Uncovering the placental mechanisms that contribute to preterm birth and risk for adverse offspring development in pregnancies complicated by suboptimal maternal body mass index

by

Hailey Scott

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Abstract

The objectives of this thesis were to understand how maternal underweight, obesity, and preterm birth alter placental development and function. Placental pathology data were obtained from an archived dataset, and qPCR and immunohistochemistry were used in a current cohort, to explore the effects of maternal body mass index (BMI) and/or preterm birth on placental development and function. Increased maternal BMI associated with increased placental inflammation, maternal vascular malperfusion, and decreased placental efficiency. Preterm placentae had increased expression of multidrug resistance transporters, and altered expression of antimicrobial peptides. These findings revealed maternal underweight and obesity are not inert conditions for the developing placenta. Upregulated placental efflux transport earlier in gestation may regulate fetal exposure to increased inflammation/infection at preterm, while altered placental defences may impair placental-mediated fetal protection. Understanding placental adaptations in these common conditions helps to uncover the mechanisms linking suboptimal maternal BMI and inflammatory states with adverse pregnancy outcomes.

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List of Abbreviations

ABC: ATP-binding cassette efflux transporters
ABCB1: gene encoded by P-glycoprotein
ABCG2: gene encoded by breast cancer resistance protein
AMP: antimicrobial peptide
AOAH: acyloxyacyl hydrolase
BCRP: breast cancer resistance protein
BMI: body mass index
CAMP: cathelicidin
CPP: Collaborative Perinatal Project
DEFB1: human β -defensin 1
GDM: gestational diabetes mellitus
GNLY: granulysin
IL: interleukin
IOM: Institute of Medicine
MVM: maternal vascular malperfusion
MDR: multidrug resistance transporter
NW: normal weight
OB: obese
OR: odds ratio
OW: overweight
P-gp: P-glycoprotein
PT: preterm
PTB: preterm birth
PTC: preterm with chorioamnionitis
r: Wilcoxon effect size
RCWIH: Research Centre for Women's and Infants' Health BioBank
SLPI: secretory leukocyte protease inhibitor
T: term
UW: underweight

Chapter 1. Introduction

1.1 Purpose of this thesis

The purpose of this thesis is to better understand how maternal underweight and obesity and preterm birth associate with altered placental development and function, to help uncover the mechanisms linking suboptimal maternal body mass index (BMI) and pro-inflammatory states with adverse pregnancy and offspring outcomes. Maternal underweight and obesity affect a large proportion of the world's population, including reproductive aged women¹⁻³. The prevalence of obesity in reproductive aged women in Canada is over 30%, and increasing globally³. These common maternal conditions have implications not only for immediate pregnancy and fetal outcomes, including increased risk for preterm birth and poor offspring growth^{4,5}, but later programming of offspring health⁶. The placenta is a critical regulator of the fetal environment, and has the ability to adapt to environmental exposures to protect the developing fetus⁷. However, these placental adaptations, or maladaptations, may result in adverse pregnancy outcomes, such as fetal exposure to inflammation⁸⁻¹², altered placental efflux transport of potentially harmful xenobiotics¹³, and preterm delivery. Preterm birth in itself is associated with adverse offspring outcomes¹⁴⁻¹⁷, and increased levels of inflammation locally at the placenta and generally in the pregnancy environment¹⁸.

While there has been research on the associations between placental pathologies and adverse pregnancy outcomes, placental histomorphological changes with maternal underweight or obesity, in the absence of other major maternal comorbidities, have not been well quantified. Additionally, the presence of placental efflux transporters in pregnancy has been established, but few studies have characterized placental efflux transport in the context of both suboptimal maternal BMI and preterm birth, in otherwise uncomplicated pregnancies. Finally, antimicrobial defence systems have been shown to be important in regulating infection and inflammation, yet their role in the placenta, and with adverse maternal environments, are poorly understood. This thesis investigates the relationships between suboptimal maternal BMI and preterm birth with the prevalence of placental pathologies, effects on placental efflux transport, and placental defence. By better understanding these relationships, the field will gain further insight into the mechanisms linking suboptimal metabolic and inflammatory states with adverse pregnancy outcomes, and how the placenta adapts or maladapts to challenging maternal environments. Characterizing placental (mal)adaptations may aid clinical decision making to better support high-risk pregnancies, allow for early interventions in offspring with poor health trajectories, and provide insight into novel therapeutic targets to optimise pregnancy, placental, and infant health.

1.2 Normal placental development and function

1.2.1 Normal development

The placenta is a critical regulator of the fetal environment, involved in oxygen and nutrient transport, waste transfer, endocrine secretion, and immunological protection of the fetus¹⁹. In

human pregnancy, the placenta begins to form approximately five days post-fertilization when the blastocyst implants into the uterine wall, developing from the trophoblast, the outer layer of the blastocyst¹⁹. Following attachment to the uterine epithelium, the trophoblast begins to differentiate into two cell layers: the outer multinucleated syncytiotrophoblast, and an inner cytotrophoblast layer¹⁹. Projections of the syncytiotrophoblast invade the maternal endometrium, which undergoes decidualization during pregnancy, allowing the blastocyst to become fully embedded within the decidua¹⁹. Lacunae then form within the syncytiotrophoblast, which become filled with maternal blood as the syncytiotrophoblast continues to erode maternal tissues¹⁹. Lacunae fuse, forming a lacunar network for maternal blood flow, and establishing uteroplacental circulation by the end of the second week of pregnancy¹⁹. To facilitate exchange between the mother and fetus, maternal uterine spiral arteries undergo remodeling by cytotrophoblast cells, producing dilated, low-resistance vessels, to optimize maternal blood supply²⁰.

The inner cytotrophoblast cell layer of the placenta also proliferates, and extends into the syncytiotrophoblast, forming primary placental chorionic villi²¹. Secondary villi develop as extraembryonic mesenchymal cells then extend into the cytotrophoblast villous core¹⁹. Finally, tertiary villi develop as fetal capillaries are formed within the extraembryonic mesoderm of the villi¹⁹. The placental chorionic villi are thus composed of four key cell layers: the outer syncytiotrophoblast, inner cytotrophoblast cell layer, connective tissue, and fetal capillaries¹⁹. Cytotrophoblast cells then penetrate the syncytiotrophoblast as extravillous cytotrophoblast columns, and form a cytotrophoblast shell across the decidua basalis, separating maternal and fetal circulations²⁰. Anchoring villi connect to the decidua basalis, while branch villi grow from the sides of these stem villi, and are surrounded by the intervillous space where maternal-fetal transfer can occur at terminal villi¹⁹.

The placenta continues to grow throughout gestation, increasing surface area for maternal-fetal exchange, and by term, measures approximately 500 grams, 15 to 20 centimeters in diameter, and two to three centimeters thick²². The functional units of the placenta, the chorionic villi, also mature to optimize exchange, so that by approximately 20 weeks, the inner cytotrophoblast cell layer is reduced and only the syncytiotrophoblast separates the maternal blood and the fetal capillary endothelium^{20,22}. The fetus is also surrounded by the amniochorionic membranes; the amnion is the innermost single cell epithelial layer in contact with the amniotic fluid, connected by extracellular matrix to the chorion trophoblast layer that lines the maternal decidua²³. The fetal membranes also play a role in mechanical and antimicrobial fetal protection, endocrine secretion, transport, and in promoting parturition²³. As the growth of the fetus relies on that of the placenta, normal development of the placenta is critical to ensure optimal fetal development.

Figure 1.1. Human placental chorionic villous. Key cell layers of the placental villi include the syncytiotrophoblast, cytotrophoblast, fetal capillaries, and connective tissue. The placenta expresses efflux transporters that regulate the transfer of inflammatory mediators, drugs, and nutrients, among others, into the intrauterine compartment, and antimicrobial peptides that may protect the developing fetus against infectious and inflammatory exposures. Icons adapted from BioRender and Microsoft PowerPoint.

1.2.2 Placental transport

As the interface between the mother and fetus, the placenta plays a critical role in the transfer of substrates to support offspring development. The syncytiotrophoblast, as the outermost cell layer of the placenta separating the maternal and fetal circulations, expresses several transporters to facilitate this exchange²⁴. The syncytiotrophoblast is an epithelial surface consisting of a microvillous plasma membrane facing the maternal circulation, and a fetal-facing basal membrane²⁵. Nutrient, drug, and gas exchange can occur across this barrier via passive and facilitated diffusion and active transport²². Gas exchange of oxygen and carbon dioxide, for example, occur via passive diffusion²². Fatty acids are mainly transferred to the fetus by simple diffusion²², but passive transport alone may be insufficient to support exchange of all nutrients, such as amino acids and glucose. Therefore, these nutrients and others are supported by active transport and facilitated diffusion²². Transporters may also be expressed in the fetal blood vessel endothelium for transport of substrates into fetal circulation^{26,27}. Placental transporters thus are critical for regulating nutrient, drug, and cytokine efflux, to mediate fetal protection and nutrition.

1.2.2.1 Nutrients

Key placental nutrient transport systems include System A (small neutral amino acids, SNATs) and System L (large neutral amino acid transport, LATs) for the transfer of amino acids^{9,28}, fatty acid transport proteins (FATP), fatty acid translocase (FAT/CD36), plasma membrane fatty acid binding protein (FABPpm), and fatty acid binding proteins (FABP) for fatty acid transport^{9,28}, and glucose (GLUT) transporters for glucose transfer from the mother to the fetus^{9,22,28}. Amino acids are essential for fetal protein synthesis, and are transferred across the placenta via active transport. Fatty acids are used by the fetus to synthesize phospholipids, biological membranes, and myelin, important for cell structure and central nervous system development, and to synthesize mediators of cell signaling, such as prostoglandins²². Fatty acids play an important role in fetal growth, including brain growth²⁸, and influence adipose tissue mass which may have implications for metabolic programming²⁹. These substrates are transported mainly by simple diffusion. Glucose, derived from the mother, is the main source of fetal energy²², and is transported via facilitated diffusion²² down a concentration gradient, as glucose levels in the mother are higher than those in the fetus³⁰.

1.2.2.2 Drugs

Placental drug transfer can be beneficial for the fetus when drugs are administered to the mother with the intention of treating the offspring, but can also be detrimental when xenobiotics are unintentionally transferred to the fetus²². Drugs in the maternal circulation are transferred into the intervillous space of the placenta, and cross the syncytiotrophoblast, connective tissue, and fetal vessel endothelium to enter the fetal circulation²². Placental drug transfer can occur via simple diffusion, which is likely the most common mechanism, facilitated diffusion, active transport, or pinocytosis. Fetal exposures to drugs may be most detrimental during the first trimester of pregnancy during organogenesis, and could have teratogenic effects and impair offspring growth and development²².

Placental efflux transporters regulate an array of endogenous and exogenous substances to mediate fetal protection and nutrition³¹. Commonly studied in the placenta are the multidrug resistance (MDR) transporters P-glycoprotein (P-gp), encoded by the gene *ABCB1*, and breast cancer resistance protein (BCRP), encoded by the gene *ABCG2*, which belong to the ATP-binding cassette (ABC) superfamily of efflux transporters¹³. P-gp is localized to the syncytiotrophoblast and cytotrophoblast cell layers of the placenta, and regulates the efflux of glucocorticoids, estrogens and progestogens, nutrients, cytokines/chemokines, antibiotics, and antiretrovirals, among other substrates^{13,32}. BCRP is localized to the syncytiotrophoblast, cytotrophoblast, and endothelial cells of fetal blood vessels, and transports endogenous steroids, antibiotics, antivirals, sulfonylureas, nutrients, and toxins from the fetus back into the maternal circulation^{13,32}. The function of these transporters is regulated in part by inflammatory molecules¹³. For example, placental P-gp and BCRP expression have been shown to be downregulated by inflammatory cytokines tumor necrosis factor-alpha (TNF- α) and interleukin (IL)-1 β in term trophoblasts, suggesting decreased fetal protection with placental inflammation²⁷.

In contrast, placental *ABCB1* mRNA has been positively correlated with IL-1 β , IL-6, and TNF- α expression at preterm³³, and P-gp/*ABCB1* and BCRP/*ABCG2* increased in preterm labour with inflammation³⁴. Thus, placental expression of MDR transporters may be altered in pregnancies complicated by maternal pro-inflammatory states, and this hypothesis should be further explored. Altered expression of transporters may impair the placenta's ability to protect the developing fetus from harmful environmental conditions, such as in suboptimal metabolic states and preterm birth, potentially contributing to adverse pregnancy and infant outcomes.

1.2.2.3 Cytokines

Increased pro-inflammatory cytokine production has been associated with adverse pregnancy outcomes and offspring development³⁵. Cytokines may be produced by the mother, fetus, or the placenta³⁵, however, whether cytokines may be directly transferred from the maternal circulation to the fetus is not well understood³⁶. While some evidence has shown transfer of IL-6 across the placenta³⁵, other studies do not corroborate this finding³⁷. Nonetheless, increased levels of pro-inflammatory mediators *in utero* have been associated with adverse fetoplacental development, and may be exacerbated by infectious or inflammatory maternal conditions³⁶.

As described above, placental MDR transporters are involved in the efflux of cytokines and chemokines^{13,32}. Specifically, P-gp has been shown to efflux pro-inflammatory mediators IL-2, interferon- γ (IFN γ), TNF- α , and chemokine (C-C motif) ligand 2, and cytokine efflux may also play a role in regulating immune response¹³.

1.2.3 Placental defences

The placenta expresses antimicrobial peptides (AMPs) to protect against infection or inflammation as part of the innate immune system, via direct and indirect immune activities³⁸⁻⁴⁰. Antimicrobial peptides are small proteins produced by epithelial surfaces and inflammatory cells³⁸. They have direct antimicrobial activity primarily through disrupting microbial membranes³⁸⁻⁴⁰, and play a role in immune modulation such as through opsonization, the production of cytokines and chemokines, and antiprotease and antiendotoxin activities³⁸⁻⁴¹. Acyloxyacyl hydrolase (AOAH), cathelicidin (CAMP), human β -defensin 1 (DEFB1), granulysin (GNLY), and secretory leukocyte protease inhibitor (SLPI), among others, are AMPs that have been identified in gestational tissues. Specifically, AOAH has been shown to be involved in microbial defense through inactivation of lipopolysaccharides by deacylation in the gastrointestinal tract⁴², though its role has not been well established in the placenta. CAMP, a broad-spectrum antimicrobial, is involved in chemotaxis and angiogenesis, among other immune modulatory functions⁴³. DEFB1 is an antimicrobial characterized to defend against both gram-positive and negative bacteria⁴⁴, and GNLY is cytolytic, prominently expressed by natural killer cells⁴⁵. Finally, SLPI has antimicrobial and antiprotease functions, and regulates inflammatory responses⁴⁶. Antimicrobial peptides are known to be important in infectious and inflammatory conditions, and thus may play a protective role in pregnancy³⁸. However, is not known how the

expression of antimicrobial peptides in gestational tissues differs with maternal BMI or gestational age.

1.2.4 Placenta regulating fetal growth

The placenta is a critical regulator of fetal growth; it is the site for nutrient, gas, and waste exchange, and is involved in endocrine signaling between the mother and fetus⁴⁷. Successful placental development and growth is key for optimal fetal growth⁴⁷. The fetus is dependent not only on nutrient availability from the mother, but the ability of the placenta to transport nutrients and other essential substrates, which is influenced by various placental factors including blood flow, area for exchange, and expression of transporters³⁰. Adequate uteroplacental blood flow may be determined early in pregnancy, during remodeling of the uterine spiral arteries⁴⁷. Transport of oxygen and carbon dioxide is mediated by placental area and blood flow⁴⁷. The endocrine role of the placenta is also important for producing hormones that can directly influence fetal growth or signal maternal adaptations in nutrient transfer⁴⁸. Inadequate placental development or function may thus have consequences for fetal development⁴⁷.

1.3 Abnormal placental development and function

The placenta adapts in response to the maternal environment, by altering its own development and function, in an attempt to optimize offspring growth²⁵. These adaptations may impact placental blood flow, nutrient transport, xenobiotic efflux, placental defence, and signaling roles, with potential consequences for fetal growth and long term offspring programming²⁵. While there are a number of factors that may adversely affect placental development and function, this thesis will focus on two common exposures: preterm birth and suboptimal maternal BMI. Preterm birth is a multifactorial condition, and may be instigated through intrauterine inflammation or infection, vascular malperfusion⁴⁹, uterine overdistention⁵⁰, or may be indicated as a result of clinical conditions such as preeclampsia or intrauterine growth restriction⁵⁰, amongst other factors, including those not yet known. Similarly, suboptimal BMI may influence placental and fetal development through various mechanisms, such as altered maternal metabolic and endocrine function^{51,52}, altered placental function, including placental nutrient transport⁵¹, with potential implications for placental growth⁵³, and with maternal obesity, increased risk for complications such as gestational diabetes mellitus, hypertension, and preterm birth^{51,52}. One pathway through which both preterm birth and maternal underweight or obesity may impair fetoplacental development is via inflammatory mechanisms.

1.3.1 Abnormal development

There is some evidence that maternal underweight and obesity may be unfavourable for placental development. Maternal obesity has been associated with chronic villitis^{54,55}, both delayed and accelerated placental villous maturation⁵⁵, and increased atheromas and villous infarcts^{55,56}, pathologies which have been linked to placental insufficiency⁵⁷, fetal growth restriction, and neurodevelopmental impairment⁵⁵. The effects of maternal undernutrition or

low BMI on placental pathologies are less well documented⁵⁸. Animal models of undernutrition have shown evidence of abnormal placental vasculature and decreased fetal blood space, labyrinth and junctional zone area⁵⁸, which may impair substrate transfer across the placenta⁵⁹. Therefore, it is important to understand even subtle placental phenotypes in these common conditions, as placental structural and functional adaptations may serve as a record of adversities experienced *in utero*, and could help reveal the mechanisms through which these adversities affect the developing offspring. However, the placental morphological and histopathologic changes induced by maternal underweight and obesity, in the absence of other comorbidities or adverse perinatal events, are poorly characterized.

Preterm birth is also associated with altered placental development. Chorioamnionitis, inflammation of the amnion and chorion, is the most frequent pathology associated with spontaneous preterm birth⁶⁰, often instigated by infection¹⁴. Histological chorioamnionitis begins as neutrophil infiltration of the decidua, and can progress as neutrophils migrate into the chorion and amnion⁶¹. Further, a fetal inflammatory response to infection is characterized by neutrophil infiltration of the umbilical cord⁶¹. Preterm birth is also commonly associated with maternal vascular malperfusion, evidenced by placental weight below the tenth percentile, placental infarcts, distal villous hypoplasia, accelerated villous maturation, and decidual arteriopathy⁶². While chorioamnionitis may be most commonly seen in extreme preterm births, maternal vascular malperfusion may be the more frequent lesion in late preterm birth⁶³. Yet, the etiology of preterm birth remains poorly understood, and a better understanding of the alterations in placental structure and function in pregnancies complicated by premature delivery may help to elucidate the underlying mechanisms and impacts on the fetus.

The mechanisms driving suboptimal placental adaptations and the development of placental pathologies are not well understood, but may involve inflammatory processes. While pregnancy itself is a controlled inflammatory process, dysregulated inflammation can result in adverse pregnancy outcomes, including impaired placental development and function. In early pregnancy, inflammation can impair spiral artery remodelling, resulting in altered utero-placental perfusion⁶⁴. Inducing maternal inflammation in animals results in structural abnormalities to the utero-placental vasculature, decreased placental blood flow, placental hypoxia⁶⁵, and the development of placental coagulopathies⁶⁶. Maternal inflammation is also associated with reduced placental weight, thickness, and area in animal models of inflammation in pregnancy, which may further have consequences for placental efficiency and perfusion⁶⁷. Thus, pro-inflammatory maternal states may be detrimental to fetoplacental development.

1.3.2 Abnormal transport

1.3.2.1 Nutrients

Fetal growth is regulated by maternal nutrition and metabolism, and suboptimal quality and quantity of nutrients available to the fetus and a pro-inflammatory state may have consequences for offspring growth development²⁵. The placenta adapts to the maternal environment, including

regulating nutrient transport, and changes in placental transporter expression can influence fetal growth and body composition, with potential for offspring programming effects²⁵. Animal models of maternal undernutrition have demonstrated decreased placental expression of nutrient transporters, including glucose and amino acid transporters, compared to controls^{53,68}, and offspring born to undernourished mothers had reduced fetal and placental weights⁵³. However, in some cases, the placenta may adapt by increasing nutrient transport to compensate for a poor maternal nutrient supply⁶⁹. In contrast, animal models of maternal obesity have shown up-regulation of placental glucose and amino acid transporter expression^{70,71}. Animals fed a high fat diet prepregnancy have also demonstrated increased fatty acid transport, including increased placental expression of fatty acid transport proteins, and increased maternal and fetal blood concentrations of cholesterol and triglycerides, where fetuses born to high fat fed dams were heavier than the controls⁷². Similarly, in humans, maternal obesity has been associated with increased placental amino acid transporter expression and with increased infant birthweight⁷³. Collectively, changes to the maternal environment induced by maternal malnutrition or suboptimal metabolic state can create a pro-inflammatory environment that adversely effects normal placental function. Insufficient or overabundance and poor quality of nutrients available to the fetus in pregnancies complicated by maternal underweight or obesity can adversely affect offspring development, and may have metabolic programming effects.

1.3.2.2 Drugs

Expression of critical placental drug efflux transporters may be altered by suboptimal maternal BMI or preterm birth, and are important as fetal drug or xenobiotic exposure may impair offspring growth and development²². While there are a lack of data on the effect of maternal BMI on placental efflux transport function, animal studies have shown increased *Abcg2*, and decreased placental *Abcb1a* mRNA expression levels in high fat fed compared to undernourished pregnant mice⁷⁴. In human pregnancy, maternal obesity has also been associated with downregulated placental *ABCB1* gene expression⁷⁵. The expression of P-gp and BCRP transporters in the placenta have also been shown to decrease with advancing gestational age^{34,76,77}, where placental *ABCB1* mRNA and syncytiotrophoblast P-gp protein levels are higher in first trimester compared to term^{77,78}, supporting the role of P-gp in fetal exchange and protection, especially at earlier stages of pregnancy⁷⁷. Increased expression of both placental P-gp and BCRP early in development is critically important given, for example, the high permeability of the fetal blood brain barrier in early pregnancy⁷⁹ and vulnerability of developing organs to exogenous insults, which may result in increased neurodevelopmental impairment in offspring exposed to inflammation at preterm⁸⁰. Expression of placental efflux transporters has also been shown to be altered by inflammation. For example, placental trophoblast cells from human pregnancies treated with inflammatory cytokines TNF- α and IL-1 β have shown decreased P-gp/*ABCB1* and BCRP/*ABCG2* transporter expression²⁷. In animal models of inflammation where pregnant mice were administered LPS, placental P-gp expression decreased, without changes in placental *Abcb1*⁸¹. Similarly, animal models administered polyinosinic-polycytidylic acid, simulating viral infection, have shown decreased placental *Abcb1a/b* and *Abcg2* mRNA, and increased maternal plasma concentrations of IFN γ , TNF- α , and IL-6⁸².

1.3.3 Abnormal defences

Altered expression of placental AMPs, such as with gestational age or suboptimal maternal BMI, may impair the placenta's ability to protect the developing fetus from environmental insults. However, it is not known how the expression of antimicrobial peptides in gestational tissues differs with maternal BMI, and placental expression of AMPs at preterm are not well understood. The effects of suboptimal maternal BMI on gestational tissue expression of the AMPs investigated in this study have not been well established. Previous studies have characterized changes in the antimicrobial peptide elafin in cervicovaginal fluid, and found that elafin concentrations increased with increasing maternal BMI⁸³. Evidence on the changing expression levels of placental antimicrobial peptides with gestational age are also conflicting. Decreased CAMP protein, but not mRNA, expression levels in the myometrium have been reported at preterm compared to term, suggesting that altered placental immune responses early in gestation may play a role in the pathogenesis of preterm birth⁴³. In contrast, CAMP in cervicovaginal fluid has been shown to be increased in women who delivered preterm compared to women who had term births, perhaps as a functional mounted response against infection/inflammation⁸³. Similarly, in normal pregnancies, amniotic fluid levels of DEFB1 have been shown to be higher at mid-trimester compared to at term⁸⁴.

1.3.4 Developmental programming

Placental structural and functional changes associated with maternal underweight and obesity may have consequences not only for immediate pregnancy and infant outcomes, but may also impact postnatal growth and health trajectories. The placenta may have the ability to adapt to its environment, such as with maternal nutrition availability in malnourished pregnancies, including upregulating nutrient transporter expression to meet fetal demand, or failing to compensate for nutrient availability and potentially contributing to fetal growth restriction⁸⁵, affecting fetal development and potentially long-term health^{9,24}. For example, poor fetal growth has been associated with non-communicable diseases later in life, including coronary heart disease, obesity⁸⁶, and neurocognitive disease⁸⁷. The placental phenotypes associated with maternal underweight and obesity may then be predictive of later health outcomes, and provide insight into the mechanisms of developmental programming⁸⁸.

Inflammation in the intrauterine compartment can additionally impair fetal growth and development, particularly key organs that are sensitive to pro-inflammatory stimuli, like the brain^{11,12}, and has been associated with risk for later neurological disease, such as autism and schizophrenia^{11,12}. Further, while inflammatory pathways are activated in normal pregnancies, particularly at the end of gestation^{18,89-91}, and are important for instigating uterine contractions and promoting rupture of fetal membranes^{18,91-93}, early activation of these pathways can result in preterm labour¹⁸. Preterm birth is a leading cause of perinatal mortality¹⁴, and individuals born preterm are at increased risk for learning disabilities^{15,16}, mood disorders¹⁵⁻¹⁷, neurological diseases including Alzheimer's and Parkinson's disease¹⁵⁻¹⁷, and metabolic diseases including obesity and type two diabetes^{94,95}.

1.4 Key knowledge gaps

Maternal underweight and obesity are global health burdens; maternal underweight remains a persisting problem, and the prevalence of obesity continues to rise¹⁻³. Both maternal underweight and obesity have been associated with increased levels of inflammation in the mother, and suboptimal quality and quantity of nutrients available to the fetus *in utero*⁹. These exposures are associated with adverse pregnancy and offspring outcomes^{8,96}, however, the mechanisms that drive these outcomes remain poorly understood.

Preterm birth is also a prevalent pregnancy complication, and is the leading cause of perinatal mortality globally¹⁴. Individuals born preterm are at increased risk for both immediate and longer term health consequences, such as neurodevelopmental and neurological conditions¹⁵⁻¹⁷ and metabolic diseases^{94,95}. Both preterm birth and suboptimal prepregnancy BMI may operate through inflammatory pathways to impair fetoplacental development, increasing risk for adverse health postnatally and long term.

The placenta is a critical regulator of the fetal environment and has the ability to adapt to environmental exposures to protect the developing fetus⁷. Placental adaptations can thus serve as a record of adversities experienced in the womb, help explain the mechanisms through which these adversities affect the developing offspring, and could be used to identify which infants are at greatest risk for poor development and health trajectories. However, the histopathological placental phenotypes elicited by maternal underweight and obesity are poorly characterized. Further, poor metabolic and inflammatory states may alter placental transport, which may influence the placenta's ability to protect the developing fetus from harmful environmental conditions, but few studies have characterized the effects of both suboptimal maternal BMI and gestational age on placental efflux transport. The effects of maternal underweight and obesity on the expression of these MDR transporters are not well understood, but are critical to study, given the pro-inflammatory^{10,97} and altered metabolic state⁹⁸ associated with suboptimal BMI, and that inflammation^{13,99} and altered hormone levels, such as glucocorticoids^{100,101}, have been shown to alter the expression of placental efflux transporters. Increased medication use often associated with comorbid infectious and inflammatory conditions may also affect placental efflux transport. Finally, antimicrobial peptides produced by gestational tissues are likely important in pregnancy given their role in modulating infection and inflammation. However, how these placental defences are altered in pregnancies complicated by maternal underweight and obesity and early birth, conditions associated with increased inflammation and susceptibility to infection, are not well understood, but may have implications for fetal defence. Further, while some data exist on the expression of AMPs in gestational tissues with preterm birth, expression in the cell layers of the placenta specifically, have not been well characterized. Quantifying placental pathologies and gene and protein expression of key placental efflux transporters and antimicrobial peptides in these common conditions helps to uncover the mechanisms linking poor maternal metabolic health and pro-inflammatory states with adverse pregnancy outcomes. Collectively, such knowledge about placental adaptations and health can aid clinical decision making to better support high-risk pregnancies and inform interventions that can optimize pregnancy, placental, and infant health.

1.5 Hypotheses and objectives

Our **goal** is to understand how common conditions in pregnancy, including maternal underweight, obesity, and/or preterm birth, which are associated with pro-inflammatory environments, alter placental development and function, pregnancy outcomes, and offspring development. We **hypothesize** that maternal underweight and obesity will increase gestational tissue inflammation and prevalence of placental pathologies, reduce gestational tissue microbial defences and transport systems important for fetal protection, and subsequently increase risk for preterm birth and suboptimal infant growth and development. We also hypothesize that preterm birth alone will be associated with impaired gestational tissue function and suboptimal infant growth and development, and that preterm infants born to women with underweight and obesity will have poorer outcomes than term infants.

My **objectives** are to:

1. Demonstrate that maternal underweight and obesity associate with impaired placental development, evidenced by increased prevalence of placental pathologies at term and preterm.
2. Demonstrate the maternal underweight and obesity associate with impaired gestational tissue function by altering expression of genes and proteins associated with transport, permeability, and microbial defence at term and preterm (with and without chorioamnionitis).
3. Demonstrate that maternal underweight and obesity associate with adverse infant outcomes, including infant birthweight and Apgar scores in term and preterm (with and without chorioamnionitis) pregnancies.

Chapter 2. Maternal underweight and obesity are associated with placental pathologies in human pregnancy

This chapter forms the basis of a manuscript under review, and a preprint available at: <https://www.medrxiv.org/content/10.1101/2021.06.01.21258127v1.full-text>

2.1 Abstract

Maternal underweight and obesity are prevalent conditions, associated with chronic, low-grade inflammation, poor fetal development, and long-term adverse outcomes for the child. The placenta senses and adapts to the pregnancy environment in an effort to support optimal fetal development. However, the mechanisms driving these adaptations, and the resulting placental phenotypes, are poorly understood. We hypothesised that maternal underweight and obesity would be associated with increased prevalence of placental pathologies in term and preterm pregnancies. Data from 12,154 pregnancies were obtained from the Collaborative Perinatal Project, a prospective cohort study conducted from 1959 to 1974. Macro and microscopic placental pathologies were analysed across maternal prepregnancy BMI to assess differences in the presence of pathologies among underweight, overweight, and obese BMI groups compared to normal weight reference BMI at term and preterm. Placental pathologies were also assessed across fetal sex. Pregnancies complicated by obesity had placentae with increased fetal inflammation at preterm and increased maternal inflammation at term. In term pregnancies, increasing maternal BMI associated with increased maternal vascular malperfusion (MVM), odds of an appropriate mature placenta for gestational age, and placental weight, and decreased placental efficiency. Male placentae, independent of maternal BMI, had increased inflammation, MVM, and placental efficiency than female placentae, particularly at term. Maternal underweight and obesity are not inert conditions for the placenta, and the histomorphological changes driven by suboptimal maternal BMI may serve as indicators of adversities experienced *in utero* and potential predictors of future health trajectories.

2.2 Introduction

Maternal underweight and obesity are global health burdens; maternal underweight remains a persisting problem, and the prevalence of obesity in pregnancy continues to rise¹⁻³. Both conditions have been associated with higher levels of inflammation in the mother, which favour increased inflammation in the placenta⁹, suboptimal nutrient availability to the fetus⁹, and adverse pregnancy and offspring outcomes^{96,102}. Yet, the mechanisms that drive these outcomes remain poorly understood.

The placenta is a critical regulator of the fetal environment and can adapt to mitigate harmful exposures, or maladapt to permit their adverse effects⁷. For example, in animals, inflammation can reduce placental area⁶⁷ and impair spiral artery remodelling⁶⁴, which can affect nutrient and gas exchange⁶⁴⁻⁶⁷ with potential consequences for the developing offspring^{8,28,74}. Additionally, there is some evidence that maternal underweight and obesity may be unfavourable for

placental development. Maternal obesity has been associated with chronic villitis^{54,55}, both delayed and accelerated placental villous maturation⁵⁵, and increased atheromas and villous infarcts^{55,56}, pathologies which have been linked to placental insufficiency⁵⁷, fetal growth restriction, and neurodevelopmental impairment⁵⁵. The effects of maternal undernutrition or low BMI on placental pathologies are less well documented⁵⁸. Animal models of undernutrition have shown evidence of abnormal placental vasculature and decreased fetal blood space, labyrinth and junctional zone area⁵⁸, which may impair placental transfer⁵⁹. The pro-inflammatory state induced by maternal underweight and obesity may also directly influence placental function, such as altered expression of placental nutrient transporters^{25,69,103}. Therefore, it is important to understand even subtle placental phenotypes in these common conditions, as placental structural and functional adaptations may serve as a record of adversities experienced *in utero*, and could help reveal the mechanisms through which these adversities affect the developing offspring. However, the placental morphological and histopathologic changes induced by maternal underweight and obesity, in the absence of other comorbidities or adverse perinatal events, are poorly characterized.

We hypothesised that, compared to women of normal weight, maternal underweight and obesity prepregnancy would be associated with increased prevalence of placental pathologies in term and preterm pregnancies, and that there would be differences in pathology prevalence based on placental sex. Using data from the Collaborative Perinatal Project (CPP), our primary objective was to determine whether placental pathologies were more prevalent in term and preterm pregnancies complicated by suboptimal maternal prepregnancy BMI. Our secondary objective was to determine whether suboptimal prepregnancy BMI associated with adverse infant outcomes at birth, including birthweight, body length, head circumference, brain-to-body weight ratio, and Apgar scores. Our findings help to quantify placental pathologies in common pregnancy conditions, and uncover the mechanisms linking poor maternal metabolic health with suboptimal fetal growth and development.

2.3 Methods

2.3.1 Study design and population

Secondary data analysis was conducted using data from the CPP, a prospective cohort study designed to identify relationships between pregnancy and perinatal risk factors and child outcomes (<https://catalog.archives.gov/id/606622>). The CPP was conducted from 1959 to 1974 at 12 hospitals across the United States, and collected pregnancy data through the prenatal period and delivery, and child outcomes for approximately 58,000 pregnancies¹⁰⁴.

The primary exposure of interest was maternal prepregnancy BMI, specifically maternal underweight, overweight, and obesity compared to normal weight as the reference category. BMI was classified according to the World Health Organization and American College of Obstetricians and Gynecologists guidelines, where maternal BMI is categorized as underweight (<18.5), normal weight (18.5–25), overweight (>25–30), or obese (>30). To evaluate the

associations between maternal BMI and placental pathologies, the study sample was restricted to pregnancies with placental pathology data available, and maternal height and prepregnancy weight available to calculate maternal prepregnancy BMI. We included only singleton births from first pregnancies (parity and gravidity of zero), where fetal sex was documented as male or female. Gestational age below 24 weeks or above 43 weeks were excluded based on the limit of viability¹⁰⁵ and morphological changes to the placenta such as those induced by cellular senescence¹⁰⁶, and increased risk for fetal complications in post-term pregnancies¹⁰⁷. The CPP calculated gestational age based on the last menstrual period to the nearest week. These selection criteria resulted in a sample of 12,154 pregnancies (Supplementary Figure 2.1).

2.3.2 Placental pathologies

The primary outcomes were macroscopic and microscopic placental pathologies (Figure 2.1). Macroscopic data included placental weight, largest and smallest diameter, thickness, and placental shape. Infant birthweight to placental weight/largest diameter/smallest diameter/thickness ratios were calculated as potential predictors of placental efficiency^{108,109}. The top and bottom 0.5% of raw infant and placental anthropometry data (birthweight, placental weight, and placental dimensions) were excluded to remove biologically implausible data. The umbilical cord was assessed for cord edema and number of vessels, given the increased incidence of a single artery cord with gestational diabetes mellitus (GDM)¹¹⁰, a covariate of interest for pregnancies with obesity. Thrombosed fetal vessels and cut surface infarcts were assessed, which may be associated with impaired placental perfusion¹¹¹. For microscopic variables: decidual vessel fibrinoids and atheroma were included, features of malperfusion¹¹². Neutrophilic infiltration of the umbilical vein, umbilical artery, cord substance, chorion and amnion membranes, and chorion and amnion of the (fetal side) placental surface were also obtained, which may be indicative of ascending maternal infection¹¹³. Cut surface calcification was included as an indicator for placental maturation¹¹⁴. Syncytium-nuclear clumping, or syncytial knots, and stromal fibrosis are signs of accelerated villous maturation and were thus included¹¹⁵. Presence of Langhans' layer, Hofbauer cells, and pathological edema were included as indicators of placental immaturity^{116,117}. A variable provided for the apparent maturity of the placenta was also included as a marker for appropriate placental development for gestational age. For multivariable analyses, categorical placental pathology variables were collapsed into binary categories (Supplementary Table 2.1).

We derived additional summary scores for placental inflammation, MVM, and placental immaturity. A maternal inflammation summary score was derived from the following variables: opacity of membranes and neutrophil infiltration of the amnion and chorion membranes and of the placental surface. Where data were available for all constituent variables, the individual variable scores were summed to create a composite score ranging from zero to fourteen, where a higher score represents increased levels of inflammation. Similarly, a fetal inflammation summary score was derived from neutrophilic infiltration of the umbilical vein, artery, and cord substance to create a composite score from zero to nine. A summary score considering features of MVM was derived from: presence of infarcts and syncytium-nuclear clumping to create a score ranging from zero to two, where a higher score indicates increased MVM. A placental immaturity

score was derived from the following variables: Langhans' layer, Hofbauer cells, stromal fibrosis, and syncytium-nuclear clumping, to create a score ranging from zero to seven where a higher score indicates a more immature placental phenotype (Supplementary Table 2.2).

2.3.3 Maternal demographics

Our primary exposure of interest, maternal prepregnancy BMI, was defined based on measured height and self-reported prepregnancy weight collected at study enrollment. In addition to prepregnancy BMI, maternal demographic data including age, race, education, marital status, income, socioeconomic index, housing density, smoking history, diabetes mellitus status, and gestational weight gain were obtained. The socioeconomic index is a composite numerical index derived from scores for education (of the head of household/chief earner), occupation (of the head of household/chief earner), and family income, ranging from 0.0-9.5, where 9.5 represents the highest socioeconomic status¹¹⁸. Smoking history was provided as the number of cigarettes smoked per day at the time of the interview, from zero cigarettes (including non-smokers) to 60 cigarettes per day. Additional smoking categories included 61 or more cigarettes per day, regular smoker but less than one cigarette per day, and irregular smoker but less than four cigarettes per month. Smoking history was presented as non-smokers (including women currently smoking zero cigarettes per day), light smokers (less than one pack of 20 cigarettes per day), and heavy smokers (one or more packs of 20 cigarettes per day). Diabetes mellitus was presented as presence or absence, where presence included diabetes mellitus reported before pregnancy, during pregnancy, both before and during, during and postpartum, or before, during, and postpartum. Based on current recommendations from the Institute of Medicine (IOM) guidelines (2009), maternal weight gain was categorized as inadequate, adequate, or excessive for singleton pregnancies based on prepregnancy BMI, where the recommended weight gain ranges are 28–40 pounds, 25–35 pounds, 15–25 pounds or 11–20 pounds¹¹⁹ for mothers who are underweight, normal weight, overweight, or have obesity, respectively.

2.3.4 Infant characteristics

Infant data were collected at the time of birth, including infant sex, gestational age, Apgar scores (at one, five, and ten minutes), and infant anthropometry including birthweight, birth length, and head circumference. The top and bottom 0.5% of raw infant anthropometry data (birthweight, birth length, head circumference) were excluded to remove biologically implausible data. Standardized birthweight, birth length, and head circumference by infant sex and gestational age were calculated using the INTERGROWTH-21st application¹²⁰. The maximum gestational age that standardized anthropometry could be calculated for was 42 weeks and six days according to the application parameters, thus standardized growth measures were not calculated for 539 (5.18%) infants in the term cohort that were delivered at 43 weeks gestation. Data for these infants were included in all other analyses. Infant brain-to-body weight ratios (BBR)¹²¹ were calculated from brain weight estimates¹²², as this may be an indicator of brain sparing during development¹²³. Brain weight estimates were calculated using the following formula: Brain weight estimate (g) =

0.037 * Head circumference (cm)^{2.57}. Brain-to-body weight ratios were then calculated with the formula: BBR = 100 * (Brain weight estimate [g])/(Birthweight [g]).

2.3.5 Statistical analyses

2.3.5.1 Univariate analyses

Univariate analysis was conducted to evaluate differences in the prevalence of placental pathologies across maternal prepregnancy BMI groups. Given that the presence of some placental pathologies are dependent on gestational age¹²⁴, we conducted all analyses stratified for term and preterm placentae. Differences between maternal BMI groups and placental measures were determined by Kruskal–Wallis test with Steel–Dwass *post hoc* for continuous nonparametric data, and Likelihood Ratio Chi Square test for categorical data. Placental pathologies were also assessed across fetal sex. Data are presented as median (interquartile range) and Wilcoxon test effect size (*r*) (95% confidence interval; CI) for non-parametric continuous data, and frequency (percentage) and Cramer’s V effect size (95% CI) or odds ratio (95% CI) (for binary fetal sex analyses) for categorical variables. Statistical significance was defined as $p < 0.05$ and all tests were two-sided. Data were analysed using JMP statistical software (14.0).

2.3.5.2 Multivariable analyses

We performed multivariable logistic and linear regression to determine the relationships between maternal prepregnancy BMI and placental pathologies at term and preterm. Logistic regression models were used to determine the associations between maternal BMI (continuous) and binary placental pathologies. Categorical placental pathology variables were collapsed into binary categories to calculate odds ratios for the pathological phenotype. Any data coded as unknown, unable to determine, or missing were excluded from regression analyses. Data are presented as unadjusted (OR) or adjusted units odds ratio (aOR) (95% CI). Linear regression models were used to determine the associations between maternal BMI (continuous) and continuous placental variables. Data are reported as adjusted beta coefficient ($a\beta$) (95% CI). Covariates of interest were identified a priori and included fetal sex (male/female), maternal race (White, Black, and Other), maternal age (continuous), smoking history (non-smoker, light smoker, heavy smoker), maximum gestational weight gain (continuous), diabetes (yes/no), maternal education (continuous), and socioeconomic index (continuous). Two regression models were defined a priori: 1) An unadjusted model was first used to identify the associations between prepregnancy BMI alone (as a continuous variable) with placental pathologies and 2) an adjusted model adjusted for the covariates defined above.

2.4 Results

2.4.1 Maternal demographics differed across maternal BMI groups

Maximum gestational weight gain among preterm (Wilcoxon test effect size, $r=-0.09$ [-0.14 to -0.04]) and term ($r=-0.05$ [-0.07 to -0.03]) pregnancies was greatest in underweight and lowest in obese BMI groups (Supplementary Tables 2.3-2.4). However, according to IOM (2009) guidelines, weight gain in underweight pregnancies was still inadequate at preterm and term, while most pregnancies with obesity had either inadequate or excessive weight gain at preterm, and excessive weight gain at term (Supplementary Tables 2.3-2.4). Maternal age, race, education, marital status, income, socioeconomic index, and housing density also differed by BMI group (Supplementary Tables 2.3-2.4).

2.4.2 Maternal obesity associated with increased neutrophil infiltration of gestational tissues

Among preterm pregnancies, fetal inflammation, characterized by the composite fetal inflammation summary score, was greater in placentae from mothers with obesity compared to all other BMI groups ($r=0.009$ [-0.04 to 0.05], Table 2.1). There were no effects of maternal BMI group on neutrophil infiltration in the preterm membranes or placental surface. Neutrophil infiltration of the umbilical vein ($aOR=1.06$ [1.01–1.12]), umbilical cord substance ($aOR=1.08$ [1.02–1.14]), and amnion membrane ($OR=1.05$ [1.01–1.10]), but not the umbilical artery, chorion membrane, or amnion and chorion of the placenta, were more likely with increasing maternal BMI (Table 2.2).

Among term pregnancies, pregnancies complicated by obesity had the greatest percentage of marked neutrophil infiltration of the umbilical vein and artery, and amnion and chorion membranes and of the placental surface, and greatest maternal inflammation summary score compared to underweight ($r=0.008$ [-0.01 to 0.03], Table 2.3). Further, neutrophilic infiltration of the umbilical vein ($aOR=1.03$ [1.01–1.05]), artery ($aOR=1.04$ [1.00–1.07]), and cord substance ($aOR=1.04$ [1.01–1.07]) increased with increasing BMI (Table 2.4). Neutrophil infiltration of the amnion membrane ($aOR=1.06$ [1.03–1.08]), chorion membrane ($aOR=1.04$ [1.02–1.06]), amnion of the placental surface ($aOR=1.04$ [1.01–1.07]), and chorion of the placental surface ($aOR=1.04$ [1.02–1.06]) were also greater with increasing BMI (Table 2.4).

2.4.3 Higher maternal BMI associated with MVM among term pregnancies

At term, increasing maternal BMI was associated with greater odds of an appropriately mature placenta based on apparent maturity of the placenta ($aOR=0.94$ [0.92–0.97]) and increased MVM ($a\beta=0.007$ [0.004–0.01], Table 2.4), and placentae from mothers with obesity had the greatest percentage of thrombosed fetal vessels compared to all other BMI groups (Cramer's $V=0.03$ [0.003–0.05], Supplementary Table 2.5). Additionally, presence of infarcts alone was more likely as maternal BMI increased ($aOR=1.03$ [1.02–1.05], Supplementary Table 2.6). There were no differences in apparent placental maturity or vasculature-related pathologies by BMI among preterm pregnancies.

2.4.4 Both maternal underweight and increased BMI influenced placental anthropometry

In preterm pregnancies where mothers were underweight, placental weight was reduced compared to placentae from mothers with overweight and obesity ($r=0.04$ [-0.006–0.09]), and smallest diameter was reduced compared to placentae from pregnancies complicated by obesity ($r=0.06$ [0.009–0.12]), but there were no differences in placental anthropometry compared to normal weight BMI (Supplementary Table 2.7). Among term pregnancies where mothers were underweight, placental weight ($r=0.08$ [0.06–0.10]) and smallest diameter ($r=0.05$ [0.03–0.07]) were reduced compared to all other BMI groups, with most prominent differences between the underweight and obese groups (Supplementary Table 2.5). At term only, birthweight to placental weight ratio was higher in mothers who were underweight compared to overweight, but not different than normal weight ($r=-0.03$ [-0.05 to -0.006], Supplementary Table 2.5). Similarly, when considering BMI as a continuous variable, placental weight and smallest diameter increased with increasing maternal BMI among preterm and term pregnancies, and birthweight to placental weight ratio decreased with increasing maternal BMI at term (Supplementary Tables 2.6, 2.8).

2.4.5 Fetal sex influenced placental pathology

Among preterm pregnancies, female placentae had a greater fetal inflammation summary score than male placentae ($r=-0.05$ [-0.10 to -0.004]), but there were no sex differences in neutrophil infiltration of the membranes or placental surface (Table 2.5). Male term placentae had greater fetal and maternal placental inflammation, including neutrophil infiltration of the umbilical vein, umbilical cord substance, amnion membrane, chorion membrane, amnion of the placenta, chorion of the placenta, and fetal and maternal inflammation summary scores (Table 2.5). Further, when considering only pregnancies with maternal obesity, at term, male placentae had greater neutrophil infiltration of the umbilical vein and chorion membrane than female placentae (Supplementary Figure 2.2).

Female preterm placentae had increased syncytium-nuclear clumping ($OR=0.53$ [0.31–0.91]), but there were no differences in presence of infarcts or MVM summary score between sexes preterm (Table 2.5). Male term placentae had increased MVM ($r=0.04$ [0.02–0.06], Table 2.5) and placental infarcts compared to female placentae ($OR=1.22$ [1.11–1.33], Table 2.6). Infant birthweight to placental weight ratio was also increased in males compared to females at preterm ($r=0.07$ [0.03–0.12]) and term ($r=0.07$ [0.05–0.09], Table 2.6). Term males had greater brain-to-body weight ratios than females ($r=0.05$ [0.03, 0.07], $p<0.0001$), but there were no differences among preterm infants by sex. Preterm males had lower 10 minute Apgar scores at birth ($r=-0.08$ [-0.12, -0.03], $p=0.002$), and at term, had lower one minute Apgar scores ($r=-0.04$ [-0.06, -0.02], $p<0.0001$), five minute Apgar scores ($r=-0.04$ [-0.06, -0.02], $p<0.0001$), and 10 minute Apgar scores than females ($r=-0.02$ [-0.04, -0.003], $p=0.02$).

2.4.6 Maternal underweight influenced infant size at birth

In preterm pregnancies, infants born to underweight mothers had reduced birthweight z-scores ($r=0.06$ [0.01, 0.11], $p=0.003$) and birth length z-scores ($r=0.09$ [0.04, 0.14], $p=0.0001$) compared to infants born to mothers with obesity (Table 2.7). Infants from underweight mothers also had

reduced head circumference z-scores compared to infants from mothers with normal weight and obesity ($r=0.09$ [0.04, 0.13], $p<0.0001$, Table 2.7). Similarly, term infants born to underweight mothers had reduced z-birthweight compared to infants born to normal weight and overweight mothers ($r=0.08$ [0.06, 0.10], $p<0.0001$), and reduced z-birth length ($r=0.07$ [0.04, 0.09], $p<0.0001$) and z-head circumference compared to all other BMI groups ($r=0.05$ [0.03, 0.07], $p<0.0001$, Table 2.8). There was no effect of maternal overweight or obesity on z-birth length or z-head circumference (Table 2.8).

Among preterm infants, z-birthweight ($a\beta=0.07$ [0.04, 0.10], $p<0.0001$), z-birth length ($a\beta=0.07$ [0.04, 0.10], $p<0.0001$), and z-head circumference ($a\beta=0.08$ [0.05, 0.11], $p<0.0001$) increased with increasing maternal BMI (Table 2.9). Similarly, among term infants, z-birthweight ($a\beta=0.04$ [0.03, 0.05], $p<0.0001$), z-birth length ($a\beta=0.03$ [0.02, 0.04], $p<0.0001$), and z-head circumference ($a\beta=0.03$ [0.02, 0.04], $p<0.0001$) also increased with increasing maternal BMI (Table 2.10).

Among term pregnancies, infant brain-to-body weight ratio was greatest in pregnancies with underweight and lowest in infants where mothers were overweight ($r=-0.06$ [-0.08, -0.04], $p<0.0001$, Table 2.8). Brain-to-body weight ratio decreased with increasing maternal BMI among term pregnancies ($a\beta=-0.03$ [-0.03, -0.02], $p<0.0001$), but there were no differences in brain-to-body weight ratio by maternal BMI among preterm pregnancies (Table 2.10).

2.5 Discussion

We evaluated the associations between maternal prepregnancy BMI and the prevalence of placental pathologies in preterm and term pregnancies, to better understand the placental mechanisms that may explain poor pregnancy and offspring outcomes in pregnancies complicated by suboptimal maternal BMI. Using data from 12,154 pregnancies from the Collaborative Perinatal Project, we found placental inflammation was increased in pregnancies complicated by maternal obesity, and notably, the inflammatory response was different based on gestational age. Maternal obesity also associated with increased MVM of the placenta, and increased maternal BMI associated with greater odds of having an appropriate mature placenta at term. Placental efficiency was highest in pregnancies where mothers were underweight and in male placentae, and pathologies also differed by fetal sex. Suboptimal maternal BMI thus alters normal gestational tissue development, with likely effects on function.

The inflammatory conditions established by maternal obesity may favour placental inflammation. In support of this, we found that higher maternal BMI associated with increased fetal (e.g. neutrophilic infiltration of the umbilical vein, artery, and cord substance) inflammation at preterm, and maternal (e.g. neutrophilic infiltration of the amnion and chorion membranes, amnion and chorion of the placenta, and opacity of membranes) gestational tissue inflammation at term. While few studies have characterized fetal- and maternal-specific placental inflammation in the context of both maternal BMI and gestational age, at term, maternal inflammation has been documented in pregnancies with obesity, evidenced by increased pro-inflammatory cytokines in the placenta¹⁰, and increased maternal, but not fetal, inflammatory lesions^{56,125}. Other studies have found no differences in placental inflammation by maternal BMI

in term pregnancies, however, these cohorts had a small number of cases^{126,127}. Less is known about the relationships between maternal obesity and specific placental inflammation at preterm, however, maternal obesity has been associated with greater risk of chorioamnionitis leading to preterm birth¹²⁸, and a combination of maternal (defined as inflammation in the chorion, amnion, and decidua) and fetal (inflammation of the umbilical cord and chorionic plate fetal vessels) gestational tissue inflammation has been linked to higher risk of extreme preterm birth than maternal gestational tissue inflammation alone¹²⁹. In the context of ascending infection, fetal placental inflammation of the umbilical cord has been associated with greater neonatal morbidity and mortality, particularly among preterm pregnancies, than maternal placental inflammation¹³⁰⁻¹³². Our study, unlike many others, considers both suboptimal maternal BMI and gestational age, and suggests that increased maternal BMI associates with fetal placental inflammation at preterm, which has been linked to adverse pregnancy and offspring outcomes^{133,134}, and maternal placental inflammation at term. Fetal inflammation of the placenta at preterm could thus be an important risk factor for adverse offspring outcomes, and maternal placental inflammation, though less frequently associated with clinical correlates¹³³, nonetheless suggests that suboptimal maternal BMI alters gestational tissue histomorphology.

Interestingly, preterm female placentae had increased fetal inflammation, while term male placentae had increased fetal and maternal inflammation. Male placentae have been observed to have higher rates of chronic inflammatory lesions in extreme preterm pregnancies¹³⁵, chronic deciduitis among extreme preterm pregnancies with preeclampsia and intrauterine growth restriction¹³⁶, and enrichment in inflammatory pathways¹³⁷. Fewer studies have characterized increased female placental inflammation compared to males, however, female placentae have been shown to have increased chronic villitis in pregnancies complicated by maternal obesity, though this study was restricted to pregnancies at or near term⁵⁴. Our findings suggest that male placentae are more susceptible to inflammation in term pregnancies, which could increase risk for impaired growth or neurodevelopment and long term health outcomes^{11,18}, while increased fetal inflammation among preterm female placentae warrants further investigation. Given the associations of maternal obesity and male sex with increased placental inflammation, term males born to mothers with obesity may be most susceptible to placental inflammation. To further investigate this, we conducted an exploratory analysis to assess whether fetal sex associated with placental inflammation among pregnancies with obesity alone. Increased inflammation in male placentae at term only, though not to the same extent as BMI inclusive differences, suggests that both maternal obesity and male sex are independent risk factors for placental inflammation at term.

We found MVM also associated with increased maternal BMI among term pregnancies, consistent with existing data¹³⁸. Previous studies have documented increased maternal vascular lesions¹²⁷ and decidual vasculopathy, but no other lesions associated with MVM, in placentae from term pregnancies complicated by obesity compared to normal weight, although this was attributed to maternal hypertensive disease⁵⁴. MVM lesions impair intervillous blood flow, altering oxygen and nutrient delivery to the fetus¹¹², and are associated with adverse offspring outcomes, including preterm birth, intrauterine growth restriction, and small for gestational age

infants¹¹². Our findings suggest that the adverse environment established by maternal obesity may impair the development and function of placental vasculature, and thus fetal development. Despite known associations of MVM and preterm birth^{112,139}, increased BMI associated with MVM only among term pregnancies in our cohort^{54,112,127}, suggesting the importance of vascular pathologies across gestation in pregnancies complicated by maternal obesity. Further, male placentae had increased MVM lesions compared to females at term in our study, consistent with other findings of increased decidual vasculopathy in males⁵⁴. Our findings suggest that the placental vasculature pathology may not be so severe as to result in early pregnancy or other adverse perinatal events, but may still have long term implications for the infant.

In contrast to previous studies, we found increased maternal BMI associated with greater odds of having an appropriately mature placenta at term. Placentae from women with obesity have been shown to have immaturity of the villous tree compared to women of normal weight¹²⁶, suggesting structural and functional maladaptation of the vasculature or decreased efficiency in maternal-fetal exchange¹²⁶. We did however observe other placental vascular pathologies, evidenced by increased MVM with higher maternal BMI. The lower number of cases of obesity in our cohort relative to the current rates, and changes to criteria used to diagnose distal villous immaturity, may in part account for these differences in apparent placental maturity.

Our findings of decreased placental size and increased infant birthweight to placental weight ratio with lower maternal BMI are consistent with reduced nutrient availability in underweight pregnancies^{52,59,102}, and suggest that the placenta may adapt to increase nutrient delivery to the fetus, whereas placentae from higher BMI pregnancies may adapt by regulating nutrient transfer to the fetus in the face of sufficient or overabundance of nutrients^{140,141}. Conversely, among term pregnancies, decreased birthweight to placental smallest diameter ratio with lower maternal BMI could suggest lower placental efficiency in pregnancies with underweight. However, placental diameter is reflective of the lateral growth of the placenta and area of the uterine lining that it encompasses, and may be influenced by inter-individual variations such as differences in placental shape, whereas placental weight captures multiple dimensions of placental growth¹⁴². Brain-to-body weight ratios were also highest in term pregnancies with underweight, and lowest where mothers were overweight. This may indicate brain sparing in pregnancies with underweight; brain sparing occurs when the fetus prioritizes oxygen and nutrient delivery to the brain in response to chronic hypoxaemia or nutrient deprivation, and is often a consequence of intrauterine growth restriction, which is most commonly caused by placental insufficiency¹⁴³⁻¹⁴⁵. Mothers that are underweight may lack sufficient nutrient delivery to the fetus, resulting in fetal brain sparing as an adaptive response¹⁴⁶, which could have adverse developmental programming effects such as hypertension later in life¹⁴⁷. Further, males had higher birthweight to placental size ratios in both preterm and term pregnancies, consistent with previous studies¹⁴⁸, suggesting greater placental efficiency than females, and had higher brain-to-body weight ratios than females at term. Male fetuses tend to grow more rapidly than females and invest greater resources in growth than placental development¹⁴⁷, and as a result, may be more susceptible to placental insults as well as adverse later health outcomes¹⁴⁷. Taken together with our findings of increased placental efficiency with lower maternal BMI, these results may suggest that males born to mothers who are underweight have the greatest placental efficiency, particularly at term.

Males also had lower Apgar scores at birth, particularly among term pregnancies, which has been previously reported¹⁴⁹⁻¹⁵¹. Low Apgar scores are related to lower vital sign measures, adjustment to the extra-uterine environment and risk of neonatal morbidity, and may be a consequence of sex differences in development^{149,151}.

Strengths of our study included the large population-based cohort, where previous studies have been limited to animal models of maternal malnutrition or inflammation to assess placental histopathology, or have lacked population size and comprehensive data. Importantly, the large sample size, collection of socioeconomic and demographic factors, deep phenotyping, and prospective nature of the dataset enables a thorough investigation of the relationships between maternal BMI and placental pathologies in a diverse population. Yet, due to the cohort's historical nature, a limitation of our study is that the prevalence of obesity was lower than current rates; only 2.63% of our cohort were classified as having obesity prepregnancy, compared to 29% of American women currently¹⁵². Rates of smoking were also increased during the time of the study compared to rates today (42-45% during the time of CPP data collection¹⁵³ compared to 16% in 2019¹⁵⁴), and no data were collected on maternal alcohol consumption¹⁵⁵. Additionally, no data were provided for GDM specifically, which is associated with adverse pregnancy outcomes and is comorbid with obesity^{156,157}, however, rates of GDM were much lower during the time of the study than current rates (0.3% in 1979¹⁵⁸ compared to 7.6% from 2007-2014¹⁵⁹), so any confounding is likely to be minimal. Still, given the population-based design, our findings may be applicable to a broad and diverse population because of the large sample size and thorough collection of demographic and placental pathology data.

Our data demonstrate that compared to normal weight, maternal underweight and obesity prepregnancy, even in the absence of other significant pregnancy complications, are not inert conditions for the developing placenta^{55,160}, which may have consequences not only for immediate pregnancy and fetal outcomes but postnatal growth and health trajectories^{108,109,161,162}. Characterizing placental (mal)adaptations to common maternal conditions using clinically-relevant indicators can help understand the mechanisms through which these conditions affect the developing offspring, and aid clinical decision making to better support high-risk pregnancies and inform interventions to optimise pregnancy, placental, and infant health.

Table 2.1. Associations between maternal prepregnancy BMI and microscopic placental pathologies in preterm pregnancies, N=1739.

Placental pathology	Prepregnancy BMI				p-value	Effect size
	UW (n=259)	NW (n=1286)	OW (n=156)	OB (n=38)		
Cord						
Neutrophil infiltration, umbilical vein					0.14	0.06 (0.00–0.07)
Not seen	222 (85.7)	1126 (87.6)	130 (83.3)	27 (71.1)		
Slight	15 (5.79)	71 (5.52)	13 (8.33)	6 (15.8)		
Moderate	0	3 (0.23)	0	0		
Marked	7 (2.70)	48 (3.73)	10 (6.41)	3 (7.89)		
Unknown/missing	15 (5.79)	38 (2.95)	3 (1.92)	2 (5.26)		
Neutrophil infiltration, umbilical artery					0.62	0.04 (0.00–0.05)
Not seen	228 (88.0)	1164 (90.5)	143 (91.7)	30 (78.9)		
Slight	9 (3.47)	45 (3.50)	3 (1.92)	3 (7.89)		
Moderate	0	1 (0.08)	0	0		
Marked	7 (2.70)	37 (2.88)	7 (4.49)	3 (7.89)		
Unknown/missing	15 (5.79)	39 (3.03)	3 (1.92)	2 (5.26)		
Neutrophil infiltration, cord substance					0.23	0.05 (0.00–0.07)
Not seen	233 (90.0)	1159 (90.1)	141 (90.38)	29 (76.3)		
Slight	8 (3.09)	58 (4.51)	6 (3.85)	5 (13.2)		
Moderate	0	2 (0.16)	0	0		
Marked	3 (1.16)	28 (2.18)	6 (3.85)	2 (5.26)		
Unknown/missing	15 (5.79)	39 (3.03)	3 (1.92)	2 (5.26)		
Fetal inflammation summary score	0 (0, 0) ^A	0 (0, 0) ^A	0 (0, 0) ^A	0 (0, 1) ^B	0.002	0.009 (-0.04 to 0.05)
Membranes						
Neutrophil infiltration, membrane, amnion					0.21	0.08 (0.03–0.10)
Not seen	208 (80.3)	1074 (83.5)	127 (81.4)	29 (76.3)		
Slight	21 (8.11)	74 (5.75)	13 (8.33)	5 (13.2)		
Moderate	0	1 (0.08)	0	1 (2.63)		
Marked	5 (1.93)	33 (2.57)	6 (3.85)	1 (2.63)		
Unknown/missing	25 (9.65)	104 (8.09)	10 (6.41)	2 (5.26)		
Neutrophil infiltration, membrane, chorion					0.65	0.04 (0.00–0.05)
Not seen	197 (76.1)	1006 (78.2)	114 (73.1)	27 (71.1)		
Slight	27 (10.4)	139 (10.8)	21 (13.5)	3 (7.89)		
Moderate	1 (0.39)	1 (0.08)	0	0		
Marked	18 (6.95)	103 (8.01)	16 (10.3)	6 (15.8)		
Unknown/missing	16 (6.18)	37 (2.88)	5 (3.21)	2 (5.26)		
Neutrophil infiltration, placental surface, amnion					0.62	0.04 (0.00–0.05)
Not seen	208 (80.3)	1062 (82.6)	127 (81.4)	31 (81.6)		

Slight	16 (6.18)	58 (4.51)	8 (5.13)	3 (7.89)		
Moderate	0	1 (0.08)	0	0		
Marked	6 (2.32)	25 (1.94)	8 (5.13)	1 (2.63)		
Unknown/missing	29 (11.2)	140 (10.9)	13 (8.33)	3 (7.89)		
Neutrophil infiltration, placental surface, chorion					0.51	0.04 (0.00–0.06)
Not seen	206 (79.5)	1061 (82.5)	127 (81.4)	29 (76.3)		
Slight	26 (10.0)	118 (9.18)	11 (7.05)	5 (13.2)		
Moderate	0	0	0	0		
Marked	12 (4.63)	67 (5.21)	14 (8.97)	2 (5.26)		
Unknown/missing	15 (5.79)	40 (3.11)	4 (2.56)	2 (5.26)		
Maternal inflammation summary score	0 (0, 1)	0 (0, 1)	0 (0, 1.25)	0 (0, 2)	0.38	0.006 (-0.05 to 0.06)
Decidua						
Decidual vessels, fibrinoid					0.24	0.05 (0.00–0.09)
Not seen	225 (86.9)	1158 (90.0)	140 (89.7)	35 (92.1)		
Present	15 (5.79)	55 (4.28)	3 (1.92)	1 (2.63)		
Unable to determine/unknown/missing	19 (7.34)	73 (5.68)	13 (8.33)	2 (5.26)		
Decidual vessels, atheroma					0.49	0.04 (0.00–0.07)
Not seen	234 (90.3)	1194 (92.8)	142 (91.0)	36 (94.7)		
Present	5 (1.93)	15 (1.17)	1 (0.64)	0		
Unable to determine/unknown/missing	20 (7.72)	77 (5.99)	13 (8.33)	2 (5.26)		
Syncytium, nuclear clumping					1.0	0.01 (0.02–0.07)
Normal	183 (70.7)	955 (74.3)	115 (73.7)	28 (73.7)		
Less than normal for term placenta	52 (20.1)	256 (19.9)	32 (20.5)	7 (18.4)		
Excessive for term placenta	10 (3.86)	42 (3.27)	6 (3.85)	1 (2.63)		
Excessive in parts	0	0	0	0		
Unable to determine/unknown/missing	14 (5.41)	33 (2.57)	3 (1.92)	2 (5.26)		
Maternal vascular malperfusion summary score	0 (0, 0)	0 (0, 0)	0 (0, 0.5)	0 (0, 0.25)	0.79	0.004 (-0.05 to 0.05)
Terminal villi						
Langhans' layer					0.24	0.04 (0.00–0.08)
Not seen	234 (90.3)	1217 (94.6)	147 (94.2)	36 (94.7)		
Present	11 (4.25)	36 (2.80)	6 (3.85)	0		
Unknown/missing	14 (5.41)	33 (2.57)	3 (1.93)	2 (5.26)		
Hofbauer cells					0.11	0.06 (0.00–0.10)
Few	227 (87.6)	1176 (91.4)	136 (87.2)	35 (92.1)		
Many	18 (6.95)	77 (5.99)	17 (10.9)	1 (2.63)		
Unknown/missing	14 (5.41)	33 (2.57)	153 (1.92)	2 (5.26)		
Stromal fibrosis					0.59	0.03 (0.00–0.07)
Not seen	231 (89.2)	1200 (93.3)	143 (91.7)	35 (92.1)		
Present	14 (5.41)	53 (4.12)	9 (5.77)	1 (2.63)		
Unable to determine/unknown/missing	14 (5.41)	33 (2.57)	4 (2.56)	2 (5.26)		

Pathological edema					0.57	0.04 (0.00–0.07)
Not seen	230 (88.8)	1199 (93.2)	145 (92.9)	35 (92.1)		
Present	15 (5.79)	53 (4.12)	8 (5.13)	1 (2.63)		
Unable to determine/unknown/missing	14 (5.41)	34 (2.64)	3 (1.92)	2 (5.26)		
Intervillous space						
Apparent maturity of placenta					0.55	0.04 (0.00–0.05)
Under 20 weeks	0	4 (0.31)	0	0		
20-27 weeks	11 (4.24)	33 (2.57)	6 (3.85)	2 (5.26)		
28-36 weeks	57 (22.0)	260 (20.2)	27 (17.3)	6 (15.8)		
37 weeks or over	177 (68.3)	952 (74.0)	119 (76.3)	28 (73.7)		
Unable to determine/unknown/missing	14 (5.41)	37 (2.88)	4 (2.56)	2 (5.26)		
Immaturity summary score	2 (2, 2)	2 (2, 2)	2 (2, 2)	2 (2, 2)	0.80	0.0008 (-0.05 to 0.05)

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables or n (%) (Likelihood Ratio Chi Square test) for categorical variables. *p<0.05. Effect sizes are Wilcoxon test effect size (r) (95% CI) for continuous variables, or Cramer's V (95% CI) for categorical variables. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Table 2.2. Multivariable analyses for associations between maternal prepregnancy BMI and microscopic placental pathologies in preterm pregnancies, N=1739.

Placental pathology	Unadjusted model			Adjusted model		
	β (95% CI)	OR (95% CI)	p-value	β (95% CI)	OR (95% CI)	p-value
Cord neutrophil infiltration						
Umbilical vein	0.07 (0.03–0.12)	1.08 (1.03–1.12)	***	0.06 (0.01–0.11)	1.06 (1.01–1.12)	**
Not seen						
Any degree of infiltration						
Umbilical artery	0.05 (-0.003 to 0.10)	1.05 (1.00–1.11)	NS	0.04 (-0.02 to 0.10)	1.04 (0.98–1.10)	NS
Not seen						
Any degree of infiltration						
Umbilical cord substance	0.08 (0.03–0.13)	1.09 (1.03–1.14)	**	0.08 (0.02–0.13)	1.08 (1.02–1.14)	**
Not seen						
Any degree of infiltration						
Fetal inflammation summary score	0.04 (0.01–0.06)	-	**	0.03 (0.003–0.05)	-	*
Membranes neutrophil infiltration						
Membrane roll, amnion	0.05 (0.006–0.10)	1.05 (1.01–1.10)	*	0.03 (-0.02 to 0.08)	1.03 (0.98–1.09)	NS
Not seen						
Any degree of infiltration						
Membrane roll, chorion	0.03 (-0.004 to 0.07)	1.03 (1.00–1.07)	NS	0.02 (-0.02 to 0.06)	1.02 (0.98–1.06)	NS
Not seen						
Any degree of infiltration						
Amnion of placenta	0.03 (-0.02 to 0.09)	1.04 (0.98–1.09)	NS	0.02 (-0.04 to 0.07)	1.02 (0.96–1.08)	NS
Not seen						
Any degree of infiltration						
Placental surface, chorion	0.03 (-0.01 to 0.07)	1.03 (0.99–1.07)	NS	0.01 (-0.03 to 0.05)	1.01 (0.97–1.06)	NS
Not seen						
Any degree of infiltration						
Maternal inflammation sum score	0.04 (0.0006–0.08)	-	*	0.02 (-0.02 to 0.07)	-	NS
Decidua						
Decidual vessels, fibrinoid	-0.03 (-0.11 to 0.04)	0.97 (0.89–1.05)	NS	-0.05 (-0.13 to 0.04)	0.96 (0.88–1.04)	NS
Not seen						
Present						
Decidual vessels, atheroma	-0.10 (-0.26 to 0.07)	0.91 (0.77–1.07)	NS	-0.12 (-0.31 to 0.07)	0.89 (0.73–1.07)	NS
Not seen						
Present						
Syncytium, nuclear clumping	0.01 (-0.10 to 0.07)	0.99 (0.91–1.07)	NS	-0.03 (-0.12 to 0.06)	0.97 (0.88–1.06)	NS
Normal/ < normal for term						
Excessive/in parts for term						

Maternal vascular malperfusion	0.006 (-0.001 to 0.01)	-	NS	0.005 (-0.002 to 0.01)	-	NS
Terminal villi						
Langhans' layer	-0.02 (-0.11 to 0.07)	0.98 (0.89–1.07)	NS	-0.04 (-0.14 to 0.06)	0.96 (0.87–1.07)	NS
Not seen						
Present						
Hofbauer cells	0.03 (-0.03 to 0.09)	1.03 (0.97–1.09)	NS	0.02 (-0.04 to 0.08)	1.02 (0.96–1.09)	NS
Few						
Many						
Stromal fibrosis	0.02 (-0.05 to 0.09)	1.02 (0.95–1.09)	NS	-0.004 (-0.08 to 0.07)	1.00 (0.93–1.07)	NS
Not seen						
Present						
Pathological edema	0.01 (-0.06 to 0.08)	1.01 (0.94–1.08)	NS	0.02 (-0.06 to 0.09)	1.02 (0.94–1.10)	NS
Not seen						
Present						
Intervillous space						
Apparent maturity of placenta	0.02 (-0.02 to 0.05)	1.02 (0.98–1.05)	NS	0.02 (-0.02 to 0.06)	1.02 (0.98–1.07)	NS
Appropriate mature						
Hyper-mature						
Immaturity summary score	0.0006 (-0.006 to 0.007)	-	NS	0.002 (-0.005 to 0.009)	-	NS

Data are β (95% CI) and unit odds ratios (95% CI) for presence of pathology from Logistic regression models with p-value from Likelihood Ratio Chi Square test for categorical variables, or β (95% CI) with p-value from Standard Least Squares models for continuous variables. The adjusted model includes fetal sex, maternal race, maternal age, smoking history, maternal education, socioeconomic status, gestational weight gain, and maternal diabetes mellitus status. NS (not significant): $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

Table 2.3. Associations between maternal prepregnancy BMI and microscopic placental pathologies in term pregnancies, N=10,145.

Placental pathology	Prepregnancy BMI				p-value	Effect size
	UW (n=1201)	NW (n=8010)	OW (n=922)	OB (n=282)		
Cord						
Neutrophil infiltration, umbilical vein					0.001	0.03 (0.01–0.04)
Not seen	1065 (88.7)	6957 (86.9)	794 (86.1)	237 (84.0)		
Slight	84 (6.99)	601 (7.50)	54 (5.86)	22 (7.80)		
Moderate	0	16 (0.20)	0	0		
Marked	24 (2.0)	197 (2.46)	35 (3.80)	16 (5.67)		
Unknown/missing	28 (2.33)	239 (2.98)	39 (4.23)	7 (2.48)		
Neutrophil infiltration, umbilical artery					0.04	0.03 (0.009–0.03)
Not seen	1141 (95.0)	7552 (94.3)	850 (92.2)	260 (92.2)		
Slight	20 (1.67)	141 (1.76)	17 (1.84)	5 (1.77)		
Moderate	0	3 (0.04)	0	0		
Marked	12 (1.0)	75 (0.94)	17 (1.84)	10 (3.55)		
Unknown/missing	28 (2.33)	239 (2.98)	38 (4.12)	7 (2.48)		
Neutrophil infiltration, umbilical cord substance					0.05	0.03 (0.007–0.03)
Not seen	1130 (94.1)	7431 (92.8)	835 (90.6)	253 (89.7)		
Slight	34 (2.83)	264 (3.30)	37 (4.01)	13 (4.61)		
Moderate	0	5 (0.06)	1 (0.11)	0		
Marked	9 (0.75)	71 (0.89)	11 (1.19)	9 (3.19)		
Unknown/missing	28 (2.33)	239 (2.98)	38 (4.12)	7 (2.48)		
Fetal inflammation summary score	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.07	0.01 (-0.008 to 0.03)
Membranes						
Neutrophil infiltration, membrane roll, amnion					0.002	0.03 (0.01–0.04)
Not seen	1028 (85.6)	6728 (84.0)	739 (80.2)	224 (79.4)		
Slight	62 (5.16)	434 (5.42)	71 (7.70)	25 (8.87)		
Moderate	0	5 (0.06)	0	1 (0.35)		
Marked	16 (1.33)	156 (1.95)	25 (2.71)	10 (3.55)		
Unknown/missing	95 (7.91)	687 (8.58)	87 (9.44)	22 (7.80)		
Neutrophil infiltration, membrane roll, chorion					0.003	0.03 (0.01–0.04)
Not seen	1032 (85.9)	6710 (83.8)	750 (81.34)	216 (76.6)		
Slight	97 (8.08)	761 (9.50)	88 (9.54)	41 (14.5)		
Moderate	2 (0.17)	9 (0.11)	0	0		
Marked	39 (3.25)	286 (3.57)	46 (4.99)	19 (6.74)		
Unknown/missing	31 (2.58)	244 (3.05)	38 (4.12)	6 (2.13)		
Neutrophil infiltration, placental surface, amnion					0.03	0.03 (0.009–0.04)
Not seen	1011 (84.2)	6683 (83.43)	747 (81.0)	237 (84.0)		

Slight	44 (3.66)	288 (3.60)	47 (5.10)	13 (4.61)		
Moderate	1 (0.08)	2 (0.02)	1 (0.11)	1 (0.35)		
Marked	9 (0.75)	95 (1.19)	17 (1.84)	8 (2.84)		
Unknown/missing	136 (11.32)	942 (11.8)	110 (11.9)	23 (8.16)		
Neutrophil infiltration, placental surface, chorion					0.04	0.03 (0.005–0.03)
Not seen	1070 (89.1)	6976 (87.1)	773 (83.8)	239 (84.8)		
Slight	85 (7.08)	620 (7.74)	83 (9.00)	22 (7.80)		
Moderate	1 (0.08)	6 (0.07)	1 (0.11)	1 (0.35)		
Marked	19 (1.58)	185 (2.31)	29 (3.15)	14 (4.96)		
Unknown/missing	26 (2.16)	223 (2.78)	36 (3.90)	6 (2.13)		
Maternal inflammation summary score	0 (0, 0) ^A	0 (0, 0) ^{AB}	0 (0, 0) ^{AB}	0 (0, 1) ^B	0.009	0.008 (-0.01 to 0.03)
Decidua						
Decidual vessels, fibrinoid					0.19	0.02 (0.00–0.04)
Not seen	1106 (92.1)	7405 (92.4)	849 (92.1)	259 (91.8)		
Present	44 (3.66)	226 (2.82)	19 (2.06)	7 (2.48)		
Unable to determine/ unknown/missing	51 (4.25)	379 (4.73)	36 (3.90)	16 (5.67)		
Decidual vessels, atheroma					0.25	0.02 (0.00–0.04)
Not seen	1127 (93.8)	7521 (93.9)	854 (92.6)	262 (92.9)		
Present	21 (1.75)	83 (1.04)	10 (1.08)	3 (1.06)		
Unable to determine/ unknown/missing	53 (4.41)	406 (5.07)	58 (6.29)	17 (6.03)		
Syncytium, nuclear clumping					0.61	0.02 (0.00–0.02)
Normal	1046 (87.1)	6962 (86.9)	799 (86.7)	253 (89.7)		
Less than normal for term placenta	97 (8.08)	556 (6.94)	60 (6.51)	13 (4.61)		
Excessive for term placenta	29 (2.41)	237 (2.96)	22 (2.39)	9 (3.19)		
Excessive in parts	5 (0.41)	41 (0.51)	5 (0.54)	1 (0.35)		
Unable to determine/unknown/missing	24 (2.00)	214 (2.67)	36 (3.90)	6 (2.13)		
Maternal vascular malperfusion summary score	0 (0, 1)	0 (0, 1)	0 (0, 1)	0 (0, 1)	0.07	0.02 (-0.004 to 0.04)
Terminal villi						
Langhans' layer					0.40	0.02 (0.00–0.03)
Not seen	1173 (97.7)	7753 (96.8)	879 (95.3)	273 (96.8)		
Present	4 (0.33)	46 (0.57)	7 (0.76)	3 (1.06)		
Unknown/missing	24 (2.00)	211 (2.63)	36 (3.90)	6 (2.13)		
Hofbauer cells					0.90	0.007 (0.00–0.02)
Few	1155 (96.2)	7660 (95.6)	872 (94.6)	271 (96.1)		
Many	22 (1.83)	140 (1.75)	13 (1.41)	5 (1.77)		
Unknown/missing	24 (2.00)	210 (2.62)	37 (4.01)	6 (2.13)		
Stromal fibrosis					0.23	0.02 (0.00–0.04)
Not seen	1135 (94.5)	7487 (93.5)	861 (93.4)	263 (93.3)		
Present	41 (3.41)	313 (3.91)	25 (2.71)	13 (4.61)		
Unable to determine/unknown/missing	25 (2.08)	210 (2.62)	36 (3.90)	6 (2.13)		

Pathological edema					0.11	0.02 (0.00–0.04)
Not seen	1135 (94.5)	7541 (94.1)	857 (93.0)	273 (96.8)		
Present	42 (3.50)	258 (3.22)	29 (3.14)	3 (1.06)		
Unable to determine/unknown/missing	24 (2.00)	211 (2.63)	36 (3.90)	6 (2.13)		
Intervillous space						
Apparent maturity of placenta					0.70	0.01 (0.00–0.01)
Under 20 weeks	1 (0.08)	6 (0.07)	0	0		
20-27 weeks	4 (0.33)	19 (0.24)	1 (0.11)	0		
28-36 weeks	92 (7.66)	543 (6.78)	55 (5.97)	19 (6.74)		
37 weeks or over	1078 (89.8)	7225 (90.2)	830 (90.0)	257 (91.1)		
Unable to determine/unknown/missing	26 (2.16)	217 (2.71)	36 (3.90)	6 (2.16)		
Immaturity summary score	2 (2, 2)	2 (2, 2)	2 (2, 2)	2 (2, 2)	0.38	-0.01 (-0.03 to 0.008)

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables or n (%) (Likelihood Ratio Chi Square test) for categorical variables. *p<0.05. Effect sizes are Wilcoxon test effect size (r) (95% CI) for continuous variables, or Cramer's V (95% CI) for categorical variables. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Table 2.4. Multivariable analyses for associations between maternal prepregnancy BMI (continuous) and microscopic placental pathologies in term pregnancies, N=10,145.

Placental pathology	Unadjusted model			Adjusted model		
	β (95% CI)	OR (95% CI)	p-value	β (95% CI)	OR (95% CI)	p-value
Cord neutrophil infiltration						
Umbilical vein	0.02 (0.004–0.04)	1.02 (1.00–1.04)	*	0.03 (0.007–0.05)	1.03 (1.01–1.05)	**
Not seen						
Any degree of infiltration						
Umbilical artery	0.05 (0.02–0.08)	1.05 (1.02–1.08)	**	0.04 (0.004–0.07)	1.04 (1.00–1.07)	*
Not seen						
Any degree of infiltration						
Umbilical cord substance	0.04 (0.02–0.07)	1.04 (1.02–1.07)	***	0.04 (0.009–0.06)	1.04 (1.01–1.07)	**
Not seen						
Any degree of infiltration						
Fetal inflammation score	0.01 (0.008–0.02)	-	****	0.01 (0.007–0.02)	-	****
Membranes and neutrophil infiltration						
Membrane roll, amnion	0.06 (0.04–0.07)	1.06 (1.04–1.08)	****	0.05 (0.03–0.08)	1.06 (1.03–1.08)	****
Not seen						
Any degree of infiltration						
Membrane roll, chorion	0.05 (0.03–0.06)	1.05 (1.03–1.07)	****	0.04 (0.02–0.06)	1.04 (1.02–1.06)	****
Not seen						
Any degree of infiltration						
Placental surface, amnion	0.04 (0.01–0.06)	1.04 (1.01–1.06)	**	0.04 (0.01–0.06)	1.04 (1.01–1.07)	**
Not seen						
Any degree of infiltration						
Placental surface, chorion	0.04 (0.02–0.06)	1.04 (1.02–1.06)	****	0.04 (0.02–0.06)	1.04 (1.02–1.06)	***
Not seen						
Any degree of infiltration						
Maternal inflammation score	0.03 (0.02–0.04)	-	****	0.03 (0.02–0.04)	-	****
Decidua						
Decidual vessels, fibrinoid	-0.03 (-0.07 to 0.01)	0.97 (0.94–1.01)	NS	-0.02 (-0.06 to 0.02)	0.98 (0.94–1.02)	NS
Not seen						
Present						
Decidual vessels, atheroma	-0.05 (-0.12 to 0.01)	0.95 (0.89–1.01)	NS	-0.06 (-0.13 to 0.004)	0.94 (0.88–1.00)	NS
Not seen						
Present						
Syncytium, nuclear clumping	0.002 (-0.03 to 0.03)	1.00 (0.71–1.03)	NS	0.001 (-0.03 to 0.04)	1.00 (0.97–1.04)	NS
Normal/ < normal for term						

Excessive/in parts for term						
Maternal vascular malperfusion	0.005 (0.002–0.008)	-	**	0.007 (0.004–0.01)	-	****
Terminal villi						
Langhans' layer	0.007 (-0.07 to 0.08)	1.01 (0.93–1.09)	NS	-0.01 (-0.09 to 0.07)	0.99 (0.91–1.07)	NS
Not seen						
Present						
Hofbauer cells	-0.03 (-0.08 to 0.02)	0.97 (0.92–1.02)	NS	-0.04 (-0.09 to 0.009)	0.96 (0.91–1.01)	NS
Few						
Many						
Stromal fibrosis	-0.006 (-0.04 to 0.03)	0.99 (0.96–1.03)	NS	0.002 (-0.03 to 0.04)	1.00 (0.97–1.04)	NS
Not seen						
Present						
Pathological edema	-0.03 (-0.07 to 0.004)	0.97 (0.93–1.00)	NS	-0.01 (-0.05 to 0.03)	0.99 (0.95–1.03)	NS
Not seen						
Present						
Intervillous space						
Apparent maturity of placenta	-0.02 (-0.05 to 0.003)	0.98 (0.96–1.00)	NS	-0.60 (-1.37 to 0.18)	0.94 (0.92–0.97)	****
Appropriate mature						
Immature						
Immaturity score	-0.0003 (-0.002 to 0.002)	-	NS	-0.0009 (-0.003 to 0.001)	-	NS

Data are β (95% CI) and unit odds ratios (95% CI) for presence of pathology from Logistic regression models with p-value from Likelihood Ratio Chi Square test for categorical variables, or β (95% CI) with p-value and from Standard Least Squares models for continuous variables. The adjusted model includes fetal sex, maternal race, maternal age, smoking history, maternal education, socioeconomic status, gestational weight gain, and maternal diabetes mellitus status. NS (not significant): $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

Table 2.5. Associations between fetal sex and microscopic placental pathologies in preterm (N=1739) and term (N=10,415) pregnancies.

Placental pathology	Preterm				Term			
	Male (n=906)	Female (n=833)	p-value	Effect size	Male (n=5357)	Female (n=5058)	p-value	Effect size
Cord								
Neutrophil infiltration, umbilical vein			0.09	0.76 (0.56–1.04)			0.0003	1.26 (1.11–1.44)
Not seen	794 (87.6)	711 (85.4)			4599 (85.9)	4454 (88.1)		
Any degree of neutrophilic infiltration	81 (8.94)	95 (11.4)			594 (11.1)	455 (9.0)		
Unknown/missing	31 (3.42)	27 (3.24)			164 (3.06)	149 (2.95)		
Neutrophil infiltration, umbilical artery			0.09	0.72 (0.49–1.05)			0.17	1.18 (0.93–1.48)
Not seen	823 (90.8)	742 (89.1)			5027 (93.8)	4776 (94.4)		
Any degree of neutrophilic infiltration	51 (5.63)	64 (7.68)			166 (3.10)	134 (2.65)		
Unknown/missing	32 (3.53)	27 (3.24)			164 (3.06)	148 (2.93)		
Neutrophil infiltration, umbilical cord substance			0.07	0.71 (0.49–1.03)			<0.0001	1.54 (1.27–1.86)
Not seen	823 (90.8)	739 (88.7)			4914 (91.7)	4735 (93.6)		
Any degree of neutrophilic infiltration	52 (5.74)	66 (7.92)			279 (5.21)	175 (3.46)		
Unknown/missing	31 (3.42)	28 (3.36)			164 (3.06)	148 (2.93)		
Fetal inflammation summary score	0 (0, 0)	0 (0, 0)	0.04	-0.05 (-0.10 to -0.004)	0 (0, 0)	0 (0, 0)	0.0005	0.03 (0.01–0.05)
Membranes								
Neutrophil infiltration, membrane roll, amnion			0.08	0.75 (0.54–1.04)			0.0004	1.30 (1.12–1.50)
Not seen	770 (85.0)	668 (80.2)			4459 (83.2)	4260 (84.2)		
Any degree of neutrophilic infiltration	74 (8.17)	86 (10.3)			464 (8.66)	341 (6.74)		
Unknown/missing	62 (6.84)	79 (9.48)			434 (8.10)	457 (9.04)		
Neutrophil infiltration, membrane roll, chorion			0.79	0.97 (0.76–1.23)			0.0008	1.21 (1.08–1.36)
Not seen	705 (77.8)	639 (76.7)			4424 (82.6)	4284 (84.7)		
Any degree of neutrophilic infiltration	173 (19.1)	162 (19.4)			772 (14.4)	616 (12.2)		
Unknown/missing	28 (3.09)	32 (3.84)			161 (3.01)	158 (3.12)		
Neutrophil infiltration, placental surface, amnion			0.22	0.80 (0.55–1.15)			0.01	1.26 (1.05–1.50)
Not seen	761 (84.0)	667 (80.1)			4460 (83.3)	4218 (83.4)		
Any degree of neutrophilic infiltration	60 (6.62)	66 (7.92)			300 (5.60)	226 (4.47)		
Unknown/missing	85 (9.38)	100 (12.0)			597 (11.1)	614 (12.1)		
Neutrophil infiltration, placental surface, chorion			0.87	1.02 (0.78–1.33)			0.002	1.22 (1.08–1.39)
Not seen	740 (81.7)	683 (82.0)			4612 (86.1)	4446 (87.9)		
Any degree of neutrophilic infiltration	134 (14.8)	121 (14.5)			596 (11.1)	470 (9.29)		
Unknown/missing	32 (3.53)	29 (3.48)			149 (2.78)	122 (2.81)		
Maternal inflammation summary score	0 (0, 1)	0 (0, 1)	0.28	-0.03 (-0.07 to 0.02)	0 (0, 0)	0 (0, 0)	0.004	0.03 (0.008–0.05)
Decidua								
Decidual vessels, fibrinoid			0.27	0.77 (0.48–1.23)			0.44	0.91 (0.72–1.15)
Not seen	818 (90.3)	740 (88.8)			4966 (92.7)	4653 (92.0)		

Present	34 (3.75)	40 (4.80)			146 (2.73)	150 (2.97)		
Unable to determine/unknown/missing	54 (5.96)	53 (6.36)			149 (2.78)	255 (5.04)		
Decidual vessels, atheroma			0.98	1.01 (0.43–2.39)			0.96	0.99 (0.69–1.43)
Not seen	837 (92.4)	769 (92.3)			5032 (93.9)	4732 (93.6)		
Present	11 (1.21)	10 (1.20)			60 (1.12)	57 (1.13)		
Unable to determine/unknown/missing	58 (6.40)	54 (6.48)			245 (4.57)	269 (5.32)		
Syncytium, nuclear clumping			0.02	0.53 (0.31–0.91)			0.94	1.01 (0.81–1.25)
Normal/Less than normal for term placenta	857 (94.6)	771 (92.6)			5028 (93.9)	4758 (94.1)		
Excessive for term placenta/ Excessive in parts	22 (2.43)	37 (4.44)			180 (3.36)	169 (3.34)		
Unable to determine/unknown/missing	27 (2.98)	25 (3.00)			149 (2.78)	131 (2.60)		
Maternal vascular malperfusion summary score	0 (0, 0)	0 (0, 0)	0.89	-0.003 (-0.05 to 0.05)	0 (0, 1)	0 (0, 1)	<0.0001	0.04 (0.02–0.06)
Terminal villi								
Langhans' layer			0.47	0.82 (0.47–1.41)			0.76	1.08 (0.65–1.80)
Not seen	854 (94.3)	780 (93.6)			5178 (96.7)	4900 (96.9)		
Present	25 (2.76)	28 (3.36)			32 (0.60)	28 (0.55)		
Unknown/missing	27 (2.98)	25 (3.00)			147 (2.74)	130 (2.57)		
Hofbauer cells			0.23	1.26 (0.86–1.86)			0.94	0.99 (0.74–1.33)
Few	814 (89.8)	760 (91.2)			5119 (95.6)	4839 (95.7)		
Many	65 (7.17)	48 (5.76)			92 (1.72)	88 (1.74)		
Unknown/missing	27 (2.98)	25 (3.00)			146 (2.73)	131 (2.59)		
Stromal fibrosis			0.17	1.39 (0.87–2.21)			0.09	0.84 (0.69–1.03)
Not seen	832 (91.8)	777 (93.3)			5026 (93.8)	4720 (93.3)		
Present	46 (5.08)	31 (3.72)			185 (3.45)	207 (4.09)		
Unable to determine/unknown/missing	28 (3.09)	25 (3.00)			146 (2.73)	131 (2.59)		
Pathological edema			0.17	1.38 (0.87–2.20)			0.11	1.20 (0.96–1.49)
Not seen	833 (91.9)	776 (93.2)			5025 (93.8)	4781 (94.5)		
Present	46 (5.08)	31 (3.72)			185 (3.45)	147 (2.91)		
Unable to determine/unknown/missing	27 (2.98)	26 (3.12)			147 (2.74)	130 (2.57)		
Intervillous space								
Apparent maturity of placenta			0.27	-			0.17	-
Under 20 weeks	2 (0.22)	2 (0.24)			1 (0.02)	6 (0.12)		
20-27 weeks	21 (2.32)	31 (3.72)			14 (0.26)	10 (0.20)		
28-36 weeks	192 (21.2)	158 (19.0)			371 (6.93)	338 (6.68)		
37 weeks or over	663 (73.2)	613 (73.6)			4819 (90.0)	4571 (90.4)		
Unable to determine/unknown/missing	28 (3.09)	29 (3.48)			152 (2.84)	133 (2.63)		
Immaturity summary score	2 (2, 2)	2 (2, 2)	0.45	0.02 (-0.03 to 0.06)	2 (2, 2)	2 (2, 2)	0.23	0.01 (-0.008 to 0.03)

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables, or n (%) (Likelihood Ratio Chi Square test) for categorical variables. *p<0.05. Effect sizes are Wilcoxon test effect size (r) (95% CI) for continuous variables, or odds ratios (95% CI) for presence of pathology in male placentae for categorical variables.

Table 2.6. Associations between fetal sex and macroscopic placental pathologies in preterm (N=1739) and term (N=10,415) pregnancies.

Placental pathology	Preterm				Term			
	Male (n=906)	Female (n=833)	p-value	Effect size	Male (n=5357)	Female (n=5058)	p-value	Effect size
Placenta ¹								
Placenta shape			0.64	-			0.17	-
Non-partite	804 (88.7)	727 (87.3)			4839 (90.3)	4553 (90.0)		
Bipartite	4 (0.44)	1 (0.12)			65 (1.21)	68 (1.34)		
Tripartite	0	0			0	2 (0.04)		
Succenturiate	12 (1.32)	11 (1.32)			61 (1.14)	53 (1.05)		
Membranous placenta	0	0			0	2 (0.04)		
Crescent shaped	0	0			2 (0.04)	0		
Irregular shape	4 (0.44)	3 (0.36)			23 (0.43)	23 (0.45)		
Unknown/missing	82 (9.05)	91 (10.9)			518 (9.67)	357 (7.06)		
Weight	382 (320, 450)	375 (320, 435)	0.17	0.03 (-0.02 to 0.08)	425 (370, 485)	420 (370, 480)	0.06	0.02 (-0.002 to 0.04)
Largest diameter	18 (17, 19)	18 (17, 19)	0.61	0.01 (-0.04 to 0.06)	19 (18, 20)	19 (18, 20)	0.03	0.02 (0.002–0.04)
Smallest diameter	16 (14, 17)	15 (14, 17)	0.41	0.02 (-0.03 to 0.07)	16 (15, 18)	16 (15, 17)	0.19	0.01 (-0.006 to 0.03)
Thickness	20 (18, 24)	20 (18, 23)	0.38	0.02 (-0.02 to 0.07)	20 (20, 25)	20 (20, 25)	0.53	0.006 (-0.02 to 0.03)
Birthweight : placenta weight	7.34 (6.52, 8.32)	7.16 (6.36, 7.93)	0.003	0.07 (0.03–0.12)	7.56 (6.78, 8.38)	7.36 (6.65, 8.17)	<0.0001	0.07 (0.05–0.09)
Birthweight : placental largest diameter	157 (140, 175)	150 (135, 166)	<0.0001	0.13 (0.09–0.18)	169 (155, 184)	165 (151, 179)	<0.0001	0.09 (0.07–0.11)
Birthweight : placental smallest diameter	183 (164, 202)	173 (156, 193)	<0.0001	0.12 (0.08–0.17)	197 (179, 214)	191 (175, 208)	<0.0001	0.09 (0.07–0.11)
Birthweight : placental thickness	140 (118, 162)	133 (116, 155)	0.003	0.07 (0.03–0.12)	147 (126, 170)	143 (125, 164)	<0.0001	0.06 (0.04–0.08)
Cord								
Cord number of vessels			0.85	0.90 (0.29–2.79)			0.10	0.66 (0.40–1.09)
Two	821 (90.6)	736 (88.4)			4965 (92.7)	4659 (92.1)		
Three	6 (0.66)	6 (0.72)			26 (0.49)	37 (0.73)		
Other/unknown/missing	79 (8.72)	91 (10.9)			366 (6.83)	362 (7.16)		
Cord edema			0.97	1.01 (0.62–1.64)			0.02	1.46 (1.07–1.99)
Not seen	781 (86.2)	700 (84.0)			4817 (89.9)	4562 (90.2)		
Present	36 (3.97)	31 (3.72)			103 (1.92)	67 (1.32)		
Unknown/missing	89 (9.82)	101 (12.1)			437 (8.16)	429 (8.48)		
Membranes and fetal surface								
Opacity of membranes			0.97	1.01 (0.69–1.46)			0.45	1.06 (0.91–1.25)
Not opaque	763 (84.2)	684 (82.1)			4648 (86.8)	4384 (86.7)		
Any degree of opacity	64 (7.06)	57 (6.84)			347 (6.48)	308 (6.09)		
Unknown/missing	79 (8.72)	92 (11.0)			362 (6.76)	366 (7.24)		
Thrombosed fetal vessels			0.93	1.05 (0.35–3.13)			0.73	0.88 (0.42–1.82)

Not seen	821 (90.6)	738 (88.6)			4983 (93.0)	4687 (92.7)		
Present	7 (0.77)	6 (0.72)			14 (0.26)	15 (0.30)		
Unknown/missing	78 (8.61)	89 (10.7)			360 (6.72)	356 (7.04)		
Cut surface								
Infarct size			0.89	0.98 (0.77–1.26)			<0.0001	1.22 (1.11–1.33)
Not applicable	660 (72.8)	593 (71.2)			3546 (66.2)	3513 (69.5)		
Any infarcts seen	164 (18.1)	150 (18.0)			1433 (26.8)	1167 (23.1)		
Unknown/missing	82 (9.05)	90 (10.8)			378 (7.06)	378 (7.47)		
Cut surface calcification			0.43	1.08 (0.89–1.32)			0.14	0.94 (0.87–1.02)
None	444 (49.0)	414 (49.7)			2051 (66.2)	1860 (36.8)		
Any calcification seen	380 (41.9)	327 (39.3)			2926 (54.6)	2819 (55.7)		
Unknown/missing	82 (9.05)	92 (11.0)			380 (7.09)	379 (7.49)		

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables or n (%) (Likelihood Ratio Chi Square test) and odds ratios (95% CI) for presence of pathology in male placentae for categorical variables. *p<0.05. Effect sizes are Wilcoxon test effect size (r) (95% CI) for continuous variables or Cramer's V (95% CI) for categorical variables. ¹The top and bottom 0.5% of raw infant and placental anthropometry data (birthweight [g], placental weight [g], placental diameter [cm], and placental thickness [mm]) were excluded to remove biologically implausible data.

Table 2.7. Infant characteristics by prepregnancy BMI in preterm pregnancies, N=1739.

	Prepregnancy BMI				p-value	Effect size
	UW (n=259)	NW (n=1286)	OW (n=156)	OB (n=38)		
Infant characteristics ¹						
Infant sex (n [%])					0.18	0.05 (0.00, 0.09)
Male	140 (54.1)	675 (52.5)	69 (44.2)	22 (57.9)		
Female	119 (45.9)	611 (47.5)	87 (55.8)	16 (42.1)		
Gestational age (weeks)	34 (32, 36)	34 (32, 36)	34 (31, 36)	34 (29.75, 36)	0.57	-0.01 (-0.06, 0.04)
Apgar scores						
1 minute	8 (6, 9)	8 (6, 9)	8 (7, 9)	7 (5, 9)	0.08	0.03 (-0.02, 0.08)
5 minutes	9 (8, 10)	9 (9, 10)	9 (9, 10)	9 (7, 9)	0.05	0.01 (-0.04, 0.07)
10 minutes	8 (7, 9)	8 (6, 9)	7 (4.5, 9)	7.5 (5.75, 9.25)	0.22	0.002 (-0.04, 0.05)
z-Birthweight	1.23 (0.17, 2.11) ^A	1.42 (0.41, 2.55) ^{AB}	1.51 (0.60, 2.82) ^{AB}	2.18 (0.97, 3.50) ^B	0.003	0.06 (0.01, 0.11)
z-Birth length	1.47 (0.12, 2.42) ^A	1.92 (0.79, 2.86) ^B	1.90 (0.70, 3.21) ^B	2.48 (1.50, 3.52) ^B	0.0001	0.09 (0.04, 0.14)
z-Head circumference	0.75 (-0.35, 1.80) ^A	1.14 (0.29, 2.16) ^B	1.16 (0.37, 2.50) ^{BC}	2.12 (1.14, 2.80) ^C	<0.0001	0.09 (0.04, 0.13)
Brain-to-body weight ratio	10.3 (9.57, 11.1)	10.3 (9.62, 11.1)	10.2 (9.45, 11.1)	10.1 (9.23, 11.1)	0.65	-0.007 (-0.06, 0.04)
Placental anthropometry						
Weight (g)	360 (310, 425) ^A	380 (320, 440) ^{AB}	405 (330, 470) ^B	440 (360, 501) ^B	0.0008	0.04 (-0.006, 0.09)
Largest diameter (cm)	18 (16, 19) ^A	18 (17, 19) ^A	18 (17, 20) ^A	19 (16, 21) ^A	0.05	0.05 (-0.004, 0.11)
Smallest diameter (cm)	15 (14, 17) ^A	16 (14, 17) ^{AB}	16 (14, 17) ^{AB}	17 (15, 18) ^B	0.005	0.06 (0.009, 0.12)
Thickness (cm)	2.0 (1.8, 2.5)	2.0 (1.8, 2.3)	2.0 (1.8, 2.5)	2.0 (1.9, 2.5)	0.77	-0.0006 (-0.05, 0.05)
Infant birthweight : placental anthropometry						
Birthweight (g) : placenta weight (g)	7.34 (6.49, 8.31)	7.22 (6.45, 8.11)	7.21 (6.41, 7.91)	6.99 (6.21, 8.26)	0.49	-0.03 (-0.07, 0.02)
Birthweight (g) : placental largest diameter (cm)	151 (138, 167)	154 (136, 170)	158 (140, 173)	163 (146, 177)	0.23	0.02 (-0.03, 0.06)
Birthweight (g) : placental smallest diameter (cm)	174 (161, 195)	178 (159, 198)	180 (162, 204)	183 (171, 204)	0.52	0.02 (-0.04, 0.07)
Birthweight (g) : placental thickness (cm)	134 (115, 156)	135 (117, 159)	140 (119, 159)	147 (119, 170)	0.23	0.03 (-0.02, 0.08)

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables. Data are n (%) (Likelihood Ratio Chi Square test) and for categorical variables. *p<0.05. Effect sizes are Wilcoxon test effect size (r) for continuous variables, and Cramer's V for categorical variables.¹The top and bottom 0.5% of raw infant and placental anthropometry data (birthweight, birth length, head circumference, placental weight, and placental dimensions) were excluded to remove biologically implausible data.

Table 2.8. Infant characteristics by prepregnancy BMI in term pregnancies, N=10145.

	Prepregnancy BMI					
	UW (n=1201)	NW (n=8010)	OW (n=922)	OB (n=282)	p-value	Effect size
Infant characteristics ¹						
Infant sex (n [%])					0.62	0.01 (0.00, 0.03)
Male	624 (52.0)	4117 (51.4)	481 (52.2)	135 (47.9)		
Female	577 (48.0)	3893 (48.6)	441 (47.8)	147 (52.1)		
Gestational age (weeks)	40 (39, 41) ^A	40 (39, 41) ^A	40 (39, 41) ^A	40 (39, 41) ^A	0.02	0.01 (-0.008, 0.03)
z-Birthweight	-0.59 (-1.33, 0.09) ^A	-0.36 (-1.04, 0.33) ^B	-0.22 (-0.91, 0.47) ^C	-0.20 (-0.90, 0.52) ^{BC}	<0.0001	0.08 (0.06, 0.10)
z-Birth length	0.16 (-0.77, 1.02) ^A	0.43 (-0.47, 1.26) ^B	0.50 (-0.47, 1.37) ^B	0.52 (-0.41, 1.47) ^B	<0.0001	0.07 (0.04, 0.09)
z-Head circumference	-0.36 (-1.16, 0.34) ^A	-0.08 (-0.99, 0.60) ^B	-0.08 (-0.92, 0.60) ^B	-0.07 (-0.78, 0.75) ^B	<0.0001	0.05 (0.03, 0.07)
Brain-to-body weight ratio	10.1 (9.48, 10.9) ^A	10.0 (9.31, 10.7) ^B	9.86 (9.15, 10.6) ^C	10.0 (9.47, 10.5) ^{ABC}	<0.0001	-0.06 (-0.08, -0.04)
Apgar scores						
1 minute	8 (7, 9)	8 (7, 9)	8 (6, 9)	8 (7, 9)	0.52	0.009 (-0.01, 0.03)
5 minutes	9 (9, 10)	9 (9, 10)	9 (9, 10)	9 (9, 10)	0.61	0.009 (-0.01, 0.03)
10 minutes	9 (8, 9)	9 (8, 10)	9 (7, 9)	8.5 (7, 9)	0.05	0.009 (-0.01, 0.03)
Placental anthropometry						
Weight (g)	400 (352, 455) ^A	420 (370, 480) ^B	440 (380, 500) ^C	446.5 (380, 500) ^{BC}	<0.0001	0.08 (0.06, 0.10)
Largest diameter (cm)	19 (17, 20) ^A	19 (18, 20) ^B	19 (18, 20) ^C	19 (18, 20) ^{BC}	<0.0001	0.06 (0.04, 0.08)
Smallest diameter (cm)	16 (15, 17) ^A	16 (15, 18) ^B	17 (15, 18) ^C	16.5 (15, 18) ^{BC}	<0.0001	0.05 (0.03, 0.07)
Thickness (cm)	2.0 (2.0, 2.5) ^A	2.0 (2.0, 2.5) ^A	2.1 (2.0, 2.5) ^A	2.0 (2.0, 2.5) ^A	0.04	0.02 (-0.0002, 0.04)
Infant birthweight : placental anthropometry						
Birthweight (g) : placenta weight (g)	7.60 (6.80, 8.41) ^A	7.46 (6.73, 8.29) ^A	7.32 (6.56, 8.12) ^B	7.29 (6.59, 8.26) ^{AB}	<0.0001	-0.03 (-0.05, -0.006)
Birthweight (g) : placental largest diameter (cm)	164 (150, 179) ^A	167 (153, 182) ^B	169 (154, 183) ^B	167 (156, 184) ^B	<0.0001	0.05 (0.03, 0.07)
Birthweight (g) : placental smallest diameter (cm)	190 (172, 210) ^A	193 (177, 212) ^B	195 (180, 214) ^B	195 (178, 214) ^B	<0.0001	0.04 (0.02, 0.07)
Birthweight (g) : placental thickness (cm)	142 (123, 163) ^A	145 (127, 167) ^B	147 (128, 170) ^B	151 (129, 174) ^B	<0.0001	0.04 (0.02, 0.06)

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) and for continuous variables. Data are n (%) (Likelihood Ratio Chi Square test) and for categorical variables. *p<0.05. Effect sizes are Wilcoxon test effect size (r) for continuous variables, and Cramer's V for categorical variables. ¹The top and bottom 0.5% of raw infant and placental anthropometry data (birthweight, birth length, head circumference, placental weight, and placental dimensions) were excluded to remove biologically implausible data.

Table 2.9. Multivariable analyses of infant characteristics by prepregnancy BMI (continuous) in preterm pregnancies, N=1739.

	Unadjusted model		Adjusted model	
	β (95% CI)	p-value	β (95% CI)	p-value
Infant characteristics				
Gestational age (weeks)	-0.06 (-0.10, -0.01)	**	-0.05 (-0.10, -0.006)	*
z-Birthweight	0.07 (0.04, 0.09)	****	0.07 (0.04, 0.10)	****
z-Birth length	0.07 (0.04, 0.10)	****	0.07 (0.04, 0.10)	****
z-Head circumference	0.07 (0.05, 0.10)	****	0.08 (0.05, 0.11)	****
Brain-to-body weight ratio	-0.01 (-0.03, 0.005)	NS	-0.02 (-0.04, 0.0001)	NS
Apgar scores				
1 minute	-0.01 (-0.05, 0.03)	NS	-0.02 (-0.06, 0.02)	NS
5 minutes	-0.02 (-0.05, 0.008)	NS	-0.02 (-0.05, 0.009)	NS
10 minutes	-0.05 (-0.13, 0.03)	NS	-0.07 (-0.15, 0.02)	NS
Placental anthropometry				
Weight (g)	3.26 (1.80, 4.73)	****	3.25 (1.72, 4.77)	****
Largest diameter (cm)	0.05 (0.01, 0.08)	**	0.04 (0.01, 0.08)	**
Smallest diameter (cm)	0.06 (0.03, 0.09)	***	0.06 (0.03, 0.09)	***
Thickness (cm)	0.05 (-0.02, 0.11)	NS	0.06 (-0.007, 0.13)	NS
Infant birthweight : placental anthropometry				
Birthweight (g) : placenta weight (g)	-0.02 (-0.04, 0.006)	NS	-0.01 (-0.04, 0.02)	NS
Birthweight (g) : placental largest diameter (cm)	0.62 (0.22, 1.02)	**	0.87 (0.45, 1.29)	****
Birthweight (g) : placental smallest diameter (cm)	0.37 (-0.11, 0.85)	NS	0.67 (0.15, 1.18)	**
Birthweight (g) : placental thickness (cm)	0.53 (-0.04, 1.10)	NS	0.49 (-0.12, 1.10)	NS

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables. Adjusted models include p-value from Standard Least Squares models. * $p < 0.05$. NS (not significant): $p > 0.05$. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$. The adjusted model includes fetal sex, maternal race, maternal age, smoking history, maternal education, socioeconomic status, gestational weight gain, and maternal diabetes mellitus status.

Table 2.10. Multivariable analyses of infant characteristics by prepregnancy BMI (continuous) in term pregnancies, N=10145.

	Unadjusted model		Adjusted model	
	β (95% CI)	p-value	β (95% CI)	p-value
Infant characteristics				
Gestational age (weeks)	0.01 (0.005, 0.02)	NS	0.03 (0.02, 0.04)	****
z-Birthweight	0.03 (0.02, 0.03)	****	0.04 (0.03, 0.05)	****
z-Birth length	0.02 (0.009, 0.03)	****	0.03 (0.02, 0.04)	****
z-Head circumference	0.02 (0.01, 0.03)	****	0.03 (0.02, 0.04)	****
Brain-to-body weight ratio	-0.02 (-0.02, -0.01)	****	-0.03 (-0.03, -0.02)	****
Apgar scores				
1 minute	-0.01 (-0.02, -0.0006)	*	-0.01 (-0.03, -0.00004)	*
5 minutes	-0.006 (-0.01, 0.001)	NS	-0.006 (-0.01, 0.002)	NS
10 minutes	-0.03 (-0.06, -0.003)	*	-0.04 (-0.07, -0.009)	**
Placental anthropometry				
Weight (g)	2.84 (2.33, 3.36)	****	3.71 (3.18, 4.25)	****
Largest diameter (cm)	0.04 (0.03, 0.06)	****	0.06 (0.04, 0.07)	****
Smallest diameter (cm)	0.04 (0.03, 0.05)	****	0.05 (0.03, 0.06)	****
Thickness (cm)	0.007 (-0.02, 0.03)	NS	0.05 (0.02, 0.07)	**
Infant birthweight : placental anthropometry				
Birthweight (g) : placenta weight (g)	-0.02 (-0.03, -0.02)	****	-0.02 (-0.03, -0.02)	****
Birthweight (g) : placental largest diameter (cm)	0.20 (0.07, 0.33)	**	0.43 (0.30, 0.57)	****
Birthweight (g) : placental smallest diameter (cm)	0.28 (0.11, 0.44)	***	0.56 (0.39, 0.73)	****
Birthweight (g) : placental thickness (cm)	0.55 (0.35, 0.76)	****	0.56 (0.34, 0.77)	****

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables. Adjusted models include p-value from Standard Least Squares models. *p<0.05. NS (not significant): p>0.05, *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001. The adjusted model includes fetal sex, maternal race, maternal age, smoking history, maternal education, socioeconomic status, gestational weight gain, and maternal diabetes mellitus status.

A

B

Figure 2.1. Prevalence of placental pathologies in term (A) and preterm (B) pregnancies. The presence or absence of categorical placental pathologies across maternal body mass index (BMI) groups, where mothers were categorized as underweight (UW), normal weight (NW), overweight (OW) or having obesity (OB) by prepregnancy BMI, in term (n=10,145; panel A) and preterm (n=1,739; panel B) pregnancies.

Supplementary Table 2.1. Binary categories for placental pathology variables for multivariable analysis.

Categorical variable	Collapsed binary categories
Cord number of vessels	Three vessels vs two vessels
Cord edema (derived from “cord description” variable)	Presence of cord edema vs absence of cord edema
Opacity of membranes	Not opaque vs any degree of opacity (partially opaque, opaque)
Thrombosed fetal vessel	Not seen vs present
Placental infarcts (derived from “infarcts size” variable)	No infarcts seen (not applicable) vs any infarcts seen (all infarcts less than three centimetres, at least one infarct measures three or more centimetres)
Cut surface calcification	Not seen (none) vs any calcification seen (maternal surface only, calcification throughout)
Neutrophilic infiltration	Not seen vs any degree of neutrophilic infiltration seen (slight, moderate, or marked)
Decidual vessels fibrinoid	Not seen vs present
Decidual vessels atheroma	Not seen vs present
Langhan’s layer	Not seen vs present
Syncytium nuclear clumping	Normal/less than normal for term placenta vs excessive for term placenta/excessive in parts
Hofbauer cells	Few vs many
Stromal fibrosis	Not seen vs present (present- not qualified further, present- rare, present- many)
Pathological edema	Not seen vs present (present- not qualified further, present- rare, present- many)
Apparent maturity of the placenta	Preterm pregnancies: appropriate mature (≤ 36 weeks) vs hyper-mature (≥ 37 weeks) Term pregnancies: appropriate mature (≥ 37 weeks) vs immature (≤ 36 weeks)

Supplementary Table 2.2. Derived summary scores variable coding.

Summary score	Variable coding
Maternal inflammation summary score	Opacity of membranes: non-opaque membranes coded as zero, partially opaque as one, and opaque as two Neutrophilic infiltration of the amnion membrane, chorion membrane, amnion of the placenta, and chorion of the placenta: no neutrophilic infiltration coded as zero, slight infiltration coded as one, moderate coded as two, and marked coded as three
Fetal inflammation summary score	Neutrophilic infiltration of the umbilical vein, artery, and cord substance: no neutrophilic infiltration coded as zero, slight infiltration coded as one, moderate coded as two, and marked coded as three
Maternal vascular malperfusion (MVM) summary score	Presence of infarcts: any infarcts seen coded as one Syncytium-nuclear clumping: excessive syncytium-nuclear clumping coded as one
Immaturity summary score	Langhans' layer: presence of Langhans' layer coded as one Hofbauer cells: many Hofbauer cells coded as one Stromal fibrosis: no stromal fibrosis coded as one Syncytium-nuclear clumping: normal or less than normal syncytium-nuclear clumping coded as one

Supplementary Table 2.3. Maternal characteristics by prepregnancy BMI in preterm pregnancies, N=1739.

	Pregpregnancy BMI					
	UW (n=259)	NW (n=1286)	OW (n=156)	OB (n=38)	p-value	Effect size
Maternal characteristics						
Pregpregnancy BMI	17.7 (17.0, 18.2) ^A	20.9 (19.8, 22.3) ^B	26.3 (25.5, 27.5) ^C	31.9 (30.2, 34.9) ^D	<0.0001	0.65 (0.62–0.67)
Gestational weight gain max (lbs)	23 (16, 28) ^A	19 (13, 26) ^B	19 (11, 28.5) ^{AB}	17 (7.5, 30) ^{AB}	0.005	-0.09 (-0.14 to -0.04)
Gestational weight gain ¹ (n [%])					<0.0001	0.23 (0.19–0.26)
Inadequate	180 (69.5)	884 (68.7)	56 (35.9)	14 (36.8)		
Adequate	67 (25.9)	305 (23.7)	50 (32.1)	9 (23.7)		
Excessive	5 (1.93)	84 (6.53)	49 (31.4)	14 (36.8)		
Unknown/missing	7 (2.70)	13 (1.01)	1 (0.64)	1 (2.63)		
Diabetes mellitus ² (n [%])					0.62	0.03 (0.00–0.06)
Yes	4 (1.54)	24 (1.87)	1 (0.64)	1 (2.63)		
No	253 (97.7)	1257 (97.7)	154 (98.7)	37 (97.4)		
Other/unknown/missing	2 (0.77)	5 (0.39)	1 (0.64)	0		
Maternal age (years)	18 (16, 20) ^A	18 (16, 20) ^A	19 (17, 22) ^B	22.5 (19, 26) ^C	<0.0001	0.04 (-0.01 to 0.09)
Maternal age grouped (years) (n [%])					<0.0001	0.13 (0.09–0.15)
≤19	177 (68.3)	880 (68.4)	84 (53.4)	15 (39.5)		
20-24	70 (27.0)	329 (25.6)	49 (31.4)	12 (31.6)		
25-29	9 (3.47)	57 (4.43)	18 (11.5)	4 (10.5)		
≥30	3 (1.16)	20 (1.56)	5 (3.21)	7 (18.42)		
Unknown/missing	0	0	0	0		
Maternal race (n [%])					0.16	0.05 (0.00–0.07)
Black	168 (64.9)	828 (64.4)	112 (71.8)	31 (81.6)		
White	71 (27.4)	339 (26.4)	32 (20.5)	5 (13.2)		
Other	20 (7.72)	119 (9.25)	12 (7.69)	2 (5.26)		
Unknown/missing	0	0	0	0		
Maternal education (years) (n [%])						
Less than high school (≤9)	103 (39.8)	419 (32.6)	45 (28.9)	13 (34.2)	0.13	0.05 (0.00–0.07)
High school (10-12)	137 (52.9)	729 (56.7)	96 (61.5)	22 (57.9)		
College and above (>12)	14 (5.41)	116 (9.02)	13 (8.33)	2 (5.26)		
Unknown/missing	5 (1.93)	22 (1.71)	2 (1.28)	1 (2.63)		
Marital status (n [%])					0.55	0.04 (0.00–0.06)
Single	120 (46.3)	602 (46.8)	77 (49.4)	19 (50.0)		
Married/common law	136 (52.5)	668 (51.9)	74 (47.4)	19 (50.0)		
Widowed/divorced/separated	3 (1.16)	16 (1.24)	5 (3.21)	0		
Unknown/missing	0	0	0	0		

Family income ³ (n [%])					0.10	0.05 (0.00–0.07)
≤\$1999	57 (22.0)	247 (19.2)	29 (18.6)	6 (15.8)		
\$2000-4999	121 (46.7)	626 (48.7)	71 (45.5)	18 (47.4)		
\$5000-7999	34 (13.1)	163 (12.7)	26 (16.7)	8 (21.1)		
≥\$8000	7 (2.70)	72 (5.60)	4 (2.56)	0		
Unknown/missing	40 (15.4)	178 (13.8)	26 (16.7)	6 (15.8)		
Socioeconomic index	3.6 (2.0, 5.0)	3.7 (2.3, 5.3)	3.7 (2.0, 5.3)	3.85 (2.55, 5.23)	0.26	0.05 (0.0002–0.10)
Socioeconomic index grouped (n [%])					0.64	0.04 (0.00–0.05)
0.0-1.9	48 (18.5)	185 (14.4)	27 (17.3)	5 (13.2)		
2.0-3.9	94 (36.3)	449 (34.9)	52 (33.3)	13 (34.2)		
4.0-5.9	61 (23.6)	365 (28.4)	46 (29.5)	13 (34.2)		
6.0-7.9	36 (13.9)	167 (13.0)	20 (12.8)	4 (10.5)		
8.0-9.5	11 (4.25)	88 (6.84)	9 (5.77)	1 (2.63)		
Unknown/missing	9 (3.47)	32 (2.49)	2 (1.28)	2 (5.26)		
Housing density (n [%])					0.67	0.03 (0.00–0.04)
<1 person	68 (26.3)	322 (25.0)	43 (27.6)	12 (31.6)		
1.0-3.0 people	177 (68.3)	897 (69.8)	109 (69.9)	25 (65.8)		
>3 people	8 (3.09)	40 (3.11)	3 (1.92)	0		
Other ⁴ /unknown/missing	6 (2.32)	27 (2.10)	1 (0.64)	1 (2.63)		
Smoking history ⁵ (n [%])					0.37	0.03 (0.00–0.05)
Non-smokers	150 (57.9)	732 (56.9)	89 (57.1)	23 (60.5)		
Light smokers (<1 pack per day)	85 (32.8)	452 (35.2)	53 (34.0)	14 (36.8)		
Heavy smokers (≥1 pack per day)	21 (8.11)	92 (7.15)	13 (8.33)	0		
Unknown/missing	3 (1.16)	10 (0.78)	1 (0.64)	1 (2.63)		
Infant characteristics						
Infant sex (n [%])					0.18	0.05 (0.00–0.09)
Male	140 (54.1)	675 (52.5)	69 (44.2)	22 (57.9)		
Female	119 (45.9)	611 (47.5)	87 (55.8)	16 (42.1)		
Gestational age (weeks)	34 (32, 36)	34 (32, 36)	34 (31, 36)	34 (29.75, 36)	0.57	-0.01 (-0.06 to 0.04)

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables, or n (%) (Likelihood Ratio Chi Square test) for categorical variables. *p<0.05. Effect sizes are Wilcoxon test effect size (r) (95% CI) for continuous variables, or Cramer's V (95% CI) for categorical variables. ¹Weight gain recommendations based on Institute of Medicine (2009) guidelines for singleton pregnancies based on prepregnancy BMI. ²Includes diabetes mellitus before pregnancy, during pregnancy, before and during pregnancy, during and post pregnancy, or before, during and post pregnancy. Does not include maternal diabetes mellitus during post partum period only. ³\$1999, \$2000-4999, \$5000-7999, ≥\$8000 equivalent to \$17315, ≤\$17800, \$17801-\$44500, \$44501-\$71200, ≥\$71201 in 2020 US dollars, respectively. ⁴Gravida in home for unwed mothers. ⁵1 pack is 20 cigarettes. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Supplementary Table 2.4. Maternal characteristics by prepregnancy BMI in term pregnancies, N=10,415.

	Pregpregnancy BMI					
	UW (n=1201)	NW (n=8010)	OW (n=922)	OB (n=282)	p-value	Effect size
Maternal characteristics						
Pregpregnancy BMI	17.8 (17.2, 18.1) ^A	21.0 (19.8, 22.3) ^B	26.5 (25.6, 27.5) ^C	32.0 (30.8, 34.4) ^D	<0.0001	0.58 (0.57–0.60)
Gestational weight gain max (lbs)	25 (20, 31) ^A	24 (18, 30) ^B	23 (15, 31) ^B	20 (13, 28.5) ^C	<0.0001	-0.05 (-0.07 to -0.03)
Gestational weight gain ¹ (n [%])					<0.0001	0.22 (0.20–0.23)
Inadequate	749 (62.4)	4317 (53.9)	232 (25.2)	77 (27.3)		
Adequate	396 (33.0)	2798 (34.9)	320 (34.7)	82 (29.1)		
Excessive	54 (4.50)	864 (10.8)	365 (39.6)	122 (43.3)		
Unknown/missing	2 (0.17)	31 (0.39)	5 (0.54)	1 (0.35)		
Diabetes mellitus ² (n [%])					0.21	0.02 (0.00–0.04)
Yes	13 (1.08)	47 (0.59)	8 (0.87)	3 (1.06)		
No	1181 (98.3)	7940 (99.1)	913 (99.0)	279 (98.9)		
Other/unknown/missing	7 (0.58)	23 (0.29)	1 (0.11)	0		
Maternal age (years)	19 (17, 21) ^A	19 (17, 22) ^A	20 (18, 22) ^B	21 (18.75, 25) ^C	<0.0001	0.05 (0.03–0.07)
Maternal age grouped (years) (n [%])					<0.0001	0.07 (0.06–0.08)
≤19	692 (57.6)	4132 (51.6)	435 (47.2)	91 (32.3)		
20-24	403 (33.6)	3030 (37.8)	355 (38.5)	120 (42.6)		
25-29	92 (7.66)	652 (8.14)	88 (9.54)	36 (12.8)		
≥30	14 (1.17)	196 (2.45)	44 (4.77)	35 (12.41)		
Missing	0	0	0	0		
Maternal race (n [%])					<0.0001	0.06 (0.04–0.07)
Black	528 (44.0)	3309 (41.3)	437 (47.4)	162 (57.5)		
White	546 (45.5)	4138 (51.7)	412 (44.7)	108 (38.3)		
Other	127 (10.6)	563 (7.03)	73 (7.92)	12 (4.26)		
Missing	0	0	0	0		
Maternal education (years) (n [%])					<0.0001	0.07 (0.05–0.08)
Less than high school (≤9)	327 (27.2)	1668 (20.8)	227 (24.6)	84 (29.8)		
High school (10-12)	663 (55.2)	4572 (57.1)	564 (61.2)	172 (61.0)		
College and above (>12)	202 (16.8)	1682 (21.0)	111 (12.0)	24 (8.51)		
Unknown/missing	9 (0.75)	88 (1.10)	20 (2.17)	2 (0.71)		
Marital status (n [%])					0.01	0.03 (0.008–0.04)
Single	389 (32.4)	2381 (29.7)	313 (34.0)	95 (33.7)		
Married/common law	785 (65.4)	5474 (68.3)	593 (64.3)	176 (62.4)		
Widowed/divorced/separated	27 (2.25)	155 (1.94)	16 (1.74)	11 (3.90)		
Unknown/missing	0	0	0	0		

Family income ³ (n [%])					0.005	0.03 (0.006–0.03)
≤\$1999	183 (15.2)	1202 (15.0)	148 (16.1)	44 (15.6)		
\$2000-4999	570 (47.5)	3572 (44.6)	422 (45.8)	140 (49.7)		
\$5000-7999	226 (18.8)	1556 (19.4)	178 (19.3)	61 (21.6)		
≥\$8000	93 (7.74)	808 (10.1)	72 (7.81)	13 (4.61)		
Unknown/missing	129 (10.7)	872 (10.9)	102 (11.1)	24 (8.51)		
Socioeconomic index	4.3 (3.0, 6.7) ^A	5.0 (3.3, 7.0) ^B	4.4 (3.0, 6.3) ^A	4.3 (2.7, 5.7) ^A	<0.0001	0.05 (0.03–0.07)
Socioeconomic index grouped (n [%])					<0.0001	0.06 (0.05–0.07)
0.0-1.9	126 (10.5)	671 (8.38)	86 (9.33)	28 (9.93)		
2.0-3.9	351 (29.2)	1990 (24.8)	281 (30.5)	86 (30.5)		
4.0-5.9	318 (26.5)	2098 (26.2)	264 (28.6)	99 (35.1)		
6.0-7.9	223 (18.6)	1776 (22.2)	184 (20.0)	46 (16.3)		
8.0-9.5	165 (13.7)	1343 (16.8)	77 (8.35)	11 (3.90)		
Unknown/missing	18 (1.50)	132 (1.65)	30 (3.25)	12 (4.26)		
Housing density (n [%])					<0.0001	0.04 (0.02–0.05)
<1 person	342 (28.5)	2679 (33.5)	331 (35.9)	91 (32.3)		
1.0-3.0 people	820 (68.3)	5095 (63.6)	557 (60.4)	185 (65.6)		
>3 people	24 (2.00)	109 (1.36)	7 (0.76)	0		
Other ⁴ /unknown/missing	15 (1.25)	127 (1.59)	27 (2.93)	6 (2.13)		
Smoking history ⁵ (n [%])					0.09	0.02 (0.00–0.03)
Non-smokers	675 (56.2)	4751 (59.3)	537 (58.24)	162 (57.5)		
Light smokers (<1 pack per day)	392 (32.6)	2511 (31.4)	281 (30.5)	82 (29.1)		
Heavy smokers (≥1 pack per day)	130 (10.8)	719 (8.98)	100 (10.9)	34 (12.1)		
Unknown/missing	4 (0.33)	29 (0.36)	4 (0.43)	4 (1.42)		
Infant characteristics						
Infant sex (n [%])					0.62	0.01 (0.00–0.03)
Male	624 (52.0)	4117 (51.4)	481 (52.2)	135 (47.9)		
Female	577 (48.0)	3893 (48.6)	441 (47.8)	147 (52.1)		
Gestational age (weeks)	40 (39, 41) ^A	40 (39, 41) ^A	40 (39, 41) ^A	40 (39, 41) ^A	0.02	0.01 (-0.00 to 0.03)

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables, or n (%) (Likelihood Ratio Chi Square test) for categorical variables. *p<0.05. Effect sizes are Wilcoxon test effect size (r) (95% CI) for continuous variables, or Cramer's V (95% CI) for categorical variables. ¹Weight gain recommendations based on Institute of Medicine (2009) guidelines for singleton pregnancies based on prepregnancy BMI. ²Includes diabetes mellitus before pregnancy, during pregnancy, before and during pregnancy, during and post pregnancy, or before, during and post pregnancy. Does not include maternal diabetes mellitus during post partum period only. ³\$1999, \$2000-4999, \$5000-7999, ≥\$8000 equivalent to \$17315, ≤\$17800, \$17801-\$44500, \$44501-\$71200, ≥\$71201 in 2020 US dollars, respectively. ⁴Gravida in home for unwed mothers. ⁵1 pack is 20 cigarettes. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Supplementary Table 2.5. Associations between maternal prepregnancy BMI and macroscopic placental pathologies in term pregnancies, N=10,145.

Placental pathology	Prepregnancy BMI				p-value	Effect size
	UW (n=1201)	NW (n=8010)	OW (n=922)	OB (n=282)		
Placenta						
Placenta shape					0.57	0.03 (0.00–0.03)
Non-partite	1059 (88.2)	7230 (90.2)	847 (91.9)	256 (90.8)		
Bipartite	14 (1.17)	107 (1.34)	8 (0.87)	4 (1.42)		
Tripartite	0	1 (0.01)	1 (0.11)	0		
Succenturiate	19 (1.58)	83 (1.04)	10 (1.08)	2 (0.71)		
Membranous placenta	0	1 (0.01)	1 (0.11)	0		
Crescent shaped	1 (0.08)	1 (0.01)	0	0		
Irregular shape	3 (0.25)	38 (0.47)	5 (0.54)	0		
Unknown/missing	105 (8.74)	549 (6.85)	50 (5.42)	20 (7.09)		
Weight (g)	400 (352, 455) ^A	420 (370, 480) ^B	440 (380, 500) ^C	447 (380, 500) ^{BC}	<0.0001	0.08 (0.06–0.10)
Largest diameter (cm)	19 (17, 20) ^A	19 (18, 20) ^B	19 (18, 20) ^C	19 (18, 20) ^{BC}	<0.0001	0.06 (0.04–0.08)
Smallest diameter (cm)	16 (15, 17) ^A	16 (15, 18) ^B	17 (15, 18) ^C	16.5 (15, 18) ^{BC}	<0.0001	0.05 (0.03–0.07)
Thickness (mm)	20 (20, 25) ^A	20 (20, 25) ^A	21 (20, 25) ^A	20 (20, 25) ^A	0.04	0.02 (-0.0002 to 0.04)
Birthweight (g) : placenta weight (g)	7.60 (6.80, 8.41) ^A	7.46 (6.73, 8.29) ^A	7.32 (6.56, 8.12) ^B	7.29 (6.59, 8.26) ^{AB}	<0.0001	-0.03 (-0.05 to -0.006)
Birthweight (g) : placental largest diameter (cm)	164 (150, 179) ^A	167 (153, 182) ^B	169 (154, 183) ^B	167 (156, 184) ^B	<0.0001	0.05 (0.03–0.07)
Birthweight (g) :placental smallest diameter (cm)	190 (172, 210) ^A	193 (177, 212) ^B	195 (180, 214) ^B	195 (178, 214) ^B	<0.0001	0.04 (0.02–0.07)
Birthweight (g) : placental thickness (mm)	142 (123, 163) ^A	145 (127, 167) ^B	147 (128, 170) ^B	151 (129, 174) ^B	<0.0001	0.04 (0.02–0.06)
Cord						
Cord number of vessels					0.80	0.01 (0.00–0.02)
Two	7 (0.58)	51 (0.64)	4 (0.43)	1 (0.35)		
Three	1088 (90.6)	7409 (92.5)	867 (94.0)	260 (92.2)		
Other/unknown/missing	106 (8.83)	550 (6.87)	51 (5.53)	21 (7.45)		
Cord edema					0.36	0.02 (0.00–0.04)
Not seen	1059 (88.2)	7232 (90.3)	837 (90.8)	251 (89.0)		
Present	16 (1.33)	128 (1.60)	18 (1.95)	8 (2.84)		
Unknown/missing	126 (10.5)	650 (8.11)	67 (7.27)	23 (8.16)		
Membranes and fetal surface						
Opacity of membranes					0.06	0.02 (0.00–0.03)
Not opaque	1023 (85.2)	6971 (87.0)	796 (86.3)	242 (85.8)		
Opaque	70 (5.8)	478 (5.97)	73 (7.9)	18 (6.38)		
Partially opaque	0	12 (0.15)	4 (0.43)	0		
Unknown/missing	108 (8.99)	549 (6.85)	49 (5.31)	22 (7.80)		

Thrombosed fetal vessels					0.02	0.03 (0.003–0.05)
Not seen	1096 (91.3)	7445 (92.9)	870 (94.4)	259 (91.8)		
Present	0	23 (0.29)	3 (0.3)	3 (1.06)		
Unknown/missing	105 (8.74)	542 (6.77)	49 (5.31)	20 (7.09)		
Cut surface						
Infarct size					0.45	0.02 (0.00–0.03)
Not applicable	817 (68.0)	5440 (67.9)	619 (67.1)	183 (64.9)		
All infarcts < 3 cms	238 (19.8)	1717 (21.4)	221 (24.0)	68 (24.1)		
>1 infarct measures ≥3cm	35 (2.9)	280 (3.50)	31 (3.36)	10 (3.55)		
Unknown/missing	111 (9.24)	573 (7.15)	51 (5.53)	21 (7.45)		
Cut surface calcification					0.18	0.02 (0.00–0.03)
None	461 (38.4)	2986 (37.3)	340 (36.9)	124 (44.0)		
Maternal surface only	473 (39.4)	3333 (41.6)	388 (42.1)	104 (36.8)		
Calcification throughout	158 (13.2)	1112 (13.9)	144 (15.6)	33 (11.7)		
Unknown/missing	109 (9.08)	579 (7.23)	50 (5.42)	21 (7.45)		

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables or n (%) (Likelihood Ratio Chi Square test) and for categorical variables. *p<0.05. Effect sizes are Wilcoxon test effect size (r) (95% CI) for continuous variables, and Cramer's V (95% CI) for categorical variables. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Supplementary Table 2.6. Multivariable analyses for associations between maternal prepregnancy BMI (continuous) and macroscopic placental pathologies in term pregnancies, N=10,145.

Placental pathology	Unadjusted model			Adjusted model		
	β (95% CI)	OR (95% CI)	p-value	β (95% CI)	OR (95% CI)	p-value
Placenta						
Weight (g)	2.84 (2.33–3.36)	-	****	3.71 (3.18–4.25)	-	****
Largest diameter (cm)	0.04 (0.03–0.06)	-	****	0.06 (0.04–0.07)	-	****
Smallest diameter (cm)	0.04 (0.03–0.05)	-	****	0.05 (0.03, 0.06)	-	****
Thickness (mm)	0.007 (-0.02 to 0.03)	-	NS	0.05 (0.02, 0.07)	-	**
Birthweight (g) : placenta weight (g)	-0.02 (-0.03 to -0.02)	-	****	-0.02 (-0.03 to -0.02)	-	****
Birthweight (g) : placental largest diameter (cm)	0.20 (0.07–0.33)	-	**	0.43 (0.30–0.57)	-	****
Birthweight (g) : placental smallest diameter (cm)	0.28 (0.11–0.44)	-	***	0.56 (0.39–0.73)	-	****
Birthweight (g) : placental thickness (mm)	0.55 (0.35–0.76)	-	****	0.56 (0.34–0.77)	-	****
Cord						
Cord number of vessels	-0.02 (-0.10 to 0.06)	0.98 (0.90–1.06)	NS	-0.03 (-0.12 to 0.06)	0.97 (0.89–1.06)	NS
Two						
Three						
Cord edema	0.03 (-0.01 to 0.07)	1.03 (0.99–1.07)	NS	0.02 (-0.03 to 0.07)	1.02 (0.97–1.07)	NS
Not seen						
Present						
Membranes and fetal surface						
Opacity of membranes	0.02 (-0.002 to 0.04)	1.02 (1.00–1.05)	NS	0.02 (-0.008 to 0.04)	1.02 (0.99–1.04)	NS
Not opaque						
Any degree of opacity						
Thrombosed fetal vessels	0.06 (-0.02 to 0.15)	1.07 (0.98–1.16)	NS	0.05 (-0.06 to 0.16)	1.05 (0.94–1.18)	NS
Not seen						
Present						
Cut surface						
Infarcts	0.02 (0.006–0.03)	1.02 (1.01–1.03)	**	0.03 (0.02–0.04)	1.03 (1.02–1.05)	****
Not applicable						
Any infarcts seen						
Cut surface calcification	-0.004 (-0.02 to 0.009)	1.00 (0.98–1.01)	NS	0.002 (-0.01 to 0.02)	1.00 (0.99–1.02)	NS
None						
Any calcification seen						

Data are unit odds ratios (95% CI) for presence of pathology and from Logistic regression models with p-value from Likelihood Ratio Chi Square test. The adjusted model includes fetal sex, maternal race, maternal age, smoking history, maternal education, socioeconomic status, gestational weight gain, and maternal diabetes mellitus status. NS (not significant): $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

Supplementary Table 2.7. Associations between maternal prepregnancy BMI and macroscopic placental pathologies in preterm pregnancies, N=1739.

Placental pathology	Prepregnancy BMI				p-value	Effect size
	UW (n=259)	NW (n=1286)	OW (n=156)	OB (n=38)		
Placenta						
Placenta shape					0.43	0.04 (0.00–0.04)
Non-partite	219 (84.6)	1135 (88.4)	142 (91.0)	35 (92.1)		
Bipartite	0	5 (0.39)	0	0		
Tripartite	0	0	0	0		
Succenturiate	5 (1.9)	16 (1.24)	1 (0.64)	1 (2.63)		
Membranous placenta	0	0	0	0		
Crescent shaped	0	0	0	0		
Irregular shape	0	7 (0.54)	0	0		
Unknown/missing	35 (13.5)	123 (9.56)	13 (8.33)	2 (5.26)		
Weight (g)	360 (310, 425) ^A	380 (320, 440) ^{AB}	405 (330, 470) ^B	440 (360, 501) ^B	0.0008	0.04 (-0.006 to 0.09)
Largest diameter (cm)	18 (16, 19) ^A	18 (17, 19) ^A	18 (17, 20) ^A	19 (16, 21) ^A	0.05	0.05 (-0.004 to 0.11)
Smallest diameter (cm)	15 (14, 17) ^A	16 (14, 17) ^{AB}	16 (14, 17) ^{AB}	17 (15, 18) ^B	0.005	0.06 (0.009–0.12)
Thickness (mm)	20 (18, 25)	20 (18, 23)	20 (18, 25)	20 (19, 25)	0.77	-0.0006 (-0.05 to 0.05)
Birthweight (g) : placenta weight (g)	7.34 (6.49, 8.31)	7.22 (6.45, 8.11)	7.21 (6.41, 7.91)	6.99 (6.21, 8.26)	0.49	-0.03 (-0.07 to 0.02)
Birthweight (g) : placental largest diameter (cm)	151 (138, 167)	154 (136, 170)	158 (140, 173)	163 (146, 177)	0.23	0.02 (-0.03 to 0.06)
Birthweight (g) : placental smallest diameter (cm)	174 (161, 195)	178 (159, 198)	180 (162, 204)	183 (171, 204)	0.52	0.02 (-0.04 to 0.07)
Birthweight (g) : placental thickness (mm)	134 (115, 156)	135 (117, 159)	140 (119, 159)	147 (119, 170)	0.23	0.03 (-0.02 to 0.08)
Cord						
Cord number of vessels					0.79	0.02 (0.00–0.05)
Two	1 (0.39)	10 (0.78)	1 (0.64)	0		
Three	223 (86.1)	1154 (89.7)	143 (91.7)	37 (97.4)		
Other/unknown/missing	35 (13.5)	122 (9.49)	12 (7.69)	1 (2.63)		
Cord edema					0.11	0.06 (0.00–0.11)
Not seen	218 (84.2)	1099 (85.5)	131 (84.0)	33 (86.8)		
Present	5 (1.93)	50 (3.90)	10 (6.41)	3 (7.89)		
Unknown/missing	36 (13.9)	137 (10.7)	15 (9.62)	2 (5.26)		
Membranes and fetal surface						
Opacity of membranes					0.28	0.06 (0.00–0.09)
Not opaque	208 (80.3)	1078 (83.8)	129 (82.7)	32 (84.2)		
Opaque	15 (5.79)	86 (6.69)	14 (8.97)	5 (13.2)		
Partially opaque	0	0	1 (0.64)	0		
Unknown/missing	36 (13.9)	122 (9.49)	12 (7.69)	1 (2.63)		

Thrombosed fetal vessels					0.26	0.05 (0.00–0.09)
Not seen	221 (85.3)	1160 (90.2)	141 (90.4)	37 (97.4)		
Present	3 (1.16)	7 (0.54)	3 (1.92)	0		
Unknown/missing	35 (13.5)	119 (9.25)	12 (7.69)	1 (2.63)		
Cut Surface						
Infarct size					0.95	0.02 (0.00–0.02)
Not applicable	178 (68.7)	936 (72.8)	111 (71.2)	28 (73.7)		
All infarcts < 3 cms	34 (13.1)	191 (14.9)	28 (17.9)	7 (18.4)		
At least one infarct measures ≥3 cms	9 (3.47)	39 (3.03)	5 (3.21)	1 (2.63)		
Unknown/missing	38 (14.7)	120 (9.33)	12 (7.69)	2 (5.26)		
Cut surface calcification					0.74	0.03 (0.00–0.05)
None	119 (45.9)	638 (49.6)	85 (54.5)	16 (42.1)		
Maternal surface only	78 (30.1)	396 (30.8)	42 (26.9)	14 (36.8)		
Calcification throughout	25 (9.65)	129 (10.0)	17 (10.9)	6 (15.8)		
Unknown/missing	37 (14.3)	123 (9.56)	12 (7.69)	2 (5.26)		

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables or n (%) (Likelihood Ratio Chi Square test) for categorical variables. *p<0.05. Effect sizes are Wilcoxon test effect size (r) (95% CI) for continuous variables, or Cramer's V (95% CI) for categorical variables. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Supplementary Table 2.8. Multivariable analyses for associations between maternal prepregnancy BMI (continuous) and macroscopic placental pathologies in preterm pregnancies, N=1739.

Placental pathology	Unadjusted model			Adjusted model		
	β (95% CI)	OR (95% CI)	p-value	β (95% CI)	OR (95% CI)	p-value
Placenta						
Weight (g)	3.26 (1.80–4.73)	-	****	3.25 (1.72–4.77)	-	****
Largest diameter (cm)	0.05 (0.01–0.08)	-	**	0.04 (0.01–0.08)	-	**
Smallest diameter (cm)	0.06 (0.03–0.09)	-	***	0.06 (0.03–0.09)	-	***
Thickness (mm)	0.05 (-0.02 to 0.11)	-	NS	0.06 (-0.007 to 0.13)	-	NS
Birthweight (g) : placenta weight (g)	-0.02 (-0.04 to 0.006)	-	NS	-0.01 (-0.04 to 0.02)	-	NS
Birthweight (g) : placental largest diameter (cm)	0.62 (0.22–1.02)	-	**	0.87 (0.45–1.29)	-	****
Birthweight (g) : placental smallest diameter (cm)	0.37 (-0.11 to 0.85)	-	NS	0.67 (0.15–1.18)	-	**
Birthweight (g) : placental thickness (mm)	0.53 (-0.04 to 1.10)	-	NS	0.49 (-0.12 to 1.10)	-	NS
Cord						
Cord number of vessels	0.03 (-0.14 to 0.19)	1.03 (0.87–1.21)	NS	0.04 (-0.13 to 0.22)	1.04 (0.88–1.24)	NS
Two						
Three						
Cord edema	0.07 (0.003–0.13)	1.07 (1.00–1.14)	NS	0.04 (-0.03 to 0.11)	1.04 (0.97–1.12)	NS
Not seen						
Present						
Membranes and fetal surface						
Opacity of membranes	0.05 (0.005–0.10)	1.06 (1.01–1.11)	*	0.05 (-0.007 to 0.11)	1.05 (0.99–1.11)	NS
Not opaque						
Any degree of opacity						
Thrombosed fetal vessels	0.04 (-0.12 to 0.19)	1.04 (0.89 to 1.20)	NS	0.02 (-0.14 to 0.18)	1.02 (0.87–1.19)	NS
Not seen						
Present						
Cut Surface						
Infarcts	0.03 (-0.005 to 0.07)	1.03 (1.00–1.07)	NS	0.03 (-0.006 to 0.08)	1.04 (0.99–1.08)	NS
Not applicable						
Any infarcts seen						
Cut surface calcification	-0.002 (-0.03 to 0.03)	1.0 (0.97–1.03)	NS	-0.009 (-0.04 to 0.02)	0.99 (0.96–1.03)	NS
None						
Any calcification seen						

Data are unit odds ratios (95% CI) for presence of pathology and from Logistic regression models with p-value from Likelihood Ratio Chi Square test. The adjusted model includes fetal sex, maternal race, maternal age, smoking history, maternal education, socioeconomic status, gestational weight gain, and maternal diabetes mellitus status. NS (not significant): $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$.

Supplementary Figure 2.1. Participant flow selection and placental pathologies of interest.

A

B

	Preterm				Term			
	Male (n=22)	Female (n=16)	OR (95% CI)	p-value	Male (n=135)	Female (n=147)	OR (95% CI)	p-value
Neutrophil infiltration								
Umbilical vein			1.00 (0.22, 4.56)	NS			2.08 (1.03, 4.22)	0.04
Not seen	15 (68.2)	12 (75.0)			107 (79.3)	130 (88.4)		
Any degree	5 (22.7)	4 (25.0)			24 (17.8)	14 (9.52)		
Unknown/missing	2 (9.09)	0			4 (2.96)	3 (2.04)		
Umbilical artery			0.76 (0.13, 4.43)	NS			0.96 (0.34, 2.72)	NS
Not seen	17 (77.3)	13 (81.3)			124 (91.9)	136 (92.5)		
Any degree	3 (13.6)	3 (18.8)			7 (5.19)	8 (5.44)		
Unknown/missing	2 (9.09)	0			4 (2.96)	3 (2.04)		
Umbilical cord substance			1.08 (0.20, 5.73)	NS			2.03 (0.82, 5.02)	NS
Not seen	16 (72.7)	13 (81.3)			117 (86.7)	136 (92.5)		
Any degree	4 (18.2)	3 (18.8)			14 (10.4)	8 (5.44)		
Unknown/missing	2 (9.09)	0			4 (2.96)	3 (2.04)		
Amnion membrane			2.33 (0.39, 14.0)	NS			1.53 (0.75, 3.12)	NS
Not seen	15 (68.2)	14 (87.5)			107 (79.3)	117 (79.6)		
Any degree	5 (22.7)	2 (12.5)			21 (15.6)	15 (10.2)		
Unknown/missing	2 (9.09)	0			7 (5.19)	15 (10.2)		
Chorion membrane			1.86 (0.38, 9.00)	NS			1.88 (1.05, 3.36)	0.03
Not seen	14 (63.6)	13 (81.3)			96 (71.1)	120 (81.6)		
Any degree	6 (27.3)	3 (18.8)			36 (26.7)	24 (16.3)		
Unknown/missing	2 (9.09)	0			3 (2.22)	3 (2.04)		
Amnion placenta			0.82 (0.10, 6.62)	NS			1.92 (0.78, 4.75)	NS
Not seen	17 (77.3)	14 (87.5)			113 (83.7)	124 (84.4)		
Any degree	2 (9.09)	2 (12.5)			14 (10.4)	8 (5.44)		
Unknown/missing	3 (13.6)	0			8 (5.93)	15 (10.2)		
Chorion placenta			2.33 (0.39, 14.0)	NS			1.72 (0.86, 3.54)	NS
Not seen	15 (68.2)	14 (87.5)			110 (81.5)	129 (87.8)		
Any degree	5 (22.7)	2 (12.5)			22 (16.3)	15 (10.2)		
Unknown/missing	2 (9.09)	0			3 (2.22)	3 (2.04)		

Supplementary Figure 2.2. Placental neutrophil infiltration in pregnancies with maternal obesity by fetal sex, n=320. Prescence or absence of neutrophil infiltration for female and male placentae among pregnancies with maternal obesity by preterm (male: n=22, female: n=16) and term (male: n=135, female: n=147) birth.

Chapter 3. Preterm birth increases placental expression of multidrug resistance transporters irrespective of prepregnancy body mass index

This chapter forms the basis of a manuscript under review, and a preprint available at: <https://www.medrxiv.org/content/10.1101/2021.07.30.21261204v1.full-text>

3.1 Abstract

Preterm birth (PTB) and suboptimal prepregnancy BMI may operate through inflammatory pathways to impair fetoplacental development. Placental efflux transporters mediate fetal protection and nutrition, however few studies consider the effect of both PTB and BMI on fetal protection. We hypothesized that PTB would alter the expression of placental multidrug resistance (MDR) transporters and selected pro-inflammatory cytokines, and that maternal underweight and obesity would further impair placental phenotype. Our objective was to determine whether placental MDR transporters P-glycoprotein (P-gp, encoded by *ABCB1*) and breast cancer resistance protein (BCRP/*ABCG2*), and pro-inflammatory cytokine levels are altered by PTB and maternal BMI. A cross-sectional study was conducted to assess the effect of PTB (+/- chorioamnionitis), or the effect of maternal prepregnancy BMI on placental MDR transporter and interleukin [IL]-6 and 8 expression in 60 preterm and 36 term pregnancies. *ABCB1* expression was increased in preterm compared to term placentae ($p=0.04$). P-gp ($p=0.008$) and BCRP ($p=0.01$) immunolabeling was increased among all preterm compared to term placentae, with P-gp expression further increased in preterm pregnancies with chorioamnionitis (PTC, $p=0.007$). Placental *IL-6* mRNA expression was decreased in PTC compared to term placentae ($p=0.0005$), and PTC associated with the greatest proportion of anti-inflammatory medications administered during pregnancy. Maternal BMI group did not influence placental outcomes. PTB and infection, but not prepregnancy BMI, alter placental expression of MDR transporters and *IL-6*. This may have implications for fetal exposure to xenobiotics that may be present in the maternal circulation in pregnancies complicated by PTB.

3.2 Introduction

Preterm birth (PTB) is a leading cause of perinatal morbidity/mortality and death among children under five years globally¹⁶³. Infants born preterm are at increased risk for both immediate and longer term health complications, including neurodevelopmental and sensory disabilities^{15,164}, mood disorders, as well as neurological^{17,164} and cardiometabolic diseases later in life^{165,166}. PTB and adverse offspring development have been associated with inflammation and infection⁹¹, however, the etiology of PTB is not well understood.

Maternal underweight and obesity are also prevalent obstetric conditions¹⁻³, and can inflict adverse effects on placental and fetal development, such as maternal vascular malperfusion, placental insufficiency, fetal growth restriction, and neurodevelopmental impairment^{8-10,55,96}. These conditions are associated with chronic, low-grade inflammation¹⁰, and suboptimal quality and quantity of nutrients available to support pregnancy nutritional demands⁹. Despite the increasing burden of these common conditions, little is known about the mechanisms that

contribute to poor fetoplacental development and adverse pregnancy outcomes such as PTB in pregnancies complicated by maternal malnutrition or suboptimal metabolic status.

Both PTB and suboptimal maternal BMI may operate through multifactorial pathways to impair fetoplacental development⁴⁹⁻⁵², including underlying inflammatory pathways^{10,91}. The placenta is a critical regulator of the fetal environment, adjusting nutrient availability to the fetus while simultaneously protecting against the entry of microbes, xenobiotics and toxins throughout pregnancy^{13,16,17}. Inflammation may impair placental function, including its ability to regulate the efflux of potentially harmful substances out of the placenta, or to undergo adaptations to protect the developing fetus from environmental insults⁸. Early activation of inflammatory pathways, including an increase in pro-inflammatory mediators in the placenta such as interleukin [IL]-6 and IL-8, may also lead to uterine contractions, cervical ripening, and membrane rupture¹⁶⁷, culminating in PTB⁹¹. Further, preterm pregnancies with chorioamnionitis (inflammation of the fetal membranes) may be at risk for altered placental parameters such as placental transport, that may contribute to placental barrier dysregulation and intrauterine inflammation. Increased gestational tissue inflammation in chorioamnionitis may adversely affect offspring growth¹⁴ in addition to the developmental consequences associated with reduced gestation length alone. The inflammatory state associated with maternal underweight and obesity also favours increased inflammation of the placenta⁹, thus pregnancies complicated by suboptimal maternal BMI and PTB may be at greatest risk for adverse outcomes.

Placental efflux transporters regulate an array of potentially harmful endogenous and exogenous substances to mediate fetal protection and nutrition³¹. The function of these transporters is regulated in part by inflammatory molecules¹³. Commonly studied in the placenta are the multidrug resistance (MDR) transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), which belong to the ATP-binding cassette (ABC) superfamily of efflux transporters¹³. P-gp is localized to the syncytiotrophoblast and cytotrophoblast cell layers of the placenta, and regulates the efflux of endogenous and synthetic glucocorticoids (cortisol; cortisone; prednisone), estrogens and progestogens, cytokines/chemokines (IL-6; interferon [IFN]- γ ; tumor necrosis factor [TNF- α]), antibiotics (amoxicillin; doxorubicin; levofloxacin; rifampicin), and antiretrovirals^{13,32}. BCRP is localized to the syncytiotrophoblast, cytotrophoblast, and endothelial cells of fetal blood vessels, and transports endogenous steroids, antibiotics (beta-lactams; doxorubicin; fluoroquinolones), antivirals, sulfonyleureas, nutrients, and toxins from the fetus back into the maternal circulation^{13,32}. The expression of placental P-gp and BCRP transporters varies throughout gestation, and may be higher in preterm placentae, and decrease with gestational age³⁴. The effects of maternal BMI on the expression of placental efflux transporters are not well understood, but are critical to study, as in many regions of the world these conditions coexist with other infectious and inflammatory diseases and increased medication use, for which MDR transporters may be affected. Altered expression of these transporters with gestational age or suboptimal maternal BMI may impair the placenta's ability to protect the developing fetus from harmful environmental conditions, potentially contributing to adverse pregnancy and infant outcomes.

Our primary study objective was to determine the effect of PTB on expression levels of MDR transporters and select inflammatory cytokines in the placenta. As both PTB and suboptimal maternal BMI are associated with increased inflammation and adverse pregnancy and infant outcomes, we then assessed the effects of maternal BMI on placental efflux transport potential within term and preterm pregnancies. We hypothesized that PTB would be associated with altered placental efflux transport and increased levels of pro-inflammatory cytokines, and that preterm pregnancies complicated by maternal underweight and obesity would show greatest changes in placental MDR expression levels.

3.3 Methods

3.3.1 Study population

Gestational tissue samples and clinical data were collected from 96 mother-infant dyads from the Research Centre for Women's and Infants' Health BioBank (RCWIH) at Mount Sinai Hospital, Toronto, Canada. Women delivered at term, preterm without chorioamnionitis, or preterm with chorioamnionitis, and were classified as underweight (UW), normal weight (NW), overweight (OW), or having obesity (OB) by prepregnancy BMI according to the World Health Organization and Society of Obstetricians and Gynaecologists of Canada¹⁶⁸ guidelines, with the exception of women classified as underweight. Due to a low prevalence of women considered underweight in the study region, a prepregnancy BMI of less than 19 was considered to be underweight, 19 to 24.9 normal weight, 25.0 to 29.9 overweight, and 30.0 or greater obese. Among 60 preterm pregnancies, 12 women were classified as underweight by prepregnancy BMI, 17 as normal weight, 18 as overweight, and 13 as having obesity, and among 36 term pregnancies, 9 were classified as underweight, 7 normal weight, 9 overweight, and 11 obese. Based on current recommendations from the Institute of Medicine (IOM) guidelines (2009), maternal weight gain was categorized as inadequate, adequate, or excessive for singleton pregnancies based on prepregnancy BMI, where the recommended weight gain ranges are 12.5-18 kilograms, 11.5-16 kilograms, 7-11.5 kilograms or 5-9 kilograms¹¹⁹ for mothers who are underweight, normal weight, overweight or who have obesity, respectively. Study inclusion criteria included singleton pregnancies and live birth with no known fetal anomalies. As our aim is to understand how PTB and low and high maternal BMI influence pregnancy outcomes and program development, our cohort exclusion criteria comprised comorbidities that may potentially confound data interpretation due to their known effects on placental development and function and the inflammatory milieu: gestational diabetes mellitus, hypertension (including pregnancy induced hypertension), lupus, antiphospholipid antibody syndrome, Crohn's disease, ulcerative colitis, colitis, Guillain-Barré syndrome, sexually transmitted infections, gastritis, urinary tract infections, smokers, documented recreational drug use during pregnancy, HELLP syndrome, pelvic inflammatory disease, and *in vitro* fertilization. Placentae were collected and snap frozen or fixed for downstream molecular and histologic analyses, according to RCWIH placental collection and storage protocols. Clinical data from the pregnancies (e.g. maternal prepregnancy BMI, medication history, mode of delivery) were obtained from chart reviews.

3.3.2 RNA extraction and qPCR

Total RNA was extracted from placental tissue using the QIAGEN RNeasy Mini Kit (CAT #74134) according to the manufacturer's instructions. Placental RNA samples were quantified and assessed for purity by Nanodrop (DeNovix DS-11 Spectrophotometer). cDNA was synthesized from RNA samples using the Bio-Rad iScript gDNA Clear cDNA Synthesis Kit (CAT #1725035) according to the manufacturer's instructions.

Real-time quantitative PCR (qPCR) was used to measure the relative placental mRNA expression levels of MDR transporter genes *ABCB1* (encoding P-gp) and *ABCG2* (encoding BCRP), and *IL-6* and *IL-8*, key pro-inflammatory cytokines involved in the pathogenesis of PTB¹⁶⁹⁻¹⁷¹ and correlated to alterations in MDR (P-gp and BCRP) expression and/or function^{99,172}. Genes of interest were normalized to the geometric mean of three stably expressed reference genes, β -actin (*ACTB*), glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), and TATA-Box binding protein (*TBP*). Primer sequences for the genes of interest and reference genes can be found in Supplementary Table 3.1. qPCR reactions were performed for each gene using 2 μ l of cDNA in 8 μ l of Master Mix, prepared with Bio-Rad SsoAdvanced Universal SYBR Green (CAT #1725274) and 5 μ M forward and reverse primer mix. Standards, samples, and a non-template control were run in triplicate using a Bio-Rad CFX384 real-time PCR detection system with the following cycling conditions: 95°C for 30 seconds, 40 cycles of 95°C for five seconds and 60°C for 20 seconds, followed by a melt curve from 65°C to 95°C at 0.5°C increments for five seconds. Relative mRNA expression was calculated using the $\Delta\Delta C_q$ method (Bio-Rad CFX Maestro 1.1 Version: 4.1.2433.1219).

3.3.3 Immunohistochemistry

Five μ m placental sections were dewaxed and rehydrated, followed by boiling/cooling series of antigen retrieval in 10 mM citric acid/sodium citrate solution at pH 6.0, by microwaving and cooling for 10 minutes on ice. Sections were washed twice with 1X PBS, then quenched in 0.3% hydrogen peroxide in 97% methanol for 30 minutes at room temperature (RT). Following two more washes in 1X PBS with 0.1% Tween, sections were blocked with serum-free protein block (DAKO) for one hour (RT). Placentae were then incubated with P-gp antibody (Abcam) at 1:400 dilution or BCRP antibody (Abcam) at 1:1200 dilution in antibody diluent (DAKO) at 4°C overnight. Antibody diluent was incubated with negative control sections instead of primary antibodies. Sections were washed three times in 1X PBS with 0.1% Tween, then incubated with Biotin-conjugated secondary antibody (SPD-060-Spring Bioscience) for one hour (RT). Following three more washes in 1X PBS with 0.1% Tween, streptavidin-horseradish peroxidase (SPD-060-Spring Bioscience) was then applied for one hour (RT). Sections were washed twice with in 1X PBS with 0.1% Tween, and DAB peroxidase substrate (Vector Laboratories) was applied for one minute (RT). Slides were counter-stained with haematoxylin (Sigma-Aldrich) prior to mounting.

Semi-quantitative scoring of staining immunolabeled area and intensity were performed for all samples across the syncytiotrophoblast, cytotrophoblast, endothelium, and connective tissue as

previously described with adaptations⁹⁹. Staining in immunolabeled areas was scored as absent (0), 1-25% (1), 26-50% (2), 51-75% (3) and 76-100% (4).

3.3.4 Statistical analyses

3.3.4.1 Univariate analyses

Univariate analyses were conducted to evaluate differences in MDR transporter and inflammatory cytokine expression across gestational age and maternal BMI groups. Differences in MDR transporter and inflammatory cytokine expression levels were first assessed between term and preterm pregnancies (inclusive of infection), as expression levels of MDR transporters and inflammatory markers are known to change over the course of pregnancy^{13,77,173}, and between term, preterm without chorioamnionitis, and preterm with chorioamnionitis pregnancies, to determine the effect of the additional impact of infection on these placental parameters. Next, the effect of maternal prepregnancy BMI, specifically maternal underweight, overweight, and obesity prepregnancy compared to normal weight as the reference category, on placental MDR transporter and cytokine expression levels was assessed in term and preterm pregnancies separately. Finally, placental data were stratified by fetal sex to assess sex differences in placental transport and inflammation in term and preterm pregnancies. Outcome measures were tested for normality and unequal variances (Levene test). Where possible, non-normal molecular data were log transformed to achieve normality. Differences between gestational age or maternal prepregnancy BMI groups and continuous outcome measures were determined by ANOVA with Tukey *post hoc* or Welch with Games-Howell *post hoc* for normally distributed data, or Kruskal–Wallis test with Steel–Dwass *post hoc* for nonparametric data. For categorical data, Likelihood Ratio Chi Square tests were used to evaluate the associations between gestational age or maternal BMI groups and categorical outcome variables, testing for differences in the distribution of participants by variable category across gestational age or maternal prepregnancy BMI group. Statistical significance was defined as $p < 0.05$. Data were analysed using JMP statistical software (Version:14.0.0). Data are presented as median (interquartile range) for non-parametric continuous data, and frequency (percentage) for categorical variables.

3.3.4.2 Multivariable analyses

We performed multivariable regression to determine the relationships between gestational age at delivery or maternal prepregnancy BMI with MDR transporter and inflammatory cytokine expression levels. Linear (Standard Least Squares) regression models were used to determine the associations between gestational age (continuous), or maternal BMI (continuous) and MDR transporter and inflammatory cytokine expression levels. For all BMI analyses, regressions were stratified by term and preterm birth. Data are reported as β (95% confidence interval) and p value from Standard Least Squares regression models. Covariates of interest were identified *a priori* and included gestational age (weeks), maternal prepregnancy BMI (continuous), infant sex, z-gestational weight gain, antibiotic use (yes/no), and chorioamnionitis status. An unadjusted model was first used to identify the associations between gestational age at delivery or prepregnancy BMI alone (as continuous variables) with placental MDR transporter and

inflammatory cytokine expression levels. An adjusted model was then used to determine associations between gestational age at delivery or prepregnancy BMI with placental transporter and cytokine expression levels, adjusting for gestational age at delivery (weeks), maternal prepregnancy BMI (continuous), infant sex, z-gestational weight gain, antibiotic use, and chorioamnionitis status.

3.4 Results

3.4.1 Maternal clinical characteristics

Inclusive of BMI, mothers who delivered preterm were younger than mothers who delivered at term (preterm, PT: 32 years [29.3, 35]; term, T: 34 years [31.3, 37.8], $p=0.005$), and had lower present weight (PT: 73 kg [63.9, 84.5]; T: 84 kg [67.2, 95], $p=0.02$) and total gestational weight gain (PT: 9.3 kg [6.68, 12.3]; T: 14 kg [12, 18], $p<0.0001$) than mothers who delivered at term. There were no differences in prepregnancy weight or prepregnancy BMI between preterm and term pregnancies. More mothers with preterm births received antibiotics (PT: $n=52$, 86.7%; T: $n=21$, 58.3%, $p=0.0002$), glucocorticoids (PT: $n=53$, 88.3%; T: $n=1$, 2.78%, $p<0.0001$), and tocolytics (PT: $n=22$, 36.7%; T: $n=0$, $p<0.0001$) than term births. All mothers who delivered preterm and had chorioamnionitis received glucocorticoids, tocolytics, and/or antibiotics during pregnancy, followed by 97% of mothers who delivered preterm but did not have chorioamnionitis, and 58% of mothers who delivered at term ($p<0.0001$, Table 3.1). Specifically, 93% of mothers who delivered preterm and had chorioamnionitis were administered antibiotics, followed by 81% of preterm pregnancies without chorioamnionitis, and 58% of term pregnancies ($p=0.0003$, Table 3.1). No mothers who delivered at term received tocolytics. All (100%) preterm pregnancies with chorioamnionitis, 77% of preterm, and one pregnancy that delivered at term (3%) were administered glucocorticoids ($p<0.0001$, Table 3.1). Twenty-nine (48%) of preterm pregnancies recruited for this study had chorioamnionitis, and 31 (52%) were preterm pregnancies without chorioamnionitis. Of the preterm pregnancies with chorioamnionitis, fetal membrane inflammation grade (defined as 0 [no inflammation], 1 [moderate clusters of neutrophils], 2 [severe, confluent neutrophils between chorion and decidua], or 3 [microabscesses between chorion and decidua]) was mostly classified as inflammation grade 2 (47%), with one membrane classified as grade 3 (5.3%). Most preterm gestational tissues without infection (65%) and at term (89%) were classified as inflammation grade 0.

Among pregnancies that delivered preterm, most mothers who were underweight, normal weight, or had obesity prepregnancy had inadequate gestational weight gain, while most mothers classified as overweight prepregnancy had adequate weight gain based on IOM (2009) guidelines by prepregnancy BMI ($p=0.02$, Table 3.2). In contrast, few mothers at term had inadequate gestational weight gain; most mothers who were underweight prepregnancy were classified as having adequate weight gain, an equal number of mothers who were normal weight had adequate or excessive weight gain, and most mothers who were overweight or had obesity had excessive gestational weight gain ($p=0.005$, Table 3.3). Mothers with obesity who delivered preterm were also more likely to receive antibiotics during pregnancy: 100% of mothers with

obesity received antibiotics, followed by 91.7% of mothers who were underweight, 88.2% who were normal weight, and 72.2% of mothers who were overweight ($p=0.04$, Table 3.2). There were no differences in antibiotic administration or other demographic or pregnancy characteristics among mothers who delivered at term by maternal BMI group (Table 3.3).

3.4.2 Placental IL-6 mRNA expression was decreased with preterm birth and chorioamnionitis infection

Placental *IL-6* mRNA expression levels were decreased in all preterm (with and without chorioamnionitis) compared to term pregnancies ($p=0.0002$, Figure 3.1A), and levels increased with advancing gestational age (unadjusted model: $\beta=0.04$ [0.02, 0.06], $p=0.001$). When stratifying by term and preterm +/- chorioamnionitis status, placental *IL-6* mRNA expression levels were lower in preterm pregnancies with chorioamnionitis compared to term pregnancies ($p=0.0005$, Figure 3.1A). There was no effect of gestational age or chorioamnionitis status on placental *IL-8* mRNA expression. Placental cytokine expression levels were also not different across maternal BMI groups at preterm or term (Supplementary Table 3.2), or when comparing male and female placentae at preterm or term (Supplementary Table 3.3).

3.4.3 MDR transporter mRNA and protein expression were increased in preterm placentae

Placental *ABCB1* mRNA expression levels were increased in all preterm pregnancies (with and without of chorioamnionitis) compared to term pregnancies ($p=0.04$, Figure 3.1B). mRNA expression levels of *ABCG2* did not differ by gestational age. There was also no effect of chorioamnionitis status on placental *ABCB1* or *ABCG2* mRNA expression levels (Figure 3.1B). Placental P-gp (PT: score of 3 [1, 4]; T: score of 1 [0, 3.25], $p=0.008$) and BCRP (PT: score of 4 [4, 4]; T: score of 4 [4, 4], $p=0.01$) protein immunolabeled areas of the syncytiotrophoblast (but not other layers of the placenta) were also increased among all preterm pregnancies compared to term pregnancies. Further, there was an effect of infection status on P-gp protein, where syncytiotrophoblast immunolabeled area of P-gp was increased in preterm pregnancies with chorioamnionitis compared to preterm without chorioamnionitis and term pregnancies ($p=0.007$, Figure 3.2A-B, Figure 3.3). There was also a small statistical difference in BCRP syncytiotrophoblast immunolabeled area across gestational age and infection groups, though the median staining value for all groups was 4 (76-100% of immunolabeled area staining): all placentae from preterm pregnancies without chorioamnionitis demonstrated 76-100% area stained, and thus scored with a 4, while staining in term pregnancies ranged from 0 (absent) to 4 (76-100% of area), suggesting increased BCRP staining in preterm placentae without chorioamnionitis ($p=0.03$, Figure 3.2A-B, Figure 3.4). There was no effect of maternal BMI group on placental mRNA or protein expression of *ABCB1*/P-gp or *ABCG2*/BCRP among term or preterm pregnancies (Supplementary Table 3.2, Figure 3.5A-C). When considering maternal BMI as a continuous variable, placental mRNA expression of *ABCG2*, but not *ABCB1*, increased with increasing maternal BMI at preterm (unadjusted and adjusted $\beta=0.008$ [0.0001, 0.02], $p=0.047$). There were no differences in placental *ABCG2* or *ABCB1* mRNA expression by maternal BMI

(continuous) at term. There was no effect of placental sex on mRNA or protein expression of *ABCB1*/P-gp or *ABCG2*/BCRP among term or preterm pregnancies (Supplementary Table 3.3).

3.4.4 Placental weight and efficiency and infant Apgar scores were decreased at preterm

Placental weight ($p < 0.0001$) and birthweight to placental weight ratio ($p < 0.0001$) were decreased in preterm pregnancies with chorioamnionitis compared to preterm without chorioamnionitis and term pregnancies, but there was no effect of maternal BMI on placental anthropometry among preterm or term pregnancies (Table 3.4, 3.5). There were no differences in standardized infant birthweight between preterm and term pregnancies, however, when stratified by maternal BMI group, standardized birthweight increased with increasing maternal BMI among preterm and term infants (Table 3.5). Apgar scores at one minute ($p = 0.0003$) were decreased in preterm pregnancies with chorioamnionitis compared to term infants, and in both preterm pregnancies with and without chorioamnionitis compared to term infants at five minutes ($p = 0.0003$, Table 3.4). Preterm pregnancies with chorioamnionitis also had the lowest median gestational age at birth, followed by preterm pregnancies without infection, and term pregnancies ($p < 0.0001$, Table 3.4).

3.5 Discussion

We examined the effect of PTB and maternal prepregnancy BMI on placental expression levels of MDR transporters and key inflammatory cytokines to better understand the placental mechanisms through which these common conditions may lead to poor pregnancy outcomes. We found a gestational age effect, where placental *ABCB1*/P-gp mRNA and protein expression levels were increased, and placental *IL-6* mRNA expression levels were decreased, in all preterm pregnancies compared to term pregnancies, effects that were amplified in preterm pregnancies with chorioamnionitis. Mothers who delivered preterm with chorioamnionitis also received the greatest proportion of glucocorticoids, antibiotics, and tocolytics during pregnancy, which may explain reductions in placental *IL-6* mRNA expression levels. There was no effect of maternal BMI group or placental sex on expression levels of placental MDR transporters or select inflammatory cytokines among term or preterm pregnancies, however there was a small significant difference where placental mRNA expression of *ABCG2* increased with increasing maternal BMI at preterm. PTB and chorioamnionitis thus alter the expression of key placental transporters and inflammatory regulators, which may impact placental development and function.

Our observations that placental transporter expression levels were increased in all preterm pregnancies is consistent with previous findings that suggest the expression of P-gp and BCRP transporters in the placenta decrease with gestational age^{34,76}. Placental *ABCB1* mRNA and syncytiotrophoblast P-gp protein levels are higher in first trimester compared to term^{77,78}, supporting the important role of P-gp in fetal exchange and protection, especially at earlier stages of pregnancy⁷⁷. In contrast, no differences in *ABCG2* mRNA levels were found across gestation. We did, however, find increased BCRP protein expression in preterm placentae, suggesting a disconnect between gene and protein level results, which could be in part explained by the semi-

quantitative nature of immunohistochemistry, or may suggest a functional adaptation in protein translation in response to other regulating factors, such as placental oxygen levels⁷⁷. Placental P-gp and BCRP expression have also been shown to be increased in mothers exhibiting high white blood cell count, which may be an indication of infection³⁴, in line with our findings of increased transporter expression in preterm pregnancies with chorioamnionitis. While results were not significant at the mRNA level, placental P-gp protein expression in the syncytiotrophoblast was highest in preterm pregnancies with chorioamnionitis compared to term pregnancies. In contrast, other studies have shown increased *ABCB1* and *ABCG2* mRNA and BCRP protein levels, but decreased P-gp protein levels with chorioamnionitis, which could be explained by post-transcriptional influences of inflammation⁹⁹. Our results suggest that P-gp protein expression is not only increased in preterm pregnancies, but is further upregulated in preterm pregnancies complicated by chorioamnionitis, likely as a functional adaptation to protect the fetus from microbial and inflammatory cytokine entry into the intrauterine compartment by increasing placental efflux mechanisms. The placenta adapts its efflux mechanisms to protect the developing fetus from increased inflammation and infection present in preterm pregnancies^{17,18}.

In our cohort, lower *IL-6* and unchanged *IL-8* mRNA expression in placentae of preterm pregnancies, including those with chorioamnionitis, may reflect a beneficial response to anti-inflammatory drug exposure to prevent PTB. *IL-6* plays a role in stimulating prostaglandin synthesis, an important hormone in the process of parturition, which may mediate the initiation of preterm labour¹⁷⁴. In addition, IL-6 is involved in acute and chronic inflammation, assisting the immune response in the recruitment and activation of leukocytes^{175,176}, and involved in infection, where placental IL-6 mRNA¹⁷⁷ and protein expression levels have been shown to increase with PTB and PTB associated with microbial invasion¹⁷⁸. Previous studies have also shown elevated levels of IL-6 protein in the amniotic fluid¹⁷⁹, maternal serum¹⁷⁷ and placental decidual cells¹⁸⁰ in pregnancies with chorioamnionitis. However, maternal medications administered during pregnancy in an effort to prevent PTB may reduce placental inflammation. Glucocorticoids can be used to reduce sterile intrauterine inflammation due to their anti-inflammatory properties, and umbilical cord plasma IL-6 concentrations have been shown to be lower in preterm pregnancies that received at least one dose of corticosteroids compared to those that received no glucocorticoids¹⁸¹. Tocolytics may be used to delay PTB and provide the opportunity to enhance fetal maturation through administration of corticosteroids, and tocolytics themselves also have anti-inflammatory properties^{182,183}. Tocolytics have been shown to suppress levels of pro-inflammatory mediators, including *IL-6* mRNA levels, in human placental cells stimulated by LPS and animal models of maternal infection¹⁸². Further, intrauterine infection may elicit an inflammatory innate immune response, which may contribute to preterm labour⁵⁰, and antibiotics may reduce amniotic infection-associated inflammation¹⁸⁴. In our cohort, mothers who delivered preterm and had chorioamnionitis received the greatest proportion of glucocorticoids, tocolytics, and antibiotics, followed by mothers who delivered preterm without chorioamnionitis, with term pregnancies receiving the lowest proportion of medications. Our data thus suggest that antenatal medications administered to prolong gestation and optimize offspring outcomes are successful in reducing some placental inflammation, though did not prevent preterm birth in this cohort.

Pregnancies with suboptimal maternal BMI may also be complicated by underlying inflammatory processes, however, in our cohort, maternal underweight and obesity did not affect the expression of placental MDR transporters or inflammatory cytokines. There was a small significant difference in placental mRNA expression levels of *ABCG2*, but not *ABCB1*, where placental mRNA expression of *ABCG2* augmented with increasing maternal BMI in preterm pregnancies. While there are a lack of data on the effect of maternal BMI on placental efflux transport function, animal studies have shown increased *Abcg2*, and decreased placental *Abcb1a* mRNA expression levels in high fat fed compared to undernourished pregnant mice⁷⁴. In human pregnancy, maternal obesity has also been associated with downregulated placental *ABCB1* gene expression⁷⁵. These alterations in placental efflux transporter expression in pregnancies complicated by maternal obesity could result in differences in placental transfer, with potential consequences for fetal drug exposure¹²⁹. Maternal obesity has also been associated with increased levels of pro-inflammatory mediators in the placenta. In contrast to our findings, placental mRNA expression levels of *IL-6* have been shown to be increased in placentae from mothers with obesity compared to lean mothers^{185,186}. *IL-8* has also been shown to be increased in placentae from women with obesity compared to non-obese placentae, but with no difference in *IL-6* placental expression¹⁰. Notably, our study criteria ensured the exclusion of maternal comorbidities such as gestational diabetes mellitus and hypertension, to better understand the effect of suboptimal BMI alone on outcomes. These comorbidities could be driving altered placental inflammation^{187,188} and efflux transport^{189,190} seen in other studies with maternal obesity and underweight. Including these conditions, and perhaps cases with more severe classes of maternal obesity, or a greater number of mothers who were underweight, may be needed to corroborate the effects of suboptimal maternal BMI on the placental pro-inflammatory phenotype.

Other strengths of our study include the cohort selection with corresponding clinical metadata; our study sample consists of data from 96 pregnancies, among term and preterm pregnancies (with and without chorioamnionitis), with further stratification by maternal underweight, normal weight, overweight, and obese BMI groups. This selection allowed for analysis not only by gestational age and infection status but by maternal BMI, which to our knowledge has not been previously done, with sufficient sample sizes. A limitation of our study is that only two select pro-inflammatory cytokines were measured, whereas the collective effect of multiple cytokines may be more indicative of the dynamic fluctuations in pro- and anti-inflammatory mediators during infection or labour. The cytokines selected for assessment have been previously demonstrated to be upregulated in PTB and maternal obesity^{185,186}, and may still be reflective of underlying inflammatory mechanisms in these conditions^{180,191}. Overall, our data will help to address gaps in knowledge on the impact of preterm birth, infection, and maternal prepregnancy BMI, in the absence of other maternal comorbidities, on placental-mediated protection of the fetus.

Our data show that preterm birth and infection status, but not maternal underweight or obesity, in otherwise uncomplicated pregnancies, alter placental expression of *IL-6* and MDR efflux transporters. Inflammation and infection have been associated with PTB and underweight/obesity, however, the underlying mechanisms linking these exposures to adverse pregnancy outcomes remain poorly understood. While current treatments for preterm delivery

aim to delay the progression of contractions and eradicate infection, increased levels of inflammation *in utero* may still have consequences for fetal development. The upregulation of placental BCRP and P-gp at preterm may have implications for the biodisposition of drugs that could affect the developing fetus⁷⁸, and should be considered in clinical treatment. Further, decreased placental *IL-6* may represent a beneficial response to anti-inflammatory medications administered for preterm labour. Altered placental expression of efflux transporters and inflammatory mediators may have implications for fetal exposure to xenobiotics in pregnancies complicated by PTB that could impair fetoplacental development.

Table 3.1. Maternal antibiotics, tocolytics, and glucocorticoids administered during pregnancy among preterm pregnancies with chorioamnionitis, preterm pregnancies without chorioamnionitis, and term pregnancies.

	Preterm Chorio (n=29)	Preterm (n=31)	Term (n=36)	p-value
Any medication received ¹ (n [%])				<0.0001
Yes	29 (100)	30 (96.8)	21 (58.3)	
No	0	1 (3.23)	15 (41.7)	
Antibiotics				
Received antibiotics (n [%])				0.0003
Yes	27 (93.1)	25 (80.6)	21 (58.3)	
No	1 (3.45)	4 (12.9)	15 (41.7)	
Missing	1 (3.45)	2 (6.45)	0	
Antibiotics given (n [%]) ²				-
Amoxicillin	3 (11.1)	1 (4.0)	0	
Ampicillin	7 (25.9)	2 (8.0)	0	
Ancef	5 (18.5)	2 (8.0)	4 (19.0)	
Cefazolin	0	3 (12.0)	7 (33.3)	
Clindamycin	3 (11.1)	2 (8.0)	0	
Erythromycin	13 (48.1)	9 (36.0)	1 (4.76)	
Flagyl	1 (3.70)	0	0	
Gentamycin	6 (22.2)	0	0	
Macrobid	0	1 (4.0)	0	
Penicillin G	13 (48.1)	10 (40.0)	1 (4.76)	
Tobramycin	2 (7.41)	0	0	
Vancomycin	1 (3.70)	2 (8.0)	1 (4.76)	
Antibiotic dose (mg) ²				-
Amoxicillin	1250 (875, 1625)	2000	-	
Ampicillin	2000 (1000, 2000)	2000	-	
Ancef	2000 (1625, 2000)	2000	2000	
Cefazolin	-	2000 (2000, 2500)	2000 (2000, 2000)	
Clindamycin	900	900	-	
Erythromycin	250	250	.	
Flagyl	.	-	-	
Gentamycin	80	-	-	
Macrobid	-	5000	-	
Penicillin G ³	2500 (2500, 3750)	5000 (2500, 5000)	.	
Tobramycin	80	-	-	
Vancomycin	500	1000	.	
Tocolytics				
Received tocolytics (n [%])				<0.0001
Yes	11 (37.9)	11 (35.5)	0	
No	17 (58.6)	20 (64.5)	36 (100)	
Missing	1 (3.45)	0	0	
Tocolytic type (n [%])				0.20
MgSO ₄	7 (63.6)	4 (36.4)	-	
MgSO ₄ and Morphine	1 (9.09)	0	-	
Adalat	3 (27.3)	6 (54.6)	-	
Adalat and Indomet	0	1 (9.09)	-	
Glucocorticoids				
Received glucocorticoids (n [%])				<0.0001

Yes	29 (100)	24 (77.4)	1 (2.78)	
No	0	7 (22.6)	35 (97.2)	
Number of courses given	2 (2, 2)	2 (2, 2)	2	0.67
Missing	n=1	n=2	-	
Gestational age given	26 (24, 28) ^A	31 (26, 32) ^B	28 ^{AB}	0.03
Missing	n=2	n=1	-	

Data are n (%) (Likelihood Ratio Chi Square test) for categorical variables or median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables. *p<0.05. ¹Received maternal antibiotics, glucocorticoids, and/or tocolytics. ²Some mothers were given multiple types of antibiotics. ³Some mothers were given multiple doses of Penicillin G. Preterm Chorio = preterm with chorioamnionitis.

Table 3.2. Maternal and pregnancy characteristics by prepregnancy BMI in preterm pregnancies.

	Pregpregnancy BMI				
	UW (n=12)	NW (n=17)	OW (n=18)	OB (n=13)	p-value
Maternal characteristics					
Prepregnancy weight (kg)	51.5 (46.5, 54.1) ^A	57 (54, 61) ^B	68.3 (64, 78.2) ^C	91 (82.2, 98.8) ^D	<0.0001
Prepregnancy BMI	18.5 (17.3, 18.8) ^A	21.2 (20.0, 23.6) ^B	26.7 (25.9, 27.8) ^C	34 (31.8, 36.9) ^D	<0.0001
Present weight (kg)	62 (54.3, 68.2) ^A	65 (63, 73) ^A	79.7 (74, 89.7) ^B	101 (87.5, 119) ^C	<0.0001
Gestational weight gain (kg)	9.3 (7, 17.0)	10 (7, 12)	10.5 (6.75, 14.4)	4.0 (0.5, 9.5)	0.10
Z-Gestational weight gain	-0.39 (-0.67, 0.53)	-0.31 (-0.67, -0.07)	-0.25 (-0.70, 0.22)	-1.03 (-1.45, -0.37)	0.10
Gestational weight gain ¹ (n [%])					0.02
Inadequate	8 (66.7)	10 (58.8)	3 (16.7)	6 (46.1)	
Adequate	3 (25.0)	5 (29.4)	6 (33.3)	1 (7.69)	
Excessive	1 (8.33)	0	5 (27.8)	2 (15.4)	
Unknown/missing	0	2 (11.8)	4 (22.2)	4 (30.8)	
Maternal age (years)	29.5 (24.5, 34)	32 (29.5, 35)	32 (29.3, 35)	33 (31, 35)	0.34
Ethnicity (n [%])					0.31
Asian	2 (16.7)	1 (5.88)	0	0	
Black	3 (25.0)	5 (29.4)	6 (33.3)	2 (15.4)	
Caucasian	4 (33.3)	6 (35.3)	8 (44.4)	8 (61.5)	
East Asian	2 (16.7)	0	0	0	
East Indian	1 (8.33)	2 (11.8)	1 (5.56)	2 (15.4)	
Latin/ Hispanic	0	1 (5.88)	1 (5.56)	0	
Middle Eastern	0	0	2 (11.1)	1 (7.69)	
Other	0	1 (5.88)	0	0	
Missing	0	1 (5.88)	0	0	
Gravidity	1.5 (1, 2.75) ^A	1 (1, 5) ^A	4 (2, 5) ^A	2 (1, 3) ^A	0.045
Parity	0.5 (0, 1)	0 (0, 1)	1 (0, 2)	0.5 (0, 2)	0.20
Pregnancy and delivery characteristics					
Received antibiotics (n [%])					0.04
Yes	11 (91.7)	15 (88.2)	13 (72.2)	13 (100)	
No	1 (8.33)	0	4 (22.2)	0	
Unknown	0	2 (11.8)	1 (5.56)	0	
Received tocolytics (n [%])					0.20
Yes	4 (33.3)	7 (41.2)	9 (50.0)	2 (15.4)	
No	8 (66.7)	9 (52.9)	9 (50.0)	11 (84.6)	

Missing	0	1 (5.88)	0	0	
Received glucocorticoids (n [%])					0.32
Yes	12 (100)	15 (88.2)	15 (83.3)	11 (84.6)	
No	0	2 (11.8)	3 (16.7)	2 (13.3)	
Mode of delivery (n [%])					0.06
C-section (labour)	1 (8.33)	5 (29.4)	3 (16.7)	5 (38.5)	
C-section (no labour)	4 (33.3)	0	5 (27.8)	2 (15.4)	
Vaginal	7 (58.3)	12 (70.6)	10 (55.6)	6 (46.2)	
Complications (n [%])					0.30
Yes	2 (16.7)	3 (17.7)	1 (5.56)	4 (30.8)	
No	10 (83.3)	14 (82.4)	17 (94.4)	9 (69.2)	

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*) for continuous variables or n (%) (Likelihood Ratio Chi Square test) for categorical variables. *p<0.05. ¹Recommendations based on IOM (2009) guidelines for gestational weight gain in singleton pregnancies based on prepregnancy BMI. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Table 3.3. Maternal and pregnancy characteristics by prepregnancy BMI in term pregnancies.

	Prepregnancy BMI				
	UW (n=9)	NW (n=7)	OW (n=9)	OB (n=11)	p-value
Maternal characteristics					
Prepregnancy weight (kg)	50 (46.5, 54.3) ^A	61 (59, 64) ^B	71.4 (60.8, 79.8) ^B	94 (76.5, 116) ^C	<0.0001
Prepregnancy BMI	18.4 (17.7, 18.7) ^A	22.4 (21.1, 24.4) ^B	26.3 (25.7, 27.5) ^C	35.5 (31.7, 44.1) ^D	<0.0001
Present weight (kg)	64.5 (63.1, 66.7) ^A	79 (72, 86) ^{AB}	87.7 (74.4, 92.5) ^{BC}	102 (90, 122) ^C	<0.0001
Gestational weight gain (kg)	14.6 ± 3.22	17.4 ± 5.98	17.4 ± 4.29	12.6 ± 5.32	0.13
Z-Gestational weight gain	0.24 ± 0.39	0.58 ± 0.72	0.57 ± 0.51	0.003 ± 0.64	0.13
Gestational weight gain ¹ (n [%])					0.005
Inadequate	1 (11.1)	1 (14.3)	0	0	
Adequate	6 (66.7)	3 (42.9)	0	3 (27.3)	
Excessive	1 (11.1)	3 (42.9)	8 (88.9)	7 (63.6)	
Unknown/missing	1 (11.1)	0	1 (11.1)	1 (9.09)	
Maternal age (years)	34.2 ± 3.70	33.4 ± 2.99	35.6 ± 3.40	34.2 ± 4.71	0.73
Ethnicity (n [%])					0.20
Asian	4 (44.4)	1 (14.3)	2 (22.2)	0	
Black	0	0	2 (22.2)	2 (18.2)	
Caucasian	3 (33.3)	6 (85.7)	4 (44.4)	5 (45.5)	
East Asian	0	0	0	0	
East Indian	1 (11.1)	0	0	1 (9.09)	
Latin/ Hispanic	0	0	0	1 (9.09)	
Middle Eastern	0	0	0	1 (9.09)	
Other	0	0	0	1 (9.09)	
Missing	1 (11.1)	0	1 (11.1)	0	
Gravidity	2 (1, 3)	2 (1, 2)	2 (1, 3.5)	3 (2, 4)	0.18
Parity	1 (0, 1)	1 (0, 1)	0 (0, 1)	1 (1, 2)	0.13
Pregnancy and delivery characteristics					
Received antibiotics (n [%])					0.24
Yes	4 (44.4)	4 (57.1)	4 (44.4)	9 (81.8)	
No	5 (55.6)	3 (42.9)	5 (55.6)	2 (18.2)	
Unknown	0	0	0	0	
Received glucocorticoids (n [%])					0.49
Yes	0	0	0	1 (9.09)	
No	9 (100)	7 (100)	9 (100)	10 (90.1)	

Mode of delivery (n [%])					0.12
C-section (labour)	0	0	1 (11.1)	2 (18.2)	
C-section (no labour)	3 (33.3)	4 (57.1)	2 (22.2)	7 (63.6)	
Vaginal	6 (66.7)	3 (42.9)	6 (66.7)	2 (18.2)	
Complications (n [%])					0.53
Yes	2 (22.2)	3 (42.9)	1 (11.1)	3 (27.3)	
No	7 (77.8)	4 (57.1)	8 (88.9)	8 (72.7)	

Data are means $\pm$ SD (ANOVA; normal distribution/equal variance) or median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*). Data are n (%) (Likelihood Ratio Chi Square test) for categorical variables. * $p < 0.05$. ¹Recommendations based on IOM (2009) guidelines for gestational weight gain in singleton pregnancies based on prepregnancy BMI. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Table 3.4. Infant characteristics in preterm pregnancies with chorioamnionitis, preterm pregnancies without chorioamnionitis, and term pregnancies.

	Gestational age and infection status			p-value
	Preterm Chorio (n=29)	Preterm (n=31)	Term (n=36)	
Infant characteristics				
Sex (n [%])				0.37
Female	13 (44.8)	12 (38.7)	20 (55.6)	
Male	16 (55.7)	19 (61.3)	16 (44.4)	
Placental weight (g)	368 (303, 463) ^A	500 (400, 650) ^B	600 (543, 743) ^C	<0.0001
Z-birthweight	0.02 (-0.34, 0.33)	0.3 (-0.71, 0.89)	0.14 (-0.49, 0.74)	0.85
Birthweight (g) : placental weight (g)	3.43 ± 1.04 ^A	4.43 ± 1.05 ^B	5.54 ± 1.05 ^C	<0.0001
Apgar score				
1 minute	8 (4, 8) ^A	8.5 (6, 9) ^{AB}	9 (8, 9) ^B	0.0003
5 minutes	8 (6, 9) ^A	9 (8, 9) ^A	9 (9, 9) ^B	0.0001
10 minutes	7 (5, 8) ^A	9 (8, 9) ^B	9 (9, 9) ^B	0.005
Gestational age (weeks)	28.1 (26.6, 31.4) ^A	34.7 (31.9, 35.6) ^B	39.1 (38.6, 39.7) ^C	<0.0001

Data are means ± SD (ANOVA; normal distribution/equal variance with Tukey *post-hoc*) or median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*). Data are n (%) (Likelihood Ratio Chi Square test) for categorical variables. *p<0.05.

Table 3.5. Infant characteristics by maternal prepregnancy BMI group in preterm and term pregnancies.

	Prepregnancy BMI				p-value
	UW (n=9-12)	NW (n=7-17)	OW (n=9-18)	OB (n=11-13)	
Preterm pregnancies					
Sex (n [%])					0.47
Female	5 (41.7)	5 (29.4)	10 (55.6)	5 (38.5)	
Male	7 (58.3)	12 (70.6)	8 (44.4)	8 (61.5)	
Placental weight (g)	410 (350, 490)	400 (330, 530)	448 (323, 690)	480 (388, 670)	0.41
Z-birthweight	-0.34 ± 0.94 ^A	-0.02 ± 0.71 ^{AB}	0.20 ± 0.65 ^{AB}	0.59 ± 1.09 ^B	0.0498
Birthweight (g) : placental weight (g)	4.39 ± 1.19	3.96 ± 1.23	3.73 ± 1.19	3.90 ± 1.00	0.52
Apgar score					
1 minute	8 (6.25, 8)	8 (4.5, 9)	8 (5, 9)	8.5 (3.75, 9)	0.93
5 minutes	8.5 (8, 9)	9 (8, 9)	9 (8, 9)	9 (8, 9)	0.87
10 minutes	8 (8, 8)	8 (2.75, 8.75)	7 (7, 9)	9 (5, 9)	0.90
Gestational age (weeks)	32.1 (29, 34.2)	31.3 (27, 34.7)	31 (27.5, 34.8)	33.3 (29.6, 35.2)	0.81
Term pregnancies					
Sex (n [%])					0.05
Female	3 (33.3)	2 (28.6)	6 (66.7)	9 (81.8)	
Male	6 (66.7)	5 (71.4)	3 (33.3)	2 (18.2)	
Placental weight (g)	500 (465, 620)	700 (563, 743)	610 (550, 780)	618 (570, 1000)	0.13
Z-birthweight	-0.31 (-0.78, 0.11) ^A	0.02 (-0.49, 0.75) ^A	0.14 (-0.27, 0.66) ^A	0.62 (0.09, 2.28) ^A	0.04
Birthweight (g) : placental weight (g)	6.11 ± 0.87	5.45 ± 1.00	5.46 ± 0.74	5.15 ± 1.35	0.25
Apgar score (n [%])					
1 minute	9 (8, 9)	9 (9, 9)	9 (8.5, 9)	9 (6.75, 9)	0.47
5 minutes	9 (9, 9)	9 (9, 9)	9 (9, 9)	9 (8.75, 9)	0.37
10 minutes	-	-	9	9 (9, 9)	1.00
Gestational age (weeks)	39.3 ± 0.52	39.3 ± 1.05	39.8 ± 1.30	38.7 ± 0.88	0.12

Data are means ± SD (ANOVA; normal distribution/equal variance with Tukey *post-hoc*) or median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*). Data are n (%) (Likelihood Ratio Chi Square test) for categorical variables. *p<0.05.

Figure 3.1. Placental mRNA expression levels of (A) *IL-6* and *IL-8* pro-inflammatory cytokines and (B) *ABCB1* and *ABCG2* efflux transporters. Placental *IL-6* mRNA expression levels were decreased in all preterm (with and without chorioamnionitis) compared to term placentae (preterm: 0.85 [0.58, 1.20]; term: 1.13 [0.99, 1.50], $p=0.0002$), and further decreased in preterm pregnancies with chorioamnionitis compared to term placentae (PTC: 0.63 [0.45-1.18]^A; PT: 0.90 [0.73-1.27]^{AB}; T: 1.13 [0.99-1.50]^B, $p=0.0005$). There was no effect of gestational age or chorioamnionitis status on placental *IL-8* mRNA expression. Placental *ABCB1* mRNA expression levels were increased in all preterm compared to term placentae (preterm: 1.02 [0.59, 1.21]; term: 0.65 [0.38, 1.00], $p=0.04$), but there was no effect of chorioamnionitis. Placental mRNA expression levels of *ABCG2* did not differ by gestational age or infection status. Data are median [IQR] with 95% confidence diamonds and p-value from ANOVA, or Welch test with Games-Howell post-hoc, * $p<0.05$. *IL-8* = interleukin 8. *IL-6* = interleukin-6. *ABCB1* = gene encoding P-glycoprotein. *ABCG2* = gene encoding breast cancer resistance protein. PTC = preterm with chorioamnionitis. PT = preterm without chorioamnionitis. T = term.

Figure 3.2. P-gp and BCRP protein staining of immunolabeled areas in preterm pregnancies with chorioamnionitis, preterm pregnancies without chorioamnionitis, and term pregnancies, across four placental regions. (A) Box plots of P-gp and BCRP staining of immunolabeled areas in preterm pregnancies with chorioamnionitis, preterm pregnancies without chorioamnionitis, and term pregnancies. P-gp immunolabeled area staining of the syncytiotrophoblast was increased in preterm pregnancies with chorioamnionitis compared to term pregnancies. BCRP immunolabeled area staining of the syncytiotrophoblast was increased in preterm pregnancies without chorioamnionitis compared to term pregnancies. There were no differences in P-gp or BCRP immunolabeling of the cytotrophoblast, fetal vessel endothelium, or connective tissue stratified by gestational age and infection group. Staining was scored as absent (0), 1-25% (1), 26-50% (2), 51-75% (3) and 76-100% (4). Semi-quantitative analysis of staining in immunolabeled area data were analysed using Kruskal–Wallis test with Steel–Dwass post hoc. $p < 0.05$. (B) Heatmap of staining of immunolabeled areas in preterm pregnancies with chorioamnionitis (PTC), preterm without chorioamnionitis (PT), and term (T) pregnancies. P-gp = P-glycoprotein. BCRP = breast cancer resistance protein. Preterm chorio = preterm with chorioamnionitis.

Figure 3.3. Representative IHC images of P-gp staining in human placentae across maternal prepregnancy BMI groups stratified by gestational age and infection status. Arrow indicates immunolabeled syncytiotrophoblast. Arrowhead indicates unlabeled cytotrophoblast. Asterisk indicates fetal blood vessels with no labeled endothelium. P-gp = P-glycoprotein. Preterm chorio = preterm with chorioamnionitis. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Figure 3.4. Representative IHC images of BCRP staining in human placentae across maternal prepregnancy BMI groups stratified by gestational age and infection status. Arrows indicate labeled syncytiotrophoblast. Arrowhead indicates cytotrophoblast. Asterisks indicate fetal blood vessels with no labeled endothelium. BCRP = Breast cancer resistance protein. Preterm chorio = preterm with chorioamnionitis. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Figure 3.5. Placental P-gp and BCRP protein staining of immunolabeled area in pregnancies with maternal underweight, normal weight, overweight, or obesity prepregnancy stratified by preterm and term pregnancies, across four placental regions. Box plots of P-gp and BCRP staining of immunolabeled areas by maternal prepregnancy BMI group in preterm (A) and term (B) pregnancies. There were no differences in placental P-gp or BCRP immunolabeled area staining by maternal prepregnancy BMI group at preterm or term. Staining was scored as absent (0), 1-25% (1), 26-50% (2), 51-75% (3) and 76-100% (4). Semi-quantitative analysis of staining in immunolabeled area data were analysed using Kruskal–Wallis test with Steel–Dwass post hoc. $p < 0.05$. (C) Heatmap of staining of immunolabeled areas across maternal prepregnancy BMI group in preterm and term pregnancies. P-gp = P-glycoprotein. BCRP = Breast cancer resistance protein. Preterm chorio = preterm with chorioamnionitis. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Supplementary Table 3.1. Primer sequences for real-time quantitative PCR gene studies.

Gene	Primer sequence
<i>ABCB1</i>	Forward: 5'-GCCCTTGTTAGACAGCCTCA-3' Reverse: 5'-GGCTTTGTCCAGGGCTTCTT-3'
<i>ABCG2</i>	Forward: 5'-TGGAATCCAGAACAGAGCTGGGGT-3' Reverse: 5'-AGAGTTCCACGGCTAAACACTGC-3'
<i>IL-6</i>	Forward: 5'-CCGCCCCACACAGACAG-3' Reverse: 5'-CCGTCGAGGATGTACCGAA-3'
<i>IL-8</i>	Forward: 5'-CTTGTCATTGCCAGCTGTGT-3' Reverse: 5'-TGACTGTGGAGTTTTGGCTG-3'
<i>ACTB</i>	Forward: 5'-AAACTACCTTCAACTCCATC-3' Reverse: 5'-ATGATCTTGATCTTCATTGT-3'
<i>GAPDH</i>	Forward: 5'-GAAGGTGAAGGTCGGAGTCAA-3' Reverse: 5'-GGAAGATGGTGATGGGATTTC-3'
<i>TBP</i>	Forward: 5'-CACGAACCACGGCACTGAT-3' Reverse: 5'-GTTGGTGGGTGAGCACAAGG-3'

Supplementary Table 3.2. Effect of maternal prepregnancy BMI on mRNA expression levels of placental MDR transporter and inflammatory genes in preterm and term pregnancies.

Placental gene	Prepregnancy BMI				p-value
	UW (n=6-8)	NW (n=6-16)	OW (n=7-18)	OB (n=8-11)	
Preterm pregnancies					
ABCB1	1.14 (0.54, 1.32)	1.10 (0.59, 1.28)	0.91 (0.58, 1.21)	0.66 (0.59, 1.05)	0.64
ABCG2	0.62 ± 0.23	0.64 ± 0.19	0.67 ± 0.20	0.75 ± 0.19	0.45
IL-6	0.85 ± 0.29	0.82 ± 0.47	0.91 ± 0.36	0.96 ± 0.41	0.83
IL-8	0.18 (0.13, 0.84)	0.33 (0.14, 1.09)	0.55 (0.28, 0.84)	0.22 (0.15, 0.44)	0.23
Term pregnancies					
ABCB1	0.65 (0.34, 1.33)	0.67 (0.38, 1.01)	0.61 (0.34, 0.76)	0.75 (0.53, 1.81)	0.30
ABCG2	0.76 ± 0.34	0.78 ± 0.23	0.70 ± 0.33	0.78 ± 0.29	0.95
IL-6	1.31 ± 0.50	1.24 ± 0.41	0.97 ± 0.19	1.38 ± 0.44	0.26
IL-8	0.26 (0.23, 0.48)	0.22 (0.13, 0.62)	0.25 (0.14, 0.34)	0.64 (0.23, 0.82)	0.21

Data are means ± SD (ANOVA; normal distribution/equal variance) or median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data). *p<0.05. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Supplementary Table 3.3. Effect of fetal sex on mRNA and protein expression levels of placental inflammatory genes and MDR transporters among preterm and term pregnancies.

	Preterm			Term		
	Male (n=34)	Female (n=22)	p-value	Male (n=14)	Female (n=18)	p-value
Gene						
<i>IL-6</i>	0.93 ± 0.39	0.76 ± 0.32	0.14	1.25 ± 0.32	1.10 ± 0.30	0.22
<i>IL-8</i>	0.32 (0.15, 0.79)	0.47 (0.18, 0.84)	0.99	0.40 (0.22, 0.62)	0.27 (0.16, 0.69)	0.57
Gene						
<i>ABCB1</i>	0.98 ± 0.47	0.92 ± 0.36	0.62	0.65 (0.39, 0.94)	0.71 (0.38, 1.16)	0.79
<i>ABCG2</i>	0.64 ± 0.19	0.72 ± 0.21	0.17	0.65 ± 0.26	0.83 ± 0.29	0.10
Protein ¹						
P-gp	3 (1, 4)	3 (1, 4)	0.64	1.5 (0, 4)	1 (0, 3)	0.76
BCRP	4 (4, 4)	4 (4, 4)	0.75	4 (3.75, 4)	4 (4, 4)	0.72

Data are means ± SD (ANOVA; normal distribution/equal variance) or median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data). *p<0.05. ¹Immunolabeled area staining of the syncytiotrophoblast.

Chapter 4. Preterm birth, but not maternal prepregnancy BMI, alters placental expression of antimicrobial peptides

4.1 Abstract

The placenta expresses antimicrobial peptides (AMPs) to protect against infection and inflammation *in utero*. Maternal underweight and obesity associate with chronic, low-grade inflammation and adverse pregnancy outcomes, including preterm birth. Preterm birth independently associates with infection and inflammation in pregnancy, however, the effects of preterm delivery or suboptimal maternal BMI on gestational tissue defences are not well understood. We hypothesized that pregnancies complicated by maternal underweight and obesity would have altered placental AMP expression at preterm and term, and that preterm birth alone would also alter placental defences. The associations between preterm birth or maternal BMI on placental expression of AMPs acyloxyacyl hydrolase (AOAH), cathelicidin (CAMP), human β -defensin 1 (DEFB1), granulysin (GNLY), and secretory leukocyte protease inhibitor (SLPI) were assessed in a cohort of 60 preterm and 36 term pregnancies using qPCR and immunohistochemistry. We also explored the effects of placental sex and relationships between placental AMP expression and infant outcomes at birth. Preterm birth associated with reduced placental mRNA expression of *AOAH*, *CAMP*, and *DEFB1*, but increased *AOAH* and *CAMP* protein expression, compared to term placentae. Placental *CAMP* mRNA expression was further decreased in preterm pregnancies with chorioamnionitis compared to term placentae. Male placentae demonstrated differential AMP expression at preterm and term compared to females. Altered placental AMP expression correlated with altered infant birthweight, Apgar scores, and placental efficiency. Maternal BMI did not affect expression levels of placental AMP mRNA or protein at preterm or term. Preterm birth, but not maternal BMI, alters the expression of placental AMPs, and AMP expression correlates with infant outcomes at birth. Our findings suggest that these innate defence systems may be impaired in preterm placentae, with implications for infant outcomes, and further supports the role of placental inflammation in adverse pregnancy outcomes.

4.2 Introduction

Maternal underweight and obesity are common maternal conditions, associated with adverse pregnancy and offspring outcomes, including preterm birth. Suboptimal maternal BMI has been associated with increased levels of inflammation in the mother and increased risk of infection during pregnancy^{9,126}. Preterm birth is also related to inflammation and infection in pregnancy, and independently associates with impaired fetoplacental development^{14,15,18,91}. The placenta is a critical immunological barrier and regulator of the intrauterine environment, that can adapt to environmental exposures to protect the developing fetus, including by regulating inflammation⁷. Placental inflammation has been increasingly implicated in a number of adverse pregnancy conditions, including preeclampsia¹⁹², fetal growth restriction¹⁹³, and preterm labour¹⁹⁴.

Among the placenta's protective mechanisms include the ability to express antimicrobial peptides (AMPs) to defend against infection and inflammation *in utero*³⁸⁻⁴¹. AMPs are small proteins produced by epithelial surfaces and inflammatory cells, that modulate infectious and inflammatory stimuli as part of the innate immune system³⁸⁻⁴⁰. Acyloxyacyl hydrolase (AOAH), cathelicidin (CAMP), human β -defensin 1 (DEFB1), granulysin (GNLY), and secretory leukocyte protease inhibitor (SLPI), among others, are AMPs that have been identified in gestational tissues. In addition to direct antimicrobial activity³⁸⁻⁴⁰, AMPs also play a role in immune modulation such as through opsonization, the production of cytokines and chemokines, and antiprotease and antiendotoxin activities³⁸⁻⁴¹. Specifically, AOAH has been shown to be involved in microbial defence through inactivation of lipopolysaccharides by deacylation in the gastrointestinal tract⁴², though its role has not been well established in the placenta. CAMP, a broad-spectrum antimicrobial, is involved in chemotaxis and angiogenesis, among other immune modulatory functions⁴³. DEFB1 is an antimicrobial characterized to defend against both gram-positive and negative bacteria⁴⁴, and GNLY is cytolytic, prominently expressed in natural killer cells⁴⁵. Finally, SLPI has antimicrobial and antiprotease functions, and regulates inflammatory responses⁴⁶. Given the associations between dysregulated inflammation and microbial invasion in the placenta and fetal membranes with adverse pregnancy outcomes such as preterm birth^{14,92,194}, AMPs may therefore play an important role in modulating infection and inflammation at the maternal-fetal interface in pregnancy³⁸.

Altered expression of placental AMPs, such as with gestational age or suboptimal maternal BMI, may impair the placenta's ability to protect the developing fetus from environmental insults. However, little is known about how placental AMP expression is altered with maternal underweight or obesity. Further, although some data exist on the expression of AMPs in gestational tissues with preterm birth, their expression in the cell layers of the placenta specifically, have not been well characterized. Deficiencies in AMPs may be permissive of prolonged inflammatory responses and adverse outcomes, while a mounted AMP response to inflammatory states may aid in the clearance of pathogens^{42,195,196}. Inflammatory exposure in the intrauterine compartment can also impair fetal growth and development, particularly key organs that are sensitive to pro-inflammatory stimuli like the brain^{11,12}, and heightened AMP expression or activity may thus improve outcomes for the offspring by reducing inflammation *in utero* and prolonging gestation. Expression of AMPs in the placenta in pregnancies complicated by preterm birth and suboptimal maternal BMI are likely to be important given the suggested role of infection and inflammation^{18,126} underlying poor pregnancy outcomes in these conditions.

Our primary study objective was to determine the effect of gestational age and infection on placental expression of AOAH, CAMP, DEFB1, GNLY, and SLPI, and to determine whether prepregnancy maternal underweight and obesity, conditions also associated with altered inflammatory and infectious pathways, altered expression of placental AMPs at preterm and term. Our secondary objective was to determine whether placental expression of AMPs was associated with newborn growth and development measures. We hypothesized that preterm birth would be associated with increased placental defences, and that preterm pregnancies complicated by maternal underweight or obesity would have further impaired gestational tissue expression of AMPs and adverse infant outcomes. Our findings uncover mechanisms linking

preterm birth and poor maternal metabolic state with impaired placental defence, and may suggest that targeting placental AMP expression could prove useful in ameliorating adverse pregnancy outcomes.

4.3 Methods

4.3.1 Study population

This study was approved by the Mount Sinai Hospital Research Ethics Board (17-0186-E) and the Carleton University Research Ethics Board (106932). Ninety-six mothers were recruited from the Research Centre for Women's and Infants' Health BioBank (RCWIH) at Mount Sinai Hospital, Toronto, Canada, the same cohort as described in *Chapter 3*. Women delivered at term (T), preterm without chorioamnionitis (PT), or preterm with chorioamnionitis (PTC), and were classified as underweight (UW), normal weight (NW), overweight (OW), or having obesity (OB) by prepregnancy BMI according to the World Health Organization and Society of Obstetricians and Gynaecologists of Canada¹⁶⁸ guidelines, with the exception of women classified as underweight. Due to a low prevalence of women considered underweight in the study region, a prepregnancy BMI of less than 19 was considered to be underweight, 19 to 24.9 normal weight, 25.0 to 29.9 overweight, and 30.0 or greater obese. The study group consisted of 60 preterm pregnancies, where women were classified as UW (n=12), NW (n=17), OW (n=18), or OB (n=13) by prepregnancy BMI, and 36 term pregnancies classified as UW (n=9), NW (n=7), OW (n=9), and OB (n=11). Study inclusion criteria included singleton pregnancies and live birth with no known fetal anomalies. To understand how preterm birth and suboptimal maternal BMI influence placental defence and pregnancy outcomes, our cohort exclusion criteria included comorbidities that may confound data interpretation due to their known effects on placental development and function and the inflammatory milieu: gestational diabetes mellitus, hypertension (including pregnancy induced hypertension), lupus, antiphospholipid antibody syndrome, Crohn's disease, ulcerative colitis, colitis, Guillain-Barré syndrome, sexually transmitted infections, gastritis, urinary tract infections, smokers, documented recreational drug use during pregnancy, HELLP syndrome, pelvic inflammatory disease, and in vitro fertilization. Placentae were collected and snap frozen or fixed according to RCWIH protocols for molecular and histologic analyses. Clinical data (e.g. maternal prepregnancy BMI, medication history, mode of delivery) and infant growth and development measures taken at the time of birth were obtained from chart reviews.

4.3.2 RNA extraction and qPCR

Total RNA was extracted from placental tissue using the QIAGEN RNeasy Mini Kit (CAT #74134) according to the manufacturer's instructions. Placental RNA samples were quantified and assessed for purity by Nanodrop (DeNovix DS-11 Spectrophotometer). cDNA was synthesized from RNA samples using the Bio-Rad iScript gDNA Clear cDNA Synthesis Kit (CAT #1725035) according to the manufacturer's instructions.

Real-time quantitative PCR (qPCR) was used to measure the relative expression levels of AMP genes *AOAH*, *CAMP*, *DEFB1*, *SLPI*, and *GNLY* in placentae (Supplementary Table 1). Genes of interest were normalized to the geometric mean of three stably expressed reference genes, β -actin (*ACTB*), glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), and hypoxanthine phosphoribosyltransferase 1 (*HPRT1*, Supplementary Table 1). qPCR reactions were performed for each gene using 2 μ l of cDNA in 8 μ l of Master Mix, prepared with Bio-Rad SsoAdvanced Universal SYBR Green (CAT #1725274) and 5 μ M forward and reverse primer mix. Standards, samples, and a non-template control were run in triplicate using a Bio-Rad CFX384 real-time PCR detection system with the following cycling conditions: 95°C for 30 seconds, 40 cycles of 95°C for five seconds and 60°C for 20 seconds, followed by a melt curve from 65°C to 95°C at 0.5°C increments for five seconds. Relative mRNA expression was calculated using the $\Delta\Delta C_q$ method (Bio-Rad CFX Maestro 1.1 Version: 4.1.2433.1219).

4.3.3 Immunohistochemistry

Five μ m placental sections were dewaxed and rehydrated, followed by boiling/cooling series of antigen retrieval in 10 mM citric acid/sodium citrate solution at pH 6.0, by microwaving and then cooling for 10 minutes on ice. Sections were washed twice with 1X PBS, then quenched in 0.3% hydrogen peroxide in 97% methanol for 30 minutes at room temperature (RT). Following two more washes in 1X PBS with 0.1% Tween, sections were blocked with serum-free protein block (DAKO) for one hour (RT). Placentae were then incubated with *AOAH* antibody (Abcam) at 1:75 dilution or *CAMP* antibody (Abcam) at 1:200 dilution in antibody diluent (DAKO) at 4°C overnight. Antibody diluent was incubated with negative control sections instead of primary antibodies. Sections were washed three times in 1X PBS with 0.1% Tween, then incubated with secondary goat biotinylated anti-mouse (Vector Laboratories) at 1:200 dilution in antibody diluent for one hour (RT). Following three more washes in 1X PBS with 0.1% Tween, streptavidin-horseradish peroxidase (Invitrogen) diluted to 1:2000 in 1X PBS was then applied for one hour (RT). Sections were washed twice with in 1X PBS with 0.1% Tween, and DAB peroxidase substrate (Vector Laboratories) was applied for one minute (RT). Slides were counter-stained with haematoxylin (Sigma-Aldrich) prior to mounting.

Images were acquired on a Evos FL Auto 2 imaging microscope (Invitrogen, Thermo Fisher Scientific). Thirty images at 20x magnification were captured at randomly selected points per each placental sample. Semi-quantitative scoring of staining intensity was performed for all samples across the syncytiotrophoblast, cytotrophoblast, endothelium, and connective tissue cell layers by one reviewer blinded to participant groups as previously described⁹⁹. Staining in immunolabeled areas was scored as absent (0), weak (1), moderate (2), strong (3) and very strong (4). Percent antibody area stained in total placental villi was quantified (Image J, v1.53e). First, the total placental villi area was quantified, then the antibody of interest area stained was quantified. Percent antibody area stained was obtained by dividing the antibody area stained by the total placental villi area. ImageJ assessments were performed by one reviewer blinded to the study groups.

4.3.4 Statistical analyses

4.3.4.1 Univariate analyses

First, differences in AMP expression levels were assessed between term and all preterm pregnancies, and between term, preterm without chorioamnionitis, and preterm with chorioamnionitis pregnancies, to determine the effect of the additional impact of infection on placental defence. Next, the effect of maternal prepregnancy BMI, specifically maternal underweight, overweight, and obesity prepregnancy compared to normal weight as the reference category, on placental AMP expression levels was assessed in term and preterm pregnancies separately. Finally, placental data were stratified by fetal sex to assess sex differences in placental defence at term and preterm. Outcome measures were tested for normality and unequal variances (Levene test). Where possible, non-normal gene data were log transformed to achieve normality. Differences between gestational age or maternal prepregnancy BMI groups and continuous outcome measures were determined by ANOVA with Tukey *post hoc* or Welch with Games-Howell *post hoc* for normally distributed data, or Kruskal–Wallis test with Steel–Dwass *post hoc* for nonparametric data. For categorical data, Likelihood Ratio Chi Square tests were used to evaluate the associations between gestational age or maternal BMI groups and categorical outcome variables, testing for differences in the distribution of participants by variable category across gestational age or maternal prepregnancy BMI group. Associations between placental antimicrobial expression levels and infant outcomes at birth were assessed by Spearman's correlation analyses at preterm and term. Statistical significance was defined as $p < 0.05$. Data were analysed using JMP statistical software (Version:14.0.0). Data are presented as median (interquartile range) for non-parametric continuous data, and frequency (percentage) for categorical variables, and Spearman's rho for correlations.

4.3.4.2 Multivariable analyses

We performed multivariable regression to determine the relationships between gestational age at delivery or maternal prepregnancy BMI with placental AMP expression levels. Linear (Standard Least Squares) regression models were used to determine the associations between gestational age (continuous), or maternal BMI (continuous) and placental AMP expression levels. For all BMI analyses, regressions were stratified by term and preterm birth. Data are reported as β (95% confidence interval) and p value from Standard Least Squares regression models. Covariates of interest were identified *a priori* and included gestational age (weeks), maternal prepregnancy BMI (continuous), infant sex, z-gestational weight gain, antibiotic use (yes/no), and chorioamnionitis status. An unadjusted model was first used to identify the associations between gestational age at delivery or prepregnancy BMI alone (as continuous variables) with placental AMP expression levels. An adjusted model was then used to determine associations between gestational age at delivery or prepregnancy BMI with placental AMP expression levels, adjusting for gestational age at delivery (weeks) or maternal prepregnancy BMI (continuous), and infant sex, z-gestational weight gain, antibiotic use, and chorioamnionitis status.

4.4 Results

4.4.1 Maternal and infant demographics

For maternal and infant characteristics, please refer to *Chapter 3. Preterm birth increases placental expression of multidrug resistance (MDR) transporters irrespective of prepregnancy body mass index (BMI)*.

4.4.2 Placental AMP mRNA expression levels were decreased, and protein expression increased at preterm

mRNA expression levels of *AOAH* were decreased in preterm placentae compared to term ($p=0.04$, Figure 4.1), while *AOAH* protein staining intensity of the syncytiotrophoblast, connective tissue, and fetal vessel endothelium decreased with increasing gestational age (unadjusted model: $p<0.05$, Table 4.1, Figure 4.2, 4.3). Placental *CAMP* mRNA expression was lower in preterm compared to term placentae ($p<0.0001$, Figure 4.1), and increased with increasing gestational age (unadjusted model: $p=0.04$, Table 4.1). In contrast, proportion of *CAMP* area stained and *CAMP* protein staining intensity in the syncytiotrophoblast, cytotrophoblast, and connective tissue decreased with increasing gestational age (unadjusted model, Table 4.1, Figure 4.2, 4.4). There was also an effect of infection status on placental *CAMP* expression levels but not on other AMPs: preterm pregnancies with chorioamnionitis had significantly lower *CAMP* expression compared to term pregnancies ($p=0.0002$, Table 4.2). *DEFB1* mRNA expression was also lower in preterm compared to term placentae ($p=0.04$, Figure 4.1), but was not altered when considering gestational age as a continuous variable. Placental *GNLY* mRNA expression levels decreased with increasing gestational age (unadjusted model: $p=0.01$, Table 4.1). There were no differences in placental *SLPI* mRNA expression levels stratified by gestational age or when considering gestational age as a continuous variable.

4.4.3 Placental AMP expression was not altered by suboptimal maternal BMI

There was no effect of maternal BMI on placental AMP mRNA or protein expression levels at preterm or term when comparing BMI groups or considering maternal BMI as a continuous variable in unadjusted and adjusted models (Table 4.3).

4.4.4 Male placentae had altered AMP expression at preterm and term

At preterm, *CAMP* cytotrophoblast staining intensity was lower in male compared to female placentae ($p=0.02$), but there were no differences in *CAMP* mRNA expression due to placental sex (Table 4.4). Placental mRNA expression levels of *DEFB1* were slightly lower in preterm male compared to female placentae ($p=0.049$, Table 4.4). There were no differences in *AOAH* protein expression, or *AOAH*, *GNLY*, or *SLPI* mRNA by placental sex at preterm. At term, mRNA expression levels of *AOAH* ($p=0.047$), but not *AOAH* protein, and *GNLY* ($p=0.03$) were higher in male

compared to female placentae (Table 4.4). There were no differences in CAMP mRNA or protein, or *SLPI* mRNA expression levels stratified by placental sex at term.

4.4.5 Placental expression of AMPs correlated with infant outcomes

Among preterm pregnancies, placental AMP mRNA expression levels did not correlate with infant outcomes. At term, placental mRNA expression of *AOAH* ($p=0.03$) and *CAMP* ($p=0.04$) positively correlated with standardized infant birthweight (Table 4.5). There were no associations between AMP mRNA expression and placental weight, birthweight to placental weight ratio, or gestational age at term.

4.5 Discussion

We evaluated the effect of preterm birth and prepregnancy maternal underweight and obesity on placental expression of antimicrobial peptides. Preterm birth associated with reduced placental mRNA expression levels of *AOAH*, *CAMP*, and *DEFB1*, but increased *AOAH* and *CAMP* protein, compared to term placentae. Preterm pregnancies with chorioamnionitis had decreased placental *CAMP* mRNA expression levels compared to term placentae. Placental sex also influenced the expression of AMPs in a gestational age dependent manner, where male placentae had decreased *DEFB1* mRNA expression and *CAMP* protein cytotrophoblast staining intensity at preterm, and increased *AOAH* and *GNLY* mRNA expression at term compared to female placentae. Suboptimal maternal BMI, however, did not associate with altered expression levels of placental AMP mRNA or protein at preterm or term. Common inflammatory and infectious exposures in pregnancy alter placental AMP defence mechanisms, but regulation of these mechanisms needs to be better understood.

AMPs may play a protective role in pregnancy by modulating placental infection and inflammation³⁸. In our cohort, preterm birth associated with decreased expression of placental *CAMP* mRNA, and increased *CAMP* protein. *CAMP* is predominantly produced by neutrophils, in addition to epithelial cells and macrophages¹⁹⁷, and the precursor peptide encoded by *CAMP* has been shown to be largely pre-synthesized and stored in neutrophil secondary granules rather than being immediately released^{43,198}. Our discrepancy in *CAMP* gene and protein expression may thus reflect increased *CAMP* storage and subsequent release from granules, supported by previous studies that have observed a similar disconnect in *CAMP* gene and protein expression in human myometrium⁴³. In support of our protein data, *CAMP* in cervicovaginal fluid has been shown to be increased in women who delivered preterm compared to women who had term births⁸³, perhaps as a functional mounted response against infection/inflammation. *CAMP* expression has been characterized in amniotic fluid¹⁹⁷, cervicovaginal fluid samples^{83,199}, myometrium⁴³, and fetal membranes²⁹, but to our knowledge, localization in the placental cell layers has not been well established. This study shows that *CAMP* staining intensity was greatest in the syncytiotrophoblast cell layer of the placenta, in line with the importance of the syncytiotrophoblast acting as a physical structural barrier and its ability to secrete immune mediators, given its direct contact with the maternal circulation²⁰⁰.

CAMP protein has also been observed to be increased in human myometrium and fetal membranes in term labour compared to non-labour^{43,201}. Given the influx of neutrophils in gestational tissues during labour, and that CAMP is predominantly produced by neutrophils, the inflammatory process of parturition may also play a role in increasing CAMP production²⁰¹. In our cohort, 84% of preterm pregnancies underwent vaginal delivery or a C-section with labour, compared to 16% of pregnancies that delivered via C-section with no labour. At term, 55% of mothers delivered vaginally or by C-section with labour, and 45% underwent a C-section without labour. Increased labouring deliveries at preterm may thus further support the increase of CAMP protein observed in preterm placentae. It has also been suggested that CAMP expression may be largely regulated by vitamin D³⁸. Preterm birth has been associated with vitamin D deficiencies, perhaps in part due to reduced vitamin D induction of CAMP to combat placental infection²⁰², however vitamin D levels were not assessed in this study.

AOAH gene expression was decreased in preterm compared to term placentae, and *AOAH* protein decreased with increasing gestational age. To date, *AOAH* expression has not been well characterized in pregnancy. In non-pregnant models, *AOAH* has been shown to inactivate gram negative bacteria endotoxin, LPS¹⁹⁵, aiding in the clearance of infection and inflammation. In a mouse model of LPS-induced acute lung injury, *AOAH* expression associated with resolution of inflammation, reduced alveolar damage, and improved morbidity and mortality, with *AOAH* deficient mice experiencing worse outcomes¹⁹⁵. *AOAH* has also been shown to play a role in inactivating LPS in colonic dendritic cells⁴² and in the urinary tract of mice¹⁹⁶, though its role in the placenta is unknown. In line with these findings of *AOAH* deficiency resulting in prolonged infection/inflammation and adverse outcomes, decreased placental *AOAH* at preterm may have implications for the progression of preterm birth, however, upregulated protein expression early in gestation may suggest post-translational modifications, or represent a limitation in semi-quantitative IHC assessment. We demonstrated that *AOAH* protein was highest in the placental syncytiotrophoblast, consistent with the role of the syncytiotrophoblast in mediating placental defence²⁰⁰, though the function and expression of *AOAH* in pregnancy should be further explored.

Placental *DEFB1* expression was also decreased at preterm compared to term. In contrast to these findings, amniotic fluid levels of *DEFB1* in healthy, term pregnancies have been shown to be higher in mid-trimester compared to at term⁸⁴. *DEFB1* is constitutively expressed with broad spectrum antimicrobial activity³⁸, and perhaps other cytolytic AMPs, such as *GNLY*, may play a greater role in placental defence earlier in pregnancy. In contrast to placental mRNA expression levels of *CAMP*, *AOAH*, and *DEFB1*, we observed a decrease in placental *GNLY* with increasing gestational age. In line with these findings, expression of *GNLY* has been shown to be down-regulated at the maternal-fetal interface in human term pregnancies²⁰³. *GNLY* is prominently expressed in natural killer cells⁴⁵, which are most abundant in the decidua in the first trimester of pregnancy and decline with advancing gestational age²⁰⁴, which may contribute to the higher placental *GNLY* expression observed in preterm placentae. *GNLY* has cytolytic properties, and has recently been shown to be selectively transferred to infected trophoblast cells from decidual NK cells to protect against infection²⁰⁵. Infection in pregnancy has implications for adverse pregnancy outcomes including preterm birth, and increased expression of placental *GNLY* may be an

important placental defence mechanism, particularly early in gestation. Although not available in time for this thesis, placental expression of *DEFB1* and *GNLY* will also be assessed at the protein level, to gain a comprehensive picture of the AMP response to preterm birth.

Separating maternal and fetal circulations, the placental barrier may be a site for transmission of infectious agents from the mother to fetus, such as group B streptococci, a common pathogen involved in chorioamnionitis³⁹. Contrary to our hypothesis, we did not see a strong effect of chorioamnionitis on placental AMP mRNA or protein expression, and *CAMP* mRNA expression was decreased with chorioamnionitis. In contrast to these findings, previous studies have shown increased levels of *CAMP* protein in amniotic fluid from pregnancies with preterm premature rupture of membranes (PPROM) with chorioamnionitis compared to PPRM without chorioamnionitis¹⁹⁷. Chorioamnionitis is characterized by neutrophil infiltration of the placenta¹⁹⁷, and *CAMP* is largely produced by neutrophils¹⁹⁷, and has been characterized to have specific antimicrobial activity against group B streptococcus³⁸. Amniotic fluid levels of *DEFB1* have also been shown to be increased in preterm births with intraamniotic inflammation and infection, compared to both women who delivered preterm without infection or inflammation and term births⁸⁴. In line with our findings, other studies have reported no differences in *DEFB1* or *GNLY* mRNA expression levels in preterm chorioamniotic membranes with chorioamnionitis⁴⁴. It is possible that placentae with a higher grading of chorioamnionitis or different strains of infection may be needed to elicit a stronger AMP response, or may suggest a failed placental adaptation to infection. In this cohort, fetal membrane inflammation grade (defined as 0 [no inflammation], 1 [moderate clusters of neutrophils], 2 [severe, confluent neutrophils between chorion and decidua], or 3 [microabscesses between chorion and decidua]) was mostly classified as inflammation grade 2 (47%) in preterm membranes with chorioamnionitis, with one membrane classified as grade 3 (5.3%). Perhaps a larger proportion of membranes with grade 3 chorioamnionitis (thus indicating a heightened inflammatory environment, at least locally) would be required show a link between inflammation and placental AMP response. AMPs have been shown to modulate immune responses, including the expression of pro-inflammatory cytokines and chemokines²⁰¹. For example, *CAMP* has been shown to induce expression of IL-6, IL-8, TNF- α , and MCP-1 in human fetal membranes and myometrium samples²⁰¹. However, *CAMP* has also been described as having both anti- and pro-inflammatory characteristics, and could therefore be beneficial in the clearance of microbes, or detrimental by increasing the inflammatory response and contributing to the inflammatory cascade associated with labour⁴³. A panel of cytokines and chemokines will be assessed in the placental samples in this cohort, to better understand the links between AMP expression and placental inflammation.

In contrast to our hypothesis, maternal BMI did not affect placental AMP expression. Literature on the expression of AMPs in pregnancies complicated by suboptimal maternal BMI are limited. Previous studies have characterized increased cervicovaginal fluid concentration of elafin, an AMP not considered here, with increasing maternal BMI⁸³. Maternal underweight prepregnancy is relatively uncommon in our catchment area/population, and few severe classes of maternal obesity were captured in our cohort. This may in part account for the lack of differences in AMP levels with both maternal UW and OB if the degree of maternal inflammation was not sufficient to elicit a placental AMP response. Study exclusion criteria also ensured the exclusion of mothers

who had comorbidities associated with inflammatory processes to allow for the assessment of maternal underweight or obesity alone, and may suggest that these conditions, in the absence of other complications, may not strongly influence placental defence. Nevertheless, our data suggest that gestational age is a stronger predictor of placental AMP expression than maternal underweight or obesity.

To our knowledge, ours is the first to explore AMP defences stratified by placental sex. Our findings of reduced placental AMPs in preterm male placentae may suggest increased immune vulnerability for males, consistent with increased risk for adverse pregnancy outcomes associated with male sex^{206,207}. In this cohort, slightly more males (60%) were born preterm than females (40%), compared to at term, where 42% of term births were male and 58% were female. This may suggest that increased susceptibility to environmental insults in male placentae early in gestation may have contributed to increased risk for preterm birth. However, at term, male placentae had increased defences compared to term female placentae, which may suggest that females may be more vulnerable to infection and inflammation at term. Other evidence has shown increased male immune response later in gestation, where male term placentae treated with LPS produced greater amounts TNF α compared to females¹³⁷. Our findings suggest a sex-dependent development of placental defence pathways, which warrants further investigation. The placenta's ability to mount a defensive response may also have implications for fetal exposure to environmental insults and offspring development, as impaired placental AMP expression may be permissive of inflammatory mediator and microbial entry into the intrauterine compartment. This may have adverse consequences for fetal growth, given associations of inflammation *in utero* and impaired fetal development, particularly key organs that are sensitive to pro-inflammatory stimuli like the brain^{11,12}.

Strengths of our study include the cohort selection with study criteria that ensured the exclusion of maternal comorbidities such as gestational diabetes mellitus and hypertension, with corresponding clinical metadata. These comorbidities associate with increased maternal inflammation, which could independently alter placental AMP expression. Our study sample also allows for stratification by both preterm and term birth (with and without infection) and by maternal BMI, where few other studies consider the effect of both preterm birth and BMI on gestational tissue defence. While immunohistochemistry allowed for localization of proteins within the placental cell layers, the discordant mRNA and protein level results could be in part attributed to the inherent limiting semi-quantitative nature of immunohistochemistry. Alternatively, qPCR is a more specific experimental method²⁰⁸, however, given the highest abundance of AMP protein located in the syncytiotrophoblast cell layer, the use of whole placental tissue in qPCR assays may have masked differences in cell-specific expression that were evident at the protein level. Overall, our data help to address gaps in knowledge on the impact of preterm birth, infection, maternal prepregnancy BMI, and placental sex, in the absence of other maternal comorbidities, on placental-mediated fetal protection and corresponding infant outcomes.

We demonstrated that gestational age and placental sex, but not suboptimal maternal prepregnancy BMI, are associated with altered gestational tissue expression of AMPs, which may

have implications for pregnancy and infant outcomes at birth. By better understanding how the placenta modulates infection and inflammation *in utero* and associations with adverse pregnancy outcomes such as preterm birth, these data could inform clinical treatment to optimize pregnancy, placental, and infant outcomes, perhaps by enhancing placental defence in pregnancy.

Table 4.1. Effect of gestational age (continuous) on mRNA and protein expression levels of placental antimicrobial peptides.

Placental AMP expression	Unadjusted			Adjusted		
	β (95% CI)	R ²	p-value	β (95% CI)	R ²	p-value
Gene						
<i>AOAH</i>	0.05 (-0.07, 0.14)	0.007	0.47	-0.005 (-0.04, 0.03)	0.07	0.78
<i>CAMP</i>	0.27 (0.01, 0.53)	0.06	0.04	0.01 (-0.003, 0.03)	0.17	0.11
<i>DEFB1</i>	-0.11 (-0.49, 0.28)	0.004	0.59	0.06 (-0.30, 0.41)	0.03	0.74
<i>GNLY</i>	-0.20 (-0.36, -0.04)	0.07	0.01	-0.22 (-0.50, 0.05)	0.14	0.10
<i>SLPI</i>	-0.14 (-0.78, 0.49)	0.003	0.66	0.09 (-0.44, 0.62)	0.008	0.75
Protein						
% Area stained						
AOAH	-0.17 (-0.38, 0.04)	0.04	0.11	-0.09 (-0.58, 0.39)	0.08	0.70
CAMP	-0.28 (-0.49, 0.07)	0.11	0.01	-0.26 (-0.72, 0.21)	0.10	0.28
Staining intensity						
AOAH						
Syncytiotrophoblast	-0.04 (-0.06, -0.01)	0.12	0.006	-0.04 (-0.09, 0.02)	0.14	0.19
Cytotrophoblast	-0.02 (-0.04, 0.001)	0.06	0.06	-0.009 (-0.05, 0.03)	0.11	0.65
Connective tissue	-0.04 (-0.06, -0.009)	0.11	0.009	-0.04 (-0.10, 0.01)	0.14	0.12
Fetal vessel endothelium	-0.02 (-0.05, -0.001)	0.07	0.04	-0.01 (-0.06, 0.04)	0.11	0.66
CAMP						
Syncytiotrophoblast	-0.06 (-0.10, -0.02)	0.12	0.008	-0.05 (-0.15, 0.04)	0.08	0.25
Cytotrophoblast	-0.04 (-0.07, -0.009)	0.10	0.01	-0.04 (-0.10, 0.02)	0.14	0.16
Connective tissue	-0.05 (-0.09, -0.02)	0.12	0.008	-0.04 (-0.13, 0.04)	0.11	0.30
Fetal vessel endothelium	-0.03 (-0.06, 0.0008)	0.06	0.06	-0.02 (-0.07, 0.04)	0.08	0.50

Data are or β (95% CI) with p-value and from Standard Least Squares models. The adjusted model includes maternal prepregnancy BMI (continuous), infant sex, z-gestational weight gain, antibiotic use (yes/no), and chorioamnionitis status (present/absent). *p<0.05.

Table 4.2. Effect of gestational age and infection status on mRNA and protein expression levels of placental antimicrobial peptides.

Placental defensin	Gestational age and infection status			
	Preterm Chorio (n=19-21)	Preterm (n=20-28)	Term (n=20-30)	p-value
Gene				
AOAH	2.47 (1.46, 3.16)	3.27 (1.33, 4.20)	4.58 (2.38, 5.94)	NS
CAMP	2.24 (0.90, 2.95) ^B	1.23 (0.64, 3.41) ^B	4.09 (2.49, 7.00) ^A	0.0002
DEFB1	1.94 (0.99, 4.33)	2.09 (1.01, 2.87)	3.11 (1.49, 6.38)	NS
GNLY	0.11 (0.03, 0.63)	0.13 (0.05, 0.66)	0.12 (0.07, 0.26)	NS
SLPI	0.66 (0.28, 2.06)	1.09 (0.29, 4.16)	0.81 (0.48, 1.60)	NS
Protein				
% Area stained				
AOAH	0.04 (0.003, 0.18)	0.01 (0.001, 0.06)	0.02 (0.003, 0.05)	0.49
CAMP	3.02 (1.98, 9.24)	2.59 (0.95, 5.33)	1.93 (0.62, 4.87)	0.29
Staining intensity				
AOAH				
Syncytiotrophoblast	0.52 (0.26, 1.08)	0.42 (0.02, 0.57)	0.15 (0.03, 0.66)	0.09
Cytotrophoblast	0.02 (0, 0.27)	0 (0, 0.09)	0 (0, 0.03)	0.15
Connective tissue	0.58 (0.31, 0.94)	0.48 (0.12, 0.69)	0.32 (0.12, 0.66)	0.16
Fetal vessel endothelium	0 (0, 0.13)	0 (0, 0.06)	0 (0, 0)	0.28
CAMP				
Syncytiotrophoblast	2.30 ± 0.96	2.06 ± 0.93	1.75 ± 0.80	0.16
Cytotrophoblast	0.5 (0.18, 1.17)	0.48 (0.12, 0.66)	0.27 (0.12, 0.43)	0.11
Connective tissue	1.77 (1.14, 2.33) ^A	1.22 (1.03, 2) ^{AB}	1.12 (0.88, 1.48) ^B	0.04
Fetal vessel endothelium	0.17 (0.07, 0.95)	0.2 (0.08, 0.29)	0.13 (0.04, 0.34)	0.57

Data are median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data, or Welch's test for normal data/unequal variance, or ANOVA; normal distribution/equal variance). NS: not significant. *p<0.05.

Table 4.3. Effect of maternal prepregnancy BMI group on mRNA and protein expression levels of placental antimicrobial peptides in preterm and term pregnancies.

Placental AMP expression	Prepregnancy BMI Group				p-value
	UW (n=5-9)	NW (n=4-15)	OW (n=6-16)	OB (n=5-13)	
PRETERM					
Gene					
AOAH	3.85 (2.01, 7.37)	3.24 (1.33, 5.97)	2.52 (1.45, 3.20)	2.53 (1.46, 4.25)	0.44
CAMP	0.75 (0.41, 1.20)	2.82 (0.98, 5.91)	1.65 (0.44, 2.66)	3.18 (0.71, 5.55)	0.07
DEFB1	0.90 (0.74, 4.85)	2.72 (1.55, 5.60)	1.80 (0.96, 2.57)	1.96 (1.35, 2.72)	0.12
GNLY	0.67 (0.15, 1.05)	0.40 (0.08, 0.63)	0.04 (0.03, 0.39)	0.08 (0.04, 0.11)	0.18
SLPI	4.16 (0.18, 5.61)	1.43 (0.52, 4.38)	0.76 (0.28, 3.27)	0.31 (0.18, 0.77)	0.08
Protein					
% Area stained					
AOAH	0.04 (0.004, 1.46)	0.003 (0.002, 0.12)	0.02 (0.003, 0.17)	0.02 (0.0004, 0.25)	0.79
CAMP	2.71 (1.63, 14.4)	2.41 (0.60, 4.39)	2.79 (0.50, 6.48)	3.93 (0.50, 10.1)	0.77
Staining intensity					
AOAH					
Syncytiotrophoblast	0.57 (0.12, 1.15)	0.33 (0.07, 0.53)	0.52 (0.43, 0.67)	0.4 (0.03, 0.67)	0.41
Cytotrophoblast	0 (0, 0.3)	0 (0, 0.1)	0.05 (0, 0.15)	0 (0, 0.09)	0.67
Connective tissue	0.6 (0.3, 1.3)	0.33 (0.03, 0.77)	0.58 (0.3, 0.86)	0.48 (0.18, 0.89)	0.61
Fetal vessel endothelium	0.1 (0, 0.88)	0 (0, 0)	0 (0, 0.1)	0 (0, 0.07)	0.18
CAMP					
Syncytiotrophoblast	2.27 ± 1.20	1.97 ± 0.51	2.16 ± 0.76	2.40 ± 1.36	0.76
Cytotrophoblast	0.57 (0.12, 0.82)	0.47 (0.2, 0.63)	0.45 (0.17, 0.72)	0.57 (0.13, 1.1)	0.98
Connective tissue	1.17 (1.05, 3.37)	1.27 (1.1, 2.07)	1.72 (1.2, 2.08)	2.03 (0.77, 2.76)	0.87
Fetal vessel endothelium	0.13 (0.03, 1.15)	0.13 (0.07, 0.23)	0.24 (0.17, 0.6)	0.18 (0.02, 0.83)	0.49
TERM					
Gene					
AOAH	4.73 ± 2.52	4.89 ± 1.97	3.12 ± 1.63	4.58 ± 2.98	0.48
CAMP	5.32 (1.86, 7.00)	3.92 (3.15, 6.51)	3.48 (2.17, 12.0)	4.26 (2.88, 7.39)	0.95
DEFB1	3.41 (1.42, 6.83)	3.59 (1.89, 6.15)	2.48 (1.65, 3.40)	2.15 (1.17, 7.62)	0.61
GNLY	0.07 (0.03, 0.09)	0.18 (0.07, 3.48)	0.15 (0.09, 0.30)	0.24 (0.10, 0.68)	0.42
SLPI	0.80 (0.56, 1.61)	1.50 (0.44, 11.7)	0.92 (0.66, 3.53)	0.97 (0.24, 2.74)	0.96
Protein					
% Area stained					
AOAH	0.03 (0.007, 0.14)	0.02 (0.002, 0.61)	0.02 (0.003, 0.05)	0.02 (0.0002, 0.05)	0.80
CAMP	4.14 (1.46, 6.30)	0.99 (0.30, 5.03)	1.93 (0.46, 3.17)	1.30 (0.60, 7.17)	0.60
Staining intensity					
AOAH					
Syncytiotrophoblast	0.23 (0.03, 0.88)	0.28 (0.009, 1.53)	0.03 (0.03, 0.72)	0.23 (0.03, 0.45)	0.81
Cytotrophoblast	0 (0, 0.07)	0.03 (0.008, 0.38)	0 (0, 0)	0 (0, 0.03)	0.09
Connective tissue	0.47 (0.17, 0.73)	0.37 (0.03, 0.49)	0.25 (0.14, 0.49)	0.37 (0.15, 0.52)	0.90
Fetal vessel endothelium	0 (0, 0)	0 (0, 0.13)	0 (0, 0)	0 (0, 0.18)	0.22

CAMP					
Syncytiotrophoblast	2.06 ± 0.84	1.85 ± 1.34	1.54 ± 0.47	1.62 ± 0.68	0.74
Cytotrophoblast	0.4 (0.05, 1.32)	0.3 (0.12, 0.46)	0.17 (0.09, 0.28)	0.33 (0.22, 0.42)	0.56
Connective tissue	1.53 (0.92, 2.45)	1.2 (0.88, 2.98)	1 (0.78, 1.22)	1.13 (0.88, 1.65)	0.50
Fetal vessel endothelium	0.17 (0, 0.42)	0.13 (0.03, 0.93)	0.12 (0.09, 0.24)	0.13 (0.02, 0.58)	1.00

Data are median (IQR; ANOVA; normal distribution/equal variance). Data are means ± SD (ANOVA; normal distribution/equal variance) or median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data, or Welch's test for normal data/unequal variance).

Table 4.4. Effect of fetal sex on mRNA and protein expression levels of placental antimicrobial peptides among preterm and term pregnancies.

	Preterm			Term		
	Male (n=34)	Female (n=22)	p-value	Male (n=14)	Female (n=18)	p-value
Gene						
<i>AOAH</i>	2.46 (1.33, 3.70)	3.16 (1.66, 4.48)	0.69	5.39 ± 1.69	3.66 ± 2.51	0.047
<i>CAMP</i>	1.21 (0.86, 2.74)	2.60 (0.49, 3.94)	0.50	5.07 ± 2.48	4.77 ± 3.21	0.79
<i>DEFB1</i>	1.49 (0.97, 3.05)	2.38 (1.80, 8.52)	0.049	4.68 (2.17, 8.21)	2.03 (1.42, 3.22)	0.14
<i>GNLY</i>	0.13 (0.04, 0.60)	0.13 (0.04, 0.67)	0.93	0.22 (0.09, 2.21)	0.10 (0.06, 0.24)	0.03
<i>SLPI</i>	0.81 (0.30, 3.88)	0.79 (0.24, 2.06)	0.82	0.69 (0.55, 5.34)	1.01 (0.44, 1.66)	0.31
Protein						
% Area stained						
AOAH	0.008 (0.001, 0.05)	0.07 (0.01, 0.56)	0.08	0.02 (0.002, 0.16)	0.02 (0.005, 0.04)	0.79
CAMP	2.69 (1.08, 6.06)	3.39 (0.81, 9.24)	0.65	1.77 (0.26, 6.08)	1.93 (0.88, 3.77)	1.00
Staining intensity						
AOAH						
Syncytiotrophoblast	0.48 (0.1, 0.69)	0.47 (0.11, 0.82)	0.96	0.05 (0.008, 0.91)	0.23 (0.03, 0.53)	0.88
Cytotrophoblast	0 (0, 0.1)	0.02 (0, 0.15)	0.78	0 (0, 0.11)	0 (0, 0)	0.24
Connective tissue	0.5 (0.2, 0.79)	0.57 (0.1, 0.89)	0.86	0.28 (0.07, 0.73)	0.32 (0.21, 0.58)	0.73
Fetal vessel endothelium	0 (0, 0.06)	0 (0, 0.1)	0.48	0 (0, 0)	0 (0, 0)	0.80
CAMP						
Syncytiotrophoblast	2.19 ± 0.97	2.17 ± 0.90	0.95	2.05 ± 1.07	1.56 ± 0.51	0.18
Cytotrophoblast	0.32 (0.14, 0.62)	0.75 (0.58, 1.17)	0.02	0.37 (0.12, 0.5)	0.25 (0.12, 0.36)	0.26
Connective tissue	1.28 (1.03, 2.29)	1.73 (1.24, 2.3)	0.36	1.42 (0.82, 2.61)	1.08 (0.91, 1.18)	0.33
Fetal vessel endothelium	0.2 (0.07, 0.38)	0.17 (0.08, 0.6)	1.00	0.1 (0, 0.44)	0.13 (0.1, 0.26)	0.64

Data are means ± SD (ANOVA; normal distribution/equal variance) or median (IQR; Kruskal-Wallis/Wilcoxon test for non-parametric data with Steel-Dwass *post-hoc*). *p<0.05.

Table 4.5. Correlations between mRNA expression levels of placental antimicrobial peptides and infant outcomes at birth in preterm and term pregnancies.

Infant outcome	Placental defensin mRNA expression									
	<i>AOAH</i>		<i>CAMP</i>		<i>DEFB1</i>		<i>GNLY</i>		<i>SLPI</i>	
	Spearman's ρ	p-value	Spearman's ρ	p-value	Spearman's ρ	p-value	Spearman's ρ	p-value	Spearman's ρ	p-value
Preterm										
Infant z-birthweight	0.25	0.08	0.21	0.16	-0.11	0.44	-0.28	0.05	0.24	0.09
Placental weight (g)	-0.09	0.51	-0.02	0.89	-0.10	0.51	-0.13	0.34	-0.21	0.14
Infant birthweight (g) : placental weight (g)	0.004	0.98	-0.006	0.97	-0.10	0.51	-0.10	0.49	0.06	0.67
Apgar score (1 minute)	-0.12	0.42	0.08	0.61	0.01	0.92	-0.10	0.49	-0.19	0.19
Gestational age (weeks)	-0.11	0.42	0.03	0.86	-0.07	0.64	-0.14	0.34	-0.08	0.59
Term										
Infant z-birthweight	0.39	0.03	0.37	0.04	-0.18	0.33	0.33	0.07	-0.09	0.63
Placental weight (g)	0.24	0.20	0.31	0.10	-0.29	0.13	-0.05	0.78	-0.13	0.49
Infant birthweight (g) : placental weight (g)	0.04	0.86	-0.12	0.54	0.24	0.21	0.24	0.22	0.11	0.58
Apgar score (1 minute)	0.004	0.98	-0.21	0.28	0.17	0.38	-0.12	0.54	-0.18	0.35
Gestational age (weeks)	-0.20	0.28	-0.31	0.10	-0.14	0.46	-0.21	0.26	0.03	0.89

Data are Spearman's rho (ρ) correlation coefficient. * $p < 0.05$.

Figure 4.1. Placental mRNA expression levels of antimicrobial peptides stratified by gestational age group. Placental *AOA* ($p=0.04$), *CAMP* ($p<0.0001$), and *DEFB1* ($p=0.04$) mRNA expression levels were decreased in all preterm (with and without chorioamnionitis) compared to term placentae. There was no effect of gestational age on placental *SLPI* or *GNLY* mRNA expression. Data were analysed by ANOVA, Welch, or Kruskal-Wallis/Wilcoxon test. * $p<0.05$.

Figure 4.2. Placental AOA and CAMP protein staining intensity in four placental cell layers (A) and percent area stained (B) in preterm and term placentae. (A). Placental AOA protein staining intensity of the syncytiotrophoblast ($p=0.006$), connective tissue ($p=0.009$), and fetal vessel endothelium ($p=0.04$) decreased with advancing gestational age. Placental CAMP protein staining intensity of the syncytiotrophoblast ($p=0.008$), cytotrophoblast ($p=0.01$) and connective tissue ($p=0.008$), also decreased with advancing gestational age. (B). Similarly, percent CAMP area stained decreased with advancing gestational age, but there were no differences in placental percent AOA area stained by gestational age.

Figure 4.3. Representative IHC images of AOA staining in human placentae across maternal prepregnancy BMI groups stratified by gestational age and infection status. Arrow indicates syncytiotrophoblast. Arrowhead indicates cytotrophoblast. Asterisks indicate fetal blood vessels with no labeled endothelium. Preterm chorio = preterm with chorioamnionitis. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Figure 4.4. Representative IHC images of CAMP staining in human placentae across maternal prepregnancy BMI groups stratified by gestational age and infection status. Arrows indicate labeled syncytiotrophoblast. Arrowhead indicates cytotrophoblast. Asterisks indicate fetal blood vessels with no labeled endothelium. Preterm chorio = preterm with chorioamnionitis. UW = underweight. NW = normal weight. OW = overweight. OB = obese.

Supplementary Table 4.1. Primer sequences for real-time quantitative PCR gene studies.

Gene	Primer sequence
<i>AOAH</i>	Forward: 5'-CGGCCAGCTAAATAAGACA-3' Reverse: 5'-ATGATTTTCATGGAAGGCAAA-3'
<i>CAMP</i>	Forward: 5'-CTCGGATGCTAACCTCTACC-3' Reverse: 5'-CGTCCTTCTTGAAGTCACAA-3'
<i>DEFB1</i>	Forward: 5'-TCGCCATGAGAACTTCCTAC-3' Reverse: 5'-GCCTTCCCTCTGTAAACAGGT-3'
<i>SLPI</i>	Forward: 5'-CCAGGGAAGAAGAGATGTTG-3' Reverse: 5'-GGCTTCCTCCTTGTTGGG-3'
<i>GNLY</i>	Forward: 5'-AGCAACCTCTGCCGGCT-3' Reverse: 5'-GACAGCAGAGGGAGTCAGGG-3'
<i>ACTB</i>	Forward: 5'-AAACTACCTTCAACTCCATC-3' Reverse: 5'-ATGATCTTGATCTTCATTGT-3'
<i>GAPDH</i>	Forward: 5'- GAAGGTGAAGGTCGGAGTCAA-3' Reverse: 5'- GGAAGATGGTGATGGGATTTC-3'
<i>HPRT1</i>	Forward: 5'-GACCAGTCAACAGGGGACATAA-3' Reverse: 5'-AAGCTTGCGACCTTGACC-3'

Chapter 5. Final discussion

We aimed to understand how common conditions in pregnancy (in the absence of other major maternal comorbidities) that are associated with a pro-inflammatory environment, alter placental development and function and offspring development. First, in a cohort of 12,154 pregnancies from the Collaborative Perinatal Project, we explored the effects of maternal BMI on placental pathology. We found that elevated maternal BMI associated with increased placental inflammation, maternal vascular malperfusion, and greater odds of an appropriate mature placenta for gestational age at term. Maternal underweight associated with smaller, but more efficient placentae, and the prevalence of pathologies also differed by placental sex. Functionally, we looked at placental expression of key efflux transport systems in pregnancy, P-gp/*ABCB1* and BCRP/*ABCG2*, that are critical for regulating drug, nutrient, and cytokine efflux, mediating fetal protection and nutrition and ensuring that potentially harmful endogenous and exogenous substances do not accumulate *in utero*. We also assessed placental expression of antimicrobial peptides, because they are known to be important in infectious and inflammatory conditions and may play a protective role in pregnancy, and both placental transporters and defences might be altered with maternal BMI and/or gestational age at birth. Maternal underweight and obesity did not impair these placental transport and defence systems in a cohort of 96 pregnancies. However, there was an effect of preterm birth on placental function. Preterm placentae had increased expression of multidrug resistance efflux transporters, but decreased pro-inflammatory cytokine expression, and altered expression levels of antimicrobial peptides compared to term placentae. Male placentae also had altered expression levels of placental defences, but not inflammatory mediators or MDR transporters. Maternal underweight and obesity may therefore impair normal placental development, with subsequent effects on function, although gestational age seemed to be the biggest driver in altered placental transport and defence in our cohort. The placental molecular and histopathological adaptations to its environment could be useful markers to identify which offspring are on course for poor postnatal development and health. Offspring born to mothers who are underweight or have obesity prepregnancy are at greater risk for poor growth^{4,209}, neurodevelopmental disorders such as autism spectrum disorders and attention deficit hyperactivity disorder^{209,210}, and non-communicable diseases such as obesity and type 2 diabetes^{94,211}, and these placental molecular and histomorphological changes could help to predict postnatal developmental. Characterizing the resulting placental phenotypes, even if subtle, may thus prove a useful tool in clinical decision making, including early clinical management and interventions in offspring with poor health trajectories to optimize newborn and child outcomes.

Figure 5.1. Placental (mal)adaptations associated with suboptimal maternal prepregnancy BMI and preterm birth. Increased maternal BMI associated with increased gestational tissue inflammation and maternal vascular malperfusion, and maternal underweight associated with smaller, but more efficient placentae. Preterm placentae had increased expression of multidrug resistance transporters, decreased *IL-6*, and altered expression of antimicrobial peptides. Icons adapted from BioRender, Connor Lab, and Microsoft PowerPoint.

5.1. Suboptimal BMI is not an inert condition for the placenta

Suboptimal maternal BMI, particularly higher BMI, may impair normal placental development. The placenta is a plastic organ that can adapt to mitigate harmful exposures, such as maternal underweight or obesity, or maladapt to permit their adverse effects⁷, which may have consequences for the offspring²¹². Our findings from a historical cohort suggest that increased maternal BMI may be detrimental to placental histomorphology, evidenced by increased gestational tissue inflammation and maternal vascular perfusion with increasing maternal BMI. Higher maternal prepregnancy BMI has been previously associated with altered placental development, including chronic villitis^{54,55}, delayed and accelerated placental villous maturation⁵⁵, and increased atheromas and villous infarcts^{55,56}, pathologies which have been linked to placental insufficiency⁵⁷, fetal growth restriction, and neurodevelopmental impairment⁵⁵. In contrast, placentae from mothers who were underweight prepregnancy exhibited greater placental efficiency, which may suggest that placentae were able to adapt to increase nutrient delivery to the fetus^{140,141} in environments which may have reduced nutrient availability^{52,59,102}. Characterizing the placental maladaptations to suboptimal maternal BMI may not only help to better understand the mechanisms through which these conditions affect the developing offspring, but the resulting placental phenotypes could serve as potential clinical

indicators at the time of birth of future health trajectories. Assessment of placental histology for pathological evaluation using haematoxylin and eosin staining is a simple and cost-effective technique that could be readily implemented in the clinical setting. Given the associations of long-term sequelae with infants born to mothers who were underweight or had obesity prepregnancy^{8,96}, identifying infants with poor health trajectories early would allow the opportunity for interventions and increased screening to optimize health outcomes.

Importantly, suboptimal maternal BMI may be most detrimental to fetoplacental development when coupled as a double burden with preterm birth (or other comorbidities). While maternal obesity has been associated with an increased prevalence of preterm birth, the combined effects of both of these exposures on placental development and function has not been well investigated. For example, we observed increased placental MDR transporter mRNA expression with increasing maternal BMI at preterm, but not term, which may support that the effects of suboptimal BMI are most prominent in pregnancies additionally challenged by preterm birth, and could represent a beneficial adaptation in placental efflux in pregnancies with the greatest levels of inflammation. Suboptimal maternal BMI, in otherwise uncomplicated pregnancies, is not inert for the developing placenta, but may be thus most detrimental for pregnancy outcomes when combined with other comorbidities. Mothers who have obesity are also at increased risk for developing gestational diabetes and preeclampsia in pregnancy, comorbidities which have also been associated with adverse placental and pregnancy outcomes²¹³. For example, gestational diabetes mellitus, a common comorbid condition with obesity, also associates with increased placental inflammation^{187,188}, and risk of fetal macrosomia and caesarean delivery has been shown to be amplified in pregnancies complicated by both maternal obesity and gestational diabetes mellitus²¹⁴. Nonetheless, maternal underweight and obesity alone are prevalent conditions¹⁻³, and it is important to understand placental development and function in these pregnancies as suboptimal maternal BMI promotes sub-clinical inflammation and altered metabolic states with likely implications for placental structure and function⁸⁻¹⁰, though may be associated with more subtle phenotypes.

5.2 Preterm birth alters placental transport and defence mechanisms

At preterm, upregulated placental efflux transporters may be an adaptive mechanism to protect the developing fetus from increased inflammation and infection present in pregnancies at risk for preterm birth^{17,1877}. Placental expression of antimicrobial peptides likely also plays a role in placental mediated fetal protection against infection and inflammation. Placental MDR transporters have been previously characterized to decrease with gestational age^{34,76}, however literature on the ontogeny of antimicrobial peptide expression with gestational is conflicting. As placental MDR transporters are also known to regulate the efflux of proinflammatory cytokines^{13,32}, it is possible that upregulation of efflux mechanisms in early pregnancy are a means to moderate inflammatory stimuli, in the face reduced placental defences. Fetal protection involves a number of complex systems, and preterm birth may work differentially target these systems.

5.3. Sex differences: underexplored but important mediators of fetoplacental outcomes

Placental sex may also contribute to altered placental structure and function, where male placentae may be more vulnerable to impaired placental development and defence, with implications for offspring growth and development. We found that male placentae had increased gestational tissue inflammation and maternal vascular malperfusion, and had altered expression levels of antimicrobial peptides. To our knowledge, we are the first to report the effect of placental sex on antimicrobial peptide expression. Males also had more efficient placentae and greater brain-to-body weight ratios than females, but lower Apgar scores. Male fetuses tend to grow more rapidly than females and invest greater resources in growth than placental development¹⁴⁷, and as a result, may be more susceptible to placental insults as well as adverse later health outcomes such as neurological and cardiovascular diseases¹⁴⁷. Evidence also suggests that female sex seems to be protective, while male placentae may be less able to adapt to environmental insults^{206,207}. These findings in both placental development and function corroborate evidence suggesting increased male vulnerability to adverse maternal environments.

5.4 Limitations

The historical nature of the dataset linking maternal BMI and placental pathologies presents limitations. For example, the prevalence of obesity was lower than current rates, and rates of smoking were increased during the time of the study. Additionally, placental pathology data were obtained directly from pathology reports, rather than having images re-assessed by a clinical pathologist. However, few studies have characterized placental histomorphology on a scale of this magnitude, allowing for strong statistical power. In our modern cohort, while we showed that anti-inflammatory medications may be successful in reducing some placental inflammation, demonstrated by decreased placental mRNA expression of *IL-6*, only two select pro-inflammatory cytokines were assessed, and a full panel of placental cytokine expression will help to better understand the mechanisms linking placental inflammation and placental and pregnancy outcomes.

5.5 Future directions

To further clarify the extent to which placental inflammatory mediators are altered by maternal underweight and obesity and preterm birth (with or without infection), and whether placental inflammation is associated with maternal medication use, immediate next steps include assessing a comprehensive panel of placental cytokines and chemokines at the protein level. Additionally, assessments of DEFB1 and SLPI protein expression and localization will provide missing information to interpret mRNA data.

Here, we considered one aspect of suboptimal maternal metabolic state by evaluating the associations between maternal BMI and placental development and function. Future directions could also include evaluating the effects of maternal gestational weight gain on fetoplacental

development, as this also associates with adverse pregnancy outcomes²¹⁵. The effects of sex and gestational age on placental defence and transport systems needs to be thoroughly explored, including for antimicrobial defences, as changes in these pathways may represent sex-dependent development of immune pathways, which could be related to known differences in risk for adverse pregnancy outcomes such as preeclampsia, placental abruption, and preterm birth with male sex^{206,207}. The role of antimicrobial peptides in preterm birth alone should also be further characterized, as there may be opportunities for the use of AMPs as novel therapeutics for preterm birth. Emerging studies show potential for the targeted gene delivery of antimicrobial peptides to augment innate immune defence against infection related preterm birth, improving pregnancy outcomes²¹⁶. Additionally, as current treatments for preterm birth have not been largely successful in preventing preterm delivery, given our findings that anti-inflammatory medications may have reduced some placental inflammation, the inflammatory pathways involved in the initiation of parturition¹⁹¹ should be further explored.

Contributions

Experiments and data analyses contained within the thesis were primarily conducted by Hailey Scott with supervision from Kristin Connor. The exceptions are listed below:

Chapter 3: Lilian M. Martinelli completed immunohistochemistry experiments for P-gp and BCRP placental efflux transporter proteins, and image capture and semi-quantitative scoring of staining intensity and staining in immunolabeled areas.

Chapter 4: Israa Zareef performed semi-quantitative scoring of staining intensity for placental AOA_H and CAMP antimicrobial peptide proteins. Hailey Scott developed the optimized methodology for percent antibody area stained, and Israa Zareef performed the semi-quantifications for placental AOA_H protein using these methods, and Hasan Abdo performed the semi-quantifications for placental CAMP protein. Allan Humphrey assisted with *AOA_H*, *CAMP*, *DEFB1*, *SLPI*, and reference gene qPCR experiments.

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