Interrogating placental function in pregnancies affected by prenatal depression

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Submitted for consideration for the degree of PhD

September 2020

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List of Abbreviations

11β-HSD2:	11β-Hydroxysteroid Dehydrogenase Type II
ADHD:	Attention Deficit Hyperactivity Disorder
AGA:	Average for Gestational Age
AIC:	Akaike Information Criterion
ALSPAC:	Avon Longitudinal Study of Parents And Children
APGAR:	Appearance, Pulse, Grimace, Activity and Respiration
ASD:	Autism Spectrum Disorder
BIC:	Bayesian Information Criterion
BMI:	Body Mass Index
BP:	Biological Processes
CBWC:	Custom Birth Weight Centile
CC:	Cellular Component
CDKN1C:	Cyclin Dependent Kinase Inhibitor 1C
cDNA:	Complementary DNA
CIs:	Confidence Intervals
CICS:	Cardiff Infant Contentiousness Scale
CSH:	Chorionic Somatomammotropin
DEG:	Differentially Expressed Gene
DLK1:	Delta-Like Non-Canonical Notch Ligand 1
DMR:	Differentially Methylated Region
dNTPs:	Deoxyribonucleotide Triphosphates
DOHaD:	Developmental Origins of Health and Disease
DSM-IV-TR:	Diagnostic and Statistical Manual of Mental Disorders-IV-Text Revision
EPDS:	Edinburgh Postnatal Depression Scale
FC:	Fold Change

FDR:	False Discovery Rate
GDM:	Gestational Diabetes Mellitus
gDNA:	Genomic DNA
GiW:	Grown in Wales
GO:	Gene Ontology
GSEA:	Gene Set Enrichment Analysis
hCG:	Human Chorionic Gonadotropin
HPA:	Hypothalamic Pituitary Adrenal
hPL:	Human Placental Lactogen
IBQ-R-SF:	Infant Behaviour Questionnaire-Revised-Short Form
ICR:	Imprinting Control Region
IGF2:	Insulin-like Growth Factor 2
IUGR:	*In Utero* Growth Restriction
L2FC:	Log2 Fold Change
Lab-TAB:	Laboratory Temperament Assessment Battery
LMICs:	Lower and Middle Income Countries
LPA:	Latent Profile Analysis
MP:	Molecular Function
NES:	Normalised Enrichment Score
PET:	Pre-Eclampsia
PCA:	Principal Component Analysis
PBS:	Phosphate Buffered Saline
PBQ:	Postnatal Bonding Questionnaire
PCR:	Polymerase Chain Reaction
PEG3:	Paternally Expressed Gene 3
PEG10:	Paternally Expressed Gene 10

PGH:	Placental Growth Hormone
PHLDA2:	Pleckstrin Homology-Like Domain Family A member 2
Prls:	Pregnancy Related Lactogenic Hormones
qPCR:	Quantitive Polymerase Chain Reaction
ROS:	Reactive Oxygen Species
SDHA:	Succinate Dehydrogenase Complex Flavoprotein Subunit A
SGA:	Small for Gestational Age
SNPs:	Single Nucleotide Polymorphisms
SRH:	Self-Reported Health
SSRI:	Selective Serotonin Reuptake Inhibitor
STAI:	State Trait Anxiety Inventory
TSC:	Trophoblast Stem Cells
UHW:	University Hospital of Wales
USVs:	Ultrasonic Vocalisations
YY1:	Yin Yang 1
YWHAZ:	Tryptophan 5-Monooxygenase Activation Protein Zeta

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Declaration

Data produced jointly:

- Samantha Garay and Katrina Savory were involved in the generation of the 12-month infant assessment data.

Data/materials provided by someone else:

- Anna Janssen established the Grown in Wales cohort.
- hPL serum data was generated by the Core Biochemical Assay Laboratory at the NIHR Cambridge Biomedical Research Centre.
- Wales Gene Park performed the RNA sequencing and generation of RNA count data.

Published Work:

- Janssen, A. B., Savory, K. A., Garay, S. M., **Sumption, L.**, Watkins, W., Garcia-Martin, I., Savory, N. et al. (2018). **Persistence of anxiety symptoms after elective caesarean delivery**. BJPsych Open.
- Savory, K. A., Garay, S. M., **Sumption, L. A.**, Kelleher, J. S., Daughters, K., Janssen, A. B., Van Goozen, S. et al. (2020). **Prenatal symptoms of anxiety and depression associated with sex differences in both maternal perceptions of one year old infant temperament and researcher observed infant characteristics**. Journal of Affective Disorders 264:383-392.
- **Sumption, L**., Garay, S. M. and John, R. M. (2020). **Low serum placental lactogen at term is associated with postnatal symptoms of depression and anxiety in women delivering female infants**. Psychoneuroendocrinology 116.

Funding:

This work was funded by:

- The Biotechnology and Biological Sciences Research Council (BBSRC) through an SWBio Doctoral Training Programme.
- The Medical Research Council (MRC).
- The Waterloo Foundation.

Acknowledgements

First I must thank Ros for her continued support back from when she was my undergraduate tutor, to my dissertation supervisor through to my PhD supervisor. Thank you for the insight, knowledge and encouragement especially over the last four years. Thank you also to the entire John group, Sam, Hannah, Raquel, Isabel, Harri and Hugo who have not only guided me the lab but made coming into work much more enjoyable.

Thanks to Anna to who established the Grown in Wales study and to the mothers who participated for whom this study would not have been possible without.

Thank you to all my friends for their support over the last four years, but especially over the last nine months which have at times been scary, but zoom quizzes have been a welcome respite to a global pandemic. Thank you also to my parents, Kate and Mark, and to my sister Faye, thank you for your continued care and love.

Finally, thank you to Marcus who I could not have done it without. There is no one I'd rather be locked down for months on end with, this is for you.

Summary

Prenatal depression is a relatively neglected yet common disorder associated with adverse outcomes for exposed infants. It is therefore imperative to identify contributing mechanisms. One key change that occurs during pregnancy is an exposure to extraordinary levels of hormones. Genetically manipulating imprinted gene expression in mice results in defects in placental endocrine lineages and changes in maternal behaviour. One human study reported lower than normal placental expression of the imprinted gene *PEG3* and hormone *hPL* in women with prenatal depression. Together, these data suggest that placental endocrine insufficiency may contribute to maternal mental health disorders.

The Grown in Wales study is an elective caesarean cohort (N= 355) with self-reported mental health scores and biological samples including placenta collected at term. Three additional postnatal questionnaires were completed and a postpartum infant assessment was performed at 12 months. Depression scores were analysed with respect to lifestyle, biological measures and both maternally-reported and independent observations of infant development.

Analysis of depression and anxiety symptom trajectories identified persistent anxiety in this population. In pregnancies with boys, transcriptional changes were identified in the placenta through RNA sequencing in relation to prenatal depression. Through qPCR, *PEG3* expression was found to be significantly lower in male placentas in relation to prenatal depression, with no disruption to other imprinted genes. In contrast, in pregnancies with girls, serum hPL was significantly associated with postnatal depression. Finally, maternally-reported temperament of girls was associated with prenatal depression, whereas independent observations of boys were associated with depression symptoms.

In summary, while the thesis did not identify a simple relationship between placental endocrine dysfunction and prenatal depression mediated by *PEG3*, it identified a sexual dimorphism in the placental transcriptomes of those with prenatal depression and in the outcomes of infants exposed *in utero*, highlighting the importance of considering fetal sex in prenatal depression.

Chapter 1: Introduction

1.1 Prenatal mental health

Pregnancy is typically a time when women experience happiness, calmness and excitement at the expectation of an upcoming birth. However, a significant number of women do not experience these feelings and instead may struggle emotionally and mentally due to the onset of a mental health disorder such as depression.

1.1.1 History

Mental illness in the perinatal period has been recognised since the time of Hippocrates in the 5th Century BCE. Hippocrates described symptoms of fever and delirium in pregnancy as puerperal fever, now recognised as puerperal sepsis caused by the group of bacterium *Streptococcus A*. However, at the time Hippocrates determined the cause as lochia which was transported to the brain causing "agitation, delirium and attacks of mania" (Ferreira 1965). At a similar time in India, the Sanskrit epic Mahabharata was written containing the passage:

"The daughter of Virata…was exceedingly afflicted by grief on account of the death of her husband…They all feared that the embryo in her womb might be destroyed" (Dipietro *et al.* 2006).

Therefore, over two millennia ago it was already known that the expectant mother's mental state was important for the health of her unborn baby. Later in the 11th century, the Trotula texts described a theory behind the cause of prenatal depression:

"If the womb is too moist, the brain is filled with water and the moisture running over to the eyes compels them to involuntarily shed tears" (Hanley 2009).

In the 19th century the French psychiatrist Louis Victor Marcé pioneered the study of perinatal psychiatry through his seminal publication "Traité de la folie des femmes enceintes" (Marcé 1858). He clinically described perinatal syndromes from more than 70 case studies and treatments of the time; predominantly incarceration in a sanatorium or asylum. Over the course of the 20th century, research into perinatal mental health prevalence, risk factors and outcomes for infants increased dramatically. However, despite this the perinatal section of the UK's Royal College of Psychiatrists only became a faculty in 2014.

1.1.2 Types

While our understanding of prenatal mental health may have moved on since the theory of a 'moist womb' and incarceration in asylums, research and awareness is still limited. This is despite prenatal mental health being one of the most common complications in pregnancy. Precise numbers of prevalence are difficult to measure due to differences in research populations, diagnostic tools and the heterogeneity of mental health disorders. The World Health Organisation estimates that 10% of the world's pregnant population suffer from a mental illness during their perinatal period, meaning each

year on average 21 million women suffer from a mental health disorder during pregnancy (Hendrick 1998). The most common mental disorders during this time are depression and anxiety.

1.1.2.1 Depression

Depressive disorders are common mental health conditions affecting an estimated 322 million people worldwide and are one of the leading causes of disability (Friedrich 2017). Depression disproportionally affects women over men, and has a median age of onset during childbearing years (Kessler *et al.* 2005). Symptoms can include sad mood, lack of energy, weight loss or gain, insomnia, restlessness, loss of interest in activities and impaired concentration. Symptoms of perinatal depression are often similar to depression symptoms outside of pregnancy. However, one study reported inconsistencies in neurological measures between perinatal depression and depression outside of pregnancy (Pawluski *et al.* 2017). Activation of the amygdala and striatum in response to a non-infant emotional stressor significantly differed between the two groups. This suggests perinatal depression is not just an extension of a depression outside of pregnancy and requires independent research.

Severity of perinatal depression can vary, but in high income countries such as the UK suicide is one of the leading causes of maternal death (Oates 2003). Often, symptoms of depression increase over pregnancy and prevalence in the UK ranges from 10% to 31% (Johanson *et al.* 2000; Hay *et al.* 2008). Reasons for this discrepancy in prevalence rates may relate to the criteria used to diagnose or the specific population studied as prevalence is often higher in lower socioeconomic populations (Bolton *et al.* 1998).

Different kinds of depressive disorders during pregnancy have been associated with different perinatal measurements. For example, according to one report, those with a major depressive disorder are more likely to have more severe symptoms than those with chronic depression, while those with chronic disorders had both a higher concentration of the stress hormone cortisol and were more strongly associated with fetal growth restriction (Field *et al.* 2007). While other measures were not different between disorders, it still outlines the heterogeneity of depressive disorders that may be difficult to disentangle.

It is commonly known that prenatal depression has adverse consequences for the exposed child (see Section 1.5), but it has been criticised that often the health of the woman is valued only to the extent that it affects the health of the newborn (Rosenfield and Maine 1985). However, women suffering from prenatal depression are often found to also have poorer self-reported health (SRH) (Haas *et al.* 2004; Lara-Cinisomo *et al.* 2018). SRH is a common predictor of mortality, consistent with objective health status and proposes a broader understanding of health than more clinical outcomes (Wu *et al.* 2013). This observed reduction in physical health may relate to those suffering from depression being less likely to reach out for support, less likely to follow doctors' orders and overall less likely to take

care over their personal health and wellbeing (Paris *et al.* 2009). Further consequences of prenatal depression can include decreased help from their support network, reduced ability to take care of oneself, poor nutrition, weight gain, substance use and relationship difficulties, all of which may increase the severity of depression and worsen the mothers' physical health (Field *et al.* 2006).

1.1.2.2 Anxiety

The reported prevalence of prenatal anxiety is higher than prenatal depression in a number of countries (Dennis *et al.* 2017). Anxiety is often characterised by worried and intrusive thoughts, along with physical symptoms such sweating, dizziness and increased blood pressure. Despite the prevalence of prenatal anxiety it has received limited research despite increasing evidence of its detrimental effects (Zietlow *et al.* 2019). Anxiety disorders during pregnancy can be separated into different categories including generalised anxiety, obsessive compulsive disorders and pregnancy related anxiety (Huizink *et al.* 2004; Huizink *et al.* 2014; Pawluski *et al.* 2017). Generalised anxiety can be further separated into state and trait anxiety (Spielberger *et al.* 1970) (Section 2.2.1.1.2).

1.1.2.3 Comorbidity

Depression and anxiety are commonly reported as comorbid in the prenatal period; in one review 30-58% of patients with major depressive disorder in pregnancy also suffered from anxiety (Field *et al.* 2010). This can make it difficult to separate the two affective disorders and thus they are often studied together. One study found comorbid depression and anxiety was associated with an increased rate of prematurity and more severe alterations in dopamine levels and sleep disturbances when compared to depression or anxiety alone (Field *et al.* 2010). However, in the same study no differences were reported for factors such as cortisol and birth weight indicating the complexity of the disorders.

It has been suggested that this comorbidity relates to the high correlation of genetic risk factors between depression and anxiety that indicate a common genetic pathway (Williamson *et al.* 2005; Kendler *et al.* 2007). However, the two disorders do have distinct symptomologies and trajectories (Penninx *et al.* 2011; Lemche *et al.* 2016). It is therefore important to study prenatal depression and anxiety as separate entities where possible.

1.1.3 Under-reported

Despite the high prevalence of depression and anxiety in the prenatal period, research into these conditions has received less attention and they are thought to be under-reported in the general population. This could be a consequence of a number of factors including societal expectations around pregnancy. Pregnant women are often described as 'glowing' and stigma still exists if these expectations are not met. Alternatively, it may relate to the misconception of a 'protection' from mental health conditions during pregnancy as a result of pregnancy hormones (Bennett *et al.* 2004). There is also a stronger focus on physical health rather than mental health at this time, and atypical depression symptoms such as fatigue or loss of appetite may be misdiagnosed as a physical complaint

instead of a mental one (Bowen and Muhajarine 2006). Given there is no recommendation for screening of prenatal mental health disorders in the UK (Solutions for Public Health 2019), women suffering from prenatal depression or anxiety may not be getting the help they need. In other high income countries only an estimated 41% of all prenatal depression cases are diagnosed and of those only an estimated 10-15% get effective treatment (Bauer *et al.* 2014; Gavin *et al.* 2015).

1.1.4 Interventions

Effective treatment is needed to help those suffering from a prenatal mental health condition. Randomised control trials are the gold standard in medicine efficiency and safety, but are complicated in pregnant women as both the mother and developing fetus need to be considered. Outside of pregnancy the most common treatments for depression and anxiety are antidepressants, the predominant class being selective serotonin reuptake inhibitors (SSRIs). In the general population they have been shown to be relatively efficacious (Cipriani *et al.* 2018). However, in the perinatal period results are more mixed (Swanson and David 2015). This is despite up to 30% of women with prenatal depression taking antidepressant medication during their pregnancy (Glover and Clinton 2016).

1.1.4.1 SSRIs

SSRIs increase intra-synaptic levels of serotonin by binding serotonin transporters (SERT) and thus blocking serotonin reuptake (Gaspar *et al.* 2003). Serotonin is a neurotransmitter produced in the brain and is present in the fetal brain from five weeks of pregnancy (Sundström *et al.* 1993). Early in development, serotonin predominantly functions as a trophic factor regulating important processes such as cell growth, differentiation, migration, dendritic pruning and synaptogenesis (Gaspar *et al.* 2003; Brummelte *et al.* 2017). In addition to acting as a growth factor, serotonin is involved in almost all levels of human behaviour as well as cognition and learning. Disturbances in serotonin signalling are associated with psychological disorders such as affective disorders, autism spectrum disorder (ASD) and schizophrenia (Sodhi and Sanders-Bush 2004). These conditions are often linked with the stress systems hypothalamic-pituitary-adrenal (HPA) axis and locus-coeruleus-norepinephrine (LC-NE), of which serotonin functions in their development and regulation (Fuller 1996; Chaouloff *et al.* 1999; Lowry 2002). Therefore, alterations in serotonin signalling while the brain is still developing has the potential to affect a wide range of mechanisms.

Studies of rodents and humans have shown that SSRIs can cross the placenta and fetal blood brain barrier. SSRIs can subsequently alter serotonin levels of the fetus which could theoretically impact serotonin signalling (Davidson *et al.* 2009; Rampono *et al.* 2009). Although literature is conflicting, exposure to SSRIs *in utero* have been shown in some studies to alter physiology and behaviour. In humans, increased prematurity and decreased birthweight have been reported, along with respiratory distress, unstable temperature regulation and sleep difficulties (Olivier *et al.* 2013; Hanley and

Oberlander 2014). In childhood, those previously exposed have been reported as having adverse motor development and language as well as an increased risk of affective disorders and ASD (Oberlander *et al.* 2010; Brandlistuen *et al.* 2015; Hanley *et al.* 2015; Hermansen *et al.* 2016; Malm *et al.* 2016). Advances in neuroimaging techniques have suggested changes in fetal blood flow which has been hypothesised to alter fetal brain microstructure (Rurak *et al.* 2011). Conversely, a number of studies have not found any relation between fetal SSRI exposure and adverse infant outcomes (Nulman *et al.* 1997; Hviid *et al.* 2013). Some studies have even reported a positive effect, buffering the consequences of maternal mental health (Weikum *et al.* 2012; Weikum *et al.* 2013b). A meta-analysis examined 23 human studies and did find an association between SSRI exposure *in utero* and prematurity, decreased birthweight and APGAR scores, though all effect sizes were small (Ross *et al.* 2013). Rodent studies should offer a more controlled environment for studying offspring exposed to SSRIs *in utero*, but have also provided inconclusive results (Glover and Clinton 2016; Kott and Brummelte 2019).

Given SSRIs are prescribed to treat mental health conditions which are well-documented in their adverse effect on infant outcomes (see Section 1.5), it is important to statistically control for this confounder which can be difficult. The association between SSRI exposure and ASD highlights this as some studies have found a risk while controlling for maternal mood, whereas others have not (Hviid *et al.* 2013; Sørensen *et al.* 2013; El Marroun *et al.* 2014; Gidaya *et al.* 2014; Harrington *et al.* 2014; Clements *et al.* 2015; Boukhris *et al.* 2016; Malm *et al.* 2016; Sujan *et al.* 2017). There are a number of other confounding factors such as single nucleotide polymorphisms (SNPs) at the serotonin transporter gene and environmental exposures (Weikum *et al.* 2013a; Dhaliwal *et al.* 2017). A number of studies have also highlighted the importance in controlling for timing of treatment, dosage and type of SSRI (Kaplan *et al.* 2016; Rotem-Kohavi and Oberlander 2017; Kott and Brummelte 2019).

Another factor not often accounted for is fetal sex. While studies often control for this and aim for roughly equal proportions in the sample, analysis is rarely separated by infant sex despite sexually dimorphic offspring outcomes related to prenatal depression (see Chapter 7). Three studies that did separate by sex found a difference between girls and boys exposed to SSRIs. Boys were associated with atypical distress to limitations at 10 months and were at a greater risk of attention deficit hyperactivity disorder (ADHD) and ASD, whereas girls had lower reelin levels in cord blood and were associated with reduced smiling and laughter at 10 months (Brummelte *et al.* 2013; Harrington *et al.* 2014; Erickson *et al.* 2019). Serotonin is also involved in sexual differentiation (Pawluski and Gemmel 2018) and by not separating analysis by fetal sex, important associations may be masked. It is also possible that in certain cases, especially when doses are low that despite altered fetal serotonin levels, serotonin signalling still functions correctly. The plasticity of the serotonin signalling pathway may mean it can remain stable under fluctuating serotonin levels.

1.1.5 Costs

The consequences of prenatal mental health conditions for the mother, child and family can extend many years. Immediate concerns relating to poor prenatal mental health include increased risk of prematurity and low birth weight (Field *et al.* 2004). Preterm birth is one of the principal causes of neonatal mortality and morbidity in higher income countries such as the USA (Armstrong 2007). Prenatal mental health is also associated with an increased risk of adverse infant development, cognition, language, motor development, temperament and internalising and externalising behaviours (Field 2011). In adolescence, children exposed to severe prenatal anxiety had a risk of mental disorders two-times that of the general UK population (O'Donnell *et al.* 2014). Longer term consequences include an increased risk of affective disorders, ASD, ADHD, schizophrenia and antisocial behaviour (Pawlby *et al.* 2009; Hay *et al.* 2010; Plant *et al.* 2015; Morgan *et al.* 2019). Non-communicable diseases such as type 2 diabetes, coronary heart disease and hypertension have also been linked with exposure to prenatal stress (Gluckman and Hanson 2004). The hypothesis encompassing this increased risk for disease after exposure to prenatal stress is called the Developmental Origins of Health and Disease (DOHaD) hypothesis (Barker 2007). It proposes that *in utero* stresses such as poor prenatal mental health, malnutrition, toxins or infections can affect short and long term health. In response to stressors the fetus adapts in an attempt to increase survival rates, but this adaptation can have lasting consequences especially if the *in utero* environment does not match the postnatal environment.

Studying the effects of prenatal mental health on exposed children can be difficult due to the confounding effects of postnatal mental health disorders. Prenatal depression and anxiety are some of the strongest predictors for postnatal depression and anxiety which can last up until a year after birth, extending the disabling period (Andersson *et al.* 2006). Prenatal and postnatal mental health have been independently linked with adverse infant outcomes suggesting different mechanisms may underlie the associations (O'Connor *et al.* 2002). Prenatal mental health may impact infant development through biological pathways, whereas postnatal disorders could act through psychological means. Another confounder when studying prenatal mental health and child development is offspring sex. While not always explored, frequent differences have been observed in development trajectories and behaviour between boys and girls exposed *in utero* to maternal depression and anxiety (see Chapter 7).

These long lasting consequences to prenatal depression and anxiety are not without a cost to the NHS and government. It is estimated that on average for every one year of births in the UK it costs £8.1 billion to deal with the impact of prenatal mental health disorders, the predominant cost relating to the exposed child (Bauer *et al.* 2014). This breaks down to approximately £76,000 per woman suffering from prenatal depression and £35,000 per woman suffering from prenatal anxiety (Bauer *et al.* 2016). However, despite this in the UK research into prenatal mental health is severely underfunded. A

recent report revealed for every £1 spent by the NHS on pregnancy care less than 1p was spent on pregnancy-related research (Guthrie *et al.* 2020). Comparatively 12p is spent on research into cancer. This pregnancy-related research covers all aspects of pregnancy including conception, contraception and both the prenatal and postnatal periods. Therefore, within this already chronically underfunded umbrella of pregnancy research, only 4% directly relates to prenatal mental health. This is despite the fact that up to 20% of all pregnant women suffer from a mental disorder during pregnancy and the lasting impacts it can have on both mother and child.

1.1.6 Causes

Why is the prevalence of mental health disorders during pregnancy so high given the adverse consequences for the exposed child? It has been suggested to be a consequence of evolution with prenatal maternal mental health disorders resulting in adaptive traits for the mother and child (Glover 2011). The adverse outcomes associated with depression and anxiety for the infant, such as ADHD, may be advantageous in an environment where a high state of awareness is protective against potential threats. Glover postulates that although depression and anxiety disorders are similar, it is likely that they have different adaptive values. Symptoms of depression such as crying may induce help from a support network and fatigue may save energy. The selection of anxiety, however, may be as a consequence of a previous way of life whereby an anxious state may help defend against real and perceived threats.

The underlying mechanisms that lead to depression and anxiety in the perinatal period are unknown. Similar to other mental health disorders it is suggested to be a combination of genetic and environmental factors that contribute. Few studies have investigated the genetic basis of prenatal depression and anxiety, but numerous studies have investigated the genetic component of these disorders outside of pregnancy. While individual studies have identified loci associated with the incidence of depression and anxiety, to date there have been no highly reproducible loci identified (Shadrina *et al.* 2018). Furthermore, studies have estimated the heritability of depression and anxiety to be between 30-50% (Gottschalk 2017; Shadrina *et al.* 2018) suggesting a substantial proportion of environmental influence.

Studies investigating environmental factors suggest similar elements are involved in depression and anxiety, including childhood trauma and stressful life events. Severity of different diseases such as cancer, diabetes and other chronic disorders are linked with the onset of depression, as well as the drugs that treat these diseases such as antihypertensive and immunomodulatory drugs (Patten and Love 1997; Nabeshima and Kim 2013). Unlike genetic loci, lifestyle and demographic factors have been investigated with respect to prenatal mental health disorders extensively (Biaggi *et al.* 2016; Dennis *et al.* 2017). While results are often conflicting, potentially due to population type and clinical

measures used, environmental factors are clearly important predictors of prenatal mental health (see Chapter 3).

The endocrine system can drastically affect key signalling pathways in the brain, and thus hormones have been implicated in the onset of depression and anxiety. Pregnancy is underlined by numerous changes to hormones ensuring the maternal body can adapt to support both mother and child. Therefore, either the drastic natural change in hormone levels, or a misfiring of pregnancy hormones could help drive the onset of perinatal mental health conditions. The HPA axis is commonly suggested to play a role in depression and anxiety (Dickens and Pawluski 2018). The HPA axis is the body's stress response and acts through its end product, the hormone cortisol (Brunton *et al.* 2008). Disruption of the pathway has been implicated in numerous mental health disorders and fetal exposure to high cortisol concentrations has been linked to adverse infant outcomes (Orta *et al.* 2017). Steroid hormones such as oestrogen and progesterone interact with the HPA axis and concentrations increase over gestation (Bloch *et al.* 2003). Independent to the HPA axis, they have also been shown to elicit changes in mood and cognition (Rubinow 2005). Steroid hormones also interact with serotonergic systems (Rubinow *et al.* 1998), which are implicated in mental health through the use of SSRIs as treatment for depression and anxiety. Numerous endocrine pathways have the potential to influence maternal mental health and almost all changes to hormone levels in pregnancy are linked to the placenta.

1.2. Placenta

The word placenta is derived from Latin for "flat cake" alluding to its shape in humans (Cross 2005). It is one of the most important organs supporting fetal life until parturition, but also one of the most poorly characterised. The placenta is predominantly constituted of cells from fetal origin that connects the growing fetus to the mother during pregnancy. As a defining characteristic of eutherian mammals, it is also the most diverse organ within the subclass (Carter and Mess 2007). However, across species the roles of the placenta in supporting fetal growth and maintaining gestation are conserved. In some lizards and snakes an extra-embryonic membrane allows for maternal-fetal exchange to support growth, but the sophisticated extra-embryonic structure of a placenta in mammals allows for a longer gestation and thus live birth of offspring (Stewart and Thompson 2003). In humans, over the course of nine months the placenta develops into a large organ weighing on average 15% of the baby's birthweight (Griffiths and Campbell 2014).

In order to fully support the developing fetus the placenta's formation is a tightly orchestrated process. In humans, at conception an oocyte and sperm cell combine to form a totipotent zygote. Five days after this event a pre-implantation embryo is formed comprised of three distinct cell lineages; the epiblast that will form the definitive germ layers, the primitive endoderm which will provide nutrient support and the trophoectoderm which gives rise to the placenta. The trophoectoderm differentiates

into trophoblast cells which subsequently divide into an inner cytotrophoblast and outer syncytiotrophoblast. Implantation happens around day six or seven of gestation at the maternal endometrium after which the surrounding endometrium is transformed into decidua. This is thought to be the beginning of placental development. During implantation the syncytiotrophoblast invades through projections into the maternal decidua. Inner cytotrophoblast cells proliferate and fuse with the syncytiotrophoblast cells causing further expansion into the decidua. At days eight to nine within syncytiotrophoblast cells, fluid filled spaces called lacunae appear. The syncytiotrophoblast subsequently penetrate and remodel maternal blood vessels and decidual glands causing maternal secretions to fill the lacunae. An anti-clotting factor prevents a wound healing response by maternal decidua to ensure continuous blood flow (Turco and Moffett 2019).

In the second week, syncytiotrophoblast begin to form more complex projections with cytotrophoblast cores called primary chorionic villi. By the third week underlying extraembryonic mesoderm, originating from the epiblast, migrate into the core of the primary villi at which point they are classed as secondary chorionic villi. By the end of the third week fetal blood vessels begin to form in the core of the villi transforming them into tertiary chorionic villi which branch out to maximise the surface area for exchange. By the fourth week, fetal blood flow is established through umbilical arteries that branch into chorionic villi through fetal capillaries. The outer layers of the villi, in contact with maternal blood, ensure separation between the maternal and fetal blood systems. The definitive placenta is formed through proliferation of villi containing fetal blood vessels, an inner layer of cytotrophoblast cells and an outer layer of syncytiotrophoblast. The latter of which are bathed in maternal blood allowing transfer of nutrients, gases and waste products through a utero-placental circulation system. This rapid development of the placenta means that by the end of the first trimester, the foundations for the placenta are laid (Turco and Moffett 2019). (Figure 1.1)

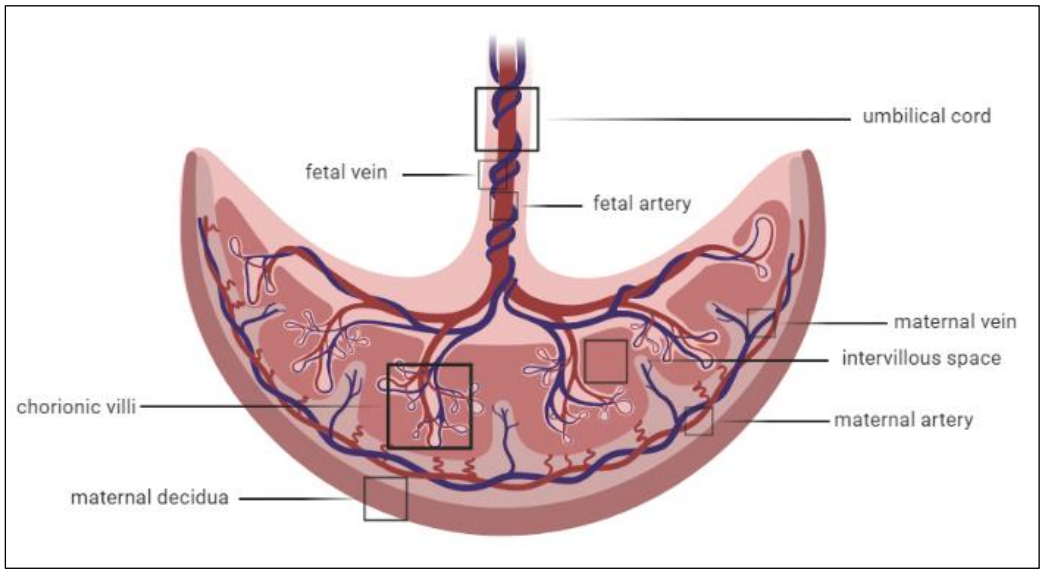

Figure 1.1: Human placenta schematic with relevant cell types identified. Created with biorender.com.

1.2.1 Functions

As the placenta is the primary organ for supporting fetal growth and development, it has numerous and highly specialised roles. The main functions of the placenta can be grouped into transport and metabolism, protection and hormone production (Gude *et al.* 2004).

The fetus is dependent on its mother for nutrition throughout pregnancy. Nutrients such as glucose are transported across the placenta, at first through maternal gland secretions and subsequently through maternal blood flow. This switch happens at 10-12 weeks when maternal blood flow is established in the placenta. At this point the exchange of oxygen and carbon dioxide between maternal and fetal circulations begins. This is essential as the placenta has high rate of oxygen consumption compared to other tissues in order to support the fetus (Gude *et al.* 2004). As previously described, the maternal and fetal blood is kept separate, in what is known as a hemochorial placenta. This is essential to protect the growing fetus from harmful substances. Xenobiotics circulating in maternal blood that have the potential to harm the fetus, are prevented from crossing the placenta through this separation. Enzymes and export pumps are also situated at the interface as a reinforcement measure for smaller xenobiotics. However, despite these systems in place, xenobiotics such as alcohol, drugs including thalidomide and SSRIs, and some viruses such as rubella can still reach the fetus and cause harm (Griffiths and Campbell 2014).

Finally, and most importantly, the placenta also acts as a super endocrine organ. As the placenta does not contain nerves, communication between mother and fetus is dependent on substance secretion in the blood. While the placenta produces substances such as cytokines and chemokines, the communication is predominantly achieved through hormones such as human chorionic gonadotropin (hCG), oestrogen, progesterone and human placental lactogen (hPL). In human placenta, the majority of hormones are produced by syncytiotrophoblast cells (Gude *et al.* 2004) although recently the extra villous cytotrophoblast cells, which are an invasive cell type, have been identified as another site of hormone production (Liu *et al.* 2018). Given the tightly orchestrated mechanisms required for fetal development, placental hormones have an array of different functions. hCG is important early in pregnancy as it stimulates cytotrophoblast fusion and differentiation of villous cytotrophoblasts. It is also suggested to prevent the degradation of the corpus luteum in the ovary that produces progesterone and oestrogen until the placenta takes over around eight weeks of pregnancy (Malassine and Cronier 2002). Progesterone and oestrogen are required to maintain the maternal decidua and thus attachment of the fetus to the uterine wall. Progesterone also prevents uterine contraction and oestrogen additionally acts as a specialised growth hormone for maternal reproductive organs such as the breasts, cervix, vagina and uterus (Gude *et al.* 2004). The hormone hPL is produced by the syncytiotrophoblast and has important functions in both mother and fetus. In the fetus it supports growth and development and stimulates the production of adrenocortical hormones. While in the

mother hPL is involved in insulin sensitivity and the induction of maternal behaviours (Handwerger and Freemark 1987) (See Chapter 6).

1.2.2 Sexual dimorphisms

As previously described the placenta is predominantly of fetal origin and thus is effectively male or female. Differences in gene expression between male and female placentas have been observed in cytotrophoblasts, syncytiotrophoblasts, arterial and venous endothelium, as well as maternal decidua (Wang *et al.* 2012b; Cvitic *et al.* 2013). Inflammatory markers, hormones and cytokines have also been reported as different between male and female placenta (Clifton 2010). It is suggested that these differences may underlie sexually dimorphic outcomes of pregnancy. For example, boys are at higher risk of prematurity, related morbidities and obstetric complications. After birth different outcomes have been reported for boys and girls, especially in relation to prenatal stressors such as depression (Sutherland and Brunwasser 2018) (see Chapter 7). Differences have also been observed in mothers carrying boys and girls; in one study mothers carrying boys were more likely to have higher anxiety in the first trimester, eat more calories, perform better at neurocognitive tasks and have lower blood pressure (DiPietro and Voegtline 2017).

Writings in the 2nd Century CE postulated that differences in the mother may result from the more vigorous and stimulating nature of male fetuses (Temkin 1991). While the exact underlying mechanisms behind these sexually dimorphic outcomes are unknown, current literature suggests a link to the placental sex. One theory relates to the presence of a foreign Y chromosome in placental cells (Gualtieri and Hicks 1985). An enrichment of graft vs host, immune and inflammation pathways observed in male placentas may be a response to maternal protection against an incompatible Y chromosome (Cvitic *et al.* 2013). A competing theory is the difference in sex steroid production. The placenta produces oestrogen, progesterone and androgens which are critical in the feminisation and masculinisation of fetal genitalia. However, these hormones are also predicted to influence maternal and fetal health and may interact with other endocrine systems to elicit sexually dimorphic changes (DiPietro and Voegtline 2017).

1.2.3 Maternal mental health

Inappropriate functioning of the placenta is linked to pregnancy complications such as pre-eclampsia and fetal growth restriction. The placenta has also been suggested to act as a mediator in the DOHaD hypothesis. A poorly functioning placenta in response to a prenatal stressor may convey this stress to the developing fetus.

As previously described, prenatal depression and anxiety are associated with adverse outcomes for exposed infants. A number of studies have investigated the placenta as a potential mediator of this association in humans. Whilst no studies have analysed the whole transcriptome of the placenta using next generation sequencing, targeted analysis has been performed, typically with a focus on the HPA

axis (Glover 2014; Zhang *et al.* 2017). In association with prenatal depression and anxiety reductions have been observed in expression of *11β-hydroxysteroid dehydrogenase type II* (*11β-HSD2*) (O'Donnell *et al.* 2012). This placental enzyme inactivates cortisol into cortisone and thus a reduction of the enzyme may increase trans-placental transfer of cortisol to the fetus. Prenatal exposure to synthetic cortisol is associated with affective disorders and neurosensory deficits after birth (Moisiadis and Matthews 2014). However, these studies are often confounded by prematurity (the primary reason for administration of synthetic cortisol) and use a synthetic compound which may act differently to endogenous cortisol (Stirrat *et al.* 2018). Other confounding factors when assessing the role of the placenta in relation to maternal mental health and infant outcomes include timing, severity and measure of prenatal stress as well as postnatal maternal care. To account for this, animal studies are used which allow such confounders to be controlled. In addition to a controlled environmental setting, animal models can be used to investigate the causality of associations and examination of underlying mechanisms.

1.2.4 Mouse studies

The association between prenatal mental health and adverse offspring outcomes in humans has been modelled in mice, with alterations in cognition, behaviour, circadian rhythm, brain structure and sleep all observed in offspring (Weinstock 2001, 2008). However, the exact phenotypes in response to stress are not always reproduced which may relate to species, type of stressor and whether analyses was separated by offspring sex. For example, evidence of learning deficits and depression- and anxiety-like symptoms have been reported as different in male and female offspring exposed to prenatal stress (Mueller and Bale 2008). However, despite these limitations, animal studies have illustrated a causal direction between prenatal mental stressors and impaired offspring outcomes.

Animal studies have also been used to investigate the mediating role of the placenta between prenatal stress and infant outcomes. Similarly to humans, stress leads to decreased expression of *11β-HSD2* and mutations in the gene cause depression- and anxiety-like behaviour in rodents (Mairesse *et al.* 2007). Furthermore, reduced enzymatic activity is linked to poor offspring outcomes such as lower birth weights and improper neurodevelopment (Trejo *et al.* 2000). Differences in *11β-HSD2* have also been described in male and female placenta, both at baseline and in response to stress (Pankevich *et al.* 2009; Cuffe *et al.* 2011) which may relate to sexually dimorphic phenotypes in prenatal stress studies.

One of the biggest criticisms of studying prenatal mental stressors in rodents is the translatability to human mental health. One alternative not commonly used is studying captive primates given their close relation to humans. In a group of rhesus monkeys, low ranking females are exposed to chronic stress during their pregnancy which offers a more natural model to study. Dramatic differences in gene methylation were observed in the placenta of these low ranking females (Massart *et al.* 2017). A

number of these methylation marks overlapped with genes previously associated with social rank in adults suggesting long-term consequences of alterations in the placenta (Tung *et al.* 2012). This highlights the ability of placental epigenetic marks to respond to stimuli and convey this stress to the offspring.

1.3 Imprinted genes

Genomic imprinting is an epigenetic process whereby genes are differentially expressed in a parent-of-origin specific manner (Surani *et al.* 1984). Expression of more than 99% of autosomal genes comes from two alleles; one inherited from the mother and one from the father. However, for imprinted genes expression is instead monoallelic and comes from either the maternal or paternal allele. Therefore, while the alleles are in a diploid state in the cell, they are functionally haploid (Surani 1998). Expression of the imprinted gene *Paternally Expressed Gene 3* (*PEG3*) in the placenta has recently been linked to prenatal depression (Janssen *et al.* 2016) providing the first evidence that members of this remarkable gene family may be relevant to our understanding of maternal mental health disorders.

1.3.1 Evolution

Imprinting is not widespread across taxa; monoallelic expression has only been observed in mammals, some seed bearing plants and two invertebrate arthropods (*Coccidae* and *Sciaridae*) (Scott and Spielman 2006). While indirect evidence exists for monoallelic expression in birds, to date no imprinted genes have been observed (Tuiskula-Haavisto and Vilkki 2007). Within mammals, imprinted genes are unique to placental mammals and not observed in egg-laying prototherians. The existence in both eutherian and marsupial mammals suggest imprinted genes may have evolved concurrently with the evolution of the placenta (Hore *et al.* 2007).

1.3.2 Discovery

Imprinting was discovered in mammals in 1984 through nucleus transplantation experiments of mouse embryos. Zygotes with either two female or male pronuclei were created. Despite comprising of two sets of parental DNA, neither were viable after implantation with extreme complications in fetal growth and placental development. This highlighted the importance of both a maternal and paternal genome for successful fetal development (McGrath and Solter 1984; Surani *et al.* 1984).

1.3.3 Mechanisms

Imprinted status is controlled by epigenetic mechanisms, both DNA methylation and histone modifications, that ‘mark’ the DNA. The Greek prefix ‘epi’ means on top of or over, and thus epigenetics refers to modifications that lie on top of chromatin and regulate genomic transcription without altering the underlying DNA. Imprinting is initially established by DNA methylation on one of the parental alleles at a specific site in either the male or female germline. This region of DNA

methylation is known as a germline differentially methylated region (gDMR). Usually imprinted genes are found in clusters of 3-12 over approximately 1 Mb and each of these regions contains one gDMR also called an imprinting control region (ICR). Deletion of the ICR results in disruption of imprinting in the entire cluster. Imprinting is commonly exerted through one of two main mechanisms; either allelic transcription of a long non-coding RNA or methylation-sensitive binding of an insulator protein. There are also a small number of imprinted genes not located within clusters that appear to be controlled by methylation at the gene's promoter (Plasschaert and Bartolomei 2014).

1.3.4 Theories

There is a strong conservation of imprinted status between humans and mice signifying their importance (Plasschaert and Bartolomei 2014). Biallelic genes protect organisms from the effects of a mutated gene as there is effectively a 'back-up' allele. Imprinted genes do not confer this advantage and mutations that inactivate the active allele are the cause of a number of genetic disorders (Butler 2009). Therefore, the existence of imprinted genes must rely on adaptive traits that outweigh the absence of this protection (Kondrashov and Crow 1991).

There are several hypotheses to account for the presence of imprinted genes. One of the most popular is the parental conflict hypothesis originally proposed in 1991 (Moore and Haig 1991). Pregnancy requires a substantial investment from the mother, diverting her own resources to support the growing fetus. As there are no costs to the male during pregnancy, this creates inequality of parental investment in the offspring. It therefore could be evolutionarily advantageous for the paternal genome to switch off fetal genes that limit fetal and/or placental growth thus promoting his offspring's growth and extracting all possible resources from the mother to increase fitness and survival. In contrast, the mother must balance resource allocation to her offspring with her own survival and ability to have offspring in subsequent pregnancies. Therefore, the maternal genome must react to this manipulation by limiting the amount of resources available to the fetus. This enables her to safeguard her own survival for postnatal nutrition and subsequent pregnancies. Both genomes are thus trying to maximise the chance to successfully pass on their own genes to the maximum number of offspring. In general, maternally expressed genes often limit fetal growth and paternally expressed genes promote it (Haig 2014).

While this is the most popular hypothesis, there are aspects of imprinting that do not fit with the theory. For example, a number of genes imprinted status persists in the adult brain, long after the perinatal period and thus parental genomes should have little investment (Wilkinson *et al.* 2007). While other theories exist, no hypothesis fully explains the mechanisms and functions of imprinted genes. It is therefore possible that imprinted genes at different loci evolved in response to different pressures and conform to different theories (Wolf and Hager 2006).

1.3.5 Functions

From the above data it is clear that imprinted genes are crucial regulators in mammalian pregnancy. Indeed the majority of imprinted genes are expressed in placental and fetal tissues and downregulated after the perinatal period (Tunster *et al.* 2013). While the overall proportion of genes expressed monoallelically is small, they have a large influence on development. Using phenotypic analyses of humans and mice with altered imprinted gene expression, the functions of individual genes have been studied. The main functions of imprinted genes include fetal and placental growth and development, postnatal development, and adult behaviour and metabolism (Charalambous *et al.* 2007; Tucci *et al.* 2019).

1.3.5.1 Fetal Growth

The first imprinted gene identified in mammals was *Insulin-like Growth Factor 2* (*Igf2)*. Dosage experiments in mice revealed *Igf2* as a positive fetal growth regulator (DeChiara *et al.* 1990). It was subsequently reported that the heterozygous mutant offspring had the same fetal growth restriction phenotype as the homozygous offspring but only when the targeted allele was inherited from the male (DeChiara *et al.* 1991). In the same year, maternally expressed genes *Igf2r* and *H19* were discovered (Barlow *et al.* 1991; Bartolomei *et al.* 1991). Since then, a number of other imprinted genes have been identified and shown to either directly or indirectly regulate fetal growth in mice such as *Phlda2*, *Peg3*, *Cdkn1c* and *Grb10* (Plasschaert and Bartolomei 2014). Altered expression of a number of these imprinted genes have also been observed in *in utero* growth restricted (IUGR) placentas. IUGR is one of the leading causes of neonatal morbidity and mortality and is associated with later life risks for metabolic disorders such as type 2 diabetes (Resnik 2002). A number of studies have identified conflicting results regarding which imprinted genes are associated with IUGR. However, this may relate to the complex nature of the disorder and its numerous potential causes including maternal malnutrition, smoking and infection (Ishida and Moore 2013). The role of imprinted genes can be further linked to fetal growth by studying imprinted disorders. Phenotypes of imprinted disorders such as Beckwith-Wiedemann Syndrome (BWS) and Silver-Russel Syndrome (SRS) include large for gestational age (LGA) and small for gestational age (AGA) infants respectively. BWS is also associated with placental overgrowth (Plasschaert and Bartolomei 2014).

1.3.5.2 Placental Development

A number of imprinted genes have been identified that regulate placental development, predominantly through mouse experiments. *Igf2* is required for placental growth and a lack of placental *Igf2* impacts nutrient transport to the fetus resulting in fetal growth restriction (Constância *et al.* 2005). *Rtl1* is a key regulator of placental capillaries for blood supply, and *Peg3*, *Ascl2*, *Phlda2* and *Cdkn1c* are all involved in the development or differentiation of the mouse placental endocrine lineages including the spongiotrophoblast (John and Hemberger 2012). Disruption of *DNMT3L*, a key regulator of DNA

methylation which maintains imprinting also leads to impaired placental development (Plasschaert and Bartolomei 2014).

1.3.5.3 Postnatal period

The role of imprinted genes continues into the postnatal period through the regulation of growth and metabolism. After birth, pups need to be able to regulate their temperature and a number of imprinted genes have been linked to the control of thermogenesis. Disruption of *Dlk1* and *Dio3* in the same imprinted cluster impairs the development of brown adipose tissue which turns food into body heat. Deletion of *Peg3* also causes dangerously low body temperatures in pups decreasing postnatal survival (Millership *et al.* 2019).

Adequate nutrition after birth is necessary for pup growth and survival, and is in part regulated by imprinted genes. Altered expression of *Magel2*, *XLas* and *Peg3* all lead to impaired suckling, postnatal growth and thus high postnatal lethality (Millership *et al.* 2019). A recent study additionally found impaired isolation-induced ultrasonic vocalisations (USVs) in *Peg3* mutant pups (McNamara *et al.* 2018). Pups signal to their mother through these USVs to elicit maternal care. Therefore, aberrant USVs may contribute to the poor postnatal growth phenotype.

1.3.5.4 Adult brain

One function of Peg3 in adult mice is in directly regulating maternal behaviour. Disrupted expression in pregnant dams was found to cause neglect of pups, impaired milk let down, reduced food intake and a failure to build adequate nests (Li *et al.* 1999; Curley *et al.* 2004; Champagne *et al.* 2009). Therefore, expression in both the mother and offspring affect postnatal offspring survival. In addition to maternal care imprinted genes also function in the regulation of metabolism, behaviour and memory. For example, mutant adult *Peg3* mice display both metabolic and behavioural disorders (Curley *et al.* 2005).

1.3.6 Endocrine dysfunction and imprinted genes

In mice, imprinted genes also indirectly influence maternal behaviour by regulating the development of placental endocrine lineages (Creeth and John 2020). Similar to human placenta, nutrient and gas exchange in mouse placenta is hemochorial in nature. However, differences exist in litter size, gestation length and in placental organisation. The fetal component of mouse placenta is separated into the labyrinth and the junctional zone (Figure 1.2). The labyrinth zone performs nutrient and gas exchange between maternal and fetal circulations similar to tertiary chorionic villi in humans. The junctional zone is the endocrine compartment and comprises of two cell types; spongiotrophoblast cells, and glycogen cells, and is lined by a single layer of parietal giant trophoblast cells. Comparable to the syncytiotrophoblast cells of human placenta, the spongiotrophoblast is the main endocrine producing lineage of murine placenta (Hemberger *et al.* 2020).

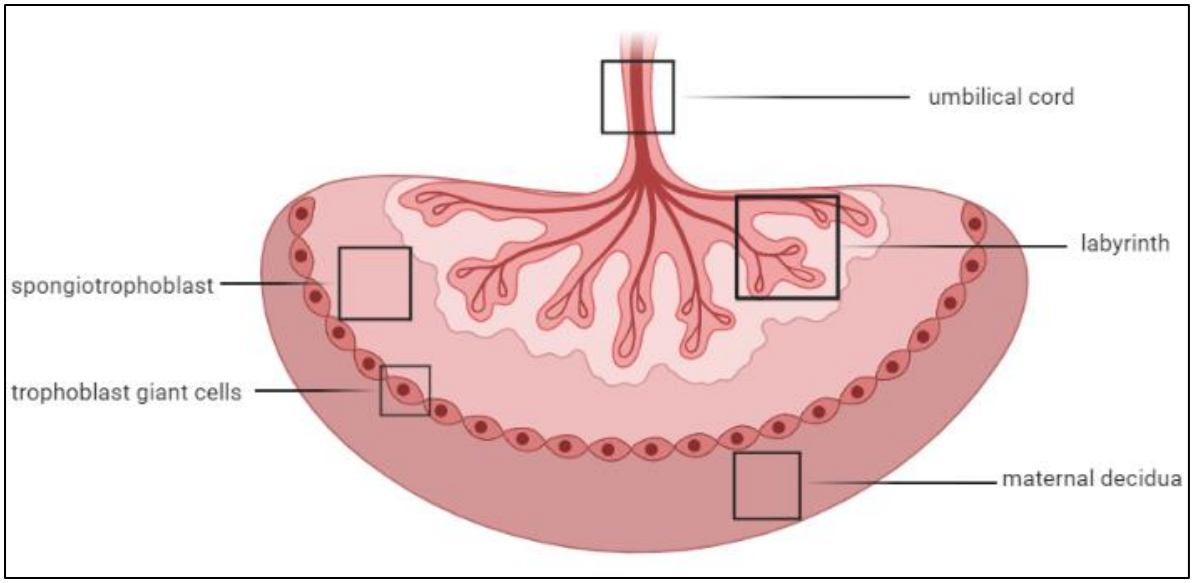

Figure 1.2: Mouse placenta schematic with relevant cell types identified. Created with biorender.com.

A small number of imprinted genes have been identified that positively or negatively regulate the placental endocrine lineages. Placental morphology analysis of transgenic mouse models have identified *Phlda2*, *Cdkn1c*, *Ascl2*, *Peg3*, *Igf2* and *Peg10* as important lineage regulators (John and Hemberger 2012; Aykroyd *et al.* 2020). The disruption of the endocrine lineages in response to aberrant imprinted gene expression consequently impacts the expression of pregnancy-related hormones (Creeth and John 2020). For example, loss of *Phlda2* expression resulted in a larger spongiotrophoblast and increased expression of pregnancy-related hormones, whereas two-fold expression of *Phlda2* was reported to restrict the size of the spongiotrophoblast lineage by 50% and reduced the expression of pregnancy-related lactogenic hormones (Prls) (Tunster *et al.* 2016). Acting antagonistically to *Phlda2*, loss of *Peg3* expression decreased the size of the spongiotrophoblast and reduced the expression of Prls (Tunster *et al.* 2018). Interestingly, this response to reduced *Peg3* expression was predominantly observed in males, as female placenta showed a more muted phenotype. Some of the hormones altered by these mutations are related to the pituitary hormone prolactin which is involved in priming the female for motherhood through stimulation of mammary glands and induction of maternal behaviour (Handwerger and Freemark 1987). Approximately halfway through gestation, prolactin is replaced by Prls secreted by the placenta. 22 Prls are produced by mouse placenta, but only a small number (Prl3d1-3 and Prl3b1) can bind and activate the prolactin receptor thus functioning as true placental lactogens (Soares *et al.* 2007). These Prls are predicted to function in the same way as prolactin and infusion of placental lactogen directly into the brain of non-pregnant females has been shown to stimulate maternal behaviour (Grattan 2011; Creeth *et al.* 2019). Indeed, it is possible the placental lactogen is more important than prolactin in the priming of maternal behaviour as mice with a mutated prolactin gene still exhibit a degree of maternal care behaviours (Horseman *et al.* 1997).

Two of the imprinted genes that regulate the endocrine spongiotrophoblast lineage, *Phlda2* and *Peg3*, have also been shown to influence maternal behaviour. Wild-type dams exposed to altered placental *Phlda2* showed modified gene expression in the prenatal maternal hypothalamus and hippocampus; two regions critical for maternal care. After birth, dams exposed *in utero* to the altered spongiotrophoblast displayed altered caregiving behaviours postpartum. In the model in which *Phlda2* was overexpressed by two fold with the smaller spongiotrophoblast lineages, dams spent less time on nursing and grooming their pups consistent with the proposed function of placental hormones in instructing maternal caregiving (Creeth *et al.* 2018). Similarly, wild-type mothers exposed to *Peg3* mutant placenta, with a smaller spongiotrophoblast and glycogen cell lineage, also showed some changes in behaviour during pregnancy and heightened anxiety-like behaviours after birth (McNamara *et al.* 2018). The more subtle changes observed during pregnancy may have been masked by the sexually dimorphic placental response to reduced *Peg3* expression. It is worth noting that one study found no link between placental *Peg3* expression and maternal behaviour in a different strain of mouse (Denizot *et al.* 2016), which could suggest a strain specific effect. However, given a relationship between *PEG3* expression and maternal mental health in humans has been observed (Section 1.3.7), a conservation of this link between species is implied. Therefore, differences in experimental design may account for the conflicting observation.

With the caveat that there are important differences between human and mouse placentas, this finding that placental endocrine insufficiency causes alterations to the behaviour of dams could thus be translated into human pregnancies. While maternal care-giving behaviours are different to maternal mental health disorders, prenatal depression and anxiety could be a presentation of misprogrammed maternal behaviour (Creeth *et al.* 2019). The work in mice demonstrated that the expression of at least two imprinted genes in the placenta can indirectly influence maternal behaviour by inducing placental endocrine insufficiency.

1.3.7 Maternal mental health disorders and imprinted genes

In humans, three genes encode placental lactogen. *Chorionic somatomammotropin* (*CSH*) 1 and 2 both encode human placental lactogen (hPL) while expression from the putative pseudogene *CSHL1* is thought to be less relevant. Similar to Prl3b1, hPL can bind and activate the prolactin receptor and is predicted to function in the same way as prolactin potentially also programming maternal behaviour in human pregnancy. If, as in mice, imprinted genes regulate the expression of placental hormones in the human placenta, this opens up the possibility that dysfunctional imprinting may contribute to maternal mental health disorders by causing placental endocrine insufficiency.

A limited number of studies have investigated prenatal mental health and imprinted genes in humans. The majority focus on methylation status as a proxy for gene expression and have identified associations between cord blood methylation and prenatal depression and anxiety symptoms (Liu *et*

al. 2012; Vangeel *et al.* 2015; Mansell *et al.* 2016). A smaller number of studies focus on placental gene expression, although despite increasing awareness in the literature, do not always separate analysis by fetal sex (Litzky *et al.* 2018). The one study that did analyse male and female placentas separately observed a significant reduction in *PEG3* expression in relation to prenatal depression, but only in those who had boys (Janssen *et al.* 2016). In this same study, reduced expression of *hPL* was also reported. The fact that the association was only found in males could correspond with the sexually dimorphic response observed in the placental analysis of *Peg3* knockout mice offspring which predominantly affected males (Tunster *et al.* 2018). As described previously, imprinted gene expression is also associated with postnatal behaviours such as learning and memory. Therefore, an altered expression of imprinted genes may additionally underlie the relationship between prenatal mental health and impaired offspring development.

1.4 Aims and hypotheses

Placental endocrine insufficiency driven by aberrant imprinted gene expression may cause maternal mental health disorders during pregnancy contributing to adverse outcomes for offspring. While evidence exists in mice, limited research has been performed in humans.

The aims of this study were to:

1) Describe the GiW cohort and identify predictors of prenatal depression and anxiety.
2) Analyse the placental transcriptome of pregnancies affected by prenatal depression.
3) Analyse targeted imprinted gene and placental hormone expression in the placenta in relation to perinatal depression and anxiety.
4) Analyse serum *hPL* expression in relation to perinatal depression and anxiety.
5) Analyse infant outcomes at 12 months in relation to prenatal depression and anxiety and biological measures.

The hypothesis to be tested were as follows:

1) A number of demographic factors will be associated with prenatal depression and anxiety.
2) The placental transcriptome of mothers with prenatal depression will differ to pregnancies uncomplicated by mental health.
3) Imprinted gene and placental hormone expression will be associated with perinatal mental health.
4) Serum hPL concentrations will be associated with perinatal mental health.
5) Imprinted gene expression will mediate a relationship between prenatal mental health and adverse infant outcomes.

Chapter 2: Methods

2.1 Recruitment

The Grown in Wales (GiW) study, funded by the Medical Research Council, was established in 2015 to examine the relationship between prenatal mental health and placental function (Janssen *et al.* 2018). Briefly, 355 women were recruited by two trained research midwives at the University Hospital of Wales (UHW) in Cardiff (Cardiff and the Vale Health Board) between 1st September 2015 and 31st November 2016. All participants were recruited at a pre-surgical appointment for an elective caesarean (ELCS) one to four days before surgery. ELCS delivery was chosen to maximise the collection of biological samples. It also ensured the placenta collected had not gone through the physiological process of labour which has been shown previously to alter gene expression (Janssen *et al.* 2015). Women were invited to participate in the study if they were pregnant with a singleton at term, had no fetal abnormalities, infections or diseases, were between the ages of 18 and 45 and were able to read English. The participants in the GiW cohort were followed up postnatally at three time points (Figure 2.1); within seven days of birth (average 4.2 days) (P1), ten weeks after birth (average 69.1 days) (P2) and at 12 months postnatally (average 405.8 days) (Y1).

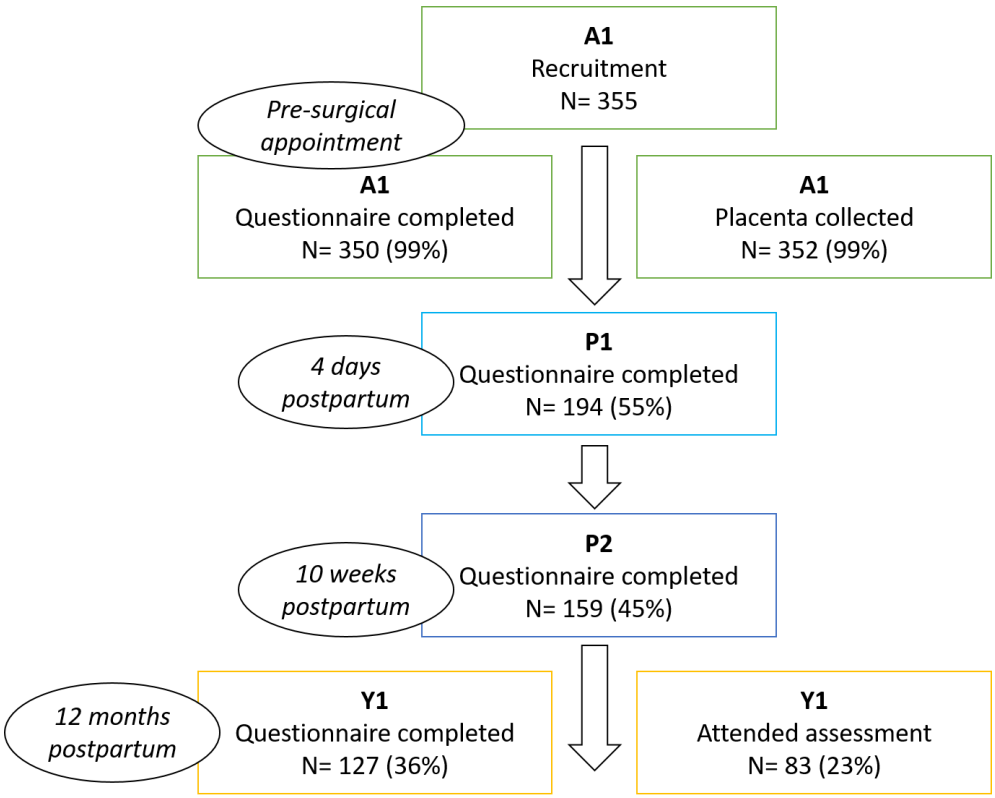

***Figure 2.1: Grown in Wales cohort study design**. Retention rates are shown as percentages in brackets.*

2.2 Data collection

2.2.1 Antenatal data collection (A1)

Participant data was collected through an extensive self-reporting questionnaire at the pre-surgical appointment and through medical notes filled in by a research midwife immediately after delivery.

2.2.1.1 Participant questionnaire

The questionnaire was separated into three sections; a demographic and lifestyle section, two nested questionnaires on current mental health, and a further food frequency questionnaire. The latter was not used in the current thesis, and thus will not be detailed further.

The first section included questions regarding maternal age, height, pre-pregnancy and current weight, ethnicity, education and income. Pre-pregnancy BMI was calculated using the maternally reported weight and height. Welsh Index of Multiple Deprivation (WIMD) scores were coded from participant's postcodes using a publicly accessible website (http://wimd.wales.gov.uk). WIMD scores are the Welsh Government's official measure of deprivation and combines income, employment, health, education, housing, physical environment, access to services and community services. The lower the WIMD score the higher the level of deprivation. Lifestyle questions included smoking and alcohol consumption in the first trimester and trimesters two and three combined. These were subsequently joined to create variables covering the whole pregnancy. Questions on mental health history and current medication for mental health were also included. Current mental health was measured using two self-reporting questionnaires designed to assess depression and anxiety symptoms.

2.2.1.1.1 Edinburgh Postnatal Depression Scale

The Edinburgh Postnatal Depression Scale (EPDS) is one of the most common screening tools for depression in the perinatal period. Introduced in 1987 for postnatal depression it has since been validated for use in pregnancy and outside of the perinatal period (Cox *et al.* 1987; Murray and Cox 1990; Cox *et al.* 1996). The EPDS questionnaire was designed to specifically differentiate depressive symptoms from typical perinatal physiological experiences, unlike more general questionnaires such as Beck's Depression Inventory (Beck *et al.* 1996; Cox *et al.* 2014). It therefore does not directly correspond to the DSM-IV-TR criteria for depression (Norhayati *et al.* 2015). The EPDS contains 10 questions relating to the last 7 days, such as '*I have been able to laugh and see the funny side of things*' and '*I have felt scared and panicky for no good reason*'. Each question is scored 0-3 on a 4 point Likert scale, with total scores ranging from 0-30 (Cox *et al.* 1987). Some studies suggest a cut off of 10 to identify anyone experiencing distress and 15 for a major depressive disorder (Khanlari *et al.* 2019). However, the majority of validation studies have determined that a cut off score of 13 and above is adequate for screening probable depression (Cox *et al.* 1987; Murray and Cox 1990; Rubertsson *et al.* 2011) and thus is used in the present thesis. The internal consistency for the present

study was measured using the Cronbach's alpha and was found to be good at 0.85. The same questionnaire was used at all time points (A1, P1, P2 and Y1). A copy of the questionnaire can be found in the Appendix.

2.2.1.1.2 State Trait Anxiety Inventory

The Speilberger State Trait Anxiety Inventory (STAI) is a self-reporting questionnaire to measure anxiety (Spielberger *et al.* 1970; Spielberger *et al.* 1983). The full questionnaire measures both state and trait anxiety. State anxiety is defined as anxiety towards a particular situation and examines present feeling whereas trait anxiety measures anxiety experienced on a day to day basis across typical situations and asks about how a participant generally feels (Julian 2011). The trait questionnaire was used in the GiW study to assess prenatal anxiety, opposed to state anxiety which may relate to the upcoming ELCS. Developed in 1970 and revised in 1983, the STAI was originally designed to measure anxiety in the general population. Since then it has been widely used and validated in the perinatal period (Gunning *et al.* 2010; Sinesi *et al.* 2019). It contains 20 questions including '*I am cool, calm and collected*' and '*I feel secure*'. The questions are scored 1-4 on a 4 point Likert scale, with total scores ranging from 20-80. While there is no certified cut off for the STAI scales, both the 75th percentile score and scores of 39-40 have been suggested to detect clinically significant symptoms (Dayan *et al.* 2002; Sinesi *et al.* 2019). In the GiW cohort the 75th percentile of the STAI-T was 40. Therefore, 40 was used as the cut off for detecting anxiety. The internal consistency for the present study was measured using the Cronbach's alpha and was found to be excellent at 0.92. The same questionnaire was used at all time points (A1, P1, P2 and Y1). A copy of the questionnaire can be found in the Appendix.

2.2.1.2 Medical notes

Research midwives collected participant data through NHS maternity records. These included the history of the participant (previous pregnancy notes and mental health history), information regarding the current pregnancy (smoking and alcohol consumption, medication, pregnancy complications and maternal weight), and birth outcomes (date and time of delivery, indication for ELCS, mode of delivery, delivery complications, birthweight, fetal sex and head circumference). Northern Meteorological Seasons defined the season of birth. Custom birthweight centiles (CBWC), that take into account maternal influences on fetal growth, were calculated using the GROW bulk centile calculator (UK) (Gardosi and Francis 2016). CBWCs take into account infant birthweight, gender and gestational age as well as maternal height, weight, ethnicity and parity.

Data on smoking and alcohol in the medical notes were compared to smoking and alcohol use taken from the self-reporting questionnaire. Rates of alcohol consumption and smoking were higher in the questionnaire. This may be because participants were more open in the questionnaires than with their

midwife or doctor. The rates of alcohol and smoking in the questionnaire were deemed more accurate and used for analysis.

History of mental health and current medication were recorded in both the self-reporting questionnaire and medical notes. There was substantial overlap in history of mental health between the two data collections. However, some participants had described previous disorders in their questionnaire and not their medical notes and for others the reverse was true. These two variables were combined to capture all available data.

Similarly, current medication for mental health disorders was not always consistent between data collections and availability of data regarding stopping medication during pregnancy was mixed. Therefore, any participant prescribed medication during pregnancy according to their medical notes were grouped together for analysis. The majority of participants taking medication for a mental health disorder took antidepressants (93%) and the predominant class of antidepressant was SSRIs (84%). Therefore, analysis focussed specifically on this group.

2.2.1.3 Postnatal data collection

2.2.1.3.1 P1 and P2

To obtain postnatal EPDS and STAI scores a P1 postnatal questionnaire was designed to be completed within the first week after delivery and a second P2 questionnaire to be completed approximately two months after delivery. Initially, the GiW study was designed according to the UHW protocol for women undergoing an ELCS, which involved staying in hospital for three-four days after surgery. This would allow the initial P1 questionnaire to be delivered to the participant on the ward and collected by the research midwife ensuring the highest completion rate. However, during participant recruitment, this protocol was changed and instead women left hospital one day after surgery. This resulted in 70% of P1 questionnaires being sent to the home address and thus reliant on the participant posting it back. This resulted in a lower than expected retention rate between A1 and P1 (55%). There was no difference in mental health scores between those who completed the questionnaire at home or in the ward.

2.2.1.3.2 Y1

To obtain postnatal mental health scores 12 months after birth, another questionnaire containing the EPDS and STAI questions was sent out to participants. In addition, follow up lifestyle questions were included regarding current working status, weight, smoking, and maternal physical or mental health problems since the birth. Questions based on the infants sleep, breastfeeding and physical problems were also included, along with validated questionnaires regarding bonding (Postnatal Bonding Questionnaire (PBQ)), and the infant's temperament (Infant Behaviour Questionnaire-Revised-Short Form (IBQ-F-SF)) and aggression (Cardiff Infant Contentiousness Scale (CICS). These validated

questionnaires will be covered in Chapter 7. At 12 months participants were invited to partake in an assessment with their infant in a laboratory setting and will also be covered in detail in Chapter 7.

2.3 Statistical analysis

All statistics were performed in RStudio (version 1.2.5033) or SPSS (version 23.0), and differences of $p < 0.05$ were considered significant. All variables were analysed for outliers and normality. Missing data was addressed using participant level mean substitution for those missing less than 20% of data for the EPDS, STAI and infant questionnaires; IBQ, PBQ and CICS. Total scores were not included in analyses if the participant was missing more than 20%. All regression models were assessed for multicolinearity using the car package in RStudio (Fox and Weisberg 2019).

Chapter specific statistical analysis and participant exclusion criteria are detailed in each research chapter. However, for all analyses participants were excluded if they later withdrew from the study (n= 8), if they did not complete the A1 EPDS or STAI questionnaires (n= 7), if they did not go on to ultimately deliver by elective caesarean (n= 10), and if they were not White (n= 30). Non-White participants represented a small proportion of the cohort (3%) and were thus excluded to minimise potential confounders introduced by heterogeneous sampling in smaller sample sizes (Bornstein *et al.* 2013; Capron *et al.* 2018).

2.4 Biological data

Maternal venous blood samples were collected for 272 participants at the pre-surgical appointment as part of an anaesthetic review. Venous blood was centrifuged to collect maternal serum before being frozen at -80°C.

Trained midwives took placental samples after delivery (within two hours) for 352 participants. Placentas were weighed and assessed for abnormalities. Three to five placental biopsies were taken from each placenta to account for differential gene expression across the organ (Janssen *et al.* 2015). Villous tissue was collected from the maternal side of the placenta, midway between the cord and distal edge. To minimise contamination of maternal decidual cells the cotyledon surface was removed. Biopsies were washed in phosphate buffered saline (PBS) (Life Technologies, UK) to remove excess maternal blood and amniotic fluid. Approximately 10 mg of each sample were stored overnight in 1 ml RNA*later* (Sigma, UK) at 4°C. Placental samples were dissected in ice cold PBS (Life Technologies) to remove blood vessels and stored long-term at -80°C. The three to five placental biopsies were combined to make a pooled sample. Using a pestle and mortar, approximately 10 mg of the pooled placental tissue was pulverised in liquid nitrogen. The pulverised placenta was then separated into two tubes, one for RNA extraction (described in Section 2.4.1), and one for DNA extraction (described in the Appendix).

2.4.1 RNA extraction

1 ml TRIzol (Life Technologies) was added to pulverised placenta samples, before being re-suspended and incubated at room temperature for five minutes. 200 µl of chloroform (Sigma) was added to each sample, mixed and incubated at room temperature for three minutes. Samples were centrifuged at 12000 g at 4°C for 15 minutes. The aqueous RNA phase was then transferred into a fresh tube before 500 µl of isopropanol (Sigma) and 1 µl of GlycoBlue™ (ThermoFisher, UK) added to precipitate and stain the RNA respectively. Tubes were mixed by inversion and incubated for ten minutes at room temperature. Tubes were then centrifuged for 10 minutes at 12000 g at 4°C. Supernatant was discarded leaving the pellet, which was washed in 75% ethanol (diluted in RNase free water) by inversion. Tubes were centrifuged at 7500 g at 4°C for 5 minutes. The ethanol was discarded and the pellet (containing the RNA) left to dry before resuspension in 30 µl RNase free water. RNA concentration was determined using a Nanodrop ND-1000 spectrophotometer and a Qubit RNA BR Assay Kit (ThermoFisher). RNA quality was assessed using a TapeStation (Agilent Technologies, USA). The minimal acceptable RIN value for inclusion in later analysis was 7. RNA was stored long term at -80°C.

2.4.2 DNase treatment

To remove genomic DNA (gDNA) contamination, extracted RNA was treated with a DNase. 1 µl of RNase-Free DNase 10X Reaction Buffer (Promega UK) and 2 µl of RNase-Free DNase (Promega) were added to 2 µg of RNA. Nuclease-free water was then added to total 10 µl. Samples were incubated at 37°C for 30 minutes. After this time, 1 µl of RQ1 RNase-Free DNase Stop Solution (Promega) was added to each tube and left at 65°C for 10 minutes to stop the reaction and inactive the DNase.

2.4.3 Reverse Transcription

DNase treated RNA samples were reverse transcribed to generate cDNA for use in PCR (Section 2.4.5) and qPCR (Section 2.4.6). 10 µl of RNA was combined with 1 µl of Random Primers (Promega), 1 µl of dNTPs (ThermoFisher) and 1 µl of RNase free water. This mix was left to incubate at 65°C for 5 minutes. 4 µl of 5X First-Strand Buffer (Invitrogen, UK), 1 µl of 0.1 M DTT and 1 µl of RNaseOUT (Invitrogen) were added to each sample along with 1 µl of SuperScript III Reverse Transcriptase (Invitrogen). Samples were then incubated at 25°C for 5 minutes, then at 50°C for 60 minutes. The reaction was terminated by heating to 70°C for 15 minutes. cDNA samples were diluted 1:5 in 10 mM Tris (pH 8.0, Sigma) before storage at -20°C. PCR was performed on all RT(+) and RT(-) samples with RT(-) samples checked for gDNA contamination (protocol in Section 2.4.5). No band on the PCR gel in the RT(-) samples indicated no gDNA contamination.

2.4.4 Primer design

Primer sequences are shown in Table 2.1. Primers included those already published and those designed for the present study. Primer3 was used for the design of novel primers and PrimerBlast was used to check for non-specific amplification. The criteria for primer pairs was: a product size between 150-300 bp, an approximate melting temperature of 60°C and binding either side of an exon boundary to prevent gDNA amplification. A PCR was performed for each primer set to check amplification of an expected size product and ensure no non-specific amplification. Melt curves generated during qPCR were assessed to confirm the presence of a single peak suggesting no primer dimers or non-specific amplification.

YWHAZ and *SDHA* were chosen as reference genes based on their stability in previous studies on human placental tissue (Meller *et al.* 2005; Drewlo *et al.* 2012).

Table 2.1: Primers used for qPCR analysis of gene expression

Target Gene	***Primer sequence***	***Reference***
YWHAZ	Forward: TTCTTGATCCCCAATGCTTC Reverse: AGTTAAGGGCCAGACCCAGT	Designed by A Janssen
SDHA	Forward: GAACATCGGAACTGCGACTC Reverse: CCTCTGCTCCGTAGATGGTC	Own design
PEG3	Forward: CTCACAACACAATCCAGGAC Reverse: TAGACCTCGACTGGTGCTTG	(Feng *et al.* 2008)
YY1	Forward: TGGAGAGAACTCACCTCCTGAT Reverse: TGGCCGAGTTATCCCTGAAC	Own design
PHLDA2	Forward: GAGAGCTGCTGGAACGCG Reverse: CGCACGGGAAGTTCTTCTG	(Apostolidou *et al.* 2007)
CDKN1C	Forward: GGAGCCTCTCGCTGACCA Reverse: CTTCTCAGGCGCTGATCTCT	Own design
PEG10	Forward: AAATTGCCTGACATGAAGAGGAGTCTA Reverse: AAGCCTAGTCACCACTTCAAAACACACTAAA	(Diplas *et al.* 2009)
IGF2	Forward: CAATGGGGAAGTCGATGCTG Reverse: GGAAACAGCACTCCTCAACG	Own design
H19	Forward: AGCGTTCGGGCTGGAGAC Reverse: CTCTGTCCTCGCCGTCACAC	(Nelissen *et al.* 2014)
HPL	Forward: CCTCGGACAGCGATGACTAT Reverse: ATGCTACTCGGGCACCTAGA	Own design
PGH	Forward: AGAGAGAAGGGGCCAGGTAT Reverse: CCTCTTGAAGCCAGGACAGG	Own design
DLK1	Forward: TGGATTCTGCGAGGATGACA Reverse: CTATCACAGAGCTCCCCGTC	Own design

2.4.5 PCR

For PCR, 14 µl of PCR mastermix was added to 1 µl of cDNA. PCR mastermix was made according to Table 2.2. Primer pairs were diluted to working solutions by adding 50 µl of 10 mM Tris (pH 8.0) to 25 µl of 100 µM forward primer and 25 µl of 100 µM reverse primer. The PCR was performed in an Applied Biosystems-Veriti 96-well Thermocycler machine using the following thermocycler conditions: i) 94°C for 3 minutes, ii) 35 cycles of 94°C for 30 seconds, iii) 60°C for 30 seconds, iv) 72°C for 30 seconds and v) 72°C for 3 minutes. PCR products were run on a 1% Tris-acetate EDTA (TAE) agarose gel containing 1 µg/ml SafeView (NBS Biologicals Ltd, UK) and visualised under ultraviolet light. A 100 bp DNA ladder (GeneFlow, UK) was run alongside PCR products to enable measurement of product size.

Table 2.2: Mastermix (1X) components for PCR

Reagents	***Volume (µl)***
RNase free water	11.5
DreamTaq Green Buffer (10X) (ThermoFisher)	1.5
10 mM dNTPs (ThermoFisher)	0.3
Primer pair	0.6
DreamTaq (ThermoFisher)	0.1

2.4.6 qPCR

cDNA was diluted by a further 1:20 dilution in 10 mM Tris (pH 8.0, Sigma). Samples were run in triplicate and on duplicate plates (six assays). Primer combinations were used as described in Section 2.4.4. 7.5 µl qPCR mastermix was added to diluted 2 µl cDNA in each well of a 384 well qPCR (ThermoFisher) plate. qPCR mastermix was made up according to Table 2.3.

Quantitive PCR (qPCR) was performed using a QuantStudio™ 5 Real-Time PCR System, 384-well machine in a 9.5 µl reaction. The reaction contained 2 µl of cDNA (diluted 1 in 100) and 7.5 µl of mastermix (Table 2.3). Reference genes *YWHAZ* and *SDHA* were run alongside the experimental genes on each plate. qPCR was performed under the following conditions: i) 3 minutes at 95°C, ii) 20 seconds at 95°C, iii) 20 seconds at 53°C, iv) 20 seconds at 72°C, and v) 20 seconds at 75°C. Steps ii-v were repeated 40 times. The melt curve was executed at 60°C to 95°C, read every 0.5°C and held for 2 seconds.

Table 2.3: Mastermix (1X) components for qPCR

Reagents	***Volume (µl)***
RNase free water	6.25
DreamTaq Buffer (10X) (ThermoFisher)	1.0
10 mM dNTPs (ThermoFisher)	0.2
Primer pair	0.4
Sybr Green (Invitrogen)	0.08
DreamTaq (ThermoFisher)	0.065

Gene expression is either presented as -ΔCT where the target gene expression is relative to the geometric mean of the reference gene expression. ΔCT values are multiplied by -1 so that higher ΔCT values represent higher concentrations. Alternatively data is expressed as fold change in expression calculated as $2^{-\Delta\Delta CT}$, where ΔΔCT is the target gene expression relative to the control defined group (Livak and Schmittgen 2001).

Chapter 3: Grown in Wales cohort

3.1 Introduction

Mental health disorders such as depression and anxiety are more common in women than men (Kessler *et al.* 2005) and prevalence rates are higher among times of great hormonal changes such as puberty, pregnancy and the menopause (Soares and Zitek 2008). The perinatal period is defined as pregnancy and the year following birth, and involves major changes in physical, psychological and social states, as well as hormone levels. Considerable attention has been paid to postnatal depression with a good level of awareness among the public (Highet *et al.* 2011; Thornsteinsson *et al.* 2014). In contrast, there is considerably less recognition of prenatal depression, despite studies often describing a higher prevalence, and evidence that prenatal depression is a strong predictor of postnatal depression (Gotlib *et al.* 1989; Heron *et al.* 2004; Andersson *et al.* 2006; Norhayati *et al.* 2015). In addition, research has tended to focus on symptoms of depression despite the serious negative effects associated with anxiety symptoms (Zietlow *et al.* 2019).

Mental health disorders are challenging to study as their presentations are variable and they are influenced by both genetic and environmental factors (Saveanu and Nemeroff 2012). While we do not know why some women are more vulnerable during the perinatal period, identifying potential predictors can help inform health services as to who may be at higher risk of developing prenatal depression or anxiety. Furthermore, it helps to identify infants exposed *in utero* who are at greater risk of adverse outcomes, as explored in Chapter 1.

A recent meta-analysis assessed 97 studies and reported a range of independent predictors for prenatal depression and anxiety (Biaggi *et al.* 2016). Due to their co-morbidity as discussed in Chapter 1, prenatal depression has been shown to be a major predictor of prenatal anxiety, and prenatal anxiety a major predictor of prenatal depression. (Edwards *et al.* 2008; Lancaster *et al.* 2010; Verreault *et al.* 2014). Additional predictors often include past experiences such as insufficient parental emotional support or care during childhood, feelings from which may resurface during pregnancy (Ammaniti and Trentini 2009; Jeong *et al.* 2013). The association between prenatal depression and anxiety, and parity is somewhat mixed (Abuidhail and Abujilban 2014; Räisänen *et al.* 2014; Ratcliff *et al.* 2015), but previous pregnancy complications have been shown to be a risk factor (Bergner *et al.* 2008). Consuming alcohol or tobacco before or during pregnancy has been shown by some to predict poor prenatal mental health, and although one study found that stopping smoking during pregnancy decreased the risk of onset, others did not (Marcus *et al.* 2003; Lee *et al.* 2007; Marcus 2009; Smedberg *et al.* 2015). Maternal age has been linked to the risk of developing a mental health disorder during pregnancy, yet some studies report those in adolescence are at a greater risk while others report older age increases risk (Räisänen *et al.* 2014; Siegel and Brandon 2014). During pregnancy, a lack of support is often found to be associated with poor maternal mental health. Being a single mother has been associated with an increased risk of depression during pregnancy, but those with unsupportive partners are at an even greater risk according to one study (Bilszta *et al.* 2008). Those from low

income families or those with less education have been found to be at greater risk of prenatal depression or anxiety, although literature is mixed (Leigh and Milgrom 2008; Jeong *et al.* 2013; Abuidhail and Abujilban 2014). One study split depression during pregnancy into major and minor groups, and found that while previous episodes of depression predicted both groups, major depression was additionally associated with conflicts with the partner, whereas minor depression was predicted by being a housewife and an unwanted pregnancy (Marchesi *et al.* 2009).

Prenatal depression and anxiety have been associated with a wide range of socio-economic factors, which may explain the disparity between findings in studies (Biaggi *et al.* 2016). Different studies focus on different populations of women, with different backgrounds and ethnicities, and thus identifying uniform predictors can be challenging. However, common factors throughout almost all studies include a history of mental illness, history of abuse, unwanted or unplanned pregnancies, pregnancy loss and adverse life events (Biaggi *et al.* 2016).

Predictors of postnatal depression have been more widely studied. There is substantial overlap between pre- and post-natal predictors of maternal mental health such as a previous history of mental health disorders and lack of support. However, they also often include factors associated with the birth such as mode of delivery and delivery complications (Field 2018). Independent predictors of postnatal depression and anxiety have been observed in some studies. For example, higher education and employment increased the risk of postnatal anxiety, whereas being a housewife increased the risk of postnatal depression (Bener *et al.* 2012). These directly opposing predictors highlight the differences between perinatal depression and anxiety.

The prevalence of prenatal depression and anxiety can vary depending on the method used to identify the disorder. Self-reporting questionnaires are most commonly used to diagnose symptoms of depression or anxiety. However, there are numerous caveats of self-reporting including social desirability bias, introspective ability and incorrect interpretation of questions (Althubaiti 2016). Furthermore, there are multiple questionnaires available for diagnosing depression symptoms including the Edinburgh Postnatal Depression Scale (EPDS) (Cox *et al.* 1987), Beck Depression Inventory (BDI) (Beck *et al.* 1996) and Center for Epidemiological Studies Depression Scale (CES-D) (Radloff 1977). There are also different questionnaires to measure anxiety symptoms including State Trait Anxiety Inventory (STAI) (Spielberger *et al.* 1983), Crown Crisp Experiential Index (CCEI) (Crown and Crisp 1979), Edinburgh Postnatal Depression Scale-Anxiety subscale (EPDS-A) (Matthey *et al.* 2013), and Pregnancy Related Anxiety Questionnaire (PRAQ) (Van den Bergh *et al.* 2005). Clinical diagnosis through structured interviews is sometimes used and can be more specific. However, the cost and time associated with interviewing patients often limits widespread use. Additionally, estimates of prevalence across studies may be inherently conservative, as women suffering from mental disorders may be less likely to participate in cohort studies.

Rates of maternal mood disorders vary widely across different countries. Lower and middle income countries (LMICs) tend to have higher rates of perinatal depression than those from higher-income countries (Woody *et al.* 2017). It is suggested that this is due to poorer access to perinatal services, and higher rates of poverty and intra-relationship violence (Rwakarema *et al.* 2015). A recent meta-analysis set out to assess the global rates of depression during pregnancy and the first four weeks of the postnatal period, and found a pooled prevalence of 11.9% (Woody *et al.* 2017). However, on average studies have reported ranges of depression from 7 to 30% in higher-income countries, and up to 47% in LMICs (Rochat *et al.* 2011; Biaggi *et al.* 2016; Woody *et al.* 2017).

Rates of anxiety during the perinatal period are often higher than rates of depression symptoms. A meta-analysis looking at 102 studies from 34 countries, reported a pooled prevalence of self-reporting prenatal anxiety of 22.9% across 70 studies, with 25% of studies from low to middle income countries (Dennis *et al.* 2017). Again, LMICs had a significantly higher pooled perinatal anxiety rate of 34%, compared to 19.4% in higher-income countries.

There are a lack of cohorts examining the prevalence of maternal mental health disorders within the UK (Table 3.1). Avon Longitudinal Study of Parents And Children (ALSPAC) is a large multi-generational cohort based in the old county of Avon. In an initial wave of recruitment in the 1990s, ALSPAC researchers reported a depression prevalence of 8-15% across the perinatal period using an EPDS cut off of ≥ 13 (Heron *et al.* 2004). However, in a second generation of participants, the prevalence of depression was 25% in pregnancy for mothers aged 18-24 compared to the 1990s prevalence of 17% for this age group (Pearson *et al.* 2018). This suggests prenatal depression is becoming increasingly common in the UK. Alternatively, the increase may be in part due to priming, as approximately half of the participants had been recruited into the study since birth, and had completed questionnaires on their mental health their whole lives. Another study set up in an area of London with a higher level of socio-economic deprivation reported a higher prenatal depression prevalence of 29% using an EPDS cut off ≥ 15, similar to the prevalence reported in LMICs (Bolton *et al.* 1998). Only ALSPAC and Howard *et al*. have reported on perinatal anxiety prevalence in a UK population (Heron *et al.* 2004; Howard *et al.* 2018). ALSPAC described a self-reported prevalence of around 15% in pregnancy, similar to the prevalence determined by diagnostic interview in Howard *et al.* 2018.

Table 3.1: UK perinatal depression and anxiety rates

Reference	***Location***	***Time point***	***Prevalence***	***Measurements***	***Notes***
Evans *et al.* 2001	Avon	2nd Trimester 3rd Trimester 8 weeks postpartum	11.8% 13.5% 9.1%	EPDS	
Bolton *et al.* 1998	London	3rd Trimester	29%	EPDS	Lower socioeconomic status
Johanson *et al.* 2000	West Midlands	3rd Trimester 3 months postpartum	9.8% 7.4%	EPDS	
Heron *et al.* 2004	Avon	2nd Trimester 3rd Trimester 8 weeks postpartum 6 months postpartum 2nd Trimester 3rd Trimester 8 weeks postpartum 6 months postpartum	11.4% 13.1% 8.9% 8.8% 14.6% 15.6% 8.1% 9.1%	EPDS CCEI	
Pearson *et al.* 2018	Avon	Prenatal	17% (1991-92) 25% (2012-16)	EPDS	Aged 18-24
Howard *et al.* 2018	London	1st Trimester	11% 15%	EPDS + SCID (depression) SCID (anxiety)	

[1] SCID: Structured Clinical Interview DSM-IV

The reported rates of mental health disorders change throughout the perinatal period. As seen in Table 3.1 there is a general increase in incidence of depression and anxiety throughout pregnancy (Johanson *et al.* 2000; Evans *et al.* 2001; Heron *et al.* 2004). Some studies report a U-shaped prevalence curve with higher rates in the first and third trimesters (Marchesi *et al.* 2009). This may reflect vulnerable times during gestation, from the immediate concerns upon finding out about the pregnancy to the later concerns about delivery and raising a child. Parturition precedes an abrupt reduction in placental hormones and drastic changes to living conditions due to the arrival of a newborn. While this could increase the vulnerability for depression, as seen in Table 3.1 the prevalence of depression often decreases between the pre- and post-natal periods. Therefore, longitudinal studies that take measurements at multiple time points can be valuable in assessing perinatal mental health conditions. Within the UK there are a limited number of birth cohorts with a longitudinal design, including the aforementioned ALSPAC. However, in Wales with a population of just over three million, prior to the GiW study, there was no pregnancy cohort specifically focussed on maternal mental health. England and Wales are often analysed together despite the Welsh Assembly having devolved responsibility of the NHS since 1999. Differences between health boards exist and results from birth cohorts in England cannot be assumed to hold true in Wales (Bevan *et al.* 2014).

The aims of the current chapter were to describe the GiW cohort, with a specific focus on the prenatal period. This description has been published in *BJPsych Open* (Janssen *et al.* 2018). For this publication I contributed in part towards the statistical analysis and manuscript preparation. The second aim was to re-assess prenatal depression predictors in the original publication using an alternative statistical method better fitting the skewed EPDS distribution and expand prediction analysis to include prenatal anxiety and postnatal mental health. In addition, the original publication described persistent anxiety symptoms after birth and this chapter set out to further explore this observation statistically. The final aim was to analyse prenatal mental health in relation to neonatal outcomes.

3.2 Methods

Methods for recruitment to the Grown in Wales study were described in Chapter 2. For ease of interpretation, collection times are summarised in Table 3.2.

Table 3.2: Data collection time points

Time	***Reference***
Day of pre-surgical appointment (antenatal)	A1
Within one week of birth (average 4.2 days)	P1
Ten weeks after birth	P2
One year after birth	Y1

3.2.1 Statistics

A description of the demographics of the entire cohort, with the exception of those who withdrew (n= 347), was carried out using percentages, or means and standard deviations where appropriate. This included both socio-demographic variables and birth outcomes. The same was performed for the dataset used for analysis in this chapter (n= 299) with the exclusions described in Section 2.3 (non-White, didn't deliver by ELCS, didn't fully complete A1 EPDS or STAI questionnaires). Valid percentages were also calculated for depression and anxiety prevalence in order to exclude missing data. Given the non-normality of the EPDS and STAI variables, Spearman's correlations were used to assess relationships with EPDS and STAI at different time points.

In order to choose the best model for predicting prenatal depression and anxiety A1 EPDS and STAI scores were entered as dependent variables into single predictor regressions to assess their relationship with potential predictors identified from the literature. Given the discrete nature and positive skew of both the EPDS and STAI scales, Poisson error distribution regressions were utilised. Any relationship between mental health scores and potential predictors with a p value< 0.15 were entered into a multivariate Poisson regression. To ensure that the Poisson error distribution was the correct family to

use, multivariate regressions using Poisson and Gaussian were compared using AIC, BIC and residual plots. Regressions using Poisson distributions fit the data better and thus used in further tests where appropriate. Due to the strong relationship between EPDS and STAI scores, the depression model was run with and without A1 STAI as a covariate to assess whether it could skew the model, and the reverse was carried out for the anxiety model. Models were then compared using the AIC, BIC, and residual plots. These indicated that the full model including the other mood score as a covariate was a better fit. Those significant at the 0.05 level in the multivariate regressions were deemed to be predictors of antenatal depression or anxiety symptoms.

Postnatal depression and anxiety predictors were analysed in the same way; first potential predictors were analysed in relation to postnatal scores in single predictor regressions, before entering those significant at 0.15 level into multivariate regressions. Additional potential predictors relating to the birth were included at the first stage. Predictors were only assessed for the first postnatal time point (P1), as lower numbers in certain groups such as 'Left before GCSE' in the education variable led to inaccurate results, meaning not all important variables could be properly assessed. Postnatal depression and anxiety are known to be strongly associated with mental health at earlier time points as well as with current anxiety and depression. However it was not possible to include both current and all previous EPDS/STAI scores due to multicolinearity. Therefore, A1 and P1 EPDS and STAI z-scores were run separately as independent variables in Poisson regressions on P1 EPDS and STAI to determine which score had the strongest effect on postnatal depression and anxiety. EPDS and STAI were standardised through creation of z-scores to allow for appropriate comparison of B scores between the two. Issues of multicolinearity and model assumptions were subsequently tested and found to be suitable.

As mental health history was a significant predictor of depression symptoms, one way ANOVAs were employed to determine whether the type of previous mental disorder significantly affected current prenatal depression.

The association of EPDS and STAI scores with delivery and birth outcomes were assessed using linear and binary regressions for continuous and categorical outcomes respectively. The relationships between outcomes and mental health scores as categorical cut offs were also assessed, data not shown here.

3.2.1.1 Longitudinal analysis

3.2.1.1.1 LPA

Latent Profile Analysis was run to determine participant depression and anxiety profiles with the tidyLPA package (Rosenberg *et al.* 2018). EPDS and STAI scores were used independently as a basis for generating profiles, however only those who completed questionnaires at all time points were able to be included (n= 86 for depression; n= 87 for anxiety). In order to find the best fitting model,

different models with a different number of classes (2-4) and different variance and covariance parameters (equal, varying, and zero) were compared using AIC, BIC and BLRT *p* values. Differences between classes were assessed using ANOVAs and Tukey Honestly Significant Difference (HSD), t-tests, and Chi-squared where appropriate.

3.2.1.1.2 Mixed effect modelling

Depression and anxiety scores were analysed over time using linear mixed effect modelling, which is routinely used for longitudinal data. It is a statistical model that allows for both fixed and random effects and thus avoiding the problem of pseudo-replication. Including random effects can allow each participant to have their own mean response trajectories over time, making more effective use of the data. Given the level of retention in this cohort, and as this analyses was to look at mental health scores over time, potential covariates were assessed for their relation to having missing EPDS or STAI scores at later time points, as done so in (Micali *et al.* 2011). Simple single predictor binomial regressions were performed for income, WIMD, maternal age, SSRI use in pregnancy, history of mental health, fetal sex, parity and BMI. Those that were significant at a 0.15 level were later included in the mixed models as confounders. Mixed models allow different numbers of time points to be completed by participants thus increasing sample size and mitigating bias by not excluding those lost to follow up. However, missing values in cofounders are not permitted in the model, therefore, when deciding on the final model, a working dataset with missing values removed was created to test different designs, to allow for appropriate comparison.

Using the glmer function of lme4 R package (Bates *et al.* 2015) and a Poisson error family generalised linear mixed model approach, a null model was first created with either EPDS or STAI score as the dependent variable, and a random effect of participant ID. Subsequent models included time as a fixed and random coefficients. Models were compared using likelihood ratio Chi-squared statistics, BIC, and AIC, and it was found that including time as a random slope and fixed effect did add to the model and thus were included.

Significant covariates associated with attrition levels were included into a final model as fixed effects. Time was the other fixed effect, and both time and participant ID were included as random effects. This allowed for analyses on the effect of time on depression and anxiety scores, whilst controlling for covariates and inter-person variance. A bobyqa optimiser was used with a maximum of 200,000 iterations in order for the model to properly converge. The final model with covariates was compared to previous models, and found to be the best fitting. Model over-dispersion and model assumptions (normality of residuals and homoscedasticity) were assessed and found to be suitable. Statistical significance for each variable was determined using likelihood ratio tests comparing models with and without a given term using a Chi-squared statistic.

3.3 Results

3.3.1 Description of cohort

Demographics for the entire dataset can be found in Table 3.3. Of the 355 women recruited into the GiW study, over 90% of them were White and over 65% were born in Wales. As is the case in other cohorts, participants had high levels of education and family income, as these are often the most likely to participate in studies. Over 10% of the women smoked at least once despite official advice being not to smoke during pregnancy, and almost 35% drank alcohol at least once. Though it should be noted, that at the time of recruitment (2015/16) the official guidelines stated that after the first trimester it was advised not to consume more than one to two units a week (IAS 2019). 28% of participants had suffered from a previous mental health disorder and 7.8% were prescribed SSRIs during their pregnancy, both of which were higher than previous reports in the UK (Charlton *et al.* 2015; Mongan *et al.* 2019). The prevalence of prenatal depression was 14% and the prevalence of prenatal anxiety was 28% (Figure 3.1).

For the purpose of this chapter, the entire GiW dataset was not used. Instead, the focus was on the subset of participants who did ultimately give birth by elective caesarean, were of White ethnicity and satisfactorily completed the initial questionnaire to allow for valid statistical analyses (n= 299). Demographics for this subset can also be found in Table 3.3. Country of birth was different as would be expected when removing those who were non-White. Gestational diabetes mellitus (GDM) was decreased in the subset which may relate to a higher prevalence of GDM in women from South Asia and South East Asia compared to those of White ethnicity (Yuen and Wong 2015). Frequency of delivery complications was also lower in the subset, as those who did not deliver by elective caesarean were excluded.

Table 3.3: Demographics of the cohort and subset

Categorical variables	***GiW cohort***	***Subset***
A1 EPDS		
<13	83.6 (85.6)	86.0 (86.2)
≥13	14.1 (14.4)	13.7 (13.8)
Missing	2.3	0.3
A1 STAI		
<40	69.3 (71.7)	70.6 (71.8)
≥40	27.3 (28.3)	27.8 (28.2)
Missing	3.4	1.6
P1 EPDS		
<13	49.7 (91.5)	52.2 (91.8)
≥13	4.6 (8.5)	4.7 (8.2)
Missing	45.7	43.1
P1 STAI		
<40	40.2 (75.3)	42.8 (76.6)
≥40	13.2 (24.7)	13.0 (23.4)
Missing	46.6	44.2
P2 EPDS		
<13	42.2 (90.7)	46.2 (91.4)
≥13	4.3 (9.3)	4.3 (8.6)
Missing	53.5	49.5
P2 STAI		
<40	33.0 (71.0)	35.5 (72.1)
≥40	13.6 (29.0)	13.7 (27.9)
Missing	53.4	50.8
Y1 EPDS		
<13	31.3 (87.9)	33.8 (87.8)
≥13	4.3 (12.1)	4.7 (12.2)
Missing	64.4	61.5
Y1 STAI		
<40	23.3 (65.3)	24.4 (63.5)
≥40	12.4 (34.7)	14.0 (36.5)
Missing	64.3	61.6
Ethnicity		
White	90.8	100
Indian/Pakistani/Bangladeshi	3.2	0
Far Eastern	1.1	0
African/Afro-Caribbean	2.3	0
South American/Hispanic	0.3	0
Middle Eastern	0.3	0
Other	1.4	0
Missing	0.6	0
Country of birth		
Wales	65.8	71.2
England	19.3	21.1
Scotland	0.6	0.7
Ireland	0.3	0.3
Other	12.6	6.0
Missing	1.4	0.7
Highest level of maternal education		
Left before GCSE	5.5	6.0
GCSE/Vocational	22.1	24.1

A Levels	11.8	12.7
University	30.7	29.1
Postgraduate	24.7	24.7
Missing	5.2	3.4
Family income		
< £18,000	8.6	8.7
£18-25,000	9.8	9.4
£25-43,000	18.4	19.7
> £43,000	46.6	48.2
Missing	16.6	14
Maternal pre-pregnancy BMI		
Underweight	1.7	1.7
Healthy	46.0	46.5
Overweight	25.0	27.1
Obese	14.4	14.0
Missing	12.9	10.7
Smoked in three months before pregnancy		
No	81.3	80.6
Yes	17.2	19.1
Missing	1.4	0.3
Smoked in pregnancy		
No	88.2	88.3
Yes	10.3	11.4
Missing	1.5	0.3
Drank alcohol in three months before pregnancy		
No	24.1	20.4
Yes	74.4	79.3
Missing	1.5	0.3
Drank alcohol in pregnancy		
No	61.8	60.2
Yes	34.5	36.8
Missing	3.7	3.0
Took illicit drugs in pregnancy		
No	97.1	98.3
Yes	0.6	0.7
Missing	2.3	1.0
Strenuous exercise in pregnancy		
Yes	15.2	16.7
No	83.0	82.6
Missing	1.8	0.7
History of mental health problems		
No	71.8	69.6
Yes	27.9	30.1
Missing	0.3	0.3
Prescribed SSRIs in pregnancy		
No	92.2	91.6
Yes	7.8	8.4
Parity		
Nulliparous	19.5	21.4
Multiparous	80.2	78.6
Missing	0.3	0
BMI at booking		
Underweight	1.4	0.7
Healthy	34.5	36.1

Overweight	32.2	33.4
Obese	23.9	24.7
Missing	8.0	5.1
Season of birth		
Spring	18.7	19.1
Summer	24.4	23.4
Autumn	35.3	35.1
Winter	20.7	22.4
Missing	0.9	0
Continuous variables	***GiW cohort***	***Subset***
Booking week	12.9 (± 3.2)	12.8 (± 3.0)
WIMD	1056.5 (± 647.6)	1072.4 (± 643.7)
Maternal age (*years*)	32.6 (± 5.1)	32.6 (± 5.1)
Pre-pregnancy BMI	25.5 (± 5.1)	25.5 (± 5.1)
Maternal birthweight (*kg*)	3.3 (± 0.6)	3.3 (± 0.6)
BMI at booking	27.2 (± 5.5)	27.2 (± 5.5)
Parity	1.2(± 1.0)	1.2 (± 1.0)
Birth outcomes		
Categorical Variables	***GiW cohort***	***Subset***
Bleeding during pregnancy		
No	89.9	89.6
Yes	9.8	10.4
Missing	0.3	0
Delivery complications		
No	90.0	90.0
Yes	9.0	10.0
Missing	1.0	0
Concerns over fetal growth		
No	77.6	78.6
Yes	19.8	19.1
Missing	2.6	2.3
CBWC category		
SGA	6.9	7.0
AGA	78.2	78.6
LGA	13.8	14.4
Missing	1.1	0
PET diagnosis		
No	98.0	99.3
Yes	0.6	0.7
Missing	1.4	0
GDM diagnosis		
No	94.5	95.7
Yes	5.5	4.3
Indication for ELCS		
Previous caesarean section	52.9	52.8
Current pregnancy complication	20.4	21.7
Previous pregnancy complication	15.2	16.4
Maternal choice	3.2	3.3
Maternal disorder (not pregnancy related)	4.3	4.3
Missing	4.0	1.5
Fetal sex		
Male	44.8	45.5
Female	54.0	54.5
Missing	1.2	0

APGAR (1 minute) Low High Missing	 5.2 93.4 1.4	 5.0 94.6 0.4
APGAR (5 minutes) Low High Missing	 0.6 98.0 1.4	 0.3 99.7 0
Continuous variables	***GiW cohort***	***Subset***
Gestational age (*days*)	274.8 (± 4.9)	274.6 (± 4.6)
Birthweight (*g*)	3547.0 (± 507.9)	3545.1 (± 520.3)
Custom birthweight centile	56.8 (± 29.0)	56.4 (±29.2)
Head circumference (*cm*)	35.2 (± 1.6)	35.3 (± 1.6)
APGAR (1 min)	8.9 (± 0.9)	8.9 (± 0.9)
APGAR (5 min)	9.8 (± 0.5)	9.8 (± 0.5)

[1] Categorical variables represented as percentages
[2] Maternal mental health scores represented as percentages (valid percentages)
[3] Continuous variables represented as means (±SD)

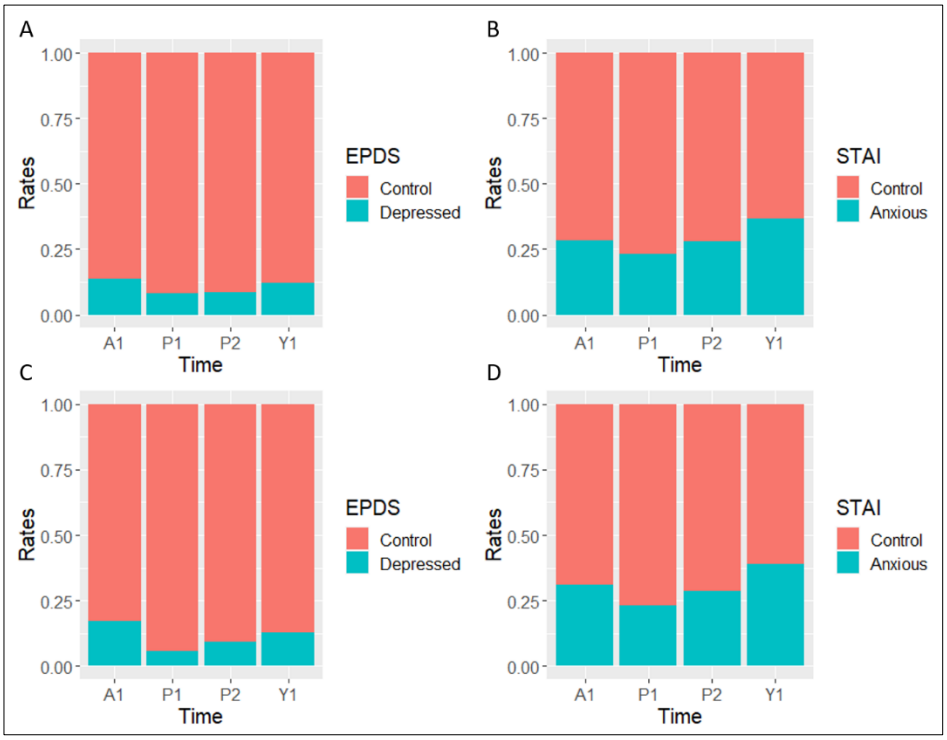

Figure 3.1: Prevalence of maternal depression and anxiety. ***A*** *and* ***B****: Total prevalence at each time point.* ***C*** *and* ***D****: Prevalence of only those who completed questionnaires at all time points (n= 86 depression, n= 87 anxiety).*

Depression and anxiety scores at each time point were strongly correlated; all correlations had a significance of $p< 0.001$ and Spearman's r correlation statistic ranged from 0.514-0.819. Both depression and anxiety scores were stable over time, with correlations ranging from 0.522-0.685 and

0.687-0.819 respectively. As seen in Table 3.4, correlations between scores closer in time had higher *r* values.

Table 3.4: Perinatal depression and anxiety score correlations

	A1 EPDS	*A1 STAI*	*P1 EPDS*	*P1 STAI*	*P2 EPDS*	*P2 STAI*	*Y1 EPDS*	*Y1 STAI*
A1 EPDS	-	-	-	-	-	-	-	-
A1 STAI	0.722	-	-	-	-	-	-	-
P1 EPDS	0.685	0.639	-	-	-	-	-	-
P1 STAI	0.696	0.819	0.785	-	-	-	-	-
P2 EPDS	0.605	0.528	0.562	0.578	-	-	-	-
P2 STAI	0.636	0.751	0.632	0.738	0.736	-	-	-
Y1 EPDS	0.577	0.514	0.522	0.563	0.573	0.592	-	-
Y1 STAI	0.584	0.722	0.548	0.687	0.544	0.775	0.726	-

[1] Spearman's *r* values
[2] All *p* values< 0.001
[3] Correlations between the same scale are highlighted in blue

3.3.2 Predictors of antenatal mental health scores

A1 EPDS and STAI were entered into single predictor regressions (Table 3.5) with potential predictors to assess their relationship before entering those with a significance level of 0.15 into a multi-variate regression (Table 3.6). Predictors significantly associated were similar between depression and anxiety scores, with alcohol in pregnancy associated with EPDS and exercise associated with STAI being the only differences. Although both the categorical and continuous BMI variables were significant, the continuous variable was taken forward to the multi-variate regression as the sample numbers were relatively small in some of the categorical groups.

Table 3.5: Prenatal depression and anxiety single predictor regressions

Categorical variables	***A1 EPDS***	***A1 STAI***
A1 EPDS	*NA*	**<0.001**
A1 STAI	**<0.001**	*NA*
Highest level of maternal education		
Left before GCSE	**<0.001**	**0.008**
GCSE/Vocational	**<0.001**	**0.015**
A Levels	**<0.001**	**0.010**
University	**<0.001**	0.0913 ɨ
Postgraduate	*ref.*	*ref.*
Family income		
< £18,000	**<0.001**	**<0.001**
£18-25,000	**0.003**	**0.004**
£25-43,000	**0.010**	**0.004**
> £43,000	*ref.*	*ref.*
Smoked in three months before pregnancy		
No	*ref.*	*ref.*
Yes	**<0.001**	**<0.001**
Smoked in pregnancy		
No	*ref.*	*ref.*
Yes	**<0.001**	**<0.001**
Drank alcohol in three months before pregnancy		
No	*ref.*	*ref.*
Yes	0.355	0.564
Drank alcohol in pregnancy		
No	*ref.*	*ref.*
Yes	0.067 ɨ	0.709
Strenuous exercise in pregnancy		
Yes	0.378	0.115 ɨ
No	*ref.*	*ref.*
History of mental health problems		
No	*ref.*	*ref.*
Yes	**<0.001**	**<0.001**
Parity		
Nulliparous	0.289	0.205
Multiparous	*ref.*	*ref.*
BMI at booking		
Underweight	0.737	0.120 ɨ
Healthy	*ref.*	*ref.*
Overweight	**<0.001**	0.188
Obese	**<0.001**	0.126 ɨ
Season of birth		
Spring	0.693	0.163
Summer	**0.012**	0.108 ɨ
Autumn	*ref.*	*ref.*
Winter	0.103 ɨ	**0.006**
Indication		
Current complication	**0.040**	0.052 ɨ
Maternal Choice	0.688	0.800
Maternal disorder	**0.039**	0.388
Previous pregnancy complication	0.972	0.758
Previous CS	*ref.*	*ref.*
Continuous variables	***A1 EPDS***	***A1 STAI***

WIMD	**<0.001**	**<0.001**
Maternal age (*years*)	0.069 ɪ	0.140 ɪ
BMI at booking	**0.001**	0.060 ɪ

[1] *p* values reported
[2] Significant values reported in bold
[3] ɪ: $p < 0.15$

In the full model, significant predictors of depression scores were current anxiety scores, maternal education, and history of mental health (Table 3.6). Lower education (left before GCSE, GCSE/Vocational, and University) were associated with higher depression scores, as did having a previous history of mental health disorders. In the full anxiety model, current depression, education and history of mental health were significantly associated (Table 3.7). However, unlike the depression model lower levels of education (Left before GCSE) were negatively associated with anxiety scores.

Table 3.6: Multivariate prenatal depression prediction model

Predictors	***Exp(B)***	***CIs***	***p value***
A1 STAI	1.047	0.039, 0.052	**<0.001**
Highest level of maternal education			
Left before GCSE	1.467	0.106, 0.653	**0.006**
GCSE/Vocational	1.213	0.025, 0.361	**0.024**
A Levels	1.041	-0.148, 0.226	0.673
University	1.228	0.068, 0.344	**0.004**
Postgraduate	*ref.*	*ref.*	*ref.*
Family Income			
< £18,000	0.942	-0.289, 0.166	0.604
£18-25,000	1.039	-0.176, 0.249	0.765
£25-43,000	1.021	-0.117, 0.158	0.723
> £43,000	*ref.*	*ref.*	*ref.*
Smoked in three months before pregnancy	1.096	-0.097, 0.275	0.334
Smoked in pregnancy	0.925	-0.322, 0.164	0.526
Alcohol in pregnancy	1.054	-0.054, 0.159	0.332
History of mental health	1.163	0.033, 0.269	**0.012**
Season of birth			
Spring	0.999	-0.148, 0.145	0.988
Summer	1.092	-0.052, 0.228	0.216
Autumn	*ref.*	*ref.*	*ref.*
Winter	0.867	-0.292, 0.006	0.061
Indication			
Current complication	1.044	-0.101, 0.184	0.554
Maternal Choice	1.106	-0.157, 0.345	0.429
Maternal disorder	1.158	-0.086, 0.367	0.204
Previous pregnancy complication	1.101	-0.054, 0.242	0.204
Previous CS	*ref.*	*ref.*	*ref.*
WIMD	1.000	>-0.001, <0.001	0.273
BMI at booking	1.002	-0.007, 0.013	0.583
Maternal age	1.003	-0.010, 0.016	0.653

[1] CIs: 95% Confidence Intervals

[2] Significant values reported in bold

Table 3.7: Multivariate prenatal anxiety prediction model

Predictors	***Exp(B)***	***CIs***	***p value***
A1 EPDS	1.034	0.029, 0.039	**<0.001**
Highest level of maternal education			
Left before GCSE	0.870	-0.274, -0.006	**0.041**
GCSE/Vocational	0.947	-0.130, 0.006	0.155
A Levels	1.001	-0.080, 0.082	0.975
University	0.947	-0.114, 0.005	0.070
Postgraduate	*ref.*	*ref.*	*ref.*
Family Income			
< £18,000	1.090	-0.019, 0.190	0.106
£18-25,000	1.065	-0.036, 0.090	0.208
£25-43,000	1.030	-0.032, 0.090	0.348
> £43,000	*ref.*	*ref.*	*ref.*
Smoked in three months before pregnancy	0.990	-0.096, 0.075	0.823
Smoked in pregnancy	1.016	-0.099, 0.131	0.781
Exercise in pregnancy	0.985	-0.077, 0.047	0.638
History of mental health	1.061	0.005, 0.113	**0.031**
Season of birth			
Spring	1.051	-0.016, 0.114	0.135
Summer	0.999	-0.063, 0.062	0.986
Autumn	*ref.*	*ref.*	*ref.*
Winter	1.045	-0.019, 0.108	0.168
Indication			
Current complication	0.953	-0.112, 0.014	0.131
Maternal Choice	1.015	-0.105, 0.132	0.802
Maternal disorder	1.009	-0.103, 0.117	0.879
Previous pregnancy complication	0.979	-0.088, 0.045	0.532
Previous CS	*ref.*	*ref.*	*ref.*
WIMD	1.000	>-0.001, <0.001	0.226
BMI at booking	0.998	-0.007, 0.002	0.303
Maternal age	1.001	-0.005, 0.007	0.791

[1] CIs: 95% Confidence Intervals

[2] Significant values reported in bold

In order to determine predictors associated with postnatal depression and anxiety, the same procedure was repeated for later time points. Postnatal depression and anxiety are known to be strongly influenced by maternal mood during pregnancy. Therefore, given this and the strong correlations between mood scores at all time points in our cohort, prenatal EPDS and STAI scores were converted into z-scores and run independently as covariates on single-predictor regressions to determine which had the larger effect on mental health at P1. All regressions were highly significant ($p< 0.001$). Based on B values, the predictor with the biggest influence on P1 EPDS score was P1 STAI score, and the biggest effect on P1 STAI was A1 EPDS (Table 3.8). Therefore, only these were taken forward into multivariate regressions to avoid multicolinearity.

Table 3.8: Effect of mental health scores on postnatal depression and anxiety

Independent variable	***P1 EPDS***	***P1 STAI***
A1 EPDS	1.533	**1.244**
A1 STAI	1.535	1.198
P1 EPDS	-	1.235
P1 STAI	**1.639**	-

[1] Exp(B) values reported

[2] Those with the largest effects reported in bold

As seen in Table 3.9, significant predictors were again similar between depression and anxiety at different time points, with education, income and a history of mental health significant in all six. Those significant at the 0.15 level were entered into final Poisson multi-variate regressions (Table 3.10, Table 3.11).

Table 3.9: Postnatal depression and anxiety single predictor regressions

Categorical variables	***P1 EPDS***	***P1 STAI***
Highest level of maternal education		
Left before GCSE	**<0.001**	**<0.001**
GCSE/Vocational	0.312	0.757
A Levels	0.316	0.720
University	0.779	0.729
Postgraduate	*ref.*	*ref.*
Family income		
< £18,000	**0.035**	**<0.001**
£18-25,000	0.059 ɪ	**0.001**
£25-43,000	0.306	**0.032**
> £43,000	*ref.*	*ref.*
Smoked in three months before pregnancy	**0.006**	**0.005**
Smoked in pregnancy	**<0.001**	**<0.001**
Drank alcohol in three months before pregnancy	**0.012**	**0.020**
Drank alcohol in pregnancy	0.082 ɪ	0.438
Strenuous exercise in pregnancy	0.109 ɪ	0.201
History of mental health problems	**<0.001**	**<0.001**
Parity	**0.004**	**0.007**
Season of birth		
Spring	**0.013**	0.218
Summer	0.585	0.633
Autumn	*ref.*	*ref.*
Winter	0.855	0.536
Indication		
Current complication	0.194	0.689
Maternal Choice	0.160	0.168
Maternal disorder	**0.002**	**0.018**
Previous pregnancy complication	0.133 ɪ	0.169
Previous CS	*ref.*	*ref.*
Continuous variables	***P1 EPDS***	***P1 STAI***
WIMD	**0.002**	**0.002**
Maternal age (*years*)	0.155	**0.026**
BMI at booking	**<0.001**	**<0.001**
Birth outcomes	***P1 EPDS***	***P1 STAI***
CBWC	0.314	0.761
Fetal sex	0.904	0.307
Delivery complications	0.669	0.083 ɪ

[1] *p* values reported

[2] Significant values reported in bold

[3] ɪ: $p < 0.15$

Table 3.10: Multivariate postnatal depression prediction model

Predictor	***Exp(B)***	***CIs***	***p value***
P1 STAI	1.056	0.046, 0.063	**<0.001**
Highest level of maternal education			
Left before GCSE	0.791	-0.752, 0.266	0.365
GCSE/Vocational	0.881	-0.385, 0.125	0.330
A Levels	0.788	-0.522, 0.037	0.094
University	0.982	-0.197, 0.162	0.845
Postgraduate	*ref.*	*ref.*	*ref.*
Family income			
< £18,000	0.724	-0.755, 0.101	0.138
£18-25,000	1.008	-0.308, 0.317	0.961
£25-43,000	0.942	-0.273, 0.149	0.576
> £43,000	*ref.*	*ref.*	*ref.*
Pre pregnancy smoking	0.840	-0.518, 0.158	0.311
Smoking in pregnancy	1.379	-0.118, 0.769	0.155
Alcohol pre pregnancy	0.964	-0.244, 0.174	0.730
Alcohol in pregnancy	1.061	-0.109, 0.227	0.490
Exercise	0.932	-0.275, 0.129	0.492
MHH	1.160	-0.040, 0.333	0.119
Parity	1.173	-0.156, 0.479	0.313
Season of birth			
Spring	1.253	0.020, 0.429	**0.030**
Summer	1.179	-0.044, 0.372	0.121
Autumn	*ref.*	*ref.*	*ref.*
Winter	1.009	-0.198, 0.214	0.465
WIMD	1.000	>-0.001, <0.001	0.996
BMI at booking	1.008	-0.007, 0.023	0.309
Indication			
Current pregnancy complication	0.939	-0.327, 0.191	0.632
Maternal choice	0.908	-0.621, 0.402	0.710
Maternal disorder	0.779	-0.672, 0.154	0.235
Previous pregnancy complication	0.878	-0.356, 0.088	0.248
Previous CS	*ref.*	*ref.*	*ref.*

[1] P1 time point

[2] Significant values reported in bold

[3] CIs: 95% Confidence Intervals

Table 3.11: Multivariate postnatal anxiety prediction model

Predictor	***Exp(B)***	***CIs***	***p value***
A1 EPDS	1.038	0.030, 0.044	**<0.001**
Highest level of maternal education			
Left before GCSE	0.968	-0.255, 0.183	0.768
GCSE/Vocational	0.907	-0.208, 0.011	0.078
A Levels	0.900	-0.220, 0.008	0.069
University	0.916	-0.164, -0.012	**0.024**
Postgraduate	*ref.*	*ref.*	*ref.*
Family income			
< £18,000	1.063	-0.115, 0.235	0.491
£18-25,000	1.163	0.016, 0.284	**0.027**
£25-43,000	0.999	-0.089, 0.085	0.973
> £43,000	*ref.*	*ref.*	*ref.*
Pre pregnancy smoking	0.929	-0.209, 0.060	0.285
Smoking in pregnancy	1.039	-0.145, 0.222	0.683
Alcohol in pregnancy	1.025	-0.041, 0.090	0.458
Exercise	0.967	-0.112, 0.045	0.404
MHH	1.026	-0.054, 0.105	0.526
Parity	0.936	-0.199, 0.068	0.333
WIMD	1.001	>-0.001, <0.001	0.802
Maternal age	0.996	-0.012, 0.004	0.361
BMI at booking	1.002	-0.004, 0.008	0.540
Indication			
Current pregnancy complication	1.106	-0.005, 0.205	0.059
Maternal choice	0.964	-0.249, 0.173	0.736
Maternal disorder	1.103	-0.084, 0.277	0.286
Previous pregnancy complication	1.036	-0.055, 0.125	0.436
Previous CS	*ref.*	*ref.*	*ref.*
Delivery complication	0.972	-0.145, 0.086	0.627

[1] P1 time point
[2] Significant values reported in bold
[3] CIs: 95% Confidence Intervals

As can be seen above, there was some overlap in the significant predictors in the final models. For all final models the other mental health score (either current or prenatal) was a highly significant predictor of depression or anxiety. It is important to remember that in single predictor regressions, mental health was highly inter-correlated, but it was not possible to include all scores due to multicolinearity. A history of mental health was significant in both prenatal depression and anxiety, in line with previous literature. While lower levels of maternal education were significantly associated with increased prenatal depressions scores, lower levels of education (compared to a postgraduate level), were associated with decreased anxiety scores both prenatally and postnatally.

3.3.3 Mental health history

As seen from both the literature and identifying the predictors in our cohort, a previous history of mental health is a highly important predictor of poor mental health in the perinatal period. Among

those who scored above the cut offs for signs of depression symptoms 68% of those had a previous history compared to 53% scoring ≥ 40 on the STAI (Table 3.12). It is important to note that this means 32% of those scoring 13 and above on the EPDS and 47% of those scoring 40 and above on the STAI had no previous reported history of a mental health disorder before developing depression or anxiety in the prenatal period. ANOVAs were performed to see if the type of previous mental health disorder had an effect on prenatal scores for those above the cut offs. However, there was no statistical difference between groups in either depression or anxiety (Depression: $F(3, 86) = 0.415$, $p = 0.743$; Anxiety: $F(3, 86) = 1.621$, $p = 0.190$).

Table 3.12: Categories of previous mental health disorder

	Depression	***Anxiety***	***Depression and Anxiety***	***Other psychotic disorder***	***No previous disorder***
EPDS	2	18	7	1	13
STAI	4	27	11	2	39

[1] Number of those with a previous history who scored above cut offs for EPDS or STAI

3.3.4 Antenatal mental health and birth outcomes

Categorical and continuous birth outcomes were entered as dependent variables into binary and linear regressions where appropriate to determine the effect of prenatal mental health on neonatal outcomes. No outcomes were significantly associated with either antenatal EPDS or STAI when using continuous mental health scores (Table 3.13) or categorical cut offs (data not shown).

Table 3.13: Single predictor regressions for birth outcomes

	A1 EPDS			***A1 STAI***		
Categorical variables (binary regressions)	***OR***	***CIs OR***	***p***	***OR***	***CIs OR***	***p***
Bleeding during pregnancy	0.953	0.872, 1.034	0.269	0.957	0.909, 1.002	0.075
Delivery complications	1.664	0.584, 4.133	0.300	0.965	0.387, 2.197	0.935
Concerns over fetal growth	0.980	0.918, 1.043	0.529	0.985	0.950, 1.018	0.380
PET diagnosis	0.842	0.518, 1.165	0.389	1.017	0.851, 1.163	0.831
GDM diagnosis	0.921	0.796, 1.045	0.234	0.994	0.928, 1.057	0.859
Fetal sex	1.026	0.978, 1.078	0.295	1.013	0.987, 1.041	0.317
APGAR (1 minute)	0.917	0.831, 1.017	0.088	0.958	0.909, 1.013	0.118
Continuous variables (linear regressions)	***B***	***CIs***	***p***	***B***	***CIs***	***p***
Gestational age (*days*)	-0.008	-0.120, 0.104	0.890	-0.015	-0.076, 0.046	0.626
Birthweight (*g*)	0.974	-11.677, 13.625	0.880	2.691	-4.107, 9.489	0.438
Custom birthweight centile	-0.119	-0.829, 0.591	0.742	0.150	-0.231, 0.530	0.442
Head circumference (*cm*)	<-0.001	-0.040, 0.040	0.994	0.012	-0.009, 0.033	0.263
APGAR (1 min)	-0.020	-0.041, 0.001	0.067	-0.009	-0.020, 0.002	0.127
APGAR (5 min)	-0.006	-0.019, 0.007	0.378	-0.003	-0.010, 0.004	0.414

[1] Categorical APGAR score at 5 minutes variable not analysed due to small numbers
[2] CIs : 95% Confidence Intervals
[3] OR: Odds Ratios

3.3.5 Longitudinal analysis

3.3.4.1 LPA

As seen in Figure 3.1, the prevalence of depression symptoms in the GiW cohort reduces over time, whereas prevalence of anxiety symptoms remain high. This was true in both the entire cohort and in only those who completed questionnaires at all time points. In order to identify different trajectory patterns, latent profiling was performed classifying participants who had similar mental health trajectories throughout the perinatal period. The best model for depression was found to be a three class model using equal variances and zero covariances. The best model for anxiety was found to be a two class model with varying variances and covariances (Figure 3.2). A brief description of a small selection of demographic variables for each subgroup can be found in Table 3.14, along with statistical comparisons between classes. Prenatal mental health scores were found to be significantly different between classes in both depression and anxiety LPA classes. There were no significant differences between anxiety classes for demographic variables. Within depression LPA classes, BMI at booking was significantly higher in class 1 compared to 3 and 2, and WIMD was significantly lower in Class 2 compared to Class 1.

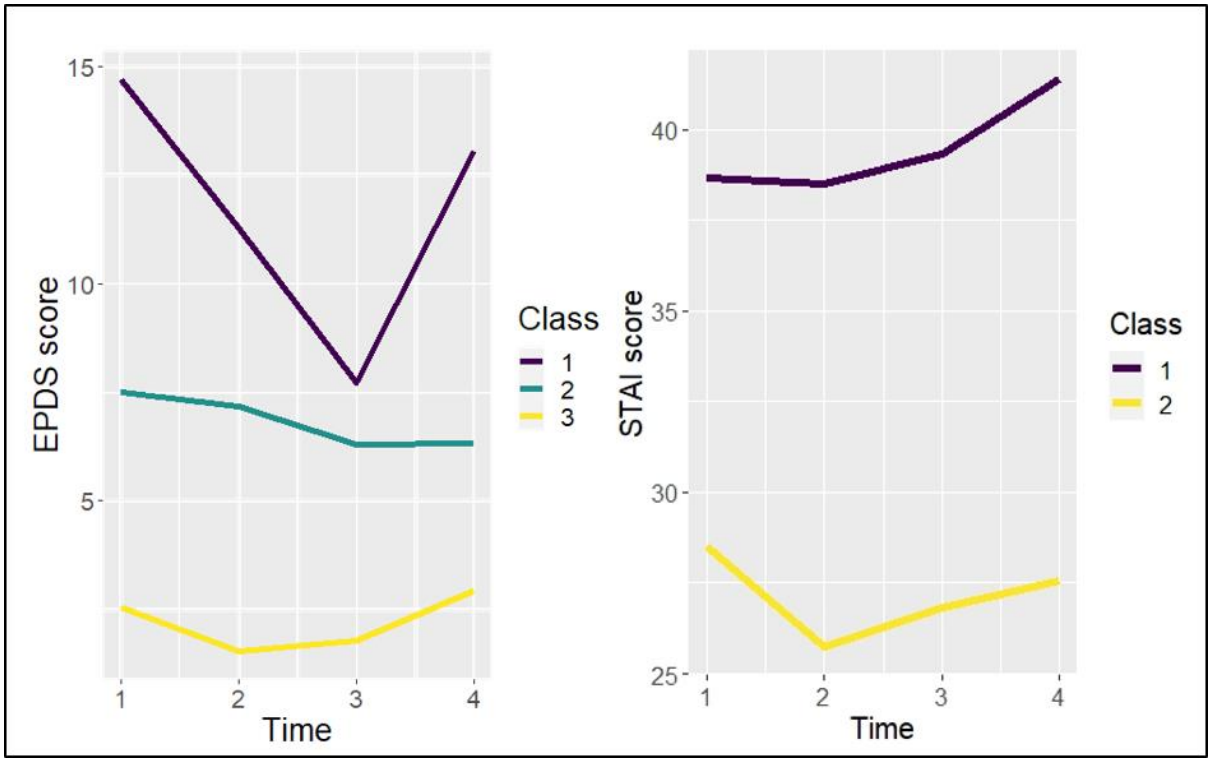

Figure 3.2: Latent Profile Trajectories*. Mental health trajectories of the best fitting models from latent profile analysis. EPDS was split into three classes and anxiety split into two. n= 86 for EPDS and n= 87 for STAI.*

Table 3.14: Comparison of LPA classes

	Depression classes				***Anxiety classes***		
	Class 1	***Class 2***	***Class 3***	***P value***	***Class 1***	***Class 2***	***P value***
Numbers	17	43	26		56	31	
A1 EPDS	14.5	7.5	2.5	**<0.001** [a] *2v1* ***<0.001*** *3v1* ***<0.001*** *3v2* ***<0.001***	9.3	4.2	**<0.001** [b]
A1 STAI	46.1	34.7	27.6	**<0.001** [a] *2v1* ***<0.001*** *3v1* ***<0.001*** *3v2* ***<0.001***	38.7	28.5	**<0.001** [b]
Maternal age	33.4	35.1	34.6	0.426 [a]	34.6	34.4	0.791 [b]
Maternal BMI at booking	31.3	25.8	25.5	**<0.001** [a] *2v1* ***<0.001*** *3v1 0.941* *3v2* ***<0.001***	27.2	25.8	0.162 [b]
WIMD	967.0	1342.1	1348.0	**0.048** [a] *2v1 0.070* *3v1 0.999* *3v2* ***0.048***	1230.6	1290.2	0.651 [b]
Fetal sex Male Female	 7 10	 18 25	 11 15	0.997 [c]	 23 33	 11 20	0.778 [c]
Previous C/S No Yes	 9 8	 21 22	 12 14	1.000 [c]	 27 29	 15 16	0.910 [c]

[1] Means reported for continuous variables and frequencies reported for categorical variables
[2] Significant values reported in bold
[3] a: One way ANOVA and Tukey HSD if significant
[4] b: t-tests
[5] c: Chi-squared

3.3.5.2 Mixed Effect Modelling

As can be seen from the mental health scores at later time points (Table 3.3), there was a substantial drop off in postnatal retention. Approximately only 55% completed the questionnaire at P1, 47% at P2 and 46% at Y1, and LPA can only be performed on a full dataset (n= 86 depression; n= 87 anxiety). To assess the change in prevalence over time and overcome this issue, generalised linear mixed models were used. In order to add relevant confounding variables to the final model, potential variables that could influence the rate of retention were assessed for each time point (Table 3.15). Prenatal mental health scores were not found to increase the rate of attrition at any time point, and neither was mental health history or SSRI prescription. WIMD, maternal age and family income were all found to be associated with attrition levels, and therefore all included in the final model.

Table 3.15: Effect on attrition rates

Independent variables	***P1 EPDS missing***	***P1 STAI missing***	***P2 EPDS missing***	***P2 STAI missing***	***Y1 EPDS missing***	***Y1 STAI missing***
A1 EPDS	0.782	0.714	0.846	0.832	0.913	0.833
A1 STAI	0.497	0.508	0.729	0.577	0.687	0.654
WIMD	**0.008**	**0.009**	**<0.001**	**0.002**	**<0.001**	**<0.001**
Maternal age	**0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
SSRI prescribed in pregnancy	0.984	0.988	0.794	0.903	0.266	0.266
Mental health history	0.260	0.588	0.511	0.920	0.221	0.334
Fetal sex	0.402	0.633	0.941	0.665	0.304	0.206
Family Income						
< £18,000	**0.007**	**0.008**	**<0.001**	**0.001**	**<0.001**	**<0.001**
£18-25,000	0.165	0.335	**0.012**	**0.009**	4**0.002**	**0.007**
£25-43,000	**0.016**	**0.021**	**0.034**	0.066	0.069	0.084
> £43,000	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*
BMI at booking	0.453	0.645	0.733	0.822	0.296	0.272

[1] *p* values from binary regressions reported
[2] Significant values reported in bold
[3] Education not included due to small numbers at later time points in some levels

EPDS score was found to be significantly negatively associated with time (Exp(B) = 0.922, $p < 0.001$) in this cohort (Table 3.16), as can be seen graphically in Figure 3.1. In contrast, time was not found to be significantly associated with STAI score (Exp(B) = 1.004, $p = 0.208$) (Table 3.17).

Table 3.16: Output of generalised linear mixed effects model for depression scores over the perinatal period

Independent variables	***Exp(B)***	***SE***	***CIs***	***p values***
Time	0.922	0.022	-0.126, -0.039	**<0.001**
WIMD	0.956	0.046	-0.136, 0.047	0.336
Maternal age	1.050	0.047	-0.044, 0.142	0.305
Family Income				
< £18,000	1.452	0.163	0.048, 0.694	
£18-25,000	1.382	0.156	0.015, 0.631	0.773
£25-43,000	1.101	0.107	-0.117, 0.306	
> £43,000	*ref.*	*ref.*	*ref.*	
	Variance	***SD***		
Random effects	0.016	0.126		

[1] Significant values reported in bold
[2] SE: Standard error
[3] CIs: 95% Confidence intervals

Table 3.17: Output of generalised linear mixed effects model for anxiety scores over the perinatal period

Independent variables	***Exp(B)***	***SE***	***CI***	***p values***
Time	1.009	0.021	-0.005, 0.023	0.208
WIMD	0.984	0.007	-0.049, 0.164	0.327
Maternal age	1.020	0.017	-0.012, 0.053	0.235
Family Income				**0.003**
< £18,000	1.226	0.059	0.087, 0.320	
£18-25,000	1.160	0.056	0.037, 0.259	
£25-43,000	1.082	0.038	0.003, 0.154	
> £43,000	*ref.*	*ref.*	*ref.*	
	Variance	***SD***		
Random effects	<0.001	0.021		

[1] Significant values reported in bold
[2] SE: Standard error
[3] CIs: 95% Confidence intervals

3.3.6 Summary of chapter results

Table 3.18: Summary of Chapter 3

Analysis	***Findings***
Description of cohort	• Prevalence of prenatal depression 14% • Prevalence of prenatal anxiety 28% • EPDS and STAI highly correlated at all time points
Predictors of prenatal mental health	• A1 STAI, education and a previous mental health disorder predicted A1 EPDS score • A1 EPDS score, education and a previous mental health disorder predicted A1 STAI
Predictors of postnatal mental health	• A1 STAI and season of birth predicted P1 EPDS • A1 EPDS, education and income predicted P1 STAI
LPA and generalised linear mixed models	• STAI scores remained high postnatally and were not associated with time • EPDS scores significantly decreased over time

3.4 Discussion

The prevalence of prenatal depression and anxiety in the GiW cohort was 14 and 27% respectively. While the rates of depression symptoms decreased over the perinatal period, the prevalence of anxiety symptoms remained high postnatally at 35% 12 months after birth. This stability of STAI scores was additionally observed in mixed effect models whereas EPDS scores were negatively associated with time. Using a different statistical model, this chapter also replicated previous findings in Janssen *et al.* 2018 where a previous history of a mental health disorder was the strongest predictor of prenatal depression and anxiety.

The Grown in Wales cohort is the first of its kind in Wales, and provides a unique snapshot of pregnancy in South East Wales for those delivering by ELCS. The cohort was set up with the

expectation of recruiting only those delivering by ELCS (ultimately> 96%) for the purpose of efficiently collecting placental biopsies. Women delivering by ELCS are an important group who are often understudied. The number of women delivering by ELCS increased from 11.8% of births in Wales in 2016 to 13.5% in 2018 (Statistics for Wales 2017, 2019). It is therefore important to research this group particularly as these women do not go through the physiological process of labour and their outcomes may differ from women delivering vaginally or by emergency caesarean. Due to the design of our study focussing on biological collection, there was not a comparable group of women in these other categories for comparison. Nonetheless, findings from this study contribute to our understanding of the causes and consequences of mental health disorders in pregnancy.

The average age of mothers in Wales in 2015-2016 was 30.4 (ONS 2017), whereas mothers in the GiW cohort were slightly older with an age of 32.6. Women delivering by ELCS tend to be multiparous and thus often older (Rydahl *et al.* 2019). Consistent with older age, the percentage of new mothers in the GiW cohort was lower than the Welsh average (20% vs 41%) (ONS 2017). Wales has one of the highest smoking rates during pregnancy in the UK at an average of 18%, with Cardiff and the Vale Health Board (UHW) slightly lower at 14.6% (Statistics for Wales 2017). In the GiW cohort only 10% reported smoking during pregnancy, which may again relate to the older age. Unlike smoking data, rates of drinking alcohol in pregnancy is not routinely collected, but a 2010 report estimated the rate of drinking at any point in pregnancy in England and Wales to be 35%, while a recent meta-analysis determined a pooled rate of 41.3% (Gartner *et al.* 2009; Popova *et al.* 2017). The GiW rates fell more in line with the former, as 35% reported drinking at any time suggesting between 2010 and 2016 prenatal alcohol consumption remained relatively stable.

Rates of mental health disorders in pregnant women were not consistently collected in Wales in 2016 when the study took place. In 2018 an estimated 24% of pregnant women had an active mental health condition (Statistics for Wales 2019), although there was no record of previous disorders. A 2019 study in Northern Ireland described the prevalence of a self-reported history of mental health disorders in pregnant women at 19% (Mongan *et al.* 2019). The prevalence of a previous mental health condition in the GiW cohort was substantially higher at 28%. Of those who scored above the cut off for depression or anxiety, 68% and 53% reported a history of mental health disorders respectively. This is important, as although a previous history of mental health disorders is a strong predictor of poor prenatal mental health, it implies a number of those with high depression or anxiety symptoms in this cohort developed them *de novo* during pregnancy. This could suggest women develop depression or anxiety in pregnancy for different reasons, highlighting the need for continued research into underlying mechanisms.

Anti-depressant use has previously been reported as particularly high in Wales. A study assessing the rate of prescription in pregnancy in Denmark, the Netherlands, Italy and the UK between 2004 and

2010 reported that Wales had the highest percentage of all regions at 4.5% (Charlton *et al.* 2015). The rest of UK had a prevalence of 3.5% compared to 2.3% in the Netherlands and 1.2% in Italy. Another report found SSRI prescription rates among the general population increased more in Wales than the rest of the UK during this time (BMA 2017), which may explain the increase seen in the Grown in Wales cohort (7.8%) compared to Charlton *et al*. 2015 (4.5%). This high prescription rate in Wales is consistent with the higher rates of mental health history in the GiW cohort compared to Northern Ireland. However, data is not currently collected across nations in a way that allows for direct comparison.

There have been a limited number of studies assessing the rates of perinatal mental health disorders within the UK, the largest being ALSPAC. Comparing prenatal scores between ALSPAC and the GiW cohort, there were similar rates of prenatal depression in the third trimester (13.5% vs 14% respectively). Anxiety in the GiW cohort was substantially higher (27% compared to 15% in ALSPAC) (Heron *et al.* 2004). The difference in anxiety prevalence could relate to the different questionnaire used to assess anxiety symptoms. Alternatively, those undergoing an ELCS may be in general more anxious as prenatal mental health is a known predictor of an ELCS delivery (McCourt *et al.* 2007). The ALSPAC group recently reported data on mothers in their second generation aged 19 to 24. In this study, the prevalence of prenatal depression increased from 17% in 1992 to 25% in 2012-16 (Pearson *et al.* 2018). This implies, at least in younger mothers, that prenatal depression rates are increasing. Given the average age of mothers in the GiW cohort is 32.6 but prenatal depression prevalence is similar to ALSPAC in 1992, prevalence of prenatal anxiety may be increasing in older mothers.

As discussed in Janssen *et al.* 2020, from graphical interpretation symptoms of depression appear to decrease over the perinatal period whereas anxiety symptoms remain high. LPA separated participants into latent profiles based on EPDS and STAI scores independently. The three depression profiles plotted those scoring high (probable depression symptoms), moderate (mild depression) and low (no depression) across the perinatal period, and map to previous severity descriptions of prenatal depression scores (McCabe-Beane *et al.* 2016). There were no demographic differences between the low scoring and mild group suggesting other factors not collected may explain the increased vulnerability. However, although profiles were not assessed in a multivariate predictor model as with the raw scores due to small group numbers, differences in demographics between the moderate and high scoring group correlate with previous literature finding different predictors for mild and moderate prenatal depression (Marchesi *et al.* 2009). STAI scores were split into two profiles; those scoring consistently high or low over the perinatal period. Together this data is consistent with the idea that those with high depression or anxiety symptoms prenatally remained consistently high up until a year after birth. As described in Janssen *et al.*, a Norwegian study linked elective caesarean delivery to high emotional distress before delivery and six months postnatally (Adams *et al.* 2012).

Given the relatively stable STAI scores for both classes, ELCS delivery does not appear to raise anxiety levels in the postnatal period but as discussed below not going through labour might prevent the resolution of anxiety for those suffering prenatally. However, as there is no control group who delivered naturally it is not possible to make final conclusions about the effect of elective caesareans. Furthermore, LPA analysis is only appropriate for participants with a 100% completion rate. Therefore, the sample size was sizeably smaller than the entire cohort and should be interpreted with caution.

Mixed effect modelling allows for the change in mental health scores over time to be explored statistically in all participants. This therefore helps in part to control for dropout rates, in a way statistical methods such as repeated measures ANOVAs and LPA cannot do. Time as a fixed effect was negatively associated with EPDS score, indicating that overall scores did decrease over time. On the other hand, STAI scores were not significantly associated with time in either direction, suggesting anxiety symptoms remained relatively steady over the course of the perinatal period. This is in contrast to other UK cohorts with data of this type, and similar to our findings using a different statistical approach (Janssen *et al.* 2018). Caesarean delivery is known to be a risk factor for postnatal anxiety (Field 2018). Not going through the physical process of labour and lack of exposure to labour-dependent hormones may maintain high anxiety levels (Janssen *et al.* 2018). This is a concern because the rates of ELCS are increasing (Rydahl *et al.* 2019), the rates of anxiety in the population are high (Remes *et al.* 2016), and being more anxious in the perinatal period is associated with adverse outcomes for children (Zietlow *et al.* 2019).

As we reported in Janssen *et al.* 2018, predictors for prenatal depression were previously assessed. This chapter additionally set out to look at predictors of prenatal anxiety, and predictors of depression and anxiety four days after birth. The current analyses used a Poisson family error distribution in generalised regressions, due to the discrete and positively skewed nature of both EPDS and STAI scores. This was in contrast to our previous study which applied Gaussian linear regressions (Janssen *et al.* 2018). Predictors of prenatal depression were similar between Janssen *et al.* 2018 and the current analyses, with the addition of maternal education ending at GCSE/Vocational or University level when compared to a postgraduate education and the loss of fetal sex. Prenatal depression scores and a family income of less than £18,000 predicted high prenatal anxiety scores. In addition, having left education before GCSE compared to a postgraduate education was negatively associated with STAI score, whereas there was a positive association for EPDS score. The same relationship with education was apparent when predicting postnatal anxiety, which replicates a previous study of a much larger cohort (Bener *et al.* 2012).

Seasons were also a significant predictor of postnatal STAI score, specifically a spring over an autumn birth, the opposite finding of a previous study set in Finland (Hiltunen *et al.* 2004). It should

be noted that in the GiW analysis, the relationship between seasons and EPDS score were assessed whereas in Hiltunen *et al*. depression cut offs were used. Another study based in Michigan, America found no significant associations between postnatal depression and season (Panthangi *et al.* 2009). The numbers in all these studies are small and given the disparity between results should be interpreted with caution. We recently reported term cortisol but not prenatal EPDS or STAI to be significantly associated with season of birth in the GiW cohort (Garay *et al.* 2019). Mothers had higher cortisol concentrations in autumn and winter, and cortisol was significantly higher in autumn compared to spring. While literature is mixed, some report significant positive associations between cortisol and depression or anxiety in the perinatal period. However, in Janssen *et al.* 2018, we showed no correlation between EPDS or STAI score and cortisol concentration. Furthermore, cortisol concentrations were higher in mothers giving birth in autumn compared to spring (Garay *et al.* 2019) yet in the present study, spring compared to autumn was associated with higher postnatal STAI scores. This indicates that cortisol may not be a marker for depression or trait anxiety in the perinatal period.

A history of mental health disorders was a strong predictor of high depression symptoms in pregnancy and in the immediate postnatal period. This relationship was further analysed by one way ANOVA to determine if the type of previous mental health disorder was significantly associated with EPDS scores in pregnancy, but no significant association was apparent. This may relate to the small sample sizes of the previous disorder groups. A recent meta-analysis pooled together predictors of prenatal depression and in line with the current analyses, observed that a previous history of poor mental health was one of the biggest predictors (Biaggi *et al.* 2016). Unfortunately, for the other most common predictors they described such as unwanted pregnancies, history of abuse and pregnancy loss, data was not available in the GiW cohort, and thus could not be examined.

Despite mixed reporting, some previous studies have described associations between poor prenatal mental health and adverse birth outcomes (Jarde *et al.* 2016; Grigoriadis *et al.* 2018). No associations reached statistical significance in this study. This could be explained by low participant numbers, but given large studies have not found any clinically significant relationships, the link between birth outcomes and prenatal depression and anxiety is still debatable (Littleton *et al.* 2007; Accortt *et al.* 2015). All of the participants in this analyses delivered by ELCS and delivery was planned in advance minimising the likelihood of complications associated with a spontaneous vaginal delivery. All the participants were recruited at term, eliminating complications associated with preterm deliveries. Together, these factors may have limited the ability to assess the relationship between mental health and adverse birth outcomes.

3.4.1 Conclusions

In summary, GiW is a longitudinal study of women from South East Wales who have all given birth by ELCS. The cohort had a similar prevalence of depression and anxiety symptoms at term relative to other studies with the same measures. Depression scores significantly decreased across the perinatal period, whereas anxiety scores were not significantly associated in any direction with time, suggesting on average anxiety levels remain elevated within the postnatal period. Both prenatal depression and anxiety were significant predictors of each other, and a history of mental health disorders prior to pregnancy was a major predictor of both. Depression and anxiety were highly correlated and, in general, shared the same predictors; maternal education and a history of previous mood disorder. Together these analyses further explore the Grown in Wales cohort, and add to the knowledge surrounding perinatal mental health.

Chapter 4: RNA sequencing

4.1 Introduction

The placenta is a transient but essential organ that develops during pregnancy to support development *in utero* (John and Hemberger 2012; Burton and Fowden 2015). To do so, the placenta performs numerous intricate tasks including the transport of nutrients to the growing fetus, removal of waste, acting as a barrier to toxins and the production of hormones that are required to initiate and sustain pregnancy. Consequently, the placenta is a key tissue for the study of pregnancy complications. Placental dysfunction can cause complications and may also arise as a consequence of a complication or an exposure during pregnancy. The placenta is fetal-derived, sharing the same genome as the fetus, and provides a record of exposures during the pregnancy which may be helpful in understanding longer term outcomes for the offspring. Identifying gene changes in the placenta can thus be helpful in identifying mechanisms underpinning aberrant placental function and predicting outcomes. High throughput techniques such as RNA sequencing (RNAseq) allows for both high specificity and sensitivity analysis of data in a relatively automated fashion.

Numerous studies have characterised the gene expression profiles of healthy placentas, identifying modules of co-expressed genes stable throughout gestation, and altered patterns of expression in different placental cell types (Sood *et al.* 2006; Buckberry *et al.* 2017). Furthermore, in healthy pregnancies placental gene expression varies between fetal sex, for both autosomal and sex chromosome genes (Cvitic *et al.* 2013; Gong *et al.* 2018; Gonzalez *et al.* 2018). In one microarray study immunity and inflammatory pathways were in enriched in male trophoblast cells compared to females (Cvitic *et al.* 2013). Cvitic *et al.* postulated that this observation may be analogous to the poorer outcomes of male fetuses often observed when compared to females. Together this research into healthy placentas helps build knowledge vital for progressing the understanding of stressed or improperly functioning placentas.

Focussing on disease states, RNAseq has shown that diseases stemming from placental malfunction such as pre-eclampsia are associated with global shifts in gene expression (Sõber *et al.* 2015; Gong *et al.* 2018). Other common pregnancy complications such as fetal growth restriction and gestational diabetes are also associated with widespread genetic changes compared to healthy pregnancies (Sõber *et al.* 2015; Alexander *et al.* 2018). Furthermore, the placental transcriptome responds to exposure of adverse stimuli such as synthetic glucocorticoids and environmental levels of arsenic (Lee *et al.* 2017; Winterbottom *et al.* 2019).

Despite the increasing number of high throughput RNAseq studies on placental tissue, limited attention has been paid to the association between maternal mental health and the placenta in a next generation-based approach. One study focused on investigating imprinted gene expression in placentas from mothers with different maternal mental health problems (Litzky *et al.* 2018). Litzky *et al.* reported 54 imprinted genes with altered placental expression in mothers with depression and 89 in

those with comorbid depression and anxiety. The majority of the altered genes in both groups were decreased in expression compared to controls. Another study investigating the response of the placental transcriptome exposed to prenatal depression used microarrays, and reported over 100 abnormally expressed genes (Olivier *et al.* 2014). Again the majority of altered genes were decreased in expression compared to controls. However, neither separated analysis by fetal sex. Given previous literature reporting differences in placental transcriptomes between fetal sexes, sex differences in response to prenatal depression may have been overlooked. Both studies found an association between taking psychiatric medication and placental gene expression, with the Olivier *et al.* study focussing specifically on SSRIs.

SSRIs are widely used to treat depression and anxiety, with approximately 13% of the UK population prescribed them (Mars *et al.* 2017). Briefly, SSRIs function to increase the extracellular concentration of serotonin by limiting its reabsorption into presynaptic cells (Sangkuhl *et al.* 2009). Serotonin, commonly known as the happiness chemical, can therefore repeatedly act on its receptors inducing downstream pathways. In addition to its role in mood-elevation, serotonin's functions are complex and manifold involving cognition, memory and vasoconstriction (Dubovicky *et al.* 2017). Serotonin is vital during pregnancy as it is involved in the development of the endocrine and nervous systems of the fetus (Herlenius and Lagercrantz 2004). Given the importance of serotonin in development, concerns have been raised over the use of SSRIs in pregnancy. SSRIs are known to cross the placenta and have been found in amniotic fluid (Hostetter *et al.* 2000; Loughhead *et al.* 2006), and there is great uncertainty on the short- and long-term effect on the exposed fetus (Marchocki *et al.* 2013; Stewart and Vigod 2018; Kott and Brummelte 2019). Therefore UK guidelines recommend stopping antidepressant use during pregnancy in mild to moderate cases of depression (NICE 2014). Nonetheless, women are still prescribed antidepressants during their pregnancy, with the most common being SSRIs. As described in Chapter 3, Wales has a particularly high prenatal prescription rate and 7.8% of participants in the GiW cohort were prescribed SSRIs during pregnancy (Charlton *et al.* 2015). Not treating depression in pregnancy can have devastating consequences for the mother and her child, but treatment with SSRIs may contribute to different adverse outcomes (Kott and Brummelte 2019). Given the close relationship of the fetus and placenta, changes in placental gene expression in association with psychiatric medication seen in Litzky *et al.* and Olivier *et al.* could mean the exposed fetus is also affected.

Mental health disorders and psychiatric medication have been associated with the altered expression of individual genes in the placenta (O'Donnell *et al.* 2012; Blakeley *et al.* 2013; Kaihola *et al.* 2015; Mina *et al.* 2015; Reynolds *et al.* 2015; Edvinsson *et al.* 2019). These approaches are hypothesis-led and rely on data from a small number of genes. An example are the studies focussing on the HPA axis which plays an important role in the stress response and involves a positive feedback loop including its hormonal end product cortisol (Duthie and Reynolds 2013). Psychological stressors such as

prenatal depression have been linked to this pathway given the introduction of the placenta which synthesises its own cortisol further stimulating the positive feedback loop (Glynn *et al.* 2013). However, hypothesis-led studies such as these do not identify the full range of changes present in the placenta and overlook novel mechanisms.

The aims of this chapter were to quantify genome-wide transcriptomic changes in term placenta in relation to mothers' reported symptoms of depression in a subset of the Grown in Wales cohort. Given previous reporting of transcriptomic differences between placentas from male and female fetuses, a direct effort was made to explore the relationship between placental gene expression and maternal mood in male and female placenta independently. Building upon differential gene expression analysis, changes in larger biological processes and pathways were investigated to gain a more complete understanding of the placental transcriptomic environment in pregnancies where mothers report symptoms of prenatal depression.

4.2 Method

Methods for the recruitment in the Grown in Wales study and RNA extraction were described in Chapter 2.

4.2.1 Sample selection

4.2.1.1 First sample set

At the start of the analysis, RNAseq data was available for a discovery set of 39 samples from the GiW study. These samples were from a study focussed on examining the expression of placental genes in relation to both fetal growth restriction and mental health balanced for a number of maternal characteristics. This sample set included 24 babies born AGA and 15 babies born SGA. Within these samples, 16 women had a prenatal EPDS score of 13 and above. These samples were chosen for a pilot analysis of gene expression in relation to prenatal depression. The remaining samples with prenatal EPDS scores below 13 were used as "controls". Two of the controls were prescribed SSRIs during their pregnancy and removed from the analysis. The final analysis involved 21 "control" (EPDS< 13) and 16 "depressed" (EPD≥ 13) samples.

4.2.1.2 Second sample set

After the analysis of the initial data, additional samples were sent for sequencing to increase the power of the study; 3 samples from mothers reporting symptoms of prenatal depression and another 21 controls with EPDS< 13. The final analysis involved 42 "control" (EPDS< 13) and 19 "depressed" (EPDS≥ 13) samples.

4.2.2 Sensitivity analysis

Only a small number of participants in the total RNAseq subset were prescribed SSRIs during their pregnancy (n= 5). Therefore, to investigate whether SSRIs masked any observations these were

removed from the analysis and a sensitivity analysis performed. The sensitivity analysis involved 42 "control" (EPDS< 13) and 14 "depressed" (EPDS≥ 13) samples.

4.2.3 RNA sequencing

Wales Gene Park performed RNA sequencing of placental samples. RNA quality was determined for all samples using an Agilent's 2100 Bioanalyzer to estimate the RNA Integrity Number (RIN) and an RNA Nano 6000 kit (Agilent Technologies). 100-900 ng of total RNA was depleted of ribosomal RNA (rRNA) before sequencing libraries were prepared using Illumina Stranded total RNA with Ribo-Zero Gold kit (Illumina Inc., USA). Steps included: rRNA depletion and clean up, RNA fragmentation, first strand cDNA synthesis, second strand cDNA synthesis, adenylation of 3'-ends, adapter ligation, PCR amplification (15 cycles) and validation. The manufacturer's protocol was followed except for the clean-up after the ribozero depletion step where AmpureXP beads (Beckman Coulter, UK) and 80% ethanol were used. Libraries were validated using the Agilent 2100 Bioanalyser with a high-sensitivity kit (Agilent Technologies) to determine insert size, and quantified with Qubit (Life Technologies). Libraries were normalised to 4 nM, pooled and clustered on cBot2 following the manufacturer's protocol. The initial 37 samples were sequenced using a 75-base paired-end (2x75 bp PE) dual index read format on the Illumina HiSeq2500 (high-output mode). The remaining 24 samples were sequenced using the same format on the Illumina HiSeq4000. The former used non-patterned flow cells and the latter used patterned flow cells. The patterned flow cells offers the advantage of being quicker, cheaper and increases the amount of usable data per flow cell.

4.2.4 RNAseq workflow

Quality control was performed on raw RNAseq paired-end sequencing reads using FastQC v0.11.2 (Andrews 2010). Raw sequencing reads were trimmed of adapters and poor quality read ends using Trim Galore v0.41 (Krueger 2012), a wrapper from Cutadapt v1.11 (Martin 2011). Trimmed reads were mapped to Gencode GRCh38 release28 primary assembly (Frankish *et al.* 2019) using STAR v2.5.1b (Dobin *et al.* 2013), then merged at sample level using Picard tools v1.45 (Broad Institute). Library strand specificity was inferred from each sequence alignment file (.bam) using RSeQC (v2.6.4) infer_experiment.py (Wang *et al.* 2012a). Read counts were summarized at exon level and aggregated gene level, specifying strandedness, using FeatureCounts (version 1.5.1) (Liao *et al.* 2014). Each sample yielded an average of 46.6 million 76-base pair paired end reads (range 17 to 115 million). In total an average exon depth coverage of 33 fold per sample was achieved. Wales Gene Park also performed this generation of count data. Quality control checks were performed on gene level counts data using RStudio and tools within the R package DESeq2 (Love *et al.* 2014).

4.2.5 Differential gene expression

Differential gene expression was tested using the Bioconductor package edgeR (v3.28) (Robinson *et al.* 2010) on FeatureCounts. To prevent biologically irrelevant genes with low counts influencing

results filterByExpr was used; based on counts per million (cpm) genes below the minimum group number were removed. Scale normalisation across libraries was performed using the default trimmed mean of M-values (TMM) normalisation method to avoid RNA composition bias. To account for differences in sequencing batch, a batch variable was incorporated into every model run. Different batches were run on different technologies, non-patterned vs patterned flow cells. Calling of differentially expressed genes was executed using generalised linear models in edgeR. *p* values were adjusted to control for false discovery rate using Benjamini-Hochberg multiple test correction (FDR), and a significance threshold of FDR< 0.05 set. No threshold was set for fold change to maximise the number of genes included. Volcano plots were created in base R packages highlighting significant adjusted *p* values and absolute log2 fold changes over one. Principal Component Analyses (PCAs) of normalised count data were generated in limma (Ritchie *et al.* 2015).

4.2.6 Confounders

Potential confounders from the literature (fetal sex, birthweight, placental weight, gestational age, maternal age, BMI, parity, WIMD, education, and smoking or drinking alcohol at any point during pregnancy) were each assessed independently on the entire dataset to determine their effect on differential gene expression. This was repeated separately for male and female placentas. For continuous variables, samples were divided at the median for comparison of groups. The effect of the variable on clustering of samples was also assessed via heatmaps. Any variables that led to more than one significant differentially expressed gene (DEG) was included as a confounder along with batch.

4.2.7 Cell type markers

In well characterised biological samples, programmes are available for cell type composition analysis. The placenta is highly heterogeneous in cell type but to date there is no reliable program for assessing cell type. Therefore, established cell type markers for trophoblast, extra villous trophoblast, cytotrophoblast and syncytiotrophoblast were examined across depression groups and fetal sex independently (Liu *et al.* 2018).

4.2.8 Gene ontology

Genes significantly altered at an FDR value< 0.05 in the DEG analysis were taken forward for Gene Ontology (GO) enrichment analysis. Genes were separated into those over and underrepresented based on their log2 fold change (L2FC) values and run separately to increase power (Hong *et al.* 2014). Limma package's goana was used to perform GO enrichment analysis for Biological Pathways, Molecular Functions, and Cellular Component ontologies (Ritchie *et al.* 2015). The package goana only supports Entrez gene IDs which were not available for all significant genes, therefore limiting the number of genes able to be analysed.

Only GO enrichments that were supported by at least five genes, and were significant at an FDR *p* value of 0.05 were taken further. REVIGO was used to remove redundant ontologies, using a medium

similarity measure and the semantic similarity measure SimRel (Supek *et al.* 2011). A circos plot was generated to visualise relationships between significant DEGs and their associated biological processes using the GOplot package (Walter *et al.* 2015).

4.2.9 Gene set enrichment analysis

Gene set enrichment analysis (GSEA) is similar to GO analyses, but can be more powerful as it takes into account global gene expression rather than just significant genes. GSEA was performed using the fgsea package on a ranked list (based on signed *p* values) of all genes to identify functionally enriched gene sets (Korotkevich *et al.* 2016). Hallmark gene sets from the Molecular Signature Database (MSigDB, Broad Institute) were analysed for enrichment. These gene sets represent stable and established biological processes (Liberzon *et al.* 2015). A Kolmogorov-Smirnov test is applied to determine significant pathways, and those with an FDR value of $p< 0.05$ were deemed significant. The level of enrichment of each pathway was expressed as normalised enrichment score (NES).

4.2.10 Weighted gene co-expression analysis

Gene co-expression analysis is a technique for describing correlation patterns among genes. In this analysis, the R package CEMiTool was performed on the TMM normalised data and a default unbiased variance-based filter used (Russo *et al.* 2018). Given the non-normality of the Q-Q plot and the high R^2 value, a Spearman's correlation and a variance stabilising transformation were used respectively. This package separates data into distinct modules, before running enrichment analysis on respective modules. Gene ontology and Hallmark pathway enrichment was performed on modules enriched in the depressed group.

4.3 Results

4.3.1 Initial RNAseq analysis

Demographics of the first RNAseq cohort can be found in Table 4.1. Birthweight was the original focus of the study that the RNAseq samples originated from and 13 SGA samples were from control mothers and 3 from depressed mothers. Fetal sex was roughly evenly split between control and depressed groups.

Table 4.1: Demographics of the first RNAseq cohort and depression subgroup

Categorical variables	***All samples (n= 37)***	***EPDS≥ 13 (n= 16)***
Highest level of maternal education		
Left before GCSE	2.7	6.2
GCSE/Vocational	37.8	37.4
A Levels	16.2	18.8
University	24.4	18.8
Postgraduate	18.9	18.8
Family income		
< £18,000	18.8	28.6
£18-25,000	6.3	7.1
£25-43,000	28.1	21.4
> £43,000	46.8	42.9
Smoked in pregnancy		
No	83.8	75.0
Yes	16.2	25.0
Drank alcohol in pregnancy		
No	51.4	50.0
Yes	48.6	50.0
History of mental health problems		
No	56.8	12.5
Yes	43.2	87.5
Prescribed SSRIs in pregnancy		
No	89.2	75.0
Yes	10.8	25.0
Parity		
Nulliparous	27.0	31.3
Multiparous	73.0	68.7
Fetal sex		
Male	51.4	56.3
Female	48.6	43.7
Continuous variables	***All samples (n= 37)***	***EPDS≥ 13 (n= 16)***
WIMD	919.6	712.3
Maternal age (*years*)	32.5	32.1
BMI at booking	27.2	28.6
Gestational age (*days*)	274.3	274.9
Birthweight (*g*)	3104.9	3278.8
Custom birthweight centile	28.1	36.4
Placental weight (*g*)	585.1	608.4
Head circumference (*cm*)	34.2	34.9

[1] Categorical variables reported as percentages and continuous variables represented as means

A PCA was generated for the 37 samples to visualise clustering before the differential gene analysis was performed (Figure 4.1). From this, fetal sex emerged as the major grouping factor and thus included as a confounder. As part of the pilot analyses, differential gene expression analysis was first performed on all 37 samples. No DEGs were identified between the control and depressed samples. The same analysis was then re-run on male and female placentas separately. Again, no genes were significantly different between the two groups. As there was no apparent differences between control and depressed groups in any analysis, this dataset was not taken further to pathway analysis.

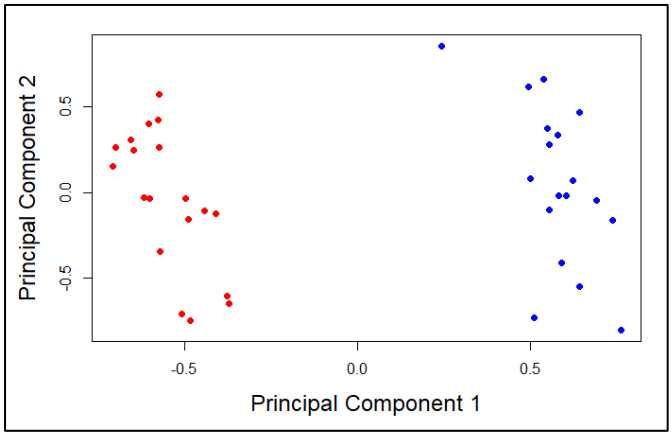

Figure 4.1: PCA of normalised counts*. Principal component analysis of normalised counts in initial RNAseq samples (n= 37). Red-male placenta, blue-female placenta.*

The initial analysis did not identify any DEGs. Therefore, 24 additional samples were identified within the GiW study to increase the number of samples. Due to the time between the two rounds of RNA sequencing, these were performed on different platforms and batch was controlled for in all analyses.

4.3.2 Combined RNAseq dataset

4.3.2.1 Demographics

Demographics of the combined dataset is provided (Table 4.2). Within this dataset 19 samples came from mothers reporting depression symptoms prenatally (EPDS≥ 13). The 19 samples in the depressed group had a mean EPDS score of 17.2, the remaining 42 samples acted as controls and had a mean EPDS of 5.3. The ratio between male and female placentas was 33 to 28. From the medical notes, five women were prescribed SSRIs during their pregnancy with a male to female ratio of four to one.

Table 4.2: Demographics of combined RNAseq cohort and depression subgroup

Categorical variables	***All samples (n= 61)***	***EPDS≥ 13 (n= 19)***
Highest level of maternal education		
Left before GCSE	8.2	15.8
GCSE/Vocational	29.5	31.6
A Levels	13.1	15.8
University	24.6	21.1
Postgraduate	24.6	15.7
Family income		
< £18,000	14.8	31.3
£18-25,000	13.0	6.3
£25-43,000	24.1	25.0
> £43,000	48.1	37.4
Smoked in pregnancy		
No	82.8	68.4
Yes	17.2	31.6
Drank alcohol in pregnancy		
No	58.7	47.4
Yes	41.3	52.6
History of mental health problems		
No	59.4	10.5
Yes	40.6	89.5
Prescribed SSRIs in pregnancy		
No	87.5	73.7
Yes	12.5	26.3
Parity		
Nulliparous	21.9	26.3
Multiparous	78.1	73.7
Fetal sex		
Male	53.1	57.9
Female	46.9	42.1
Continuous variables	***All samples (n= 61)***	***EPDS≥ 13 (n= 19)***
WIMD	980.8	689.6
Maternal age (*years*)	32.6	31.8
BMI at booking	26.3	28.7
Gestational age (*days*)	274.0	274.4
Birthweight (*g*)	3285.3	3316.3
Custom birthweight centile	41.1	38.5
Placental weight (*g*)	630.6	619.5
Head circumference (*cm*)	34.7	34.8

[1] Categorical variables reported as percentages and continuous variables represented as means

4.3.2.2 Confounders

To control for technical variation across the two sequencing rounds, a batch variable was added into all statistical models. To assess the need to add further confounding variables into the models, potential confounders were analysed individually on the entire dataset (n= 61) (Table 4.3). Infant characteristics (fetal sex, birthweight, placental weight, and gestational age) and maternal demographics (maternal age, BMI, parity, WIMD, education, and smoking or drinking alcohol at any

point during pregnancy) were independently assessed for effects on differential gene expression. Family income was not assessed due to missing data. Fetal sex was associated with the most DEGs (n= 72), 57% of which were genes located on sex chromosomes such as *XIST* and *ZFY*. The remaining 43% were autosomal. In line with a previous study on first trimester placental tissue, *FRG1JP* was higher in male placenta suggesting it remains higher throughout pregnancy although its function is unknown (Gonzalez *et al.* 2018). *GPANK1*, thought to play a role in immunity, was also significantly higher in male placenta in the current study. To investigate this further, an analysis was performed to compare only "control" male and "control" female placentas ensuring depression was not confounding results. In this analysis 50 DEGs were identified. Of these, 78% were also identified in the former analysis (Table 4.4). Gene ontology analysis was performed on the 50 DEGs and epigenetic ontologies such as histone modification and covalent chromatin modification were significantly enriched (Table 4.3).

From the other potential confounders assessed maternal smoking at any point in pregnancy was associated with three DEGs. This included two in the *CYP* family of genes (*CYP1A1* and *CYP1B1*) which were both over expressed compared to placentas from mothers who did not smoke (Table 4.3). This was consistent with previous literature (Bruchova *et al.* 2010; Suter *et al.* 2010; Huuskonen *et al.* 2016).

Given the large number of DEGs between fetal sexes, potential confounders were also assessed separately between male and female placentas. No variables had any significant gene changes within male placenta, and within female placentas there were two significant gene changes between nulliparous and multiparous women (Table 4.3) (*SNORD3C* and *TMEM74B*; both over expressed). Therefore along with controlling for batch, when analysing all samples together fetal sex and smoking were controlled for, and when analysing females smoking was included as a confounder.

Table 4.3: Differential gene expression results of confounders

Model	***Variable***	***DEGs***
---All samples---		
All samples	Fetal sex *(ref= female)*	Overexpressed: *UTY; USP9Y; DDX3Y; RPS4Y1; ZFY; TTTY15; KDM5D; TXLNG2P; EIF1AY; GYG2P1; PRKY; AC006032.1; PCDH11Y; FRG1JP; CA2; FRG1EP; ARTN; CXCR4; ST18; AC092066.1; GUCA2A; RPS6KA6; ARMCX3; PAK3; RP11-150O12.3; CORO6; GPRASP1; GNPDA1; GOT1; HIRIP3; GPANK1; AMMECR1; HDAC6*
		Under expressed: *RHBDD1; CHM; BRCC3; RPS4X; UBA1; OSMR; MBTPS2; ASAP2; CXorf23; CA5BP; HSD17B10; CA5B; SMC1A; EIF2S3; TOMM5; NAA10; DDX3X; ZFX; EIF1AX; C12orf45; TBC1D16; ACE2; PLCE1; BIN2; SH3TC1; CXorf36; KDM5C; PHOSPHO1; STS; CTD-2541M15.1; KDM6A; NPFFR2; PUDP; CELF2; RP11-676F20.1; CGB8; IL13RA2; DLGAP1; XIST*
All samples	Smoking *(ref= no)*	Overexpressed*: CYP1A1; CYP1B1, MYH14*
		-
Female samples	Parity *(ref= nulliparous)*	Overexpressed: *SNORD3C; TMEM74B*
		-

[1] Only results where > 1 gene are significantly differentially expressed are reported
[2] DEG analysis performed on all potential confounders independently in three groups; all samples (N= 61), male samples only (n= 33) and female samples only (n= 28)

Table 4.4: Differential gene expression and gene ontology results by fetal sex

Model	***Variable***	***DEGs***
---Control samples only---		
All samples	Fetal sex *(ref= female)*	Overexpressed: *DDX3Y; USP9Y; UTY; TTTY15; ZFY; RPS4Y1; KDM5D; EIF1AY; TXLNG2P; PRKY; AC006032.1; PCDH11Y; ATP2C2; RIC3; NEXMIF; FRG1JP; RP6-170F5.2; FRG1EP; CORO6; GPRASP1; RPS6KA6; RP11-150O12.3; NRCAM; GNPDA1; PDK4; GPANK1; AKAP8L; C2orf49; HDAC6*
		Under expressed: *EEF2K; SMC1A; UBA1; EIF2S3; HSD17B10; DDX3X; ZFX; NAA10; EIF1AX; ASAP2; KDM5C; CXorf36; KDM6A; STS; BIN2; PHOSPHO1; PUDP; ACTG2; COL12A1; IL13RA2; XIST*
Model	***Variable***	***Significantly enriched GOs***
All samples	Fetal sex *(ref= female)*	Cellular protein metabolic process; Organonitrogen compound metabolic process; Macromolecule modification; Cellular amide metabolic process; Amide biosynthetic process; Organonitrogen compound biosynthetic process; Chromosome organization; Oxidation-reduction process; Chromatin organization; Covalent chromatin modification; Histone modification

[1] n= 42

4.3.2.3 Maternal contamination

The placental biopsies were taken below the membrane on the maternal side to minimise maternal contamination. However, as a precaution the level of contamination of maternal cells was measured

by *XIST* expression. *X-inactive specific transcript* (*XIST*) is an X chromosome gene involved in the X-activation process, and thus expression in males is nominal. *XIST* was minimally expressed in all of the male samples (108 maximum normalised count), compared to female samples (6218 median normalised count) (Figure 4.2).

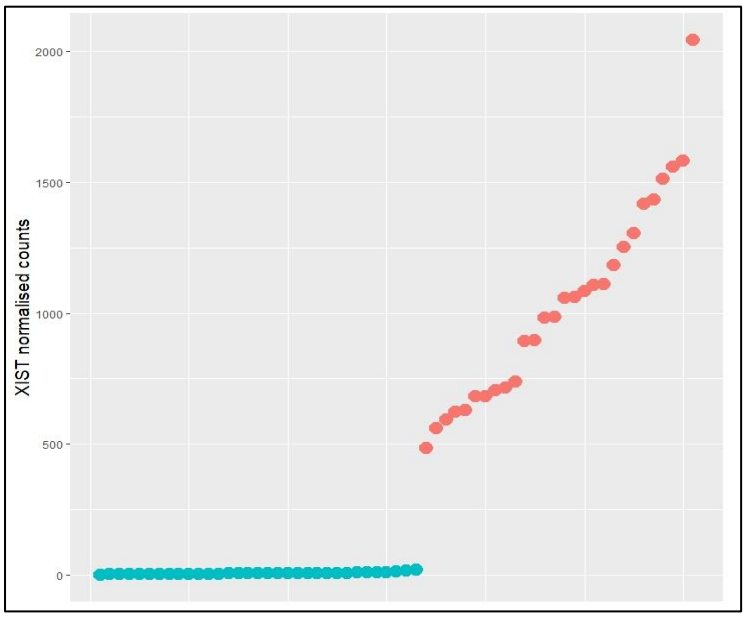

Figure 4.2:* XIST *expression across samples. *Normalised expression counts are plotted. Blue-male samples, pink-female samples.*

4.3.2.4 Cell type markers

Despite the detailed protocol for collecting placental biopsies to ensure only trophoblast cells were sampled, the placenta is highly heterogeneous in cell type. Therefore, established markers of different placental cell types were analysed across depression groups and fetal sexes. No significant differences were found in any cell type (Table 4.5; Figure 4.3). This implies there was no difference in cell type between groups.

Table 4.5: Differential gene expression results for placental cell type markers

Cell type	***Gene***	***Control v Depressed***		***Male v Female***	
		L2FC	***FDR***	***L2FC***	***FDR***
Trophoblast (TB)	*KRT7*	0.181	0.247	0.175	0.449
	KRT8	0.190	0.290	0.211	0.515
	GCM1	0.019	0.937	0.126	0.612
	CYP19A1	0.322	0.132	0.047	0.937
	ERVW-1	-0.33	0.062	-0.04	0.911
	FLT1	-0.12	0.792	-0.011	0.994
Extra villous trophoblast (EVT)	*ADAM12*	0.077	0.784	0.201	0.574
	DIO2	-0.36	0.723	-0.743	0.551
	TAC3	0.151	0.950	0.115	0.982
	HLA-G	0.524	0.710	-0.636	0.766
	PSG2	0.384	0.349	0.331	0.600
	ITGA5	0.160	0.366	-0.094	0.761
	PSG5	0.227	0.420	0.036	0.962
	MCAM	0.092	0.860	-0.191	0.774
	MMP2	-0.174	0.638	-0.120	0.844
	CGB5	-0.649	0.468	1.155	0.166
Cytotrophoblast (CTB)	*SEPP1*	0.052	0.854	-0.063	0.881
	ERVFRD-1	-0.33	0.136	-0.226	0.509
	TEAD4	>-0.001	0.999	-0.155	0.655
Syncytiotrophoblast (SYT)	*CSH2*	0.211	0.516	0.011	0.992
	CSHL1	0.083	0.791	0.156	0.717
	GH2	0.173	0.560	0.033	0.966
	CGA	0.076	0.863	-0.308	0.509
	CGB3	-0.190	0.859	-0.876	0.389
	CGB5	-0.649	0.468	-1.155	0.166
	PSG4	-0.156	0.785	0.535	0.411

[1] L2FC: Log2 Fold Change

[2] FDR: Adjusted *p* values

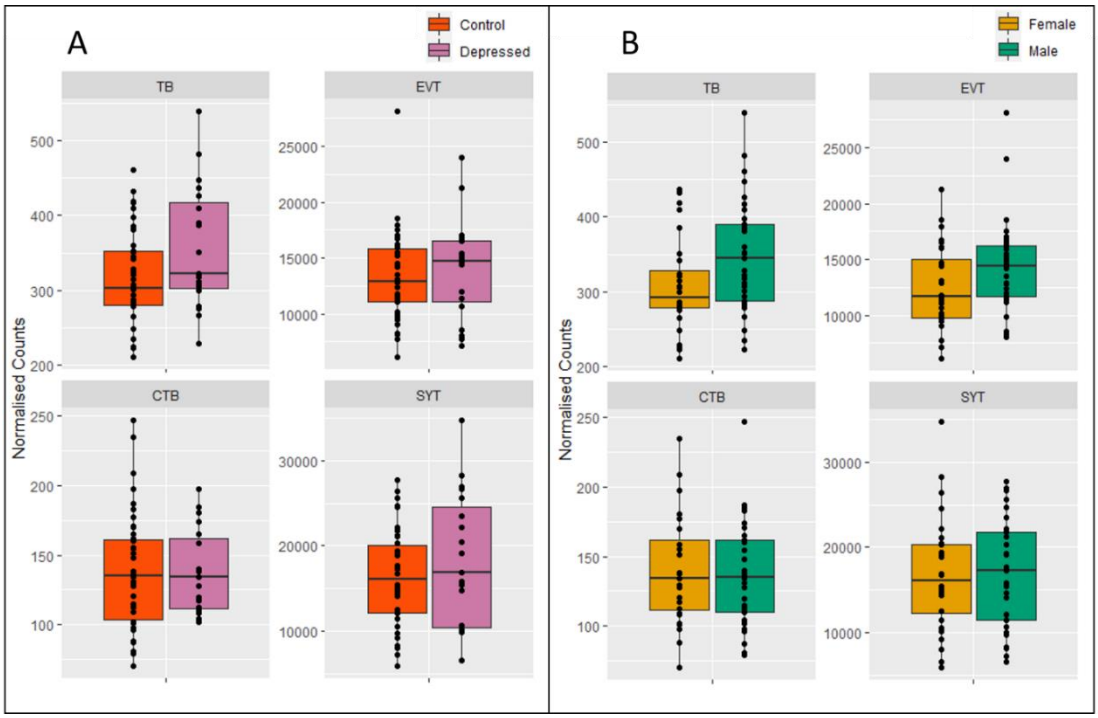

***Figure 4.3: Normalised counts of cell type markers**. **A**: Control v Depressed analysis, n= 42v19. **B**: Male v Female analysis, n= 33v28. TB-Trophoblast (KRT7 gene); EVT-Extra villous trophoblast (ADAM12); CTB-Cytotrophoblast (SEPP1); SYT-Syncytiotrophoblast (CSH2). Lower and upper box boundaries 25th and 75th percentiles respectively. Line inside box is median. Error bars represent 1.5* IQR from the edges of box.*

4.3.2.5 Differential gene expression

Differential gene expression was analysed in conjunction with depression in two ways. Firstly samples were split into groups and examined categorically (EPDS< 13 vs ≥ 13). This approach identified 141 DEGs (42 up, 99 down). Gene expression was then examined continuously across the spectrum of EPDS values. This approach identified 304 DEGs (102 up, 202 down) (Table 4.6, Table 4.7 and Figure 4.4.A/B). Absolute L2FCs in the categorical analysis were substantially higher, as L2FC values for continuous analyses are more akin to a linear regression; expression resulting from one unit of change in EPDS. 67% of DEGs in the categorical analysis were also significant in the continuous analysis, and increasing the categorical FDR value to 0.1 and 0.15, 80% and 95% respectively of the DEGs in the continuous were also significant in the categorical. Identification of essentially the same genes by the two approaches provided the validation that the most significant gene changes were identified. Further analysis was performed on the DEGs identified by the categorical approach to maximise the L2FCs.

Table 4.6: Number of differentially expressed genes

Dependent variable	***Number of significant DEGs***		
	All samples	***Male***	***Female***
Depression	141	2	0
EPDS score	304	6	0

[1] Confounders for all samples: project, sex + smoking
[2] Confounders for male samples: project
[3] Confounders for female samples: project + parity

Table 4.7: Differentially expressed genes in all samples

Model	***DEGs***	***Log2FC***	***DEGs***	***Log2FC***	***DEGs***	***Log2FC***	***DEGs***	***Log2FC***	***DEGs***	***Log2FC***
All	KCNE1	1.477	HSBP1	0.284	AAK1	-0.346	MROH1	-0.461	SLC25A34	-0.693
	RF00100	1.087	ATP5H	0.279	ZNF841	-0.347	PRSS16	-0.467	ATG9B	-0.696
	RN7SKP48	1.075	CAPZA2	0.279	RBM25	-0.349	SRRM1	-0.469	AC009120.6	-0.703
	RN7SKP255	0.936	ELOC	0.269	FMN1	-0.355	ATXN2L	-0.478	ATN1	-0.708
	RN7SKP9	0.788	GLUD1	0.250	RP11-414K1.3	-0.357	BCL9L	-0.479	MT-CO1	-0.716
	BST1	0.582	COMMD2	0.228	TRA2A	-0.372	CTC-358I24.1	-0.492	ST7-OT4	-0.726
	MINPP1	0.572	MAPK1	0.227	ZNF611	-0.372	WDR60	-0.495	RP5-1112D6.8	-0.735
	TMEM99	0.558	INTS2	0.222	RAB11B	-0.378	BRD4	-0.498	MT-CO2	-0.793
	SCPEP1	0.546	FBXO21	0.222	DXO	-0.380	COL28A1	-0.504	RP11-274B21.2	-0.802
	HSPA1B	0.536	PPP2CB	0.219	RALGPS1	-0.381	BCORL1	-0.509	RP11-192H23.4	-0.808
	RCAN1	0.502	ACOX1	0.204	ANP32B	-0.386	ZNF662	-0.525	AC005301.9	-0.835
	ARPC5	0.464	NFKB1	0.202	ZNF586	-0.386	KDM6B	-0.534	RP11-274B21.4	-0.838
	TMEM70	0.456	ZKSCAN1	-0.208	RERE	-0.388	ENOSF1	-0.542	AC097500.2	-0.845
	CLDN4	0.417	NEMF	-0.230	TFAP2A	-0.393	SRRM2	-0.545	RP11-421E14.2	-0.849
	PSAP	0.393	PSPC1	-0.258	KMT2B	-0.393	GET4	-0.557	LINC00997	-0.874
	TMEM14C	0.365	NFATC2IP	-0.270	ZNF432	-0.395	ATXN7L2	-0.558	RP11-429G19.3	-0.894
	NDUFA8	0.363	PRCC	-0.275	EP400	-0.397	PRR12	-0.568	RP11-794G24.1	-0.902
	NPC2	0.357	TCF25	-0.276	GATA3	-0.404	XXbac-BPG283O16.9	-0.578	RP1-47A17.1	-0.926
	MMADHC	0.327	HSPBAP1	-0.277	KMT2E	-0.405	AP000689.8	-0.579	RP11-312P12.2	-0.939
	BCAS2	0.325	NXF1	-0.280	SERINC2	-0.410	RP11-83A24.2	-0.580	RP11-380G5.3	-0.953
	AVEN	0.316	PCNX4	-0.297	CTD-2270P14.1	-0.423	TMC1	-0.593	LL22NC03-2H8.5	-1.638
	DYNC1LI1	0.315	POM121	-0.297	NPRL3	-0.431	RP13-507I23.1	-0.614		
	GPN1	0.312	SYT7	-0.300	PHF12	-0.435	AC000123.2	-0.621		
	SLC25A11	0.306	NHSL1	-0.304	PPP1R13L	-0.439	GLTSCR1	-0.640		
	ERH	0.305	SETD5	-0.307	CNOT3	-0.446	ZNF385A	-0.643		
	PEX5	0.304	CARS2	-0.313	SHANK2	-0.448	MIR34AHG	-0.654		
	PLPP3	0.299	MAST4	-0.313	ATXN2	-0.451	MBD6	-0.671		
	KEAP1	0.298	DMTF1	-0.315	PER1	-0.451	RP11-773D16.1	-0.674		
	RECQL	0.289	ANKRD11	-0.320	POM121C	-0.453	RP11-468H14.2	-0.682		
	C19orf12	0.285	SAMD4B	-0.341	PPP1R3E	-0.457	EGFEM1P	-0.691		

[1] N= 61

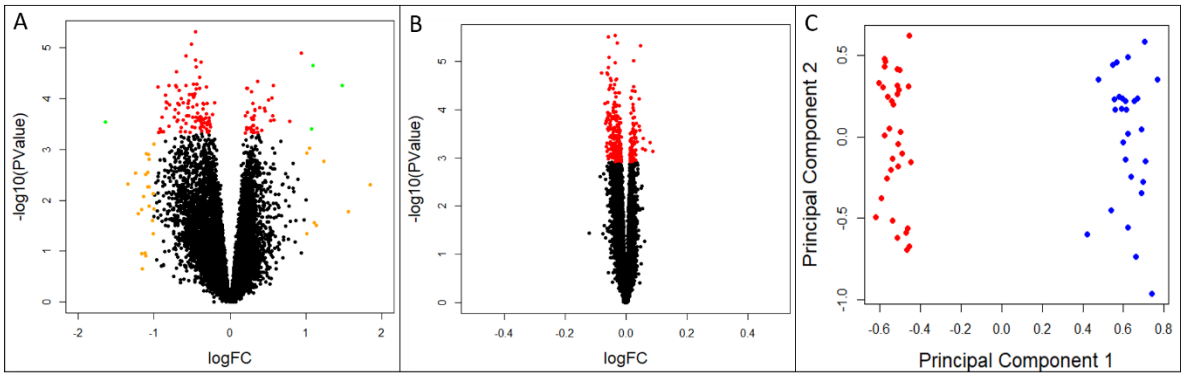

Figure 4.4: Differential gene expression. ***A*** *and* ***B****: Volcano plots.* ***A****: Control vs depressed (EPDS< 13 v EPDS≥ 13) analysis, n= 42 vs 19.* ***B****: Continuous EPDS DEG analysis, n= 61. Red-p< 0.05; orange-log2FC> 1; green-p< 0.005 + log2FC >1.* ***C****: Principal component analysis of normalised counts, red-male, blue-female.*

4.3.2.6 Gene ontology analysis

Gene ontology (GO) analysis was first performed for all the samples to understand the significance of the placental DEGs. Biological processes (BP) (n= 81) were enriched more so over cellular component (CC) (n= 40) and molecular function (MF) ontologies (n= 18). Within significantly upregulated genes, BP ontologies involving metabolic processes and stress responses were enriched. Among BP ontologies of down regulated genes metabolic process ontologies were also enriched as well as ontologies involving epigenetic regulation such as ‘chromatin organisation’, ‘histone modification’ and ‘chromosome organisation’ (Figure 4.5).

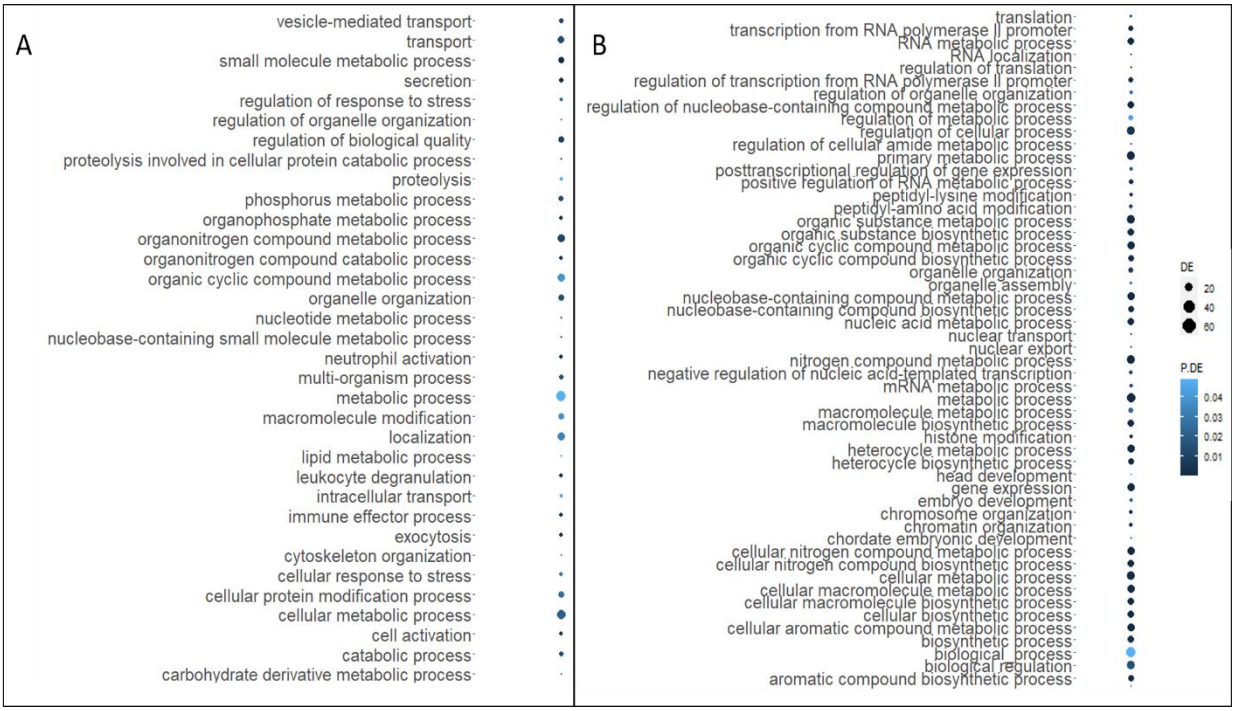

Figure 4.5: Gene ontology analysis. *Gene ontology analysis of significant differentially expressed genes in the combined RNAseq dataset (n= 61). Colour represents p value for over-representation of the GO term in the set. Size of dot represents number of genes in ontology present in significant genes.* ***A****: Biological Processes ontologies enriched in upregulated genes.* ***B****: Biological processes ontologies enriched in downregulated genes.*

4.3.2.7 Gene set enrichment analysis

To further functionally characterise the altered environment in the placenta a GSEA was performed on the entire dataset. A ranked gene list of all genes was generated according to signed *p* values and used as the input file for GSEA to identify functionally enriched pathways in the Hallmark gene set. Pathways significantly enriched included those involved in inflammation such as 'oxidative phosphorylation', 'interferon gamma response', 'inflammatory response', 'complement' and 'apoptosis' (Figure 4.6). Markers of endoplasmic reticulum (ER) stress 'hypoxia' and 'unfolded protein response' were also significantly enriched.

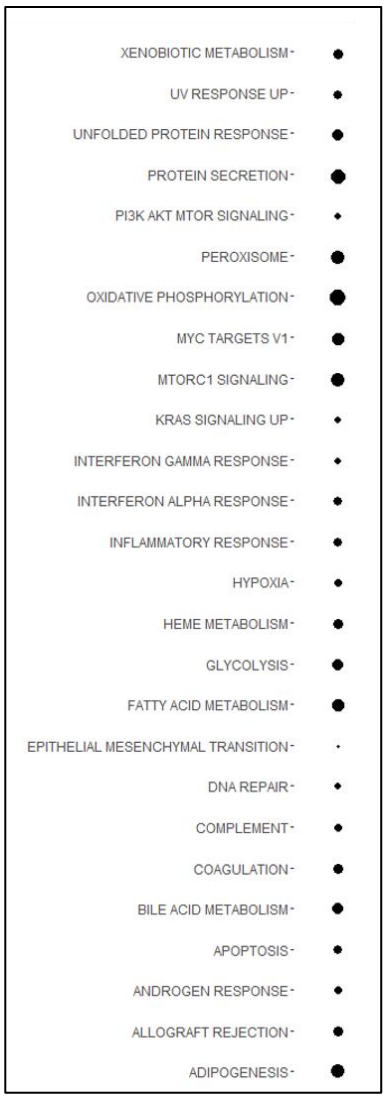

Figure 4.6: Gene set enrichment analysis*. Gene set enrichment of pathways in the Hallmark gene set of all samples in the combined dataset (n= 61). Size of dot represents normalised enrichment score.*

4.3.2.8 Analysis of male and female data separately

When the PCA plot of the count data was generated, fetal sex was the clear grouping factor (Figure 4.4.C). Therefore, the DEG analyses was repeated separating by fetal sex. There were no DEGs between female placenta exposed to depression and controls, and only two DEGs in male placentas (*KCNE1* and *MGLL*) (Table 4.6). *KCNE1* is involved in regulating potassium channels and *MGLL* catalyses the production of free fatty acids and glycerol from monoacylglycerides.

4.3.2.9 Sensitivity analysis

4.3.2.9.1 DEG analysis

To ask whether SSRIs were obscuring the identification of gene changes associated with depression, a sensitivity approach was used excluding samples from women prescribed SSRI treatment during

pregnancy. When SSRI-exposed samples were excluded and all samples were re-analysed, 152 DEGs were identified (Table 4.8). Of these, 95 were genes found in the original analysis when SSRI-exposed samples were included. When increasing the FDR to 0.1, 100% of genes identified in the initial analyses were also identified in the sensitivity analysis. When the samples were separated by fetal sex, as before there were no significant DEGs in the female analyses. However, within the female samples, only one participant was prescribed SSRIs during her pregnancy, i.e. only one sample was removed. In the male only analysis, 89 DEGs were identified (Table 4.9). This was in contrast to the initial analysis of all samples where only 2 DEGs were identified. Within the 89 DEGs, *NFKB1* as well as *DNMT3A* and the two genes encoding syncytin-1 and 2 (*ERVW-1* and *ERVFD-1* respectively) were altered.

Table 4.8: Differentially expressed genes in all samples within the sensitivity analysis

Model	***DEGs***	***Log2FC***	***DEGs***	***Log2FC***	***DEGs***	***Log2FC***	***DEGs***	***Log2FC***	***DEGs***	***Log2FC***
All	*KCNE1*	1.296	*TJAP1*	-0.247	*FNBP4*	-0.412	*CTD-2270P14.1*	-0.529	*GLTSCR1*	-0.709
	RF00100	1.100	*DCAF8*	-0.269	*ZNF611*	-0.416	*BCORL1*	-0.531	*STRADA*	-0.714
	RN7SKP255	0.936	*PCNT*	-0.270	*FMN1*	-0.422	*WDR60*	-0.535	*ATN1*	-0.725
	RN7SKP9	0.790	*DNMT3A*	-0.283	*DXO*	-0.423	*MROH1*	-0.538	*SLC25A34*	-0.731
	BST1	0.608	*PRCC*	-0.287	*CH17-472G23.4*	-0.425	*ENOSF1*	-0.555	*ST7-OT4*	-0.735
	SCPEP1	0.608	*NXF1*	-0.291	*SHANK2*	-0.425	*CCDC57*	-0.571	*RP11-574K11.20*	-0.763
	TMEM99	0.593	*PDE8A*	-0.292	*HCG18*	-0.426	*SRRM2*	-0.572	*C6orf163*	-0.789
	MINPP1	0.532	*MAST4*	-0.312	*KMT2B*	-0.434	*KDM6B*	-0.575	*AC009120.6*	-0.794
	RCAN1	0.521	*TCF25*	-0.313	*POM121C*	-0.434	*COL28A1*	-0.582	*MIR34AHG*	-0.804
	BTN3A3	0.426	*SAFB2*	-0.317	*LZTS3*	-0.436	*RP11-83A24.2*	-0.585	*ATG9B*	-0.818
	SERPINH1	0.398	*NFATC2IP*	-0.318	*ANKMY1*	-0.446	*FTX*	-0.598	*RP11-236F9.4*	-0.830
	PSAP	0.383	*ZNF248*	-0.320	*CNOT3*	-0.455	*AP000689.8*	-0.599	*RP11-274B21.2*	-0.831
	BCAS2	0.368	*CCNL2*	-0.327	*PHF12*	-0.459	*RP5-1159O4.1*	-0.602	*EGFEM1P*	-0.843
	TMEM14C	0.361	*TRAPPC9*	-0.328	*DIP2A*	-0.460	*ATXN7L2*	-0.605	*AC005301.9*	-0.844
	MMADHC	0.345	*DMTF1*	-0.333	*PPP1R3E*	-0.460	*GET4*	-0.610	*RP5-882C2.2*	-0.888
	DYNC1LI1	0.324	*ANKRD11*	-0.336	*SRCAP*	-0.462	*XXbac-BPG283O16.9*	-0.618	*RP11-421E14.2*	-0.908
	ERH	0.323	*ZSCAN30*	-0.339	*SRRM1*	-0.462	*SRCIN1*	-0.620	*TMEM247*	-0.912
	UROD	0.323	*CARS2*	-0.346	*ATXN2*	-0.465	*PKD1P6*	-0.623	*AP000347.2*	-0.932
	PLPP3	0.323	*GIGYF1*	-0.356	*PPP1R13L*	-0.473	*PRR12*	-0.647	*RP11-380G5.3*	-0.949
	RECQL	0.313	*SAMD4B*	-0.356	*MLLT6*	-0.475	*RP13-507I23.1*	-0.648	*RN7SL141P*	-0.989
	PEX5	0.301	*SPIRE2*	-0.363	*BCL9L*	-0.476	*CTD-2547E10.2*	-0.652	*RP11-312P12.2*	-1.005
	CAPZA2	0.295	*AAK1*	-0.365	*MICAL3*	-0.480	*RP11-705C15.2*	-0.658	*RP1-47A17.1*	-1.022
	GLUD1	0.279	*PCSK7*	-0.377	*PER1*	-0.489	*SGK494*	-0.662	*RP11-192H23.4*	-1.087
	MAPK1	0.267	*ZNF841*	-0.378	*TNS4*	-0.497	*RP11-773D16.1*	-0.664	*RP4-723E3*.1	-1.534
	NFKB1	0.250	*MED15*	-0.381	*WDR90*	-0.497	*TMEM155*	-0.676		
	FBXO21	0.246	*RP11-414K1.3*	-0.382	*MZF1*	-0.513	*LENG8*	-0.679		
	PPP2CB	0.242	*AC024560.3*	-0.382	*CTC-358I24.1*	-0.516	*MT-CO1*	-0.681		
	COMMD2	0.231	*RBM25*	-0.385	*BRD4*	-0.521	*ZNF385A*	-0.682		
	ACOX1	0.214	*SLC2A11*	-0.389	*MNT*	-0.522	*CTD-2574D22.4*	-0.687		
	ZKSCAN1	-0.224	*RALGPS1*	-0.390	*ATXN2L*	-0.522	*RP3-476K8.3*	-0.699		
	ACBD6	-0.226	*KMT2E*	-0.395	*DNAH1*	-0.523	*RP13-204A15.3*	-0.701		
	NCBP3	-0.231	*EP400*	-0.411	*ZNF432*	-0.528	*MBD6*	-0.707		

[1] N= 56

Table 4.9: Differentially expressed genes in male samples within the sensitivity analysis

Model	***DEGs***	***Log2FC***	***DEGs***	***Log2FC***	***DEGs***	***Log2FC***
Male	*CTC-205M6.2*	1.846	*ZKSCAN1*	-0.331	*ATXN2*	-0.709
	KCNE1	1.837	*ACBD6*	-0.335	*SRRM1*	-0.740
	RF00100	1.508	*KIAA2026*	-0.350	*BCORL1*	-0.743
	RN7SKP255	1.229	*DNMT3A*	-0.383	*ERVFRD-1*	-0.776
	TM4SF18	1.047	*NEMF*	-0.390	*KMO*	-0.822
	TIPARP	0.678	*SYNJ1*	-0.422	*FRA10AC1*	-0.825
	TMEM147	0.663	*MAST4*	-0.431	*PKD1P6*	-0.831
	TMEM70	0.634	*SRSF11*	-0.469	*XXbac-BPG283O16.9*	-0.844
	RPS29	0.628	*ZNF354B*	-0.469	*CTD-2547E10.2*	-0.913
	NPC2	0.610	*ZNF226*	-0.474	*ZNF385A*	-0.916
	TMEM208	0.603	*NECAB3*	-0.508	*LINC00470*	-0.930
	RPS12	0.570	*MYO6*	-0.548	*ATN1*	-1.045
	SLC31A2	0.551	*SMARCE1*	-0.579	*ANKRD20A5P*	-1.053
	ESYT1	0.550	*DDX55*	-0.583	*SLC25A34*	-1.054
	RRAGA	0.546	*RBM25*	-0.583	*RP11-274B21.2*	-1.059
	HSPE1	0.545	*FNBP4*	-0.588	*RP11-454L1.2*	-1.062
	PLA2G15	0.535	*IGSF5*	-0.607	*RP11-255C15.1*	-1.077
	EXOSC6	0.514	*TRA2A*	-0.609	*GS1-124K5.14*	-1.107
	MGLL	0.514	*ERVW-1*	-0.620	*RP11-727A23.5*	-1.110
	FSTL1	0.508	*RP11-414K1.3*	-0.626	*ATG9B*	-1.127
	PEX5	0.461	*KMT2B*	-0.629	*RP11-192H23.4*	-1.215
	RECQL	0.443	*ZNF611*	-0.633	*RP11-142L16.2*	-1.302
	ERH	0.439	*PPP1R3E*	-0.636	*C12orf56*	-1.351
	SYNGR2	0.429	*CNOT3*	-0.641	*PCLO*	-1.361
	STOML2	0.422	*ZNF432*	-0.652	*RN7SL141P*	-1.429
	CAPZA2	0.398	*PHF12*	-0.655	*RP11-421E14.2*	-1.467
	MAPK1	0.383	*CBWD1*	-0.656	*RP11-267N12.3*	-1.504
	PPP2CB	0.361	*ZNF471*	-0.677	*RP11-757O6.2*	-1.606
	ICMT	0.360	*SRCAP*	-0.699	*LL22NC03-2H8.5*	-2.582
	NFKB1	0.351	*NSRP1*	-0.702		

[1] N= 29

4.3.2.9.2 GO analysis

Gene ontology analysis was repeated for the DEGs when all placentas were analysed and for just the male placentas (Figure 4.7). Similar ontologies were enriched in all samples compared to original results including an enrichment of metabolic processes and epigenetic modifications. Immune ontologies 'immune system process' and 'leukocyte mediated immunity' were also enriched within upregulated genes. In the male analysis numerous metabolic pathways were enriched, as were ontologies relating to stress response.

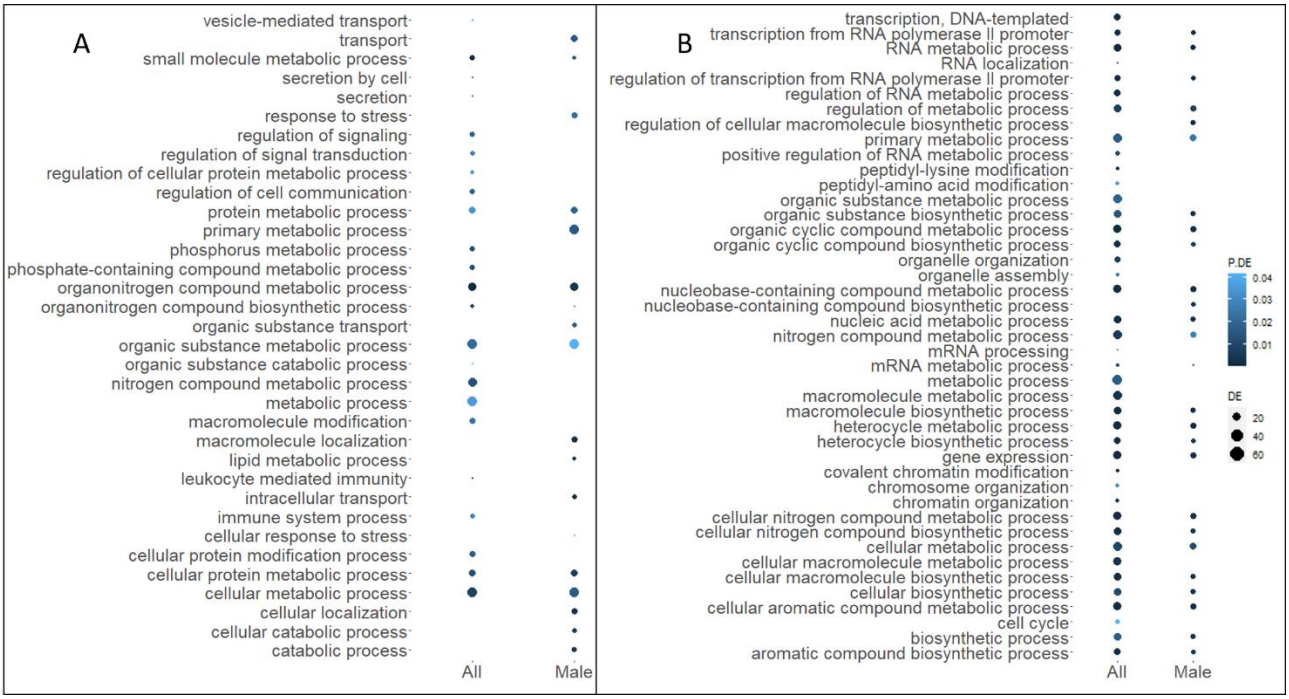

Figure 4.7: Gene ontology analysis*. Gene ontology analysis of significant differentially expressed genes in the sensitivity analysis, both all samples (n= 56) and male samples (n= 18). Colour represents p value for over-representation of the GO term in the set. Size of dot represents number of genes in ontology present in significant genes.* ***A****: Ontologies enriched in upregulated genes.* ***B****: Ontologies enriched in downregulated genes.*

Due to the indistinct nature of gene ontology analyses, single genes are often assigned to multiple terms. To illustrate this a circos plot was generated for the significant genes in the male samples to visualise the relationship between genes and GO terms, with genes ordered by absolute L2FC (Figure 4.8).

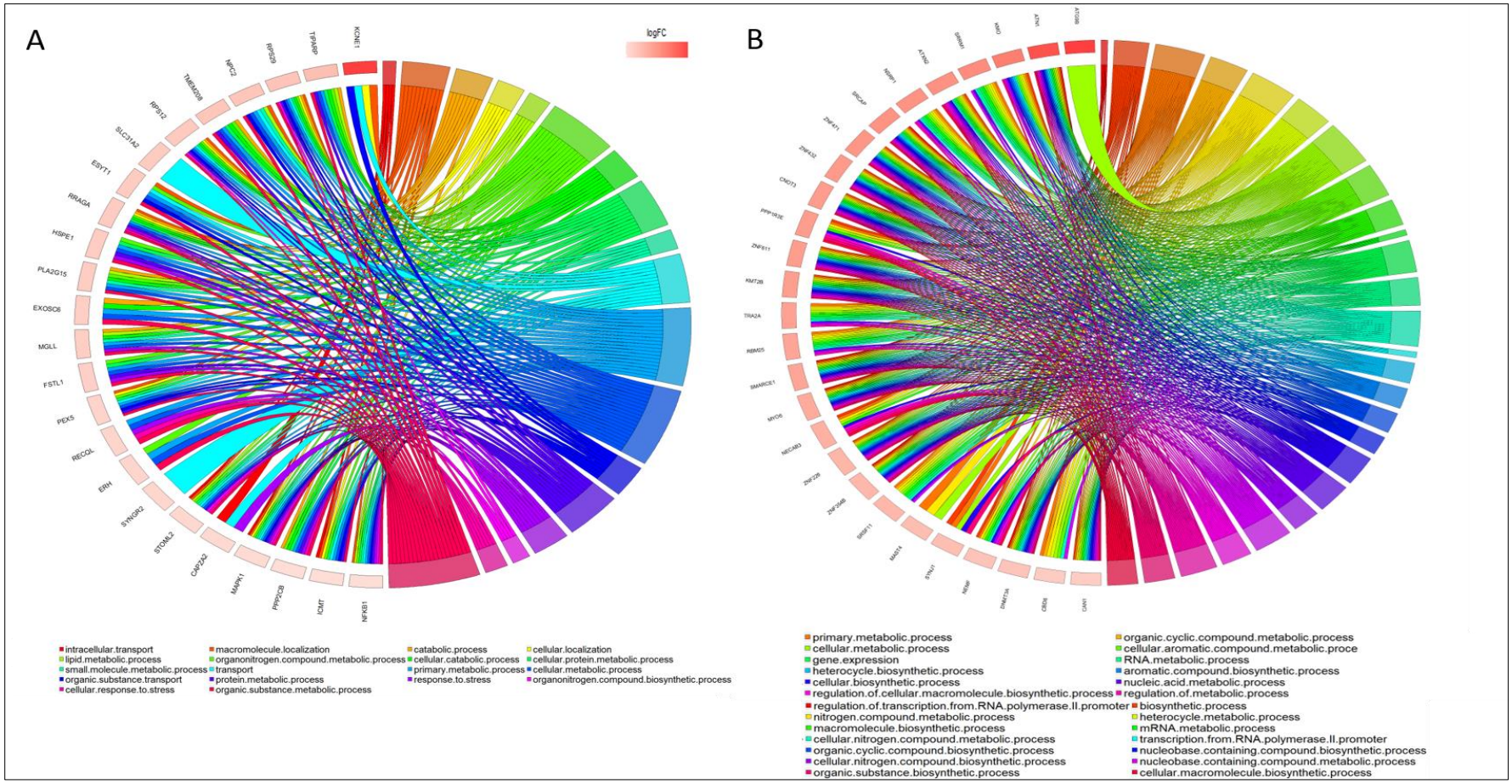

Figure 4.8: Circos plot to indicate the relationship between genes and GO terms in male samples excluding those prescribed SSRIs in pregnancy. Signifcant differentially expressed genes presented on left and their log2 fold changes of gene expression indicated as coloured squares. Coloured lines represent involvement in GO term. ***A****: Upregulated genes,* ***B****: Down regulated genes*

4.3.2.9.3 GSEA analysis

GSEA was rerun on all the sensitivity analyses data to gain a more precise biological understanding of pathways in the placenta associated with prenatal depression (Figure 4.9). Within all samples, enriched pathways were very similar to the original analyses, with 'IL6 JAK STAT3 signalling', 'IL2 STAT5 signalling', 'E2F targets' and 'cholesterol homeostasis' additionally enriched. There was also substantial overlap between pathways enriched in all placentas and only male placentas in the sensitivity analyses including infection and immunity related pathways. Male placentas were additionally enriched in 'TNFα signalling via NFKB', 'MYC targets V2', 'estrogen response late', 'DNA repair', 'angiogenesis' and 'androgen response'. Despite the lack of DEGs, female placentas were analysed through GSEA to identify subtle changes that may not have reached significance in differential gene expression analysis. A small number of pathways were significantly enriched across all comparisons, including unfolded protein response, protein secretion and oxidative phosphorylation. While almost all of the enriched pathways in the female placentas were also enriched in the other comparisons, a much smaller total number were significantly enriched and only the 'G2M' pathway was uniquely enriched in female placenta.

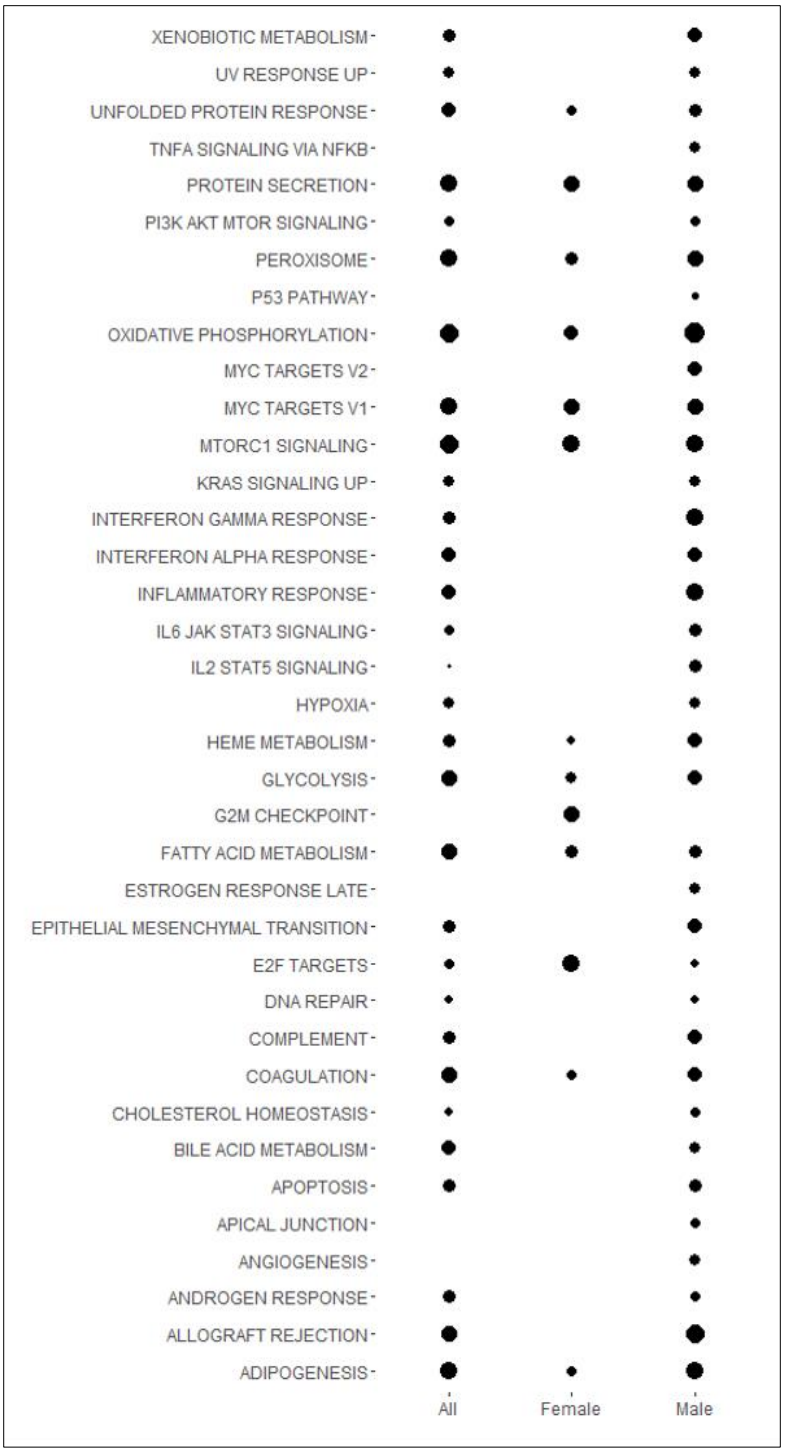

Figure 4.9: Gene set enrichment analysis. *Gene set enrichment of pathways in the Hallmark gene set of within the sensitivity analysis. Including all samples (n= 56), female samples (n= 19) and male samples (n= 18). Size of dot represents normalised enrichment score.*

4.3.2.10 Weighted gene co-expression network

To identify key genomic modules in placentas exposed to prenatal depression, a weighted gene co-expression network was performed for samples from women who had boys, excluding those

prescribed SSRIs during pregnancy. This analysis was chosen as the confounding effects of females and SSRIs were not included. The network comprised of 6 modules, with sizes ranging from 61 (M6) to 310 (M1) genes (Figure 4.10). Two modules (M1 and M6) were enriched in depressed samples, wheras the remaining four were enriched in the control samples. Module 1 was enriched in 'positive regulation of MHC class II biosynthetic process' and 'regulation of ribosome biogenesis'. Module 6 was enriched in ten ontologies including 'oxidative phosphorylation', consistent with previous GSEA analysis. In addition 'regulation of hormone metabolic process' and 'intrinsic apoptotic signalling pathway by p53 class mediator' onotlogies were also enriched in the depressed group. GSEA was performed on modules 1 and 6, with 'epithelial mesenchymal transition' enriched in the former and inflammation-related pathways 'interferon gamma response' and 'coagulation' in the latter.

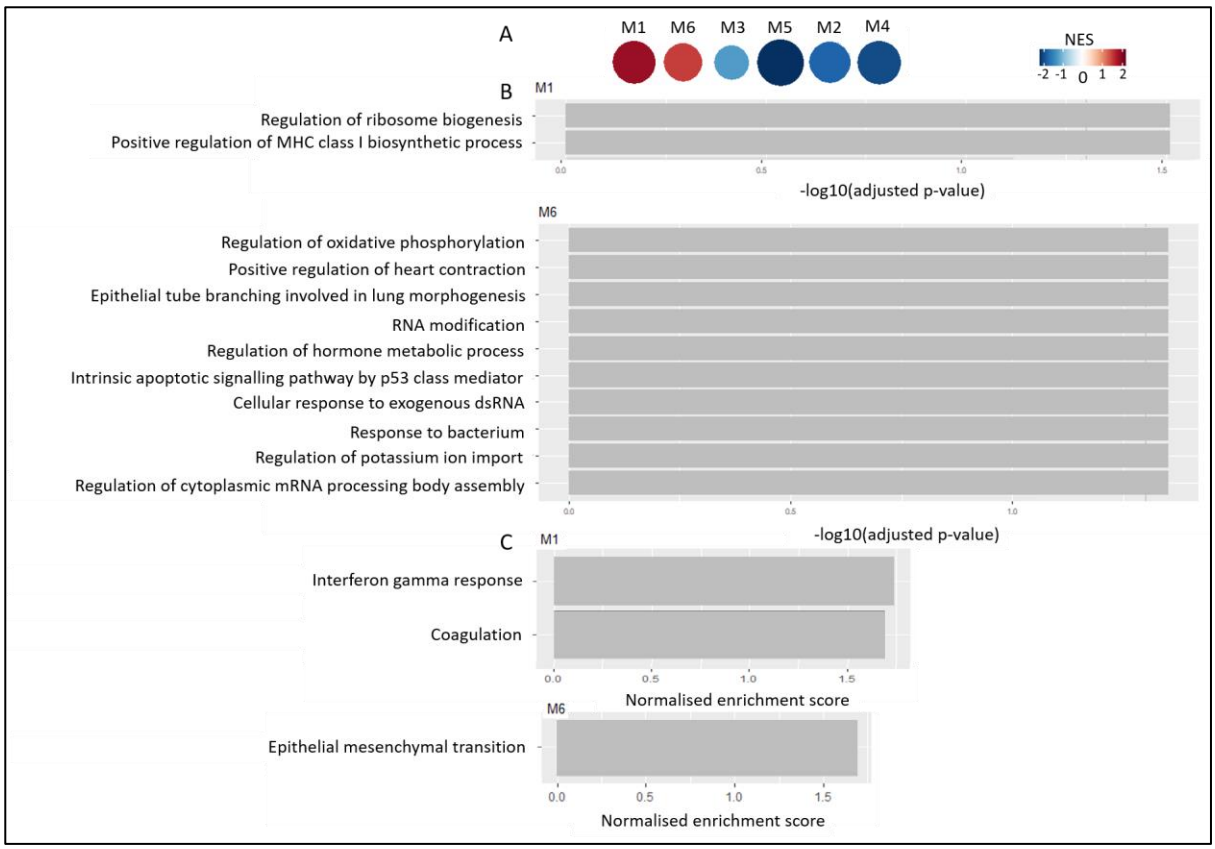

Figure 4.10: CEMiTool analysis. CEMiTool analysis of male samples from the sensitivity analysis (n= 18). ***A****: Module enrichment in depressed group, only modules 1 and 6 were enriched for GSEA. Size and colour or the points represents the normalised enrichment score (NES)* ***B****: Significantly enriched gene ontologies for modules 1 and 6.* ***C****: Significantly enriched pathways in the Hallmark gene set for modules 1 and 6.*

4.3.3 Summary of chapter results

Table 4.10: Summary of Chapter 4

	All placenta	***Male placenta***	***Female placenta***
Original RNAseq data (n= 37)			
DEGs	0	0	0
Combined RNAseq data (n= 61)			
DEGs	141	2	0
Sensitivity analysis on combined RNAseq data (n= 56)			
DEGs	152	89	0
GO	64	44	0
GSEA	22	34	8

4.4 Discussion

This analysis set out to determine genome-wide transcriptional changes in term placenta exposed to maternally reported symptoms of depression, with a focus on fetal sex-specific responses. A key finding from this work was the association between prenatal depression symptoms and significant placental gene changes. Remarkably, gene changes were found predominantly in male placenta exposed to depression, but only when samples from participants prescribed SSRIs were removed from the analysis. 89 differentially expressed genes were identified in male placentas from mothers reporting prenatal depression symptoms and no DEGs were identified in female placentas.

Through the processes of determining potential confounders to include in the models of the main analyses, a number of genes were found to be differentially expressed between "control" male and female placentas. Naturally occurring differences between male and female placental gene expression in humans (Cvitic *et al.* 2013; Gong *et al.* 2018; Gonzalez *et al.* 2018) and rodents (O'Connell *et al.* 2013; Kalisch-Smith *et al.* 2017) have been reported and it is now widely accepted that the male and female placenta differ even under normal conditions. In this study, gene ontology analysis of the DEGs in the control samples revealed that a number of genes were related to epigenetic processes. A key difference between males and females is the presence of sex-chromosomes. While not all the identified DEGs were physically linked to these chromosomes, female cells inactivate one copy of their X-chromosome which requires extensive epigenetic processes (Yang *et al.* 2011). Differences in epigenetic processes may contribute to DEGs at autosomal loci. These inherent epigenetic differences may also underlie differences in response to prenatal depression seen here, and to other stressors described in the literature (Gabory *et al.* 2012; Mina *et al.* 2015; Rosenfeld 2015; Bale 2016). Genes relating to different metabolic processes were also altered, which given male fetuses and placentas are on average larger than females (Rosenfeld 2015), may correlate with an increased energy expenditure.

When all control and depressed samples were compared over 140 DEGs were identified. Among those expressed at a higher level in the depressed group were a number of inflammatory related genes. Significantly higher in the depressed group was *NFKB1,* a transcription factor activated by stimuli such as cytokines and oxidant free radicals, which itself activates further immune pathways (Lingappan 2019). *RCAN1* which acts on calcineurin an important inflammatory response regulator (Junkins *et al.* 2013), was also overexpressed. Moreover there were signs of increased mitochondrial metabolism as expression of some mitochondrial genes were increased and mitochondrial ontologies within the cellular component class of the GO analysis were enriched. Increased levels of reactive oxygen species (ROS) can be a by-product of stress and increased metabolic drive (Murphy 2009), and results from the DEG analysis of depression-exposed placentas correspond with this pathway. HSP70 is a stress-induced protein and can be induced by increased ROS levels (Jacquier-Sarlin *et al.* 1994). Encoded by three paralogs; *HSPA1A*, *HSPA1L*, and *HSPA1B* (Dvorakova *et al.* 2017), the latter of which was significantly higher in the depressed samples, and the other two significant at a *p* value< 0.05 but not an FDR *p*< 0.05. Furthermore *PEX5*, which helps clear ROS was also increased in this group, indicative of increased ROS levels (Wang and Subramani 2017). In addition, pathways in the GSEA relating to the immune response and inflammation such as 'interferon gamma response', 'inflammatory response' and 'complement' were also enriched. Although underlying mechanisms are not well understood, depression and inflammation appear closely linked (Raison and Miller 2013; Dahl *et al.* 2014; Amodeo *et al.* 2017). Together, these data suggest that placentas from mothers who self-report prenatal depression symptoms have a stressed placental environment.

PER1 expression was significantly lower in placentas from the depressed group. *PER1* is a circadian clock gene involved in mammalian circadian rhythm (Spencer *et al.* 2013). In humans the master circadian clock is found in the suprachiasmatic nucleus (SCN) of the hypothalamus and acts upon peripheral oscillators in other tissues through influence on the HPA axis (Rusak and Zucker 1979; Waddell *et al.* 2012). The placenta has been suggested to act as a peripheral oscillator as placental clock gene expression has been shown to vary throughout the day in humans and rodents (Pérez *et al.* 2014). Disruptions in peripheral oscillators are associated with diseases such as cancer and diabetes, as well as psychological disorders (Bildt and Michélsen 2002; Horikawa *et al.* 2005; Conlon *et al.* 2007). Furthermore pregnancy is known to alter maternal circadian rhythm and clock gene expression (Cousins *et al.* 1983; Schrader *et al.* 2011). Knockout *Per1* mouse models are associated with increased stress and anxiety (Spencer *et al.* 2013) and decreased placental *PER1* has been putatively associated with increased anxiety scores in humans (Sheehan *et al.* 2018). Although the present study focussed on prenatal depression, as previously discussed in Chapter 1, depression and anxiety are often comorbid. 17 out of the 19 women reporting depression symptoms in this study also reported high anxiety symptoms. Together, these data suggests a potential link between prenatal mental health and impaired circadian rhythm specifically in the placenta that could be explored further.

In addition to identifying gene changes in the placenta associated with depression symptoms, a second key finding from this study was in relation to SSRIs. In the initial analysis of male placenta, only 2 DEGs were identified between controls (n= 22) and depressed (n= 11) groups. However, when samples from pregnancies in which participants prescribed SSRIs were removed, 89 DEGs were identified (n= 22 male controls vs 7 male depressed). The sensitivity analyses revealed a similar number of DEGs in a comparison of all samples (n= 42 control vs 14 depressed; DEGs= 152). These data suggest that SSRIs mask the relationship between prenatal depression and the placenta, and removing them from the analysis uncovers the association. Given SSRIs can cross the placenta (Hostetter *et al.* 2000), and exposure to other chemicals in pregnancy can alter placental transcription (Winterbottom *et al.* 2019), it is plausible that the medication may have an effect. Given the increased number of DEGs when only untreated depression was analysed, SSRIs may prevent some of the alterations induced by depression, despite women reporting active symptoms with potential beneficial outcomes for the fetus. A similar finding has recently been reported in a qPCR analysis on the Swedish Biology, Affect, Stress, Imaging, Cognition (BASIC) cohort, although significant results did not pass multiple correction (Edvinsson *et al.* 2019). In this study the genes analysed were chosen based on previous associations with depression. Our approach was unbiased and illustrates the importance of hypothesis generating RNAseq approaches. The protective effect of SSRIs in the present study is extremely preliminary but, if validated and also found in fetal tissues, would support the use of SSRIs in pregnancy.

Female placenta appeared to be resilient when exposed to prenatal depression symptoms in all analyses. As explored in Chapter 7, sexually dimorphic outcomes for children exposed to prenatal depression have previously been reported (Glover and Hill 2012) and, as will be reported in Chapter 7, we found a similar male-specific impact in the GiW infant study (Savory *et al.* 2020). Global transcriptome level sexually dimorphic differences in the placenta have been described in response to a number of maternal stressors such as asthma, obesity, poor maternal diet, and socio-economic adversity in human and rodent studies (Mao *et al.* 2010; Bale 2011; Dunn *et al.* 2011; Osei-Kumah *et al.* 2011; Howerton and Bale 2012; Appleton *et al.* 2013; Bronson and Bale 2014; Davis and Pfaff 2014). This study, however is the first to investigate the association between maternally reported symptoms of depression and the genome-wide transcriptome changes analysing male and female placenta separately, with striking findings for the male placenta. Why male placentas appear to respond to depression and female placentas remain relatively unaffected is unclear. Female mouse placentas have higher levels of DNA methylation in comparison to male placentas which has been suggested to be protective against potential stressors (Gallou-Kabani *et al.* 2010; Shallie and Naicker 2019). DNA methylation differences between male and female human placenta have previously been reported (Martin *et al.* 2017). It may be that these epigenetic differences explain the apparent sexually dimorphic response of the placenta to prenatal depression.

In the sensitivity analysis where SSRI samples were removed, a number of DEGs were identified in the comparison of all untreated depressed samples and the comparison of male untreated depressed samples such as *NFKB1* and *PEX5*. Additionally, there were genes that were only identified as significantly altered in the male-only analysis, one of which was *DNMT3A* which was expressed significantly lower in the depressed samples. *DNMT3A* is a *de novo* methyltransferase that adds methyl groups to previously unmethylated DNA. DNA methylation is an epigenetic regulator of gene expression (Moore *et al.* 2012) and therefore it is possible that this change in *DNMT3A* expression contributes to the altered gene expression described. Methyltransferases can be regulated by a number of factors including cytokines and growth factors such as p53 and E2F (Logan *et al.* 2013). Pathways involving these growth factors were significantly enriched in this model. Aberrant expression of *DNMTs* have been implicated in a number of outcomes including dysregulation of trophoblast cells (Logan *et al.* 2013). Two other genes significantly decreased in expression were *ERVW-1* and *ERVFD-1* which encode syncytin 1 and 2 respectively. These genes are critical for syncytiotrophoblast development and regulation, and are suggested to be important in a number of placental pathologies including pre-eclampsia (Vargas *et al.* 2009). Therefore, a mechanism involving aberrant *DNMT3A* and syncytin-1/2 levels could lead to an adverse syncytiotrophoblast environment.

Enriched pathways were also very similar between all and male untreated samples. All pathways significantly enriched in all untreated samples were also enriched in male untreated samples. A small number of pathways were specifically enriched in the untreated depressed male placenta including 'TNFα signalling by NFKB', 'p53 pathway' and 'IL6 JAK STAT3' signalling. The IL6/JAK/STAT3 pathway is a pro-inflammatory pathway often observed in chronic inflammation (Hanada and Yoshimura 2002), and in one study where mice were exposed to stress in pregnancy IL-6 was only increased in male placenta (Bronson and Bale 2014). Furthermore, in mice the IL6/JAK/STAT3 pathway has been shown to act on the spongiotrophoblast region of the placenta which is the main endocrine compartment (Creeth and John 2020), and thus may alter expression of placental hormones (Hsiao and Patterson 2011). Adequate levels of placental hormones are critical for driving physiological changes during pregnancy, and play a role in maternal adaptation and both fetal and placental development (John 2013). As described in Chapter 1, placental hormones have been shown in mice to induce maternal behaviour (Creeth *et al.* 2019) and our group has demonstrated that placental endocrine dysfunction can drive alterations in the behaviour of mouse mothers (Creeth *et al.* 2018; McNamara *et al.* 2018). Therefore, alterations to placental hormones in response to prenatal depression may mediate the relationship between maternal mental health and adverse infant outcomes. The RNAseq analysis did not identify any placental hormones that were significantly differentially expressed between the groups. However, the sample size was small (7 vs 22). As will be reported in Chapter 5 and Chapter 6, targeted approaches uncovered a relationship between placental lactogen and mental health symptoms.

A small number of significantly enriched pathways in the male untreated depressed group were also significant in the female analyses such as 'oxidative phosphorylation' and 'glycolysis'. This indicates that both male and female placenta can respond to the prenatal depression. It may be that the response in female placenta is attenuated or it is possible that there is more heterogeneity within the female samples. A larger sample size is needed to more fully understand the impact of exposure to prenatal depression on female placentas.

Through the systems biology approach of co-expression analysis it was possible to identify two modules that were significantly enriched in male placentas exposed to prenatal depression within the sensitivity analysis. Modules identified contain groups of genes highly correlated across samples and through post hoc characterisation can provide a deeper understanding of underlying processes. 'Oxidative phosphorylation' and 'intrinsic apoptotic signalling pathway by p53 class mediator' ontologies were significantly enriched in module six, mirroring results from the previous GSEA analysis. 'Regulation of hormone metabolic processes' was also enriched in male placentas exposed to prenatal depression. Again, although individual hormones were not identified among the DEGs, these data suggest the possibility that placental endocrine insufficiency is a factor in prenatal mood disorders.

Two previous studies have investigated the relationship between placental gene expression and prenatal depression using RNAseq or microarray, albeit analysing male and female placentas together. Litzky *et al.* focussed solely on imprinted gene expression and reported 89 DEGs in response to prenatal depression in their cohort (Litzky *et al.* 2018). No imprinted genes were found among the DEGs in any comparison in the current analyses. The RNAseq sample size may have been too small to pick up the fluctuations reported by Litzky *et al.* (n= 458). The second study to assess placental gene expression in relation to maternal depression used a similar number of samples to our study, and first used microarrays before validation by qPCR in a smaller group (Olivier *et al.* 2014). There was no overlap in DEGs between this study and the current analyses. This may be due to population differences, different criteria used to define depression, or potentially differences in the way in which samples were collected. Placenta collected for the GiW cohort were all from ELCS deliveries and therefore representative of placentas in pregnancy. Olivier *et al.* did not report the delivery method of participants, and thus it cannot be assumed they delivered by elective caesarean, and 48% of the participants in Litzky *et al.* delivered vaginally. Placentas delivered through ELCS and placentas that have gone through the process of labour have different transcriptomic patterns which may account for the lack of similarity between studies (Lee *et al.* 2010; Janssen *et al.* 2015).

4.4.1 Limitations

A limitation of this study is the relatively small sample sizes for the depressed groups. Furthermore, the study was not originally designed to take into account the confounding effect of SSRIs, and it was not possible to determine the direct effect of the medication on the placenta, hence the sensitivity

analysis approach was used. Also, only one mother who delivered a girl was on medication for her mood and thus little was gained by removing the one sample. A further limitation was that the RNAseq data was generated in two batches more than one year apart. Although batch was controlled for, samples were processed on two different sequencing platforms. Finally, validation of the RNAseq findings by qPCR was not undertaken given the relatively small size of the GiW cohort. Therefore, this analysis should be considered as a pilot study requiring further validation in larger cohorts.

4.4.2 Conclusions

In summary, this chapter reports on the analysis of RNAseq data of term placenta in relation to self-reported symptoms of prenatal depression. A key finding was the presence of a number of gene changes in male placenta exposed to depression in comparison to female placenta, uncovered when samples potentially exposed to SSRIs were removed. These data highlight the specific sensitivity of male placenta with pathway analysis indicating a significant stress response. Data further suggests that SSRI use in pregnancy may provide protection against the detrimental consequence of prenatal depression on the placenta, with the caveats highlighted. The lack of consistent data on SSRIs in pregnancy makes it extremely challenging for clinicians to decide whether to prescribe them in pregnancy, and for women to make the decision to take them. Replicating the biological data presented in this study will help scientifically underpin clinical decisions on treatment pathways for women with prenatal depression.

Chapter 5: Imprinted gene expression and maternal mental health

5.1 Introduction

Mouse models demonstrate that imprinted genes expressed in the placenta, including *Peg3* influence maternal behaviour during the perinatal period by regulating the development of the endocrine lineages of the placenta (Creeth and John 2020). This is consistent with our knowledge that placental hormones induce maternal behaviours in rodents enabling the mother to support her offspring in pregnancy and after birth (Grattan 2011). Our group previously reported a reduction in the expression of *PEG3* in placentas of mothers with prenatal depression in a sex-specific manner (Janssen *et al.* 2016). Expression of the placental hormone *hPL* was also decreased consistent with the findings that *Peg3* regulates placental hormones in mice (Tunster *et al.* 2018). Together, these data suggest that low placental *PEG3* might contribute to prenatal depression in human pregnancies by altering the expression of placental hormones.

PEG3 is a paternally expressed gene located in a 500 kb imprinted domain found in humans on chromosome 19q13.4 (Figure 5.1) (Kuroiwa *et al.* 1996). Within the domain there is additionally partial evidence for imprinting of *APeg3*, *Zim1-3*, *Zfp264* and *Usp29* (He and Kim 2014). While the protein coding capacity of these genes vary across placental mammals, *Peg3* is well conserved across all placental mammals highlighting its significance (He and Kim 2014). In humans *PEG3* is strongly expressed in the ovaries, hypothalamus and placenta, with expression also detected in the adrenal gland, endometrium and testis (Kim *et al.* 1997). Within human placenta, *PEG3* expression is localised to the villous cytotrophoblast, a population of proliferative cells that give rise to syncytiotrophoblast and interstitial cytotrophoblasts (Hiby *et al.* 2001).

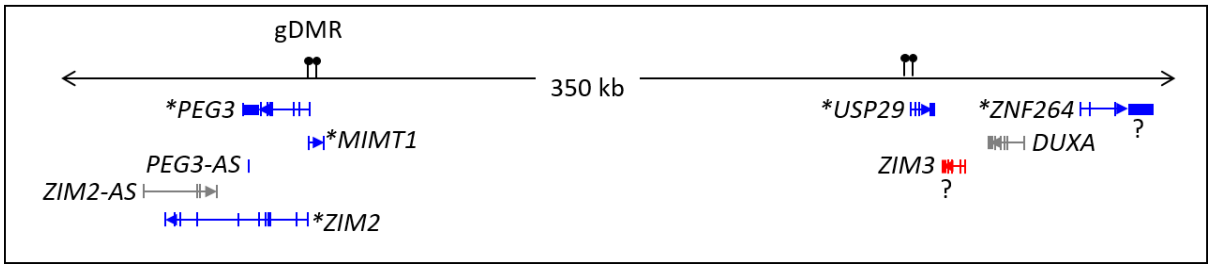

Figure 5.1: Map of human chromosome 19q13.43* PEG3 *imprinted domain. *Confirmed paternally expressed transcripts in blue with direction of transcription indicated by arrow. Predicted maternal (red) and paternal (blue) imprinted status indicated by '?'. Unknown imprinted status indicated by a grey colour. Asterisks indicate known expression in human placenta. Differentially methylated regions (DMRs) indicated by filled lollipops. Minor transcripts not shown. Adapted from NCBI Genome Data Viewer.*

Transcriptional control of *Peg3* has predominantly been studied in mice with likely multiple regulatory elements at play. A 4 kb DMR spanning the *Peg3* promoter was demonstrated to act as an

imprinting control region (ICR) and to be essential for maintaining mono-allelic expression of a number of the domain's genes (Kim *et al.* 2012). The DMR is methylated on the maternal allele resulting in mono-allelic expression from the paternal allele. The exact mechanism of this acquired allele-specific methylation is unknown, but *Dnmt3a*$^{-/-}$ and *Dnmt3b*$^{+/-}$ mice fail to acquire any methylation at the *Peg3* DMR (Hata *et al.* 2002). In mice, paternal deletion of the DMR results in complete loss of expression of *Peg3* and *Usp29*, and biallelic expression of *Zim1*/*2*. However, loss of the maternal DMR leads to a two-fold increase in *Peg3* and *Usp29* expression on the paternal allele (Kim *et al.* 2012). This indicates that the maternal allele, although silenced through methylation, is still functional and involved in a trans-allelic regulation of gene expression (Bretz and Kim 2018).

Peg3 is a transcription factor and is predicted to function in a number of contexts including as a transcriptional repressor of genes such as those involved in cellular metabolism (Thiaville *et al.* 2013; Lee *et al.* 2015). It is highly expressed in the placenta and like other imprinted genes is important in pregnancy and the perinatal period (Hiby *et al.* 2001). Mutant mouse models have suggested *Peg3* as a positive regulator of placental and fetal growth because loss of function of *Peg3* results in lighter fetuses and placenta (Li *et al.* 1999; Kim *et al.* 2013; Denizot *et al.* 2016). These lower birthweights are more pronounced in male pups (Kim *et al.* 2013). A double dosage of *Peg3* was shown to increase birthweight but only in female pups (Bretz *et al.* 2018). Within the placenta, a loss of function of *Peg3* reduces the number of spongiotrophoblast and glycogen cells (Tunster *et al.* 2018). The spongiotrophoblast is the endocrine compartment of murine placenta, and thus a reduction in the number of cells results in reduced expression of a number of placental hormones. In a separate study, Peg3 was identified as a transcriptional repressor of a subset of placental hormones (Kim *et al.* 2013). Therefore, in the placenta of mice, Peg3 can both up and down regulate placental hormone synthesis through two distinct mechanisms. The impact of loss of function of *Peg3* on the placenta has sexually dimorphic consequences with male placentas showing a greater loss of spongiotrophoblast and glycogen cells and female placenta showing few changes in placental hormone expression (Tunster *et al.* 2018). In the mouse, female placenta appear to be able to compensate for a loss of *Peg3*.

Loss of function of *Peg3* has been shown to impact maternal behaviour in two ways. When *Peg3* was originally targeted, dams with loss of function of *Peg3* displayed deficits in maternal behaviour. *Peg3* deficient dams also had a reduction in the number of oxytocin producing neurones responsible for a number roles in priming females for motherhood (Li *et al.* 1999). In a different study of *Peg3* mutant pups, dams failed to adequately prepare for motherhood as seen through impaired food intake, maternal behaviours and mammary gland development (Curley *et al.* 2004). Postnatally, mutant pups also had impaired suckling, weight gain and growth. When *Peg3* expression was instead lost in dams, remarkably similar phenotypes were observed such as impaired milk let down, maternal food intake and nest building (Curley *et al.* 2004). In one study, wild-type mouse mothers carrying loss-of-function *Peg3* pups were shown to exhibit maternal care deficits alongside increased anxiety-like

behaviours (McNamara *et al.* 2018). In this study, loss of function of *Peg3* was confined to the offspring. Taken together with the data on Peg3 function regarding placental hormones, this suggests that the abnormal expression of *Peg3* in the placenta influences the behaviour of mothers by modulating the programming of maternal behaviour (Creeth *et al.* 2019). Together, rodent studies have shown the importance of *Peg3* in a successful pregnancy for mother and offspring, although the mechanism behind its regulation remains uncertain.

Yin Yang 1 (YY1) was suggested to be involved in regulating *Peg3* expression due to the presence of multiple YY1 binding sites in the *Peg3* DMR (Kim *et al.* 2003). These binding sites are conserved between mice, cows and humans (Kim *et al.* 2003). YY1 is a ubiquitous Gli-Krupple type zinc finger transcription factor with both activator and repressor functions (Thomas and Seto 1999). It has been experimentally shown that YY1 does not bind the methylated region and the binding sites on the unmethylated paternal allele are able to bind YY1 (Kim *et al.* 2003). However, studies outlining the function of YY1 upon *Peg3* expression are conflicting. Reductions in *YY1* were associated with both hypomethylation and hypermethylation at the *Peg3* DMR (Kim *et al.* 2007; Kim and Kim 2008; Kim *et al.* 2009), whereas removal of the YY1 binding sites did not affect DMR methylation (He *et al.* 2017). Reduction in *YY1* levels have been associated with an increased expression of *Peg3* (Kim and Kim 2008; Perera *et al.* 2015), whereas removal of YY1 binding sites either downregulated *Peg3* (He *et al.* 2017) or both increased and decreased expression depending on the number of binding sites removed (Kim *et al.* 2008). Furthermore, removal of YY1 binding sites resulted in sexually dimorphic phenotypic outcomes (He *et al.* 2017). The sexually dimorphic behaviour observed in *YY1* and *Peg3* mouse models, as well as the multiple functions of YY1 in different biological processes complicates the relationship between the two genes and makes interpretation of results in mice difficult. Still, no study has assessed the relationship between placental *PEG3* expression and *YY1* expression in humans.

Few studies have reported on the expression of *PEG3* in human placenta in relation to complications of pregnancy. In contrast to mouse studies, no correlation with birthweight has been reported (Kumar *et al.* 2012; Moore *et al.* 2015; Janssen *et al.* 2016). An altered DNA methylation status at *PEG3* DMR in cord blood was reported in association with infant temperament (Fuemmeler *et al.* 2016), but not prenatal stress (Vidal *et al.* 2014) or depression early in pregnancy (Liu *et al.* 2012). A number of other parental factors have been linked to altered DNA methylation at *PEG3* including fetal alcohol syndrome (Masemola *et al.* 2015), maternal antibiotic use (Vidal *et al.* 2013), parental obesity (Soubry *et al.* 2015; Dalgaard *et al.* 2016), toxic metals (Vidal *et al.* 2015), and maternal folate intake *(Haggarty et al. 2013)*. Reduced *PEG3* expression due to hypermethylation of the DMR has also been observed in breast and ovarian cancers (Dowdy *et al.* 2005; Feng *et al.* 2008; Chen *et al.* 2011). Together, these data suggest imprinting of *PEG3* can be unstable and may be sensitive to environmental exposures.

Our original findings of reduced *PEG3* and *hPL* expression in placentas from mothers with clinically diagnosed and/or self-reporting depression symptoms was undertaken in pregnancy cohorts with a relatively low number of participants (Janssen *et al.* 2016). The GiW cohort was established to explore this original finding in a larger cohort, and to further investigate biological factors linked to prenatal depression. Neither *PEG3* nor any other imprinted gene were found to be significantly altered in the RNAseq analysis (Chapter 4). The aims of the current chapter were to apply a targeted approach (qPCR) to ask whether *PEG3* expression was lower in relation to prenatal depression symptoms in a larger number of placental samples. To expand on the Janssen *et al*. paper, additional aims were to ask whether lower *PEG3* was associated with postnatal depression symptoms. Rodent studies showed changes in anxiety-like behaviour in response to altered *Peg3* expression (McNamara *et al.* 2018). Therefore, differences between prenatal anxiety and depression in relation to *PEG3* expression in humans were studied using a subset of women reporting anxiety symptoms but not depression symptoms. Another aim was to explore the mechanism behind placental *PEG3* expression changes in response to prenatal depression by investigating *YY1* expression levels and pyrosequencing of the *PEG3* DMR. Furthermore, to determine if the reduction in *PEG3* was part of a widespread disruption of imprinted genes, three other paternally and three maternally expressed imprinted genes were analysed. The final aim was to determine if the reduction in *hPL* reported by Janssen *et al*. was also present in prenatal depression cases in the Grown in Wales cohort.

5.2 Methods

Methods for the recruitment in the Grown in Wales study and processing of RNA through to qPCR were described in Chapter 2. For ease of interpretation, collection times are summarised in Table 5.1.

Table 5.1: Data collection time points

Time	***Reference***
Day of pre-surgical appointment (antenatal)	A1
Within one week of birth (average 4.2 days)	P1
Ten weeks after birth	P2
One year after birth	Y1

5.2.1 Sample selection

As previously reported, the prevalence of prenatal depression in the GiW cohort was 14% based on self-reporting EPDS scores. Of those reporting depression symptoms, 41 were White and ultimately delivered by elective caesarean. For the depression analysis, these 41 samples were chosen alongside an equal number of biologically matched controls not reporting depression symptoms (EPDS< 8) or anxiety symptoms (STAI< 40), nor prescribed antidepressants during pregnancy. For the anxiety analysis, 20 participants reporting high levels of prenatal anxiety (STAI≥ 40) and low levels of

depression (EPDS< 8) were chosen. Data from these samples were analysed against biologically matched samples from the controls for the depression study. After quality control checks outlined in Section 2.4.1, a number of samples were eliminated due to degradation. Within the depression subset 33 samples from participants reporting symptoms of prenatal depression passed the quality control check along with 33 matched controls Out of these, 29 samples were also analysed in Chapter 4 (13 control, 13 depressed). Within the anxiety-only subset 16 samples from participants reporting high anxiety symptoms passed control checks. 16 biologically matched controls were thus chosen from the depression subset control group. 8 of these control samples were also analysed in Chapter 4, but no anxiety-only samples went through RNAseq analysis.

5.2.2 Primer design

Process for designing primers described in Section 2.4.4.

Originally, gene expression was to be examined for all genes in the *PEG3* domain, and a number of imprinted genes in the *IGF2*/*H19* and *KCNQ1* imprinted domains. Multiple primer pairs were designed for the *PEG3* domain genes *MIMT1*, *ZIM2*, *PEG3-AS*, *USP29* and *ZNF264*, as well as the *KCNQ1* domain gene *SLC22A18*. However, these primer sets did not successfully amplify products by standard qPCR suggesting these genes were expressed at too low levels for meaningful analysis. The final genes analysed were *PEG3*, *YY1*, *PEG10*, *DLK1*, *IGF2*, *H19*, *PHLDA2*, *CDKN1C*, *hPL* and *PGH*.

5.2.3 Statistics

Gene expression is presented in one of two ways. Firstly as fold change relative to control groups and normalised to the two reference genes *YWHAZ* and *SDHA*. Alternatively data is expressed as '-DCT' scores, again normalised to the two reference genes. For ease of interpretation the original DCT scores were multiplied by -1 so higher scores represented higher expression levels.

Normalised DCT values were checked for normality using Shapiro-Wilk tests, Kolmogorov-Smirnov tests, histograms and normal Q-Q plots and no genes met a normal distribution. Therefore, all tests used in this chapter relating to gene expression utilised non-parametric distributions.

A description of the maternal demographics and birth outcomes of the two subsets (depression subset n= 66 and anxiety-only subset n= 32) was carried out using percentages or means, for categorical and continuous variables respectively.

To assess placental gene expression in relation to prenatal EPDS and STAI score Spearman's correlations were used. Comparing placental gene expression in those above and below the EPDS or STAI cut off was performed using Mann Whitney U tests. Analyses were repeated each time splitting samples based on fetal sex. *PEG3* expression was also analysed with respect to postnatal depression scores. Given the smaller numbers in the postnatal data, expression was only analysed with respect to

EPDS score and not the categorical cut off as inferences can be made with fewer data points in continuous analyses.

Regressions were used to further explore the relationship between postnatal depression score and *PEG3* expression whilst controlling for prenatal depression score. A continuous analysis rather than categorical cut off of 13 and above was used as the sample size was reduced for postnatal scores. Both linear and Poisson error distributions were assessed to ensure the correct model was chosen to appropriately fit the data. Assumptions of each model were assessed and models compared using AIC scores. A Poisson error family distribution was deemed the best fit and thus results reported used this error kind.

Spearman's correlations, Mann Whitney U tests and one Way ANOVAs were used to assess placental *PEG3* gene expression in relation to maternal demographics and birth outcomes. *PEG3* could not be analysed with respect to SSRI prescription during pregnancy due to small sample sizes (male: three participants prescribed SSRIs; female: six participants prescribed SSRIs).

To compare gene expression between placentas of different fetal sex, only "control" male and female placentas were compared to remove the potentially confounding effect of maternal mental health. Mann Whitney U tests were used to compare groups.

Given the number of tests performed, an adjustment for multiple comparisons was executed on all categorical and continuous analysis using an FDR correction to control for type I errors.

5.3 Results

5.3.1 Demographics

Demographics for the 66 participants in the depression subset are provided (Table 5.2). Control and depressed groups were matched for all demographics and birth outcomes, except history of mental health and SSRI prescription. The mean A1 EPDS score for control and depressed groups were 3.2 and 15.8 respectively. The mean A1 STAI score for control and depressed groups were 27.4 and 47.3 respectively. After birth, data was available for 41 participants of the depression subset at P1, and 28 participants at P2. Postnatal EPDS scores stayed relatively high in the depressed group (P1 EPDS mean of 11.4 and a P2 EPDS mean of 10.8) compared to the control group (P1 EPDS mean of 3.7 and a P2 EPDS mean of 2.8).

Table 5.2: Demographics of depression subset

Categorical variables	***All samples (n= 66)***	***EPDS≥ 13 (n= 33)***
Highest level of maternal education		
Left before GCSE	10.9	12.1
GCSE/Vocational	21.9	27.3
A Levels	20.3	24.2
University	29.7	27.3
Postgraduate	17.2	9.1
Family income		
< £18,000	13.8	17.9
£18-25,000	20.7	17.9
£25-43,000	22.4	25.0
> £43,000	43.1	39.2
Smoked in pregnancy		
No	83.3	78.8
Yes	16.7	21.1
Drank alcohol in pregnancy		
No	93.8	93.8
Yes	6.2	6.2
History of mental health problems		
No	59.1	30.3
Yes	40.9	69.7
Prescribed SSRIs in pregnancy		
No	86.4	72.7
Yes	13.6	27.3
Parity		
Nulliparous	21.2	21.2
Multiparous	78.8	78.8
Fetal sex		
Male	36.4	36.4
Female	63.6	63.6
Continuous variables	***All samples (n= 66)***	***EPDS≥ 13 (n= 33)***
WIMD	997.9	911.5
Maternal age (*years*)	31.9	32.0
BMI at booking	28.5	28.9
Gestational age (*days*)	275.3	274.7
Birthweight (*g*)	3502.0	3488.2
Custom birthweight centile	51.8	51.2
Placental weight (*g*)	669.7	664.8
Head circumference (*cm*)	35.2	35.2

[1] Categorical variables represented as percentages and continuous variables represented as means

In order to determine whether changes in gene expression were related to depression or anxiety symptoms, an anxiety-only subset was created (n= 32), demographics presented in Table 5.3. Again, control and anxious groups were matched for all demographics and birth outcomes, except history of mental health and SSRI prescription. Within the anxious group, women did not additionally report high depression symptoms (mean 6.5). The mean A1 STAI score for control and anxious groups were 24.9 and 42.2 respectively.

Table 5.3: Demographics of anxiety-only subset

Categorical variables	***All samples (n= 32)***	***EPDS≥ 13 (n= 16)***
Highest level of maternal education		
Left before GCSE	3.3	0
GCSE/Vocational	16.7	18.8
A Levels	10.0	12.5
University	36.7	31.3
Postgraduate	33.3	37.4
Family income		
< £18,000	9.7	6.7
£18-25,000	3.2	0
£25-43,000	22.6	26.7
> £43,000	64.5	66.6
Smoked in pregnancy		
No	87.5	81.3
Yes	12.5	18.7
Drank alcohol in pregnancy		
No	93.8	87.5
Yes	6.2	12.5
History of mental health problems		
No	81.2	68.8
Yes	18.8	31.2
Prescribed SSRIs in pregnancy		
No	96.9	93.8
Yes	3.1	6.2
Parity		
Nulliparous	18.8	25.0
Multiparous	81.2	75.0
Fetal sex		
Male	50.0	50.0
Female	50.0	50.0
Continuous variables	***All samples (n= 37)***	***EPDS≥ 13 (n= 16)***
WIMD	1192.6	1122.4
Maternal age (*years*)	32.5	33.6
BMI at booking	25.8	25.7
Gestational age (*days*)	275.0	274.3
Birthweight (*g*)	3575.9	3583.2
Custom birthweight centile	60.6	64.6
Placental weight (*g*)	699.5	689.5
Head circumference (*cm*)	35.2	35.1

[1] Categorical variables represented as percentages and continuous variables represented as means

5.3.2 *PEG3*

5.3.2.1 PEG3 *and prenatal mental health*

Placental *PEG3* expression was first analysed with respect to prenatal mental health (Figure 5.2). Placental *PEG3* expression was significantly lower in mothers reporting prenatal depression symptoms (Fold Change (FC) = 0.834, p= 0.035). When splitting the analysis by fetal sex the

reduction in *PEG3* was only significant in male placenta (male: FC= 0.728, *p*= 0.017). *PEG3* was also significantly correlated with EPDS score but only in male placenta (male: *r*= -0.439, *p*= 0.032). Within the anxiety-only subgroup *PEG3* expression was not associated with anxiety scores through fold change or continuous analyses. This result was the same for male and female placentas analysed separately.

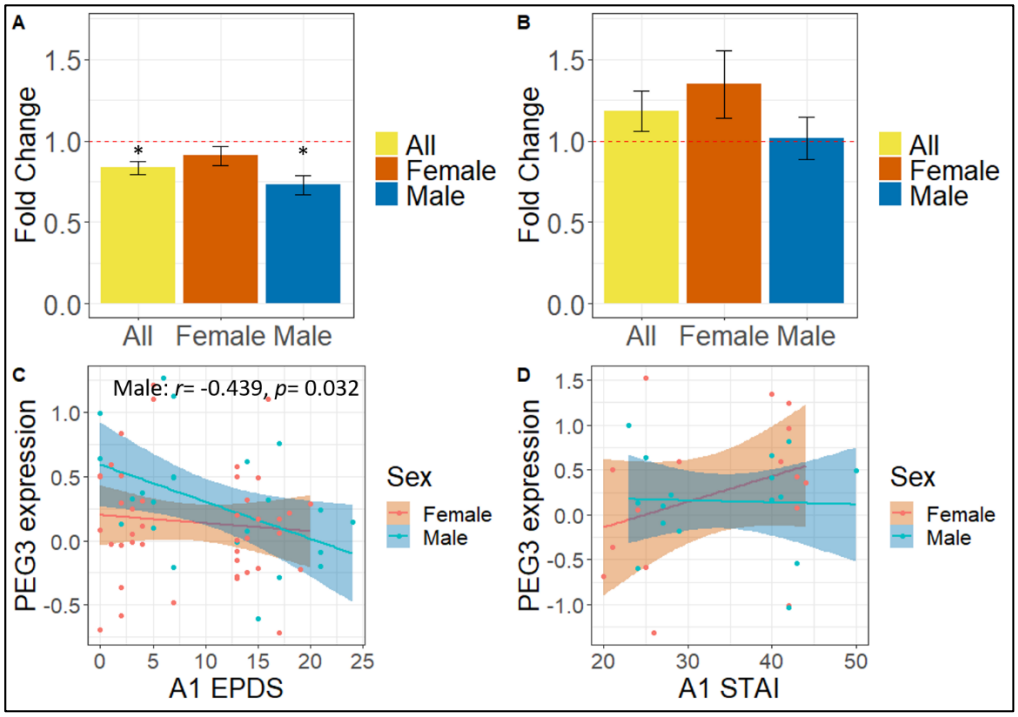

Figure 5.2:* PEG3 *expression and prenatal mental health*.** ***A: PEG3 *was significantly decreased in placentas from mothers reporting prenatal depression* (p= *0.035), and specifically in male placentas* (p= *0.017) but not female.* ***B:*** PEG3 *was not significantly associated with prenatal anxiety in all samples, or in male or placentas separately.* ***C:*** PEG3 *expression was associated with EPDS score in male placentas of the depression subset* (p= *0.032) only.* ***D:*** PEG3 *expression was not associated with STAI score in male or female placentas of the anxiety subset. Numbers: Depression subset (All: 33 vs 33; Male: 12 vs 12; Female: 21 vs 21); Anxiety-only subset (All: 16 vs 16; Male: 8 vs 8; Female: 8 vs 8). Red dotted line represents control samples fold change. Error bars represent standard error.*

5.3.2.2 PEG3 *and postnatal mental health*

To determine if there was an association between postnatal depression symptoms and placental *PEG3* expression correlations were performed (Figure 5.3). When all samples were analysed together *PEG3* expression was significantly associated with P1 EPDS score (*r*= -0.354, *p*= 0.025). This was not significant when split by gender (male: *r*= -0.490, *p*= 0.064; female: *r*= -0.212, *p*= 0.308). P2 EPDS score was not associated with *PEG3* expression in any correlation.

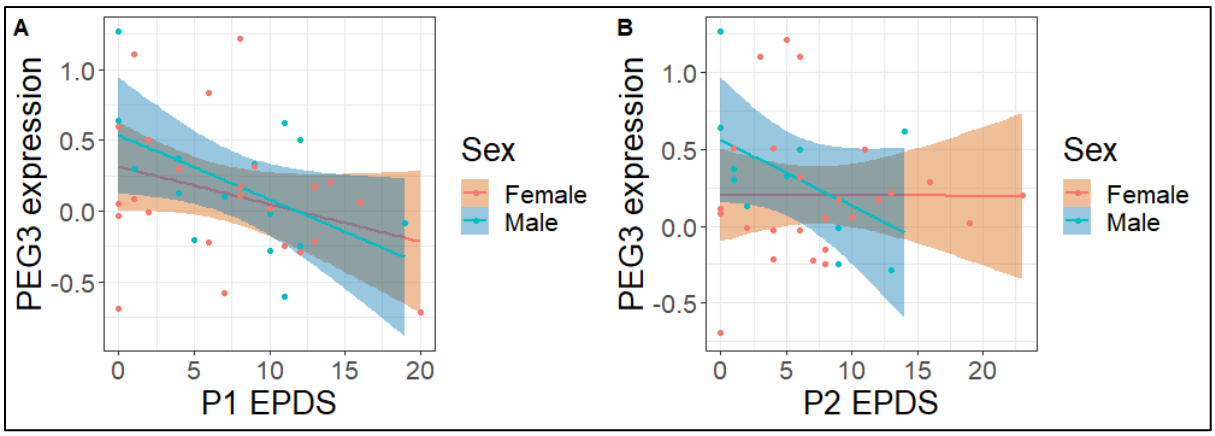

Figure 5.3:* PEG3 *expression and postnatal mental health*.** ***A: *PEG3 expression was significantly correlated with P1 (four days after birth) EPDS (p= 0.025), but not when split by fetal sex.* ***B:*** *PEG3 expression was not associated with EPDS at P2 (ten weeks after birth) in any analyses. Numbers: P1 time point (Male: 15, Female: 25); P2 time point (Male: 11; Female: 25).*

To explore the relationship between *PEG3* expression and P1 EPDS score further, regressions were used to determine if this association was still significant after controlling for EPDS at A1. *PEG3* expression was significantly associated with the dependent variable P1 EPDS score after controlling for depression score at A1 (Exp(B)= 0.754, *p*= 0.046, CI= -0.563, -0.009, n= 40). Again neither male nor female *PEG3* expression was significantly associated with P1 EPDS score individually.

5.3.2.3 PEG3 *expression and demographics*

PEG3 expression was analysed in relation to maternal demographics such as maternal age and BMI at booking, as well as birth outcomes such as placental weight and birthweight (Table 5.4). *PEG3* expression was not significantly associated with any maternal socio-economic demographics or birth outcomes when all samples were analysed together. When splitting samples by fetal sex, *PEG3* expression was significantly lower in placentas from mothers of males infants with a mental health history (*p*= 0.020). As described in Chapter 3, prenatal EPDS score was strongly associated with mental health history. A regression to determine if *PEG3* expression was still associated with A1 EPDS score after controlling for a history of mental health in male placentas was not significant (*p*= 0.106). However, given the strong relationship between the A1 EPDS and history of mental health, and the small sample size of the data limited conclusions can be drawn.

Table 5.4: PEG3 *expression in relation to maternal socio-demographic factors and birth outcomes*

Maternal demographic	***All samples***	***Male samples***	***Female samples***
Categorical variables		***p value***	
Highest level of maternal education*	0.706	0.812	0.255
Family income*	0.263	0.886	0.401
Season of birth*	0.245	0.926	0.180
Smoked in pregnancy	0.938	0.406	0.449
Drank alcohol in pregnancy	0.535	0.797	0.585
History of mental health problems	0.769	**0.026**	0.214
Parity	0.627	0.970	0.760
Continuous variables		***p value***	
WIMD	0.474	0.433	0.594
Maternal age (*years*)	0.262	0.310	0.542
Maternal BMI	0.268	0.231	0.620
Parity	0.227	0.777	0.351
Birth outcomes			
Categorical variables		***p value***	
Indication for ELCS*	0.254	0.639	0.194
Fetal sex	0.225	-	-
Continuous variables		***p value***	
Placental weight (*g*)	0.496	0.692	0.326
Gestational age (*days*)	0.387	0.146	0.999
CBWC	0.477	0.862	0.410
Birthweight (*g*)	0.301	0.651	0.352
Head circumference (*cm*)	0.228	0.766	0.135

[1] Significant values reported in bold

[2] Spearman's correlations for continuous correlations, Mann Whitney U tests for categorical variables with two factors, One Way ANOVAs for categorical variables with more than two factors (*)

[3] Numbers: All= 66, male= 24, female= 42

5.3.3 Other imprinted genes and maternal mood

Additional imprinted genes were analysed with respect to prenatal depression and anxiety (Figure 5.4, Figure 5.5). The paternally expressed genes *PEG10*, *IGF2* and *DLK1* were not significantly different in placentas from mothers reporting symptoms of depression. The maternally expressed genes *H19*, *PHLDA2* and *CDKN1C* were also not significantly associated with prenatal depression.

When these same genes were analysed with respect to prenatal anxiety, *IGF2*, *PHLDA2* and *CDKN1C* were significantly higher in the anxious group (*IGF2*: FC= 1.634, *p*= 0.016; *PHLDA2*: FC= 1.724, *p*= 0.012; *CDKN1C*: FC= 1.250, *p*= 0.023). When splitting by fetal sex both groups had higher *IGF2* but only females were significantly higher (male: FC= 1.418, *p*= 0.251; female: FC= 1.927, *p*= 0.022). Likewise both sexes had higher *PHLDA2* and *CDKN1C* but only in male placentas were they significantly higher (*PHLDA2*: male: FC= 1.759, *p*= 0.011; female: FC= 1.702, *p*= 0.09; *CDKN1C*: male: FC= 1.364, *p*= 0.038; female: FC= 1.171, *p*= 0.195).

When analysing gene expression with respect to A1 STAI score *IGF2* was not significantly correlated. However, *PHLDA2* and *CDKN1C* were significantly positively associated with STAI

(*PHLDA2*: *r*= 0.386, *p*= 0.029; *CDKN1C*: *r*= 0.372, *p*= 0.036). Again only male placentas were significant in further analyses (*PHLDA2*: *r*= 0.513, *p*= 0.042; *CDKN1C*: *r*= 0.547, *p*= 0.028).

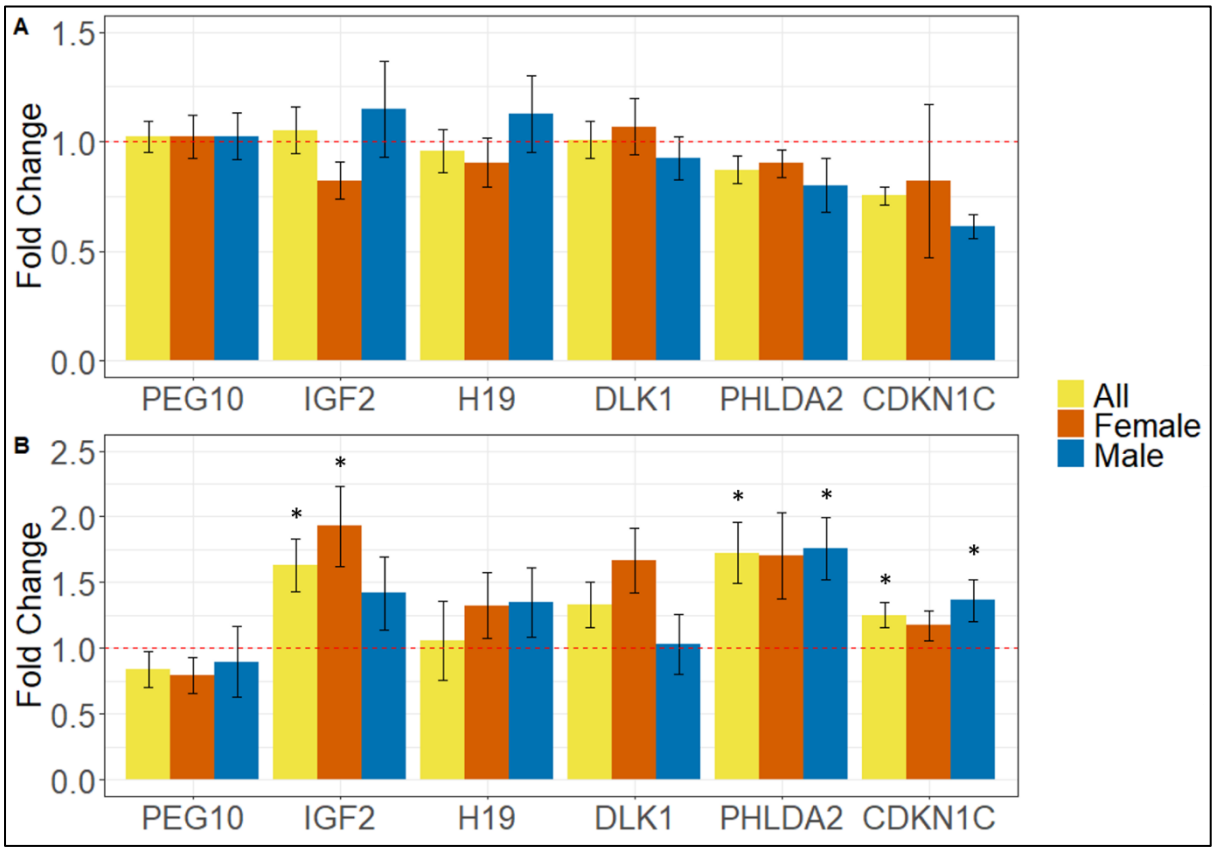

Figure 5.4: Imprinted gene expression and prenatal depression and anxiety. ***A****: Depression subset, imprinted gene expression was not significantly different in placentas of women reporting high depressive symptoms.* ***B****: Anxiety-only subset:* IGF2 *was significantly higher in placentas of those reporting high anxious symptoms (*p= *0.016), and specifically only in female placentas (*p= *0.022).* PHLDA2 *and* CDKN1C *was significantly higher in those reporting high anxious symptoms (*p= *0.012,* p= *0.023 respectively), and specifically only in male placenta (*p= *0.011,* p= *0.028 respectively.* PEG10, H19 *and* DLK1 *were not significantly altered in placentas from mothers reporting high anxious symptoms. Numbers: A: Depression subset (all: 33 vs 33, male: n= 12 vs 12, female: n= 21 vs 21). G-L: Anxiety-only subset (all: 16 vs 16, male: n= 8 vs 8, female: n= 8 vs 8). Red dotted line represents control samples fold change. Error bars represent standard error.*

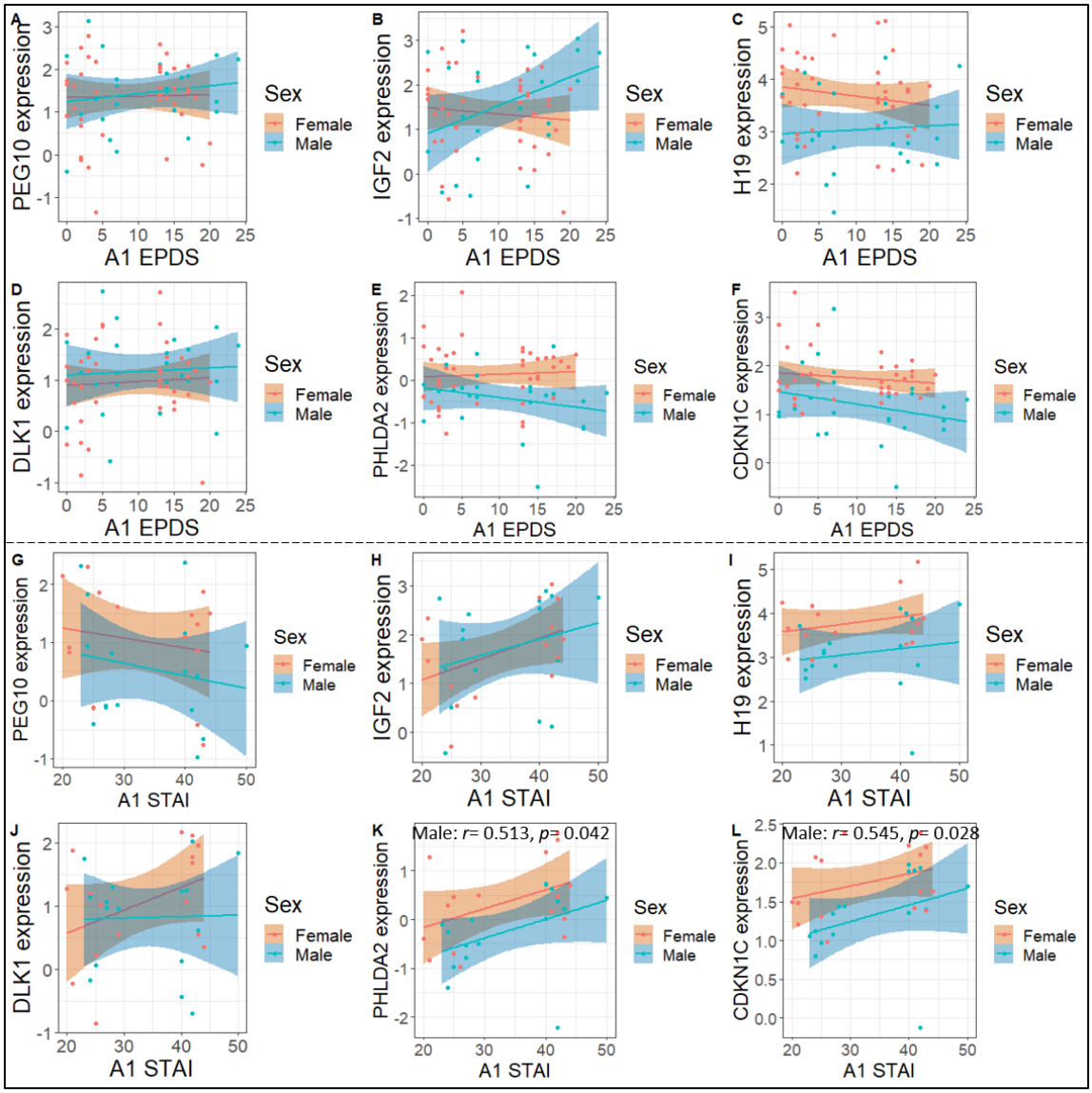

Figure 5.5: Imprinted gene expression and prenatal EPDS and STAI scores. ***A-F****: A1 EPDS was not associated with A1 EPDS score in placentas from either fetal sex.* ***G-J****: A1 STAI was not associated with* PEG10, IGF2, H19 *or* DLK1 *expression in the anxiety-only subset in placentas from either fetal sex.* ***K****: A1 STAI was significantly associated with* PHLDA2 *expression only in male placenta* (p= *0.042).* ***L****: A1 STAI was significantly associated with* CDKN1C *expression only in male placenta* (p=*0.028). Numbers: A-F: Depression subset (male: n= 24, female: n=42). G-L: Anxiety-only subset (male: n= 16, female: n= 16).*

5.3.4 *YY1* expression and maternal mental health

The relationship between *YY1* and maternal mental health was investigated as a potential mechanism to explain the reduction in *PEG3* seen in male placentas from mothers with depression (Figure 5.6). *YY1* was expressed at significantly higher levels in the depressed group (FC= 1.246, *p*= 0.040) when all placentas were analysed together (Figure 5.6.A). Although similarly elevated, the data was not significant when male placentas were analysed separately (Figure 5.6.A). *YY1* expression was not

significantly associated with EPDS score (Figure 5.6.B). Within the anxiety-only subset, *YY1* was not associated with prenatal anxiety either by anxiety group (Figure 5.6.C) or with STAI score as a continuous variable (Figure 5.6.D) when all, male-only or female-only samples were analysed.

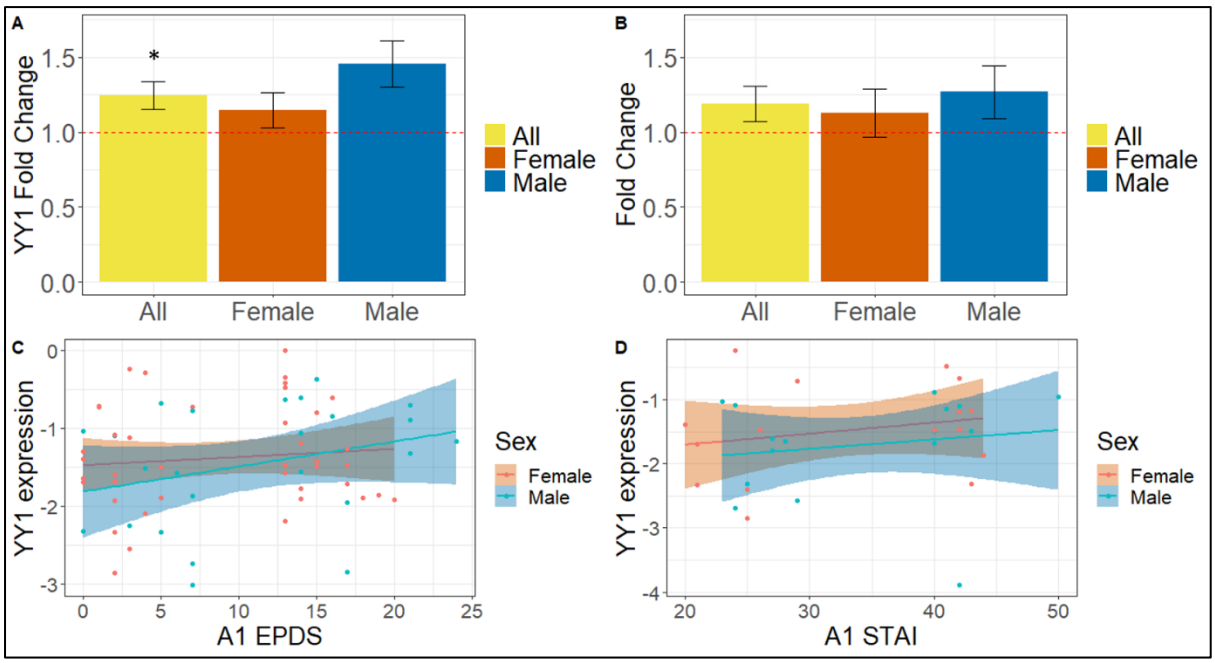

Figure 5.6:* YY1 *expression and prenatal mental health. ***A****: YY1 expression was significantly higher in placentas of mothers reporting high symptoms of depression (*p= *0.046), but not in male or female placentas separately.* ***B****: YY1 was not significantly different in any analyses in the anxiety-only subset.* ***C****: YY1 expression was not significantly associated with A1 EPDS score in the depressive subset.* ***D****: YY1 expression was not significantly associated with A1 STAI score in the anxiety-only subset. Numbers: Depression subset (All: 33 vs 33; Male: 12 vs 12; Female: 21 vs 21); Anxiety-only subset (All: 16 vs 16; Male: 8 vs 8; Female: 8 vs 8). Red dotted line represents control samples fold change. Error bars represent standard error.*

To investigate the relationship between *YY1* and *PEG3* expression, a correlation analysis was used on samples in the depression subset. There was no significant association between the two genes in all samples or when expression was analysed in either sex.

5.3.5 Placental hormones and maternal mental health

PGH and *hPL* expression were examined to investigate whether placental hormone gene expression was associated with prenatal depression or anxiety (Figure 5.7). *PGH* was not associated with depression or anxiety in either subset by any analysis approach. *hPL* was not associated with depression symptoms in the depression subset. In the anxiety-only subset, *hPL* expression was significantly correlated with STAI score (r= 0.423, p= 0.016) and was significantly higher in those

reporting anxious symptoms (FC= 1.467, *p*= 0.032). Upon further analyses, this relationship was only apparent in female placentas (female: *r*= 0.569, *p*= 0.021; FC= 1.836, *p*= 0.038). There was also no significant relationship between *hPL* and *PEG3* expression in either sex.

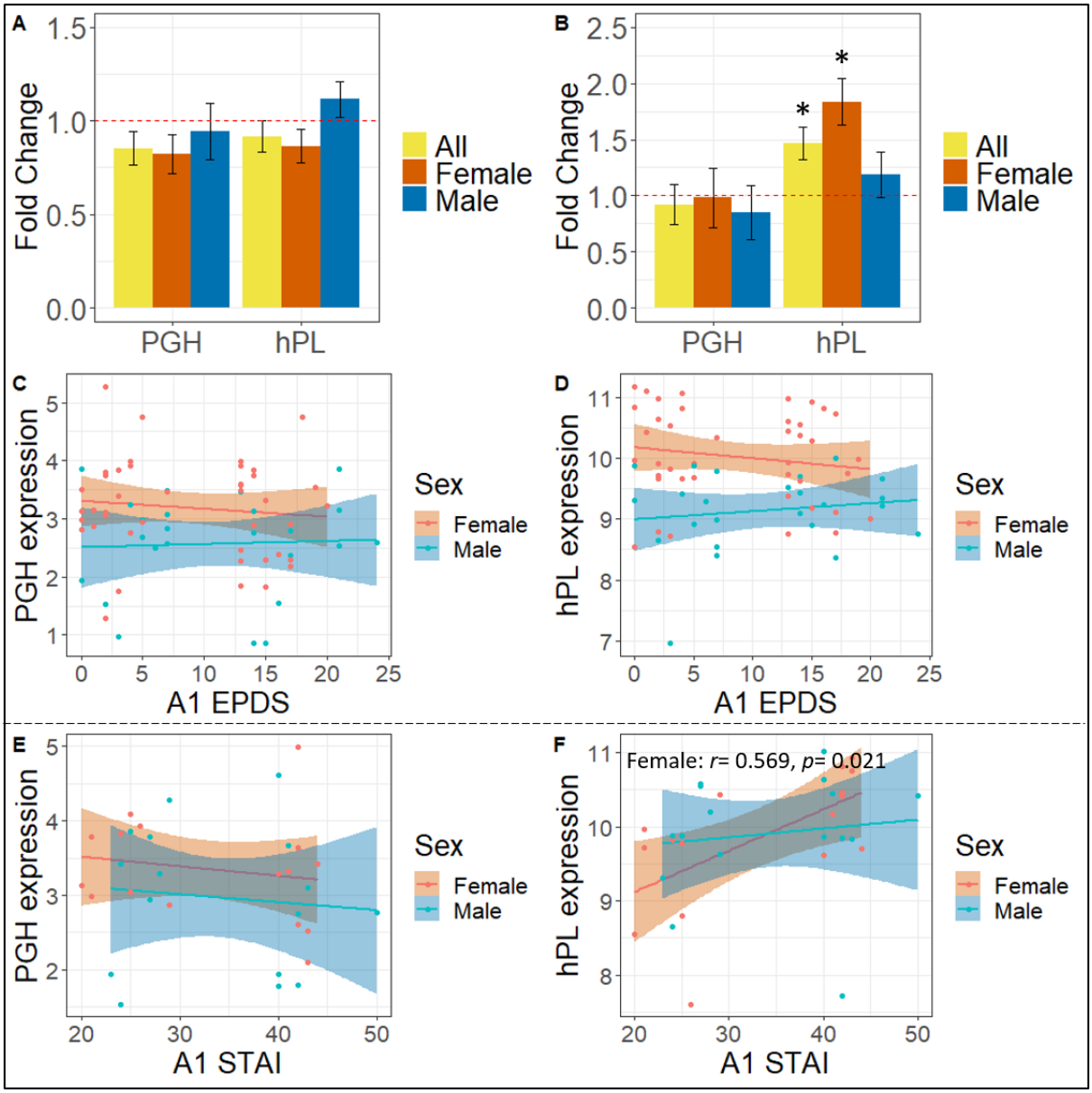

Figure 5.7: Placental hormone expression and prenatal mental health. ***A****:* PGH *and* hPL *expression were not significantly different in placentas from mothers with high depressive symptoms in the depression subset.* ***B****: In the anxiety-only subset* PGH *was not significantly different in placentas from mothers with high anxiety symptoms.* hPL *was significantly higher in placentas from anxious mothers when all placentas were analysed together (*p*= 0.032) and in female only placentas (*p*= 0.038).* ***C****:* PGH *expression was not significantly associated with A1 EPDS in the depression subset.* ***D****:* hPL *expression was not significantly associated with A1 EPDS score in the depression subset.* ***E****:* PGH *was not significantly associated with A1 STAI score in the anxiety-only subset.* ***F****:* hPL *was significantly associated with A1 STAI score in the anxiety-only subset, but only*

*in female placenta (*p*=0.013). Numbers: Depression subset (All: 33 vs 33; Male: 12 vs 12; Female: 21 vs 21); Anxiety-only subset (All: 16 vs 16; Male: 8 vs 8; Female: 8 vs 8). Red dotted line represents control samples fold change. Error bars represent standard error.*

5.3.6 Male and female placental gene expression

The expression levels of all the genes analysed above were compared between male and female placentas (Table 5.5). Only control samples were compared to eliminate the potential confounding effect of maternal mental health. There was no significant difference in maternal socio-demographics or birth outcomes for the two groups (*data not shown*). The two placental hormones *PGH* and *hPL* were significantly higher in control female placentas (p= 0.030 and p= 0.001 respectively), as was *H19* (p= 0.006).

Table 5.5: Gene expression of control male and female samples

Gene	***Male***	***Female***	***p value***
PEG3	0.503	0.197	0.082
YY1	-1.760	-1.493	0.449
PEG10	1.304	1.264	1.000
DLK1	1.155	0.901	0.494
IGF2	1.198	1.441	0.593
H19	2.923	3.778	**0.006**
PHLDA2	-0.205	0.130	0.291
CDKN1C	1.477	1.832	0.141
PGH	2.637	3.308	**0.030**
hPL	9.001	10.110	**0.001**

[1] -DCT values presented
[2] Significant values reported in bold

5.3.7 SSRI analysis

Given the findings in Chapter 4 where SSRIs were shown to have an impact on placental gene expression, it was possible that *PEG3* was expressed at lower levels due to SSRI exposure rather than prenatal depression. To explore this further, participants prescribed SSRIs during pregnancy were removed from the analysis. Nine participants in the depression subset (three with boys, six with girls) and one participant in the anxiety-only subset (with a boy) were prescribed SSRIs during their pregnancies. Within the depression subset *PEG3* was no longer significantly correlated with A1 EPDS in male placenta (r= -0.339, p= 0.133) and no longer significantly lower in male placenta from mothers reporting prenatal depressive symptoms (FC= 0.775, p= 0.095). There was no change in results in the female placentas or in the anxiety-only subset.

Within the depression subset, *PEG3* expression was compared between those prescribed and not prescribed SSRIs, irrespective of EPDS score. *PEG3* was lower in placentas from those prescribed SSRIs but this was not significant (FC=0.869, *p*= 0.336, n= 57 vs 9). When analysis was separated by fetal sex, *PEG3* expression in male placentas from mothers prescribed SSRIs was significantly lower (FC= 0.649, p= 0.016, n= 21 vs 3), although only three samples were in the SSRI group and thus should be interpreted with as extremely preliminary.

5.3.8 Multiple correction

Given the number of different tests in the analyses of the current chapter an adjustment for multiple comparisons was run. No significant *p* values in the above analyses remained significant after adjustment for FDR.

5.3.9 Summary of chapter results

Table 5.6: Summary of Chapter 5

Analysis	***Subset***	***Findings***	***Notes***
PEG3 and prenatal mental health	Depression	• Placental *PEG3* down in mothers with high depressive symptoms	Specifically in male placenta
	Anxiety only	• No relationship between *PEG3* and anxiety symptoms only	
PEG3 and postnatal mental health	Depression	• Placental *PEG3* significantly associated with EPDS score four days after birth	After controlling for depression in pregnancy
YY1 as mechanism behind *PEG3* reduction	Depression	• *YY1* up in all placentas from mothers with depressive symptoms • Not significant in male placentas alone • Not associated with A1 EPDS or *PEG3* expression	
Remaining imprinted genes and prenatal mental health	Depression	• No relationship between other imprinted genes and prenatal depression	
	Anxiety only	• *PHLDA2* and *CDKN1C* positively associated with A1 STAI score	Specifically in female placenta
Placental hormones and prenatal mental health	Depression	• *hPL* or PGH expression not associated with A1 EPDS	
	Anxiety only	• *hPL* significantly associated with A1 STAI	Specifically in female placenta

5.4 Discussion

This chapter provides further evidence that the paternally expressed gene *PEG3* is expressed at lower levels in term placenta from pregnancies where mothers report high levels of depression symptoms. Critically, lower *PEG3* was only found in those pregnancies where mothers had boys. Lower *PEG3* in male placenta was found to be associated specifically with exposure to prenatal depression symptoms and not prenatal anxiety. The association between low *PEG3* expression and high maternal EPDS scores remained significant four days after birth after adjusting for depression scores in pregnancy. Analyses of other imprinted genes suggested that this abnormal expression is confined to *PEG3* and is not part of a larger disruption of paternally imprinted genes. The association between *PEG3* and *hPL* expression previously reported (Janssen *et al.* 2016) was not reproduced.

Loss of *Peg3* expression in the placenta has been shown to cause alterations in maternal behaviour in mice, with specifically changes in anxiety-like behaviour postnatally (McNamara *et al.* 2018). While there was no evidence of depression-like symptoms in the mouse mode, depression-like behaviour is relatively difficult to measure in mice. In this study, lower placental expression of *PEG3* was found in the group with depression and not the group specifically reporting anxiety. However, depression and anxiety are commonly co-morbid in human pregnancies and within the depression subset in the current analyses 85% of those reporting high depression symptoms also reported high anxiety symptoms. It is also important to note the smaller sample size in the anxiety-only group. These results suggest, at least in humans, the relationship is stronger between *PEG3* and either prenatal depression or co-occurring prenatal depression and anxiety than prenatal anxiety alone.

This is the first study to explore the relationship between imprinted genes in human placenta and postnatal mental health. Four days after birth *PEG3* expression was significantly inversely correlated with EPDS scores after controlling for depression during pregnancy. No relationship existed two months postnatally. It is important to note the smaller sample size and thus replicating the analyses in a larger cohort is crucial. In human studies it is not possible to determine the direction of causality between *PEG3* expression and maternal depression symptoms. However, given that altered maternal behaviours were observed postnatally in wild-type dams carrying pups with loss of *Peg3* expression (Curley *et al.* 2004; McNamara *et al.* 2018), placental *PEG3* expression in humans could be causing changes in maternal mood both in pregnancy and in the postnatal period.

A significant reduction of *PEG3* expression was only associated with symptoms of depression in placentas from male offspring. Sex-biased outcomes have been reported in response to altered *Peg3* expression in mice, with males often more severely affected. In males altered sizes of placental lineages and aberrant placental hormone expression have been reported, and a malnutrition study described an increase in placental *Peg3* expression only in males (Radford *et al.* 2012; Tunster *et al.* 2018). The current analysis is the second report of a sex-biased outcome relating to placental *PEG3*

expression in humans (Janssen *et al.* 2016). Although higher, placental *PEG3* expression was not significantly different between male and female placentas from control pregnancies in the current analysis (p= 0.084). In mice, placental *Peg3* has been reported as significantly higher in male mice compared to female pups (Faisal *et al.* 2014). The reason behind the sexually dimorphic association with prenatal depression seen in the current analyses is unknown, although may relate to the differences observed in *Peg3* regulatory control in mice (Perera and Kim 2016b; He *et al.* 2017).

Although work from the mouse model suggests that reduced *PEG3* may be causing mental health symptoms in a human pregnancy, it is important to note that the male placenta were relatively more globally impacted by prenatal depression (Chapter 4). This raises the possibility that lower *PEG3* is a consequence of exposure to prenatal depression, rather than a cause. Further studies in mice will be useful in determining whether *Peg3* responds to prenatal stressors, as has been shown for the imprinted *Cdkn1c* gene (Van de Pette *et al.* 2017). It will also be possible in larger data sets to ask whether specific maternal lifestyles are associated with reduced *PEG3*.

The *Peg3* DMR contains multiple tandem repeats serving as YY1 binding sites that are conserved between humans and mice, and thus suggested to function in *Peg3* regulation. Whilst conflicting results exist on whether YY1 acts as a repressor or activator on *Peg3* expression in mice, there is a consensus that directly or indirectly the transcription factor is important (Kim *et al.* 2007; Kim and Kim 2008; Kim *et al.* 2008; Kim *et al.* 2009; He *et al.* 2017). The association between *YY1* and *PEG3* expression has not previously been studied in human placenta. The current analyses showed *YY1* expression was significantly higher in placentas from mothers reporting high depression symptoms, suggesting an inverse correlation between *PEG3* and *YY1*. However, *YY1* expression did not correlate with prenatal EPDS score, nor with *PEG3* expression. Furthermore, the increase in *YY1* from mothers reporting high depression symptoms was not male-specific. Therefore, although *YY1* expression appears important in *Peg3* regulation in mice, this may not be conserved in humans.

Whilst different studies have reported contradictory findings for YY1 on *Peg3* expression, the role of the *Peg3* DMR on *Peg3* imprinted status is more defined. Methylation on the maternal allele silences *Peg3* expression resulting in mono-allelic expression from the paternal allele. One of the aims of this chapter was to investigate methylation as a potential mechanism behind the reduction in *PEG3* expression in male placenta. Pyrosequencing was chosen to examine methylation status at four points within two CpG islands spanning the *Peg3* DMR and analysed with respect to *PEG3* expression. Primer design and optimisation of the protocol can be found in the Appendix. Unfortunately, lockdowns due to COVID-19 meant the final analysis of samples could not be performed. A significant association between methylation at one or more of the CpG islands and *PEG3* expression would have suggested that improper methylation repressed *PEG3* expression in mothers with high symptoms of depression. Given methylation is predominantly established and maintained through

DNA methyltransferases (DNMTs) (Hendrich and Bird 2000), this could be a potential driver. However, if CpG methylation had not been associated with *PEG3* expression it would suggest an alternative regulatory factor was controlling expression. *Peg3* has at least two alternative promoters in mice and one in humans that are close in proximity to supposed transcriptional enhancers (Perera and Kim 2016a). Therefore, long range cis-regulatory elements may be involved in *Peg3* expression. Furthermore, the expression of mouse *Peg3* promoters are sexually biased (Perera and Kim 2016b) and thus may also be a mechanism behind the increased vulnerability of males to aberrant *Peg3* expression.

In addition to pyrosequencing being halted by COVID-19, western blots were also planned to assess protein levels of PEG3 and YY1. mRNA expression does not always correlate with final protein levels as translation- and degradation-rates can alter final protein concentrations (Brockmann *et al.* 2007). Therefore, protein levels of PEG3 were to be measured to assess whether changes in gene expression did translate to protein level. YY1 was also to be analysed by western blot to assess the relationship between the two genes. Optimisation of the Western blots had been partly completed and the protocol found in the Appendix.

In Chapters 4 and 6, removing those prescribed SSRIs from analyses uncovered significant findings which had been potentially masked by the medication. SSRI medication has been associated with significant gene expression changes in human placenta (Olivier *et al.* 2014), although prenatal mental health was found to be a greater influence on specifically imprinted gene expression in the placenta than psychiatric medication (Litzky *et al.* 2018). Neither study found a relationship between *PEG3* expression and either prenatal depression or medication, although neither separated analyses by fetal sex. A sensitivity analysis was performed within the current chapter for the relationship between *PEG3* and prenatal mental health. *PEG3* was no longer associated with prenatal depression in male placenta, and again no relationship with anxiety was observed. The lack of significance observed in the depression analysis is likely due to decreased numbers because there was still a general reduction in placental *PEG3* from mothers reporting depression symptoms, but it was not significant (p= 0.095). Given the small number of participants prescribed SSRIs it was not possible to statistically determine if the effect observed between *PEG3* and prenatal depression in male placentas within the original analysis related to prenatal depression or the treatment. *PEG3* expression was significantly lower in male placenta from women prescribed SSRIs compared to those not prescribed antidepressants (p= 0.016), although only three samples were included in the former group and when male and female placentas were analysed together the reduction was not significant (p= 0.336). It would therefore be interesting in a larger cohort with a higher number of participants prescribed SSRIs during pregnancy to determine the effect of SSRIs on *PEG3* expression.

While the focus of the current analyses has been on the expression of *PEG3*, a number of other imprinted genes were also analysed with respect to prenatal mental health. Unlike *PEG3*, the other paternally expressed genes *IGF2*, *PEG10* and *DLK1* were not associated with prenatal depression. This suggests the link between *PEG3* and prenatal depression was not part of a wider disruption of paternally expressed genes. Placental expression of an additional paternally expressed gene, *Mest*, has previously been implicated in the maternal behaviour of mice and its imprinted status is conserved in humans (Kobayashi *et al.* 1997; Lefebvre *et al.* 1998). However, it was it was not investigated in the present study as to date there are no links between *Mest* and the control of the endocrine compartment in mouse placenta. Reductions in both *IGF2* and *DLK1* along with maternally expressed *H19* have previously been associated with comorbid prenatal depression and anxiety in an RNAseq analysis (Litzky *et al.* 2018). These observations were not replicated in the current analysis. It is important to note that Litzky *et al.* had a substantially larger sample size and thus increased power to detect alterations in expression levels of genes. Furthermore, comparing placental gene expression between cohorts should be done so with caution as populations studied comprised of different modes of delivery; Litzky *et al*. studied placentas where 48% of which were from vaginal deliveries (Litzky *et al.* 2018). The expression of imprinted genes can vary across modes of delivery (Janssen *et al.* 2015) and thus placental gene expression may not be directly comparable between the two studies. Although *IGF2* was not associated with prenatal depression, in the anxiety-only subgroup *IGF2* was significantly higher in placentas from anxious mothers specifically in female placentas. An increase in placental *IGF2* has previously been associated with maternal depression at 28 weeks in mothers who had girls but not boys (Mina *et al.* 2015). However, in the current analysis *IGF2* was not significantly associated with A1 STAI, questioning the relationship between the gene and prenatal anxiety.

Two maternally imprinted genes, *PHLDA2* and *CDKN1C*, were also analysed with respect to maternal mental health. In mice, genetic modification of *Phlda2* expression in the placenta has been linked to alterations in maternal behaviour (Creeth *et al.* 2018) and *Cdkn1c* has been identified as another imprinted gene that regulates placental hormone lineages (Tunster *et al.* 2011). Whilst the expression of neither gene was associated with prenatal depression, both genes were positively correlated with prenatal anxiety in the anxiety-only subset. Furthermore, the relationship was only significant in male placentas. *PHLDA2* and *CDKN1C* are located in the same imprinted domain and under transcriptional control of the same ICR. The results observed in the current study may thus correspond with changes observed in mice. Whilst depression and anxiety are often comorbid, an increasing number of studies have identified biological responses unique to different psychological states in pregnancy (Evans *et al.* 2008; Qiu *et al.* 2013; Wen *et al.* 2017; Scheinost *et al.* 2020). This may explain the difference in results between the anxiety-only and depression subsets. The sample size of the anxiety cohort is also smaller than the depression cohort, and thus analyses should be repeated in a larger cohort to disentangle the relationship between *PHLDA2*, *CDKN1C* and prenatal mental health.

In Janssen *et al*. 2016, the first study describing reduced placental *PEG3* expression in relation to prenatal depression, reductions in the placental hormone *hPL* were also reported (Janssen *et al.* 2016). There was a significant reduction in *hPL* in all three cohorts, and a correlation between *PEG3* and *hPL* in two of the three cohorts. Thus *hPL* was hypothesised to be a potential mediator between *PEG3* and prenatal depression. However, a reduction in *hPL* expression in relation to prenatal depression was not observed in the present study, and like in the Queen Charlotte's cohort in Janssen *et al*., there was no significant relationship between *PEG3* and *hPL* in the current analysis. Furthermore, mediation analysis in the previous study did not find *PEG3* to have an indirect effect on maternal EPDS through *hPL* expression (Janssen *et al.* 2016). Together this questions the relationship between *PEG3* expression and *hPL* in human placentas. *Peg3* acts upon the spongiotrophoblast in mice and directly affects placental hormone expression (Tunster *et al.* 2018). However, in mice *Peg3* expression occurs in all trophoblast cells, while in humans expression is restricted to the villous cytotrophoblast (Hiby *et al.* 2001). Therefore, given different expression patterns, placental *PEG3* may act via different mechanisms in mice and humans.

Whilst no association was apparent between *hPL* and prenatal depression, *hPL* expression was significantly higher in placentas from mothers reporting anxious symptoms in the anxiety-only subset. When splitting analyses by fetal sex this association was only present in female placenta. A study on a mutant *Peg3* mouse model reported increased expression of the prolactin gene family in the brains of *Peg3* knockout pups, specifically in female pups (Kim *et al.* 2013). However, *PEG3* expression was not associated with anxiety in this group in the current analysis. Analysis of *PEG3*, *hPL* and prenatal anxiety could be performed in a larger sample to further investigate this finding.

hPL, *PGH* and *H19* were the only genes significantly different between "control" male and female samples. All three genes were significantly higher in female placenta. While the difference in *H19* has not been reported before, sexual dimorphisms in placental *PGH* (Coutant *et al.* 2001) and maternal serum *hPL* have previously been described (Houghton *et al.* 1984). Higher levels of the two placental hormones have been linked to a potentially higher hormone production within the syncytiotrophoblast in female placentas (Lacroix *et al.* 2002). Therefore, a difference in endocrine production between male and female placentas may underline sexually dimorphic pregnancy outcomes.

5.4.1 Limitations

It is important to note that the correlations and categorical analyses in this chapter did not pass correction for multiple testing and thus results should be interpreted with caution. Despite this, the observation of a reduction in *PEG3* in placentas from women reporting prenatal depression symptoms has been reported previously in three separate cohorts, indicating the result's validity. Sample sizes were relatively small in this analyses, particularly in the anxiety-only subset but these samples were taken from a much larger cohort (n= 355) and represented the majority of samples where women reported significant depression symptoms. In all four cohorts, the majority of participants were White,

and in three of the cohorts' placenta was collected by ELCS. It is therefore important to repeat the analyses performed in this chapter in other cohorts representing different populations and modes of delivery to see if results are replicated.

5.4.2 Conclusions

This is the fourth pregnancy cohort study to show a reduction in placental *PEG3* expression in mothers reporting high depression symptoms. For the first time, the association between *PEG3* and depression symptoms was shown to be stronger than with prenatal anxiety symptoms alone, and remained significant postnatally. *Peg3* mutant mouse models often affect males more severely than females, and a sexually dimorphic outcome was again observed here. This aberrant *PEG3* expression did not appear to be part of a widespread misexpression of paternally expressed imprinted genes, although the mechanism of transcriptional changes remains unknown. Finally the hypothesis of *hPL* acting as an intermediary between *PEG3* expression and prenatal depression was not supported by the findings, suggesting other mechanisms may be at play.

Chapter 6: Maternal serum hPL and maternal mental health

6.1 Introduction

Chapter 5 replicated a previous finding from our group that *PEG3* expression was lower in male placentas from mothers reporting higher symptoms of prenatal depression (Janssen *et al.* 2016). The original study also reported a reduction in placental mRNA expression of the hormone *hPL* in mothers with higher depression scores. In mice, *Peg3* regulates the development of the placental endocrine compartment and expression of placental hormones including those functioning as placental lactogens (Tunster *et al.* 2018). These data suggested that changes in the expression of placental *PEG3* might contribute to prenatal depression by regulating the expression of *hPL* and the production of placental lactogen. However, in the GiW cohort, placental *hPL* expression was not associated with either *PEG3* expression or symptoms of depression (Chapter 5). While *hPL* expression was not associated with prenatal depression symptoms, there was a significant positive correlation between expression and prenatal anxiety symptoms alone. This relationship was only significant in female placenta. This chapter sought to further investigate the relationship between hPL and maternal mental health focussing on serum hPL levels.

hPL is encoded by two identical genes, *CSH1* and *CSH2*, which are both members of the Growth Hormone gene family located on chromosome 17.q23.3 (Harper *et al.* 1982; Chen *et al.* 1989; Cattini *et al.* 2006; Männik *et al.* 2010). In humans, this gene family arose from multiple duplications of the growth hormone gene and there is strong sequence conservation between family members (approximately 95%) (Chen *et al.* 1989). Expression of four of the five genes that make up this family (*CSHL1*, *CSH1*, *CSH2* and *GH2*) occurs predominantly in the placental villous syncytiotrophoblast, but a recent single cell RNAseq analysis showed additional expression of *CSH1* and *CSH2* in extra villous trophoblast cells (Liu *et al.* 2018). The remaining gene *human growth hormone* (*hGH*) is expressed in the pituitary gland (Cattini *et al.* 2020). hGH can cross the blood brain barrier when concentrations are high (Pan *et al.* 2005) and hPL levels steadily increase over gestation to peak at term. hPL has been detected in human cerebrospinal fluid (Peake *et al.* 1983; Walker *et al.* 1990) and it is presumed that this hormone directly acts on the maternal brain to induce maternal behaviour (Grattan 2011).

Prolactin is structurally and functionally similar to hPL and has a well-established role in regulating rodent maternal behaviour (Handwerger and Freemark 1987; Creeth *et al.* 2019). Prolactin receptors are found in brain regions associated with emotion and behaviour, and knockout prolactin receptor (PRL-R) dams lack appropriate maternal behaviours (Lucas *et al.* 1998; Woodside *et al.* 2008; Patil *et al.* 2014). In rats, prolactin is associated with the activation of neural progenitors resulting in new olfactory neurones, crucial for offspring recognition (Shingo *et al.* 2003). Reduced neurogenesis as a result of suppressed prolactin has been associated with postnatal anxiety in dams (Larsen and Grattan 2010). Although both prolactin and hPL levels rise over human pregnancies, hPL is considerably more abundant in maternal serum near term (5-7 vs 0.15-0.18 µg/ml) (Newbern and Freemark 2011).

hPL also has a stronger affinity for prolactin receptors than prolactin itself, and thus also suggested to function in maternal behaviour (Freemark 2010; Newbern and Freemark 2011).

A small number of studies have reported on a link between prolactin and maternal mental health in humans (Asher *et al.* 1995; Ingram *et al.* 2003; Groer and Morgan 2007; Gurtunca and Sperling 2017). There is also limited indirect evidence supporting a link between hPL and maternal mental health. Both expression levels and maternal serum concentrations of hPL have been negatively associated with fetal growth restriction which has been independently linked with prenatal depression (Roh *et al.* 2005; Diego *et al.* 2009; Männik *et al.* 2010). Maternal obesity has been described as a risk factor for poor prenatal mental health (Molyneaux *et al.* 2014). Obesity is also associated with a reduction in placental *hPL* expression (Cattini *et al.* 2006; Vakili *et al.* 2013). The previous finding of an association between *hPL* and prenatal depression symptoms is the first study investigating *hPL* and perinatal mental health directly (Janssen *et al.* 2016). However, this relationship was only analysed for placental expression of *hPL*, and not hPL serum concentrations. Together, these studies suggest a potential role for hPL in maternal mental health.

As we previously reported, 7.8% of women in the Grown in Wales cohort were prescribed SSRIs during their pregnancy to treat poor mental health (Janssen *et al.* 2018). In mice SSRIs are predicted to stimulate prolactin synthesis through increasing serotonin levels (Kamberi *et al.* 1971; Gil-ad *et al.* 1976) and a large pharmacoepidemiological study found SSRIs were associated with a rise in prolactin in humans (Petit *et al.* 2003). Therefore, any relationship between hPL and maternal mental health may be masked by the action of SSRIs. This was not previously assessed in Janssen *et al*. 2016 as there was insufficient data available on participant's medication history.

Placental *hPL* expression was analysed with respect to prenatal mental health in Chapter 5. The aim of the current chapter was to investigate serum hPL in the Grown in Wales cohort by first comparing the relationship between placental *hPL* expression and maternal serum hPL. Secondly, the relationship between maternal serum hPL and maternally reported symptoms of depression and anxiety in the perinatal period were investigated. Analyses were separated by fetal sex given the male-specific reduction in *hPL* previously reported (Janssen *et al.* 2016). A secondary sensitivity analyses was performed to examine the relationship between hPL and maternal mental health without the potentially confounding effect of SSRIs. The analysis of maternal serum hPL and perinatal mental health presented in this chapter was published in *Pychoneuroendocrinology* (Sumption *et al.* 2020), and the chapter includes and extends the results reported in this paper.

6.2 Methods

Methods for the recruitment in the Grown in Wales (GiW) study and maternal blood collection were described in Chapter 2.

For ease of interpretation, collection times are summarised in Table 6.1.

Table 6.1: Data collection time points

Time	***Reference***
Day of pre-surgical appointment (antenatal)	A1
Within one week of birth (average 4.2 days)	P1
Ten weeks after birth	P2
One year after birth	Y1

6.2.1 Participant selection

355 women were recruited into the GiW cohort, of which 272 had maternal serum hPL measurements available. Of these, 233 were White, had sufficient prenatal depression and anxiety data (A1) and ultimately delivered by elective caesarean. The current chapter focused on this group of women. Not all of these participants completed mental health questionnaires at the later time points (Table 6.2).

Table 6.2: Numbers of participants at each time point

	All	***Male infants***	***Female infants***
A1 EPDS	233	106	127
A1 STAI	233	106	127
P1 EPDS	133	62	71
P1 STAI	135	62	73
P2 EPDS	124	56	68
P2 STAI	122	54	68
Y1 EPDS	95	36	59
Y1 STAI	96	36	60

6.2.2 Human placental lactogen

hPL concentrations were measured using the Leinco Technologies Human Placental Lactogen Micro-ELISA test kit (Universal Biologicals product code T115-96 tests). Samples were measured in duplicate. The NIHR Cambridge Biomedical Research Centre, Core Biochemical Assay Laboratory, performed these measurements.

6.2.3 Statistical analysis

Normality of hPL was assessed using Shapiro-Wilk tests, Kolmogorov-Smirnov tests, histograms and normal Q-Q plots, and was found not to be normally distributed. Therefore, non-parametric tests were used where appropriate. Frequencies and means were reported for categorical and continuous demographic variables respectively.

Description of the generation of placental hPL measures is provided in Chapter 5. The relationship between the two hPL measurements was analysed for those participants with both expression and serum data available (n= 71). hPL serum measurements and hPL expression data from the depression subset were compared to maternal demographics and birth outcomes (n= 233 and n= 66 respectively). Spearman's correlations, Mann Whitney U tests and ANOVAs were used where appropriate.

hPL serum data was available for a large number of participants, and thus a different analysis approach was performed compared to Chapter 5 when subsets were used. Maternal serum hPL was analysed with respect to maternal mental health at all four time points using unadjusted and adjusted regressions. Continuous EPDS and STAI values were used for analysis rather than a categorical cut off of 13 and above and 40 and above respectively to account for smaller sample sizes in postnatal data. Potential confounders from the literature were analysed in relation to prenatal depression and anxiety scores. Confounders that were associated with A1 EPDS or A1 STAI at a p value< 0.15 in single predictor models were taken forward to multiple regressions with A1 EPDS or A1 STAI respectively. Backward selection was chosen to create the final model due to the number of confounders in a limited dataset. Confounders significant with either A1 EPDS or STAI were taken forward to adjusted linear regressions to simplify analyses and ensure the same confounders were used throughout. All unadjusted and adjusted regressions were additionally separated by fetal sex.

To ensure relationships between hPL and prenatal mood were not confounded by SSRI use in pregnancy, a secondary sensitivity analyses was performed. Women prescribed SSRIs at any point during their pregnancy were removed from the dataset and both unadjusted and adjusted analyses repeated. To compare the relationship between hPL and depression symptoms, and hPL and anxiety symptoms, EPDS and STAI scores were converted into standardised z-scores. Comparison of serum hPL levels between participants carrying either boys or girls was performed using a Mann Whitney U test.

Placental expression of *hPL* was additionally measured with respect to postnatal depression, as was performed for *PEG3* expression in Chapter 5. In line with the analysis in the previous chapter, analysis was only performed for postnatal depression in the depressive subset and not separated by fetal sex due to smaller sample sizes. Spearman's correlations were used to compare expression and postnatal EPDS scores up until 10 weeks postnatally.

6.3 Results

6.3.1 Demographics

Demographics of the 233 participants with hPL maternal serum data available, and for the 71 who had hPL measured through both serum and mRNA expression are provided (Table 6.3). Demographics of the depression subset and anxiety-only subset for the hPL expression data can be found in Chapter 5.

Table 6.3: Demographic and birth outcomes

Demographics	***% or median***	
Categorical variables	***All maternal serum (n= 233)***	***Both maternal serum and expression (n= 71)***
Highest education level		
Left before GCSE	6.2	5.9
GCSE or vocational	23.3	22.3
A-level	12.8	17.8
University	30.8	32.6
Postgraduate	26.9	22.4
Family income		
< £18,000	8.3	9.0
£18-25,000	11.7	14.9
£25-43,000	22.0	25.4
> £43,000	58.0	50.7
Season of birth		
Spring	21	28.2
Summer	23.2	19.7
Autumn	33.9	28.2
Winter	21.8	23.9
Smoked in pregnancy		
No	89.7	87.3
Yes	10.3	12.7
Drank alcohol in pregnancy		
No	93.0	92.9
Yes	7.0	7.1
History of mental health problems		
No	69.5	64.8
Yes	30.5	35.2
Prescribed SSRIs in pregnancy		
No	91.0	87.3
Yes	9.0	12.7
Parity		
Nulliparous	19.3	18.3
Multiparous	80.7	81.7
Fetal sex		
Female	45.5	42.3
Male	54.5	57.7
Continuous variables		
A1 EPDS	7.3	8.3
A1 STAI	34.4	36.7
WIMD score	1270.0	1860.0
Maternal age (*years*)	33.0	33.0
BMI at booking	26.4	27.2
Gestational age *(days)*	274.0	274.0
Custom birthweight centile	50.7	58.0
Birth weight *(g)*	3519.3	3553.7
Placental weight *(g)*	654.0	672.9
Head circumference *(cm)*	35.5	35.4

[1] Categorical variables represented as percentages and continuous variables represented as means

6.3.2 Comparison of hPL measurements

hPL was measured at the placental expression level and at the maternal serum level. For participants who had both hPL measurements available (n= 71), maternal serum and placental expression of *hPL* were compared. There was no significant association between the two measurements (Figure 6.1).

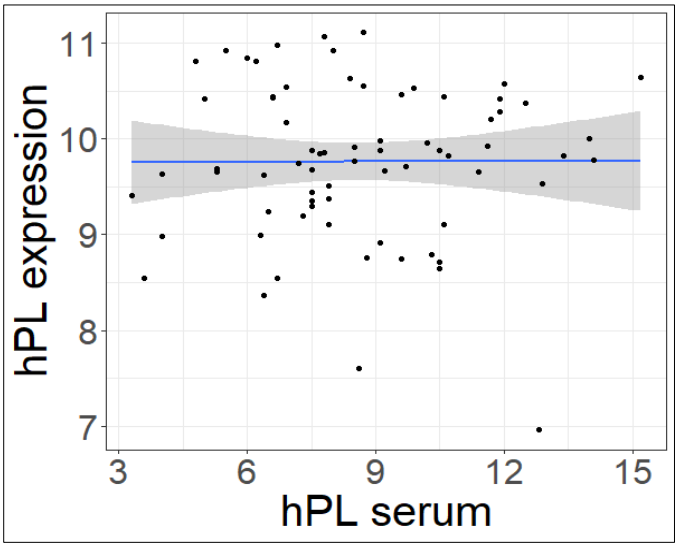

Figure 6.1: Comparison of hPL measurements*. Placental hPL mRNA expression was compared to hPL concentration in maternal serum. There was no significant association between the two measurements. n= 71.*

6.3.3 hPL and demographics

Both hPL measures were compared to maternal demographics and birth outcomes (Table 6.4). In line with previous literature, hPL serum was significantly positively associated with growth measures; birthweight, head circumference and placental weight (Roh *et al.* 2005; Männik *et al.* 2010) and negatively associated with maternal BMI (Vakili *et al.* 2013). hPL serum was additionally positively associated with WIMD score, a measure of deprivation as determined by postcode. WIMD score takes into account indicators such as employment status, health, educational achievement and income. However, individually mental health history, education and income were not significantly associated with serum hPL. Contrasting with serum measurements, *hPL* as measured by mRNA expression was not significantly associated with any maternal demographics or birth outcomes, except for fetal sex, as reported in Chapter 5.

Table 6.4: hPL measurements in relation to maternal socio-demographic factors and birth outcomes

Maternal demographic	***hPL serum***	***Placental hPL expression***
Categorical variables		
Highest level of maternal education*	0.443	0.374
Family income*	0.909	0.206
Season of birth*	0.621	0.058
Smoked in pregnancy	0.785	0.661
Drank alcohol in pregnancy	0.182	0.535
History of mental health problems	0.408	0.597
SSRI prescription	0.879	0.786
Parity	0.348	0.754
Continuous variables		
WIMD	**0.002**	0.542
Maternal age (*years*)	0.278	0.145
Maternal BMI	**0.009**	0.871
Birth outcomes		
Categorical variables		
Indication for ELCS*	0.554	0.506
Fetal sex	0.436	**<0.001**
Continuous variables		
Placental weight (*g*)	**<0.001**	0.925
Gestational age (*days*)	0.238	0.574
CBWC	**<0.001**	0.793
Birthweight (*g*)	**<0.001**	0.250
Head circumference (*cm*)	**0.002**	0.585

[1] *p* values reported
[2] Significant values reported in bold
[3] Spearman's correlations performed for continuous variables, Mann Whitney U tests for categorical variables with two factors, One Way ANOVAs for categorical factors with more than two factors (*)
[4] Numbers: maternal serum= 233, placental mRNA expression= 66

6.3.4 Serum hPL and perinatal mental health

In line with the overall cohort (n= 299, Chapter 3), A1 EPDS and A1 STAI scores recorded at term were strongly associated with EPDS and STAI scores recorded at all three postnatal time points ($p<$ 0.001 for all comparisons; Table 6.5). Hence, prenatal mental health scores were used to determine the confounding variables for testing to simplify analyses.

Table 6.5: Correlations between perinatal mental health scores

	A1 EPDS	***A1 STAI***	***P1 EPDS***	***P1 STAI***	***P2 EPDS***	***P2 STAI***	***Y1 EPDS***	***Y1 STAI***
A1 EPDS	-	-	-	-	-	-	-	-
A1 STAI	0.722; **<0.001**	-	-	-	-	-	-	-
P1 EPDS	0.644; **<0.001**	0.595; **<0.001**	-	-	-	-	-	-
P1 STAI	0.693; **<0.001**	0.793; **<0.001**	0.763; **<0.001**	-	-	-	-	-
P2 EPDS	0.577; **<0.001**	0.523; **<0.001**	0.538; **<0.001**	0.584; **<0.001**	-	-	-	-
P2 STAI	0.633; **<0.001**	0.752; **<0.001**	0.587; **<0.001**	0.697; **<0.001**	0.739; **<0.001**	-	-	-
Y1 EPDS	0.615; **<0.001**	0.548; **<0.001**	0.580; **<0.001**	0.593; **<0.001**	0.607; **<0.001**	0.541; **<0.001**	-	-
Y1 STAI	0.598; **<0.001**	0.729; **<0.001**	0.561; **<0.001**	0.661; **<0.001**	0.536; **<0.001**	0.727; **<0.001**	0.711; **<0.001**	-

[1] Correlation coefficient and *p* values reported
[2] Significant values reported in bold
[3] Taken from Sumption *et al*. 2020

Relationships between prenatal A1 EPDS and STAI scores and potential confounders were assessed separately using single predictor regressions (Table 6.6). Confounders that were associated with A1 EPDS or STAI at $p< 0.15$ were taken forward into multiple linear regression analyses, with A1 EPDS and STAI as dependent variables. A backward selection process was performed to determine the final confounders. 'History of mental health' and 'highest maternal education level' were significantly associated with A1 EPDS. 'History of mental health' and 'WIMD' were significantly associated with A1 STAI. All three variables were taken forward to the adjusted models.

Table 6.6: Confounders and prenatal mental health scores

Demographics	***A1 EPDS***	***A1 STAI***
Categorical variables		
Highest education level		
Left before GCSE	**0.033**	0.177
GCSE or vocational	0.686	0.191
A-level	0.661	0.131
University	**0.025**	0.401
Postgraduate	*Ref.*	*Ref.*
Family income		
< £18,000	**0.003**	**0.003**
£18-25,000	0.355	0.182
£25-43,000	0.374	0.219
> £43,000	*Ref.*	*Ref.*
Season of birth		
Spring	0.846	0.355
Summer	0.130	0.286
Autumn	*Ref.*	*Ref.*
Winter	0.235	0.084
Smoking in pregnancy	**0.025**	**0.040**
Alcohol in pregnancy	0.073	0.069
History of mental health	**<0.001**	**<0.001**
Parity	0.996	0.557
Continuous variables		
Maternal BMI	0.139	0.265
WIMD score [a]	**0.029**	**0.004**
Maternal age (*years*)	0.347	0.476
Gestational age *(days)*	0.692	0.844

[1] *p* values reported
[2] Significant values reported in bold
[3] Taken from Sumption *et al.* 2020

Regressions were used to investigate the relationship between hPL and perinatal depression and anxiety (Table 6.7). In the unadjusted model there were no significant relationships when all samples were analysed together. The analyses was then repeated, splitting by fetal sex. In mothers who had girls, term anxiety scores were associated with reductions in hPL concentration. At ten weeks after birth, depression and anxiety scores were both negatively associated with hPL concentration. When controlling for history of mental health, education and WIMD only the association between hPL and mental health scores ten weeks postnatally remained significant.

Table 6.7: Unadjusted and adjusted multiple linear regression models analysing association between hPL concentration and perinatal maternal mental health scores

Mood scores	***All***			***Male***			***Female***		
	B	***CIs***	***p***	***B***	***CIs***	***p***	***B***	***CIs***	***p***
Unadjusted linear regressions									
A1 EPDS	-0.179	-0.396, 0.038	0.106	-0.040	-0.383, 0.303	0.817	-0.278	-0.560, 0.004	0.053
A1 STAI	-0.172	-0.587, 0.242	0.413	0.357	-0.282, 0.996	0.270	-0.561	-1.100, -0.023	**0.041**
P1 EPDS	-0.158	-0.418, 0.103	0.233	0.005	-0.409, 0.419	0.980	0.308	-0.642, 0.026	0.070
P1 STAI	-0.393	-0.946, 0.160	0.162	-0.349	-1.195, 0.497	0.413	-0.453	-1.190, 0.283	0.224
P2 EPDS	-0.188	-0.473, 0.096	0.193	0.034	-0.457, 0.526	0.889	-0.361	-0.700, -0.022	**0.037**
P2 STAI	-0.485	-1.107, 0.137	0.125	-0.106	-1.166, 0.955	0.842	-0.775	-1.538, -0.012	**0.047**
Y1 EPDS	-0.104	-0.465, 0.257	0.567	0.048	-0.626, 0.721	0.887	-0.195	-0.632, 0.241	0.374
Y1 STAI	-0.358	-1.166, 0.451	0.382	0.073	-1.458, 1.603	0.923	-0.583	-1.549, 0.383	0.232
Adjusted linear regressions									
A1 EPDS	-0.069	-0.280, 0.143	0.523	0.094	-0.233, 0.422	0.569	-0.216	-0.500, 0.069	0.136
A1 STAI	0.012	-0.385, 0.409	0.953	0.513	-0.094, 1.119	0.097	-0.438	-0.966, 0.090	0.103
P1 EPDS	-0.118	-0.382, 0.146	0.377	0.064	-0.373, 0.501	0.771	-0.311	-0.660, 0.037	0.079
P1 STAI	-0.234	-0.797, 0.329	0.413	-0.069	-0.949, 0.811	0.876	-0.510	-1.285, 0.265	0.193
P2 EPDS	-0.101	-0.375, 0.173	0.466	0.250	-0.217, 0.716	0.287	-0.367	-0.679, -0.056	**0.022**
P2 STAI	-0.325	-0.925, 0.274	0.285	0.171	-0.861, 1.203	0.740	-0.776	-1.475, -0.077	**0.030**
Y1 EPDS	0.077	-0.269, 0.423	0.659	0.271	-0.294, 0.836	0.335	-0.117	-0.546, 0.311	0.585
Y1 STAI	-0.032	-0.852, 0.788	0.939	0.420	-1.004, 1.844	0.551	-0.544	-1.518, 0.429	0.429

[1] Significant values reported in bold
[2] Adjusted for history of mental health, WIMD + highest education
[3] CIs: 95% confidence intervals
[4] Taken from Sumption *et al*. 2020

6.3.5 Sensitivity analysis

To account for the possibility that SSRIs were masking the association between perinatal mental health scores and hPL serum concentrations, a sensitivity analyses was run (Table 6.8, Figure 6.2). Out of the 233 participants in the current analysis, 21 were prescribed SSRIs at any point in their pregnancy. Of these 13 had boys and 8 had girls. These participants were removed from the analysis and the unadjusted and adjusted models were re-run for the remaining 212 participants. Within the unadjusted models in this secondary analyses, hPL concentration was significantly associated with A1 EPDS and STAI, P1 EPDS and STAI and P2 EPDS and STAI scores in only mothers who had girls.

In the adjusted model, hPL was significantly negatively associated with EPDS and STAI scores at P1 and P2 time points, again only in mothers with girls.

Table 6.8: Unadjusted linear regression assessing relationship between hPL and perinatal mental health scores, in women not prescribed SSRIs

Mood scores	***All***			***Male***			***Female***		
	B	***CIs***	***p***	***B***	***CIs***	***p***	***B***	***CIs***	***p***
Unadjusted linear regressions									
A1 EPDS	-0.194	-0.405, 0.016	0.070	-0.045	-0.381, 0.291	0.791	-0.297	-0.569, -0.026	**0.032**
A1 STAI	-0.182	-0.569, 0.205	0.356	0.307	-0.321, 0.935	0.334	-0.527	-1.012, -0.042	**0.034**
P1 EPDS	-0.183	-0.435, 0.068	0.151	0.045	-0.356, 0.447	0.822	-0.385	-0.707, -0.062	**0.020**
P1 STAI	-0.530	-1.039, -0.020	**0.042**	-0.323	-1.131, 0.486	0.427	-0.728	-1.389, -0.066	**0.032**
P2 EPDS	-0.181	-0.470, 0.107	0.216	0.084	-0.422, 0.590	0.740	-0.378	-0.720, -0.037	**0.030**
P2 STAI	-0.554	-1.118, 0.010	0.054	-0.177	-1.204, 0.850	0.731	-0.825	-1.477, -0.173	**0.014**
Y1 EPDS	-0.098	-0.447, 0.252	0.580	0.036	-0.681, 0.752	0.919	-0.182	-0.577, 0.213	0.359
Y1 STAI	-0.489	-1.237, 0.260	0.198	-0.191	-1.720, 1.338	0.801	-0.641	-1.496, 0.213	0.138
Adjusted linear regressions									
A1 EPDS	-0.090	-0.301, 0.121	0.400	0.098	-0.236, 0.432	0.561	-0.239	-0.521, 0.043	0.095
A1 STAI	-0.025	-0.413, 0.363	0.901	0.499	-0.134, 1.131	0.121	-0.434	-0.928, 0.060	0.085
P1 EPDS	-0.165	-0.425, 0.097	0.215	0.040	-0.402, 0.483	0.855	-0.357	-0.698, -0.015	**0.041**
P1 STAI	-0.363	-0.902, 0.175	0.184	-0.059	-0.941, 0.822	0.893	-0.718	-1.433, -0.004	**0.049**
P2 EPDS	-0.069	-0.350, 0.212	0.626	0.180	-0.842, 1.202	0.724	-0.737	-1.387, -0.086	**0.027**
P2 STAI	-0.336	-0.898, 0.226	0.238	.334	-0.144, 0.812	0.166	-0.337	-0.658, -0.016	**0.040**
Y1 EPDS	0.065	-0.280, 0.411	0.708	.264	-0.359, 0.888	0.391	-0.077	-0.489, 0.334	0.707
Y1 STAI	-0.288	-1.076, 0.499	0.468	.037	-1.417, 1.491	0.959	-0.588	-1.510, 0.335	0.207

[1] Significant values reported in bold
[2] Adjusted for history of mental health, WIMD + highest education
[3] CIs: 95% confidence intervals
[4] Taken from Sumption *et al*. 2020

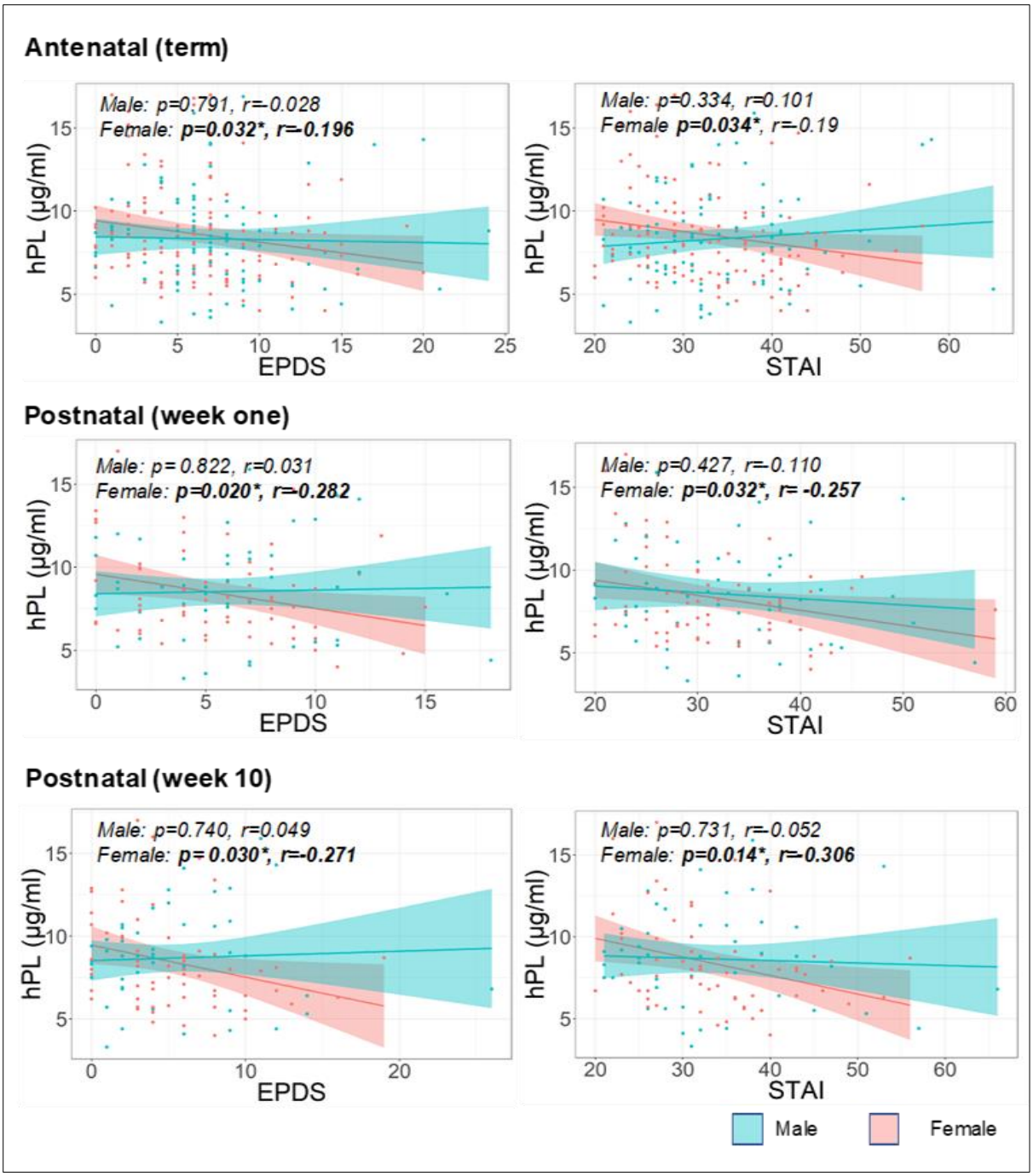

Figure 6.2: Maternal serum hPL values correlate with EPDS and STAI scores for mothers of girls. *Spearman's correlation of maternal serum hPL and perinatal mental health scores in women not prescribed SSRIs in pregnancy. Male and female data analysed separately. Line of fit is linear model. Taken from Sumption* et al. *2020.*

To compare the relationship between hPL and the different postnatal mental health scores, standardised z-scores were created for EPDS and STAI scores. Comparing B-values of adjusted regressions, hPL had a greater influence over P1 EPDS score than P1 STAI score (-0.073 vs -0.006

respectively). At the P2 time point, hPL had a similar influence over EPDS and STAI scores (-0.071 vs -0.068 respectively.

6.3.6 hPL serum and fetal sex

The sexually dimorphic relationship observed was not a consequence of inherently different serum hPL concentrations between mothers carrying boys and girls. For those scoring below the cut offs for depression and anxiety excluding those prescribed SSRIs, the mean hPL serum from mothers carrying boys was 8.291 and in those carrying girls was 8.795 (p= 0.448).

6.3.7 Controlling for prenatal mental health

In order to simplify analysis, the same confounders were used when analysing perinatal depression and anxiety. Therefore, prenatal mental health was not controlled for in the postnatal analysis. Within the sensitivity analysis samples, prenatal depression and anxiety scores were included as additional confounders in postnatal anxiety and depression models respectively. No multivariate regressions remained significant after controlling for prenatal mental health. However, as seen in Chapter 3, maternal mental health and previous mental health history were strongly correlated introducing strong multicolinearity into the model between independent variables.

6.3.8 hPL serum and *PEG3* expression

hPL expression and *PEG3* expression were not associated in Chapter 5. To determine if this lack of association was limited to expression levels, serum hPL concentrations were analysed with respect to *PEG3* expression. There was no significant correlation between hPL serum and *PEG3* expression including or excluding participants prescribed SSRIs.

6.3.9 *hPL* expression and postnatal depression

The analysis of the serum data revealed a relationship between serum hPL and postnatal mental health, but not prenatal mental health. *hPL* expression data from Chapter 5 was analysed with respect to postnatal depression in the depression subset (Table 6.9). There was no relationship between postnatal depression and placental *hPL* gene expression when all samples were analysed or when participants prescribed SSRIs were removed.

Table 6.9: Correlations between hPL *expression and postnatal depression*

All samples	***r***	***p***	***n***
P1 EPDS	-0.141	0.385	40
P2 EPDS	0.016	0.927	36
Sensitivity analysis			
P1 EPDS	-0.118	0.504	34
P2 EPDS	0.115	0.553	29

6.3.10 Summary of chapter results

Table 6.10: Summary of Chapter 6

Finding	***Notes***
Serum hPL and mRNA expression *hPL* not correlated	
Serum hPL associated with postnatal depression and anxiety scores at 10 weeks postnatally	Only in women who had girls
Serum hPL associated with postnatal depression and anxiety scores at 4 days and 10 weeks postnatally	Only in women who had girls and were not prescribed SSRIs in pregnancy
No significant difference in hPL serum concentration between women carrying boys or girls	

6.4 Discussion

This chapter set out to further characterise the relationship between hPL and perinatal mental health in the Grown in Wales cohort. A key finding from this analyses was that serum hPL was associated with postnatal depression and anxiety symptoms, but only in mothers who had girls. hPL was negatively associated with EPDS and STAI scores four days and ten weeks after birth, but only when samples from participants prescribed SSRIs were removed from the analysis. There was no relationship between maternal serum hPL and perinatal mental health in mothers with boys. Finally there was no association between maternal serum hPL and expression levels of placental *hPL*.

Results of the analysis between serum hPL and perinatal mental health has been published (Sumption *et al.* 2020). Briefly, this was the first report of a relationship between serum levels of hPL and maternal mental health in humans. Though units of change were small, serum hPL was negatively associated with maternal postnatal depression and anxiety scores up until ten weeks after birth. This is consistent with a previous finding of lower placental *hPL* expression in mothers with prenatal depression (Janssen *et al.* 2016). Although Janssen *et al*. did not assess anxiety, depression and anxiety are often comorbid (Gorman 1996) and it is likely that the women in the earlier study were both depressed and anxious. Both placental lactogen and the related pituitary hormone prolactin have well-established roles in the priming of rodent maternal behaviours (Handwerger and Freemark 1987; Creeth *et al.* 2019). Administration of prolactin stimulates caregiving behaviours and dams with blocked prolactin receptors do not adequately prepare for motherhood (Bridges *et al.* 1990; Lucas *et al.* 1998). hPL and prolactin are structurally similar and hPL can bind with a strong affinity to the prolactin receptor. It is thus hypothesised to have similar downstream effects (Freemark 2010; Newbern and Freemark 2011). Consistent with the current findings of a relationship between hPL and postnatal anxiety scores in human mothers, reduced prolactin has previously been linked with postnatal anxiety in mice (Larsen and Grattan 2010). A previous study showed that changes to the placental hormone-producing cells in the mouse placenta can alter the behaviour of wild-type mouse

mothers (Creeth *et al.* 2018). In a another study, a reduction in the placental endocrine compartment was associated with increased postnatal anxiety in mice (McNamara *et al.* 2018). Together, these data suggest that low hPL, as observed in the present study, plays a causal role in driving postnatal depression and anxiety.

An additional significant finding of this chapter was that removing samples from women prescribed SSRIs during pregnancy strengthened the association between serum hPL and postnatal mental health. SSRIs block the reuptake of serotonin by pre-synaptic neurones and increase the available serotonin (Sangkuhl *et al.* 2009). In rodents, serotonin is thought to increase prolactin concentrations by indirectly stimulating the pituitary gland (Kamberi *et al.* 1971; Gil-ad *et al.* 1976). In humans the literature is more conflicting (Sagud *et al.* 2002; Coker and Taylor 2010) but independently all SSRIs have been associated with hyperprolactinemia (Emiliano and Fudge 2004) and a large pharmacoepidemiological study described a relationship between SSRIs and an increase in serum prolactin levels (Petit *et al.* 2003). The relationship between maternal hPL and SSRIs had not previously been studied in the perinatal period. A mechanism of SSRIs increasing prolactin levels could compensate for reduced hPL in women reporting symptoms of depression and anxiety. This could explain the stronger relationship between hPL and maternal mental health in the sensitivity analysis once the confounding action of SSRIs was removed.

The analysis in Chapter 5 revealed a positive association between prenatal anxiety and placental *hPL* expression scores, specifically in pregnancies where the mother was carrying a girl. While hPL serum was not significantly associated with prenatal scores after adjustment, hPL serum was negatively associated with postnatal anxiety and depression. In addition there was no correlation between serum and expression levels of hPL in participants that had data available for both measurements. Differences in expression and serum levels of placental hormones have previously been reported in human studies (Koutsaki *et al.* 2011). Multiple factors can affect final protein concentrations and it has been estimated that mRNA levels may only account for 20-40% of differences in protein concentrations (Brockmann *et al.* 2007). Posttranscriptional modifications, regulation of translation, rate of secretion and protein stability may all affect final serum hPL concentrations. For example *in vitro*, an influx of calcium ions causes an increased and sustained secretion of hPL (Petit *et al.* 1993; Meuris *et al.* 1994; Petit and Belisle 1995) and the immunosuppressive glycoprotein glycodelin A suppresses hPL secretion (Jeschke *et al.* 2002). Therefore, it is possible that another factor may additionally influence maternal hPL serum levels. Alternatively, the lack of correlation between *hPL* gene expression and protein may be due to the sample size of the qPCR expression analysis. Generating qPCR data for all the sample for which the serum data was available would address this question.

The relationship between serum hPL and postnatal mental health was only observed in mothers who had girls. It was not the case that serum levels were intrinsically lower in females as no difference was observed in the levels of serum hPL between "control" mothers carrying boys and girls. However, there were higher levels of placental *hPL* expression in female placentas (Chapter 5). This could suggest that the female placenta is working harder to manufacture *hPL* mRNA but failing to generate enough hPL protein. Sexually dimorphic relationships between hPL and other pregnancy complications have previously been reported. In SGA pregnancies where the baby was female, lower hPL serum was observed (Lagerström *et al.* 1990). Additionally, in mothers who smoked during pregnancy hPL was significantly lower but again only in those who had girls (Bremme *et al.* 1990). These sexually-biased outcomes may reflect endocrine responses inherent to male or female placentas when under stress.

6.4.1 Limitations

A key limitation of this study was the sample size, particularly at the later time points (P2= 124, Y1= 95). No association between serum hPL at term and mental health 12 months after birth was apparent. However, these numbers are smaller and it will be important to repeat this study within a larger cohort. The significant associations between hPL and mental health were only seen in women who carried girls. This group at each time point had more samples than those carrying boys. Increasing the sample size for boys might uncover the same significant relationships in this group. Secondly, serum hPL was only measured at one time point, just prior to an ELCS. Given hPL concentrations change over time (Peake *et al.* 1983), it will be important to measure levels over the course of pregnancy. The final limitation of this analysis is the small sample size used to compare *hPL* expression and hPL serum concentration. Although results appear contradictory, there were demographic differences between datasets which may explain this. Alternatively, there may be a feedback mechanism between serum hPL and gene expression in the placenta, which would be interesting to pursue in a future study.

6.4.2 Conclusions

This chapter set out to assess the relationship between serum hPL and maternally reported symptoms of perinatal depression and anxiety. Serum hPL was negatively associated with postnatal depression and anxiety scores, and this relationship was only significant in mothers carrying girls and not boys. Removing participants who were prescribed SSRIs during pregnancy through a sensitivity analysis strengthened this relationship. However, there was no association between placental *hPL* expression and hPL serum concentrations suggesting posttranscriptional mechanisms may affect final serum concentrations.

Chapter 7: Infants

7.1 Introduction

It is well documented that children exposed *in utero* to maternal stress are at increased risk of adverse outcomes including low birth weight, impaired development and atypical behaviour (Field 2011; Kingston *et al.* 2012). Prenatal psychological stressors such as depression and anxiety have been associated with increased crying, difficult behaviours early in life, and impaired attention, cognition and language (Huizink *et al.* 2002; Laplante *et al.* 2004; Van den Bergh *et al.* 2005; Wurmser *et al.* 2006; Talge *et al.* 2007; Keim *et al.* 2011; Van Batenburg-Eddes *et al.* 2012; Raikkonen *et al.* 2015; Lahti *et al.* 2017). The impact of exposure to these stressors continues past infanthood with impaired attention observed at seven years and in adolescence an increased risk of violent behaviours and affective disorders such as depression and anxiety (Gutteling *et al.* 2006; Pawlby *et al.* 2009; Hay *et al.* 2010; Plant *et al.* 2015). Prenatal psychosocial stress studies extending beyond offspring adolescence are rare, but impaired temperament and internalising behaviours earlier in life have been independently linked to adult eating disorders, leadership potential, depression recurrence, poor SRH, chronic stress, substance abuse, less life satisfaction and poor coping skills (Wright-Guerina *et al.* 2011; Naicker *et al.* 2013; Essaua *et al.* 2014; Burt *et al.* 2015). Whilst the presence of prenatal depression, determined through clinical diagnosis or self-reporting questionnaires, has been linked to adverse infant outcomes, the severity of the stressor is also associated with infant impairment (Brennan *et al.* 2000). Furthermore, subclinical levels of prenatal depression have been linked with adverse infant phenotypes (Meaney 2018) suggesting the relationship acts on a spectrum.

Variability of diagnosis criteria, time of exposure and statistical analysis approach can lead to conflicting reports on the effects of prenatal depression and anxiety on exposed children (Field 2011; Kingston *et al.* 2012). For example, exposure to prenatal depression in the second trimester was associated with childhood cortical thickness in regions linked to inhibition and attention, but not when the exposure was experienced in the first or third trimesters (Lebel *et al.* 2016). This suggests there may be sensitive time points within pregnancy that correspond with neuronal development. Rodent studies can remove certain confounding effects such as time of exposure and heritable characteristics. Studies have shown that prenatal stress in dams causes impaired sleep and social interactions in offspring as well as anxiety and depression-like behaviour (Weinstock 2001; Darnaudery and Maccari 2008). However, unlike humans a significant portion of rodent offspring neurodevelopment occurs postnatally (Maier *et al.* 1997; Matthews 2002). Therefore, postnatal maternal behaviour will have a bigger influence, thus questioning the translatability of rodent studies for this field of research.

As reported in Chapter 3, prenatal depression and anxiety are a strong predictors of postnatal mental health. Postnatal depression is associated with similar infant outcomes to prenatal depression including impaired motor development, cognition and social development (Cornish *et al.* 2005; Netsi *et al.* 2018; Slomian *et al.* 2019). Poor mother-infant bonding has been suggested as a mediator between postnatal depression and infant behaviour, separate to prenatal mental health and infant

outcomes (Fransson *et al.* 2020). Furthermore, studies have shown that the effects of poor prenatal mental health are independent and additive to the effects of postnatal mental health upon infant development (O'Connor *et al.* 2002; Hentges *et al.* 2019). Therefore, prenatal mental health disorders likely impacts offspring development through different mechanistic pathways to postnatal mental health disorders.

An additional confounder to understanding the relationship between prenatal depression and infant development is infant sex. Differences in fetal and placental development between sexes have previously been reported in healthy pregnancies (Cvitic *et al.* 2013; Gong *et al.* 2018; Gonzalez *et al.* 2018), and described in relation to stressors (Bale 2011; Dunn *et al.* 2011; Osei-Kumah *et al.* 2011; Howerton and Bale 2012) and Chapters 4 & 5. However, only a limited proportion of the current literature separates analysis by fetal sex. Those that have found boys exposed to prenatal depression had worse motor and regulatory control, increased attention deficits and externalising behaviours, and poorer cognitive development when exposed to prenatal depression and anxiety (Murray *et al.* 2010; Gerardin *et al.* 2011; Glover and Hill 2012). One study reported that girls and boys exposed to prenatal depression were equally likely to present cognitive delays at 18 months (Deave *et al.* 2008), but a different study showed by 16 years only boys had poorer cognition (Murray *et al.* 2010). This suggests longer-term consequences of exposure to prenatal stress for boys compared to girls. Boys are often reported as more at risk from ADHD and ASD when exposed to prenatal stress (Kinney *et al.* 2007; Li *et al.* 2010; Ronald *et al.* 2011). However, this has been challenged as symptoms in girls are often misinterpreted resulting in a bias in clinical diagnosis between boys and girls (Au-Yeung *et al.* 2019; Vildalen *et al.* 2019; Hull *et al.* 2020). Alternatively, girls exposed to prenatal stress have higher observed fear at eight months and an increased risk of internalising behaviours and affective disorders such as depression and anxiety in adolescence (Boyle and Pickles 1997; Quarini *et al.* 2016; Soe *et al.* 2016; Nolvi *et al.* 2019). Current literature does not indicate that either sex is more sensitive to prenatal stressors, potentially again due to the heterogeneity of data collected.

Prenatal depression and anxiety are a complex disorders, and the mechanism behind their relationship with adverse infant outcomes is not understood. However, there is a link between prenatal mental health and alterations in placental gene expression (Janssen *et al.* 2016; Litzky *et al.* 2018; Edvinsson *et al.* 2019) and Chapter 4. Placental dysfunction may influence neurodevelopment by restricting nutrient supply to the rapidly developing fetal brain (Miller *et al.* 2016). In addition, the placenta acts as a super-endocrine organ secreting hormones to support the mother during the perinatal period which can influence maternal behaviour as shown in mice (Creeth *et al.* 2018; McNamara *et al.* 2018). Hormones produced by the placenta may also directly influence fetal brain development, as has been shown for placental serotonin in mice (Bonnin *et al.* 2011). Therefore, there are many routes whereby the placenta could influence infant neurodevelopment. Previously studies have shown that alterations in placental HPA axis genes and mitochondrial genes were associated with prenatal

psychosocial stress and adverse infant temperament (O'Donnell *et al.* 2009; Lambertini *et al.* 2015). However, given the complexity and heterogeneity of both maternal psychological stressors and infant development, mediation may occur through multiple mechanistic pathways.

Imprinted genes have been proposed to act as mediators between the prenatal environment and later life outcomes as they can respond to environmental stimuli and a number of genes are co-expressed in the placenta and developing hypothalamus (Keverne 2014). Imprinted genes expressed in the placenta have roles in the regulation of birthweight (Kappil *et al.* 2015) which is commonly concurrent with prenatal depression and anxiety (Diego *et al.* 2006; Field *et al.* 2006). The expression of ten imprinted genes have been associated with neonatal neurodevelopment, although the relationship with prenatal mental health was not analysed (Green *et al.* 2015). To date one study has investigated imprinted gene expression in relation to both prenatal stress and neonatal development but found no mediating effect (Litzky *et al.* 2018). However, later infant phenotypes were not tested and analysis was not separated by infant sex. Chapter 5 replicated a previous finding that placental expression of the imprinted gene *PEG3* was decreased in relation to prenatal depression (Janssen *et al.* 2016). Furthermore, methylation of *PEG3* in infant cord blood has independently been linked to infant temperament (Fuemmeler *et al.* 2016). *PEG3* is therefore a candidate linking prenatal stress and adverse infant outcomes. This could be a direct relationship with lower *PEG3* influencing fetal brain development and behaviour, as has been suggested from mouse studies (Li *et al.* 1999). Alternatively, as *PEG3* is hypothesised to regulate the placental hormone *hPL* (Janssen *et al.* 2016) and in Chapter 6, lower serum hPL was associated with postnatal mental health symptoms, there might be an indirect relationship whereby placental endocrine insufficiency acts via maternal mood to influence the offspring's neurodevelopment.

The aims of the current chapter were to assess the relationship between prenatal mental health scores and infant development through maternally reported questionnaires and independent observations. Given the differences reported with respect to offspring sex in placental gene expression and adverse infant outcomes previously described, the impact of fetal sex was explored. This analysis has been published in the *Journal of Affective Disorders* (Savory *et al.* 2020). For this publication I contributed in part towards infant assessments, data analysis and manuscript preparation. The second aim of the current chapter was to assess infant outcomes observed in the first aim in relation to biological data. Placental expression of *PEG3*, and placental expression and maternal serum concentrations of the placental hormone hPL were examined.

7.2 Methods

Methods for the recruitment in the Grown in Wales (GiW) study were described in Chapter 2.

For ease of interpretation, collection times are summarised in Table 7.1.

Table 7.1: Data collection time points

Time	***Reference***
Day of pre-surgical appointment (antenatal)	A1
Within one week of birth (average 4.2 days)	P1
Ten weeks after birth	P2
One year after birth	Y1

7.2.1 Y1 Questionnaire

Approximately 12 months postpartum, GiW participants completed a further questionnaire containing EPDS and STAI scales, general demographic questions and questions regarding the mother's perception of her infant. Of the 126 participants who returned this questionnaire, 6 were not of White ethnicity, 3 did not ultimately deliver by ELCS, 1 infant was visually impaired, 1 was born preterm, 1 infant did not have their age recorded and 1 infant was 18 months at the time of completion. The remaining 113 participants were included in the analysis of the questionnaire.

7.2.1.1 Bonding

Postnatal bonding between mother and infant was assessed by the Postnatal Bonding Questionnaire (PBQ) (Brockington *et al.* 2006). This is a 25 item questionnaire comprising of four clinically relevant factors and an overall total reflecting a mother's feelings or attitudes towards her baby. It was designed to identify disturbances in bonding that could lead to a poor mother-infant relationship. Items are scored on a six-point Likert scale from '*always'* to '*never'* with lower scores indicative of better bonding between the dyad.

Factor one identifies general problems with bonding, for example "*I feel close to my baby*" and "*my baby winds me up*". Factor two assesses rejection and anger, for example "*I feel distant from my baby*" and "*my baby annoys me*". Factor three measures infant-focussed anxiety about care, for example "*I am afraid of my baby*" and "*I feel confident when caring for my baby*". Factor four measures risk of abuse and was not included as it has been shown to have little validity and questions are of an extreme nature (Wittkowski *et al.* 2007). In the current analysis factors one, two and three, and the overall total were analysed. The PBQ has acceptable internal consistency with one validation study reporting the Cronbach's alpha for the total PBQ to be 0.76 (Wittkowski *et al.* 2007). For the present study, the Cronbach's alpha was good at 0.83.

7.2.1.2 Aggression

Infant aggression was measured using the Cardiff Infant Contentiousness Scale (CICS) (Hay *et al.* 2006). CICS was developed to identify early manifestations of aggression as childhood aggression has been linked with later life behavioural problems (Cummings *et al.* 1989). The questionnaire is comprised of 5 questions embedded inside a list of 20 other typical infant development questions.

Higher scores represent higher levels of aggression. For the present study, the Cronbach's alpha of the CICS was 0.73.

7.2.1.3 Temperament

Infant temperament was assessed using the Infant Behavioural Questionnaire Revised-Short Form (IBQ-R-SF) (Rothbart 1981; Gartstein and Rothbart 2003; Putnam *et al.* 2014). The IBQ-R-SF is comprised of 91 questions measuring 14 subscales such as 'smiling' and 'orienting' that load onto three main dimensions; Negative Affect, Regulatory Capacity and Surgency (extroversion) (Rothbart 2011). Questions report on the frequency of a behaviour (such as crying, laughing or smiling) over the previous week in different situations (such as whilst bathing, playing or changing) on a seven-point Likert scale. The IBQ-R-SF has good reliability and validity across populations with Cronbach's alpha ranging from 0.64 to 0.86 in previous studies (Putnam *et al.* 2014). For the present study, the average Cronbach's alpha for the 14 subscales was 0.76, with scores for individual subscales reported in Table 7.2. Four questions from the soothability subscale were unintentionally removed during printing of the questionnaire.

Table 7.2: Inter-reliability measures for IBQ-R-SF subscales

Measure	***Cronbach's alpha***
Activity	0.73
Distress	0.80
Fear	0.80
Orienting	0.74
Smiling	0.73
High Intensity Pleasure	0.85
Low Intensity Pleasure	0.75
Soothability	0.71
Falling Reactivity	0.83
Cuddliness	0.65
Sensitivity	0.86
Sadness	0.75
Approach	0.63
Vocal Reactivity	0.78

7.2.2 Infant Laboratory Assessment

In addition to the questionnaire, at twelve months after birth participants were invited to attend a laboratory based assessment with their infants. Of the 83 participants that attended an assessment, seven were removed from the analysis as either they were not White, did not deliver by ELCS, the child was born prematurely or the father attended. Of these 76 participants, 71 also completed the Y1 questionnaire.

7.2.2.1 Setting

Mothers and infants were assessed in an experimental testing room by two trained researchers unaware of prenatal mental health scores. The assessment was recorded using a digital camera placed in the corner of a room to allow for subsequent coding. Infant behaviour was coded using Laboratory Temperament Assessment Battery (Lab-TAB) (Goldsmith and Rothbart 1996; Planalp *et al.* 2017) and the Bayley's Scales of Infant Development Third Edition (Michalec 2011).

7.2.2.2 Lab-TAB

The Lab-TAB coding system measures infant responses in different tasks within a controlled setting by independent researchers. Episodes are standardised and designed to measure five dimensions of temperament; fear, persistence, anger, activity and joy. Six episodes were carried out in the assessment; Free Play, Collaboration, Sustained Attention, Maternal Separation, Novel Toy, and Joy. For analysis in the current chapter Maternal Separation, Novel Toy and Sustained Attention were analysed. Coding of these tasks was in line with published Lab-TAB coding (Planalp *et al.* 2017).

7.2.2.3 Novel Toy Task

The Novel Toy Task is an adaptation of the Unpredictable Mechanical Toy Task from the Lab-TAB manual which was designed to elicit fear (Goldsmith and Rothbart 1996). Infants were sat in a chair and a remote-controlled robot toy was brought into the room. The mother sat behind the infant and was asked to remain uninvolved with the task. The robot was placed approximately 1 M from the infant's chair. It then walked towards the infant stopping approximately 20 cm from the chair, pausing for 10 seconds, walking backwards to the starting position and again pausing for 5 seconds. This was repeated three times. Afterwards the infant was able to interact with the robot. If the task was stopped due to the infant's distress, coding was continued with the score in the previous epoch. From this task, 'facial fear', 'distress', 'bodily fear', 'escape', 'startle' and 'parent behaviour' was measured.

7.2.2.4 Sustained Attention Task

The Sustained Attention Task was an adaptation of the Lab-TAB Repeated Visual Stimulation Task that measures how long an infant gazes at or manipulates an object (Goldsmith and Rothbart 1996). In this task a wind-up carousel was used. The infant was sat in a chair with the mother behind who was again requested to remain uninvolved. The carousel was placed 40 cm from the chair and set to play for approximately 3 minutes. If the task was stopped due to the infant's distress, coding was continued with the score in the previous epoch. From this task, 'facial interest', 'duration of looking', 'gestures', 'parent behaviour', 'infant positive affect', 'infant negative affect' and 'latency to look away' from the carousel was measured.

7.2.2.5 Maternal Separation Task

In the Maternal Separation Task, mothers were asked to leave the room in a manner they would normally do at home, for example by saying goodbye or walking out silently. One of the researchers

left the room with the mother and together watched from behind a one-way mirror. One researcher remained in the room for safety but did not interact with the infant. The infant had two minutes to play with toys available to them in the assessment room. From this task, 'facial fear', 'distress', 'latency to fear', 'bodily fear' and 'escape' were coded.

7.2.2.6 Infant Neurodevelopment

Age-standardised cognition, receptive language and expressive language were measured using the Bayley Scales of Infant Development Third Edition (BSID-III). Cognition was measured after the Lab-TAB tasks and language was measured throughout the assessment. The Bayley Scales are commonly used to measure infant development through standardised tasks. Each of the three composite scores; cognition, receptive language and expressive language, were created from five raw scores and standardised to infant age.

7.2.3 Statistical Analysis

Results in the current chapter have already been published (Savory *et al.* 2020). The following statistical analysis was performed in the published study.

Normality was assessed using Shapiro-Wilk tests, Kolmogorov-Smirnov tests, histograms and normal Q-Q plots, and was found not to be normally distributed. Therefore, non-parametric tests were used where appropriate. Frequencies and means were reported for categorical and continuous demographic variables respectively.

Chi-squared tests and Spearman's correlations were used to analyse demographic data for categorical and continuous data respectively.

Continuous scales of all measurements were used over categorical cut offs to maximise statistical power. Relationships between prenatal mental health scores (EPDS and STAI) and questionnaire data (PBQ, CICS and IBQ-R-SF) were analysed using partial correlations adjusted for infant age at the time of the questionnaire. Partial correlations were repeated separating by infant sex. Relationships significant at $p< 0.05$ were subsequently analysed through linear regressions controlling for infant age and maternal parity. Parity has previously been suggested to affect early mother-infant relationships (Fish and Stifler 1993).

The relationship between prenatal mental health scores and assessment data (Bayley and Lab-TAB) was analysed in the same manner; partial correlations controlling for infant age at time of assessment and linear regressions additionally controlling for parity for significant results. As the sample size was too small to add additional confounding variables, the analysis of assessment data was re-run using postnatal EPDS and STAI scores at 12 months to assess the time point with the most influence over infant outcomes.

The following analysis is separate to the published data (Savory *et al.* 2020).

The relationship between prenatal EPDS and STAI scores, and maternal demographics and infant outcomes 12 months postpartum were assessed. Partial correlations and logistic regressions were used for continuous and categorical variables, respectively, whilst controlling for infant age.

Questionnaire and assessment variables were analysed in relation to placental gene expression of *PEG3* and *hPL* and maternal serum measurements of hPL. Placental gene expression methods are available from Chapters 2 and 5. *PEG3* and *hPL* mRNA expression was available for 70 participants who completed the questionnaire and 63 who took part in an assessment. 95 participants who completed a questionnaire and 66 participants who took part in an assessment had hPL serum measurements.

Questionnaire and assessment variables were analysed in relation to gene expression using the same statistical procedure as used in the published paper (Savory *et al.* 2020); partial correlations controlling for infant age and subsequently linear regressions controlling for maternal parity for significant partial correlations.

7.3 Results

7.3.1 Demographics

Demographics for the 113 participants who completed a Y1 questionnaire, and for the 76 who participated in an assessment are provided (Table 7.3).

Table 7.3: Demographics and birth outcomes

Demographics	***% or median***	
	Questionnaire Responders (n= 113)	***Assessment Attendees (n= 76)***
Categorical variables		
Highest education level		
Left before GCSE	0.0	0.0
GCSE or vocational	17.3	13.7
A-level	12.7	12.3
University	35.5	37.0
Postgraduate	34.5	37.0
Family income		
< £18,000	2.0	1.4
£18-25,000	5.9	4.3
£25-43,000	21.6	17.4
> £43,000	70.6	76.8
Season of birth		
Spring	20.4	26.3
Summer	21.2	21.1
Autumn	36.3	35.5
Winter	22.1	17.1
Smoked in pregnancy		
No	95.6	97.4
Yes	4.4	2.6
Drank alcohol in pregnancy		
No	53.2	56.8
Yes	46.8	43.2
History of mental health problems		
No	71.7	69.7
Yes	28.3	30.3
Prescribed SSRIs in pregnancy		
No	93.8	93.4
Yes	6.2	6.6
Parity		
Nulliparous	26.5	25.0
Multiparous	73.5	75.0
Fetal sex		
Female	59.3	53.9
Male	40.7	46.1
Occupation status		
Working	81.3	73.9
Not working	18.8	26.1
Gene expression data available		
Yes	61.9	82.9
No	38.1	17.1
hPL maternal serum data available		
Yes	84.1	86.8
No	15.9	13.2
Continuous variables		
A1 EPDS	7.0	6.0
A1 STAI	33.0	33.0
WIMD score	1535	1352.0
Maternal age (*years*)	36.0	36.0

BMI at booking	26.4	26.3
Gestational age *(days)*	274.0	274.0
Custom birthweight centile	59.9	59.9
Birth weight *(g)*	3540.0	3460.0
Placental weight *(g)*	681.0	678.5
Head circumference *(cm)*	35.5	35.0
Infant age at year 1 (*months*)	12.0	12.0

[1] Categorical variables represented as percentages, continuous variables represented as means
[2] Adapted from Savory et al. 2020

7.3.2 A1 scores and maternal characteristics at Y1

Depression and anxiety scores at term were assessed for their relationship with maternal variables 12 months after birth (Table 7.4). Both EPDS and STAI were significantly associated with an increased risk of excess weight retention (≥ 5 kg) from pre-pregnancy to 12 months postpartum (p= 0.001 and p= 0.003 respectively). Both mental health scores were also significantly associated with an increased risk of developing a mental health disorder after birth (EPDS p< 0.001, STAI p= 0.001). Prenatal STAI scores were significantly associated with whether the mother breastfed or not only in those who had boys (p= 0.037).

Table 7.4: Prenatal mental health scores and maternal outcomes at 12 months postpartum

Variable	***A1 EPDS***			***A1 STAI***		
	All	***Male***	***Female***	***All***	***Male***	***Female***
Categorical Variables						
Retention of ≥ 5kg from pre-pregnancy to Y1	1.198; **0.001**	1.242; **0.025**	1.185; **0.017**	1.103; **0.003**	1.101; 0.052	1.113; **0.023**
Working status at Y1	1.062; 0.216	1.129; 0.112	1.010; 0.895	1.050; 0.072	1.066; 0.117	1.046; 0.237
Mood disorder since birth of child	0.793; **<0.001**	0.644; **0.008**	0.834; **0.007**	0.900; **0.001**	0.819; **0.012**	0.926; **0.035**
Breastfed	0.920; 0.050	0.885; 0.094	0.940; 0.251	0.956; 0.063	0.917; **0.037**	0.980; 0.498

[1] Exp(B) and p values reported
[2] Significant values reported in bold
[3] Controlling for infant age at time of questionnaire

7.3.3 Prenatal mental health and infant anthropometric measures

Maternal mental health scores at A1 were also assessed for their relationship with infant outcomes at 12 months postpartum (Table 7.5). There were no significant relationships between prenatal mental health and postnatal infant growth when all participant data was analysed, or when separated by infant sex. There was additionally no relationship with the onset of an infant health problem after birth.

Table 7.5: Prenatal mental health scores and infant anthropometric measures at 12 months postpartum

Variable	***A1 EPDS***			***A1 STAI***		
	All	***Male***	***Female***	***All***	***Male***	***Female***
Continuous Variables-Partial correlations						
Infant weight	0.044; 0.705	0.035; 0.844	0.194; 0.229	0.084; 0.473	0.183; 0.301	0.108; 0.508
Infant growth (*kg*)	0.047; 0.691	0.043; 0.811	0.170; 0.294	0.076; 0.517	0.147; 0.406	0.112; 0.493
Infant AC (*cm*)	-0.050; 0.670	-0.153; 0.387	0.064; 0.697	-0.080; 0.497	-0.194; 0.272	0.039; 0.813
Infant HC (*cm*)	0.098; 0.414	0.037; 0.838	0.177; 0.288	0.150; 0.209	0.144; 0.424	0.218; 0.188
Categorical Variables-Binomial regressions						
Health problems since birth of child	0.958; 0.290	1.041; 0.562	0.909; 0.076	0.977; 0.301	1.005; 0.884	0.956; 0.140

[1] *r* and *p* values reported for continuous variables
[2] Exp(B) and p values for categorical variables
[3] Significant values reported in bold
[4] Controlled for infant age at time of questionnaire

7.3.4 Prenatal mental health and infant outcomes

7.3.4.1 Questionnaire

Partial correlations were performed for A1 mental health scores against maternally reported infant variables (Table 7.6). When all samples were analysed together, EPDS and STAI scores were significantly associated with PBQ1 and PBQ2 of the postnatal bonding questionnaire and with the CICS aggression score. Within the IBQ-R-SF, EPDS and STAI were associated with the Negativity dimension and individual subscales of 'sadness', 'distress', and 'soothability'. When results were analysed separately by infant gender, only female infant bonding, aggression and Negativity were associated with prenatal depression and anxiety scores. For boys, 'approach' and 'vocal reactivity' within the IBQ-R-SF were associated with prenatal depressive symptoms.

Table 7.6: Prenatal mental health scores and maternal perception of infants at 12 months postpartum

	A1 EPDS			***A1 STAI***		
	All	***Male***	***Female***	***All***	***Male***	***Female***
PBQ1	**0.001**	0.916	**<0.001**	**0.012**	0.310	**0.035**
PBQ2	0.083	0.847	0.074	0.071	0.115	0.397
PBQ3	**0.048**	0.542	**0.042**	**<0.001**	0.104	**0.001**
CICS	**0.004**	0.525	**0.003**	**0.013**	0.117	0.055
NEG	**0.002**	0.102	**0.016**	**0.006**	0.097	**0.047**
Sadness	**0.002**	0.359	**0.002**	**0.002**	0.093	**0.010**
Distress	**0.007**	0.592	**0.011**	**0.002**	0.356	**0.016**
Fear	0.339	0.082	0.594	0.495	0.228	**0.595**
Falling Reactivity	**0.030**	0.285	0.052	0.156	0.528	0.178
SUR	0.308	0.973	0.118	0.741	0.255	0.479
Approach	0.237	**0.047**	0.665	0.284	0.170	0.914
Vocal Reactivity	0.068	**0.044**	0.312	0.610	0.531	0.684
High Intensity Pleasure	0.865	0.656	0.502	0.068	0.077	0.677
Smiling	0.215	0.301	0.442	0.349	0.752	0.337
Activity	0.101	0.745	0.085	0.326	0.805	0.149
Sensitivity	0.976	0.273	0.195	0.967	0.230	0.137
REG	0.655	0.789	0.783	0.383	0.524	0.527
Low Intensity Pleasure	0.776	0.990	0.597	0.913	0.759	0.839
Cuddliness	0.471	0.586	0.581	0.277	0.557	0.318
Orienting	0.090	0.494	0.098	0.094	0.302	0.239
Soothability	**0.007**	0.301	**0.013**	**0.003**	0.054	**0.035**

[1] *p* values reported
[2] Significant values reported in bold
[3] Controlling for infant age at time of questionnaire
[4] Adapted from Savory *et al.* 2020

Significant relationships in the partial correlation analysis were analysed by multiple linear regressions to additionally control for parity. With the exception of 'vocal reactivity' in the IBQ-R-SF with EPDS, and Negativity with STAI in females, all relationships remained significant.

For mothers with girls, A1 EPDS scores were significantly associated with PBQ1 ($p< 0.001$, B= 0.39, CI 0.22, 0.56) and PBQ3 ($p= 0.041$, B= 0.10, CI<0.00, 0.19). EPDS was also associated with the CICS ($p= 0.003$, B= 0.19, CI 0.07, 0.31), the Negativity dimension within the IBQ-R-SF ($p= 0.017$, B= 0.05, CI 0.01, 0.09), and for individual subscales of 'sadness' ($p= 0.002$, B= 0.08, CI 0.03, 0.13), 'distress' ($p= 0.012$, B= 0.08, CI 0.02, 0.13) and 'soothability' ($p= 0.012$, B= -0.06, CI -0.10, -0.01). For mothers with girls, prenatal STAI remained significant for 'sadness' ($p= 0.012$, B= 0.04, CI 0.01, 0.07), 'distress' ($p= 0.017$, B= 0.04, CI 0.01, 0.07) and 'soothability' ($p= 0.039$, B= -0.03, CI -0.05, <0.00). In boys, when controlling for infant age and parity prenatal EPDS scores were only significant with 'approach' on the IBQ-R-SF ($p= 0.043$, B= -0.04, CI -0.09, <0.00).

7.3.4.2 Assessment

Prenatal mental health scores were subsequently analysed with respect to infant behaviour objectively assessed in a laboratory assessment (Table 7.7). A1 EPDS was significantly associated with cognition

from the Bayley's task, 'intensity of escape' from the Novel Toy Task, and 'distress' from the Maternal Separation Task. A1 STAI was only significantly associated with receptive language. When analyses was repeated separating by infant sex, prenatal depression scores were associated with female 'intensity of escape' in the Novel Toy Task only. Conversely, for males prenatal depression and anxiety scores were associated with 'facial interest' in the Sustained Attention Task and 'facial fear' in the Maternal Separation Task. Both EPDS and STAI scores were significantly associated with male expressive language, and STAI score was additionally associated with male receptive language.

Table 7.7: Prenatal mental health scores and independent assessments of infants at 12 months postpartum

	A1 EPDS			***A1 STAI***		
	All	***Male***	***Female***	***All***	***Male***	***Female***
Bayley's						
Cognitive	**0.021**	0.184	0.079	0.160	0.543	0.213
Receptive language	0.052	0.075	0.398	**0.018**	**0.032**	0.244
Expressive language	0.232	**0.018**	0.735	0.102	**0.008**	0.925
Novel Toy						
Facial Fear	0.439	0.367	0.073	0.560	0.102	0.574
Distress	0.310	0.458	0.072	0.602	0.182	0.751
Bodily Fear	0.534	0.580	0.107	0.549	0.149	0.534
Intensity of Escape	**0.035**	0.914	**0.018**	0.442	0.643	0.252
Startle Response	0.489	0.304	0.821	0.268	0.262	0.701
Parent Behaviour	0.536	0.196	0.631	0.127	**0.044**	0.888
Sustained Attention						
Facial Interest	0.112	**0.015**	0.889	0.189	**0.038**	0.855
Duration of Looking	0.529	0.330	0.878	0.516	0.316	0.771
Gestures	0.965	0.632	0.998	0.577	0.854	0.791
Parent Behaviour	0.072	0.206	0.170	0.312	0.188	0.882
Infant Positive Affect	0.634	0.917	0.576	0.738	0.866	0.076
Infant Negative Affect	0.747	0.946	0.444	0.947	0.872	0.948
Latency to Look Away	0.703	0.342	0.557	0.777	0.665	0.257
Maternal Separation						
Facial Fear	0.349	**0.039**	0.480	0.189	**0.002**	0.719
Distress	**0.042**	0.155	0.214	0.036	0.070	0.263
Latency to Fear Response	0.365	0.152	0.983	0.906	0.126	0.803
Bodily Fear	0.431	0.482	0.448	0.549	0.429	0.607
Escape	0.395	0.379	0.688	0.610	0.550	0.773

[1] *p* values reported
[2] Significant values reported in bold
[3] Controlling for infant age at time of assessment
[4] Adapted from Savory *et al.* 2020

Linear regressions were performed for significant partial correlations to allow maternal parity to be controlled for. All relationships remained significant with the exception of male 'facial interest' in the Sustained Attention Task with A1 STAI score. For females there was a significant correlation between prenatal EPDS and 'intensity of escape' in the Novel Toy Task (*p*= 0.024, B= 0.05, CI 0.01,

0.09). In boys, EPDS scores were significantly associated with expressive language (p= 0.009, B= -0.19, CI -0.33, -0.05), 'facial fear' on the Maternal Separation Task (p= 0.039, B= 0.09, CI) and 'facial interest' on the Sustained Attention Task (p= 0.023, B= -0.02, CI -0.03, <0.00). Prenatal STAI scores were significantly associated in boys with receptive (p= 0.024, B= -0.08, CI -0.14, -0.01) and expressive language (p= 0.005, B= -0.11, CI -0.17, -0.04). Also in male infants, STAI scores were associated with 'facial fear' on the Maternal Separation Task (p= 0.003, B= -0.02, CI -0.05, <0.00) and 'parent behaviour' (p= 0.044, B= -0.02, CI -0.05, <0.00).

7.3.4.2.1 Postnatal mental health scores

Partial correlations analysing infant assessment scores separately by infant sex were repeated using EPDS and STAI scores from the Y1 time point (Table 7.8).

Table 7.8: Postnatal mental health scores and independent assessments of infants at 12 months postpartum

	Y1 EPDS		***Y1 STAI***	
	Male	***Female***	***Male***	***Female***
Bayley's				
Cognitive	0.158	0.512	0.209	0.932
Receptive language	0.260	0.633	**0.041**	0.465
Expressive language	0.063	0.411	**0.041**	0.747
Novel Toy				
Facial Fear	0.253	0.127	0.076	0.091
Distress	0.882	0.380	0.263	0.305
Bodily Fear	0.429	0.118	0.172	0.097
Intensity of Escape	0.800	0.379	0.576	0.177
Startle Response	0.677	0.345	0.421	0.805
Parent Behaviour	0.602	0.630	0.314	0.255
Sustained Attention				
Facial Interest	**<0.001**	0.907	**0.003**	0.886
Duration of Looking	0.103	0.293	0.211	0.599
Gestures	0.268	0.822	0.686	0.597
Parent Behaviour	0.114	0.421	0.155	0.909
Infant Positive Affect	0.935	0.990	0.291	0.594
Infant Negative Affect	0.968	0.927	0.987	0.930
Latency to Look Away	0.454	0.479	0.513	0.541
Maternal Separation				
Facial Fear	0.666	0.873	0.239	0.302
Distress	0.626	0.179	0.281	0.126
Latency to Fear Response	0.300	0.943	0.306	0.349
Bodily Fear	0.659	0.894	0.989	0.547
Escape	0.767	0.684	0.445	0.734

[1] p values reported
[2] Significant values reported in bold
[3] Controlling for infant age at time of assessment
[4] Adapted from Savory *et al.* 2020

Multiple linear regressions were performed on significant partial correlations to control for parity and infant age at the time of assessment. All significant partial correlations remained significant at the regression level, with Y1 EPDS associated with 'facial interest' on the Sustained Attention Task (p= 0.001, B= -0.02, CI -0.03, -0.01) in the boys. Y1 STAI was associated with receptive (p= 0.040, B= -0.06, CI= -0.12, <0.00) and expressive (p= 0.042, B- -0.07, CI= -0.13, <0.00) language and facial interest in the Sustained Attention Task (p= 0.007, B= -0.01, CI -0.02 <0.00) in the boys.

7.3.5 Biological measures and infant outcomes

7.3.5.1 Questionnaire

Partial correlations were performed between maternally reported infant outcomes and placental gene expression of *PEG3* and *hPL*, as well as maternal serum measurements of hPL (Table 7.9). When all samples were combined, *hPL* gene expression was associated with the Negativity domain of the IBQ-R-SF. Separating this analysis by infant sex this association was only apparent in female infants with additional significant relationships between *hPL* and 'sadness', 'distress' and 'falling reactivity'. Placental expression of *hPL* in male placenta was associated with the Regulatory domain of the IBQ-R-SF and additionally 'sensitivity' and 'vocal reactivity' subscales. hPL serum was significantly associated with the 'orienting' subscale in female infants. *PEG3* expression was significantly associated with male infant outcomes for PBQ3, 'sadness' and 'soothability'.

Table 7.9: Biological measurements and maternally reported infant outcomes

	PEG3			***hPL-Expression***			***hPL-Serum***		
	All	***Male***	***Female***	***All***	***Male***	***Female***	***All***	***Male***	***Female***
PBQ-total	0.967	0.458	0.847	0.798	0.637	0.760	0.182	0.939	0.317
PBQ1	0.783	0.619	0.460	0.698	0.227	0.784	0.115	0.219	0.430
PBQ2	0.324	0.245	0.755	0.491	0.063	0.463	0.534	0.851	0.564
PBQ3	0.052	**0.022**	0.468	0.787	0.253	0.778	0.171	0.303	0.398
CICS	0.760	0.519	0.870	0.689	0.116	0.559	0.112	0.153	0.355
IBQ									
NEG	0.110	0.083	0.340	**0.015**	0.724	**0.012**	0.242	0.289	0.663
Sadness	0.089	**0.027**	0.494	0.074	0.699	**0.044**	0.559	0.714	0.688
Distress	0.419	0.347	0.552	0.065	0.880	**0.020**	0.179	0.497	0.383
Fear	0.425	0.675	0.476	0.069	0.165	0.331	0.689	0.576	0.606
Falling Reactivity	0.151	0.308	0.297	0.100	0.706	**0.015**	0.306	0.215	0.663
SUR	0.607	0.905	0.569	0.144	0.060	0.520	0.450	0.889	0.252
Approach	0.919	0.265	0.552	0.102	0.117	0.469	0.301	0.857	0.201
Vocal Reactivity	0.159	0.939	0.137	0.068	**0.022**	0.544	0.695	0.813	0.512
High Intensity Pleasure	0.954	0.615	0.701	0.176	0.110	0.635	0.905	0.904	0.306
Smiling	0.634	0.285	0.842	0.493	0.868	0.772	0.763	0.875	0.548
Activity	0.862	0.857	0.956	0.846	0.497	0.467	0.748	0.473	0.985
Sensitivity	0.250	0.405	0.431	0.281	**0.045**	0.914	0.407	0.869	0.107
REG	0.701	0.280	0.394	0.313	**0.025**	0.459	0.731	0.123	0.171
Low Intensity Pleasure	0.791	0.371	0.971	0.711	0.231	0.148	0.731	0.388	0.299
Cuddliness	0.874	0.336	0.772	0.498	0.076	0.798	0.803	0.354	0.841
Orienting	0.090	0.543	0.157	0.057	0.051	0.821	0.233	0.532	**0.042**
Soothability	0.624	**0.045**	0.653	0.787	0.623	0.820	0.505	0.307	0.973

[1] *p* values reported
[2] Significant values reported in bold
[3] Controlling for infant age at time of questionnaire

Significant associations were further analysed in multiple linear regressions controlling for infant age and parity. *hPL* expression remained significantly associated with the Negativity domain (p= 0.018, B= 0.385, CI 0.070, 0.70), 'distress' (p= 0.026, B= 0.529, CI 0.068, 0.989) and 'falling reactivity' (p= 0.015, B= -0.497, CI -0.894, -0.101) in girls. In boys *hPL* expression was significantly associated with 'sensitivity' (p= 0.042, B= 0.521, CI 0.020, 1.022) and 'vocal reactivity' (p= 0.007, B= 0.512, CI 0.159, 0.866). hPL serum was still significantly associated with orienting in females (p= 0.044, B= -0.094, CI -0.185, -0.003). Finally *PEG3* expression only remained significantly associated with PBQ3 in boys (p= 0.019, B= 2.191, CI 0.399, 3.983).

hPL expression and prenatal mental health were both associated with maternally reported female Negativity after controlling for infant age and parity. However, prenatal depression and anxiety were not associated with *hPL* expression for those with girls in the current sample (EPDS: r= -0.020, p= 0.896; STAI: r= 0.141, p= 0.362). These data did not support a role for *hPL* gene expression in

mediating the relationship between prenatal mental health and maternally reported infant Negativity in female infants.

7.3.5.2 Assessment

Biological measures were additionally analysed with respect to infant outcomes measured by independent assessments (Table 7.10). *PEG3* expression was significantly associated with 'infant positive affect' in the Sustained Attention Task for all samples. *hPL* gene expression was not correlated with any component. Serum measurements were associated with 'infant negative affect' in the Sustained Attention Task in girls, and with 'latency to look away' in the Sustained Attention Task for boys.

Table 7.10: Biological measurements and independently assessed infant outcomes

	PEG3			***hPL-Expression***			***hPL-Serum***		
	All	***Male***	***Female***	***All***	***Male***	***Female***	***All***	***Male***	***Female***
Bayley's									
Cognitive	0.899	0.701	0.869	0.830	0.797	0.959	0.274	0.478	0.493
Receptive language	0.316	0.534	0.265	0.296	0.420	0.547	0.836	0.458	0.490
Expressive language	0.122	0.144	0.266	0.566	0.879	0.591	0.410	0.220	0.444
Novel Toy									
Facial Fear	0.751	0.992	0.506	0.629	0.388	0.691	0.562	0.514	0.948
Distress	0.719	0.926	0.506	0.757	0.443	0.675	0.294	0.178	0.774
Bodily Fear	0.822	0.793	0.738	0.663	0.433	0.425	0.939	0.574	0.386
Intensity of Escape	0.109	0.499	0.143	0.721	0.920	0.782	0.242	0.238	0.478
Startle Response	0.814	0.795	0.483	0.614	0.592	0.801	0.794	0.995	0.790
Parent Behaviour	0.531	0.949	0.526	0.188	0.691	0.150	0.461	0.408	0.225
Sustained Attention									
Facial Interest	0.184	0.255	0.271	0.276	0.486	0.396	0.493	0.839	0.352
Duration of Looking	0.731	0.685	0.947	0.194	0.251	0.499	0.449	0.530	0.057
Gestures	0.322	0.127	0.796	0.588	0.118	0.891	0.141	0.539	0.072
Parent Behaviour	0.185	0.313	0.361	0.141	0.330	0.304	0.711	0.920	0.502
Infant Positive Affect	**0.034**	0.135	0.173	0.788	0.738	0.974	0.317	0.629	0.254
Infant Negative Affect	0.152	0.313	0.263	0.354	0.577	0.450	0.295	0.622	**0.040**
Latency to Look Away	0.878	0.949	0.983	0.732	0.737	0.877	0.585	**0.035**	0.175
Maternal Separation									
Facial Fear	0.385	0.396	0.626	0.868	0.273	0.284	0.161	0.108	0.770
Distress	0.786	0.129	0.212	0.497	0.905	0.215	0.980	0.936	0.952
Latency to Fear Response	0.310	0.187	0.916	0.312	0.532	0.419	0.161	0.141	0.537
Bodily Fear	0.688	0.610	0.531	0.791	0.679	0.551	0.353	0.431	0.314
Escape	0.498	0.964	0.320	0.194	0.454	0.311	0.418	0.409	0.474

[1] *p* values reported
[2] Significant values reported in bold
[3] Controlling for infant age at time of assessment

Significant partial correlations were subsequently analysed through multiple linear regressions controlling for infant age and maternal parity. *PEG3* remained significantly associated with 'infant positive affect' in the Sustained Attention Task (p= 0.043, B= -0.252, CI -0.495, -0.008). hPL serum concentrations only remained significantly associated with 'latency to look away' in the Sustained Attention Task for boys (p= 0.040, B= -0.388, CI -0.755, -0.020).

7.3.6 Sensitivity analysis

A sensitivity analysis was performed to assess whether maternal SSRI use in pregnancy affected the relationship between biological measures and infant outcomes (Table 7.11). Given the small number of participants prescribed SSRIs (assessment n= 5, questionnaire n= 7), a sensitivity analysis was performed for the key composite infant scores. Significant relationships were observed between *hPL* expression and the Negativity dimension, specifically in females, and for the Regulatory dimension in males. No additional significant relationships were observed and no significant associations were lost.

Table 7.11: Sensitivity analysis for biological measurements and key composite infant scores

	PEG3			***hPL-Expression***			***hPL-Serum***		
	All	***Male***	***Female***	***All***	***Male***	***Female***	***All***	***Male***	***Female***
Infant Questionnaire									
SUR	0.657	0.847	0.566	0.166	0.072	0.492	0.414	0.949	0.203
NEG	0.122	0.092	0.355	**0.012**	0.740	**0.007**	0.223	0.295	0.581
REG	0.804	0.243	0.412	0.405	**0.031**	0.406	0.468	0.176	0.089
PBQ	0.755	0.525	0.899	0.770	0.126	0.432	0.159	0.412	0.387
CICS	0.977	0.484	0.838	0.745	0.665	0.661	0.216	0.996	0.167
Assessment									
Cognitive	0.888	0.701	0.882	0.857	0.797	0.998	0.352	0.368	0.783
Receptive	0.316	0.534	0.265	0.296	0.420	0.547	0.918	0.579	0.490
Expressive	0.122	0.144	0.266	0.566	0.879	0.591	0.431	0.256	0.444

[1] p values reported
[2] Significant values reported in bold
[3] Controlling for infant age

7.3.7 Summary of chapter results

Table 7.12: Summary of Chapter 7

Analysis	***Findings***
Prenatal mental health and maternal perception of infant	Predominant number of associations seen in girls but not boys • Mother-infant bonding • Infant aggression • Infant Negativity
Prenatal mental health and independent assessment of infant	Predominant number of associations seen in boys but not girls • Cognition • Language • Facial responses
Biological measures and infant outcomes	Minimal number of associations, no clear findings

7.4 Discussion

This chapter set out to investigate the relationship between prenatal mental health and infant development at 12 months in the GiW cohort. A key finding from this analysis was that prenatal depression and anxiety symptoms were associated with maternal perception of infant temperament and bonding in girls but not boys. This relationship was not found in the independent observations recorded by researchers. However, there was a relationship between higher prenatal mental health symptoms and impaired infant behaviour, cognition and language in boys. Placental genes *PEG3* and *hPL* did not appear to be driving this relationship.

Prenatal depression and anxiety scores were positively correlated with impaired bonding for mothers with girls only, suggesting prenatal mental health affects postnatal attachment. A relationship between prenatal mental health and postnatal bonding has previously been reported, although analysis was not separated by infant sex (Goecke *et al.* 2012; Dubber *et al.* 2014; Ohoka *et al.* 2014; Rossen *et al.* 2016). Female infant aggression and negative affectivity were also associated with prenatal mental health scores. Negative affect commonly manifests as increased anger, crying and fear when exposed to a novel stimulus, and is regularly associated with prenatal depression and anxiety (Austin *et al.* 2005; McGrath *et al.* 2008; Glynn *et al.* 2018). Maternally reported negative affect in infants has shown to be relatively stable later in childhood (Lee and Bates 1985; Putnam *et al.* 2008) and is associated with an increased risk for psychopathologies such as depression and anxiety later in life (Nigg 2006). Consistent with findings for negative affect only in daughters, girls exposed to prenatal mental health are at a greater risk of these affective disorders in adolescence (Boyle and Pickles 1997; Quarini *et al.* 2016; Soe *et al.* 2016; Nolvi *et al.* 2019). On the other hand, there was no relationship between prenatal mental health and bonding or infant aggression in boys and very little association with temperament. Within the questionnaire subset, there were more participants who had daughters than sons and this smaller sample size may explain the lack of significant findings in the boys.

Alternatively, impaired bonding may drive the difference in maternally-reported infant perceptions between girls and boys in relation to maternal mood. Bonding has been shown to influence postnatal infant development (Le Bas *et al.* 2019) and impaired bonding may lead the mother to perceive her infant differently.

In contrast with associations between prenatal mental health and maternally reported infant measures being predominantly apparent in daughters, prenatal depression and anxiety were not widely associated with independent observations in girls. Only prenatal depression symptoms and 'intensity of escape' in the Novel Toy Task were significantly associated. However in boys, prenatal depression and anxiety symptoms were associated with poor language and cognitive development, as well as facial responses to the Maternal Separation and Sustained Attention Tasks. The language deficit observed related to both expressive and receptive language suggesting an overall limited ability to verbally communicate in infants exposed to higher prenatal symptoms of depression and anxiety. This association has previously been reported (Laplante *et al.* 2004; Sohr-Preston and Scaramella 2006), as has an increased risk for male infants specifically (Kurstjens and Wolke 2001). An impaired language development may account for the alterations observed in facial responses as communication relies on a less developed form. Similar numbers of sons and daughters were analysed in the independent assessment suggesting a smaller sample size does not explain the lack of significant findings in the girls.

There was a distinct difference in infant outcomes in relation to prenatal mental health between maternally reported outcomes and independent observations. There are a number of potential explanations for this disparity. Firstly, the two may not measure the same domains of infant development; while sustained attention and fear in the laboratory assessments have parallels with 'orienting' and 'fear' subscales in the IBQ-R-SF respectively, it is debated whether or not they are directly comparable (Planalp *et al.* 2017). Furthermore, one study suggested that the Novel Toy Task elicits fear in less than half of infants studied and may elicit curiosity in others (Buss and Goldsmith 1998). In addition to this, a number of measures significant in either the questionnaire or assessment were not measured in the other, such as language and bonding. In the current study there are also fewer participants who completed the assessment compared to the questionnaire and thus the sample size may be inadequate to detect all significant relationships. An alternative or additional factor relates to the maternally-reported nature of the questionnaire. Due to societal expectations of girls and boys, even at a young age, maternal perception of her infant may differ depending if she has a son or daughter. This may manifest as an interpretation of problems that do not exist, especially when confounded by the effect of poor prenatal mental health. Again, poor mother-infant bonding reported in the questionnaire between mothers and daughters could further confound this.

An alternative explanation for this sex-specific observation could be an altered *in utero* environment. The placenta is predominantly of fetal origin and sex-dependent differences in gene expression and hormone production have previously been reported (Cvitic *et al.* 2013; Gong *et al.* 2018; Gonzalez *et al.* 2018). *PEG3* is an imprinted gene expressed in the placenta and its expression has been negatively associated with prenatal depression, but in only women who had boys (Janssen *et al.* 2016) and Chapter 5. Methylation of *PEG3* in infant cord blood has also been positively associated with infant negative affectivity and externalising behaviours at 14 months (Fuemmeler *et al.* 2016). As the silenced *PEG3* maternal allele is methylated, an increase in methylation would suggest a decrease in mRNA expression. In another study, reduced placental expression of *Peg3* in mice altered pup USVs which have been linked with autistic-like tendencies in humans (McNamara *et al.* 2018). From this it was proposed that placental *PEG3* could mediate the relationship between maternal mental health and adverse infant phenotypes. In the current study *PEG3* expression was only associated with limited infant measures. Expression was negatively associated with 'infant positive affect' in the Sustained Attention Task. This is in contrast to increased methylation at the *PEG3* promoter (thus suggesting decreased expression) which was positively associated with negative affectivity (Fuemmeler *et al.* 2016). This disparity could be explained by the differences in the tissue type, the mode of delivery of participants, time point of follow up or the maternal-reporting measures of infant negativity in Fuemmeler *et al*. opposed to the objective measures of infant positivity in the current study. Neither of the measures associated with *PEG3* expression in the current study were also associated with prenatal mental health. While *PEG3* expression was significantly associated with prenatal depression in Chapter 5, not all participants responded to subsequent data collection at 12 months postpartum. Although prenatal mental health did not significantly correspond with drop-out rates at 12 months postpartum (Chapter 3), a different sample population was analysed which could underlie the lack of associations.

The placental hormone hPL was also analysed with respect to infant outcomes. Placental *hPL* expression was associated with the Negativity dimension in the IBQ-R-SF as well as 'distress' and 'falling reactivity' in girls. In boys, expression correlated with 'sensitivity' and 'vocal reactivity'. Maternal serum hPL was associated with 'orienting' behaviours in girls and 'latency to look away' in boys. There was no similarity between results for *PEG3* or either hPL measurement. Whilst prenatal depression and anxiety were also associated with the Negativity dimension in females, the mental health scores did not correlate with *hPL* expression thus suggesting mediation is not occurring in this instance. This is in line with a previous study that found no mediation effect of imprinted gene expression and neonatal outcomes (Litzky *et al.* 2018). However, the lack of associations does not exclude the potential for a relationship as not all temperament dimensions are obviously apparent in younger children (Nigg 2006). For example, while negativity is frequently observed in younger infants, regulatory behaviour is often more apparent in older children. However, results presented here

and previous studies to date suggest that there is not a strong case for a mechanism of *PEG3* and hPL acting as mediators between prenatal mental health and infant development.

Treatment of prenatal depression and anxiety with SSRIs has also been associated with impaired postnatal infant development (Kott and Brummelte 2019). Although results are conflicting, lower development scores, increased social avoidance, depression, anxiety and psychomotor deficits have all been observed in human and rodent offspring exposed *in utero* to SSRIs (Glover *et al.* 2015; Boukhris *et al.* 2016; Malm *et al.* 2016; Gustafsson *et al.* 2018). Different outcomes have also been reported for male and female infants (Kott and Brummelte 2019). In addition, Chapter 6 describes how maternal SSRI use in pregnancy alters maternal postnatal mental health which can impact infant temperament and development. Given the small number of participants prescribed SSRIs during their pregnancy in the current study, a sensitivity analysis was performed for gene expression and key composite infant development scores. No differences in significant results were observed suggesting SSRIs were neither driving nor masking any associations between gene expression and infant outcomes. However, it is important to note the very low numbers prescribed SSRIs in this group, and thus limited conclusions should be drawn.

In addition to infant behavioural and neurodevelopmental outcomes examined, prenatal mental health was analysed with respect to maternal characteristics and infant anthropometric measurements at 12 months. There was a significant association between prenatal mental health scores and the occurrence of a mental health problem postnatally. This is consistent with prenatal mental health being a strong predictor of postnatal depression and anxiety (Chapter 3). Prenatal anxiety scores were associated with breastfeeding but only if mothers had sons. A number of studies have highlighted a relationship between prenatal mental health and breastfeeding, but do not separate analyses by infant sex (Insaf *et al.* 2011; Adedinsewo *et al.* 2014; Figueiredo *et al.* 2014). Postnatal depression is also a risk factor for not breastfeeding, and one study reported that mothers who had boys were at a greater risk of postnatal depression than those with girls (Myers and Johns 2019), but this was not observed in the current study (Chapter 3). Prenatal mental health did not impact upon infant growth or infant health problems. Studies that report an association between prenatal mental health and anthropometric measures are commonly from LMICs suggesting alternative confounding factors may underlie the relationship (Stewart 2007).

7.4.1 Limitations

It is well documented that prenatal depression and anxiety are risk factors for poor postnatal mental health (Gotlib *et al.* 1989; Heron *et al.* 2004; Andersson *et al.* 2006) and Chapter 3. Postnatal depression and anxiety are also a risk factors for poor infant development and behaviour, although studies have reported that the effects of prenatal mental health on infant development are distinct from those after birth (O'Connor *et al.* 2002; O'Hara and McCabe 2013; Myers and Johns 2019). Given the

relatively small sample size of the groups, models were not additionally controlled for postnatal mental health. Regressions were repeated with postnatal depression and anxiety scores as independent variables and fewer associations were found to be significant. However, Y1 STAI scores remained significant with receptive and expressive language and thus it is not possible to determine which time point had a greater effect. Similarly it is not possible to attribute findings to solely anxiety or depression scores, as within the GiW cohort they are highly comorbid. Finally, although independent researchers not biased to prenatal mental health scores performed the Lab-TAB assessments, they were performed in a novel environment by strangers. Therefore assessments in familiar settings such as at home may uncover additional infant characteristics.

7.4.2 Conclusions

In summary, this chapter reports on the relationship between prenatal mental health and infant temperament and development in the GiW cohort. A key finding was the relationship between prenatal mental health scores and maternally reported infant negativity in girls alone. This was in contrast to assessments made by independent researchers where prenatal mental health was predominantly associated with infant development of males. This disparity may relate to impaired mother-daughter bonding in those with poor prenatal mental health. However, the relationship between prenatal mental health and adverse infant outcomes at 12 months does not appear to be mediated by *PEG3* expression or the placental hormone hPL.

Chapter 8: General discussion

Exposure to prenatal depression is commonly associated with adverse outcomes for infants including low birth weight and abnormal temperament. A number of these associations are sexually dimorphic in nature; for example boys are often at a greater risk of prematurity and poor cognitive development (Field 2011). One hypothesis linking these associations is a placental endocrine insufficiency driven by aberrant expression of imprinted genes (Creeth and John 2020). In mice, altered imprinted gene expression in the placenta causes disruptions in the placental endocrine lineages and subsequent misexpression of placental hormones. Specifically a reduction in the imprinted gene *Peg3* led to a more severe placental phenotype in male placenta, potentially analogous to the sexually dimorphic associations to prenatal depression and infant outcomes in humans (Tunster *et al.* 2018). Alterations in maternal behaviour during pregnancy and postpartum were also observed in this mouse model (McNamara *et al.* 2018). The overarching aims of the current thesis were to investigate gene expression in human placenta in relation to prenatal mental health and placental hormones. Gene expression and placental hormones were additionally analysed with respect to infant behaviour and development to assess for a mediatory role.

8.1 Main findings

Prenatal mental health is frequently understudied in comparison to postnatal mental health despite an often higher prevalence. The GiW study was the first cohort to measure the rates of prenatal depression and anxiety in South Wales with a prevalence of 14% and 28% respectively. Despite all participants delivering by ELCS, these prevalence rates were relatively in line with previous UK cohorts that sampled different modes of delivery. However, unlike other cohorts the rates of anxiety remained high postnatally. While there was no directly equivalent cohort who delivered vaginally for comparison, this may relate to the mode of delivery and an absence of the physical process of labour as explored in Chapter 3. The prevalence of prenatal depression and anxiety observed in the GiW cohort reinforces the importance of studying these disorders.

Placental transcriptomes of participants with high prenatal depression symptoms were analysed through RNA sequencing in Chapter 4. Numerous differentially expressed genes were observed in relation to prenatal depression, a number of which were associated with inflammation. A key finding from this analysis was the striking sexually dimorphic gene expression changes. While female placentas appeared relatively resilient, 89 genes were differentially expressed in male placentas in relation to prenatal depression, but only when those prescribed SSRIs were removed from the analyses. This is the first time this placental sexual dimorphism has been observed in pregnancies with prenatal depression through RNA sequencing. Across all RNAseq analysis, no imprinted genes were significantly altered in placentas of participants with high prenatal depression scores.

In Chapter 5 a targeted approach was subsequently used to analyse imprinted gene expression in relation to prenatal depression symptoms. A reduction in *PEG3* was significantly associated with

higher prenatal depression symptoms, specifically in male placentas. This replicated a previous finding by our group (Janssen *et al.* 2016). Developing on previous work, the association remained significant four days after birth and did not appear to be part of a larger disturbance of paternally expressed genes. However, unlike Janssen *et al.* 2016, *PEG3* expression was not associated with expression of the placental hormone *hPL*. To expand on this, in Chapter 6 maternal serum concentrations of hPL were analysed with respect to perinatal mental health. After controlling for confounders, serum hPL was not associated with prenatal mental health, but was associated with postnatal depression and anxiety scores. In contrast to findings in Janssen *et al.* these associations were only significant in mothers who had girls and not boys. This is the first time maternal serum hPL has been linked to perinatal mental health. Furthermore, the results were strengthened by the exclusion of participants who were prescribed SSRIs during their pregnancy. Interestingly *hPL* expression and serum measurements were not correlated in the analysis of this chapter.

Finally, infant outcomes at 12 months were analysed with respect to prenatal mental health and biological measurements; *PEG3* expression, *hPL* mRNA expression and hPL concentrations in maternal serum. Again sexually dimorphic outcomes were observed in this analyses. For female infants, prenatal depression and anxiety scores were associated with maternally reported adverse infant temperament, bonding and aggression, while few associations were observed in male infants. Interestingly, these relationships were not observed in independent infant measures made by researchers. However, a number of these independent observations were significantly associated with prenatal mental health scores in male infants alone. In contrary to the hypothesised association, biological measurements did not mediate the relationship between prenatal mental health scores and infant outcomes.

8.2 Sexual dimorphisms

One of the most striking findings from this thesis was the sexually dimorphic observations in relation to prenatal mental health. Historically, the study of the perinatal period has at worst focussed solely on male offspring, and at best aimed for equal representation of males and females in sampling. Increasingly, a number of studies are separating analyses by fetal sex and finding clear differences (Kott and Brummelte 2019). In the current study, differences were seen at the level of placental gene expression, hormone concentrations, and both maternally reported and independent 12 month infant observations (Figure 8.1).

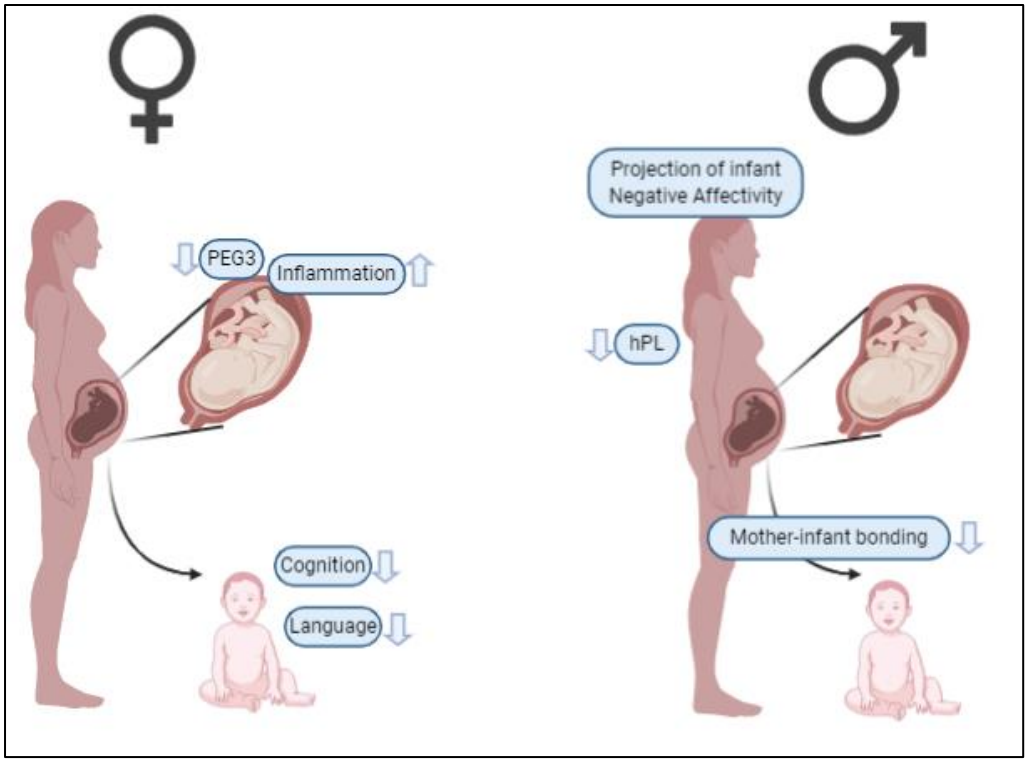

Figure 8.1: Sexually dimorphic effects observed when the mother had prenatal depression. When the mother had a boy, placental changes in gene expression and adverse infant development at 12 months was recorded. When the mother had a girl, changes in a maternal hormone was seen alongside changes to the relationship between mother and daughter and the way the mother perceived her daughter. Created with biorender.com.

Overall, more disruption was seen in male placentas in relation to prenatal depression than female placentas. As well as a reduction in *PEG3* expression, the overall number of differentially expressed genes through RNAseq was higher in male placentas. At 12 months, in independent observations, impairments in cognition and language were associated with high prenatal mood scores but only in male infants. Therefore, the disruptions observed in the male placenta in relation to prenatal depression may be associated with altered developmental pathways after birth. While it is important to note that *PEG3* expression was not associated with infant development, the sample size was reduced and different participants were analysed in Chapters 5 and 7 due to participant drop out.

Alternatively the changes observed in the RNAseq, specifically in the inflammatory-related genes and pathways may relate to altered offspring development. Inflammation has long been linked to depression in the prenatal period (Amodeo *et al.* 2017) and inflammatory responses in the placentas of humans and rodents have been linked to adverse infant outcomes including ASD (Kim *et al.* 2015). Furthermore, in line with the current findings male rodents have been shown to be more vulnerable to the effects of stress-induced placental inflammation than females with altered locomotor activity persisting into adulthood (Bronson and Bale 2014). Another gene altered in the male placenta of participants with prenatal depression symptoms was *ANKRD11*. Placental methylation of *ANKRD11* has previously been associated with neonatal attention (Paquette *et al.* 2016). Poor attention of newborns has been correlated with delays in language and motor skills at aged three (Liu *et al.* 2010).

Therefore, the altered expression of *ANKRD11* could be associated with the male-specific outcomes observed in Chapter 7. Together these results suggest different potential pathways through which the placenta could mediate the relationship between prenatal depression and infant outcomes.

Contrasting with male placentas, no genes were differentially expressed in relation to prenatal depression in the RNAseq or qPCR analysis of female placentas. However, in pregnancies where the baby was female, maternal serum concentrations of the placental hormone hPL were significantly associated with postnatal depression scores. Although there was no change in expression levels of *hPL* in relation to perinatal mental health, expression and serum levels of hPL were not correlated. At 12 months, maternal reports of bonding, aggression and temperament of female infants was significantly associated with prenatal depression scores, but this relationship was not apparent in independent observations. It is again important to note that hPL serum measurements were not associated with these infant observations. However, together these data could imply that prenatal depression has a more severe impact upon the mother when the child is female. Alternatively, when the child is male the predominant effects are observed in the placenta and offspring.

The hypothesis for the current thesis relates to observations in reduced placental *Peg3* mouse models. Postnatal anxiety-like symptoms were observed in wild-type mothers exposed to reduced placental *Peg3* (McNamara *et al.* 2018). However, prenatally the only differences in maternal phenotype were subtle changes to novel reactivity. This may correspond with the difference in placental phenotypes of male and female mutant *Peg3* fetuses (Tunster *et al.* 2018). Mixed-sex litters in mouse pregnancies could mean the muted placental phenotype of *Peg3* females attenuate the more severe effects in male placenta. Future work to address this could involve the generation of single sex litters as recently reviewed in (Douglas and Turner 2020). This may allow Peg3 functionality studies in a model more similar to human pregnancies. Despite the subtle prenatal phenotype observed in mice, in humans *PEG3* expression has now to date been significantly associated with prenatal depression in four cohorts (Janssen *et al.* 2016). Furthermore, the association was only in male placenta, corresponding to the more acute placental phenotype observed in male mice (Tunster *et al.* 2018). In previous human and mouse studies, *PEG3* expression was associated with placental lactogenic hormone expression (Janssen *et al.* 2016; Tunster *et al.* 2018). This was suggested as a mechanism behind the relationship between *PEG3* and prenatal depression.

The current thesis replicated the male specific reduction in placental *PEG3* expression in mothers with prenatal depression (Janssen *et al.* 2016). However, further results in the thesis do not directly support *PEG3* influencing *hPL* expression or serum levels. Neither hPL measurement was significantly associated with prenatal depression in pregnancies with male infants. The disparity between the current thesis and mouse studies may relate to the expression patterns of *PEG3* in human and mouse placenta. In humans placental *PEG3* expression is limited to the villous cytotrophoblast,

whereas in mice *Peg3* is expressed in all trophoblast cells (Hiby *et al.* 2001). Furthermore, of the three cohorts analysed in Janssen *et al.* only two recruited solely ELCS participants and thus has comparable placental expression to the GiW cohort (Janssen *et al.* 2016). Of these two, only in one cohort was *PEG3* significantly associated with *hPL* expression. Together these data suggest the relationship between *PEG3* and hPL in humans is more complex than the relatively simplistic mechanism identified in mice.

Another explanation for the lack of associations between hPL measurements and prenatal depression symptoms in pregnancies with male infants could correspond to the disrupted placental phenotype observed in the RNAseq analysis. The expression of a number of genes and pathways were altered in male placentas exposed to prenatal depression. A disruption of the placental environment could potentially affect hPL production masking any association with prenatal depression. Interestingly, the protein secretion pathway was enriched in male placenta in association with prenatal depression, but was also enriched in female placenta. However, enrichment of inflammation pathways exclusively in male placentas could interact with the protein secretion pathway impacting the secretion of hPL specifically in male placentas.

The male-specific alterations observed in the RNAseq analysis could also suggest that changes in the transcriptomes of male trophoblastic cells drive differences in placental development and subsequently impact *PEG3* expression. Altered expression of the syncytiotrophoblast development genes syncytin-1 and 2 support this hypothesis. It is important to note that *PEG3* expression was not significantly altered in the RNAseq data. This may be a consequence of different participants and a smaller sample size. Alternatively, RNAseq may be broader in its approach and be unable to detect all changes, especially when controlling for multiple testing in smaller sample sizes.

While the results indicate *PEG3* does not impact *hPL* expression in human pregnancies, due to the limitations of human studies it still remains to be tested if *PEG3* can affect the endocrine lineages as is observed in mice. Establishing causality can be difficult in humans, especially during gestation, hence the commonly used mouse model. While it is beyond the scope of this study, recent reports have highlighted new potential avenues to approach causality studies in humans.

Molecular and functional analyses of mouse trophoblast lineages have used mouse trophoblast stem cells (TSC) for over 20 years. However, until recently human TSC cultures had not been successfully established. In 2018 human TSCs were derived from blastocysts which could give rise to distinct trophoblast lineages (Okae *et al.* 2018). This experimental technique was subsequently used in conjunction with genetic manipulation of the imprinted gene *CDKN1C* in the study of hydatidiform moles (Takahashi *et al.* 2019). This manipulation of human TSCs could be an invaluable tool to study the direct effect of imprinted gene expression on human endocrine lineages. Alternatively the use of placental organoids would remove the need for *in vivo* studies. Trophoblastic placental organoids are

functional models of the early placenta and have similar transcriptomic profiles to first trimester placental tissue (Turco *et al.* 2018). The *in vitro* experimental design allows for relatively simple genetic manipulation techniques that could be used to investigate the impact of imprinted gene expression.

8.3 Different dimensions of prenatal depression

In the future, causality studies linking imprinted gene expression to placental endocrine lineages could be approached using genetic manipulation techniques *in vitro*. However, causality studies linking imprinted gene expression to prenatal depression in humans are more complicated, in part due to the complex nature of mental health disorders. As discussed in Chapter 1, there are different types of depressive disorder including major and chronic depression. These different disorders are associated with different outcomes for the exposed infant, but the design of the GiW cohort did not allow for separation within the EPDS questionnaire. Furthermore, as explored in Chapter 3 there are a number of factors that can influence the emergence of prenatal depression. In line with prior studies a history of a previous mental health disorder was the strongest predictor of prenatal depression and anxiety symptoms (Biaggi *et al.* 2016). This could suggest an underlying vulnerability to mental disorders in this group. However, 32% of those with high depression scores did not have a recorded history of a previous mental health disorder. While this does not rule out the same underlying vulnerability as those with a history of mental health it raises the possibility of different mechanisms underlying the susceptibility to prenatal depression. It is likely that within a population women are vulnerable to the onset of prenatal depression for different reasons. This heterogeneity could further extend to individuals, with a combination of factors including current stressful life events impacting depression. It is possible that one of these factors may relate to a placental endocrine insufficiency, but given the relatively small sample size studied in the current thesis the analysis was not able to capture this link. Clustering techniques in a considerably larger cohort could help to identify different underlying vulnerabilities.

Differences in underlying vulnerabilities to prenatal depression between women could also explain the often conflicting results in infant studies. While an association between prenatal depression and general infant outcomes is well established, the exact phenotype observed often varies between human studies. While this could relate to the sample demographics or mode of delivery, the mothers underlying vulnerability to prenatal depression could additionally affect it. Different pathways could be acting in the mothers and placentas which impact the fetus postnatally. This could explain the lack of findings between biological measures and infant outcomes in Chapter 7 of the current thesis.

The main focus of the current thesis was prenatal depression, but 85% of those with depression symptoms in the GiW cohort also exhibited anxiety symptoms. Comorbidity has been shown to have differing effects on neonatal outcomes when compared to depression or anxiety alone (Field *et al.*

2010). As observed in Chapter 3 whilst similar, trajectories of depression and anxiety have been shown to be different (Penninx *et al.* 2011). While broadly parallel, predictors of prenatal depression and anxiety were different in Chapter 3, and subtle differences were seen in their relationships with infant development at 12 months in Chapter 7. The size of the GiW cohort meant that only limited numbers of participants reported either high depression or anxiety symptoms but not both. 20 participants reported only anxiety symptoms, of which only 16 had acceptable placental mRNA quality and were studied in the qPCR analysis of Chapter 5. While significant associations were observed in this group between STAI scores and gene expression, limited conclusions can be drawn due to the small sample size when split by fetal sex. It is therefore important to acknowledge that when EPDS and STAI scores are analysed, comorbidity exists for the majority of participants. Future work with a larger cohort may allow for better separation of the two prenatal disorders.

8.4 Strengths and Limitations

The structure of the Grown in Wales cohort can be seen as both a limitation and a strength. Of the 355 women recruited 91% were White. This is higher than the Cardiff local authority area which had a White population of 84% in 2016 (Statistics for Wales 2017). This discrepancy may relate to the requirement for English-speaking participants during recruitment. Given the high percentage of White participants and thus low proportion of women representing other ethnicities, White participants were selected for all analysis. Different cultures may affect maternal lifestyle during the perinatal period and thus potentially maternal mental health. Differences have also been observed in imprinted gene expression of women from different ethnicities (King *et al.* 2015), and thus this group was chosen to limit variation. A study investigating SNPs from over 2000 British individuals identified those born in Wales to be the second most different to the rest of the British population after those born in the Orkney Isles (Leslie *et al.* 2015). 72% of the participants included in the analysis of this thesis were born in Wales further reducing genetic variation. However, it is important to be aware that the results presented in this thesis may not be attributable to those from other ethnicities and replication of results in an ethnically diverse population should be performed in the future.

The homogenous nature of recruitment to the GiW cohort can be seen as another strength. Two trained midwives, at one location, recruited all the participants at the same time point in their pregnancies. This ensured all women completed the initial EPDS and STAI questionnaires under the same conditions. However, as is often the case with cohort studies, there was a high proportion of women recruited with high income and education levels. This group of women are less likely to suffer from mental health disorders, and thus interpreting the prevalence of depression and anxiety should be done with caution. However, given other UK birth cohorts often face the same limitation, comparisons between UK regions should still be appropriate.

Participants were recruited at their pre-surgical appointment for an ELCS. This was initially chosen to increase the rates of placenta collected, but ELCS is also an increasingly common mode of delivery in Wales (Statistics for Wales 2017, 2019). As previous findings show, imprinted gene expression changes when the placenta goes through the process of labour (Janssen *et al.* 2015). Therefore, placenta delivered through an ELCS is more akin to the placenta during pregnancy. Consequently, gene expression analysis cannot necessarily be directly compared between studies that were not solely comprised of ELCS-delivered placentas. Rates of depression and anxiety may also be higher in this group as a previous history of mental health is a risk factor for an ELCS (McCourt *et al.* 2007). Also any analysis involving data collected after postpartum may be affected by the ELCS. Unfortunately there was no directly comparable cohort to determine if the ELCS affected postnatal variables such as infant temperament or maternal mental health. Therefore, the generalisability of results is limited by the ELCS and it is important to replicate the findings in a cohort with mixed modes of delivery.

Another limitation relates to the self-reporting nature of the mental health questionnaires. The gold standard in assessing depression and anxiety levels would be to have qualified psychiatrists perform DSM interviews. Therefore, the subjective nature of the questionnaires should be considered when assessing results. However, given the prohibitive cost of a psychiatrist for diagnosing participants, the majority of other studies use self-reporting questionnaires to determine rates of depression and anxiety and thus are comparable to the present study.

An additional limitation with the initial data collection is the data regarding SSRI use. Medication during pregnancy was not originally planned to be an integral part of the GiW study and thus little data was collected regarding its use. A number of previous studies have reported that different types of SSRI affect offspring outcomes as well as the timing of maternal administration (Dubovicky *et al.* 2017). As the NHS recommends stopping SSRI use in pregnancy for those with mild depression (NICE 2014), a number of participants recorded that they stopped taking the medication during gestation. However, details regarding this cessation of antidepressants was not part of the questionnaire and therefore not collected from all participants. In future studies, measuring SSRI concentrations in maternal blood serum may be a more accurate way of determining SSRI usage. The specific type and dosage of SSRI was not always recorded and the sample size of those prescribed SSRIs was too small to separate further. Therefore, it would be interesting to repeat the analysis relating to SSRI use in pregnancy in a larger cohort and separate analysis by SSRI type.

A final limitation of the study is that both hPL measurements were not available for all samples. This could explain the lack of correlation between serum and mRNA expression. Alternatively, as explored in Chapter 6, it could relate to differences in posttranslational modifications. In addition, the analyses of biological data in relation to infant outcomes could not be performed for the same group of participants as in Chapters 5 and 6 as not all participants completed a Y1 questionnaire or attended an

assessment. Therefore, comparison of analyses between chapters should be done so with caution. Despite the decrease in numbers at the Y1 time point significant observations were still apparent, particularly when splitting analyses by infant sex. The clear strength of the analyses at 12 months was the inclusion of both maternally reported and independent infant observations which revealed remarkably sexually dimorphic links to prenatal depression symptoms.

8.5 Concluding remarks

This thesis contributes to the growing body of literature supporting sexually dimorphic outcomes in response to prenatal depression. Initially highlighting the importance of studying mental health disorders during pregnancy, the prevalence of prenatal depression in a South Wales ELCS cohort was 14%. The thesis provides new evidence for placental gene expression modifications in relation to prenatal depression, specifically in male placenta. In association with perinatal depression the maternal serum concentrations of the placental hormone hPL was significantly altered in pregnancies where the infant was female. Finally, sexually dimorphic differences were observed in infant behaviour, temperament and development at 12 months in relation to prenatal depression scores. It is important to note that these associations were observed only in pregnancies delivered by ELCS and thus additional investigation should be performed with different modes of delivery before assuming the results can be generalised to the rest of the population. The results from this thesis did not support the simple mechanism reported in mice whereby Peg3 influences placental lactogenic hormones and indirectly influences maternal behaviour. However, *PEG3* expression and hPL concentrations were significantly altered in relation to prenatal depression. Given the transcriptomic disruption in RNAseq analysis a more complex mechanism may underlie the role of the placenta in prenatal depression.

Appendix

s1. EPDS Questionnaire

DIRECTIONS: Please UNDERLINE the answer which comes closest to how you have felt in the **past week**, not just how you feel today. Here is an example already completed:
I have felt happy:

Yes, all the time
Yes, most of the time
No, not very often
No, not at all

This would mean: I have felt happy most of the time in the past few days.
Please complete the other questions in the same way. Do not take too long over it and make sure you answer all the questions.

IN THE PAST WEEK

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 had occurred to me
   Yes, quite often
   Sometimes
   Hardly ever
   Never

s2. STAI Questionnaire

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then tick in the appropriate box on the right to indicate how you **generally** feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you **generally** feel, even before pregnancy.

	In general….	*Almost never*	*Sometimes*	*Often*	*Almost always*
1.	I feel pleasant				
2.	I feel nervous and restless				
3.	I feel satisfied with myself				
4.	I wish I could be as happy as others seem to be				
5.	I feel like a failure				
6.	I feel rested				
7.	I am "calm, cool and collected"				
8.	I feel that difficulties are piling up so that I cannot overcome them				
9.	I worry too much over something that really doesn't matter				
10.	I am happy				
11.	I have disturbing thoughts				
12.	I lack self-confidence				
13.	I feel secure				
14.	I make decisions easily				
15.	I feel inadequate				
16.	I am content				
17.	Some unimportant thought runs through my mind and bothers me				
18.	I take disappointments so keenly that I can't put them out of my mind				
19.	I am a steady person				
20.	I get in a state of tension or turmoil as I think over my recent concerns and interests				

s3. DNA extraction

Pulverised pooled placenta was generated as in Section 2.4. Samples were added to 1 ml of lysis buffer (see Table s1) and 20 µl of Proteinase K (ThermoFisher). Samples were left overnight at 55°C. The following day 1 ml of phenol (Sigma) was added to each sample. Tubes were mixed for 10 minutes, incubated at room temperature for 5 minutes and mixed again for a further 10 minutes. Samples were centrifuged for 10 minutes at 12000 rpm and the clear phase transferred to a fresh tube. An equal volume of chloroform (Sigma) was added to each tube, mixed for 10 minutes and centrifuged again for 10 minutes at 12000 rpm. The top phase was transferred to a fresh tube and 1/30th volume of NaOAc (pH 6.0) (ThermoFisher) and 1 volume of 100% ethanol added. Tubes were slowly inverted to precipitate the DNA. The precipitate was transferred to a tube containing 70% ethanol and washed by inversion. The ethanol was removed from the tube and pellet left to air dry before resuspension in 80 µl water.

Table s1: Lysis buffer reagents

Reagents	***Volume (µl)***
TE Buffer (10 mM Tris, 1 mM EDTA)	650
NaCl (1.5 M)	300
SDS (0.5%)	50

s4. Bisulphite conversion

PEG3 has two CpG islands in its promoter region, the first (CpG1) is between the first exon and the first intron of *PEG3* and the second (CpG2) is in the first intron of the gene. The pyrosequencing primers were designed by Dr Raquel Boqué-Sastre to analyse DNA methylation at three points in CpG1 and at one point in CpG2. The three primers designed for CpG1 targeted upstream, downstream and central sections of the island. The primer sequences can be found in Table s2.

Table s2: Pyrosequencing primers

Primer name	***Primer region***	***Forward primer***	***Reverse primer***	***Product size***
Primer 1	CpG island 1, upstream	GGGAGGGGAAAGAAAATTTTTATAGGT	ACACCAATACTATCCCTATTACC	187 bp
Primer 2	CpG island 1 downstream	GTTATTAGTTTAGGGTGGATATT	CCCTTTTCCAAACCTAACTTTAAAAT	80 bp
Primer 3	CpG island 1, middle	GGTGGTTTTAGGTTAGTTAGAAAGG	ACCAAACTATAAATAATTAATACCTTTT	68 bp
Primer 4	CpG island 2, upstream	TGGTGAATAAAGTTTTGGTTAGGTAATA	AACTCAATCCCCCCATCTAAAAAAACC	31 bp

Bisulphite conversion was performed using a Zymo kit (Cambridge Bioscience, UK). The CT conversion reagent was prepared according to manufacturer's instructions (900 µl water, 300 µl Dilution buffer and 50 µl Dissolving buffer vortexed for 10 minutes). 130 µl of CT conversion reagent was added to 20 µl of DNA (1 µg). Samples were heated to 98°C for 10 minutes before incubated overnight at 50°C. 600 µl of M-Binding buffer was added to Zymo-Spin IC columns which were placed into collection tubes. The samples from the previous day added to each column and inverted to mix. Tubes were centrifuged at 13000 rpm for 30 seconds and flow-through discarded. 100 µl of M-Wash buffer was added to each column and again centrifuged at 13000 rpm for 30 seconds. 200 µl of M-Desulphonation buffer was added to each column and incubated at room temperature for 15 minutes. Samples were centrifuged for 30 seconds at 13000 rpm, before columns were washed in 200 µl of M-Wash buffer, this step was repeated once. Zymo-Spin IC columns were transferred into a 1.5 ml microcentrifuge tube and 20 µl of M-Elution buffer pipetted directly onto the column. Samples

were incubated at room temperature for 5 minutes before a final centrifuge at 13000 rpm for 30 seconds.

1.5 µl of bisulphonated DNA was added to a 28.5 µl of PCR mastermix outlined in Table s3. The samples were run in a thermocycler at i) 95°C for 3 minutes, ii) 95°C for 30 seconds, iii) 52°C for 30 seconds, iv) 72°C for 30 seconds, v) repeat ii-iv 40 times, vi) 72°C for 3 minutes. 5 µl of the PCR product was run on an agarose gel as described in Section 2.4.5.

Table s3: Mastermix (1X) components for bisulphite PCR

Reagents	***Volume (µl)***
RNase free water	23.5
DreamTaq Buffer (10X) (ThermoFisher)	3
10 mM dNTPs (ThermoFisher)	0.6
Primer pair	1.2
DreamTaq (ThermoFisher)	0.2

s5. Pyrosequencing

Pyrosequencing was performed using all Qiagen (UK) reagents. 65 µl of immobilisation buffer (Table s4) was added to wells of a 96 well PCR plate alongside 15 µl of PCR product generated in Section s2. The plate was sealed and vortexed for 10 minutes. The samples were then immediately washed in 70% ethanol, Denaturation solution and Wash buffer using the PyroMark Q96 Vacuum Workstation. Using the vacuum, samples were transferred into a PyroMark Q96 Plate Low containing 36 µl of Annealing buffer and 3 µl of 10 µM sequencing primer (Table s5) in each well. This plate was heated at 80°C for 2 minutes before cooling at room temperature for 5 minutes. The PyroMark reagents and cartridge were prepared according to manufacturer's instructions. The samples were run in the Q96 PyroMark ID machine according to the settings generated by the sequencing primers.

Table s4: Immobilisation buffer reagents

Reagent	***Volume (µL)***
Streptavidin Sepharose HP	1.5
PyroMark Binding Buffer	40
Water	23.5

Table s5: Sequencing primers

Primer	***Sequencing primer***
Primer 1	AAATTTTTATAGGTAGGATAGTT
Primer 2	ATTTTTTTTTTAGTAGTTGTTTAGA
Primer 3	AGATTTTGTAGTAGTTTTTTAGATT
Primer 4	ATTTTGTTTTTAAGTATAAATGGT

s6. Western blot

Pulverised pooled placenta was generated as in Section 2.4. Samples were denatured and reduced in 5 µl beta-mercaptoethanol (Bio-Rad, UK) and 500 µl of 2X Laemmli Loading Buffer (Bio-Rad) in a heat block at 100°C for 5 minutes.

Samples were briefly centrifuged and 15 µl were loaded into Mini-PROTEAN TGX (4-20%) Gels (Bio-Rad) alongside 5 µl of ladder-precision plus protein dual colour standards (Bio-Rad). The gel was immersed in running buffer (500 ml water and 50 ml 10X TGS (Bio-Rad)) and was resolved in a Bio-Rad tank at 200 V for approximately 30 minutes, until the dye had reached the end of the gel.

The gel was then transferred between the sheets of Trans-Blot Turbo Transfer Packs (Bio-Rad) and run on a Trans-blot Turbo Transfer System (Bio-Rad) for seven minutes using a manufacturer-programmed setting. The transfer of ladder onto the membrane indicated successful transfer of protein. Submersion of the used gel in GelCode (ThermoFisher) allowed comparison of approximate levels of protein loaded between wells.

The membrane was washed in TBS-T (500 ml TBS (pH 8.0) (Bio-Rad) and 5 ml of 20% Tween (Sigma)) for 5 minutes at room temperature 3 times. The membrane was then blocked in 4% milk (Marvel) for 1 hour at room temperature with gentle agitation. The membrane was then exposed to specific primary antibodies diluted in 4 ml of 4% milk at 4°C overnight. GAPDH control samples were run alongside the experimental primary antibody. The membrane was again washed three times in TBS-T for a total of 30 minutes. The membrane was then exposed to the secondary horseradish peroxidase (HRP) conjugated antibody diluted in 5 ml of 4% milk for 1 hour at room temperature. The membrane was washed in TBS-T 3 times for a total of 30 minutes.

Immunologically reactive proteins were visualised using ECL Western Blotting Substrate (Promega). Chemiluminescent images of bands were obtained on sensitive autoradiography X-ray films exposed for different time periods (20 seconds, 30 seconds, 1 minute).

References

Abuidhail, J. and Abujilban, S. (2014). Characteristics of Jordanian depressed pregnant women: a comparison study. *Journal of Psychiatric and Mental Health Nursing* **21:**573-579.

Accortt, E. E., Cheadle, A. C. and Dunkel Schetter, C. (2015). Prenatal depression and adverse birth outcomes: an updated systematic review. *Maternal Child Health Journal* **19:**1306-1337.

Adams, S. S., Eberhard-Gran, M., Sandvik, Å. R. and Eskild, A. (2012). Mode of delivery and postpartum emotional distress: a cohort study of 55,814 women. *British Journal of Obstetrics and Gynaecology* **119:**298-305.

Adedinsewo, D. A., Fleming, A. S., Steiner, M., Meaney, M. J., Webb Girard, A. and MAVAN team. (2014). Maternal Anxiety and Breastfeeding: Findings From the MAVAN (Maternal Adversity, Vulnerability and Neurodevelopment) Study. *Journal of Human Lactation* **30:**102-109.

Alexander, J., Teague, A. M., Chen, J., Aston, C. E., Leung, Y. K., Chernausek, S., Simmons, R. A. *et al.* (2018). Offspring sex impacts DNA methylation and gene expression in placentae from women with diabetes during pregnancy. *PLoS One* **13:**e0190698.

Althubaiti, A. (2016). Information bias in health research: definition, pitfalls, and adjustment methods. *Journal of Multidisciplinary Healthcare* **9:**211-217.

Ammaniti, M. and Trentini, C. (2009). How New Knowledge About Parenting Reveals the Neurobiological Implications of Intersubjectivity: A Conceptual Synthesis of Recent Research. *Psychoanalytic Dialogues* **19:**537-555.

Amodeo, G., Trusso, M. A. and Fagiolini, A. (2017). Depression and Inflammation: Disentangling a Clear Yet Complex and Multifaceted Link. *Neuropsychiatry* **7:**448-457.

Andersson, L., Sundström-Poromaa, I., Wulff, M., Åström, M. and Bixo, M. (2006). Depression and anxiety during pregnancy and six months postpartum: a follow-up study. *Obstetrics and Gynaecology* **85:**937-944.

Andrews, S. (2010). FastQC: a quality control tool for highthroughput sequence data [Online]. Available at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/ [Accessed: 26/07/2017].

Apostolidou, S., Abu-Amero, S., O'Donoghue, K., Frost, J., Olafsdottir, O., Chavele, K. M., Whittaker, J. C. *et al.* (2007). Elevated placental expression of the imprinted PHLDA2 gene is associated with low birth weight. *Journal of Molecular Medicine* **85:**379-387.

Appleton, A. A., Armstrong, D. A., Lesseur, C., Lee, J., Padbury, J. F., Lester, B. M. and Marsit, C. J. (2013). Patterning in placental 11-B hydroxysteroid dehydrogenase methylation according to prenatal socioeconomic adversity. *PLoS One* **8:**e74691.

Armstrong, J. (2007). Progesterone for preterm birth prevention: A potential 2 billion dollar opportunity. *Americal Journal of Obstetrics and Gynecology* **196:**194-195.

Asher, I., Kaplan, B., Modai, I., Neri, A., Valevski, A. and Weizman, A. (1995). Mood and hormonal changes during late pregnancy and puerperium. *Clinical and Experimental Obstetrics and Gynecology* **22:**321-325.

Au-Yeung, S. K., Bradley, L., Robertson, A. E., Shaw, R., Baron-Cohen, S. and Cassidy, S. (2019). Experience of mental health diagnosis and perceived misdiagnosis in autistic, possibly autistic and non-autistic adults. *Autism* **23:**1508-1518.

Austin, M. P., Hadzi-Pavlovic, D., Leader, L., Saint, K. and Parker, G. (2005). Maternal trait anxiety, depression and life event stress in pregnancy: relationships with infant temperament. *Early Human Development* **81:**183-190.

Aykroyd, B. R. L., Tunster, S. J. and Sferruzzi-Perri, A. N. (2020). Igf2 deletion alters mouse placenta endocrine capacity in a sexually dimorphic manner. *Journal of Endocrinology* **246:**93-108.

Bale, T. L. (2011). Sex differences in prenatal epigenetic programming of stress pathways. *Stress* **14:**348-356.

Bale, T. L. (2016). The placenta and neurodevelopment: sex differences in prenatal vulnerability. *Dialogues in Clinical Neuroscience* **18:**459-464.

Barker, D. J. (2007). The origins of the developmental origins theory. *Journal of International Medicine* **261:**412-417.

Barlow, D. P., Stoger, R., Herrmann, B. G., Saito, K. and Schweifer, N. (1991). The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature* **349:**84-87.

Bartolomei, M. S., Zemel, S. and Tilghman, S. M. (1991). Parental imprinting of the mouse H19 gene. *Nature* **351:**153-155.

Bates, D., Maechler, M., Bolker, B. and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. *Journal of Statistical Software* **67:**1-48.

Bauer, A., Knapp, M. and Parsonage, M. (2016). Lifetime costs of perinatal anxiety and depression. *Journal of Affective Disorders* **192:**83-90.

Bauer, A., Parsonage, M., Knapp, M., Iemmi, V. and Adelaja, B. (2014). The costs of perinatal mental health problems. London: London School of Economics and Political Science.

Beck, A., Steer, R. and Brown, G. (1996). Beck Depression Inventory. Second ed San Antonio, TX: Psychological Corporation.

Bener, A., Gerber, L. M. and Sheikh, J. (2012). Prevalence Of psychiatric disorders and associated risk factors in women during their postpartum period: A major public health problem and global comparison. *International Journal of Women's Health* **4:**191-200.

Bennett, H. A., Einarson, A., Taddio, A., Koren, G. and Einarson, T. R. (2004). Prevalence of depression during pregnancy: systemic review. *Obstetrics and Gynaecology* **103:**698-709.

Bergner, A., Beyer, R., Klapp, B. F. and Rauchfuss, M. (2008). Pregnancy after early pregnancy loss: a prospective study of anxiety, depressive symptomatology and coping. *Journal of Psychosomatic Obstetrics and Gynaecology* **29:**105-113.

Bevan, G., Karanikolos, M., Exley, J., Nolte, E., Connolly, S. and Mays, N. (2014). The four health systems of the UK: How do they compare?

Biaggi, A., Conroy, S., Pawlby, S. and Pariante, C. M. (2016). Identifying the women at risk of antenatal anxiety and depression: A systematic review. *Journal of Affective Disorders* **191:**62-77.

Bildt, C. and Michélsen, H. (2002). Gender differences in the effects from working conditions on mental health: a 4-year follow-up. *Environmental Health* **75:**252-258.

Bilszta, J. L., Tang, M., Meyer, D., Milgrom, J., Ericksen, J. and Buist, A. E. (2008). Single motherhood versus poor partner relationship: outcomes for antenatal mental health. *Australian and New Zealand Journal of Psychiatry* **42:**56-65.

Blakeley, P. M., Capron, L. E., Jensen, A. B., O'Donnell, K. J. and Glover, V. (2013). Maternal prenatal symptoms of depression and down regulation of placental monoamine oxidase A expression. *Journal of Psychosomatic Research* **75:**341-345.

Bloch, M., Daly, R. C. and Rubinow, D. R. (2003). Endocrine factors in the etiology of postpartum depression. *Comprehensive Psychiatry* **44:**234-246.

BMA. (2017). Breaking down barriers – the challenge of improving mental health outcomes.

Bolton, H. L., Hughes, P. M., Turton, P. and Sedgwick, P. (1998). Incidence and demographic correlates of depressive symptoms during pregnancy in an inner London population. *Journal of Psychosomatic Obstetrics and Gynaecology* **19:**202-209.

Bonnin, A., Goeden, N., Chen, K., Wilson, M. L., King, J., Shih, J. C., Blakely, R. D. *et al.* (2011). A transient placental source of serotonin for the fetal forebrain. *Nature* **472:**347-350.

Bornstein, M. H., Jager, J. and Putnick, D. L. (2013). Sampling in developmental science: situations, shortcomings, solutions, and standards. *Developmental Review* **33:**257-270.

Boukhris, T., Sheehy, O., Mottron, L. and Bérard, A. (2016). Antidepressant Use During Pregnancy and the Risk of Autism Spectrum Disorder in Children. *JAMA Pediatrics* **170:**117-124.

Bowen, A. and Muhajarine, N. (2006). Antenatal depression. *The Canadian Nurse* **102:**26-30.

Boyle, M. H. and Pickles, A. (1997). Maternal depressive symptoms and ratings of emotional disorder symptoms in children and adolescents. *Journal of Child Psychology and Psychiatry* **38:**981-992.

Brandlistuen, R. E., Ystrom, E., Eberhard-Gran, M., Nulman, I., Koren, G. and Nordeng, H. (2015). Behavioural effects of fetal antidepressant exposure in a Norwegian cohort of discordant siblings. *International Journal of Epidemiology* **44:**1397-1407.

Bremme, K., Lagerstrom, M., Andersson, O., Johansson, S. and Eneroth, P. (1990). Influences of maternal smoking and fetal sex on maternal serum oestriol, prolactin, hCG, and hPL levels. *Archives of Gynecology ad Obstetrics* **247:**95-103.

Brennan, P. A., Hammen, C., Andersen, M. J., Bor, W., Najman, J. M. and Williams, G. M. (2000). Chronicity, severity, and timing of maternal depressive symptoms: relationships with child outcomes at age 5. *Developmental Psychology* **36:**759-766.

Bretz, C. L., Frey, W. D., Teruyama, R. and Kim, J. (2018). Allele and dosage specificity of the Peg3 imprinted domain. *PLoS One* **13:**e0197069.

Bretz, C. L. and Kim, J. (2018). Trans-allelic mutational effects at the Peg3 imprinted locus. *PLoS One* **13:**e0206112.

Bridges, R. S., Numan, M., Ronsheim, P. M., Mann, P. E. and Lupini, C. E. (1990). Central prolactin infusions stimulate maternal behavior in steroid-treated, nulliparous female rats. *PNAS* **87:**8003-8007.

Brockington, I. F., Fraser, C. and Wilson, D. (2006). An evaluation of two bonding questionnaires: A comparison of the Mother-to-Infant Bonding Scale with the Postpartum Bonding Questionnaire in a sample of primiparous mothers. *Archives of Womens Mental Health* **9:**233-242.

Brockmann, R., Beyer, A., Heinisch, J. J. and Wilhelm, T. (2007). Posttranscriptional Expression Regulation: What Determines Translation Rates? *PLoS Computational Biology* **23:**e57.

Bronson, S. L. and Bale, T. L. (2014). Prenatal stress-induced increases in placental inflammation and offspring hyperactivity are male-specific and ameliorated by maternal antiinflammatory treatment. *Endocrinology* **155:**2635-2646.

Bruchova, H., Vasikova, A., Merkerova, M., Milcova, A., Topinka, J., Balascak, I., Pastorkova, A. *et al.* (2010). Effect of maternal tobacco smoke exposure on the placental transcriptome. *Placenta* **31:**186-191.

Brummelte, S., Galea, L. A., Devlin, A. M. and Oberlander, T. F. (2013). Antidepressant use during pregnancy and serotonin transporter genotype (SLC6A4) affect newborn serum reelin levels. *Developmental Psychobiology* **55:**518-529.

Brummelte, S., Mc Glanaghy, E., Bonnin, A. and Oberlander, T. F. (2017). Developmental changes in serotonin signaling: implications for early brain function, behavior and adaptation. *Neuroscience* **342:**212-231.

Brunton, P. J., Russell, J. A. and Douglas, A. J. (2008). Adaptive Responses of the Maternal Hypothalamic-Pituitary-Adrenal Axis during Pregnancy and Lactation. *Journal of Neuroendocrinology* **20:**764-776.

Buckberry, S., Bianco-Miotto, T., Bent, S. J., Clifton, V., Shoubridge, C., Shankar, K. and Roberts, C. T. (2017). Placental transcriptome co-expression analysis reveals conserved regulatory programs across gestation. *BMC Genomics* **18:**10.

Burt, N. M., Boddy, L. E. and Bridgett, D. J. (2015). Contribution of temperament to eating disorder symptoms in emerging adulthood: Additive and interactive effects. *Eating Behaviours* **2015:**30-35.

Burton, G. J. and Fowden, A. L. (2015). The placenta: a multifaceted, transient organ. *Philosophical Transactions of the Royal Society of London, Series B Biological Sciences* **370:**1663.

Buss, K. A. and Goldsmith, H. H. (1998). Fear and anger regulation in infancy: Effects on the temporal dynamics of affective expression. *Child Development* **69:**359-374.

Butler, M. G. (2009). Genomic imprinting disorders in humans: a mini-review. *Journal of Assisted Reproduction and Genetics* **26:**477-486.

Capron, L. E., Ramchandani, P. G. and Glover, V. (2018). Maternal prenatal stress and placental gene expression of NR3C1 and HSD11B2: The effects of maternal ethnicity. *Psychoneuroendocrinology* **87:**166-172.

Carter, A. M. and Mess, A. (2007). Evolution of the placenta in eutherian mammals. *Placenta* **28:**259-262.

Cattini, P. A., Jin, Y., Jarmasz, J. S., Noorjahan, N. and Bock, M. E. (2020). Obesity and regulation of human placental lactogen production in pregnancy. *Journal of Neuroendocrinology* e12859.

Cattini, P. A., Yang, X., Jin, Y. and Detillieux, K. A. (2006). Regulation of the human growth hormone gene family: possible role for Pit-1 in early stages of pituitary-specific expression and repression. *Neuroendocrinology* **83:**145-153.

Champagne, F. A., Curley, J. P., Swaney, W. T., Hasen, N. S. and Keverne, E. B. (2009). Paternal influence on female behaviour: the role of Peg3 in exploration, olfaction, and neuroendocrine regulation of maternal behaviour of female mice. *Behavioural Neuroscience* **123:**469-480.

Chaouloff, F., Berton, O. and Mormède, P. (1999). Serotonin and stress. *Neuropsychopharmacology* **21:**28-32.

Charalambous, M., da Rocha, S. T. and Ferguson–Smith, A. C. (2007). Genomic imprinting, growth control and the allocation of nutritional resources: consequences for postnatal life. *Current Opinion in Endocrinolgy, Diabetes and Obesity* **14:**143-112.

Charlton, R. A., Jordan, S., Pierini, A., Garne, E., Neville, A. J., Hansen, A. V., Gini, R. *et al.* (2015). Selective serotonin reuptake inhibitor prescribing before, during and after pregnancy: a population-based study in six European regions. *British Journal of Obstetrics and Gynaecology* **122:**1010-1020.

Chen, E. Y., Liao, Y. C., Smith, D. H., Barrera-Saldana, H. A., Gelinas, R. E. and Seeburg, P. H. (1989). The human growth hormone locus: nucleotide sequence, biology, and evolution. *Genomics* **4:**479-497.

Chen, M. Y., Liao, W. S., Lu, Z., Bornmann, W. G., Hennessey, V., Washington, M. N., Rosner, G. L. *et al.* (2011). Decitabine and suberoylanilide hydroxamic acid (SAHA) inhibit growth of ovarian cancer cell lines and xenografts while inducing expression of imprinted tumor suppressor genes, apoptosis, G2/M arrest, and autophagy. *Cancer* **117:**4424-4438.

Cipriani, A., Furukawa, T. A., Salanti, G., Chaimani, A., Atkinson, L. Z., Ogawa, Y., Leucht, S. *et al.* (2018). Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *The Lancet* **391:**1357-1366.

Clements, C. C., Castro, V. M., Blumenthal, S. R., Rosenfield, H. R., Murphy, S. N., Fava, M., Erb, J. L. *et al.* (2015). Prenatal antidepressant exposure is associated with risk for attention deficit-hyperactivity disorder but not autism spectrum disorder in a large health system. *Molecular Psychiatry* **20:**727-734.

Clifton, V. L. (2010). Review: Sex and the human placenta: mediating differential strategies of fetal growth and survival. *Placenta* **31:**S33-39.

Coker, F. and Taylor, D. (2010). Antidepressant-induced hyperprolactinaemia: incidence, mechanisms and management. *CNS Drugs* **24:**563-574.

Conlon, M., Lightfoot, N. and Kreiger, N. (2007). Rotating shift work and risk of prostate cancer. *Epidemiology* **18:**182-183.

Constância, M., Angiolini, E., Sandovici, I., Smith, P., Smith, R., Kelsey, G., Dean, W. *et al.* (2005). Adaptation of nutrient supply to fetal demand in the mouse involves interaction between the Igf2 gene and placental transporter systems. *PNAS* **102:**19219-19224.

Cornish, A. M., McMahona, C. A., Ungerer, J. A., Barnett, B., Kowalenko, N. and Tennantd, C. (2005). Postnatal depression and infant cognitive and motor development in the second postnatal year: The impact of depression chronicity and infant gender. *Infant Behavior and Development* **28:**407-417.

Cousins, L., Rigg, L., Hollingsworth, D., Meis, P., Halberg, F., Brink, G. and Yen, S. S. (1983). Qualitative and quantitative assessment of the circadian rhythm of cortisol in pregnancy. *Americal Journal of Obstetrics and Gynecology* **145:**411-416.

Coutant, R., Boux de Casson, F., Douay, O., Mathieu, E., Rouleau, S., Beringue, F., Gillard, P. *et al.* (2001). Relationships between Placental GH Concentration and Maternal Smoking, Newborn Gender, and Maternal Leptin: Possible Implications for Birth Weight. *Journal of Clinical Endocrinology and Metabolism* **86:**4854-4859.

Cox, J., Holden, J. and Henshaw, C. (2014). Perinatal Mental Health: The Edinburgh Postnatal Depression Scale (EPDS) Manual (2nd edition). RCPsych Publications.

Cox, J. L., Chapman, G., Murray, D. and Jones, P. (1996). Validation of the Edinburgh Postnatal Depression Scale (EPDS) in non-postnatal women. *Journal of Affective Disorders* **39:**185-189.

Cox, J. L., Holden, J. M. and Sagovsky, R. (1987). Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *The British Journal of Psychiatry* **150:**782-786.

Creeth, H. D. J. and John, R. M. (2020). The placental programming hypothesis: Placental endocrine insufficiency and the co-occurrence of low birth weight and maternal mood disorders. *Placenta* 10.1016/j.placenta.2020.1003.1011.

Creeth, H. D. J., McNamara, G. I., Isles, A. R. and John, R. M. (2019). Imprinted genes influencing the quality of maternal care. *Frontiers in Neuroendocrinology* **53:**100732.

Creeth, H. D. J., McNamara, G. I., Tunster, S. J., Boque-Sastre, R., Allen, B., Sumption, L., Eddy, J. B. *et al.* (2018). Maternal care boosted by paternal imprinting in mammals. *PLoS Biology* **16:**e2006599.

Cross, J. C. (2005). How to make a placenta: Mechanisms of trophoblast cell differentiation in mice – A Review. *Placenta* **26:**S3-9.

Crown, S. and Crisp, A. H. (1979). Manual of the Crown-Crisp Experiential Index. London: Hodder and Stoughton.

Cuffe, J. S., Dickinson, H., Simmons, D. G. and Moritz, K. M. (2011). Sex specific changes in placental growth and MAPK following short term maternal dexamethasone exposure in the mouse. *Placenta* **32:**981-989.

Cummings, E. M., Iannotti, R. J. and Zahn-Waxler, C. (1989). Aggression between peers in early childhood: Individual continuity and change. *Child Development* **60:**887–895.

Curley, J. P., Barton, S., Surani, A. and Keverne, E. B. (2004). Coadaptation in mother and infant regulated by a paternally expressed imprinted gene. *Proc Biol Sci* **271:**1303-1309.

Curley, J. P., Pinnock, S. B., Dickson, S. L., Thresher, R., Miyoshi, N., Surani, M. A. and Keverne, E. B. (2005). Increased body fat in mice with a targeted mutation of the paternally expressed imprinted gene Peg3. *FASEB J* **19:**1302-1304.

Cvitic, S., Longtine, M. S., Hackl, H., Wagner, K., Nelson, M. D., Desoye, G. and Hiden, U. (2013). The human placental sexome differs between trophoblast epithelium and villous vessel endothelium. *PLoS One* **29:**e79233.

Dahl, J., Ormstad, H., Aass, H. C. D., Malt, U. F., Bendz, L. T., Sandvik, L., Brundin, L. *et al.* (2014). The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. *Psychoneuroendocrinology* **45:**77-86.

Dalgaard, K., Landgraf, K., Heyne, S., Lempradl, A., Longinotto, J., Gossens, K., Ruf, M. *et al.* (2016). Trim28 Haploinsufficiency Triggers Bi-stable Epigenetic Obesity. *Cell* **164:**353-364.

Darnaudery, M. and Maccari, S. (2008). Epigenetic programming of the stress response in male and female rats by prenatal restraint stress. *Brain Research Reviews* **57:**571-585.

Davidson, S., Prokonov, D., Taler, M., Maayan, R., Harell, D., Gil-Ad, I. and Weizman, A. (2009). Effect of exposure to selective serotonin reuptake inhibitors in utero on fetal growth: potential role for the IGF-I and HPA axes. *Pediatric Research* **65:**236-241.

Davis, E. P. and Pfaff, D. (2014). Sexually dimorphic responses to early adversity: implications for affective problems and autism spectrum disorder. *Psychoneuroendocrinology* **49:**11-25.

Dayan, J., Creveuil, C., Herlicoviez, M., Herbel, C., Baranger, E., Savoye, C. and Thouin, A. (2002). Role of anxiety and depression in the onset of spontaneous preterm labor. *American Journal of Epidemiology* **155:**293-301.

Deave, T., Heron, J., Evans, J. and Emond, A. (2008). The impact of maternal depression in pregnancy on early child development. *British Journal of Obstetrics and Gynaecology* **115:**1043-1051.

DeChiara, T. M., Efstradiatis, A. and Robertson, J. (1990). A growth-dc:liciency pbenotype in beterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* **345:**78-80.

DeChiara, T. M., Robertson, E. J. and Efstratiadis, A. (1991). Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* **64:**849-859.

Denizot, A. L., Besson, V., Correra, R. M., Mazzola, A., Lopes, I., Courbard, J. and Marazzi, G. (2016). A novel mutant allele of Pw1/Peg3 does not affect maternal behaviour or nursing behaviour. *PLoS Genetics* **12:**e1006053.

Dennis, C., Falah-Hassani, K. and Shiri, R. (2017). Prevalence of antenatal and postnatal anxiety: Systematic review and meta-analysis. *British Journal of Psychiatry* **210:**315-323.

Dhaliwal, G., Weikum, W., Jolicoeur-Martineau, A., Brain, U., Grunau, R. and Oberlander, T. (2017). Household chaos, maternal depression and prenatal ssri exposure interact to affect executive function of 6 year old children. *Paediatrics & Child Health* **22:**e8-9.

Dickens, M. J. and Pawluski, J. L. (2018). The HPA Axis During the Perinatal Period: Implications for Perinatal Depression. *Endocrinology* **159:**3737-3746.

Diego, M. A., Field, T., Hernandez-Reif, M., Schanberg, S., Kuhn, C. and Gonzalez-Quintero, V. H. (2009). Prenatal depression restricts fetal growth. *Early Human Development* **85:**65-70.

Diego, M. A., Jones, N. A., Field, T., Hernandez-Reif, M., Schanberg, S., Kuhn, C. and Gonzalez-Garcia, A. (2006). Maternal Psychological Distress, Prenatal Cortisol, and Fetal Weight. *Psychosomatic Medicine* **68:**747-753.

Dipietro, J. A., Novak, F. S. M., Costigan, K. A., Atella, L. D. and Reusing, S. P. (2006). Maternal psychological distress during pregnancy in relation to child development at age two. *Child Development* **77:**573-587.

DiPietro, J. A. and Voegtline, K. M. (2017). The gestational foundation of sex differences in development and vulnerability. *Neuroscience* **342:**4-20.

Diplas, A. I., Lambertini, L., Lee, M. J., Sperling, R., Lee, Y. L., Wetmur, J. and Chen, J. (2009). Differential expression of imprinted genes in normal and IUGR human placentas *Epigenetics* **4:**235-240.

Dobin, A., Davis, C. A., Schlesigner, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P. *et al.* (2013). STAR: Ultrafast universal RNA-Seq aligner. *Bioinformatics* **29:**15-21.

Douglas, C. and Turner, J. M. A. (2020). Advances and challenges in genetic technologies to produce single-sex litters. *PLoS Genetics* **16:**e1008898.

Dowdy, S. C., Gostout, B. S., Shridhar, V., Wu, X., Smith, D. I., Podratz, K. C. and Jiang, S. W. (2005). Biallelic methylation and silencing of paternally expressed gene 3 (PEG3) in gynecologic cancer cell lines. *Gynecologic Ontology* **99:**126-134.

Drewlo, S., Levytska, K. and Kingdom, J. (2012). Revisiting the housekeeping genes of human placental development and insufficiency syndromes. *Placenta* **33:**952-954.

Dubber, S., Reck, C., Müller, M. and Gawlik, S. (2014). Postpartum bonding: the role of perinatal depression, anxiety and maternal–fetal bonding during pregnancy. *Archives of Womens Mental Health* **18:**187-195.

Dubovicky, M., Belovicova, K., Csatlosova, K. and Bogi, E. (2017). Risks of using SSRI / SNRI antidepressants during pregnancy and lactation. *Interdisciplinary Toxicology* **10:**30-34.

Dunn, G. A., Morgan, C. P. and Bale, T. L. (2011). Sex-specificity in transgenerational epigenetic programming. *Hormones and Behaviour* **59:**290-295.

Duthie, L. and Reynolds, R. M. (2013). Changes in the maternal hypothalamic-pituitary-adrenal axis in pregnancy and postpartum: influences on maternal and fetal outcomes. *Neuroendocrinology* **98:**106-115.

Dvorakova, L., Ivankova, K., Krofta, L. and Hromadnikova, I. (2017). Expression profile of heat shock proteins in placental tissues of patients with preterm prelabor rupture of membranes and spontaneous preterm labor with intact membranes. *Americal Journal of Reproductive Immunology* **78:**4.

Edvinsson, A., Hellgren, C., Kallak, T. K., Åkerud, H., Skalkidou, A., Stener-Victorin, E., Fornes, R. *et al.* (2019). The effect of antenatal depression and antidepressant treatment on placental tissue: a protein-validated gene expression study. *BMC Pregnancy and Childbirth* **19:**479.

Edwards, B., Galletly, C., Semmler-Booth, T. and Dekker, G. (2008). Antenatal psychosocial risk factors and depression among women living in socioeconomically disadvantaged suburbs in Adelaide, South Australia. *Australian and New Zealand Journal of Psychiatry* **42:**45-50.

El Marroun, H., White, T. J. H., van der Knaap, N. J. F., Homberg, J. R., Fernández, G., Schoemaker, N. K., Jaddoe, V. W. V. *et al.* (2014). Prenatal Exposure to Selective Serotonin Reuptake Inhibitors and Social Responsiveness Symptoms of Autism: Population-Based Study of Young Children. *British Journal of Psychiatry* **205:**95-102.

Emiliano, A. B. and Fudge, J. L. (2004). From galactorrhea to osteopenia: rethinking serotonin-prolactin interactions. *Neuropsychopharmacology* **29:**833-846.

Erickson, N. L., Hancock, G. R., Oberlander, T. F., Brain, U., Grunauc, R. E. and Gartsteind, M. A. (2019). Prenatal SSRI antidepressant use and maternal internalizing symptoms during pregnancy and postpartum: Exploring effects on infant temperament trajectories for boys and girls. *Journal of Affective Disorders* **258:**179-194.

Essaua, C. A., Lewinsohn, P. M., Olaya, B. and Seeley, J. R. (2014). Anxiety disorders in adolescents and psychosocial outcomes at age 30. *Journal of Affective Disorders* **163:**125-132.

Evans, J., Heron, J., Francomb, H., Oke, S. and Golding, J. (2001). Cohort study of depressed mood during pregnancy and after childbirth. *British Medical Journal* **232:**257.

Evans, L. M., Myers, M. M. and Monk, C. (2008). Pregnant women's cortisol is elevated with anxiety and depression — but only when comorbid. *Archives of Womens Mental Health* **11:**239.

Faisal, M., Kim, H. and Kim, J. (2014). Sexual differences of imprinted genes' expression levels. *Gene* **533:**434-438.

Feng, W., Marquez, R. T., Lu, Z., Liu, J., Lu, K. H., Issa, J. P., Fishman, D. M. *et al.* (2008). Imprinted tumor suppressor genes ARHI and PEG3 are the most frequently down-regulated in human ovarian cancers by loss of heterozygosity and promoter methylation. *Cancer* **112:**1489-1502.

Ferreira, A. J. (1965). Emotional Factors in Prenatal Environment: A Review. *The Journal of Nervous and Mental Disease* **141:**108–118.

Field, T. (2011). Prenatal depression effects on early development: a review. *Infant Behavior and Development* **34:**1-14.

Field, T. (2018). Postnatal anxiety prevalence, predictors and effects on development: A narrative review. *Infant Behavior and Development* **51:**24-32.

Field, T., Diego, M., Dieter, J., Hernandez-Reif, M., Schanberg, S., Kuhn, C., Yando, R. *et al.* (2004). Prenatal depression effects on the fetus and the newborn. *Infant Behavior and Development* **27:**216-229.

Field, T., Diego, M. and Hernandez-Reif, M. (2006). Prenatal depression effects on the fetus and newborn: a review. *Infant Behaviour and Development* **29:**445-455.

Field, T., Diego, M., Hernandez-Reif, M., Figueiredo, B., Ascencio, A., Schanberg, S. and Kuhn, C. (2007). Prenatal dysthymia versus major depression disorder effects on maternal cortisol and fetal growth. *Depression and Anxiety* **25:**11-16.

Field, T., Diego, M., Hernandez-Reif, M., Figueiredo, B., Deeds, O., Ascencio, A., Schanberg, S. *et al.* (2010). Comorbid depression and anxiety effects on pregnancy and neonatal outcome. *Infant Behavior and Development* **33:**23-29.

Figueiredo, B., Canário, C. and Field, T. (2014). Breastfeeding is negatively affected by prenatal depression and reduces postpartum depression. *Psychological Medicine* **44:**927-936.

Fish, M. and Stifler, C. A. (1993). Mother parity as a main and moderating influence on early mother-infant interaction. *Journal of Applied Developmental Psychology* **14:**557-572.

Fox, J. and Weisberg, S. (2019). An R Companion to Applied Regression, Third Edition. Thousand Oaks, California: Sage.

Frankish, A., Diekhans, M., Ferreira, A. M., Johnson, R., Jungreis, I., Loveland, J., Mudge, J. M. *et al.* (2019). GENCODE reference annotation for the human and mouse genomes. *Nucleic Acids Research* **47:**766-773.

Fransson, E., Sörensen, F., Kallak, T. K., Ramklint, M., Eckerdal, P., Heimgärtner, M., Krägeloh-Mann, I. *et al.* (2020). Maternal perinatal depressive symptoms trajectories and impact on toddler behavior – the importance of symptom duration and maternal bonding. *Journal of Affective Disorders* **273:**541-551.

Freemark, M. (2010). Placental hormones and the control of fetal growth. *Journal of Clinical Endocrinology and Metabolism* **95:**2054-2057.

Friedrich, M. J. (2017). Depression is the leading cause of disability around the world. *JAMA* **317:**1517.

Fuemmeler, B. F., Lee, C. T., Soubry, A., Iversen, E. S., Huang, Z., Murtha, A. P., Schildkraut, J. M. *et al.* (2016). DNA Methylation of Regulatory Regions of Imprinted Genes at Birth and Its Relation to Infant Temperament. *Genetics and Epigenetics* **8:**59-67.

Fuller, R. W. (1996). Mechanisms and Functions of Serotonin Neuronal Systems. *Annals of the New York Academy of Sciences* **780:**176-184.

Gabory, A., Ferry, L., Fajardy, I., Jouneau, L., Gothié, J. D., Vigé, A., Fleur, C. *et al.* (2012). Maternal diets trigger sex-specific divergent trajectories of gene expression and epigenetic systems in mouse placenta. *PLoS One* **7:**e47986.

Gallou-Kabani, C., Gabory, A., Tost, J., Karimi, M., Mayeur, S., Lesage, J., Boudadi, E. *et al.* (2010). Sex- and diet-specific changes of imprinted gene expression and DNA methylation in mouse placenta under a high-fat diet. *PLoS One* **5:**e14398.

Garay, S. M., Savory, K. A., Sumption, L. A., Penketh, R. J. A., Jones, I. R., Janssen, A. B. and John, R. M. (2019). Seasonal variation in salivary cortisol but not symptoms of depression and trait anxiety in pregnant women undergoing an elective caesarean section. *Psychoneuroendocrinology* **108:**14-19.

Gardosi, J. and Francis, A. (2016). Customised Centile Calculator. UK: GROW.

Gartner, A., Cosh, H., Gibbon, R. and Lester, N. (2009). A profile of alcohol and health in Wales.

Gartstein, M. A. and Rothbart, M. K. (2003). Studying infant temperament via the Revised Infant Behavior Questionnaire. *Infant Behavior and Development* **26:**64-86.

Gaspar, P., Cases, O. and Maroteaux, L. (2003). The developmental role of serotonin: news from mouse molecular genetics. *Nature Reviews Neuroscience* **4:**1002-1012.

Gavin, N. I., Meltzer-Brody, S., Glover, V. and Gaynes, B. N. (2015). Is Population-Based Identification of Perinatal Depression and Anxiety Desirable? Wiley-Blackwell.

Gerardin, P., Wendland, J., Bodeau, N., Galin, A., Bialobos, S., Tordjman, S., Mazet, P. *et al.* (2011). Depression during pregnancy: is the developmental impact earlier in boys? A prospective case-control study. *Journal of Clinical Psychiatry* **72:**378-387.

Gidaya, N. B., Lee, B. K., Burstyn, I., Yudell, M., Mortensen, E. L. and Newschaffer, C. J. (2014). In Utero Exposure to Selective Serotonin Reuptake Inhibitors and Risk for Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders* **44:**2558-2567.

Gil-ad, I., Zambotti, F., Carruba, M. O., Vicentini, L. and Muller, E. E. (1976). Stimulatory role for brain serotoninergic system on prolactin secretion in the male rat. *Proceedings of the Society for Experimental Biology and Medicine* **151:**512-518.

Glover, M. E. and Clinton, S. M. (2016). Of rodents and humans: A comparative review of the neurobehavioral effects of early life SSRI exposure in preclinical and clinical research. *International Journal of Developmental Neuroscience* **51:**50-72.

Glover, M. E., Pugh, P. C., Jackson, N. L., Cohen, J. L., Fant, A. D., Akil, H. and Clinton, S. M. (2015). Early-life Exposure to the SSRI Paroxetine Exacerbates Depression-Like Behavior in anxiety/depression-prone Rats. *Neuroscience* **284:**775-797.

Glover, V. (2011). Annual Research Review: Prenatal stress and the origins of psychopathology: an evolutionary perspective. *Journal of Child Psychology and Psychiatry* **51:**356-367.

Glover, V. (2014). Maternal depression, anxiety and stress during pregnancy and child outcome; what needs to be done. *Best Practice & Research: Clinical Obstetrics & Gynaecology* **28:**25-35.

Glover, V. and Hill, J. (2012). Sex differences in the programming effects of prenatal stress on psychopathology and stress responses: an evolutionary perspective. *Physiology Behaviour* **106:**736-740.

Gluckman, P. D. and Hanson, M. A. (2004). The developmental origins of the metabolic syndrome. *Trends in Endocrinology & Metabolism* **15:**183-187.

Glynn, L. M., Davis, E. P. and Sandman, C. A. (2013). New insights into the role of perinatal HPA-axis dysregulation in postpartum depression. *Neuropeptides* **47:**363-370.

Glynn, L. M., Howland, M. A., Sandman, C. A., Davis, E. P., Phelan, M., Baram, T. Z. and Stern, H. S. (2018). Prenatal maternal mood patterns predict child temperament and adolescent mental health. *Journal of Affective Disorders* **228:**83-90.

Goecke, T. W., Voigt, F., Faschingbauer, F., Spangler, G., Beckmann, M. W. and Beetz, A. (2012). Prenatal Attachment and Other Correlates of Postnatal Maternal Attachment to Twins. *Archives of Gynecology ad Obstetrics* **286:**309-316.

Goldsmith, H. H. and Rothbart, M. K. (1996). Prelocomotor and Locomotor Laboratory Temperament Assessment Battery, Lab-TAB, version 3.0. Technical Manual. Department of Psychology, University of Wisconsin, Madison, WI.

Gong, S., Sovio, U., Aye, I. L., Gaccioli, F., Dopierala, J., Johnson, M. D., Wood, A. M. *et al.* (2018). Placental polyamine metabolism differs by fetal sex, fetal growth restriction, and preeclampsia. *JCI Insight* **3:**e120723.

Gonzalez, T. L., Sun, T., Koeppel, A. F., Lee, B., Wang, E. T., Farber, C. R., Rich, S. S. *et al.* (2018). Sex differences in the late first trimester human placenta transcriptome. *Biology of Sex Differences* **9:**4.

Gorman, M. D. (1996). Comorbid depression and anxiety spectrum disorders. *Depression and Anxiety* **4:**160-168.

Gotlib, I. H., Whiffen, V. E., Mount, J. H., Milne, K. and Cordy, N. I. (1989). Prevalence rates and demographic characteristics associated with depression in pregnancy and the postpartum. *Journal of Consulting and Clinical Psychology* **57:**269-274.

Gottschalk, M. G. (2017). Genetics of generalized anxiety disorder and related traits. *Dialogues in Clinical Neuroscience* **19:**159-168.

Grattan, D. (2011). A Mother's Brain Knows. *Journal of Neuroendocrinology* **23:**1188-1189.

Green, B. B., Kappil, M., Lambertini, L., Armstrong, D. A., Guerin, D. J., Sharp, A. J., Lester, B. M. *et al.* (2015). Expression of imprinted genes in placenta is associated with infant neurobehavioral development. *Epigenetics* **10:**834-841.

Griffiths, S. K. and Campbell, J. P. (2014). Placental structure, function and drug transfer. *Continuing Education in Anaesthesia Critical Care and Pain* **15:**84-89.

Grigoriadis, S., Graves, L., Peer, M., Mamisashvili, L., Tomlinson, G., Vigod, S. N., Dennis, C. L. *et al.* (2018). Maternal anxiety during pregnancy and the association with adverse perinatal outcomes: Systematic review and meta-analysis. *Journal of Clinical Psychiatry* **79:**17r12011.

Groer, M. W. and Morgan, K. (2007). Immune, health and endocrine characteristics of depressed postpartum mothers. *Psychoneuroendocrinology* **32:**133-139.

Gualtieri, T. and Hicks, R. (1985). An immunoreactive theory of selective male affliction. *Behavioural Brain Science* **8:**427-441.

Gude, N. M., Roberts, C. T., Kalionis, B. and King, R. G. (2004). Growth and function of the normal human placenta. *Thrombosis Research* **114:**297-407.

Gunning, M. D., Denison, F. C., Stockley, C. J., Ho, S. P., Sandhu, H. K. and Reynolds, R. M. (2010). Assessing maternal anxiety in pregnancy with the State-Trait Anxiety Inventory (STAI): issues of validity, location and participation. *Journal of Reproductive and Infant Psychology* **10:**266-273.

Gurtunca, N. and Sperling, M. A. (2017). Growth Hormone, Prolactin, and Placental Lactogen in the Fetus and Newborn.*Fetal and Neonatal Physiology (Fifth Edition)*. Vol. 2. pp. 1470-1476.

Gustafsson, H. C., Goodman, S. H., Feng, T., Choi, J., Lee, S., Newport, D. J., Knight, B. *et al.* (2018). Major depressive disorder during pregnancy: Psychiatric medications have minimal effects on the fetus and infant yet development is compromised. *Developmental Psychopathology* **30:**773-785.

Guthrie, S., Lichten, C. A., Leach, B., Pollard, J., Parkinson, S. and Altenhofer, M. (2020). Pregnancy research review: Policy report. Santa Monica, California: California: RAND Corporation.

Gutteling, B. M., de Weerth, C., Zandbelt, N., Mulder, E. J. H., Visser, G. H. A. and Buitelaar, J. K. (2006). Does Maternal Prenatal Stress Adversely Affect the Child's Learning and Memory at Age Six? *Journal of Abnormal Child Psychology* **34:**787-796.

Haas, J. S., Jackson, R. A., Fuentes-Afflick, E., Stewart, A. L., Dean, M. L., Brawarsky, P. and Escobar, G. J. (2004). Changes in the Health Status of Women During and After Pregnancy. *Journal of General Internal Medicine* **20:**45-51.

Haggarty, P., Hoad, G., Campbell, D. M., Horgan, G. W., Piyathilake, C. and McNeill, G. (2013). Folate in pregnancy and imprinted gene and repeat element methylation in the offspring. *Americal Journal of Clinical Nutrition* **97:**94-99.

Haig, D. (2014). Coadaptation and conflict, misconception and muddle, in the evolution of genomic imprinting. *Heredity* **113:**96-103.

Hanada, T. and Yoshimura, A. (2002). Regulation of cytokine signaling and inflammation. *Cytokine & Growth Factor Reviews* **13:**413-421.

Handwerger, S. and Freemark, M. (1987). Role of Placental Lactogen and Prolactin in Human Pregnancy. In: Mahesh, V.B. and Dhindsa, D.S. and Anderson, E. and Kalra, S.P. (eds.) *Regulation of Ovarian and Testicular Function. Advances in Experimental Medicine and Biology*. Boston, MA: Springer, pp. 399-420.

Hanley, G. E., Brain, U. and Oberlander, T. F. (2015). Prenatal exposure to serotonin reuptake inhibitor antidepressants and childhood behavior. *Pediatric Research* **78:**174-180.

Hanley, G. E. and Oberlander, T. F. (2014). The effect of perinatal exposures on the infant: antidepressants and depression. *Best Practice & Research: Clinical Obstetrics & Gynaecology* **28:**37-48.

Hanley, J. (2009). Perinatal Mental Health: A Guide for Health Professionals and Users. Wiley Blackwell.

Harper, M. E., Barrera-Saldana, H. A. and Saunders, G. F. (1982). Chromosomal localization of the human placental lactogen-growth hormone gene cluster to 17q22-24. *American Journal of Human Genetics* **34:**227-234.

Harrington, R. A., Lee, L.-C., Crum, R. M., Zimmerman, A. W. and Hertz-Picciotto, I. (2014). Prenatal SSRI Use and Offspring With Autism Spectrum Disorder or Developmental Delay. *Pediatrics* **133:**e1241-1248.

Hata, K., Okano, M., Lei, H. and Li, E. (2002). Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development* **129:**1983-1993.

Hay, D. F., Pawlby, S., Waters, C. S., Perra, O. and Sharp, D. (2010). Mothers' antenatal depression and their children's antisocial outcomes. *Child Development* **81:**149-165.

Hay, D. F., Pawlby, S., Waters, C. S. and Sharp, D. (2008). Antepartum and postpartum exposure to maternal depression: different effects on different adolescent outcomes. *Journal of Child Psychology and Psychiatry, and Allied Disciplines* **49:**1079-1088.

Hay, D. F., Waters, C. S., Perra, O., Swift, N., Kairis, V., Phillips, R. and van Goozen, S. (2006). Precursors to aggression are evident by 6 months of age. *Developmental Science* **17:**471-480.

He, H. and Kim, J. (2014). Regulation and function of the peg3 imprinted domain. *Genomics Informatics* **12:**105-113.

He, H., Ye, A., Perera, B. P. U. and Kim, J. (2017). YY1's role in the Peg3 imprinted domain. *Nature Scientific Reports* **7:**6427.

Hemberger, M., Hanna, C. W. and Dean, W. (2020). Mechanisms of early placental development in mouse and humans. *Nature Reviews Genetics* **21:**27-43.

Hendrich, B. and Bird, A. (2000). Mammalian methyltransferases and methyl-CpG-binding domains: proteins involved in DNA methylation. *Current Topics in Microbiology and Immunology* **249:**55-74.

Hendrick, V. (1998). Evaluation of mental health and depression during pregnancy. *Psychopharmacol Bulletin* **34:**297-299.

Hentges, R. F., Graham, S. A., Plamondon, A., Tough, S. and Madigan, S. (2019). A Developmental Cascade From Prenatal Stress to Child Internalizing and Externalizing Problems. *Journal of Pediatric Psychology* **44:**1057-1067.

Herlenius, E. and Lagercrantz, H. (2004). Development of neurotransmitter systems during critical periods. *Experimental Neurology* **190:**S8-21.

Hermansen, T. K., Røysamb, E., Augusti, E. M. and Melinder, A. (2016). Behavior and inhibitory control in children with prenatal exposure to antidepressants and medically untreated depression. *Psychopharmacology* **233:**1523-1535.

Heron, J., O'Connor, T. G., Golding, J., Glover, V. and Team, A. S. (2004). The course of anxiety and depression through pregnancy and the postpartum in a community sample. *Journal of Affective Disorders* **80:**65-73.

Hiby, S. E., Lough, M., Keverne, E. B., Surani, M. A., Loke, Y. W. and King, A. (2001). Paternal monoallelic expression of *PEG3* in the human placenta. *Human Molecular Genetics* **10:**1093-1100.

Highet, N. J., Gemmill, A. W. and Milgrom, J. (2011). Depression in the perinatal period: awareness, attitudes and knowledge in the Australian population. *Australian and New Zealand Journal of Psychiatry* **45:**223-231.

Hiltunen, P., Jokelainen, J., Ebeling, H., Szajnberg, N. and Moilanen, I. (2004). Seasonal variation in postnatal depression. *Journal of Affective Disorders* **78:**111-118.

Hong, G., Zhang, W., Li, H., Shen, X. and Guo, Z. (2014). Separate enrichment analysis of pathways for up- and downregulated genes. *Journal of the Royal Society Interface* **11:**20130950.

Hore, T. A., Rapkins, R. W. and Graves, G. A. (2007). Construction and evolution of imprinted loci in mammals. *Trends in Genetics* **23:**440-448.

Horikawa, K., Minami, Y., Iijima, M., Akiyama, M. and Shibata, S. (2005). Rapid damping of food-entrained circadian rhythm of clock gene expression in clock-defective peripheral tissues under fasting conditions. *Neuroscience* **134:**335-343.

Horseman, N. D., Zhao, W., Montecino-Rodriguez, E., Tanaka, M., Nakashima, K., Engle, S. J., Smith, F. *et al.* (1997). Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. *EMBO Journal* **16:**6926-6935.

Hostetter, A., Stowe, Z. N., Strader, J. R. J., McLaughlin, E. and Llewellyn, A. (2000). Dose of selective serotonin uptake inhibitors across pregnancy: clinical implications. *Depression and Anxiety* **11:**51-57.

Houghton, D. J., Shackleton, P., Obiekwe, B. C. and Chard, T. (1984). Relationship of maternal and fetal levels of human placental lactogen to the weight and sex of the fetus. *Placenta* **5:**455-458.

Howard, L., Ryan, E., Trevillion, K., Anderson, F., Bick, D., Bye, A., Byford, S. *et al.* (2018). Accuracy of the Whooley questions and the Edinburgh Postnatal Depression Scale in identifying depression and other mental disorders in early pregnancy. *British Journal of Psychiatry* **212:**50-56.

Howerton, C. L. and Bale, T. L. (2012). Prenatal programing: at the intersection of maternal stress and immune activation. *Hormones and Behaviour* **62:**237-242.

Hsiao, E. Y. and Patterson, P. H. (2011). Activation of the maternal immune system induces endocrine changes in the placenta via IL-6. *Brain, Behaviour, and Immunity* **25:**604-615.

Huizink, A. C., Menting, B., Oosterman, M., Verhage, M. L., Kunseler, F. C. and Schuengel, C. (2014). The interrelationship between pregnancy-specific anxiety and general anxiety across pregnancy: a longitudinal study. *Psychosomatic Obstetrics and Gynaecology* **35:**92-100.

Huizink, A. C., Mulder, E. J., Robles de Medina, P. G., Visser, G. H. and Buitelaar, J. K. (2004). Is pregnancy anxiety a distinctive syndrome? *Early Human Development* **79:**81-91.

Huizink, A. C., Robels de Medina, P. G., Mulder, E., J. H., Visser, G. H. A. and Buitelaar, J. K. (2002). Psychological Measures of Prenatal Stress as Predictors of Infant Temperament. *Adolescent Psychiatry* **41:**1078-1085.

Hull, L., Petrides, K. V. and Mandy, W. (2020). The Female Autism Phenotype and Camouflaging: a Narrative Review. *Review Journal of Autism and Developmental Disorders* https://doi.org/10.1007/s40489-40020-00197-40489.

Huuskonen, P., Amezaga, M. R., Bellingham, M., Jones, L. H., Storvik, M., Häkkinen, M., Keski-Nisula, L. *et al.* (2016). The human placental proteome is affected by maternal smoking. *Reproductive Toxicology* **63:**22-31.

Hviid, A., Melbye, M. and Pasternak, B. (2013). Use of selective serotonin reuptake inhibitors during pregnancy and risk of autism. *New England Journal of Medicine* **369:**24016-24015.

IAS. (2019). Alcohol Guidelines for Pregnant Women.

Ingram, J., Greenwood, R. and Woolridge, M. (2003). Hormonal predictors of postnatal depression at 6 months in breastfeeding women. *Journal of Reproductive and Infant Psychology* **21:**61-68.

Insaf, T. Z., Fortner, R. T., Pekow, P., Dole, N., Markenson, G. and Chasan-Taber, L. (2011). Prenatal Stress, Anxiety, and Depressive Symptoms as Predictors of Intention to Breastfeed Among Hispanic Women. *Journal of Womens Health* **20:**1183-1192.

Ishida, M. and Moore, G. E. (2013). The role of imprinted genes in humans. *Molecular Aspects of Medicine* **34:**826-840.

Jacquier-Sarlin, M. R., Fuller, K., Dinh-Xuan, A. T., Richard, M. J. and Polla, B. S. (1994). Protective effects of hsp70 in inflammation. *Experientia* **50:**1031-1038.

Janssen, A. B., Capron, L. E., O'Donnell, K., Tunster, S. J., Ramchandani, P. G., Heazell, A. E., Glover, V. *et al.* (2016). Maternal prenatal depression is associated with decreased placental expression of the imprinted gene PEG3. *Psychological Medicine* **46:**2999-3011.

Janssen, A. B., Savory, K. A., Garay, S. M., Sumption, L., Watkins, W., Garcia-Martin, I., Savory, N. *et al.* (2018). Persistence of anxiety symptoms after elective caesarean delivery. *BJPsych Open* **4:**354-360.

Janssen, A. B., Tunster, S. J., Savory, N., Holmes, A., Beasley, J., Parveen, S. A., Penketh, R. J. *et al.* (2015). Placental expression of imprinted genes varies with sampling site and mode of delivery. *Placenta* **36:**790-795.

Jarde, A., Morais, Kingston, D., Giallo, R., MacQueen, G. M., Giglia, L., Beyene, J. *et al.* (2016). Neonatal Outcomes in Women With Untreated Antenatal Depression Compared With Women Without Depression. *JAMA Psychiatry* **73:**826-837.

Jeong, H. G., Lim, J. S., Lee, M. S., Kim, S. H., Jung, I. K. and Joe, S. H. (2013). The association of psychosocial factors and obstetric history with depression in pregnant women: focus on the role of emotional support. *General Hospital Psychiatry* **35:**354-358.

Jeschke, U., Richter, D.-U., Walzel, H., Bergemann, C., Mylonas, I., Sharma, S., Keil, C. *et al.* (2002). Stimulation of hCG and inhibition of hPL in isolated human trophoblast cells in vitro by glycodelin A. *Archives of Gynecology ad Obstetrics* **268:**163-167.

Johanson, R., Chapman, G., Murray, D., Johnson, I. and Cox, J. (2000). The North Staffordshire Maternity Hospital prospective study of pregnancy-associated depression. *Journal of Psychosomatic Obstetrics and Gynaecology* **21:**93-97.

John, R. M. (2013). Epigenetic regulation of placental endocrine lineages and complications of pregnancy. *Biochemical Society Transactions* **41:**701-709.

John, R. M. and Hemberger, M. (2012). A placenta for life. *Reproductive Biomedicine Online* **25:**5-11.

Julian, L. J. (2011). Measures of Anxiety. *Arthritis Care Research* **63:**S467-S472.

Junkins, R. D., MacNeil, A. J., Wu, Z., McCormick, C. and Lin, T.-J. (2013). Regulator of Calcineurin 1 Suppresses Inflammation during Respiratory Tract Infections. *Journal of Immunology* **190:**5178-5186.

Kaihola, H., Olivier, J., Sundström Poromaa, I. and Åkerud, H. (2015). The Effect of Antenatal Depression and Selective Serotonin Reuptake Inhibitor Treatment on Nerve Growth Factor Signaling in Human Placenta. *PLoS One* **10:**e0116459.

Kalisch-Smith, J. I., Simmons, D. G., Pantaleon, M. and Moritz, K. M. (2017). Sex differences in rat placental development: from pre-implantation to late gestation. *Biology of Sex Differences* **8:**17.

Kamberi, I. A., Mical, R. S. and Porter, J. C. (1971). Effects of melatonin and serotonin on the release of FSH and prolactin. *Endocrinology* **88:**1288-1293.

Kaplan, Y. C., Keskin-Arslan, E., Acar, S. and Sozmen, K. (2016). Prenatal selective serotonin reuptake inhibitor use and the risk of autism spectrum disorder in children: a systematic review and meta-analysis. *Reproductive Toxicology* **66:**31-43.

Kappil, M. A., Green, B. B., Armstrong, D. A., Sharp, A. J., Lambertini, L., Marsit, C. J. and Chen, J. (2015). Placental expression profile of imprinted genes impacts birth weight. *Epigenetics* **10:**842-849.

Keim, S. A., Daniels, J. L., Dole, N., Herring, A. H., Siega-Riz, A. M. and Scheidt, P. C. (2011). A prospective study of maternal anxiety, perceived stress, and depressive symptoms in relation to infant cognitive development. *Early Human Development* **87:**373-380.

Kendler, K. S., Gardner, C. O., Gatz, M. and Pederson, N. L. (2007). The sources of comorbidity between major depression and generalized anxiety disorder in a Swedish national twin sample. *Psychological Medicine* **3:**453-462.

Kessler, R. C., Berglund, P., Demler, M. A., Jin, R., Merikangas, K. R. and Walters, E. E. (2005). Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *JAMA Psychiatry* **62:**593-602.

Keverne, E. B. (2014). Genomic imprinting, action, and interaction of maternal and fetal genomes. *PNAS* **112:**6834-6840.

Khanlari, S., Barnett Am, B., Ogbo, F. A. and Eastwood, J. (2019). Re-examination of perinatal mental health policy frameworks for women signalling distress on the Edinburgh Postnatal Depression Scale (EPDS) completed during their antenatal booking-in consultation: a call for population health intervention. *BMC Pregnancy and Childbirth* **19:**221.

Kim, D. R., Bale, T. L. and Epperson, C. N. (2015). Prenatal Programming of Mental Illness: Current Understanding of Relationship and Mechanisms. *Current Psychiatry Reports* **17:**5.

Kim, J., Ashworth, L., Branscomb, E. and Stubbs, L. (1997). The human homolog of a mouse-imprinted gene, Peg3, maps to a zinc finger gene-rich region of human chromosome 19q13.4. *Genome Research* **7:**532-540.

Kim, J., Ekram, M. B., Kim, H., Faisal, M., Frey, W. D., Huang, J., M., Tran, K. *et al.* (2012). Imprinting control region (ICR) of the Peg3 domain. *Human Molecular Genetics* **21:**2677-2687.

Kim, J., Frey, W. D., He, H., Kim, H., Ekram, M. B., Bakshi, A., Faisal, M. *et al.* (2013). Peg3 mutational effects on reproduction and placenta-specific gene families. *PLoS One* **8:**e83359.

Kim, J. and Kim, J. D. (2008). In vivo YY1 knockdown effects on genomic imprinting. *Human Molecular Genetics* **17:**391-401.

Kim, J., Kollhoff, A., Bergmann, A. and Stubbs, L. (2003). Methylation-sensitive binding of transcription factor YY1 to an insulator sequence within the paternally expressed imprinted gene, Peg3. *Human Molecular Genetics* **12:**233-245.

Kim, J. D., Hinz, A. K., Choo, J. H., Stubbs, L. and Kim, J. (2007). YY1 as a controlling factor for the Peg3 and Gnas imprinted domains. *Genomics* **89:**262-269.

Kim, J. D., Kang, K. and Kim, J. (2009). YY1's role in DNA methylation of Peg3 and Xist. *Nucleic Acids Research* **37:**5656-5664.

Kim, J. D., Yu, S., Choo, J. H. and Kim, J. (2008). Two evolutionarily conserved sequence elements for Peg3/Usp29 transcription. *BMC Molecular Biology* **9:**108.

King, K., Murphy, S. and Hoyo, C. (2015). Epigenetic regulation of Newborns' imprinted genes related to gestational growth: patterning by parental race/ethnicity and maternal socioeconomic status. *Journal of Epidemiology and Community Health* **69:**639-647.

Kingston, D., Tough, S. and Whitfield, H. (2012). Prenatal and Postpartum Maternal Psychological Distress and Infant Development: A Systematic Review. *Child Psychiatry & Human Development* **43:**683-714.

Kinney, D. K., Miller, A. M., Crowley, D. J., Huang, E. and Gerber, E. (2007). Autism Prevalence Following Prenatal Exposure to Hurricanes and Tropical Storms in Louisiana. *Journal of Autism and Developmental Disorders* **38:**481-488.

Kobayashi, S., Kohda, T., Miyoshi, N., Kuroiwa, Y., Aisaka, K., Tsutsumi, O., Kaneko-Ishino, T. *et al.* (1997). Human PEG1/MEST, an imprinted gene on chromosome 7. *Human Molecular Genetics* **6:**781-786.

Kondrashov, A. S. and Crow, J. F. (1991). Haploidy or diploidy: Which is better? *Nature* **351:**314-315.

Korotkevich, G., Sukhov, V. and Sergushichev, A. (2016). An algorithm for fast preranked gene set enrichment analysis using cumulative statistic calculation. *BioRxiv* Article 060012.

Kott, J. and Brummelte, S. (2019). Trick or treat? Evaluating contributing factors and sex-differences for developmental effects of maternal depression and its treatment. *Hormones and Behaviour* **111:**31-45.

Koutsaki, M., Sifakis, S., Zaravinos, A., Koutroulakis, D., Koukoura, O. and Spandidos, D. A. (2011). Decreased placental expression of hPGH, IGF-I and IGFBP-1 in pregnancies complicated by fetal growth restriction. *Growth Hormone and IGF Research* **21:**31-36.

Krueger, F. (2012). Trim Galore!

Kumar, N., Leverence, J., Bick, D. and Sampath, V. (2012). Ontogeny of growth-regulating genes in the placenta. *Placenta* **33:**94-99.

Kuroiwa, Y., Kaneko-Ishino, T., Kagitani, F., Kohda, T., Li, L. L., Tada, M., Suzuki, R. *et al.* (1996). Peg3 imprinted gene on proximal chromosome 7 encodes for a zinc finger protein. *Nature Genetics* **12:**186-190.

Kurstjens, S. and Wolke, D. (2001). Effects of maternal depression on cognitive development of children over the first 7 years of life. *Journal of Child Psychology and Psychiatry* **42:**623-636.

Lacroix, M. C., Guibourdenche, J., Frendo, J. L., Muller, F. and Evain-Brion, D. (2002). Human Placental Growth Hormone—A Review. *Placenta* **23:**S87-94.

Lagerström, M., Bremme, K. and Eneroth, P. (1990). Maternal serum levels of estriol, prolactin, human placental lactogen and chorionic gonadotrophin related to fetal sex in normal and abnormal pregnancies. *Gynecologic and Obsetetric Investigations* **30:**198-203.

Lahti, M., Savolainen, K., Tuovinen, S., Pesonen, A. K., Lahti, J., Heinonen, K., Hamalainen, E. *et al.* (2017). Maternal Depressive Symptoms During and After Pregnancy and Psychiatric Problems in Children. *Journal of American Academy of Child & Adolescent Psychiatry* **56:**30-39.

Lambertini, L., Chen, J. and Nomura, Y. (2015). Mitochondrial Gene Expression Profiles Are Associated with Maternal Psychosocial Stress in Pregnancy and Infant Temperament. *PLoS One* **10:**e0138929.

Lancaster, C. A., Gold, K. J., Flynn, H. A., Yoo, H., Marcus, S. M. and Davis, M. M. (2010). Risk factors for depressive symptoms during pregnancy: a systematic review. *American Journal of Obstetrics and Gynaecology* **202:**5-14.

Laplante, D. P., Barr, R. G., Brunet, A., Galbaud du Fort, G., Meaney, M. L., Saucier, J. F., Zelazo, P. R. *et al.* (2004). Stress during pregnancy affects general intellectual and language functioning in human toddlers. *Pediatric Research* **56:**400-410.

Lara-Cinisomo, S., Swinford, C., Massey, D. and Hardt, H. (2018). Diabetes, Prenatal Depression, and Self-Rated Health in Latina Mothers. *Diabetes Spectrum* **31:**159-165.

Larsen, C. M. and Grattan, D. R. (2010). Prolactin-induced Mitogenesis in the Subventricular Zone of the Maternal Brain During Early Pregnancy Is Essential for Normal Postpartum Behavioral Responses in the Mother. *Endocrinology* **151:**3805-3814.

Le Bas, G., Youssef, G. J., Macdonald, J. A., Rossen, L., Teague, S. J., Kothe, E. J., McIntosh, J. E. *et al.* (2019). The role of antenatal and postnatal maternal bonding in infant development: A systematic review and meta-analysis. *Social Development* **29:**3-20.

Lebel, C., Walton, M., Letourneau, N., Giesbrecht, G. F., Kaplan, B. J. and D., D. (2016). Prepartum and postpartum maternal depressive symptoms are related to children's brain structure in preschool. *Biological Psychiatry* **80:**859-868.

Lee, A. M., Lam, S. K., M., S. M. L. S., Chong, C. S., Chui, H. W. and Fong, D. Y. (2007). Prevalence, course, and risk factors for antenatal anxiety and depression. *Obstetrics and Gynaecology* **110:**1102-1112.

Lee, C. L. and Bates, J. E. (1985). Mother-child interaction at age two years and perceived difficult temperament. *Child Development* **56:**1314-1325.

Lee, J. Y., Yun, H. J., Kim, C. Y., Cho, Y. W., Lee, Y. and Kim, M. H. (2017). Prenatal exposure to dexamethasone in the mouse induces sex-specific differences in placental gene expression. *Development, Growth & Differentiation* **59:**515-525.

Lee, K. J., Shim, S. H., Kang, K. M., Kang, J. H., Park, D. Y., Kim, S. H., Farina, A. *et al.* (2010). Global gene expression changes induced in the human placenta during labor. *Placenta* **31:**698-704.

Lee, S., Ye, A. and Kim, J. (2015). DNA-Binding Motif of the Imprinted Transcription Factor PEG3. *PLoS One* **10:**e0145531.

Lefebvre, L., Viville, S., Barton, S. C., Ishino, F., Keverne, E. B. and Surani, M. A. (1998). Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene Mest. *Nature Genetics* **20:**163-169.

Leigh, B. and Milgrom, J. (2008). Risk factors for antenatal depression, postnatal depression and parenting stress. *BMC Psychiatry* **8:**24.

Lemche, E., Surguladze, S. A., Brammer, M. J., Phillips, M. L., Sierra, M., David, A. S., Williams, S. C. *et al.* (2016). Dissociable brain correlates for depression, anxiety, dissociation, and somatization in depersonalization-derealization disorder. *CNS Spectrums* **21:**35-42.

Leslie, S., Winney, B., Hellenthal, G., Davison, D., Boumertit, A., Day, T., Hutnik, K. *et al.* (2015). The fine scale genetic structure of the British population. *Nature* **519:**309-314.

Li, J., Olsen, J., Vestergaard, M. and Obel, C. (2010). Attention-deficit/hyperactivity disorder in the offspring following prenatal maternal bereavement: a nationwide follow-up study in Denmark. *European Child and Adolescent Psychiatry* **19:**747-753.

Li, L., Keverne, E. B., Aparicio, S. A., Ishino, F., Barton, S. C. and Surani, M. A. (1999). Regulation of maternal behaviour and offspring growth by paternally expressed *Peg3*. *Science* **284:**330-333.

Liao, Y., Smyth, G. K. and Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30:**923-930.

Liberzon, A., Birger, C., Thorvaldsdóttir, H., Ghandi, M., Mesirov, J. P. and Tamayo, P. (2015). The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Systems* **1:**417-425.

Lingappan, K. (2019). NF-κB in Oxidative Stress. *Current Opinion in Toxicology* **7:**81-86.

Littleton, H. L., Radecki Breitkopf, C. and Berenson, A. B. (2007). Correlates of anxiety symptoms during pregnancy and association with perinatal outcomes: a meta-analysis. *Americal Journal of Obstetrics and Gynecology* **196:**424-432.

Litzky, J. F., Deyssenroth, M. A., Everson, T. M., Lester, B. M., Lambertini, L., Chen, J. and Marsit, C. J. (2018). Prenatal Exposure to Maternal Depression and Anxiety on Imprinted Gene Expression in Placenta and Infant Neurodevelopment and Growth. *Pediatric Research* **83:**1075-1083.

Liu, J., Bann, C., Lester, B., Tronick, E., Das, A., Lagasse, L., Bauer, C. *et al.* (2010). Neonatal neurobehavior predicts medical and behavioral outcome. *Pediatrics* **125:**90-98.

Liu, Y., Fan, X., Wang, R., Lu, X., Dang, Y. L., Wang, H., Lin, H. Y. *et al.* (2018). Single-cell RNA-seq reveals the diversity of trophoblast subtypes and patterns of differentiation in the human placenta. *Cell Research* **28:**819-832.

Liu, Y., Murphy, S. K., Murtha, A. P., Fuemmeler, B. F., Schildkraut, J., Huang, Z., Overcash, F. *et al.* (2012). Depression in pregnancy, infant birth weight and DNA methylation of imprint regulatory elements. *Epigenetics* **7:**735-746.

Livak, K. J. and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitive PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25:**402-408.

Logan, P. C., Mitchell, M. D. and Lobie, P. E. (2013). DNA methyltransferases and TETs in the regulation of differentiation and invasiveness of extra-villous trophoblasts. *Frontiers in Genetics* **4:**265.

Loughhead, A. M., Fisher, A. D., Newport, D. J., Ritchie, J. C., Owens, M. J., DeVane, C. L. and Stowe, Z. N. (2006). Antidepressants in amniotic fluid: another route of fetal exposure. *American Journal of Psychiatry* **163:**145-147.

Love, M. I., Huber, W. and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15:**550

Lowry, C. A. (2002). Functional subsets of serotonergic neurones: Implications for control of the hypothalamic-pituitary-adrenal axis. *Journal of Neuroendocrinology* **14:**911-923.

Lucas, B. K., Ormandy, C. J., Binart, N., Bridges, R. S. and Kelly, P. A. (1998). Null Mutation of the Prolactin Receptor Gene Produces a Defect in Maternal Behavior *Endocrinology* **139:**4102-4107.

Maier, S. E., Chen, W. J., Miller, J. A. and West, J. R. (1997). Fetal alcohol exposure and temporal vulnerability regional differences in alcohol-induced microencephaly as a function of the timing of binge-like alcohol exposure during rat brain development. *Alcoholism: Clinical and Experimental Research* **21:**1418-1428.

Mairesse, J., Lesage, J., Breton, C., Bréant, B., Hahn, T., Darnaudéry, M., Dickson, S. L. *et al.* (2007). Maternal stress alters endocrine function of the feto-placental unit in rats. *American Journal of Physiology* **292:**E1526-1533.

Malassine, A. and Cronier, L. (2002). Hormones and human trophoblast differentiation: a review. *Endocrine* **19:**3-11.

Malm, H., Brown, A. S., Gissler, M., Gyllenberg, D., Hinkka-Yli-Salomäki, S., McKeague, I. W., Weissman, M. *et al.* (2016). Gestational exposure to selective serotonin reuptake inhibitors and offspring psychiatric disorders: a national register-based study. *Journal of the American Academy of Child and Adolescent Psychiatry* **55:**359-366.

Männik, J., Vaas, P., Rull, K., Teesalu, P., Rebane, T. and Laan, M. (2010). Differential expression profile of growth hormone/chorionic somatomammotropin genes in placenta of small- and large-for-gestational-age newborns. *Journal of Clinical Endocrinology and Metabolism* **95:**2433-2442.

Mansell, T., Novakovic, B., Meyer, B., Rzehak, P., Vuillermin, P., Ponsonby, A.-L., Collier, F. *et al.* (2016). The effects of maternal anxiety during pregnancy on IGF2/H19 methylation in cord blood. *Translational Psychiatry* **6:**e765.

Mao, J., Zhang, X., S ieli, P. T., Falduto, M. T., Torres, K. E. and Rosenfeld, C. S. (2010). C ontrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. *Proceedings of the National Academy of Sciences of the United States of America* **107:**5557-5562.

Marcé, L. V. (1858). Traité, de la folie des femmes enceintes des nouvelles accouchées et des nourrices et considérations médico-légales qui se rattachent à ce sujet. Paris, France: JB Baillière.

Marchesi, C., Bertoni, S. and Maggini, C. (2009). Major and minor depression in pregnancy. *Obstetrics and Gynaecology* **113:**1292-1298.

Marchocki, Z., Russell, N. E. and O' Donoghue, K. (2013). Selective serotonin reuptake inhibitors and pregnancy: A review of maternal, fetal and neonatal risks and benefits. *Obstetric Medicine* **6:**155-158.

Marcus, S. M. (2009). Depression during pregnancy: rates, risks and consequences--Motherisk Update 2008. *Canadian Journal of Clinical Pharmacology* **16:**e15-22.

Marcus, S. M., Flynn, H. A., Blow, F. C. and Barry, K. L. (2003). Depressive symptoms among pregnant women screened in obstetrics settings. *Journal of Womens Health* **12:**373-380.

Mars, B., Heron, J., Kessler, D., Davies, N. M., Martin, R. M., Thomas, K. H. and Gunnell, D. (2017). Influences on antidepressant prescribing trends in the UK: 1995-2011. *Social Psychiatry and Psychiatric Epidemiology* **52:**193-200.

Martin, E., Smeester, L., Bommarito, P. A., Grace, M. R., Boggess, K., Kuban, K., Karagas, M. R. *et al.* (2017). Sexual epigenetic dimorphism in the human placenta: implications for susceptibility during the prenatal period. *Epigenomics* **9:**267-278.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* **17:**10-12.

Masemola, M. L., van der Merwe, L., Lombard, Z., Viljoen, D. and Ramsay, M. (2015). Reduced DNA methylation at the PEG3 DMR and KvDMR1 loci in children exposed to alcohol in utero: a South African Fetal Alcohol Syndrome cohort study. *Frontiers in Genetics* **6:**85.

Massart, R., Suderman, M. J., Nemoda, Z., Sutti, S., Ruggiero, A. M., Dettmer, A. M., Suomi, S. J. *et al.* (2017). The Signature of Maternal Social Rank in Placenta Deoxyribonucleic Acid Methylation Profiles in Rhesus Monkeys. *Child Development* **88:**900-918.

Matthews, S. G. (2002). Early programming of the hypothalamo–pituitary–adrenal axis. *Trends in Endocrinology & Metabolism* **13:**373-380.

Matthey, S., Fisher, J. and Rowe, H. (2013). Using the Edinburgh postnatal depression scale to screen for anxiety disorders: conceptual and methodological considerations. *Journal of Affective Disorders* **146:**224-230.

McCabe-Beane, J. E., Segre, L. S., Perkhounkova, Y., Stuart, S. and O'Hara, M. W. (2016). The identification of severity ranges for the Edinburgh Postnatal Depression Scale. *Journal of Reproductive and Infant Psychology* **34:**293-303.

McCourt, C., Weaver, J., Statham, H., Beake, S., Gamble, J. and Creedy, D. K. (2007). Elective cesarean section and decision making: a critical review of the literature. *Birth* **34:**65-79.

McGrath, J. and Solter, D. (1984). Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* **37:**179-183.

McGrath, J. M., Records, K. and Rice, M. (2008). Maternal depression and infant temperament characteristics. *Infant Behavior and Development* **31:**71-80.

McNamara, G. I., Creeth, H. D. J., Harrison, D. J., Tansey, K. E., Andrews, R. M., Isles, A. R. and John, R. M. (2018). Loss of offspring Peg3 reduces neonatal ultrasonic vocalisations and increases maternal anxiety in wild type mothers. *Human Molecular Genetics* **27:**440-450.

Meaney, M. J. (2018). Perinatal maternal depressive symptoms as an issue for population health. *Americal Journal of Psychiatry* **175:**1084-1093.

Meller, M., Vadachkoria, S., Luthy, D. A. and Williams, M. A. (2005). Evaluation of housekeeping genes in placental comparative expression studies. *Placenta* **26:**601-607.

Meuris, S., Polliotti, B., Robyn, C. and Lebrun, P. (1994). Ca2+ entry through L-type voltage-sensitive Ca2+ channels stimulates the release of human chorionic gonadotrophin and placental lactogen by placental explants. *Biochimica and Biophysica Acta* **1220:**101-106.

Micali, N., Simonoff, E. and Treasure, J. (2011). Pregnancy and post-partum depression and anxiety in a longitudinal general population cohort: The effect of eating disorders and past depression. *Journal of Affective Disorders* **131:**150-157.

Michalec, D. (2011). Bayley Scales of Infant Development: Third Edition. Boston, MA.: Springer.

Miller, S. L., Huppi, P. S. and Mallard, C. (2016). The consequences of fetal growth restriction on brain structure and neurodevelopmental outcome. *Journal of Physiology* **594:**807-823.

Millership, S. J., Van De Pette, M. and Withers, D. J. (2019). Genomic imprinting and its effects on postnatal growth and adult metabolism. *Cellular and Molecular Life Sciences* **76:**4009-4021.

Mina, T. H., Räikkönen, K., Riley, S. C., Norman, J. E. and Reynolds, R. M. (2015). Maternal distress associates with placental genes regulating fetal glucocorticoid exposure and IGF2: Role of obesity and sex. *Psychoneuroendocrinology* **59:**112-122.

Moisiadis, V. G. and Matthews, S. G. (2014). Glucocorticoids and fetal programming part 1: outcomes. *Nature Reviews Endocrinology* **10:**391-402.

Molyneaux, E., Poston, L., Ashurst-Williams, S. and Howard, L. M. (2014). Obesity and mental disorders during pregnancy and postpartum: a systematic review and meta-analysis. *Obstetrics and Gynecology* **123:**857-867.

Mongan, D., Lynch, J., Hanna, D., Shannon, C., Hamilton, S., Potter, C., Gorman, C. *et al.* (2019). Prevalence of self-reported mental disorders in pregnancy and associations with adverse neonatal outcomes: a population-based cross-sectional study. *BMC Pregnancy and Childbirth* **19:**412.

Moore, G. E., Ishida, M., Demetriou, C., Al-Olabi, L., Leon, L. J., Thomas, A. C., Abu-Amero, S. *et al.* (2015). The role and interaction of imprinted genes in human fetal growth. *Philosophical Transactions of the Royal Society of London, Series B Biological Sciences* **370:**20140074.

Moore, L. D., T., L. and Fan, G. (2012). DNA Methylation and Its Basic Function. *Neuropsychopharmacology* **38:**23-28.

Moore, T. and Haig, D. (1991). Genomic imprinting in mammalian development: a parental tug-of-war. *Trends in Genetics* **7:**45-49.

Morgan, J. E., Channon, S., Penny, H. and Waters, C. S. (2019). Longitudinal studies examining the impact of prenatal and subsequent episodes of maternal depression on offspring antisocial behaviour. *European Child and Adolescent Psychiatry* **10:**1007.

Mueller, B. R. and Bale, T. L. (2008). Sex-specific programming of offspring emotionality after stress early in pregnancy. *Neuroscience* **28:**9055-9065.

Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochemical Journal* **417:**1-13.

Murray, D. and Cox, J. L. (1990). Screening for depression during pregnancy with the Edinburgh depression scale (EDDS). *Journal of Reproductive and Infant Psychology* **8:**99-107.

Murray, L., Arteche, A., Fearon, P., Halligan, S., Croudace, T. and Cooper, P. (2010). The effects of maternal postnatal depression and child sex on academic performance at age 16 years: a developmental approach. *Journal of Child Psychology and Psychiatry* **51:**1150-1159.

Myers, S. and Johns, S. E. (2019). Male infants and birth complications are associated with increased incidence of postnatal depression. *Social Science and Medicine* **220:**56-64.

Nabeshima, T. and Kim, H.-C. (2013). Involvement of Genetic and Environmental Factors in the Onset of Depression. *Experimental Neurobiology* **22:**235-243.

Naicker, K., Galambos, N. L., Zeng, Y., Senthilselvan, A. and Colman, I. (2013). Social, Demographic, and Health Outcomes in the 10 Years Following Adolescent Depression. *Journal of Adolescent Health* **52:**533-538.

Nelissen, E. C. M., Dumoulin, J. C. M., Busato, F., Ponger, L., Eijssen, L. M., Evers, J. L. H., Tost, J. *et al.* (2014). Altered gene expression in human placentas after IVF/ICSI. *Human Reproduction* **29:**2821-2831.

Netsi, E., Pearson, R. M., Murray, L., Cooper, P., Craske, M. G. and Stein, A. (2018). Association of persistent and severe postnatal depression with child outcomes. *JAMA Psychiatry* **75:**247-253.

Newbern, D. and Freemark, M. (2011). Placental hormones and the control of maternal metabolism and fetal growth. *Current Opinion in Endocrinology, Diabetes and Obesity* **18:**409-416.

NICE. (2014). Antenatal and postnatal mental health: clinical management and service guidance.

Nigg, J. T. (2006). Temperament and developmental psychopathology. *Journal of Child Psychology and Psychiatry* **47:**395-422.

Nolvi, S., Bridgett, D. J., Korja, R., Kataja, E. L., Junttila, N., Karlsson, H. and L., K. (2019). Trajectories of maternal pre- and postnatal anxiety and depressive symptoms and infant fear: Moderation by infant sex. *Journal of Affective Disorders* **257:**589-597.

Norhayati, M. N., Hazlina, N. H., Asrenee, A. R. and Emilin, W. M. (2015). Magnitude and risk factors for postpartum symptoms: a literature review. *Journal of Affective Disorders* **175:**34-52.

Nulman, I., Rovet, J., Stewart, D. E., Wolpin, J., Gardner, A., Theis, J. G., Kulin, N. *et al.* (1997). Neurodevelopment of children exposed in utero to antidepressant drugs. *Neuroscientist* **336:**258-262.

O'Connell, B. A., Moritz, K. M., Walker, D. W. and Dickinson, H. (2013). Sexually dimorphic placental development throughout gestation in the spiny mouse (Acomys cahirinus). *Placenta* **34:**119-126.

O'Connor, T. G., Heron, J. and Glover, V. (2002). Antenatal anxiety predicts child behavioral/emotional problems independently of post-natal depression. *Journal of American Academy of Child & Adolescent Psychiatry* **41:**1470-1477.

O'Donnell, K., O'Connor, T. G. and Glover, V. (2009). Prenatal Stress and Neurodevelopment of the Child: Focus on the HPA Axis and Role of the Placenta. *Developmental Neuroscience* **31:**285-292.

O'Donnell, K. J., Glover, V., Barker, E. D. and O'Connor, T. G. (2014). The persisting effect of maternal mood in pregnancy on childhood psychopathology. *Developmental Psychopathology* **26:**393-403.

O'Donnell, K. J., Jensen, A. B., Freeman, L., Khalife, N., O'Connor, T. G. and Glover, V. (2012). Maternal prenatal anxiety and downregulation of placental 11β-HSD2. *Psychoneuroendocrinology* **37:**818-826.

O'Hara, M. W. and McCabe, J. E. (2013). Postpartum depression: current status and future directions. *Annual Review of Clinical Psychology* **9:**379-407.

Oates, M. (2003). Suicide: The leading cause of maternal death. *The British Journal of Psychiatry* **183:**279-281.

Oberlander, T. F., Papsdorf, M., Brain, U. M., Misri, S., Ross, C. and Grunau, R. E. (2010). Prenatal effects of selective serotonin reuptake inhibitor antidepressants, serotonin transporter promoter genotype (SLC6A4), and maternal mood on child behavior at 3 years of age. *Archives of Pediatric and Adolescent Medicine* **164:**444-451.

Ohoka, H., Koide, T., Goto, S., Murase, S., Kanai, A., Masuda, T., Aleksic, B. *et al.* (2014). Effects of maternal depressive symptomatology during pregnancy and the postpartum period on infant–mother attachment. *Psychiatry and Clinical Neurosciences* **68:**631-639.

Okae, H., Toh, H., Sato, T., Hiura, H., Takahashi, S., Shirane, K., Kabayama, Y. *et al.* (2018). Derivation of Human Trophoblast Stem Cells. *Cell Stem Cell* **22:**50-63.e56.

Olivier, J. D. A., Akerud, H., Kaihola, H., Pawluski, J. L., Skalkidou, A., Högberg, U. and Sundström-Poromaa, I. (2013). The effects of maternal depression and maternal selective serotonin reuptake inhibitor exposure on offspring. *Frontiers in Cellular Neuroscience* **7:**73.

Olivier, J. D. A., Åkerud, H., Skalkidou, A., Kaihola, H. and Sundström-Poromaa, I. (2014). The effects of antenatal depression and antidepressant treatment on placental gene expression. *Frontiers in Cellular Neuroscience* **8:**465.

ONS. (2017). Births by parents' characteristics in England and Wales: 2016.

Orta, O. R., Gelaye, B., Bain, P. A. and Williams, M. A. (2017). The association between maternal cortisol and depression during pregnancy, a systematic review. *Archives of Womens Mental Health* **21:**43-53.

Osei-Kumah, A., Smith, R., Jurisica, I., Caniggia, I. and Clifton, V. L. (2011). Sex-specific differences in placental global gene expression in pregnancies complicated by asthma. *Placenta* **32:**570-578.

Pan, W., Yu, Y., Cain, C. M., Nyberg, F., Couraud, P. O. and Kastin, A. J. (2005). Permeation of growth hormone across the blood-brain barrier. *Endocrinology* **146:**4898-4904.

Pankevich, D. E., Mueller, B. R., Brockel, B. and Bale, T. L. (2009). Prenatal stress programming of offspring feeding behavior and energy balance begins early in pregnancy. *Physiology and Behaviour* **98:**94-102.

Panthangi, V., West, P., T., S.-M. R., Geeta, M. and Reickert, E. (2009). Is seasonal variation another risk factor for postpartum depression? *Journal of the American Board of Family Medicine* **22:**492-497.

Paquette, A. G., Houseman, E. A., Green, B. B., Lesseur, C., Armstrong, D. A., Lester, B. and Marsita, C. J. (2016). Regions of variable DNA methylation in human placenta associated with newborn neurobehavior. *Epigenetics* **11:**603-613.

Paris, R., Bolton, R. E. and Weinberg, M. K. (2009). Postpartum depression, suicidality, and mother-infant interactions. *Archives of Womens Mental Health* **12:**309-321.

Patil, M. J., Henry, M. A. and Akopian, A. N. (2014). Prolactin receptor in regulation of neuronal excitability and channels. *Channels (Austin)* **8:**193-202.

Patten, S. B. and Love, E. J. (1997). Drug-induced depression. *Psychotherapy and Psychosomatics* **66:**63-73.

Pawlby, S., Hay, D. F., Waters, C. S., Sharp, D. and O'Keane, V. (2009). Antenatal depression predicts depression in adolescent offspring: Prospective longitudinal community-based study. *Journal of Affective Disorders* **113:**236-243.

Pawluski, J. L. and Gemmel, M. (2018). Perinatal SSRI medications and offspring hippocampal plasticity: interaction with maternal stress and sex. *Hormones* **17:**15-24.

Pawluski, J. L., Lonstein, J. S. and Fleming, A. S. (2017). The Neurobiology of Postpartum Anxiety and Depression. *Trends in Neurosciences* **40:**106-120.

Peake, G. T., Buckman, M. T., Davis, L. E. and Standefer, J. (1983). Pituitary and Placentally Derived Hormones in Cerebrospinal Fluid during Normal Human Pregnancy. *Journal of Clinical Endocrinology and Metabolism* **56:**46-52.

Pearson, R. M., Carnegie, R. E. and Cree, C. (2018). Prevalence of Prenatal Depression Symptoms Among 2 Generations of Pregnant Mothers. *JAMA Network Open* **1:**e180725.

Penninx, B. W., Nolen, W. A., Lamers, F., Zitman, F. G., Smit, J. H., Spinhoven, P., Cuijpers, P. *et al.* (2011). Two-year course of depressive and anxiety disorders: results from the Netherlands Study of Depression and Anxiety (NESDA). *Journal of Affective Disorders* **133:**76-85.

Perera, B. P. and Kim, J. (2016a). Alternative promoters of Peg3 with maternal specificity. *Scientific Reports* **6:**24438.

Perera, B. P., Teruyama, R. and Kim, J. (2015). Yy1 gene dosage effect and bi-allelic expression of Peg3. *PLoS One* **10:**e0119493.

Perera, B. P. U. and Kim, J. (2016b). Sex and Tissue Specificity of Peg3 Promoters. *PLoS One* **11:**e0164158.

Pérez, S., Murias, L., Fernández-Plaza, C., Díaz, I., González, C., Otero, J. and Díaz, E. (2014). Evidence for clock genes circadian rhythms in human full-term placenta. *Systems Biology in Reproductive Medicine* **61:**360-366.

Petit, A. and Belisle, S. (1995). Stimulation of intracellular calcium concentration by adenosine triphosphate and uridine 5'-triphosphate in human term placental cells: evidence for purinergic receptors. *Journal of Clinical Endocrinology and Metabolism* **80:**1809-1815.

Petit, A., Gallot-Payet, N., Vaillencourt, C., Bellabarba, D., Lehoux, J. G. and Belisle, S. (1993). A role for extracellular calcium in the regulation of placental lactogen release by angiotensin-2 and dopamine in human term trophoblastic cells. *Journal of Clinical Endocrinology and Metabolism* **70:**670-676.

Petit, A., Piednoir, D., Germain, M. L. and Trenque, T. (2003). Drug-induced hyperprolactinemia: a case-non-case study from the national pharmacovigilance database. *Therapie* **58:**159-163.

Planalp, E. M., Van Hulle, C., Gagne, J. R. and Hill Goldsmith, H. (2017). The Infant Version of the Laboratory Temperament Assessment Battery (Lab-TAB): Measurement Properties and Implications for Concepts of Temperament. *Frontiers in Psychology* **8:**846.

Plant, D. T., Pariante, C. M., Sharp, D. and Pawlby, S. (2015). Maternal depression during pregnancy and offspring depression in adulthood: Role of child maltreatment. *The British Journal of Psychiatry* **207:**213-220.

Plasschaert, R. N. and Bartolomei, M. S. (2014). Genomic imprinting in development, growth, behavior and stem cells. *Development* **141:**1805-1813.

Popova, S., Lange, S., Probst, C., Gmel, G. and Rehm, J. (2017). Estimation of national, regional, and global prevalence of alcohol use during pregnancy and fetal alcohol syndrome: a systematic review and meta-analysis. *The Lancet* **5:**290-299.

Putnam, S. P., Helbig, A. L., Gartstein, M. A., Rothbart, M. K. and Leerkes, E. J. (2014). Development and assessment of short and very short forms of the infant behavior questionnaire-revised. *Journal of Personality Assessment* **96:**445-458.

Putnam, S. P., Rothbart, M. K. and Gartstein, M. A. (2008). Homotypic and heterotypic continuity of fine-grained temperament during infancy, toddlerhood, and early childhood. *Infant and Child Development* **17:**387–405.

Qiu, A., Rifkin-Graboi, A., Chen, H., Chong, Y. S., Kwek, K., Gluckman, P. D., Fortier, M. V. *et al.* (2013). Maternal anxiety and infants' hippocampal development: timing matters. *Translational Psychiatry* **3:**e306.

Quarini, C., Pearson, R. M., Stein, A., Ramchandani, P. G., Lewis, G. and Evans, J. (2016). Are female children more vulnerable to the long-term effects of maternal depression during pregnancy? *Journal of Affective Disorders* **189:**329-335.

Radford, E. J., Isganaitis, E., Jimenez-Chillaron, J., Schroeder, J., Molla, M., Andrews, S., Didier, N. *et al.* (2012). An Unbiased Assessment of the Role of Imprinted Genes in an Intergenerational Model of Developmental Programming. *PLoS Genetics* **8:**e1002605.

Radloff, L. S. (1977). The CES-D scale: A self report depression scale for research in the general population. *Applied Psychological Measurements* **1:**385-401.

Raikkonen, K., Pesonen, A. K., O'Reilly, J. R., Tuovinen, S., Lahti, M., Kajantie, E., Villa, P. *et al.* (2015). Maternal depressive symptoms during pregnancy, placental expression of genes regulating glucocorticoid and serotonin function and infant regulatory behaviors. *Psychological Medicine* **45:**3217-3226.

Räisänen, S., Lehto, S. M., Nielsen, H. S., Gissler, M., Kramer, M. R. and Heinonen, S. (2014). Risk factors for and perinatal outcomes of major depression during pregnancy: a population-based analysis during 2002–2010 in Finland. *BMJ Open* **4:**e004883.

Raison, C. L. and Miller, A. H. (2013). Malaise, melancholia and madness: the evolutionary legacy of an inflammatory bias. *Brain, Behaviour and Immunity* **31:**1-8.

Rampono, J., Simmer, K., Ilett, K., Hackett, L. P., Doherty, D. A., Elliot, R., Kok, C. H. *et al.* (2009). Placental Transfer of SSRI and SNRI Antidepressants and Effects on the Neonate. *Pharmacopsychiatry* **42:**95-100.

Ratcliff, B. G., Sharapova, A., Suardi, F. and Borel, F. (2015). Factors associated with antenatal depression and obstetric complications in immigrant women in Geneva. *Midwifery* **31:**971-978.

Remes, O., Brayne, C., van der Linde, R. and Lafortune, L. (2016). A systematic review of reviews on the prevalence of anxiety disorders in adult populations. *Brain and Behavior* **6:**e00497.

Resnik, R. (2002). Intrauterine growth restriction. *Obstetrics and Gynaecology* **99:**490-496.

Reynolds, R. M., Pesonen, A. K., O'Reilly, J. R., Tuovinen, S., Lahti, M., Kajantie, E., Villa, P. M. *et al.* (2015). Maternal depressive symptoms throughout pregnancy are associated with increased placental glucocorticoid sensitivity. *Psychological Medicine* **40:**2023-2030.

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W. and Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* **43:**e47.

Robinson, M. D., McCarthy, D. J. and Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26:**139-140.

Rochat, T. J., Tomlinson, M., Bärnighausen, T., Newel, M. and Stein, A. (2011). The prevalence and clinical presentation of antenatal depression in rural South Africa. *Journal of Affective Disorders* **135:**362-373.

Roh, C. R., Budhraja, V., Kim, H. S., Nelson, D. M. and Sadovsky, Y. (2005). Micro-array based identification of differentially expressed genes in hypoxic term human trophoblasts and in placental villi of pregnancies with growth restricted fetuses. *Placenta* **26:**319-328.

Ronald, A., Pennell, C. E. and Whitehouse, A. J. O. (2011). Prenatal maternal stress associated with ADHD and autistic traits in early childhood. *Frontiers in Developmental Psychology* **1:**233.

Rosenberg, J. M., Beymer, P. N., Anderson, D. J., Van Lissa, C. J. and Schmidt, J. A. (2018). tidyLPA: An R Package to Easily Carry Out Latent Profile Analysis (LPA) Using Open-Source or Commercial Software. *Journal of Open Source Software* **3:**978.

Rosenfeld, C. S. (2015). Sex-Specific Placental Responses in Fetal Development. *Endocrinology* **156:**3422-3434.

Rosenfield, A. and Maine, D. (1985). Maternal mortality—a neglected tragedy, where is the M in MCH? *Lancet* **2:**83-85.

Ross, L. E., Grigoriadis, S., Mamisashvili, L., VonderPorten, E. H., Roerecke, M., Rehm, J. and Cheung, A. (2013). Selected pregnancy and delivery outcomes after exposure to antidepressant medication: a systematic review and meta-analysis. *JAMA Psychiatry* **70:**436-443.

Rossen, L., Hutchinson, D., Wilson, J., Burns, L., Olsson, C. A., Allsop, S., Elliott, E. J. *et al.* (2016). Predictors of postnatal mother-infant bonding: the role of antenatal bonding, maternal substance use and mental health. *Archives of Womens Mental Health* **19:**609-622.

Rotem-Kohavi, N. and Oberlander, T. F. (2017). Variations in Neurodevelopmental Outcomes in Children with Prenatal SSRI Antidepressant Exposure. *Birth Defects Research* **109:**909-923.

Rothbart, M. K. (1981). Measurement of temperament in infancy. *Child Development* **52:**569-578.

Rothbart, M. K. (2011). Becoming who we are: Temperament and personality in development. Guilford Press.

Rubertsson, C., Börjesson, K., Berglund, A., Josefsson, A. and Sydsjö, G. (2011). The Swedish validation of Edinburgh postnatal depression scale (EPDS) during pregnancy. *Nordic Journal of Psychiatry* **65:**414-418.

Rubinow, D. R. (2005). Reproductive steroids in context. *Archives of Women's Mental Health* **8:**1-5.

Rubinow, D. R., Schmidt, P. J. and Roca, C. A. (1998). Estrogen-serotonin interactions: implications for affective regulation. *Biological Psychiatry* **44:**839-850.

Rurak, D., Lim, K., Sanders, A., Brain, U., Riggs, W. and Oberlander, T. F. (2011). Third trimester fetal heart rate and doppler middle cerebral selective serotonin reuptake inhibitor exposure. *Pediatric Research* **70:**96-101.

Rusak, B. and Zucker, I. (1979). Neural regulation of circadian rhythms. *Physiological Reviews* **59:**499-526.

Russo, P. S. T., Ferreira, G. R., Cardozo, L. E., Bürger, M. C., Arias-Carrasco, R., Maruyama, S. R., Hirata, T. D. C. *et al.* (2018). The Molecular Signatures Database (MSigDB) hallmark gene set collection. *BMC Bioinformatics* **19:**56.

Rwakarema, M., Premji, S. S., Nyanza, E. C., Riziki, P. and Palacios-Derflingher, L. (2015). Antenatal depression is associated with pregnancy-related anxiety, partner relations, and wealth in women in Northern Tanzania: a cross-sectional study. *BMC Womens Health* **15:**68.

Rydahl, E., Declercq, E., Juhl, M. and Maimburg, R. D. (2019). Cesarean section on a rise—Does advanced maternal age explain the increase? A population register-based study. *PLoS One* **14:**e0210655.

Sagud, M., Pivac, N., Mück-Seler, D., Jakovljević, M., Mihaljević-Peles, A. and Korsić, M. (2002). Effects of sertraline treatment on plasma cortisol, prolactin and thyroid hormones in female depressed patients. *Neuropsychobiology* **45:**139-143.

Sangkuhl, K., Klein, T. and Altman, R. (2009). Selective Serotonin Reuptake Inhibitors (SSRI) Pathway. *Pharmacogenetics and Genomics* **19:**907-909.

Saveanu, R. V. and Nemeroff, C. B. (2012). Etiology of Depression: Genetic and Environmental Factors. *Psychiatric Clinics* **35:**51-71.

Savory, K. A., Garay, S. M., Sumption, L. A., Kelleher, J. S., Daughters, K., Janssen, A. B., Van Goozen, S. *et al.* (2020). Prenatal symptoms of anxiety and depression associated with sex differences in both maternal perceptions of one year old infant temperament and researcher observed infant characteristics. *Journal of Affective Disorders* **264:**383-392.

Scheinost, D., Spann, M. N., McDonough, L., Peterson, B. S. and Monk, C. (2020). Associations between different dimensions of prenatal distress, neonatal hippocampal connectivity, and infant memory. *Neuropsychopharmacology* **45:**1272-1279.

Schrader, J. A., Nunez, A. A. and Smale, L. (2011). Site-specific changes in brain extra-SCN oscillators during early pregnancy in the rat. *Journal of Biological Rhythms* **26:**363-367.

Scott, R. J. and Spielman, M. (2006). Genomic imprinting in plants and mammals: how life history constrains convergence. *Cytogenetic Genome Research* **113:**53-67.

Shadrina, M., Bondarenko, E. A. and Slominsky, P. A. (2018). Genetics Factors in Major Depression Disease. *Frontiers in Psychiatry* **9:**334.

Shallie, P. D. and Naicker, T. (2019). The placenta as a window to the brain: A review on the role of placental markers in prenatal programming of neurodevelopment. *International Journal of Developmental Neuroscience* **73:**41-49.

Sheehan, P. M., Bousman, C., Komiti, A., Judd, F., Newman, L., Tonge, B., Castle, D. *et al.* (2018). Assessment of Placental Cortisol Pathway Gene Expression in Term Pregnant Women with Anxiety. *Neuropsychobiology* **77:**1-7.

Shingo, T., Gregg, C., Enwere, E., Fujikawa, H., Hassam, R., Geary, C., Cross, J. C. *et al.* (2003). Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. *Science* **299:**117-120.

Siegel, R. S. and Brandon, A. R. (2014). Adolescents, pregnancy, and mental health. *Journal of Pediatric and Adolescent Gynecology* **2014:**138-150.

Sinesi, A., Maxwell, M., O'Carroll, R. and Cheyne, H. (2019). Anxiety scales used in pregnancy: systematic review. *BJPsych Open* **5:**e5.

Slomian, J., Honvo, G., Emonts, P., Reginster, J.-V. and Bruyère, O. (2019). Consequences of maternal postpartum depression: A systematic review of maternal and infant outcomes. *Womens Health (London)* **15:**1745506519844044.

Smedberg, J., Lupattelli, A., Mårdby, A. C., Øverland, S. and Nordeng, H. (2015). The relationship between maternal depression and smoking cessation during pregnancy--a cross-sectional study of pregnant women from 15 European countries. *Archives of Womens Mental Health* **18:**73-84.

Soares, C. N. and Zitek, B. (2008). Reproductive hormone sensitivity and risk for depression across the female life cycle: A continuum of vulnerability? *Journal of Psychiatry and Neuroscience* **33:**331-343.

Soares, M. J., Konno, T. and Alam, S. M. (2007). The prolactin family: effectors of pregnancy-dependent adaptations. *Trends in Endocrinology & Metabolism* **18:**114-121.

Sõber, S., Reiman, M., Kikas, T., Rull, K., Inno, R., Vaas, P., Teesalu, P. *et al.* (2015). Extensive shift in placental transcriptome profile in preeclampsia and placental origin of adverse pregnancy outcomes. *Scientific Reports* **5:**13336.

Sodhi, M. S. K. and Sanders-Bush, E. (2004). Serotonin and brain development. *International Review of Neurobiology* **59:**111-174.

Soe, N. N., Wen, D. J., Poh, J. S., Li, Y., Broekman, B. F., Chen, H., Chong, Y. S. *et al.* (2016). Pre- and Post-Natal Maternal Depressive Symptoms in Relation with Infant Frontal Function, Connectivity, and Behaviors. *PLoS One* **11:**e0152991.

Sohr-Preston, S. L. and Scaramella, L. V. (2006). Implications of Timing of Maternal Depressive Symptoms for Early Cognitive and Language Development. *Clinical Child and Family Psychology Review* **9:**65-83.

Solutions for Public Health. (2019). Screening for antenatal and postnatal mental health problems. UK NSC, Wellington House, London, SE1 8UG.

Sood, R., Zehnder, J. L., Druzin, M. L. and Brown, P. O. (2006). Gene expression patterns in human placenta. *PNAS* **103:**5478-5483.

Sørensen, M. J., Grønborg, T. K., Christensen, J., Parner, E. T., Vestergaard, M., Schendel, D. and Pedersen, L. H. (2013). Antidepressant Exposure in Pregnancy and Risk of Autism Spectrum Disorders. *Clinical Epidemiology* **5:**449-459.

Soubry, A., Murphy, S. K., Wang, F., Huang, Z., Vidal, A. C., Fuemmeler, B. F., Kurtzberg, J. *et al.* (2015). Newborns of obese parents have altered DNA methylation patterns at imprinted genes. *International Journal of Obstetrics* **39:**650-657.

Spencer, S., Falcon, E., Kumar, J., Krishnan, V., Murkherjee, S., Birnbaum, S. G. and McClung, C. A. (2013). Circadian genes Period 1 and Period 2 in the nucleus accumbens regulate anxiety-related behavior. *European Journal of Neuroscience* **37:**242-250.

Spielberger, C., D., Gorusch, R. L. and Lushene, R. E. (1970). STAI manual for the State-Trait Anxiety Inventory. Pao Alto, California: Consulting Psychologists Press Inc.

Spielberger, C., Gorsuch, R., Lushene, R., Vagg, P. and Jacobs, G. (1983). Manual for the State-trait Anxiety Inventory. Palo Alto, C. A.: Consulting Psychologists Press. Inc.

Statistics for Wales. (2017). Maternity Statistics Wales, 2015-16.

Statistics for Wales. (2019). Maternity and Birth Statistics, Wales 2018.

Stewart, D. and Vigod, S. (2018). Antenatal use of antidepressants and risk of teratogenicity and adverse pregnancy outcomes: Selective serotonin reuptake inhibitors (SSRIs). *UpToDate*.

Stewart, R. C. (2007). Maternal depression and infant growth – a review of recent evidence. *Maternal and Child Nutrition* **3:**94-107.

Stewart, R. L. and Thompson, M. B. (2003). Evolutionary transformations of the fetal membranes of viviparous reptiles: a case study of two lineages. *Journal of Experimental Zoology Part A: Comparative Experimental Biology Journal* **299:**13-32.

Stirrat, L. I., Sengers, B. G., Norman, J. E., Homer, N. Z. M., Andrew, R., Lewis, R. M. and Reynolds, R. M. (2018). Transfer and Metabolism of Cortisol by the Isolated Perfused Human Placenta. *Journal of Clinical Endocrinology and Metabolism* **103:**640-648.

Sujan, A. C., Rickert, M. E., Öberg, A. S., Quinn, P. D., Hernández-Díaz, S., Almqvist, C., Lichtenstein, P. *et al.* (2017). Associations of Maternal Antidepressant Use During the First Trimester of Pregnancy With Preterm Birth, Small for Gestational Age, Autism Spectrum Disorder, and Attention-Deficit/Hyperactivity Disorder in Offspring. *JAMA* **317:**1553-1562.

Sumption, L., Garay, S. M. and John, R. M. (2020). Low serum placental lactogen at term is associated with postnatal symptoms of depression and anxiety in women delivering female infants. *Psychoneuroendocrinology* **116:**104655.

Sundström, E., Kölare, S., Souverbic, F., Samuelsson, E.-B., Pschera, H., Lunell, N. O. and Seigera, Å. (1993). Neurochemical differentiation of human bulbospinal monoaminergic neurons during the first trimester. *Developmental Brain Research* **75:**1-12.

Supek, F., Bošnjak, M., Škunca, N. and Šmuc, T. (2011). REVIGO summarizes and visualizes long lists of gene ontology terms. *PLoS One* **6:**e21800.

Surani, M. A. (1998). Imprinting and the initiation of gene silencing in the germ line. *Cell* **93:**309-312.

Surani, M. A., Barton, S. C. and Norris, M. L. (1984). Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. *Nature* **308:**548-550.

Suter, M., Abramovici, A., Showalter, L., Hu, M., Shope, C. D., Varner, M. and Aagaard-Tillery, K. (2010). In utero tobacco exposure epigenetically modifies placental CYP1A1 expression. *Metabolism* **59:**1481-1490.

Sutherland, S. and Brunwasser, S. M. (2018). Sex Differences in Vulnerability to Prenatal Stress: A Review of the Recent Literature. *Current Psychiatry Reports* **20:**102.

Swanson, A. M. and David, A. L. (2015). Animal models of fetal growth restriction: Considerations for translational medicine. *Placenta* **36:**623-630.

Takahashi, S., Okae, H., Kobayashi, N., Kitamura, A., Kumada, K., Yaegashi, N. and Arima, T. (2019). Loss of p57KIP2 expression confers resistance to contact inhibition in human androgenetic trophoblast stem cells. *PNAS* **116:**26606-26613.

Talge, N. M., Neal, C. and Glover, V. (2007). Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *Journal of Child Psychology and Psychiatry* **48:**245-261.

Temkin, O. (1991). Soranus' Gynecology. Baltimore: Johns Hopkins University Press.

Thiaville, M. M., Huang, J. M., Kim, H., Ekram, M. B., Roh, T. Y. and Kim, J. (2013). DNA-binding motif and target genes of the imprinted transcription factor PEG3. *Gene* **512:**314-320.

Thomas, M. J. and Seto, E. (1999). Unlocking the mechanisms of transcription factor YY1: are chromatin modifying enzymes the key? *Gene* **236:**197-208.

Thornsteinsson, E. B., Loi, N. M. and Moulynox, A. L. (2014). Mental health literacy of depression and postnatal depression: A community sample. *Open Journal of Depression* **3:**101-111.

Trejo, J. L., Cuchillo, I., Machín, C. and Rúa, C. (2000). Maternal adrenalectomy at the early onset of gestation impairs the postnatal development of the rat hippocampal formation: effects on cell numbers and differentiation, connectivity and calbindin-D28k immunoreactivity. *Journal of Neuroscience Research* **62:**644-647.

Tucci, V., Isles, A. R., Kelsey, G., Ferguson-Smith, A. C. and Erice Imprinting Group. (2019). Genomic Imprinting and Physiological Processes in Mammals. *Cell* **176:**952-965.

Tuiskula-Haavisto, M. and Vilkki, J. (2007). Parent-of-origin specific QTL—a possibility towards understanding reciprocal effects in chicken and the origin of imprinting. *Cytogenetic Genome Research* **117:**305-312.

Tung, J., Barreiro, L. B., Johnson, Z. P., Hansen, K. D., Michopoulos, V., Toufexis, D., Michelini, K. *et al.* (2012). Social environment is associated with gene regulatory variation in the rhesus macaque immune system. *Proc Natl Acad Sci U.S.A* **109:**6490-6495.

Tunster, S. J., Boqué-Sastre, R., McNamara, G. I., Hunter, S. M., Creeth, H. D. J. and John, R. M. (2018). Peg3 Deficiency Results in Sexually Dimorphic Losses and Gains in the Normal Repertoire of Placental Hormones. *Frontiers in Cell and Developmental Biology* **6:**123.

Tunster, S. J., Creeth, H. D. and John, R. M. (2016). The imprinted Phlda2 gene modulates a major endocrine compartment of the placenta to regulate placental demands for maternal resources. *Developmental Biology* **409:**251-260.

Tunster, S. J., Jenson, A. B. and John, R. M. (2013). Imprinted genes in mouse placental development and the regulation of fetal energy stores. *Reproduction* **145:**117-137.

Tunster, S. J., Van de Pette, M. and John, R. M. (2011). Fetal overgrowth in the Cdkn1c mouse model of Beckwith-Wiedemann syndrome. *Disease Models and Mechanisms* **4:**814-821.

Turco, M. Y., Gardner, L., Kay, R. G., Hamilton, R. S., Prater, M., Hollinshead, M. S., McWhinnie, A. *et al.* (2018). Trophoblast organoids as a model for maternal–fetal interactions during human placentation. *Nature* **564:**263-267.

Turco, M. Y. and Moffett, A. (2019). Development of the human placenta. *Development* **146:**163428.

Vakili, H., Jin, Y., Menticoglou, S. and Cattini, P. A. (2013). CCAAT-enhancer-binding protein β (C/EBPβ) and downstream human placental growth hormone genes are targets for dysregulation in pregnancies complicated by maternal obesity. *Journal of Biological Chemistry* **288:**22849-22861.

Van Batenburg-Eddes, T., Brion, M. J., Henrichs, J., Jaddoe, V. W. V., Hofman, A., Verhulst, F. C., Lawlor, D. A. *et al.* (2012). Parental depressive and anxiety symptoms during pregnancy and attention problems in children: a cross-cohort consistency study. *Journal of Child Psychology and Psychiatry* **54:**591-600.

Van de Pette, M., Abbas, A., Feytout, A., McNamara, G., Bruno, L., To, W. K., Dimond, A. *et al.* (2017). Visualizing Changes in Cdkn1c Expression Links Early-Life Adversity to Imprint Mis-regulation in Adults. *Cell Reports* **18:**1090-1099.

Van den Bergh, B. R., J., M. E., Mennes, M. and Glover, V. (2005). Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: links and possible mechanisms. A review. *Neuroscience and Biobehavioural Reviews* **29:**237-258.

Vangeel, E. B., Izzi, B., Hompes, T., Vansteelandt, K., Lambrechts, D., Freson, K. and Claes, S. (2015). DNA methylation in imprinted genes *IGF2* and *GNASXL* is associated with prenatal maternal stress. *Genes, Brain and Behaviour* **14:**573-582.

Vargas, A., Moreau, J., Landry, S., LeBellego, F., Toufaily, C., Rassart, E., Lafond, J. *et al.* (2009). Syncytin-2 Plays an Important Role in the Fusion of Human Trophoblast Cells. *Journal of Molecular Biology* **392:**301-318.

Verreault, N., Da Costa, D., Marchand, A., Ireland, K., Drista, M. and Khalifé, S. (2014). Rates and risk factors associated with depressive symptoms during pregnancy and with postpartum onset. *Journal of Psychosomatic Obstetrics and Gynaecology* **35:**84-91.

Vidal, A. C., Murphy, S. K., Murtha, A. P., Schildkraut, J. M., Soubry, A., Huang, Z., Neelon, S. E. *et al.* (2013). Associations between antibiotic exposure during pregnancy, birth weight and aberrant methylation at imprinted genes among offspring. *International Journal of Obstetrics* **37:**907-913.

Vidal, A. C., Neelon, B., Liu, Y., Tuli, A. M., Fuemmeler, B. F., Hoyo, C., Murtha, A. P. *et al.* (2014). Maternal stress, preterm birth, and DNA methylation at imprint regulatory sequences in humans. *Genetics and Epigenetics* **6:**37-44.

Vidal, A. C., Semenova, V., Darrah, T., Vengosh, A., Huang, Z., King, K., Nye, M. D. *et al.* (2015). Maternal cadmium, iron and zinc levels, DNA methylation and birth weight. *BMC Pharmacology and Toxicology* **16:**20.

Vildalen, V. U., Brevik, E. J., Haavik, J. and Lundervold, A. J. (2019). Females With ADHD Report More Severe Symptoms Than Males on the Adult ADHD Self-Report Scale. *Journal of Attention Disorders* **23:**959-967.

Waddell, B. J., Wharfe, M. D., Crew, R. C. and Mark, P. J. (2012). A rhythmic placenta? Circadian variation, clock genes and placental function. *Placenta* **33:**533-539.

Walker, W. H., Fitzpatrick, S. L. and Saunders, G. F. (1990). Human placental lactogen transcriptional enhancer. Tissue specificity and binding with specific proteins. *Journal of Biological Chemistry* **265:**12940-12948.

Walter, W., Sánchez-Cabo, F. and Ricote, M. (2015). GOplot: an R package for visually combining expression data with functional analysis. *Bioinformatics* **31:**2912-2914.

Wang, L., Wang, S. and Li, W. (2012a). RSeQC: quality control of RNA-seq experiments. *Bioinformatics* **28:**2184-2185.

Wang, W. and Subramani, S. (2017). Role of PEX5 ubiquitination in maintaining peroxisome dynamics and homeostasis. *Cell Cycle* **16:**2037-2045.

Wang, Y., Pringle, K. G., Sykes, S. D., Marques, F. Z., Morris, B. J., Zakar, T. and Lumbers, E. R. (2012b). Fetal sex affects expression of renin-angiotensin system components in term human decidua. *Endocrinology* **153:**462-468.

Weikum, W. M., Brain, U., Chau, C. M. Y., Grunau, R. E., Thomas Boyce, W., Diamond, A. and Oberlander, T. F. (2013a). Prenatal serotonin reuptake inhibitor (SRI) antidepressant exposure and serotonin transporter promoter genotype (SLC6A4) influence executive functions at 6 years of age. *Frontiers in Cellular Neuroscience* **7:**180.

Weikum, W. M., Mayes, L. C., Grunau, R. E., Brain, U. and Oberlander, T. F. (2013b). The impact of prenatal serotonin reuptake inhibitor (SRI) antidepressant exposure and maternal mood on mother-infant interactions at 3 months of age. *Infant Behaviour and Development* **36:**485-493.

Weikum, W. M., Oberlander, T. F., Hensch, T. K. and Werker, J. F. (2012). Prenatal exposure to antidepressants and depressed maternal mood alter trajectory of infant speech perception. *Proc Natl Acad Sci U.S.A* **109:**17221-17227.

Weinstock, M. (2001). Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Progress in Neurobiology* **65:**427-451.

Weinstock, M. (2008). The long-term behavioural consequences of prenatal stress. *Neuroscience & Behavioural Reviews* **32:**1073-1086.

Wen, D. J., Poh, J. S., Ni, S. N., Chong, Y. S., Chen, H., Kwek, K., Shek, L. P. *et al.* (2017). Influences of prenatal and postnatal maternal depression on amygdala volume and microstructure in young children. *Translational Psychiatry* **7:**e1103.

Wilkinson, L. S., Davies, W. and Isles, A. R. (2007). Genomic imprinting effects on brain development and function. *Nature Reviews Neuroscience* **4:**1-19.

Williamson, D. E., Forbes, E. E., Dahl, R. E. and Ryan, N. D. (2005). A genetic epidemiologic perspective on comorbidity of depression and anxiety. *Child and Adolescent psychiatric Clinics of North America* **4:**707-726.

Winterbottom, E. F., Ban, Y., Xiaodian, S., Capobianco, A. J., Marsit, C. J., Wang, L., Karagas, M. R. *et al.* (2019). Transcriptome-wide analysis of changes in the fetal placenta associated with prenatal arsenic exposure in the New Hampshire Birth Cohort Study. *Environmental Health* **18:**100.

Wittkowski, A., Wieck, A. and Mann , S. (2007). An evaluation of two bonding questionnaires: A comparison of the Mother-to-Infant Bonding Scale with the Postpartum Bonding Questionnaire in a sample of primiparous mothers. *Archives of Womens Mental Health* **10:**171-175.

Wolf, J. B. and Hager, R. (2006). A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *PLoS Biology* **4:**e380.

Woodside, B., Augustine, R. A., Ladyman, S. R., Naef, L. and Grattan, D. R. (2008). Role of prolactin in the metabolic adaptations to pregnancy and lactation. In: Bridges, R.S. (ed.) *Neurobiology of the Parental Brain*. USA: Elsevier Inc.

Woody, C. A., Ferrari, A. J., Siskind, D. J., Whiteford, H. A. and Harris, M. G. (2017). A systematic review and meta-regression of the prevalence and incidence of perinatal depression. *Journal of Affective Disorders* **219:**86-92.

Wright-Guerina, D., Oliver, P. H., Gottfried, A. W., Gottfried, A. E., Reichard, R. J. and Riggioe, R. E. (2011). Childhood and adolescent antecedents of social skills and leadership potential in adulthood: Temperamental approach/withdrawal and extraversion. *The Leadership Quarterly* **22:**482-494.

Wu, S., Wang, R., Zhao, Y., Ma, X., Wu, M., Yan, X. and He, J. (2013). The relationship between self-rated health and objective health status: a population-based study. *BMC Public Health* **13:**320.

Wurmser, H., Rieger, M., Domogalla, C., Kahnt, A., Buchwald, J., Kowatsch, M., Kuehnert, N. *et al.* (2006). Association between life stress during pregnancy and infant crying in the first six months postpartum: A prospective longitudinal study. *Early Human Development* **82:**341-349.

Yang, C., Chapman, A. G., Kelsey, A. D., Minks, J., Cotton, A. M. and Brown, C. J. (2011). X-chromosome inactivation: molecular mechanisms from the human perspective. *Human Genetics* **130:**175-185.

Yuen, L. and Wong, V. W. (2015). Gestational diabetes mellitus: Challenges for different ethnic groups. *World Journal of Diabetes* **6:**1024-1032.

Zhang, W., Finik, J., Dana, K., Glover, V., Ham, J. and Nomura, Y. (2017). Prenatal Depression and Infant Temperament: The Moderating Role of Placental Gene Expression. *Infancy* **23:**211-231.

Zietlow, A. L., Nonnenmacher, N., Reck, C., Ditzen, B. and Müller, M. (2019). Emotional Stress During Pregnancy – Associations With Maternal Anxiety Disorders, Infant Cortisol Reactivity, and Mother–Child Interaction at Pre-school Age. *Frontiers in Psychology* **10:**2179.