Investigating Placental Inflammation as a Mediator of Maternal Obesity and Risk of Preterm Birth

by

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Placental Inflammation as a Potential Mediator of Maternal Obesity and Risk of Preterm Birth

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Preterm birth (<37 weeks' gestation) is the leading cause of infant mortality worldwide. Pre-pregnancy obesity is the most prevalent and potentially modifiable risk factor of preterm birth, which may partially be attributed to placental inflammation. However, evidence of a pro-inflammatory effect of obesity on the placenta in pregnancy has been conflicting due to limited tissue-level biomarkers of the placenta and bias attributed to study inclusion criteria. My dissertation uses a combination of placental histopathology and transcriptomic data to address these limitations and determine how pre-pregnancy obesity predisposes women to preterm birth through placental dysfunction.

My first manuscript leverages placental histopathology reports from a large, contemporary US birth cohort to evaluate if pre-pregnancy obesity increases the risk of acute and chronic placental inflammation in term pregnancies. Obesity was associated with a lower risk of acute placental inflammation, but a higher risk of chronic placental inflammation. After accounting for selection bias, obesity was still associated with risk of chronic but not acute placental inflammation. In my second manuscript, we performed a cluster analysis of placental features extracted from histopathology reports to classify early (<32 weeks' gestation) and late (32 to <37 weeks' gestation) preterm births into placental pathological phenotypes. We found that pre-pregnancy obesity may predispose women to preterm birth through placental vascular impairment and that obesity mainly affects placental health in more severe, early preterm births. In the third

manuscript, we analyzed placental transcriptomic data from two pregnancy cohorts to elucidate inflammatory and non-inflammatory molecular pathways contributing to placental dysfunction in preterm births. Applying high-dimensional mediation analyses, we observed the interleukin 1 receptor-like 1 gene to be a mediator of a positive association between pre-pregnancy BMI and higher gestational age. Interleukin 1 receptor-like 1 is a known inhibitory gene of acute inflammatory pathways.

Contrary to our hypothesis, obesity may predispose women to preterm birth through mechanisms other than acute placental inflammation. We applied various analytic methods to identify preterm birth subtypes most susceptible to the adverse effects of obesity and have characterized nuanced relationships between obesity and placental inflammation to inform future preterm interventions.

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Preface

My dissertation summarizes the work of three different projects all originally intended to answer the question, "*How does maternal obesity contribute to the risk of preterm birth through the placenta?"* Yet in the face of results that conflicted with my assumptions and understanding of maternal obesity and pregnancy, this work exemplifies my experience learning and applying the scientific method. This dissertation is a testament to my growth as a clinician-epidemiologist and I am grateful for the support of my department, committee members, friends, and family throughout my training.

I would like to give a special thanks to my advisor Dr. Janet Catov, who has been an incredible mentor to me since I started the M.D.-Ph.D. program. She exemplifies the collaborative spirit of research and has instilled in me the importance of developing research hypotheses that are intended for the betterment of the community. As a mentor, she has fostered a learning environment where I could freely pursue my own research goals and she has provided me the resources and expertise to exact them.

I have also been fortunate to have the tremendous support of my other three committee members. There is no one I can think of who rivals the enthusiasm for learning like Dr. James Roberts. From the start of my training, he has been an advocate for my growth as a physician-scientist and I am sincerely appreciative for the countless meetings, opportunities, and hours of feedback he has spent for my benefit. I am grateful to Dr. Marnie Bertolet who has the amazing talent of taking my abstract ideas and translating them into substantive testable research questions. Her guidance (and patience) has been integral to my understanding and application of many of the methods used in my dissertation. I wish to thank Dr. Jennifer Adibi, for being the creative catalyst

on my committee. She has encouraged me to approach my research from different perspectives and using novel methods. Dr. Adibi has a knack for bringing multiple disciplines together for collaborative research, and I admire her ability to make every member of her research team feel like they have a voice worth sharing. I would also like to thank collaborators, Drs. Jiebiao Wang and Tony Parks. Their guidance has made the fields of bioinformatics and perinatal pathology seem approachable (almost) to me, which I would never have imagined at the start of this program. Lastly, I'd like to thank members of the Adibi lab, the Pregnancy Adaptation Group at Magee-Womens Research Institute, and my MSTP cohort for their technical help, feedback, and levity through the highs and lows of my training.

I wish to thank my long-time research mentor, Dr. Julia Finkelstein. I would have never considered pursing the MD-PhD program if it weren't for the opportunities and training she provided me. Her enthusiasm for research is infectious and her gift for mentorship are qualities I strive to emulate.

I thank my family who I am forever indebted to for their love and countless support throughout my life. My parents' unfaltering belief in me has been a source of motivation throughout my training. The work ethic, resilience, and humility I've relied on throughout this degree, I owe to them. I also thank my brother for his sarcasm, which balances out our parents' support to keep me grounded.

This degree would not have been possible without the support of my wife, Song My Hoang. We started dating the week before I joined the PhD program. I am breaking a cardinal rule in epidemiology to infer causation from correlation, but I attribute my success in this program to her. I am thankful for her patience, her love, and for feigning interest whenever I talk about data. She is a constant reminder that there is more to life than work.

1.0 Introduction

1.1 Specific Aims

Preterm birth (<37wks) is the leading determinant of newborn mortality globally. There are few preventive measures for preterm birth because of its heterogenous clinical presentation and etiology. Interventions targeting risk factors that are prevalent, modifiable, and generalizable to all preterm births will have the greatest impact. Pre-pregnancy obesity is the most prevalent potentially modifiable risk factor for preterm birth. To identify efficacious obesity interventions a clear understanding of how obesity predisposes women to preterm birth is desperately needed.

Pre-pregnancy obesity may increase the risk of preterm birth through placental inflammation. Obesity is a chronic pro-inflammatory state that we hypothesize may disrupt the delicate inflammatory environment in pregnancy promoting preterm delivery or increasing the susceptibility of pregnant women to clinical indications for preterm birth. To date, population evidence of a pro-inflammatory effect of obesity on the placenta has been conflicting. Though these findings may indicate that obesity acts through mechanisms other than inflammation, we suspect that a reliance on indirect circulating blood biomarkers in population studies has been a barrier to interrogating the inflammatory effects of obesity on the placenta.

Thus, we leverage clinically-available placental histopathology data and transcriptomic data of the placenta to investigate the effect of pre-pregnancy obesity on placental inflammation in term and preterm births using tissue-level biomarkers. We hypothesize that pre-pregnancy obesity increases the risk of preterm birth through histological placental inflammation and the upregulation of molecular inflammatory pathways in the placenta. Since pre-pregnancy obesity

may also predispose women to preterm birth through non-inflammatory mechanisms, we also apply empirical approaches to analyze placental histopathological and transcriptomic data to identify other possible mechanisms of placental dysfunction.

Elucidating the underlying pathophysiology of obesity and preterm birth may identify placental pathways targetable for pharmacological or nutritional preterm birth interventions. Further, placental histopathology is an underutilized data source for population research due to concerns of bias resulting from restricting analyses to pregnancies with placental data originally collected for clinical purposes. This work offers a framework to addressing this form of selection bias to improve how others investigate clinical data in epidemiological studies.

The scientific aims of this work are:

1. To investigate whether pre-pregnancy obesity increases the risk of histologic placental inflammation in term pregnancies, using a large, contemporary US birth cohort with available placental histopathology.
    a. A sub-aim is to quantify how susceptible our findings are to selection bias.
2. To assess if pre-pregnancy obesity is association with inflammatory and non-inflammatory placental dysfunction in early (<32 weeks') and late (32 to <37weeks) preterm births using an empirical clustering analysis of placental histopathology in the same US birth cohort.
3. To identify placental genes and cell types that may mediate the association between obesity and risk of preterm birth using high-dimensional mediation analyses and cell deconvolution of several publicly available placental transcriptomic datasets.

1.2 The Persistent Public Health Burden of Preterm Birth

1.2.1 Epidemiology of Preterm Birth

Preterm birth is defined as the delivery of a baby prior to 37 weeks' gestation.[1] In 2014, there were an estimated 14.84 million preterm births globally, corresponding to 10.6% of all births.[2] While preterm birth disproportionately affects low and middle income countries (LMICs),[2, 3] some high-income countries, such as the United States have high preterm birth rates. In 2019 the US prevalence of preterm birth was 10.02%.[4]

The high prevalence of preterm birth is a major public health concern because preterm birth is the leading cause of neonatal mortality worldwide, resulting in 965,000 deaths annually.[5] Preterm birth also contributes to 15% of deaths of children less than 5 years of age and is associated with long-term morbidity for children, including neurodevelopmental impairment and respiratory complications.[5-8] Mortality rates of preterm birth increase with decreasing gestational age at delivery. Thus, the severity of prematurity is a key component of mortality associated with preterm birth.[9]

The consequences of infant mortality and morbidity of preterm birth also pose an economic burden.[10] In the US alone, the societal costs of preterm birth are estimated to be $26 billion.[6] However, costs of preterm birth may be higher as these estimates do not account for all long-term morbidities of preterm birth (e.g. asthma and learning disabilities) nor the lifetime costs to caregivers.[6] Caregivers experience indirect economic costs from loss of employment caring for preterm infants. The economic impact of preterm birth for US families likely varies depending on accessibility and quality of healthcare, but limited resources to manage long-term complications of preterm birth may exacerbate the economic burden.

Despite the well-documented health and economic concerns of preterm birth, there has been little change in the prevalence of preterm birth in the US since 1990.[4, 11] A study of birth certificate data found that from 1990 to 2006 the rate of preterm birth rose from 10.62% to 12.80% then steadily declined to 11.39% by 2013.[11] More recent trends from the March of Dimes, indicate that the rate of preterm birth from 2007 to 2016 declined from 10.4% to 9.6% and has since risen to 10.2% by 2019.[12]

The lack of progress in reducing the burden of preterm birth does not indicate that the primary risk factors of preterm birth have not changed. Instead, the attributable risk of preterm birth from certain risk factors (smoking, alcohol use, teen pregnancy) have decreased since 1990 while others have increased (obesity, assisted reproductive therapy) due to changing maternal and pregnancy characteristics. Research efforts should focus on the most prevalent of risk factors of preterm birth and we must recognize that prevention of preterm birth is a moving target closely linked to other current public health issues such as obesity.

Overall trends in preterm birth also obfuscate another key point. Preterm birth is getting worse specifically for Black women (**Figure 1)**. As of 2018, the US prevalence of preterm birth among non-Hispanic black women was 14.1% compared to 9.1% in non-Hispanic white women, 8.6% in Asian women, 11.5% in American Indian or Alaskan Native women, and 9.7% in women of Hispanic ethnicity.[4] Racial disparities in preterm birth reflect adverse socio-economic and cultural consequences of slavery that impact Black women's experience receiving healthcare and overall maternal health.[13, 14] Black women compared to white women are less likely to initiate prenatal care in 1st trimester, more likely to deliver at hospitals with higher maternal mortality rates and more likely to have negative interactions with healthcare staff.[15, 16] These systemic racial disparities in healthcare warrant changes in health policy but are more difficult to implement than

targeting individual risk factors that may also explain race disparities in preterm birth. The health of Black women is negatively impacted by living in neighborhoods with higher rates of air pollution,[17] racial segregation[18] and sparce greenspace[17, 19] compared to white women. These environmental differences are associated with a higher risk of indications for preterm birth (e.g. preeclampsia),[20] and are potentially mediated by preventable risk factors like pre-pregnancy obesity that disproportionately affect Black women.[21] Thus, preterm birth interventions should target risk factors that are not only prevalent in the US overall but are targeted towards Black women to have the greatest impact.

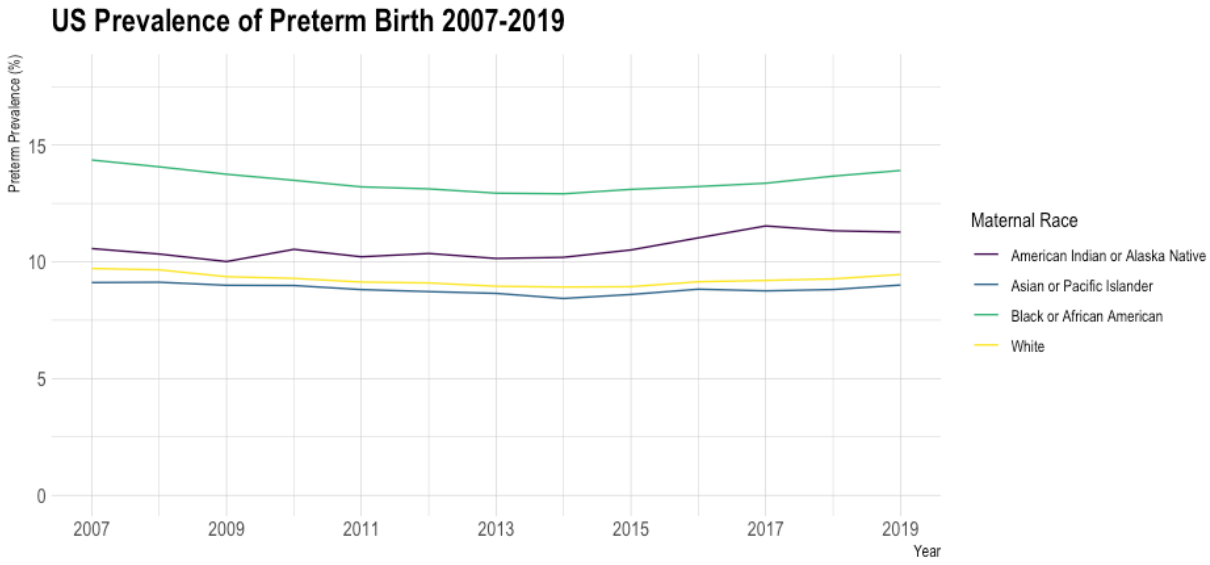


Figure 1: US trends in preterm birth by maternal self-reported race

1.2.2 The Importance of Classifying Preterm Birth

Preterm birth is a clinical diagnosis that is defined by time rather than pathophysiology.[22] Multiple phenotypic classification systems (**Table 1)** have been developed to understand the

etiology, sequelae or clinical management of preterm birth.[22, 23] The first classification of preterm birth subgroups preterm deliveries by gestational age into extreme preterm birth (<28weeks), very preterm birth (28 to <32 weeks), and moderate or late preterm birth (32 to <37weeks gestation).[9] Alternative gestational age cut-offs include early (<32 weeks) and late preterm birth (32 to 36 weeks).[22] Stratifying by gestational age ranges helps evaluate risk of preterm neonatal morbidity and mortality[6] as earlier gestation is associated with worse neonatal outcomes.

The second classification of preterm birth is based on clinical presentation of spontaneous or indicated preterm birth. Spontaneous preterm births result from the idiopathic onset of labor or premature rupture of membranes (PPROM) surrounding the fetus. Indicated births result from pregnancy complications (e.g. preeclampsia) requiring physician-initiated early delivery.[1, 9] In the US, 70% of preterm births are spontaneous (45% spontaneous preterm labor and 25% PPROM) and 30% are provider-initiated.[1] Preterm classification based on clinical presentation reflects the pathway to delivery including clinical management of indicated preterm births.[24]

The third classification is based on proposed etiologic classification of preterm births by maternal, fetal, or placental origin.[24] Maternal causes may include infection, trauma, worsening pre-existing conditions, uterine rupture and preeclampsia. Fetal causes may include intrauterine growth restriction, fetal anomalies, polyhydramnios, fetal infection or fetal inflammatory response and complications of twin pregnancies. Placental etiologies include placental infections (e.g., chorioamnionitis), placental abruption, and other placental abnormalities. However, classification of etiology requires further refinement as preterm births have can have overlapping maternal, fetal, and placental origin[22] and 30% of preterm births have no clear etiology.[6]

Although no current classification approach is ideal, sub-classifying preterm births have clinical, public health, and research implications. Clinically, classifying a preterm pregnancy by

gestational age will inform clinical management of the neonate and classifying preterm births by clinical presentation will determine if preterm birth interventions will be effective in future pregnancies. Current interventions to reduce the risk of preterm birth in future pregnancies are specific to women with a history of spontaneous preterm birth.[25] From a public health perspective, understanding the geographic and demographic distribution of extreme, very, and moderate preterm births will inform funding and priority of public health efforts at the local, state, and national level. Classification by etiology is informative for preterm birth research. Identifying sub-groups of preterm births with shared etiology or pathophysiology guides which risk factors to target for preterm prevention. For this dissertation, we focus on pre-pregnancy obesity as a risk factor for preterm birth as it a risk factor of extreme preterm birth irrespective of clinical presentation[26] that may increase the risk of multiple etiologies of preterm birth (e.g. preeclampsia, intrauterine growth restriction, fetal anomalies, and exacerbation of pre-existing conditions).[27] Further, refining preterm classifications by etiology is a secondary aim of this dissertation.

Table 1: Classification paradigms of preterm birth

Gestational Age	Clinical Phenotype	Etiology

• Extreme preterm birth (<28weeks)
• Very preterm birth (28 to <32 weeks)
• Moderate/late preterm birth (32 to <37weeks)
	• Spontaneous preterm birth
	  ○ spontaneous preterm labor
	  ○ premature rupture of membranes
	• Indicated preterm birth
		• Maternal origin
		• Fetal origin
		• Placental origin

1.2.3 Limited Treatments of Preterm Birth

Currently, clinical strategies to reduce the risk of preterm birth in singleton pregnancies include: 1) intramuscular (IM) progesterone caproate in women with a history of spontaneous preterm birth, 2) cervical cerclage (sewing the cervix closed) in women with a history of preterm delivery and short cervical length, and 3) vaginal progesterone in women with a short cervical length before 24 weeks of gestation.[25] These therapies however have limited benefit. IM progesterone caproate is only indicated for women with a history of spontaneous preterm birth and recurrent preterm birth affects only 15% of women with a prior history of spontaneous preterm birth.[25, 28] The recent PROLONG multi-site clinical trial found no benefit from IM progesterone caproate to reduce recurrent risk of preterm birth, leading the FDA and American College of Obstetrics and Gynecology to request additional studies to test the effectiveness of IM progesterone caproate.[25] Cervical cerclage and vaginal progesterone are specific to pregnancies affected by short cervical length, limiting the generalizability of these preterm interventions. Short cervical length (less than 25 mm) occurs in less than 2% of all pregnancies and in only 10% of pregnancies with a previous spontaneous preterm birth.[29, 30]

The limited scope of interventions hinders public health efforts to reduce preterm birth and maternal factors such as obesity may limit the efficacy of certain interventions. In a secondary analysis of the Maternal-Fetal Medicine Units Network Progesterone Trial of women with a history of spontaneous preterm birth, IM progesterone caproate was associated with a non-significant higher risk of preterm birth in obese women (RR: 1.55, 95% CI: 0.83-2.89).[31] Research on causes of preterm birth with shared pathophysiology preterm birth will inform interventions of greater scope and efficacy.

1.2.4 Pathophysiology of Preterm Birth

Preterm birth is a clinical syndrome defined by shorter gestational age and multifactorial etiology. Causes of preterm birth require an understanding of how normal pregnancy can go wrong and can broadly be classified as maternal, fetal, or of placental origin (**Figure 2**).

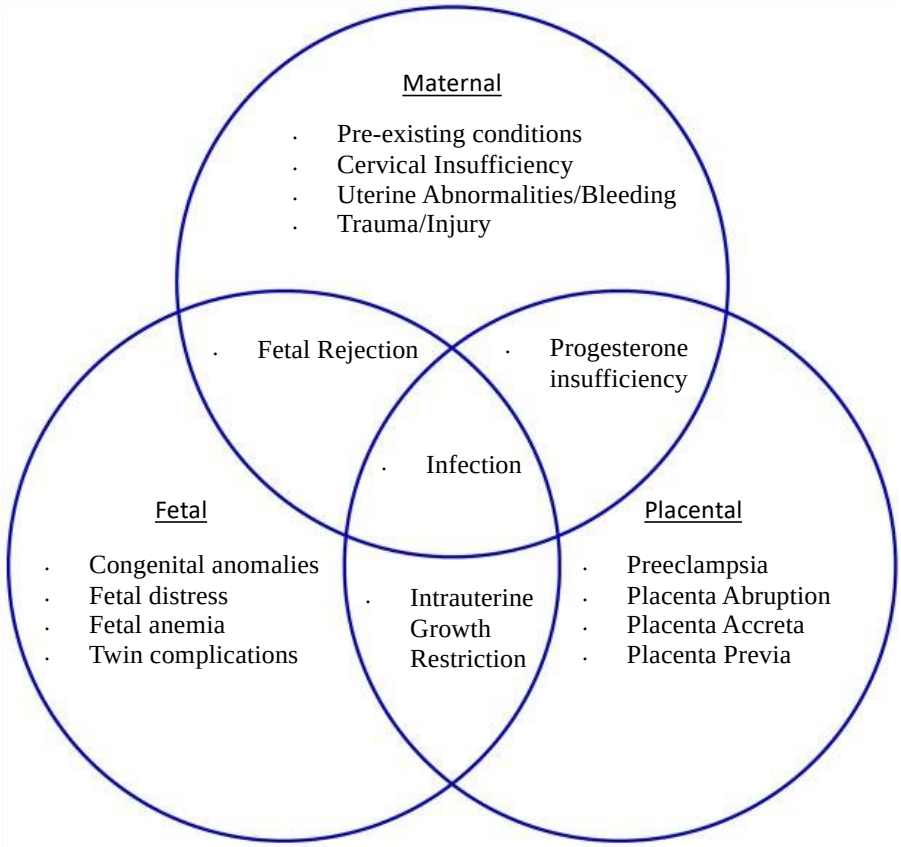


Figure 2: Maternal, fetal, and placental causes of preterm birth

1.2.4.1 Maternal Factors

During pregnancy, women undergo physiologic changes in both uterine and extrauterine tissues. Increased production of progesterone from the corpus luteum and the placenta stimulate growth of the uterine lining and uterine vascularization to support fetal development.[32] Structural

abnormalities or abnormal bleeding of the uterus may result in a spontaneous or physician-initiated preterm delivery.[6] Immune and hormonal signaling during pregnancy keeps uterine muscle in a quiescent state to prevent premature labor contractions[32] and maintains immunotolerance during pregnancy to prevent fetal rejection.[33] Imbalances in hormone and immune control may lead to premature labor, fetal rejection, or rupture of the membranes surrounding the fetus.[34, 35] In addition to the uterus, the cervix is maintained in a closed rigid state with cervical canal occluded by a thick mucus plug to prevent ascending infections from the vagina.[1, 36] Cervical insufficiency in which the cervix may be prematurely shortened or dilated is a well-documented cause of preterm birth.[6, 37] However, there is no effective method to predict cervical insufficiency in nulliparous women prior to pregnancy,[38] and a history of cervical insufficiency does not predict cervical length in the following pregnancy.[36]

Physiologic adaptations by the placenta and uterus have downstream effects on non-reproductive tissues. Increases in fluid volume and vasculature of the uterus affect cardiac load and renal filtering of metabolic waste and nutrients.[39] Pregnant women have an increased metabolic rate that the body adapts to by increasing ventilation to increase oxygen intake as well as shifting to an insulin resistant state to optimize glucose utilization by the fetus.[39] Thus, underlying maternal cardiovascular, pulmonary, renal, and endocrine conditions can put a woman at higher risk of preterm birth.[6, 35]

Maternal etiologies of preterm birth unrelated to physiologic changes of pregnancy include maternal injury or trauma to the uterus.[6] Severe extrauterine infections, such as from influenza, may necessitate an indicated preterm birth to prevent maternal or neonatal death.[40]

1.2.4.2 Fetal Factors

The health of the fetus is one determinant of indicated preterm births. A preterm delivery is often necessary to prevent fetal demise.[41] Healthcare providers use fetal ultrasound and electronic fetal heart rate monitoring (cardiotocography) to detect fetal growth restriction, congenital abnormalities (e.g. Down's syndrome and neural tube defects), fetal anemia,[42] abnormal fetal heart rate, and reduced fetal movements.[43] All these fetal signs are indications for preterm birth.[6, 24, 35, 41] While these etiologies are considered fetal factors, underlying causes are often unknown or result from maternal and placental conditions (e.g. preeclampsia), emphasizing the opportunity to identify shared pathophysiology for preterm birth interventions. Other fetal indications of preterm birth include complications from twin pregnancies, including demise of one of the fetuses and twin-twin transfusion syndrome, in which abnormal connections between twins in pregnancy result in life-threatening imbalances in amniotic fluid.[44]

1.2.4.3 Placental Factors

Proper placental function is essential for a healthy pregnancy. During first trimester, the placenta implants into the uterine wall causing significant vascular remodeling to allow for proper maternal-fetal transport of nutrients and metabolic waste products.[45, 46] Abnormal implantation from failed maternal spiral artery remodeling may contribute to intrauterine growth restriction and preeclampsia,[46] two leading causes of provider-initiated preterm birth.[6, 41] Failed spiral artery remodeling may also contribute to spontaneous preterm births by causing placental ischemia.[47] Conversely, placenta cells called trophoblasts that invade too far into the uterine tissue can necessitate a preterm delivery to avoid life threatening bleeding during labor from a condition called placenta accrete.[48] Other placenta implantation disorders necessitating preterm delivery

include premature detachment from the uterus called placental abruption,[49] and placenta previa, a condition where the placenta implants over the cervix preventing vaginal delivery.[50]

After implantation, the placenta is the predominant source of progesterone by late first trimester.[45] Progesterone promotes uterine wall thickness, inhibits uterine contractions until labor onset, and modifies the uterine immune environment to prevent fetal rejection.[51, 52] Lower serum progesterone concentrations, altered placental expression of progesterone receptor A and B, and changes in placental progesterone metabolism may increase the risk of preterm birth.[52] Lastly, inflammation of the placental chorion and fetal membranes (chorioamnionitis) is a sign of intrauterine infection that increases the risk of preterm birth.[53] Chorioamnionitis is reported in approximately 40-70% of all spontaneous preterm births.[53]

While I've highlighted etiologies as of maternal, fetal or placental origin, many of these complications have overlapping pathophysiology (e.g., preeclampsia, IUGR, fetal rejection, and premature rupture of membranes, spontaneous preterm birth). Abnormal inflammation (impaired or excessive) is a potential cause of preterm birth that affect can affect the mother, fetus, and placenta. Excessive inflammation may lead to fetal rejection, premature rupture of fetal membranes, abnormal uterine contractions, and cervical insufficiency though precise mechanisms remain unclear. Further, prediction of infectious causes of preterm birth is poor and confirmed only after delivery. While 30% of preterm births have no known cause, inflammation from either subclinical infection or non-infectious causes may play a role.[35] Despite the heterogeneous etiologies of preterm birth, focusing on risk factors associated with inflammation from either infectious (e.g. bacterial vaginosis) or non-infectious causes (e.g. obesity) will potentially have the most impact at reducing the burden of preterm birth. Further, to understand the genetic and

metabolic inflammatory mechanisms targetable for preterm interventions, studies leveraging advances in high-throughput technologies are warranted.[6, 10, 54]

1.2.5 Risk Factors of Preterm Birth

While the etiologic pathways of preterm birth are poorly understood there are many well-documented risk factors for preterm birth. Risk factors are broadly categorized as sociodemographic characteristics, pregnancy characteristics, and potentially modifiable characteristics. These are summarized in **Table 2.**

Table 2: Summary of risk factors of preterm birth

Sociodemographic Factors	Odds Ratio	Pregnancy Factors	Odds Ratio	Modifiable Factors	Odds Ratio
•Age > 40 years	1.20	•Preterm birth history	2.00	•Smoking	2.00
•Black race (experience of racism)	2.00	•Cervical/uterine anomalies	2.00	•Underweight BMI	1.50
•Low SES	1.20	•Multiple gestation	12.8	•Obese BMI	1.50-2.50
•Low educational attainment	1.30	•ART	1.50	•Birth spacing < 6 months	1.33
•Limited prenatal care	2.50	• Stress/depression	~1.50	•Chronic Hypertension	3.81
		•Bacterial vaginosis	3.00	•Pre-existing Diabetes	3.51
		•Periodontal disease	4.00		

1.2.5.1 Sociodemographic Characteristics

Sociodemographic characteristics include both maternal and neighborhood level factors. Maternal age has a "U-shaped" relationship with risk of preterm birth.[55] There is a reported higher risk of preterm birth among women less than 25 years old (OR: 1.08, 95% CI: 1.01-1.15) and women over 40 years of age (OR: 1.20, 95% CI: 1.06-1.36) compared to women 30-34 years old even after adjusting for parity. Black women have a 2-fold greater risk of preterm birth compared

to white women.[56] Societal level factors associated with preterm birth include lower inadequate prenatal care and socioeconomic care.[6, 57] The American College of Obstetrics and Gynecology recommend attending prenatal visits every 4 weeks until 28 weeks of gestation, every 2 weeks 28-36 weeks of gestation, and every week from 36 weeks of gestation to delivery.[57] Compared to women receiving the recommended number of prenatal care visits, women receiving no prenatal care had a 7 times greater risk of preterm birth and women receiving less than 25% of the recommended prenatal care visits had a 2.5 fold higher risk of preterm birth.[57] Women living below the US federal poverty line relative to those above the poverty line had a modestly higher odds of preterm birth <35 weeks (OR:1.15, 95% CI: 0.96-1.39) and very preterm birth <32 weeks (OR: 1.23, 95% CI: 1.03-1.46).[58] In a US cohort, a 1.3 times higher odds of preterm birth was observed among women without a high school diploma/GED compared to women without a college degree.[59] The association between education attainment and risk of preterm birth was not replicated in a Dutch cohort,[60] perhaps suggesting the economic value of education in the US is distinct from that in European countries. More recently, air pollution has been associated with higher risk of preterm birth. A recent systematic review reported that exposure to ozone and $PM_{2.5}$ was associated with a median 11.5% (2.0-19.0%) higher risk of preterm birth.[61]

1.2.5.2 Pregnancy Characteristics

The reproductive history of a pregnant women is informative of a woman's risk of preterm birth. A prior preterm birth less than 32 weeks is associated with a 2-fold higher risk of preterm birth.[1, 62] Between 15 to 50% of women with a prior preterm birth will have a recurrent preterm birth in the next pregnancy.[1] Additionally, women who have a history of cervical biopsies or uterine abnormalities have a 2-fold higher risk of preterm birth compared to those with no cervical or uterine complications.[1, 63]

Clinical factors during the current pregnancy are also prognostic for preterm birth risk. Twin or multiples gestation are associated with 12.8 times higher risk of preterm birth than singleton pregnancies.[64] Approximately 60% of all twin pregnancies will be delivered preterm.[1] Assisted reproductive technology increases the likelihood of twin pregnancies; therefore, there is an associated higher risk of preterm birth with use of assisted reproductive technology.[1] However, even among singleton pregnancies, assisted reproductive technology (for male or female infertility) compared to pregnancies without use of assisted reproductive technology is associated with a 1.5-fold higher risk of preterm birth.[65] Other clinical risk factors include infection and maternal psychological health. Bacterial vaginosis is associated with a 2 to 3-fold higher risk of preterm birth and periodontal disease is associated with a 4-fold higher risk of preterm birth.[66, 67] However, use of antibiotics pre-pregnancy and during pregnancy is not associated with a lower risk of preterm birth and has been shown to increase the risk of preterm birth in some studies.[68, 69]

1.2.5.3 Modifiable Characteristics

Several individual risk factors are potentially modifiable factors for preterm birth interventions. Tobacco and alcohol use are two traditional risk factors of preterm birth.[1, 6] However, mild alcohol use during pregnancy is not associated with preterm birth and the decline in cigarette smoking to 11-13% of pregnant women in developed countries due to anti-tobacco legislation has lowered the population attributable risk of smoking for preterm birth.[1, 70] Another maternal factor associated with preterm birth is pre-pregnancy BMI. There is a "J-shaped" association between BMI and preterm birth.[26] Compared to women with a lean BMI (18.5 to <25kg/m^2), underweight BMI (<18.5kg/m^2) is associated with approximately a 1.5-fold increase in spontaneous preterm birth, while obese BMI (>30kg/m^2), is associated with a 1.5 to 2.5-fold increase in indicated-preterm births and spontaneous preterm birth < 32 weeks of gestation.[26] The

adverse effects of obesity may be exacerbated by excessive weight gain during pregnancy.[71] In a pooled cohort of pregnancies from Europe, North America, and Oceania, high gestational weight gain Z-scores normalized for gestational age (≥1 standard deviation) compared to normal gestational weight gain Z-scores (-1.0 to 0.9 standard deviations) was associated with 2.14 times higher odds (95% CI: 1.86-2.46) of preterm birth.[71] Co-morbidities of obesity are also significant risk factors of preterm birth. In a large retrospective cohort study, pre-pregnancy hypertension was associated with a 3.81 times risk of preterm birth <37 weeks (95% CI: 3.54-4.10) and 6.31 times high risk of preterm birth <34 weeks (95% CI: 5.53-7.19).[72] Likewise, pre-pregnancy diabetes was associated with a 3.51-fold higher risk of preterm birth <37 weeks (95% CI: 3.25-3.78) and a 3.29-fold higher risk of preterm birth <34 weeks (95% CI: 2.76-3.93).[72] Chronic hypertension and pre-existing hypertension were most strongly associated with medically-indicated preterm births and preterm births with preeclampsia, but these co-morbidities were also significant risk factors for spontaneous preterm births.

Birth spacing duration or the timing between a previous delivery and the conception of the current pregnancy, is another risk factor of preterm birth. In women with a birth spacing interval of less than 6 months compared to women with a birth spacing interval of 18-23 months, there is a modest 1.33 times greater odds of moderate preterm birth (32-36 weeks) and a 1.68 times higher odds of very preterm birth (<32 weeks).[73]

1.2.6 Targeting Maternal Obesity for Preterm Birth Prevention

To summarize, preterm birth is the most common adverse pregnancy outcome in the US with lasting health consequences for the child. To date, little progress has been made to reduce the burden of preterm birth in the US. Public health efforts should prioritize addressing modifiable

risk factors that would reduce a large portion of preterm births if prevented (i.e., a high population attributable risk) in pregnant women in the general US population and high-risk pregnancy groups such as Black women. Ideally, risk factors are generalizable to all preterm births irrespective of classification and have shared pathophysiology across the multiple etiologies of preterm birth. Finally, targeting risk factors that have existing interventions or prevention strategies for other conditions, allows for a faster transition from research to public health action.

Using these criteria, we believe pre-pregnancy obesity is a suitable target for preterm birth prevention. Based on 2017 National Vital Statistics Birth cohort, pre-pregnancy obesity is a potentially modifiable risk factor with a higher population attributable fraction **(Figure 3)** than other known modifiable risk factors in the US. Further obesity contributes to other strong risk factors of preterm birth including pre-existing hypertension and diabetes. The adverse effects of obesity for preterm birth may be more pronounced in Black women, as the prevalence of obesity is 10% higher in Black pregnant women compared to white pregnant women (40% vs 29%).[74]

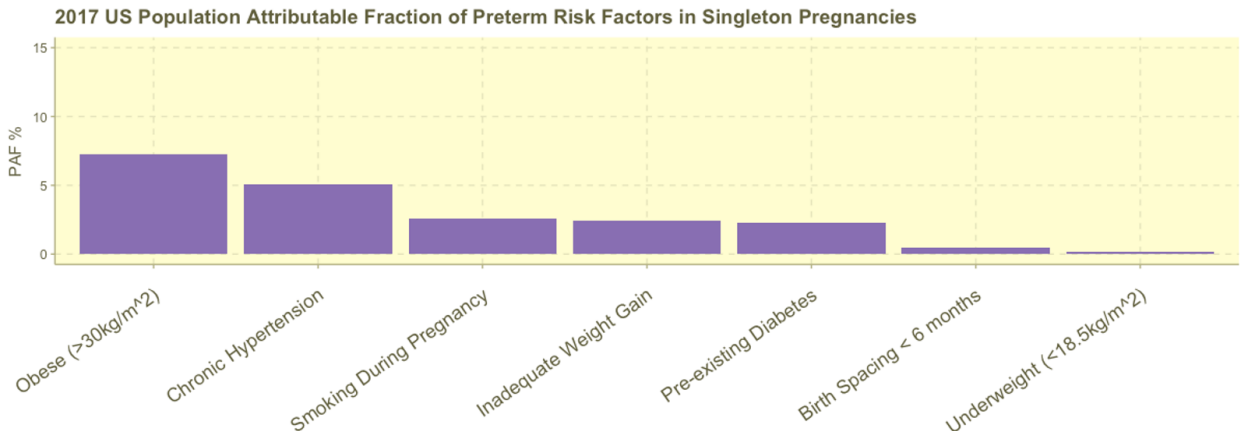


Figure 3: 2017 US population attributable fraction of modifiable preterm risk factors

The population attributable fraction (PAF%) describes the proportion of preterm births attributable to each modifiable maternal risk factor in the US. The PAF% was estimated based on 2017 US Vital Statistics Data and published risk ratios.[72, 73, 75-78]

Pre-pregnancy obesity has been reported to increase the risk of both spontaneous and medically indicated preterm birth, with the strongest association in extreme preterm births (<28weeks gestation).[26] Likewise, pre-pregnancy obesity has already been the target of prevention strategies for preeclampsia,[79-82] a hypertensive disorder of pregnancy and medical indication for preterm delivery. Active interventions for prevention of preeclampsia relevant to obesity include lipid-lowering (pravastatin) and glycemic-lowering (metformin) drugs,[81] energy and lipid-lowering diets,[80] fish oil supplementation,[80] bariatric surgery prior to pregnancy,[83] interpregnancy weight reduction,[84] and reducing weight gain during pregnancy by diet and exercise.[82] However, to determine if these interventions will translate to preterm birth, a better understanding of the pathophysiology between pre-pregnancy BMI and preterm birth is warranted.

1.3 Maternal Obesity and Risk of Preterm Birth

1.3.1 Epidemiology of Maternal Obesity in Pregnancy

In the US, the prevalence of obesity among women 18-44 years of age has steadily increased from 8.9% in 1990 to 30.5% in 2018 (**Figure 4**).[85] With approximately one third of all women of reproductive age being obese, the US is among the countries with the highest rates of maternal obesity. Similar to preterm birth, there are notable racial disparities for pre-pregnancy obesity in the US. Non-Hispanic Black and Native American women have a 1.5 times higher odds of obesity than white women.[86] Lower socioeconomic status and lower education attainment[87, 88]

are also associated with higher risk of maternal obesity, suggesting disadvantaged populations are at higher risk for maternal obesity.

Maternal obesity is associated with higher risk of short-term and long-term complications for the mother and infant (**Table 3)**. In pregnancy, the primary concern of obesity are complications related to diabetes and hypertension. In a US 2011-2016 national cohort of obese women 20 to 44 years of age, the prevalence of hypertension and type 2 diabetes was 17.2% and 8.9% respectively.[89] Obese women with pre-existing diabetes are at a 2 times higher risk for complications in pregnancy such as diabetic retinopathy.[27] In women without pre-existing conditions maternal obesity is associated with increased risk of both gestational diabetes and preeclampsia.[90] Overweight BMI ($\geq$25kg/m^2) is associated with a 2-fold higher odds of preeclampsia, and pre-pregnancy obesity ($\geq$30 kg/m^2) is associated with a 3-fold higher odds of preeclampsia.[91] Likewise, maternal obesity is associated with a 4 times higher odds of gestational diabetes and severe obesity ($\geq$40kg/m^2) is associated with an 8 to 9 times higher odds of gestational diabetes.[92, 93] US data from 7 states from 2004-2006, found that the population attributable fraction of gestational diabetes from an overweight and obese BMI is 46%, meaning nearly half of gestational diabetes cases would have been prevented if overweight and obese BMIs were reduced to a lean BMI.[94] The US prevalence of maternal obesity has increased from 20.2% in 2004 to 31.6% in 2019,[85] meaning obesity may contribute to an even larger proportion of gestational diabetes cases today. Pregnancy complications such as preeclampsia are also an indication for preterm birth. As I will discuss in more detail shortly, obese women, particularly women with a BMI $\geq$40kg/m^2, have an associated 2 to 3-fold increased risk of both indicated preterm birth and early spontaneous preterm birth (<32 weeks) compared to lean women.[27, 93] Other maternal complications include delivery outcomes. Compared to lean women, obese women have an

associated 2-fold higher risk of caesarean section and a 1.2 times higher risk of post-partum hemorrhage.[27, 93, 95]

For the infant, maternal obesity can impact fetal viability and development during pregnancy. Obese women are 25-30% more likely than lean women to have a miscarriage[93, 96] and 50% more likely to have a stillbirth in later pregnancy.[93] In a meta-analysis, obesity was associated with a higher odds of birth defects including neural tube defects of any kind (OR: 1.87, 95% CI: 1.62-2.15), spina bifida (OR: 2.24, 95% CI: 1.86-2.69), and hydrocephaly (OR: 1.68; 95% CI, 1.19-2.36).[97] Maternal obesity compared to a lean BMI also increases the risk of a large-for-gestational age (LGA) baby; there is a 1.74-fold higher risk of LGA for obesity class I (≥30kg/m^2; RR: 1.74, 95% CI 1.65–1.83) which increases to a 2.32-fold higher risk of LGA for obesity class III (≥40kg/m^2; RR: 2.32, 95% CI: 2.14-2.52).[98] Consequently, a higher risk of a larger baby also increases the risk of delivery complications for the fetus, specifically shoulder dystocia (a labor complication that can result in nerve damage or collar bone fracture in the neonate).[27]

Table 3: Short-term and long-term complications of maternal obesity

Maternal Complications	**Pre-conception**	• Infertility/ Time to Conception
	Pregnancy/Delivery	• Exacerbation of co-morbidities (e.g. diabetic retinopathy, nephropathy) • Miscarriage/ Stillbirth • Preeclampsia, Gestational hypertension • Preterm Birth • Cesarean Delivery • Post-partum hemorrhage Infection wound disruption
	Long-term Postpartum	• Weight Retention/ Persistent Obesity • Type II diabetes • Cardiovascular Disease • Dyslipidemia • All- cause mortality
Child Complications	**Birth**	• Congenital malformations (neural tube defects, cardiac malformations, hydrocephaly • LGA or macrosomia (birthweight>4000g)

		• Shoulder Dystocia
	Later-life complications	• Childhood Obesity • Insulin resistance/ diabetes • Dyslipidemia

1.3.2 Maternal Obesity and Risk of Preterm Birth in Population Studies

The relationship between obesity and risk of preterm birth is well-documented. Yet, findings are inconsistent across diverse populations and due to variation in cut-offs for both obesity and preterm birth. I conducted a narrative review of the literature to summarize studies since 2010 on obesity and risk of preterm birth. I applied the search strategy in **Table 4** on March 4, 2022, to identify studies in PubMed. Additional studies were found using Google Scholar. I included only human studies of singleton pregnancies with an available pre-pregnancy BMI or first trimester BMI, preterm birth as an outcome, and term births as a comparator group. Cross-sectional, case-control, and cohort studies were all acceptable study designs. I included studies that classified preterm birth as gestational age <37 weeks, stratified as spontaneous or indicated, or stratified by gestational age ranges. Studies are summarized in **Appendix Table 1**.

Table 4: Pubmed search strategy

Search Strategy	Number of Studies
(("pre-pregnancy BMI"[All Fields] OR "obesity, maternal"[MeSH Terms] OR "pre-pregnancy obesity"[All Fields] OR "maternal BMI"[All Fields] OR "maternal obesity"[All Fields]) AND ("obstetric labor, premature"[MeSH Terms] OR "Premature Birth"[MeSH Terms] OR ("preterm premature rupture of the membranes"[Supplementary Concept] OR "preterm premature rupture of the membranes"[All Fields] OR "pprom"[All Fields] OR "pproms"[All Fields]) OR "preterm birth"[All Fields] OR "prematurity"[All Fields] OR "preterm labo*"[All Fields])) AND (2010:2022[pdat])	378

1.3.2.1 Preterm Births (<37 weeks)

There were 34 studies that reported on pre-pregnancy obesity and risk of preterm birth. Odds ratios from 20 studies on the association between obesity (≤30kg/m^2) and odds of preterm birth (<37 weeks) compared to lean women are graphically represented in **Figure 4.** Overall, study findings suggest a modest effect of obesity on risk of preterm birth <37 weeks of gestation. Obesity was associated with a 1.05 to 1.79 times higher odds of preterm birth compared to lean women, which was statistically significant in 9 of the 20 studies.[76, 81, 99-116] The lack of a significant association in several studies may be a consequence of a small sample size,[100, 106, 108, 114] or reflect variation in the study population. One study excluded pregnancies by artificial reproductive technology despite obesity being a well-documented cause of infertility.[106] Another study with non-significant findings used data from the 1980s when the prevalence of obesity was less than 10%.[115] Despite only a modest effect, the severity of obesity may exacerbate the associated risk of preterm birth. In four studies, the association between obesity and odds of preterm birth increased from obese class I (30-34.9kg/m^2) to obese class III (≥40kg/m^2),[103, 107, 117, 118] suggesting a dose-response of pre-pregnancy BMI on risk of preterm birth.

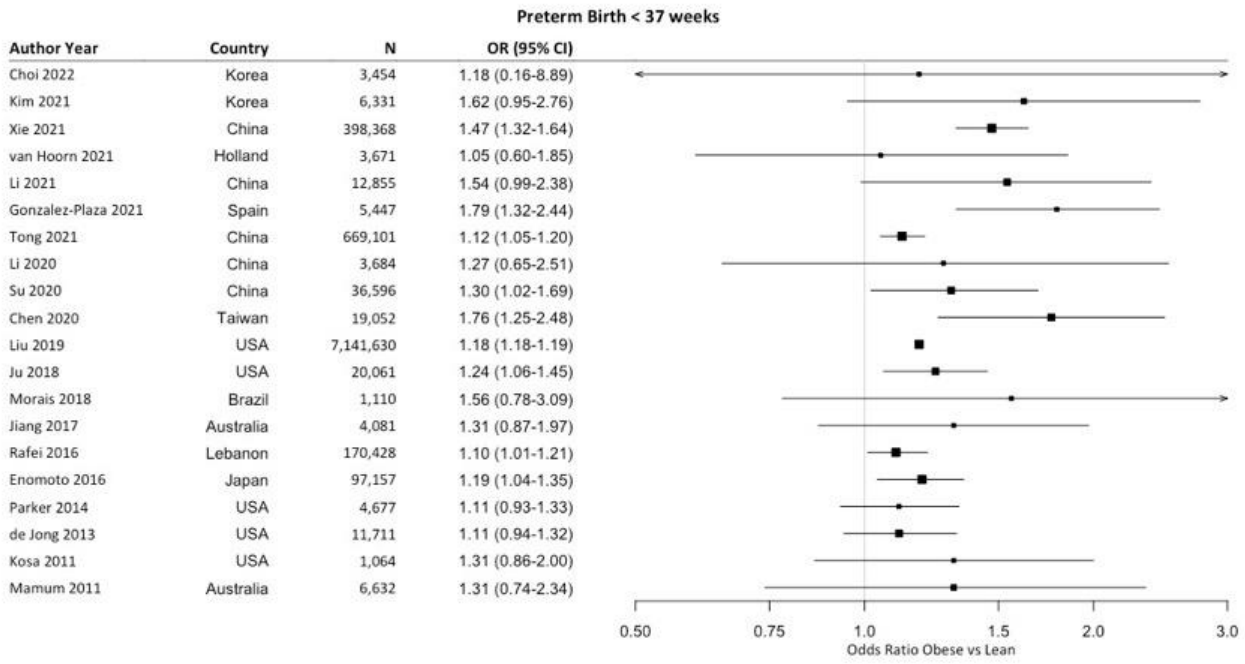


Figure 4: Summary of findings on obesity and preterm birth <37 weeks

1.3.2.2 Spontaneous and Indicated Preterm Birth

There were ten studies that specified whether preterm births were spontaneous or indicated. Studies generally found that obesity was associated with indicated preterm-births, while findings on spontaneous preterm birth differed by gestational age (**Figure 5)**. In five studies, pre-pregnancy obesity was associated with a 1.5 to 3-fold higher odds of indicated preterm birth.[26, 104, 117, 119, 120] The magnitude of this association is stronger with more severe obesity and earlier gestational age at delivery. *Cnattingius et al.* found that among indicated preterm births, obesity class II (35 to <40kg/m^2) compared to lean women was associated with a 2.74 times higher odds of extremely preterm birth (<28wks), a 2.52 times higher odds of very preterm birth (28 to <32wks), and 2.00 times higher odds of moderate preterm birth (32 to 36wks).[26] Likewise, women with obesity class III compared to lean women was associated with a 3.84 times higher odds of extremely preterm birth, 4.16 times higher odds of very preterm birth, and 2.45 times higher odds of moderate preterm

birth among indicated deliveries. Comparable trends were reported in a North American pregnancy cohort by *Ram et al.*[117]

Data on BMI and spontaneous preterm birth are conflicting. In studies that did not stratify spontaneous preterm birth as extremely, very, or moderate preterm, there were no significant associations between obesity and spontaneous preterm birth.[113, 119] However, studies that further classified spontaneous preterm birth by gestational age report that an obese BMI is associated with a higher odds of extremely (<28wks) and very spontaneous preterm birth (<32weeks),[26, 117, 120-122] and either a null or lower odds of moderate spontaneous preterm birth compared to lean women.[26, 117, 120, 122]

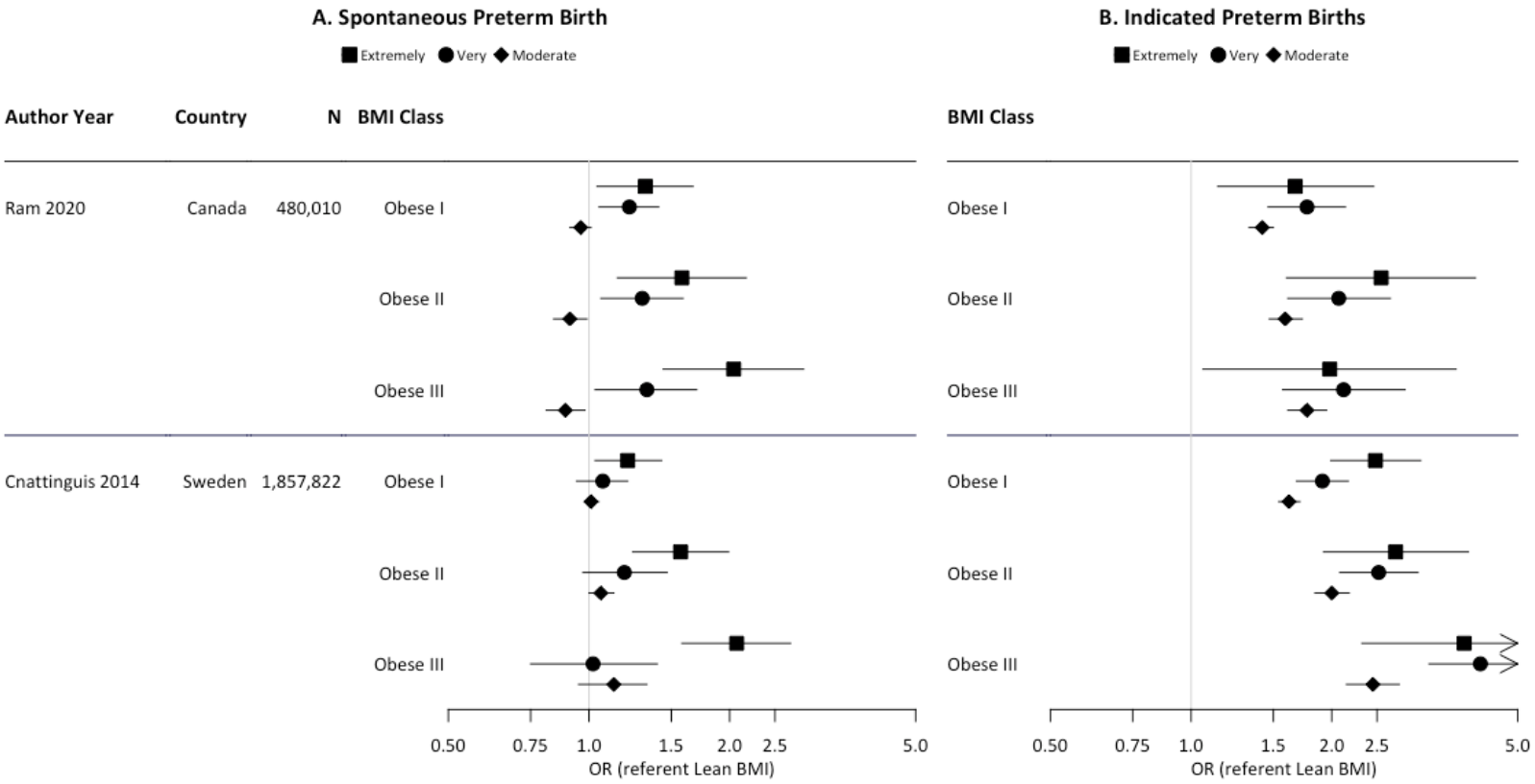


Figure 5: Association between obesity and preterm birth stratified by clinical presentation and gestational age.

The different shaped boxes indicate the odds ratio estimates for preterm birth based on severity of preterm birth as extremely (<28weeks), very (28 to 32 weeks), and moderate preterm birth (32 to <37 weeks). Obesity classes are defined as class I (30 to <35 kg/m^2), class II (35 to <40kg/m^2), and class III (≥40kg/m^2).

1.3.2.3 Severity of Preterm Birth by Gestational Age

Grouping preterm births by gestational age is a classification for severity of prematurity. There were thirteen studies that classified preterm births by gestational age cut-offs. Two studies compared early (<32weeks) and late (>32weeks) spontaneous preterm births.[121, 123] Neither study reported significant findings between obesity and risk of early spontaneous preterm births and one of these studies reported only a weak association between obesity and increased risk of late spontaneous preterm birth (OR: 1.26, 95% CI: 1.03-1.54).[121] Another two studies measured associations in early (<32 or 33weeks) and late (>32 or 33 weeks) preterm births of any subtype.[111, 118] In both studies, obese women compared to lean women had a significantly higher risk of early preterm birth (ORs: 1.33 and 1.66) and a modest higher risk of late preterm birth (ORs: 1.16 to 1.33). A fifth study classified early preterm birth as less than 34 weeks and late preterm birth as 34-36 weeks and stratified by clinical presentation (spontaneous and indicated).[116] Obesity was associated with a 1.78 higher odds of early indicated preterm birth compared to lean women and a 1.49 higher odds of late preterm birth. Among spontaneous preterm births, there was no significant association in either early or late preterm birth. Lastly, a study that restricted to late preterm births (32-36 weeks) observed a 2-fold higher odds of late preterm birth (OR: 2.16, 95% CI: 1.16-4.04) among extremely obese women ($\geq$50kg/m^2) compared to lean women.[124] These finding generally indicate obesity is associated with more severe preterm birth.

The remaining seven studies, classified preterm births as extremely (<28weeks), very (28 to <32 weeks) and moderate (32 to <37weeks) preterm birth.[26, 76, 104, 105, 117, 122, 125] In four studies that didn't stratify between spontaneous and indicated preterm births, there was a higher odds of preterm birth compared to lean women and the strength of this association increased with more severe prematurity (extremely and very preterm birth).[76, 104, 105, 125] Three studies further stratified

preterm births by clinical presentation.[26, 117, 122] Among indicated pregnancies, obesity was associated with a higher odds of preterm birth compared to lean women and the strength of this association increased with more severe prematurity (extremely and very preterm birth). Among spontaneous preterm births, two studies found obesity was only associated with a higher odds of extremely preterm birth,[26, 122] while the third study reported a higher odds of both extreme preterm birth (ORs obese I: 1.32, obese II: 1.58, obese III: 2.40), and very preterm birth (ORs obese I: 1.22, obese II: 1.30, and obese III: 1.33).[117] Findings from these 13 studies demonstrate that the associated higher odds of preterm birth increases in magnitude with earlier gestation and more severe obesity (e.g. BMI ≥40kg/m^2). A recent meta-analysis similarly reported the obesity was more strongly associated with early preterm birth (<32 weeks; OR: 1.40 95% CI: 1.20-1.63) than preterm birth <37 weeks (OR: 1.17, 95% CI: 1.13-1.21).[126] Further, the association between obesity and risk of preterm birth is more pronounced in indicated pregnancies, but an association between obesity and odds of spontaneous preterm birth exists for extremely and very preterm birth.

1.3.2.4 Racial/Ethnic Stratifications for Preterm Birth

In five US studies, pregnancies were stratified by race and ethnicity. In the US, preterm birth disproportionately affects Black women,[54] and Black women are more likely to be obese than white women.[127] Yet, in three studies obesity was associated with a lower odds of preterm birth in Black women.[112, 123] The protective effect was strongest for preterm births before 32 weeks of gestation (OR: 0.23, 95% CI: 0.08-0.70). In contrast, obesity was associated with a higher risk of preterm birth among white women (OR: 1.40-1.80). These contrasts in risk of preterm birth from obesity are striking but should be interpreted with caution. One study found that the association between obesity and odds of preterm birth in Black women depended on the severity of obesity.[107] Obesity I (BMI 30-34.9kg/m^2) was associated with a lower odds of preterm birth (OR: 0.95, 95%

CI: 0.93-0.97), obesity II (35-39.9kg/m^2) was not associated with preterm birth, and obesity III (≥40kg/m^2) was associated with a higher odds of preterm birth (OR: 1.05, 95% CI: 1.02-1.08). Another study by *Shaw et al.,* found obesity increased the risk of spontaneous preterm births <28 weeks and lowered the risk of spontaneous preterm births between 32 to 36 weeks for all racial and ethnic groups.[122] While there were some differences in effect size in Black and Latina women compared to white women, the magnitude of race/ethnic differences were not as strong as in spontaneous preterm births. Future studies are needed to look at severity and type of preterm birth across racial and ethnic groups. The attenuated effect of obesity on risk of preterm birth in Black women may also correlate with differences in cultural attitudes towards weight and psychosocial environmental factors in Black women compared to white women.[128] A careful interrogation of factors associated with pre-pregnancy BMI, body-image and adverse pregnancy outcomes is warranted to understand racial differences in obesity-associated pregnancy outcomes. Further, differences in the effect of obesity and odds of preterm birth may also exist between white women and other racial/ethnic groups in the US including Asian-Americans, Pacific-Islanders, and Latinx women;[103, 107, 112, 122] however additional studies are warranted.

For my dissertation, race is not only a pertinent confounder of associations between pre-pregnancy BMI and preterm birth, but also placental mechanisms between obesity and risk of preterm birth may vary between Black and white women. While our goal is to identify risk factors and placental mechanisms generalizable to all pregnant women, the pregnancy experience of Black women is inherently different from white women due to structural and individual discrimination and possibly differences in genetic ancestry.[16, 129, 130] Thus, we will test for Black-white interactions with pre-pregnancy obesity on risk of placental dysfunction measured by histopathology and placental gene expression.

1.3.3 The Placenta: A Key Mediator of Obesity and Risk of Preterm Birth

Obesity may increase the risk of preterm birth either through placental mechanisms or mechanisms independent of the placenta (**Figure 6)**. Mechanisms independent of the placenta consist of obesity-associated chronic conditions including chronic hypertension, cardiovascular disease, and type II diabetes that may be exacerbated during pregnancy necessitating a preterm delivery.[27] This pathway seems unlikely to explain a major proportion of preterm births without involvement of the placenta, as cardiovascular events in women of reproductive age and life-threatening consequences of hypertension and diabetes during pregnancy are rare. We suspect, placental-mediated pathophysiology is the predominant pathway between pre-pregnancy obesity and preterm birth as the placenta is the key organ that regulates nutrient transport, hormone control, and inflammation during pregnancy.[45, 131] The placenta may predispose obese women to preterm birth through placental inflammation and placental vascular impairment. Though specific mechanisms have yet to be fully elucidated, growing literature on several hypothesized mechanisms offer promise for future preterm birth interventions.

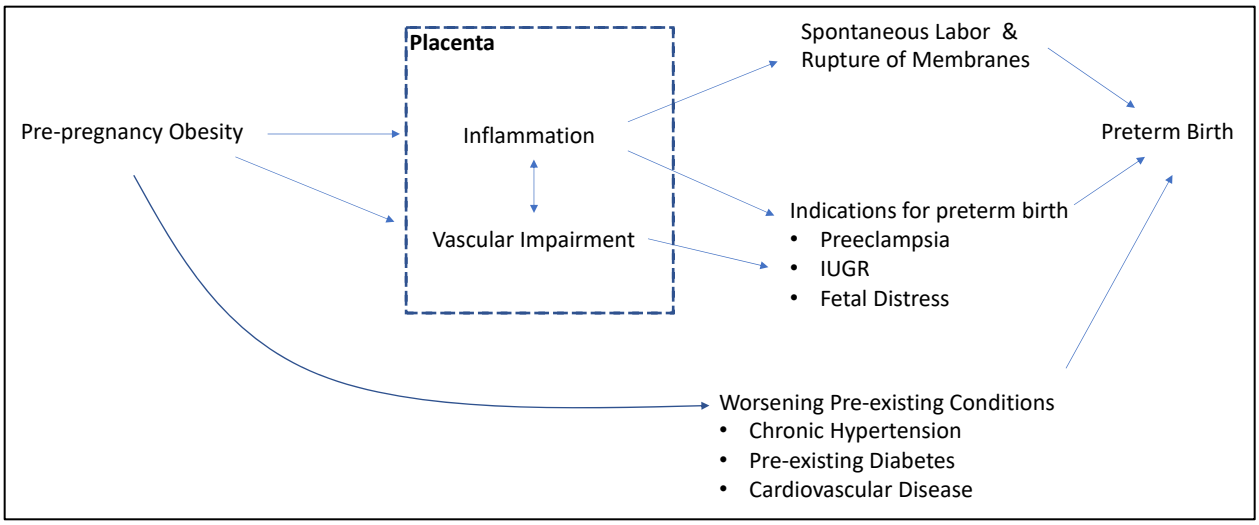


Figure 6: Biological framework of obesity and risk of preterm birth

1.3.3.1 Placental Inflammation

Placental inflammation is required for proper placental growth, protection against infection and initiation of labor.[33, 132] However, excess placental inflammation may increase risk of preterm delivery by impairing maternal-fetal nutrient transport, promoting rupture of membranes that protect the fetus and prematurely stimulating contractions of labor.[133, 134] Although placental inflammation from infection is a well-studied cause of preterm birth, most preterm births are not the result of infection.[135] In many cases, the pathophysiology of preterm birth may be the result of sterile placental inflammation (i.e. inflammation from non-infectious causes).[131] Obesity may be a cause of excessive sterile inflammation, predisposing women to preterm birth, spontaneous or indicated.[136, 137] Obesity may contribute to placental inflammation through upregulation of inflammatory cytokines, lipotoxicity (damage from the accumulation of lipids) and microbiome changes.

Placental trophoblasts, placental macrophages (Hoffbauer cells) and adipose tissue produce similar inflammatory adipokines (leptin, resistin) and cytokines (IL-6, TNF-alpha, MCP-1).[134] Obesity and high-fat diets are reported to increase expression of pro-inflammatory cytokines in adipose and placental tissue.[134, 138] An increase in pro-inflammatory gene expression may promote the rupture of fetal membranes around the fetus and stimulate labor increasing the risk of preterm birth.[139] Notably, leptin gene expression in placental tissue can active metalloproteinases, which are remodeling enzymes that promote weakening of fetal membranes and ripen the cervix for dilation.[139] Increased leptin expression may also impair placental angiogenesis. Impaired angiogenesis may contribute to the increased risk of placental conditions like preeclampsia and fetal growth restriction, both indications for preterm birth.[140] Increased cytokine expression of TNF-alpha and IL-6 may predispose an obese women to preterm labor by promoting uterine muscle contractility.[139] Increased expression of TNF-alpha also increases insulin resistance in pregnancy along with the adipokine resistin.[134] Insulin resistance can further exacerbate conditions during pregnancy such as diabetes and preeclampsia that may lead to an indicated preterm birth.

Excess maternal adiposity may contribute to placental lipotoxicity. Obesity is correlated with higher concentrations of lipids including saturated fatty acids and triglycerides.[141-143] Lipidomic and metabolomic analyses of obese women with spontaneous preterm birth are characterized by signs of dyslipidemia, oxidized lipids, and pro-inflammatory lipids (lipoxygenase and metabolites of arachidonic acid).[144] Under normal conditions the placenta stores lipids and uses fatty acids as a source of energy particularly in late pregnancy.[137] However, in high concentrations, fatty acids can be lipotoxic to the placenta by causing oxidative damage from excess fatty acid oxidation and impairing mitochondrial function.[137, 145, 146] Impaired mitochondrial

function increases insulin resistance, inflammation, and oxidative damage which could increase a women's risk of preterm birth.[145]

High fatty acid concentrations can also act as a ligand for TLRs 2 and 4 on placental villous tissue, the part of the placenta in direct contact with maternal blood.[45, 137, 147, 148] Activated TLRs trigger adaptor proteins such as myeloid differentiation primary response gene 88 (MyD88), to upregulate cytokine expression (IL-1B and IL-6) in placental villi.[149] TLR-associated inflammation is hypothesized to contribute to the initiation of labor as illustrated in **Figure 7**.[139] Bacteria normally activate TLR2 and TLR4 through LPS, but saturated fatty acids are similar to LPS, leading to comparable TLR activation.[149, 150] Activation of TLRs and their downstream proteins (MyD88, Il-1ß, Il-6) are associated with higher preterm birth risk.[134, 151-155] Higher expression of TLRs may allow lower levels of LPS and fatty acids to bind to TLRs triggering placental inflammation and preterm labor. In term births, pre-pregnancy obesity is associated with higher TLR4 expression in placental villous tissue.[156] It is not known if obesity is associated with TLR2 or TLR4 expression in preterm births, however, this is a possible contributor to preterm birth risk in obese women.

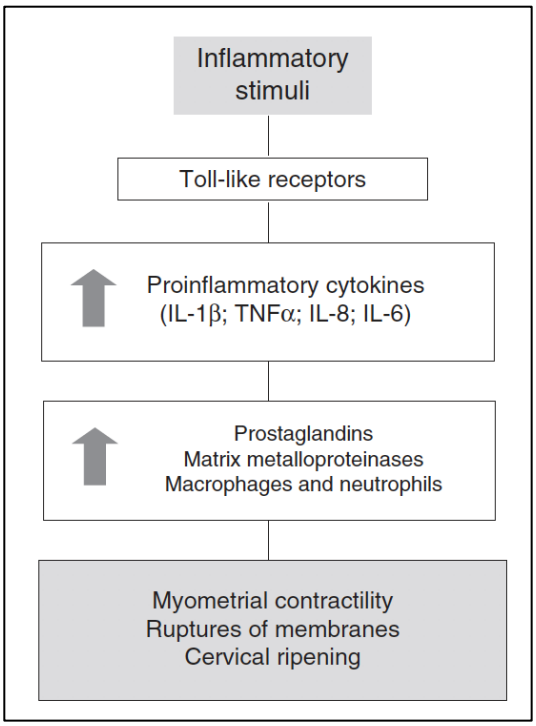


Figure 7: Mechanism of action of toll-like receptors and intiation of labor (figure from *Challis et al. 2009*)

A relatively newer hypothesis for underlying mechanisms of obesity and preterm birth risk are changes in the gut and placenta microbiome.[157-159] The gut microbiome is effected by diet and is known to differ in bacteria phyla between lean and obese individuals.[160] Changes in gut microbiome can lead to "leaky" intestinal barrier function allowing for more pro-inflammatory bacteria in the gut.[161] Two studies have demonstrated that obesity and a high-fat diet are associated with higher circulating concentrations of LPS, a component of bacteria that cause inflammation through TLRs.[161, 162] The one study also showed that higher LPS concentrations were associated with higher systemic inflammation in obese pregnant women. Greater circulating LPS may increase placental inflammation through TLRs expressed on placental tissue. To date no study has directly measured how gut microbiome changes may mediate the risk of preterm birth in obese

women compared to lean women nor if gut microbiome changes in obese women may trigger placental inflammation in term and preterm pregnancies by activation of TLR4 in the placenta. Alternatively, obesity may shift the placenta microbiome to be more proinflammatory,[159] predisposing a pregnancy to preterm birth; however data is limited to one study.

1.3.3.2 Placental Vascular Impairment

The strongest associations between pre-pregnancy obesity and prematurity are for indicated preterm births.[26] The primary placental indication for preterm birth is the hypertensive disorder of pregnancy, preeclampsia.[41, 163] Obesity is associated with a 3-fold increased risk of preeclampsia,[90] and in population studies of obesity and indicated preterm births, the exclusion of participants with hypertensive disorders of pregnancy, attenuates the association to the null.[26, 163] A hallmark feature of preterm preeclampsia is impaired spiral artery remodeling, which normally extends from the placenta to the inner third of the myometrium.[46] Pregnancies with preeclampsia have a higher prevalence of placental features of maternal vascular malperfusion than pregnancies without preeclampsia.[164, 165]

In addition to inflammatory signaling, placental angiogenic factors are needed for coordinating placental spiral artery remodeling.[166] The angiogenic factors, vascular endothelial growth factor, angiopoietin and placental growth factor are highly expressed in early pregnancy for placental vascularization while the antiangiogenic factor, soluble FMS-like tyrosine kinase I is overexpressed in the placentas of women with preeclampsia.[166] The angiogenic factor, VEGF is also proinflammatory and inflammatory cells such as natural killer cells regulate placental expression of angiogenic factors.[167] Thus, cross-talk between inflammatory signaling and angiogenic signaling occurs in normal pregnancy, and metabolic conditions like obesity may disrupt the balance between these signaling pathways.[167]

The interplay of different physiologic pathways makes identifying a single placental mechanism that explains the association between obesity and preterm birth challenging. On the other hand, overlapping pathways allow for multiple targets for preterm birth interventions in obese women. The use of high-throughput technologies now allow for studies to measure multiple molecular pathways simultaneously and assess how multiple pathways can interact with each other.[168] Investigating molecular targets where both placental inflammatory and non-inflammatory mechanisms converge offer the greatest promise for interventions in obese women generalizable to a larger portion of preterm births irrespective of clinical presentation or etiology.

1.4 Novel Methods for Assessing Placental Inflammation in Epidemiological Studies

Biologic evidence supporting placental inflammation as a mechanism between obesity and risk of preterm birth has been inconsistent in population studies. Obesity is well-documented to increase systemic inflammation outside of pregnancy;[169] and animal and *in-vitro* studies support placental inflammatory signaling as a key driver of preterm labor and premature rupture of membranes.[137, 140] Yet, population studies on obesity and placental inflammation in term and preterm birth have been conflicting.[170] Most of these studies rely on maternal circulating biomarkers of inflammation as a proxy for placental inflammation. Yet, inflammatory biomarkers in maternal circulation do not necessarily correlate with placental inflammation.[171] The reliance on circulating inflammatory biomarkers is driven by timing of sampling, cost, and feasibility.

Circulating blood biomarkers can be sampled prior to preterm birth allowing for evidence of a temporal sequence between a biological change in pregnancy and preterm birth. In human studies, the placental cannot be sampled during pregnancy for ethical concerns for the safety of

the fetus. One promising solution to measure *in-utero* placental dysfunction is quantification of placental extracellular vesicles. These are lipid-bound components from the placenta measurable in the blood prior to delivery.[172] To date, variability in sampling of extracellular vesicles and poor understanding of vesicle content reflective of placental dysfunction has limited the utility of extracellular vesicles for epidemiological research.[172] Thus, epidemiological biomarkers of the placenta must rely on circulating blood biomarkers or placental tissue collected at delivery. Tissue-specific biomarkers of placental inflammation require skilled and immediate sampling of placental tissue at the time of delivery.[173] In research settings, proper sampling of placental tissue can be costly and infeasible as clinical management is the main priority for any pregnancy.

Yet, placental histopathology is a tissue-level measure of placental health collected as part of obstetric care for select pregnancies. Data on placental gene expression is also becoming more accessible with lower costs of measuring the placental transcriptome and the increased public availability of transcriptomic datasets. Placental histopathology and transcriptomics offer alternative measures to blood biomarkers to study placental pathophysiology in epidemiological studies. The application and limitations of these approaches are discussed.

1.4.1 Placental Histopathology

1.4.1.1 Application

Placental histopathology is an underutilized clinical source of tissue-level data on the placenta that can be leveraged for population research. Placental histopathological evaluations are conducted by highly-trained perinatal pathologists for preterm pregnancies as well as for fetal, pregnancy, and labor-associated indications in term pregnancies.[173] Placental histopathological evaluations measure gross and histologic morphology of the placenta which characterize

inflammatory, vascular, and developmental pathology.[173, 174] Detection of many histopathology lesions including lesions of acute inflammation (e.g. acute chorioamnionitis, fetal vasculitis, and funisitis) can be highly reproducible across pathologists, limiting the potential of measurement bias across studies.[175] Further, despite histopathology reports measuring the placenta only at the time of delivery, knowledge of the cell types present in the placenta as well as the combination of certain placental lesions (e.g. thrombosis of umbilical cord vessels and downstream obliteration of fetal vessels) allows pathologists to make inferences about the chronicity and sequence of lesions. Placental histopathology can also localize where dysfunction is occurring in the placenta, adding a spatial dimension to understanding the pathophysiology of pregnancy conditions that circulating biomarkers cannot provide. By automating abstraction of pathology reports, placental pathological features can be linked to large birth cohorts to leverage histopathology data in population research.[174]

1.4.1.2 Findings on Obesity and Histologic Inflammation

To date, histopathology studies of maternal exposures on placental inflammation have been limited. There have been 10 histopathology studies on maternal obesity and placental inflammation (**Table 5)**. With the exception of one study that did not provide definitions for histopathological lesions,[176] acute inflammation was considered of maternal origin (acute chorioamnionitis, maternal inflammatory response or neutrophil infiltration of placental chorion) or fetal origin (acute vasculitis, funisitis, or neutrophil infiltration of the umbilical cord) and was measured by neutrophil infiltration (innate immune cells) of the chorioamnion and/or umbilical cord. Neutrophil infiltration of the chorion is considered a maternal response as neutrophils from decidual tissue migrate into the chorion. While neutrophil infiltration of the umbilical cord or fetal vessels is a fetal response as neutrophils migrate from umbilical veins or fetal vessels of the chorionic plate

into the surrounding parenchyma.[177] Chronic inflammation was defined as chronic villitis or villitis of unknown etiology, which was measured as lymphocyte infiltration (adaptive immune cells) of placental villous tissue.

There was considerable heterogeneity in the included pregnancy population across studies with three studies considering all singleton pregnancies,[178-180] three studies oversampling pregnancy complications (hypertensive disorders of pregnancy, diabetes, and fetal growth restriction),[181, 182] two studies excluding pregnancy complications,[183, 184] 1 study restricting to pregnancies delivered after 35 weeks,[185] and 1 study randomly selecting pregnancies from a sleep disordered breathing cohort.[186] The variation in study populations and placental pathology definitions likely explain the disparate findings for associations between obesity and acute and chronic placental inflammation. Obesity was associated with higher frequency or odds of acute placental inflammation in 4 studies and no association in 5 studies. Similarly, obesity was associated with an increased frequency or odds of chronic placental inflammation in 3 studies and no association in 6 studies. Thus, while histopathology offers unique tissue-level clinical biomarkers of placental inflammation, the application of histopathology for epidemiological research requires thoughtful consideration of the potential sources of bias and variability in study design.

Table 5: Summary of histopathology studies on obesity and placental inflammation

Study	Pregnancy Population	BMI Comparisons	Placental Inflammation Measures	Results
Zhang 2022[180]	1,849 singleton pregnancies	obese (BMI ≥30kg/m^2) vs non-obese (BMI <30kg/m^2) at time of delivery	Acute Inflammation •MIR (Acute Chorioamnionitis) •FIR (Acute Vasculitis/Funisitis) Chronic Inflammation •Chronic Villitis	•obese women compared to non-obese women did not have significantly different frequencies of MIR (30.5% vs 31.6%, p=0.65), FIR (12.4% vs 11.3%, p=0.52) or chronic villitis (18.2% vs 19.0%, p=0.71)
Rosado-Yepez 2021[184]	114 singleton pregnancies excluding women with chronic hypertension, diabetes, gestational hypertension, preeclampsia, hyper- or hypothyroidism, GDM, or infants with congenital defects	•self-reported pre-pregnancy BMI classified as normal (18.5-24.99kg/m^2), overweight (25-29.99kg/m^2), and obesity (≥30kg/m^2)	•infection/inflammatory lesions (not specified) •immune/idiopathic inflammatory lesions (not specified)	•no inflammatory lesions were reported among any of the pregnancies
Scott 2021[179]	12,154 primigravida singleton pregnancies (10,145 term and 1739 preterm births) •pregnancies are from the 1959-1974 CPP cohort of 59,391 pregnancies	•self-reported pre-pregnancy BMI	Acute Inflammation •neutrophil infiltration of the umbilical vein, artery, cord substance, amnion membrane, chorion membrane, amnion placental surface, or chorion placental surface chronic inflammation •many Hoffbauer cells in the terminal villi (relative to few Hoffbauer cells in the terminal villi)	Term Births •1-unit increase BMI is associated with higher odds of neutrophilic infiltration in the umbilical vein (OR:1.03, 95% CI 1.01-1.05), umbilical artery (1.04, 1.00-1.07), umbilical cord substance (1.04, 101-1.07), amnion membrane (1.06, 1.03-1.08), amnion placental surface (1.04, 1.02-1.06), chorion membrane (1.04, 1.02-1.06), and chorion placental surface (1.04, 1.02-1.06), but not presence of Hoffbauer cells in the terminal villi (0.96, 0.91-1.01) Preterm Births •a 1-unit increase BMI was associated with a higher odds of neutrophilic infiltration in the umbilical vein (OR:1.06, 95% CI 1.01-1.12), and umbilical cord substance (1.08, 1.02-1.14), but not neutrophilic infiltration of the umbilical artery (1.04, 0.98-1.10), amnion membrane (1.03, 0.98-1.09), amnion placental surface (1.02, 0.96-1.08), chorion membrane (1.02, 0.98-1.09), and chorion placental surface (1.01,

				0.97-1.06), or Hoffbauer cells in the terminal villi (1.02, 0.96-1.09)
Brouwers 2019[183]	382 spontaneous singleton term pregnancies excluding women with GDM, FGR or preeclampsia	•self-reported pre-pregnancy BMI classified as normal (<24.9kg/m^2), overweight (25-29.9kg/m^2), and obese (≥30kg/m^2)	Acute Inflammation •MIR ≥ stage 2 (Acute Chorioamnionitis) •FIR ≥ stage 2 (Acute Funisitis) Chronic Inflammation •High-Grade Chronic Villitis	•obese women compared to normal BMI women were associated with a higher odds of chronic villitis (OR: 18.1, 95% CI: 1.6-205.2), but not MIR ≥ stage 2 (1.6, 0.4-5.6) or FIR ≥ stage 2 (2.2, 0.4-10.8)
Leon-Garcia 2016[185]	423 singleton pregnancies ≥35 weeks of gestation	•early-pregnancy BMI (<14 weeks) classified as obese (≥30 kg/m^2) and normal (20-24.9kg/m^2)	Acute Inflammation •Acute Chorioamnionitis •Fetal Vasculitis Chronic Inflammation •Chronic Villitis	•obese women compared to lean women had higher frequencies of chronic villitis (26.0% vs 16.1%, p=0.014,), but not chorioamnionitis (22.9% vs 27.1%, p=0.326), or vasculitis (8.7% vs 11.5%, p=0.338) •obese women compared to lean women had a higher odds of chronic villitis (OR: 1.96, 95% CI: 1.18-3.27)
He 2016[186]	92 pregnant women from a cohort designed to study sleep disordered breathing	•measured BMI at first prenatal visit classified as obese (≥30kg/m^2) or normal (BMI <25kg/m^2)	Acute Inflammation •Acute Chorioamnionitis Only •Chorioamnionitis and Fetal Vasculitis Chronic Inflammation •Villitis of unknown etiology	•obese women compared to lean women had a higher frequency of chorioamnionitis and fetal vasculitis (27.7% vs 8.9%, p=0.03), but not chorioamnionitis only (19.2% vs 26.7%, p=0.50), or villitis of unknown etiology (4.3% vs 0.0%, p=0.50)
Kovo 2015[182]	1047 singleton term pregnancies with complications (hypertensive disorders of pregnancy, chronic hypertension, GDM, pregestational diabetes, fetal growth restriction, and non-reassuring fetal heart rate necessitating emergency C-section) and 186 uncomplicated term pregnancies; women with fever or clinical chorioamnionitis were excluded	•pre-pregnancy BMI classified as normal (18-24.9kg/m^2) or obese (≥30kg/m^2)	Acute Inflammation •MIR stages 1-3 (stage 1: 5-10 neutrophils in high powered field of subchorionic space, stage 2: 11-30 neutrophils/high powered field in lower chorionic plate, and stage 3: >30 neutrophils/high powered field throughout the chorionic plate) •FIR stages 1-3 (stage 1: early umbilical phlebitis, stage 2: umbilical arteritis, stage 3: concentric umbilical perivasculitis) Chronic Inflammation •Chronic Villitis	Uncomplicated Pregnancies •obese women compared to lean women did not have significant differences in frequencies of MIR (19.3% vs 19.3%, p>0.99), FIR (3.2% vs 6.4%, p=0.387) or chronic villitis (1.6% vs 8.0%, p=0.078) Complicated Pregnancies •obese women compared to lean women did not have significant differences in frequencies of MIR (24.4% vs 30.6%, p=0.085), FIR (7.7% vs 11.7%, p=0.097) or chronic villitis (3.2% vs 6.5%, p=0.065)

Huang 2014[178]	39,774 singleton pregnancies from the 1959-1974 CPP cohort of 59,391 pregnancies •ancillary analysis of 31,331 pregnancies excluding women with complications (preterm birth, GDM, chronic diabetes, chronic hypertension, and gestational hypertension)	•self-reported pre-pregnancy BMI classified as underweight (<18.5kg/m^2), normal (18.5-24.9kg/m^2), overweight (25-29.9kg/m^2), and obese (≥30.0kg/m^2)	Acute Inflammation •fetal neutrophilic infiltration: neutrophilic infiltration of umbilical vessels, cord substance, or chorion/amnion membrane roll or placental surface of umbilical cord Chronic Inflammation •maternal lymphocytic infiltration of capsularis or basalis or at margin	All Pregnancies •obese women compared to normal BMI women had a higher odds of fetal neutrophilic infiltration (OR: 1.26, 95% CI: 1.08-1.48), but not maternal lymphocytic infiltration (0.79, 0.52-1.19) Pregnancies Excluding Complications •obese women compared to normal BMI women had a higher odds of fetal neutrophilic infiltration (OR: 1.30, 95% CI: 1.04-1.62), but not maternal lymphocytic infiltration (0.66, 0.35-1.23)
Barr et al 2012[181]	56 singleton term pregnancies (26 with preeclampsia, GDM or fetal growth restriction)	•pre-pregnancy BMI classified as non-obese (<30kg/m^2) or obese (≥30kg/m^2)	Acute Inflammation •MIR: same definition as *Kovo 2015* •FIR: same definition as *Kovo 2015* Chronic Inflammation •Chronic Villitis	•obese women compared to lean women had a higher frequency of MIR (42.8% vs 3.6%, p<0.001), but there were no significant differences in frequencies of FIR (14.3% vs 0.0%, p=0.119) or chronic villitis (0.0% vs 0.0%, p<0.001)
Becroft et al 2004[187]	529 live-born singleton term pregnancies with SGA births	•self-reported pre-pregnancy BMI classified as <20kg/m^2, 20-21.99kg/m^2, 22-24.99kg/m^2, >25kg/m^2	Chronic Inflammation •Villitis of unknown etiology	•women with BMI >25kg/m^2 compared to women with a BMI 20-21.99kg/m^2 had a 2.15 higher odds of villitis of unknown etiology after adjusting for SES, ethnicity, gravidity, maternal age, pregnancy induced hypertension, late coryza, kidney/urinary problems (OR: 2.15, 95% CI: 0.97-4.78)

Abbreviations: CPP, Collaborative Perinatal Project; FGR, fetal growth restriction; FIR, fetal inflammatory response; GDM, gestational diabetes; MIR, maternal inflammatory response; SGA, small for gestational age.

1.4.1.3 Limitations of Placental Histopathology for Population Research

The studies summarized in **Table 5** on maternal obesity and placental inflammation highlight many of the current barriers of research that I aimed to address in this dissertation. To make causal inferences about associations between pre-pregnancy obesity and risk of preterm birth requires limiting biases present in epidemiologic studies: selection bias, measurement error, and confounding biases.

Across histopathological studies of obesity and placental inflammation, there was considerable variation in the study population inclusion criteria. Differences in study populations could be of clinical interest to the investigators such as analyzing the effects of obesity on placental inflammation only in women with pregnancy complications;[181, 182] but often analyses are restricted to pregnancies where data on the maternal exposure (pre-pregnancy BMI) and placental histopathology are available.[178-180] This may induce selection bias, as placental histopathology in clinical practice is collected for pregnancy complications.[174, 188, 189] While studies focused on pregnancy complications (preterm birth, preeclampsia, FGR) will have complete data, in studies intended to reflect healthy term pregnancies, pregnancy complications will be oversampled. Using directed acyclic graphs (**Figure 8A)**, we can see that oversampling pregnancy complications may result in unanticipated pathways between a maternal exposure (denoted as A) and a placental outcome (denoted as Y). Indications can occur prior to pregnancy (U1; e.g. pre-existing health conditions), during pregnancy (U2; e.g. preeclampsia, intrauterine infection), or after delivery (U3; e.g. low 5-min Apgar score). Existing studies have attempted to address these concerns by excluding pregnancy complications from analysis,[178, 183, 184] but excluding complications related to the exposure such as pre-pregnancy BMI and preeclampsia may lead to underestimation of associations between the exposure and placental outcome (**Figure 8B)**. Analytic methods that

minimize selection bias by approaches other than exclusion are needed to account for oversampling of pathology indications in epidemiological studies. We directly address this concern in the first paper of this dissertation using weighting and imputation methods.

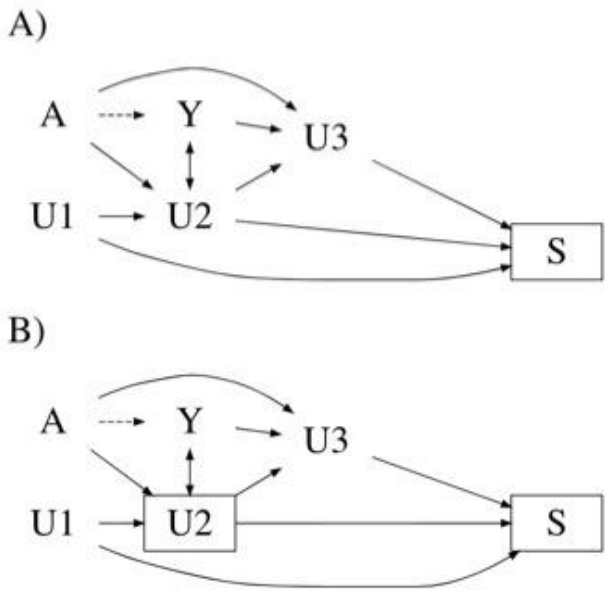


Figure 8: Causal diagrams on selection bias for the association between pre-pregnancy obesity (A) and risk of histologic placental inflammation (Y).

A) The scenario in which a study restricts analysis to deliveries with placental histopathology but does not correct for factors (U1-U3) associated with likelihood of a histopathology evaluation. B) The scenario in which pregnancy factors (U2) are excluded.

A second limitation of adapting placental histopathology for population research, is that considering individual placental lesions may oversimplify pathology in the placenta. Pathologists usually make clinical inferences about the placental pathophysiology of pregnancy conditions by considering the combinations of placental lesions in the placenta. Isolated placental pathological findings may be incidental or reflect normal physiologic processes in pregnancy. Inflammation is

necessary for both placental implantation and normal initiation of labor at term,[33, 139] and a high proportion (28-78%) of uncomplicated term pregnancies are reported to have at least one histopathological feature.[190, 191] To avoid capturing incidental findings, studies to date have considered the severity of placental lesions[183] or rely on anticipated groupings of histopathology features (e.g. inflammatory or vascular impairment) using correlative measures, factor analysis and pre-defined groups based on expert opinion.[192-195] These approaches are not ideal as grading of placental pathology is variable across pathologists inducing measurement bias, and presupposing placental pathology groups may miss key histopathological features and/or lack measures to assess accuracy of groupings.

In the second aim of my thesis, we use an empirical based approach to cluster placental pathology lesions of preterm birth to avoid incidental findings or bias by pre-supposing pathology groups. Another advantage of clustering placental pathology features is that it allows us to consider how pre-pregnancy obesity may impact inflammatory and non-inflammatory mechanisms. Considering the pathology of the entire placenta will allow us to use an unbiased, data-driven approach to under how the placenta may mediate associations between pre-pregnancy obesity and preterm birth.

Lastly, most histopathology studies to date have been small in sample size. Small sample sizes mean most studies are underpowered to adjust for confounders or consider effect modifiers of associations between pre-pregnancy obesity and placental inflammation. The lack of confounder adjustment despite maternal obesity and placental inflammatory lesions sharing common causes including parity, maternal race, smoking exposure, and sociodemographic factors may lead to biased associations. While two large studies were conducted on histopathology data from the Collaborative Perinatal Project (CPP), this cohort is from the 1950s-1960s,[178, 179] where

less than 10% of pregnancies were obese. Given that 30% of pregnant women today are obese,[74] the CPP cohort is not contemporary enough to adequately reflect current maternal exposures.

For the first two aims of this cohort, we utilized the Magee Obstetric Maternal and Infant (MOMI) database, a large pregnancy cohort with placental histopathology data available on deliveries between 2008 and 2012 (n=21,584 pregnancies) at Magee-Womens Hospital in Pittsburgh, PA. The MOMI database utilized extensible markup language and server integration services packages for an automated approach to extract text from pathology reports, codify text into variables, and link to medical record, ICD-9, ultrasound and state birth record data on pregnancies.[174] This automated approach for abstracting pathology data enabled for a large dataset with extensive histopathology and clinical data available on pregnancies for thorough assessment of confounders, effect modification, and analytic approaches to handle missing data.

1.4.2 Placental Omics Analyses

1.4.2.1 Application

The long-term goal of elucidating the pathophysiology of obesity and risk of preterm birth is to identify biologic targets for interventions. The challenge of identifying genes targetable for preterm interventions in obese women is that obesity causes multi-organ system changes.[136, 196] The desire for a singular gene target that accounts for the complex interdependence of inflammatory, cardiovascular, hormonal, and metabolic pathways dysregulated in obesity is unlikely. There are approximately 30,000 genes in the human genome that code for proteins which are regulated by thousands of additional non-protein coding genes.[176] Further, coding and non-coding genes interact with each other, allowing for potentially millions of combinations of genes that could contribute to preterm birth in obese women.[176] Intervening on isolated molecular targets

without considering all molecular pathways can contribute to unintended adverse consequences for childhood development (e.g. DES and vaginal cancer in daughters).[197] The complexity and ethical considerations of identify molecular targets warrants alternative approaches to low-dimensional molecular analyses.

Omics technologies allow for genes at the DNA, mRNA or protein level to be simultaneously analyzed in a sample.[168, 198] It is now possible to consider thousands of genes that are differentially expressed by obesity and preterm birth status. Sets of genes that are differentially expressed by a phenotype like obesity or preterm birth, can elucidate molecular pathways that are upregulated or downregulated using methods such as gene set enrichment analyses,[199] ingenuity pathway analyses,[200] and hierarchal clustering.[201] Characterizing shared molecular pathways of obesity and preterm birth in the placenta, can give a better understanding of how placental dysfunction may be a mediator of obesity and risk of preterm birth. While we suspect obesity to modify inflammatory pathways in pregnancy, omics studies can identify other non-inflammatory mechanisms relevant to obesity and preterm birth. An agnostic approach to identify other potential placental mechanisms of preterm birth in obese women will help inform novel preterm birth interventions.

1.4.2.2 Summary of Findings

To date, the bulk of omics studies on maternal obesity and preterm birth have focused on DNA methylomics and transcriptomics. Obesity is a potential epigenetic exposure. An epigenetic exposure is an environmental factor that changes gene expression by altering chromosome structure without modifying the DNA sequence.[202] Epigenetic changes include methylation or acetylation of DNA that inhibit or promote gene transcription by changing the DNA structure to a more stable (inhibitory) or unstable (promote) state.[202] Methylomics may inform how obesity

regulates DNA transcription while transcriptomics measures if the amount of mRNA produced by DNA transcription is different by maternal obesity status.

To our knowledge there has been only 1 published methylomic study and 3 transcriptomic studies of placental tissue that have compared DNA methylation and gene expression between obese and lean women.[159, 203-205] Methylomic data of placental villous tissue on a second set of pregnancies is publicly available (GSE120062); however, no study has been published on this dataset. In the one methylomic study of 20 uncomplicated term pregnancies, obese women had increased placental methylation and lower mRNA expression of a gene cluster related to growth hormone and chorionic-somatotrophin hormone.[205] The investigators postulate that these placental hormones regulate maternal metabolism as a feedback loop between mother and placenta. In transcriptomic studies, obesity was generally associated with lower gene expression[159, 203] and differentially expressed genes were involved in pathways related to extracellular matrix organization,[159, 204] retinol metabolism,[159, 203] inflammation[159, 203] and developmental pathways (trophoblast, bone and blood vessels).[159, 204] These findings indicate that while obesity may modify placental inflammatory pathways, pathways related to placental tissue remodeling and development are also affected.

Unlike obesity, there have many methylomic and transcriptomic studies of placental tissues investigating preterm birth. These studies have been quite heterogenous. Studies have investigated genes specific to advancing gestational age (1 study),[206] preterm labor with concomitant chorioamnionitis (2 studies),[207, 208] idiopathic spontaneous preterm labor (6 studies),[209-214] PPROM (1 study),[215] and early-onset preeclampsia (6 studies).[216-221] The study on advancing gestational age, found that differentially methylated regions of DNA from the placenta and cord blood were correlated with longer gestational age.[206] Not surprisingly studies investigating preterm labor

associated with PPROM or chorioamnionitis were associated with inflammatory processes including neutrophil activity,[208] cytokine signaling,[211, 215] viral infections, and bacterial infections.[215] Idiopathic spontaneous preterm labor was associated with a variety of pathways: trophoblast development pathways (via insulin-like growth factor binding protein),[211, 214] iron transport,[209] nitric oxide pathway,[209] fatty acid metabolism,[214] RNA stabilization,[212] extracellular matrix organization,[212] and inflammatory pathways.[209, 211, 214] Early-onset preeclampsia was associated with biological pathways related to cell signaling (TGF-B, MAP kinase, insulin-growth factor),[211, 216, 219, 220] renin-angiotensin signaling,[217, 220] oxidative stress,[220] and inflammation.[216, 219]

Variability across preterm birth studies likely reflects heterogeneity in both the preterm birth subtypes analyzed, omics platform, and study objectives. Some studies focused on using differential methylation or transcriptomic patterns to cluster preterm pregnancies or pregnancies with preeclampsia into distinct groups.[219] Other studies aimed to predict preterm birth or early gestational age.[206, 212] While other studies aimed to identify specific inflammatory or signaling pathways.[210] Despite study variation, methylomic and transcriptomic studies found general trends related to modification of inflammation and cell-signaling pathways that may be shared across preterm birth subtypes. Shared pathophysiology across preterm birth subtypes needs further validation, but could inform molecular targets for interventions that would prevent preterm births from multiple high-risk groups.

1.4.2.3 Research Gaps

There is a paucity of transcriptomic and methylomic studies on maternal obesity during pregnancy. With the exception of one transcriptomic analysis of 183 pregnancies,[204] the other three cohorts conducted transcriptomic or methylomic analyses each on 25 pregnancies or fewer. Smaller sample sizes limit the ability to account for potential confounders between obesity status

and placental gene expression such as parity, smoking status, and ethnicity.[222] Additionally, fetal sex, labor and ancestry may modify associations between obesity and placental gene expression.[130, 211, 222] Larger omics studies powered to look at confounding and effect modifiers of obesity and gene expression are warranted.

Second, no study to date has directly measured placental gene mediators of obesity and risk of preterm birth. Transcriptomic and methylomic analyses have relied on comparisons across a single phenotype such as preterm birth status. These studies have identified molecular pathways and differentially expressed genes of preterm birth related to fatty acid metabolism, leptin signaling, and inflammation. However, without direct testing of placental gene mediation between obesity and preterm birth, inferring shared molecular pathways between obesity and preterm birth is speculative at best.

High-dimensional mediation analyses have recently been developed to identify gene mediators between an exposure and outcome.[223, 224] High-dimensional mediation analysis first screens genes associated with the outcome (e.g. preterm birth), applies a penalty to ß-coefficients estimated between gene expression and outcome, and then uses joint significant testing of the coefficients between the exposure and gene expression and gene expression and outcome to test statistical significance of the placental gene mediator.[224] This approach accounts for multiple hypothesis testing of thousands of genes and provides an estimate for the proportion of the exposure and outcome mediated by the gene. Thus, this approach not only identifies genes as significant mediators of obesity and risk of preterm birth but can also delineate the direct effect of pre-pregnancy BMI on risk of preterm birth from the proportion of the effect of BMI mediated by placental gene expression.

Another major limitation of transcriptomic and methylomic studies is that the placenta is a heterogenous tissue with multiple tissue and cell types. As mentioned above, the acute inflammatory changes in chorionic membrane can cause preterm labor. Cell type differences are generally studied within placental villi only. Differential gene expression in placental villous tissue may be driven by changes in cell fractions rather than individual genes.[225] Several single cell RNA-seq studies have been conducted to identify the gene expression patterns of placental cells.[225-228] Cell deconvolution is a method that leverages single cell expression patterns from reference datasets to estimate cell proportions in bulk placental tissue.[229, 230] Cell deconvolution is advantageous for population studies where bulk tissue is typically collected. Combining findings on individual genes and cell types helps elucidate if pathophysiologic changes occur globally across the placenta or are localized to cell types. Localizing the source of pathology is necessary to understanding biological targets for preterm birth interventions.

1.4.3 Dissertation Objectives

The overarching hypothesis of this dissertation is that pre-pregnancy obesity predisposes women to preterm birth through placental inflammation. Population studies on placental mechanisms of obesity and preterm birth have been conflicting. There is a reliance on maternal circulating biomarkers as proxies for placental tissue-level biomarkers. In studies that measure placental tissue, methodological constraints for study inclusion criteria and analyses may bias study findings. Additionally, existing transcriptomic analyses on obesity and preterm birth have not directly measured placental genes as mediators between obesity and risk of preterm birth. Therefore, to address these limitations this dissertation utilizes several birth cohorts with placental histopathology and transcriptomic data for the following manuscripts:

1. **Manuscript 1:** To investigate whether pre-pregnancy obesity increases the risk of acute and chronic histologic placental inflammation in term pregnancies, using a large, contemporary US birth cohort with available placental histopathology.
    a. A sub-aim is to quantify how susceptible associations between obesity and placental inflammation are to selection bias.
2. **Manuscript 2:** To assess if pre-pregnancy obesity is associated with inflammatory and non-inflammatory placental dysfunction in early (<32 weeks') and late (32 to <37weeks) preterm births using an empirical clustering analysis of placental histopathology in the same US birth cohort.
3. **Manuscript 3:** To identify placental genes and cell types that may mediate the association between obesity and risk of preterm birth using high-dimensional mediation analyses and cell deconvolution in two placental transcriptomic datasets.

2.0 Original Research

2.1 Manuscript 1: Pre-pregnancy Obesity and Risk of Placental Inflammation at Term: A selection bias analysis

2.1.1 Abstract

Purpose: Studies utilizing placental histopathology oversample adverse pregnancies possibly biasing findings. We examine the association between pre-pregnancy obesity (risk factor for systemic inflammation) and histologic placental inflammation (biomarker correlated with impaired neonatal neurodevelopment) and how selection bias may influence the association.

Methods: Singleton term deliveries between 2008-2012 from the Magee Obstetric Maternal and Infant database were analyzed. Pre-pregnancy BMI was categorized as underweight, lean (referent), overweight and obese. Outcomes were pathological diagnoses of acute (acute chorioamnionitis and fetal inflammation) and chronic placental inflammation (chronic villitis). The relative risk of placental inflammation in obese vs lean women were estimated by log-binomial regression. Selection bias was assessed by comparing estimates using complete case, exclusion of pregnancy complications, multiple imputation, and inverse probability weighting. Risk ratios were adjusted for hypothetical bias factors to examine how results were impacted by residual selection bias.

Results: Across methods, obesity was associated with an 8-15% lower risk of acute chorioamnionitis, a 7-14% lower risk of acute fetal inflammation and a 12-30% higher risk of

chronic villitis relative to lean women. Residual selection bias could strongly bias risk estimates for acute inflammation above or below the null but only modestly for chronic villitis.

Conclusions: Obesity may contribute to chronic placental inflammation and we highlight robust methods to analyze clinical data susceptible to selection bias.

2.1.2 Introduction

Placental histopathology reports (gross and microscopic examination of placental tissue) are a clinical data source useful for studying placental etiologies of pregnancy outcomes. Histopathology reports provide tissue-level biomarkers of the placenta, potentially more correlated with the pathophysiology of pregnancy complications than blood biomarkers. A caveat of utilizing pathology reports for research is placentas are often evaluated for clinical indications,[231] which may induce selection bias by oversampling high-risk pregnancies. Studies have primarily accounted for this by excluding pregnancies with complications.[178, 184, 232, 233] This approach may introduce bias if certain complications are on the causal pathway between the exposure and the placental outcome.

Alternative methods to excluding pregnancies include multiple imputation by chained equations (MICE) and inverse probability weighting (IPW) for minimizing selection bias.[234-239] MICE estimates missing data based on the distribution of observed data and is an effective method when variables with complete data are strongly correlated with whether a variable is observed or missing.[235] IPW creates a pseduo-population reflective of the target popuation, by weighting observations based on the probability for the indication for missing data (e.g., indication for placental histopathology). IPW is effective at reducing selection bias when the causal relationship between the observed variables and the indication for missing data are correctly specified. MICE

and IPW are both robust analytic methods but rely on different missing data assumptions potentially leading to variable findings in population studies with complex missing data patterns. Though we and others in cancer epidemiology have applied IPW in histopathology studies,[240, 241] both MICE and IPW are not routinely used in pregnancy research.

Addressing selection bias may inform studies on obesity and placental inflammation. Obesity is the most prevalent preventable risk factor for adverse pregnancy outcomes (gestational diabetes, preeclampsia, stillbirth) in term pregnancies perhaps partially mediated by placental inflammation.[26, 242-244] Placental inflammatory lesions are prevalent in term pregnancies (15-40%).[190, 245] Acute chorioamnionitis is a reproducible histologic marker of acute placental inflammation.[174, 175] Chronic villitis is a histologic placental finding plausibly indicative of chronic inflammatory exposures including obesity.[246, 247] Findings on pre-pregnancy obesity and risk of acute chorioamnionitis in term pregnancies have been conflicting,[178, 248, 249] while obesity has been consistently associated with higher risk of chronic villitis.[185, 187, 246, 250, 251] Restricting analyses to women with histopathology may explain the variability across studies of acute inflammation, but to our knowledge no formal analysis of selection bias has been conducted.

Therefore, we compared various methods for handling selection bias to evaluate associations between obesity and histologic inflammation (complete case analysis, exclusion of pregnancy complications, MICE and IPW). We then applied recently developed sensitivity analyses[252, 253] to assess the potential magnitude of residual selection bias to interpret the robustness of the association between pre-pegnancy obesity and placental inflammation. We hypothesized that associations with acute inflammation would be more suceptible to bias than chronic villitis given inconsistent findings on obesity and chorioamnionitis, but a well-documented positive association between obesity and chronic villitis.

The purpose of this study is two-fold. 1) To test the hypothesis that pre-pregnancy obesity increases the risk of placental inflammation. Clarifying the relationship between obesity and placenta inflammation using histologic data will inform our understanding of the pathophysiology of adverse pregnancy outcomes in obese women. 2) To quantify the bias of estimates on obesity and risk of placental inflammation that result from restricting studies to pregnancies with available placental histopathology. Our goal is to inform how future studies that leverage pregnancy data originally collected for clinical purposes can address selection bias.

2.1.3 Methods

2.1.3.1 Study Population

We use data from the Magee Obstetrics Maternal and Infant (MOMI) database. MOMI contains extensive maternal and pregnancy data on all pregnancies at Magee-Womens Hospital in Pittsburgh, PA since 1995 and is linked to placental histopathology records for pregnancies between 2008 and 2012. Histopathology data was collected on a subset of term births (38%) that oversampled pregnancy complications, making MOMI a suitable cohort to study the consequences of selection bias. There were 41,864 viable singleton term births (37-42 weeks' gestation) between 2008 and 2012 in MOMI (**Appendix Figure 1)**. Chronic inflammatory health conditions may cause weight loss and increase placental inflammation; therefore, we excluded 421 deliveries with chronic inflammatory conditions (HIV, tuberculosis, hepatitis B and C; transplant history, and autoimmune disorders: lupus, rheumatoid arthritis, multiple sclerosis). The number of pregnancies included for analysis depended on selection bias strategy. For complete case analysis, there were 9,632 pregnancies after excluding 31,811 pregnancies missing data on pre-pregnancy BMI, placental inflammation and study covariates. For complete case analysis of uncomplicated

pregnancies, there were 4,049 included pregnancies after further excluding 5,583 pregnancies from the complete case analysis cohort. For MICE, there were 41,443 included pregnancies (allowed for missing data on BMI, histopathology, and covariates). For IPW, there were 15, 571 pregnancies after excluding 25,872 pregnancies without histopathology evaluations (allowed for missing data on BMI and covariates). The University of Pittsburgh IRB approved this project (STUDY20050303); no consent was needed as data were de-identified.

2.1.3.2 Measures

Pre-pregnancy BMI was calculated as a ratio of weight (kg) to height (m) squared (kg/m^2) using self-reported data at first prenatal visit. Self-reported pre-pregnancy weight is highly correlated with first measured weight in pregnancy (r=0.99) at Magee-Womens Hospital.[254] BMI weight classes were calculated using standard classifications (underweight, $<18.5 kg/m^2$; lean, 18.5 to $<25 kg/m^2$; overweight, 25 to $<30 kg/m^2$; and obese, $\geq 30 kg/m^2$).[255]

Placental pathological features of acute and chronic inflammation were extracted from pathology reports conducted by two placental pathologists and linked to the MOMI database by an automated process previously described and validated.[174] Briefly, pathology data were extracted from pathology reports by extensible markup language, extracted text was converted into variables, and linked to delivery data. Placental inflammatory lesions were categorized using conventional pathology definitions (**Appendix Table 2**). Markers of acute inflammation included acute chorioamnionitis and acute fetal inflammation (fetal vasculitis and/or funisitis); villitis was a marker of chronic inflammation. High interobserver agreement for diagnoses of chorioamnionitis (weighted kappa: 0.79), funisitis (0.70), vasculitis (0.82) and villitis (0.83) has previously been reported,[175] and there was high agreement between the clinical pathology reports and blinded re-reviews by the pathologist (W.T.P.) for inflammation/infection (82% agreement).[193]

Maternal characteristics were extracted from medical records. Covariates included maternal age, self-reported race (white, Black, and other races), maternal education (some college education vs high school education or less), insurance coverage (public vs private), parity (nulliparous/parous) and smoking status during pregnancy (yes/no).

Additional pregnancy characteristics determined by ICD-9 codes, unless otherwise noted, were used to impute missing data and generate IPW weights and are detailed in **Appendix A.** Maternal characteristics included self-reported maternal substance use during pregnancy (yes/no), chronic hypertension (yes/no), pre-existing diabetes (yes/no), maternal comorbidities (yes/no), complication in last pregnancy (yes/no; nulliparous pregnancies defined as no), and history of multiple abortions (yes/no; includes both terminations and miscarriages).

Pregnancy characteristics included gestational hypertension (yes/no), preeclampsia, HELLP or eclampsia (yes/no), gestational diabetes, gestational weight gain (maternal weight at delivery minus pre-pregnancy weight), suspected intrauterine infection (yes/no) and placental/labor complications (yes/no). Gestational age was determined using best obstetric estimate, which was estimated by ultrasound during first and second trimester in conjunction with last menstrual period. A delivery with labor was defined as any pregnancy that had spontaneous onset of contractions, an induced delivery or a delivery with a recorded start and end time for labor. Deliveries without labor were pregnancies by scheduled caesarean-sections.

Fetal characteristics included biologic fetal sex (male/female; intersex were not specified in MOMI dataset for years 2008-2012), fetal distress (yes/no), small for gestational age (yes/no; birthweight $<10^{th}$ percentile using Alexander birthweight curve; SGA), large for gestational age (yes/no; birthweight $\geq 90^{th}$ percentile; LGA)[256] and 5-minute Apgar score.

2.1.3.3 Statistical Analysis

Pregnancy characteristics across BMI classes and the prevalence of poor fetal health indicators between women with and without placental inflammatory lesions are described using means and standard deviations for continuous variables and proportions for categorical variables.

Log-binomial regression was used to calculate risk ratios (RR) and 95% confidence intervals (CI) of placental inflammatory lesions among obese, overweight, and underweight women compared to lean women. The dose-response relationships between pre-pregnancy BMI and risk of placental inflammatory lesions were visualized by predicted probability plots derived from regression models. Models were adjusted for maternal age, race, insurance, education, and smoking status. Pregnancies to women who did not identify as white or Black (5.4%) were comparable in pregnancy characteristics to white women. Therefore, we dichotomized self-reported race to Black vs not Black. Regression models were measured using generalized estimating equations with robust standard errors to account for within-cluster variation for pregnancies from the same woman (5,642 women with >1 pregnancy in MOMI).

We examined whether associations between placental inflammatory lesions and obesity varied by presence of labor at delivery, race, and fetal sex by testing for an interaction. Labor requires placental inflammatory signaling that may be disrupted in obese women.[257] Black women have higher rates of acute placental inflammation and obesity-associated placental conditions (e.g. preeclampsia) than white women.[258] Differential placental gene expression by fetal sex may upregulate inflammatory pathways in obese women.[222, 259] Stratified results are presented for any significant interaction by the Wald test ($p<0.10$).

Causal diagrams were used to summarize potential sources of selection bias from standard indications for placental histopathology (**Appendix Table 3**).[236] Associations between pre-

pregnancy obesity and acute and chronic placental inflammation were assessed by complete case analysis, exclusion of pregnancy complications, MICE and IPW.

For complete case analysis, pregnancies missing data on obesity, placental inflammation and covariates were excluded. For exclusion of pregnancy complications, RRs were estimated after excluding 5,583 deliveries with complications often excluded in prior studies: preeclampsia, gestational diabetes, SGA, fetal complications, suspected intrauterine infection, placental/labor complications.[178, 184, 232, 260]

MICE handles missing data by assigning values to missing observations based on the distribution of observed data. MICE accounts for statistical uncertainty in assigning values to missing data by imputing missing values multiple times and then pooling estimates across imputations.[234, 235] This approach assumes that data missing on prepregnancy BMI and placental histopathology can be estimated based on observed maternal, pregnancy, and delivery characteristics (i.e. missing at random). Auxiliary variables associated with missingness on pre-pregnancy BMI and placental histopathology were included to improve efficiency of imputation.[235] Continuous variables were imputed by predictive mean matching, binary variables by logistic regression, and variables with ≥2 categories by polytomous regression. RRs were estimated after imputation of missing data on BMI status, placental histologic lesions and covariates (20 imputations and 20 iterations) and pooling estimates across imputations by Rubin's Rules.[234, 261]

IPW creates a pseudo-population representative of all term births, by weighting pregnancies using the inverse of the probability of having a placental histopathology evaluation based on data collected prior to delivery.[240] Since IPW is addressing selction bias from missing data on the outcome of placental inflammation and not pre-pregnancy BMI (34.4% missing) or

other variables considered for IPW (16.6% missing), we first imputed data on these variables by MICE (10 imputations, 20 iterations) as recommended by others.[237, 262] Inverse probability weights were then estimated from the probability of a pregnancy having a placenta sent for pathology evaluation based on maternal and pregnancy characteristics using logistic regression.

RRs by complete case, exclusion of complications, MICE, and IPW were graphically compared by forest plots. Additional details on methods for imputing variables by MICE, variable selection for IPW, and diagnostics for IPW are described in **Appendix A**.

Unmeasured factors that may distinguish pregnancies lacking placental histopathology from pregnancies sent for placental histopathology may bias associations between pre-pregnancy BMI and placental inflammation. To approximate residual selection bias, we estimated the extent of hypothetical bias (a bias factor) attributed to indications for placental histopathology with available data.[253] We reasoned any unmeasured factor would at most bias our observed RRs by as much as the strongest measured factors in our study. RRs were divided by these bias factors to determine the strength of residual selection necessary to explain away our associations. Calculations of bias factors are summarized in **Appendix A**.

All statistical analyses were conducted using R Studio.[263]

2.1.4 Results

2.1.4.1 Maternal, Obstetric and Placental Characteristics

Obese women compared to underweight, lean, and overweight women tended to be older and were more likely to have chronic hypertension and pre-existing diabetes and less likely to be nulliparous and to deliver with labor (**Table 6**). The frequency of gestational diabetes, hypertensive disorders of pregnancy, and LGA infants were higher among obese women compared to the other

BMI classes. Obese women compared to lean women had lower frequencies of acute chorioamnionitis (34.0% vs 42.5%) and acute fetal inflammation (18.3% vs 22.9%) and a higher frequency of chronic villitis (16.6% vs 14.1%). Frequencies of placental lesions for underweight and overweight women were comparable to lean women.

Placental lesions were associated with worse fetal health. Infants born to women with acute chorioamnionitis or acute fetal inflammation had higher proportions of fetal distress and 5-min Apgar score <7 than those born to women without these features (**Appendix Table 4**). Women with chronic villitis had a higher prevalence of SGA infants compared to women without chronic villitis.

Table 6:Maternal and obstetric characteristics[a,b]

	Missing N	Underweight (N=394)	Lean (N=4707)	Overweight (N=2377)	Obese (N=2290)
Maternal Characteristics					
Maternal Age (yrs)	0	26.6 (± 5.67)	28.3 (± 6.07)	28.7 (± 5.95)	29.0 (± 5.85)
Maternal Race n (%)	55				
Not Black		312 (80.6%)	3931 (84.1%)	1854 (78.3%)	1669 (73.0%)
Black		75 (19.4%)	743 (15.9%)	513 (21.7%)	616 (27.0%)
Education, n (%)	40				
High School/GED or Less		181 (46.1%)	1334 (28.5%)	649 (27.4%)	708 (31.1%)
Some College		212 (53.9%)	3350 (71.5%)	1723 (72.6%)	1571 (68.9%)
Smoking During Pregnancy, n (%)	37	113 (28.8%)	766 (16.3%)	358 (15.1%)	353 (15.5%)
Medical Insurance, n (%)	25				
Medicare/Medicaid		129 (32.7%)	1110 (23.7%)	561 (23.7%)	597 (26.1%)
Private/Self-Pay		265 (67.3%)	3583 (76.3%)	1810 (76.3%)	1688 (73.9%)
Nulliparity, n (%)	7	244 (61.9%)	2928 (62.3%)	1373 (57.8%)	1170 (51.1%)
Diabetes Status, n (%)	25				
No Diabetes		367 (93.1%)	4334 (92.4%)	2083 (87.9%)	1760 (77.0%)
Gestational Diabetes		25 (6.35%)	317 (6.75%)	246 (10.4%)	425 (18.6%)
Pre-existing Diabetes		2 (0.508%)	42 (0.895%)	42 (1.77%)	100 (4.38%)
Pre-existing Hypertension, n (%)	25	1 (0.254%)	65 (1.39%)	74 (3.12%)	222 (9.72%)
Gestational Hypertension, n (%)	45	20 (5.08%)	249 (5.31%)	204 (8.61%)	242 (10.6%)
Preeclampsia, HELLP, Eclampsia, n (%)	45	22 (5.58%)	415 (8.85%)	311 (13.1%)	339 (14.9%)
Delivery Characteristics					

	Missing N	Underweight (N=394)	Lean (N=4707)	Overweight (N=2377)	Obese (N=2290)
IOM Weight Gain, n (%)	525				
Adequate		151 (40.2%)	1614 (35.8%)	461 (20.5%)	419 (19.8%)
Excessive		96 (25.5%)	2030 (45.1%)	1571 (70.0%)	1245 (58.8%)
Inadequate		129 (34.3%)	860 (19.1%)	213 (9.49%)	454 (21.4%)
Gestational Age (wks)	0	39.0 (± 1.20)	39.2 (± 1.21)	39.1 (± 1.24)	39.0 (± 1.22)
Gestational Age at Delivery (wks), n (%)	0				
37-38 wks		127 (32.2%)	1314 (27.9%)	716 (30.1%)	733 (32.0%)
39-40 wks		231 (58.6%)	2742 (58.3%)	1341 (56.4%)	1289 (56.3%)
41-42 wks		36 (9.14%)	651 (13.8%)	320 (13.5%)	268 (11.7%)
Labor Delivery, n (%)	11	361 (91.6%)	4329 (92.1%)	2133 (89.8%)	1870 (81.7%)
Placental Weight (g)	24	423 (± 94.1)	454 (± 98.8)	470 (± 104)	487 (± 107)
Weight for Gestational Age, n (%)	6				
AGA		271 (68.8%)	3679 (78.2%)	1805 (76.0%)	1733 (75.7%)
LGA		14 (3.55%)	342 (7.27%)	266 (11.2%)	345 (15.1%)
SGA		109 (27.7%)	683 (14.5%)	303 (12.8%)	212 (9.26%)
Male Fetal Sex, n (%)	0	205 (52.0%)	2445 (51.9%)	1215 (51.1%)	1132 (49.4%)
Placental Pathology Lesions					
Acute Chorioamnionitis, n (%)	0	158 (40.1%)	2002 (42.5%)	971 (40.8%)	778 (34.0%)
Acute Fetal Inflammation, n (%)	0	79 (20.1%)	1078 (22.9%)	563 (23.7%)	420 (18.3%)
Chronic Villitis, n (%)	0	58 (14.7%)	663 (14.1%)	361 (15.2%)	379 (16.6%)

[a]Table uses original data in MOMI for women with complete data on pre-pregnancy BMI and placental histopathology (n=9,768).
[b]Continuous variables are represented as mean ± standard deviation.

2.1.4.2 Risk of Acute and Chronic Inflammation

RR estimates of acute chorioamnionitis, acute fetal inflammation, and chronic villitis were consistent across all four analytic methods (complete case, exclusion of pregnancy complications, MICE, and IPW), though IPW slightly attenuated results towards the null (**Figure 9**). After adjusting for covariates, obese women compared to lean had an 8-15% lower risk of acute chorioamnionitis (weakest estimate by IPW RR: 0.92; 95% CI 0.86, 0.99; strongest estimate by complete case RR: 0.85; 0.79, 0.91). Similarly, obesity was associated with a 7-14% lower risk of acute fetal inflammation (IPW RR: 0.93; 0.83,1.04; complete case RR: 0.86; 0.78, 0.95). In contrast, obese women compared to lean women had a 12-30% higher risk of chronic villitis (IPW RR: 1.12; 0.98,1.28; exclusion of pregnancy complications RR: 1.30; 1.08, 1.56). Underweight and overweight BMIs were not associated with risk of placental inflammatory lesions. Comparable trends were reported when measuring pre-pregnancy BMI as a continuous variable (**Appendix Figure 2**).

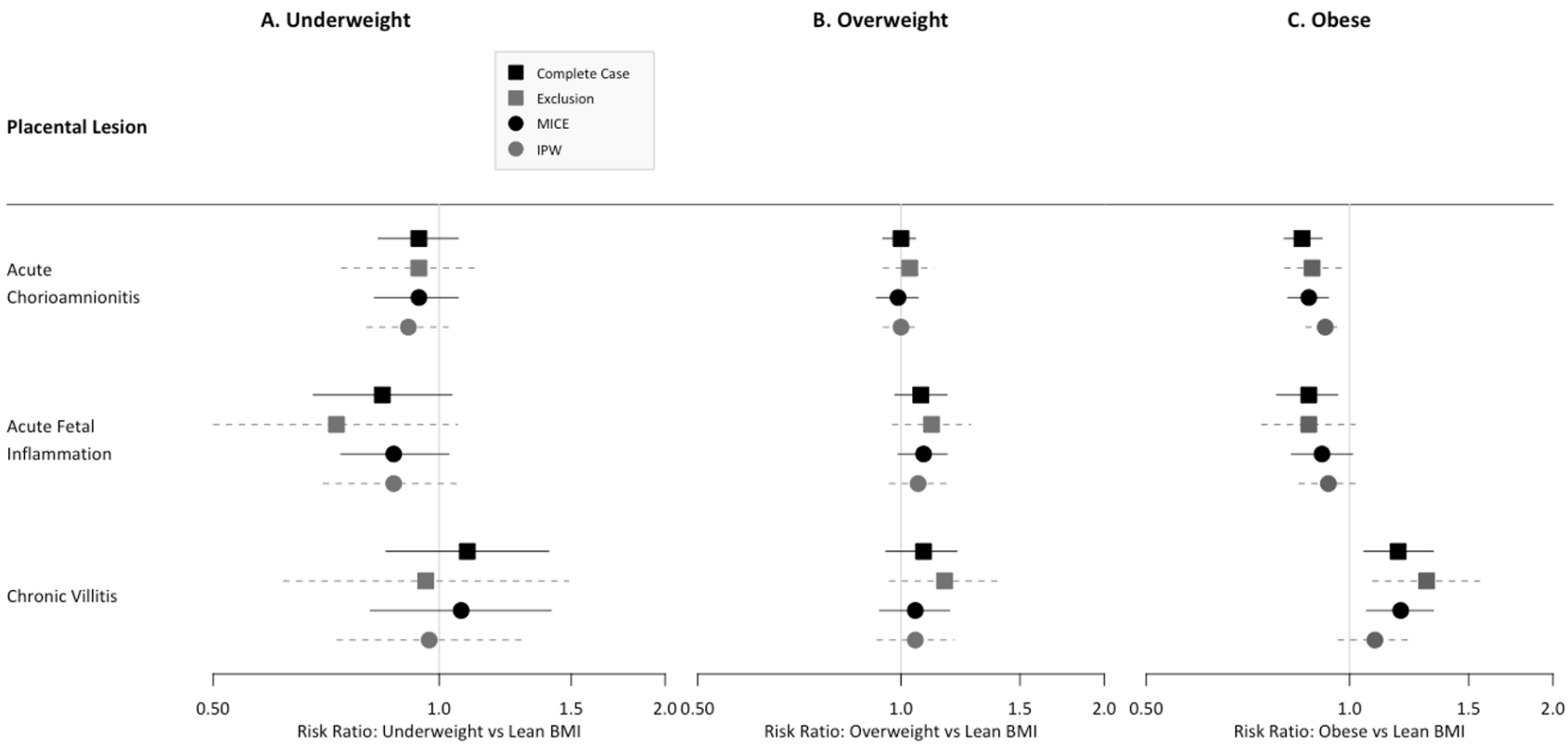


Figure 9: Risk ratios of placental inflammatory lesions in A) underweight (<18.5kg/m^2), B) overweight (25 to <30kg/m^2), C) and obese women (≥30kg/m^2) relative to lean women (18.5 to <25kg/m^2).

Symbols of different shapes and colors represent different analytic strategies: complete case (black box), exclusion of pregnancy complications (grey box), multiple imputation by chained equations (black circle), inverse probability weighting (grey circle). Risk ratios were adjusted for maternal age, race, insurance, education, nulliparity and smoking.

RRs for all placental inflammatory lesions were consistent when excluding pregnancy complication individually (**Appendix Figure 3)**. Interactions between pre-pregnancy BMI with maternal race, fetal sex, and labor were not significant ($p>0.10$) for any of the placental inflammatory lesions. There was little change in the distribution of inverse probability weights and RR estimates by adding interactions or higher order functions for continuous variables to IPW models **(Appendix Table 5)**. Although women with histopathology were more likely to have pregnancy complications (gestational diabetes, hypertensive disorders of pregnancy, suspected intrauterine infection) than women without histopathology evaluations; after IPW, pregnancies with fewer complications were upweighted so that pregnancies with histopathology had characteristics comparable to the general population **(Appendix Table 6)**.

2.1.4.3 Sensitivity Analyses for Selection Bias

Restricting analysis to pregnancies with placental histopathology (S) for an association between pre-pregnancy obesity (A) and placental histologic inflammation (Y) could be biased by factors associated with placental histopathology evaluation pre-pregnancy (U1), during pregnancy (U2), and post-pregnancy (U3). The pre-pregnancy, pregnancy, and post-pregnancy factors we considered are outlined in **Appendix Figure 4.**

Figure 10 presents IPW RR estimates for obesity corrected for bias factors from measured pregnancy complications. Residual selection bias could strongly skew the association between obesity and risk of acute placental inflammation. Selection bias from chronic hypertension, diabetes, preeclampsia, congenital defects, SGA, gestational hypertension, and substance use could all explain away the protective effect of obesity on acute chorioamnionitis and acute fetal inflammation. While adverse pregnancy history, intrauterine infection, labor/fetal complications, and maternal comorbidities could all bias the protective effect of obesity further from the null

(**Appendix Figure 5)**. In contrast, RR estimates for chronic villitis changed only modestly after adjusting for selection bias from pregnancy complications.

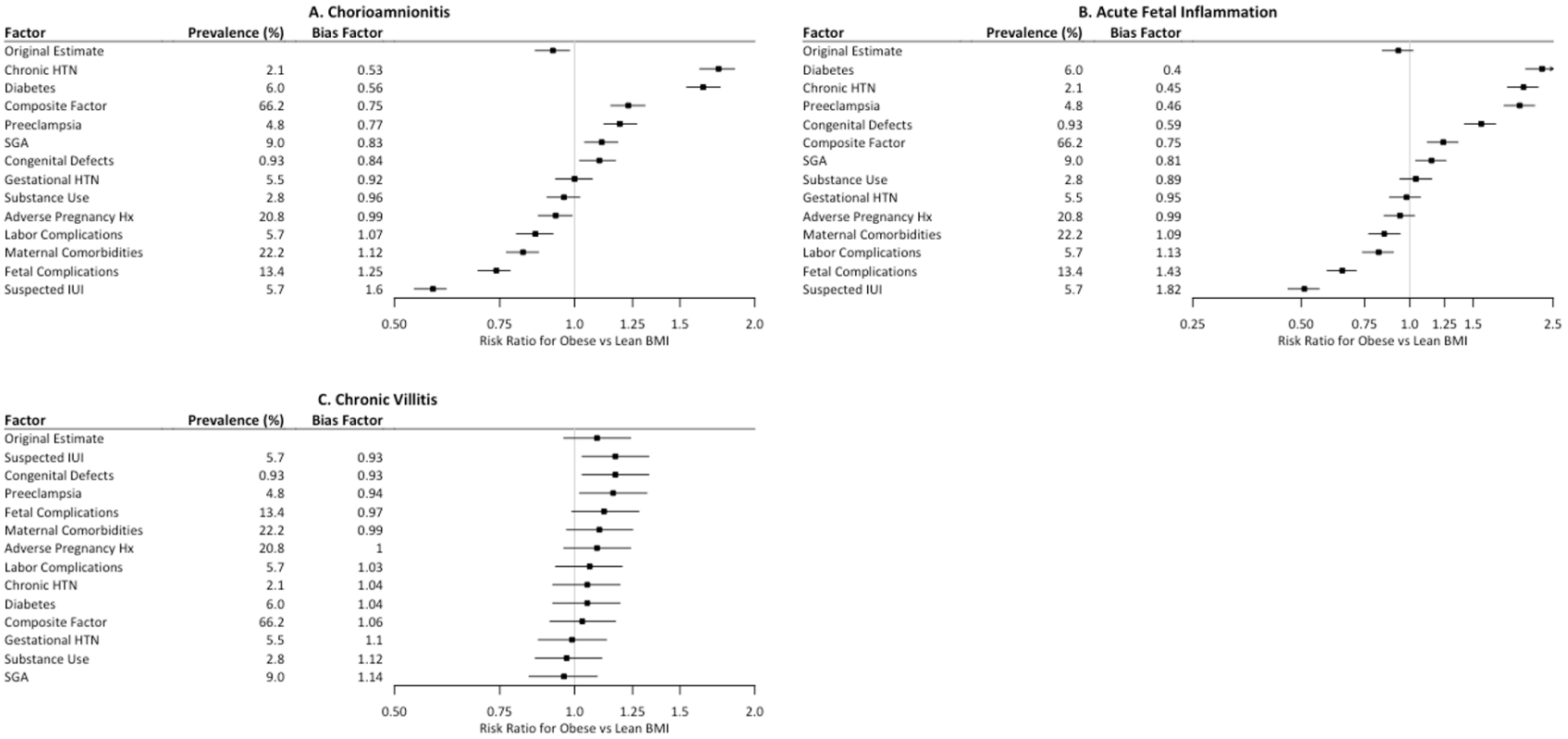


Figure 10: Risk ratios for A) acute chorioamnionitis, B) acute fetal inflammation, and C) chronic villitis in obese women compared to lean women corrected for hypothetical residual selection bias.

The prevalence refers to the prevalence of each pregnancy complication among term births in MOMI (n=41,443). The bias factor is the amount of bias for a given pregnancy complication and risk ratios are adjusted by dividing the bias factor from the original risk ratio estimate. The original estimate is the risk ratio measured by inverse probability weighting and adjusted for maternal age, race, insurance, education, nulliparity and smoking. Congenital defects included neural and cardiovascular congenital defects and chromosomal aneuploidy. Labor complications included fetal hydrops, oligohydramnios, placental accreta, placenta

abruption, placenta previa, prolonged second stage of labor, prolonged membrane rupture. Maternal comorbidities included seizure disorders, thyroid disorders, vascular disorders, and uterine anomalies/cervical surgeries. Fetal complications included fetal arrhythmias or a general clinical diagnosis of fetal distress. Adverse pregnancy history included stillbirth, preterm birth, or miscarriage. Abbreviations: HTN, hypertension; Hx, history; IUI, intrauterine infection; SGA, small for gestational age.

2.1.5 Discussion

Contrary to what we hypothesized, obesity was associated with lower risk of acute placental inflammation, but higher risk of chronic placental inflammation in term pregnancies. These findings were consistent across all analytic approaches (complete case, IPW, or exclusion of complications). Sensitivity analyses indicated residual selection bias could be large enough to explain away the observed association between obesity and acute inflammatory lesions. In contrast, the positive association between obesity and risk of chronic villitis was robust to potential selection bias.

Consistent with our findings, the association we detected between maternal obesity and increased risk of chronic villitis is well-established.[185, 250, 251] Obesity decreases expression of the histocompatibility antigen, HLA-G required for immunotolerance,[159, 264] and promotes macrophage recruitment to placental villi, a diagnostic feature of chronic villitis.[246, 247] Our finding supports the hypothesis that maternal obesity is a chronic inflammatory condition.

In contrast to other studies, we report maternal obesity was associated with lower risk of acute placental inflammation. Acute placental inflammation in term pregnancies has previously been reported to be associated with labor, epidural use, augmentation of labor, and infection.[177, 233, 265] We did not demonstrate a significant interaction between BMI and the presence of labor on placental inflammation and accounting for intrauterine infection by exclusion or down-weighting these pregnancies by IPW, did not change our findings. Differential management of labor between obese and lean women could partially explain the protective effect of obesity on risk of acute inflammation. Obese women are more likely to have delayed onset of labor, prolonged labor, and labor complications compared to lean women.[257, 266, 267] Further research is needed to understand

how factors associated with obstetric management of labor influences placental outcomes in obese women.

These findings may also be susceptible to selection bias. Our use of causal diagrams and sensitivity analyses directly addresses the possibility of selection bias and helps disentangle biased from accurate estimates. In our study, a subclinical outcome like acute histologic inflammation may be affected differently by various indications for histopathology. It is also unclear if some histopathology indications such as preeclampsia cause placental inflammation or if placental inflammation causes preeclampsia. The potential for reverse causation makes it difficult to minimize selection bias by exclusion of pregnancies with histopathology indications.

Nine prior studies have been conducted on pre-pregnancy obesity and risk of acute placental inflammation in term births.[178, 180, 184-186, 232, 233, 248, 249] Like ours, all nine studies are susceptible to selection bias. Two studies showing a pro-inflammatory effect of obesity on acute placental inflammation came from the Collaborative Perinatal Project, a cohort of 54,390 deliveries between 1959-1966.[268, 269] These studies excluded 27% of deliveries missing placental pathology or pre-pregnancy BMI data.[178, 248] Another study of 56 term pregnancies found obese women compared non-obese women had a higher frequency of placentas with acute maternal inflammation, but women with intrauterine infection were excluded and half the cohort had preeclampsia, GDM, or fetal growth restriction.[249] A fourth study showing a positive association between obesity and acute inflammation was a secondary analysis of pregnant women with sleeping disorders.[186] The other five studies had null findings but similar concerns for selection bias by excluding pregnancy complications or using data from biobanks that oversampled adverse pregnancies.[180, 184, 185, 232, 233]

Large clinical registries like the MOMI database offer unique opportunities to study pathophysiologic aspects of pregnancy but require analytic methods to address limitations. Collection of all placentas in a hospital for histopathological evaluation is not feasible and research priorities are always ancillary to the clinical priorities of a safe delivery.[173] Thus, recruiting a sample representative of all pregnancies is challenging and robust methods are needed to address selection bias. In other clinical fields, IPW and MICE are common strategies for handling missing data.[241, 261, 270] Despite complex missing data patterns related to pre-pregnancy, pregnancy, and post-pregnancy indications for histopathology, estimates for associations between obesity and histologic placental inflammation by IPW and MICE were comparable suggesting both approaches may similarly handle selection bias in histopathology studies of obesity.

Unmeasured pathology indications (e.g. abnormal cord blood gases)[231] or hard to define measures (e.g. physician decision-making) may also influence which pregnancies have a placental evaluation,[173] leading to biased estimates even with MICE and IPW.[237, 261, 270] We applied a recently developed sensitivity analysis method to assess residual selection bias using measured clinical complications as a proxy for unmeasured factors.[253] Associations between obesity and acute placental inflammatory lesions were strongly biased (negatively and positively) such that the direction of association between obesity and acute inflammation may not be stable. In contrast, clinical indications did not strongly modify the association between obesity and chronic villitis. Our sensitivity analysis may indicate why there are inconsistencies across studies for the association between obesity and acute inflammatory lesion, but less so for chronic villitis.

There were several strengths to this study. The MOMI dataset is among the largest contemporary cohorts with placental histopathology data since the Collaborative Perinatal Project of the 1960s.[178] Inflammatory pathology features were extracted from pathology evaluations

reported by two pathologists using a standardized protocol and there was excellent agreement for review of placental slides between clinical pathology reports and a pathologist blinded for features of placental inflammation/infection. Our study measured BMI by clinical cut-offs and as a continuous variable. There were many maternal and pregnancy variables to leverage for analytic assessment of selection bias.

Our study also had several limitations. Pre-pregnancy BMI was self-reported, which can lead to measurement bias from misclassification of weight status. An analysis of BMI misclassification in the MOMI dataset concluded that despite some misclassification of obese BMI, obesity was significantly associated with all adverse pregnancy outcomes measured.[271] We were unable to assess labor duration, medical induction, epidural use, or abnormal cord gases which could partially explain the protective effect of obesity on acute inflammation or be sources of selection bias. Sensitivity analyses helped estimate the potential magnitude of residual selection bias. An extension of IPW known as modularized IPW may be more efficient at handling multiple patterns of missing data in clinical studies, but these methods are still emerging.[237] Finally, we did not apply sensitivity analyses for residual measurement error and confounding, as these were beyond the scope of this paper.

2.1.5.1 Conclusion

Placental histopathology allows for tissue-level measures of the placenta in population studies, but the oversampling of pregnancies with adverse outcomes and under-ascertainment of pregnancies without complications limits the generalizability of findings and may introduce bias. Using several analytic approaches to minimize selection bias and sensitivity analyses to assess the impact of residual selection bias, we report that obesity may modestly contribute to chronic

placental inflammation. Further our study highlights methods to robustly deploy clinical data to study pregnancy and placental health in future research.

2.2 Manuscript 2: Latent class analysis of placental histopathology: a novel approach to classifying early and late preterm births.

2.2.1 Abstract

Background: Neonatal morbidity attributable to prematurity predominantly occurs among early preterm births (<32 weeks), rather than late preterm births (32 to <37 weeks). Methods to distinguish early and late preterm births are lacking given heterogeneity in pathophysiology and risk factors, including maternal obesity. While preterm births are often characterized by clinical presentation (spontaneous or clinically indicated), classifying deliveries by placental features detected on histopathology reports may help identify subgroups of preterm births with similar etiology and risk factors. Latent class analysis is an empirical approach to characterize preterm births based on observed combinations of placental features.

Objective: To identify histopathologic markers that can distinguish early (<32 weeks) and late preterm births (32 to <37 weeks) that are also associated with maternal obesity and neonatal outcomes.

Study Design: Women with a singleton preterm birth at Magee-Womens Hospital (Pittsburgh, PA) in 2008-2012 and a placental evaluation (89% of preterm births) were stratified into early (n=900, 61% spontaneous) and late preterm births (n=3,362, 57% spontaneous). Pre-pregnancy BMI was self-reported at first prenatal visit and 16 abstracted placental features were

analyzed. Placental subgroups (i.e. latent classes) of early and late preterm births were determined separately by latent class analysis of placental features. The optimal number of latent classes was selected by comparing fit statistics. The probability of latent class membership across pre-pregnancy BMI was estimated in early preterm births and in late preterm births by an extension of multinomial regression called pseudo-class regression adjusting for race, smoking, education and parity. The frequencies of severe neonatal morbidity (composite outcome: respiratory distress, bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis, periventricular leukomalacia, patent ductus arteriosus, and retinopathy of prematurity), small for gestational age and length of NICU stay were compared across latent classes by chi-squared and Kruskal-Wallis tests.

Results: Early preterm births grouped into 4 latent classes based on placental histopathological features: acute inflammation (38% of cases), maternal vascular malperfusion with inflammation (29%), maternal vascular malperfusion (25%), and fetal vascular thrombosis with hemorrhage (8%). As BMI increased from 20 to 50kg/m^2, the probability of maternal vascular malperfusion and fetal vascular thrombosis with hemorrhage increased while the probability of maternal vascular malperfusion with inflammation decreased. There was minimal change in the probability of acute inflammation with increasing BMI. Late preterm births also had 4 latent classes: maternal vascular malperfusion (22%), acute inflammation (12%), fetal vascular thrombosis with hemorrhage (9%) and low risk pathology (58%). BMI was not associated with major changes in likelihood of the latent classes in late preterm births. Associations between BMI and likelihood of the latent classes were not modified by type of delivery (spontaneous or indicated) in early or late preterm births. Maternal malperfusion and fetal vascular thrombosis with

hemorrhage were associated with greater neonatal morbidity than the other latent classes in early and late preterm births.

Conclusions: Obesity may predispose women to early but not late preterm birth through placental vascular impairment. Latent class analysis of placental histopathological data provides an evidenced-based approach to group preterm births with shared underlying etiology and risk factors.

2.2.2 Introduction

In the US, approximately 80% of preterm births (PTBs) are late PTBs (32 to <37 weeks of gestation), but 75% of neonatal deaths among PTBs occur in early PTBs (<32 weeks).[272] Early and late PTB may have distinct risk factors (e.g. maternal obesity) with variable pathophysiology.[26] Additionally, PTBs are classified as spontaneous or clinically indicated PTBs.[273] Yet, this classification is not informative of PTB etiology and findings between clinical classification and neonatal morbidity risk are conflicting.[273-275] Placental histopathological evaluations are routinely conducted to inform a clinician's assessment for a cause of PTB.[173] These evaluations are an underutilized resource to understand the pathophysiology and neonatal sequelae of early and late PTBs.

Interpreting placental histopathology is challenging because findings can be incidental or related to physiologic processes like labor.[265, 276, 277] A high proportion (28-78%) of uncomplicated term pregnancies are reported to have at least one histopathological feature.[190, 191] In the absence of an approach to distinguish healthy from complicated placentas, histopathology offers limited insight for pediatric follow-up. Clusters of histopathological features may be more reflective than single measures of true pathology and prognostic of neonatal outcomes.

Various approaches have previously classified PTBs by patterns of histopathological features. Prior approaches have been based on anticipated groupings of histopathology (e.g. inflammatory or vascular impairment) using correlative measures, factor analysis and pre-defined groups based on expert opinion.[192-195] These methods may presuppose placental pathology groups, miss key histopathological features and/or lack strong associations with clinical outcomes. Empirical classification, as applied here, may identify novel placental histopathological patterns in PTB.[278-280]

Latent class analysis (LCA) is a statistical method that classifies individuals into groups based on different patterns of observed data.[281] Groups are called latent classes because they are estimated, not directly measured. For example, grouping individuals into personality types based on survey response patterns. LCA is appealing over other clustering methods (e.g. hierarchal or k-means clustering) because standard model fit statistics inform the appropriate number of latent classes, LCA allows for missing data, and latent classes are easy to interpret.[280, 281] Covariates can be added to LCA models to test if risk factors like obesity predict latent class membership.[281-283]

We aimed 1) to classify early and late PTBs into placental latent classes based on observed patterns of placental histopathological features by LCA and 2) to determine if pre-pregnancy BMI was associated with specific latent classes. We focus on pre-pregnancy BMI as our approach may help understand inconsistent findings on obesity and PTB.[4] Evidence suggests obesity is associated with an increased risk of early PTB (<32 weeks), but not late PTB.[26, 90] Obesity may alter the risk of early and late PTBs by different placental mechanisms.[136, 137, 140] For clinical relevance of our classification approach, we compared the prevalence of severe neonatal morbidity (composite outcome: respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis, patent ductus arteriosus, periventricular leukomalacia,

retinopathy of prematurity), length of neonatal intensive care unit (NICU) stay and small for gestational age (SGA) across latent classes.

2.2.3 Methods

2.2.3.1 Study Participants

Delivery data were collected from the Magee Obstetric Medical and Infant (MOMI) database. We included live singleton early (20 to <32 weeks) and late PTBs (32 to <37 weeks) delivered between 2008-2012 with available placental pathology data (94% of early PTBs and 88% of late PTBs). We excluded stillbirths (0.5% of PTBs) because our automated placental report abstraction approach did not reliably distinguish placental findings. Women with multifetal gestations were excluded as placental findings in multifetal pregnancies are different from singleton pregnancies, and placental findings were unable to be linked to each fetus.[284, 285] The University of Pittsburgh IRB approved this project (STUDY20050303), and no consent was needed as data were de-identified.

2.2.3.2 Placental Data

Placental histopathological features considered for LCA were extracted from pathology reports conducted by two placental pathologists following a standardized protocol and linked to the MOMI database by an automated process previously described.[174] Histopathology definitions were adapted from the 2014 Amsterdam Criteria (**Appendix Table 7**).[231, 286] Pathological features included markers of inflammation (acute chorioamnionitis, vasculitis, funisitis, deciduitis, villitis, intervillitis), maternal vascular malperfusion (MVM: villous infarct, intraparenchymal hemorrhage, subchorionic hemorrhage, advanced villous maturation, decidual vasculopathy,

villous agglutination, intervillous thrombus), fetal vascular malperfusion (avascular villi, stromal vascular karyorrhexis, fetal vascular thrombosis, chorangiosis) and other markers (placental growth, chorioangioma, chorangiomatosis, delayed villous maturation, dysmaturity). Stromal-vascular karyorrhexis and avascular villi were combined as stromal-vascular karyorrhexis progresses to avascular villi.[287] There was excellent agreement for review of placental slides between clinical pathology reports and a pathologist blinded to all clinical information except gestational age (W.T.P.) for features of inflammation (82%)[193] and for features of MVM (kappa=0.78).[288]

2.2.3.3 Anthropometry

Pre-pregnancy BMI was calculated as a ratio of weight (kg) to height (m) squared (kg/m^2) using self-reported data at first prenatal visit. Self-reported pre-pregnancy weight is highly correlated with first measured weight in pregnancy (r=0.99) at Magee-Womens Hospital.[254]

2.2.3.4 Pregnancy Characteristics

Gestational age was determined using best obstetric estimate based on first/second trimester ultrasound in conjunction with last menstrual period.[289] Pregnancy and neonatal outcomes were extracted from medical records based on ICD-9 codes provided in the **Appendix B**. Pregnancy complications include gestational diabetes, gestational hypertension, preeclampsia/eclampsia, cervical shortening, clinical chorioamnionitis, and preterm premature rupture of membranes (PPROM). PTBs were classified as early PTB for deliveries <32 weeks of gestation and late PTB for deliveries 32 to <37 weeks.[273] Early PTBs were not further classified as there were few PTBs <28weeks (n=374, 7.8% of PTBs) and PTBs <32 weeks are postulated to have similar etiologies.[9, 273] A spontaneous PTB was defined as a pregnancy with spontaneous

onset of contractions or premature rupture of fetal membranes before 37 weeks (irrespective of induction or cesarean section after labor); and a clinically indicated PTB was defined as an induced pregnancy or cesarean section before 37 weeks.[1] Adverse neonatal outcomes included small for gestational age (birthweight <10th percentile using Alexander birthweight curve; SGA),[256] length of NICU stay (infant hospital discharge date minus date of delivery) and a composite score for severe neonatal morbidity: respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis, periventricular leukomalacia, patent ductus arteriosus, and retinopathy of prematurity.

2.2.3.5 Statistical Analyses

Frequencies of individual placental features were compared between early and late PTBs by chi-squared and Fisher's exact tests. Early (<32 weeks) and late (32 to <37weeks) PTBs were analyzed separately for: 1) grouping deliveries into classes reflective of distinct placental pathology, 2) predicting class membership across pre-pregnancy BMI and 3) comparing clinical outcomes across classes.

Deliveries were classified based on observed patterns of 16 placental histopathological features using LCA. We call these groups placental latent classes since they are empirically derived from observed histopathology patterns, and not directly measured by the pathologist. Rare placental features (< 1% of PTBs) were excluded due to limited numbers. The optimal number of placental latent classes in early and late PTBs were determined by comparing 6 models ranging from 1 to 6 latent classes using model fit statistics. We excluded models that generated rare latent classes (<5%) and models with poor entropy (<0.70), a measure of how accurately pregnancies are classified.[280, 290] Latent classes were labeled based on the combination of placental features with probabilities of occurring >25% within that class. Among late PTBs, we excluded deliveries at 36

weeks as a sensitivity analysis to assess the effect of PTB misclassification due to potential inaccurate dating. Few women (4.5%) had more than one pregnancy in the cohort. Restricting to the first pregnancy did not change the number or composition of placental features within latent classes. Therefore, we included all pregnancies in the analysis.

We evaluated if pre-pregnancy BMI was associated with likelihood of the placental latent classes by four regression methods (pseudo-class, most-likely class, probability-weighted, and single-step latent class regression) described in the **Appendix B**.[282, 291] Briefly, latent classes are statistically estimated; therefore, pregnancies may be misclassified into the wrong class. Misclassification adds variability that is accounted for by these regression methods. Assessing consistency in findings across methods helps identify if errors were introduced by the estimation method. Pseudo-class regression is the preferred method as it adequately accounts for variability from potential latent class misclassification and is flexible in handling missing data.[282] Associations were visualized by predicted probability plots. Models were adjusted for maternal race, education, parity and smoking. We examined whether associations between BMI and likelihood of latent classes were modified by interaction with clinical presentation of PTB (spontaneous vs indicated), race and fetal sex.[292, 293] For any variable found to be an effect modifier ($p<0.10$), stratified results are presented.

We compared the proportions of pregnancy complications and neonatal morbidities across placental latent classes (deliveries assigned to most-likely latent class) by chi-squared and Fisher's exact tests for categorical outcomes and Kruskal-Wallis tests for continuous outcomes. An alpha level of 0.05 was assumed for nominal significance and a Bonferroni-corrected alpha was used for significance after multiple comparisons of pregnancy ($0.05/8 = 0.006$) and neonatal outcomes ($0.05/10 = 0.005$).

Pre-pregnancy BMI were missing in 42% of PTBs. Women with and without a reported pre-pregnancy BMI had comparable maternal and pathology characteristics (**Appendix Tables 8 and 9**). Missing data were imputed by multiple imputation with chained equations.[234] We compared regression estimates by complete case analysis and by imputation to assess sensitivity to missingness. Additional details are provided in the **Appendix B**. Analyses were conducted in R Studio.[263]

2.2.4 Results

There were 4,262 liveborn singleton PTBs with placental histopathology at Magee-Womens Hospital between 2008 and 2012; 20% were early PTBs (<32 weeks) and 80% were late PTBs (32 to <37 weeks; **Figure 11**). Women with early PTB were younger, more likely to be black, less likely to have a college education, and less likely to have diabetes (pre-existing or gestational) compared to women with late PTB **(Table 7)**. Women with early PTB compared to women with late PTB were more likely to have PPROM, spontaneous PTB, and less likely to have an SGA baby. There were 797 (89%) early PTBs and 2,392 (71%) late PTBs with at least one placental histopathological feature. Early PTBs had higher frequencies of histopathological features of acute inflammation and MVM than late PTBs (**Figure 12)**.

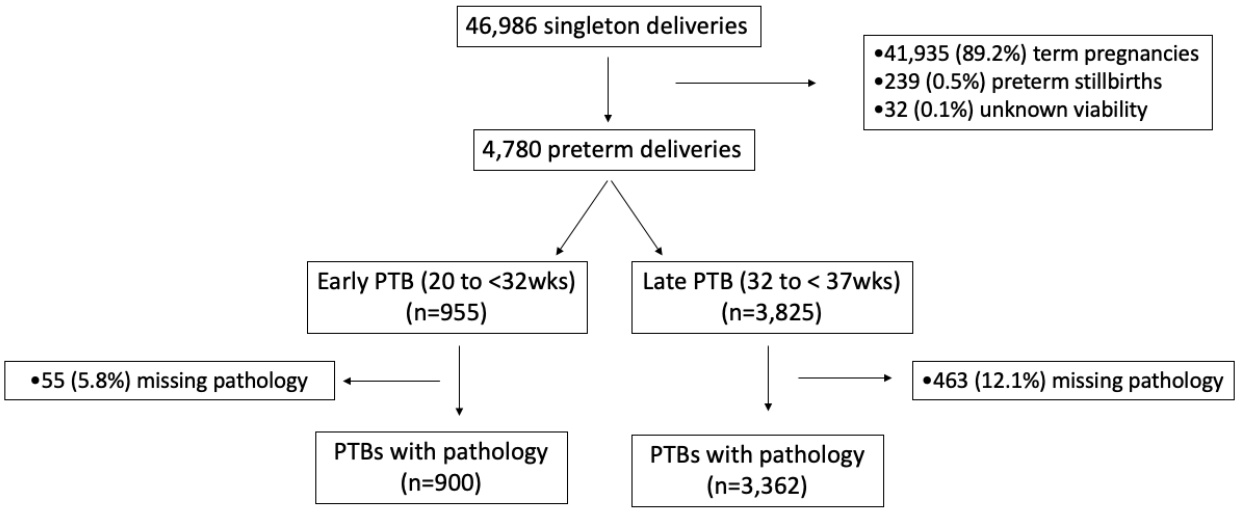


Figure 11: Study selection criteria.

Inclusion and exclusion criteria for study population. Abbreviations: PTB, preterm birth.

Table 7: Maternal and delivery characteristics for early (n=900) and late preterm births (n=3,362)[a,b]

Maternal Characteristics	**Missing (n)**	**Early PTB (20 to <32 weeks)**	**Missing (n)**	**Late PTB (32 to <37 weeks)**	**p-value**
Maternal Age (years)	0	27.3 ± 6.3	0	28.4 ± 6.2	<0.001
Race, n (%)	5		14		0.003
White		613 (68.5)		2434 (72.7)	
Black		255 (28.5)		780 (23.3)	
Other		27 (3.0)		134 (4.0)	
Education, n (%)	20		33		<0.001
High School /GED or less		432 (49.1)		1263 (37.9)	
Some College		448 (50.9)		2066 (62.1)	
Pre-pregnancy BMI, kg/m^2	445	26.9 ± 7.5	1365	26.3 ± 6.8	0.274
Weight Status, n (%)	445		1365		0.162
Underweight (<18.5kg/m^2)		30 (6.6)		109 (5.5)	
Lean (18.5 to <25kg/m^2)		203 (44.6)		939 (47.0)	
Overweight (25 to <30kg/m^2)		93 (20.4)		465 (23.3)	
Obese (>30kg/m^2)		129 (28.4)		484 (24.2)	
Smoking in Pregnancy, n (%)	12	214 (24.1)	27	774 (23.2)	0.608
Nulliparous at Enrollment, n (%)	0	463 (51.4)	5	1593 (47.5)	0.037
Multiple Abortion History, n (%)	0	140 (15.6)	5	487 (14.5)	0.462
Diabetes, n (%)	0		0		<0.001

No Diabetes		821 (91.2)		2851 (84.8)	
Pre-existing Diabetes		47 (5.2)		314 (9.3)	
Gestational Diabetes		32 (3.6)		197 (5.9)	
Chronic Hypertension, n (%)	0	98 (10.9)	0	280 (8.3)	0.020
Hypertensive Disorders, n (%)	2		7		0.207
No Hypertensive Disorders		626 (69.7)		2390 (71.2)	
Gestational Hypertension		26 (2.9)		125 (3.7)	
Preeclampsia, HELLP, Eclampsia		246 (27.4)		840 (25.0)	
Delivery Characteristics					
PPROM, n (%)	0	345 (38.3)	0	988 (29.4)	<0.001
Clinical Presentation, n (%)	3		2		0.034
Indicated		350 (39.0)		1446 (43.0)	
Spontaneous		547 (61.0)		1914 (57.0)	
Gestational Weight Gain					
Weight Gain Mean (kg)	494	8.9 ± 6.6	1476	12.8 ± 6.8	<0.001
Weight Gain Z-score	505	-0.21 ± 1.22	1505	-0.10 ± 1.10	0.032
Infant Birthweight (g)	26	1138 ± 459	14	2493 ± 560	<0.001
Small for Gestational Age, n (%)	21	113 (12.9)	13	581 (17.3)	0.002
Male Fetal Sex, n (%)	0	504 (56.0)	0	1821 (54.2)	0.345

Abbreviations: HELLP, Hemolysis, Elevated Liver enzymes, Low Platelets; PPROM, preterm premature rupture of membranes; PTB, preterm birth.

[a] Continuous variables are represented as mean ± standard deviation.

[b] Continuous variables were compared by t-tests for normally distributed variables and Mann-Whitney U tests for skewed data. Categorical variables were compared by chi-squared tests.

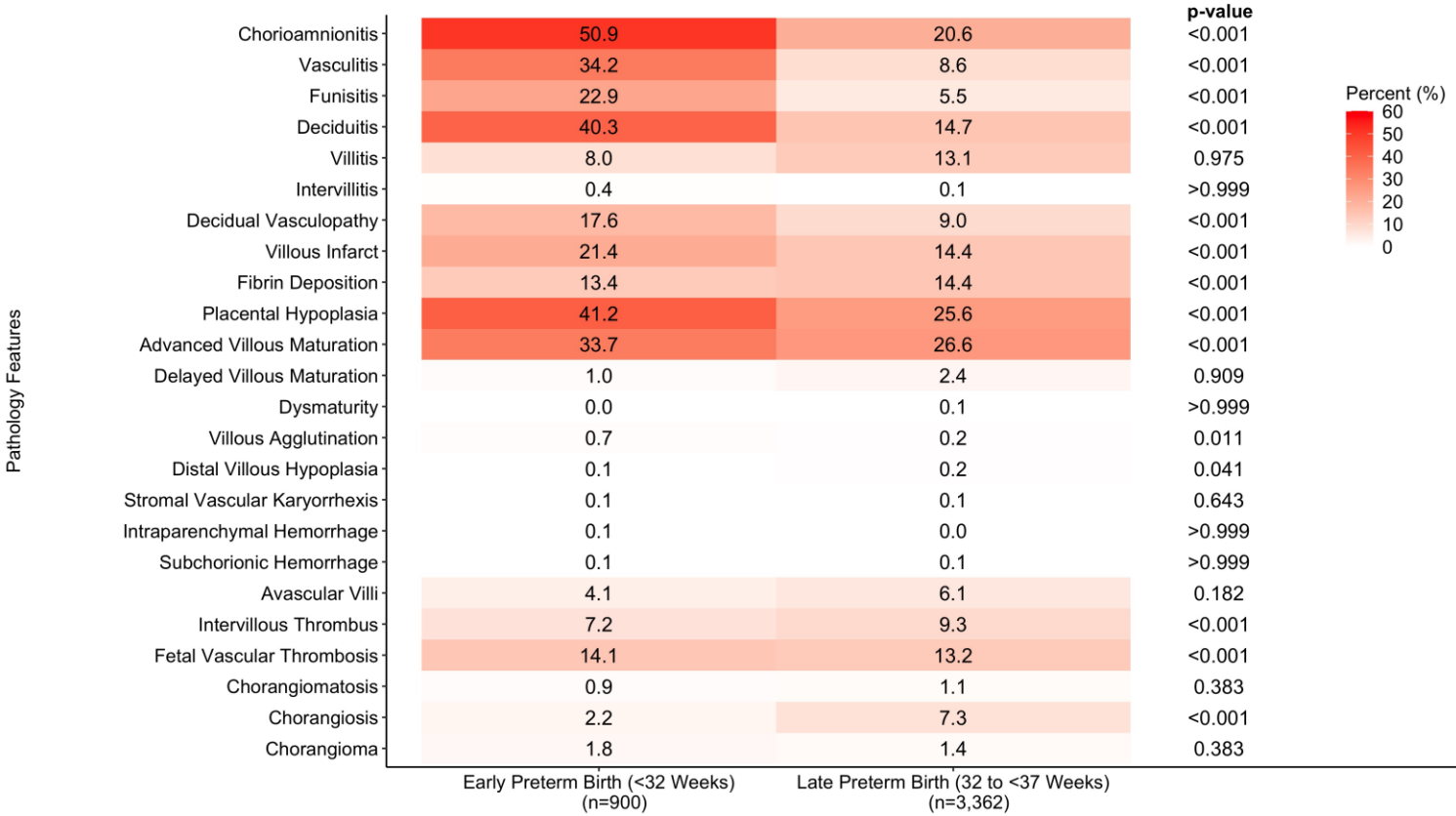


Figure 12: Distribution of placental lesions in early and late preterm births.

Proportions of placenta features in early and late preterm births are represented by percentages with higher percentages graphically represented with a darker red color. 175 (4.1%) pregnancies were missing data on placental hypoplasia.

Based on fit statistics, class size, and classification accuracy, early PTBs grouped into 4 placental latent classes by LCA (**Appendix Figures 6 and 7**)**.** Among early PTBs, 38% had acute inflammation, 29% had MVM with chorioamnionitis, 25% had MVM and 8% had fetal vascular thrombosis (FVT) with hemorrhage (**Figure 13A)**. Late PTBs also grouped into 4 latent classes (**Appendix Figures 6 and 8).** Among late PTBs, 58% had low risk pathology (no pathological features with >25% probability of occurring for the latent class), 22% had MVM, 12% had acute inflammation and 9% had FVT with hemorrhage **(Figure 13B)**. Excluding late PTBs at 36 weeks did not affect the optimal number of latent classes (**Appendix Figure 9)**.

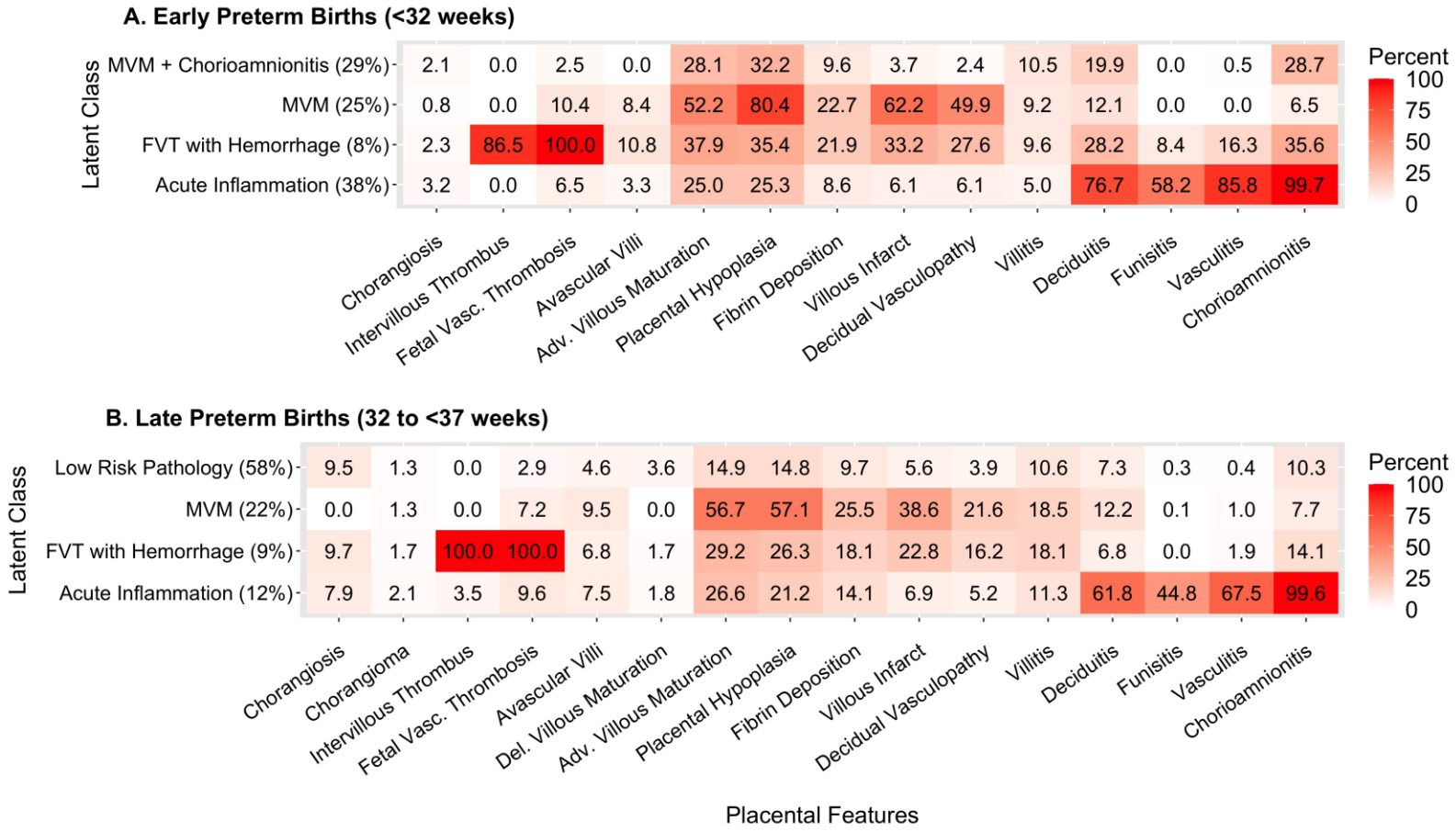


Figure 13: Probabilities of placental features in latent classes of early (A) and late (B) preterm births.

Latent classes and conditional probabilities of placental histopathological features were identified by latent class analysis. Labels for the different latent classes are based on the combination of placental features that have conditional probabilities greater than 25% within the latent class. Abbreviations: adv, advanced; del, delayed; FVT, fetal vascular thrombosis; MVM, maternal vascular malperfusion; vasc, vascular.

Among early PTB, increasing pre-pregnancy BMI from 20 to 50kg/m^2 was associated with a higher probability of MVM (20.2% to 37.0%) and FVT with hemorrhage (6.6% to 14.9%), and a lower probability of MVM with chorioamnionitis (35.0% to 13.2%) after adjusting for race, education, parity, and smoking **(Figure 14)**. There was minimal change in the probability of acute inflammation (38.2% to 34.8%) with increasing BMI in early PTBs. In late PTBs, increasing BMI from 20 to 50kg/m^2 was associated with an increased probability of FVT with hemorrhage (7.4% to 14.9%) and a decreased probability of low risk pathology (60.1% to 53.0%). There was minimal change in the probabilities of MVM (21.4% to 21.7%) and acute inflammation (11.2% to 10.4%) with increasing BMI in late PTBs. There were no interactions between BMI with fetal sex, race, or clinical presentation of PTB (spontaneous vs indicated) for early or late PTB. Associations were consistent across early or late PTBs irrespective of regression method or imputation (**Appendix Table 10**).

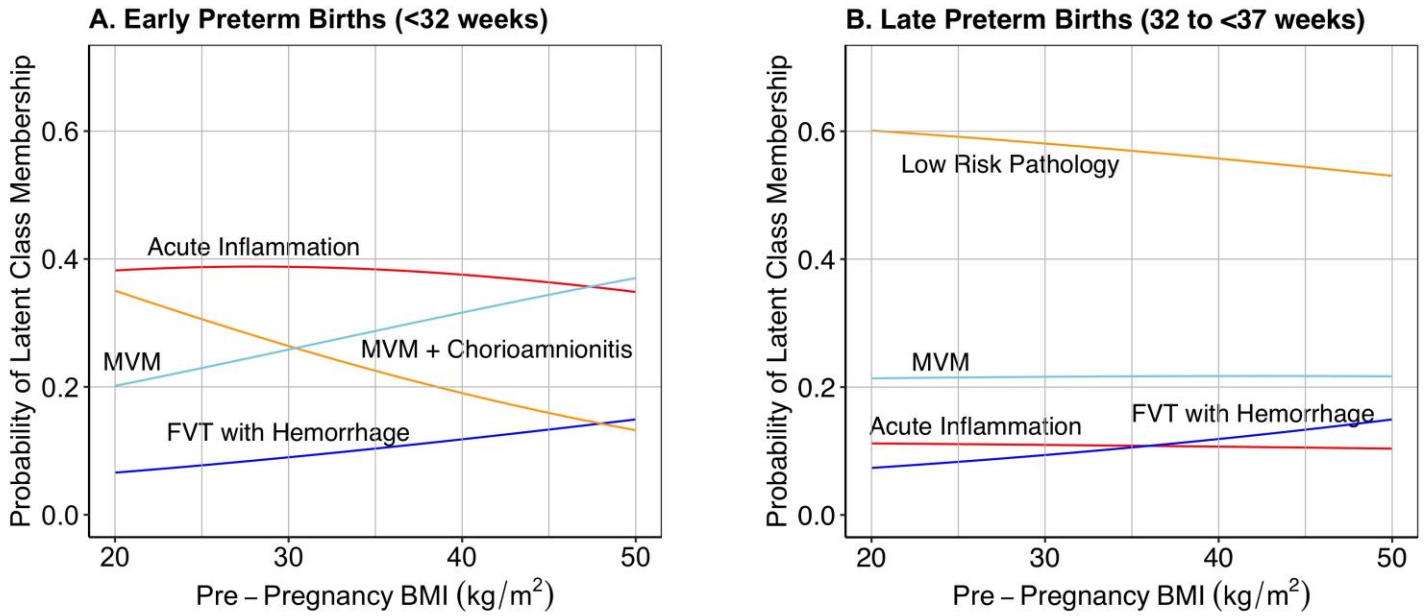


Figure 14: Predicted probabilities of placental latent class membership across pre-pregnancy BMI (kg/m^2) in early (A) and late preterm births (B).

Predicted probabilities are derived from pseudo-class regression models adjusted for maternal race, education, smoking and parity. Predicted probabilities use the average values for the covariates and are based on multiply imputed data (early preterm birth, n=900; late preterm birth, n=3,362). Abbreviations: FVT, fetal vascular thrombosis; MVM, maternal vascular malperfusion.

Among early PTBs, women with acute inflammation were most likely to have a spontaneous delivery (87.5%), PPROM (60.6%) and clinical chorioamnionitis (38.8%) relative to the other latent classes (**Table 8)**. Early PTBs with MVM had the highest frequencies of preeclampsia, eclampsia, and HELLP (79.7%); severe neonatal morbidity (82.0%) and SGA (31.8%) relative to the other latent classes. Among late PTBs, women with acute inflammation had the highest frequencies of spontaneous delivery (75.0%), PPROM (43.6%), and clinical chorioamnionitis (8.7%) compared to the other latent classes (**Table 9)**. Late PTBs with MVM, had the highest prevalence of preeclampsia, eclampsia, and HELLP (53.1%); SGA (42.1%) and longer median NICU stay (9 days, IQR: 18 days), while women with FVT with hemorrhage had the highest frequency of severe neonatal morbidity (19.9%) relative to the other latent classes.

Table 8: Pregnancy and neonatal outcomes based on placental pathology in early preterm births (n=900)[a,b,c]

Clinical Outcomes	Acute Inflammation (n=343)	FVT with Hemorrhage (n=71)	MVM (n=202)	MVM with Chorioamnionitis (n=284)	p-value
Pregnancy Outcomes					
Diabetes, n (%)					0.115
No Diabetes	318 (92.7)	65 (91.5)	179 (88.6)	259 (91.2)	
Pre-existing Diabetes	19 (5.5)	2 (2.8)	15 (7.4)	11 (3.9)	
Gestational Diabetes	6 (1.8)	4 (5.6)	8 (4.0)	14 (4.9)	
Hypertensive Disorders, n (%)					<0.001**
No Hypertensive Disorders	318 (93.0)	39 (54.9)	39 (19.3)	230 (81.3)	
Gestational Hypertension	13 (3.8)	5 (7.0)	2 (1.0)	6 (2.1)	
Preeclampsia, HELLP, Eclampsia	11 (3.2)	27 (38.0)	161 (79.7)	47 (16.6)	
Cervical Shortening, n (%)	11 (3.2)	0 (0.0)	0 (0.0)	9 (3.2)	0.014*
Clinical Chorioamnionitis, n (%)	134 (38.8)	8 (11.3)	5 (2.5)	25 (8.8)	<0.001**
PPROM, n (%)	208 (60.6)	22 (31.0)	20 (9.9)	95 (33.5)	<0.001**

Delivery Method, n (%)					<0.001**
Indicated	43 (12.5)	39 (56.5)	169 (83.7)	99 (35.0)	
Spontaneous	300 (87.5)	30 (43.5)	33 (16.3)	184 (65.0)	
Cesarean Section, n (%)	118 (35.9)	48 (68.6)	166 (82.2)	152 (55.9)	<0.001**
Male Fetal Sex, n (%)	188 (54.8)	41 (57.7)	104 (51.5)	171 (60.2)	0.262
Neonatal Outcomes					
Small for Gestational Age, n (%)	15 (4.5)	14 (20.3)	63 (31.8)	21 (7.6)	<0.001**
Severe Neonatal Morbidity, n (%)[d]	228 (68.1)	49 (71.0)	159 (82.0)	214 (76.2)	0.004**
Respiratory Distress, n (%)	200 (59.7)	43 (62.3)	140 (72.2)	191 (68.0)	0.020*
Bronchopulmonary Dysplasia, n (%)	74 (22.1)	12 (17.4)	32 (16.5)	47 (16.7)	0.269
Intraventricular Hemorrhage, n (%)	90 (26.2)	16 (22.5)	39 (19.3)	60 (21.1)	0.245
Necrotizing Enterocolitis, n (%)	32 (9.3)	2 (2.8)	23 (11.4)	24 (8.5)	0.162
Patent Ductus Arteriosus, n (%)	58 (16.9)	15 (21.1)	46 (22.8)	57 (20.1)	0.389
Periventricular Leukomalacia, n (%)	12 (3.5)	0 (0.0)	3 (1.5)	7 (2.5)	0.299
Retinopathy of Prematurity, n (%)	42 (12.2)	10 (14.1)	20 (9.9)	26 (9.2)	0.479
Median Days in NICU (IQR)	30.0 (52.8)	33.0 (35.5)	36.0 (28.0)	31.0 (38.0)	0.130

Abbreviations: FVT, Fetal Vascular Thrombosis; HELLP, Hemolysis, Elevated Liver enzymes, Low Platelets; IQR, interquartile range; MVM, maternal vascular malperfusion; NICU, neonatal intensive care unit; PPROM, preterm premature rupture of membranes; PTB, preterm birth.

[a] Categorical variables were compared across groups by chi-squared and Fisher's exact tests. Days in NICU was compared across groups based on Kruskal-Wallis test.
[b] a * denotes nominal significance at $p<0.05$ and ** denotes statistical significance after adjusting for multiple pregnancy (0.05/8 outcomes=0.006) and neonatal outcomes (0.05/10 = 0.005).
[c] Data were missing for hypertensive disorders of pregnancy (n=2), delivery method (n=3), cesarean section (n=27), small for gestational age (n=21), severe neonatal morbidity (n=21), respiratory distress (n=21), bronchopulmonary dysplasia (n=21) and median days in NICU (n=136).
[d] Severe neonatal morbidity is a composite outcome of respiratory distress, bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis, patent ductus arteriosus, periventricular leukomalacia and retinopathy of prematurity.

Table 9: Pregnancy and neonatal outcomes based on placental pathology in late preterm births (n=3,362)[a,b,c]

Clinical Outcomes	Acute Inflammation (n=401)	FVT with Hemorrhage (n=299)	Low Risk Pathology (n=2,088)	MVM (n=574)	p-value
Pregnancy Outcomes					
Diabetes, n (%)					0.365
No Diabetes	345 (86.0)	247 (82.6)	1762 (84.4)	497 (86.6)	
Pre-existing Diabetes	39 (9.7)	29 (9.7)	203 (9.7)	43 (7.5)	
Gestational Diabetes	17 (4.2)	23 (7.7)	123 (5.9)	34 (5.9)	
Hypertensive Disorders, n (%)					<0.001**
No Hypertensive Disorders	335 (84.0)	191 (63.9)	1621 (77.8)	243 (42.4)	
Gestational Hypertension	11 (2.8)	11 (3.7)	77 (3.7)	26 (4.5)	
Preeclampsia, HELLP, Eclampsia	53 (13.3)	97 (32.4)	386 (18.5)	304 (53.1)	
Cervical Shortening, n (%)	4 (1.0)	2 (0.7)	4 (0.2)	3 (0.5)	0.033*
Clinical Chorioamnionitis, n (%)	35 (8.7)	1 (0.3)	30 (1.4)	3 (0.5)	<0.001**
PPROM, n (%)	175 (43.6)	62 (20.7)	631 (30.2)	120 (20.9)	<0.001**

Delivery Method, n (%)					<0.001**
Indicated	100 (25.0)	167 (55.9)	807 (38.6)	372 (64.9)	
Spontaneous	300 (75.0)	132 (44.1)	1281 (61.4)	201 (35.1)	
Cesarean Section, n (%)	91 (23.2)	138 (47.3)	700 (34.2)	270 (47.7)	<0.001**
Male Fetal Sex, n (%)	219 (54.6)	141 (47.2)	1151 (55.1)	310 (54.0)	0.081
Neonatal Outcomes					
Small for Gestational Age, n (%)	58 (14.5)	54 (18.1)	228 (11.0)	241 (42.1)	<0.001**
Severe Neonatal Morbidity, n (%)[d]	44 (11.3)	58 (19.9)	263 (12.9)	91 (16.5)	0.001**
Respiratory Distress, n (%)	33 (8.5)	44 (15.1)	219 (10.8)	60 (10.9)	0.053
Bronchopulmonary Dysplasia, n (%)	0 (0.0)	0 (0.0)	5 (0.3)	4 (0.7)	0.190
Intraventricular Hemorrhage, n (%)	14 (3.5)	8 (2.7)	45 (2.2)	26 (4.5)	0.016*
Necrotizing Enterocolitis, n (%)	2 (0.5)	4 (1.3)	10 (0.5)	9 (1.6)	0.026*
Patent Ductus Arteriosus, n (%)	3 (0.8)	6 (2.0)	24 (1.2)	11 (1.9)	0.229
Periventricular Leukomalacia, n (%)	1 (0.3)	1 (0.3)	4 (0.2)	1 (0.2)	0.793
Retinopathy of Prematurity, n (%)	0 (0.0)	2 (0.7)	1 (0.0)	5 (0.9)	0.002
Median Days in NICU (IQR), n (%)	6.0 (13.8)	2.0 (12.0)	1.0 (9.0)	9.0 (18.0)	<0.001

Abbreviations are described in the first footnote of Table 2.

[a] See footnote [a] in Table 2 for how variables were compared across latent classes.

[b] See footnote [b] in Table 2 for denoting statistical significance.
[c] Data were missing for hypertensive disorders of pregnancy (n=7), delivery method (n=2), cesarean section (n=63), small for gestational age (n=13), severe neonatal morbidity (n=95), respiratory distress (n=95), bronchopulmonary dysplasia (n=95) and median days in NICU (n=404).
[d] See footnote [d] in Table 2 for definition of severe neonatal morbidity.

2.2.5 Discussion

2.2.5.1 Principal Findings

Early PTBs were classified as having acute inflammation, MVM, FVT with hemorrhage, and features of MVM and chorioamnionitis. In contrast, over half of late PTBs had minimal placental pathology with the remaining late PTBs having acute inflammation, MVM and FVT with hemorrhage. Pre-pregnancy BMI was associated with an increased likelihood of MVM in early but not late PTB, and this association was not modified by clinical presentation of PTB (spontaneous or indicated). MVM and FVT with hemorrhage were associated with greater neonatal morbidity than the other latent classes in early and late PTBs.

2.2.5.2 Results in the Context of What is Known

Among early PTBs, acute inflammation was the most frequent placental latent class perhaps reflective of intrauterine infection. Infection causes 25-40% of PTBs and risk of infection increases with earlier delivery.[1, 294] Acute inflammation had the highest prevalence of clinical chorioamnionitis, spontaneous labor, and PPROM relative to the other classes. In exploratory analyses, no differences in placental latent classes were observed by PPROM status in early spontaneous PTBs to suggest differing pathophysiology. MVM and FVT with hemorrhage were two other classes in early PTBs. Placental features in the MVM latent class (decidual vasculopathy, villous infarct, placental hypoplasia, advanced villous maturation) aligned with the Amsterdam Pathology Criteria.[165, 295] Placental features of FVT with hemorrhage (including fetal vascular thrombosis, intervillous thrombus, villous infarct, acute chorioamnionitis) did not align with the Amsterdam criteria, but perhaps reflect PTBs with endothelial injury of placental vessels due to inflammation.[296] A third of early PTBs had co-occurring chorioamnionitis and malperfusion

(advanced villous maturation and placental hypoplasia). This aligns with findings on the same cohort of PTBs that *a priori* measured the co-occurrence of MVM with intrauterine infection[193] and findings from a cohort of 109 PTBs (<34 weeks).[297] Vascular impairment from maternal morbidity may predispose the placenta to infection, but future studies are warranted.[193]

The majority of late PTBs had low risk of any placental pathology. A study from the same cohort found that 31% of late PTBs (34-36 weeks) had no placental histopathology findings.[193] Another study found that 30% of PTBs had no severe maternal, fetal or placental conditions (e.g. preeclampsia or fetal growth-restriction).[135] Inaccurate gestational dating of term births as PTBs is unlikely to explain our findings as excluding pregnancies at 36 weeks did not change the latent classes. It also unlikely that late PTBs were from iatrogenic causes by a healthcare provider as 61% of the low risk pathology latent class experienced spontaneous labor. Late PTBs may occur for reasons not mediated by the placenta (e.g., maternal or fetal conditions) or from placental dysfunction at a molecular level not detected by placental histopathology.

Increasing pre-pregnancy BMI was associated with a higher likelihood of MVM in early PTB, but no placental pathology in late PTB. MVM had the highest prevalence of hypertensive disorders of pregnancy in early PTBs. Obesity may predispose women to early PTB through vascular impairment and our findings align with studies that have reported obesity to be a risk factor of early, but not late PTB.[26, 120] Higher gestational weight gain was also associated with MVM in exploratory analyses of early PTBs. Other adiposity measures are worth investigating.

MVM and FVT with hemorrhage were associated with greater neonatal morbidity compared to the other latent classes in early and late PTBs. Prior literature has shown MVM is associated with fetal growth restriction, neonatal thrombocytopenia, and intraventricular

hemorrhage,[164, 165, 193, 298, 299] and fetal thrombotic vasculopathy is associated with fetal demise, growth restriction and cardiac anomalies.[287, 300]

2.2.5.3 Research and Clinical Implications

LCA can aid pathologists in refining diagnostic criteria for placental pathology set by the Amsterdam Placental Workshop Group. The co-occurrence of MVM with acute inflammation may reflect understudied inflammatory pathology in women with underlying vascular impairment. Alternatively, placental hypoplasia and advanced villous maturation were present across all latent classes of early PTBs possibly indicating less precise measures of MVM.

Current preterm interventions (cerclage, progesterone) are effective in a subset of pregnancies. Quantifying associations between maternal factors and placental subtypes of PTBs may inform management of other high-risk subgroups of pregnancies. We found BMI to be associated with early PTBs with MVM. Future studies could also assess risk factors (autoimmune disorders, infection) predictive of PTBs with acute placental inflammation. Risk factors aligning with specific placental mediators of PTB may be targetable for intervention. Classifying PTBs by placental subtypes may also be more prognostic of neonatal morbidity than other PTB classifications. MVM and FVT with hemorrhage were associated with neonatal morbidity.

LCA offers an approach to predict risk of PTB and other syndromes (e.g. stillbirth) using clinical data collected in early pregnancy. Studies have predicted risk of cardiovascular events and cancer based on LCA of self-reported depressive symptoms[301] and metabolic biomarkers, respectively.[302]

2.2.5.4 Strengths and Limitations

Strengths of this study include the generalizability of our findings to the majority of PTBs in Pittsburgh, PA, as placental evaluations were available on 89% of all singleton PTBs delivered at Magee-Womens Hospital. Placental histopathology was collected using a standardized protocol. Groupings of placental features were determined by a data-driven method to minimize bias from *a priori* knowledge.

Study limitations included an inability to evaluate chronicity (e.g. chronic chorioamnionitis) and severity of placental features as these aspects were not validated for automated extraction,[174] and may have variable detection. Except for some placental features of inflammation and MVM, reporting agreement of other placental features was not measured and may vary across pathologists.[175, 193, 288, 303] Further, agreement between pathology reports and manual review of placental features (advanced villous maturation) commonly reported at earlier gestation may be impacted by lack of blinding to gestational age; the pathologist was blinded to all other clinical data. Another limitation was 42% of deliveries were missing data on pre-pregnancy BMI. However, the direction of associations between BMI and likelihood of latent classes were consistent across complete case and multiple imputation analyses. Pre-pregnancy BMI was self-reported, though BMI misclassification did not impact findings in a prior study of this cohort.[271] SGA based on birthweights was a proxy for fetal growth restriction because of missing fetal ultrasound data. Lastly, only 37% of term births had histopathology evaluations and term pregnancies with complications are oversampled making comparisons to PTBs unfeasible.

2.2.5.5 Conclusions

LCA of placental features offers an empirical approach to group PTBs into classes possibly reflective of etiology. We found an understudied cluster of early PTBs with features of

inflammation and MVM and report that early PTBs with MVM are associated with worse neonatal outcomes compared to PTBs with other pathology. Further, we report maternal obesity to be associated with early PTBs with MVM. The translation of these placental phenotypes to risk factors measurable in early pregnancy may inform earlier identification of PTB risk and opportunities for intervention.

2.3 Manuscript 3: Identification of Placental Transcriptome Mediators for the Association Between Maternal Obesity and Gestational Age

2.3.1 Abstract

Background: Maternal obesity may modify the risk of preterm birth (<37 weeks' gestation) through upregulation of placental inflammatory pathways or changes in inflammatory cell populations in the placenta. High dimensional mediation and cell deconvolution are recently developed methods for transcriptomic analyses to identify placental genes and cell types that may mediate the association between obesity and preterm birth.

Objective: We aimed to identify placental genes and cell types that may explain associations between maternal BMI and gestational age in the ENVIRONAGE birth cohort with publicly available placental transcriptomic data.

Methods: Placental RNA microarray data was available on 183 mother-neonate pairs enrolled at delivery without planned cesarean section. First trimester BMI and gestational age were extracted from medical records. BMI was further categorized as underweight (<18.5kg/m^2), lean (18.5 to <25kg/m^2), overweight (25 to <30kg/m^2) and obese (≥30kg/m^2), and preterm birth was

defined as a delivery <37 weeks of gestation. Gene expression was compared between lean and obese women and by preterm birth status using linear regression models adjusted for maternal age, parity, smoking status, and neonatal ethnicity. Gene set enrichment analysis was used to test if pathways of toll-like receptor mediated inflammation, leptin signaling, or trophoblast development were upregulated by obesity or preterm birth status. High-dimensional mediation analysis was used to identify placental gene mediators of maternal BMI and gestational age. Placental cell types were estimated from publicly available single-cell RNA-seq data by cell deconvolution methods. Estimated cell types were compared across BMI classes and preterm birth status by two-way ANOVAs. Pathways co-expressed with placental gene mediators identified in the ENVIRONAGE cohort were further characterized in a second cohort of 10 term pregnancies with available RNA-Seq data on placental villous tissue and chorionic membranes by negative binomial regression models and gene set enrichment analyses.

Results: Gene set enrichment analyses of targeted inflammatory pathways showed toll-like receptor and adipocytokine pathways in obese vs lean women to be upregulated with nominal significance, but not for preterm birth. Maternal BMI was associated with longer gestational age at delivery (ß=0.09 weeks, SE=0.03). High-dimensional mediation analyses identified placental expression of the inflammation regulating gene, IL1RL1, to mediate 15% of the total effect of obesity on gestational age. In a second cohort, increased IL1RL1 expression was associated with decreased expression of gene sets for neutrophil degranulation and preeclampsia in placental villous tissue. Cell deconvolution revealed extravillous cells in placental villous tissues were significantly lower in preterm compared to term births, but no placental cell types differed by first trimester BMI.

Conclusion: The immune-regulatory gene, IL1RL1, may partially mediate the association between pre-pregnancy BMI and longer gestational age at delivery possibly by decreased activity of genes related to neutrophil degranulation and preeclampsia.

2.3.2 Background

Obesity (>30kg/m^2) affects 30% of pregnant women in the US[304] and is the most prevalent modifiable risk factor for adverse pregnancy outcomes.[126] While obesity is consistently associated with preeclampsia, gestational diabetes, and macrosomia, the relationship between obesity and risk of preterm birth varies by severity and clinical presentation.[26, 117] Obesity may increase the risk of preterm births before 32 weeks of gestation, but have a protective effect in preterm births with spontaneous labor between 32-36 weeks.[26, 117, 122] Studies characterizing the underlying pathophysiology of obesity and preterm birth will help understand the heterogeneous effect of obesity on preterm birth and allow for targeted preterm prevention methods in obese women.

Placental inflammation is hypothesized to mediate the effects of maternal obesity and gestational age at delivery. Excessive adipose tissue can promote inflammation through leptin signaling,[134, 305, 306] and higher maternal fatty acid concentrations can cause oxidative damage in the placenta[144, 146] or activate placental toll-like receptor (TLR) 2 and 4 pathways.[148, 150, 156] Higher TLR2 and TLR4 expression,[151-154] higher saturated fatty acids,[144, 307] and genetic polymorphisms in leptin genes[308] have all been associated with a higher risk of preterm birth. Despite these findings, population studies have not universally shown obesity to increase placental inflammation[309, 310] and obesity may even reduce certain mechanisms of acute placental inflammation.[311] Obesity may also contribute to placental dysfunction through non-inflammatory mechanisms such as angiogenesis or developmental pathways.[140]

High-dimensional omics approaches can simultaneously test if maternal obesity is associated with inflammatory and non-inflammatory placental pathways. To date, few studies have measured the effect of obesity on the placenta transcriptome and methylome.[146, 159, 203-205] Findings across these studies have shown that obesity is generally associated with downregulation of placental genes[159] by increased global methylation of placental DNA.[205] However, molecular pathways of acute inflammation[146, 159, 203] and trophoblast differentiation[159] may be upregulated.

Inferring how obesity-associated placental mechanisms affect gestational age is difficult. No study has directly assessed placental gene mediation between obesity and risk of preterm birth. It is also unclear whether increased gene expression is due to global upregulation of placental genes or due to changes in cell composition in the placenta. Recently developed mediation analyses are now able to directly test for placental gene mediators of obesity and preterm birth in high-dimensional settings.[224] Likewise, cell deconvolution methods allow for estimation of placental cell types in bulk placental tissue based on existing single-cell RNA-Seq datasets.[226-228, 230] Cell deconvolution and high-dimensional mediation analyses allow for direct testing of placental mediators of obesity and higher risk of preterm birth.

Our objective was to identify placental genes and placental cell types that mediate the association between obesity and gestational age to inform future preterm interventions in obese women. We apply high-dimensional mediation analysis on a publicly available placental microarray dataset[204] for an agnostic approach to identify placental gene mediators of obesity and gestational age. We also apply gene enrichment analyses for a targeted approach to test if hypothesized placental inflammatory pathways and trophoblast developmental pathways are enriched by obesity and preterm birth status. Lastly, we use reference RNA-Seq data of placental cells to estimate placental cell types associated with obesity and preterm birth.

2.3.3 Methods

2.3.3.1 Study Population and Microarray Analysis

This was a secondary analysis of placental microarray data from the Belgian birth cohort, ENVIRONAGE (ENVIRonmental influence ON early AGEing). The birth cohort was originally designed to study lifestyle factors (including maternal BMI) on molecular signatures of child health early in life.[312] The cohort included 183 mother-infant pairs from women without a planned cesarean section at the time of delivery from the East-Limburg Hospital in Genk, Flanders. Pregnancies missing covariate data, birthweights <1000g, and low-quality placental RNA (RNA integrity <6) were excluded.

Data on first-trimester weight, delivery weight, height, gestational age and fetal sex were extracted from medical records. Maternal weight was measured at first antenatal visit (7-9 weeks of gestation) and first trimester BMI was calculated as the weight in kilograms divided by maternal height in meters squared. Gestational age was determined by last menstrual period and verified with ultrasound data. Preterm birth was defined as a gestational age less than 37 weeks of gestation. Detailed information about newborn ethnicity (European or non-European), maternal age, maternal smoking status during pregnancy and parity were obtained from questionnaires. Placental sampling was conducted within one hour of delivery in quadruplicate from the fetal-side of the placenta and stored in RNAlater. RNA was extracted from the placenta, gene expression was analyzed by the Agilent Whole Human Genome 8x60k microarray (Agilent technologies, Santa Clara, US), and results were deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE128381. There were 14,040 genes included in the publicly available dataset. Additional details on the study population, tissue sampling and data processing have previously been published.[204]

2.3.3.2 Low-Dimensional Analysis

Linear regression was used to measure the association between first trimester BMI and gestational age adjusting for parity, newborn ethnicity (a proxy for maternal ethnicity), maternal smoking status, and maternal age. We tested for effect modification on the additive scale of the association between BMI and gestational age by newborn ethnicity, nulliparity, and fetal sex. Interactions with p-values <0.10 by the Wald test were considered significant. We also measured associations between first trimester BMI measured by BMI classes using standard WHO cut-offs (underweight: <18.5kg/m^2, lean: 18.5 to <25kg/m^2, overweight: 25 to <30kg/m^2, and obese: ≥30kg/m^2) and odds of preterm birth using logistic regression models.

2.3.3.3 Linear models for gene expression analyses

We identified genes with differential expression in the placenta with first trimester BMI and gestational age by linear regression models using the *limma* package in R Studio.[313] BMI and gestational age were modeled as continuous variables and using categorical cut-offs for BMI classes and preterm birth status. We adjusted regression models for the same covariates (ethnicity, nulliparity, smoking, and maternal age) as in the low-dimensional analysis. We similarly tested for significant interactions ($p<0.05$) between neonatal ethnicity, fetal sex, and nulliparity with BMI.

We considered significance after multiple hypothesis testing using the Benjamini-Hochberg false discovery rate (FDR) at 5%. Differentially expressed genes were visualized using Volcano plots. Significantly differentially expressed genes were used to identify upregulated biological and disease pathways of first trimester BMI and gestational age by Gene Ontology and Kyoto Encyclopedia of Genes and Genomes at an Benjamini-Hochberg FDR of <0.05.[314, 315]

2.3.3.4 Gene Set Enrichment Pathways

Based on existing literature, obesity may predispose women to preterm birth through TLR2/TLR4 inflammatory response, leptin signaling, or insulin-like growth factor binding signaling activity.[148, 153, 156, 203, 211, 305, 308] Therefore, we were interested in seeing if these mediating placental gene sets vary by the maternal exposure of obesity or by the outcome, preterm birth status. We identified 12 placental gene sets from Molecular Signatures Database (http://www.gsea-msigdb.org/gsea/msigdb/index.jsp) on TLR 2/4 signaling (Molecular Signature Database IDs: M5932, M11500, M15121, M11084, M23240, M3261), leptin signaling (M10462, M16799, M27195, M18053) and insulin-like growth factor binding signaling (M12608, M27285).[316, 317] We determined if these placental gene sets were enriched by obesity status (obese vs lean women) and by preterm birth status (preterm vs term birth) in the ENVIRONAGE cohort using gene set enrichment analysis (Broad Institute, San Diego, California).[199, 318] A normalized enrichment score was calculated for gene sets and gene sets were considered positively (increased expression) or negatively (decreased expressed) enriched with a nominal p-value <0.05 and statistically significant at a false discovery rate (FDR) adjusted p-value less than 5%.

2.3.3.5 High-Dimensional Mediation Analysis

We used the high-dimensional mediation method described by *Zhang et al* to identify potential gene mediators between first trimester BMI and gestational age using the *HIMA* package in R.[223, 224] This is a 3-step process that identifies potential gene mediators between first trimester BMI and gestational age by first screening genes strongly associated with gestational age, then selecting a subset of the screened gene using the minimax concave penalty, and lastly applies joint significance testing of the association between BMI and gene expression and the association between gene expression and gestational age to determine if these genes are significant

mediators.[224] We assumed that the only exposure-mediator confounders and mediator-outcome confounders were nulliparity, neonatal ethnicity, and maternal smoking status. We considered significance of genes after multiple hypothesis testing using the Benjamini-Hochberg FDR at 5%. To identify if placental gene mediators between BMI and gestational age varied by parity status, neonatal ethnicity, and fetal sex, we preformed stratified analyses by these variables. This method only allows for continuous exposures, so we estimated the effects of 1st trimester BMI on gestational age, with gestation age measured as a continuous variable or as preterm vs term birth.

2.3.3.6 Cell Deconvolution

We used cell deconvolution with the *EpiSCORE* package in R Studio to estimate cell types in placental tissues.[230] First, a reference expression dataset for placental cell types was estimated using published single-cell RNA-seq data of placental cell types by *Suryawanshi et al.*[226] Cell types were then estimated by comparing the gene expression profiles of bulk placental tissue samples in the ENVIRONAGE cohort to cell-specific gene expression patterns from the single-cell reference dataset. Differences in estimated cell proportions were assessed across BMI classes and preterm birth status by two-way ANOVAs. The significance of the interaction between BMI and preterm birth status was tested by linear mixed regression with an interaction term between BMI as a continuous variable and preterm birth due to the limited number of obese women with preterm birth (n=1). We determined if estimated cell-types mediated the association between BMI and gestational age using the *mediate* package in R Studio.[319] Exposure-mediator covariates included nulliparity, maternal age, smoking status and neonatal ethnicity; and mediator-outcome covariates included nulliparity, maternal age, neonatal ethnicity, and smoking status.

2.3.3.7 Exploration of Gene Mediator Molecular Pathways

Few studies measure the placental transcriptome in placental villous tissue and chorionic membranes despite observed global differences in gene expression, morphology and function in pregnancy.[197, 222, 225] The significant gene mediator, IL1RL1, of 1st trimester BMI and gestational age identified in the ENVIRONAGE cohort was further explored in placental villous tissue and chorionic membranes from pregnancies in The Infant Development and Environment Study (TIDES).[320] This exploration dataset included ten uncomplicated term births (>37weeks) delivered in San Francisco with available pre-pregnancy BMI, gestational age, and placental transcriptomic data.

Placental biopsies were sampled from placental villous tissue and chorionic membranes in duplicate. Total RNA from the tissue replicates was pooled, a total RNA library was prepared, and RNA sequencing was performed by NextSeq (Illumina, San Diego, USA). The Galaxy workflow was used to convert raw fastq reads into count data in R Studio (https://training.galaxyproject.org/training-material/topics/transcriptomics/tutorials/rna-seq-reads-to-counts/tutorial.html).

Negative binomial regression was used to compare normalized IL1RL1 expression by placental tissue type using the *DESeq2* package in R.[321] Negative binomial regression was also used to identify other genes co-expressed with increasing IL1RL1 expression in placental villous tissue and in chorionic membranes separately. Genes that were differentially expressed at an FDR cut-off of <5% were used to identify upregulated biological and disease pathways of first trimester BMI and gestational age by Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, and Disease Ontology gene set enrichment.[314, 315, 322] We similarly tested if the inflammatory gene sets

for TLR2/4, leptin, and trophoblast development were upregulated by targeted Gene set enrichment analysis as previously described.

Lastly, we compared estimated cell proportions across tissue types and by IL1RL1 expression in placental villous tissue and chorionic membrane tissue by cell deconvolution as previously described. Significant differences in cell proportions by tissue type were determined by Wilcoxon signed rank test using a nominal p-value of <0.05. The distribution of cell proportions across IL1RL1 expression was graphically visualized by scatter plots using *ggplot2* in R Studio and associations between IL1RL1 expression and cell proportions were quantified by linear regression models.

2.3.4 Results

2.3.4.1 Low-Dimensional Analyses

There were 183 pregnancies in this cohort; 4% of women were underweight, 55% were lean, 26% were overweight, and 15% were obese. Pregnancy characteristics are compared by BMI class in **Table 10**. Women across BMI classes had comparable maternal characteristics including for maternal age, smoking status and parity. There were statistical differences in pregnancy characteristics across BMI classes for gestational hypertension, gestational age, preterm birth, and birthweight.

A 1kg/m^2 increase in first trimester BMI was significantly associated with 0.09 weeks (~1 day) longer gestational age at delivery in multivariate analyses. This association was significantly modified by parity status (p=0.003**, Appendix Figure 10**). Adjusting for maternal age, smoking status, and neonatal ethnicity, for every 1kg/m^2 increase in first trimester BMI, there was a 0.18

week increase in gestational age among nulliparous pregnancies, and a 0.02 week increase in multiparous pregnancies.

Table 10: Maternal and pregnancy characteristics[a,b]

	Underweight (N=8)	Lean (N=100)	Overweight (N=48)	Obese (N=27)	p-value
Maternal Age, years	27.00 (4.000)	30.00 (5.000)	29.50 (6.000)	29.00 (6.000)	0.10
Smoking Status					0.26
Never Smoker	6 (75.0%)	70 (70.0%)	30 (62.5%)	17 (63.0%)	
Quit Before Pregnancy	0 (0%)	25 (25.0%)	13 (27.1%)	8 (29.6%)	
Smoke During Pregnancy	2 (25.0%)	5 (5.0%)	5 (10.4%)	2 (7.4%)	
Nulliparous					0.15
	6 (75.0%)	53 (53.0%)	19 (39.6%)	11 (40.7%)	
1st Trimester BMI, kg/m^2	18.00 (0.58)	21.95 (3.13)	27.05 (2.63)	34.00 (4.45)	<0.001
Gestational Diabetes	0 (0%)	3 (3.0%)	1 (2.1%)	2 (7.4%)	0.57
Gestational HTN					
	0 (0%)	1 (1.0%)	6 (12.5%)	1 (3.7%)	0.015
Gestational Weight Gain, kg	11.50 (8.25)	13.00 (7.58)	14.60 (7.15)	11.00 (9.20)	0.20
Neonatal Ethnicity					0.12
European	8 (100%)	90 (90.0%)	37 (77.1%)	22 (81.5%)	
Non-European	0 (0%)	10 (10.0%)	11 (22.9%)	5 (18.5%)	
Fetal Sex					0.13
Female	3 (37.5%)	41 (41.0%)	28 (58.3%)	16 (59.3%)	
Male	5 (62.5%)	59 (59.0%)	20 (41.7%)	11 (40.7%)	
Gestational Age, weeks	36.50 (4.500)	39.00 (2.000)	40.00 (1.000)	40.00 (1.500)	0.002
Preterm Birth (<37wks)					0.01
	4 (50.0%)	15 (15.0%)	4 (8.3%)	1 (3.7%)	
Birthweight, grams	2615 (612.5)	3353 (756.3)	3370 (633.8)	3640 (430.0)	0.002

[a]Continuous variables are represented as median (IQR).
[b]Significance of continuous variables across BMI classes was compared by Kruskal-Wallis tests and categorical variables by chi-squared and fisher exact tests.

2.3.4.2 Differential Gene Expression Patterns

We determined if any placental gene differed by maternal first trimester BMI or gestational age using linear regression models adjusted for maternal age, parity, smoking status, and neonatal ethnicity. Only 1 gene (ZBTB16) was significantly upregulated with 1kg/m^2 increase in first trimester BMI and no genes were differentially expressed between obese and lean women (**Appendix Figure 11)**. There were 1,377 genes that were differentially expressed for a 1-week increase in gestational age (700 upregulated and 677 downregulated) and 1,514 differentially expressed genes (795 upregulated and 719 downregulated) in preterm compared to term pregnancies (**Appendix Figures 12 and 13)**. Only the IL1RL1 gene was differentially expressed by preterm birth status by more than a 2-fold change **(Appendix Figure 12**). All other significant genes had expression changes less than 2-fold. The top 20 genes ranked by FDR-adjusted p-value for preterm birth and gestational age are listed in **Table 11**. No genes were differentially expressed for interactions between BMI and preterm birth status with nulliparity, fetal sex, or neonatal ethnicity.

Table 11: Top 20 differentially expressed genes by preterm birth and gestational age.[a,b]

Preterm v Term Birth			1-Week Increase Gestational Age		
Gene	**Log2 Fold Change**	**P value**	**Gene**	**Log2 Fold Change**	**P value**
BRP44	0.38	0.0001095887	HBB	0.11	1.520405e-07
ANKRD9	-0.42	0.0001095887	SLC22A	-0.13	1.831658e-06
SMAGP	0.45	0.0001330503	**ANKRD**	0.07	3.140365e-06
NR2C2	-0.32	0.0001330503	IL1RL1	0.34	3.140365e-06
ILDR2	-0.60	0.0001490998	CA1	0.24	1.358006e-05
XLOC_002956	-0.69	0.0001505766	HBD	0.16	2.049823e-05
NEAT1	-0.81	0.0001505766	BTNL9	0.25	2.381025e-05
DAPK1	-0.57	0.0002376953	PSMG1	-0.07	4.719310e-05
LOC441455	-0.27	0.0002376953	**BRP44**	-0.06	5.476916e-05
SLC27A2	0.66	0.0002376953	**SMAGP**	-0.07	6.012763e-05
MKRN1	-0.26	0.0002376953	**NEAT1**	0.13	1.133633e-04
SSPN	0.45	0.0002440247	NXPH4	-0.12	1.604950e-04
SBF1P1	-0.37	0.0002542687	GINS4	-0.07	1.944769e-04
HPDL	0.70	0.0002571705	**SSPN**	-0.07	2.049181e-04
SLC22A18	0.65	0.0002839508	**ILDR2**	0.13	2.416583e-04
CDC42BPG	-0.39	0.0002847179	ADAMT	-0.12	2.416583e-04
COL11A2	-0.62	0.0002949491	ATP5G1	-0.07	3.350643e-04
EFEMP1	0.44	0.0003270521	**LOC441455**	-0.07	3.757910e-04
CKAP2	0.46	0.0003270521	CLEC18B	0.09	3.842292e-04
KAT8	-0.27	0.0003484174	**NR2C2**	-0.08	3.842292e-04

[a]Genes ranked from lowest to highest FDR adjusted p-value.
[b]Bolded genes were among the top 20 differentially expressed genes for both preterm birth and gestational age.

Based on the subset of differentially expressed genes by preterm birth status, there were 144 pathways that were significantly enriched in the KEGG database (FDR <0.05) and 69 biological pathways in the GO database (FDR <0.05). Of the 25 most significant KEGG enriched pathways, 11 pathways of neurodegenerative disorders, oxidative processes, diabetic cardiomyopathy and non-alcoholic fatty liver disease had gene sets that clustered together (**Appendix Figure 14).** Other enriched pathways included pathways related to cancer (3 pathways), infection (5 pathways), and cellular processes (6 pathways). There was considerable heterogeneity in the 25 enriched biological pathways in the GO database (**Appendix Table 11)**. These included pathways related to protein catabolism, neutrophil degranulation, ribosomal metabolism, cellular respiration, and cell signaling.

2.3.4.3 Targeted Gene Enrichment Analysis

In addition to the agnostic approach of identifying enriched gene sets of only differentially expressed genes in the previous section, we tested if 12 hypothesized genes related to TLR2/4 inflammation, leptin signaling, and trophoblast development were enriched by gene set enrichment analyses. Only 3 pathways were positively enriched in obese compared to lean women at a nominal p-value <0.05 (**Table 12**). Two pathways were related to TLR-mediated inflammation and one pathway related to leptin/adipokine signaling. No pathways were enriched for preterm pregnancies vs term pregnancies.

Table 12: Gene set enrichment in obese vs lean women for pre-selected gene sets[a]

ID	Pathway	Enriched Genes	Nominal p-value	FDR q-value
M3261	Toll Like Receptor Signaling	22/68	0.026	0.098
M10462	Adipocytokine Signaling	11/47	0.040	0.083
M5932	Hallmark Inflammation	55/136	0.044	0.074

[a]ID refers to the Molecular Signature Database IDs.

2.3.4.4 High-Dimensional Mediation Analyses

We identified two placental gene mediators of first trimester BMI and gestational age by high dimensional mediation analysis. In unadjusted analyses, expression of IL1RL1 mediated 15% (p=0.047) of the total effect of the positive association between a 1-unit increase in BMI and gestational age (**Table 13**). This association was not statistically significant (p=0.057) after adjusting for parity, maternal age, smoking, and neonatal ethnicity.

GALNT15 significantly mediated 28.2% of the total effect of pre-pregnancy BMI on log-odds of preterm birth($p<0.001$) in unadjusted analyses. GALNT15 also significantly mediated the association after adjusting for covariate, but the overall association between BMI and log odds of preterm was not significant. In stratified analyses by fetal sex, PTX3 was a significant mediator of BMI and odds of preterm birth among women with a female fetus. RBP3, TIMP4, SMIM3, were all significant placental gene mediators of BMI and odds of preterm birth when restricting to women with neonates of European descent.

Table 13: Placental gene mediators of maternal BMI and gestational age [a,b,c,d]

	Univariate Analysis 1st Trimester BMI and Gestational Age				
Gene	**Total Effect of BMI in weeks (SE)**	$\hat{\alpha}$	$\hat{\beta}$	**Proportion Mediated (%)**	**FDR P-value**
IL1RL1	0.10 (0.03)**	0.06	0.25	15.1	**0.047**
	Multivariate Analysis 1st Trimester BMI and Gestational Age				
Gene	**Total Effect of BMI in weeks (SE)**	$\hat{\alpha}$	$\hat{\beta}$	**Proportion Mediated (%)**	**FDR P-value**
IL1RL1	0.09 (0.03)**	0.06	0.29	18.3	0.057
	Univariate Analysis 1st Trimester BMI and Log odds of Preterm Birth				
Gene	**BMI Total Effect in log odds (SE)**	$\hat{\alpha}$	$\hat{\beta}$	**Proportion Mediated (%)**	**FDR P-value**
GALNT15	-0.11 (0.06)*	0.04	-0.79	28.2	0.004
	Multivariate Analysis 1st Trimester BMI and Log Odds of Preterm Birth				
Gene	**BMI Total Effect in log odds (SE)**	$\hat{\alpha}$	$\hat{\beta}$	**Proportion Mediated (%)**	**FDR P-value**
GALNT15	-0.09 (0.06)	0.04	-1.23	51.8	0.0006

[a] $\hat{\alpha}$ is the effect of BMI on gene expression.
[b] $\hat{\beta}$ is the effect of gene expression on gestational age.
[c] Exposure-mediator and mediator-outcome models were adjusted for preterm birth**.**
[d] Stars indicate significance of total effect of BMI on gestational age or preterm birth. * denotes p<0.05, ** p<0.01, and **p<0.001.

2.3.4.5 Cell Deconvolution

Using the *Suryawanshi et al.* single-cell reference dataset, we found that placental samples in the ENVIRONAGE cohort were on average comprised of villous cytotrophoblasts (60%), followed by vascular endothelial cells (21%), extravillous trophoblasts (10%), and fibroblasts (7%; **Appendix Figure 15)**. There were no significant differences in cell types by BMI status (**Figure 15**). Villous cytotrophoblasts were significantly lower in preterm births compared to term births (9.3% vs 10.4% p=0.03). No other cell types differed by preterm birth status and no cell types significantly mediated the associations between BMI and gestational age either as continuous variables or using categorical variables.

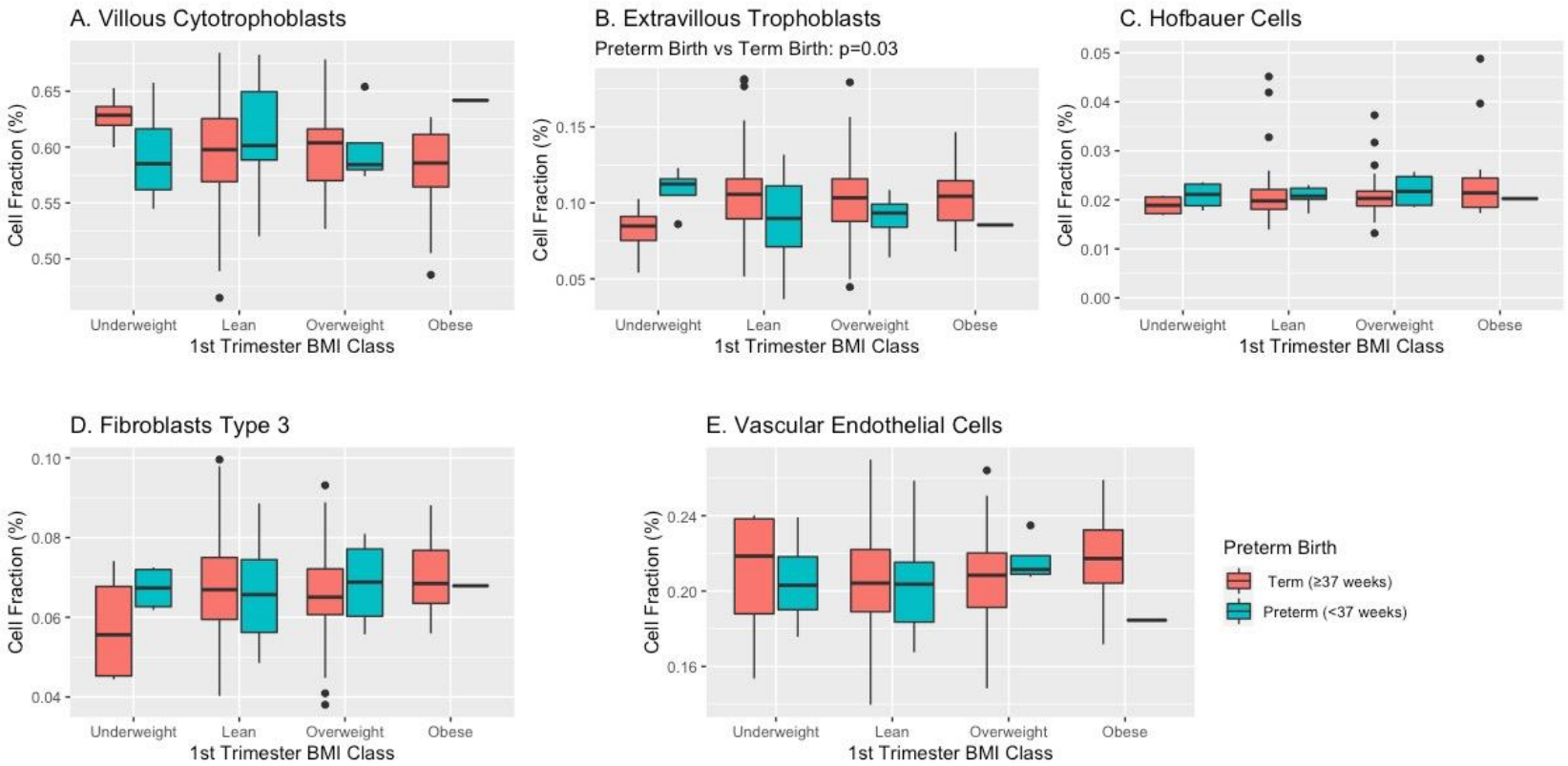


Figure 15: Estimated cell types by BMI class and preterm birth status.

Differences in placental celll types by pre-pregnancy BMI class and preterm birth status were conducted by two-way ANOVAs. Linear mixed regression with an interaction between first trimester BMI and preterm birth status was used to test the significance for the interaction between BMI and preterm birth given the limited number of preterm births among obese women (n=1).

2.3.4.6 Exploratory Pathway Analyses of IL1RL1

Details on the 10 uncomplicated term pregnancies from the TIDES cohort are provided in **Appendix Table 12.** Gene expression of IL1RL1 did not vary between placental villous and chorionic membrane tissue (p=0.63; **Appendix Figure 16A**). With increased expression of IL1RL1, there were 852 genes (278 upregulated and 574 downregulated) differentially expressed in placental villous tissue and 105 genes (66 upregulated and 39 downregulated) differentially expressed in chorionic membranes. NOP2/Sun RNA Methyltransferase Family Member 7 was the only shared upregulated gene (**Appendix Figure 16B)** and epiphycan was the only shared downregulated gene between placental villous tissue and chorionic membranes (**Appendix Figures 16B and 16C)**.

Gene enrichment analyses of genes significantly co-expressed with IL1RL1 revealed that increased expression of IL1RL1 was associated with 44 GO biological pathways and 2 Disease Ontology gene sets in placental villous tissue. Of the 44 GO pathways, 4 were significantly enriched with FDR adjusted p-value <0.001 and gene sets for all four pathways had decreased expression (**Figure 16A)**. The 4 biological pathways included cell signaling, multi-multicellular organism processes, extracellular matrix organization, and neutrophil degranulation and the 2 down-regulated disease gene sets were for Alzheimer's Disease and preeclampsia. Given the relevance of neutrophil degranulation and preeclampsia to preterm birth the expression of the genes in these pathways is provided in **Figures 16B and 16C**. In chorionic membrane tissue, there were 5 enriched GO biological pathways (**Figure 17A)** and 1 Disease Ontology gene set associated with IL1RL1 expression (**Figure 17B)**. The GO pathways included leukocyte mediated cytotoxicity, positive regulation of cell killing, retinoid metabolic process, multi-multicellular organism processing, and protein processing. Though these pathways were enriched, they were

not clearly upregulated or downregulated. The Disease Ontology pathway was for preeclampsia and most genes (8/10 genes) were increased in expression.

Cell deconvolution did not reveal any differences in cell types with increased IL1RL1 expression in either placental villous tissue or chorionic membranes. However, there were significant differences between villous tissue and chorionic membranes for median cell fractions of synctiotrophoblasts (21.2% vs 0.0%, p=0.009), extravillous cytotrophoblasts (17.0% vs 49.4%, p=0.002), Hofbauer cells (0.6% vs 4.9%, p=0.002), fibroblasts subtype 2 (5.0% vs 1.4%, p=0.006), fibroblasts subtype 3 (22.8% vs 30.1%, p=0.027), and vascular endothelial cells (14.0% vs 4.0%, p=0.002).

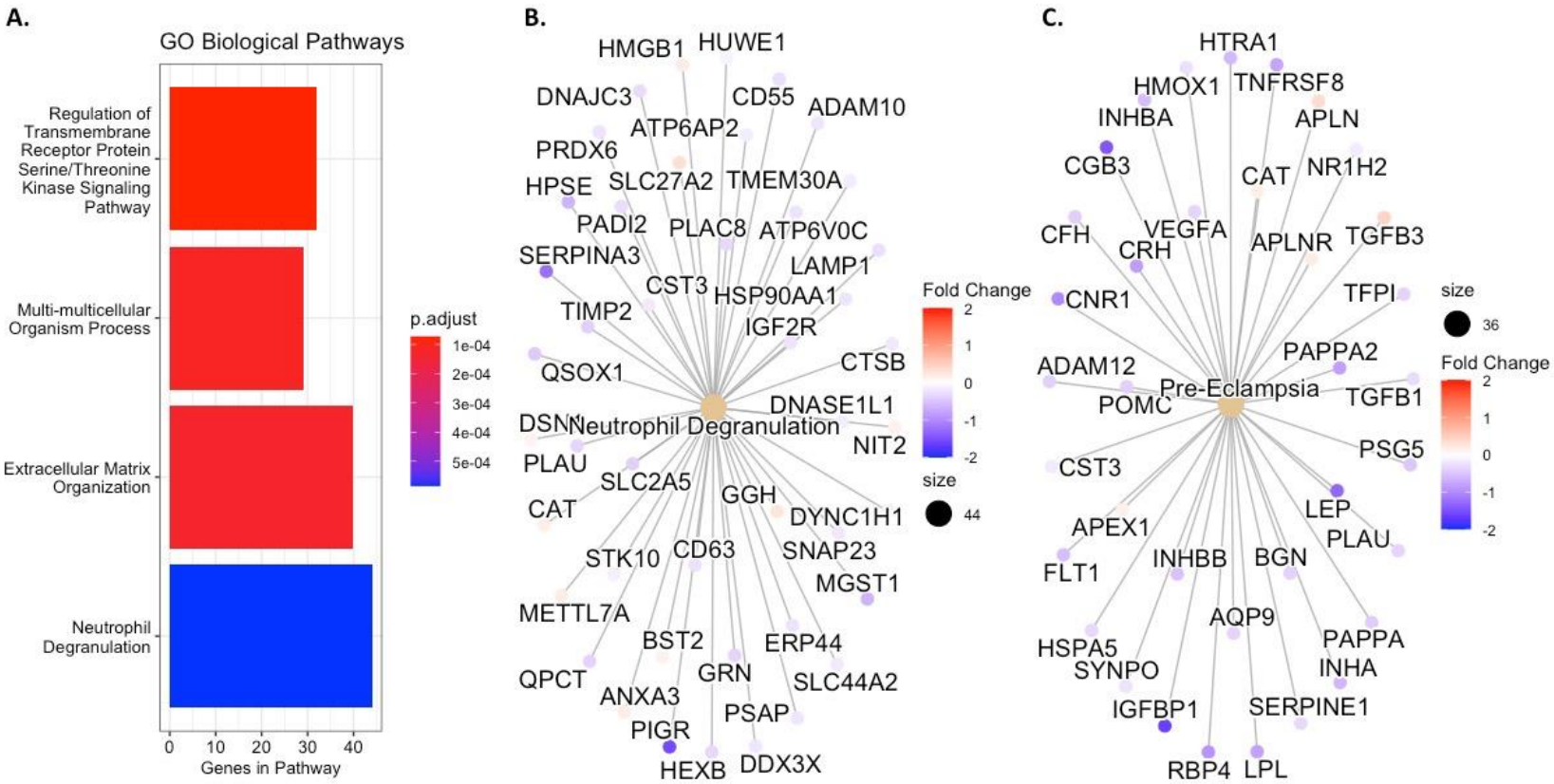


Figure 16: Gene enriched pathways co-expressed with IL1RL1 in placental villous tissue.

A) 4 enriched Gene Ontology pathways associated with IL1RL1 expresssion. B) Differentially expressed genes in the Neutrophil Degranulation Gene Ontology pathway. C) Differentially expressed genes in the preeclampsia Disease Ontology pathway. The size of the tan nodes indicate the number genes within a pathway. The shading of the smaller nodes indicate the fold change in gene expression with red reprsenting increased gene expression and blue representing decresed gene expression.

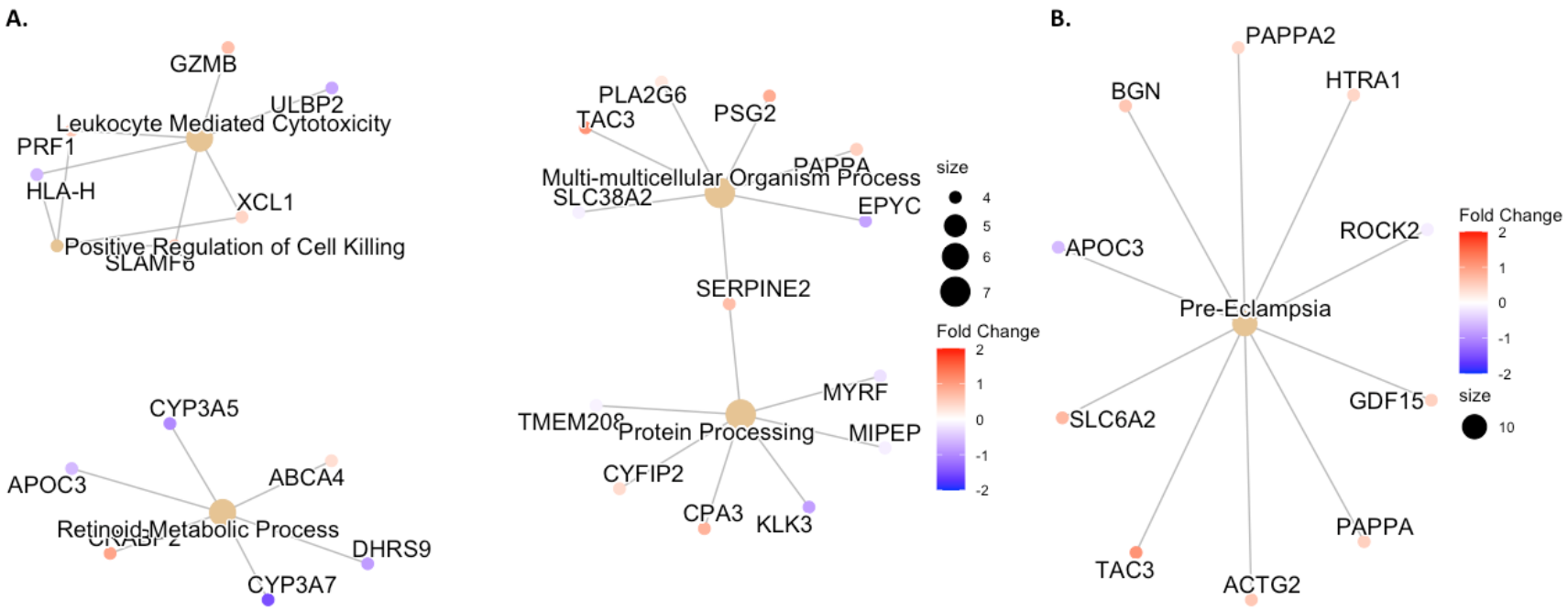


Figure 17: Gene enriched pathways co-expressed with IL1RL1 in chorionic membrane tissue.

A) Differentially expressed genes in the 5 enriched Gene Ontology pathways associated with IL1RL1 expresssion. B) Differentially expressed genes in the 1 enriched Disease Ontology pathway. The size of the tan nodes indicate the number genes within a pathway. The shading of the smaller nodes indicate the fold change in gene expression with red reprsenting increased gene expression and blue representing decresed gene expression.

2.3.5 Discussion

Contrary to the hypothesized pro-inflammatory effect of obesity on higher risk of early labor, an inflammation-regulating gene, IL1RL1, partially mediated the association between first-trimester BMI and longer gestational age. Exploratory analyses in a second cohort indicate that increased IL1RL1 expression in placental villous tissue is associated with decreased expression of genes related to neutrophil degranulation and preeclampsia. Cell deconvolution did not reveal any placental cell types to be significant mediators of the association between maternal BMI and gestational age.

2.3.5.1 Results in the Context of What is Known

We found that increased expression of the inflammation regulating gene, IL1RL1, to be a mediator of the association between BMI and longer gestational age. IL1RL1 encodes a transmembrane protein receptor for the inflammatory cytokine IL-33 and soluble decoy receptor for IL-33.[323, 324] The two proteins are the result of alternative splicing and can only be distinguished when measured as proteins. IL-33 is highly expressed in placental endothelial cells and chorioamniotic membranes.[323] Increased IL1RL1 expression has been shown in term pregnancies without chorioamnionitis compared to preterm pregnancies complicated by either clinical or histologic chorioamnionitis.[324] Histologic chorioamnionitis is defined by the presence of neutrophils in the placental chorion and amnion.[177] This aligns with our finding that increased IL1RL1 expression is associated with decreased expression of genes related to neutrophil degranulation in placental tissue. Prior placental transcriptomic analyses have also identified differential IL1RL1 expression associated with spontaneous preterm labor.[212, 214] Like our study, ILRL1 was found to cluster with lower expression of genes associated with extracellular matrix

remodeling in villous tissue and retinoid metabolic processes in chorionic membranes.[212, 214] Low maternal plasma retinoid metabolites has recently been proposed as a predictive biomarker for preterm birth[325] and extracellular membrane remodeling occurs with chorioamnionitis membrane rupture.[139, 326] We also found increased expression of IL1RL1 to be associated with decreased placental villous expression of genes in preeclampsia. SNPs in the IL1RL1 gene are associated with preeclampsia,[327] but further analysis of placental gene and protein expression of IL1RL1 is needed. Thus, IL1RL1 is a promising gene target for future studies to identify a molecular signature of preterm births. This gene is possibly involved in multiple molecular pathways in spontaneous preterm births and preterm births due to preeclampsia.

High-dimensional analysis also identified GALNT15, a transferase protein of oligosaccharides, to be a potential gene mediator of obesity and preterm birth. GALNT15 has not previously been shown to be associated with preterm birth, but was found to have increased expression in complete hydatidiform moles compared to normal first trimester placental tissue.[328] Though GALNT15 may be a potential mediator of obesity and lower risk of preterm birth, this finding should be interpreted with caution as the overall association between obesity and preterm birth was not significant in multivariate analyses. Other potential mediators in stratified analyses by fetal sex and neonatal ethnicity included PTX3, TIMP4, RBP1 and SMIM3. PTX3 is a pattern recognition molecule of innate immune response to infection[329] associated with preeclampsia[330] and spontaneous preterm labor.[210, 331] TIMP4 is an inhibitor of metalloproteinases and RBP1 is a retinol binding protein which may also indicate an important role of molecular pathways related to extracellular matrix remodeling and retinoid metabolism. SMIM3 is not well studied in the context of obesity and preterm birth. All five of the genes require further validation in future studies.

Similar to the original analysis of the ENVIRONAGE cohort,[204] we did not find placental genes to be differentially expressed between obese and lean women using linear regression models. Analyzing differential gene expression for each gene individually may miss modest biological effects and pathways where sets of genes act together.[199] Targeted gene set enrichment analyses did show nominal significance of genes sets related to TLR-mediated inflammation, but this was not a statistically significant finding after adjusting for multiple hypothesis testing. The lack of a strong association between obesity and overexpression of inflammatory genes in the placenta may reflect the birth cohort studied. This cohort enrolled pregnant women without planned c-section at the time of delivery and only two preterm births were at 31 weeks of gestation. The positive association between BMI and gestational age may reflect the observed protective association between obesity and late spontaneous preterm births.[117] It is possible that a cohort including obese women with early preterm births (<32 weeks) and more indicated preterm births may have revealed other inflammatory genes and pathways associated with obesity. Alternatively, pre-pregnancy exposures such as maternal obesity may not correlate well with transcriptomic analyses of the placenta at delivery. Molecular effects of pre-pregnancy BMI may be dynamic across pregnancy or unstable over time, explaining away associations with the placental transcriptome.

In this cohort, there were no significant differences in placental cell types by BMI status, and there was only a modest lower cell proportion of extravillous trophoblasts in preterm births compared to term births. The similar cell proportions across BMI classes are not surprising given the lack of differentially expressed genes by BMI status. To our knowledge only one prior study has compared placental cell types between preterm pregnancies and term pregnancies.[225] The proportion of extravillous trophoblasts appeared to differ between pregnancies with preterm labor and term labor, but the authors did not statistically test for differences. In another study comparing

placental cell types by preeclampsia status, women with preeclampsia had almost a 2-fold higher proportion of extravillous trophoblasts than women without preeclampsia.[229] The authors suggest that increased extravillous trophoblasts in preeclampsia may reflect an arrest in trophoblast differentiation. The implications of changes in cell fractions warrant further evaluation, but even modest changes in cell proportions of extravillous trophoblasts may drive differences in gene expression in preterm birth.

2.3.5.2 Strengths and Limitations

The ENVIRONAGE birth cohort is one of the largest population-based cohorts with placental microarray data. This allowed for us to adjust for potential confounders and test for effect modification in this analysis. The original analysis was designed to study the effects of maternal obesity on low birth weight, an outcome related to gestational age. First trimester BMI was measured rather than based on self-reported data. We applied both hypothesis-driven gene set enrichment analyses of inflammatory pathways in combination with agnostic approaches. We also utilized recently developed high-dimensional mediation analyses and cell deconvolution approaches to directly measure placental mediators of obesity and preterm birth.

Limitations in this study included a lack of data on variables that could modify placental gene expression including labor induction, labor duration, and PPROM status. Transcriptomic analyses were conducted by microarray analyses instead of by RNA-Seq. As the single-cell reference dataset was based on RNA-Seq data, some gene markers of placental cell types may not have been measured in the ENVIRONAGE cohort, decreasing the efficiency of cell deconvolution. The approximate distribution of placental cell types estimated in the ENVIRONAGE cohort aligns with placental cell proportions measured in a prior study.[332] Further, there are few markers of syncytiotrophoblasts and erythroblasts available from the reference dataset. Future work pooling

multiple single cell reference datasets will allow for more accurate estimation of placental cell types. Despite, the ENVIRONAGE cohort being one of the largest pregnancy cohorts with placental transcriptomic data, only 1 obese woman had a preterm delivery. Pooling placental datasets that include more severe preterm births (i.e., <32 weeks' gestation) and medically-indicated preterm births may identify placental gene mediators in a subset of obese women with a higher risk of preterm birth.

2.3.5.3 Conclusions

Using high-dimensional mediation analyses we found inflammation regulatory gene, IL1RL1, to partially mediate the association between higher maternal BMI and longer gestational age at delivery. IL1RL1 expression was associated with lower expression of acute inflammation pathways and genes related to preeclampsia in placental villous tissue, perhaps indicating IL1RL1 to be a key regulator of a subset of spontaneous preterm births and preterm births due to preeclampsia. By directly measuring genes of the placental transcriptome as mediators of first trimester BMI and gestational age, we provide evidence to support identifying upstream regulators of IL1RL1 for preterm birth prevention. Future studies should investigate other factors associated with obesity including diet, exercise, plasma lipid profiles as potential regulators of IL1RL1 for preterm interventions. Our findings may also help understand the pathophysiology of chorioamnionitis a key risk factor of preterm birth and neonatal sequalae. High-dimensional mediation analyses is an effective tool to leverage the high resolution of transcriptomic analyses for population research on maternal risk factors of preterm birth.

3.0 Conclusions

We originally hypothesized that obesity may predispose women to preterm birth through placental inflammation irrespective of length of gestation or clinical manifestation (spontaneous or medically-indicated). Contrary to our hypothesis, we found that maternal obesity may be modestly protective against acute placental inflammation in late preterm births (32-36weeks) and in healthy term pregnancies. Instead, maternal obesity was associated with increased placental vascular impairment in preterm births <32 weeks. By leveraging histopathology as a tissue-level biomarker of the placenta, applying novel empirical methods of preterm classification, and using high-dimensional mediation analyses of placental transcriptomic data, our findings offer a more comprehensive characterization of placental dysfunction associated with obesity during pregnancy.

In **manuscript 1**, we addressed the major limitation of selection bias in histopathology studies to estimate an association between pre-pregnancy obesity and histologic placental inflammation in term pregnancies. We found that obesity increased the risk of chronic placental inflammation but had a protective effect against acute inflammation. The protective effect of obesity on acute inflammation was contradictory to previous studies. We suspected that differences across studies on obesity and acute inflammation was a consequence of restricting analyses to term pregnancies where there is an indication for placenta pathology evaluation. By using causal diagrams, analytic methods, and sensitivity analyses we conducted a critical evaluation of how the nature of inclusion into a study using histopathology data can contribute to variability in the association between obesity and acute inflammation in both magnitude and direction. Thoughtful

understanding of potential sources of bias will improve reproducibility in placental histopathology research.

Manuscript 2 extended work in manuscript 1 to assess the effects of maternal pre-pregnancy BMI more broadly on placental dysfunction in preterm births. Rather than considering individual placental lesions of placental inflammation like traditional approaches, combinations of placental features were agnostically clustered to classify preterm births into placental pathology phenotypes. This approach addressed concerns of classifying incidental placental findings as pathological by considering all placental features and without presupposing which placental features group together. In doing so, we demonstrated that pre-pregnancy BMI increases vascular impairment in early preterm births, and we also provided a novel classification of preterm births potentially more prognostic of neonatal outcomes than existing preterm birth classifications.

To complement the histopathological findings in the first two papers, we analyzed publicly available placental transcriptomic data from a birth cohort in **manuscript 3**. Existing transcriptomic studies of placentas have characterized differentially expressed genes and molecular pathways by preterm birth status and obesity status separately. Without measuring both obesity and preterm birth together, we cannot make direct inferences about the placental pathophysiology of obesity and risk of preterm birth. Therefore, we extended these studies by directly measuring placental genes as mediators of the association between BMI and gestational age. We found that the inflammation regulating gene, IL1RL1, mediated 15% of the association between BMI and longer gestational age. We further identified that increased expression of IL1RL1 in a second birth cohort was associated with downregulation genes for neutrophil activity, extracellular membrane remodeling and preeclampsia in placental villous tissue. Despite a protective effect of obesity for preterm birth in this cohort, we identified a placental gene and

related molecular pathways that are associated with obesity and may be intervention targets for a subset of preterm births.

3.1 Future Direction

3.1.1 Minimizing Biases of Histopathology

Findings from manuscript 1 indicated that residual selection bias may have explained the protective association between obesity and risk of acute placental inflammation. We suspect that the inverse probability weighting and multiple imputation models applied to reduce selection bias may not have sufficiently minimized bias from selection into the study. For inverse probability weighting we estimated weights by only one model for missing placental histopathology data. It is possible that there are multiple missing data patterns for placental histopathology, maternal pre-pregnancy BMI, and other maternal characteristics. Efforts to further minimize selection bias may therefore be needed. Future studies should try to model multiple missing data patterns by estimating several weights by recently proposed modularized IPW to better account for missing data.[174]

IPW methods that account for different missing data patterns may also allow for future studies to combine histopathology data in term and preterm pregnancies. A major limitation for investigating placental dysfunction as a mediator of obesity and risk of preterm birth has been the inability to study preterm births and term births together due to differences in indications for placental histopathology. Preterm birth is an indication for placental histopathology evaluation for pregnancies included in the MOMI cohort,[174] while term pregnancies only have histopathology

evaluations for pregnancies with complications. Thus 93% of preterm births have histopathology reports compared to 37% of term births. Modularized IPW could adjust for different missing data patterns in preterm and term births to study the effects of obesity on placental inflammation or other lesions in preterm and term births. This may allow for term pregnancies to act as a control for preterm births in histopathology studies if analytic models for selection bias are correctly specified.

We also applied sensitivity analyses using bias factors to estimate how robust histopathology findings were to residual selection bias. Bias factor analyses relied on observed pregnancy variables to provide a crude approximation of whether an unobserved variable of equivalent strength would explain away associations between obesity and risk of acute or chronic inflammation.[253] This approach may not be reasonable if observed variables are not good approximations of selection bias for unobserved factors. Deterministic quantitative sensitivity analyses proposed by *Lash et al* could be implemented to provide adjusted estimates for associations between obesity and placental outcomes under different assumptions of how strong and prevalent an unmeasured variable contributes to selection bias.[333] Another possibility is to quantify selection bias between obesity and a surrogate outcome, called a negative outcome.[334] The negative outcome would be hypothesized to have the same form of selection bias as the placental histopathological outcome, but would be measurable in both those with and without histopathology data. The degree of variability between estimates for the negative outcome with and without histopathology would provide stronger evidence for the presence or absence of selection bias. Further advances in quantifying and minimizing selection bias will help understand variability across population studies for obesity and histopathology findings.

Another commonly reported limitation of histopathology studies is variability in detection of placental histopathology lesions between institutions and pathologists. Variability in detection is a form of measurement error that may also bias associations between obesity and placental histopathology outcomes. Misclassification of placental lesions as present or absent could bias associations between obesity and a placental lesion towards or away from the null, mask curvilinear associations, and increase statistical variability (i.e. reduce precision) associations.[335] Only a handful of studies have tested interobserver agreement in detection of placental lesions within hospitals.[175, 336] Even with high agreement (80-90%) in detection in one hospital, the same agreement may not extend to other hospitals or institutions with variable diagnostic criteria, pathologist experience, and number of pathology reports conducted. Pathologists at many hospitals including Magee-Womens Hospital, are not blinded to maternal and clinical characteristics on pregnancies. Knowledge of clinical features known to be associated with placental histopathology features (e.g., preeclampsia and decidual vasculopathy; preterm birth and advanced villous maturation) may lead to overreporting of placental histopathology lesions by pathologists resulting in measurement error.

In addition to selection bias, quantitative bias analyses can be used to account for measurement error of placental histopathology lesions.[335] Under different assumptions of false positives (i.e. incorrectly detecting a placental lesion) and false negatives (i.e. failing to detect a placental lesion), associations between obesity and acute and chronic histological inflammation can be adjusted for measurement error.[333] More complex methods of adjusting for measurement error across multiple lesions could also be applied to the study in manuscript 2. This may include simulation studies where a proportion of observations on placental lesions are randomly assigned to being detected or not detected to see how misclassification error may affect clustering of

placental lesions. Alternatively, in studies that cluster placental lesions by presupposed histopathological groups (e.g. lesions of MVM or FVM), latent class analysis can determine how well individual lesions align with the overall histopathological group.[335]

Advancing these methodological approaches to handling bias will allow for greater reproducibility across histopathology studies. Large birth cohorts that link placental histopathology reports with clinical data have great potential to study risk factors and adverse pregnancy conditions possibly mediated by the placenta. However, using data originally collected for clinical use requires thoughtful consideration of potential bias for population research.

3.1.2 Follow-up Transcriptomic Analyses in Preterm Births

We identified placental gene mediators and molecular mechanisms of obesity and gestational age using placental transcriptomic data from a European birth cohort in manuscript 3.[204] Despite this birth cohort having a large sample size for placental transcriptomic analyses, this cohort was limited to primarily European women without planned caesarean sections at delivery. Preterm births were more likely to occur by spontaneous labor than by indications necessitating delivery. Further only 2 preterm births occurred prior to 32 weeks' gestation. Population studies have shown maternal obesity to be associated with a higher risk of early preterm births (<32 weeks)[26, 104, 117] and we observed maternal obesity to be associated with maternal vascular impairment in early, but not late preterm births (32-36 weeks' gestation). Therefore, our analysis in the third manuscript may have missed a subset of preterm births where obesity would have had the strongest adverse effect on placental molecular mechanisms.

Ideally, subsequent transcriptomic analyses should include larger birth cohorts with medically-indicated preterm births and preterm births less than 32 weeks' gestation. Including

preterm births likely associated with obesity will potentially identify other placental genes and molecular pathways perhaps related to vascular placental pathology like we observed in manuscript 2.

Further studies could also merge multiple placental transcriptomic datasets to increase sample size and include a more ethnically diverse population with more early preterm births. Prior studies have merged multiple transcriptomic datasets to improve clustering analyses and prediction of preeclampsia.[337] Pooling datasets can be computationally challenging due to variation in collection of pregnancy characteristics and measurement of the placental transcriptome across different platforms. Pooling studies may also bias associations between obesity and risk of preterm birth, which could impact mediation analyses. Despite these limitations, utilizing multiple transcriptomic datasets may produce findings more generalizable to preterm birth than a single cohort to inform biological targets for preterm birth interventions.

In addition to pooling transcriptomic analyses of bulk placental tissue, pooling publicly available single cell RNA-Seq datasets would allow for more accurate estimation of cell types by cell deconvolution (https://randel.github.io/EnsDeconv/reference/index.html). A limitation of our study in manuscript 3 was estimation of placental cells based on one reference cohort.[226] In the European birth cohort the proportion of placental syncytiotrophoblasts was estimated to be 0% in the cohort. In contrast, the cell fraction of syncytiotrophoblasts of placental villous tissue in the TIDES cohort was estimated to be about ~20%. This may indicate that there were insufficient genes from the microarray data from the European birth cohort relative to RNA-Seq data from TIDES to match on to the reference single cell RNA-Seq data. Additional genes that distinguish syncytiotrophoblasts from other placental cell-types may have improved estimation of synctiotrophoblasts in the European cohort. Other single cell cohorts have also included placental

cell types collected later in pregnancy,[225, 227, 338] distinguish between cell types of maternal and fetal origin,[225, 227] include cells collected from chorionic membranes,[225] and include placental cells from preterm and term pregnancies.[225] Combining reference datasets will provide better estimation of how maternal obesity affects placental cell fractions and whether pathological changes in placental cell fractions are of maternal or fetal origin.

3.1.3 Future Analyses of IL1RL1

In manuscript 3, the placental gene, IL1RL1, was found to be significant placental mediator of obesity and longer gestational age. Higher IL1RL1 expression was also associated with lower placental villous tissue expression of genes in pathways related to neutrophil degranulation and extracellular matrix organization. Prior studies have also shown IL1RL1 inhibits toll-like receptor 2 and 4 mediated inflammation.[323, 339] These findings support a general protective effect of obesity on acute histologic placental inflammation as we observed in term births, but validation of these findings in both villous tissue and chorionic membranes is required.

Future work should measure placental gene expression in combination with placental histopathology. A simple experiment would be to compare placental villous and chorionic membrane gene expression of IL1RL1, its ligand, IL33, and genes from the TLR and neutrophil degranulation pathways by quantitative PCR across obese and lean women with and without histological chorioamnionitis. Increased gene expression of IL1RL1, in obese women compared to lean, women without histologic chorioamnionitis compared to women with histological chorioamnionitis, and an interaction effect between BMI and chorioamnionitis status would provide supporting evidence for the inhibitory role of IL1RL1 for acute inflammation in the placenta. Differential expression of the ligand for IL1RL1, IL33, or downstream targets of IL1RL1

(i.e. TLR2/TLR4, or genes of neutrophil degranulation) would indicate if other genes related to IL1RL1 pathways could explain the protective effect of obesity on acute inflammation.

The IL1RL1 gene encodes both a transmembrane receptor associated with a type-2 helper T cell immune response and a soluble protein that inhibits acute inflammatory pathways.[323, 339] A previous study found that preterm labor with intrauterine infection lowered amniotic fluid concentrations of soluble IL1RL1, but did not measure the transmembrane IL1RL1 protein in placental chorion or amnion tissue.[324] Therefore, to validate the effect of obesity on IL1RL1 expression, we would compare soluble IL1RL1 protein concentrations in amniotic fluid by ELISA and IL1RL1 protein abundance in placental villous and chorionic membrane tissue by western blotting between the four groups: obese women with chorioamnionitis, obese women without chorioamnionitis, lean women with chorioamnionitis, and lean women without chorioamnionitis. We would expect that obese women without histologic chorioamnionitis would have the highest amniotic fluid concentrations of soluble IL1RL1 compared to the other groups, but there would be no significant difference in transmembrane IL1RL1 protein abundance across groups.

Finally, *in-vitro* studies are necessary to test if higher IL1RL1 expression in obese women compared to lean women blunts an inflammatory response in placental tissue. Villous trophoblasts, extravillous trophoblasts, and human umbilical vein endothelial cells could be isolated and cultured from placentas of obese and lean women. Cells could then be cultured in media with 4 different inflammatory conditions: 1) the ligand of IL1RL1, IL-33, 2) IL-33 and LPS, 3) LPS alone, and 4) IL-1B. The inflammatory response could be measured by inflammatory cytokines IL-6 and IL-8 cell culture concentrations and by measurement of the pro-inflammatory transcription factor, nuclear factor kappa B, in its phosphorylated form (active form) in placental cells. If placentas from obese women compared to lean women produce more soluble IL1RL1,

placental cells from obese women treated with IL-33 should have a blunted inflammatory response compared to lean women. The contrast in an inflammatory response should be even greater between obese and lean women for the combined treatment of IL-33 and LPS, as IL-33 upregulates the LPS receptors, TLR2 and TLR4. In contrast, the inflammatory response from LPS alone and the non-specific pro-inflammatory cytokine, IL-1B should not differ between obese and lean women. Testing across different placental cell types may also help localize where obesity affects the IL1RL1 pathway.

Even if increased IL1RL1 expression in obese women is protective against a subset of preterm births, validating IL1RL1 activity, by qPCR, protein concentrations, and in-vitro assays could inform a potential target for spontaneous preterm birth prevention. If validation studies support an association between obesity and increased gene expression of IL1RL1 and IL1RL1 is found to decrease placental inflammation, the next step would be to look upstream of IL1RL1 activity. Determining if excess adiposity or other factors associated with obesity such as lipid concentrations, endocrine signaling, diet, or exercise modify IL1RL1 expression would provide a more targeted approach to increasing IL1RL1 expression. Studies could also focus on pharmacological agents that increase IL1RL1 activity or similarly block IL-33 activity. Pre-clinical trials are underway for monoclonal antibodies and soluble decoy receptors for IL-33 to mimic IL1RL1 activity in the context of asthma, atopic dermatitis, cardiovascular disease, acute kidney injury, and colitis.[340] Similar drug antagonists of IL-33 could be tested in animal models of preterm birth and clinical chorioamnionitis.

3.1.4 Evaluation of other markers of metabolic syndrome

A major limitation of obesity and preterm birth research is that it is unclear what underlying metabolic changes predispose obese women to preterm birth. BMI is a non-invasive clinical measure that helps predict women at higher risk of adverse pregnancy outcomes, but it is not clear what clinically should be intervened upon. Metabolic syndrome defined by high central adiposity (waist circumference >80cm), high triglycerides (≥150mg/dL), low HDL, high fasting glucose (≥100mg/dL), low HDL (≤50mg/dL), and high blood pressure (≥130/≥85mmHg) may be more informative of the metabolic factors associated with increased risk of preterm birth.[341, 342]

A cohort of pregnant women that measured both BMI and different components of metabolic syndrome in early pregnancy (<15 weeks) found that metabolic syndrome was associated with almost a 3-fold higher risk of preterm birth compared to women without metabolic syndrome.[342] High triglycerides, low HDL, and high blood pressure were the primary factors of metabolic syndrome that were associated with preterm birth. The same study found no association between obesity and risk of preterm birth or insulin resistance and risk of preterm birth. Another study of spontaneous preterm birth found no association between metabolic syndrome and risk of preterm birth. Further, the association between pre-pregnancy BMI and risk of spontaneous preterm birth was not significantly modified by metabolic syndrome (BMI x metabolic syndrome interaction p-value=0.50).[343] The only metabolic factor associated with higher risk of spontaneous preterm birth was triglycerides ≥150mg/dL compared to triglyceride <150mg/dL (RR: 1.35, 95% CI: 1.02-1.77). Metabolic syndrome may have similar relationships between pre-pregnancy BMI and risk of preterm birth that vary by gestation and clinical presentation. However, individual metabolic factors such as high triglycerides, may increase risk of preterm birth irrespective of

preterm classification. Additional population studies are needed to understand the relationship between metabolic factors (triglycerides, insulin resistance, cholesterol) and risk of preterm birth.

A future direction of this dissertation would be to analyze metabolic syndrome and individual components of metabolic syndrome with risk of placental histopathology features in term and preterm births. This work would be an extension of our assessment of obesity on risk of placental dysfunction in manuscripts 1 and 2. A similar protective effect of metabolic syndrome on acute histologic inflammation in term births would provide stronger biological evidence that obesity decreases acute placental inflammation. Likewise, demonstrating metabolic syndrome to be associated with a higher risk of maternal vascular pathology in early preterm birth but not late preterm birth, would also provide stronger evidence for obesity to be a risk factor of early but not late preterm birth. Discordant results would also be informative, as it would indicate that obesity is a heterogeneous phenotype that may predispose women to preterm birth differently depending on the associated metabolic changes. Discordant results may also provide biological evidence for specific metabolic risk factors like high triglycerides that are associated with preterm birth. Pinpointing the metabolic risk factor that predispose women to preterm birth may inform preterm birth interventions that are more feasible and safer than weight loss or weight gain restriction during pregnancy.

3.1.5 Addressing Race Disparities in Obesity and Risk of Preterm Birth

Recent population studies from the US indicate that self-reported race may modify the association between obesity and risk of preterm birth.[107, 112, 122, 123] Despite Black women having a higher prevalence of obesity and a higher risk of preterm birth than white women, four studies have shown that the association between obesity and risk of preterm birth is attenuated or even

reversed in Black women compared to white women.[107, 112, 122, 123] In *Liu et al 2019*, the authors demonstrated that the protective effect of obesity on risk of preterm birth in Black women was only in observed in women <25 year of age, and that obesity had a comparable higher risk of preterm birth in Black and white women ≥30 years of age.[107] The authors did not provide an explanation for this trend with age. *De Jongh et al 2014*, suggested the racial differences in the effect of obesity on risk of preterm birth may relate to differences in total fat mass and central fat mass, but this was not tested.[112]

Identifying risk factors of preterm birth in Black women is necessary to reducing race disparities in maternal and child health. Clarifying Black-white differences for obesity and risk of preterm birth will inform if public health interventions should prioritize obesity for preterm birth prevention. It is possible that culturally patterned ideas about weight or cultural variations in diet may explain the attenuated effect of obesity on risk of preterm birth in Black women.[128, 344] Understanding racial differences in obesity and risk of preterm birth may also lead to new hypotheses about why Black women have a higher risk of preterm birth.

In manuscripts 1 and 2 we tested to see if the association between obesity and placental dysfunction measured by placental histopathology was modified by maternal self-reported race (Black vs not Black) by interaction. Maternal race did not significantly modify associations between obesity and placental histopathological outcomes in either study, though we may not have been powered to appreciate race-BMI interactions in preterm births. In latent class analysis of placental histopathology features in early and late preterm births, Black women had a high frequency of the acute inflammation latent class compared to women who did not identify as Black. Future histopathology studies should compare socioeconomic, behavioral, and environmental risk factors of preterm births across placental latent classes in Black women. The effects of race-related

stress and other environmental factors on placental health are not well understood, but may clarify race disparities in preterm birth.

In manuscript 3, 80% of the birth cohort was of European ancestry and the other 20% were defined as of non-European ancestry. The reliance of transcriptomic studies on white women or women of European ancestry limits the generalizability of findings to women of other ethnic groups. Even if race is a social construct weakly associated with genetic ancestry,[345] molecular findings in the placenta of white women may not be the same for Black women due to differences in epigenetic factors (socioeconomic status, environmental factors, stress). A follow-up study of manuscript 3 would be to repeat the transcriptomic analysis in a more ethnically diverse population.

3.1.6 Target Trials for Preterm Birth Prevention in Obese Women

My dissertation work investigated placental mechanisms of obesity and risk of preterm birth with the broader goal of informing targeted interventions for preterm birth in obese women. Even though further elucidation of the pathophysiology of obesity and risk of preterm birth is warranted before clinical trials can be conducted, causal inference methods can be leveraged to frame observational data as interventions.[346, 347] A study that analyzes observational data as a randomized trial is called a target trial. Target trials frame observational data with eligibility criteria, a treatment strategy, an allocation strategy, an outcome, and a follow-up period similar to an intervention.[348] Analytic methods are used to adjust for confounding variables to make individuals across treatments similar in all characteristics except for the treatment, mimicking randomization.[346] Assuming the analytic models used to emulate randomization are correctly specified, the treatment is well-defined, and individuals across all strata of treatment and

confounders exist, a causal relationship between treatment and outcome can be estimated like an intention to treat or per protocol analysis in a randomized trial. Target trials allow for larger sample sizes than clinical trials and can provide quicker answers about interventions if clinical trials are ongoing or have yet to be conducted.

For example, a target trial was conducted using health claims data to emulate randomization of women with a history of infertility to an infertility treatment: letrozole or clomiphene.[349] The probability of a live pregnancy and the risk of adverse pregnancy outcomes (e.g. preterm birth, congenital malformations) was then compared between women on the two infertility treatments by an intention to treat analysis.[349] The target trial found that the probability of pregnancy was comparable between infertility treatments but letrozole was associated with a higher risk of congenital malformations, NICU admission, and preterm births than clomiphene. Using a target trial framework, observational data on women planning to get pregnant was used to compare the efficacy and risk of adverse events from infertility treatments that had only previously been tested in small clinical trials underpowered to compare the two infertility treatments.

We could analyze birth registry data from US Vital Statistics or pregnancy data from large birth cohorts such as MOMI or Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-be (nuMoM2b) to emulate preterm birth interventions related to obesity. Findings from manuscript 2 indicated that obesity increased maternal vascular impairment in early preterm births. Target trials could emulate randomization to anti-hypertensive medication or aspirin compared to no medication to test for a reduction in risk of preterm birth. Likewise, target trials could measure if lipid-lower medications such as pravastatin lower the risk of preterm birth.

Target trials can also be designed to frame general metabolic exposures in an intervention framework.[350] For example, rather than specifying treatment to a statin, a target trial can emulate

randomizing women to HDL >50mg/dL vs HDL ≤50mg/dL in first trimester. A reduction in preterm birth in women "randomized" to HDL>50mg/dL compared to women "randomized" to HDL≤50mg/dL would suggest that any intervention (e.g. diet, medication, exercise) that increases HDL to >50mg/dL would reduce the risk of preterm birth. Likewise, a target trial that observes a lower risk of preterm birth by "randomizing" women to a pre-pregnancy BMI of 20-25kg/m^2 or to a BMI of ≥30kg/m^2 would indicate that any reduction in pre-pregnancy BMI from obese to lean would lower the risk of preterm birth.

Target trials are not without limitations. Unlike randomized clinical trials, target trials require statistical models to adjust for confounding bias. If residual confounding bias is still present the target trial will provide biased estimates that would likely vary from results in a clinical trial. Target trials also require simulating "time zero" where pregnant women are simultaneously enrolled, allocated to an intervention, and followed-up.[347] Unlike clinical trials, clinical decision making determines when a pregnant woman initiates a medication. A medication may also be stopped, modified in dose, or changed for a new medication. Observational data may also have limited repeated measures during pregnancy to feasibly assess the intervention of interest throughout pregnancy like a clinical trial. These caveats can bias the result of target trials and limit their applicability.

Still, target trials allow for evidence more aligned with an intervention framework than case-control studies, cohort studies and meta-analyses of observational studies. Aligning biological findings on the pathophysiology of obesity and preterm birth with findings from target trials would provide stronger evidence for preterm birth interventions in obese women than biological data alone.

3.2 Public Health Significance

My dissertation work contributes to public health by improving reproducibility of population studies of histopathology, identifying preterm birth subtypes more prognostic of neonatal morbidity, and characterizing potential placental biological targets for preterm birth prevention.

Epidemiological studies that leverage placental histopathology data have the potential to improve prediction of long-term childhood outcomes, later-life maternal health outcomes and outcomes in future pregnancies.[351, 352] From a public health perspective, the opportunity to predict future health outcomes for mother and child years prior to adverse outcomes allows for earlier intervention. For example, maternal vascular lesions detected during pregnancy are associated with risk factors of later life cardiovascular disease and cognitive impairment.[353, 354] Early identification and management of cardiovascular or cognitive risk factors could prolong life without clinical disease and disability.

Despite the clear public health benefit of placental histopathology, authors in a recent commentary in *Obstetrics and Gynecology* questioned the clinical utility of placental histopathology.[355] The authors cite the paucity of population-level studies of histopathology, the reliance on highly select cohorts, and lack of statistical methods to handle confounding bias. My work analyzes a large birth cohort with placental histopathology data to study the adverse effects of maternal obesity on placental health in term and preterm births. In addition to adjusting for confounders, we apply methods to identify potential bias from restricting analyses to women with available placental histopathology reports using causal diagrams, adjust for selection bias, and quantify concerns for residual selection bias. Our analysis helps explain differences in associations between obesity and placental inflammation across studies with different inclusion criteria. The

methods applied in this cohort will help improve reproducibility in population-level studies of placental histopathology so that clinicians and public health officials will have more confidence in interpreting placental histopathology reports for maternal and child health.

Interventions to predict preterm birth have had limited benefit because preterm birth is a multi-modal syndrome of early gestation.[1, 23] In manuscript 2, we clustered preterm births based on placental histopathology features. Our approach grouped early and late preterm births into distinct placental phenotypes, identified preterm births with maternal vascular malperfusion to have greater neonatal morbidity, and found pre-pregnancy obesity to be a risk factor for early preterm births with maternal vascular malperfusion. Our approach not only identified clusters of preterm birth prognostic of adverse neonatal outcomes but also identified preterm births in which intervention on obesity would be beneficial. Our work has the potential to improve prediction and prevention of preterm birth and will inform clinical management of neonates possibly to minimize debilitating sequelae in children.

The long-term public health goal of this dissertation is to inform future preterm birth interventions in obese women. Research to date has focused on targeting placental inflammation as a cause of preterm birth possibly due to maternal factors such as obesity.[131] Surprisingly obesity was associated with lower risk of acute placental inflammation based on histopathology and transcriptomic analyses. Instead, pre-pregnancy obesity increased placental maternal vascular malperfusion in early preterm births <32 weeks with many of these pregnancies complicated by preeclampsia. Though our findings require replication in other populations, our work suggests that prioritizing preterm birth interventions that address cardiovascular risk factors in obese women may be most beneficial.

The positive association between pre-pregnancy BMI and longer gestational age mediated by placental expression of IL1RL1 in manuscript 3, may also inform preterm birth prevention in a subset of preterm births associated with infection and preeclampsia. We found IL1RL1 expression in placental villous tissue to downregulate neutrophil degranulation, a biological process in preterm births with intrauterine infection.[323, 356] Increased placental villous expression of IL1RL1 was also associated with a lower expression of genes associated with pre-eclampsia. Thus, pharmacological drugs or dietary interventions that increase IL1Rl1 expression or are agonists for IL1RL1 activity, may address shared etiologies of preterm births from infection and preeclampsia. Prevention of shared pathophysiology of preterm birth will have the greatest impact at reducing preterm birth at the population level.

Appendix Table 1: Summary of evidence since 2010 for associations between obesity and preterm birth

Study	Country (Years)	Study Design	Population	BMI Measure	BMI Classification	Preterm Definition	Main Findings
Choi et al. (2022)[100]	Korea (2013-2017)	Prospective Cohort	3,454 singleton pregnancies	•BMI measured during 1st trimester	• underweight (<18.5kg/m^2), lean (18.5-22.9kg/m^2), overweight (23-24.9kg/m^2), and obese (≥25kg/m^2)	•preterm birth: GA <37 weeks determined by self-report questionnaire	•obese women compared to lean BMI was not associated with preterm birth (OR: 1.18, 95% CI: 0.16-8.89)
Kim et al. (2021)[104]	Korea (2016-2018)	Prospective Cohort	6,331 singleton pregnant Korean women	•pre-pregnancy BMI extracted from medical records	• underweight (<18.5kg/m^2), lean (18.5-22.9kg/m^2), overweight (23-24.9kg/m^2), and obese I (25-29.9kg/m^2), obese II (30-34.9kg/m^2), obese III (≥35kg/m^2)	•GA extracted from medical records •preterm delivery GA <37 weeks, GA <34 weeks and GA<28 weeks •stratified as spontaneous and indicated	•obese I women compared to lean women had a higher odds of indicated preterm delivery <37 weeks (OR: 1.97, 95% CI: 1.17-3.32) but no associations with overall preterm delivery < 37 weeks, <34 weeks, <28 weeks or spontaneous preterm delivery <37 weeks •obese II/III women (combined) compared to lean women had a higher odds of preterm delivery <34 weeks (OR: 3.54, 95% CI: 1.55-8.06) and indicated preterm delivery (OR: 3.15, 95% CI: 1.52-6.49) but not preterm delivery <37weeks, <28weeks, or spontaneous preterm delivery
Xie et al. (2021)[157]	China (2017-2019)	Retrospective Cohort	398,368 singleton pregnancies with no pre-existing diabetes, hypertension, thyroid disease, viral infection, tuberculosis, or history of c-section	•pre-pregnancy BMI extracted from medical records	•underweight (<18.5kg/m^2), lean (18.5 to <24.0kg/m^2), overweight (24 to <28.0kg/m^2), and obese (≥28.0kg/m^2)	•GA extracted from medical records •preterm delivery: GA <37 weeks	•obese women compared to lean women had a higher odds of preterm birth (OR: 1.47, 95% CI: 1.32-1.64)
van Hoorn et	Holland (2012-2014)	Prospective Cohort	3,671 singleton pregnancies	•self-reported pre-pregnancy	•women classified as class I (BMI	•GA determined by first trimester ultrasound	•pre-pregnancy BMI class VII was not associated with higher risk of preterm birth (RR: 1.05, 95%

al. (2021)[109]			without fetal demise <24 weeks or chromosomal abnormalities	BMI in early pregnancy	<18.5kg/m^2), class II (18.5-19.9kg/m^2), class III (20.0-22.9kg/m^2), and class IV (23.0-24.9kg/m^2), class V (25.0-27.4kg/m^2), class VI (27.5-29.9kg/m^2), class VII (obese class; ≥30kg/m^2)	•preterm birth: GA <37 weeks	CI: 0.60-1.85) compared to women in BMI class III
Li et al. (2021)[105]	China (2018-2020)	Retrospective Cohort	12, 855 singleton pregnancies excluding women <18 years or with major medical illness	•self-reported pre-pregnancy BMI at first prenatal visit	•underweight (BMI <18.5kg/m^2), lean (18.5-23.99kg/m^2), overweight (24-29.99kg/m^2), and obese (≥30.00kg/m^2)	•GA extracted from medical records •preterm birth: GA <37 weeks	•obese women compared to lean women had a higher odds of preterm birth (OR: 1.54, 95% CI: 0.99-2.38)
Gonzalez-Plaza et al. (2021)[101]	Spain (2015-2016)	Cross-sectional Study	5447 singleton pregnancies with delivery after 23 weeks of gestation	•self-reported pre-pregnancy BMI extracted from medical records	•underweight (BMI <18.5kg/m^2), lean (18.5-23.99kg/m^2), overweight (24-29.99kg/m^2), and obese (≥30.00kg/m^2)	•GA extracted from medical records •preterm birth: GA <37 weeks	•obese women compared to lean women had a higher odds of preterm birth (OR: 1.79, 95% CI: 1.32-2.44)
Tong et al. (2021)[357]	China (2013-2017)	Retrospective Cohort	669,101 singleton pregnancies without chronic diseases (anemia, hypertension, heart disease,	•measured pre-pregnancy BMI at preconception visit	•underweight (<18.5kg/m^2), lean (18.5-23.9kg/m^2), overweight (24-27.9kg/m^2), and obese (≥28.0kg/m^2)	•GA extracted from medical records •preterm birth: GA between 28 to <37 weeks	•obese women compared to lean women had a higher risk of preterm birth (IRR: 1.12, 95% CI: 1.05-1.20)

			hepatitis B, epilepsy, thyroid, chronic nephritis, cancer and diabetes)				
Li et al. (2020)[106]	China (2015)	Case-control Study	3,684 singleton nulliparous pregnancies (322 preterm and 3,362 term births) excluding pregnancies by artificial reproductive technology and pregnancies to infants with congenital defects/ neurological damage	•pre-pregnancy BMI extracted from medical records	•underweight (<18.5kg/m^2), lean (18.5-23.9kg/m^2), overweight (24-27.9kg/m^2), and obese (≥28.0kg/m^2)	•GA extracted from medical records •preterm delivery: GA <37 weeks •moderately preterm: GA 32-36 weeks •very preterm: GA 28-31 weeks •extremely preterm: GA <28 weeks	•obese women compared to lean women had a higher odds of extremely preterm birth (OR: 15.1, 95% CI: 1.32-172.13), but not moderately preterm birth (OR: 1.23, 95% CI: 0.58-2.59), very preterm birth (OR: 0.77, 95% CI: 0.10-5.77), or any preterm birth <37 weeks (OR: 1.27, 0.65-2.51)
Su et al. (2020)[76]	China (2015-2018)	Retrospective Cohort	36,596 singleton pregnancies 22 to 44 weeks of gestation excluding women with pre-existing diabetes and hypertension	•pre-pregnancy BMI extracted from medical records	•underweight (<18.5kg/m^2), lean (18.5 to <23kg/m^2), overweight (23 to <27.5kg/m^2), and obese (≥27.5kg/m^2)	•GA determined by self-reported LMP •preterm birth: GA <37 weeks •moderately preterm: GA 32-36 weeks •very preterm: GA 28-31 weeks •extremely preterm: GA <28 weeks	•obese women compared to lean women had a higher risk of preterm birth <37 weeks (RR: 1.30, 95% CI: 1.02-1.69), and extremely preterm birth (RR: 8.26, 95% CI: 1.63-41.88), but not moderate (RR: 1.23, 95% CI: 0.94-1.61) or very preterm birth (RR: 2.67, 95% CI: 0.79-9.08)

Pratt et al. (2020)[124]	Australia (2011-2016)	Retrospective Cohort	18,518 singleton pregnancies excluding women weighing >180kg	•BMI at first prenatal visit extracted from medical records	•underweight (<18kg/m^2), lean (19-24kg/m^2), overweight (25-29kg/m^2), obese (30-49kg/m^2), and morbidly obese (≥50kg/m^2)	•GA extracted from medical records •late preterm birth: GA 32-36 weeks	•compared to lean women there was an increased odds of late preterm birth for morbidly obese women (≥50kg/m^2; OR: 2.16, 95% CI: 1.16-4.04), but not obese 30-49kg/m^2 women (OR: 0.88, 95% CI: 0.75-1.04)
Ram et al. (2020)[117]	Canada (2012-2016)	Retrospective Cohort	487,870 women (480,010 singleton and 7,860 twin pregnancies) excluding births <24 weeks of gestation, women with pre-existing medical conditions (hypertension, diabetes, renal disease, auto-immune disorders), twins with twin-to-twin transfusion syndrome, and monoamniotic twins	•pre-pregnancy BMI extracted from medical records	•underweight (<18.5kg/m^2), lean (18.5-24.9kg/m^2), overweight (25-29.9kg/m^2), and obese I (30-34.9kg/m^2), obese II (35-39.9kg/m^2), obese III (≥40kg/m^2)	•GA extracted from medical records •early preterm birth: GA <32 weeks •preterm birth <37 weeks •preterm birth <34 weeks •preterm birth <28 weeks •preterm births stratified as indicated or spontaneous	<u>Singleton Pregnancies</u> •compared to lean women, there was a higher risk of preterm births <32 weeks for women of obese I (RR: 1.38, 95% CI: 1.23-1.55), obese II (RR: 1.53, 95% CI: 1.31-1.89), and obese III women (RR: 1.57, 95% CI:1.29-1.99) •compared to lean women obese I, II, and III women had a higher risk of preterm births < 37 weeks (RR: 1.12 to 1.20), <34 weeks (RR:1.20 to 1.42), <28 weeks (RR: 1.40 to 2.02), spontaneous preterm birth <32weeks (RR:1.22 to 1.33), spontaneous preterm births <28 weeks (RR: 1.32 to 2.04), and indicated preterm births at any gestational age cut-off •compared to lean women, there was an associated lower risk of spontaneous preterm births <37 weeks for obese II and III women (RR: 0.91, 95% CI: 0.84-0.99 and 0.89, 0.81-0.98) <u>Twin Pregnancies</u> •compared to lean women, obese I women had a lower risk of spontaneous preterm births <37 weeks (RR: 0.81, 95% CI: 0.79-0.95) and a higher risk of provider-initiated preterm births <37 weeks (RR: 1.19, 95% CI: 1.08-1.32) •compared to lean women, obese II women had an associated higher risk of indicated preterm births <37 weeks (RR: 1.21, 95% CI 1.05-1.39) •no other comparisons were significant for twin pregnancies

Kutchi et al. (2020)[358]	India (2016-2017)	Prospective Cohort	200 pregnancies (100 BMI ≥25kg/m^2 and 100 women with BMI <25 kg/m^2)	•measured during 1st trimester	•BMI ≥25 kg/m^2 vs BMI <25kg/m^2	•GA cut-off not defined •stratified preterm as spontaneous and indicated	•women with a BMI ≥25 kg/m^2 compared to women with a BMI <25 kg/m^2 had a higher odds of preterm birth of any type (OR: 4.63, 95% CI: 1.47-14.51) and indicated preterm birth (OR: 6.95, 95% CI: 1.42-30.57) •odds ratio for spontaneous birth not estimated
Chen et al. (2020)[99]	Taiwan (2005)	Prospective Cohort	19,052 singleton pregnancies	•self-report pre-pregnancy from survey 6-18 months postpartum	•underweight (<18.5 kg/m^2), lean (18.5-25 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥30 kg/m^2)	•preterm birth: GA <37wks determined by questionnaire 6 to 18 postpartum	•obese women compared to lean women had a higher odds of preterm birth (OR: 1.76, 95% CI: 1.25-2.48)
Slack et al. (2019)[125]	England (1990-2007)	Retrospective Cohort	479,864 singleton pregnancies with live births >20 weeks of gestation	•measured BMI at first antenatal visit (~12 weeks of gestation)	•underweight (<18.5kg/m^2), lean (18.5-25kg/m^2), overweight (25-29.9kg/m^2), and obese I (30-34.9kg/m^2), obese II (35-39.9kg/m^2), obese IIIa (40-49.9kg/m^2), and obese IIIb (≥50kg/m^2)	•GA extracted from medical records •extreme preterm birth: GA 20-27 weeks •very preterm birth: 28-31 weeks •moderately preterm: GA 32-36 weeks	•compared to lean women: -obese I had a higher odds of extreme preterm birth (OR: 1.20, 95% CI 1.03-1.40), very preterm birth (OR: 1.21, 95% CI: 1.07-1.37), and moderately preterm birth (OR: 1.07, 95% CI: 1.02-1.12) -obese II had a higher odds of extreme preterm birth (OR:1.39, 95% CI: 1.13-1.71), very preterm birth (OR:1.35, 95% CI: 1.12-1.61), and moderately preterm birth (OR: 1.13, 95% CI: 1.05-1.22) -obese IIIa had a higher odds of extreme preterm birth (OR: 1.52, 95% CI: 1.14-2.03), but not very preterm (OR: 1.04, 95% CI: 0.76-1.42) or moderate preterm birth (OR: 1.11, 95% CI: 0.99-1.25) -obese class IIIb had a higher odds of extreme preterm birth (OR: 2.80, 95% CI: 1.31-5.98) and moderately preterm birth (OR: 2.18, 95% CI: 1.58-2.99), but not very preterm (OR: 1.59, 95% CI: 0.65-3.88)
Liu et al. (2019)[359]	USA (2016-2017)	Retrospective Cohort	•7,141,630 live singleton births excluding women with	•self-reported pre-pregnancy BMI extracted	•underweight (<18.5kg/m^2), lean (18.5-25kg/m^2), overweight (25-	•GA determined by best obstetric estimate	<u>All Pregnancies</u> •compared to lean women, there was a higher odds of preterm birth <37 weeks for obese women ≥30kg/m^2 (OR:1.18, 95% CI: 1.18-1.19), obese I (OR:1.12, 95% CI: 1.11-1.13), obese II (OR: 1.21,

			pre-existing diabetes or hypertension	from medical records	29.9kg/m^2), and obese I (30-34.9kg/m^2), obese II (35-39.9kg/m^2), obese III (≥40kg/m^2)	•preterm birth: GA < 37 weeks •extreme preterm birth: GA 20-27 weeks •very preterm birth: 28-31 weeks •moderately preterm: GA 32-36 weeks	95% CI: 1.20-1.23), and obese III (OR: 1.34, 95% CI: 1.32-1.35) Hispanic Women •compared to lean women, there was a higher odds of preterm birth <37 weeks for obese women ≥30kg/m^2 (OR:1.20, 95% CI: 1.18-1.22), obese class I (OR: 1.14, 95% CI: 1.12-1.16), obese II (OR:1.24, 95% CI: 1.22-1.27), and obese III (OR: 1.38, 95% CI: 1.34-1.41) Non-Hispanic White Women •compared to lean women, there was a higher odds of preterm birth <37 weeks for obese women ≥30kg/m^2 (OR:1.23, 95% CI: 1.22-1.25), obese class I (OR: 1.15, 95% CI: 1.13-1.16), obese II (OR: 1.26, 95% CI: 1.24-1.28), and obese III (OR: 1.45, 95% CI: 1.43-1.48) Non-Hispanic Black Women •compared to lean women, there was no association with preterm birth for obese ≥30kg/m^2 (OR: 0.99, 95% CI: 0.97-1.00) or obese II (OR: 1.02, 95% CI: 0.99-1.04), a lower risk with obese class I (OR: 0.95, 95% CI: 0.93-0.97), and higher risk with obese III (OR: 1.05, 95% CI: 1.02-1.08) Women of Other Race/Ethnicity •compared to lean women, there was a higher odds of preterm birth for obese ≥30kg/m^2 (OR: 1.26, 95% CI: 1.23-1.29), obese class I (OR: 1.25, 95% CI: 1.21-1.28), obese II (OR:1.26, 95% CI: 1.20-1.31), and obese III (OR: 1.32, 95% CI: 1.25-1.39)
Ratnasiri et al. (2019)[118]	USA (2007-2016)	Cross-sectional Study	4,621,082 singleton pregnancies	•self-report pre-pregnancy extracted from birth records	•underweight (<18.5 kg/m^2), lean (18.5 to 25 kg/m^2), overweight (25-29.9 kg/m^2), obese I (30-34.9 kg/m^2), obese II (35-39.9 kg/m^2),	•GA determined by obstetric estimate •preterm birth: GA <37wks •very preterm birth: GA < 32 wks	•compared to lean women there was an increased odds of preterm birth in women of obese I (OR: 1.16, 95% CI: 1.14-1.17), obese II (OR: 1.24 95% CI: 1.22-1.26), and obese III (OR: 1.33, 95% CI: 1.30-1.36) •compared to lean women there was an increased odds of very preterm birth in women of obese I (OR: 1.43, 95% CI: 1.38-1.47), obese II (OR:1.61, 95% CI: 1.54-1.68), and obese III (OR: 1.66, 95% CI: 1.58-1.75)

					obese III (≥40 kg/m^2)		
Granese et al. (2019)[360]	Italy (2010-2016)	Case-control Study	8,179 singleton pregnancies (7,315 term births and 639 preterm births)	•extracted from medical records	•BMI cut-offs not defined •BMI grouped as underweight, normal, overweight and obese	•GA extracted from medical records •preterm birth: GA ≤ 36 wks 6/7days	•among preterm birth cases, 35% were obese compared to 12% of term birth controls •BMI classes differed significantly between preterm and term births (p<0.0001)
Ju et al. (2018)[103]	USA (2001-2011)	Cross-sectional Study	20,061 pregnant women from Hawaii	•self-reported pre-pregnancy BMI extracted from birth records	•underweight (<18.5kg/m^2), lean (18.5-24.9kg/m^2), overweight (25-29.9kg/m^2), obese (30-39.9kg/m^2), extremely obese (≥40kg/m^2)	•GA determined by best obstetric estimate •preterm birth: GA <37 weeks	<u>All Women</u> •compared to lean women, there was a higher odds of preterm birth among obese ≥30kg/m^2 women (OR: 1.24, 95% CI: 1.06-1.45), obese 30-39.9kg/m^2 women (OR: 1.17, 95% CI: 1.00-1.39) and extremely obese women (OR: 1.68, 95% CI: 1.21-2.34) <u>Native Hawaiian/Pacific Islander</u> •compared to lean women, there was no association with preterm birth among all obese ≥30kg/m^2 women (OR: 1.04, 95% CI: 0.83-1.29), obese 30-39.9kg/m^2 women (OR: 0.99, 95% CI: 0.79-1.26) and extremely obese women (OR: 1.29, 95% CI: 0.86-1.95) <u>Non-Native Hawaiian/Pacific-Islander</u> •compared to lean women, there was a higher odds of preterm birth among all obese ≥30kg/m^2 women (OR: 1.40, 95% CI: 1.13-1.74), obese 30-39.9kg/m^2 women (OR: 1.31, 95% CI:1.04-1.65) and extremely obese women (OR: 2.10, 95% CI: 1.25-3.51)
Mocking et al. (2018)[361]	Indonesia and Ghana (2012-2014)	Prospective cohort	Indonesia: 433 pregnant women Ghana: 946 pregnant women	•Indonesia: self-report pre-pregnancy BMI •Ghana: BMI measured at enrollment	•BMI stratified into tertitles	•GA determined by LMP •preterm birth: GA <37 wks	•Indonesia: no difference in preterm birth prevalence between low (12.5 to 20.6 kg/m^2; 4.5%), middle (20.7 to 24.3 kg/m^2; 4.5%) and upper (24.9 to 39.2 kg/m^2; 5.7%) BMI tertiles (p=0.89) •Ghana: no difference in preterm birth prevalence between low (15.4 to 23.1 kg/m^2; 7.4%), middle (23.1 to 27.0 kg/m^2; 8.9%), upper (27.1 to 42.3 kg/m^2; 8.0%) BMI tertiles (p=0.83)

				(median GA: 12 wks)			
Morais et al. (2018)[108]	Brazil (2011-2014)	Cross-sectional Study	1,110 singleton pregnancies	•BMI at first prenatal visit extracted from medical records	•BMI classified by Atalah reference curve normalized for GA: low weight, adequate weight, overweight, or obese	•GA extracted from medical records •preterm birth: GA < 37wks	•women with preterm birth had a non-significant higher odds of an obese BMI at first prenatal visit (OR: 1.56; 95% CI: 0.78-3.09) compared to term births
Granese et al. (2017)[362]	Italy (2013-2014)	Retrospective Cohort	2048 singleton pregnancies	•BMI extracted from birth records, but timing not reported	•BMI classified as <18, 18-25, and ≥25kg/m^2 and as a continuous variable	•data extracted from medical records •GA ≤ 36 wks 6/7days	•a 1-unit increase in maternal BMI was associated with lower odds of preterm birth (OR: 0.91 95% CI: 0.87-0.96)
Jiang et al. (2017)[102]	Australia (2011-2015)	Retrospective Cohort	4081 singleton pregnancies	•BMI at first prenatal visit extracted from medical records	•underweight (<18.5 kg/m^2), lean (18.5-25 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥30 kg/m^2)	•GA extracted from medical records •preterm birth: GA < 37 wks	•in obese women compared to lean women the unadjusted odds ratio for preterm birth was 1.31 (95% CI: 0.87-1.97) and 1.25 (OR: 0.83-1.89) after adjusting for gestational diabetes and smoking status
El Rafei et al. (2016)[363]	Lebanon (2001-2012)	Retrospective Cohort	170,428 singleton pregnancies between 28 and 42 weeks of gestation	•self-reported pre-pregnancy BMI based on interviews at delivery	•underweight (<18.5 kg/m^2), lean (18.5-25 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥30 kg/m^2)	•GA determined by ultrasound in late 1st or early 2nd trimester •very preterm birth: GA <33weeks •preterm birth: GA <37weeks	•obese women compared to lean women had a higher odds of very preterm birth (OR: 1.33, 95% CI: 1.02-1.74) and preterm birth <37weeks (OR: 1.10, 95% CI: 1.01-1.21)
Enomoto et al. (2016)[113]	Japan (2013)	Cross-sectional Study	97,157 singleton pregnancies delivering after 22 weeks	•pre-pregnancy BMI extracted from medical records	•underweight (<18.5 kg/m^2), lean (18.5-25 kg/m^2), overweight (25-29.9 kg/m^2), and	•gestational age determined by LMP •preterm birth: GA <37 weeks •stratified as spontaneous and	•obese women compared to lean women had a higher odds of any preterm birth (OR: 1.19, 95% CI: 1.04-1.35) and induced preterm birth (OR: 1.57, 95% CI: 1.23-2.01), but not spontaneous preterm birth (OR: 1.09, 95% CI: 0.94-1.26)

					obese (≥30 kg/m^2)	medically indicated	
Cosson et al. (2015)[364]	France (2002-2010)	Cross-sectional Study	15,551 non-Asian women with singleton pregnancies	•self-reported pre-pregnancy BMI surveyed at delivery	•lean (18.5 to 25kg/m^2), overweight (25-29.9kg/m^2), and obese (≥30kg/m^2)	•not reported how GA was estimated •preterm birth: GA <37 wks	•there was no significant difference in preterm birth across BMI classes (7.9% among lean, 7.3% among overweight, and 8.2% among obese women; p>0.05)
Shaw et al. (2014)[122]	USA (2007-2009)	Cross-sectional Study	42,771 spontaneous singleton births (21 to <41wks)	•pre-pregnancy BMI extracted from medical records	•underweight (<18.5 kg/m^2), lean (18.5 to 25 kg/m^2), overweight (25-29.9 kg/m^2), obese I (30-34.9 kg/m^2), obese II (35-39.9 kg/m^2), obese III (≥40 kg/m^2)	•GA extracted from birth certificates and defined by obstetric estimate •preterm birth: GA <37 wks •preterm birth further classified as: 20-23wks, 24-27wks, 28-31wks, and 32-36wks	<u>Primiparous Pregnancies</u> •compared to lean women, there was a higher odds of preterm birth at 20-23wks for: -white women of obese I, (RR: 2.69, 95% CI: 1.56-4.65), obese II (RR: 4.02, 95% CI: 2.07-7.78) and obese III (RR: 6.29, 95%: 3.06-12.9) -black women of obese I (OR: 1.89, 95% CI: 1.07-3.34), obese II (OR: 2.47, 95% CI: 1.26-4.84), and obese III (OR: 4.34, 95% CI: 2.30-8.16) -Latina women of obese I (OR: 2.76, 95% CI: 1.97-3.87), obese II (OR: 2.65, 95% CI: 1.58-4.46), obese III (OR: 4.45, 95% CI: 2.53, 7.82) •compared to lean women, there was a higher odds of preterm birth at 24-27wks for: -white women of obese II (RR: 3.79, 95% CI: 2.48-5.78) and obese III (RR: 2.06, 95% CI: 0.96-4.42) -black women of obese I (OR: 1.70, 95% CI: 1.07-2.68), and obese III (OR: 3.86 95% CI: 2.26-6.58) -Latina women of obese I (OR: 2.04, 95% CI: 1.63-2.56), obese II (OR: 2.84, 95% CI: 2.08-3.87), and obese III (OR: 2.94, 95% CI: 1.92-4.51) -compared to lean women, there was a higher odds of preterm birth at 28-31wks for Latina women of obese II (RR: 1.66, 95% CI: 1.21-2.27) -there was no association in any racial/ethnic group between BMI and risk of preterm birth at 32 to 36weeks

							Multiparous Pregnancies •compared to lean women, there was a higher risk of preterm birth at 24-27 weeks for white women of obese I (RR: 1.65, 95% CI: 1.15-2.36) and Latina women of obese I (RR: 1.43, 95% CI: 1.17-1.75) •compared to lean women, obese women of any class had a lower risk of preterm birth at 32-36wks (risk ratios from 0.60 to 0.90) among white, black, and Latina women
Sharashova et al. (2014)[121]	Russia (2006-2011)	Retrospective Cohort	29,709 singleton spontaneous births	•BMI measured at first prenatal visit (before 12 wks)	•underweight (<18.5 kg/m^2), lean (18.5-25 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥30 kg/m^2)	•GA estimated by LMP •preterm birth: GA < 37 wks •very preterm birth: GA <32wks •moderate preterm birth: GA 32 to <37wks	•obese women compared to lean women had a higher odds of preterm birth (OR: 1.29, 95% CI:1.08-1.56), very preterm birth (OR: 1.49, 95% CI: 0.94- 2.37), and moderate preterm birth (OR: 1.26, 95% CI: 1.03-1.54)
Parker et al. (2014)[120]	USA (1998-2002)	Case-control Study	4677 singleton pregnancies (1396 preterm and 3281 race and age-matched term birth)	•self-reported pre-pregnancy BMI recorded at delivery	•underweight (<18.5 kg/m^2), lean (18.5-25 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥30 kg/m^2)	•GA estimated by LMP and confirmed by ultrasound •preterm birth: GA <37 weeks •early preterm birth: GA <34 wks •late preterm: GA 34-36wks •stratified as spontaneous and indicated	•obese women compared to lean women had a higher odds of indicated (OR:1.59, 95% CI: 1.23-2.05), but not spontaneous preterm birth (OR: 0.90, 95% CI: 0.73-1.12), or preterm birth of any type (OR: 1.11; 95% CI: 0.93-1.33) •obese compared to lean women had a higher odds of early (OR: 1.78, 95% CI: 1.19-2.66) and late indicated preterm birth (OR: 1.49, 95% CI: 1.09-2.04) •obesity was not associated with odds of early (OR: 1.25, 95% CI: 0.90-1.72) or late spontaneous preterm birth (OR: 0.76, 95% CI: 0.58-0.98)
Cnattingius et al. (2013)[26]	Sweden (1992-2010)	Retrospective Cohort	1,857,822 singleton pregnancies	•BMI measured at first prenatal visit (8-12wks gestation)	•underweight (<18.5 kg/m^2), lean (18.5 to 25 kg/m^2), overweight (25-29.9 kg/m^2), and obese I (30-34.9	•GA estimated by ultrasound (81%), LMP (13%), and post-natal assessment (6%) •preterm birth classified as	Spontaneous Preterm Births •compared to lean women, there was a higher odds of extremely preterm birth for women of obese I (OR: 1.21, 95% CI: 1.03-1.43), obese II (OR: 1.57, 95% CI: 1.24-1.99), and obese III (OR: 2.07, 95% CI: 1.58-2.70)

					kg/m^2), obese II (35-39.9 kg/m^2), obese III (≥40 kg/m^2)	extremely preterm (GA: 22-27wks), very preterm (GA: 28-31 wks and moderate preterm (GA:32-36wks) •stratified as spontaneous/ indicated	•obesity was not associated with odds of very preterm birth or moderate preterm birth <u>Medically Indicated Preterm Births</u> •compared to lean women, there was a higher odds of extremely preterm birth for women of obese I (OR: 2.43, 95% CI: 1.99-3.10), obese II (OR: 2.74, 95% CI: 1.92-3.92), obese III (OR: 3.84, 95% CI: 2.32-6.38) •compared to lean women, there was a higher odds of very preterm birth for women of obese I (OR: 1.91, 95% CI: 1.68-2.17), obese II (OR: 2.52, 95% CI: 2.08-3.06), obese III (OR: 4.16, 95% CI: 3.23-5.36) •compared to lean women, there was a higher odds of moderate preterm birth in women of obese I (OR: 1.62, 95% CI: 1.54-1.71), obese II (OR: 2.00, 95% CI: 1.84-2.18), obese III (OR: 2.45, 95% CI: 2.15-2.79)
de Jongh et al. (2013)[112]	USA (2009-2011)	Retrospective Cohort	11,711 singleton pregnancies	•pre-pregnancy BMI extracted from medical records	•lean (18.5-25 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥30 kg/m^2)	•GA extracted from medical records •preterm birth: GA <37 wks	•entire cohort, there was no association for odds of preterm birth in obese women compared to lean women (OR: 1.11, 95% CI: 0.94-1.32) •among white women, obese women had a higher odds of preterm birth (OR: 1.40, 95% CI: 1.12-1.75) compared to lean women •among black women, obese women compared to lean women had a lower odds of preterm birth (OR: 0.87, 95% CI: 0.68-1.19) •among Hispanic women, obese women compared to lean women had a higher odds of preterm birth (OR: 2.20, 95% CI: 1.23-3.95) •among Asian women, obese women compared to lean women had a higher odds of preterm birth (OR: 3.07, 95% CI: 1.16-8.13)
Torloni et al. (2012)[123]	USA (2003-2009)	Case-control Study	1762 viable singleton pregnant women with spontaneous vaginal delivery: 447	•self-reported pre-pregnancy BMI at first prenatal visit	•underweight (<18.5 kg/m^2), lean (18.5-25 kg/m^2), overweight (25-29.9 kg/m^2), and	•GA estimated by LMP and confirmed by ultrasound •preterm birth: GA <37 weeks •moderate preterm	•entire cohort: obese compared to lean BMI was not associated preterm birth <37wks (OR: 1.01, 95% CI: 0.78-1.31), moderate preterm birth (OR: 0.96, 95% CI: 0.72-1.28), or early preterm birth (OR: 1.16, 95% CI: 0.72-1.85) •African-American women: obese compared to lean BMI was associated with a lower odds of any

			preterm (145 African-American and 302 Caucasian) and 1315 (522 African-American and 793 Caucasian)		obese (≥30 kg/m^2)	birth: 32 to <37 wks •early preterm: <32wks	preterm birth <37wks (OR: 0.72, 95% CI: 0.38-1.40), moderate preterm birth (OR: 0.73, 95% CI: 0.45-1.19) and early preterm birth (OR: 0.23, 95% CI: 0.08-0.70) •Caucasian women: obese compared to lean BMI had a higher odds preterm birth <37 wks (OR: 1.84, 95% CI: 1.15-2.95), moderate preterm birth (OR: 1.20, 95% CI: 0.84-1.72) and early preterm birth (OR: 2.30, 95% CI: 1.32-4.00)
Kosa et al. (2011)[114]	USA (2002-2003)	Nested Case-control Study	1064 singleton pregnancies (354 preterm and 710 non-low birth weight term births; frequency matched for race and birth month)	•self-reported pre-pregnancy BMI based on survey data collected during the first trimester	•underweight (<18.5 kg/m^2), lean (18.5-25 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥30 kg/m^2) •BMI also measured as continuous	•GA is based off of obstetric estimate •preterm birth: GA <37 wks	•obese women compared to lean women had a non-significant increased odds of preterm birth (OR: 1.31, 95% CI: 0.86-2.00) •a pre-pregnancy BMI of 34kg/m^2 compared to a BMI of 24kg/m^2 was associated with a higher odds of preterm birth (OR: 2.01, 95% CI: 1.20-3.39)
Mamum et al. (2011)[115]	Australia (1981-1983)	Retrospective Cohort	6632 viable singleton pregnancies	•self-reported pre-pregnancy BMI based on survey at first prenatal visit	•underweight (<18.5 kg/m^2), lean (18.5-25 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥30 kg/m^2)	•GA extracted from medical records •preterm birth: 21 < GA ≤36 wks	•an obese BMI compared to a lean BMI was not associated with preterm birth after adjusting for age (OR: 1.31, 95% CI: 0.74-2.34), and after adjusting for age, education, race, smoking, alcohol consumption and high risk pregnancy (OR:1.08, 95% CI: 0.60-1.95)
Wise et al. (2010)[169]	USA (1995-2003)	Prospective Cohort	7,841 singleton viable pregnancies to African-American women	•self-reported BMI from a survey administered 0-2 years before pregnancy	•underweight (<18.5 kg/m^2), lean (18.5 to 25 kg/m^2), overweight (25-29.9 kg/m^2), obese I (30-34.9 kg/m^2), obese II (35-39.9 kg/m^2), obese III (≥40 kg/m^2)	•GA estimated by questionnaire administered up to 2 years after delivery •preterm birth: GA <37 wks •women asked if preterm birth was spontaneous or	<u>Spontaneous Preterm Birth</u> •compared to lean women, there was no significant odds of preterm birth for women of obese I (OR:1.17, 95% CI: 0.91-1.52), and obese II (OR: 1.16, 95% CI: 0.80-1.69), obese III (OR:1.42, 95% CI: 0.97-2.08) <u>Indicated Preterm Birth</u> •compared to lean women, there was a higher odds of preterm birth for women of obese I (OR: 2.18, 95% CI:1.67-2.83), obese II (OR: 1.92, 95% CI:

						indicated on questionnaire	1.33-2.78), and obese III (OR: 1.70, 95% CI: 1.14-2.51)

Abbreviations: CI, confidence interval; GA, gestational age; LMP, last menstraul period; OR, odds ratio; RR, risk ratio.

Appendix Table 2: Magee-Womens hospital 2008-2012 diagnostic criteria for placental inflammatory lesions

Placental Lesion	Diagnostic Criteria
Chronic Villitis	Lymphohistiocytic inflammation of stem villi, intermediate villi, or terminal villi
Acute Chorioamnionitis	A maternal response to infected amniotic fluid characterized by presence of linear accumulation of neutrophils within subchorionic fibrin or chorionic plate itself.
Acute Fetal Lesions	Acute fetal lesions include fetal vasculitis and funisitis. Fetal vasculitis is characterized by neutrophils within or emerging from fetal vessels of the chorionic plate or umbilical cord. Funisitis is defined as acute vasculitis of umbilical cord vessels in which neutrophils traverse through vessel wall into the surrounding Wharton's jelly.

Appendix Table 3: Indications for placental pathology evluations adapted from *Langston et al.*[189]

Maternal Indication	Definition	Relevance to Study
Maternal Co-morbidities	Systemic disorders with clinical concerns for mother or infant. Examples include severe diabetes, impaired glucose metabolism, hypertensive disorders, collagen diseases, maternal seizures, or severe anemia (<9g)	Selection Bias
Premature Delivery	delivery <37 weeks of gestation; irrespective if spontaneous or medically-indicated delivery	Excluded
Peripartum Fever/ Infection	clinical signs and symptoms of infection include maternal pyrexia in labor, increased maternal C-reactive protein and maternal sepsis	Selection bias
Infection during pregnancy	infections of clinical concern during pregnancy (e.g. human immune deficiency virus, syphilis, cytomegalovirus, primary herpes, toxoplasma, rubella)	No suspected role
Unexplained Bleeding	unexplained bleeding or excessive bleeding >500cm^3	Selection bias
Oligohydramnios	ammonitic fluid volume less than minimum expected for gestational age	Excluded
Recurrent Pregnancy Complications	unexplained or recurrent pregnancy complications (intrauterine growth restriction, stillbirth, spontaneous abortion, premature birth)	Excluded (except for growth restriction)
Invasive Procedures with Placental Injury	Not defined in dataset	No suspected role
Abruption	partial or complete detachment of placenta prior to delivery	No suspected role
Nonelective Pregnancy Termination	Not defined in dataset	Excluded
Thick/Viscous Meconium	turbid or viscous meconium present in ammonitic fluid	No suspected role
Polyhydramnios	ammonitic fluid volume greater than maximum expected for gestational age	No suspected role
History of Substance Use	broad term that includes use of nicotine, alcohol, cannabis, prescription drugs (e.g., opioids, sedatives), illicit drugs (e.g., amphetamines), and drugs used to treat maternal opioid use disorder (e.g., methadone).	Selection bias
Post-term pregnancy	gestational age ≥42 weeks	Excluded

Severe Maternal Trauma	non-specific maternal trauma during pregnancy necessitating medical intervention	No suspected role
Prolonged Membrane Rupture	rupture of membranes >24 hours prior to delivery	Mediator or selection bias
Fetal/Neonatal Indication	**Definition**	**Relevance to Study**
Admission to NICU	Admission or transfer to a nursery other than level 1	No suspected role
Stillbirth	unexplained nonviable intrauterine pregnancy after 20 weeks of gestation	Excluded
Compromised Clinical Condition	cord blood pH <7.0, 5-min Apgar score ≤6, ventilator assistance >10 minutes, severe anemia, or hematocrit <35%	Selection bias
Fetal Hydrops	abnormal fluid accumulation in two or more fetal serous cavities (e.g., ascites, pleural effusion)	No suspected role
Small for Gestational Age	birthweight <10th percentile for gestational age	Selection bias
Large for Gestational Age	birthweight >95th percentile for gestational age	Selection bias
Neonatal Seizures	seizures or epileptic fits of the neonate within the first 28 days of life	No suspected role
Infection/sepsis	sepsis or bacteremia of the neonate within the first 28 days of life	No suspected role
Congenital Anomalies	neural and cardiovascular congenital defects of the fetus or chromosomal aneuploidy	Selection bias
Twin Placentas	twin pregnancies dichorionic placentas or in twin pregnancies where the chorionicity is unclear at delivery	Excluded
Asymmetric Growth	disproportionate growth of one fetus relative to another fetus in twin pregnancies	Excluded
Vanishing Twin	the disappearance of a twin in the uterus during pregnancy because of miscarriage of on twin	Excluded
Placental Indication	**Definition**	**Relevance to Study**
Physical Placental Abnormality	infarct, mass, vascular thrombosis, retroplacental hematoma, amnion nodosum, abnormal coloration or malodor of the placenta	No suspected role
Abnormal placental shape	A variety of macroscopic lesions obvious at delivery of placenta including bilobed, membranous placenta, large chorangioma, infarct, subchorionic hematoma, and excess calcification	No suspected role
Small/large placenta	placental weight less than 10th percentile or greater than 10th percentile for gestational age	Selection bias

Umbilical cord lesion	thrombosis, torsion, true knot, single artery, or absence of Wharton's jelly within the umbilical cord	No suspected role
Short Umbilical cord	umbilical cord < 32cm at term	No suspected role
Long Umbilical Cord	umbilical cord >100cm	No suspected role
Marginal Cord Insertion	insertion of the umbilical cord at the margins of the placenta	No suspected role

Appendix Table 4: Neonatal outcomes among women with and without histologic placental inflammation[a,b]

Histologic Acute Chorioamnionitis			
	Lesion Present (N=3909)	**No Lesion (N=5859)**	**P-value**
Small for Gestational Age, n (%)	415 (10.6%)	892 (15.2%)	< 0.001
Large for Gestational Age, n (%)	398 (10.2%)	569 (9.7%)	0.47
Low 5-Minute Apgar Score (<7), n (%)	78 (2.0%)	68 (1.2%)	0.001
Fetal Distress, n (%)[b]	1039 (26.6%)	1115 (19.0%)	< 0.001
Histologic Acute Fetal Inflammation			
	Lesion Present (N=2140)	**No Lesion (N=7628)**	**P-value**
Small for Gestational Age, n (%)	216 (10.1%)	1091 (14.3%)	< 0.001
Large for Gestational Age, n (%)	219 (10.2%)	748 (9.8%)	0.59
Low 5-Minute Apgar Score (<7), n (%)	59 (2.8%)	87 (1.1%)	< 0.001
Fetal Distress, n (%)	631 (29.5%)	1523 (20.0%)	< 0.001
Histologic Chronic Inflammation			
	Lesion Present (N=1461)	**No Lesion (N=8307)**	**P-value**
Small for Gestational Age, n (%)	243 (16.6%)	1064 (12.8%)	< 0.001
Large for Gestational Age, n (%)	127 (8.7%)	840 (10.1%)	0.10
Low 5-Minute Apgar Score (<7), n (%)	22 (1.5%)	124 (1.5%)	>0.99
Fetal Distress, n (%)	315 (21.6%)	1839 (22.1%)	0.66

[a]Comparisons of neonatal outcomes were made among term pregnancies with complete data on pre-pregnancy BMI and placental histopathology (n=9768).
[b]Fetal distress refers to fetal arrhythmias or a general clinical diagnosis of fetal distress.

Appendix Table 5: Inverse probability weighting model selection[a,b,c]

#	Model Variables	Estimated Weights		Estimated Risk of Acute Chorioamnionitis		Estimated Risk of Acute Fetal Inflammation		Estimated Risk of Chronic Villitis	
		Mean (SD)	Min, Max	RR	95% CI	RR	95% CI	RR	95% CI
1	**Clinical Indications for Pathology**: Maternal Substance Use, Maternal Comorbidities, Chronic HTN, Diabetes, Adverse Pregnancy History, Preeclampsia, Suspected Intrauterine Infection, Congenital Abnormalities, Fetal Distress, SGA, Labor/Placental Complications, 5-min Apgar Score	1.31 (0.54)	0.38, 1.98	0.92	0.86, 0.98	0.93	0.83, 1.03	1.15	1.01, 1.31
2	**Model 1 + Pregnancy Characteristics:** Maternal Age, Race, Education, Insurance, Pre-pregnancy BMI, Nulliparity, Smoking Status, Marital Status, History of Multiple Abortion, Weight Gain, Fetal Sex, Gestational Age at Delivery, Labor Status	1.43 (0.80)	0.38, 6.15	0.92	0.86, 0.99	0.93	0.83, 1.04	1.12	0.98, 1.28
3	**Model 2 + Higher Order Terms and Interactions:** Restricted Cubic Splines for continuous variables , Race x Preeclampsia, Race x Diabetes, BMI X Preeclampsia, Age x Preeclampsia, Gestational Age x Preeclampsia, Gestational Age x SGA, Labor x Suspected Intrauterine Infection, Fetal Distress x SGA	1.45 (0.82)	0.38, 6.38	0.92	0.85, 0.99	0.93	0.83, 1.03	1.12	0.98, 1.28
4	**Variable Selection Approach**: Stepwise Regression of Model 3 Variables	1.45 (0.82)	0.38, 6.38	0.92	0.85, 0.99	0.93	0.83, 1.03	1.12	0.98, 1.28

[a]Missing data on model variables were first imputed by multiple imputation with chained equations.
[b]Stabilized weights were calculated for inverse probability weighted models.
[c]Risk ratio estimates are adjusted for maternal age, race, education, insurance status, nulliparity, and smoking status.

Abbreviations: BMI, body mass index; HTN, hypertension; SGA, small for gestational age.

Appendix Table 6: Comparison of maternal and delivery characteristics between women with and without placental pathology

Variable[a,b]	Missing N	No Pathology Available (N=25,872)	Pathology Available (N=15,571)	IPW Up-weighted Pregnancies (n=3,914)
Maternal Age (yrs)	**9**	28.8 (± 5.8)	28.4 (± 6.0)	29.3 ± 5.3
Maternal Race n (%)	**307**			
Not Black		21075 (82.1)	12081 (78.1)	3345 (85.5)
Black		4598 (17.9)	3382 (21.9)	569 (14.5)
Education, n (%)	**437**			
High School/GED or Less		6597 (25.8)	4957 (32.1)	812 (20.7)
Some College		18988 (74.2)	10464 (67.9)	3102 (79.3)
Medical Insurance, n (%)	**115**			
Medicare/Medicaid		7873 (30.5)	4042 (26.0)	1332 (34.0)
Private/Self-Pay		17923 (69.5)	11490 (74.0)	2582 (66.0)
Pre-pregnancy BMI (kg/m^2)	**14,271**	24.9 (± 5.6)	26.4 (± 6.6)	24.7 ± 5.4
Pre-pregnancy BMI Classes, n (%)	**14,271**			
Underweight (<18.5kg/m^2)		807 (4.6)	394 (4.0)	202 (5.2)
Lean (18.5 to <25kg/m^2)		10042 (57.7)	4707 (48.2)	2302 (58.8)
Overweight (25 to <30kg/m^2)		3818 (21.9)	2377 (24.3)	840 (21.5)
Obese (≥30kg/m^2)		2737 (15.7)	2290 (23.4)	570 (14.6)
Smoking During Pregnancy, n (%)	**383**	2852 (11.1)	2663 (17.3)	309 (7.9)
Nulliparity, n (%)	**161**	10490 (40.8)	8937 (57.5)	1541 (39.4)
History of Multiple Abortion, n (%)	**161**	2619 (10.2)	1707 (11.0)	377 (9.6)
Maternal Indications				
Maternal Substance Use	**115**	331 (1.3)	819 (5.3)	0 (0.0)
Maternal Comorbidities[c]	**241**	5738 (22.3)	3398 (22.0)	857 (21.9)
Adverse Pregnancy History[d]	**5144**	4378 (19.6)	3176 (22.8)	759 (19.4)
Diabetes Status, n (%)	**115**			
No Diabetes		25351 (98.3)	13514 (87.0)	3,914 (100.0)

Variable[a,b]	Missing N	No Pathology Available (N=25,872)	Pathology Available (N=15,571)	IPW Up-weighted Pregnancies (n=3,914)
Gestational Diabetes		404 (1.57)	1661 (10.7)	0(0.0)
Pre-existing Diabetes		41 (0.2)	357 (2.3)	0 (0.0)
Pre-existing Hypertension, n (%)	**115**	248 (1.0)	604 (3.9)	2 (0.1)
Gestational Hypertension, n (%)	**156**	1083 (4.2)	1177 (7.6)	81 (2.1)
Preeclampsia, HELLP, Eclampsia, n (%)	**156**	231 (0.9)	1757 (11.3)	0 (0.0)
Suspected Intrauterine Infection, n (%)	**115**	788 (3.1)	1549 (10.0)	14 (0.4)
Pregnancy and Delivery Characteristics[c]				
IOM Weight Gain, n (%)	**15,737**			
Adequate		5604 (34.0)	2645 (28.6)	1380 (35.3)
Excessive		8100 (49.2)	4942 (53.5)	1904 (48.6)
Inadequate		2759 (16.8)	1656 (17.9)	630 (16.1)
Gestational Age (wks)	**0**	39.2 (± 1.1)	39.1 (± 1.2)	39.2 ± 1.1
Labor Delivery, n (%)	**63**	22153 (85.8)	13793 (88.7)	3320 (84.8)
Male Fetal Sex, n (%)	**188**	12962 (50.5)	7982 (51.3)	1968 (50.3)
Placental, Labor and Neonatal Indications for Placental Pathology				
Labor/Placenta Complications, n (%)[e]	**115**	749 (2.9)	1591 (10.2)	10 (0.3)
Fetal Complications, n (%)[f]	**115**	2054 (8.0)	3467 (22.3)	43 (1.1)
Congenital Abnormalities, n (%)[g]	**826**	113 (0.4)	267 (1.7)	0 (0.0)
5-Min Apgar Score (<4), n (%)	**332**	147 (0.6)	109 (0.7)	1 (0.0)
Small for Gestational Age, n (%)	**70**	1589 (6.2)	2121 (13.6)	120 (3.1)

[a]This table compares term pregnancies with available BMI that had placental pathology collected compared to term pregnancies that did not have placental pathology collected.
[b]Continuous variables are represented as mean ± standard deviation
[c]Maternal comorbidities included seizure disorders, thyroid disorders, vascular disorders, and uterine anomalies/cervical surgeries.
[d]Adverse pregnancy history included stillbirth, preterm birth, or miscarriage.
[e]Labor/placenta complications included fetal hydrops, oligohydramnios, placental accreta, placenta abruption, placenta previa, prolonged second stage of labor, prolonged membrane rupture.
[f]Fetal complications included fetal arrhythmias or a general clinical diagnosis of fetal distress.
[g]Congenital abnormalities included neural and cardiovascular congenital defects and chromosomal aneuploidy

Appendix Table 7: Pathology definitions for placental lesions

Pathology Finding	Description
Acute Chorioamnionitis	A maternal response to infected amniotic fluid characterized by the presence of a linear accumulation of neutrophils within the subchorionic fibrin or the chorionic plate itself.
Acute Vasculitis	A fetal response to infected amniotic fluid characterized by neutrophils within or emerging from the vessels of the fetal chorionic plate or umbilical cord
Acute Funisitis	Acute vasculitis of the umbilical cord vessels in which the neutrophils traverse through the vessel wall into the surrounding Wharton's jelly.
Villitis of Unknown Etiology	Lymphohistiocytic infiltrate within clusters of villi with no evidence for an underlying infectious etiology. Variable degrees of villous destruction are often present.
Intervillitis	Infiltrate of maternal mononuclear cells in the intervillous space, usually accompanied by an increase in perivillous fibrin
Acute Deciduitis	A significant linear accumulation of neutrophils within the decidual tissues of the placental basal plate or the extraplacental membranes
Villous Infarct	Devitalization of a region of placental villi due to obstruction of the underlying maternal blood flow. Characterized by a geographically limited loss of staining, often with collapse of the intervening maternal blood space.
Intraparenchymal Hemorrhage	Accumulation of blood within the intervillous space
Subchorionic Hemorrhage	Accumulation of blood that underlies a large portion of the chorionic plate
Decidual Vasculopathy	Incomplete, pathologically abnormal remodeling of maternal vessels supplying the placenta, with four often co-occurring manifestations. Absence of vascular remodeling is defined by the presence of a smooth muscle wall in at least one decidual vessel of the placental basal plate. Mural hypertrophy of decidual arterioles is characterized by thickening of the muscle wall of a decidual vessel from any location, with the thickened muscle wall leaving a luminal diameter of less than 30% of the total vessel diameter. Fibrinoid necrosis of vessel walls presents as a waxy, intense red degeneration of at least one decidual vessel wall from any location. Atherosis is defined by the presence of foamy macrophages within at least one decidual vessel wall. Fibrinoid necrosis and atherosis commonly co-occur.
Distal Villous Hypoplasia	A paucity of villi in relation to the surrounding stem villi. Stem villi appear thin and relatively elongated with an increased abundance of syncytial knots. Diagnosis made when present in ≥30% of a full-thickness slide of parenchyma.
Stromal Vascular Karyorrhexis	Karyorrhectic degeneration of the intravascular, endothelial and adjacent stromal nuclei.
Advanced/ Accelerated Villous Maturation	Characterized by the presence of at least two specific pathologic changes in the villous architecture. Advanced villous maturation is

	typically characterized by an increase in the percentage of villi containing a syncytial knot (increased syncytial knots), a decrease in the percentage of intermediate villi and/or distal villous hypoplasia (zones of abnormally long, thin, unbranched terminal villi).
Dysmaturity	Abnormal villous maturation that does not fit the patterns of accelerated or delayed villous maturation.
Fibrin Deposition	Small foci of fibrinoid maternal tightly encasing the entrapped villi (perivillous deposition) or an increased percentage (3% is the upper limit) of small foci of fibrinoid material within or adjacent to villi (intervillous deposition). A small amount of perivillous fibrin is acceptable in the upper third of the placental parenchyma.
Avascular Villi	Characterized by the loss of villous vessels, often with compaction and hyalinization of the villous stroma.
Villous Agglutination	A form of placental parenchymal injury seen in MVM and consists of small foci of villi that clump together in association with degenerative changes in the covering villous trophoblast; they are smaller than infarcts, and the affected villi maintain nuclear viability in their stromal elements.
Fetal Vascular Thrombosis	The presence of thromboses identified in fetal vessels or non-occlusive thrombus involving one chorionic plate vessel. Non-occlusive thrombi manifest as crescentic deposits of dense fibrin within the vascular lumen. Occlusive thrombi completely fill the vessel lumen, sometimes with extravasation of red cells or red cell fragments.
Intervillous Thrombus	A localized area of thrombosis within the intervillous space that is generally polygonal in shape, displaces adjacent trophoblastic villi and contains straight parallel laminations.
Chorioangioma	A benign well-circumscribed nodular lesion composed of fetal capillary vascular channels and supporting stroma, surrounded by trophoblast.
Chorangiosis	Diffuse increase in the number of vessels in the terminal villi. Diagnosed by 10 or more villi containing 10 or more capillary cross sections in several different regions of the placenta
Chorioangiomatosis	Patchy to diffuse network of small anastomosing capillaries surrounded by prominent pericytes and stromal cells primarily affecting immature intermediate and stem villi.
Delayed Villous Maturation	A monotonous villous population (≥ 10 villi) with reduced numbers of vasculosyncytial membranes for gestational age, a continuous cytotrophoblast layer and centrally placed capillaries. Diagnosis made when present in ≥30% of a full-thickness slide of parenchyma.
Placental Weight	Weight of placenta in grams
Placental Hypoplasia	Placental weight below the 10^{th} percentile for gestational age and/or an umbilical cord below the 10^{th} percentile.

Appendix Table 8: Maternal characteristics in women with and without pre-pregnancy BMI reported[a]

	Early Preterm Birth (<32 weeks)			Late Preterm Birth (32 to <37 weeks)		
Pregnancy Characteristics	Available BMI (n=455)	Missing BMI (n=445)	p-value	Available BMI (n=1,997)	Missing BMI (n=1,365)	p-value
Maternal Age (years)[b]	27.1 ± 6.4	27.4 ± 6.3	0.518	28.4 ± 6.2	28.4 ± 6.3	0.673
Race, n (%)			0.876			0.360
White	308 (68.1)	305 (68.8)		1461 (73.3)	973 (71.8)	
Black	128 (28.3)	127 (28.7)		463 (23.2)	317 (23.4)	
Other	16 (3.5)	11 (2.5)		69 (3.5)	65 (4.8)	
Education, n (%)			0.071			0.009
High School /GED or less	208 (46.0)	224 (52.3)		718 (36.1)	545 (40.6)	
Some College	244 (54.0)	204 (47.7)		1270 (63.9)	796 (59.4)	
Smoking in Pregnancy, n (%)	119 (26.4)	95 (21.7)	0.124	461 (23.2)	313 (23.3)	0.992
Nulliparous at Enrollment, n (%)	231 (50.8)	232 (52.1)	0.732	962 (48.2)	631 (46.3)	0.282
Multiple Abortion History, n (%)	70 (15.4)	70 (15.7)	0.959	287 (14.4)	200 (14.7)	0.859
Diabetes, n (%)			0.374			0.075
No Diabetes	421 (92.5)	400 (89.9)		1710 (85.6)	1141 (83.6)	
Pre-existing Diabetes	14 (3.1)	18 (4.0)		102 (5.1)	95 (7.0)	
Gestational Diabetes	20 (4.4)	27 (6.1)		185 (9.3)	129 (9.5)	
Chronic Hypertension, n (%)	54 (11.9)	44 (9.9)	0.397	152 (7.6)	128 (9.4)	0.079
Hypertensive Disorders, n (%)			0.247			0.023
No Hypertensive Disorders	305 (67.2)	321 (72.3)		1455 (73.0)	935 (68.7)	
Gestational Hypertension	14 (3.1)	12 (2.7)		73 (3.7)	52 (3.8)	
Preeclampsia, HELLP, Eclampsia	135 (29.7)	111 (25.0)		466 (23.4)	374 (27.5)	
Male Fetal Sex, n (%)	261 (57.4)	243 (54.6)	0.444	1068 (53.5)	753 (55.2)	0.354

Abbreviations: HELLP, Hemolysis, Elevated Liver enzymes, Low Platelets.

[a] Categorical variables were compared between deliveries with a recorded pre-pregnancy BMI and deliveries missing a pre-pregnancy BMI by chi-squared tests in early and late preterm births separately. Maternal age was compared across groups by t-tests.

[b] Maternal age is represented as mean ± standard deviation.

Appendix Table 9: Distribution of placental lesions in women with and without pre-pregnancy BMI data available among early and late preterm births[a]

	Early Preterm Birth (<32 Weeks)			Late Preterm Birth (32 to <37 Weeks)		
Placental Features	Available BMI (n=455)	Missing BMI (n=445)	p-value	Available BMI (n=1,997)	Missing BMI (n=1,365)	p-value
Chorioamnionitis, n (%)	230 (50.5)	228 (51.2)	0.889	410 (20.5)	282 (20.7)	0.962
Vasculitis, n (%)	153 (33.6)	155 (34.8)	0.756	160 (8.0)	128 (9.4)	0.185
Funisitis, n (%)	92 (20.2)	114 (25.6)	0.065	103 (5.2)	81 (5.9)	0.371
Deciduitis, n (%)	179 (39.3)	184 (41.3)	0.585	285 (14.3)	210 (15.4)	0.398
Villitis, n (%)	42 (9.2)	30 (6.7)	0.210	266 (13.3)	173 (12.7)	0.621
Intervillitis, n (%)	3 (0.7)	1 (0.2)	0.624	2 (0.1)	1 (0.1)	>0.999
Decidual Vasculopathy, n (%)	87 (19.1)	71 (16.0)	0.246	178 (8.9)	124 (9.1)	0.913
Villous Infarct, n (%)	101 (22.2)	92 (20.7)	0.634	275 (13.8)	209 (15.3)	0.230
Fibrin Deposition, n (%)	69 (15.2)	52 (11.7)	0.152	293 (14.7)	190 (13.9)	0.575
Placental Hypoplasia, n (%)	182 (41.7)	171 (40.6)	0.791	486 (25.3)	342 (26.1)	0.626
Advanced Villous Maturation, n (%)	155 (34.1)	148 (33.3)	0.853	538 (26.9)	355 (26.0)	0.574
Delayed Villous Maturation, n (%)	5 (1.1)	4 (0.90)	>0.999	53 (2.7)	28 (2.1)	0.303
Dysmaturity, n (%)	0 (0.0)	0 (0.0)	>0.999	3 (0.2)	1 (0.1)	0.651
Villous Agglutination, n (%)	3 (0.7)	3 (0.7)	>0.999	2 (0.1)	6 (0.4)	0.069
Distal Villous Hypoplasia, n (%)	0 (0.0)	1 (0.2)	0.494	2 (0.1)	6 (0.4)	0.069
Stromal Vascular Karyorrhexis, n (%)	1 (0.2)	0 (0.0)	>0.999	3 (0.2)	0 (0.0)	0.276
Intraparenchymal Hemorrhage, n (%)	0 (0.0)	1 (0.2)	0.494	1 (0.1)	0 (0.0)	>0.999
Subchorionic Hemorrhage, n (%)	1 (0.2)	0 (0.0)	>0.999	2 (0.1)	0 (0.0)	0.518
Avascular Villi, n (%)	18 (4.0)	19 (4.3)	0.945	117 (5.9)	89 (6.5)	0.476
Intervillous Thrombus, n (%)	38 (8.4)	27 (6.1)	0.232	191 (9.6)	121 (8.9)	0.531
Fetal Vascular Thrombosis, n (%)	75 (16.5)	52 (11.7)	0.049	236 (11.8)	148 (10.8)	0.742
Chorangiomatosis, n (%)	4 (0.9)	4 (0.9)	>0.999	15 (0.8)	23 (1.7)	0.019
Chorangiosis, n (%)	10 (2.2)	10 (2.3)	>0.999	133 (6.7)	112 (8.2)	0.104
Chorangioma, n (%)	8 (1.8)	8 (1.8)	>0.999	20 (1.0)	28 (2.1)	0.019

[a] All placental features are represented as n (%) and are compared between women with and without pre-pregnancy BMI data available by chi-squared and Fisher's exact tests.

Appendix Table 10: Odds ratios of placental latent classes with increasing pre-pregnancy BMI applying different regression methods by complete case analysis and multiple imputation[a,b]

Early Preterm Births (<32 weeks)			
Outcomes	**Regression Method**	**Complete Case (n=455) OR (95% CI)**	**Multiple Imputation (n=900) OR (95% CI)**
MVM + Chorioamnionitis (ref)	-	-	-
Acute Inflammation	1-step latent class regression	1.09 (1.04-1.14)	NA
	Most-likely class regression	1.06 (1.02-1.10)	1.04 (1.01-1.07)
	Probability-weighted regression	1.06 (1.02-1.10)	1.04 (1.00-1.08)
	Pseudo-class regression	1.04 (1.00-1.08)	1.03 (1.00-1.07)
MVM	1-step latent class regression	1.15 (1.09-1.22)	NA
	Most-likely class regression	1.09 (1.05-1.13)	1.07 (1.03-1.11)
	Probability-weighted regression	1.09 (1.05-1.14)	1.07 (1.04-1.11)
	Pseudo-class regression	1.07 (1.02-1.12)	1.05 (1.02-1.09)
Fetal Vascular Thrombosis with Hemorrhage	1-step latent class regression	1.07 (0.93-1.23)	NA
	Most-likely class regression	1.09 (1.04-1.14)	1.07 (1.02-1.12)
	Probability-weighted regression	1.09 (1.03-1.15)	1.07 (1.02-1.13)
	Pseudo-class regression	1.07 (1.02-1.13)	1.06 (1.01-1.11)
Late Preterm Births (32 to <37weeks)			
Outcomes	**Regression Methods**	**Complete Case (n=1,997) OR (95% CI)**	**Multiple Imputation (n=3,362) OR (95% CI)**
Low Risk Pathology (ref)	-	-	-
Acute Inflammation	1-step latent class regression	1.01 (0.98-1.03)	NA
	Most-likely class regression	1.00 (0.99-1.03)	1.00 (0.98-1.02)
	Probability-weighted regression	1.01 (0.99-1.03)	1.00 (0.98-1.02)

	Pseudo-class regression	1.01 (0.98-1.03)	1.00 (0.98-1.02)
MVM	1-step latent class regression	1.00 (0.98-1.03)	NA
	Most-likely class regression	1.01 (0.99-1.03)	1.01 (0.99-1.02)
	Probability-weighted regression	1.01 (0.99-1.03)	1.01 (0.99-1.03)
	Pseudo-class regression	1.00 (0.98-1.02)	1.00 (0.99-1.02)
Fetal Vascular Thrombosis with Hemorrhage	1-step latent class regression	1.03 (1.00-1.05)	NA
	Most-likely class regression	1.03 (1.01-1.05)	1.03 (1.01-1.05)
	Probability-weighted regression	1.03 (1.00-1.05)	1.03 (1.01-1.05)
	Pseudo-class regression	1.03 (1.00-1.05)	1.03 (1.01-1.05)

Abbreviations: MVM, maternal vascular malperfusion; NA, not applicable; ref, reference.

[a] Odds ratios represent the likelihood of a placental latent class relative to the likelihood of the reference class for each 1kg/m^2 increase in pre-pregnancy BMI. Models were adjusted for maternal race, education, smoking status and parity.

[b] Multiple imputation could not be used for single-step latent class regression.

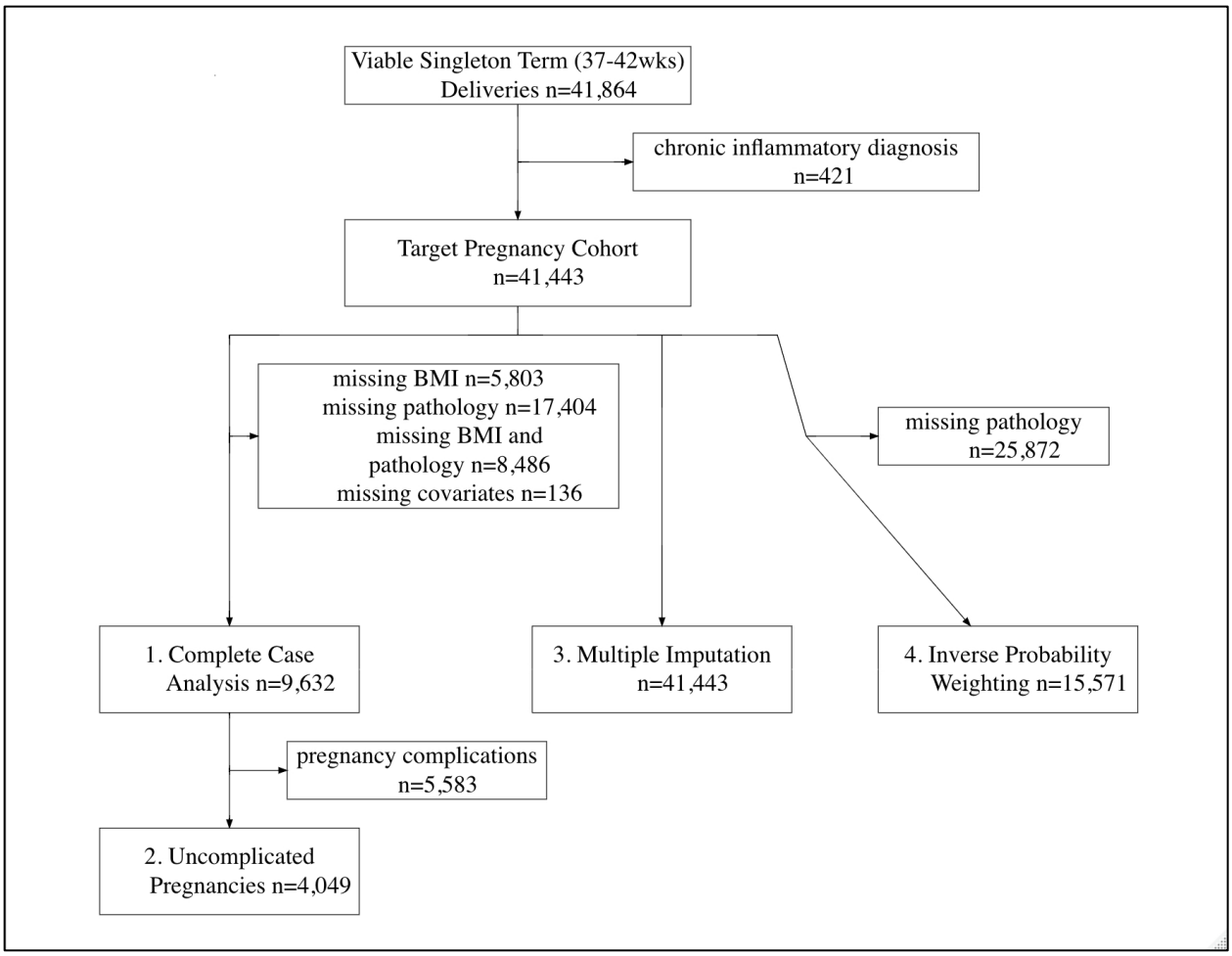
Appendix Table 11: Gene ontology biological pathways of preterm birth

Pathways	Number of Genes	FDR Adjusted P-value
Proteasomal Protein Catabolic Process	402	<0.0001
Neutrophil Degranulation	390	<0.0001
ncRNA Metabolic Process	389	<0.0001
Macroautophagy	259	<0.0001
Ribosome Biogenesis	258	<0.0001
Viral Life Cycle	279	<0.0001
Myeloid Cell Differentiation	337	<0.0001
Golgi Vesicle Transport	298	<0.0001
Mitochondrial Transplantation	125	<0.0001
ATP Metabolic Process	252	<0.0001
Electron Transport Chain	161	<0.0001
Response to Endoplasmic Reticulum Stress	239	<0.0001
Cell Cycle G2/M Phase Transition	223	<0.0001
Antigen Processing and Presentation	189	<0.0001
Regulation of Protein Stability	233	<0.0001
RNA localization	190	<0.0001
In-utero Embryonic Development	288	<0.0001
Regulation of Binding	273	<0.0001
Regulation of GTPase Activity	340	<0.0001
Negative Regulation of Transferase Activity	219	<0.0001
Protein Folding	175	<0.0001
Response to Starvation	156	<0.0001
Morphogenesis of a Polarized Epithelium	114	<0.0001
Regulation of Response to Biotic Stimulus	286	<0.0001
Aging	277	<0.0001

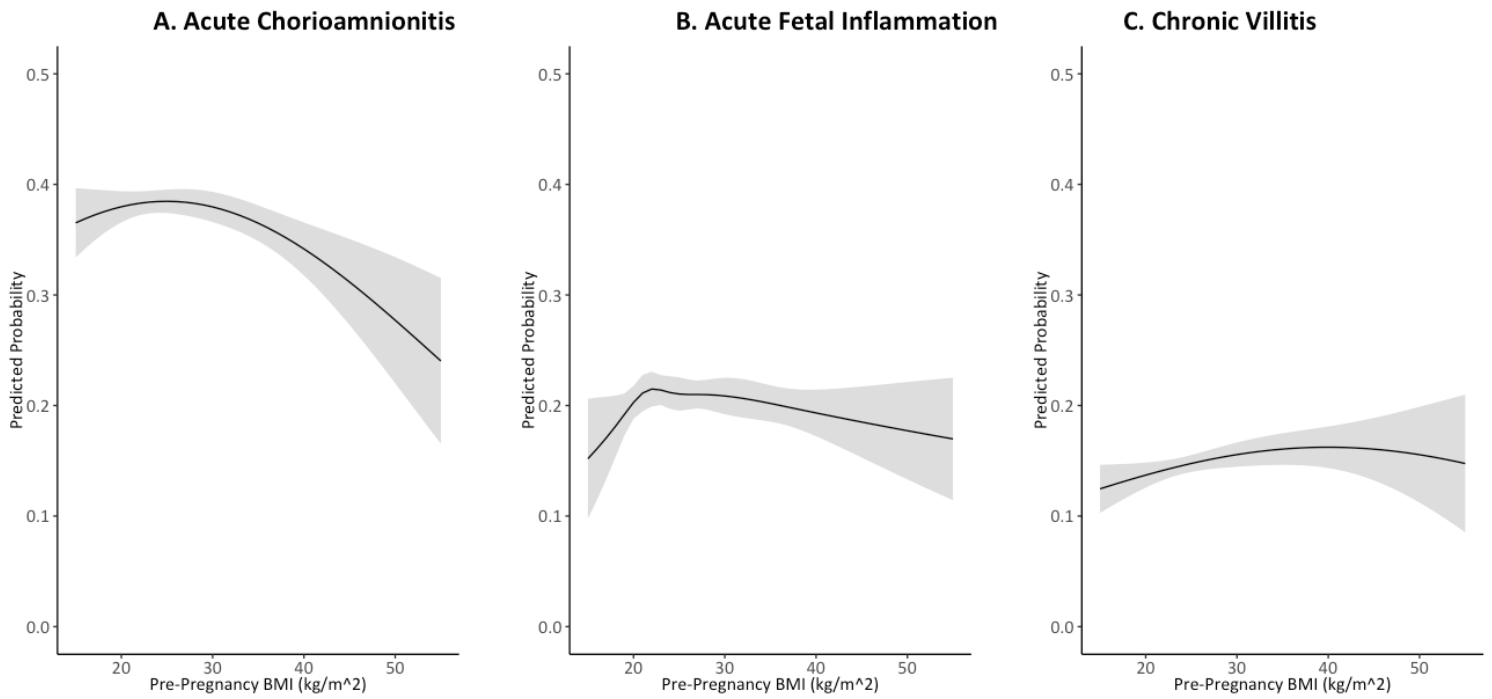
Appendix Table 12: Pregnancy characteristics of TIDES cohort[a,b]

	Overall (N=10)
Maternal Age, yrs	34.87 (3.057)
Maternal Race	
White	8 (80.0%)
Black	1 (10.0%)
Asian	1 (10.0%)
Marital Status	
Married	9 (90.0%)
Single	1 (10.0%)
Smoking During Pregnancy	
No	10 (100%)
Highest Education Attainment	
High-School	1 (10.0%)
Some College	9 (90.0%)
Number of Stressful Life Events	
None	7 (70.0%)
One Event	2 (20.0%)
Two Events	1 (10.0%)
Pre-Pregnancy BMI, kg/m^2	23.33 (2.544)
Overweight (>25kg/m^2)	2 (20.0%)
History of Infertility	4 (40.0%)
Nulliparous	3 (30.0%)
Gestational Weight Gain, lbs	22.50 (6.000)
Gestational Age, wks	40.21 (1.643)
Fetal Sex	
Female	5 (50.0%)
Male	5 (50.0%)

[a]Continuous Variables are represented as median (IQR) and categorical variables as n (%).

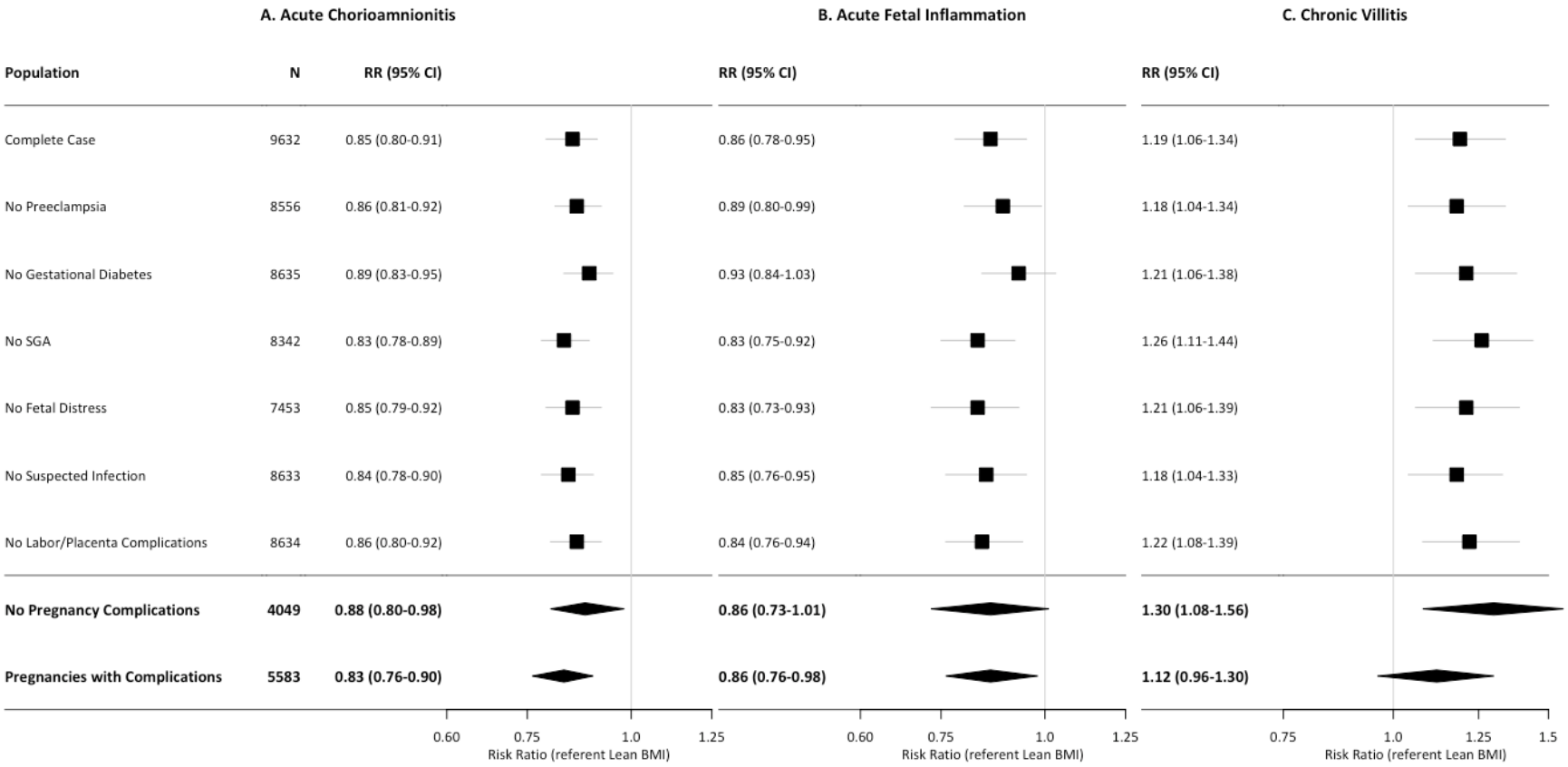


Appendix Figure 1: Study selection criteria and analysis plan



Appendix Figure 2: Predicted probability plots of associations between pre-pregnancy BMI and risk of A) acute chorioamnionitis, B) acute fetal inflammation and C) chronic villitis.

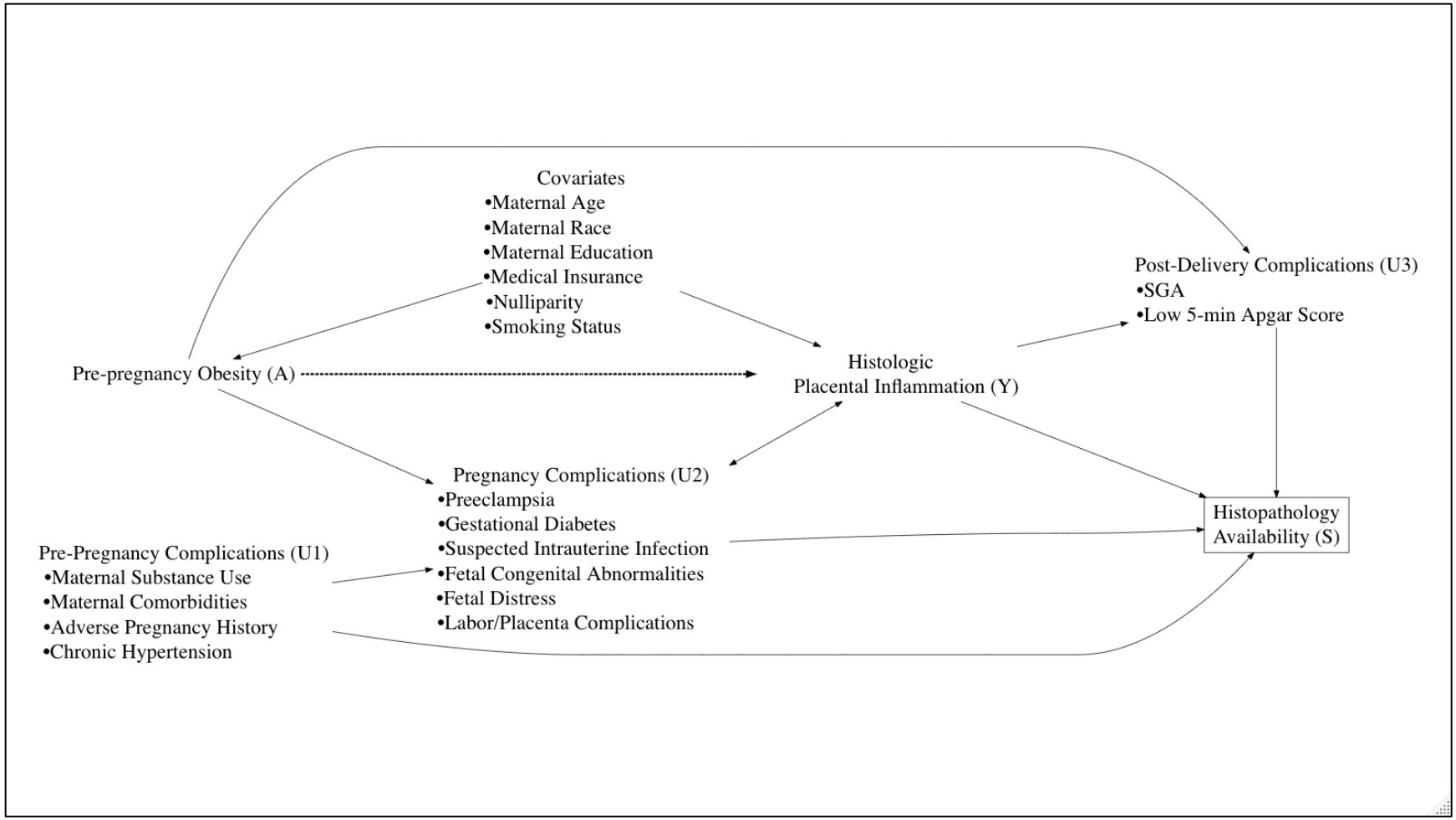
Predicted probability plots were derived from log-binomial models using inverse probability weights and models were adjusted for maternal age, race, insurance, education, nulliparity and smoking.



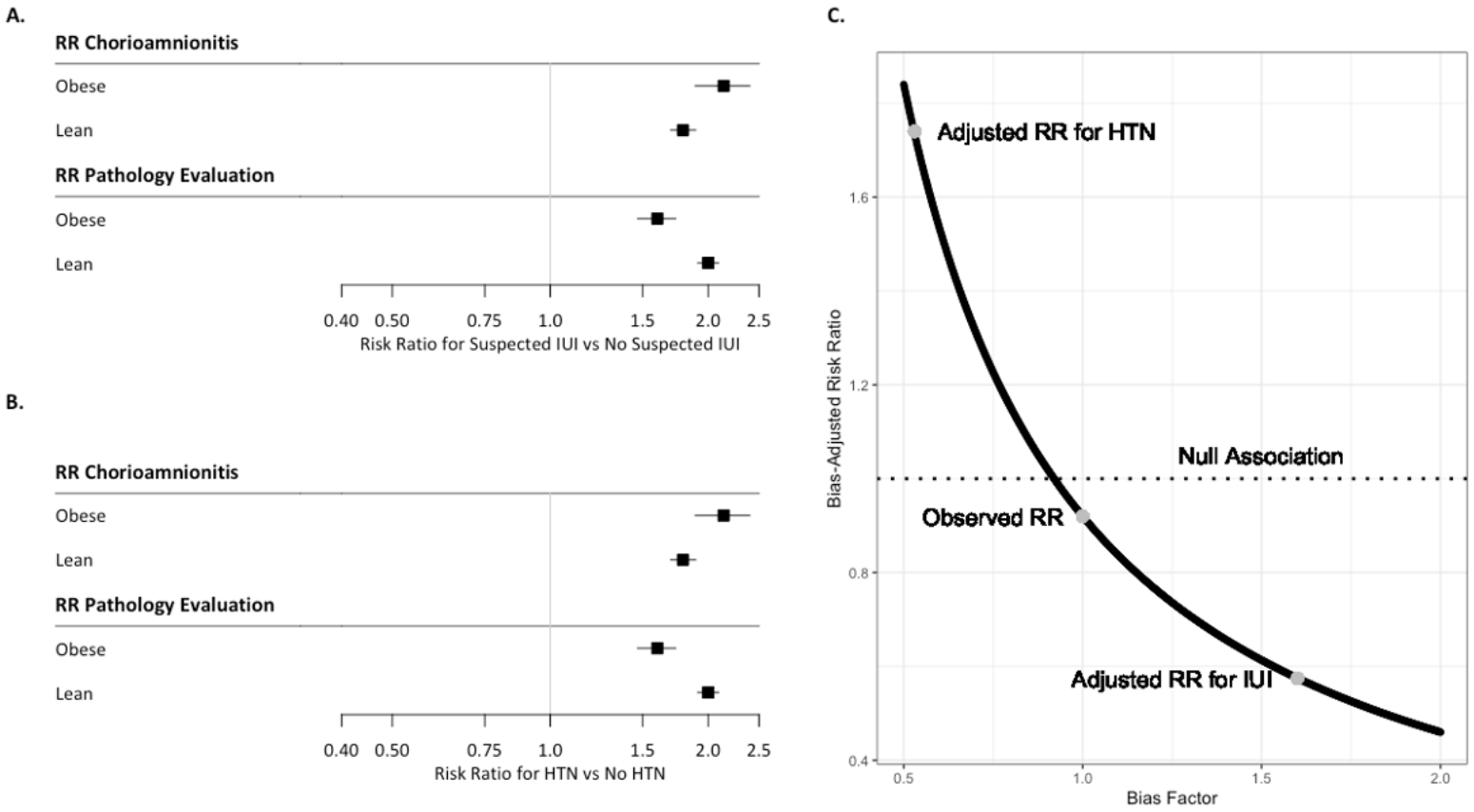
Appendix Figure 3: Risk ratios for A) acute chorioamnionitis, B) acute fetal inflammation, and C) chronic villitis in obese women compared to lean women after excluding pregnancy complications.

Risk ratios are adjusted for maternal age, race, education, insurance status, nulliparity, and smoking status.

Abbreviations: SGA; small for gestational age.



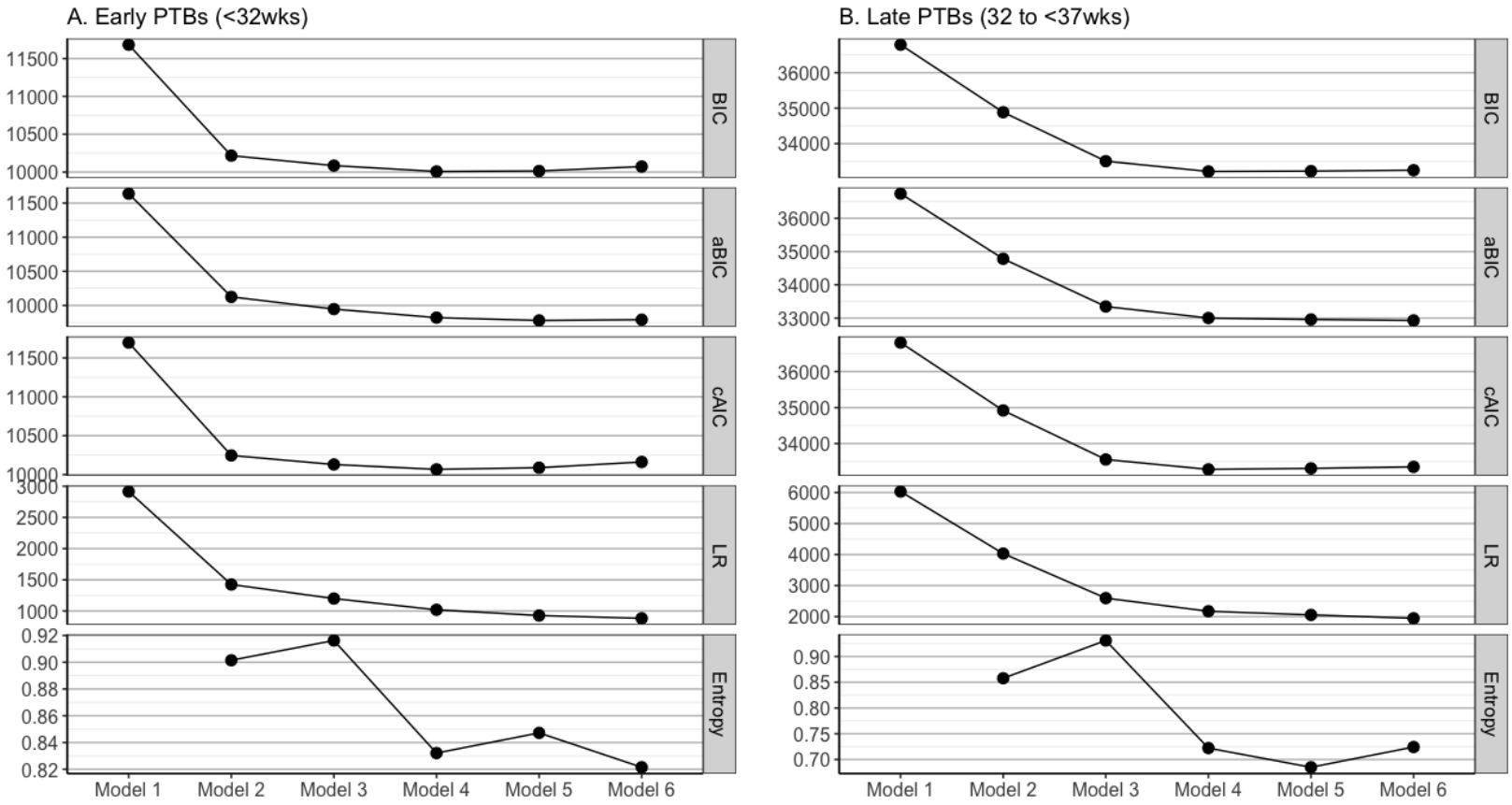
Appendix Figure 4: Causal diagram for research question



Appendix Figure 5: Bias factor derivation for pre-pregnancy obesity and risk of acute chorioamnionitis using suspected intrauterine infection and chronic hypertension as case examples.

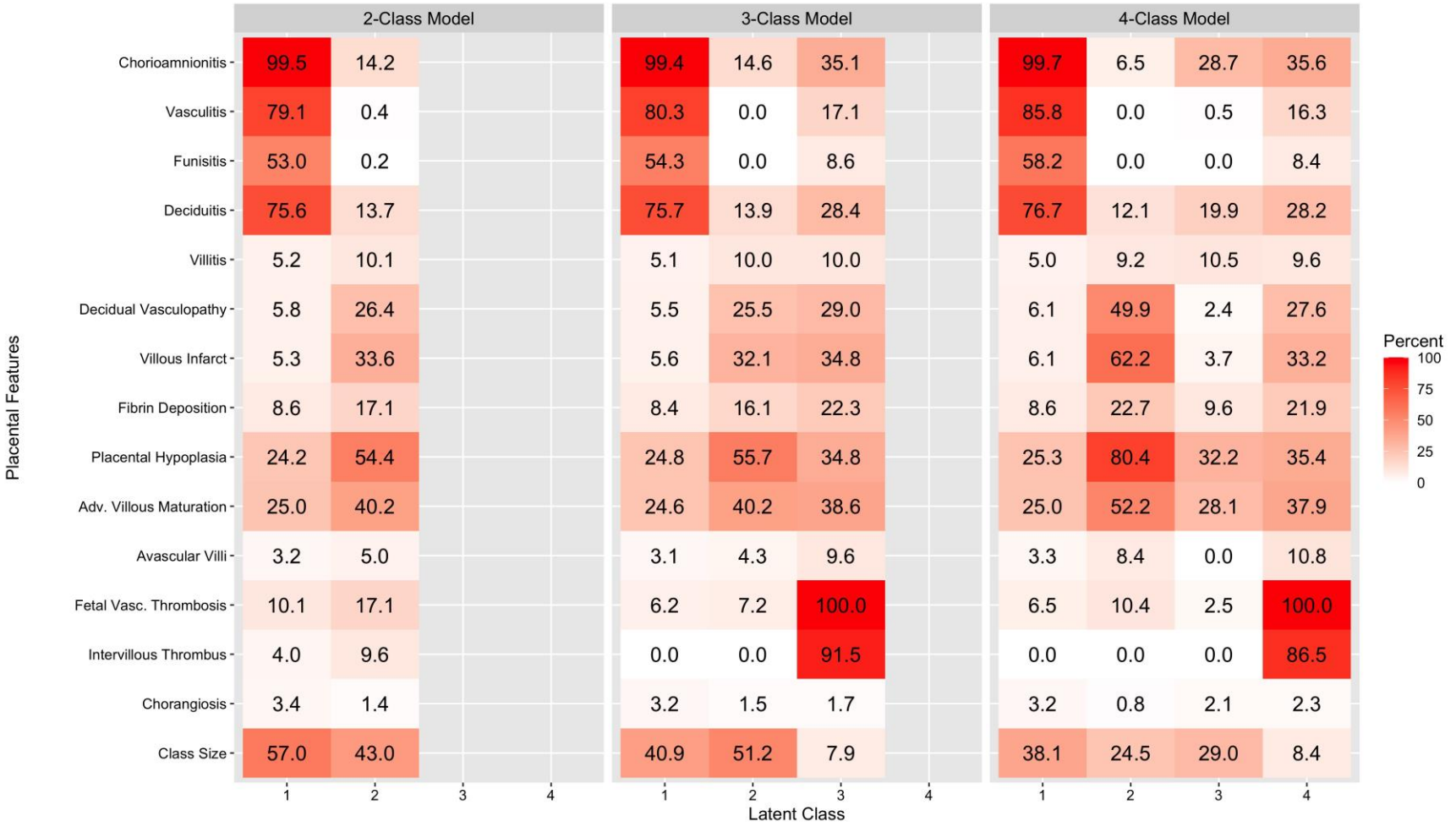
Forest plots demonstrate the RRs for chorioamnionitis and likelihood of placental histopathology stratified in obese and lean women with and without suspected intrauterine infection (A) as well as with and without chronic hypertension (B) required to calculate bias factors. Plot (C) shows the observed RR for pre-pregnancy obesity and risk of chorioamnionitis using inverse probability weights, the observed RR adjusted for the bias factor for suspected intrauterine

infection, and the observed RR adjusted for the bias factor for chronic hypertension. Risk ratios in forest plots A and B were estimated by univariate log-binomial regression.



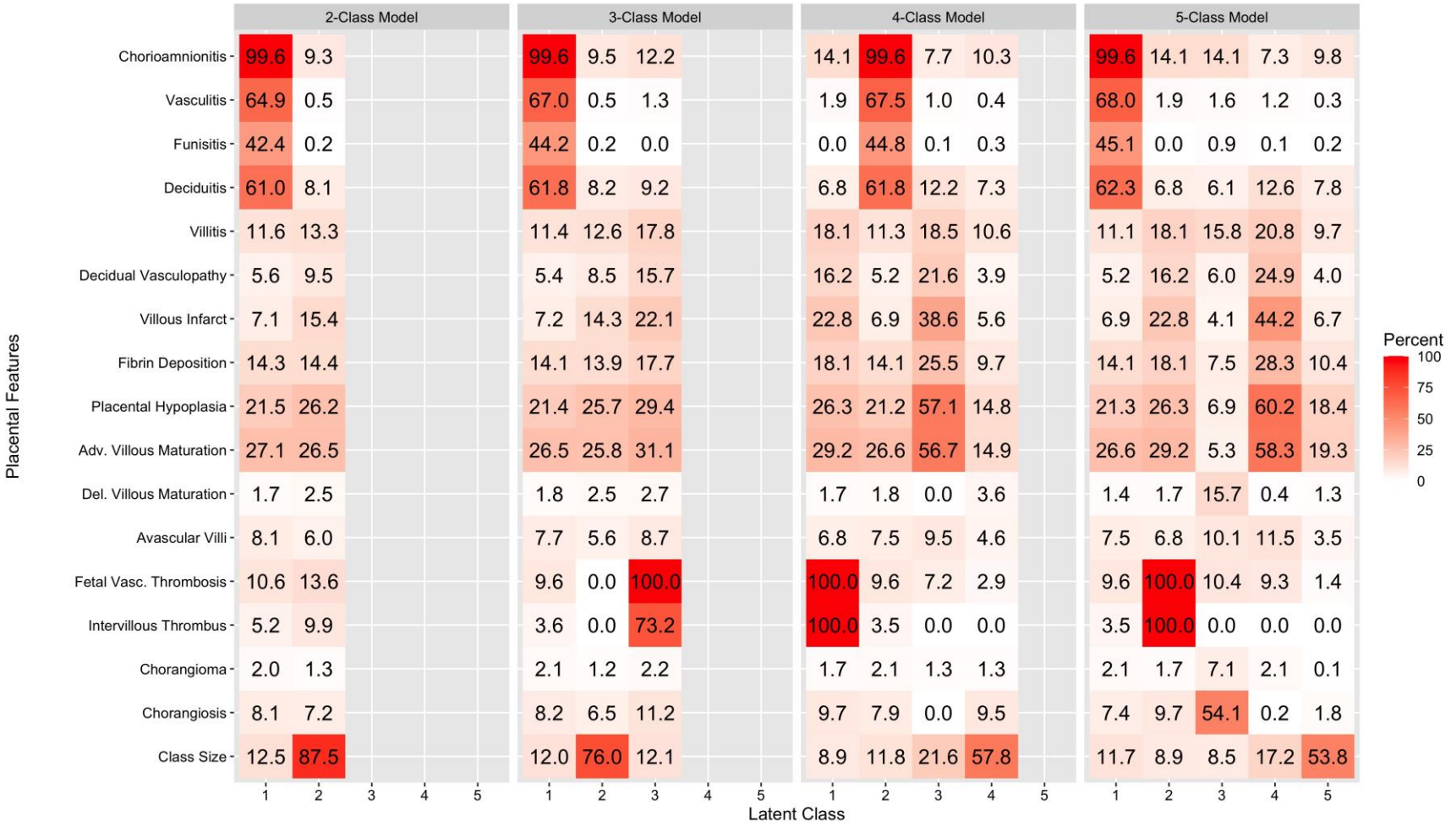
Appendix Figure 6: Scree plots for LCA models with 1 to 6 latent classes in A) early and B) late preterm births.

Models 1 to 6 indicate LCA models with 1 to 6 latent classes. Lower scores for BIC, aBIC, cAIC, and LR indicate a better fitting model. A higher entropy score indicates higher accuracy in separating PTBs into latent classes. Latent class 6 in the early preterm birth models did not converge, which could result in inaccurate values for empirical statistics. Abbreviations: aBIC, adjusted Bayesian Information Criterion; BIC, Bayesian Information Criterion; cAIC, corrected Akaike Information Criterion; LR, likelihood-ratio test, PTB, preterm birth.



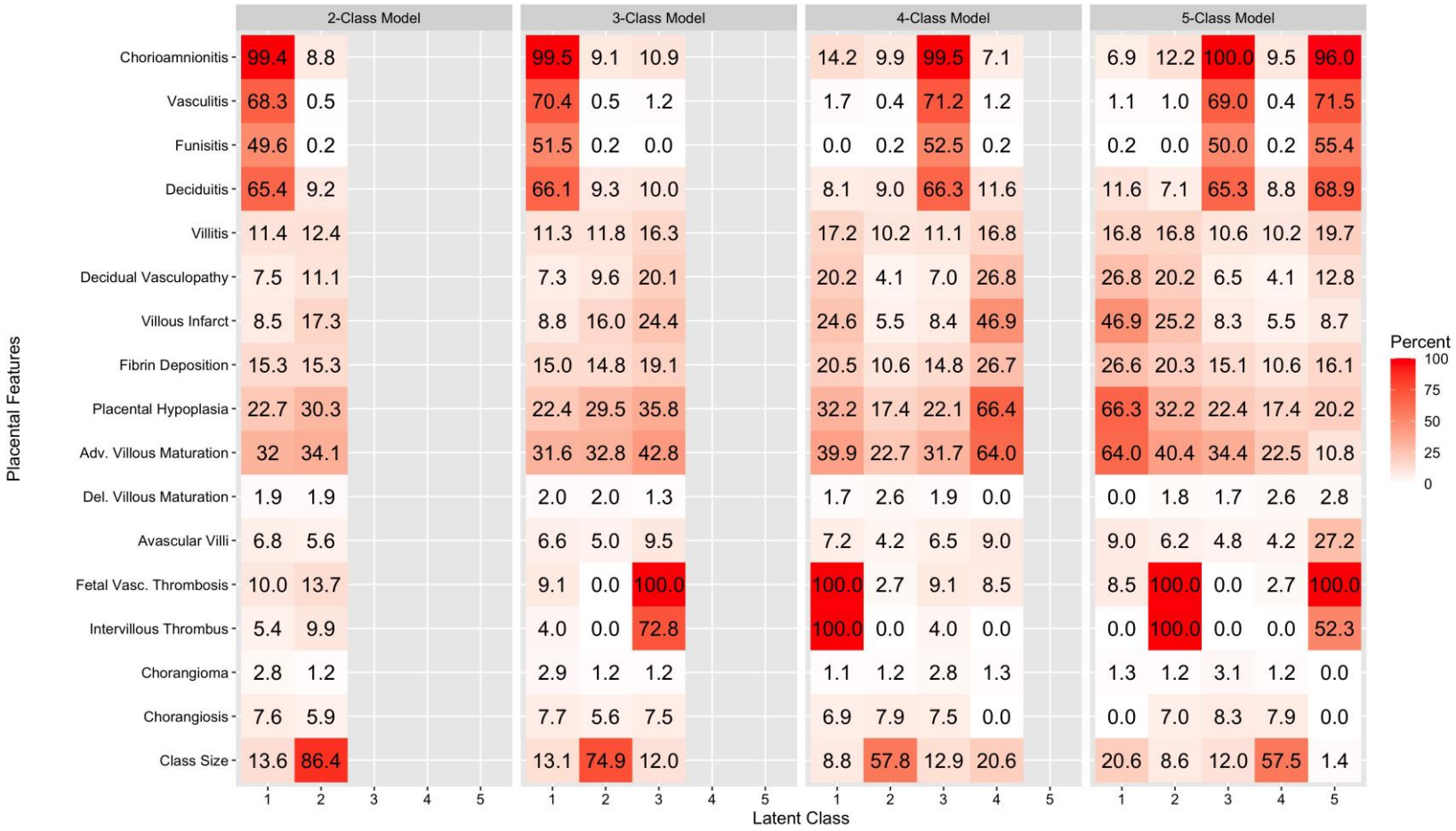
Appendix Figure 7: Heat map of placental features across latent class models in early preterm births (n=900).

Each panel represents a model with 2 to 4 latent classes and each column within a panel represents one of the latent classes within the model. Values in each cell represent the conditional probability of a placental feature occurring in a particular class. The cells in the bottom row indicate the proportion of early preterm births in each latent class. Abbreviations: Adv, advanced; Vasc, vascular.



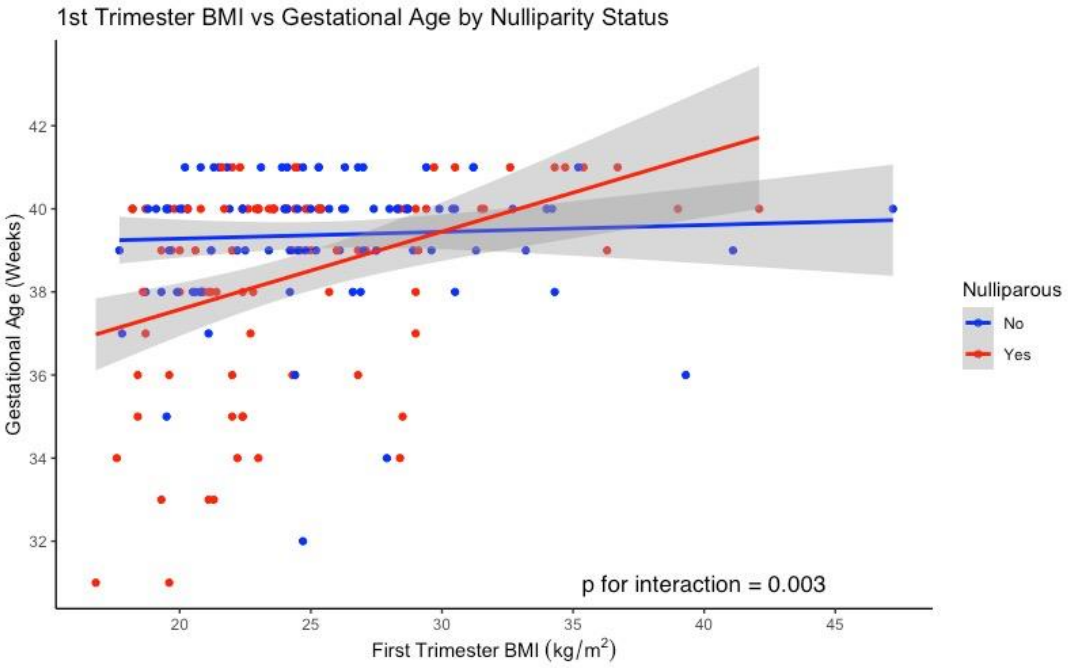
Appendix Figure 8: Heat map of placental features across latent class models in late preterm births (n=3,362).

Each panel represents a model with 2 to 5 latent classes and each column within a panel represents one of the latent classes within the model. Values in each cell represent the conditional probability of a placental feature occurring in a particular class. The cells in the bottom row indicate the proportion of late preterm births in each latent class. Abbreviations: Adv, advanced; Del, delayed; Vasc, vascular.



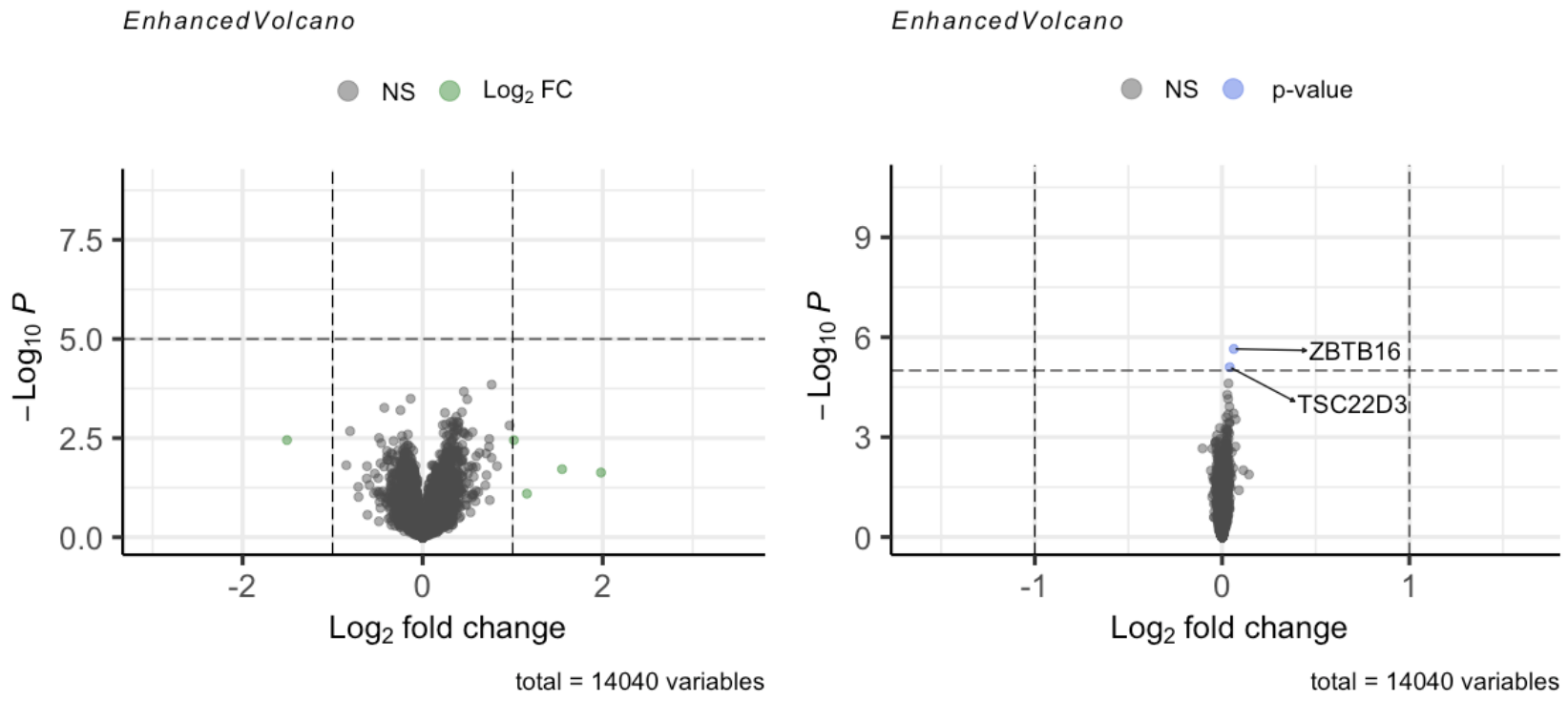
Appendix Figure 9: Supplementary Figure 4: Proportion of placental features across latent class models in late preterm births excluding 36 weeks (n=1,995).

Each panel represents a model with 2 to 5 latent classes and each column within a panel represents one of the latent classes within the model. Values in each cell represent the conditional probability of a placental feature occurring in a particular class. The cells in the bottom row indicate the proportion of late PTBs in each latent class. Abbreviations: Adv, advanced; Del, delayed; Vasc, vascular.



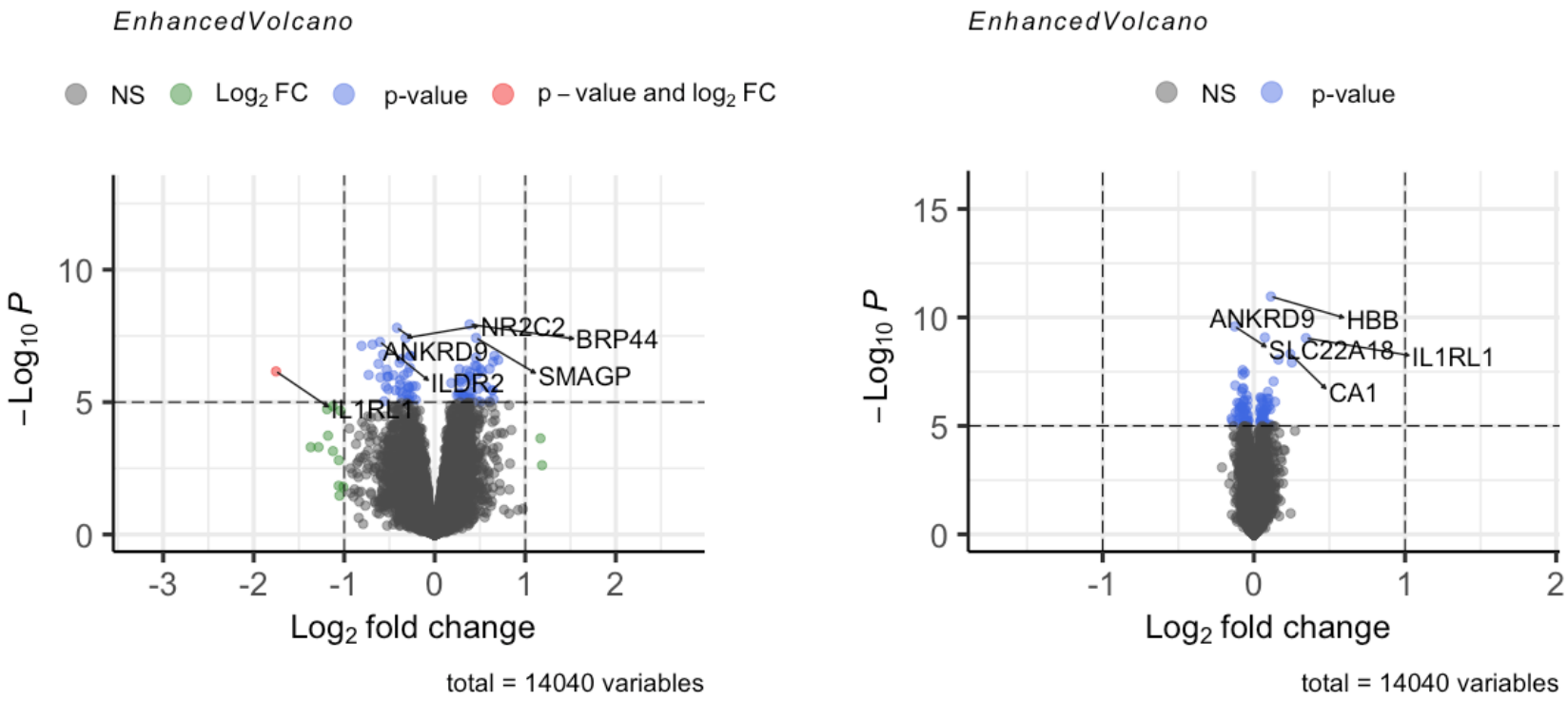
Appendix Figure 10: First trimester BMI and gestational age by parity status

Associations were measured by linear regression models adusted by maternal age, smoking status, and neonatal ethnicity. The joint affect of BM and nulliparity status on gestational age was modeled by an interaction term in linear regression models (BMI x nulliparity interaction p-value =0.003).



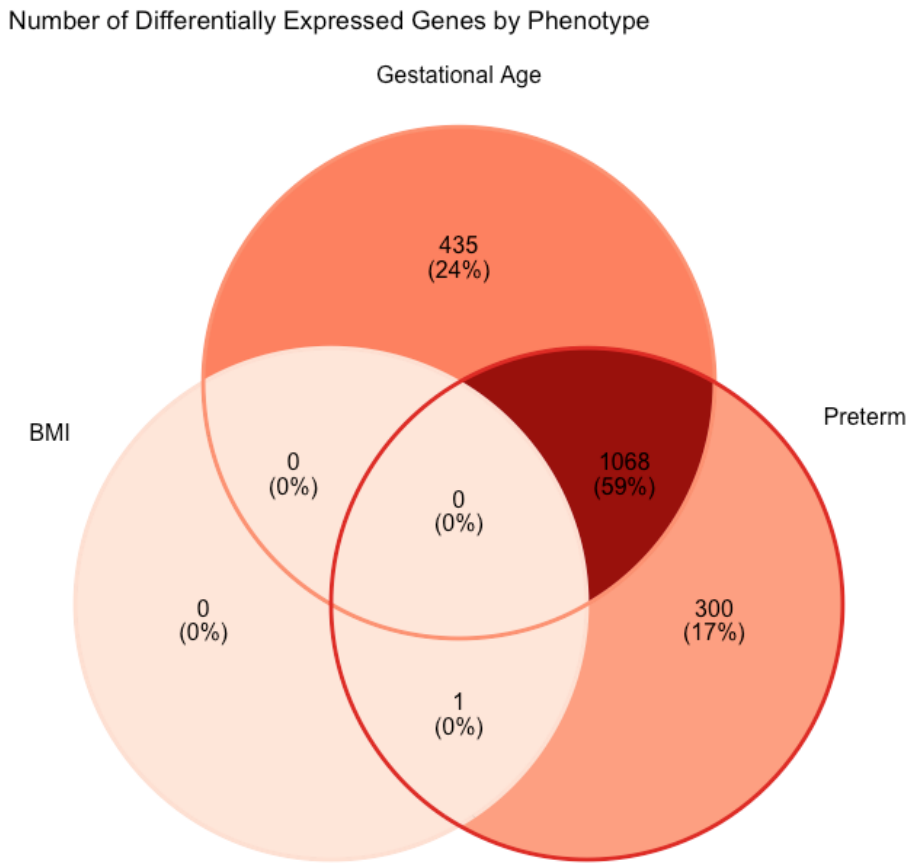
Appendix Figure 11: Volcano plots of differentially expressed genes by maternal BMI

Left Plot) Volcano plot of differentially expressed genes by obesity status. Right Plot) Volcano plot of differentially expressed genes for a 1kg/m^2 increase in first trimester BMI. For the Volcano plots green dots indicate genes have greater than a log2 fold change of 1 in gene expression but are not statistically significant at the expresssion an FDR adjusted p-value <0.00001, blue dots indicate genes that have statistically signifiant differential gene expression at adjusted p<0.0001 but the log2-fold change is less than 1 in absolute magnitude, and red dots indicate genes that have a log2 fold change greater than 1 and are statistically significant at an adjusted p-value <0.00001.



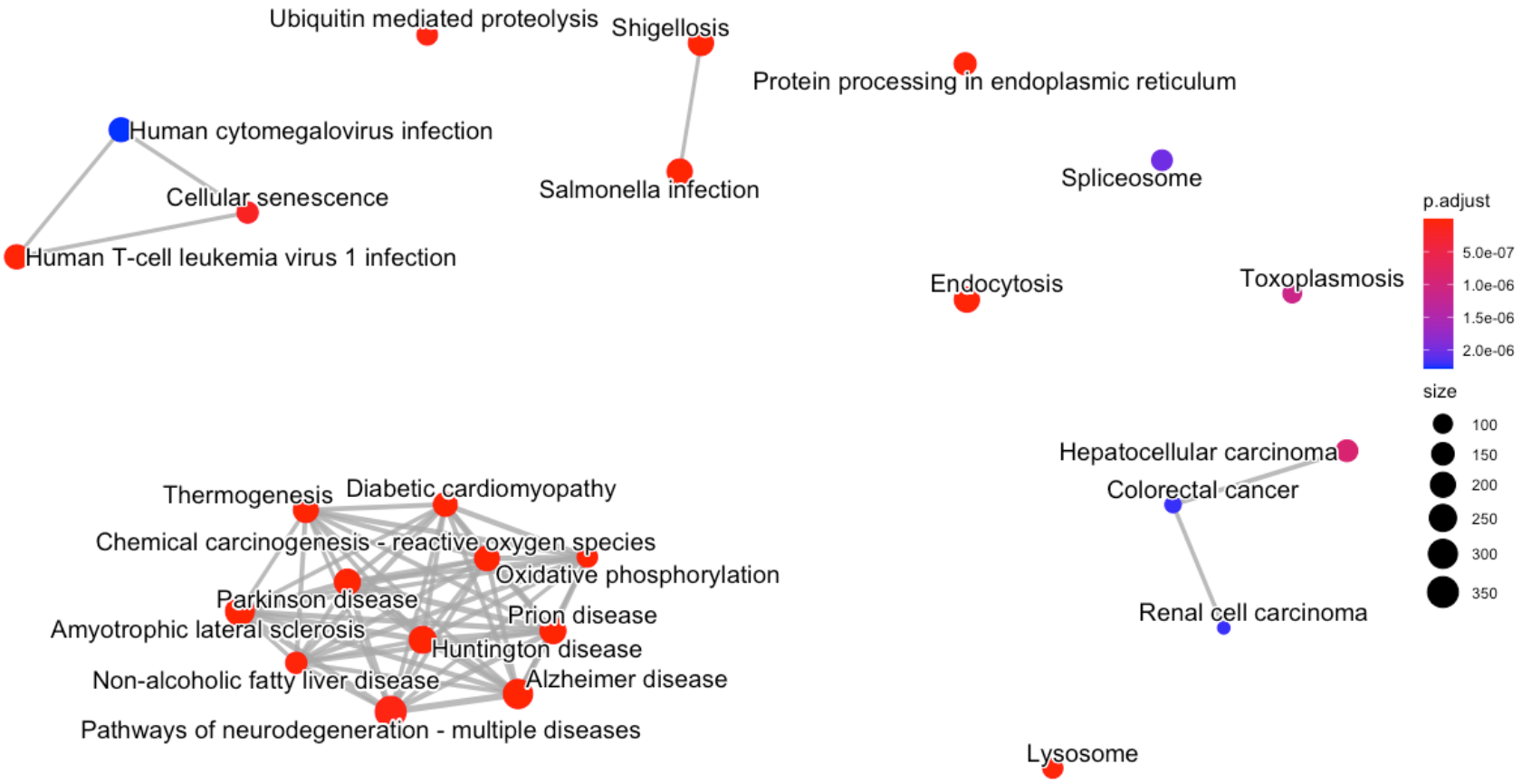
Appendix Figure 12: Volcano plots of differentially expressed genes by gestational age

Left Plot) Volcano plot of differentially expressed genes by preterm birth status. Right Plot) Volcano plot of differentially expressed genes for a 1-week increase in gestational age at delivery. For the Volcano plots green dots indicate genes have greater than a log2 fold change of 1 in gene expression but are not statistically significant at the expresssion an FDR adjusted p-value <0.00001, blue dots indicate genes that have statistically signifiant differential gene expression at adjusted p<0.0001 but the log2-fold change is less than 1 in absolute magnitude, and red dots indicate genes that have a log2 fold change greater than 1 and are statistically significant at an adjusted p-value <0.00001.



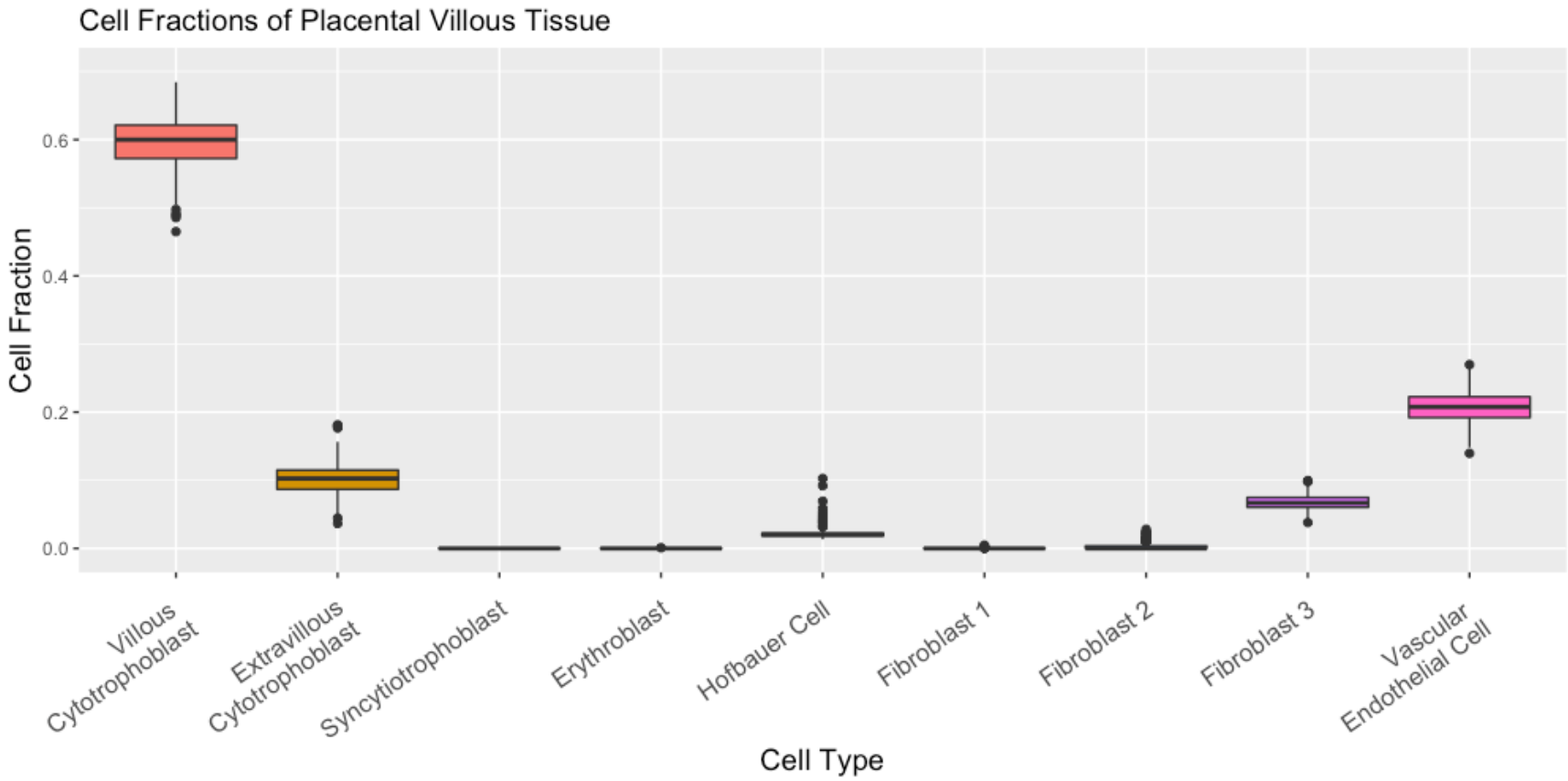
Appendix Figure 13: Overlap in differentially expressed genes by BMI and gestational age

The venn-diagram depicts the number of differentially expressed genes for a 1-kg/m^2 increase in BMI (left circle), a 1-week increase in gestational age at delivery (top circle) and between preterm and term prengnancies (right circle). Where 2-circles overlap indicates genes that are differentially expressed between two of the three phenotypes.



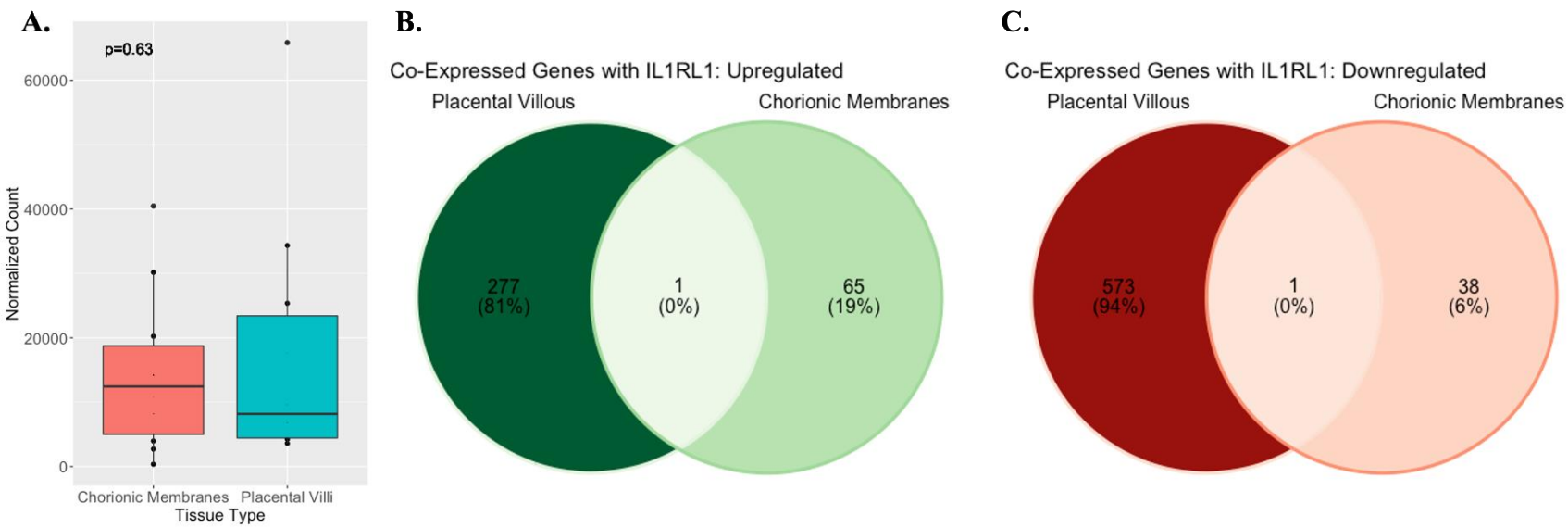
Appendix Figure 14: Enrichment map of KEGG enriched pathways in preterm vs term pregnancies

The size of the nodes indicates the number of genes eriched in a pathway. The color of the nodes indicate the FDR adjusted p-value for each gene set with red indicaing a lower adjusted p-value. Clustering of nodes indicates pathways with overlapping genes.



Appendix Figure 15: Estimated placental cell proportions in ENVIRONAGE birth cohort.

Estimated placental cell proportions for all 183 pregnancies are depicted using box plots based on the *Suryawanshi et al 2018* single cell RNA-Seq reference dataset. The bold horizontal line for each box represents the median cell-proportions.



Appendix Figure 16: Genes co-expressed with IL1RL1 expression in placental villous and chorion membrane tissue

A) Difference in gene epxression of IL1RL1 in chorionic membranes and placental villous tissue by Wilcoxon signed rank test. B) Venn-diagram indicating the number of upregulated genes with with increasing IL1RL1 expression in placental villous tissue and chorionic membranes.. C) Venn-diagram indicating the number of downregualted genes with with increasing IL1RL1 expression in placental villous tissue and chorionic membranes. Where 2-circles overlap indicates genes that are differentially expressed in the same direction for both tissue types. Genes were considered significantly differentially expressed using an FDR adjusted p-value <0.05.

Appendix A Supplemental Methods for Manuscript 1

Appendix A.1.1 Selection Bias Variables

Maternal characteristics and respective ICD-9/ICD-10 codes included maternal substance use, chronic hypertension (ICD-9: 642.00-642.24; ICD-10: O10.02-O10.93, O11.1-O11.3) pre-existing diabetes (ICD-9: 250.00-250.93, 648.00-648.04, ICD-10: O24.011-O24.013, O24.111-O24.12, O24.311-O24.33, O24.811-024.93) and other maternal comorbidities. Maternal substance use was defined as self-reported use of alcohol, cocaine, narcotic, marijuana, or other substances (amphetamines, barbiturate, anti-depressants, hallucinogens; ICD-9: 304.00-305.93; ICD-10: F10.10-F19.99). Maternal comorbidities included hyperthyroidism (ICD-9: 242.00-242.41, 242.80-242.91), hypothyroidism (243.00, 244.00-244.30, 244.80, 244.90), seizure disorders (ICD-9: 345.00-345.91, 649.40-649.42, 780.39; ICD-10: G40.A01-G40.A19, G40.401-G40.519, G40.804, G40.822-G40.824 G40.901, G40.909, O99.350-O99.355, R56.9), uterine anomalies (ICD-9: 218.0-218.9, 654.10-654.00-654.93, 694.9, 752.2-752.752.39; ICD-10: D25-D25.9, O34.00-O34.29, O34.80-O34.90, Q51.0-Q51.818), cervical abnormality (ICD-9: 654.51-654.54; ICD-10: O34.30-O34.43), and vascular conditions. Vascular conditions comprised of pulmonary embolism (ICD-9: 415.11-415.19, 416.2; ICD-10: I26.01-I26.99, I27.82), heart operation, (ICD-9 procedure code: 362, 370, 390, 391, 3500-3799, 3922-3999), structural heart disease (ICD-9: 391.0-39.899, 414.8, 422.90-422.99, 424.0-424.43, 424.90-424.99, 425.0-425.9, 4 745.0-746.9, V433; ICD-10: I01.0-I09.9, I25.5, I40.0-I43, I34.0-I39, Q20.0-Q24.8, Z952), and thrombocytopenia (ICD-9: 641.30-641.33, 649.30-649.34, 666.30-666.34; ICD-10: D65, O45.021-045.23, O46.009-O46.029, O46.099, O99.111-O99.13).

Pregnancy outcomes and corresponding ICD-9/ICD-10 codes included gestational diabetes diagnosed by the Carpenter-Coustan criteria (ICD-9: 648.80-648.84; ICD-10: O24.410-O24.439, O99.810-O99.815), gestational hypertension (ICD-9: 642.30-642.34; ICD-10: O13.3-O13.5), preeclampsia/eclampsia (ICD-9: 642.40-642.74; ICD-10: O14.00-O14.25, O15.00-O15.9, O11.1-O11.9). Labor and delivery outcomes included a clinical diagnosis of fetal distress or fetal arrhythmia (ICD-9: 659.70-659.73; ICD-10: O36.8990, O76), suspected intrauterine infection defined as clinical chorioamnionitis (ICD-9: 658.40-658.43, ICD-10: O411.039, O411.210-O411.239), premature rupture of membrane (ICD-9: 658.10-658.33; ICD-10: O42.011-O42.02, O42.911-O42.92), maternal sepsis (ICD-9: 380-389, 670.20-670.24, 995.91, 995.92; ICD-10: A40.0-A41.9, O85, O86.04, R65.20, T80.211A, T81.4XXA, T81.44XA, T81.44XD, T81.44XS) or maternal fever during labor (ICD-9: 659.20-659.23, ICD-10: O75.2); and labor/placental complications: prolonged rupture of membranes (ICD-9: 65.820-658.23, ICD-10: O42.10-O42.113, O421.2, O42.90), prolonged second stage of labor (ICD-9: 662.20; ICD-10: O63.1), uterine rupture (ICD-9: 665.00-665.11, ICD-10: O71.00-O7.13), placenta previa (ICD-9: 641.00-641.13, ICD-10: O44.00-O44.13), oligohydramnios (ICD-9: 658.00-658.03, ICD-10: O41.00X0-O41.03X9), placental abruption (ICD-9: 641.20-641.23; ICD-10: O45.001-O45.93), or placenta accreta, increta, percreta (ICD-9: 656.70-656.73, 666.00- 667.04; ICD-10: O43.101-O43.92, O72.0, O73.0, O73.1). Congenital defects were defined as structural heart defects (ICD-9: 424.0, 424.1, 745.10-747.11; ICD-10: I340, I348, Q20.03, Q2.10-Q25.4), congenital anatomical central nervous system abnormalities (ICD-9: 740.00-742.9, ICD-10: Q00.0-Q07.9) and chromosomal abnormalities (ICD-9: 758.0-759.9, 759.81; ICD-10: Q87.1, Q90.0-Q98.9). Variables in the MOMI database and corresponding ICD-9/ICD-10 codes can be publicly accessed at http://35.227.121.98:5000/data-dictionary.

Appendix A.1.2 Model Fitting for Continuous Variables

We measured the dose-response relationships between pre-pregnancy BMI and risk of placental inflammatory lesions. Our rationale was to ensure we did not miss any curvilinear associations between the exposures measured as continuous variables and risk of acute and chronic placental inflammation. We determined the proper functional form for pre-pregnancy BMI by comparing three different generalized estimating equation models: 1) a model with the exposures measured as linear terms, 2) a model with the exposures measured using a quadratic polynomial and 3) a model with the exposures measured using restricted cubic splines with 5 knots located at the 0.05, 0.275, 0.5, 0.725, and 0.95 quantiles. We considered quadratic terms, as both lower and upper extremes of pre-pregnancy BMI may increase placental inflammation. We considered restricted cubic splines with 5 knots because these parameters offer sufficient flexibility for sample sizes larger than 100 observations. The optimal models for pre-pregnancy BMI were determined based on the quasi-likelihood under the independence criterion (QIC) and QICu statistics, with lower QIC and QICu values representing better fitting models. For any discrepancies between the QIC and QICu statistics, the more parsimonious model was selected.

Appendix A.1.3 Additional details on IPW

We determined the variables, functional forms of variables, and interaction terms to include in the logistic regression model by comparing RRs and distribution of weights across models of varying complexity.[10] Maternal and pregnancy characteristics were compared between women

without placental histopathology, women with histopathology data, and women with histopathology with inverse probability weights in the highest quartile (>75th percentile) to evaluate if upweighted deliveries after IPW were similar to deliveries without placental histopathology.

Appendix A.1.4 Derivation of Residual Selection Bias Factor

Risk ratio estimates were adjusted for by a selection bias factor based on the methods described in *Smith and VanderWeele 2019*.[253] The authors posit that the amount of residual bias is reflected by dividing the observed risk ratio by the true risk ratio (*Equation 1).*

$$\textit{Equation 1B} = \frac{RR_{AY}^{obs}}{RR_{AY}^{true}}$$

Since the true risk ratio between the exposure (A) and the outcome (Y) is not known, the bias factor caused by some factor (U) can be estimated by the equation (*Equation 2)*:

$$\textit{Equation 2B} \leq \left(\frac{RR_{UY|(A=1)}\ x\ RR_{SU|(A=1)}}{RR_{UY|(A=1)} + RR_{SU|(A=1)} - 1}\right) \text{ x } \left(\frac{RR_{UY|(A=0)}\ x\ RR_{SU|(A=0)}}{RR_{UY|(A=0)} + RR_{SU|(A=0)} - 1}\right)$$

Thus, for a given factor (U), the amount of bias (B) can be derived by four risk ratio estimates

- risk ratio between U and the outcome (Y) in the exposed (A=1); $RR_{UY|(A=1)}$
- risk ratio between U and the outcome (Y) in the unexposed (A=0); $RR_{UY|(A=0)}$
- risk ratio between U and selection into the study (S) in the exposed (A=1); $RR_{SU|(A=1)}$

- risk ratio between U and selection in the study (S) in the unexposed (A=0); $RR_{SU|(A=0)}$

The bias factor (B) can then be divided from the observed estimate to get the true risk ratio estimate. We reasoned any unmeasured factor in our study would at most bias our observed risk ratio estimates by as much as the strongest measured factors in our study. Therefore, by calculating the adjusted risk ratio estimates due to observed pregnancy indications for placental histopathology we were able to get a range of plausible risk ratio estimates accounting for residual confounding.

To help with the interpretation of residual selection bias we will use two examples of clinical indications that may bias the association between pre-pregnancy obesity and risk of acute chorioamnionitis. The first example assumes that there is residual selection bias from oversampling pregnancies with suspected intrauterine infection (IUI), a placental histopathology indication associated with a higher risk of chorioamnionitis. We can estimate the bias due to IUI by calculated the four aforementioned RRs:

- RR between IUI and chorioamnionitis in obese women $RR_{UY|(A=1)}$ = 2.14
- RR between IUI and chorioamnionitis in lean women $RR_{UY|(A=0)}$ = 1.76
- RR between IUI and having a histopathology evaluation in obese women $RR_{SU|(A=1)}$ = 1.60
- RR between IUI and having a histopathology evaluation in lean women $RR_{SU|(A=0)}$ = 2.12

These RR are used to calculate a bias factor:

$$B \leq \left(\frac{RR_{UY|(A=1)}\ x\ RR_{SU|(A=1)}}{RR_{UY|(A=1)}\ +\ RR_{SU|(A=1)} - 1}\right) \text{ x } \left(\frac{RR_{UY|(A=0)}\ x\ RR_{SU|(A=0)}}{RR_{UY|(A=0)}\ +\ RR_{SU|(A=0)} - 1}\right)$$

$$\text{B} \leq \left(\frac{2.14\ x\ 1.60}{2.14 + (1.60-1)}\right) \text{ x } \left(\frac{1.76\ x\ 2.12}{1.76 + (2.12-1)}\right) \leq 1.60$$

The observed RR between obesity and risk of acute chorioamnionitis is adjusted for the bias factor to give the true bias-adjusted RR, which shifted to a stronger protective effect (RR_{obs}: 0.92 to RR_{true} = 0.58)

$$RR_{True} = \frac{RR_{obs}}{B} = \frac{0.92}{1.60} = 0.58$$

The second example, assumes that there is residual selection bias from the oversampling of women with chronic hypertension (HTN), a placental histopathology indication associated with a lower risk of chorioamnionitis. Calculating the same risk ratios:

- RR between HTN and chorioamnionitis in obese women $RR_{UY|(A=1)}$ = 0.52
- RR between HTN and chorioamnionitis in lean women $RR_{UY|(A=0)}$ = 0.50
- RR between HTN and having a histopathology evaluation in obese women $RR_{SU|(A=1)}$ = 1.60
- RR between HTN and having a histopathology evaluation in lean women $RR_{SU|(A=0)}$ = 2.21

These RR are used to calculate a bias factor:

$$B \leq \left(\frac{0.52\ x\ 1.60}{0.52+\ (1.60-1)}\right) \text{ x } \left(\frac{0.50\ x\ 2.21}{0.50+\ (2.21-1)}\right) = 0.53$$

The observed RR between obesity and risk of acute chorioamnionitis is adjusted for the bias factor to give the true bias-adjusted RR, which shifts the RR from an anti-inflammatory to a pro-inflammatory association (RR_{obs}: 0.92 to RR_{true} = 1.74).

$$RR_{True} = \frac{RR_{obs}}{B} = \frac{0.92}{0.53} = 1.74$$

A visual representation of these examples are provided in **Appendix Figure 5.**

Appendix A.1.5 R packages

Diagrams for Figures 2, Supplemental Figure 1, and Supplemental Figure 4 were created using *DiagrammeR*. Forest plots for Figure 1 , Figure 3, and Supplemental Figure 3 were created using *forestplot.* Generalized estimating equations for risk ratio calculations were conducted by *geepack* and predicted probabilities were estimated by *emmeans*. Multiple imputation for missingness was conducted by *mice*. Bias factors for residual selection bias were calculated using the *EValue*. Predicted probability plots for Supplemental Figure 2 were created using the *ggplot2* and *cowplot* packages.

Appendix B Supplemental Methods for Manuscript 2

Appendix B.1.1 ICD-9 Codes for Clinical Outcomes

Pregnancy complications and adverse birth outcomes were extracted from medical records. The pregnancy outcomes and corresponding ICD-9 codes included: gestational diabetes diagnosed by the Carpenter-Coustan criteria (ICD-9 code 648.83), gestational hypertension (642.3), preeclampsia/eclampsia (642.4-642.7) cervical shortening (649.70, 649.71, 649.73), clinical chorioamnionitis (762.7) and preterm premature rupture of membranes (PPROM; 658.11). Adverse birth outcomes included small for gestational age and length of NICU stay, which are both described in the main text. A composite score for severe neonatal morbidity was also measured and consisted of the following adverse neonatal outcomes: respiratory distress syndrome (ICD-9 codes 769, 770.6, 518.82), bronchopulmonary dysplasia (770.7), intraventricular hemorrhage (772.10-772.14), necrotizing enterocolitis (777.50-777.53), periventricular leukomalacia (779.7), patent ductus arteriosus (747.0), and retinopathy of prematurity (362.20, 362.22-362.27).

Appendix B.1.2 Model Fit Statistics for Latent Class Analysis

Model fit statistics used to determine the optimal number and composition of latent classes included measures of global and local fit. Global fit refers to the statistical goodness of fit of the overall latent class model. Latent class models with varying numbers of classes (1 to 6 classes) were compared using goodness of fit statistics to determine the model with the best global fit: log-

likelihood, likelihood-ratio test, Bayes Information Criterion, adjusted Bayes Information Criterion, and corrected Akaike Information Criterion. Lower values for all of these statistical measures indicate a model with better global fit.

Local fit refers to how well each variable (e.g. placental feature) fits a particular latent class model. Bivariate residuals were used to identify origins of poor local fit. Bivariate residuals are indicators of the remaining degree of correlation between placental pathology features in latent class models, with higher values indicating higher residual correlation (i.e. poor local fit). Poor local fit was considered a reduction in bivariate residuals less than 90% after latent class analysis. Placental features that had poor local fit with the majority of other placental features were excluded and latent class models were rerun.

Appendix B.1.3 Regression Analyses

We analyzed the associations between pre-pregnancy BMI and likelihood of placental latent class membership by pseudo-class regression. Pseudo-class regression accounts for bias from misclassification of individuals into incorrect classes by taking random samples from the distributions of the posterior probabilities derived from a latent class model to assign class membership. Prior research indicates 18 imputations of random samples be drawn for each additional latent class in a model; for example, 4 latent classes would require 72 imputations. Estimates of the associations between pre-pregnancy BMI and likelihood of class membership for each imputation are pooled by applying Rubin's Rules.

Several alternative methods to pseudo-class regression were used for measuring associations between pre-pregnancy BMI and likelihood of latent class membership: single-step latent class regression, most-likely class regression and probability-weighted regression. In single-

step regression, covariates were added to a latent class model along with placental histopathology variables to simultaneously construct placental latent classes and estimate the association between pre-pregnancy BMI and probability of a latent class. While single-step regression outperforms pseudo-class regression in estimating standard errors for associations, missing data on pre-pregnancy BMI could change the optimal number and composition of latent classes. For most-likely class regression, after conducting latent class analysis, pregnancies were assigned to their most likely latent class. Multinomial regression was then conducted to measure the likelihood of class membership with increasing pre-pregnancy BMI. This approach uses a hard partitioning of pregnancies into latent classes (assigning a pregnancy to only 1 class), which ignores error from misclassification of individuals into incorrect latent classes. Lastly, probability weighted regression was conducted by the same approach as most-likely class regression, except pregnancies are weighted by their posterior probability of class membership to adjust for bias in point estimates due to misclassification of classes. However, probability weighted regression does not adjust standard errors for potential misclassification of classes resulting in narrower confidence intervals than expected. Odds ratios and confidence intervals with a 1-unit increase in pre-pregnancy BMI were used to assess consistency of estimates across methods in **Supplemental Table 4**.

Appendix B.1.4 Handling of Missing Data

Data on pre-pregnancy BMI were missing in 42% of preterm births due to missingness on self-reported weight. We used multiple imputation by chained equations to account for missing data in pseudo-class regression models.[43] We assume imputing BMI based on observed data by the established multiple imputation method is sufficient, but violation of this assumption (i.e.

missingness not at random) can never be ruled out. Missing data for continuous variables were imputed by predictive mean matching and for categorical variables by logistic regression. In addition to pre-pregnancy, variables considered for the imputation model included baseline characteristics (maternal race, education, smoking status, parity), pregnancy variables (fetal sex, clinical presentation of PTB as spontaneous or indicated), histopathology features, and auxiliary variables for imputing pre-pregnancy BMI (maternal age, 1-minute Apgar score, and 5-minute Apgar score). Auxiliary variables were correlated with pre-pregnancy BMI and predictive of missingness for pre-pregnancy BMI. Multiple imputation was conducted using the *mice* package in R Studio using 72 imputations and 20 max iterations.

Bibliography

1.Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet (London, England)*. Jan 5 2008;371(9606):75-84. doi:10.1016/s0140-6736(08)60074-4

2.Chawanpaiboon S, Vogel JP, Moller A-B, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *The Lancet Global Health*. 2019;7(1):e37-e46. doi:10.1016/S2214-109X(18)30451-0

3.Vogel JP, Chawanpaiboon S, Moller A-B, Watananirun K, Bonet M, Lumbiganon P. The global epidemiology of preterm birth. *Best Practice & Research Clinical Obstetrics & Gynaecology*. 2018/10/01/ 2018;52:3-12. doi:https://doi.org/10.1016/j.bpobgyn.2018.04.003

4.Martin JAH, Brady E; Osterman, Michelle J.K.; Driscoll, Anne K. . *Births: Final Data 2018*. Vol. 68. 2019.

5.Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. *The Lancet*. 2015/01/31/ 2015;385(9966):430-440. doi:https://doi.org/10.1016/S0140-6736(14)61698-6

6.Frey HA, Klebanoff MA. The epidemiology, etiology, and costs of preterm birth. *Seminars in Fetal and Neonatal Medicine*. 2016/04/01/ 2016;21(2):68-73. doi:https://doi.org/10.1016/j.siny.2015.12.011

7.Ream MA, Lehwald L. Neurologic Consequences of Preterm Birth. *Current Neurology and Neuroscience Reports*. 2018/06/16 2018;18(8):48. doi:10.1007/s11910-018-0862-2

8.Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. *Lancet*. Jan 19 2008;371(9608):261-9. doi:10.1016/s0140-6736(08)60136-1

9.Blencowe H, Cousens S, Chou D, et al. Born too soon: the global epidemiology of 15 million preterm births. *Reprod Health*. 2013;10 Suppl 1(Suppl 1):S2-S2. doi:10.1186/1742-4755-10-S1-S2

10.Purisch SE, Gyamfi-Bannerman C. Epidemiology of preterm birth. *Seminars in Perinatology*. 2017/11/01/ 2017;41(7):387-391. doi:https://doi.org/10.1053/j.semperi.2017.07.009

11.Martin JA, Hamilton BE, Osterman MJ, Curtin SC, Matthews TJ. Births: final data for 2013. *Natl Vital Stat Rep*. Jan 15 2015;64(1):1-65.

12.Statistics NCfH. Preterm Birth: United States, 2007-2018 March of Dimes; 2020.

13.Morning A, Brückner H, Nelson A. SOCIALLY DESIRABLE REPORTING AND THE EXPRESSION OF BIOLOGICAL CONCEPTS OF RACE. *Du Bois Review: Social Science Research on Race*. 2019:1-17. doi:10.1017/S1742058X19000195

14.Krieger N. Discrimination and health inequities. *Int J Health Serv*. 2014;44(4):643-710. doi:10.2190/HS.44.4.b

15.Howell EA. Reducing Disparities in Severe Maternal Morbidity and Mortality. *Clin Obstet Gynecol*. 2018;61(2):387-399. doi:10.1097/GRF.0000000000000349

16.Davis D-A. *Reproductive Injustice*
Racism, Pregnancy, and Premature Birth. vol 7. NYU Press; 2019.

17.Weber KA, Yang W, Lyons E, Stevenson DK, Padula AM, Shaw GM. Greenspace, Air Pollution, Neighborhood Factors, and Preeclampsia in a Population-Based Case-Control Study in California. *Int J Environ Res Public Health*. May 12 2021;18(10)doi:10.3390/ijerph18105127

18.Salow AD, Pool LR, Grobman WA, Kershaw KN. Associations of neighborhood-level racial residential segregation with adverse pregnancy outcomes. *Am J Obstet Gynecol*. Mar 2018;218(3):351.e1-351.e7. doi:10.1016/j.ajog.2018.01.022

19.Tiako MJN, McCarthy C, Meisel ZF, Elovitz MA, Burris HH, South E. Association between Low Urban Neighborhood Greenness and Hypertensive Disorders of Pregnancy. *Am J Perinatol*. Aug 27 2021;doi:10.1055/s-0041-1733786

20.Stanhope KK, Adeyemi DI, Li T, Johnson T, Boulet SL. The relationship between the neighborhood built and social environment and hypertensive disorders of pregnancy: A scoping review. *Ann Epidemiol*. Dec 2021;64:67-75. doi:10.1016/j.annepidem.2021.09.005

21.Fasanya HO, Hsiao CJ, Armstrong-Sylvester KR, Beal SG. A Critical Review on the Use of Race in Understanding Racial Disparities in Preeclampsia. *J Appl Lab Med*. Jan 12 2021;6(1):247-256. doi:10.1093/jalm/jfaa149

22.Kramer MS, Papageorghiou A, Culhane J, et al. Challenges in defining and classifying the preterm birth syndrome. *American Journal of Obstetrics and Gynecology*. 2012/02/01/ 2012;206(2):108-112. doi:https://doi.org/10.1016/j.ajog.2011.10.864

23.Goldenberg RL, Gravett MG, Iams J, et al. The preterm birth syndrome: issues to consider in creating a classification system. *American Journal of Obstetrics and Gynecology*. 2012/02/01/ 2012;206(2):113-118. doi:https://doi.org/10.1016/j.ajog.2011.10.865

24.Villar J, Papageorghiou AT, Knight HE, et al. The preterm birth syndrome: a prototype phenotypic classification. *American Journal of Obstetrics and Gynecology*. 2012/02/01/ 2012;206(2):119-123. doi:https://doi.org/10.1016/j.ajog.2011.10.866

25.Breslin N, Gyamfi-Bannerman C. Current Preterm Birth Prevention Strategies. *Clin Perinatol*. 2020/12/01/ 2020;47(4):705-717. doi:https://doi.org/10.1016/j.clp.2020.08.001

26.Cnattingius S, Villamor E, Johansson S, et al. Maternal Obesity and Risk of Preterm Delivery. *Jama*. 2013;309(22):2362-2370. doi:10.1001/jama.2013.6295

27.Poston L, Caleyachetty R, Cnattingius S, et al. Preconceptional and maternal obesity: epidemiology and health consequences. *The Lancet Diabetes & Endocrinology*. 2016;4(12):1025-1036.

28.Mazaki-Tovi S, Romero R, Kusanovic JP, et al. Recurrent Preterm Birth. *Seminars in Perinatology*. 2007/06/01/ 2007;31(3):142-158. doi:https://doi.org/10.1053/j.semperi.2007.04.001

29.van Os MA, Kleinrouweler CE, Schuit E, et al. Influence of cut-off value on prevalence of short cervical length. *Ultrasound in Obstetrics & Gynecology*. 2017;49(3):330-336. doi:10.1002/uog.15967

30.Mancuso MS, Owen J. Prevention of preterm birth based on a short cervix: cerclage. *Seminars in perinatology*. 2009;33(5):325-333. doi:10.1053/j.semperi.2009.06.005

31.Heyborne KD, Allshouse AA, Carey JC. Does 17-alpha hydroxyprogesterone caproate prevent recurrent preterm birth in obese women? *American journal of obstetrics and gynecology*. 2015;213(6):844. e1-844. e6.

32.Talati AN, Hackney DN, Mesiano S. Pathophysiology of preterm labor with intact membranes. *Seminars in Perinatology*. 2017/11/01/ 2017;41(7):420-426. doi:https://doi.org/10.1053/j.semperi.2017.07.013

33.Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. *Nat Rev Immunol*. Aug 2017;17(8):469-482. doi:10.1038/nri.2017.64

34.Gomez-Lopez N, StLouis D, Lehr MA, Sanchez-Rodriguez EN, Arenas-Hernandez M. Immune cells in term and preterm labor. *Cellular & Molecular Immunology*. 2014/11/01 2014;11(6):571-581. doi:10.1038/cmi.2014.46

35.Romero R, Dey SK, Fisher SJ. Preterm labor: One syndrome, many causes. *Science*. 2014;345(6198):760-765. doi:10.1126/science.1251816

36.Nott JP, Bonney EA, Pickering JD, Simpson NAB. The structure and function of the cervix during pregnancy. *Translational Research in Anatomy*. 2016/03/01/ 2016;2:1-7. doi:https://doi.org/10.1016/j.tria.2016.02.001

37.Manuck TA, Esplin MS, Biggio J, et al. The phenotype of spontaneous preterm birth: application of a clinical phenotyping tool. *American journal of obstetrics and gynecology*. 2015;212(4):487.e1-487.e11. doi:10.1016/j.ajog.2015.02.010

38.Chandiramani M, Shennan AH. Cervical insufficiency: prediction, diagnosis and prevention. *The Obstetrician & Gynaecologist*. 2008;10(2):99-106.

39.Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. *Cardiovasc J Afr*. Mar-Apr 2016;27(2):89-94. doi:10.5830/CVJA-2016-021

40.Nadeau HC, Subramaniam A, Andrews WW. Infection and preterm birth. *Semin Fetal Neonatal Med*. Apr 2016;21(2):100-5. doi:10.1016/j.siny.2015.12.008

41.Ananth CV, Vintzileos AM. Maternal-fetal conditions necessitating a medical intervention resulting in preterm birth. *American Journal of Obstetrics and Gynecology*. 2006/12/01/ 2006;195(6):1557-1563. doi:https://doi.org/10.1016/j.ajog.2006.05.021

42.Prefumo F, Fichera A, Fratelli N, Sartori E. Fetal anemia: Diagnosis and management. *Best Practice & Research Clinical Obstetrics & Gynaecology*. 2019/07/01/ 2019;58:2-14. doi:https://doi.org/10.1016/j.bpobgyn.2019.01.001

43.Gravett C, Eckert LO, Gravett MG, et al. Non-reassuring fetal status: Case definition & guidelines for data collection, analysis, and presentation of immunization safety data. *Vaccine*. 2016;34(49):6084-6092. doi:10.1016/j.vaccine.2016.03.043

44.Simpson LL. Twin-twin transfusion syndrome. *Am J Obstet Gynecol*. Jan 2013;208(1):3-18. doi:10.1016/j.ajog.2012.10.880

45.Gude NM, Roberts CT, Kalionis B, King RG. Growth and function of the normal human placenta. *Thrombosis Research*. 2004/01/01/ 2004;114(5):397-407. doi:https://doi.org/10.1016/j.thromres.2004.06.038

46.Roberts JM, Escudero C. The placenta in preeclampsia. *Pregnancy hypertension*. 2012;2(2):72-83. doi:10.1016/j.preghy.2012.01.001

47.Romero R, Kusanovic JP, Chaiworapongsa T, Hassan SS. Placental bed disorders in preterm labor, preterm PROM, spontaneous abortion and abruptio placentae. *Best Pract Res Clin Obstet Gynaecol*. 2011;25(3):313-327. doi:10.1016/j.bpobgyn.2011.02.006

48.Bartels HC, Postle JD, Downey P, Brennan DJ. Placenta Accreta Spectrum: A Review of Pathology, Molecular Biology, and Biomarkers. *Dis Markers*. 2018;2018:1507674-1507674. doi:10.1155/2018/1507674

49.Ananth CV, Kinzler WL. Placental abruption: Pathophysiology, clinical features, diagnosis, and consequences.

50.Lockwood CJ, Russo-Stieglitz MK, Berghella V. Placenta previa: Epidemiology, clinical features, diagnosis, morbidity and mortality.

51.Kumar P, Magon N. Hormones in pregnancy. *Nigerian medical journal: journal of the Nigeria Medical Association*. 2012;53(4):179.

52.Di Renzo GC, Tosto V, Giardina I. The biological basis and prevention of preterm birth. *Best Practice & Research Clinical Obstetrics & Gynaecology*. 2018/10/01/ 2018;52:13-22. doi:https://doi.org/10.1016/j.bpobgyn.2018.01.022

53.Tita ATN, Andrews WW. Diagnosis and management of clinical chorioamnionitis. *Clinics in Perinatology*. 2010;37(2):339-354. doi:10.1016/j.clp.2010.02.003

54.Manuck TA. Racial and ethnic differences in preterm birth: a complex, multifactorial problem. Elsevier; 2017:511-518.

55.Fuchs F, Monet B, Ducruet T, Chaillet N, Audibert F. Effect of maternal age on the risk of preterm birth: A large cohort study. *PLoS One*. 2018;13(1):e0191002-e0191002. doi:10.1371/journal.pone.0191002

56.Schaaf JM, Liem SM, Mol BW, Abu-Hanna A, Ravelli AC. Ethnic and racial disparities in the risk of preterm birth: a systematic review and meta-analysis. *Am J Perinatol*. Jun 2013;30(6):433-50. doi:10.1055/s-0032-1326988

57.Debiec KE, Paul KJ, Mitchell CM, Hitti JE. Inadequate prenatal care and risk of preterm delivery among adolescents: a retrospective study over 10 years. *American Journal of Obstetrics and Gynecology*. 2010/08/01/ 2010;203(2):122.e1-122.e6. doi:https://doi.org/10.1016/j.ajog.2010.03.001

58.DeFranco EA, Lian M, Muglia LJ, Schootman M. Area-level poverty and preterm birth risk: A population-based multilevel analysis. *BMC Public Health*. 2008/09/15 2008;8(1):316. doi:10.1186/1471-2458-8-316

59.El-Sayed AM, Galea S. Temporal changes in socioeconomic influences on health: maternal education and preterm birth. *Am J Public Health*. 2012;102(9):1715-1721. doi:10.2105/AJPH.2011.300564

60.Jansen PW, Tiemeier H, Jaddoe VWV, et al. Explaining educational inequalities in preterm birth: the generation r study. *Archives of Disease in Childhood - Fetal and Neonatal Edition*. 2009;94(1):F28-F34. doi:10.1136/adc.2007.136945

61.Bekkar B, Pacheco S, Basu R, DeNicola N. Association of Air Pollution and Heat Exposure With Preterm Birth, Low Birth Weight, and Stillbirth in the US: A Systematic Review. *JAMA Network Open*. 2020;3(6):e208243-e208243. doi:10.1001/jamanetworkopen.2020.8243

62.Yang J, Baer RJ, Berghella V, et al. Recurrence of Preterm Birth and Early Term Birth. *Obstet Gynecol*. 2016;128(2):364-372. doi:10.1097/AOG.0000000000001506

63.Jakobsson M, Gissler M, Sainio S, Paavonen J, Tapper A-M. Preterm Delivery After Surgical Treatment for Cervical Intraepithelial Neoplasia. *Obstetrics & Gynecology*. 2007;109(2):309-313. doi:10.1097/01.Aog.0000253239.87040.23

64.Fuchs F, Senat MV. Multiple gestations and preterm birth. *Semin Fetal Neonatal Med*. Apr 2016;21(2):113-20. doi:10.1016/j.siny.2015.12.010

65.Dunietz GL, Holzman C, McKane P, et al. Assisted reproductive technology and the risk of preterm birth among primiparas. *Fertility and Sterility*. 2015/04/01/ 2015;103(4):974-979.e1. doi:https://doi.org/10.1016/j.fertnstert.2015.01.015

66.Manns-James L. Bacterial Vaginosis and Preterm Birth. *Journal of Midwifery & Women's Health*. 2011;56(6):575-583. doi:10.1111/j.1542-2011.2011.00086.x

67.Khader YS, Ta'ani Q. Periodontal Diseases and the Risk of Preterm Birth and Low Birth Weight: A Meta-Analysis. *Journal of Periodontology*. 2005;76(2):161-165. doi:10.1902/jop.2005.76.2.161

68.Nguyen MH, Fornes R, Kamau N, et al. Antibiotic use during pregnancy and the risk of preterm birth: a population-based Swedish cohort study. *Journal of Antimicrobial Chemotherapy*. 2022;doi:10.1093/jac/dkac053

69.McClure EM, Goldenberg RL. Use of antibiotics to reduce preterm birth. *Lancet Glob Health*. Jan 2019;7(1):e18-e19. doi:10.1016/s2214-109x(18)30543-6

70.Ion R, Bernal AL. Smoking and Preterm Birth. *Reproductive Sciences*. 2015/08/01 2014;22(8):918-926. doi:10.1177/1933719114556486

71.Santos S, Voerman E, Amiano P, et al. Impact of maternal body mass index and gestational weight gain on pregnancy complications: an individual participant data meta-analysis of European, North American and Australian cohorts. *BJOG: An International Journal of Obstetrics & Gynaecology*. 2019;126(8):984-995.

72.Berger H, Melamed N, Davis BM, et al. Impact of diabetes, obesity and hypertension on preterm birth: Population-based study. *PLoS One*. 2020;15(3):e0228743. doi:10.1371/journal.pone.0228743

73.Nerlander LM, Callaghan WM, Smith RA, Barfield WD. Short interpregnancy interval associated with preterm birth in U S adolescents. *Matern Child Health J*. 2015;19(4):850-858. doi:10.1007/s10995-014-1583-z

74.Statistics NCfH. Natality public-use data, 2020. Public-use data file and documentation. Accessed March 2022. https://www.cdc.gov/nchs/data_access/Vitalstatsonline.htm

75.Statistics NCfH. Natality public-use data, 2017. Public-use data file and documentation. Accessed March 2021. https://www.cdc.gov/nchs/data_access/Vitalstatsonline.htm

76.Su XJ, Huang SJ, Li X, Du QL. Prepregnancy Overweight and Obesity Are Associated with an Increased Risk of Preterm Birth in Chinese Women. *Obes Facts*. 2020;13(2):237-244. doi:10.1159/000506688

77.Sharma AJ, Vesco KK, Bulkley J, et al. Associations of gestational weight gain with preterm birth among underweight and normal weight women. *Matern Child Health J*. 2015;19(9):2066-2073.

78.Smith LK, Draper ES, Evans TA, et al. Associations between late and moderately preterm birth and smoking, alcohol, drug use and diet: a population-based case–cohort study. *Archives of Disease in Childhood-Fetal and Neonatal Edition*. 2015;100(6):F486-F491.

79.Maric-Bilkan C, Abrahams VM, Arteaga SS, et al. Research Recommendations From the National Institutes of Health Workshop on Predicting, Preventing, and Treating Preeclampsia. *Hypertension*. 2019;73(4):757-766. doi:doi:10.1161/HYPERTENSIONAHA.118.11644

80.Allen R, Rogozinska E, Sivarajasingam P, Khan KS, Thangaratinam S. Effect of diet- and lifestyle-based metabolic risk-modifying interventions on preeclampsia: a meta-analysis. *Acta Obstet Gynecol Scand*. Oct 2014;93(10):973-85. doi:10.1111/aogs.12467

81.Tong S, Kaitu'u-Lino TuJ, Hastie R, Brownfoot F, Cluver C, Hannan N. Pravastatin, proton-pump inhibitors, metformin, micronutrients, and biologics: new horizons for the prevention or treatment of preeclampsia. *American Journal of Obstetrics and Gynecology*. 2022/02/01/ 2022;226(2, Supplement):S1157-S1170. doi:https://doi.org/10.1016/j.ajog.2020.09.014

82.Staff AC, Umans JG, Jeyabalan A. Chapter 18 - Prediction and Prevention of Preeclampsia. In: Taylor RN, Conrad KP, Davidge ST, Staff AC, Roberts JM, eds. *Chesley's Hypertensive Disorders in Pregnancy (Fifth Edition)*. Academic Press; 2022:405-417.

83.Maggard MA, Yermilov I, Li Z, et al. Pregnancy and Fertility Following Bariatric Surgery: A Systematic Review. *JAMA*. 2008;300(19):2286-2296. doi:10.1001/jama.2008.641

84.Mostello D, Jen Chang J, Allen J, Luehr L, Shyken J, Leet T. Recurrent preeclampsia: the effect of weight change between pregnancies. *Obstet Gynecol*. Sep 2010;116(3):667-672. doi:10.1097/AOG.0b013e3181ed74ea

85.Systems BRFS. *Obesity among women of childbearing age: United States, 1990-2019*. Accessed April 16, 2020. www.marchofdimes.org/peristats

86.Singh GK, DiBari JN. Marked Disparities in Pre-Pregnancy Obesity and Overweight Prevalence among US Women by Race/Ethnicity, Nativity/Immigrant Status, and Sociodemographic Characteristics, 2012–2014. *Journal of obesity*. 2019;2019

87.Gaillard R, Durmuş B, Hofman A, Mackenbach JP, Steegers EAP, Jaddoe VWV. Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. *Obesity*. 2013;21(5):1046-1055. doi:10.1002/oby.20088

88.Bombard JM, Dietz PM, Galavotti C, et al. Chronic Diseases and Related Risk Factors among Low-Income Mothers. *Matern Child Health J*. 2012/01/01 2012;16(1):60-71. doi:10.1007/s10995-010-0717-1

89.Azeez O, Kulkarni A, Kuklina EV, Kim SY, Cox S. Peer Reviewed: Hypertension and Diabetes in Non-Pregnant Women of Reproductive Age in the United States. *Preventing chronic disease*. 2019;16

90.Jeyabalan A. Epidemiology of preeclampsia: impact of obesity. *Nutr Rev*. 2013;71 Suppl 1(0 1):S18-S25. doi:10.1111/nure.12055

91.Bodnar LM, Ness RB, Markovic N, Roberts JM. The Risk of Preeclampsia Rises with Increasing Prepregnancy Body Mass Index. *Annals of Epidemiology*. 2005/08/01/ 2005;15(7):475-482. doi:https://doi.org/10.1016/j.annepidem.2004.12.008

92.Chu SY, Callaghan WM, Kim SY, et al. Maternal obesity and risk of gestational diabetes mellitus. *Diabetes care*. 2007;30(8):2070-2076.

93.Marchi J, Berg M, Dencker A, Olander EK, Begley C. Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews. *Obes Rev*. Aug 2015;16(8):621-38. doi:10.1111/obr.12288

94.Kim SY, England L, Wilson HG, Bish C, Satten GA, Dietz P. Percentage of gestational diabetes mellitus attributable to overweight and obesity. *Am J Public Health*. 2010;100(6):1047-1052. doi:10.2105/AJPH.2009.172890

95.Heslehurst N, Simpson H, Ells LJ, et al. The impact of maternal BMI status on pregnancy outcomes with immediate short-term obstetric resource implications: a meta-analysis. *Obesity reviews : an official journal of the International Association for the Study of Obesity*. Nov 2008;9(6):635-83. doi:10.1111/j.1467-789X.2008.00511.x

96.Lashen H, Fear K, Sturdee DW. Obesity is associated with increased risk of first trimester and recurrent miscarriage: matched case–control study. *Human Reproduction*. 2004;19(7):1644-1646. doi:10.1093/humrep/deh277

97.Stothard KJ, Tennant PWG, Bell R, Rankin J. Maternal Overweight and Obesity and the Risk of Congenital Anomalies: A Systematic Review and Meta-analysis. *JAMA*. 2009;301(6):636-650. doi:10.1001/jama.2009.113

98.Kim SS, Zhu Y, Grantz KL, et al. Obstetric and Neonatal Risks Among Obese Women Without Chronic Disease. *Obstet Gynecol*. 2016;128(1):104-112. doi:10.1097/AOG.0000000000001465

99.Chen C-N, Chen H-S, Hsu H-C. Maternal Prepregnancy Body Mass Index, Gestational Weight Gain, and Risk of Adverse Perinatal Outcomes in Taiwan: A Population-Based Birth Cohort Study. *Int J Environ Res Public Health*. 2020;17(4):1221. doi:10.3390/ijerph17041221

100.Choi H, Lim J-Y, Lim N-K, et al. Impact of pre-pregnancy body mass index and gestational weight gain on the risk of maternal and infant pregnancy complications in Korean women. *International Journal of Obesity*. 2022/01/01 2022;46(1):59-67. doi:10.1038/s41366-021-00946-8

101.González-Plaza E, Bellart J, Martínez-Verdú M, Arranz Á, Luján-Barroso L, Seguranyes G. Pre-pregnancy overweight and obesity prevalence and relation to maternal and perinatal outcomes. *Enferm Clin (Engl Ed)*. Jun 19 2021;doi:10.1016/j.enfcli.2021.04.004

102.Jiang S, Chipps D, Cheung WN, Mongelli M. Comparison of adverse pregnancy outcomes based on the new IADPSG 2010 gestational diabetes criteria and maternal body mass index. *Australian and New Zealand Journal of Obstetrics and Gynaecology*. 2017;57(5):533-539. doi:10.1111/ajo.12628

103.Ju AC, Heyman MB, Garber AK, Wojcicki JM. Maternal Obesity and Risk of Preterm Birth and Low Birthweight in Hawaii PRAMS, 2000–2011. *Matern Child Health J*. 2018/06/01 2018;22(6):893-902. doi:10.1007/s10995-018-2464-7

104.Kim SY, Oh SY, Sung JH, et al. Validation of a Strict Obesity Definition Proposed for Asians to Predict Adverse Pregnancy Outcomes in Korean Pregnant Women. *J Korean Med Sci*. Nov 15 2021;36(44):e281. doi:10.3346/jkms.2021.36.e281

105.Li H, Miao C, Xu L, et al. Maternal pre-pregnancy body mass index, gestational weight gain trajectory, and risk of adverse perinatal outcomes. *Int J Gynaecol Obstet*. Sep 9 2021;doi:10.1002/ijgo.13922

106.Li L, Chen Y, Lin Z, et al. Association of pre-pregnancy body mass index with adverse pregnancy outcome among first-time mothers. *PeerJ*. 2020;8:e10123-e10123. doi:10.7717/peerj.10123

107.Liu B, Xu G, Sun Y, et al. Association between maternal pre-pregnancy obesity and preterm birth according to maternal age and race or ethnicity: a population-based study. *Lancet Diabetes Endocrinol*. Sep 2019;7(9):707-714. doi:10.1016/s2213-8587(19)30193-7

108.Morais SS, Nascimento SL, Godoy-Miranda AC, Kasawara KT, Surita FG. Body Mass Index Changes during Pregnancy and Perinatal Outcomes - A Cross-Sectional Study. *Rev Bras Ginecol Obstet*. Jan 2018;40(1):11-19. Mudancas do indice de massa corporal na gravidez e resultados perinatais - um estudo transversal. doi:10.1055/s-0037-1608885

109.van Hoorn F, de Wit L, van Rossem L, et al. A prospective population-based multicentre study on the impact of maternal body mass index on adverse pregnancy outcomes: Focus on normal weight. *PLoS One*. 2021;16(9):e0257722. doi:10.1371/journal.pone.0257722

110.Xie D, Yang W, Wang A, et al. Effects of pre-pregnancy body mass index on pregnancy and perinatal outcomes in women based on a retrospective cohort. *Scientific Reports*. 2021/10/06 2021;11(1):19863. doi:10.1038/s41598-021-98892-y

111.El Rafei R, Abbas HA, Charafeddine L, et al. Association of Pre-Pregnancy Body Mass Index and Gestational Weight Gain with Preterm Births and Fetal Size: an Observational Study from Lebanon. *Paediatr Perinat Epidemiol*. Jan 2016;30(1):38-45. doi:10.1111/ppe.12249

112.de Jongh BE, Paul DA, Hoffman M, Locke R. Effects of Pre-pregnancy Obesity, Race/Ethnicity and Prematurity. *Matern Child Health J*. 2014/04/01 2014;18(3):511-517. doi:10.1007/s10995-013-1296-8

113.Enomoto K, Aoki S, Toma R, Fujiwara K, Sakamaki K, Hirahara F. Pregnancy Outcomes Based on Pre-Pregnancy Body Mass Index in Japanese Women. *PLoS One*. 2016;11(6):e0157081-e0157081. doi:10.1371/journal.pone.0157081

114.Kosa JL, Guendelman S, Pearl M, Graham S, Abrams B, Kharrazi M. The Association Between Pre-pregnancy BMI and Preterm Delivery in a Diverse Southern California Population of Working Women. *Matern Child Health J*. 2011/08/01 2011;15(6):772-781. doi:10.1007/s10995-010-0633-4

115.Mamun AA, Callaway LK, O'Callaghan MJ, et al. Associations of maternal pre-pregnancy obesity and excess pregnancy weight gains with adverse pregnancy outcomes and length of

hospital stay. *BMC Pregnancy and Childbirth*. 2011/09/06 2011;11(1):62. doi:10.1186/1471-2393-11-62

116.Parker MG, Ouyang F, Pearson C, et al. Prepregnancy body mass index and risk of preterm birth: association heterogeneity by preterm subgroups. *BMC pregnancy and childbirth*. 2014;14:153-153. doi:10.1186/1471-2393-14-153

117.Ram M, Berger H, Lipworth H, et al. The relationship between maternal body mass index and pregnancy outcomes in twin compared with singleton pregnancies. *Int J Obes (Lond)*. Jan 2020;44(1):33-44. doi:10.1038/s41366-019-0362-8

118.Ratnasiri AWG, Lee HC, Lakshminrusimha S, et al. Trends in maternal prepregnancy body mass index (BMI) and its association with birth and maternal outcomes in California, 2007-2016: A retrospective cohort study. *PLoS One*. 2019;14(9):e0222458-e0222458. doi:10.1371/journal.pone.0222458

119.Wise LA, Palmer JR, Heffner LJ, Rosenberg L. Prepregnancy body size, gestational weight gain, and risk of preterm birth in African-American women. *Epidemiology*. 2010;21(2):243-252. doi:10.1097/EDE.0b013e3181cb61a9

120.Parker MG, Ouyang F, Pearson C, et al. Prepregnancy body mass index and risk of preterm birth: association heterogeneity by preterm subgroups. *BMC Pregnancy and Childbirth*. 2014/04/30 2014;14(1):153. doi:10.1186/1471-2393-14-153

121.Sharashova EE, Anda EE, Grjibovski AM. Early pregnancy body mass index and spontaneous preterm birth in Northwest Russia: a registry-based study. *BMC Pregnancy and Childbirth*. 2014/09/04 2014;14(1):303. doi:10.1186/1471-2393-14-303

122.Shaw GM, Wise PH, Mayo J, et al. Maternal Prepregnancy Body Mass Index and Risk of Spontaneous Preterm Birth. *Paediatr Perinat Epidemiol*. 2014;28(4):302-311. doi:10.1111/ppe.12125

123.Torloni MR, Fortunato SJ, Betrán AP, et al. Ethnic disparity in spontaneous preterm birth and maternal pre-pregnancy body mass index. *Archives of Gynecology and Obstetrics*. 2012/04/01 2012;285(4):959-966. doi:10.1007/s00404-011-2102-8

124.Pratt A, Howat P, Hui L. Maternal and perinatal outcomes for women with body mass index ≥50 kg/m(2) in a non-tertiary hospital setting. *Aust N Z J Obstet Gynaecol*. Jun 2020;60(3):361-368. doi:10.1111/ajo.13064

125.Slack E, Best KE, Rankin J, Heslehurst N. Maternal obesity classes, preterm and post-term birth: a retrospective analysis of 479,864 births in England. *BMC Pregnancy and Childbirth*. 2019/11/21 2019;19(1):434. doi:10.1186/s12884-019-2585-z

126.Vats H, Saxena R, Sachdeva MP, Walia GK, Gupta V. Impact of maternal pre-pregnancy body mass index on maternal, fetal and neonatal adverse outcomes in the worldwide populations: A systematic review and meta-analysis. *Obes Res Clin Pract*. Nov-Dec 2021;15(6):536-545. doi:10.1016/j.orcp.2021.10.005

127.McIntosh MS, Kumar V, Kalynych C, et al. Racial Differences in Blood Lipids Lead to Underestimation of Cardiovascular Risk in Black Women in a Nested observational Study. *Glob Adv Health Med*. 2013;2(2):76-79. doi:10.7453/gahmj.2012.076

128.Agyemang P, Powell-Wiley TM. Obesity and Black Women: Special Considerations Related to Genesis and Therapeutic Approaches. *Curr Cardiovasc Risk Rep*. 2013;7(5):378-386. doi:10.1007/s12170-013-0328-7

129.Bryant AS, Worjoloh A, Caughey AB, Washington AE. Racial/ethnic disparities in obstetric outcomes and care: prevalence and determinants. *American journal of obstetrics and gynecology*. 2010;202(4):335-343. doi:10.1016/j.ajog.2009.10.864

130.Ramos BRdA, Mendes ND, Tanikawa AA, et al. Ancestry informative markers and selected single nucleotide polymorphisms in immunoregulatory genes on preterm labor and preterm premature rupture of membranes: a case control study. *BMC pregnancy and childbirth*. 2016;16:30-30. doi:10.1186/s12884-016-0823-1

131.Nadeau-Vallée M, Obari D, Palacios J, et al. Sterile inflammation and pregnancy complications: a review. *Reproduction*. December 1, 2016 2016;152(6):R277-R292. doi:10.1530/rep-16-0453

132.PrabhuDas M, Bonney E, Caron K, et al. Immune mechanisms at the maternal-fetal interface: perspectives and challenges. *Nat Immunol*. 2015;16(4):328-334. doi:10.1038/ni.3131

133.Heerema-McKenney A. Defense and infection of the human placenta. *Apmis*. Jul 2018;126(7):570-588. doi:10.1111/apm.12847

134.Hauguel-de Mouzon S, Guerre-Millo M. The Placenta Cytokine Network and Inflammatory Signals. *Placenta*. 2006/08/01/ 2006;27(8):794-798. doi:https://doi.org/10.1016/j.placenta.2005.08.009

135.Barros FC, Papageorghiou AT, Victora CG, et al. The Distribution of Clinical Phenotypes of Preterm Birth Syndrome: Implications for Prevention. *JAMA Pediatrics*. 2015;169(3):220-229. doi:10.1001/jamapediatrics.2014.3040

136.King JC. Maternal obesity, metabolism, and pregnancy outcomes. *Annual review of nutrition*. 2006;26:271-91. doi:10.1146/annurev.nutr.24.012003.132249

137.Myatt L, Maloyan A. Obesity and Placental Function. *Seminars in reproductive medicine*. Jan 2016;34(1):42-9. doi:10.1055/s-0035-1570027

138.Roberts KA, Riley SC, Reynolds RM, et al. Placental structure and inflammation in pregnancies associated with obesity. *Placenta*. Mar 2011;32(3):247-54. doi:10.1016/j.placenta.2010.12.023

139.Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF, Petraglia F. Inflammation and Pregnancy. *Reproductive Sciences*. 2009;16(2):206-215. doi:10.1177/1933719108329095

140.Denison FC, Roberts KA, Barr SM, Norman JE. Obesity, pregnancy, inflammation, and vascular function. 2010;140(3):373. doi:10.1530/rep-10-0074

141.Arner P, Rydén M. Fatty Acids, Obesity and Insulin Resistance. *Obes Facts*. 2015;8(2):147-155. doi:10.1159/000381224

142.Geraghty AA, Alberdi G, O'Sullivan EJ, et al. Maternal and fetal blood lipid concentrations during pregnancy differ by maternal body mass index: findings from the ROLO study. *BMC pregnancy and childbirth*. 2017;17(1):360-360. doi:10.1186/s12884-017-1543-x

143.Zhou H, Urso CJ, Jadeja V. Saturated Fatty Acids in Obesity-Associated Inflammation. *J Inflamm Res*. 2020;13:1-14. doi:10.2147/JIR.S229691

144.Borkowski K, Newman JW, Aghaeepour N, et al. Mid-gestation serum lipidomic profile associations with spontaneous preterm birth are influenced by body mass index. *PLoS One*. 2020;15(11):e0239115. doi:10.1371/journal.pone.0239115

145.Engin AB. What Is Lipotoxicity? In: Engin AB, Engin A, eds. *Obesity and Lipotoxicity*. Springer International Publishing; 2017:197-220.

146.Saben J, Lindsey F, Zhong Y, et al. Maternal obesity is associated with a lipotoxic placental environment. *Placenta*. 2014/03/01/ 2014;35(3):171-177. doi:https://doi.org/10.1016/j.placenta.2014.01.003

147.Håversen L, Danielsson KN, Fogelstrand L, Wiklund O. Induction of proinflammatory cytokines by long-chain saturated fatty acids in human macrophages. *Atherosclerosis*. 2009;202(2):382-393.

148.Yang X, Haghiac M, Glazebrook P, Minium J, Catalano PM, Hauguel-de Mouzon S. Saturated fatty acids enhance TLR4 immune pathways in human trophoblasts. *Human reproduction (Oxford, England)*. 2015;30(9):2152-2159. doi:10.1093/humrep/dev173

149.Amirchaghmaghi E, Taghavi SA, Shapouri F, Saeidi S, Rezaei A, Aflatoonian R. The role of toll like receptors in pregnancy. *International journal of fertility & sterility*. Oct-Dec 2013;7(3):147-154.

150.Hwang DH, Kim J-A, Lee JY. Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. *European journal of pharmacology*. 2016;785:24-35. doi:10.1016/j.ejphar.2016.04.024

151.Li L, Kang J, Lei W. Role of Toll-like receptor 4 in inflammation-induced preterm delivery. *Molecular Human Reproduction*. 2010;16(4):267-272. doi:10.1093/molehr/gap106

152.Riley JK, Nelson DM. Toll-like Receptors in Pregnancy Disorders and Placental Dysfunction. journal article. *Clinical Reviews in Allergy & Immunology*. December 01 2010;39(3):185-193. doi:10.1007/s12016-009-8178-2

153.Koga K, Mor G. Toll-Like Receptors at the Maternal-Fetal Interface in Normal Pregnancy and Pregnancy Disorders. *American Journal of Reproductive Immunology*. 2010;63(6):587-600. doi:10.1111/j.1600-0897.2010.00848.x

154.Kumazaki K, Nakayama M, Yanagihara I, Suehara N, Wada Y. Immunohistochemical distribution of Toll-like receptor 4 in term and preterm human placentas from normal and complicated pregnancy including chorioamnionitis. *Human Pathology*. 2004/01/01/ 2004;35(1):47-54. doi:https://doi.org/10.1016/j.humpath.2003.08.027

155.Pineda A, Verdin-Teran SL, Camacho A, Moreno-Fierros L. Expression of toll-like receptor TLR-2, TLR-3, TLR-4 and TLR-9 is increased in placentas from patients with preeclampsia. *Archives of medical research*. Jul 2011;42(5):382-91. doi:10.1016/j.arcmed.2011.08.003

156.Yang X, Li M, Haghiac M, Catalano PM, O'Tierney-Ginn P, Hauguel-de Mouzon S. Causal relationship between obesity-related traits and TLR4-driven responses at the maternal-fetal interface. *Diabetologia*. Nov 2016;59(11):2459-2466. doi:10.1007/s00125-016-4073-6

157.Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The Placenta Harbors a Unique Microbiome. *Science Translational Medicine*. 2014;6(237):237ra65-237ra65. doi:10.1126/scitranslmed.3008599

158.Staude B, Oehmke F, Lauer T, et al. The Microbiome and Preterm Birth: A Change in Paradigm with Profound Implications for Pathophysiologic Concepts and Novel Therapeutic Strategies. *Biomed Res Int*. 2018;2018:7218187-7218187. doi:10.1155/2018/7218187

159.Sureshchandra S, Marshall NE, Wilson RM, et al. Inflammatory Determinants of Pregravid Obesity in Placenta and Peripheral Blood. Original Research. *Frontiers in Physiology*. 2018-August-07 2018;9(1089)doi:10.3389/fphys.2018.01089

160.John GK, Mullin GE. The Gut Microbiome and Obesity. *Current Oncology Reports*. 2016/06/02 2016;18(7):45. doi:10.1007/s11912-016-0528-7

161.Cani PD, Amar J, Iglesias MA, et al. Metabolic Endotoxemia Initiates Obesity and Insulin Resistance. *Diabetes*. 2007;56(7):1761-1772. doi:10.2337/db06-1491

162.Basu S, Haghiac M, Surace P, et al. Pregravid Obesity Associates With Increased Maternal Endotoxemia and Metabolic Inflammation. *Obesity*. 2011;19(3):476-482. doi:10.1038/oby.2010.215

163.Gould JB, Mayo J, Shaw GM, Stevenson DK, Medicine tMoDPRCaSUSo. Swedish and American studies show that initiatives to decrease maternal obesity could play a key role in reducing preterm birth. *Acta Paediatrica*. 2014;103(6):586-591. doi:https://doi.org/10.1111/apa.12616

164.Roberts DJ, Post MD. The placenta in pre-eclampsia and intrauterine growth restriction. *Journal of Clinical Pathology*. 2008;61(12):1254-1260. doi:10.1136/jcp.2008.055236

165.Ernst LM. Maternal vascular malperfusion of the placental bed. *Apmis*. Jul 2018;126(7):551-560. doi:10.1111/apm.12833

166.Karumanchi SA, Rana S, Taylor RN. Chapter 6 - Angiogenesis and Preeclampsia. In: Taylor RN, Roberts JM, Cunningham FG, Lindheimer MD, eds. *Chesley's Hypertensive Disorders in Pregnancy (Fourth Edition)*. Academic Press; 2015:113-132.

167.Redman CWG, Sargent IL, Taylor RN. Chapter 8 - Immunology of Normal Pregnancy and Preeclampsia. In: Taylor RN, Roberts JM, Cunningham FG, Lindheimer MD, eds. *Chesley's Hypertensive Disorders in Pregnancy (Fourth Edition)*. Academic Press; 2015:161-179.

168.Sirdeshmukh R, Jayaram S, Gupta MK, et al. Integration of Transcriptomic and Proteomic Data for Disease Insights. *Current Proteomic Approaches Applied to Brain Function*. Springer; 2017:325-356.

169.Wisse BE. The Inflammatory Syndrome: The Role of Adipose Tissue Cytokines in Metabolic Disorders Linked to Obesity. *Journal of the American Society of Nephrology*. 2004;15(11):2792-2800. doi:10.1097/01.Asn.0000141966.69934.21

170.Pendeloski KPT, Ono E, Torloni MR, Mattar R, Daher S. Maternal obesity and inflammatory mediators: A controversial association. *American journal of reproductive immunology (New York, NY : 1989)*. May 2017;77(5)doi:10.1111/aji.12674

171.Pantham P, Aye ILMH, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta*. 2015/07/01/ 2015;36(7):709-715. doi:https://doi.org/10.1016/j.placenta.2015.04.006

172.Block LN, Bowman BD, Schmidt JK, Keding LT, Stanic AK, Golos TG. The promise of placental extracellular vesicles: models and challenges for diagnosing placental dysfunction in utero†. *Biol Reprod*. Jan 4 2021;104(1):27-57. doi:10.1093/biolre/ioaa152

173.Turowski G, Tony Parks W, Arbuckle S, Jacobsen AF, Heazell A. The structure and utility of the placental pathology report. *APMIS*. 2018;126(7):638-646. doi:https://doi.org/10.1111/apm.12842

174.Catov JM, Peng Y, Scifres CM, Parks WT. Placental pathology measures: Can they be rapidly and reliably integrated into large-scale perinatal studies? *Placenta*. Jun 2015;36(6):687-92. doi:10.1016/j.placenta.2015.03.001

175.Beebe, Cowan, Hyde, Altshuler. Methods to improve the reliability of histopathological diagnoses in the placenta. *Paediatric and Perinatal Epidemiology*. 2000;14(2):172-178. doi:https://doi.org/10.1046/j.1365-3016.2000.00253.x

176.Salzberg SL. Open questions: How many genes do we have? *BMC Biology*. 2018/08/20 2018;16(1):94. doi:10.1186/s12915-018-0564-x

177.Kim CJ, Romero R, Chaemsaithong P, Chaiyasit N, Yoon BH, Kim YM. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance.

American journal of obstetrics and gynecology. 2015;213(4 Suppl):S29-S52. doi:10.1016/j.ajog.2015.08.040

178.Huang L, Liu J, Feng L, Chen Y, Zhang J, Wang W. Maternal prepregnancy obesity is associated with higher risk of placental pathological lesions. *Placenta*. 2014/08/01/ 2014;35(8):563-569. doi:https://doi.org/10.1016/j.placenta.2014.05.006

179.Scott H, Grynspan D, Anderson LN, Connor KL. Maternal underweight and obesity are associated with placental pathologies in human pregnancy. *medRxiv*. 2021:2021.06.01.21258127. doi:10.1101/2021.06.01.21258127

180.Zhang P, Haymar T, Al-Sayyed F, et al. Placental pathology associated with maternal age and maternal obesity in singleton pregnancy. *J Matern Fetal Neonatal Med*. Feb 27 2022:1-10. doi:10.1080/14767058.2022.2044777

181.Bar J, Schreiber L, Saruhanov E, Ben-Haroush A, Golan A, Kovo M. Placental histopathological findings in obese and nonobese women with complicated and uncomplicated pregnancies. *Arch Gynecol Obstet*. Dec 2012;286(6):1343-7. doi:10.1007/s00404-012-2450-z

182.Kovo M, Zion-Saukhanov E, Schreiber L, et al. The Effect of Maternal Obesity on Pregnancy Outcome in Correlation With Placental Pathology. *Reproductive Sciences*. 2015/12/01 2015;22(12):1643-1648. doi:10.1177/1933719115592712

183.Brouwers L, Franx A, Vogelvang TE, Houben ML, van Rijn BB, Nikkels PG. Association of Maternal Prepregnancy Body Mass Index With Placental Histopathological Characteristics in Uncomplicated Term Pregnancies. *Pediatr Dev Pathol*. Jan-Feb 2019;22(1):45-52. doi:10.1177/1093526618785838

184.Rosado-Yépez PI, Chávez-Corral DV, Reza-López SA, et al. Relation between pregestational obesity and characteristics of the placenta. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet*. Oct 2020;33(20):3425-3430. doi:10.1080/14767058.2019.1573222

185.Leon-Garcia SM, Roeder HA, Nelson KK, et al. Maternal obesity and sex-specific differences in placental pathology. *Placenta*. 2016/02/01/ 2016;38:33-40. doi:https://doi.org/10.1016/j.placenta.2015.12.006

186.He M, Curran P, Raker C, Martin S, Larson L, Bourjeily G. Placental findings associated with maternal obesity at early pregnancy. *Pathol Res Pract*. Apr 2016;212(4):282-7. doi:10.1016/j.prp.2016.01.006

187.Becroft DM, Thompson JM, Mitchell EA. Placental villitis of unknown origin: epidemiologic associations. *Am J Obstet Gynecol*. Jan 2005;192(1):264-71. doi:10.1016/j.ajog.2004.06.062

188.Zhou YY, Ravishankar S, Luo G, Redline RW. Predictors of High Grade and Other Clinically Significant Placental Findings by Indication for Submission in Singleton Placentas

From Term Births. *Pediatr Dev Pathol*. Aug 2020;23(4):274-284. doi:10.1177/1093526620904801

189.Langston C, Kaplan C, Macpherson T, Manci E. Practice guideline for examination of the placenta. *Archives of Pathology & Laboratory Medicine*. 1997;121(5):449.

190.Romero R, Kim YM, Pacora P, et al. The frequency and type of placental histologic lesions in term pregnancies with normal outcome. *J Perinat Med*. 2018;46(6):613-630. doi:10.1515/jpm-2018-0055

191.Pathak S, Lees CC, Hackett G, Jessop F, Sebire NJ. Frequency and clinical significance of placental histological lesions in an unselected population at or near term. *Virchows Archiv*. 2011;459(6):565-572.

192.Kelly R, Holzman C, Senagore P, et al. Placental vascular pathology findings and pathways to preterm delivery. *Am J Epidemiol*. Jul 15 2009;170(2):148-58. doi:10.1093/aje/kwp131

193.Catov JM, Scifres CM, Caritis SN, Bertolet M, Larkin J, Parks WT. Neonatal outcomes following preterm birth classified according to placental features. *American journal of obstetrics and gynecology*. 2017;216(4):411. e1-411. e14.

194.Kramer MS, Chen MF, Roy I, et al. Intra- and interobserver agreement and statistical clustering of placental histopathologic features relevant to preterm birth. *Am J Obstet Gynecol*. Dec 2006;195(6):1674-9. doi:10.1016/j.ajog.2006.03.095

195.Salafia CM, Pezzullo JC, Ghidini A, Lopèz-Zeno JA, Whittington SS. Clinical correlations of patterns of placental pathology in preterm pre-eclampsia. *Placenta*. 1998/01/01/ 1998;19(1):67-72. doi:https://doi.org/10.1016/S0143-4004(98)90100-X

196.Denison FC, Roberts KA, Barr SM, Norman JE. Obesity, pregnancy, inflammation, and vascular function. *Reproduction*. Sep 2010;140(3):373-85. doi:10.1530/rep-10-0074

197.Adibi JJ, Layden AJ, Birru RL, et al. First trimester mechanisms of gestational sac placental and foetal teratogenicity: a framework for birth cohort studies. *Human Reproduction Update*. 2021;27(4):747-770. doi:10.1093/humupd/dmaa063

198.Chambers DC, Carew AM, Lukowski SW, Powell JE. Transcriptomics and single-cell RNA-sequencing. *Respirology*. 2019;24(1):29-36. doi:https://doi.org/10.1111/resp.13412

199.Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences*. 2005;102(43):15545-15550. doi:doi:10.1073/pnas.0506580102

200.Krämer A, Green J, Pollard J, Jr, Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics*. 2013;30(4):523-530. doi:10.1093/bioinformatics/btt703

201.Oyelade J, Isewon I, Oladipupo F, et al. Clustering Algorithms: Their Application to Gene Expression Data. *Bioinform Biol Insights*. 2016;10:237-253. doi:10.4137/BBI.S38316

202.Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. *Nature Reviews Genetics*. 2016/08/01 2016;17(8):487-500. doi:10.1038/nrg.2016.59

203.Altmäe S, Segura MT, Esteban FJ, et al. Maternal Pre-Pregnancy Obesity Is Associated with Altered Placental Transcriptome. *PLoS One*. 2017;12(1):e0169223-e0169223. doi:10.1371/journal.pone.0169223

204.Cox B, Tsamou M, Vrijens K, et al. A Co-expression Analysis of the Placental Transcriptome in Association With Maternal Pre-pregnancy BMI and Newborn Birth Weight. Original Research. *Frontiers in Genetics*. 2019-April-29 2019;10(354)doi:10.3389/fgene.2019.00354

205.Mitsuya K, Parker AN, Liu L, Ruan J, Vissers MCM, Myatt L. Alterations in the placental methylome with maternal obesity and evidence for metabolic regulation. *PLoS One*. 2017;12(10):e0186115. doi:10.1371/journal.pone.0186115

206.Falick Michaeli T, Spiro A, Sabag O, et al. Determining gestational age using genome methylation profile: A novel approach for fetal medicine. *Prenat Diagn*. Oct 2019;39(11):1005-1010. doi:10.1002/pd.5535

207.Ackerman WEt, Buhimschi IA, Eidem HR, et al. Comprehensive RNA profiling of villous trophoblast and decidua basalis in pregnancies complicated by preterm birth following intra-amniotic infection. *Placenta*. Aug 2016;44:23-33. doi:10.1016/j.placenta.2016.05.010

208.Konwar C, Price EM, Wang LQ, Wilson SL, Terry J, Robinson WP. DNA methylation profiling of acute chorioamnionitis-associated placentas and fetal membranes: insights into epigenetic variation in spontaneous preterm births. *Epigenetics & Chromatin*. 2018/10/29 2018;11(1):63. doi:10.1186/s13072-018-0234-9

209.Schuster J, Uzun A, Stablia J, Schorl C, Mori M, Padbury JF. Effect of prematurity on genome wide methylation in the placenta. *BMC Med Genet*. 2019;20(1):116-116. doi:10.1186/s12881-019-0835-6

210.Bukowski R, Sadovsky Y, Goodarzi H, et al. Onset of human preterm and term birth is related to unique inflammatory transcriptome profiles at the maternal fetal interface. *PeerJ*. 2017;5:e3685. doi:10.7717/peerj.3685

211.Brockway HM, Kallapur SG, Buhimschi IA, et al. Unique transcriptomic landscapes identified in idiopathic spontaneous and infection related preterm births compared to normal term births. *PLoS One*. 2019;14(11):e0225062. doi:10.1371/journal.pone.0225062

212.Chim SSC, Lee WS, Ting YH, Chan OK, Lee SWY, Leung TY. Systematic identification of spontaneous preterm birth-associated RNA transcripts in maternal plasma. *PLoS One*. 2012;7(4):e34328-e34328. doi:10.1371/journal.pone.0034328

213.Rinaldi SF, Makieva S, Saunders PT, Rossi AG, Norman JE. Immune cell and transcriptomic analysis of the human decidua in term and preterm parturition. *Mol Hum Reprod*. Oct 1 2017;23(10):708-724. doi:10.1093/molehr/gax038

214.Lien Y-C, Zhang Z, Cheng Y, et al. Human Placental Transcriptome Reveals Critical Alterations in Inflammation and Energy Metabolism with Fetal Sex Differences in Spontaneous Preterm Birth. *Int J Mol Sci*. 2021;22(15):7899. doi:10.3390/ijms22157899

215.Luo X, Shi Q, Gu Y, et al. LncRNA pathway involved in premature preterm rupture of membrane (PPROM): an epigenomic approach to study the pathogenesis of reproductive disorders. *PLoS One*. 2013;8(11):e79897. doi:10.1371/journal.pone.0079897

216.Liang M, Niu J, Zhang L, et al. Gene expression profiling reveals different molecular patterns in G-protein coupled receptor signaling pathways between early- and late-onset preeclampsia. *Placenta*. Apr 2016;40:52-9. doi:10.1016/j.placenta.2016.02.015

217.Broekhuizen M, Hitzerd E, van den Bosch TPP, et al. The Placental Innate Immune System Is Altered in Early-Onset Preeclampsia, but Not in Late-Onset Preeclampsia. *Front Immunol*. 2021;12:780043. doi:10.3389/fimmu.2021.780043

218.Wang Y, Lumbers ER, Arthurs AL, et al. Regulation of the human placental (pro)renin receptor-prorenin-angiotensin system by microRNAs. *Mol Hum Reprod*. Sep 1 2018;24(9):453-464. doi:10.1093/molehr/gay031

219.Wilson SL, Leavey K, Cox BJ, Robinson WP. Mining DNA methylation alterations towards a classification of placental pathologies. *Hum Mol Genet*. Jan 1 2018;27(1):135-146. doi:10.1093/hmg/ddx391

220.Herzog EM, Eggink AJ, Willemsen SP, et al. Early- and late-onset preeclampsia and the tissue-specific epigenome of the placenta and newborn. *Placenta*. Oct 2017;58:122-132. doi:10.1016/j.placenta.2017.08.070

221.Lykoudi A, Kolialexi A, Lambrou GI, et al. Dysregulated placental microRNAs in Early and Late onset Preeclampsia. *Placenta*. Jan 2018;61:24-32. doi:10.1016/j.placenta.2017.11.005

222.Sood R, Zehnder JL, Druzin ML, Brown PO. Gene expression patterns in human placenta. *Proceedings of the National Academy of Sciences*. 2006;103(14):5478-5483. doi:doi:10.1073/pnas.0508035103

223.Zhang H, Zheng Y, Hou L, Zheng C, Liu L. Mediation analysis for survival data with High-Dimensional mediators. *Bioinformatics*. Aug 3 2021;37(21):3815-21. doi:10.1093/bioinformatics/btab564

224.Zhang H, Zheng Y, Zhang Z, et al. Estimating and testing high-dimensional mediation effects in epigenetic studies. *Bioinformatics*. 2016;32(20):3150-3154. doi:10.1093/bioinformatics/btw351

225.Pique-Regi R, Romero R, Tarca AL, et al. Single cell transcriptional signatures of the human placenta in term and preterm parturition. *eLife*. 2019/12/12 2019;8:e52004. doi:10.7554/eLife.52004

226.Suryawanshi H, Morozov P, Straus A, et al. A single-cell survey of the human first-trimester placenta and decidua. *Sci Adv*. Oct 2018;4(10):eaau4788. doi:10.1126/sciadv.aau4788

227.Tsang J, Vong J, Ji L, et al. Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics. PNAS 114 E7786–E7795. 2017.

228.Vento-Tormo R, Efremova M, Botting RA, et al. Single-cell reconstruction of the early maternal–fetal interface in humans. *Nature*. 2018/11/01 2018;563(7731):347-353. doi:10.1038/s41586-018-0698-6

229.Campbell KA, Colacino JA, Puttabyatappa M, et al. Placental gene expression-based cell type deconvolution: Cell proportions drive preeclampsia gene expression differences. *bioRxiv*. 2021:2021.07.29.454041. doi:10.1101/2021.07.29.454041

230.Teschendorff AE, Zhu T, Breeze CE, Beck S. EPISCORE: cell type deconvolution of bulk tissue DNA methylomes from single-cell RNA-Seq data. *Genome Biol*. Sep 4 2020;21(1):221. doi:10.1186/s13059-020-02126-9

231.Khong TY, Mooney EE, Nikkels PG, Morgan TK, Gordijn SJ. *Pathology of the placenta: a practical guide*. Springer; 2018.

232.Brouwers L, Franx A, Vogelvang TE, Houben ML, van Rijn BB, Nikkels PG. Association of Maternal Prepregnancy Body Mass Index With Placental Histopathological Characteristics in Uncomplicated Term Pregnancies. *Pediatric And Developmental Pathology*. Jan-Feb 2019;22(1):45-52. doi:10.1177/1093526618785838

233.Roberts DJ, Celi AC, Riley LE, et al. Acute Histologic Chorioamnionitis at Term: Nearly Always Noninfectious. *PLoS ONE*. 2012;7(3):e31819. doi:10.1371/journal.pone.0031819

234.Azur MJ, Stuart EA, Frangakis C, Leaf PJ. Multiple imputation by chained equations: what is it and how does it work? *Int J Methods Psychiatr Res*. 2011;20(1):40-49. doi:10.1002/mpr.329

235.Hughes RA, Heron J, Sterne JAC, Tilling K. Accounting for missing data in statistical analyses: multiple imputation is not always the answer. *International Journal of Epidemiology*. 2019;48(4):1294-1304. doi:10.1093/ije/dyz032

236.Hernán MA, Hernández-Díaz S, Robins JM. A structural approach to selection bias. *Epidemiology*. Sep 2004;15(5):615-25. doi:10.1097/01.ede.0000135174.63482.43

237.Peskoe SB, Arterburn D, Coleman KJ, Herrinton LJ, Daniels MJ, Haneuse S. Adjusting for selection bias due to missing data in electronic health records-based research. *Statistical Methods in Medical Research*. 2021;30(10):2221-2238. doi:10.1177/09622802211027601

238.Cole SR, Hernán MA. Constructing inverse probability weights for marginal structural models. *Am J Epidemiol*. Sep 15 2008;168(6):656-64. doi:10.1093/aje/kwn164

239.Seaman SR, White IR. Review of inverse probability weighting for dealing with missing data. *Statistical Methods in Medical Research*. 2013;22(3):278-295. doi:10.1177/0962280210395740

240.Assibey-Mensah V, Parks WT, Gernand AD, Catov JM. Race and risk of maternal vascular malperfusion lesions in the placenta. *Placenta*. 2018/09/01/ 2018;69:102-108. doi:https://doi.org/10.1016/j.placenta.2018.07.017

241.Liu L, Nevo D, Nishihara R, et al. Utility of inverse probability weighting in molecular pathological epidemiology. *Eur J Epidemiol*. 2018;33(4):381-392. doi:10.1007/s10654-017-0346-8

242.Hadley EE, Discacciati A, Costantine MM, et al. Maternal obesity is associated with chorioamnionitis and earlier indicated preterm delivery among expectantly managed women with preterm premature rupture of membranes. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Socie*. Sep 22 2017:1-8. doi:10.1080/14767058.2017.1378329

243.King JC. Maternal obesity, metabolism, and pregnancy outcomes. *Annual review of nutrition*. 2006;26:271-91. doi:10.1146/annurev.nutr.24.012003.132249

244.Poston L. Obesity in pregnancy; Where are we, where should we go? *Midwifery*. 2017/06/01/ 2017;49:4-6. doi:https://doi.org/10.1016/j.midw.2017.01.007

245.Pathak S, Sebire NJ, Hook L, et al. Relationship between placental morphology and histological findings in an unselected population near term. *Virchows Archive*. 2011/07/01 2011;459(1):11-20. doi:10.1007/s00428-011-1061-6

246.Redline RW. Villitis of unknown etiology: noninfectious chronic villitis in the placenta. *Human Pathology*. 2007/10/01/ 2007;38(10):1439-1446. doi:https://doi.org/10.1016/j.humpath.2007.05.025

247.Tamblyn JA, Lissauer DM, Powell R, Cox P, Kilby MD. The immunological basis of villitis of unknown etiology - review. *Placenta*. Oct 2013;34(10):846-55. doi:10.1016/j.placenta.2013.07.002

248.Scott H, Grynspan D, Anderson LN, Connor KL. Maternal underweight and obesity are associated with placental pathologies in human pregnancy. *medRxiv*. 2021;

249.Bar J, Schreiber L, Saruhanov E, Ben-Haroush A, Golan A, Kovo M. Placental histopathological findings in obese and nonobese women with complicated and uncomplicated pregnancies. journal article. *Archives of Gynecology and Obstetrics*. December 01 2012;286(6):1343-1347. doi:10.1007/s00404-012-2450-z

250.Becroft DM, Thompson JM, Mitchell EA. Placental villitis of unknown origin: Epidemiologic associations. *American journal of obstetrics and gynecology*. 2005/01/01/ 2005;192(1):264-271. doi:https://doi.org/10.1016/j.ajog.2004.06.062

251.Redline RW, Abramowsky CR. Clinical and pathologic aspects of recurrent placental villitis. *Human Pathology*. 1985;16(7):727-731.

252.Smith LH, Mathur MB, VanderWeele TJ. Multiple-bias Sensitivity Analysis Using Bounds. *Epidemiology*. 2021;32(5):625-634. doi:10.1097/ede.0000000000001380

253.Smith LH, VanderWeele TJ. Bounding Bias Due to Selection. *Epidemiology*. 2019;30(4):509-516. doi:10.1097/EDE.0000000000001032

254.Catov JM, Abatemarco D, Althouse A, Davis EM, Hubel C. Patterns of gestational weight gain related to fetal growth among women with overweight and obesity. *Obesity*. 2015;23(5):1071-1078. doi:10.1002/oby.21006

255.World Health Organization. *Obesity: preventing and managing the global epidemic*. World Health Organization; 2000.

256.Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. *Obstetrics & Gynecology*. Feb 1996;87(2):163-8. doi:10.1016/0029-7844(95)00386-x

257.Carlson NS, Hernandez TL, Hurt KJ. Parturition dysfunction in obesity: time to target the pathobiology. *Reproductive Biology and Endocrinology*. 2015;13:135-135. doi:10.1186/s12958-015-0129-6

258.Chen Y, Huang L, Zhang H, Klebanoff M, Yang Z, Zhang J. Racial disparity in placental pathology in the collaborative perinatal project. *International Journal of Clinical and Experimental Pathology*. 2015;8(11):15042.

259.Myatt L, Maloyan A. Obesity and Placental Function. *Seminars in reproductive medicine*. Jan 2016;34(1):42-9. doi:10.1055/s-0035-1570027

260.Roberts K, Riley S, Reynolds R, et al. Placental structure and inflammation in pregnancies associated with obesity. *Placenta*. 2011;32(3):247-254.

261.Austin PC, White IR, Lee DS, van Buuren S. Missing Data in Clinical Research: A Tutorial on Multiple Imputation. *Canadian Journal of Cardiology*. 2021/09/01/ 2021;37(9):1322-1331. doi:https://doi.org/10.1016/j.cjca.2020.11.010

262.Seaman SR, White IR, Copas AJ, Li L. Combining multiple imputation and inverse-probability weighting. *Biometrics*. Mar 2012;68(1):129-37. doi:10.1111/j.1541-0420.2011.01666.x

263.*R: A language and environment for statistical computing.* . R Foundation for Statistical Computing; 2020. https://www.R-project.org

264.Challier JC, Basu S, Bintein T, et al. Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. *Placenta*. Mar 2008;29(3):274-81. doi:10.1016/j.placenta.2007.12.010

265.Mi Lee S, Romero R, Lee KA, et al. The frequency and risk factors of funisitis and histologic chorioamnionitis in pregnant women at term who delivered after the spontaneous onset of labor. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Socie*. 2011;24(1):37-42. doi:10.3109/14767058.2010.482622

266.Gunatilake RP, Perlow JH. Obesity and pregnancy: clinical management of the obese gravida. *American journal of obstetrics and gynecology*. 2011/02/01/ 2011;204(2):106-119. doi:https://doi.org/10.1016/j.ajog.2010.10.002

267.Devlieger R, Benhalima K, Damm P, et al. Maternal obesity in Europe: where do we stand and how to move forward?: A scientific paper commissioned by the European Board and College of Obstetrics and Gynaecology (EBCOG). *Eur J Obstet Gynecol Reprod Biol*. Jun 2016;201:203-8. doi:10.1016/j.ejogrb.2016.04.005

268.Klebanoff MA. The Collaborative Perinatal Project: a 50-year retrospective. *Paediatric and perinatal epidemiology*. 2009;23(1):2-8. doi:10.1111/j.1365-3016.2008.00984.x

269.Hardy JB. The Collaborative Perinatal Project: lessons and legacy. *Ann Epidemiol*. May 2003;13(5):303-11. doi:10.1016/s1047-2797(02)00479-9

270.Pedersen AB, Mikkelsen EM, Cronin-Fenton D, et al. Missing data and multiple imputation in clinical epidemiological research. *Clin Epidemiol*. 2017;9:157-166. doi:10.2147/CLEP.S129785

271.Bodnar LM, Siega-Riz AM, Simhan HN, Diesel JC, Abrams B. The impact of exposure misclassification on associations between prepregnancy BMI and adverse pregnancy outcomes. *Obesity*. 2010;18(11):2184-2190.

272.Services USDoHaH. Linked Birth / Infant Death Records 2017-2018, as compiled from data provided by the 57 vital statistics jurisdictions through the Vital Statistics Cooperative Program. Accessed June 27, 2021. http://wonder.cdc.gov/lbd-current-expanded.html

273.Kramer MS, Papageorghiou A, Culhane J, et al. Challenges in defining and classifying the preterm birth syndrome. *Am J Obstet Gynecol*. Feb 2012;206(2):108-12. doi:10.1016/j.ajog.2011.10.864

274.Ray JG, Park AL, Fell DB. Mortality in Infants Affected by Preterm Birth and Severe Small-for-Gestational Age Birth Weight. *Pediatrics*. Dec 2017;140(6)doi:10.1542/peds.2017-1881

275.Tita AT, Doherty L, Roberts JM, et al. Adverse Maternal and Neonatal Outcomes in Indicated Compared with Spontaneous Preterm Birth in Healthy Nulliparas: A Secondary Analysis of a Randomized Trial. *Am J Perinatol*. 2018;35(7):624-631. doi:10.1055/s-0037-1608787

276.Park HS, Romero R, Lee SM, Park CW, Jun JK, Yoon BH. Histologic chorioamnionitis is more common after spontaneous labor than after induced labor at term. *Placenta*. Sep 2010;31(9):792-5. doi:10.1016/j.placenta.2010.06.013

277.Ganer Herman H, Schreiber L, Miremberg H, Ben Zvi M, Bar J, Kovo M. Histological chorioamnionitis at term according to labor onset: a prospective controlled study. *Journal of Perinatology*. 2019/04/01 2019;39(4):581-587. doi:10.1038/s41372-019-0327-8

278.Stanek J, Biesiada J. Clustering and classical analysis of clinical and placental phenotypes in fetal growth restriction and constitutional fetal smallness. *Placenta*. 2016/06/01/ 2016;42:93-105. doi:https://doi.org/10.1016/j.placenta.2016.04.012

279.Stanek J, Biesiada J, Trzeszcz M. Clinicoplacental phenotypes vary with gestational age: an analysis by classical and clustering methods. *Acta Obstetricia et Gynecologica Scandinavica*. 2014;93(4):392-398. doi:10.1111/aogs.12350

280.Schreiber JB. Latent Class Analysis: An example for reporting results. *Research in Social and Administrative Pharmacy*. 2017/11/01/ 2017;13(6):1196-1201. doi:https://doi.org/10.1016/j.sapharm.2016.11.011

281.Linzer DA, Lewis JB. poLCA: An R package for polytomous variable latent class analysis.

282.Clark S, Muthén B. Relating Latent Class Analysis Results to Variables not Included in the Analysis. 01/01 2009;

283.Vermunt JK. Latent Class Modeling with Covariates: Two Improved Three-Step Approaches. *Political Analysis*. 2017;18(4):450-469. doi:10.1093/pan/mpq025

284.Murray SR, Stock SJ, Cowan S, Cooper ES, Norman JE. Spontaneous preterm birth prevention in multiple pregnancy. *The Obstetrician & Gynaecologist*. 2018;20(1):57-63. doi:https://doi.org/10.1111/tog.12460

285.Weiner E, Dekalo A, Feldstein O, et al. The placental factor in spontaneous preterm birth in twin vs. singleton pregnancies. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 2017/07/01/ 2017;214:1-5. doi:https://doi.org/10.1016/j.ejogrb.2017.04.035

286.Catov J, Muldoon M, Reis S, et al. Preterm birth with placental evidence of malperfusion is associated with cardiovascular risk factors after pregnancy: a prospective cohort study. *BJOG: An International Journal of Obstetrics & Gynaecology*. 2018;125(8):1009-1017. doi:https://doi.org/10.1111/1471-0528.15040

287.Redline RW, Ravishankar S. Fetal vascular malperfusion, an update. *Apmis*. 2018;126(7):561-569.

288.Shaaban CE, Rosano C, Cohen AD, et al. Cognition and Cerebrovascular Reactivity in Midlife Women With History of Preeclampsia and Placental Evidence of Maternal Vascular Malperfusion. *Front Aging Neurosci*. 2021;13:637574-637574. doi:10.3389/fnagi.2021.637574

289.Committee opinion no 611: method for estimating due date. *Obstet Gynecol*. Oct 2014;124(4):863-6. doi:10.1097/01.AOG.0000454932.15177.be

290.Celeux G, Soromenho G. An entropy criterion for assessing the number of clusters in a mixture model. *Journal of classification*. 1996;13(2):195-212.

291.Wang C-P, Hendricks Brown C, Bandeen-Roche K. Residual Diagnostics for Growth Mixture Models. *Journal of the American Statistical Association*. 2005/09/01 2005;100(471):1054-1076. doi:10.1198/016214505000000501

292.Manuck TA. Racial and ethnic differences in preterm birth: A complex, multifactorial problem. *Semin Perinatol*. 2017;41(8):511-518. doi:10.1053/j.semperi.2017.08.010

293.Peelen MJCS, Kazemier BM, Ravelli ACJ, et al. Impact of fetal gender on the risk of preterm birth, a national cohort study. *Acta Obstetricia et Gynecologica Scandinavica*. 2016;95(9):1034-1041. doi:https://doi.org/10.1111/aogs.12929

294.Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science (New York, NY)*. Aug 15 2014;345(6198):760-5. doi:10.1126/science.1251816

295.Khong TY, Mooney EE, Ariel I, et al. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. *Arch Pathol Lab Med*. Jul 2016;140(7):698-713. doi:10.5858/arpa.2015-0225-CC

296.Redline RW. Inflammatory response in acute chorioamnionitis. *Semin Fetal Neonatal Med*. Feb 2012;17(1):20-5. doi:10.1016/j.siny.2011.08.003

297.Chisholm KM, Norton ME, Penn AA, Heerema-McKenney A. Classification of preterm birth with placental correlates. *Pediatric and Developmental Pathology*. 2018;21(6):548-560.

298.Wright E, Audette MC, Ye XY, et al. Maternal Vascular Malperfusion and Adverse Perinatal Outcomes in Low-Risk Nulliparous Women. *Obstetrics & Gynecology*. 2017;130(5)

299.Kovo M, Schreiber L, Ben-Haroush A, et al. The placental factor in early- and late-onset normotensive fetal growth restriction. *Placenta*. 2013/04/01/ 2013;34(4):320-324. doi:https://doi.org/10.1016/j.placenta.2012.11.010

300.Saleemuddin A, Tantbirojn P, Sirois K, et al. Obstetric and Perinatal Complications in Placentas with Fetal Thrombotic Vasculopathy. *Pediatric and Developmental Pathology*. 2010/11/01 2010;13(6):459-464. doi:10.2350/10-01-0774-OA.1

301.Kaptein KI, De Jonge P, Van Den Brink RH, Korf J. Course of depressive symptoms after myocardial infarction and cardiac prognosis: a latent class analysis. *Psychosomatic Medicine*. 2006;68(5):662-668.

302.Santaolalla A, Garmo H, Grigoriadis A, et al. Metabolic profiles to predict long-term cancer and mortality: the use of latent class analysis. *BMC molecular and cell biology*. 2019;20(1):1-15.

303.Kramer MS, Chen MF, Roy I, et al. Intra- and interobserver agreement and statistical clustering of placental histopathologic features relevant to preterm birth. *American journal of*

obstetrics and gynecology. 2006/12/01/ 2006;195(6):1674-1679. doi:https://doi.org/10.1016/j.ajog.2006.03.095

304.Systems BRFS. *Obesity among women of childbearing age: United States, 1990-2018*. Accessed April 16, 2020. www.marchofdimes.org/peristats

305.Obeidat RA, Abdo N, Sakee B, et al. Maternal and fetal serum leptin levels and their association with maternal and fetal variables and labor: A cross-sectional study. *Ann Med Surg (Lond)*. Dec 2021;72:103050. doi:10.1016/j.amsu.2021.103050

306.Obradovic M, Sudar-Milovanovic E, Soskic S, et al. Leptin and Obesity: Role and Clinical Implication. Review. *Frontiers in Endocrinology*. 2021-May-18 2021;12doi:10.3389/fendo.2021.585887

307.Aung MT, Ashrap P, Watkins DJ, et al. Maternal lipidomic signatures in relation to spontaneous preterm birth and large-for-gestational age neonates. *Scientific Reports*. 2021/04/14 2021;11(1):8115. doi:10.1038/s41598-021-87472-9

308.Salem H, Rosenfeld T, Altarescu G, Grisaru-Granovsky S, Birk R. Maternal and neonatal leptin and leptin receptor polymorphisms associated with preterm birth. *Gene*. 2016/10/10/ 2016;591(1):209-213. doi:https://doi.org/10.1016/j.gene.2016.07.014

309.Layden AJ, Bertolet M, Parks WT, Roberts JM, Adibi JJ, Catov JM. Latent class analysis of placental histopathology: a novel approach to classifying early and late preterm births. *American Journal of Obstetrics and Gynecology*. 2022/03/11/ 2022;doi:https://doi.org/10.1016/j.ajog.2022.03.012

310.Fakor F, Sharami SH, Milani F, et al. The association between level of maternal serum leptin in the third trimester and the occurrence of moderate preterm labor. *J Turk Ger Gynecol Assoc*. 2016;17(4):182-185. doi:10.5152/jtgga.2016.16121

311.Nogues P, Dos Santos E, Couturier-Tarrade A, et al. Maternal Obesity Influences Placental Nutrient Transport, Inflammatory Status, and Morphology in Human Term Placenta. *J Clin Endocrinol Metab*. Mar 25 2021;106(4):e1880-e1896. doi:10.1210/clinem/dgaa660

312.Janssen BG, Madhloum N, Gyselaers W, et al. Cohort Profile: The ENVIRonmental influence ON early AGEing (ENVIRONAGE): a birth cohort study. *International Journal of Epidemiology*. 2017;46(5):1386-1387m. doi:10.1093/ije/dyw269

313.Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. Apr 20 2015;43(7):e47. doi:10.1093/nar/gkv007

314.Wu T, Hu E, Xu S, et al. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *The Innovation*. 2021;2(3)doi:10.1016/j.xinn.2021.100141

315.Yu G, Li F, Qin Y, Bo X, Wu Y, Wang S. GOSemSim: an R package for measuring semantic similarity among GO terms and gene products. *Bioinformatics*. 2010;26(7):976-978. doi:10.1093/bioinformatics/btq064

316.Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst*. 2015;1(6):417-425. doi:10.1016/j.cels.2015.12.004

317.Liberzon A, Subramanian A, Pinchback R, Thorvaldsdóttir H, Tamayo P, Mesirov JP. Molecular signatures database (MSigDB) 3.0. *Bioinformatics*. 2011;27(12):1739-1740. doi:10.1093/bioinformatics/btr260

318.Mootha VK, Lindgren CM, Eriksson K-F, et al. PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nature Genetics*. 2003/07/01 2003;34(3):267-273. doi:10.1038/ng1180

319.Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. Mediation: R package for causal mediation analysis. 2014;

320.Barrett ES, Sathyanarayana S, Janssen S, et al. Environmental health attitudes and behaviors: findings from a large pregnancy cohort study. *Eur J Obstet Gynecol Reprod Biol*. 2014;176:119-125. doi:10.1016/j.ejogrb.2014.02.029

321.Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*. 2014/12/05 2014;15(12):550. doi:10.1186/s13059-014-0550-8

322.Yu G, Wang LG, Yan GR, He QY. DOSE: an R/Bioconductor package for disease ontology semantic and enrichment analysis. *Bioinformatics*. Feb 15 2015;31(4):608-9. doi:10.1093/bioinformatics/btu684

323.Topping V, Romero R, Than NG, et al. Interleukin-33 in the human placenta. *J Matern Fetal Neonatal Med*. Mar 2013;26(4):327-38. doi:10.3109/14767058.2012.735724

324.Stampalija T, Chaiworapongsa T, Romero R, et al. Soluble ST2, a modulator of the inflammatory response, in preterm and term labor. *J Matern Fetal Neonatal Med*. Jan 2014;27(2):111-21. doi:10.3109/14767058.2013.806894

325.You Y-A, Hwang S-Y, Kim S-M, et al. Identification of Indicators for Preterm Birth Using Retinoid Metabolites. *Metabolites*. 2021;11(7):443. doi:10.3390/metabo11070443

326.Tency I, Verstraelen H, Kroes I, et al. Imbalances between matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) in maternal serum during preterm labor. *PLoS One*. 2012;7(11):e49042-e49042. doi:10.1371/journal.pone.0049042

327.Chen P, Zhang K, Zhou B, et al. The variations in the IL1RL1 gene and susceptibility to preeclampsia. *Immunol Invest*. 2014;43(5):424-35. doi:10.3109/08820139.2013.879173

328.King JR, Wilson ML, Hetey S, et al. Dysregulation of Placental Functions and Immune Pathways in Complete Hydatidiform Moles. *Int J Mol Sci*. 2019;20(20):4999. doi:10.3390/ijms20204999

329.Porte R, Davoudian S, Asgari F, et al. The Long Pentraxin PTX3 as a Humoral Innate Immunity Functional Player and Biomarker of Infections and Sepsis. Review. *Front Immunol*. 2019-April-12 2019;10doi:10.3389/fimmu.2019.00794

330.Angeli F, Angeli E, Trapasso M, Verdecchia P. Pentraxin-3 and the pathogenesis of preeclampsia. *Hypertension Research*. 2020/09/01 2020;43(9):979-981. doi:10.1038/s41440-020-0466-5

331.Cruciani L, Romero R, Vaisbuch E, et al. Pentraxin 3 in maternal circulation: an association with preterm labor and preterm PROM, but not with intra-amniotic infection/inflammation. *J Matern Fetal Neona*. 2010;23(10):1097-1105. doi:10.3109/14767050903551509

332.Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M. Omega-3 fatty acid addition during pregnancy. *The Cochrane database of systematic reviews*. 2018;11(11):CD003402-CD003402. doi:10.1002/14651858.CD003402.pub3

333.Lash TL, Fox MP, Fink AK. *Applying Quantitative Bias Analysis to Epidemiologic Data*. Springer Publishing Company, Incorporated; 2009.

334.Infante-Rivard C, Cusson A. Reflection on modern methods: selection bias—a review of recent developments. *International Journal of Epidemiology*. 2018;47(5):1714-1722. doi:10.1093/ije/dyy138

335.van Smeden M, Lash TL, Groenwold RHH. Reflection on modern methods: five myths about measurement error in epidemiological research. *International Journal of Epidemiology*. 2019;49(1):338-347. doi:10.1093/ije/dyz251

336.Scifres CM, Parks WT, Feghali M, Caritis SN, Catov JM. Placental maternal vascular malperfusion and adverse pregnancy outcomes in gestational diabetes mellitus. *Placenta*. 2017/01/01/ 2017;49:10-15. doi:https://doi.org/10.1016/j.placenta.2016.11.004

337.Leavey K, Benton SJ, Grynspan D, Kingdom JC, Bainbridge SA, Cox BJ. Unsupervised Placental Gene Expression Profiling Identifies Clinically Relevant Subclasses of Human Preeclampsia. *Hypertension*. Jul 2016;68(1):137-47. doi:10.1161/hypertensionaha.116.07293

338.Piliszek A, Grabarek JB, Frankenberg SR, Plusa B. Cell fate in animal and human blastocysts and the determination of viability. *MHR: Basic science of reproductive medicine*. 2016;22(10):681-690.

339.Liu J, Buckley JM, Redmond HP, Wang JH. ST2 negatively regulates TLR2 signaling, but is not required for bacterial lipoprotein-induced tolerance. *J Immunol*. May 15 2010;184(10):5802-8. doi:10.4049/jimmunol.0904127

340.Chen WY, Tsai TH, Yang JL, Li LC. Therapeutic Strategies for Targeting IL-33/ST2 Signalling for the Treatment of Inflammatory Diseases. *Cellular Physiology and Biochemistry*. 2018;49(1):349-358. doi:10.1159/000492885

341.Han TS, Lean ME. A clinical perspective of obesity, metabolic syndrome and cardiovascular disease. *JRSM Cardiovasc Dis*. 2016;5:2048004016633371-2048004016633371. doi:10.1177/2048004016633371

342.Chatzi L, Plana E, Daraki V, et al. Metabolic Syndrome in Early Pregnancy and Risk of Preterm Birth. *American Journal of Epidemiology*. 2009;170(7):829-836. doi:10.1093/aje/kwp211

343.Grieger JA, Bianco-Miotto T, Grzeskowiak LE, et al. Metabolic syndrome in pregnancy and risk for adverse pregnancy outcomes: A prospective cohort of nulliparous women. *PLoS Med*. Dec 2018;15(12):e1002710. doi:10.1371/journal.pmed.1002710

344.Hendley Y, Zhao L, Coverson DL, et al. Differences in weight perception among blacks and whites. *J Womens Health (Larchmt)*. Dec 2011;20(12):1805-11. doi:10.1089/jwh.2010.2262

345.Brueckner H, Morning A, Nelson A. The expression of biological concepts of race.

346.Labrecque JA, Swanson SA. Target trial emulation: teaching epidemiology and beyond. *Eur J Epidemiol*. 2017;32(6):473-475. doi:10.1007/s10654-017-0293-4

347.Hernán MA, Robins JM. Using Big Data to Emulate a Target Trial When a Randomized Trial Is Not Available. *American journal of epidemiology*. 2016;183(8):758-764. doi:10.1093/aje/kwv254

348.Moreno-Betancur M. The Target Trial: A Powerful Device Beyond Well-defined Interventions. *Epidemiology*. 2021;32(2)

349.Yland JJ, Chiu YH, Rinaudo P, Hsu J, Hernán MA, Hernández-Díaz S. Emulating a target trial of the comparative effectiveness of clomiphene citrate and letrozole for ovulation induction. *Hum Reprod*. Jan 20 2022;doi:10.1093/humrep/deac005

350.Amarenco P, Kim JS, Labreuche J, et al. Treat stroke to target trial design: First trial comparing two LDL targets in patients with atherothrombotic strokes. *Eur Stroke J*. 2019;4(3):271-280. doi:10.1177/2396987319838100

351.Parks WT, Catov JM. The Placenta as a Window to Maternal Vascular Health. *Obstetrics and Gynecology Clinics of North America*. 2020/03/01/ 2020;47(1):17-28. doi:https://doi.org/10.1016/j.ogc.2019.10.001

352.Matsika A. Clinical correlates of histopathological entities of the placenta. *Aust J Gen Pract*. Jan-Feb 2021;50(1-2):62-69. doi:10.31128/ajgp-11-19-5154

353.Shaaban CE, Rosano C, Cohen AD, et al. Cognition and Cerebrovascular Reactivity in Midlife Women With History of Preeclampsia and Placental Evidence of Maternal Vascular

Malperfusion. Brief Research Report. *Frontiers in Aging Neuroscience*. 2021-May-04 2021;13doi:10.3389/fnagi.2021.637574

354.Catov JM, Muldoon MF, Reis SE, et al. Preterm birth with placental evidence of malperfusion is associated with cardiovascular risk factors after pregnancy: a prospective cohort study. *BJOG*. 2018;125(8):1009-1017. doi:10.1111/1471-0528.15040

355.Polnaszek BE, Clark SL, Rouse DJ. Pathologic Assessment of the Placenta: Evidence Compared With Tradition. *Obstetrics & Gynecology*. 9900;

356.Valeff N, Juriol L, Quadrana F, et al. Expression of IL-33 Receptor Is Significantly Up-Regulated in B Cells During Pregnancy and in the Acute Phase of Preterm Birth in Mice. *Front Immunol*. 2020;11:446-446. doi:10.3389/fimmu.2020.00446

357.Tang J, Zhu X, Chen Y, et al. Association of maternal pre-pregnancy low or increased body mass index with adverse pregnancy outcomes. *Sci Rep*. Feb 15 2021;11(1):3831. doi:10.1038/s41598-021-82064-z

358.Kutchi I, Chellammal P, Akila A. Maternal Obesity and Pregnancy Outcome: in Perspective of New Asian Indian Guidelines. *The Journal of Obstetrics and Gynecology of India*. 2020/04/01 2020;70(2):138-144. doi:10.1007/s13224-019-01301-8

359.Liu B, Xu G, Sun Y, et al. Association between maternal pre-pregnancy obesity and preterm birth according to maternal age and race or ethnicity: a population-based study. *Lancet Diabetes Endocrinol*. 2019;7(9):707-714. doi:10.1016/S2213-8587(19)30193-7

360.Granese R, Gitto E, D'Angelo G, et al. Preterm birth: seven-year retrospective study in a single centre population. *Italian Journal of Pediatrics*. 2019/04/11 2019;45(1):45. doi:10.1186/s13052-019-0643-9

361.Mocking M, Savitri AI, Uiterwaal CSPM, et al. Does body mass index early in pregnancy influence the risk of maternal anaemia? An observational study in Indonesian and Ghanaian women. *BMC Public Health*. 2018;18(1):873-873. doi:10.1186/s12889-018-5704-2

362.Granese R, Mantegna S, Mondello S, et al. Preterm birth: incidence, risk factors and second trimester cervical length in a single center population. A two-year retrospective study. *Eur Rev Med Pharmacol Sci*. Oct 2017;21(19):4270-4277.

363.El Rafei R, Abbas HA, Charafeddine L, et al. Association of Pre-Pregnancy Body Mass Index and Gestational Weight Gain with Preterm Births and Fetal Size: an Observational Study from Lebanon. *Paediatr Perinat Epidemiol*. 2016;30(1):38-45. doi:10.1111/ppe.12249

364.Cosson E, Cussac-Pillegand C, Benbara A, et al. Pregnancy adverse outcomes related to pregravid body mass index and gestational weight gain, according to the presence or not of gestational diabetes mellitus: A retrospective observational study. *Diabetes & Metabolism*. 2016/02/01/ 2016;42(1):38-46. doi:https://doi.org/10.1016/j.diabet.2015.06.001