (SD: 8.83)	U= 1941.00	0.51
BMI (kg/metre^2)	Mean 21.77 (SD: 2.75)	Mean: 21.30 (SD: 3.30)	U= 1449.50	0.12
Gestational Age (weeks)	Mean: 35.59 (SD: 2.93)	Mean: 35.68 (SD: 3.09)	U= 2111.00	0.66

Table 14 Associations between medical, obstetric & biological sample characteristics and prenatal depression.

Prenatal Depression: Associations with Gender related Variables

Prenatal depression was not associated with the number of the female children from previous births (Table 15). Prenatal depression was also not associated with the participant's or her family's preference for a son.

Gender Related Characteristics	Sample Characteristics		Inter-group Comparisons	
	Controls N = 66	High Scorers N = 67	Test Statistic	p value
Number of Previous Female Children	Mean: 0.25 (SD: 0.53)	Mean: 0.25 (SD: 0.56)	U = 22.00	0.83
Number of Previous Male Children	Mean: 0.08 (SD: 0.27)	Mean: 0.10 (SD: 0.31)	U = 2152.50	0.60
Participant's Preference for a Male Child	n=7 (10.6%)	n=12 (17.9%)t Statistic	$\chi^2 = 2.68$	0.26
Family's Preference for a Son	n=9 (13.6%)	n=15 (22.4%)	$\chi^2 = 3.02$	0.22

Table 15 Associations between gender related characteristics and prenatal depression.

Prenatal Depression and Social Support

The findings of the association between prenatal depression and the levels of social support in the sample are presented in Table 16.

i. Primary Analysis

Prenatal depression was associated with low levels of social support, and high rates of verbal and physical abuse by the partner/spouse and alcohol abuse in a member of the family.

Compared to controls, high scorers reported higher rates of verbal abuse by their partners (χ^2=16.10, p<0.001).

Compared to controls, significantly higher rates of spousal physical abuse were reported in high scorers. Mothers reporting physical abuse had higher EPDS and K10 scores (Mean EPDS score: 14.29 (6.34); Mean K10 score: 12.43 (7.18)) compared to those who did not report physical abuse (Mean EPDS score: 7.44 (6.54) and mean K10 score: 6.10 (6.64); U=192, p=0.01 for EPDS and U=204.5, p=0.02 for K10 scores).

Prenatal depression was associated with excessive alcohol intake by a member of the family living in the same household as the mother (χ^2=5.85, p=0.02). This association did not differ between the group of mothers where the alcohol abuser was the participant's spouse and the group where the alcohol abuser was another member of the family such as the father-in-law (χ^2=0.22, p=0.64).

Social Support Characteristics	**Sample Characteristics**		**Inter-Group Comparisons**	
	Controls N = 66	**High Scorers N = 67**	**Test Statistic**	**p value**
Social Support Score	Mean: 11.55 (SD: 7.14)	Mean: 18.79 (SD: 9.18)	**U=1265.50**	**<0.01**
Verbal Abuse	n=4 (6.1%)	n=23 (34.3%)	**χ^2 =16.10**	**<0.01**
Physical Abuse	n=1 (1.5%)	n=6 (8.9%)	χ^2 =3.69	0.06
Excessive Alcohol Intake by a Family Member	n=8 (12.1%)	n=20 (29.9%)	**χ^2 = 5.85**	**0.02**

Table 16 Associations between levels of social support and prenatal depression.

ii. Secondary Analysis

Significantly lower levels of social support (Mean: 21.62 SD: 9.45) were reported by cases as compared to high scorers without MDD (Mean: 15.95 SD: 8.91) and controls (Mean: 11.55 SD: 7.14; $F=15.390$, $p<0.01$).

5.1.3.3 ATTRITION

Of the 133 mothers who were assessed prenatally and invited for follow-up, details on birth outcomes were available for 107 mothers who returned to Snehalaya Hospital for their deliveries. At 1.5-3 months post-birth, 58 mother-infant dyads returned for the postnatal assessment. The attrition rate at the time of birth was thus 19.5%. The attrition rate at the postnatal assessment was 56.40% (Fig. 26).

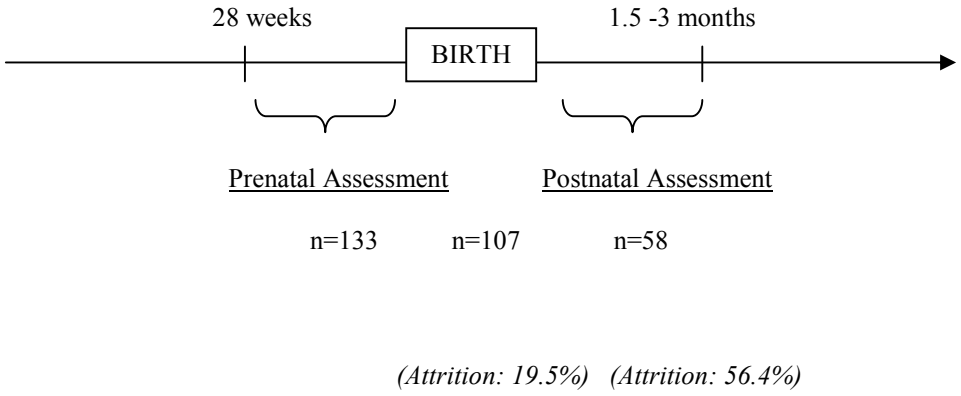

Fig. 26 Schematic diagram showing attrition rates across the three phases of assessment.

Of the 58 mother-infant dyads that returned for the postnatal assessment, 25 (43.1%) had elevated symptoms of depression prenatally and 33 (56.1%) were controls. There is thus some evidence of selective attrition in the sample that returned for the postnatal assessment.

Attrition Sample: Characteristics

The characteristics of the attrition sample are presented in Table 17. Other than an increased rate of alcohol abuse in a family member in the attrition sample compared to the postnatal sample, no differences were noted between the two. There was no difference in maternal age, parity or socio-economic status between the attrition sample and follow-up sample. Mothers in the attrition group did not live further away from the health centre than mothers who returned for follow-up. There were no differences in prenatal EPDS and K10 scores between the two groups.

Reasons for Attrition:

The reasons for attrition could not be ascertained because the attrition sample could not be contacted through regular follow-up methods. Many mothers did not have a telephone number or a complete postal address as in the case with rural populations in the developing world. This made follow-up phone calls and letters extremely difficult.

In addition, it is possible that some mothers immunised their babies at local health clinics or the children were immunised by local health workers during domiciliary postnatal visits at the participants' homes. In such cases, it may be presumed that mothers did not return to the study location for the immunisation of their babies thus possibly contributing to attrition in this sample.

Variable	Sample Characteristics		Statistical Test
	Attrition Sample (n=75)	Postnatal Sample (n=58)	
Mother's Age (years)	Mean (SD): 21.6 (2.9)	Mean (SD): 21.5 (2.4)	t = -0.20, p = 0.84
Mother's Educational Status	Illiterate: n=4 Primary education: n=17 Secondary education: n=34 High school: n=13 University education: n=7	Illiterate: n=0 Primary education: n=10 Secondary education: n=32 High school: n=10 University education: n= 6	χ^2 = 4.24, p = 0.38
Husband's Age (years)	Mean (SD): 28.4 (5.9)	Mean (SD): 27.4 (6.3)	t = -0.99, p = 0.33
Husband's Educational Status	Illiterate: n=5 Primary education: n= 26 Secondary education: n= 30 High school: n=7 University education: n=7	Illiterate: n= 4 Primary education: n=9 Secondary education: n=26 High school: n=11 University education: n= 8	χ^2 = 7.56, p = 0.11
Husband's Occupation	Daily wage earner: n=32 Skilled/semi-skilled worker: n=43	Daily wage earner: n=25 Skilled/semi-skilled worker: n= 33	χ^2 = 0.03, p = 0.96
Marital Status	Married: n= 74 Unmarried: n=1	Married: n=56 Unmarried: n=2	χ^2 = 0.66, p = 0.42
Family Income (INR)	Mean (SD): 4202.97 (5818.49)	Mean (SD): 3534.48 (2694.80)	t = -0.81, p = 0.42

Presence of Debt	n = 12	n = 7	$\chi^2 = 0.41$, p = 0.52
Experience of Hunger in the Past 6 Months	n = 11	n = 10	$\chi^2 = 0.16$, p = 0.69
Gestational Age at Assessment (weeks)	Mean (SD): 35.82 (3.04)	Mean (SD): 35.36 (3.04)	t = -0.86, p = 0.39
Obstetric Score	Primi: n = 35 Multi: n = 23	Primi: n = 47 Multi: n = 28	$\chi^2 = 0.08$, p = 0.79
Illness in the Family	n = 16	n = 18	$\chi^2 = 0.22$, p = 0.64
Alcoholism in a Family Member	n = 23	n = 5	**$\chi^2 = 9.56$**, p<0.01**
Distance of Home from Study Location	In the same district: n=36 (48.00%) Within 45 kms.: n=38 (50.60%) >45 kms.: n=1 (1.30%)	In the same district: n=29 (50.00%) Within 45 kms.: n= 29 (50.00%) >45 kms.: n = 0	$\chi^2 = 4.25$, p = 0.24
Prenatal EPDS Scores	Mean (SD): 8.88 (6.75)	Mean (SD): 7.02 (7.16)	t = -1.54, p = 0.13

Prenatal K10 Scores	Mean (SD): 7.27 (6.81)	Mean (SD): 6.12 (7.81)	t = -0.90, p = 0.37
% of High Scorers	n = 43 (57.3%)	n = 25 (43.1%)	χ^2 = 2.65, p = 0.10
(n) High Scorers and Clinical Cases of Prenatal Depression	High scorers: n = 25 Cases: n = 18	High scorers: n = 14 Cases: n = 11	χ^2 = 2.68, p = 0.26

Table 17 Comparisons between attrition sample and follow-up sample.

5.1.3.4 POSTNATAL CHARACTERISTICS: PREVALENCE OF POSTNATAL DEPRESSION

Twenty six of fifty-seven mothers (45.6%) scored high for symptoms of postnatal depression on screening questionnaires. Six mothers (10.3%) met criteria for a diagnosis of postnatal MDD.

Of the mothers with elevated scores for postnatal depression, 12 mothers (46.2%) also had elevated depression scores during pregnancy. Thus, of the twenty-five prenatal high scorers that returned for follow-up, twelve (50%) scored high for depression postnatally. Fourteen mothers (53.9%) developed high levels of depressive symptoms de-novo in the postnatal period.

Four of the six mothers with postnatal MDD (66.7%) also met a diagnosis for MDD during pregnancy. The remaining two mothers (33.3%) were prenatal controls who developed clinical depression following child-birth.

Summary of Findings:

- Twenty nine mothers (14.9%) met a diagnosis of major depression during pregnancy while sixty seven mothers (34.5%) had elevated symptoms of prenatal depression.
- Prenatal depression was associated with hunger, low social support, verbal and physical abuse by the spouse, and living with an alcohol abuser.
- The rate of attrition at postnatal follow-up was 56.4%.

5.2 PRENATAL DEPRESSION: ASSOCIATIONS WITH FOETAL STRESS RESPONSIVITY

5.2.1 INTRODUCTION

A number of studies have provided evidence for an association between prenatal maternal depression and postnatal child development (Talge, Neal et al. 2007). It is important to know whether these effects are due to the continuity of prenatal depression into the postnatal period as postnatal depression (PND) or whether they are attributable to the direct effects of prenatal depression on the foetus.

Some studies have reported prenatal depression to be associated with poor foetal growth and disturbances in foetal heart rate, movement and sleep (Monk, Fifer et al. 2000; Field, Diego et al. 2004; Field, Diego et al. 2006). However investigations into the association between prenatal depression and foetal responses to stress are limited and the underlying mechanisms are as yet not well understood. As discussed in Section 2.2, this is due to the methodological limitations of assessing behavioural and stress responsivity patterns in foetuses using non-invasive techniques. The few inquiries into the association between prenatal depression and foetal stress responses have employed measures of foetal heart rate (FHR) during induced maternal stress as a paradigm to study foetal stress responsivity. The premise underlying this approach is that FHR is regulated by the autonomic nervous system (the sympathetic

component of which forms part of the offspring's stress response mechanism). Thus, measurable changes in FHR occur in response to a stressor.

A number of studies have provided evidence of a linear association between prenatal depression and FHR responses to induced maternal stress. This includes an increase in mean FHR and a decrease in FHR variability during and after foetal exposure to a stressor (Monk, Fifer et al. 2000; Allister 2001; DiPietro, Costigan et al. 2003; Monk, Myers et al. 2003). Further details on these studies are presented in Table 2 on pp. 41- 43. Interestingly, some authors suggest the possibility of a threshold level of maternal stress beyond which adverse effects are observed on foetal maturation and offspring development (DiPietro, Novak et al. 2006; DiPietro, Kivlighan et al. 2010).

Sandman and colleagues argue that the association between neuroendocrinological markers of prenatal psychopathology and foetal stress responsivity is curvi-linear (cubic) in nature (Sandman, Wadhwa et al. 1999; Sandman, Glynn et al. 2003). Another study exploring this issue has reported better cognitive performance in a sample of 2 year olds exposed to high levels of prenatal maternal anxiety compared to controls (DiPietro, Novak et al. 2006). Evidence from animal literature provides some support for these findings and a number of studies report foetal exposure to mild prenatal stress to be associated with better neuronal differentiation and spatial learning in rodent offspring (Meek, Burda et al. 2000; Fujioka, Fujioka et al. 2001; Fujioka, Fujioka et al. 2006). These investigations suggest that the relationship between prenatal maternal depression and foetal stress responsivity may not be linear but is possibly non-linear, or curvi-linear, in nature.

The following section presents the results of this thesis examining the relationship between prenatal depression and foetal cardiac response to a stressor, i.e. repeated vibroacoustic

stimulation. The findings of the relationship between prenatal depression and the total magnitude of foetal response to vibroacoustic stimulation, foetal habituation and the change in FHR in response to the stimulus are reported.

5.2.2 STATISTICAL ANALYSES

All foetal heart data was coded in MATLAB R2009b by two researchers blind to the prenatal depression status of the participants. The details of the data extraction and coding procedures are described on pp. 125-128 in Section 4.1.

5.2.2.1 DEFINITIONS

The definitions of the concepts related to foetal stress responses used in this thesis are as follows:

Foetal Heart Rate Acceleration: An increase in FHR of at least 15 beats per minute above the baseline FHR sustained for at least 15 seconds is classified as an acceleration in foetal heart rate (Freeman, Garite et al. 2003).

Foetal Heart Rate Habituation: The failure of a foetus, that has previously responded to at least one stimulus, to respond by means of a FHR acceleration to the 9^{th} and 10^{th} vibroacoustic stimulus.

Response score: The total number of vibroacoustic stimuli to which the foetus responds by means of a FHR acceleration irrespective of when the stimulus was given during the 10 minute stimulation period.

Using the definition of FHR habituation, foetuses were classified as habituated or not habituated. If a foetus habituated, a response score was calculated accordingly. For example, a foetus that responded to the 1st, 2nd, 5th, 6th, 8th, 9th and 10th stimuli was given a response score of 7 and classified as 'not habituated'. If a foetus did not show a sufficient response to any of the ten vibroacoustic stimuli to meet criteria for a FHR acceleration it was classified as unresponsive. The details of the FHR assessment are described on pp. 120-128 in Section 4.1.

5.2.2.2 OUTCOME VARIABLES

Two sets of outcome variables were constructed to test associations between foetal responsivity and prenatal depression as described below. The variables in Set I were selected as the primary outcome variables as they represent the foetus' response to the vibroacoustic stimulus. They reflect the integrated foetal response to stress following inputs from both the sympathetic and parasympathetic nervous system.

Set I: Foetal Stress Responsivity Variables:

i. Summation of the area under the curve (ΣAUC): In order to obtain an index of the total magnitude of foetal response to stimulation, ΣAUC was calculated for each foetus by adding the AUC for each FHR acceleration following stimulation.
ii. Stimulation FHR: This is the mean FHR during the period when the foetus was exposed to vibroacoustic stimulation.
iii. Change in FHR: This represents the change in FHR from baseline to post-stimulation levels and reflects the net increase or decrease in FHR due to exposure to the stimulus. It was calculated by subtracting the mean baseline FHR from the mean post-stimulation FHR for each foetus.

iv. Foetal habituation: Foetuses were classified as habituated or not-habituated using the definition of habituation described above. Every foetus was assigned a response score and for those foetuses that habituated, the number of trials to habituation was recorded.

Set II: Other Foetal Heart Rate Variables

1. Baseline mean foetal heart rate and variability (beat to beat variability, BBV).
2. Post-stimulation mean FHR and variability.

BBV was not selected as a primary outcome because it is regulated only by the parasympathetic nervous system and not by both sympathetic and parasympathetic components of the autonomic nervous system (ANS). An investigation of the parasympathetic control of the foetal heart is beyond the scope of this thesis.

5.2.2.3 STATISTICAL TESTS

An exploratory data analysis was conducted to determine the distribution of foetal cardiac responses and FHR in the sample. Change in FHR and post-stimulation FHR were normally distributed. All other FHR variables were not normally distributed. Correlations between various FHR variables were determined. The following statistical analyses were then performed to test associations between prenatal depression and foetal stress responsivity:

1. Primary Comparisons

Foetal stress responsivity was compared between the foetuses of high scorers for prenatal depression and the foetuses of controls. Parametric (independent sample t

test) and non-parametric tests (Mann-Whitney tests) were used for normally and non-normally distributed outcome variables as appropriate. Habituation was compared between the two groups using the Chi-Square test.

2. Secondary Comparisons

(a) Mothers with elevated symptoms of prenatal depression were split into two groups: mothers with MDD (cases) and high scorers without MDD. Foetal responsivity in these two groups was compared against the foetuses of control mothers using the analysis of variance (ANOVA). Habituation was compared between the three groups using the Chi-Square test.

(b) Inter-quintile Comparisons for Primary Outcome Variables: Prenatal depression (measured as continuous scores on the EPDS) was split into quintiles. The mean values of foetal responsivity in each of these quintiles were plotted for the four primary outcome variables. Curve estimation techniques were used to determine whether a linear or a quadratic solution explained the association best. Mean FHR values in the highest responding quintiles were then compared against FHR values in the other quintiles using Mann-Whitney tests. Effect sizes for these differences were also calculated.

Quintiles were chosen over continuous EPDS scores to explore the association between prenatal depression and foetal responsivity. This is because categorisation has been reported to have practical advantages in small sample sizes and the interpretation of parameter estimates (Turner, Dobson et al. 2010). Although some statisticians argue that categorisation may result in the loss of statistical efficacy, it has been widely used in epidemiological research. It is particularly useful when clinically relevant thresholds are to be considered and

when results are to be interpreted by medical and public health professionals (Turner, Dobson et al. 2010). Steps 1 and 2 (a) were repeated to test associations between FHR at baseline and post-stimulation, and prenatal depression.

5.2.3 RESULTS

5.2.3.1 SAMPLE CHARACTERISTICS

Foetal Heart Rate Characteristics

The foetal heart rate characteristics of the sample are presented in Table 18. Complete FHR data was available for 131 participants. Stimulation and recovery period FHR data was not available for two participants who declined to undergo vibroacoustic stimulation.

The sample had a mean baseline FHR of 143.81 (SD: 9.25) beats per minute which is within the normal range of FHR [120-160 beats per minute (Volpe 2008)]. Mean post-stimulation FHR (Mean: 146.28 SD: 9.93) was higher than baseline levels but within normal limits. The FHR characteristics of this sample are consistent with similar inquiries in western populations (Allister 2001; Monk, Myers et al. 2003).

Fifty nine foetuses (45%) habituated to the vibroacoustic stimulus and 37 foetuses (28.24%) failed to habituate. Thirty five foetuses (26.72%) did not show a sufficient response to the vibroacoustic stimulus to meet criteria for an acceleration in FHR and were thus categorised as unresponsive.

FHR Variables: Inter-correlations

There were strong correlations between FHR at baseline, during stimulation and post-stimulation (further details may be found in Appendix IVB).

	N	Mean (SD)	Range
Foetal Stress Responsivity Variables			
∑AUC	131	88395.24 (5861.42)	69, 364.81-1,05,990.47
Stimulation FHR	131	147.29 (10.53)	113.67-179.33
Change in FHR	131	2.66 (7.59)	14.05-22.70
Response Score	131	3.15 (3.20)	0-10
Other FHR Variables			
Baseline FHR	133	143.81 (9.25)	117.12-170.11
Baseline BBV	133	3.59 (2.61)	0.33-16.73
Post-stimulation FHR	131	146.28 (9.93)	109.82-171.24
Post-stimulation BBV	131	4.47 (2.77)	0.75-16.11

Table 18 FHR Sample characteristics.

5.2.3.2 PRENATAL DEPRESSION: ASSOCIATIONS WITH FOETAL STRESS RESPONSIVITY

I. Primary Comparisons: Foetal Stress Responsivity in High-Scoring mothers for Prenatal Depression and Controls

Foetal responsivity to stress did not differ between the foetuses of mothers who scored high for symptoms of prenatal depression and the foetuses of controls on any of the 4 main FHR variables under test (Table 19).

The distribution of the foetuses that habituated is presented in Table 20. There were no differences in habituation between the foetuses of high-scoring mothers and controls

(χ^2=1.72, p=0.42). Fifty nine (61.45%) of the 96 foetuses that responded to vibroacoustic stimulation habituated. Twenty eight (42.4%) foetuses habituated in the group of mothers who scored high for symptoms of prenatal depression (n=66). Thirty one foetuses (47.7%) habituated in the control group (n=65). The absence of differences in habituation between the foetuses of high scoring mothers and controls remained when unresponsive foetuses were excluded from the analyses (χ^2=1.31, p=0.25).

Foetal Stress Responsivity Variables	**Total Sample (n=131)**	**Foetuses of Controls (n=65)**	**Foetuses of High Scorers (n=66)**	**Inter-Group Comparisons**	
	Mean (SD)	**Mean (SD)**	**(Mean SD)**	**Test Statistic**	**p value**
∑AUC	88395.24 (5861.42)	88374.01 (5769.04)	88416.14 (5995.14)	U = 2097.00	0.82
Stimulation FHR	147.29 (10.53)	146.94 (10.34)	147.64 (10.79)	U = 2040.00	0.63
Change in FHR	2.66 (7.59)	2.35 (7.51)	2.96 (7.72)	t = -0.46	0.64
Response Score	3.15 (3.20)	2.97 (3.19)	3.33 (3.23)	U = 1948.50	0.36

Table 19 Primary Comparisons: Foetal responsivity to stress in high-scoring mothers and controls.

In addition, continous depression scores (on the EPDS and K10) were also examined between the groups of mothers whose foetuses habituated and those that did not. No differences were

found between these groups (For EPDS scores: U=1061.5, p=0.82; for K10 scores: U=1058, p=0.80).

There were no differences in either foetal stress responsivity or foetal habituation based on foetal sex and the gestational age of the foetus at assessment (Appendix IVC and Appendix IVD).

Prenatal Depression		**Habituation**			**Total**
		Non-Habituating Foetuses	**Habituating Foetuses**	**Un-Responsive Foetuses**	
Foetuses of Non-depressed Mothers (Controls)	n	15	31	19	65
Foetuses of Depressed Mothers	n	22	28	16	66
Total	n	37	59	35	131
	% of Total	28.24 %	45.04 %	26.71 %	100.0%

Table 20 Foetal habituation in high-scoring mothers and controls.

II. Secondary Comparisons

(a) Foetal Stress Responsivity in Controls, High Scorers without MDD and Cases

Foetal responsivity to stress was compared between the foetuses of controls, high scorers without MDD and cases of prenatal depression (Table 21). There were no differences in foetal responsivity between these groups.

Foetal Stress Responsivity Variables	Foetuses of Controls (n=65)	Foetuses of High Scorers (n=38)	Foetuses of Cases (n=28)	Inter-Group Comparisons	
	Mean (SD)	Mean (SD)	Mean (SD)	Test Statistic	p value
∑AUC	88374.01 (5769.04)	87876.62 (6115.607)	89104.50 (5871.48)	F = 0.35	0.70
Stimulation FHR	146.94 (10.34)	146.37 (10.77)	149.25 (10.78)	F = 0.68	0.51
Change in FHR	2.35 (7.51)	-.25 (0.86)	4.30 (4.82)	F = 0.91	0.50
Response Score	2.97 (3.19)	2.59 (2.67)	4.28 (3.66)	F = 2.51	0.09

Table 21 Foetal responsivity to stress in cases of prenatal MDD, high-scoring mothers and controls.

(b) Inter-Quintile Comparisons

In order to explore whether a dose dependent relationship existed between prenatal depression and FHR; prenatal maternal EPDS scores were split into five quintiles. The characteristics of these quintiles are presented in Table 22.

The mean of the four foetal responsivity variables in each quintile was plotted. The pattern of association between prenatal depression and foetal stress responsivity was found to be U shaped (Fig. 27-30). The foetuses of mothers in the first and fifth quintiles showed the highest magnitudes of FHR responses to the vibroacoustic stimulus. The lowest foetal responsivity to the stimulus was shown by the foetuses of mothers in the third quintile (EPDS scores 4-9).

Curve estimation techniques found a quadratic solution to explain 98% of the variance for the stimulation FHR and the change in FHR ($p<0.05$) (Table 23).

Quintiles	EPDS Scores	Cumulative % of Sample	n
1	0-1	21.8%	29
2	2-3	37.6%	21
3	4-9	59.4%	29
4	10-15	82.0%	30
5	16-27	100%	24

Table 22 Prenatal depression quintiles, split based on EPDS scores.

	Linear Model		Quadratic Model	
	R2	Sig.	R2	Sig.
∑AUC	0.00	0.89	0.01	0.67
Stimulation FHR	0.12	0.56	0.98	0.02*
Change in FHR	0.12	0.57	0.98	0.02*
Response Score	0.01	0.67	0.02	0.33

*$p<0.05$

Table 23 Curve estimation for prenatal depression and foetal stress responsivity.

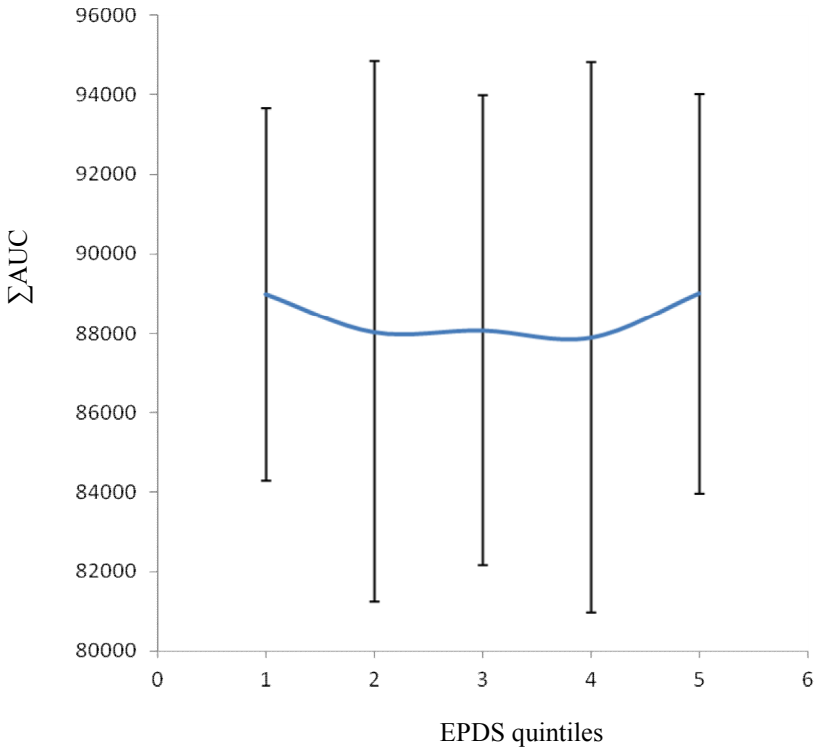

Fig. 27 Association between prenatal depression and total magnitude of foetal response to stimulation (∑AUC).
Note: The black bars denote the standard deviation for each data point.

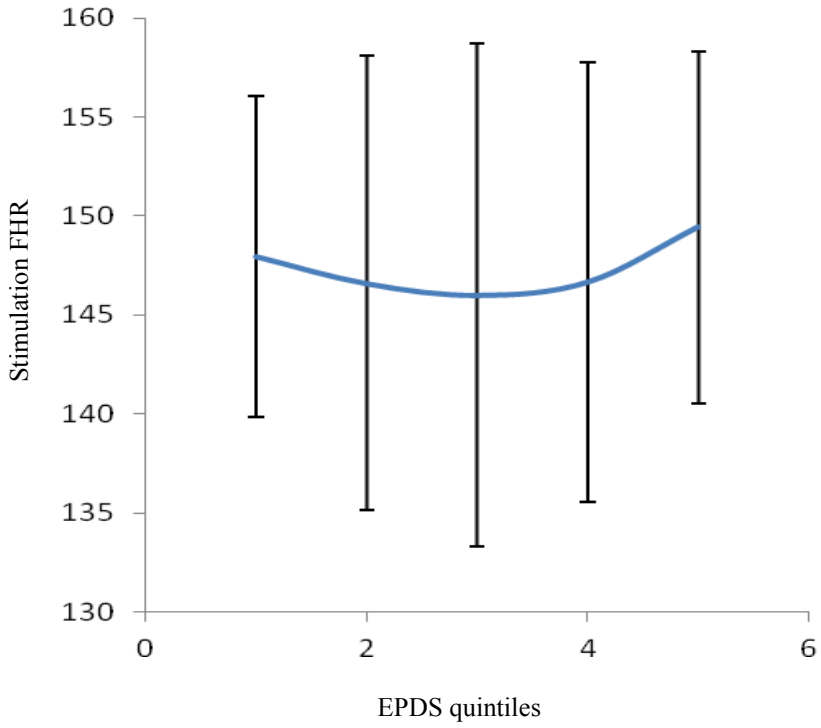

Fig. 28 Association between prenatal depression and stimulation FHR.
Note: The black bars denote the standard deviation for each data point.

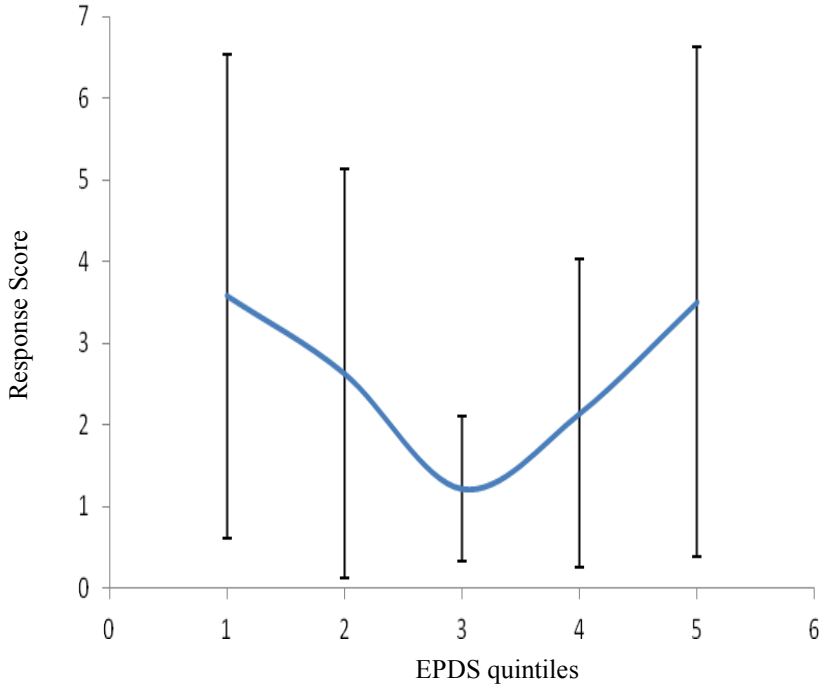

Fig. 29 Association between prenatal depression and response score. Note: The black bars denote the standard deviation for each data point.

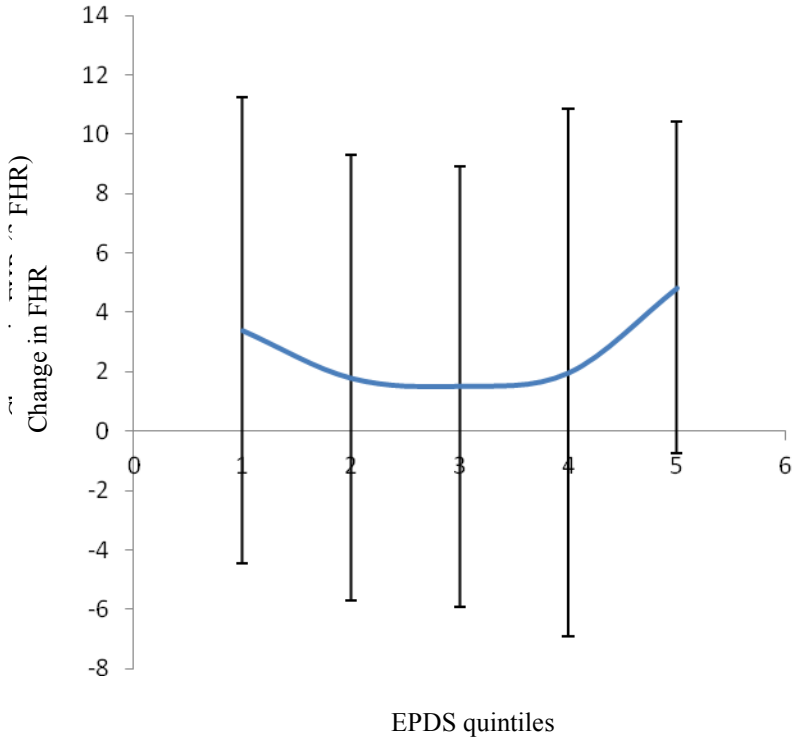

Fig. 30 Association between prenatal depression and change in FHR from baseline to post-stimulation levels (δ FHR). Note: The black bars denote the standard deviation for each data point.

Supplementary Analysis

As foetal stress responsivity was highest in quintiles 1 and 5, a supplementary analysis was conducted to explore the differences in foetal stress responsivity between the foetuses exposed to moderate levels of prenatal depression (corresponding to the trough of the U shaped curve) and the foetuses exposes to very high and very low levels of prenatal depression (corresponding to the ascending arms of the curve).

The quintiles were therefore reconstituted into three groups as follows:

Quintile 1:	Group 1	corresponding to low levels of prenatal depression.
Quintile 2,3,4:	Group 2	corresponding to moderate levels of prenatal depression.
Quintile 5:	Group 3	corresponding to high levels of prenatal depression.

Mean FHR parameters were compared between groups 1 and 2, and groups 2 and 3 (Table 24).

Although there were no significant differences were detected in foetal responsivity between groups 1 and 2, and groups 2 and 3, near significant differences were detected in total foetal response to stimulation between groups 1 and 2. The foetuses in group 2 showed lower responses to the vibroacoustic stimulus compared to group 1 correspondingto a small effect size of 0.17. Foetuses in group 2 also a smaller δ FHR than the foetuses in group 3 corresponding to a moderate effect size of 0.40.

FHR Variable	Group 1 Mean (SD)	Group 2 Mean (SD)	Group 3 Mean (SD)	Pooled SD	Inter-Group Comparisons		Effect Size Groups 1 vs. 2	Effect Size Groups 2 vs. 3
					Groups 1 vs. 2	Groups 2 vs. 3		
Stimulation FHR	147.95 (8.10)	146.42 (11.73)	149.43 (8.71)	10.53	t=0.65 p=0.52	t=1.14 p=0.26	0.14	0.29
Response Score	3.35 (3.33)	2.89 (3.35)	3.83 (3.51)	3.40	U=1105 p=0.78	U=766.5 p=0.25	0.14	0.28
Change in FHR (δFHR)	3.39 (7.84)	1.76 (7.95)	4.82(5.48)	7.59	t=0.95 p=0.34	t=1.73 p=0.09	0.21	0.40

Table 24 Inter-group comparisons and effect sizes for foetal stress responsivity.

5.2.3.3 PRENATAL DEPRESSION: ASSOCIATIONS WITH OTHER FHR VARIABLES

FHR in High-Scoring mothers for Prenatal Depression and Controls: There were no associations between FHR and BBV, and prenatal maternal depression, during baseline and post-stimulation periods (Table 25).

FHR in Control Mothers, High Scorers without MDD and Cases: There were no differences in mean FHR and BBV during the baseline and post-stimulation periods between the foetuses of controls, high scorers for prenatal depression without MDD and cases (Table 26).

Further, there were no differences in baseline FHR (and BBV), and post-stimulation FHR (and BBV) based on the sex of the foetus and the gestational age at assessment (Appendix IVC and Appendix IVD).

Other FHR Variables	Total Sample (n=133)	Foetuses of Controls (n=67)	Foetuses of High Scorers (n=66)	Inter-Group Comparisons	
	Mean (SD)	Mean (SD)	(Mean SD)	Test Statistic	p value
Baseline FHR	143.81 (9.25)	144.09 (9.37)	143.16 (8.87)	U = 2204.00	0.98
Baseline BBV	3.59 (2.61)	3.55 (2.43)	3.68 (7.95)	U = 2127.00	0.95
Post-stimulation FHR	146.28 (9.93)	146.43 (8.86)	146.13 (10.95)	t = 0.18	0.86
Post-stimulation BBV	4.47 (2.77)	4.72 (2.78)	4.23 (2.76)	U = 1869.00	0.20

Table 25 FHR in the foetuses of high-scoring mothers and controls.

FHR Variable	Total Sample (n=131) Mean (SD)	Foetuses of Controls (n=65) Mean (SD)	Foetuses of High Scorers without MDD (n=38) Mean (SD)	Foetuses of Cases (n=28) Mean (SD)	Inter-Group Comparisons Test Statistic	p value
Baseline FHR	143.81 (9.25)	144.09 (9.37)	144.07 (10.23)	142.83 (7.68)	F = 0.20	0.81
Baseline BBV	3.59 (2.61)	3.55 (2.43)	3.49 (2.76)	3.84 (2.86)	F = 0.17	0.84
Post-stimulation FHR	146.28 (9.93)	146.43 (8.86)	145.37 (11.35)	147.13 (10.53)	F = 0.28	0.76
Post-stimulation BBV	4.47 (2.77)	4.72 (2.78)	3.83 (2.23)	4.74 (3.28)	F = 1.42	0.25

Table 26 FHR in the foetuses of controls, high scores without MDD and cases.

5.2.4 DISCUSSION

There are two key findings reported from this sub-section. First, there was no evidence of a linear association between prenatal depressive symptoms and altered foetal cardiac responses to vibroacoustic stimulation (including delayed habituation in foetal cardiac responses). Second, the data indicate a curvilinear relationship between prenatal depression and foetal cardiac responses to vibroacoustic stimulation, suggesting that foetuses of mothers who score either high or very low for depressive symptoms have an elevated response to the stimulus compared to the foetuses of mothers with moderate levels of prenatal depression.

These results are at variance with some studies from the western world which have reported linear associations between prenatal depression and anxiety, and disturbances in FHR responsivity to a stressor (Monk, Fifer et al. 2000; Allister 2001; DiPietro, Costigan et al. 2003; Monk, Myers et al. 2003). Elevated neuroendocrinological markers of prenatal psychopathology have been found to be associated with delayed foetal habituation (Sandman, Wadhwa et al. 1999; Sandman, Glynn et al. 2003). However, the authors argue that a non-linear (cubic) solution explains their findings better than a linear solution. In addition, DiPietro and colleagues report positive associations between pregnancy specific hassles, and heightened FHR variability and foetal motor activity (DiPietro, Kivlighan et al. 2010).

It is important to note that this curvilinear association between prenatal depression and foetal responsivity was not evident under baseline conditions in this thesis, but in response to a potential stressor. Further, the U shaped association was found to persist into the post-stimulation period.

The results are therefore consistent with reports of a complex, cubic relationship between elevated prenatal neuroendocrinology and increased foetal responsivity (Sandman, Wadhwa et al. 1999; Sandman, Glynn et al. 2003). In addition, the findings are congruent with evidence of a U shaped association between stress and reactivity reported in other biological systems. Wilke and colleagues have reported evidence of an inverted U shaped relationship between stress and productivity in adult life (Wilke, Gmelch et al. 1985). Similar U shaped associations have been reported between birth weight and total urinary excretion of glucocorticoid metabolites in infants (Clark, Hindmarsh et al. 1996); birth weight and type II diabetes mellitus (McCance, Pettitt et al. 1994) and maternal nutrition and foetal growth (Wu, Bazer et al. 2004). Supporting evidence has also been provided from animal literature where non-linear relationships between stress and optimal functioning in response to challenging situations have been reported (Yerkes and Dodson 1908). Although these findings may appear counterintuitive, they are consistent in that they suggest that some exposure to stimulation is necessary to promote neural development both before and after birth (DiPietro, Kivlighan et al. 2010). Interestingly, there is supportive evidence from human inquiries that exposure to moderate levels of prenatal maternal stress during intra-uterine life is associated with better foetal performance and higher Bayley scores at 2 years post-birth (DiPietro, Novak et al. 2006; DiPietro, Kivlighan et al. 2010).

The curvilinear association between prenatal depression and foetal reactivity also appears to be consistent with the emerging concept of biological sensitivity to context. This theory contends that in both extremely supportive and unsupportive developmental conditions, children maintain high levels of stress reactivity (Boyce and Ellis 2005; Belsky and Pluess 2009). The authors argue that this is a susceptibility factor and reflects organismic plasticity with a “curvilinear or U shaped relation emerging between levels of supportiveness versus

stressfulness in early childhood environments" (J. Belsky & M. Pluess 2009; p. 887). In such a situation, high reactivity profiles emerge in both highly stressful and highly supportive postnatal social contexts. It is important to emphasise that to date the idea of biological sensitivity has been considered in a postnatal context. It is plausible that such sensitivity may exist during intra-uterine life. It could thus be hypothesised that intra-uterine exposure to moderate amounts of prenatal stress would result in the matching of intra-uterine and extra-uterine environmental influences. Such an intra-uterine milieu confers a developmental advantage to the developing foetus by way of optimal functionality in a post-natal environment which will often involve exposure to multiple moderately stressful life experiences.

In this study, the foetuses of mothers with high levels of prenatal depression showed elevated responses to the vibroacoustic stimulus. This is likely to be because of the programming influence of prenatal maternal depression on the foetus' developing HPA axis. In addition, the foetuses of mothers with very low levels of prenatal depression also showed elevated responses to stimulation. It is possible that these foetuses are exposed to a relatively non-stressful intra-uterine environment. It could be suggested that in such a situation, the presentation of a moderately stressful stressor (such as the vibroacoustic stimulus) would be extremely unfamiliar and the foetuses would therefore show elevated stress responses to the same. DiPietro and colleagues suggest that this pattern supports a downward extension to the foetal period of the notion commonly applied to postnatal performance and development, that both too little and too much stress may be maladaptive, but moderate amounts can be facilitative (DiPietro, Novak et al. 2006; p. 585). In other words, it is possible that exposure to moderate amounts of stress is necessary to prepare the foetus for real life experiences.

There are two key ways in which this study differs from previous work, and these could explain the variance between these findings and some previous reports. First, direct foetal stimulation was utilised to elicit foetal responses in contrast to indirect methods of maternal stimulation. Although some inquiries have utilised maternal cognitive stimulation as a means to stimulate the foetus, it is possible that these findings are influenced by maternal cognitions and maternal mood (Monk, Fifer et al. 2000; DiPietro, Costigan et al. 2003; Monk, Myers et al. 2003). Second, the results must be considered in the context of the sample i.e. a non-western developing world population. It is possible that different patterns of stress are experienced in these settings. Of particular note is the absence of smoking and alcohol consumption among the women in the sample, which are likely to act as significant confounders in previous studies.

Strengths and limitations

There are a number of strengths to this study. It is the first to investigate the association between prenatal depression and foetal responsivity in the developing world. The study was carried out in an entirely non-smoking, non-alcohol consuming prenatal population of women and thus the findings are not confounded by the effects of these influences on foetal development. Foetuses were stimulated directly through vibroacoustic stimulation and not indirectly through maternal cognitive stimulation. An attempt was made to measure complex patterns of foetal responsivity by quantifying the total magnitude of foetal response to vibroacoustic stimulation rather than relying on the measurement of mean values of FHR.

There are several limitations to consider. First, a quarter of the foetuses in this sample did not show sufficient response to the vibroacoustic stimulus to be assessed for habituation. The reason for this insufficient response is unclear although this group did not differ significantly

from foetuses who responded with regard to gestational age, prenatal mood of the mother, maternal age and nutritional status. It is possible that the stimulus used was not strong enough to elicit a response in some foetuses and a stronger stimulus may need to be considered for future studies. Second, the total duration of the FHR assessment was relatively short (30 minutes) and foetuses were exposed to a series of only 10 stimuli. It is possible that this period of exposure was not sufficient to observe habituation responses completely in some foetuses.

Conclusion

In conclusion, the findings of this study provide evidence to support a curvilinear or U shaped relationship between prenatal depression and foetal responsivity to vibroacoustic stimulation. These results support the possibility that exposure to a certain amount of stress may be necessary for optimal foetal development in the setting of this study. However, further investigation with larger samples and different stimuli in different population groups is needed.

Summary of Findings:

- There were no linear associations between prenatal depression and foetal responsivity to stress.
- The association between prenatal maternal depression and foetal stress responsivity was U shaped with the foetuses of mothers with both very high and very low levels of prenatal depression showing elevated cardiac responses to vibroacoustic stimulation compared to the foetuses of moderately depressed mothers.

5.3 PRENATAL DEPRESSION: ASSOCIATIONS WITH INFANT CORTISOL RESPONSIVITY

5.3.1 INTRODUCTION

Cortisol responsivity to a stressor has been widely used as an indicator of the functioning of the hypothalamic-pituitary adrenal (HPA) axis in response to stress (Egliston, McMahon et al. 2007). The HPA axis has been implicated as one of the key neuronal substrates for the possible programming effects of prenatal maternal depression on offspring development (Glover, O'Connor et al. 2010). Prenatal depression has been associated with increased infant cortisol reactivity to stressful tasks under laboratory conditions (Brennan, Pargas et al. 2008; deBruijn, Bakel et al. 2009). Prenatal stress has been found to predict cortisol responses to the first day of school in a sample of 5 year olds (Gutteling, Weerth et al. 2005) and on children's awakening and afternoon cortisol levels at 10 years of age (O'Connor, Ben-Shlomo et al. 2005). However, one study on preterm infants reported intra-uterine exposure to glucocorticoids to be associated with a dampening of infant cortisol responses to immunisation (Glover, Miles et al. 2005). The association between prenatal depression and infant cortisol response to immunisation in healthy, term infants has never been explored. In addition, all studies investigating this relationship have been carried out in western populations. The findings of this thesis examining the relationship between prenatal depression and infant cortisol responsivity to a common stressor i.e. immunisation, in a sample from rural South India, are presented in this section.

5.3.2 STATISTICAL ANALYSIS

A descriptive analysis was conducted on infant and maternal salivary cortisol data. A new variable, cortisol response (δ cortisol), was constructed by subtracting baseline cortisol from post-immunisation cortisol for each infant. Cortisol response represents the infant's cortisol response to immunisation and reflects the change in the infant's cortisol levels from baseline to post-immunisation values.

The standardized z score method was used to identify outliers using a cut off of $\pm$2.5 SD (Mickey, Dunn et al. 2004). These outliers were excluded in all further analysis.

The following analyses were conducted to examine the association between prenatal depression and infant cortisol responsivity:

i. **Primary Comparisons:** Infant cortisol responsivity was compared between the infants of prenatally depressed mothers (high-scorers) and controls using the independent sample t tests and Mann Whitney tests for parametrically and non-parametrically distributed data.
ii. **Secondary Comparisons:** The group of high scorers was split into those meeting a diagnosis for a major depressive disorder (MDD) during pregnancy (cases) and high-scorers without MDD. Infant cortisol responsivity was compared between these groups and the infants of controls using ANOVAs.
iii. The association between postnatal depression (PND) and, infant and maternal cortisol was also investigated using independent sample t tests and Mann Whitney tests.
iv. **Regression Analyses:** A regression analysis was performed to examine whether prenatal depression predicted infant cortisol response independent of PND and

maternal cortisol. In this model, maternal cortisol served as a proxy control for genetic influences on infant cortisol levels.

v. **Inter-Quintile Comparisons:** To investigate the relationship between prenatal depression and infant cortisol responsivity, prenatal maternal depression (EPDS) scores were categorised into quintiles (further details may be found on p. 180). Infant cortisol responsivity in each quintile was plotted. Curve estimation was used to determine whether a linear or quadratic solution explained the association best. Inter-quintile comparisons were carried out using Mann Whitney tests. The analysis was repeated for infant baseline and post-immunisation cortisol.

5.3.3 RESULTS

5.3.3.1 INFANT SALIVARY CORTISOL: SAMPLE CHARACTERISTICS

Sample Size and Attrition:

Fifty-eight of 133 mother-infant dyads returned for the postnatal assessment (attrition rate = 56.4%). Infants were assessed at a mean age of 57.7 days (SD: 23.2 days, Range: 15-114 days).

Baseline infant saliva was collected for 58 infants. However, due to inadequate sampling, cortisol values were obtained in 56 infants. Following the elimination of outliers (n=2), baseline infant cortisol data was available for 54 infants.

Post-immunisation infant saliva was collected in 43 infants. In the 15 missing cases, mothers had immunised their children in other local clinics and the infants did not receive their dose of

the DPT vaccine at the study site. Hence, post-immunisation saliva could not be collected in these infants. Post-immunisation cortisol values were not obtained in 3 infants because of inadequate quantity of the sample. Upon eliminating the sole outlier, post-immunisation cortisol analysis was conducted on 39 infants.

Cortisol response (δ Cortisol) was calculated for the sample of 39 infants for whom both baseline and post-immunisation cortisol data was available. Cortisol response analysis was conducted on 38 infants after excluding the sole outlier (n=1).

Maternal postnatal saliva was collected for 57 mothers. One mother had abandoned her baby at the study location leaving the child in the care of the orphanage run within Snehalaya Hospital. Saliva samples could not be obtained from this mother. Cortisol values were not obtained for 4 mothers due to inadequate sampling. After excluding outliers (n=1), maternal cortisol analysis was conducted on 52 mothers.

Sample Characteristics:

The characteristics of infant and postnatal maternal cortisol are presented in Table 27 (a). The mean baseline and δ cortisol values of the sample are in concordance with similar reports from western populations (Gutteling, Weerth et al. 2005).

Descriptive Characteristics	Infant Cortisol Values (nmol/l)			Postnatal Maternal Cortisol (nmol/l)
	Baseline Cortisol	Post-immunisation Cortisol	Cortisol Response (δ Cortisol)	
No. of Saliva Samples Collected	58	43	39	57
Missing Data	2	3		4
No. of Outliers	2	1	1	1
Final Sample Size	54	39	38	52
Mean	9.35	29.31	19.32	21.24
SD	10.27	15.15	18.58	36.45
Min	0.50	6.45	-35.23	0.79
Max	50.87	63.86	63.27	217.00

Table 27(a) Infant and Postnatal Maternal Cortisol: Sample characteristics.

5.3.3.2 PRENATAL DEPRESSION AND INFANT CORTISOL RESPONSE

The characteristics of cortisol response in the infants of prenatal high scorers and controls are presented in Table 27 (b) and (c). Thirty six of thirty eight infants (94.74%) experienced a rise in cortisol following immunisation.

Fig. 31 depicts the change in cortisol from baseline to post-immunisation levels in the infants of prenatal high scorers and controls. The slope for the cortisol response to immunisation is higher in the infants of high scoring mothers (28.24) compared to controls (13.53).

Cortisol Variable (nmol/l)	Prenatal Control		Prenatal High Scorer		Inter-Group Comparison	
	N	Mean (SD)	N	Mean (SD)	Test Statistic	p value
Infant Baseline Cortisol	30	10.63 (10.41)	24	7.74 (10.09)	U = 284.00	p = 0.19
Infant Post-immunisation cortisol	22	24.16 (12.86)	17	35.98 (15.62)	t = -2.59	**p = 0.01****
Infant Cortisol Response	23	14.49 (19.79)	15	26.71 (14.14)	t = -2.07	**p = 0.05***
Maternal Postnatal Cortisol	30	20.43 (41.35)	22	22.34 (29.35)	U = 293.00	p = 0.46

*p is significant at 0.05 level, **p is significant at 0.01 level

Table 27 (b) Infant and maternal cortisol characteristics in prenatal high scorers and controls.

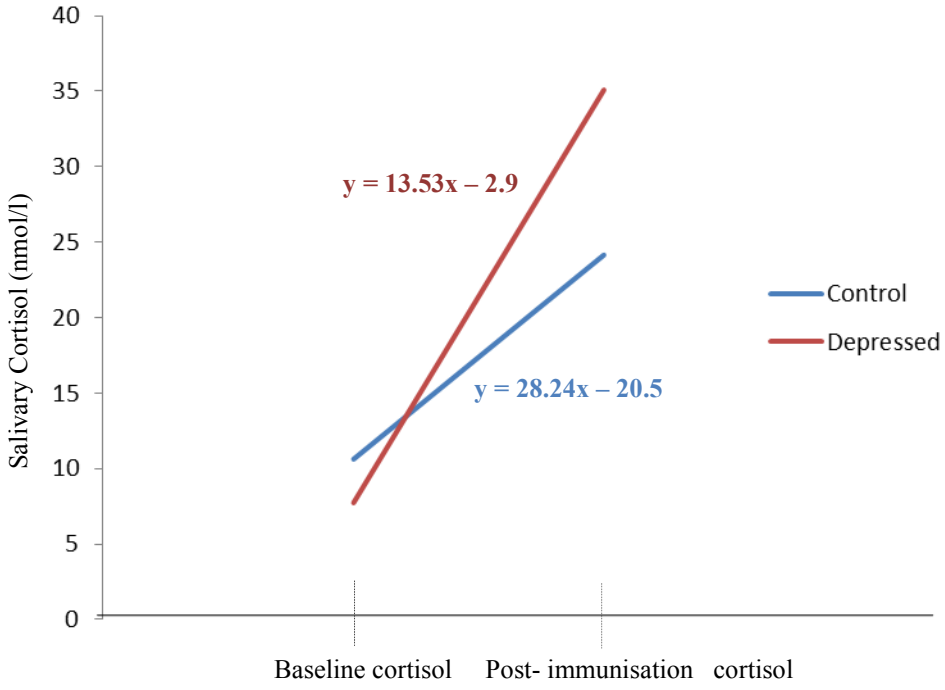

Fig. 31 Plot of baseline and post-immunisation cortisol in the infants of prenatal high scorers mothers and controls.

Primary Comparisons: The results of the primary comparisons are presented in Table 27(b). Compared to controls (Mean: 14.49 nmol/l), the infants of prenatal high scorers showed a higher cortisol response to immunisation (Mean: 26.71 nmol/l; t = -2.07, p = 0.05).

Secondary Comparisons: Compared to controls and cases, the infants of high scorers without MDD showed an elevated cortisol response to immunisation (Table 27 (c); F = 4.27, p = 0.05).

Cortisol Variables (nmol/l)	**Prenatal Control**		**Prenatal High Scorers without MDD**		**Cases of Prenatal Depression**		**Inter-Group Comparison**	
	N	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**Test Statistic**	**p value**
Infant Baseline Cortisol	30	10.63 (10.41)	14	7.07 (4.82)	10	8.68 (15.01)	F = 1.05	0.31
Infant Post-immunisation Cortisol	22	24.16 (12.86)	11	36.32 (13.70)	6	35.36 (20.12)	F = 3.28	0.05*
Infant Cortisol Response	23	14.49 (19.79)	9	28.89 (12.77)	6	23.45 (16.66)	F = 4.27	0.05*
Maternal Postnatal Cortisol	30	20.43 (41.35)	13	24.44 (20.12)	9	19.31 (40.46)	F = 0.00	0.99

*p is significant at 0.05 level, **p is significant at 0.01 level

Table 27 (c) Infant and maternal cortisol characteristics in prenatal controls, high scorers without MDD and cases.

5.3.3.3 Prenatal Depression: Associations with Infant Baseline and Post-Immunisation Cortisol and Maternal Postnatal Cortisol

The results of the comparisons in infant and maternal cortisol between high scorers for prenatal depression and controls are presented in Table 27 (b). The findings of these comparisons between controls, high scorers without MDD and cases are presented in Table 27 (c).

Infant Baseline Cortisol

There were no differences in infant baseline cortisol between infants of prenatal high scorers (Mean: 7.74 nmol/l) and controls (Mean: 10.63 nmol/l; U=284.00, p=0.19). In addition, an analysis of variance did not detect differences in baseline cortisol between the infants of controls, high scorers without MDD and cases (F=1.05, p=0.31).

Infant Post-Immunisation Cortisol

Compared to controls (Mean: 24.16 nmol/l), the infants of mothers with prenatal depression showed elevated post-immunisation cortisol levels (Mean: 35.98 nmol/l; t=-2.59, p=0.01). The infants of high scorers without MDD showed the highest post-immunisation compared to the infants of cases and controls (F=3.28, p=0.05).

Maternal Postnatal Cortisol

Postnatal maternal cortisol did not differ between high scorers for prenatal depression and controls (U= 293.00, p=0.46). In addition, there were no differences in postnatal maternal cortisol between controls, high scorers without MDD and cases (F=0.01, p=0.99).

5.3.3.4 *Postnatal Depression and Infant Cortisol Reactivity*

The sample characteristics of infant and maternal cortisol, categorised according to the postnatal depression (PND) status of the mother are presented in Table 28. PND was not associated with infant baseline (U = 311, p = 0.49); infant post-immunisation (t = -0.82, p = 0.42) and infant δ cortisol (t = -0.54, p = 0.59). Mothers with PND had elevated postnatal cortisol levels (Mean: 27.78 nmol/l) compared to mothers without PND (Mean: 24.15 nmol/l; U = 224, p = 0.04).

	Infant Baseline Cortisol		**Infant Post-immunisation Cortisol**		**Infant δ Cortisol**		**Maternal Postnatal Cortisol**	
	No PND	**PND**	**No PND**	**PND**	**No PND**	**PND**	**No PND**	**PND**
N	16	17	16	17	16	17	28	29
Mean	10.07	6.89	28.09	29.05	18.02	22.16	20.99	21.55
Median	7.19	6.71	24.21	25.53	18.96	22.12	9.82	7.03
SD	9.35	4.27	14.08	15.80	15.14	15.97	21.72	48.90
Min	0.50	1.77	9.34	6.45	-14.45	3.63	2.27	0.79
Max	37.08	15.33	55.14	62.25	43.99	54.02	76.05	217

PND = Postnatal depression

Table 28 Infant and maternal cortisol characteristics in postnatally depressed and non-depressed groups.

5.3.3.5 *Infant Sex and Age at Assessment: Associations with Infant Cortisol Reactivity*

Baseline cortisol was significantly higher in boys (U=479.00, p = 0.04) and cortisol reactivity was significantly higher in girls (t = 2.36, p=0.02). There were no associations between the

infant's age at assessment and cortisol reactivity. The findings of this analysis are presented in Appendix IVE.

5.3.3.6 Regression Analyses: The Effect of Prenatal Depression on Infant Cortisol Responsivity Independent of Postnatal Depression

To examine if prenatal depression predicted infant cortisol response to immunisation independent of PND, postnatal maternal cortisol and infant sex, a three step linear regression model was conducted as follows:

Step 1: Prenatal depression was entered as a predictor

Step 2: Postnatal depression was added to the model

Step 3: Maternal cortisol and sex of the infant were added to the model.

In this model, prenatal depression predicted infant cortisol response independent of postnatal depression (B = 0.41, p = 0.02). When maternal cortisol was added to the model, prenatal depression continued to predict infant cortisol response independent of PND and maternal cortisol B=0.39, p=0.02; Table 29). Female sex remained a significant predictor of infant cortisol reactivity after controlling for pre- and postnatal depression and maternal cortisol.

Regression Model for Infant Cortisol Response		Standardized Coefficients (β)	Sig.
1	(Constant)		0.00
	Prenatal depression	0.41	0.01
2	(Constant)		0.00
	Prenatal depression	0.41	0.02*
	Postnatal depression	0.03	0.84
3	(Constant)		0.01
	Prenatal depression	0.41	0.01*
	Postnatal depression	0.05	0.74
	Maternal postnatal cortisol	0.19	0.22
	Infant sex (female)	0.32	0.02*

*p is significant at 0.05 level, **p is significant at 0.01 level

Table 29 Results of regression analysis for infant cortisol response.

5.3.3.7 Inter-Quintile Comparisons and Curve Estimation

Prenatal Depression and Infant Cortisol Response

The findings of inter-quintile comparisons and curve estimation techniques examining the relationship between prenatal depression and infant cortisol response to immunisation are presented in Fig. 32 and Table 30 respectively.

There was a U shaped association between prenatal depression and infant cortisol responsivity. Infants of mothers in quintiles 1, 4 and 5 (EPDS scores of 0-1 and 10-27) showed higher rises in cortisol following immunisation compared to the infants of mothers in quintiles 2 and 3 (EPDS scores of 2-9). A quadratic solution was found to explain the association better than a linear solution (R^2=0.20, p=0.02).

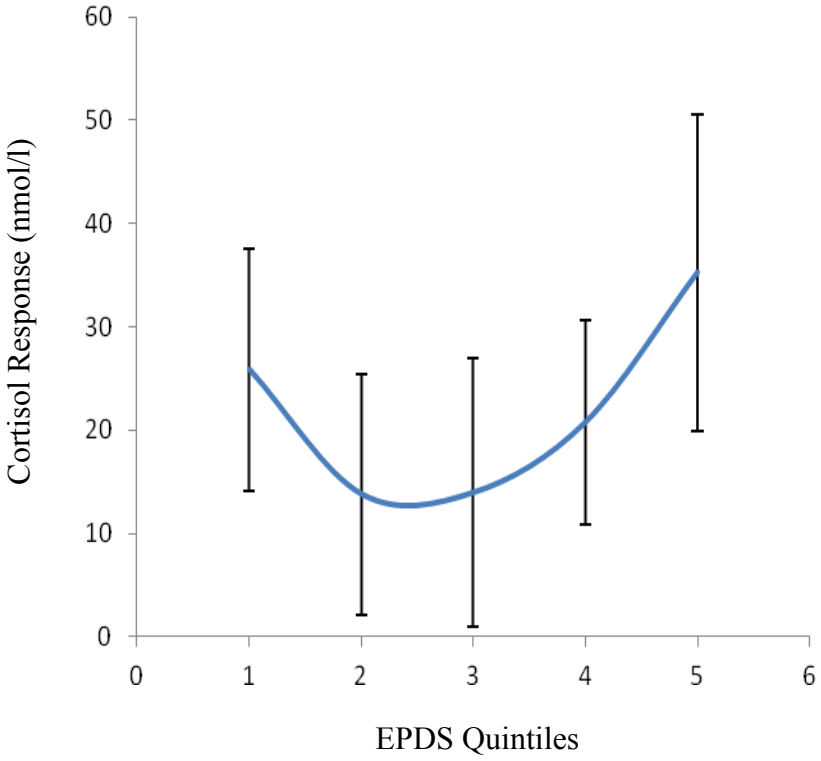

Fig. 32 Infant cortisol response curve plotted against EPDS quintiles. Note: The black bars denote the standard deviation for each data point.

Prenatal Depression: Associations with Infant Baseline and Post-Immunisation Cortisol

There was no particular pattern of association between infant baseline cortisol and prenatal depression (EPDS) quintiles as seen in Fig 33. A U shaped association was found to exist between prenatal depression and infant post-immunisation cortisol (Fig. 34). The infants of mothers in EPDS quintiles 1 and 5 showed elevated post-immunisation cortisol levels compared to the infants of mothers in quintiles 2, 3 and 4. A quadratic solution (R^2=0.39, $p<0.01$) was found to explain this association better than a linear solution (Table 30).

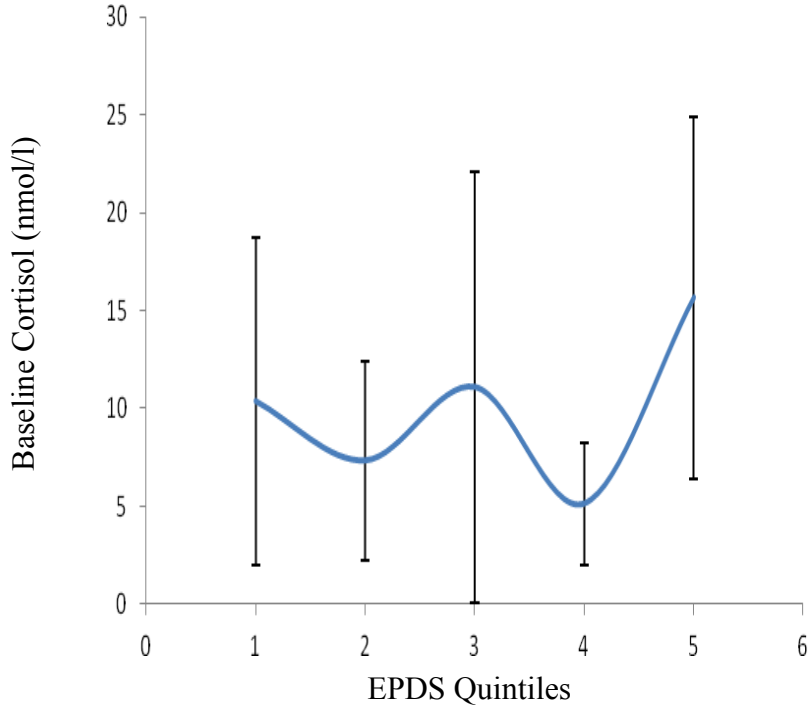

Fig. 33 Infant baseline cortisol curve plotted against EPDS quintiles.
Note: The black bars denote the standard deviation for each data point.

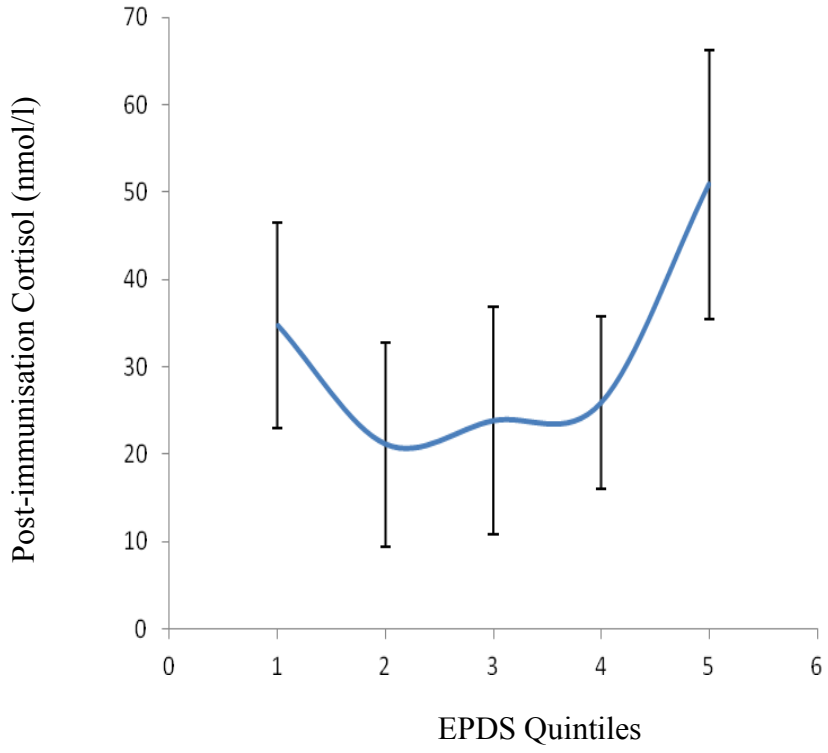

Fig. 34 Infant post-immunisation cortisol curve plotted against EPDS quintiles.
Note: The black bars denote the standard deviation for each data point.

Cortisol x Prenatal Depression	Linear Model		Quadratic Model	
	R^2	Sig.	R^2	Sig.
Baseline Cortisol	0.00	0.64	0.03	0.43
Post-immunisation Cortisol	0.08	0.08	0.39	**<0.01****
Cortisol Response (δ Cortisol)	0.07	0.12	0.20	**0.02***

*p is significant at 0.05 level, **p is significant at 0.01 level

Table 30 Findings of curve estimation techniques for the association between prenatal depression and infant cortisol: Linear and quadratic models.

Inter-Quintile Comparisons: The non-linear association between prenatal depression and infant cortisol suggested that infants of mothers with prenatal depression scores in the first and fifth quintiles performed differently from those in quintiles 2, 3 and 4. Accordingly, the quintiles were reconstituted into three groups as follows:

Quintile 1 → Group 1

Quintile 2, 3, 4 → Group 2

Quintile 5 → Group 3

The findings of the inter-group comparisons are presented in Table 31. Compared to group 2, infant cortisol response to immunisation was higher in group 3. There were significant differences between groups 1 and 2, and groups 2 and 3 for post-immunisation cortisol. The findings are further supported by the large effect sizes noted in the differences in post-immunisation cortisol and cortisol response between groups 2 and 3.

Cortisol	Mean (SD)			Inter-group Comparisons			Effect Sizes	
	Group 1	**Group 2**	**Group 3**	**Group 1 vs. 2**	**Group 2 vs. 3**	**Group 1 vs. 3**	**Group 1 vs. 2**	**Group 2 vs. 3**
Baseline	11.18 (11.54)	7.00 (7.32)	11.00 (16.55)	U=207 p=0.18	U=127 p=0.78	U=53.5 p=0.52	0.41	0.08
Post-Immuni-sation	34.72 (11.76)	23.33 (11.42)	48.95 (15.37)	t=2.3* p=0.03	t=4.65** p<0.01	t= -1.90 p= 0.09	0.75	2.23
δ Cortisol	20.57 (16.81)	15.73 (13.54)	35.23 (18.23)	t = 0.84 p=0.41	t = 2.80 p=0.01*	t= -1.48 p=0.17	0.31	1.22

δ Cortisol = cortisol response to immunisation *p is significant at 0.05 level, **p is significant at 0.01 level.

Table 31 Effect sizes and results of inter-group comparisons.

5.3.4 DISCUSSION

In this study, prenatal depression predicted elevated infant cortisol responses to immunisation independent of postnatal depression, postnatal maternal cortisol and infant sex. In addition, a U shaped association was found to exist between prenatal depression and infant cortisol responsivity. The infants of mothers with very high and very low levels of prenatal depression showed elevated cortisol responses to immunisation compared to the infants of mothers with moderate levels of prenatal depression. Further, compared to the infants of controls, the infants of prenatally depressed mothers showed lower baseline and elevated post-immunisation cortisol levels in this study.

Comparison of Findings with Previous Literature

The findings of this study are consistent with a number of western reports of an association between prenatal depression and elevated infant cortisol response to a stressor. Maternal perinatal depression has been reported to predict elevated infant cortisol reactivity in a US based sample of infants at 6 months post-birth (Brennan, Pargas et al. 2008). Gutteling and colleagues found prenatal stress to predict cortisol response to the first day of school in 29 Dutch 5-year olds (Gutteling, Weerth et al. 2005). In another Dutch sample, prenatal depression predicted elevated levels of cortisol following exposure to stressful laboratory tasks in female children but not in male children at 3 years of age (deBruijn, Bakel et al. 2009). However, neither of these studies controlled for the influence of postnatal maternal mood on infant cortisol responsivity. A study by Glover et al found prenatal exposure to glucocorticoids to predict dampened cortisol response to immunisation at 4 months in a sample of 45 preterm babies independent of exposure to postnatal steroids (Glover, Miles et al. 2005). The findings of this study are at variance with this report and further research is needed to explain this discrepancy. It is however important to note that the inquiry by Glover and colleagues differs from this study in two key ways. First, the authors did not assess prenatal depression but rather prenatal glucocorticoid exposure. Second, the study was conducted on preterm infants who spent their first month of life in intensive care and not healthy, term infants.

The findings of an elevation in post-immunisation cortisol in the infants of prenatally depressed mothers compared to controls are congruent with previous reports from western populations of an association between prenatal depression and elevated post-stress levels of cortisol in offspring (Gutteling, Weerth et al. 2005; Brennan, Pargas et al. 2008).

Findings of sex differences in cortisol levels have been reported in previous literature however, a further investigation of the same is beyond the scope of this thesis (O'Connor, Ben-Shlomo et al. 2005).

Prenatal Depression and Infant Cortisol Responsivity: A Curvilinear Relationship

The finding of a U shaped association between prenatal depression and infant cortisol responsivity to immunisation reported in this study is a novel one. There have been no reports of this association in previous literature exploring the association of prenatal depression with infant cortisol. This finding suggests that infants exposed to both very high levels and very low levels of maternal depression during intra-uterine life show elevated stress responses compared to those exposed to moderate levels of maternal depression.

In a three-group comparison however, infant cortisol response was found to be higher in the infants of high-scoring mothers compared to the infants of both controls and clinical cases of prenatal depression. Although this finding appears to be inconsistent with the U shaped association between prenatal depression and infant cortisol responsivity, a further exploration of the group of high scorers (n=9) revealed that a third (n=3) were categorised as quintile 3 (n = 3, Mean δ Cortisol = 20.76 nmol/l), a third as quintile 4 (n=3, Mean δ Cortisol = 27.04 nmol/l) and a third as quintile 5 (n=3, Mean δ Cortisol = 38.87 nmol/l). The infants in quintile 3 showed the lowest cortisol response to immunisation compared to those in quintiles 4 and 5 and those in quintiles 1 and 2 (Table 31). Thus while the precise reason for the discrepancy between the three-group comparison and the five-group (quintile) comparison cannot be ascertained from the data due to the small size of the postnatal sample, it is possible that this may be accounted for by the mixed nature of the high-scoring group.

Nevertheless, similar U shaped associations have however been reported in other biological processes. In a sample of 190 British nine year olds, the association between birth weight and urinary excretion of glucocorticoid metabolites was reported to be U shaped (Clark, Hindmarsh et al. 1996). In this study, children with both very low and very high birth weights showed increased adrenocortical function than those of moderate birth weight after controlling for sex, age and current weight. A similar association has been reported between:

(i) birth weight and non-insulin dependent diabetes mellitus (McCance, Pettitt et al. 1994)

(ii) prenatal maternal nutrition and foetal growth (Wu, Bazer et al. 2004) and

(iii) stress and productivity in adult life (Wilke, Gmelch et al. 1985).

In a study of the relationship between maternal neuroendocrinological markers of prenatal psychopathology and foetal stress responsivity, Sandman and colleagues found a complex cubic solution to explain the association better than a linear one (Sandman, Wadhwa et al. 1999; Sandman, Glynn et al. 2003). In a study reporting positive associations between moderate levels of prenatal stress and better cognitive functioning in children, DiPietro and colleagues suggested that such associations may represent an extension of the programming hypothesis in that exposure to both very high and very low levels of prenatal stress may be detrimental to offspring development, whereas exposure to moderate levels of prenatal stress may possible facilitate offspring development (DiPietro, Novak et al. 2006; DiPietro, Kivlighan et al. 2010).

Underlying Mechanisms

Although the mechanisms underlying the association between prenatal depression and infant cortisol responsivity are as yet not well understood, recent literature provides evidence in support of a number of hypotheses. These are discussed in detail in Chapter 6. In brief, the underlying mechanisms include the possible programming influence of prenatal depression on

the developing offspring's HPA axis (Talge, Neal et al. 2007), the influence of prenatal stress on utero-placental blood flow and subsequent foetal growth (Steele, Warren et al. 1993; Field, Diego et al. 2004) and the influence of high risk behaviours such as smoking, alcohol consumption and poor nutrition in depressed mothers compared to controls (Goldenberg 1991). In addition the contribution of genetic influences to the heritability of HPA axis functioning cannot be ignored (Bartels, Van den Berg et al. 2003).

Strengths and Limitations

There are a number of limitations to consider in this study. First, prenatal maternal cortisol was not controlled for. Second, it was not possible to control for the infant's daytime sleeping which is known to influence cortisol levels (Gunnar, Malone et al. 1985; Weerth and Geert 2002). Third, the data was limited to a single pre-stress and post-stress sample of cortisol when multiple post-stress levels may have afforded a better understanding of the stress response process. Fourth, the final postnatal sample size for infant cortisol responsivity was small (n=38).

Nevertheless, there are a number of strengths to this study. It is the first to investigate the association between prenatal depression and infant cortisol responsivity in a developing world population and in a non-smoking, non-alcohol consuming prenatal population. A pre-stress/post-stress paradigm was employed to study HPA axis response to stress. Immunisation was utilised as the stressful stimulus rather than laboratory induced tasks which are possibly influenced by the infant's age and temperament. All saliva samples were collected in a hospital based setting and were not contaminated by breastfeeding (Egliston, McMahon et al. 2007).

Conclusion

In this study, prenatal depression predicted elevated infant cortisol response to immunisation independent of PND, postnatal maternal cortisol and infant sex. Further a U shaped association existed between prenatal depression and infant cortisol responsivity with infants of mothers with very high and very lows of prenatal depression showing elevated cortisol responses compared to the infants of mothers with moderate levels of prenatal depression. This finding, although intriguing, must be interpreted with caution. Replication studies in different populations, using serial measurements of cortisol before and after exposure to the stressor and careful control for diurnal fluctuations are needed before the association can be clearly understood.

Summary of Findings:

- Prenatal depression predicted elevated infant cortisol responses to immunisation independent of postnatal depression.
- A U shaped association existed between prenatal depression and infant cortisol responsivity to immunisation.

5.4 PRENATAL DEPRESSION: ASSOCIATIONS WITH INFANT TEMPERAMENT

5.4.1 INTRODUCTION

Studies in western populations have reported prenatal depression to be associated with increased reports of difficult infant temperament. For example, prenatal depression and anxiety were associated with a 3.06 increase in the odds of difficult temperament in an Australian sample of 4 month olds (Austin, Hadzi-Pavlovic et al. 2005). Similar associations have been reported from the USA and Europe (Brouwers, van Baar et al. 2001; Huizink, Robles De Medina et al. 2002; Field, Diego et al. 2004; Mohler, Parzer et al. 2006; Field, Diego et al. 2008c). A summary of this literature and an overview of the possible mechanisms underlying this association are presented in Section 2.4.

This chapter reports the findings of this thesis exploring the associations between prenatal maternal depression and infant temperament in a sample of 1.5-3 month olds from rural South India. It is important to note that none of the mothers in this study smoked, consumed alcohol, drugs of abuse or psychotropic medication during their pregnancies.

5.4.2 STATISTICAL ANALYSES

5.4.2.1 FACTOR ANALYSIS AND RELIABILITY TESTING

A factor analysis was conducted for each of the 6 dimensions of temperament as measured on the Infant Behaviour Questionnaire [IBQ; (Rothbart 1981)] in SPSS v.15.0. The Cronbach's alpha for each dimension was calculated. The correlations of each item within each dimension of temperament, and the Cronbach's alpha if each of the items were to be deleted, were also calculated.

For the purpose of this study, and because the IBQ has not been previously validated for use in a rural Indian population, a Cronbach's alpha of >0.5 was considered satisfactory. Items found to significantly lower the Cronbach's alpha for each dimension of temperament were deleted. Items not found to be correlated with the other constituents for each dimension were also deleted. However, care was taken to ensure that the minimum possible numbers of items (if any) were deleted from each temperament dimension. The scores for each dimension of temperament were then re-calculated.

5.4.2.2 OUTCOME VARIABLES

The outcome variables whose associations with prenatal depression are reported in this section are as follows:

1. Six dimensions of infant temperament: These include activity level, distress to limitations, distress and latency to approach sudden and novel stimuli (DLSNS), duration of orienting, smiling and laughter and soothability.

2. Three composites of infant temperament: The six dimensions were collapsed into three composites (Miceli, Whitman et al. 1998) for each infant as follows:

   i. The Negative Reactivity Composite (NRC): The NRC reflects higher levels of negative reactivity in the infant (Miceli, Whitman et al. 1998). It is constructed by averaging the scores of the dimensions related to negative aspects of infant temperament i.e. activity level, distress to limitations and distress and latency to approach novel and sudden stimuli.

   ii. The Positive Reactivity Composite (PRC): PRC indicates the level of positive reactivity demonstrated by the infant (Miceli, Whitman et al. 1998). The dimensions of smiling and laughter, duration of orienting and soothability are averaged to obtain a PRC score.

   iii. The Global Reactivity Composite (GRC): The GRC score indicates the infant's overall negative reactivity, relative to that infant's positive reactivity (Miceli, Whitman et al. 1998). It is computed by subtracting positive reactivity from negative reactivity. A high GRC score indicates an overall higher negative reactivity in the infant.

5.4.2.3 STATISTICAL TESTS

An exploratory data analysis was conducted to determine the distribution of the six dimensions and three composites of infant temperament. Statistical tests were carried out to examine the association between prenatal depression and infant temperament as follows:

1. **Correlations:** Associations between prenatal depression scores (EPDS) and infant temperament were tested using continuous correlations.

2. **Primary Comparisons:** Associations between prenatal maternal depression and infant temperament were compared between the infants of mothers with elevated symptoms of prenatal depression (high scorers) and the infants of controls using parametric (Independent sample t test) and non-parametric tests (Mann-Whitney tests) for normally and non-normally distributed dimensions of temperament respectively.

3. **Secondary Comparisons:** Prenatal high scorers were categorised into those who met a clinical diagnosis of a major depressive disorder (MDD) during pregnancy (cases) and those who did not (high scorers without MDD). Temperament scores of the infants of these two groups were compared against the infants of controls using the analysis of variance (ANOVA).

4. **Inter Quintile Comparisons and Curve Estimation:** Prenatal depression (EPDS) scores were categorised into quintiles and mean infant NRC and GRC scores in each of these quintiles were plotted. Curve estimation techniques were used to examine whether a linear or quadratic solution explained the relationship between prenatal depression and the composites of infant temperament.

5. **Association of infant temperament with potential confounders:** Associations between infant temperament scores and the following variables were tested using continuous correlations, independent sample t tests and Mann-Whitney tests as appropriate: postnatal depression (PND), birth weight, infant age and sex, weight percentile, breastfeeding status and infant health status at assessment.

6. **Supplementary Analysis:** Associations between intra-uterine (foetal) and extra-uterine (infant) responsivity were studied by examining correlations between the following groups of variables:

   I. Foetal Responsivity:

   a. Foetal heart rate during exposure to vibroacoustic stimulation (Stimulation FHR)

   b. Change in FHR from baseline to post-stimulation levels (Delta FHR or δ FHR)

   c. The total magnitude of foetal response to stimulation (ΣAUC)

   II. Infant responsivity:

   a. Infant Physiological Responsivity: Infant cortisol response to immunisation (Delta Cortisol or δ Cortisol)

   b. Infant Psychological Responsivity: Negative Reactivity Composite (NRC) and Positive Reactivity Composite (PRC) of Infant Temperament

5.4.3 RESULTS

5.4.3.1 INTERNAL CONSISTENCY OF THE INFANT BEHAVIOUR QUESTIONNAIRE

The factor analysis of the six dimensions of infant temperament constructed from the IBQ is shown in Table 32. One item from the 'distress to limitations' sub-scale and one from the 'soothability' sub-scale were found to increase the Cronbach's α of the respective dimensions of infant temperament considerably if not included and were thus deleted.

The positive dimensions of infant temperament, namely duration of orienting, smiling and laughter and soothability were found to correlate reasonably with each other. There were no correlations between the negative dimensions of infant temperament i.e. activity level, distress

to limitations and DLSNS (Table 33).

Dimension	No. of Items	Cronbach's α	Items Deleted	Cronbach's α After Item Deleted
Activity Level	13	0.631	0	0.631
Distress to Limitations	12	0.591	1 (q19)	0.622
DLSNS	5	0.535	0	0.535
Duration of Orientating	1	Not run as only 1 item	N/A	Not run as only 1 item
Smiling and Laughter	4	0.600	0	0.600
Soothability	11	0.540	1 (q44)	0.579

**p<0.05

Table 32 Table showing factor analysis of the dimensions of infant temperament measured on the IBQ.

Dimension	Activity Level	Distress to Limit-ations	DLSNS	Duration of Orient-ing	Smiling & Laughter	Sooth-ability
Activity Level	-	0.14	-0.90	0.33	0.20	0.17
Distress to Limitations		-	-0.07	-0.10	-0.01	0.18
DLSNS			-	-0.19	-0.08	0.06
Duration of Orientating				-	0.27*	0.36*
Smiling and Laughter					-	0.35*
Soothability						-

*p<0.05

Table 33 Table showing inter-correlations between dimensions of infant temperament.

5.4.3.2 *Infant Temperament in Rural South India: Sample Characteristics*

The mean scores for each of the dimensions and composites of infant temperament are presented in Table 34. Of the six dimensions of infant temperament; activity level and DLSNS were distributed normally while the other four were not. Of the three composites; NRC and GRC were normally distributed.

Components of Infant Temperament (n=58)		**Mean (SD)**	**Range**
Dimensions	Activity Level	3.21 (0.64)	2.00 - 4.54
	Distress to Limitations	3.47 (0.60)	2.36 - 5.27
	DLASNS	2.55 (1.26)	1.00 - 6.00
	Duration of Orienting	2.72 (1.53)	1.00 - 6.00
	Smiling & Laughter	2.77 (0.92)	1.00 - 5.00
	Soothability	3.26 (0.58)	2.11 - 4.78
Composites	Negative Reactivity Composite	3.08 (0.50)	2.19 - 4.66
	Positive Reactivity Composite	2.93 (0.77)	1.62 - 4.85
	Global reactivity composite	0.16 (0.90)	-2.29 - 2.08

Table 34 Table showing sample characteristics of infant temperament.

5.4.3.3 *Prenatal Depression: Associations with Infant Temperament*

The findings of the association between prenatal depression and infant temperament are presented in Table 35.

Components of Infant Temperament		Temperament Scores Mean (SD)		Corr-elations with Prenatal EPDS scores	Inter-Group Differences	
		Infants of Controls (n=33)	Infants of High Scorers (n=25)		Primary Comparisons: Controls vs. High Scorers	Secondary Comparisons: Controls, High Scorers & Cases
Dimensions	**Activity Level**	3.19 (0.63)	3.24 (0.66)	r_p = 0.10 p = 0.46	t = -0.30 p = 0.77	F= 0.04 p = 0.96
	Distress to Limitations	3.45 (0.59)	3.48 (0.62)	r_s = -0.01 p = 0.95	U= 426.50 p = 0.77	F= 0.16 p = 0.85
	DLASNS	2.29 (1.25)	2.92 (1.21)	r_p = 0.15 p = 0.25	t = -1.90 p = 0.06	F= 1.84 p =0.17
	Duration of Orienting	2.88 (1.51)	2.50 (1.56)	r_s = -0.14 p = 0.29	U= 350.30 p = 0.35	F= 0.44 p = 0.65
	Smiling & Laughter	2.66 (0.92)	2.92 (0.90)	r_s = 0.03 p = 0.85	U= 480.50 p = 0.25	F= 0.55 p = 0.58
	Soothability	3.20 (0.56)	3.36 (0.60)	r_s = -0.02 p = 0.90	U= 468.50 p = 0.34	F= 0.55 p = 0.58
Composites	**NRC**	2.98 (0.48)	3.22 (0.50)	r_p = 0.17 p = 0.20	t = -1.82 p = 0.07	F= 1.63 p = 0.21
	PRC	2.91 (0.79)	2.93 (0.74)	r_s = -0.10 p = 0.49	U= 407.50 p = 0.99	F= 0.01 p = 0.99
	GRC	0.67 (0.89)	0.29 (0.93)	r_p = 0.15 p = 0.26	t = -0.93 p = 0.36	F = 0.42 p = 0.66

Table 35 Table showing associations between prenatal depression and infant temperament with mean scores in each group and inter-group differences.

Primary Comparisons: There were no differences in temperament scores between the infants of high scorers for symptoms of prenatal depression and controls. Similarly, there were no differences in negative, positive and global reactivity between the two groups.

Secondary Comparisons: There were no differences in temperament scores between the infants of controls, high scorers without MDD and cases. Similarly, there were no differences in the reactivity composites between these groups.

5.4.3.4 INTER QUINTILE COMPARISONS

Prenatal maternal EPDS scores were split into quintiles (Table 36) and mean infant NRC and PRC scores in each of these quintiles were plotted. The resultant curves obtained are depicted in Figures 35 and 36.

Quintile Number	EPDS Scores	% of Prenatal Sample	Prenatal n	% of Postnatal Sample	Postnatal n
1	0-1	21.8%	29	29.3%	17
2	2-3	37.6%	21	50.0%	12
3	4-9	59.4%	29	65.5%	9
4	10-15	82.0%	30	86.2%	12
5	16-27	100%	24	100.0%	8

Table 36 Table showing distribution of quintiles based on prenatal maternal EPDS scores.

While the relationship between infant negative reactivity (NRC) and prenatal depression was 'S' shaped, the association between infant positive reactivity (PRC) and prenatal depression was 'U' shaped.

Although curve estimation techniques found a quadratic model to fit these associations better than a linear model, none of these solutions reached significance (Table 37).

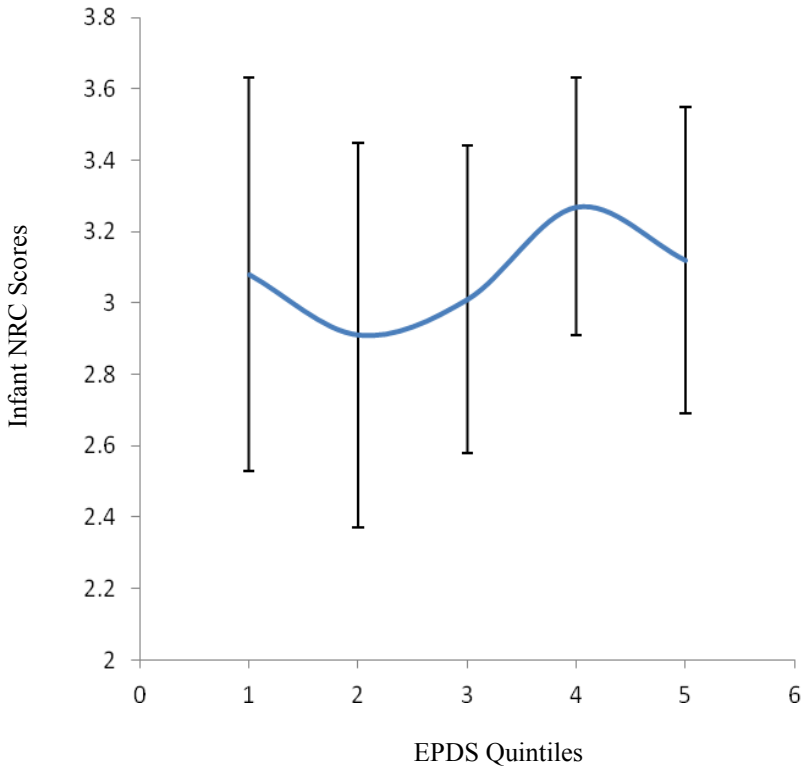

Fig. 35 Graph showing the relationship between infant negative reactivity (NRC) scores and prenatal maternal depression (EPDS) quintiles. Note: The black bars denote the standard deviation for each data point.

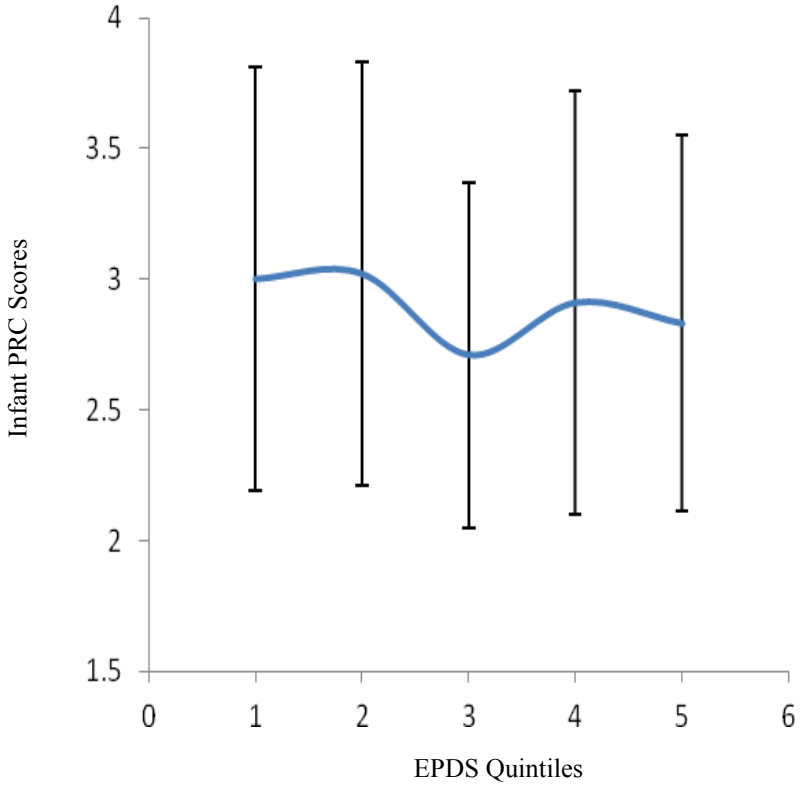

Fig. 36 Graph showing the relationship between infant positive reactivity (PRC) scores and prenatal maternal depression (EPDS) quintiles. Note: The black bars denote the standard deviation for each data point.

Component of Temperament	Nature of Curve	Linear Model		Quadratic Model	
		R^2	Sig.	R^2	Sig.
Negative Reactivity Composite	S shaped	0.27	0.33	0.42	0.59
Positive Reactivity Composite	U shaped	0.31	0.33	0.42	0.59

Table 37 Table showing curve estimation for components of temperament showing a U or S shaped association with prenatal depression.

5.4.3.5 *Association of Infant Temperament with Potential Confounders*

The results of the analysis examining the association of infant temperament with potential confounding variables are presented in Table 38.

Postnatal depression was not associated with infant temperament. Younger infants were overall more negatively reactive and had higher global reactivity scores as compared to older infants. Smiling and laughter and soothability were positively correlated to the infants' age with older infants having higher scores on these dimensions.

Breastfed infants with higher weight percentiles had higher positive reactivity scores. Breastfed infants and infants with higher weight percentiles were also more soothable than non-breastfed and underweight infants. Infants with health problems in the past two weeks (fever, diarrhoea and cough) had higher negative reactivity scores.

Construct	PND	Sex of infant	Inf-ant Age (r)	Birth Wt. (r)	Wt. perc-entile (r)	Breast feeding	Health status of infant
Dimensions of Infant Temperament							
Activity Level	t= 0.40	t= 0.31	0.21	0.01	0.08	t= 1.44	t= -0.93
Distress to Limitations	U=329.0	U=380.0	-1.32	0.15	-0.11	U=27.5	U=361.0
DLSNS	t= -1.08	t= 0.73	-0.07	-0.01	0.14	t= 1.25	t= -1.84
Duration of Orienting	U=393.5	U=381.5	0.04	0.13	**0.34****	U=9.00	U=397.5
Smiling & Laughter	U=372.0	U=384.0	**0.43***	0.11	0.22	U=8.5	U=384.0
Soothability	U=372.5	U=406.5	**0.27***	0.23	**0.29***	**U=0.00***	U=387.0
Infant Temperament Composites							
NRC	t= -1.19	t = 0.50	-0.02	0.04	0.09	t= 1.77	**t= -2.24***
PRC	U=402.5	U=384.0	0.25	0.21	**0.41****	**U=0.00***	U=410.5
GRC	t= -0.93	t= 0.68	**-0.30****	-0.03	-0.24	t= -0.49	t= -1.57

*p<0.05 **p<0.01 r = correlation coefficient

Table 38 Table showing associations between postnatal depression and potential confounders.

5.4.3.6 Supplementary Analysis: Associations between Intra-Uterine and Extra-Uterine Reactivity

The findings of the associations between intra-uterine (foetal) and extra-uterine (infant) reactivity are presented in Table 39. There were strong correlations between intra-uterine and extra-uterine physiological measures of offspring responsivity. Foetal cardiac responses to

vibroacoustic stimulation at 9 months of gestation were correlated with infant cortisol responsivity to immunisation at 1.5-3 months post-birth (r=0.36-0.40, p<0.05).

Infant psychological responsivity (NRC and PRC) did not correlate with physiological measures of offspring responsivity during intra-uterine and extra-uterine life. Importantly, infant temperament did not correlate with infant cortisol response to immunisation at 1.5-3 months post birth.

		Extra-uterine (infant) Responsivity					
		Physiological responsivity		**Psychological responsivity**			
		δ Cortisol		**NRC**		**PRC**	
		r	**p value**	**r**	**p value**	**r**	**p value**
Intra-uterine (foetal) responsivity	**i. Stim. FHR**	0.36*	0.02	-0.11	0.41	0.08	0.57
	ii. ∑AUC	0.37*	0.02	-0.12	0.36	0.04	0.79
	iii. δ FHR	0.40*	0.01	0.16	0.25	0.08	0.58
Extra-uterine (infant) physiological responsivity	**δ Cortisol**	-	-	-0.05	0.74	-0.06	0.71

Stim.FHR=Stimulation FHR *p<0.05 **p<0.01

Table 39 Correlations between intra-uterine and extra-uterine responsivity in offspring.

5.4.4 DISCUSSION

In this study, prenatal depression was not associated with maternal reports of difficult infant temperament. Interestingly, postnatal depression was also not associated with maternal reports of difficult infant temperament. Older age, weight percentile of the infant at assessment and

breastfeeding were associated with positive dimensions of infant temperament, while poor infant health was associated with negative infant reactivity.

Comparison with Previous Literature

The findings of this study are at variance with fourteen inquiries from the western world which report associations between prenatal depression and reports of difficult infant temperament (For further details on the 14 studies, see Table 4 pages 81-84). These studies have found prenatal depression to be associated with lower neonatal scores on the Brazelton scale (Lundy, Jones et al. 1999; Brouwers, van Baar et al. 2001; Field, Diego et al. 2001; Diego, Field et al. 2005) , negative infant reactivity (Huizink, Robles De Medina et al. 2002; Davis, Glynn et al. 2007), difficult infant temperament (McGrath, Records et al. 2008) and increased infant fussing (Van den Bergh 1990; Werner, Myers et al. 2007). However, the findings of two western reports vary from these inquiries and report no independent effect of prenatal depression on infant temperament (Austin, Hadzi-Pavlovic et al. 2005; Kaplan, Evans et al. 2008). In the only study from the developing world exploring the association between prenatal psychopathology and infant cognitive development, prenatal mental disorders were not found to predict Bayley scores in 12 month old Ethiopian infants (Servili, Medhin et al. 2010).

There are a number of reasons that may account for the variance in findings between this study and those of previous western inquiries. First, the sample size at follow-up was small and infant temperament data was available for only 58 infants. There was also some evidence of selective attrition in the sample. Second, infants were assessed at a very young age, i.e. 1.5-3 months. It is possible that, despite the established validity of the IBQ at 2 weeks and 2 months of life (Worobey and Blajda 1989), this is too early for the nuances of infant

behaviours assessed by the IBQ to become evident to the mother. In addition, a single postnatal assessment reduces the possibility of tapping into the narrow repertoire of behavioural patterns in very young infants. Third, this study was carried out in an entirely non-smoking, non-alcohol consuming prenatal population. It is possible that the association between prenatal depression and infant temperament reported in western literature is confounded by low to moderate levels of smoking and alcohol reported in 18-20% of the prenatal population in these settings (Brouwers, van Baar et al. 2001; Huizink, Robles De Medina et al. 2002; Werner, Myers et al. 2007). Fourth, despite rigorous efforts at translation, back-translation and piloting as per the WHO World Mental Health Initiative Interview Translation Guidelines (Harkness, Pennell et al. 2008) with strict emphasis on obtaining a conceptually accurate, culturally acceptable translation, it is possible that the use of a western measure of infant temperament such as the IBQ did not function appropriately in non-western settings. Difficulties were experienced in the comprehension of certain culture-specific items by rural women such as item 45, 'In the last two weeks, how often have you tried the following method to soothe your baby: offering baby his/her dummy or security object?'. Difficulty was also experienced with item 44 under soothing techniques, 'In the last two weeks, how often did you try to soothe your baby by offering him/her food or liquid?'. This is because breastfeeding as a method of soothing is culturally encouraged and widely practised among rural Indian women irrespective of postnatal mood, maternal nutrition and socio-cultural adversity. In addition, low levels of literacy in the study population necessitated an interviewer-administered design although the IBQ was designed to be self-reporting in nature. Further, it may be that a cultural bias against reporting behavioural problems in children, especially to persons in authority such as health personnel, may exist among rural Indian women. Finally, the possibility that indeed there is no association between prenatal

depression and infant temperament in these settings cannot be ignored and further research in developing world populations is needed to explore this issue.

Correlations between Intra-Uterine and Extra-Uterine Responsivity

The findings of a correlation in the physiological measures of prenatal and postnatal responsivity i.e. foetal cardiac and infant cortisol responsivity are in congruence with previous reports of associations between foetal and infant heart rates (DiPietro, Costigan et al. 2000), behaviour (Patrick, Campbell et al. 1982; Robertson, Dierker et al. 1982; Almli, Ball et al. 2001) and sleep (Kurjak, Stanojevic et al. 2004). These findings are plausible given that pre- and postnatal assessments may be considered prospective assessments of the same individual at different time-points and in different environments. Differences in continuity would not be expected unless the individual was subjected to a dramatic life-changing experience between these two points of time.

Infant temperament was however not found to be correlated with either infant cortisol responsivity or foetal cardiac responsivity. This is at variance with some previous work reporting associations between increased foetal movements and difficult infant behaviour (Madison, Madison et al. 1986; DiPietro and Bornstein 2002a). It is possible that the IBQ did not function adequately to assess infant temperament in this setting despite careful translation, back translation and piloting of the questionnaire.

Underlying Mechanisms

An overview of the possible mechanisms underlying the association between prenatal depression and infant temperament is presented on pages 75-77 of Section 2.4. Broadly, these include the possible programming influence of prenatal depression on the developing offspring's hypothalamic-pituitary-adrenal (HPA) axis, the influence of maternal depression

on postnatal caregiving and the contributory role of maternal smoking, alcohol consumption and genetics to the association.

Conclusion

Prenatal depression was not associated with maternal reports of difficult infant temperament in this sample of mothers and infants from rural south India. Nevertheless, this is the first study to explore the association between prenatal depression and infant temperament in the developing world and in a non-smoking, non-alcohol consuming prenatal population. This section highlights the need for future research to address infant temperament in the context of prenatal depression in the developing world. It also draws to attention the need to design robust measures of infant temperament for use in low-income settings.

Summary of Findings:

- Prenatal depression is not associated with maternal reports of difficult infant temperament in rural south India.
- There is evidence of a correlation between physiological measures of foetal and infant responsivity.

5.5 PRENATAL DEPRESSION: ASSOCIATIONS WITH OTHER INFANT OUTCOMES

5.5.1 INTRODUCTION

In this section, the findings of this thesis exploring the association with the following outcomes are presented:

i. Birth outcome assessed through birth weight and preterm births

ii. Puerperal Complications defined as:

   (a) Complications during labour: These are complications occurring during the first stage of labour before the delivery of the baby and include, but are not limited to, the failure of induction, malpresentations, foetal distress and oligohydramnios.

   (b) Complications during delivery: These are complications occurring during the second and third stage of labour during the delivery of the baby and the placenta. These include tears (vaginal, clitoral, perineal and rectal) and the presence of the umbilical cord around the baby's neck.

   (c) Immediate postnatal complications: These are complications developing within the first hour of delivery and include postpartum haemorrhage, adherent placenta and hematomas.

iii. Infant physical growth measured as infant weight, length and head circumference at the time of the postnatal assessment

iv. Infant health at the postnatal assessment

v. Breastfeeding status at the postnatal assessment

A summary of the previous literature exploring the association of prenatal depression with these outcomes is presented in Section 2.5 on page 85.

5.5.2 STATISTICAL ANALYSES

For all preterm births and puerperal complications the outcomes were dichotomised and compared between depressed mothers (high scorers) and non-depressed mothers using the Chi-Square Test. Prenatal EPDS and K10 scores were compared between these groups using the Mann-Whitney Test as the data was not normally distributed. Birth weight was compared between depressed and non-depressed groups of mothers using the independent sample t test and ANOVAs. Correlations between birth weight and prenatal EPDS and K10 scores are also reported.

Infant physical growth, infant health and breastfeeding was compared between the infants of controls and prenatally depressed mother (high scorers) using independent sample t tests, Mann Whitney tests and Chi-square tests as appropriate. The group of prenatal high scorers was then categorised as those diagnosed with a major depressive episode (MDD) during pregnancy (cases) and those without MDD (high scorers without MDD). These outcomes were compared between these 2 groups and the infants of controls using ANOVAs and Chi-square tests as appropriate.

A supplementary analysis was conducted to determine the association between infant growth, infant heath and breastfeeding, and postnatal depression (PND).

5.5.3 RESULTS

5.5.3.1 SAMPLE CHARACTERISTICS

Data on birth outcome was available for 107 of the 133 foetuses assessed prenatally (80.45%). Data was not available on 26 babies as their mothers did not return to the study location either for their deliveries or for follow-up post-birth. The mean birth weight for babies in the sample was 2840 grams (SD 400 grams). Sixteen babies (15%) were below the 2500 grams cut-off for low birth weight as defined on page 130, while 3 babies were born prematurely (2.8%). The sample characteristics for birth outcome and puerperal complications are presented in Table 40.

Obstetric variable	**n (total n = 107)**	**%**
Preterm births	3	2.8%
Low Birth Weight (≤2500 g)	16	15%
Type of delivery	Normal = 75	70.10 %
	Operational/Instrumental = 32	29.90%
Complications during labour	26	24.3%
Complications during delivery	17	15.9%
Postnatal complications	15	14.0%
Birth weight (grams)	Mean (SD) 2840 (40)	Range 1600-3900

Table 40 Sample characteristics for birth outcome & puerperal complications.

Data on infant physical growth, infant health and breastfeeding was available for 58 infants. The age at assessment of infants of mothers with high scores for prenatal depression did not

differ from the infants of controls (U = 369.00. p = 0.49). Infant age also did not differ between mothers who scored high for symptoms of postnatal depression and controls (U = 348.00, p = 0.29)

The sample characteristics of infant physical growth are presented in Table 41. The mean infant age at assessment was 57.7 days (SD: 23.2). The mean infant weight and length were 4.94 kgs (1.08) and 58.35 cms (4.96) respectively.

Infant Anthropometry Variables		**Total Sample (n=58)**	**Prenatal Control (n = 32)**	**Prenatal High Scorer (n=25)**
		Mean (SD)	**Mean (SD)**	**Mean (SD)**
Infant Weight	Weight	4.94 (1.08)	5.06 (0.89)	4.76 (1.30)
	Centile	38.21 (27.32)	40.28 (28.30)	33.76 (6.45)
Infant Length	Length	58.35 (4.96)	58.06 (3.57)	58.68 (6.45)
	Centile	50.78 (33.25)	44.09 (32.57)	57.84 (32.95)
Infant Head Circumference	Head Circumference	38.27 (2.07)	38.45 (1.76)	38.04 (2.42)
	Centile	29.21 (25.92)	27.69 (26.09)	31.16 (26.09)
Infant age at assessment (days)		57.7 (23.2)	59.8 (21.6)	55.687 (25.5)

Table 41 Sample characteristics for infant physical growth.

The sample characteristics of infant health are presented in Table 42. Twenty nine infants experienced illness: 16 (27.6%) had symptoms of cough, 5 (8.6%) had diarrhoea, 4 (6.9%)

had fever, 2 (3.4%) had vomiting and 2 (3.4%) cried excessively at the time of the postnatal assessment.

Infant Health Variables	Total Sample (n=58)	Prenatal Depression	
	n (%)	Control (n=33)	Prenatal High Scorer (n=25)
Sex of the infant	Female: 26 (44.8%)	15	11
	Male: 32 (55.2%)	18	14
Admitted to intensive care unit immediately post-birth	6 (10.3%)	2	4
Hospitalised post-neonatal period	1 (1.7%)	0	1
Ill-health in the period following birth until assessment	29 (50%)	16	13
Infant immunised for age	58 (100%)	33	25

Table 42 Sample characteristics for infant physical health.

The sample characteristics of the breastfeeding status of the infants are presented in table 43. The majority of the mothers in the postnatal sample (n=57; 98.3%) breastfed their infants with 51 out of 58 infants (87.9%) being exclusively breastfed. Infants were breastfed for an average of 9 times per day with each feed lasting a mean of 14.53 minutes (SD: 6.47). Five mothers (8.6%) experienced problems breastfeeding their infants. Of these, 4 complained of insufficient lactation and 1 had a breast abscess. Fourteen infants (24.1%) were reported as experiencing difficulty in being breastfed by their mothers. Mothers reported that this was because the infant vomited after breastfeeding (n = 7, 12.1%), the infant cried excessively during feeding (n = 6, 10.3%) and the infant stopped sucking very quickly (n = 1, 1.7%).

Variable	n (%)	n (% within breastfeeding variable)	
	Postnatal Sample	Prenatal Controls	Prenatal High Scorers
Breastfeeding Status			
Currently breastfed	57 (98.3%)	33 (57.9%)	24 (42.1%)
Exclusively breastfed	51 (87.9%)	31 (60.8%)	20 (39.2%)
Initiation of breastfeeding			
Initiation of breastfeeding following birth (hours)	Mean (SD): 5.30 (12.12)	Mean (SD): 3.95 (9.27)	Mean (SD): 7.09 (15.11)
Prelacteal feeds	6 (10.3%)	3 (50%)	3 (50%)
Type of breastfeeding:			
Demand:	40 (69%)	25 (62.5%)	15 (37.5%)
Timed:	5 (8.6%)	1 (20%)	4 (80%)
Mixed:	13 (22.4%)	7 (53.8%)	6 (46.2%)
Quantity of breastfeeding			
Times per day the infant is breastfed	Mean (SD): 9.48 (3.54)	Mean (SD): 9.55 (3.33)	Mean (SD): 9.40 (3.87)
Duration per feed (minutes)	Mean (SD): 14.53 (6.47)	Mean (SD): 14.24 (5.88)	Mean (SD): 14.92 (7.29)
Hours per day the infant is breastfed	Mean (SD): 2.24 (1.25)	Mean (SD): 2.19 (1.02)	Mean (SD): 2.30 (1.52)
Problems associated with breastfeeding			
Maternal problems with breastfeeding	5 (8.6%)	2 (40%)	3 (60%)
Infant problems in being breastfed	14 (24.1%)	6 (42.9%)	8 (57.1%)

Table 43 Sample characteristics for breastfeeding.

5.5.3.2 *INFANT OUTCOMES: ASSOCIATION WITH PRENATAL DEPRESSION*

Prenatal Depression: Associations with Birth Outcome

The results exploring the association between prenatal depression and birth outcome are presented in Appendix IVF. There were no differences in birth weight between the babies of high scorers for prenatal depression and controls (t=0.52, p=0.60). In addition, birth weight did not differ between the babies of controls, high scorers without MDD and cases (F=0.25, p=0.78).

Prenatal depression was associated with preterm deliveries (χ^2=11.93, p<0.01) with all preterm births (n=3) occurring in mothers with prenatal MDD. However this result must be interpreted with caution as only three preterm deliveries occurred in the sample.

Prenatal Depression: Associations with Puerperal Complications

The findings of the association between prenatal depression and puerperal complications are presented in Appendix IVG. Compared to controls, an increased number of puerperal complications were reported high scorers for prenatal depression (χ^2=5.48, p=0.02).

There was no association between prenatal depression and the type of delivery (χ^2=0.13, p=0.72).

Prenatal Depression: Associations with Infant Physical Growth

Appendix IVH presents the findings of the analysis exploring the relationship between prenatal depression and infant physical growth**.** There were no associations between prenatal depression and infant growth centiles standardised for age (For weight: U=343.00, p=0.28; for length: U=329.00, p=0.19; for head circumference: U=357.00, p=0.49).

There were no differences in growth centiles between the infants of controls, high scorers without MDD and cases of MDD (For weight: F=1.05, p=0.36; for length: F=1.03, p=0.37; for head circumference: F=0.39, p=0.68).

Prenatal Depression: Associations with Infant Health

The findings of the analysis exploring the association between prenatal depression and infant health are presented in Appendix IVI. Prenatal depression was not associated with infant ill-health (χ^2=0.07, p=0.79). There were no differences in health between the infants of controls, high scorers without MDD and cases (χ^2=0.12, p=0.94).

Prenatal Depression: Associations with Breastfeeding

Appendix IVJ presents the findings of the analysis exploring the relationship between prenatal depression and breastfeeding.

Prenatal depression was associated with increased maternal reports of the infant experiencing difficulty in being breastfed. This was significantly higher in mothers who suffered from clinical depression during pregnancy as compared to high-scoring mothers and controls (χ^2 = 12.85, p = 0.04). Six of eleven mothers who suffered from prenatal MDD (54.54%) reported breastfeeding difficulties in their infants while only two of fourteen high scorers without MDD (14.29%) and six of thirty-three controls (18.18%) reported difficulties in breastfeeding their infants.

Prenatal depression was not associated with the nature, duration or the frequency of breastfeeding or with the mother experiencing problems in breastfeeding.

5.5.3.3 SUPPLEMENTARY ANALYSIS: ASSOCIATION OF POSTNATAL DEPRESSION WITH INFANT GROWTH, HEALTH AND BREASTFEEDING

The findings of the analysis exploring the relationship between postnatal depression and infant growth, health and breastfeeding are presented in Appendix IVK-M.

PND was not associated with infant weight, length and head circumference at the time of postnatal assessment. In addition, there was no association between PND and current infant health problems ($\chi 2=1.83$, $p=0.40$).

PND was associated with the number of times and the total duration per day that the infant was breastfed. Cases of PND (with postnatal MDD) breastfed their infants more (Mean: 12.67 times/day SD: 5.35 and Mean: 3.97 hours/day SD: 2.54) compared to high scorers for PND (without postnatal MDD) (Mean: 8.65 times/day SD: 2.70 and Mean: 1.96 hours/day SD: 0.68) and mothers without PND (Mean: 9.41 times/day SD:3.40 and mean: 2.09 hours/day SD:1.03). PND was not associated with the mother and infant experiencing problems during breastfeeding.

A regression analysis was conducted to explore whether prenatal depression predicted breastfeeding difficulties independent of infant age, sex, health and PND. The findings of this analysis are presented in Appendix IVN. Prenatal depression did not predict the frequency or duration of breastfeeding and difficulties with breastfeeding after controlling for these confounders. PND however predicted the duration of breastfeeding and maternal reports of difficulty with breastfeeding independent of prenatal depression, infant age, sex and health.

5.5.4 DISCUSSION

In this study, prenatal depression was associated with an increased report of puerperal complications, but not with birth weight. Although prenatal depression was found to be associated with preterm deliveries, this finding must be interpreted with caution as the number of preterm deliveries was small (n=3). Prenatal depression was not associated with infant physical growth and infant health. It was associated with increased reports of breastfeeding difficulties but this association disappeared after controlling for PND, infant age, sex and health.

Comparison with Previous Literature

The findings of an association between preterm deliveries and prenatal depression are consistent with previous western reports. Previous studies carried out in the western world have reported associations between prenatal depression and preterm births (Wadhwa, Culhane et al. 2001b). Steer and colleagues have reported a 3.39 increase in the likelihood of preterm delivery in prenatally depressed women (95% CI: 3.24 to 3.56) (Steer, Scholl et al. 1992). Wadhwa et al reported a decrease of 3 days in gestational age at birth for every unit increase in prenatal anxiety (Wadhwa, Sandman et al. 1993). Prenatal depression was found to be associated with prematurity in a sample of African-American women (adjusted OR 1.96, 95% CI; 1.04 to 3.72) (Orr, James et al. 2002) and Brazilian women (RR = 2.32, p = 0.01) (Rondó, Ferreira et al. 2003).

The finding of an absence of association between birth weight and prenatal depression are at variance with some reports from the developing world. In a study in Pakistan, babies of mothers with prenatal depression in the third trimester of pregnancy were 2 times more likely to have low birth weight (95% CI: 1.1 to 3.3) than the babies of controls (Rahman, Iqbal et al.

2004). Similar associations have been reported from Goa, India (Patel and Prince 2006) and in western literature (Wadhwa, Sandman et al. 1993; Sandman, Wadhwa et al. 1997; Hoffman and Hatch 2000). Nevertheless, not all previous reports are consistent with this and Andersson and colleagues report no associations between prenatal depression and low birth weight in Swedish mothers (OR 1.41, 95% CI: 0.58 to 3.49)(Andersson, Sundstram-Poroma et al. 2004). Further investigation into the association between prenatal depression and birth weight in the developing world is necessary before this relationship can be clearly understood in these settings. It is possible that because all the mothers in this sample were relatively well nourished, birth weight was not associated with prenatal depression.

In this study, there were no associations between prenatal depression and infant physical growth. This is at variance with some reports from India, Pakistan, Vietnam, Brazil and Nigeria where poorer growth has been reported in the infants of depressed mothers compared to controls (Black, Baqui et al. 2009). In the only study from India, compared to controls the infants of depressed mothers were found to be underweight (RR 2.3, 95% CI: 1.1-4.7) and stunted (RR 2.9, 95% CI: 1.3-6.8) at 6 months of age (Patel, DeSouza et al. 2003). However, not all previous work is consistent with this and no association between maternal depression and infant growth has been reported from South Africa, Peru and Ethiopia (Harpham, Huttly et al. 2005; Tomlinson, Cooper et al. 2006). In addition, there were no associations between pre- or postnatal depression, and infant health, in this study. These findings differ from previous reports of higher rates of hospital admissions and illness (Patel, Rodrigues et al. 2002; Rahman, Iqbal et al. 2004) in the infants of depressed mothers compared to controls. Although further research in low-income settings is required to explain these findings, it is possible that because 57 of the 58 infants were breastfed, it was unlikely that they would suffer from undernutrition. This is because breast milk contributes to all the calorific and

protein requirements of infants up to 6 months of age. Further, it is possible that as mothers who returned for follow-up were motivated to promote the health of their children; infant health did not differ between the groups of prenatal high scorers and controls.

In this sample, postnatal maternal depression, and not prenatal depression, predicted breastfeeding. This is consistent with previous breastfeeding literature reporting an association between PND and increased difficulty in the infant being breastfed (Patel, Rodrigues et al. 2002). Results from studies conducted in Finland (Tamminen 1988) and Barbados (Galler, Harrison et al. 2006) have shown that mothers with depressive symptoms have more negative attitudes towards breastfeeding and are less likely to breastfeed their infants than controls. It is possible that the efficacy of breastfeeding is influenced more by current (postnatal) maternal mood rather than by past (prenatal) mood. Interestingly, in this study mothers with PND breastfed their infants for a longer duration per day than mothers without PND. Although this finding may appear counterintuitive, it is possible that the infants of depressed mothers are more difficult and cry more than the infants of non-depressed mothers. In this case, breastfeeding may be employed to soothe the infant. In addition, postnatally depressed mothers reported their infants as having increased difficulty in being breastfed. Thus it may be that the findings reflect a compensatory increase in breastfeeding in postnatally depressed women.

Conclusions

Prenatal depression was associated with preterm deliveries but was not associated with birth weight, infant physical growth, infant health and breastfeeding difficulties. Further, the finding of an association between prenatal depression and preterm delivery should be interpreted with caution as the number of preterm deliveries in the sample was small (n=3).

These results are at variance from some previous literature from the developing and western world. Nevertheless, this is the first inquiry into the relationship between prenatal depression and infant growth, infant health and breastfeeding from the developing world. Further research into this association in low-income countries is necessary, before the relationship between prenatal depression and infant growth and development in these settings can be clearly understood.

Summary of Findings:

- Prenatal depression is associated with a higher incidence of preterm births, however the number of preterm deliveries in the sample was small (n=3).
- Prenatal depression is not associated with birth weight, infant physical growth.

CHAPTER 6
DISCUSSION

6 DISCUSSION

This thesis set out to test three specific hypotheses, in a rural sample in South India, namely that prenatal maternal depression is associated with:

i. Elevated foetal stress responsivity to vibroacoustic stimulation measured through foetal cardiac responses,
ii. Elevated infant cortisol responsivity to immunisation and,
iii. Difficult infant temperament.

The three key findings from this thesis are:

i. There is an association between prenatal depression and foetal stress responsivity and it is curvilinear (U shaped),
ii. Prenatal depression predicts infant cortisol responsivity independent of postnatal depression (PND), the association between the two also being curvilinear and,
iii. There was no association between prenatal maternal depression and infant temperament in this sample from rural south India.

Taken together, these findings suggest that the potential influence of prenatal depression on offspring development begins very early, even before birth, with correlations in the patterns of reactivity to stressors between intra-uterine and extra-uterine life.

In this section, the findings of this thesis are discussed in the context of pre-existing literature and the underlying mechanisms governing the association of prenatal depression with foetal

and infant development. The detailed discussion pertaining to each specific finding is presented at the end of each corresponding sub-section of the results chapter.

6.1 SUMMARY OF FINDINGS

The overall prevalence of major depressive disorder (MDD) during pregnancy in this sample of mothers from rural South India was 15% (29 out of 194 participants). Thirty five percent (67 out of 194) of the participants had elevated symptoms of prenatal depression.

Primary Hypotheses: Findings

i. There were no linear associations between prenatal depression and foetal responsivity to stress, including foetal habituation to repeated vibroacoustic stimulation. A curvilinear (U shaped) association however existed between prenatal maternal depression and foetal stress responsivity with foetuses of mothers with very high and very low levels of prenatal depression showing elevated responses to vibroacoustic stimulation compared to the foetuses of mothers with moderate levels of prenatal depression.

ii. Prenatal depression predicted infant cortisol response to immunisation independent of PND, postnatal maternal cortisol and infant sex. Compared to the infants of non-depressed mothers, the infants of high scorers for prenatal depression showed elevated cortisol responses to immunisation at 1.5-3 months after birth. A curvilinear (U shaped) association existed between prenatal maternal depression and infant cortisol response to immunisation with the infants of mothers with very high and very low

levels of prenatal depression showing elevated cortisol responses to immunisation compared to the infants of mothers with moderate levels of prenatal depression.

iii. Prenatal maternal depression was not associated with maternal reports of difficult infant temperament. PND was also not associated with infant temperament in this sample.

Further, prenatal (foetal) stress responsivity was correlated with postnatal (infant) stress responsivity. However, neither foetal nor infant stress responsivity was associated with maternal reports of infant temperament.

Secondary Hypotheses: Findings

Prenatal maternal depression was associated with a higher incidence of preterm deliveries but was not associated with birth weight. Prenatal depression was not associated with infant weight, length and physical health at 1.5-3 months post-birth. Prenatal depression was not associated with the mother's ability to breastfeed her baby although PND was found to predict the duration of breastfeeding and infant difficulty in being breastfed. This association remained significant after controlling for confounding influences.

6.2 COMPARISON OF FINDINGS WITH PREVIOUS LITERATURE

Prevalence of Prenatal Depression

The prevalence rate of a diagnosis of prenatal MDD in this sample was 14.9% while 34.5% of participants had elevated symptoms of prenatal depression. This is consistent with previous reports from the Indian sub-continent. A study from the south Indian state of Tamil Nadu

reported a prevalence of prenatal MDD of 16.2% (Chandran, Tharyan et al. 2002). An inquiry from Pakistan reported 25% of rural women to have met ICD-10 criteria for clinical prenatal depression (Rahman 2007). Inquiries from Goa, India; Pakistan and Bangladesh, have reported prevalence rates of prenatal depressive symptomatology of 42.1%, 42.7% and 33% respectively (Patel, Rodrigues et al. 2002; Gausia, Fisher et al. 2009; Imran and Haider 2010).

Importantly, the prevalence of prenatal depression in this study, and in other studies from the Indian sub-continent, are higher than estimates from the western world [7.7% and 21.7% for prenatal MDD and symptoms of prenatal depression respectively in western populations (Bennett, Einarson et al. 2004)].

Prenatal Depression: Associations with Foetal Responsivity

I. Linear Associations between Prenatal Depression and Foetal Responsivity

There were no linear associations between prenatal depression and foetal responsivity to stress detected in this sample. This is at variance from some western reports where compared to control foetuses, the foetuses of mothers with prenatal depression and anxiety have been found to display increases in foetal heart rate (FHR) following maternal cognitive stimulation (Monk, Fifer et al. 2000; DiPietro, Costigan et al. 2003; Monk, Myers et al. 2003). Prenatal depression has also been reported to be associated with delays in the return of FHR to baseline following vibroacoustic stimulation in one study (Allister 2001).

II. Prenatal Depression and Foetal Habituation

In this study there was no association between prenatal depression and delayed foetal habituation. Two western studies have however reported delayed foetal habituation to vibroacoustic stimulation to be associated with elevated neuro-endocrinological markers of

prenatal psychopathology in mothers (Sandman, Wadhwa et al. 1999; Sandman, Glynn et al. 2003). However, not all western literature is consistent with this and positive associations between pregnancy specific stress and increased foetal neurological maturation have also been reported (DiPietro, Kivlighan et al. 2010).

III. Prenatal Depression and Foetal Responsivity: U Shaped Association

The finding of a curvilinear (U shaped) association between prenatal depression and foetal stress responsivity is relatively novel. Importantly, this pattern of association was not observed at baseline and only emerged upon the presentation of a stressful stimulus.

Similar U shaped associations between stress and responsivity have been reported in other biological systems. In a study of 190 nine year olds from Salisbury, UK, the relationship between urinary excretion of total glucocorticoid metabolites and birth weight was U-shaped with the highest mean excretion being reported in children with low and heavy birth weights (Clark, Hindmarsh et al. 1996). A similar association between birth weight and type II diabetes was reported from a sample of 1179 Pima Indians (McCance, Pettitt et al. 1994). Sandman and colleagues have reported evidence of a complex, non-linear (cubic) association between maternal neuroendocrinological markers of prenatal psychopathology (ACTH, BE and CRH) and FHR responses to vibroacoustic stimulation (Sandman, Wadhwa et al. 1999; Sandman, Glynn et al. 2003). DiPietro and colleagues report evidence linking moderate levels of prenatal stress with accelerated neurological development in foetuses (DiPietro, Kivlighan et al. 2010). Results from a previous inquiry by the same authors have reported increased cognitive performance in the infants of mothers with high levels of prenatal anxiety and depression compared to the infants of non-depressed mothers at 2 years of age (DiPietro, Novak et al. 2006).

Belsky and Pleuss suggest that high reactivity profiles of child development emerge in both extremely stressful and extremely supportive postnatal environments and are indicative of a U shaped relationship between environmental stress and child functioning in the postnatal period (Belsky and Pluess 2009). A U shaped association between psychosocial stress and productivity has also been reported during adult life (Wilke, Gmelch et al. 1985). Evidence from rodent literature has established U shaped associations between stress and optimal functioning in response to challenging situations (Yerkes and Dodson 1908) and between prenatal maternal nutrition and, foetal growth and utero-placental blood flow (Wu, Bazer et al. 2004).

In the context of this thesis, there are several reasons to explain why no linear associations were detected between prenatal depression and foetal stress responsivity. First, a vibroacoustic stimulus was used as a potential stressor to elicit foetal responses. Previous studies have utilised maternal cognitive stimulation through the Stroop task as a means to elicit foetal responses (Monk, Fifer et al. 2000; DiPietro, Costigan et al. 2003; Monk, Myers et al. 2003). As these studies have relied on maternal cognition to stimulate the foetus, it is possible that the findings have been influenced by maternal responses to the task in the context of maternal mood. Only one study has utilised vibroacoustic stimulation and reported elevations in FHR at baseline, during stimulation and post-stimulation in the foetuses of prenatally depressed mothers compared to controls (Allister 2001). Further, in this thesis, 35 of the 131 foetuses (26.72%) exposed to vibroacoustic stimulation did not show a response sufficient enough to be classified as a FHR acceleration. Thus, habituation could not be ascertained in these foetuses. While the reason for this insufficient response could not be determined, it is possible that the strength of the VAS was not sufficient to elicit a response in some cases. Nevertheless, the findings of a curvilinear association between prenatal

depression and foetal stress responsivity remained the same whether or not this group of foetuses was included in the analysis. Second, none of the women smoked or consumed alcohol during pregnancy in this study. It is possible that the findings from western populations have been confounded by the teratogenic effects of prenatal smoking and alcohol consumption on foetal neurodevelopment (Little and Streissguth 1981; Stein and Kline 1983; Jacobson, Jacobson et al. 1994). Finally, this study was carried out in a prenatal population from the rural, developing world. It is possible that the patterns of association between prenatal depression and foetal responsivity in this setting may be different from those observed in western populations.

The findings of a curvilinear relationship between prenatal maternal depression and foetal responsivity to stress suggest that very high and very low levels of prenatal stress may be maladaptive to offspring development whereas exposure to moderate amounts of prenatal stress may facilitate offspring development (DiPietro, Novak et al. 2006). It could be hypothesised that exposure to a moderately stressful intra-uterine environment, as seen in mothers with moderate levels of prenatal depression, could result in an optimal matching of the intra- and extra-uterine environment and thus confer the offspring with a developmental advantage from the evolutionary perspective (Glover, 2011).

The elevated stress responses observed in foetuses exposed to high levels of maternal depression could be attributed to the programming effects of prenatal depression on the foetal HPA axis. The elevated stress responses observed in the foetuses of mothers with very low levels of prenatal depression could represent the unfamiliarity of this group to moderate levels of intra-uterine stimulation. It is possible that the higher stress responses in this group were because the foetuses were experiencing the effects of a stressor out of the normal range that

were potentially accustomed to. This finding suggests that exposure to small to moderate amounts of stimulation in-utero is necessary for the development of the foetal stress response mechanism. Thus, the findings of such U shaped associations may represent genetically derived responsivity patterns that aid survival and reflect an optimisation in the nature-nurture interface (Calabrese and Baldwin 2001). Importantly, the findings in this thesis are not confounded by the teratogenic effects of maternal smoking, alcohol consumption, recreational drug use or psychotropic medication during pregnancy.

The U shaped association between prenatal depression and foetal stress responsivity reported in this study will need replication in larger samples before it can be further understood. The findings are suggestive of a programming effect of maternal depression on offspring stress responsivity even before birth and despite employing indirect measures of foetal responsivity. Further, it is possible that this curvilinear association is observed not only in the context of the programming influence of prenatal depression but, as Calabrese and Baldwin suggest, the U shaped association may be woven into the fabric of all biological processes (Calabrese and Baldwin 2001).

Prenatal Depression: Associations with Infant Cortisol Response to Immunisation

The findings of elevated cortisol responses to stress (immunisation) in the infants of prenatal high scorers compared to the infants of prenatal controls are consistent with previous reports from the western world. Prenatal maternal depression has been reported to predict infant cortisol reactivity to stressful tasks at 6 months (deBruijn, Bakel et al. 2009) and 5 years of age (Gutteling, Weerth et al. 2005). This effect has been independent of the influence of postnatal maternal mood. Similar findings of an association between prenatal maternal stress and elevated ACTH responses to stressors in offspring have also been reported in animal

literature (Takahashi, Kalin et al. 1988; Takahashi and Kalin 1991; Takahashi, Haglin et al. 1992). However, a study of preterm infants found intra-uterine exposure to exogenous glucocorticoids to be associated with a dampened response to immunisation at 4 and 12 months post-birth (Glover, Miles et al. 2005). This is in contrast to the finding of elevated cortisol responses to immunisation in the infants of high scorers for prenatal depression reported in this thesis and further research is needed to understand this discrepancy.

The finding of a curvilinear (U shaped) association between prenatal maternal depression and infant cortisol response to immunisation is a novel one and no similar associations have been reported in pre-existing animal and human literature. However, evidence linking moderate levels of prenatal depression to accelerated foetal neurodevelopment and increased infant performance during cognitive tasks does exist (DiPietro, Novak et al. 2006; DiPietro, Kivlighan et al. 2010). Similar reports of a U shaped relationship have been reported in other biological processes as described above.

Despite being intriguing, the findings of a U shaped association between prenatal depression and infant cortisol responsivity to immunisation need to be interpreted with caution given the relatively small size of the postnatal sample. Further investigation in larger samples and different population groups is needed before this pattern of association can be clearly understood. Nevertheless this study does provide general support for a relationship between prenatal maternal depression and infant cortisol responsivity to stress. The findings add to the existing body of evidence suggesting the possibility of a programming effect of prenatal maternal depression on the offspring's HPA axis. Importantly, these findings are independent of the effects of maternal smoking, alcohol consumption and drug abuse on the development of the foetus.

Prenatal Depression: Association with Infant Temperament

There were no associations between prenatal depression and any of the six dimensions or three composites of infant temperament in this study. In addition, PND was also not found to be associated with infant temperament in this sample.

These findings are at variance with a number of studies from the western world which report associations between prenatal maternal depression and maternal reports of difficult infant temperament. Prenatal maternal depression has been associated with lower neonatal scores on habituation, orientation, motor and autonomic stability clusters and higher withdrawal and depression scores on the Brazelton Scale (Field, Diego et al. 2001; Field, Diego et al. 2004). Compared to the infants of non-depressed mothers, the infants of prenatally depressed mothers have been reported to have elevated negative reactivity and a more difficult temperament (Davis, Glynn et al. 2007; McGrath, Records et al. 2008). A few studies have however attributed the effect of prenatal mood on infant temperament solely to prenatal anxiety and not to prenatal depression (Van den Bergh 1990; Huizink, Robles De Medina et al. 2002; Austin, Hadzi-Pavlovic et al. 2005). In addition, two studies of 4-6 month old infants from the USA and Australia found no independent effect of prenatal depression on infant temperament (Austin, Hadzi-Pavlovic et al. 2005; Kaplan, Evans et al. 2008). Further details of the previous reports on the association between prenatal maternal depression and infant temperament are presented in Table 4 on pages 80-84 of this thesis.

There are several possible reasons which may account for the variance in the findings of this thesis with previous reports from high-income countries. First, data on infant temperament was available for only a relatively small proportion of the prenatal sample (58 out of 133).

Further, there was some evidence of selective attrition in the sample with 62.7% of prenatally depressed mothers (n = 42) not returning for follow up compared to 50% of control mothers (n = 33). Second, infants were assessed at a young age (1.5-3 months) and it is possible that an assessment of infant temperament at less than 3 months post birth is too early for the nuances of infant behaviours assessed through maternal reports to become evident. Third, the study sample was specific to rural, south India and high risk behaviours seen in prenatally depressed mothers from the western world, such as increased levels of smoking and alcohol consumption, were not reported in this sample. Fourth, despite the fact that careful translation, back translation and piloting of the temperament questionnaire was carried out as per the WHO World Mental Health Initiative Interview Translation Guidelines (Harkness, Pennell et al. 2008), it is possible that the use of a western questionnaire based on maternal report did not function adequately in a rural developing world setting. Finally, the possibility that there is indeed no association between prenatal depression and infant temperament in this population cannot be ignored. Nevertheless, this is the first study to investigate the association between prenatal maternal depression and infant temperament in the developing world. More studies investigating this issue in low and middle income countries (LAMICs) need to be undertaken. Further, the development of measures to assess infant temperament in these settings also needs to be addressed.

Continuity in Stress Responsivity from Intra-Uterine to Extra-Uterine Life

The findings of an association between foetal cardiac responsivity and infant cortisol responsivity to stress are consistent with previous reports of congruence between measures of foetal and infant physiological activity. Consistencies in heart rate patterns, behavioural state

organisation and patterns of sleep have been reported from intra-uterine to extra-uterine life (Groome, Singh et al. 1997a; DiPietro, Costigan et al. 2000; Kurjak, Stanojevic et al. 2004). No associations between both foetal cardiac or infant cortisol responsivity, and infant temperament were found in this sample. This is in contrast with the findings of DiPietro and colleagues who report an association between foetal behaviour and increased infant irritability (DiPietro and Bornstein 2002a; DiPietro, Ghera et al. 2008). Evidence linking infant temperament with levels of infant cortisol is conflicting and while some studies suggest a positive association between infant behaviour and cortisol responsivity to stress (Lewis and Thomas 1990), others report negative or absent associations (Gunnar, Connors et al. 1988; Ramsay and Lewis 2003).

There are three possible reasons to explain the findings of the absence of an association between physiological and temperament measures of offspring reactivity in this study. First, the reliability of a western measure in assessing infant temperament in a different language and a culturally different setting is questionable. Second, infants were assessed at a young age and it is possible that a questionnaire based on maternal report is unable to tap into the nuances of infant behaviour displayed at this age. Further, it is possible that a single postnatal assessment is not adequate to assess infant temperament at this early age. Finally, a social bias against acknowledging difficult behaviours in their children may exist among rural Indian mothers.

The findings of the study nevertheless provide evidence of continuity in physiological measures of stress responsivity from intra-uterine to extra-uterine life. This suggests that the programming influence of prenatal maternal depression on offspring stress responses begins before birth and continues from foetal to postnatal life.

Prenatal Depression: Associations with Other Infant Outcomes

The finding of an association between prenatal depression and preterm deliveries in this study is consistent with previous reports from the western and developing world (Wadhwa, Sandman et al. 1993; Wadhwa, Culhane et al. 2001b; Orr, James et al. 2002; Rondó, Ferreira et al. 2003). Nevertheless, as the number of preterm deliveries occurring in this sample was small (n=3), this findings must be interpreted with caution. However, prenatal depression was not associated with birth weight in this sample. This is inconsistent with previous reports from the Indian subcontinent of lower birth weights in the neonates of prenatally depressed mothers compared to non-depressed mothers (Rahman, Iqbal et al. 2004; Patel and Prince 2006).

In this thesis, prenatal depression did not predict infant physical growth, infant health and breastfeeding. This is at variance with previous inquiries from India, Pakistan, Vietnam, Brazil and Nigeria which report higher levels of undernutrition, stunting, hospital admissions and diarrhoea in the children of depressed mothers compared to controls (Black, Baqui et al. 2009; Ross, Hanlon et al. 2011).

There have been no previous reports examining the association between prenatal depression and breastfeeding. PND has however been associated with an early cessation of breastfeeding (Cooper, Murray et al. 1993; Henderson, Evans et al. 2003), increased difficulty in breastfeeding (Patel, Rodrigues et al. 2002) and more negative attitudes towards breastfeeding (Tamminen 1988; Galler, Harrison et al. 2006). In this PND was associated with increased duration of breastfeeding and increased maternal reports of infant difficulty in being breastfed.

The discrepancy between the findings of this thesis and pre-existing literature may be due to the small size of the postnatal sample. Further, there may have been some selective attrition

suggesting that mothers who returned for follow-up were likely to be highly motivated to promote the health of their infants.

6.3 PRENATAL DEPRESSION AND OFFSPRING DEVELOPMENT: ASSOCIATION OR CAUSATION?

This thesis provides evidence linking prenatal depression to aberrations in foetal and infant stress responsivity. The question as to whether this relationship implies causation or merely reflects an association between prenatal depression and offspring development is addressed based on the nine causal criteria put forth by Sir Bradford Hill (Hill 1965). An analysis of the findings of this thesis according to these criteria is presented in Table 44.

There appears to be sufficient evidence from this thesis and pre-existing literature to support the possibility of a causal relationship between prenatal depression and elevated cortisol responses to stress in infants. The relationship between prenatal depression and foetal responsivity may be regarded as an association and further investigation is needed to support causation in this context. There was no association between prenatal depression and infant temperament found in this thesis.

Bradford-Hill Criteria	Concordance of the Findings of this Thesis with these Criteria		
	Foetal Responsivity	**Infant Cortisol Responsivity**	**Infant Temperament**
Strength of the association	+/-	+	-
Consistency of findings	+/-	+	-
Specificity of findings	Needs further investigation	Needs further investigation	Needs further investigation
Temporality of findings	Suggestive, needs further investigation	+	-
Evidence of a biological gradient	+	+	-
Coherence of findings	+	+	-
Experiment	Needs further investigation	Needs further investigation	Needs further investigation
Consideration of alternative explanations	Needs further investigation	Needs further investigation	Needs further investigation
Plausibility	+	+	+

+ = Yes, - = No, +/- = uncertain

Table 44 Consideration of the findings of the 3 key hypotheses of this thesis in the context of the Bradford-Hill Criteria.

6.4 UNDERLYING MECHANISMS

The potential mechanisms underlying the relationship between foetal and infant stress responsivity, and prenatal maternal depression, reported in this thesis are described below. The specific mechanisms concerning each individual hypothesis is discussed at the end of the respective sub-sections of Chapter 5.

Broadly speaking, the associations between prenatal depression and offspring stress responses demonstrated in this study may be attributed to the programming influences prenatal maternal depression on the offspring's HPA axis. However, it is possible that other mechanisms may also account for or contribute to these effects. A broad overview of these mechanisms is presented below. This is followed by an explanation of the findings of this thesis in the context of these mechanisms.

Mechanism 1: The Programming Influence of Prenatal Depression on the Foetal HPA Axis

One of the key neuronal substrates for the programming effects of prenatal depression is the foetal HPA axis. This is because the developing HPA axis is sensitive to changes in the intra-uterine environment, particularly the levels of cortisol in the amniotic fluid and in foetal circulation.

High levels of maternal cortisol are observed in prenatally depressed mothers above the physiological increase in maternal cortisol found in normal states of pregnancy. This, coupled with a down regulation of the placental barrier enzyme, 11 beta hyrdoxy steroid dehydrogenase 2 (11BHSD2), in prenatally depressed mothers, increases placental permeability to cortisol (Mairesse, Lesage et al. 2007; Glover, Bergman et al. 2009). The amount of cortisol in the intra-uterine environment and in foetal circulation is higher in the foetuses of depressed and anxious mothers compared to controls (Glover, Bergman et al. 2009). Sandman and colleagues argue that elevated levels of cortisol potentially exert programming influences on areas in the foetal brain with high concentrations of glucocorticoid receptors i.e. the foetal hippocampus, HPA axis and parahippocampal regions (Sandman, Wadhwa et al. 1999). As these structures are concerned with the stress response,

memory, learning and emotional processing, it is possible that the programming influence of prenatal depression and elevated maternal cortisol on these structures may account for the disturbances in related offspring outcomes observed in prenatally depressed mothers.

Mechanism 2: Transplacental Transport of Maternal Glucocorticoids and the Role of the Placenta

The placenta and the barrier enzyme, 11BHSD2, regulate foetal exposure to maternal cortisol by inactivating the larger proportion (80%) of maternal cortisol attempting to cross the placenta (O'Donnell, O'Connor et al. 2009). Thus, while maternal cortisol may contribute to approximately 40% of the variance in foetal cortisol concentrations (Gitau, Cameron et al. 1998), only about 20% of maternal cortisol reaches the foetus. Compared to non-depressed mothers, a down-regulation in the expression and activity of 11BSHD2 is reported in mothers with prenatal depression. In addition, placental CRH causes the placenta to secrete cortisol into the intra-uterine milieu during pregnancy (Glover, Bergman et al. 2009). These biological processes could explain the increased foetal exposure to cortisol in the foetuses of depressed mothers, potentially increasing the risk of their vulnerability to the programming influences of prenatal depression.

Mechanism 3: Activation of the Maternal Sympathetic Nervous System in Response to Prenatal Depression

Prenatal depression induces activation of the maternal sympathetic nervous system resulting in the release of catecholamines. Although the placenta is impermeable to adrenaline and noradrenaline, their vasoconstrictive effects on the maternal cardiovascular system may indirectly influence the foetus (O'Donnell, O'Connor et al. 2009).

Uterine artery vasoconstriction induced by the activation of the maternal sympathetic nervous system in response to prenatal maternal depression results in a decrease in utero-placental blood flow (Copper, Goldenberg et al. 1996). This limits the supply of oxygen and nutrients to the foetus. The compromise in foetal nutrition and oxygenation could be responsible for the increased risk of adverse neurodevelopmental outcomes reported in the offspring of prenatally depressed mothers compared to controls.

Mechanism 4: The Effect of Prenatal Depression on Postnatal Caregiving

It is already well established that PND is associated with problems in child development, poor mother-child interactions and less sensitive parenting (Murray and Cooper 1997; Beck 1998). In approximately two-thirds of women, prenatal depression continues into the postnatal period (Chandran, Tharyan et al. 2002; Patel, Rodrigues et al. 2002; Rahman, Iqbal et al. 2003). It is therefore possible that the influence of prenatal depression on child development may simply be because of its continuation into the postnatal period as PND.

Mechanism 5: High Risk Behaviours in Prenatally Depressed Mothers

Compared to controls, higher rates of smoking, alcohol consumption, recreational drug use and the consumption of psychotropic medication are reported in prenatally depressed mothers (Wolkind 1981a; Brouwers, van Baar et al. 2001). Further, prenatally depressed mothers show decreased health seeking behaviours and poorer levels of nutrition than non-depressed mothers (Wolkind 1981b). A low protein diet has been found to decrease the expression and the activity of the placental enzyme 11BHSD2 (Bertram, Trowern et al. 2001).

The extent to which high risk behaviour profiles in prenatally depressed mothers contribute to the adverse neurodevelopmental outcomes observed in offspring is not known. This is of particular importance in the western world. In two independent studies from the Netherlands,

12-17% and 15-18% of mothers were reported to have smoked and consumed alcohol respectively during pregnancy (Brouwers, van Baar et al. 2001; Huizink, Robles De Medina et al. 2002).

Mechanism 6: Heritability of HPA Axis Function

Twin studies have provided evidence in support of the influence of genetics and inter-generational transmission of HPA axis activity (Bartels, Van den Berg et al. 2003; Federenko, Nagamine et al. 2004). A simultaneous analysis of 5 comparable twin studies reported a maximum heritability of HPA axis functioning of 62% (Bartels, Van den Berg et al. 2003). Another study found genetic factors to account for 40-45% of the variance in cortisol in monozygotic twins (Young, Aggen et al. 2000). Nevertheless, environmental factors, including those relating to prenatal depression, could account for the remaining 45-60% of the variance in stress responsivity and cortisol production.

Associations between Prenatal Maternal Depression and Foetal and Infant Development in Rural South India: Possible Mechanisms

Mechanisms 1, 2 and 3 attribute the overall association between prenatal maternal depression during pregnancy and disturbances in offspring development to the direct effects of prenatal depression and maternal glucocorticoids on the foetus. Mechanism 4 proposes an indirect effect of prenatal depression on offspring development via its continuity as PND and its effect on mothering.

The overall findings relating to the three main hypotheses of this thesis provide evidence in support of mechanisms 1-3. First, this study reports associations between prenatal maternal depression and foetal stress responsivity suggesting a direct effect of prenatal depression on

foetal development. Second, prenatal depression predicted infant cortisol responsivity to stress independent of the influence of maternal PND. Further, as none of the study participants smoked, consumed alcohol, recreational drugs or psychotropic medication before, during or after their pregnancies, it is unlikely that the offspring outcomes observed in this sample may be attributed to these confounders. Further, the mean body mass index of the sample was within the normal range and did not differ significantly between the depressed and non-depressed groups of mothers reducing the possibility that poor levels of maternal nutrition may have contributed to the associations between prenatal depression and offspring outcomes observed in this study.

Finally, although mechanisms 1, 2 and 3 could potentially explain the key findings of this thesis, the role of genetics in HPA axis functioning (Mechanism 6) cannot be ruled out.

6.5 LIMITATIONS

The limitations of this study are considered below:

6.5.1 LIMITATIONS IN THE OVERALL STUDY DESIGN

There are several limitations of the study design to acknowledge.

i. The study was observational and not experimental in design. Although it addresses patterns of association between prenatal depression and offspring stress responsivity, it is not able to determine causal relationships (Hill 1965).
ii. The number of women who met criteria for prenatal MDD (n=28) was relatively small to explore complex effects .

iii. Questionnaire measures designed for western populations were employed to assess maternal depression and infant temperament in a rural, developing world setting. Although all questionnaires were carefully translated, back translated and piloted, difficulties were encountered in the administration of the Infant Behaviour Questionnaire (IBQ) because of the unfamiliarity of the rural populace with certain items (such as the use of a security object to soothe the infant). Further, although the questionnaires were designed to be self-reporting in nature, low levels of literacy in the study population necessitated an interview-administered design.

iv. High levels of attrition were encountered (56.4%) and only 58 mothers returned for the postnatal assessment. Furthermore, there was some evidence of selective attrition with a larger proportion of prenatal controls compared to high scorers returning for follow-up.

v. The study was limited to a single prenatal and a single postnatal assessment. Multiple pre- and postnatal assessments may have provided more information on the programming effects of prenatal depression during multiple sensitive periods of gestation.

vi. The postnatal assessment was conducted when the infant was very young (1.5-3 months). It is possible that this is too early for the nuances in infant behaviour to have become evident to facilitate an assessment of the same through maternal reports.

vii. Issues pertaining to the co-morbidity of depression with anxiety were not addressed in this sample.

6.5.2 *Limitations of the Prenatal Assessment*

i. A quarter of foetuses did not show a sufficient response to the vibroacoustic stimulus to be assessed for habituation. The reason for this insufficient response was not known. Furthermore, the group of foetuses who responded to the stimulus was similar to the group that did not with respect to gestational age and prenatal mood of the mother. It is, therefore, possible that the strength of the VAS was not sufficient to elicit responses in all foetuses.

ii. The total duration of the foetal assessment was relatively short (30 minutes). Foetal stimulation was limited to exposure to 10 vibroacoustic stimuli.

iii. An assessment of foetal behavioural states using ultrasound techniques over a longer duration may have provided more information about foetal stress responsivity than 30 minutes of FHR monitoring.

6.5.3 *Limitations of the Postnatal Assessment*

i. Maternal cortisol levels during pregnancy were not controlled for in the analysis of infant cortisol responsivity to immunisation.

ii. The data was limited to a single pre- and post-immunisation cortisol sample when multiple assessments of infant cortisol both before and after immunisation may have contributed to a better understanding of the effect of prenatal maternal depression on the functioning of the infant's HPA axis.

iii. The postnatal assessment was conducted at 1.5 – 3 months post-birth. Thus although very young infants were assessed (see point vi in Section 6.5.1), the range of infant age at assessment was large.

iv. The size of the postnatal sample was small. Only 58 mothers returned for postnatal follow-up, and data on post-immunisation cortisol was available for only 38 infants.

v. Infants older than 1.5 months had already received their first dose of the DPT vaccine, and the immunisation during the postnatal assessment represented their second exposure.

vi. Infant daytime sleeping was not controlled for when measuring infant cortisol.

vii. Despite using a meticulous translation-back translation protocol, difficulties were encountered in the comprehension of certain items of the IBQ by the rural population. This is because:

(a) The rural populace was unfamiliar with certain items of the questionnaire,

(b) Some mothers reported that the infant was too young to display certain behaviours (for example, anger when being left alone in his/her cot) and,

(c) Mothers were unfamiliar with Likert scales and so the IBQ had to be administered by a researcher.

Furthermore, it may also be that rural Indian mothers are reluctant to report their babies as being 'difficult' or 'poorly behaved' to trained medical personnel.

6.6 STRENGTHS

There are several important strengths of the study to consider:

i. This is the first study to investigate the association between prenatal depression and foetal and infant responsivity to stress in the developing world. Importantly, the study was carried out in a rural (and not urban) prenatal sample in India. As over 70% of India's population resides in villages, the findings of this study may be generalisable to this population (Dyson and Visaria 2004; Ratna 2007).

ii. This is the first study to be carried out in an entirely non-smoking non-alcohol consuming prenatal population.

iii. The study was prospective in design and used a combination of measures of FHR, infant cortisol and infant temperament to assess offspring outcomes.

iv. A combination of self-report questionnaires and a clinical interview were used to assess mothers for pre- and postnatal depression in this study.

v. Foetal stress responsivity was studied using direct foetal stimulation by a vibroacoustic stimulus rather than indirectly through maternal cognitive stimulation.

vi. Infant cortisol was measured before and after immunisation in order to study infant cortisol responsivity (indicative of HPA axis functioning) to a common stressor.

vii. Care was taken to control for a number of confounding influences including PND, maternal nutrition and postnatal maternal cortisol.

viii. All FHR data was coded independently by two researchers blind to the prenatal mood of the mother.

6.7 IMPLICATIONS

There are several important implications of the findings of this study. These are discussed below.

Implications for Research

The findings of this thesis have three important implications for research. First, the findings provide an understanding of the patterns of association between prenatal maternal depression and offspring responsivity suggesting that this association may be U shaped rather than linear, and provide an insight into the potential underlying mechanisms. The results provide evidence

in support of the hypothesis that the programming influence of prenatal depression on offspring development begins before birth. Second, this thesis draws attention to the importance of carrying out future research in low and middle income countries where high fertility rates, high rates of maternal depression and different contextual stressors exist compared to western populations. Third, this thesis highlights the importance of combining psychological assessments with physiological measures when assessing mothers and infants.

Implications for Public Health and Clinical Medicine

The findings highlight the high prevalence of prenatal depression in rural south India and the need for the inclusion of a screening protocol for prenatal maternal depression during routine prenatal care in primary health care settings. The findings of this thesis reiterate the dual risks to mother and child associated with prenatal depression. Further, the findings of this study suggest that disturbances in foetal responsivity may be precursors of later difficulties. This thesis therefore highlights the importance of early identification and treatment of prenatal depression to clinicians, midwives and obstetric nurses.

Importantly, the finding of a curvilinear association between prenatal depression and child outcomes raises questions of public health importance as to whether all mothers found to score high for symptoms of prenatal depression need to be treated or as to whether only severe cases of prenatal depression need treatment.

6.8 SCOPE FOR FURTHER INVESTIGATION

There are many ways in which further investigation in this area may be undertaken. All existing inquiries into the association between prenatal maternal depression and child

outcomes have been carried out in North America, Europe and Australia despite the fact that the majority (80%) of global pregnancies occur in the developing world. Further, high rates of socio-economic adversity and poorer levels of nutrition are reported from these settings. It is important that future studies address the needs of families in the developing world. It is also important that future research focuses on the development of questionnaire measures adapted to low-resource developing world settings to assess both maternal depression and child outcomes.

Further research into the mechanisms governing the association between prenatal maternal depression and child outcome, particularly offspring stress responsivity, needs to be undertaken. Inquiries should investigate the possibility of patterns of association between prenatal depression and child outcome that are not necessarily linear in nature. The possibilities of a threshold of intra-uterine exposure to maternal depression or stress, and a dose-dependent relationship between prenatal maternal depression and child outcome, need to be addressed. Future studies should investigate these issues in large samples in different population groups in order to gain a clearer understanding of the underlying mechanisms in different contexts. This would inform the design of effective prenatal interventions and help researchers better understand the complex hormonal and biological environment within which the foetus develops.

A chasm in the knowledge of foetal programming and prenatal depression influences on offspring development is the lack of information about the contributory role of genetics. Further, it is known that similar levels of prenatal stress do not affect all children in the same way (Talge, Neal et al. 2007). Future investigations involving twin studies and adoption studies need to be undertaken before the genetic and vulnerability factors, the various risk and

protective factors in the pre- and postnatal environments, and mechanisms of resilience can be elucidated.

Finally, intervention strategies to treat prenatal depression and the use of randomised controlled trials (RCTs) to test these interventions need to be undertaken. RCTs would not only contribute to the development of effective treatments for prenatal depression but may be able to provide the necessary experimental evidence to establish a causal association between prenatal depression and offspring outcomes.

CHAPTER 7
CONCLUSIONS

7 CONCLUSIONS

This thesis represents the first inquiry into the association between prenatal maternal depression and, foetal and infant development in the rural, developing world. This is the first study to investigate this issue in an entirely non-smoking, non-alcohol consuming prenatal sample. Fifteen percent of mothers met ICD-10 criteria for a major depressive episode during pregnancy while 35.5% of mothers scored high for symptoms of prenatal depression. This suggests that prenatal depression is a significant clinical issue in this setting.

Three key hypotheses were tested in this thesis. They concerned the association of prenatal maternal depression with:

(i) Foetal stress responsivity measured through foetal cardiac responses to vibroacoustic stimulation,

(ii) Infant cortisol responsivity to immunisation and,

(iii) Infant temperament.

Although there were no linear associations between prenatal maternal depression and foetal stress responsivity, the relationship was found to be curvilinear (U shaped) with foetuses exposed to both very high and very low levels of maternal depression showing elevated cardiac responses to repeated vibroacoustic stimulation compared to the foetuses exposed to moderate levels of prenatal maternal depression. Importantly, this pattern of association was not observed in foetuses at baseline but only upon the presentation of a stressor. A similar association existed between prenatal maternal depression and infant cortisol response to immunisation at 1.5-3 months post-birth. Furthermore, prenatal maternal depression predicted

higher levels of infant cortisol responsivity to immunisation independent of postnatal maternal depression, postnatal maternal cortisol and infant sex. Baseline cortisol levels were not associated with prenatal depression. Prenatal depression was not associated with maternal reports of difficult infant temperament. Foetal stress responsivity correlated with infant cortisol responsivity in this study, suggesting a continuity in physiological responses to stress from intra-uterine to extra-uterine life.

In contrast to previous studies investigating the effect of prenatal depression on postnatal child outcomes, the results of this thesis suggest that the influence of prenatal depression on offspring can be observed even before birth. The findings of this thesis suggest that this association may be attributable, in part, to the programming influence of prenatal maternal depression on the developing offspring's hypothalamic-pituitary-adrenal axis. The nature of this sample rules out the possibility that the observed associations could be attributed to the confounding influences of prenatal smoking and alcohol consumption on foetal and infant development. Further, the findings of this thesis suggest that the association between prenatal maternal depression and offspring stress responsivity may not always be linear as previously presumed, but may be non-linear or even curvilinear in nature.

Concluding Comments:

The results of this study, although intriguing, must be interpreted with caution and replication in larger samples and different population groups is required. It is important to note that while associations between exposure to prenatal depression and disturbances in offspring stress responsivity have been reported in this thesis, they do not imply causality. Larger experimental designs and intervention studies are required to confirm a causal relationship.

Nevertheless this study is the first of its kind to be carried out in the developing world and in an entirely non-smoking non-alcohol consuming prenatal population. The findings have implications for future research, particularly on the need for further investigation into the influence of prenatal depression on child outcomes in socio-economically disadvantaged populations.

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APPENDICES

Appendix I: Participant Information

APPENDIX IA: PARTICIPANT INFORMATION SHEET (ENGLISH)

DEPARTMENT OF PSYCHIATRY
UNIVERSITY OF OXFORD

ST. JOHN'S RESEARCH INSTITUTE
ST. JOHN'S NATIONAL ACADEMY OF HEALTH SCIENCES

Solur Baby Project
Snehalaya Socio-Medical Relief Centre
Solur Village
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Does Prenatal Maternal Depression Predict Foetal and Infant Development?

A Study of Mothers and Infants in Rural India

INFORMATION SHEET

You are invited to take part in a research project about the effect of the mother's mental state during pregnancy on the development of her foetus and infant. Anxiety and depression in a pregnant woman affect her unborn baby and these effects are seen in infancy and childhood, however the nature and extent of these effects is poorly understood. This is especially so in pregnant Indian women and therefore we want to find out about how the mental state of pregnant women in India effects their foetus and child. This study involves 2 phases: one

during the last few weeks of your pregnancy and one when your baby is 6 to 14 weeks old i.e. during the time of DPT vaccination.

If you decide to take part in this study, a researcher (Michelle Fernandes) will meet with you at Snehalaya Hospital, Solur at a time convenient to you. In the first part of the study, she will ask you questions about your mental state, your family life and your eating habits. She will also ask you to lie down and record the heart rate of your baby using a machine specially designed for this purpose. During this time, she will also place a noise producing device on your abdomen a few times to determine how your baby responds to the same. This whole process will not take more than an hour and a half.

Should you decide to take part in the first part of the study, the researcher may invite you to meet her again when your child is 6 – 14 weeks old (1.5 to 3.5 months old) when you visit Snehalaya hospital for the immunisation of your baby with DPT vaccine. During this time she will ask you questions about your mental state, the breastfeeding of your baby and the way in which your baby behaves and reacts to you. She will also take your baby's length, weight and head measurements and will also take the fingerprints of your baby. She will also collect a small amount of your baby's saliva using a cotton bud like device, once before immunisation and once after immunisation. This whole process will take about one hour.

All information that you give us will be treated in the strictest of confidence and is not discussed with your doctor, nurse or family members unless you specifically ask for this to happen. The baby's heart graphs, fingerprints and saliva samples will be stored securely and labeled with a code so that they cannot be identified with an individual. All information you

give us will be used to understand how the mother's mental state during pregnancy effects her child and no comments will be able to be traced back to you.

You do not have to take part in this study if you don't want to. If you do decide to take part you may withdraw at any time without having to give a reason. If you feel uncomfortable or distressed at any point of time you have every right to tell us and we shall stop the procedure/interview immediately. Taking part in the first part of the study in no way compels you to take part in the second part of the study even if you are invited to do so. Your decision whether or not to take part does not affect your care and management in any way, as the study is entirely separate.

Thank you for taking the time to read/listen to this. If you are willing to take part in the study, please fill in the attached form and the researcher (Michelle Fernandes) will contact you shortly.

DR. PAUL G. RAMCHANDANI DR. MICHELLE C. FERNANDES

APPENDIX IA: PARTICIPANT INFORMATION SHEET (KANNADA)

ಗರ್ಭಾವಸ್ಥೆಯಲ್ಲಿ ತಾಯಿಯ ಖಿನ್ನತೆಯು ಭ್ರೂಣದ ಮತ್ತು ಮಗುವಿನ ಬೆಳವಣಿಗೆಯ ಮೇಲೆ ಪರಿಣಾಮ ಬೀರುತ್ತದೇಯೇ ?

ಭಾರತದ ಗ್ರಾಮೀಣ ಪ್ರದೇಶದ ತಾಯಿ ಮತ್ತು ಮಗುವಿನ ಪೂರ್ವ ಭಾವಿ ಅಧ್ಯಯನ.
ಪತ್ರ : ಮಹಿಳೆಯು ಗರ್ಭೀಣಿಯಾಗಿದ್ದಾಗ ಅವಳ ಮಾನಸಿಕ ಸ್ಥಿತಿಯು ಭ್ರೂಣ ಮತ್ತು ನವಜಾತಿಶಿಶು ನ ಬೆಳವಣಿಗೆಯ ಮೇಲೆ ಪರಿಣಾಮ ಬೀರುತ್ತದೆಯೇ ಎಂಬ ಪ್ರಾಯೋಗಿಕ ಅಧ್ಯಯನದಲ್ಲಿ ಸ್ವಇಚ್ಛೆಯಿಂದ ಭಾಗವಹಿಸಲು ನಿಮ್ಮನ್ನು ಆಹ್ವಾನಿಸಲಾಗಿದೆ. ಗರ್ಭಿಣಿಯ ಉದ್ವೇಗ ಮತ್ತು ಖಿನ್ನತೆಯಿಂದ ಅವಳ ಹುಟ್ಟದೇ ಇರುವ ಮಗುವಿನ ಮೇಲೆ ಆಗುವ ಪರಿಣಾಮ ಮತ್ತು ನವಜಾತ ಶಿಶುವಿನ ಮತ್ತು ಮಗುವಿನ ಬಾಲ್ಯದ ಮೇಲೆ ಆಗುವ ಪರಿಣಾಮವನ್ನು ಕಂಡಿದ್ದರು ಸಹ, ಅದು ಯಾವ ರೀತಿ ಮತ್ತು ಎಷ್ಟರ ಮಟ್ಟಿಗೆ ಪರಿಣಾಮ ಬೀರುತ್ತದೆ ಎಂಬುದನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡಿರುವ ಪ್ರಮಾಣ ಬಹಳ ಕಡಿಮೆ . ಈ ಅರಿವು ಕಡಿಮೆ ಇರುವುದು ಪ್ರಮುಖವಾಗಿ ಭಾರತೀಯ ಗರ್ಭಿಣಿ ಹೆಂಗಸರಲ್ಲಿ ಮಾತ್ರ ಅದ್ದರಿಂದ ನಾವು ಭಾರತೀಯ ಗರ್ಭಿಣಿ ಮಹಿಳೆಯ ಮಾನಸಿಕ ಸ್ಥಿತಿಯು ಭ್ರೂಣ ಮತ್ತು ಮಗುವಿನ ಬೆಳವಣಿಗೆಯ ಮೇಲೆ ಯಾವ ರೀತಿ ಪರಿಣಾಮ ಬೀರುತ್ತದೆ ಎಂಬುದನ್ನು ಕಂಡುಹಿಡಿಯಲು ಇಚ್ಚಿಸುತ್ತೇವೆ. ಈ ಅಧ್ಯಯನವು ೨ ಹಂತಗಳನ್ನು ಒಳಗೊಂಡಿರುತ್ತದೆ. ಮೊದಲನೇ ಹಂತವು ನಿಮ್ಮ ಗರ್ಭಾವಸ್ಥೆಯ ಕೊನೆಯ ಕೆಲವು ವಾರಗಳಲ್ಲಿ ಇರುತ್ತದೆ. ಮತ್ತು ಮತ್ತೊಂದು ಯು ನಿಮ್ಮ ಮಗುವಿಗೆ ೬ ರಿಂದ ೧೪ ವಾರ ಇರುವಾಗ. ಇದು ನೀವು ಮಗುವಿಗೆ ಪಿ.ಟಿ. ಚುಚ್ಚುಮದ್ದು ಕೊಡಿಸಲು ಬರುವ ವೇಟೆಯಲ್ಲಿರುತ್ತದೆ.

ನೀವು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಇಚಿಸಿದರೆ, ಸಂಶೋಧಕ (ಮೆ ಕೆಲ್ ಫರ್ನ್ಯಾಂಡಿಸ್) ನಿಮ್ಮನ್ನು ನಿಮಗೆ ಅನುಕೂಲವಾದ ಸಮಯದಲ್ಲಿ ಸ್ನೇಹಾಲಯ ಆಸ್ಪತ್ರೆ, ಸೋಲೂರಿನಲ್ಲಿ ಭೇಟಿಯಾಗುತ್ತಾರೆ. ಅಧ್ಯಯನದ ಮೊದಲಿನ ಹಂತದಲ್ಲಿ (ಭಾಗದಲ್ಲಿ) ಇವರು ನಿಮ್ಮ ಮಾನಸಿಕ ಸ್ಥಿತಿ, ನಿಮ್ಮ ಕೌಟುಂಬಿಕ ಮತ್ತು ಆಹಾರ ಅಭ್ಯಸಕ್ಕೆ ಸಂಬಂಧಿಸಿದಂತೆ ಕೆಲವು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳುತ್ತಾರೆ ಮತ್ತು ಇವರ ನಿಮ್ಮನ್ನು ಮಲಗಿಕೊಳ್ಳುವಂತೆ ಹೇಳಿ, ನಿಮ್ಮ ಮಗುವಿನ ಹೃದಯ ಬಡಿತವನ್ನು ಅದಕ್ಕಾಗಿಯೇ ವಿಶೇಷವಾಗಿ ರೂಪಿಸಲಾಗಿರುವ ಯಂತ್ರದ ಮೂಲಕ ಮತ್ತು ಇವರು ಶಬ್ದವನ್ನು ಹೊರ ಚೆಲ್ಲುವ ಉಪಕರಣವನ್ನು ನಿಮ್ಮ ಹೊಟ್ಟೆಯ ಮೇಲೆ ಕೆಲವು ಸಮಯ ಇಟ್ಟು ನಿಮ್ಮ ಮಗು ಅದಕ್ಕೆ ಹೇಗೆ ಪ್ರತಿಕ್ರಿಯಿಸುತ್ತದೆ ಎಂಬುದನ್ನು ಪರೀಕ್ಷಿಸುತ್ತಾರೆ. ಈ ಪೂರ್ತಿ ಪ್ರಕ್ರಿಯೆ ನಿಮ್ಮ ಒಂದುವರೆ (1 1/2) ಗಂಟೆಗಿಂತ ಹೆಚ್ಚು ಸಮಯ ತೆಗೆದುಕೊಳ್ಳುವುದಿಲ್ಲ.

ನೀವು ಅಧ್ಯಯನದ ಮೊದಲ ಹಂತದಲ್ಲಿ ಭಾಗವಹಿಸಲು ತೀರ್ಮಾನಿಸಿದರೆ, ಸಂಶೋಧಕರು ನಿಮ್ಮನ್ನು ಮತ್ತೊಂದು ಭಾರಿ ಭೇಟಿಯಾಗಲು ಆಹ್ವಾನಿಸುತ್ತಾರೆ, ಈ ಭೇಟಿಯು ನಿಮ್ಮ ಮಗುವಿಗೆ 6 – 14 ವಾರ ಇರುವಾಗ (1.5 – 3.5 ತಿಂಗಳವರೆಗೆ) ಮತ್ತು ಈ ಭೇಟಿಯು ನಿಮ್ಮ ಮಗುವಿಗೆ ಡಿ.ಪಿ.ಟಿ ಚುಚ್ಚುಮದ್ದು ಕೊಡಿಸಲು ಬಂದಿರುವಾಗ ಆಗಿರುತ್ತದೆ. ಈ ಯಲ್ಲಿ ಸಂಶೋಧಕರು ನಿಮ್ಮನ್ನು ನಿಮ್ಮ ಮಾನಸಿಕ ಸ್ಥಿತಿಯ ಬಗ್ಗೆ, ಮಗುವಿಗೆ ಎದೆಯಾಲು ಉ ಸುತ್ತಿರುವ ಬಗ್ಗೆ ಮತ್ತು ನಿಮ್ಮ ಮಗುವಿನ ವರ್ತನೆ ಮತ್ತು ಮಗು ಪ್ರತಿಕ್ರಿಯಿಸತ್ತಿರುವುದಕ್ಕೆ ಸಂಬಂಧಿಸಿದಂತೆ ಕೆಲವು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳುತ್ತಾರೆ, ಸಂಶೋಧಕರು ನಿಮ್ಮ ಮಗುವಿನ ಉದ್ದ , ತೂಕ , ಮತ್ತು ತಲೆಯ ಸುತ್ತಳತೆಯನ್ನು ಮತ್ತು ನಿಮ್ಮ ಮಗುವಿನ ಬೆರಳಿನ ಗುರುತನ್ನು ತೆಗೆದುಕೊಳ್ಳುತ್ತಾರೆ. ಮತ್ತು ಇವರು ನಿಮ್ಮ ಮಗುವಿನ ಜೊಲ್ಲಿನ ಸ್ವಲ್ಪ ಪ್ರಮಾಣವನ್ನು ಹತ್ತಿಯಲ್ಲಿ ತೆಗೆದುಕೊಳ್ಳುತ್ತಾರೆ . ಜೊಲ್ಲನ್ನು ಚುಚ್ಚು ಮದ್ದು ಕೊಡುವ ಮೊದಲು ಮತ್ತು ಚುಚ್ಚು ಮದ್ದು ಕೊಟ್ಟ ನಂತರ ಎರಡು ಭಾರಿ ತೆಗೆದುಕೊಳ್ಳುತ್ತಾರೆ. ಈ ಪೂರ್ತಿ ಪ್ರಕ್ರಿಯೆಗೆ ಸುಮಾರು 1 ಗಂಟೆ ತೆಗೆದುಕೊಳ್ಳಬಹುದು.

ನೀವು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಕೊಡುವ ಎಲ್ಲಾ ಮಾಹಿತಿಗಳನ್ನು ಖಂಡಿತವಾಗಿಯೂ ಗೌಪ್ಯವಾಗಿ ಇಡಲಾಗುತ್ತದೆ. ಈ ವಿಷಯವನ್ನು ನಿಮ್ಮ ವೈದ್ಯರು, ಶುಶ್ರೂಷಕಿ ಮತ್ತು ಕುಟುಂಬದ ಸದಸ್ಯರೊಂದಿಗೂ ಸಹ ಚರ್ಚಿಸಲಾಗುವುದಿಲ್ಲ. ನೀವು ಚರ್ಚಿಸಲು ಹೇಳಿದಂತಹ ಸಂದರ್ಭದ ಮಾತ್ರ ಇವರೊಂದಿಗೆ ಚರ್ಚಿಸಲಾಗುತ್ತದೆ. ಮಗುವಿನ ಹೃದಯದ ಪಟ್ಟಿ , ಬೆರೆಳಿನ ಗುರುತು ಮತ್ತು ಲ್ಲನ್ನು ಜೊಪಾನವಾಗಿ ಶೇಖರಿಸಿ ಇಡಲಾಗುತ್ತದೆ. ಇವುಗಳನ್ನು ಗುರುತಿನ ಸಂಖ್ಯೆ ನೀಡುವ ಮೂಲಕ ಹೆಸರಿಸಿ ಇಡಲಾಗುತ್ತದೆ. ಅದ್ದರಿಂದ ಇದನ್ನು ಯಾವುದೇ ವ್ಯಕ್ತಿ ಗುರುತು ಹಿಡಿಯುವುದು ಸಾಧ್ಯವಿಲ್ಲ . ನೀವು ಕೊಡುವ ಮಾಹಿತಿ ಎಲ್ಲವನ್ನು ಗರ್ಭಿಣಿಯ ಮಾನಸಿಕ ಸ್ಥಿತಿಯಿಂದ ಮಗುವಿನ ಮೇಲಾಗುವ ಪರಿಣಾಮ ತಿಳಿಯುವುದಕ್ಕೆ ಮಾತ್ರ ಉಪಯೋಗಿಸಲಾಗುತ್ತದೆ ಹೊರತು ಯಾವುದೇ ರೀತಿಯ ವಿಮರ್ಶೆ ನಿಮ್ಮ ಮೇಲೆ ಇದರಿಂದ ಆಗುವುದಿಲ್ಲ.

ನಿಮಗೆ ಇಚ್ಛೆ ಇಲ್ಲದಿದ್ದರೆ ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವ ಅವಶ್ಯಕತೆ ಇಲ್ಲ. ನೀವು ಭಾಗವಹಿಸಲು ನಿರ್ಧರಿಸಿದ್ದರೂ ಸಹ, ಯಾವ ಸಮಯದಲ್ಲಿ ಬೇಕಾದರೂ ಒಪ್ಪಿಗೆಯನ್ನು ಯಾವ ಕಾರಣವನ್ನು ನೀಡದೆ ಹಿಂತೆಗೆದುಕೊಳ್ಳಬಹುದು.ಯಾವ ಸಮಯದಲ್ಲಾದರೂ ನಿಮಗೆ ಅಸಮಾ

ಸ್ಥಿತಿ ಒದಗುತ್ತಿದೆ ಅಥವಾ ಒತ್ತಡಕ್ಕೆ ಒಳಾಗಾಗುತ್ತಿದ್ದೇನೆ ಎಂದೆನಿಸಿದರೆ ನೀವು ನಮಗೆ ತಿಳಿಸುವ ಹಕ್ಕನ್ನು ಹೊಂದಿರುವೀರಿ ಮತ್ತು ಆ ಕ್ಷಣದಲ್ಲಿಯೇ ನಾವು ಮೌಖಿತ ಪ್ರಶ್ನೆಯನ್ನು / ಪರೀಕ್ಷೆ ನಿಲ್ಲಿಸುತ್ತೇವೆ. ನೀವು ಮೊದಲನೆಯ ಹಂತದಲ್ಲಿ ಭಾಗವಹಿಸಿರುವುದರಿಂದ , ನಾವು ಆಹ್ವಾನಿಸಿದರೂ ನೀವು ಎರಡನೇ ಹಂತದಲ್ಲಿ ಭಾಗವಹಿಸಬೇಕೆಂಬ ಒತ್ತಡವೇನು ಇರುವುದಿಲ್ಲ . ನೀವು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವುದರಿಂದಾಗಲಿ ಅಥವಾ ಭಾಗವಹಿಸದೇ ಇರುವುದರಿಂದಾಗಲಿ ನಿಮಗೆ ಇಲ್ಲಿ ಸಿಗುತ್ತಿರುವ ಉಪಚಾರ ಮತ್ತು ಮೇಲ್ವಿಚಾರಣೆಯಲ್ಲಿ ಯಾವ ಬದಲಾವಣೆಯು ಇರುವುದಿಲ್ಲ, ಈ ಅಧ್ಯಯನವು ಇದರಿಂದ ಸಂಪೂರ್ಣವಾಗಿ ಬೇರೆಯಾಗಿದೆ.

ಇದನ್ನು ಓದಲು / ಕೇಳಲು ನೀವು ತೆಗೆದುಕೊಡಿರುವ ನಿಮ್ಮ ಸಮಯಕ್ಕೆ ಬಹಳ ಧನ್ಯ ದಗಳು. ನೀವು ಈ ಅಧ್ಯಾಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಇಚ್ಛಿಸಿ ರೆ ಇದರೊಂದಿಗೆ ಲಗತ್ತಿಸಿರುವ ಅರ್ಜಿಯನ್ನು ಭರ್ತಿ ಮಾಡಿ ಮತ್ತು ಸಂಶೋಧಕ (ಮೆಕಲ್ ಫರ್ ನ್ಯಾಂಡಿಸ್) ಶೀಘ್ರದಲ್ಲಿಯೇ ನಿಮ್ಮನ್ನು ಸಂಪರ್ಕಿಸುತ್ತಾರೆ.

APPENDIX IB: INFORMED CONSENT FORM (ENGLISH)

DEPARTMENT OF
PSYCHIATRY
UNIVERSITY OF
OXFORD

ST. JOHN'S RESEARCH INSTITUTE
ST. JOHN'S NATIONAL ACADEMY
OF HEALTH SCIENCES

Solur Baby Project
Snehalaya Socio-Medical Relief Centre
Solur Village, Karnataka
India

Does Prenatal Maternal Depression Predict Foetal and Infant Development?
A Study of Mothers and Infants in Rural India
(Investigator: Michelle Fernandes)

CONSENT FORM – Confidential

Have you read/been read the information sheet? Yes/No

Have you had the opportunity to ask questions and discuss the study? Yes/No

Have you received satisfactory answer to all your questions? Yes/No

Who have you spoken to? ……

Do you understand that you are free to withdraw from this study:

- at any time
- without having to give a reason for withdrawing
- and without it affecting your future medical care or that of your child? Yes/No

Do you agree to take part in this study? Yes/No

Do you agree to allow your baby's heart rate to be measured using a machine designed for this purpose? Yes/No

Do you agree to allow the researcher to place a sound producing device on your abdomen to measure you baby's reaction? Yes/No

Do you agree to allow your baby's weight, length, head and fingerprints to be measured? Yes/No

Do you agree to allow the researcher to collect some of your baby's saliva before and after the DPT vaccine is given? Yes/No

Do you agree to allow the researcher(s) to examine your medical records? Yes/No

Signed: …… Left thumb impression:

Name in block letters: ……..

Date: …….

Principal Investigator signature: ……

Date:……..

APPENDIX IB: INFORMED CONSENT FORM (KANNADA)

ಒಪ್ಪಿಗೆ ಪತ್ರ : ಗೌಪ್ಯತೆ

ಹಂತ ಒಂದು : ಗರ್ಭವಸ್ಥೆಯ ವಿಚಾರಣೆ

- ಒಪ್ಪಿಗೆ ಪತ್ರವನ್ನು ನೀವೇ ಓದಿರುವೀರಾ / ಬೇರೆಯವರಿಂದ ಓದಿ ತಿಳಿದಿರುವೀರಾ ?
  ಹೌದು / ಇಲ್ಲ

- ನಿಮಗೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಮತ್ತು ಅಧ್ಯಯನದ ಬಗ್ಗೆ ಚರ್ಚಿಸಲು ಅವಕಾಶದೊರತಿವೇಯೇ ? ಹೌದು / ಇಲ್ಲ

  ನಿಮ್ಮ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೂ ಸಮಾಧಾನಕರ ಉತ್ತರ ದೊರತಿದ್ದೆಯೇ ?
  ಹೌದು / ಇಲ್ಲ

  ನೀವು ಯಾರಹೊಂದಿಗೆ ಮಾತನಾಡಿದ್ದೀರಿ ? _____

  ನೀವು ಯಾವಾಗ ಬೇಕಾದರೂ ಅಧ್ಯಯಾನದಿಂದ ಹೊರ ಬರಬಹುದೆಂದು ನಿಮಗೆ ತಿಳಿದಿದೇಯೆ?

- ಯಾವ ಸಂದರ್ಭವಲ್ಲಾದರೂ
- ಯಾವುದೇ ಕಾರಣ ನೀಡದೇ ಹೊರ ಬರಬಹುದು
- ಇದರಿಂದ ನಿಮಗೆ ದೊರೆಯುವ ಮುಂದಿನ ಉಪಚಾರ ಮತ್ತು ವೈದ್ಯಕೀಯ ಚಿಕಿತ್ಸೆಯಲ್ಲಿ ಯಾವುದೇ ಬದಲಾವಣೆಗೆ ಇರುವುದಿಲ್ಲ
- ಹೌದು / ಇಲ್ಲ

ನೀವು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಇಚ್ಚಿಸುತ್ತೀರಾ ?
ಹೌದು / ಇಲ್ಲ

- ನೀವು ನಿಮ್ಮ ಮಗುವಿನ ಹೃದಯ ಬಡಿತದ ಪ್ರಮಾಣವನ್ನು ಅದಕ್ಕಾಗಿಯೇ ರೂಪಿಸಿರುವ ಯಂತ್ರದ ಮೂಲಕ ಪರಿಶೀಲಿಸಲು ಒಪ್ಪಿಗೆ ನೀಡುತ್ತೀರಾ ?
  ಹೌದು / ಇಲ್ಲ

- ನೀವು ಸಂಶೋಧಕರನ್ನು ನಿಮ್ಮ ಹೊಟ್ಟೆಯ ಮೇಲೆ ಶಬ್ದ ಹೊರ ಚಲ್ಲುವ ಉಪಕರಣವನ್ನಿಟ್ಟ ನಿಮ್ಮ ಮಗುವಿನ ಪ್ರತಿಕ್ರಿಯೆಯನ್ನು ಪರೀಕ್ಷಿಸಲು ಒಪ್ಪಿಗೆ ನೀಡುತ್ತೀರಾ?
  ಹೌದು / ಇಲ್ಲ

- ನೀವು ನಿಮ್ಮ ವೈದ್ಯಕೀಯ ಮಾಹಿತಿ ಪತ್ರಗಳನ್ನು ಸಂಶೋಧಕರು ಪರೀಕ್ಷಿಸಲು ಒಪ್ಪಿಗೆ ನೀಡುತ್ತೀರಾ ?
  ಹೌದು / ಇಲ್ಲ

- ನೀವು ನಿಮ್ಮ ಮಗುವಿನ ತೂಕ, ಉದ್ದ , ತಲೆ ಮತ್ತು ಬೆರಳಿನ ಗುರುತನ್ನು ಅಳತೆ ಮಾಡಲು ಒಪ್ಪಿಗೆ ನೀಡುತ್ತೀರಾ?
  ಹೌದು / ಇಲ್ಲ

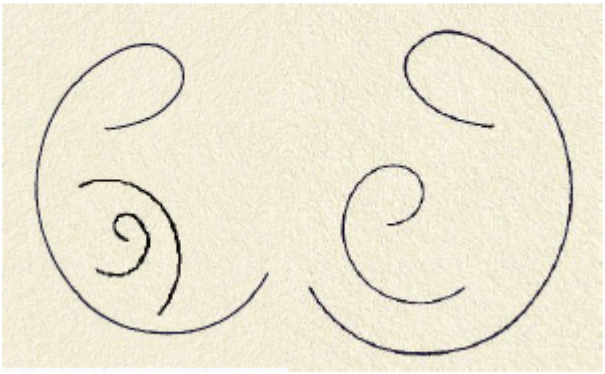
- ನೀವು ನಿಮ್ಮ ಮಗುವಿನ ಮಗುವಿನ ಜೊಲ್ಲಿನ ಸ್ವಲ್ಪ ಪ್ರಮಾಣವನ್ನು ಡಿ.ಪಿ.ಟಿ ಚುಚ್ಚು ಮದ್ದಿನ ಮೊದಲು ಮತ್ತು ನಂತರ ಸಂಶೋಧಕರು ತೆಗೆದುಕೊಳ್ಳಲು ಒಪ್ಪಿಗೆ ನೀಡುತ್ತೀರಾ?
  ಹೌದು / ಇಲ್ಲ

Signed: …… Left thumb impression:

Name: …… Date:…….

APPENDIX IC: FOLLOW UP CARD (ENGLISH)

THE SOLUR MOTHER AND BABY PROJECT



I AM PART OF THE SOLUR MOTHER AND BABY PROJECT

ABOUT THE MOTHER'S HEALTH DURING PREGNANCY AND THE BABY'S GROWTH

MY STUDY NUMBER IS

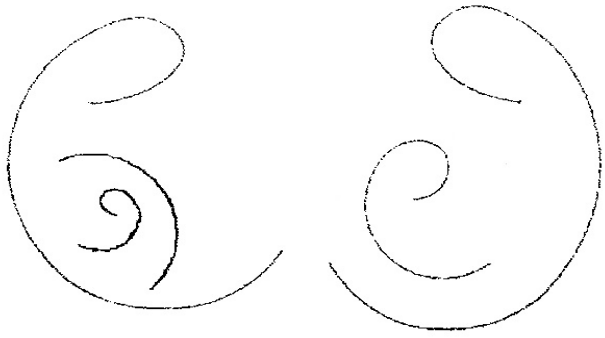
MY NEXT VISIT IS ON:

_____

Study contact:
Dr. Michelle Fernandes
Tel: 9535081508

APPENDIX IC: FOLLOW UP CARD (KANNADA)

ಸೋಲುರ್ ತಾಯಿ ಮತ್ತು ಮಗುವಿನ ಪ್ರಜೆಕ್ಟ್



ಸೋಲುರ್ ತಾಯಿ ಮತ್ತು ಮಗುವಿನ ಪ್ರಜೆಕ್ಟ್ ನಾನು ಇದರಲ್ಲಿ ಭಾಗವಹಿಸುತ್ತಿದ್ದೇನೆ.

ಇದು ಗರ್ಭಿಣಿಯರ ಆರೋಗ್ಯ ಮತ್ತು ಮಗುವಿನ ಬೆಳವಣಿಗೆ ಬಗ್ಗೆ

ನನ್ನ ಪ್ರಜೆಕ್ಟ್ ಸಂಖ್ಯೆ:

_____

ನಾನು ಮತ್ತೆ ಈ ದಿನಾಂಕದಲ್ಲಿ ಬರುತ್ತೇನೆ.

_____

ಪ್ರಜೆಕ್ಟ್ ಮಾಡುವವರು
ಡಾ: ಎಮ್. ಫರ್ನಾಂಡೆಸ್
ದೂರವಾಣಿ: 9535081508

APPENDIX ID: PARTICIPANT FOLLOW UP LETTER (ENGLISH)

From: The Solur Mother & Baby Project

Snehalaya Hospital, Solur Post,

Date:

Dear Mrs. ……..,

Subject: Phase II Assessment on ……..

Congratulations on the birth of your baby……..! I am contacting you because you took part in a study about how your mood affects your baby's growth during your prenatal check-up at Snehalaya Hospital, Solur. Thank you very much for your participation in this study.

As you were informed at the time of the assessment during your pregnancy, we require you to bring your baby for another assessment at Snehalaya Hospital to complete the study. Your baby's next vaccination for DPT is due on …….. If this date is convenient for you, I will assess your baby as per the second part of the study on this date.

During this assessment, I will check your baby's weight, collect a little of your baby's saliva and ask you some questions on how your baby reacts to different situations. I will also ask you some questions on your mood and your health. Participation in this study is not compulsory, but we would appreciate your co-operation very much.

Please bring your follow-up card or this letter along with you to Snehalaya Hospital if you wish to participate in the second phase of this study. You will be reimbursed Rs. 100.00

towards your bus fare, meal charges and the cost of the immunization of your baby. Please contact me on 9535081508 if you have any questions.

I look forward to seeing you and your baby at Snehalaya Hospital on ……..

Yours sincerely,

Dr. Michelle Fernandes

The Solur Mother & Baby Project

Snehalaya Hospital, Solur (P),

Magadi (T), Ramnagara (D),

Karnataka – 5462 127

Ph: 9535081508

APPENDIX ID: PARTICIPANT FOLLOW UP LETTER (KANNADA)

ಸ್ನೇಹಾಲಯ ಆಸ್ಪತ್ರೆ
ಸೋಲೂರು-562127
ದಿನಾಂಕ ಃ

ಮಾನ್ಯರೆ,

_____

ವಿಷಯ ಃ ಪಾಪುವಿಗೆ ಪರೀಕ್ಷೆ ಮಾಡಿಸಲು ದಿನಾಂಕ ———— ರಂದು ಬರುವ ಬಗ್ಗೆ

ನಿಮ್ಮ ಮಗು ಹುಟ್ಟಿರುವುದಕ್ಕೆ ನಿಮಗೆ ಧನ್ಯವಾದಗಳು.

ನೀವು ಸ್ನೇಹಾಲಯ ಆಸ್ಪತ್ರೆಯಲ್ಲಿ ಗರ್ಬಿಣಿ ಪರೀಕ್ಷೆ ಮಾಡಿಸಿದಾಗ ನಿಮ್ಮ ಮಗುವಿನ ಬಡಿತವನ್ನು ಪರೀಕ್ಷಿಸಿದ್ದೀವಿ, ಪಾಪುವಿನ ಚಟುವಟಿಕೆ ಸಾದಾರಣವಾಗಿತ್ತು, ಏನು ತೊಂದರೆ ಇರಲಿಲ್ಲ.

ನಿಮ್ಮ ಮಗು ಈಗ ಯಾವ ರೀತಿ ಬೆಳವಣಿಗೆಯಲ್ಲಿದೆ ಎಂದು ನೋಡಬೇಕು ಮತ್ತು 2ನೇ ಸಲ ಪರೀಕ್ಷಿಸಿ ಯಾವ ತೊಂದರೆ ಇಲ್ಲ ಎಂದು ಖಚಿತಪಡಿಸಿಕೊಳ್ಳಲು ಒಂದು ಸಲ ಆಸ್ಪತ್ರೆಗೆ ಬರಬೇಕು. 2ನೇ ಸಲ ಪರೀಕ್ಷೆಗೆ ದಿನಾಂಕ ———— ದಲ್ಲಿ ಬರಬೇಕು, ನೀವು ಆದಷ್ಟು ಸೋಮವಾರ ಬರುವ ಪ್ರಯತ್ನ ಮಾಡಿ. ಇಲ್ಲ ಬುಧವಾರ ಮತ್ತು ಶುಕ್ರವಾರ ದಿನಗಳಲ್ಲಿ ಬರಬೇಕು.

ಪಾಪುವಿನ ಬೆಳವಣಿಗೆ ಹೇಗಿದೆ ನೋಡಬೇಕು ಮತ್ತು ಪಾಪುವಿನ ಎಂಜಲನ್ನು ಪರೀಕ್ಷಿಸಬೇಕು. ನಿಮಗೆ ಉಪಯೋಗವಾಗುವ ಸ್ವಲ್ಪ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಬೇಕಾಗುತ್ತದೆ. ಪಾಪುವಿನ ಆರೋಗ್ಯದ ಬಗ್ಗೆ ಪ್ರಶ್ನೆ ಕೇಳಬೇಕಾಗುತ್ತದೆ.

ನಿಮಗೆ ನಾವು ರೂ.100 ನ್ನು ಕೊಡುತ್ತೇವೆ. ಅದು ನೀವು ಬರುವ ಬಸ್ ಚಾರ್ಜ್ ಮತ್ತು ಆಸ್ಪತ್ರೆಯ ಖರ್ಚು. ನಿಮಗೆ ಏನಾದ್ರು ತೊಂದರೆ ಇದ್ದರೆ ನೀವು ಇವರನ್ನು ಸಂಪರ್ಕಿಸಿ- ಡಾ ಃ ಫರ್ನಾಂಡಸ್ (Ph: 9535081508).

ನಿಮ್ಮನ್ನು ಮತ್ತು ಮಗುವನ್ನು ಈ ದಿನಾಂಕದಲ್ಲಿ ನೋಡಲಾಗುತ್ತಿದೆ ಃ ————

ಧನ್ಯವಾದಗಳು,

ಡಾ ಃ ಎಮ್.ಸಿ. ಫರ್ನಾಂಡಸ್
ಸೋಲೂರು ತಾಯಿ ಮತ್ತು ಮಗುವಿನ ಪ್ರಾಜೆಕ್ಟ್,
ಸ್ನೇಹಾಲಯ ಆಸ್ಪತ್ರೆ, ಸೋಲೂರು ಅಂಚೆ,
ಮಾಗಡಿ (ತಾ), ರಾಮನಗರ (ಜಿ)- 562127
Phಃ 9535081508

Appendix II: Measures

APPENDIX IIA: PRENATAL ASSESSMENT DATA SHEET

DEPARTMENT OF
PSYCHIATRY
UNIVERSITY OF OXFORD

ST. JOHN'S RESEARCH
INSTITUTE
ST. JOHN'S NATIONAL
ACADEMY OF HEALTH
SCIENCES

SOLUR BABY PROJECT

Does Prenatal Maternal Depression Predict Foetal and Infant Development?
A Study of Mothers and Infants in Rural India

Mother's Name: ……..

Mother's Address: ……..

Telephone Number: ……..

Study ID Number: ☐

Date: ……..

(To be detached and stored separately)

DEPARTMENT OF
PSYCHIATRY
UNIVERSITY OF OXFORD

ST. JOHN'S RESEARCH
INSTITUTE
ST. JOHN'S NATIONAL
ACADEMY OF HEALTH
SCIENCES

PHASE I – PRENATAL ASSESSMENT

SOLUR BABY PROJECT

Does Prenatal Maternal Depression Predict Foetal and Infant Development?
A Study of Mothers and Infants in Rural India

Study ID Number: ☐

Date: ……

(To be retained on questionnaire pack)

SOCIODEMOGRAPHIC DATA SHEET

i. Age:

ii. Education: a. Uneducated b. Primary School c. Secondary School
d. Higher Secondary School e. Higher education

iii. Occupation:

iv. Husband's age:

v. Husband's education: a. Uneducated b. Primary School
c. Secondary School d. Higher Secondary School
e. Higher education

vi. Husband's occupation:

vii. Marital Status: married/unmarried

viii. Age at marriage:

ix. Consanguinity:

xii. Religion:

xiii. How many people live in the same house as you?

xiv. Type of family: a. Nuclear b. Joint
c. Three generation family d. Other

xv. Approximate total family income per month:

xvi. Is this enough to meet your daily needs?

xvii. Are you or your family in debt at present?

xviii. Have you ever been hungry in the past 6 months because you could not afford to buy food?

PRENATAL & ROUTINE MEDICAL DATA

To be extracted from hospital records:

I. From the Prenatal Hospital Record:

1. Last menstrual period:
2. Estimated date of delivery (EDD):
3. Most recent hemoglobin value:
4. Height at present (in cm.):
5. Weight at present (in kg.): Body Mass Index:
6. Current gestational age:
7. History of medical illness during pregnancy:
   a. Hypertension □
   b. Preeclampsia/Eclampsia (fits) □
   c. Gestational diabetes □
   d. Bleeding per vaginum □
   e. Decreased foetal movements □
   f. Premature contractions □
   g. Fever □
   h. Other
   …….....
8. Obstetric score: Gravida □ Para □ Abortion □
   Living □ Dead □

II. From the Last Trimester Ultrasound Scan

LMP: EDD: Gestational Age:
Presentation: Anomalies:
Amniotic fluid volume:
Estimated foetal weight: Crown Rump Length: Evidence of intra uterine growth retardation: Other:

To be answered by the participant:

1. Is this your first pregnancy? Yes/No

2. If no, could you please give us some details about your previous pregnancies, abortions and miscarriages:

Maternal Age	Outcome of pregnancy	Type of delivery	Sex of child	Current status

Rate outcome of pregnancy as follows:
1 = Live birth
2 = Still birth/dead on birth
3 = Abortion
4 = Miscarriage

Rate type of delivery as follows:
1 = Normal vaginal delivery
2 = LSCS
3 = Forceps
4 = Vacuum extraction

3. Is this current pregnancy a planned pregnancy? Yes/No

4. Did you conceive spontaneously or did you have difficulty conceiving?

5. Have you attended checkups with the doctor at least once every 3 months during your pregnancy? Yes/No

6. Have any of your children ever died? Yes/No
   If yes, at what age? …….
   What was the cause?.....

7. Have you suffered from any medical illness in the past? Yes/No
   Epilepsy □ Asthma □
   Heart Disease □ Hypertension □
   Diabetes □ Other ……..

8. Have you ever suffered from any mental illness? Yes/No
   If yes:
   Depression □ Fear □ Anxiety □
   Schizophrenia □ Psychoses □

   How long ago was it? …….

How many episodes do you suffer from in a year? …….
Did you visit a doctor and receive medical attention and/or treatment
for your condition? Yes/No
Are you currently on any medication? Yes/No
Please give details of the same ……..

9. Do you smoke? Yes/No
   a. How much did you smoke per day before you became pregnant? ……
   b. How much do you smoke per day now? ……

10. Do you drink alcohol? Yes/No
    a. How much alcohol did you consume per day before you became pregnant?
    ……

    b. How much do you drink per day now?.....

11. Has anyone in your family been ill in the past 6 months? Yes/No
    Details…….
    .

12. Has anyone in your family ever suffered from mental illness? Yes/No
    If yes, how is this person related to you? ……..
    What did this person suffer from? ……..……
    Was this person treated or is this person undergoing treatment? Yes/No

13. Does anyone in your family drink alcohol
    so much that it bothers you? Yes/No
    If yes, how is this person related to you? ……..
    How much on an average does this person drink a day? ……...

14. What sex would you prefer your baby to be?

    Male □ Female □

APPENDIX IIB: THE EDINBURGH POSTNATAL DEPRESSION SCALE (ENGLISH)

We are now going to ask you about how you have felt in the past 7 days. Please indicate the answer for each question that comes closest to describing how you have felt in the past 7 days.

1. I have been able to laugh and see the funny side of things
   - □ As much as I always could
   - □ Not quite so much now
   - □ Definitely not so much now
   - □ Not at all

2. I have looked forward with enjoyment to things
   - □ As much as I ever did
   - □ Rather less than I used to
   - □ Definitely less than I used to
   - □ Hardly at all

3. I have blamed myself unnecessarily when things went wrong
   - □ Yes, most of the time
   - □ Yes, some of the time
   - □ Not very often
   - □ No, never

4. I have been anxious or worried for no good reason
   - □ No, not at all
   - □ Hardly ever
   - □ Yes, sometimes
   - □ Yes, very often

5. I have felt scared or panicky for no very good reason
   - □ Yes, quite a lot
   - □ Yes, sometimes
   - □ No, not much
   - □ No, not at all

6. Things have been getting on top of me
   - □ Yes, most of the time I haven't been able to cope at all
   - □ Yes, sometimes, I haven't been coping as well as usual
   - □ No, most of the time, I have coped quite well
   - □ No, I have been coping as well as ever

7. I have been so unhappy that I have had difficulty sleeping
   - □ Yes, most of the time

- Yes, sometimes
- Not very often
- No, not at all

8. I have felt sad or miserable
   - Yes, most of the time
   - Yes, quite often
   - Not very often
   - No, not at all

9. I have been so unhappy that I have been crying
   - Yes, most of the time
   - Yes, quite often
   - Only occasionally
   - No, never

10. The thought of harming myself has occurred to me
    - Yes, quite often
    - Sometimes
    - Hardly ever
    - Never

APPENDIX IIB: THE EDINBURGH POSTNATAL DEPRESSION SCALE (KANNADA)

ಪ್ರಶ್ನೆ 1.

ಮುಂಚೆ ಒಂದು ವಾರದ ದಿನಗಳು:

1. ನೀವು ಏನಾದರು ತಮಾಷೆ ವಸ್ತು ನೋಡುವಾಗ ಮುಂಚೆತರ ನಗುತ್ತೀರಾ.
   . ಮುಂಚೆತರಾ
   . ಸ್ವಲ್ಪ ಕಡಿಮೆ
   . ಜಾಸ್ತಿ ಕಡಿಮೆ
   . ಇಲ್ಲವೆ ಇಲ್ಲ

2. ಮುಂದೆ ಬರುವ ವಸ್ತುವನ್ನು ನೋಡಿ ಸಂತೋಷ ಪಡುವುದು.
   . ನಾನು ಯಾವಾಗಲು ಮಾಡದೇ ಇರುವುದು
   . ಅದಕ್ಕಿಂತ ಕಡಿಮೆ ಉಪಯೋಗಿಸುವುದು.
   . ಕಡಿಂತವಾಗ ಕಡಿಮೆಗಿಂತ ಉಪಯೋಗಿಸುವುದು.
   . ಇಲ್ಲವೇ ಇಲ್ಲ.

3. ಕಾರಣವಿಲ್ಲದೆ ತಪ್ಪು ನಡೆದಾಗ ನನ್ನ ತಪ್ಪನು ನಾನೆ ತಿಳಿಸಿಕೊಳ್ಳುತ್ತೆಲೆ
   . ಹೌದು, ಬಹಳ ಸಮಯ
   . ಹೌದು, ಕೆಲವು ಸಮಯ
   . ಇಲ್ಲ, ಕಲವೊಮ್ಮೆ
   . ಇಲ್ಲ, ಇಲ್ಲವೆ ಇಲ್ಲ

4. ಕಾರಣವಿಲ್ಲದೆ ನಾನು ಕಾತರಗೊಳ್ಳುವುದು ಅಥವಾ ಚಿಂತೆ ಪಡುವುದು
   . ಇಲ್ಲ, ಇಲ್ಲೆವೆ ಇಲ್ಲ,
   . ಅಪರೂಪ
   . ಹೌದು, ಕಲವೊಮ್ಮೆ
   . ಹೌದು, ಯಾವಾಗಲು

5. ಕಾರಣವಿಲ್ಲದೆ ಕಾತರಗೊಳ್ಳುವುದು ಅಥವಾ ಭಯಪಡುವುದು
   . ಹೌದು, ಸ್ವಲ್ಪ ಜಾಸ್ತಿ
   . ಹೌದು, ಕಲವೊಮ್ಮೆ
   . ಇಲ್ಲ, ತುಂಬ ಇಲ್ಲ
   . ಇಲ್ಲ, ಇಲ್ಲವೇ ಇಲ್ಲ.

6. ಕಷ್ಟಗಳು ನನ್ನ ಕೈ ಮೀರಿ ಬಂದಾಗ
   . ಹೌದು, ಬಹಳ ಸಮಯ ನನ್ನ ಕಷ್ಟವನ್ನು ನಾನೇ ಬಗೆಹರಿಸಲು ಆಗುವುದಿಲ್ಲ
   . ಹೌದು ಕಲವೊಮ್ಮೆ ಅವರ ಜೊತೆ ಏಗುವುದಕ್ಕೆ ಆಗುವುದಿಲ್ಲ.
   . ಇಲ್ಲ, ಬಹಳ ಸಮಯ ಅವರ ಜೊತೆ ಸಹಕರಿಸಿದ್ದೇನೆ
   . ಇಲ್ಲ, ನಾನು ಯಾವಾಗಲು ಸಹಕರಿಸುತ್ತೇನೆ.

7. ನಾನು ನಿದ್ದೆ ಮಾಡಲು ಕಷಪಡುತ್ತಿದೆ. ಆದ್ದರಿಂದ ನಾನು ಬಹಳ ಉದ್ವಗ್ನಗೊಂಡೆ
   . ಹೌದು, ಬಹಳ ಸಮಯ
   . ಹೌದು, ಕೆಲವು ಸಮಯ
   . ಇಲ್ಲ, ಕಲವೊಮ್ಮೆ
   . ಇಲ್ಲ, ಇಲ್ಲವೆ ಇಲ್ಲ

8. ನನ್ನಗೆ ಬಹಳ ಬೇಸರಗೊಂಡಾಗ
   . ಹೌದು, ಬಹಳ ಸಮಯ
   . ಹೌದು, ಸ್ವಲ್ಪ ಸಮಯ
   . ಬಿಟ್ಟು, ಬಿಟ್ಟು
   . ಇಲ್ಲವೇ ಇಲ್ಲ

9. ನಾನು ಬಹಳ ಅಳುತ್ತೀದ್ದೆ. ಆದ್ದರಿಂದ ನಾನು ಬಹಳ ಉದ್ವಗ್ನಗೊಂಡೆ
   . ಹೌದು, ಬಹಳ ಸಮಯ
   . ಹೌದು, ಸ್ವಲ್ಪ ಸಮಯ
   . ಬಿಟ್ಟು ಬಿಟ್ಟು.
   . ಇಲ್ಲವೇ ಇಲ್ಲ.

10. ನನಗೆ ನಾನೇ ನೋವು ಉಂಟುಮಾಡಿಕೊಂಡಾಗ ಅದು ನನಗೆ ನೋವು ಉಂಟಾಗುತ್ತದೆ.
   . ಹೌದು, ಬಟ್ಟು ಬಿಟ್ಟು
   . ಕೆಲವೊಮ್ಮೆ
   . ಯಾವಾಗಲು ಇಲ್ಲ
   . ಇಲ್ಲವೇ ಇಲ್ಲ.

APPENDIX IIC: THE KESSLER 10 SCALE OF PSYCHOLOGICAL DISTRESS (ENGLISH)

The next questions are about how you have been feeling in the past 4 weeks. Kindly indicate the answer that comes closest to how you have felt in the past 4 weeks.

In the past 4 weeks:	None of the time	A little of the time	Some of the time	Most of the time	All of the time
How often did you feel worn out for no good reason?					
About how often did you feel nervous?					
About how often did you feel so nervous that nothing could calm you down?					
About how often did you feel hopeless?					
About how often did you feel restless or fidgety?					
About how often did you feel so restless you could not sit still?					
About how often did you feel depressed?					
About how often did you feel that everything was an effort?					
About how often did you feel so sad that nothing could cheer you up?					
About how often did you feel worthless?					

APPENDIX IIC: THE KESSLER 10 SCALE OF PSYCHOLOGICAL DISTRESS (KANNADA)

ಪ್ರಶ್ನಾವಳಿ - 2

ಕಳೆದ 4 ವಾರಗಳಲ್ಲಿ ನಿಮಗಾದ ಅನುಭವವನ್ನು ಈ ಕೆಳಗಿನ 10 ಪ್ರಶ್ನೆಗಳನ್ನು ಉತ್ತರಿಸುವುದರ ಮೂಲಕ ತಿಳಿಸಿರಿ.

		ಯಾವಾಗಲೂ ಇಲ್ಲ	ಕೆಲವು ಸಾರಿ	ಹೆಚ್ಚು ಸಾರಿ	ಸ್ವಲ್ಪ	ಎಲ್ಲಾ ಸಮಯದಲ್ಲಿ
1	ಕಳೆದ 4 ವಾರಗಳಲ್ಲಿ ನಿಮಗೆ ಎಷ್ಟು ಸಾರಿ ನಿಮಗೆ ಸತ್ತಾಳ (ಶಕ್ತಿಗುಂದಿದ) ಹಾಗೆ ಆಯಿತು ?					
2	ಕಳೆದ 4 ವಾರಗಳಲ್ಲಿ ನಿಮಗೆ ಎಷ್ಟು ಸಾರಿ ಧೈರ್ಯ ಹೀನವಾಯಿತು ?					
3	ಕಳೆದ 4 ವಾರಗಳಲ್ಲಿ ನಿಮಗೆ ಹೀನವಾಗಿ ಯಾವುದೇ ಸಂಗತಿ ಸಮಾಧಾನ ಪಡಿಸದೇ ಇರುವಂತಾದು ?					
4	ಕಳೆದ 4 ವಾರಗಳಲ್ಲಿ ನಿಮಗೆ ಎಷ್ಟು ಸಾರಿ ನಿರಾಶೆಯಾಯಿತು ?					
5	ಕಳೆದ 4 ವಾರಗಳಲ್ಲಿ ನಿಮಗೆ ಎಷ್ಟು ಸಾರಿ ಅಶಾಂತವೆನಿಸಿತು ?					
6	ಕಳೆದ 4 ವಾರಗಳಲ್ಲಿ ನಿಮಗೆ ಅಶಾಂತವಾಗಿ ಸುಮ್ಮನೆ ಕೂತುಕೊಳ್ಳದೆ ಇರುವಂತಹ ಸ್ಥಿತಿ ?					
7	ಕಳೆದ 4 ವಾರಗಳಲ್ಲಿ ನಿಮಗೆ ಎಷ್ಟು ಸಾರಿ ನಿರುತ್ಸಾಹವೆನಿಸಿತು ?					
8	ಕಳೆದ ವಾರದಲ್ಲಿ ನಿಮಗೆ ಎಷ್ಟು ಎಲ್ಲವೂ ಪ್ರಯತ್ನವೆನಿಸಿತು ?					
9	ಕಳೆದ ವಾರದಲ್ಲಿ ಎಷ್ಟು ಸಾರಿ ನಿಮಗೆ ಯಾವುದು ಪ್ರೋತ್ಸಾಹಿಸುವುದಿಂದೆನಿಸಿತು ?					
10	ಕಳೆದ ವಾರದಲ್ಲಿ ಎಷ್ಟು ಸಾರಿ ನಿಮಗೆ ಪ್ರಯೋಜನವಿಲ್ಲಂದೆನಿಸಿತು ?					

APPENDIX IID: THE MINI INTERNATIONAL NEUROPSYCHIATRIC INTERVIEW V.5.0.0 – MODULE FOR MAJOR DEPRESSIVE DISORDER

A. Major Depressive Disorder

(➡ means go to the diagnostic boxes, circle NO in all diagnostic boxes and move to the next module)

A1	a	Have you **ever** been consistently depressed or down, most of the day, nearly every day, for at least two weeks? If A1a = YES	NO	YES
	b	Have you been consistently depressed or down, most of the day, nearly every day, for the past 2 weeks?	NO	YES
A2	a	Have you **ever** been much less interested in most things or much less able to enjoy the things you used to enjoy most of the time over at least 2 weeks? If A2a = YES	NO	YES
	b	In the past 2 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 **A1a** or **A2a** coded YES?	NO ➡	YES

IF CURRENTLY DEPRESSED (A1b OR A2b = YES) , EXPLORE THE CURRENT DEPRESSIVE EPISODE AND THE MOST SYMPTOMATIC PAST EPISODE OTHERWISE EXPLORE THE MOST SYMPTOMATIC PAST EPISODE

A3		Over the 2 week period when you felt depressed or uninterested,	Current Episode		Past Episode	
	a	Was your appetite decreased or increased nearly every day? Did your weight decrease or increase without trying intentionally (i.e. by ± 5% of body weight or ± 8lbs. or ± 3.5 kgs. for a 160 lbs./70 kgs. person in a month)?	NO	YES	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	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	NO	YES
	d	Did you feel tired or without energy almost every day?	NO	YES	NO	YES
	e	Did you feel worthless or guilty almost every day?	NO	YES	NO	YES

	f	Did you have difficulty concentrating or making decisions almost every day?	NO	YES	NO	YES
	g	Did you repeatedly consider hurting yourself, feel suicidal, or wish that you were dead?	NO	YES	NO	YES

IF A3e=YES: ASK FOR AN EXAMPLE; THE EXAMPLE IS CONSISTENT WITH A DELUSIONAL IDEA
□ No □ Yes

A4		Are **3** or more **A3** answers coded **YES** (or **4 A3** answers if **A1a** or **A2a** are coded **NO** for the past episode or if **A1b** or **A2b** are coded **NO** for the current episode)?	NO	YES	NO ➡	YES
		VERIFY IF THE POSITIVE SYMPTOMS OCCURRED DURING THE SAME 2 WEEK TIME FRAME				
		IF A4 IS CODED **NO** FOR CURRENT EPISODE THEN EXPLORE **A3a-A3g** FOR MOST SYMPTOMATIC PAST EPISODE				
A5		Did 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
A6		Are the symptoms due entirely to the loss of a loved one (bereavement) and are they similar in severity, level of impairment, and duration to what most others would suffer under similar circumstances? If so, this is uncomplicated bereavement.				
		HAS UNCOMPLICATED BEREAVMENT BEEN RULED OUT?			NO ➡	YES
A7	a	Were you taking any drugs or medicines just before these symptoms began? □No □Yes				
	b	Did you have any medical illness just before these symptoms began? □No □Yes				
		IN THE CLINICIAN'S JUDGEMENT, ARE THESE LIKELY TO BE DIRECT CAUSES OF THE PATIENT'S DEPRESSION? IF NECESSARY ASK ADDITIONAL OPEN-ENDED QUESTIONS				
		A7 (SUMMARY): HAS AN ORGANIC CAUSE BEEN RULED OUT?	NO	YES	UNCERTAIN	

A8 Code **YES** if **A7(SUMMARY) = YES** or **UNCERTAIN**
SPECIFY IF THE EPPISODE IS CURRENT
AND/OR PAST OR BOTH (RECURRENT)

NO	**YES**
Major Depressive Episode	
Current □ Past □	

A9 Code **YES** if **A7b = YES** And **A7(SUMMARY)=NO**
SPECIFY IF THE EPPISODE IS CURRENT
AND/OR PAST OR BOTH (RECURRENT)

NO	**YES**
Mood disorder due to a general medical condition	
Current □ Past □	

A10 Code **YES** if **A7a = YES** And **A7(SUMMARY)=NO**
SPECIFY IF THE EPPISODE IS CURRENT
AND/OR PAST OR BOTH (RECURRENT)

NO	**YES**
Substance Induced Mood Disorder	
Current □ Past □	

CHRONOLOGY:

A11 How old were you when you first began having symptoms of depression? _____ age

A12 During your lifetime, how many distinct times did you have these symptoms of depression (daily for at least 2 weeks)?

_____

A13 Is there any family history of bipolar disorder or any relative ever treated with a mood stabilizer? Yes/No

APPENDIX IIE: SOCIAL SUPPORT QUESTIONNAIRE

We will now ask you some questions about the help your family, friends and neighbours give you in particular circumstances and about your relationship with your partner.

(1) Do you feel that there are enough people in your environment that would help you with your daily chores if you were sick (like cooking, cleaning, shopping, fetching water)?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(2) Do you feel there are enough people in your environment that would look after your children / your needs at home if you were called away for an emergency?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(3) Do you feel there are enough people in your environment that would lend or give you something that you need (like food, clothing, money)?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(4) Do you feel there are enough people in your environment that would take you or your child / your family members to the doctor in case of an emergency?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(5) Do you feel there are enough people in your environment to give you advice (e.g., specific suggestions on what to do when your child/family member has a health problem, or advice on your household management or financial matters?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(6) Do you feel there are enough people in your environment to give you the information you need (e.g., people that can tell you about all the possible people you can go to if your child/family member needs a tutor, or that can tell you what

the options are for helping your sick family member: going to the doctor, eating a particular type of food?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(7) Do you feel there are enough people in your environment to talk about things that are very personal and private, like difficulties in your relationship with your husband, family matters, physical complaints, family planning?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(8) Do you feel there are enough people in your environment that listen to you when you want to talk about your sorrows?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(9) Do you feel there are enough people that can comfort you when you feel unhappy about your daily life?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(10) Do you feel there are enough people in your environment that show interest and concern in your well-being (e.g., when you are sick)?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(11) Do you feel there are enough people in your environment that tell you that you did a good job in handling a problem (e.g., your child's difficult behaviour or your child's health problem/family member, or a problem at work or in your household)?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(12) Do you feel there are enough people in your environment that express their respect for one of your personal qualities (e.g., your personal strength in facing

difficulties, being a very friendly person, helping other people when they have problems)?

- Definitely not enough
- Not enough
- Enough
- Definitely enough

(13) Does your partner scream or yell at you?

- Never
- Rarely (once a year or less)
- Occasionally (once in a few months)
- Frequently (once a month or more but not on a regular basis)
- Very frequently (more than once a week or everyday)

(14) Has your partner ever hit you? Yes/No

If yes continue with questions (15) to (20). If no, go to Questionnaire 4.

(15) Does your partner punch you with his fists?

- Never
- Rarely (once a year or less)
- Occasionally (once in a few months)
- Frequently (once a month or more but not on a regular basis)
- Very frequently (more than once a week or everyday)

(16) Does your partner slap your face and/or head?

- Never
- Rarely (once a year or less)
- Occasionally (once in a few months)
- Frequently (once a month or more but not on a regular basis)
- Very frequently (more than once a week or everyday)

(17) Does your partner kick/punch you in the abdomen?

- Never
- Rarely (once a year or less)
- Occasionally (once in a few months)
- Frequently (once a month or more but not on a regular basis)
- Very frequently (more than once a week or everyday)

(18) Does your partner threaten you with a weapon e.g. knife, stick, belt, iron rod?

- Never
- Rarely (once a year or less)

- Occasionally (once in a few months)
- Frequently (once a month or more but not on a regular basis)
- Very frequently (more than once a week or everyday)

(19) Does your partner beat you so badly that you have had to visit a doctor or nurse?

- Never
- Rarely (once a year or less)
- Occasionally (once in a few months)
- Frequently (once a month or more but not on a regular basis)
- Very frequently (more than once a week or everyday)

(20) Does your partner frighten you?

- Never
- Rarely (once a year or less)
- Occasionally (once in a few months)
- Frequently (once a month or more but not on a regular basis)
- Very frequently (more than once a week or everyday)

APPENDIX IIF: POSTNATAL ASSESSMENT DATA SHEET

DEPARTMENT OF
PSYCHIATRY
UNIVERSITY OF OXFORD

ST. JOHN'S RESEARCH
INSTITUTE
ST. JOHN'S NATIONAL
ACADEMY OF HEALTH
SCIENCES

PHASE II – POSTNATAL ASSESSMENT

SOLUR BABY PROJECT

Does Prenatal Maternal Depression Predict Foetal and Infant Development?
A Study of Mothers and Infants in Rural India

Study ID Number: ☐

Date: …….

(To be retained on questionnaire pack)

POSTNATAL AND OBSTERIC OUTCOME DATA SHEET

I. Maternal Postnatal Data

*to be extracted from hospital records

1. Date of delivery*:
2. Type of delivery*: a. Normal b. LSCS c. Forceps
   d. Vacuum extraction e. Breech presentation
3. Outcome of pregnancy*: a. Healthy baby b. Still born c. Congenital abnormalities
4. Labour complications*: a. Prolonged labour b. Premature rupture of membranes
   c. Abnormal presentation d. Foetal distress
   e. Other…….
5. Postnatal complications: a. Prolonged bleeding following delivery b. Fever
   c. Urinary tract infection d. Other……..
6. Have you been admitted to hospital after your delivery? Yes/No
   If yes, what was the reason for your admission to hospital?.....
   ……
   How long were you admitted in hospital for? ……

II. Baby's Data

7. Baby's sex:
8. Gestational age at birth*:
   a. Preterm (<37 weeks)
   b. Term (37-40 weeks)
   c. Post term (>40 weeks)
9. Birth weight (in gm.)*:
10. Birth length (in cm.)*:
11. Head circumference at birth (in cm.)*:
12. Following delivery, has your baby suffered from: Fever □ Birth asphyxia □
    Respiratory distress □ Seizures □ Meconium aspiration □ Jaundice □
13. Was your baby admitted to the neonatal intensive care unit following delivery?
    Yes/No

If yes, what was the reason for the admission? ……

How long was your baby admitted there for? ……..

14. In the past 1-3 months, has your baby had:
    Fever □ Diarrhoea □ Vomiting □ Cough □ Excessive crying □

15. Has your baby been admitted to hospital after delivery? Yes/No
    If yes, what was the reason for the admission? ……
    How long was your baby admitted there for? ……..

16. Are you happy with the sex of your baby? Yes/No

17. Is your family happy with the sex of your baby? Yes/No

18. If no, does your family give you a hard time and/or
    blame you for your baby's sex? Yes/No

19. Do you plan to have more children? Yes/No

20. If yes, which sex would you prefer your next baby to be? Male □ Female □

21. Has your infant been immunised with the following vaccines?

Age of infant→ Vaccines↓	At birth	6 weeks	10 weeks	14 weeks
	BCG	DPT - 1	DPT - 2	DPT - 3
	OPV – 0 dose	OPV – 1	OPV – 2	OPV – 3

APPENDIX IIG: THE INFANT BEHAVIOUR QUESTIONNAIRE (ENGLISH)

We will now ask you a few questions about your infant's behaviour and how your baby responds to you.

We are interested to see how your baby has been behaving in certain situations in the **LAST WEEK**.

Please indicate how often the baby did this during the **LAST WEEK**

1 Never	2 Very rarely	3 Less than half the time	4 About half the time	5 More than half the time	6 Almost always	7 Always	X Does not apply

The "does not apply" column is used when you did not see your baby in that situation.

"Never" should be circled if you did see your baby in that situation but the baby never engaged in the behaviour.

Please **indicate** an option for every item.

Feeding									
When having to wait for food or liquids during the last week, how often did your baby:									
1.	Seem not bothered	1	2	3	4	5	6	7	X
2.	Show mild fussing	1	2	3	4	5	6	7	X
3.	Cry loudly	1	2	3	4	5	6	7	X
During feeding, how often did your baby:									
4.	Lie or sit quietly?	1	2	3	4	5	6	7	X
5.	Squirm or kick?	1	2	3	4	5	6	7	X
6.	Wave arms?	1	2	3	4	5	6	7	X
7.	Fuss or cry?	1	2	3	4	5	6	7	X
When given a new food or liquid, how often did your baby:									
8.	Accept it immediately?	1	2	3	4	5	6	7	X
9.	Reject it by spitting out, closing mouth etc.?	1	2	3	4	5	6	7	X
10.	Not accept it no matter how many times it was offered?	1	2	3	4	5	6	7	X

Sleeping									
Before falling asleep at night during the last week, how often did your baby:									
11.	Show no fussing or crying	1	2	3	4	5	6	7	X
During sleep how often did your baby:									
12.	Toss about?	1	2	3	4	5	6	7	X
13.	Move from the middle of the cot to the end of the cot?	1	2	3	4	5	6	7	X
14.	Sleep in one position only?	1	2	3	4	5	6	7	X
On waking, how often did your baby:									
15.	Fuss or cry immediately?	1	2	3	4	5	6	7	X
16.	Lie quietly in his/her cot?	1	2	3	4	5	6	7	X
17.	Coo or “talk”?	1	2	3	4	5	6	7	X
18.	Cry within a few minutes?	1	2	3	4	5	6	7	X
How often did your baby?									
19.	Seem angry if left in his/her cot?	1	2	3	4	5	6	7	X
20.	Seem happy if left in his/her cot?	1	2	3	4	5	6	7	X
21.	Cry or fuss before going to sleep?	1	2	3	4	5	6	7	X

Bathing and Dressing									
When being dressed or undressed, during the last week, how often did your baby:									
22.	Wave his/her arms and kick?	1	2	3	4	5	6	7	X
23.	Squirm?	1	2	3	4	5	6	7	X
24.	Smile or laugh?	1	2	3	4	5	6	7	X
When put into the bath how often did your baby:									
25.	Startle? (gasp, throw out arms)	1	2	3	4	5	6	7	X
26.	Smile?	1	2	3	4	5	6	7	X
27.	Splash or kick?	1	2	3	4	5	6	7	X
28.	Look surprised?	1	2	3	4	5	6	7	X
When placed in/on a mat/swing/cot/chair, how often did your baby:									
29.	Wave arms and kick?	1	2	3	4	5	6	7	X

30.	Squirm and turn body?	1	2	3	4	5	6	7	X
31.	Lie or sit quietly?	1	2	3	4	5	6	7	X
32.	Show distress at first then quiet down?	1	2	3	4	5	6	7	X
When you returned from having been away, and your baby was awake, how often did s/he:									
33.	Smile or laugh?	1	2	3	4	5	6	7	X
When introduced to a strange person, how often did your baby:									
34.	Refuse to go to the stranger?	1	2	3	4	5	6	7	X
35.	Never "warm up" to the stranger?	1	2	3	4	5	6	7	X
36.	Smile?	1	2	3	4	5	6	7	X

Soothing techniques									
Have you tried any of the following soothing techniques in the last two weeks? If so, how often did the method soothe your baby? Mark X if you did not try the technique in the LAST TWO WEEKS									
37.	Rocking	1	2	3	4	5	6	7	X
38.	Holding	1	2	3	4	5	6	7	X
39.	Singing or talking	1	2	3	4	5	6	7	X
40.	Walking with your baby	1	2	3	4	5	6	7	X
41.	Giving the baby a toy	1	2	3	4	5	6	7	X
42.	Showing the baby something to look at	1	2	3	4	5	6	7	X
43.	Patting or gently rubbing some part of your baby's body	1	2	3	4	5	6	7	X
44.	Offering food or liquid	1	2	3	4	5	6	7	X
45.	Offering baby his/her dummy or security object	1	2	3	4	5	6	7	X
46.	Changing your baby's position	1	2	3	4	5	6	7	X
47.	Other	1	2	3	4	5	6	7	X

APPENDIX IIG: THE INFANT BEHAVIOUR QUESTIONNAIRE (KANNADA)

IBQ ROTHBART

ಪ್ರಶ್ನಾವಳಿ-4

ನಾವು ಈಗ ನಿಮ್ಮ ಮಗುವಿನ ನಡೆತೆ ಮತ್ತು ಹ್ಯಾಗೆ ನಿಮ್ಮ ಮಗುವಿನ ಪ್ರತಿಕ್ರಿಯೆ ನಿಮಗೆ ಆಯಿತೆಂದು ಕೆಲವು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳುತ್ತೇವೆ

ನಿಮ್ಮ ಮಗುವಿನ ನಡೆತೆ ಕೆಲವು ಸಂದರ್ಭದಲ್ಲಿ ಹ್ಯಾಗೆ ಇತ್ತು ಎಂಬುದಾಗಿ ನೋಡಲು ನಾವು ಆಸಕ್ತಿ ನೋಡುತ್ತೇವೆ.

1	2	3	4	5	6	7	×
ಯಾವಾಗಲೂ ಇಲ್ಲ	ತುಂಬಾ ಅಪರೂಪ	ಅರ್ಧಕ್ಕಿಂತ ಹೆಚ್ಚು	ಅರ್ಧ ಸಮಯ	ಅರ್ಧ ಸಮಯಕ್ಕಿಂತ ಹೆಚ್ಚು	ಹೆಚ್ಚಾಗಿ ಯಾವಾಗಲು	ಯಾವಾಗಲು	ನಿಮಗೆ ಅನ್ವಯಿಸುವುದಿಲ್ಲ

ತಿನ್ನಿಸುವುದು

ನಿಮ್ಮ ಮಗುವಿನ ಆಹಾರ ಅಥವಾ ಪಾನೀಯ ಕೊಡಲು ಕಾಯುವಾಗ ಕಳೆದ ವಾರ ಎಷ್ಟು ಸಾರಿ ?

		1	2	3	4	5	6	7	×
1	ಯಾವ ಗಲಾಟೆ ಮೊದಲಿಲ್ಲ								
2	ಸ್ವಲ್ಪ ಗಲಾಟೆ ಮಾಡುವುದು								
3	ಜೋರಾಗಿ ಅಳುವುದು ತಿನ್ನಿಸುವ ಸಮಯದಲ್ಲಿ ಎಷ್ಟು ಸಲ								
4	ಬಿದ್ದುಕೊಳ್ಳುವುದು ಅಥವಾ ಮೌನದಲ್ಲಿ ಕುಳಿತುಕೊಳ್ಳುವುದ ?								
5	ಮೈ ಮುರಿಯುವುದು ಅಥವಾ ಒದೆಯುವುದು								
6	ಕೈ ಅಲ್ಲಾಡಿಸುವುದ ?								

7	ಗಲಾಟೆ ಅಥವಾ ಕೂಗುವುದು ನಿಮ್ಮ ಮಗುವಿನ ಹೊಸ ಆಹಾರ ಅಥವಾ ಪಾನಿಯ ಕೊಡುವಾಗ ಎಷ್ಟು ಸಾರಿ								
8	ಕೂಡಲೆ ಸ್ವೀಕಾರ ಮಾಡುವುದ ?								
9	ಉಗುಳಿ ಹೊರಗೆ ಹಾಕು ಬಾಯಿ ಮುಚ್ಚಿಕೊಂಡಿರುವುದಾ ?								
10	ಎಷ್ಟು ಸಾರಿ ಕೊಟ್ಟರೂ ಸ್ವೀಕಾರ ಸ್ವೀಕಾರ ಮಡುವುದಿಲ್ಲವಾ								

ನಿದ್ರಿಸುವುದು

ಕಳೆದ ವಾರ ರಾತ್ರಿ ನಿದ್ದೆ ಮಾಡುವ ಮೊದಲು ಎಷ್ಟು ಸಾರಿ ಎದ್ದೇಳಿತು

		1	2	3	4	5	6	7	x
11	ಗಲಾಟೆ ಅಥವಾ ಕೂಗುವುದು ನಿದ್ರೆಯಲ್ಲಿದ್ದಾಗ ಎಷ್ಟು ಸಲ ಏನು ಮಾಡಿತು								
12	ಅಲ್ಲಾಡಿದೆ								
13	ಮಂಚದ ನಡುವಿನಿಂದ ಕೊನೆಗೆ								
14	ಒಂದೇ ಸ್ಥಾನದಲ್ಲಿ ಮಾತ್ರ ಮಲಗಿದೆ ಎಬ್ಬಿಸಿದಾಗ ನಿನ್ನ ಎಷ್ಟು ಸಾರಿ								
15	ಗಲಾಟೆ ಅಥವಾ ಕೂಡಲೆ ಕೂಗಿದೆ								
16	ಮೌನವಾಗಿ ಅವನ / ಅವಳ ಮಂಚದಲ್ಲಿ ಬಿದ್ದಿರುವುದು								

17	ಹೂಹೂ ಅಥವಾ ಮಾತನಾಡು								
18	ಸ್ವಲ್ಪ ನಿಮಿಷದಲ್ಲೇ ಕೂಗಿತು ಎಷ್ಟು ಸಾರಿ ನಿನ್ನ ಮಗು ?								
19	ಅವನನ್ನು / ಅವಳನ್ನು ಮಂಚದಲ್ಲಿ ಹಾಕಿದರೆ ಕೋಪದಲ್ಲಿ ಅಳುತ್ತದಾ ?								
20	ಒಂದು ವೇಳೆ ಮಂಚದಲ್ಲಿ ಬಿಟ್ಟರೆ ಸಂತೋಷ ಇದ್ದ ಹಾಗೆ ಇರುತ್ತದಾ ?								
21	ನಿದ್ದೆ ಮಾಡುವ ಮೊದಲು ಗಲಾಟೆ ಅಥವಾ ಕೂಗುತ್ತದಾ ?								

ಸ್ನಾನ ಮತ್ತು ಧರಿಸುವುದು

ಬಟ್ಟೆ ತೆಗೆಯುವಾಗ ಹಾಕುವಾಗ ಎಷ್ಟು ಸಾರಿ ನಿನ್ನ ಮಗು

22	ಅವನ / ಅವಳ ಕೈ ಅಲ್ಲಾಡಿಸು ಮತ್ತು ಒದೆಯುತ್ತದೆ								
23	ಮೈಮುರಿಯುವುದು								
24	ನಗೆ ಅಥವಾ ಕಿರುಚು ಸ್ನಾನ ಮಾಡುವಾಗ ಎಷ್ಟು ಸಾರಿ ನಿನ್ನ ಮಗು								
25	ಹೆದರಿಸು								
26	ನೆಗೆ								
27	ಸಿಡಿಯುವುದು ಅಥವಾ ಒದೆಯುತ್ತದೆ								
28	ಆಶ್ಚರ್ಯಕರವಾಗಿ ಕೂಗುತ್ತದಾ ?								

ಯಾವಾಗಲೂ ಕಾರ್ ಅಥವಾ ಮಗುವಿನ ಕುರ್ಚಿಯಲ್ಲಿ ಇಟ್ಟಾಗ ಎಷ್ಟು ಸಲ

29	ಕೈ ಅಲ್ಲಾಡಿಸು ಮತ್ತು ಒಡೆಯುತ್ತದೆ ?								
30	ಮೈ ಮುರಿದ ತಿರುಗಿದ								
31	ಮೌನವಾಗಿ ಬಿದ್ದು ಕೊಂಡಿದೆ ಅಥವಾ ಇಳಿದೆ								
32	ಮೊದಲು ಹೆದರಿಕೆ ಹೆಚ್ಚು ನಂತರ ಮೌನವಾಗಿರುತ್ತದೆ								

ನೀವು ಹೊರಗೆ ಹೋಗಿ ಬಂದಾಗ ಮಗು ಎಚ್ಚರಿಕೆ ಇದ್ದಾಗ ಎಷ್ಟು ಸಾರಿ ಅವನ / ಅವಳ .

33	ನೆಗೆದು ಕಿರುಚುವುದು								
34	ಪರಿಚಯ ಇದ್ದವರ ಹತ್ತಿರ ಹೋಗಲು ನಿರಾಕರಿಸುವಿರಾ ?								
35	ನೆಗೆ ಅಥವಾ ಕಿರುಚು								
36	ನೆಗಾಡು								

ಸಂತೈಸುವ ಕಲಾಕೌಶಲ್ಯ

ಕಳೆದ 2 ವಾರಗಳಲ್ಲಿ ಈ ಕೆಳಗಿನ ಕಲಾಕೌಶಲ್ಯದನು ಯಾವಾಗಲಾದರೂ ಪ್ರಯತ್ನಿಸಿದ್ದೀರಾ ? ಒಂದು ವೇಳೆ ಹೌದೆಂದಾದರೆ ಎಷ್ಟು ಸಾರಿ ನಿಮ್ಮ ಮಗುವಿಗೆ ಸಂತೈಸುವಿರಿ ? ಪ್ರಯತ್ನಿಸದಿದ್ದರೆ x ಚಿಹ್ನೆ ಹಾಕಿರಿ.

37	ಅಲ್ಲಾಡಿಸು								
38	ಹಿಡಿಯುವುದು								
39	ಹಾಡುವುದು ಅಥವಾ ಮಾತನಾಡುವುದು								

40	ಮಗುವಿನೊಂದಿಗೆ ಸಿಡಿಯುವುದಾ								
41	ಮಗುವಿಗೆ ಅಲ್ಪ ಸಾಮಾನು ಕೊಡುವುದು								
42	ಮಗುವಿಗೆ ಏನಾದರೂ ನೋಡುವ ಹಾಗೆ ಮಾಡುವುದಾ								
43	ಮಗುವಿಗೆ ದೇಹದ ಮೇಲೆ ಚುರುಕಾಗಿ ತಪ್ಪುವುದಾ								
44	ಆಹಾರ ಅಥವಾ ಪಾನಿಯ ಕೊಡುವುದಾ								
45	ಮಗುವಿಗೆ ಕೈಗೊಂಬೆ ಅಥವಾ ರಕ್ಷಣೆಯ ವಸ್ತು ಕೊಡುವುದು								
46	ನಿಮ್ಮ ಮಗುವಿನ ಸ್ಥಾನ ಬದಲಾವಣೆ								
47	ಇನ್ನಿತರ								

APPENDIX IIH: THE BREASTFEEDING QUESTIONNAIRE

We will now ask you some questions about the breastfeeding of your infant.

1. **Do you breastfeed your baby?**
   a. Yes
   b. No
   c. If no, what is the reason for not breastfeeding your baby?
   ……
   If no, go to question 10
   If yes, go to question 2

2. **How long after the delivery of your baby did you initiate breastfeeding?**.....

3. **Did you give your bay any prelacteal feeds?** Yes/No
   If yes, what was your baby given?
   a. Water
   b. Honey
   c. Cow's milk
   d. Other:

4. **Is your baby exclusively breastfed?** Yes/No
   If not, what do you feed your baby other than breast milk?
   ……

5. **Do you breastfeed your baby according to a particular time schedule (timed breastfeeding) or whenever your baby cries (demand feeding)?**
   a. Timed breastfeeding
   b. Demand breastfeeding
   c. A mix of both

6. **Approximately, how many times a day do you feed your baby?**.....

7. **How long (approximately) does each feed last?**
   ……..

8. **Do you have any of the following problems with breastfeeding your baby:**
   a. Insufficient milk
   b. Sore nipples
   c. Painful breast/breast abscess
   d. Other:

9. **Do you feel that your baby has the following problems with being breastfed:**
   a. Difficulty sucking
   b. Stops sucking very soon
   c. Refuses to suck
   d. Cries after breastfeeding
   e. Vomits after breastfeeding

Appendix III: Further Information on Data Processing

APPENDIX IIIA: SALIVARY ASSAY PROTOCOL

The following appendix presents the summary of the assay protocol as per the guideline of Salimetrics Inc. published in 'High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit' ©Salimetrics LLC, PA, USA 2011

Assay Summary

1. Bring all reagents to room temperature and mix before use.
2. Bring plate to room temperature and prepare for use with NSB wells. (Use of NSB wells is optional.)
3. Prepare 1X wash buffer.
4. Prepare tube with 24 mL of assay diluent for conjugate dilution, which will be made later.
5. Pipette 25 µL of standards, controls, and unknowns into appropriate wells.
6. Pipette 25 µL of assay diluent into zero and NSB wells.
7. Make final 1:1600 dilution of conjugate (15 µL into 24 mL assay diluent), mix, and immediately pipette 200 µL into each well. Note any pH indicator colour changes.
8. Mix plate for 5 minutes at 500 rpm. Incubate for an additional 55 minutes at room temperature.
9. Wash plate 4 times with 1X wash buffer. Blot.
10. Add 200 µL TMB solution to each well.
11. Mix plate for 5 minutes at 500 rpm. Incubate in dark at room temperature for 25 additional minutes.
12. Add 50 µL stop solution to each well. Mix for 3 minutes at 500 rpm.
13. Wipe plate bottom clean and read within 10 minutes of adding stop.

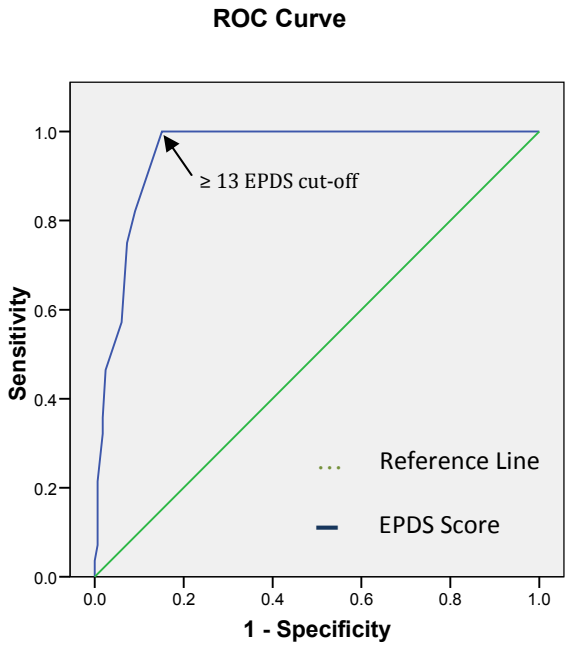
Calculations

1. Compute the average optical density (OD) for all duplicate wells.
2. Subtract the average OD for the NSB wells (if used) from the average OD of the zero, standards, controls, and unknowns.
3. Calculate the percent bound (B/Bo) for each standard, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo).
4. Determine the concentrations of the controls and unknowns by interpolation using software capable of logistics. We recommend using a 4-parameter sigmoid minus curve fit.
5. If a dilution of the sample is used, multiply the results by the dilution factor. Samples with cortisol values greater than 3.0 µg/dL (82.77 nmol/L) should be diluted with assay diluent and rerun for accurate results. When running multiple plates, or multiple sets of strips, a standard curve should be run with each individual plate and/or set of strips.

APPENDIX IV: RESULTS

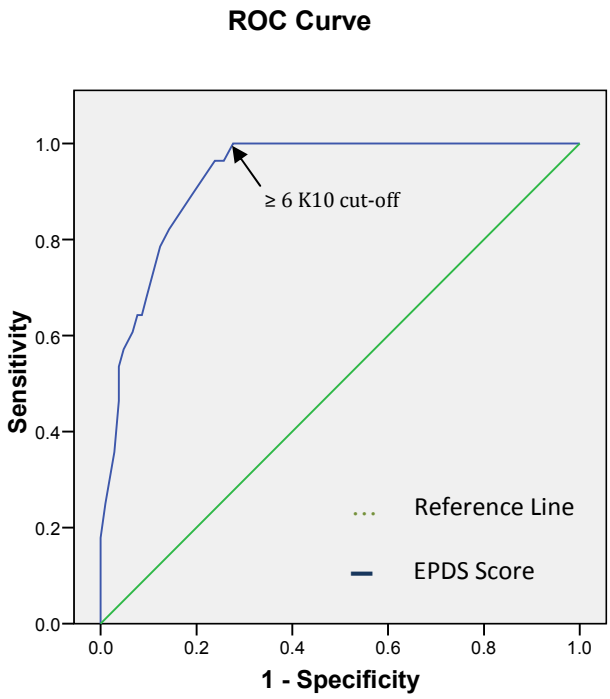
APPENDIX IVA: RECEIVER OPERATING CHARACTERISTICS (ROC) OF THE EPDS AND K10

ROC Curve for the EPDS predicting clinically diagnosed prenatal depression.



This Figure shows the Receiver Operating characteristic (ROC) Curve for the EPDS predicting clinically diagnosed depression – sensitivity vs. 1-specificity. Area under the curve = 0.95. Reference line indicates the performance of a test with no discrimination ability between cases and non-cases above that of chance.

ROC Curve for the K10 predicting clinically diagnosed prenatal depression.



This Figure shows the Receiver Operating characteristic (ROC) Curve for the K10 predicting clinically diagnosed depression – sensitivity vs. 1-specificity. Area under the curve = 0.95

APPENDIX IVB: TABLE SHOWING CORRELATIONS BETWEEN FHR VARIABLES

The correlations between FHR variables are presented below.

		Baseline FHR	Base. BBV	Stim. FHR	Stim. BBV	Sum AUC	Resp-onse Score	Post-stim.ion FHR	Post-stim. BBV	Delta FHR	Delta BBV
Baseline FHR	r	1	0.10	**0.72****	-0.14	**0.80****	-.17	**0.69****	-0.11	**-0.30****	**-0.17***
	Sig.		0.24	<0.01	0.15	<0.01	0.06	<0.01	0.21	<0.01	0.05
Baseline BBV	r			0.10	0.13	**0.18***	0.14	0.09	0.12	-0.02	**-0.64****
	Sig.			0.27	0.15	0.05	0.12	0.29	0.16	0.81	<0.01
Stim. FHR	r				0.03	**0.95****	**0.43****	**0.82****	**0.17***	**0.21***	0.06
	Sig.				0.74	<0.01	<0.01	<0.01	0.05	0.02	0.48
Stim.BBV	r					0.08	**0.34****	0.08	**0.38****	**0.27****	**0.20***
	Sig.					0.35	<0.01	0.38	<0.01	0.01	0.02
SumAUC	r						**0.35****	**0.81****	0.12	0.10	-0.03
	Sig.						<0.01	<0.01	0.16	0.25	0.72
Response Score	r							**0.21***	**0.42****	**0.47****	**0.23****
	Sig.							0.02	<0.01	<0.01	0.01
Post-stimulation FHR	r								0.11	**0.49****	0.02
	Sig.								0.22	<0.01	0.87
Post-stimulation BBV	r									**0.27****	**0.69****
	Sig.									0.01	<0.01
Delta FHR	r										**0.23****
	Sig.										0.01

**Correlation is significant at the 0.01 level
*Correlation is significant at the 0.05 level

Base. = baseline; stim. = stimulation r = correlation coefficient (Spearman)

APPENDIX IVC: FOETAL RESPONSIVITY: ASSOCIATIONS WITH FOETAL SEX (TABLE)

This table presents the findings of the analysis of the association between foetal responsivity and foetal sex. Determination of foetal sex is prohibited by law in India; therefore data pertaining to foetal sex was available only for the 58 infants that returned for post-natal follow-up at 1.5-3 months of age.

Foetal Responsivity Variables	Test Statistics	p value
∑AUC	U = 413.00	0.67
Stimulation FHR	t = 0.03	0.97
Change in FHR	t =-0.10	0.92
Response Score	U = 372.00	0.80
Habituation	χ^2 =1.00	1.00
Baseline FHR	U = 443.00	0.67
Baseline BBV	U = 487.00	0.27
Post-stimulation FHR	t = -0.54	0.59
Post-stimulation BBV	U = 444.00	0.35

APPENDIX IVD: FOETAL RESPONSIVITY: ASSOCIATIONS WITH FOETAL AGE (TABLE)

This table presents the findings of the analysis of the association between foetal responsivity and foetal gestational age at assessment (n=131).

Foetal Responsivity Variables	Test Statistic	p value
∑AUC	$r_s = -0.02$	0.85
Stimulation FHR	$r_p = -0.04$	0.67
Change in FHR	$r_p = -0.07$	0.46
Response Score	$r_s = 0.01$	0.94
Habituation	$t = -0.77$	0.44
Baseline FHR	$r_s = -0.06$	0.49
Baseline BBV	$r_s = 0.26$	0.30
Post-stimulation FHR	$r_p = -0.09$	0.33
Post-stimulation BBV	$r_s = -0.02$	0.82

APPENDIX IVE: INFANT SEX AND AGE AT ASSESSMENT: ASSOCIATIONS WITH CORTISOL REACTIVITY (TABLE)

This table presents the findings of the analysis of the association between infant sex and age, and infant cortisol variables at 1.5 – 3 months post-birth.

Infant Cortisol Variable (nmol/l)	**N**	**Infant Sex**		**Comparisons in infant cortisol between male and female infants**	**Infant cortisol correlations with infant age at assessment**
		Female	**Male**		
		Mean (SD)	Mean (SD)		
Baseline Cortisol	54	7.22 (6.01)	11.34 (12.38)	U = 479.00 p = 0.04*	r_s = -0.12 p = 0.37
Post-immunisation Cortisol	39	31.78 (16.80)	26.52 (14.39)	t = 1.08 p = 0.29	r_p = -0.19 p = 0.26
Infant Cortisol Response (δ)	38	24.56 (15.74)	15.18 (13.15)	t = 2.36 p = 0.02*	r_p = -0.09 p = 0.60

*Correlation is significant at the 0.05 level

APPENDIX IVF: PRENATAL DEPRESSION: ASSOCIATION WITH BIRTH OUTCOME (TABLE)

This table presents the findings of the analysis of prenatal depression with birth outcomes.

Obstetric Variable	Control (n)	Prenatal Depression (n)	Prenatal EPDS Scores	Prenatal K10 Scores	Inter-group Comparisons	
					Depressed Mothers & Controls	Controls, High Scorers Without MDD & Cases
Preterm Births	0	3	U =10.00 **p <0.01****	U = 33.00 **p = 0.02***	$\chi^2 = 3.14$ p = 0.08	$\chi^2 = 11.93$ **p <0.01**
LBW	6	10	r = -0.03 p = 0.77	r = -0.01 p = 0.77	t = 0.52 p = 0.60	F = 0.25 p = 0.78
Type of delivery	Normal: 37 Operational/ Instrumental : 17	Normal: 38 Operational/ Instrumenta l : 15	U = 630.50 p = 0.39	U = 676.00 p = 0.65	$\chi^2 = 1.27$ p = 0.26	$\chi^2 = 1.71$ p = 0.43
Birth weight (gms)	Mean (SD): 2860 (320)	Mean (SD): 2820 (470)	U = 657.50 p = 0.77	U = 654.00 p = 0.74	$\chi^2 = 0.06$ p = 0.81	$\chi^2 = 0.06$ p = 0.97

*p<0.05 **p<0.01

APPENDIX IVG: PRENATAL DEPRESSION: ASSOCIATION WITH PUERPERAL COMPLICATIONS (TABLE)

This table presents the findings of the analysis of prenatal depression with puerperal complications.

Obstetric Variable	Control (n)	Prenatal Depression (n)	Prenatal EPDS Scores	Prenatal K10 Scores	Inter-group Comparisons	
					Depressed Mothers & Controls	Controls, High Scorers Without MDD & Cases
Complications during labour	15	11	U = 1179.50 p = 0.89	U = 1195.50 p = 0.98	$\chi^2 = 0.13$ p = 0.72	$\chi^2 = 2.98$ p = 0.23
Complications during delivery	13	4	U = 958.50 p = 0.49	U = 950.50 p = 0.45	$\chi^2 = 0.72$ p = 0.40	$\chi^2 = 0.78$ p = 0.67
Postnatal complications	8	7	U = 569.00 p = 0.09	U = 553.50 p = 0.07	$\chi^2 = 5.48$ **p = 0.02***	$\chi^2 = 5.53$ p = 0.06

*p<0.05 **p<0.01

APPENDIX IVH: PRENATAL DEPRESSION: ASSOCIATION WITH INFANT GROWTH (TABLE)

This table presents the findings of the analysis of prenatal depression with infant physical growth.

Inter-group comparisons	Infant Weight		Infant Length		Infant Head Circumference	
	Absolute	**Centile**	**Absolute**	**Centile**	**Absolute**	**Centile**
Mothers with Prenatal Depression vs. Controls	t = 1.11 p = 0.27	U = 343.00 p = 0.28	t = -0.43 p = 0.67	U = 329.00 p = 0.19	t = 0.74 p = 0.46	U = 357.00 p = 0.49
Prenatal Controls, High Scorers Without MDD & Cases	F = 1.05 p = 0.36	F = 0.80 p = 0.45	F = 0.11 p = 0.90	F = 1.03 p = 0.37	F = 1.89 p = 0.16	F = 0.39 p =0.68
Continuous Prenatal Depression Scores						
Prenatal EPDS Scores	r_p = -0.16 p = 0.23	r_s = -0.19 p = 0.15	r_p = 0.16 p = 0.23	r_s = 0.22 p = 0.10	r_p = -0.01 p = 0.93	r_s = 0.15 p = 0.26
Prenatal K10 scores	r_p = -0.08 p = 0.56	r_s = -0.16 p = 0.23	r_p = 0.15 p = 0.25	r_s = 0.17 p = 0.21	r_p = 0.06 p = 0.63	r_s = 0.11 p = 0.44

r_p = Pearson correlation (for normally distributed data)
r_s = Spearman correlation (for non-normally distributed data)
t = independent sample t test statistic, U = Mann Whitney statistic F = ANOVA statistic

APPENDIX IVI: PRENATAL DEPRESSION: ASSOCIATION WITH INFANT HEALTH (TABLE)

This table presents the findings of the analysis of prenatal depression with infant health.

	Inter-group Comparisons		Correlations	
	Prenatal Depression & Controls	**Prenatal Clinical Cases, High Scorers & Controls**	**Prenatal EPDS Scores**	**Prenatal K10 Scores**
Current Infant Health Problems	$\chi^2 = 1.35$ p = 0.51	$\chi^2 = 4.42$ p = 0.35	F = 0.25 p = 0.78	F = 0.54 p = 0.59

χ^2 = Chi square statistic, F = ANOVA statistic

APPENDIX IVJ: PRENATAL DEPRESSION: ASSOCIATION WITH BREASTFEEDING

This table presents the findings of the analysis of prenatal depression with breastfeeding.

Breastfeeding Variables	Inter-group Comparisons		Correlations	
	Prenatal Depression & Controls	Prenatal Clinical Cases, High Scorers & Controls	Prenatal EPDS Scores	Prenatal K10 Scores
Breastfeeding Status				
Currently breastfed	$\chi^2 = 1.34$ p = 0.25	$\chi^2 = 4.35$ p = 0.11	U = 0.00 p = 0.08	U = 0.00 p = 0.08
Exclusively breastfed	$\chi^2 = 2.60$ p = 0.11	$\chi^2 = 2.67$ p = 0.26	U = 102.5 p = 0.07	U = 98.00 p = 0.05
Initiation of Breastfeeding				
Duration to initiation of breastfeeding	U = 313.50 p = 0.11	F = 0.96 p = 0.33	$r_s = 0.09$ p = 0.40	$r_s = 147.5$ p = 0.83
Prelacteal feeds	$\chi^2 = 0.13$ p = 0.72	$\chi^2 = 0.31$ p = 0.86	U = 152.5 p = 0.93	U = 147.5 p = 0.83
Type of breastfeeding	$\chi^2 = 3.34$ p = 0.19	$\chi^2 = 3.78$ p = 0.44	F = 0.96 p = 0.39	F = 1.73 p = 0.19
Quantity of breastfeeding				
Times/day infant is breastfed	t = 0.15 p = 0.88	F = 0.03 p = 0.88	$r_p = -0.03$ p = 0.84	$r_p = 0.01$ p = 0.97
Duration per feed	U = 402.00 p = 0.86	F = 0.15 p = 0.70	$r_s = 0.13$ p = 0.32	$r_s = 0.20$ p = 0.13
Total duration/day infant is breastfed	U = 394.00 p = 0.77	F = 0.12 p = 0.74	$r_s = 0.13$ p = 0.30	$r_s = 0.15$ p = 0.28
Problems associated with breastfeeding				
Maternal problems with breastfeeding	$\chi^2 = 0.64$ p = 0.43	$\chi^2 = 0.85$ p = 0.65	U = 65.50 p = 0.06	U = 98.50 p = 0.34
Infant problems in being breastfed	$\chi^2 = 1.48$ p = 0.22	$\chi^2 = 6.94$ p = 0.03*	U = 212.5 p = 0.08	U = 261.5 p = 0.39

*p<0.05 **p<0.01; *t = independent sample t test statistic, U = Mann Whitney statistic, F = ANOVA statistic*

APPENDIX IVK: POSTNATAL DEPRESSION: ASSOCIATION WITH INFANT GROWTH

This table presents the findings of the analysis of maternal postnatal depression (PND) with infant growth.

Inter-Group Comparisons	Infant Weight		Infant Length		Infant Head Circumference	
	Absolute	**Centile**	**Absolute**	**Centile**	**Absolute**	**Centile**
No PND, PND	t = -0.73 p = 0.47	U = 393.00 p = 0.72	t = -0.26 p = 0.79	U = 397.50 p = 0.77	t = -0.68 p = 0.50	U = 394.5 p = 0.89
No PND, High Scorers for PND, Clinical Cases of PND	F = 1.04 p = 0.36	F = 0.76 p = 0.47	F = 1.43 p = 0.25	F = 3.22 p = 0.04*	F = 2.87 p = 0.06	F = 2.35 p = 0.10
Continuous Scores						
Postnatal EPDS Scores	r_p = 0.17 p = 0.22	r_s = 0.06 p = 0.63	r_p = 0.14 p = 0.31	r_s = 0.04 p = 0.79	r_p = 0.25 p = 0.06	r_s = 0.09 p = 0.53
Postnatal K10 scores	r_p = 0.20 p = 0.13	r_s = 0.05 p = 0.72	r_p = 0.16 p = 0.22	r_s = 0.01 p = 0.95	r_p = 0.23 p = 0.08	r_s = -0.01 p = 0.99

PND: Postnatal depression, t = independent sample t test statistic, U = Mann Whitney statistic, F = ANOVA statistic, r_p = Pearson correlation co-efficient r_s = Spearman correlation co-efficient, *p <0.05 **p<0.01

APPENDIX IVL: POSTNATAL DEPRESSION: ASSOCIATION WITH INFANT HEALTH

This table presents the findings of the analysis of maternal postnatal depression (PND) with infant health.

	Inter-group Comparisons	
	PPD & Controls	**PPD Clinical Cases, High Scorers & Controls**
Current Infant Health Problems	$\chi^2 = 1.83$; p = 0.40	$\chi^2 = 2.74$; p =0.60

χ^2 = Chi square statistic

APPENDIX IVM: POSTNATAL DEPRESSION: ASSOCIATION WITH BREASTFEEDING

This table presents the findings of the analysis of maternal postnatal depression (PND) with breastfeeding.

	Inter-Group Comparisons	
Breastfeeding Variables	**PND & Controls**	**PND Cases, High Scorers & Controls**
Breastfeeding Status		
Currently breastfed	$\chi^2 = 0.83$; p = 0.36	$\chi^2 = 0.83$; p = 0.67
Exclusively breastfed	$\chi^2 = 0.01$; p = 0.91	$\chi^2 = 0.20$; p = 0.90
Initiation of Breastfeeding		
Duration to initiation of breastfeeding	U = 382.50; p = 0.59	F = 0.13; p = 0.88
Prelacteal feeds	$\chi^2 = 0.07$; p = 0.79	$\chi^2 = 1.19$; p = 0.55
Type of breastfeeding	$\chi^2 = 0.68$; p = 0.71	$\chi^2 = 2.13$; p = 0.71
Quantity of breastfeeding		
Times/day infant is breastfed	t = -0.18; p = 0.86	F = 3.22; **p = 0.04***
Duration per feed	U = 367.50; p = 0.41	F = 2.00; p = 0.15
Total duration/day infant is breastfed	U = 366.5; p = 0.44	F = 8.10; **p <0.01****
Problems associated with breastfeeding		
Maternal problems with breastfeeding	$\chi^2 = 1.36$; p = 0.24	$\chi^2 = 1.51$; p = 0.47
Infant problems in being breastfed	$\chi^2 = 2.83$; p = 0.09	$\chi^2 = 3.83$; p = 0.15

PND: Postnatal depression, t = independent sample t test statistic, U = Mann Whitney statistic, F = ANOVA statistic, χ^2 = Chi square statistic , *p <0.05 **p<0.01

APPENDIX IVN: REGRESSION ANALYSIS – PRENATAL DEPRESSION ASSOCIATIONS WITH BREASTFEEDING

This table presents the findings of the regression analysis exploring the association between prenatal depression and breastfeeding independent of maternal postnatal depression (PND) and other confounders.

Linear Regression Model		Unstandardized Coefficients	Standardized Coefficients	p value
		B	Beta	
Times/day the infant is breastfed	(Constant)	8.34		0.00
	Infant's age	0.01	0.05	0.72
	Infant's sex	0.36	0.05	0.71
	Prenatal depression	0.12	0.03	0.85
	Postnatal depression	0.78	0.15	0.30
Total duration/day infant is breastfed	(Constant)	2.02		0.00
	Infant age	-0.01	-0.10	0.44
	Infant sex	0.22	0.09	0.50
	Prenatal depression	0.16	0.10	0.45
	Postnatal depression	.569	0.31	**0.03***
Maternal reports of infant difficulty in being breastfed	(Constant)	0.30		0.32
	Infant sex	0.16	0.11	0.42
	Infant's age	-0.01	-0.12	0.37
	Current infant health problems	-0.45	-0.23	0.08
	Prenatal depression	0.15	0.16	0.24
	Postnatal depression	0.31	0.29	**0.04***

*p <0.05 **p<0.01

APPENDIX V: MISCELLANEOUS

APPENDIX VA: ICD-10 CRITERIA FOR THE DIAGNOSIS OF A MAJOR DEPRESSIVE EPISODE

F32.2 Severe/Major depressive episode without psychotic symptoms

Note: If important symptoms such as agitation or retardation are marked, the patient may be unwilling or unable to describe many symptoms in detail. An overall grading of severe episode
may still be justified in such a case.

A. General features of depression as follows:

(1) reduced concentration and attention;
(2) reduced self-esteem and self-confidence;
(3) ideas of guilt and unworthiness (even in a mild type of episode);
(4) bleak and pessimistic views of the future;
(5) ideas or acts of self-harm or suicide;
(6) disturbed sleep;
(7) diminished appetite.

B. All three of these symptoms must be present:

(1) Depressed mood to a degree that is definitely abnormal for the individual, present for most of the day and almost every day, largely uninfluenced by circumstances, and sustained for at least 2 weeks.

(2) Loss of interest or pleasure in activities that are normally pleasurable;

(3) Decreased energy or increased fatiguability.

C. An additional symptom or symptoms from the following list should be present, to give a total of at least eight:

(1) Loss of confidence and self-esteem;

(2) Unreasonable feelings of self-reproach or excessive and inappropriate guilt;

(3) Recurrent thoughts of death or suicide, or any suicidal behaviour;

(4) Complaints or evidence of diminished ability to think or concentrate, such as indecisiveness or vacillation;

(5) Change in psychomotor activity, with agitation or retardation (either subjective or objective);

(6) Sleep disturbance of any type;

(7) Change in appetite (decrease or increase) with corresponding weight change).

A fifth character may be used to specify the presence or absence of the "somatic syndrome": F32.00 Without somatic syndrome or F32.01 With somatic syndrome

C. There must be no hallucinations, delusions, or depressive stupor

This information is taken from the World Health Organisation International Classification of Diseases version 10 (ICD-10) (http://apps.who.int/classifications/apps/icd/icd10online/)

APPENDIX VB: STUDY PROTOCOL APPROVED BY THE ETHICS COMMITTEES OF THE UNIVERSITY OF OXFORD AND ST. JOHN'S MEDICAL COLLEGE HOSPITAL, BANGALORE

Aim of the Study

The aim of the study is to investigate the nature and extent of the effect of prenatal maternal anxiety and depression on foetal and infant development. The first phase of the study seeks to determine the prevalence and socio-cultural aetiology of maternal negative affect states in the prenatal population of rural India and to investigate its relationship to foetal growth and physiological responses. The second phase of the study carried out postnatally aims to ascertain the effect of prenatal stress on obstetric outcome, the physical growth of the baby and infant temperament and stress responsiveness. The effect of maternal negative affect states on breastfeeding practices and fluctuating asymmetry in infant dermatoglyphic patterns will also be investigated at this stage.

The results of this study will used to reconstruct the aetiological link between prenatal maternal anxiety and depression and infant development through the intrauterine programming of the foetus in response to elevated levels of maternal stress during pregnancy. Prenatal maternal mental health remains unaddressed in the developing world, particularly in the rural context. This prospective study would serve as the first comprehensive inquiry into the same, the results of which will provide the long overdue necessary evidence for the incorporation of a brief assessment of maternal mental health into routine prenatal care.

Objectives

Primary Objective

- To determine, in a rural sample in India, the association between depression during pregnancy and
    i. Foetal stress responses measured through foetal cardiac responses to vibroacoustic stimulation
    ii. Infant temperament and stress responsiveness
    iii. Infant physical growth

Secondary Objectives

- To determine the prevalence of prenatal maternal depression in a rural sample and its relationship to socio-demographic variables
- To determine the association between prenatal depression and obstetric outcome
- To determine the association between prenatal depression and infant growth, health and breastfeeding
- To determine the association between prenatal maternal negative affect states and dermatoglyphic irregularities in infants

Study Hypothesis

Primary Hypotheses:

- Prenatal depression is associated with disturbances in foetal stress responses
- Prenatal depression is associated with disturbances in infant cortisol response to immunisation
- Prenatal depression is associated with maternal reports of temperamental disturbances in infancy

Secondary Hypotheses

- Prenatal depression is prevalent in sizeable proportions in the rural prenatal population and is associated with the socio-demography of the mother, particularly intimate partner violence and poor social support
- Prenatal depression is associated with lower birth weights and a higher incidence of preterm births
- Prenatal maternal depression is associated with impaired physical growth during infancy
- Prenatal stress is associated with poorer infant health and altered patterns of breastfeeding during infancy
- Increased fluctuating asymmetry will be noted in the fingerprints of infants of mothers found to have high levels of depression/anxiety during pregnancy.

Research Methods

Study Setting and Population

The study will be conducted at Solur Village in the Magadi taluk of Bangalore Rural District, Karnataka, India. Solur is located 76 kilometres from Bangalore city, the capital of the Karnataka state in south India. Karnataka is the eight most populous state in the country with a total population of 52.73 million, 66 % of which live in villages. The population is largely of Kannada speaking people though some may speak Hindi, Tamil, Tulu or Urdu as well. The predominant source of livelihood is agriculture though some may engage in sericulture (silk farming), mining, unskilled labour and small businesses.

Snehalaya Socio-Medical Relief Centre is a 40-bedded charitable, missionary run secondary level hospital for obstetrics and gynaecology run by the religious congregation of the Sisters of Charity. It provides free/concessional obstetric, gynaecologic and paediatric care to rural Indian women and children, with more than 150 babies being delivered per month at this hospital. It is located in Solur Village and caters to the population of 3 primary health centres i.e. 90,000. 160 to 200 pregnant rural women present to its prenatal clinic per week. These are held twice a week along with the immunization clinic for children. The hospital also caters to paediatric and general medical care of the rural populace.

Collaborations

This study will be carried out in collaboration with:

i. The Department of Psychiatry, St. John's Medical College Hospital, Bangalore, Karnataka, India
ii. The St. John's Research institute, Bangalore, Karnataka, India
iii. Snehalaya Socio-Medical Relief Centre, Solur Village, Magadi Taluk, Ramnagara, Karnataka, India

Sample Characteristics

Women attending prenatal clinics at Snehalaya Socio-Medical Relief Centre, Karnataka, India will be recruited and assessed in their third trimester of pregnancy; and mother-infant dyads will be reassessed while attending immunization clinics at the same at 6-14 weeks post birth.

Inclusion criteria at Recruitment:

- Pregnant women in their third trimester of pregnancy with a singleton foetuses with no known congenital abnormality as detected by ultrasound investigation
- Mothers who speak one of the study languages (Kannada, Hindi or English)
- Mothers with no known history of severe psychiatric morbidity namely schizophrenia and frank psychosis

Exclusion Criteria at Follow Up:

- Mothers whose pregnancy outcomes are intrauterine death, still birth, birth of a malformed child or birth of a child suffering from neonatal hypoxia or any other significant medical/surgical condition

Study design

Prospective, observational study

Sample Size and Power Calculations

There is limited data on which to base the estimation of power for this study, particularly because of the heterogeneity of similar studies carried out in the past and also because of a large amount of variability in the methods used to assess maternal psychological stress and infant outcomes. It was therefore decided to establish a design where there will be a difference substantial enough to be considered clinically significant - a moderately large effect size of 0.6 or greater was selected for this study. In support of this, findings from a study conducted by Bergman and colleagues (Bergman 2008) show a correlation of -0.39

between prenatal stress and child developmental outcomes, corresponding to an effect size of 0.6 (standardised difference).

To detect an effect size of 0.6, the sample size of 66 per group will allow for 32% attrition, while still providing power in excess of 80% at the significance level of 5% (one-tailed).

Methodology

The Prenatal assessment

Mothers meeting inclusion criteria for the study will be recruited in the third trimester of pregnancy. Mothers will be explained the nature, purpose and methodology of the study and will be invited to participate in the same. After obtaining informed consent, mothers will be screened for depression using the Edinburgh Postnatal Depression Scale (EPDS; Cox 1987) and Kessler – 10 scale (K-10; Kessler 1994, 2000) after which they will be asked to complete a Social Support Questionnaire (SSQ, modified from K. Srinivasan et al, 2009) and a 24 hour routine/holiday dietary recall specially designed for the rural populace in Karnataka by St. John's Research Institute, Bangalore, India. Socio-demographic and routine prenatal medical data, including details of foetal growth extracted from the last trimester ultrasound scan sheet, will also be collected. A careful record of smoking and alcohol consumption during pregnancy, complications including decreased foetal movements and bleeding per vaginum during pregnancy, a family history or a past history of psychiatric illness, a history of present and past medical ailments and the consumption of therapeutic and/or illicit drugs will be kept and adjusted for during analyses. Mothers found to have EPDS and K-10 scores consistent with depression/psychological distress will then be interview using the Mini International Neuropsychiatric Interview Plus, Version 5.0.0 (MINI Plus; Sheehan & Lecrubier 2006) by a trained researcher. The MINI Plus will also be administered to 66 low scorers who will serve as controls for the post natal assessment.

The 66 high scores and 66 controls will then be asked to lie supine in bed in a quiet room and the foetal heart rate (FHR) will be recorded for a duration of 10 minutes using a foetal heart rate monitor. After the baseline FHR recording is obtained, a vibro-acoustic stimulus using an electronic artificial larynx (EAL) will be applied to the mother's abdomen in close proximity to the foetal head. Each stimulus will be applied for a duration of 3 seconds and 10 such stimuli will be applied 1 minute apart. The accelerations so obtained will be indicated on the graph following which the un-stimulated FHR will be recorded for a further10 minutes**.** The graph so obtained will be added to the mother's data file. This will be later coded by a researcher blind to other details of the study and used to compare the physiological response of the foetuses of prenatally stressed and non-stressed mothers. The recording of FHR, FHR variability and accelerations using a foetal heart rate monitor is a routinely employed method of assessment of foetal well-being in hospitals. Further, findings from past literature support our ancillary hypothesis that prenatal stress is associated with prolonged foetal habituation as measured by FHR accelerations in response to a vibro-acoustic stimulus.

Vibroacoustic stimulation using an electronic artificial larynx is widely employed in the routine prenatal assessments of foetal well-being. The device produces a broad-band noise at a fundamental frequency of 65 Hz ±7%, with sound pressure levels measured at 1 m in air averaged at 68 dB[53]. Although there have been some concerns about the safety of the VAS in the past, apart from momentary, reversible physiological changes in foetal behavioural state and heart rate, there is no evidence that vibro-acoustic stimulation is associated with harmful effects on neurological or hearing in children exposed in utero[54-56].

The 66 mothers found to be score high on the screening questionnaires and the 66 controls will then be invited to partake in the postnatal assessment at 6 to 14 weeks post birth, (coincident with the immunization of the infant in accordance with the National Immunization Schedule of India to reduce attrition).

The Postnatal assessment

Data about obstetric outcome (including birth weight, length and head circumference at birth) will be extracted from hospital records for all 132 mothers. Mothers returning for follow-up at 1.5-3 months post-birth will be asked to complete the EPDS, K-10 and a breastfeeding questionnaire adapted from Patel's study of postnatally depressed mothers in Goa, India (Patel 2002). Given the difficulty in assessing behaviour and social/emotional responses at 6-14 weeks of age, an assessment of the infant's temperament will be employed using the Infant Behaviour Questionnaire (M. K. Rothbart, 1981). The infant's physical growth (weight, length and head circumference) will also be recorded

During the period when he infant is in the quiet, alert phase of activity, the first salivary sample to assess baseline cortisol will be collected.

Following this, the infant's fingerprints will be obtained using an inkless pad and photosensitive paper. Digital scans of the fingerprints will be obtained and coded using the popular procedure described by Holt (1969) at a later date.

The second salivary sample will be collected from the infant after immunisation (where the needle prick presents an acute stressful stimulus) and assessed for cortisol levels at a later date.

All saliva samples will be collected between 9.00 am and 12.00 pm using sorbettes. All samples will be collected in duplicate, centrifuged and stored at temperatures below minus twenty degrees centigrade. The samples will be transported in a vaccine carrier over ice at just below zero degrees Celsius from Solur to St. John's Medical College, Bangalore, where they will be refrozen and analysed using the salivary cortisol enzyme immunoassay kit developed by Salimetrics Europe at a later date.

Following prenatal data collection each mother's data file will be tagged with a study number which will be used to match prenatal and postnatal maternal and infant data for data storage

purposes. All scales will be administered by trained interviewers, research assistants and researchers in Kannada.

The following section provides a brief overview of the constructs that will be assessed and the methods of assessment:

		Construct	Method of Assessment
Prenatal Assessment	**Mental & Social Health**	Depression	Edinburgh Postnatal Depression Scale (Cox, 1987)
		Anxiety/Psychological Distress	Kessler – 10 scale (Kessler, 1994, 2000)
		Clinical Psychiatric Interview	The Mini International Neuropsychiatric Interview Plus Version 5.0.0 (Sheehan & Lecrubier 2006)
	Social Variables	Social Support	Social Support Questionnaire (modified from Srinivasan, 2009 [as yet unpublished])
		Socio-demographic data	Socio-demographic data sheet
	Medical Morbidity & Nutrition	Routine prenatal medical data	Prenatal data sheet
		Nutritional Status Assessment	24 hour usual/holiday dietary recall (rural) (St. John's Research Institute), Hemoglobin Level, Body Mass Index
	Intra-uterine Foetal Response	Foetal Physiological Response & Habituation	Baseline foetal heart rate (FHR) using FHR monitor, habituation studied as FHR acceleration in response to a vibro-acoustic stimulus
Postnatal Assessment	**Mental & Social Health**	Depression	Edinburgh Postnatal Depression Scale (Cox, 1987)
		Anxiety/Psychological Distress	Kessler – 10 scale (Kessler, 1994, 2000)
	Medical Morbidity & Breastfeeding	Obstetric outcome & Postnatal complications	Postnatal data sheet including birth outcome and birth weight
		Breastfeeding	Breastfeeding Questionnaire (modified from Patel 2002)
		Temperament	Infant Behaviour Questionnaire (M. K. Rothbart, 1981)
		Anthropometry	Weight, length and head circumference

	Infant Outcomes	Fluctuating dermatoglyphic asymmetry	Infant fingerprints using inkless pad and photosensitive paper
		Stress Responsiveness	Pre and post immunization salivary cortisol

Planned Analysis and Predicted Results

A P-P plot will be used to check the normality of distribution for all numerical data. Non-normally distributed variables will be log transformed. Both parametric and nonparametric tests will be used to test associations. Differences between foetal and infant outcomes in prenatally stressed and non-stressed groups of mothers will be compared using appropriate tests depending upon the distribution of the variables. Multiple regression analyses will be used to test the study hypothesis controlling for covariates.

It is expected that after controlling for confounding influences, an association between prenatal maternal depression and altered physiological foetal response, temperament abnormalities and increased stress responsiveness in infants and impaired physical growth in the same will be established. Further, an association between prenatal anxiety/depression and decreased efficacy of breastfeeding is expected. Finally, we expect to report higher fluctuating dermatoglyphic asymmetry in the infants of prenatally stressed mothers.

The results of this study will be disseminated to the community through reports and will be published in peer-reviewed journals.

APPENDIX VC: PICTURES OF THE STUDY LOCATION AND SAMPLE

Village center, Solur, Karnataka, India.

Typical village dwelling, Solur.

Farmlands, Solur.

Outdoor kitchen, Solur.

In-patient building of Snehalaya Hospital, Solur.

Prenatal clinic, Snehalaya Hospital, Solur.

Assessment room for the study, Snehalaya Hospital, Solur.

Participating mother and baby: The Solur Mother and Baby Project, Solur, India.

APPENDIX VD: PUBLISHED WORK FROM THIS THESIS

- Assessing prenatal depression in the rural developing world: a comparison of two screening measures

  Michelle Caroline Fernandes, Krishnamachari Srinivasan, Alan L. Stein, Gladys Menezes, R. S. Sumithra and Paul G. Ramchandani

  Archives of Women's Mental Health (2011) Volume 14, Number 3, p.209-216