ABSTRACT

Title of Dissertation: EXAMINING THE IMPACT OF PRECONCEPTION AND EARLY PREGNANCY SERUM LEVELS OF MATERNAL VITAMIN D ON CLINICAL MARKERS OF IMPLANTATION AND PREECLAMPSIA

Zeina Mohamad Alkhalaf, Doctor of Philosophy, 2023

Dissertation directed by: Associate Professor Marie E. Thoma
Department of Family Science

Vitamin D is a hormone rather than a vitamin, that is essential for overall health and wellbeing, including but not limited to the reproductive system. Although vitamin D is available through several sources, such as natural ultraviolet sunlight, food, and supplements, low circulating 25-hydroxyvitamin D (25(OH)D) levels of <30 ng/mL are common among pregnant women, with up to 69% of the US population suffering from the condition. Epidemiologic studies have suggested that low maternal serum 25(OH)D levels may be associated with adverse pregnancy outcomes, such as early pregnancy loss and preeclampsia, which may be initiated early in the pregnancy process during implantation and placentation.

From a life course perspective, the periconception and early pregnancy period marks a critical time for establishing a healthy pregnancy. Implantation and placentation occur early in pregnancy and involve a complex process that relies on optimal endometrial receptivity and a host of hormonal and immunologic signaling events. Disruptions to this process may be indicated by early clinical markers of pregnancy complications (e.g., vaginal bleeding or subchorionic hemorrhage) and associated with later adverse outcomes (e.g., preeclampsia). In contrast, higher

Human Chorionic Gonadotropin (hCG) levels, which have been linked to nausea and vomiting, may be markers of robust implantation and placentation. Therefore, I sought to investigate the preconception and early gestation maternal serum 25(OH)D levels on: (i) vaginal bleeding and subchorionic hemorrhage; (ii) nausea and vomiting; (iii) preeclampsia.

In Aim 1, an analysis of medical record documentation of vaginal bleeding and subchorionic hemorrhage found that women who were persistently deficient/insufficient in maternal serum 25(OH)D at both preconception and 8-week gestation had 2.18 times higher (95% CI: 1.13, 4.20) odds of having subchorionic hemorrhage compared to women who remained sufficient across both time periods, even after adjustment for potential confounders. Additionally, an analysis of daily diaries showed women with deficient 25(OH)D levels had a higher odds (OR: 3.02, 95% CI: 1.13, 8.13) of moderate/heavy bleeding versus none compared to women with sufficient 25(OH)D levels based on self-reported daily diaries on vaginal bleeding at the start of pregnancy. In Aim 2, women with persistently deficient 25(OH)D levels at both preconception and early gestation had lower odds (OR: 0.34, 95% CI: 0.20, 0.60) of experiencing nausea and vomiting based on medical records. In comparison, women who increased their 25(OH)D levels early in pregnancy (i.e., were deficient/insufficient at preconception then became sufficient at 8-week gestation) had 1.71 (95% CI: 1.12, 2.61) times higher odds of nausea and vomiting compared to those who were persistently sufficient across both time periods. Based on self-reported nausea and vomiting symptoms from daily diaries, deficient 25(O)D was associated with lower odds (OR 0.65; 95% CI 0.40, 1.06) of both nausea and vomiting when comparing to sufficient 25(OH)D levels. In Aim 3, women who had deficient 25(OH)D at preconception had an increased risk (RR: 1.45, 95% CI: 0.64, 3.29) of preeclampsia (as identified from medical records), although results were insignificant. Linear spline models demonstrated that the risk of preeclampsia declined with

each 1 ng/mL increase of 25(OH)D levels up to 40-45 ng/mL (RR: 0.97, 95% CI: (0.93, 1.00), but that levels beyond this threshold show an increase in the risk of preeclampsia for each 1 ng/mL increase in 25(OH)D (RR: 1.03; 95% CI: 1.00, 1.06).

This research highlights the importance of exploring the maternal serum levels of 25(OH)D at both preconception and early gestation and how it may affect adverse pregnancy outcomes, such as vaginal bleeding, subchorionic hemorrhage, preeclampsia, and pregnancy outcomes that signify a robust implantation response, such as nausea and or vomiting. It further underscores the importance of assessing maternal serum 25(OH)D levels prior to critical time of implantation and placentation and potential biologic mechanisms that may lead to adverse pregnancy outcomes. Supporting healthy implantation and placentation is of utmost importance as this may guide the remainder of the health of the pregnancy, and any disruption to this process may increase the mother and infant's risk of maternal morbidity and mortality (e.g., preeclampsia, vaginal bleeding, subchorionic hemorrhage). Future studies are needed with more diverse, larger sample sizes, and both paternal and maternal nutrition to further assess preconception nutritional risk factors on adverse and robust pregnancy outcomes. Accordingly, this research is vital as it may aid in identifying early factors that may reduce adverse maternal and infant health outcomes.

EXAMINING THE IMPACT OF PRECONCEPTION AND EARLY PREGNANCY SERUM LEVELS OF MATERNAL VITAMIN D ON CLINICAL MARKERS OF IMPLANTATION AND PREECLAMPSIA

by

Zeina Mohamad Alkhalaf

Dissertation submitted to the Faculty of Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2023

Advisory Committee:

Associate Professor Dr. Marie Thoma (Chair)
Professor Sunni Mumford (Special Member)
Professor Sandra Quinn
Professor Edmond Shenassa
Associate Professor Typhanye Dyer (Dean's Representative)
Associate Professor Jamie Trevitt

Dedication

This dissertation and research are dedicated to my late son, Mohamad Abdullah Alhauli. Losing you has been the hardest experience of my life, and I wish you were with us every single day. If there is one thing I hope to achieve in life, it is to make one less parent feel the same pain that we feel every single day. Everything I do, I do for you. You are always missed, and forever in our hearts.

Acknowledgements

The work of this dissertation could not have been done without acknowledging support from my advisors, family, friends, and colleagues. First, to my chair, advisor, and mentor, Marie. The amount of respect, dedication, and mentorship you have provided me through this journey has been insurmountable. You care deeply about others around you, and for your compassion and empathy, I am extremely grateful. You have always supported and encouraged me even before I began this program. I hope one day, I can become half of the researcher, mentor, and advisor you are today. To my mentor Sunni, you have planted a seed of curiosity in me from the first day I met you, and for that I am forever grateful. You have continuously supported and guided me through my own growth as a researcher. You are an incredible mentor and teacher and I have no doubt that the students at the University of Pennsylvania feel as lucky to have you. I would like to thank the rest of my committee members whose dedication, support, and guidance have been profound in my completion of this dissertation: Drs. Edmond Shenassa, Sandra Quinn, Typhanye Dyer, Jamie Trevitt. I would like to thank the Department of Family Science for supporting me through my doctoral program, especially through COVID and all the uncertainties that we have faced over the last 4 years.

To my mom, dad, brothers, sisters, and in-laws- thank you for believing in me and supporting me through my doctoral program. I know it wasn't an easy road for many of you with us, and your support has been a blessing in my life. To Abe, thank you for continuously checking in on me and asking about my dissertation, trying to understand the complex background and ideas, for that I am grateful. You showed love and care at a time that is most isolating and difficult, and I will always cherish that. Mom and dad, you moved halfway across the world and faced many difficulties and challenges in your mid-thirties, to give your three children a chance at a better life.

I hope you both feel reassured that your challenges and sacrifices have not been in vain, and that without your decision to immigrate and start a new life with your three young children, I wouldn't be here today defending my dissertation. To my dearest Hiba, thank you for being the most supportive of friends during this difficult time, and always encouraging me to move forward.

To my children, Faye, Yusef, and Fares. Faye, you have watched me grow into the person I am today, as we sacrificed many things to complete my degrees. I hope that if you do take away anything from this experience, it is that you can do anything you set your mind to. I thank you for always being by my side and supporting me through one of the equally biggest hurdle and achievement of my life. To Yusef, you were born right before my doctoral program and grew up alongside this doctoral journey with me. You have warmed our souls and hearts when we needed it the most. You were the light that allowed me to keep going even when I felt that I couldn't. To Fares, you came at the end of this doctoral journey, and I couldn't have prayed for a more wonderful final addition to our family. We are eternally blessed that you have joined us. I love each of you dearly- you are going to change the world. You are the greatest blessings, and I want to thank you for always loving each other and being more than just siblings, but friends.

And finally, to my husband, best friend, supporter, mentor, confidant, and the most incredible human I have ever known, Abdullah. There aren't enough words to express the level of love and support you have provided me through this journey. You believed in me more than anyone in the world, and many times even when I doubted myself. You pushed, guided, and loved me when I needed it the most. We did the impossible and worked through completing our doctoral programs together, all the while taking care of and raising our three children. We've experienced the deepest of losses, and the highest of gains together on this journey called life, and I wouldn't want to share this journey with anyone else in the world, except for you. You are the best thing to

have ever happened to our children and I, and I will eternally be grateful for the gift of having you in my life.

Table of Contents

List of Tables

List of Supplemental Tables

List of Figures

List of Abbreviations

NICHD- Eunice Kennedy Shriver National Institute of Child Health and Human Development

CRP- C-Reactive Protein

DRIs- Dietary Reference Intakes

EAGeR- Effects of Aspirin in Gestation and Reproduction Trial

LDA- Low-Dose Aspirin

25(OH)D- 25 Hydroxyvitamins D2 and D3

DUA- Data User Agreement

LBW- Low Birth Weight

IVF- In Vitro Fertilization

HCG- Human Chorionic Gonadotropin

HG- Hyperemesis Gravidarum

GDF15- Growth Differentiation Factor 15

VDRs- Vitamin D Receptors

DEQAS- Vitamin D External Quality Assessment Scheme

CONSORT- Consolidated Standards of Reporting Trials

AIC- Akaike Information Criterion

BIC- Bayes Information Criterion

RR- Risk Ratio

OR- Odds Ratio

BMI- Body Mass Index

DAG- Directed Acyclic Graph

CI- Confidence Interval

PPT- Positive Pregnancy Test

Chapter 1: INTRODUCTION

1.1. Background and Significance

Vitamin D, a hormone rather than a vitamin, is essential for overall health and wellbeing, including but not limited to the reproductive system.[1–5] Vitamin D influences an array of health systems including those affecting the bone, respiratory, reproductive, and chronic disease outcomes.[6–8] While vitamin D is generally available through several sources such as natural ultraviolet sunlight, food, and supplements, a large proportion of the global population is still at risk of vitamin D deficiency.[6,8,9] Consequently, it is important to assess the prevalence and risk factors of vitamin D deficiency and insufficiency, alternatively called hypovitaminosis D, and consequent health outcomes for subgroups of health vulnerable populations, such as pregnant women.[9,3,4,10] Epidemiologic studies have suggested that hypovitaminosis D may be associated with adverse pregnancy outcomes such as early pregnancy loss and preeclampsia.[11–14] Therefore, assessment of hypovitaminosis D during the preconception and early pregnancy period is an important public health concern that must be addressed.

Low circulating 25-hydroxyvitamin D (25(OH)D) levels of <30 ng/mL are common among pregnant women, with up to 69% of the US population suffering from the condition.[9,10] The Endocrine Society has recommended that a minimum level of 30ng/mL of circulating vitamin D is critical to support reproductive health.[1] The American College of Obstetricians and Gynecologists recommends women who are considering becoming pregnant to take prenatal supplementation to ensure sufficient vitamin D intake before and during pregnancy.[15] These recommendations are suggested to increase circulating levels of vitamin D. Currently, the levels of vitamin D recommended in prenatal vitamins provided by the Dietary Reference Intakes (DRIs) are 600IU/day.[16] However, since the majority of the US population have hypovitaminosis D, newer data suggests that these intakes are too low to increase circulating vitamin D levels to support a

healthy pregnancy.[17] A randomized control trial of vitamin D supplementation during pregnancy found that a safe threshold is between 2000-4000IU/day, with 4,000IU/day being the most effective in maintaining sufficient vitamin D levels.[17]

The periconception and early pregnancy period marks a critical time for establishing a healthy pregnancy.[18] Successful implantation and placentation involve a complex process that relies on optimal endometrial receptivity and a host of hormonal and immunologic signaling events.[18] Disruptions to this process may be indicated by early clinical markers of pregnancy complications (e.g. subchorionic hemorrhage) and associated with later adverse outcomes (e.g. preeclampsia).[19–21] It is postulated that maternal nutrient stores may play a critical role in this process.[18] A growing body of evidence suggests that circulating vitamin D is associated with several important reproductive health processes that impact both preconception and pregnancy outcomes.[3] These processes include modulation of inflammation for key reproductive organs such as the ovaries, uterus, and placenta (or cells that give rise to the placenta during development).[3]

Several in vitro studies using mouse and human cells have examined the role of vitamin D in implantation and placentation.[22–24] It is suggested that vitamin D may influence endometrial receptivity through the expression of homeobox gene HOXA10 in endometrial stroma cells, which are essential for endometrial development and uterine receptivity for implantation.[25] Additionally, vitamin D has been shown to exert immunosuppressive components in the early stages of pregnancy and suppress cytokines, which may lower inflammation and further support successful implantation.[26,27] Trophoblasts are cells that form the outer layer of a blastocyst, giving rise to a large portion of the placenta.[28] Placental trophoblasts support the production of growth factors and hormone secretion, cellular proliferation and modulation of maternal immune responses and vascularization of the placenta during pregnancy.[28] Studies using both mouse and human cells

have found high levels of vitamin D receptors in trophoblastic cells in the placenta that are hypothesized to provide anti-inflammatory effects that can support a successful pregnancy.[29,30] This suggests that vitamin D may modulate inflammatory processes through vitamin D receptors that are located in key reproductive organs.[29,30] Accordingly, hypovitaminosis D may increase the maternal risk of inflammatory pregnancy disorders, such as preeclampsia, which poses an increased health risk for both the mother and baby.[12,21,31–33]

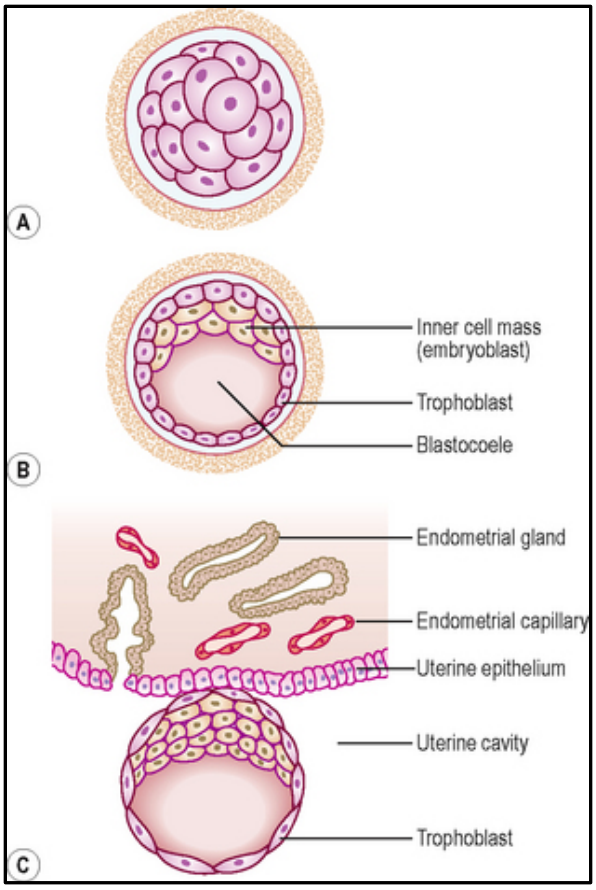

Figure 1.1. The early stages of the implantation period. This diagram expresses the process of the embryo implanting in the uterine wall. Adapted from: Themes, U. F. O. (2016, June 16).

These clinical outcomes are consistent with several epidemiologic studies that show associations between insufficient prenatal vitamin D status and adverse perinatal outcomes, including pregnancy loss, preterm birth, fetal growth restriction, and preeclampsia, which are outcomes that have also been linked to disruptions in implantation and placentation.[11,12,32,34–37] However, many of these earlier observational studies were limited to cross-sectional measurement of vitamin D late in pregnancy rather than during the critical periconception window;[12,32,34–37] and only one found associations between low preconception vitamin D and pregnancy loss.[11] More recently, an IVF study found sufficient preconception vitamin D increased the success of implantation and placentation, therefore leading to a successful pregnancy.[38] However, randomized controlled trials that have examined vitamin D supplementation express mixed results

with some studies showing an association between timing of vitamin D supplementation, such as earlier in pregnancy, and dose, such as higher dosage, and reduced risk of preeclampsia.[17,39–44] Other RCTs found no association between vitamin D supplementation and reduced risk of adverse pregnancy outcomes such as preeclampsia.[39,42] The inconsistent findings may be attributed to supplementation initiated later in pregnancy, rather than before pregnancy and/or in the early stage around the time of implantation. Accordingly, further research is needed to examine the critical preconception and early pregnancy period in which serum 25(OH)D levels may impact both early and later pregnancy complications. Identifying these critical exposure periods for vitamin D have significant public health implications given that up to 69% of pregnant women in the United States have vitamin D insufficiency and deficiency (defined as serum concentrations of 25(OH)D less than <30 ng/mL).[9,10]

1.2 Specific Aims & Hypotheses

Given the limitations of prior studies, this research uses data from the Effects of Aspirin in Gestation and Reproduction Trial (EAGeR) to examine the effects of maternal serum vitamin D during preconception versus 8-week gestation on perinatal outcomes (i.e. vaginal bleeding/subchorionic hemorrhage, nausea/vomiting, and preeclampsia). EAGeR is a prospective preconception longitudinal study that followed women with a history of pregnancy loss while attempting and during pregnancy, if they conceived.[48–50] A data use agreement has been approved by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), and this research will build on prior studies using this unique data source.[5,51–53] As such, I will be able to address knowledge gaps on maternal serum vitamin D exposure windows and associations with early clinical indicators of disrupted or robust implantation (vaginal bleeding, subchorionic hemorrhage and nausea, respectively) and preeclampsia. I hypothesize that

insufficient maternal serum vitamin D levels during these critical time windows, specifically prior to conception, may impact successful implantation and placentation, as well as both short- and long-term health outcomes of pregnancy. Specifically, I aim to:

Aim 1) Examine the association between pre-pregnancy and 8-week gestation serum 25(OH)D levels on risk of vaginal bleeding/subchorionic hemorrhage. *Hypothesis: Insufficient pre-pregnancy and early pregnancy serum 25(OH)D will increase the odds of vaginal bleeding/subchorionic hemorrhage compared to sufficient levels.*

Aim 2) Examine the association between pre-pregnancy and 8-week gestation serum 25(OH)D levels on the likelihood of nausea/vomiting. *Hypothesis: Insufficient pre-pregnancy and early pregnancy serum 25(OH)D will be inversely associated with nausea/vomiting compared to sufficient levels.*

Aim 3) Examine the association between pre-pregnancy and 8-week gestation serum 25(OH)D levels on risk of preeclampsia. *Hypothesis: Insufficient pre-pregnancy and early pregnancy serum 25(OH)D will increase the risk of preeclampsia compared to sufficient levels.*

Chapter 2: LITERATURE REVIEW

2.1. Sources and forms of Vitamin D

Vitamin D is supplied through sun exposure, diet, and supplementation.[54] Exposure to sunlight is the most common and readily available form of vitamin D.[6] Vitamin D precursor (7-dehydrocholesterol) is produced through exposure to UV rays and synthesized through the skin to Previtamin D3 and then converted to cholecalciferol (vitamin D3).[3] Sun exposure for approximately 15 minutes over the entire body would produce the equivalence of 10,000 IU of cholecalciferol.[7] However, the American Academy of Dermatology has recommended against sun exposure as a source of vitamin D due to its possible risk factors for becoming a skin carcinogen.[55,56] Therefore, recommendations have been to increase consumption through foods that are naturally rich in vitamin D, or fortified foods and beverages.[56] Consumption of plant and animal food sources such as fish, eggs and provide additional sources of both vitamin D2 and D3.[7] Vitamins D2 and D3 are synthesized through the liver, then form into 25(OH)D (calcidiol), which is measured through serum vitamin D due to its stability.[57] Afterwards, 25(OH)D is then synthesized in the kidneys, which then binds to vitamin D receptors in reproductive organs such as the ovaries, uterus, and placenta.[3]

Serum levels of 25-hydroxyvitamin D (25(OH)D) measures individual vitamin D levels, which includes both vitamin D_2 and D_3.[4] The most active form of vitamin D is $1,25(OH)_2D$, converted from 25(OH)D, which is then expressed in target tissues to create a biological immune response.[4] Dietary guidelines for vitamin D consumption are between 400-800 international units (IU), but daily are not usually met by the general population.[58] Furthermore, the levels of vitamin D in prenatal vitamins recommended by the Dietary Reference Intakes (DRIs) are 600 IU/day.[16] In addition to supplementation, fortified milk and dairy have been used as an approach to improve vitamin D status in the general population within the United States, Canada, Finland, and India in

the past 10 years.[59,60]

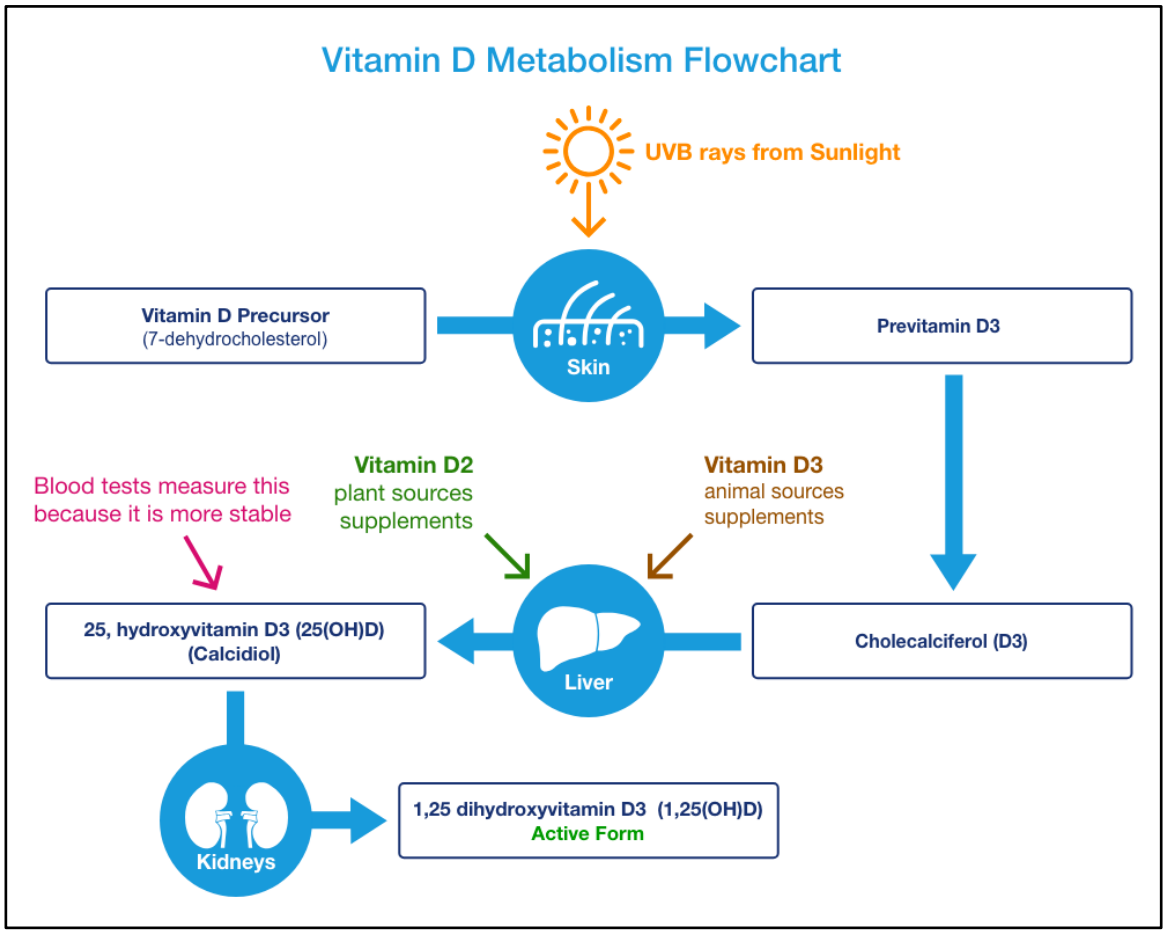

Figure 2.1. Flowchart expressing the process of vitamin D metabolism through the conversion of vitamin D2 and D3. The liver is vital for the conversion of vitamin D to 25(OH)D, which is then further metabolized by the kidney to 1,25(OH)2D. [61]

2.1.1. Factors that affect absorption and metabolism of Vitamin D

It is important to note that there are varying factors that impact vitamin D exposure and metabolism, including genetic, environmental, and lifestyle factors.[61,62] Environmental factors such access to sunlight exposure during specific seasons, often referred to as seasonality, may affect an individual's vitamin D status.[61,63–65] Studies conducted on women in Europe found that 25(OH)D measurements varied seasonally and that the spring and summer months provided up to 80% of the total vitamin D intake for the women.[61] Previous twin studies have shown that serum 25(OH)D is genetically driven and may further explain differences in 25(OH)D concentrations

within certain populations.[66,67] Melanin, which is a pigmentation of the skin, absorbs ultraviolet radiation (UVR) and reduces the absorption of vitamin D, thus reducing vitamin D synthesis in the skin.[68] In addition, certain lifestyle factors such as increased time spent indoors rather than outdoors, may also affect an individual's serum 25(OH)D levels, such as decreased physical activity, indoor jobs, higher education, decreased sun exposure (e.g., head and body covering of skin).[69,70] Some of the lifestyle factors may be modifiable by increasing time spent outdoors, eating foods with naturally occurring or fortified vitamin D, and using supplements to increase individual serum vitamin D levels.[6]

2.2. Measurement of Serum Vitamin D

Serum 25(OH)D measurement is the gold standard for vitamin D measurement.[57] Two main sources of serum vitamin D measurement are 25-hydroxyvitamin D (25(OH)D), which measures an individual's vitamin D levels, while 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) is the most active form of vitamin D.[4] 25(OH)D is the main vitamin D metabolite to determine individual vitamin D levels because it is the major circulating form of vitamin D.[4] The half-life of 25(OH)D is approximately 2-3 weeks, while the circulating half-life of 1,25(OH)D is 4-6 hours.[57] Additionally, 25(OH)D measures both vitamin D intake through sources such as diet and supplementation ($25(OH)D_2$), as well as sun exposure ($25(OH)D_3$).[57] There are several ways to measure 25(OH)D to determine an individual's vitamin D levels. 25(OH)D can be measured through liquid chromatography-tandem mass spectrometry (LC-MS) and immunoassays (including radioimmunoassays).[57] The different measurements of 25(OH)D may yield different results, however, both measurements have been established as adequate in measuring 25(OH)D levels.[57] Therefore, serum measurement of 25(OH)D is the preferred measure for evaluating individual vitamin D levels.[57]

Clinical evaluation of vitamin D is based on recommendations established by the Endocrine Society. The Endocrine Society put together a task force to provide guidelines to clinicians to support the evaluation, treatment, and prevention of vitamin D deficiency in individuals who may be at risk of hypovitaminosis D.[9,71] The task force made final recommendations based on systematic reviews of evidence and discussions with six experts and a methodologist.[9] These conclusions were recommended based on supplementation guidelines and tolerable upper limit levels, which vary based on clinical circumstances or ages presented.[9] In addition, recommendations for vitamin D serum level testing were suggested to be 25(OH)D due to reliability in diagnoses of hypovitaminosis D.[9]

Based on 25(OH)D levels, the Endocrine Society's Clinical Guidelines for vitamin D cutoffs are as follows, sufficiency is defined as levels ≥30 ng/mL (75 nmol/L), insufficiency is defined as 21-29 ng/mL (52-<72 nmol/L), and deficiency is defined as ≤20ng/mL (<50 nmol/L).[9] These recommendations were based on supporting bone and fall prevention, and currently, there are no clinical recommendations for reproductive health. Further research on vitamin D levels and/or supplementation to optimize reproductive health are needed.[9]

Table 2.1. Summarizes The Endocrine Society's Clinical Guidelines regarding vitamin D cutoffs.[9]

The Endocrine Society's Clinical Guidelines Subcommittee	
25(OH)D Levels	Levels ng/mL (nmol/L)
Sufficiency	≥ 30 ng/mL (≥75 nmol/L)
Insufficiency	21-29 ng/mL (52-<72 nmol/L)
Deficiency	≤ 20ng/mL (≤50 nmol/L)

2.3. Prevalence and Risk Factors for Hypovitaminosis D

Hypovitaminosis D (vitamin D insufficiency and deficiency) has been recognized as a global epidemic for over a decade.[8] Over a billion people worldwide are either vitamin D insufficient or deficient.[8] Vitamin D synthesis through sun exposure is one of the major sources of vitamin D for humans.[8] While the majority of vitamin D is synthesized through the skin as cholecalciferol, there are several risk factors that influence hypovitaminosis D.[72] Some of these risk factors include limited skin exposure to UVB rays, nutritional deficiency, and insufficient metabolism of vitamin D (Table 2.2).[73]

Countries that have an abundance of sunshine throughout the year have been suggested to have higher vitamin D synthesis, but recent research has found that countries abundant in sunshine such as the Middle East have the highest rates of hypovitaminosis D worldwide.[74,75] This may be due to increased head and body covering due to customary cultural practices, as well as spending more time indoors due to a very hot climate year round.[74(p),75] A recent systematic review found the prevalence in the Middle East for vitamin D deficiency to be between 30-90%.[76] Several studies conducted in Saudi Arabia also found a prevalence of low vitamin D in over 90% of the population.[77,78] In addition, countries less abundant in sunshine such as Europe, have higher levels of vitamin D.[63,64(p25),65,79–82] Roughly, <20% of the population in Northern Europe have vitamin D deficiency.[73,83–86] Additionally, 30-60% of Western, Southern, and Eastern Europe suffer from vitamin D deficiency.[63,64(p25),65,79–82]

Table 2.2. Summarizes risk factors which influence environmental and dietary effects of vitamin D.[72]

Risk Factors for Low 25(OH)D Concentrations
Risk Factors that limit skin exposure to UVB rays
Latitudes above 40° north

Winter season Exposure in early morning and evening (before 10 AM, after 4 PM) Cloud cover and atmospheric pollution Limited time spent outdoors Customary dress that conceals large portions of the body Sunscreen use Dark skin pigmentation Older age
Risk Factors that limit dietary exposure to vitamin D Low dietary intake of oily fish and egg yolks Vegetarian diets Low/no dietary intake of vitamin D fortified foods Exclusive breastfeeding in infants No intake of vitamin D supplements
Other risk factors that alter vitamin D supply or metabolism Vitamin D status of infant depends on vitamin D status of mother during pregnancy Low dietary calcium intake Obesity Genetic factors that affect vitamin D physiology and requirements Poor renal function Liver disease and cholestasis Chronic disease Malabsorption (coeliac, inflammatory bowel disease, cystic fibrosis, etc.)

In addition to demographic variation in the prevalence of hypovitaminosis D, there are several socioeconomic and biological factors that may increase that risk for the general population. Low socioeconomic status is a risk factor for hypovitaminosis D and may suggest a disproportion in nutrition through limited consumption of vitamin D rich foods such as fresh fruits, vegetables, fish, poultry, meat, and dairy.[87] Low socioeconomic status is associated with low diet quality due

to societal inequalities, such as food deserts, which are neighborhoods with limited access to nutritional foods, and food swamps, which are neighborhoods with an abundance of unhealthy food.[88] In the United States, previous studies have found that Non-Hispanic Black populations have higher prevalence of vitamin D deficiency than Non-Hispanic White populations.[89–91] This may be due to biologic and sociologic factors. First, a higher proportion of non-Hispanic Black individuals in the U.S. live in areas that are considered food deserts.[92,93] Additionally, genetic factors may affect the absorption and metabolism of vitamin D in the body.[94] Vitamin D synthesis requires vitamin D binding protein polymorphic alleles which are 1F, 1S, and 2.[94] Polymorphic refers to multiple variant forms of a DNA sequence which may occur in different individuals within a population, where an allele is defined as one of the genetic variant forms.[95] Non-Hispanic Black have primarily the 1F allele, where Non-Hispanic White populations have the 1S and 2.[94] These genetic differentiations between vitamin D binding proteins greatly lower the amount of vitamin D metabolism as seen in 25(OH)D measurement, although it is still not fully understood how these differences in metabolism may occur.[94] Additionally, Non-Hispanic Black populations have increased melanin, which inhibitors vitamin D synthesis, and increases the risk of hypovitaminosis D.[96] Therefore, Non-Hispanic Black populations are more likely to have hypovitaminosis D and may require increased sources of vitamin D to allow for the recommended levels for maintaining reproductive health.[96]

In addition, pregnant women are a unique sub-group of individuals who are at a higher risk of being vitamin D deficient or insufficient than the general population.[97–99] Levels of vitamin D increase during pregnancy, specifically through higher demand for calcium to support fetal skeletal growth.[100,101] While hypovitaminosis has been associated with rickets due to poor bone development during pregnancy and in infancy, additional consequences are recently being

recognized.[100,101] Furthermore, vitamin D status at preconception and during pregnancy has been recently associated with both reproductive and pregnancy disorders that may impact both inception of pregnancy as well as pregnancy outcomes.[5,11,12,14,97,102] Therefore, vitamin D status is critical to be further studied and assessed in this unique vulnerable population.

2.4. Vitamin D's Role in Implantation and Placentation

Women of reproductive-age and pregnant women, specifically, face unique risk factors that may place them at greater risk for hypovitaminosis D, and in turn, have implications on reproductive health and pregnancy outcomes.[3,100,103] A growing body of literature suggests vitamin D may play a role in implantation and placentation, leading to improved pregnancy and birth outcomes.[2–4,11,18,29,104] Vitamin D receptors are a class of proteins within cells that regulate the expression of certain genes that reduce inflammation and support homeostasis.[105] Vitamin D receptors are found in the immune and reproductive system, including the ovary, uterus, and placenta (specifically, trophoblasts that form the placenta).[104]

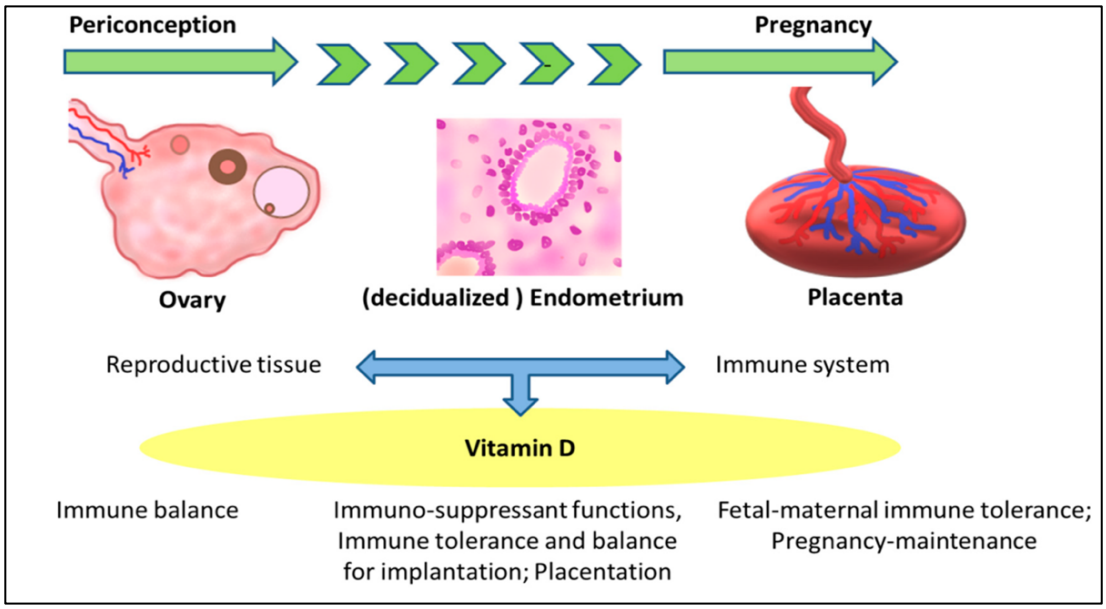

Figure 2.2. Vitamin D's immunomodulatory effects on reproductive systems from periconception to pregnancy period.[3]

The maternal immune system is vital for the regulation of inflammation and inflammatory stimuli within a pregnancy.[106] It also plays a critical role in establishing, maintaining, and supporting a healthy pregnancy to term, including supporting healthy implantation and placentation.[106] Several mediating pathways support the maternal immune system and regulation of inflammation to support a pregnancy to term.[107] Vitamin D receptors (VDRs) have been shown to impact the immune system, which helps regulate inflammation when there are acute or chronic inflammatory responses during pregnancy.[107] Vitamin D receptors are found in pregnancy organs such as the uterus and placenta, which impact hormone secretion, pregnancy implantation, and placental immune modulations for a healthy pregnancy.[1,28] Additionally, during pregnancy VDRs influence immune regulation and insulin secretion.[28] Therefore, VDR expression may significantly impact implantation and placenta alteration that is often associated with pregnancy disorders, such as preeclampsia.[100,108,] While the biological mechanisms of maternal vitamin D levels on these pregnancy disorders have not been completely understood, there are pathways through VDRs located in reproductive organs that have been shown to reduce inflammation or regulate endocrine functions and may help better explain the association between low maternal vitamin D status and risk of adverse pregnancy outcomes.[34,109–111]

Inflammation involves inflammatory cytokines which are released during various inflammatory diseases. [21,29,107,110] Increasing secretions of cytokines are essential in immune cell modulation, which helps in tissue and cell repair during times of inflammation.[21,29,107,110,111] Cytokines during pregnancy have been shown to be decreased by maternal vitamin D levels, which helps support immunomodulation and exert immunosuppressive components in the early stages of pregnancy to support successful implantation.[26,27] The reduction of cytokine production through $1,25(OH)_2D$ binding to vitamin D receptors located in the female reproductive organs lower

maternal inflammatory pathways to support implantation and a healthy pregnancy.[3] Under the appropriate environment, through the reduction of cytokine production and lower maternal inflammatory pathways, the embryo will implant into the endometrium and start secreting human chorionic gonadotropin (hCG).[112]

During the periconception period, vitamin D plays a vital role in providing immune balance and endocrine regulation to support healthy endometrial activity.[3] Vitamin D then provides immune-suppressant functions and balance for successful implantation and furthermore healthy placentation.[3] During the placentation process, vitamin D provides fetal-maternal immune tolerance to maintain the pregnancy, specifically through VDRs located on the trophoblasts that form the placenta.[18] In addition to influencing endocrine systems and local anti-inflammatory responses, vitamin D may also play a role in reducing systemic inflammatory responses.[110] Mice studies have found high levels of vitamin D receptors in trophoblastic cells in the placenta that are hypothesized to provide anti-inflammatory effects in the organ for a successful pregnancy.[29] This is due to $1,25(OH)_2D$ binding to the VDRs within various tissues and cells found in the ovaries, endometrium, and placenta which support immune function and reduction of inflammation.[3]

Trophoblasts are tissues that regulate the endocrine system and support appropriate hormone secretion, cellular proliferation, and maintains modulation of maternal immune responses during pregnancy.[104] Additionally, trophoblasts are located the outer thin layer of cells to help an embryo implant to the uterus successfully, which then helps form the placenta.[104] Binding of the vitamin D to VDRs on the trophoblasts allows for normal production of estrogen and progesterone to maintain the pregnancy until the placenta is formed.[112] Additionally, the lining of the human endometrium contain VDRs, which may also facilitate implantation by regulating the immune system as seen previously in IVF trials.[113] Furthermore, placentas of preeclamptic women with

isolated trophoblasts have one-tenth of CYP28B1, which is an enzyme activity of trophoblasts, compared with levels found in uncomplicated pregnancies.[114] Although it is suggested that vitamin D lowers the risk of preeclampsia, it is still unclear of the direct pathway.[2,12,21] One hypothesis is that low vitamin D may impair cytokine balance, thus causing abnormal implantation and placentation.[2] Thus, the immune system, particularly inflammation through cytokine expression, play a key role in the prenatal and pregnancy period.[3]

Several pregnancy hormones are thought to regulate immune function and sustain pregnancies and may be modulated by VDRs located on reproductive organs.[112,113,115,116] Progesterone is a vital steroid pregnancy hormone that is produced in the ovaries, placenta, and adrenal glands.[115,116] Progesterone is thought to improve embryo implantation and reduce the risk of both miscarriage and premature labor due to its anti-inflammatory cytokine production.[115,116] During early pregnancy, the corpus luteum increases progesterone production in pregnant women until the placenta is formed, and typically gradually rises to 175-811 nmol/L in the third trimester in comparison to 35-50 nmol/L in non-pregnant women within the follicular and mid-luteal phases of their menstrual cycle.[115,116] Estradiol is another important steroid pregnancy hormone, produced by the ovaries, that is thought to induce anti-inflammatory cytokines that reduce the risk of miscarriage and help maintain a pregnancy.[117–119] It is thought that estradiol levels in early pregnancy are reflective of the quality of the dominant follicle as well as supports the function of corpus luteum post-ovulation.[118] HCG is the first hormonal secretion by an embryo, and thus an early pregnancy marker.[112] HCG has been suggested as a clinical marker of implantation, although, differentiating levels of hCG are clinically used to determine a normal versus abnormal pregnancy.[120] It is determined that low levels or slow-rising levels of hCG are suggestive of an

potential miscarriage.[112,118] While it is suggestive that hCG supports maternal immune response to embryo implantation and placentation, the biological mechanisms are still not fully understood.[112]

Recent research suggests that vitamin D is a regulator of genetic and epigenetic factors and may significantly impact placental development.[121] DNA methylation is critical in ensuring placental development through regulation of trophoblast invasion during the placentation process.[122] Placental dysfunction is a critical marker for preeclampsia, and DNA methylation through epigenetic changes may lead to the dysfunction of implantation and placentation.[122] Studies measuring the effects of vitamin D supplementation and cord blood DNA methylation have found differences in DNA methylation in mothers who supplemented with low (600IU) versus high (3,800IU) vitamin D, which suggests potential genetic and epigenetic changes during pregnancy.[40] This is critical for our understanding of how epigenetic changes through insufficient maternal vitamin D may impact DNA methylation, and impact the implantation and placentation process which may lead to the development of preeclampsia.[40,122] Furthermore, previous studies have referenced the potential of gene expression pathways that involve the dysregulation of immune response due to early pregnancy vitamin D insufficiency and leading to potential adverse pregnancy outcomes.[13,14,109,123]

The implantation and placentation consist of stages that occur through particular biological mechanisms that may aid in the clinical guidance on best timing to measure successful implantation and placentation assessment. The implantation period consists of three stages in which 1) contact is made between the blastocyst and implantation site within the endometrium, 2) the trophoblast cells connect to the endometrial wall from the blastocyst, 3) trophoblast cells invade the endometrial stroma.[124] This process occurs during the timing of what is known as "window of implantation" which occurs between week 3-4 of gestation.[124–126] The placentation

period occurs around week 8 of gestation and is vital in providing the source of oxygen and nutrients, known as oxygenation, to the fetus.[124–126] Around week 8 of gestation, membranes of the chorion begin to form, which is then mediated by extravillous trophoblastic cells (EVT) into the placenta formation, which is also modulated by levels of oxidative stress in the villi, or also known as fibrous stroma.[127,128] The placenta is then fully formed by 20 weeks of gestation.[129] Any disruptions at this stage in implantation and placentation may cause abnormal processes that impact future development of the pregnancy and lead to adverse pregnancy outcomes.[127,128]

2.5. Hypothesized Mechanisms Between Maternal Vitamin D and Clinical Markers of Implantation/Placentation and Preeclampsia

2.5.1. Vaginal Bleeding/Subchorionic Hemorrhage

Vaginal bleeding during the first trimester is one of the most common pregnancy complications and is experienced by 15-25% of women.[130] Subchorionic hemorrhage occurs when the chorion membranes that are connected to the uterus partially detach and cause abnormal bleeding during pregnancy.[19] Roughly 11% of women experience subchorionic hemorrhage during pregnancy.[19] First trimester vaginal bleeding and subchorionic hemorrhages are biologically connected to several factors that may prevent proper implantation early in pregnancy and, accordingly, are considered potential clinical markers of disruption to implantation, placentation, or pregnancy loss.[130] These implantation disruptions include human tissue that are expressed in trophoblasts, which are located in the outer thin layer of cells to help an embryo implant to the uterus successfully.[28] These cells then help form the placenta.[28] Since high levels of vitamin D receptors are found in trophoblastic cells within the placenta, vitamin D is hypothesized to provide anti-inflammatory effects in the uterus and placenta that may mitigate some of this disruption.[29] Furthermore, vaginal bleeding and subchorionic hemorrhage are clinically significant due to their

common occurrence. Bleeding during pregnancy may be a clinical presentation of disrupted implantation and/or pregnancy loss, cause considerable stress and anxiety for both women and clinicians alike, and therefore may prompt additional testing and monitoring.[130,131]

2.5.2. Nausea

Nausea is a very common pregnancy symptom, which affects between 50-70% of pregnant women.[132] It has been previously established that nausea during early pregnancy is a clinical marker for successful implantation and placental function, as it may be indicative of higher hCG and other hormones that maintain pregnancy.[133] Previous studies have acknowledged that nausea during early pregnancy was associated with a lower risk of miscarriage, preterm birth, low birth weight (LBW), and perinatal death.[134] It has been suggested that early placental growth stimulates secretion of hCG and thyroxine, which may manifest clinically as a more heightened nausea response.[132] As such, successful implantation and healthy placental function result in higher hCG being secreted, which leads to greater nausea symptoms.[132] Therefore, nausea is a commonly reported symptom during pregnancy and may be indicative of a more robust implantation response.[132] This provides an important clinical outcome in which to examine my hypothesis in the absence of other biomarkers related to the complex process of implantation.

2.5.3. Preeclampsia

Preeclampsia is a leading cause of maternal morbidity, mortality, and preterm birth.[33,135] Preeclampsia is a maternal hypertensive disorder and previous studies have shown significantly lower vitamin D levels in women who are diagnosed.[12,13,31,34,136] Early onset and severe preeclampsia have been shown to be associated with placental insufficiency.[28] Placental insufficiency is characterized by impaired placentation and decreased trophoblast invasion, which facilitates oxygenation of the placenta.[20]

Preeclampsia has been associated with increased inflammatory cytokines, which further promotes an inflammatory state.[21] These particular inflammatory cytokines are seen in women with preeclampsia and are associated with placental ischemia, which is a vascular disorder that results in poor placental circulation.[21] The imbalance of pro-inflammatory cytokine excretion further lead to placental inflammation and increase the maternal risk of pregnancy complications.[21] Additionally, women who develop preeclampsia have impaired interactions between trophoblasts within the cells that become the placenta and endometrial lining which contributes to abnormal placentation.[3] This inflammatory pathway through vitamin D receptors and trophoblast and endometrial cells have been seen in previous studies that have shown a 5-fold increased risk of preeclampsia in women who had vitamin D levels <15ng/mL.[32] Previous studies have seen similar associations of low maternal vitamin D and increased risk of preeclampsia.[9,14,32,137] Studies have largely examined vitamin D in pregnancy, rather than prior to pregnancy, which may influence vitamin D measurement due to biologic changes during pregnancy. [13,14,109,123]

2.5.4. Gaps in Prior Research

There is limited research on the relationship between vitamin D and pregnancy outcomes, particularly related to implantation. Although several studies have assessed the association between maternal (during pregnancy) vitamin D and risk of preeclampsia, there are few studies that examine outcomes in the early pregnancy period, which may set the stage for the development of preeclampsia later in pregnancy.[12,13,34,136] Epidemiologic studies have shown maternal serum vitamin D levels <15ng/mL had a 5-fold increased risk of preeclampsia.[12] Inflammation has also been shown to increase risk of preeclampsia and studies have linked CRP, a biomarker of inflammation, to the prediction of preeclampsia.[138] Given vitamin D's immunomodulatory effects,

it has been hypothesized that sufficient vitamin D may reduce inflammation and, consequently, risk for preeclampsia, but few studies have examined this pathway.[139,140]

Another gap in prior research is the assessment of vitamin D exposure or supplementation prior to pregnancy. Previous studies examining the association between vitamin D and adverse outcomes have been mixed, which may be attributed to when vitamin D was assessed or administered during pregnancy (that is, before or after implantation).[12,31,32,34,123,136] This may distort potential causal relationships, because serum 25(OH)D has been shown to increase in pregnancy due to physiologic changes and, thus, assessment during pregnancy may not represent the critical exposure window for optimizing pregnancy outcomes. A previous study using the EAGeR data by Mumford et al. found an increased risk of pregnancy loss with lower preconception vitamin D versus at 8-week gestation, highlighting the importance of assessing the critical period prior to implantation and the role of vitamin D in supporting this process.[11]

2.6. Conceptual Framework: The Life Course Perspective

The pregnancy period and early life are critical windows that predict one's future of health and disease.[88] The life course framework is postulated to operate under three mechanisms: sensitive and critical periods of development, cumulative risk model, and the pathways model.[142] The first two models (sensitive/critical periods and cumulative risk) can help us to understand the relationship between vitamin D and early pregnancy outcomes and pre-eclampsia to be examined in this dissertation.

The sensitive and critical windows model posits that exposures during these critical windows may alter or change the development of the fetus and affect their short and long term health, potentially leading to adverse pregnancy outcomes.[143] The Barker Hypothesis is an example of the sensitive and critical time periods in which exposures, such as nutrients, during

early life may have lasting effects on later life health.[144] The Barker Hypothesis theorized that adverse nutrition prenatally and in early pregnancy may increase the risk of adverse health outcomes later in life, which include obesity, diabetes, insulin sensitivity, hypertension, hyperlipidemia, coronary heart disease, and stroke.[141] The most documented example of the connection between maternal nutrient deficiency and fetal development is the connection between folate deficiency and neural tube defects.[145] The development of the fetal neural tube occurs at approximately 3 weeks of pregnancy, in which at 28 days post conception, the neural tube is closed.[145] Most women during this time are often unaware of their pregnancies, and therefore a folate deficiency may be present due to multiple factors such as not taking a prenatal supplement.[145] If maternal nutrition, such as folate, in the early critical periods of development may alter the health trajectory of the infant and increase their risk of spina bifida,[145] then vitamin D, which is a source of anti-inflammatory support for the placenta and uterus lining may also have an impact in the early critical periods of implantation and the health trajectory of the pregnancy.[29,107,110,146,147] Given the formation of the neural tube occurs early in pregnancy, the critical exposure window for folic acid is prior to pregnancy (preconception).[145] Similarly, preconception and early pregnancy vitamin D may have an important role in early development during the period of implantation and placental formation through the mechanisms described in Section 2.4 and 2.5 and outlined in Figure 1 (see Appendix I). Specifically, vitamin D deficiency disrupts functioning of reproductive organs that leads to adverse pregnancy outcomes.[37,112,116,118] This results through the disruption of hormones (estrogen and progesterone), which help to maintain a pregnancy, and local and systemic immune functioning, which facilitate endometrial receptivity and implantation.[37,112,116,118] Clinical markers of disrupted implantation include vaginal bleeding and subchorionic hemorrhage.[19,130,131,148,149] If implantation is successful, it results in

production of hCG, which has been linked to a greater nausea response, and facilitates further development of the placenta and maintenance of the pregnancy.[132–134,150] Vitamin D receptors on trophoblasts that form the placenta can also facilitate this development given sufficient vitamin D levels.[18,22,112] In the absence of a robust implantation/placentation, women may be more susceptible to the development of pre-eclampsia and other adverse pregnancy outcomes, such as intrauterine growth restriction.[13,18,20,22]

Another life course framework, the cumulative risk model, may also explain the development of vitamin D insufficiency prior to pregnancy.[142,151] This model posits that over time reproductive health declines due to exposures that increase the body's allostatic load.[152] The best example of this life course pathway is known as the "Weathering Hypothesis," which was proposed by Arlene Geronimus to explain the disparities in race and gender, particularly in African American women, who biologically start to age at a faster rate than white women due to increased allostatic load from socioeconomic disadvantages early on in life.[153] Similarly, the accumulation of insufficient maternal vitamin D, while not directly being evaluated in my study, can develop through a lack of exposure to sources of vitamin D over time (e.g., sunlight or food). Additionally, the cumulative risk framework assesses the risk of accumulation of a certain exposure, or lack thereof, such as vitamin D.[142,151] In addition, nutritional deficiencies, lack of supplement use, or cultural practices, such as covering up for Muslim and religious individuals, may increase vitamin D deficiency or insufficiency risk.[74,90,154–156] Accordingly, this lack of vitamin D exposure may accumulate over time, resulting in insufficient vitamin D stores prior to and during pregnancy.

In sum, the life course perspective is important in examining preconception and prenatal maternal serum vitamin D on pregnancy outcomes proposed in this dissertation. These pathways are critical to understanding how the biological mechanisms of vitamin D may affect pregnancy

outcomes, such as nausea, vaginal bleeding/subchorionic hemorrhage and preeclampsia.[93] Any adverse exposure occurring during the critical period of early fetal development or that may accumulate over time will result in a chain of events that may increase risk of adverse pregnancy outcomes.[151] Specifically, previous studies assessing maternal serum vitamin D and adverse pregnancy outcomes have not been able to fully differentiate these critical windows and distinguish ideal timing for optimizing vitamin D stores to improve pregnancy outcomes.[12,13,31,42,157] Findings from this dissertation can be used to inform the need for access to early and simple interventions, such as vitamin D supplementation, to promote maternal and pregnancy health outcomes.

2.6.1. Directed Acyclic Graphs (DAGs) for Aims 1, 2, and 3

Vitamin D levels can be influenced by both dietary sources and sun exposure and absorption (Chapter 2). The DAGs represent the different covariates considered and their distinct pathways related to both the exposure and outcomes under study in each aim (Chapters 4, 5, and 6). Each of the paths represents associations between covariates and the exposure (vitamin D) and outcomes (vaginal bleeding/subchorionic hemorrhage, nausea, and preeclampsia). White circles represent covariates adjusted for in the analysis. Green empty circles represent unobserved variables. Green circles with a triangle inside represent the exposure of interest. Blue empty circles represent ancestors of outcomes. Finally, blue circles with a line inside represent the outcomes of interest. For each aim, all covariates inform adjustment to minimize confounding bias when analyzing the association between the exposure (vitamin D) and each of our outcomes (vaginal bleeding/subchorionic hemorrhage, nausea, and preeclampsia). After adjustment, no biasing pathways remain. These DAG adjustments were determined using the DAGitty website.[158]

2.6.2. Covariate relationships with vitamin D and outcomes under study

I was interested in several potential confounding factors including age, BMI, smoking, physical activity, vitamin use, diet/nutrition, SES status, parity, season of blood draw, sun exposure, and stress. It has been determined that there are complex relationships between these confounders and the exposures and outcomes of interest. Previous research has shown racial and ethnic differences in vitamin D levels within individuals in the United States, in particular affecting those who are African American.[159] These differences may be due to differences in factors that affect sun exposure, but may also be due to biologic factors that influence vitamin D absorption.[159] Specifically, darker skin contains more melanin which inhibits the absorption of vitamin D through the skin, which is the most bioavailable form of vitamin D.[91] In addition, race and ethnicity has been shown to be associated with stress either related to socioeconomic factors or racism, which has been shown to be associated with numerous perinatal outcomes, including pregnancy loss and hypertensive disorders during pregnancy.[159–164] Parity has also been seen as a predictor of vitamin D deficiency and insufficiency, in particular among pregnant women.[165] Parity may increase stress (i.e., socioeconomic factors), which may also affect the implantation process of pregnancy and inflammation.[166–168] Dietary factors and vitamin consumption, which are additional sources of nutrients that would affect vitamin D levels, may also be affected by parity,.7/28/23 12:51:00 PM A nutrient deficient diet has also been linked to adverse perinatal outcomes (e.g., folic acid deficiency), particularly early in gestation.[145,171–173] In addition, parity may affect physical activity, as having more children is associated with less time for physical activity, which in turn may increase risk for higher BMI.[174] BMI has been previously sited as a predictor of vitamin D deficiency and insufficiency, and nutrition, diet, and vitamins may be associated with lower levels vitamin D.[58,175] In addition, while we do not have specific nutrition and diet information, vitamins

are used as a proxy for a source of exposure for vitamin D. Similar to parity, socioeconomic status (SES) has been associated with stress, nutrition, diet and vitamin consumption, and physical activity, since SES is highly correlated with resources that enable individuals to exercise, consume adequate nutrition, and minimize stress.[176]

Physical activity, nutrition and BMI have relationships with vitamin D and the outcome.[177–179] Physical activity is associated with a higher likelihood of being outdoors and, thus, an increase in sun exposure, which affects vitamin D.[180] Physical activity has a direct link with diet and nutrition behaviors, which both affect BMI.[177–179] Lifestyle factors like physical activity tend to decline as we age, which increases the risk of higher BMI and overall fertility health, such as those of embryo quality, which leads to disrupted implantation, and or implantation failure.[181–183] BMI has been previously sited as a predictor of vitamin D deficiency and insufficiency, and nutrition, diet, and vitamins may be associated with lower levels vitamin D.[58,175] Diet is also a distinct source of vitamin D separate from BMI and physical activity. In this analysis, vitamins were used as a proxy for a dietary source of exposure for vitamin D.

Due to aspirin's blood thinning effects, women who use aspirin during pregnancy may be at a higher risk of vaginal bleeding.[184] However, previous studies have shown that aspirin has been associated with a decreased risk of pregnancy loss and disrupted implantation.[185,186] Treatment status is not a confounder in these analyses, but is an antecedent of the outcome. Adjustment for antecedents of the outcome can, in some cases, increase precision of covariate estimates.[187] Finally, aspirin has been shown to increase the incidence of robust implantation and therefore decrease the risk of pregnancy loss.[185,186] Finally, previous studies have shown that aspirin has been associated with a decreased risk of inflammation and preeclampsia.[184–186,188,189]

Description of mechanism between vitamin D and outcomes

For Aim 1, assessing the relationship between vitamin D and vaginal bleeding/subchorionic hemorrhage, I hypothesize that this effect is due to low levels of vitamin D being associated with pregnancy loss (Chapter 4).[11,19,112,131,150,190,191] This DAG describes the mechanism and potential confounding factors considered in my analysis for Aim 1 (Figure 2.3).

For Aim 2, assessing the relationship between vitamin D and nausea, I hypothesize that this effect is due to robust implantation (Chapter 5). Higher levels of vitamin D have been associated with robust implantation, which then may lead to a higher hCG response, increasing a woman's risk of nausea and decreasing the risk of pregnancy loss (Figure 2.4).[11,112,150,169,190]

For Aim 3, assessing the relationship between vitamin D and preeclampsia, I predict that low levels of vitamin D may increase the risk of preeclampsia (Chapter 6). In addition, vitamin D has been associated with lower levels of inflammation, which may be measured through c-reactive protein (CRP).[192,193] Low levels of vitamin D have been associated with disrupted implantation, which then may lead to placental insufficiency and an increased risk of preeclampsia (Figure 2.5).[14,22,104,109,127,190,194]

*Note: Although the results for Aim 3 were suggestive, the relationship between preconception vitamin D and preeclampsia was insignificant. Therefore, we did not do the mediation analysis. The preeclampsia DAG has been updated to express the mediating pathways, but the mediator outcome and mediator exposure confounders have been removed for simplicity.

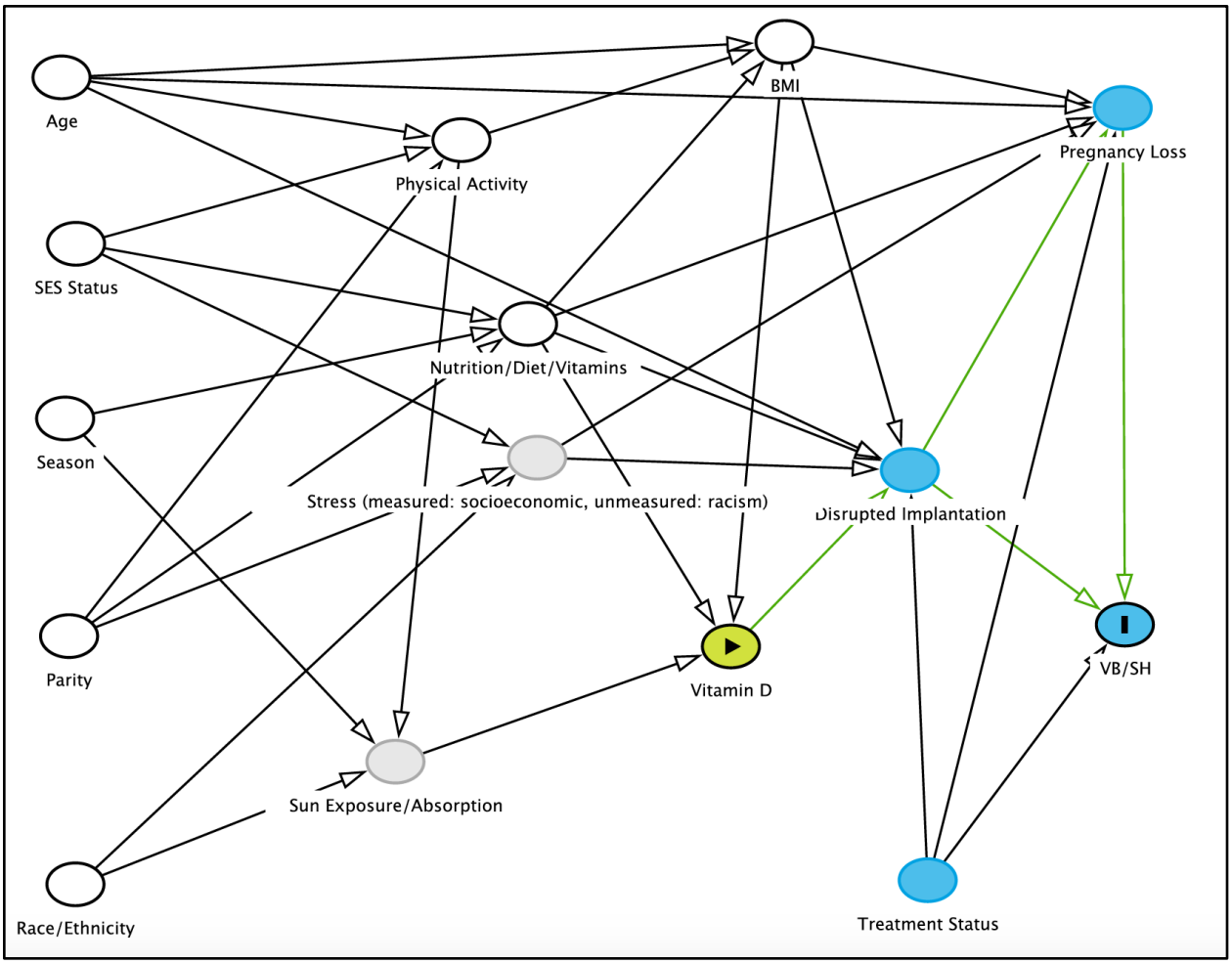

Figure 2.3. Directed Acyclic Graph (DAG) assessing maternal serum vitamin D and vaginal bleeding/subchorionic hemorrhage.

*VB: Vaginal Bleeding, SH: Subchorionic Hemorrhage

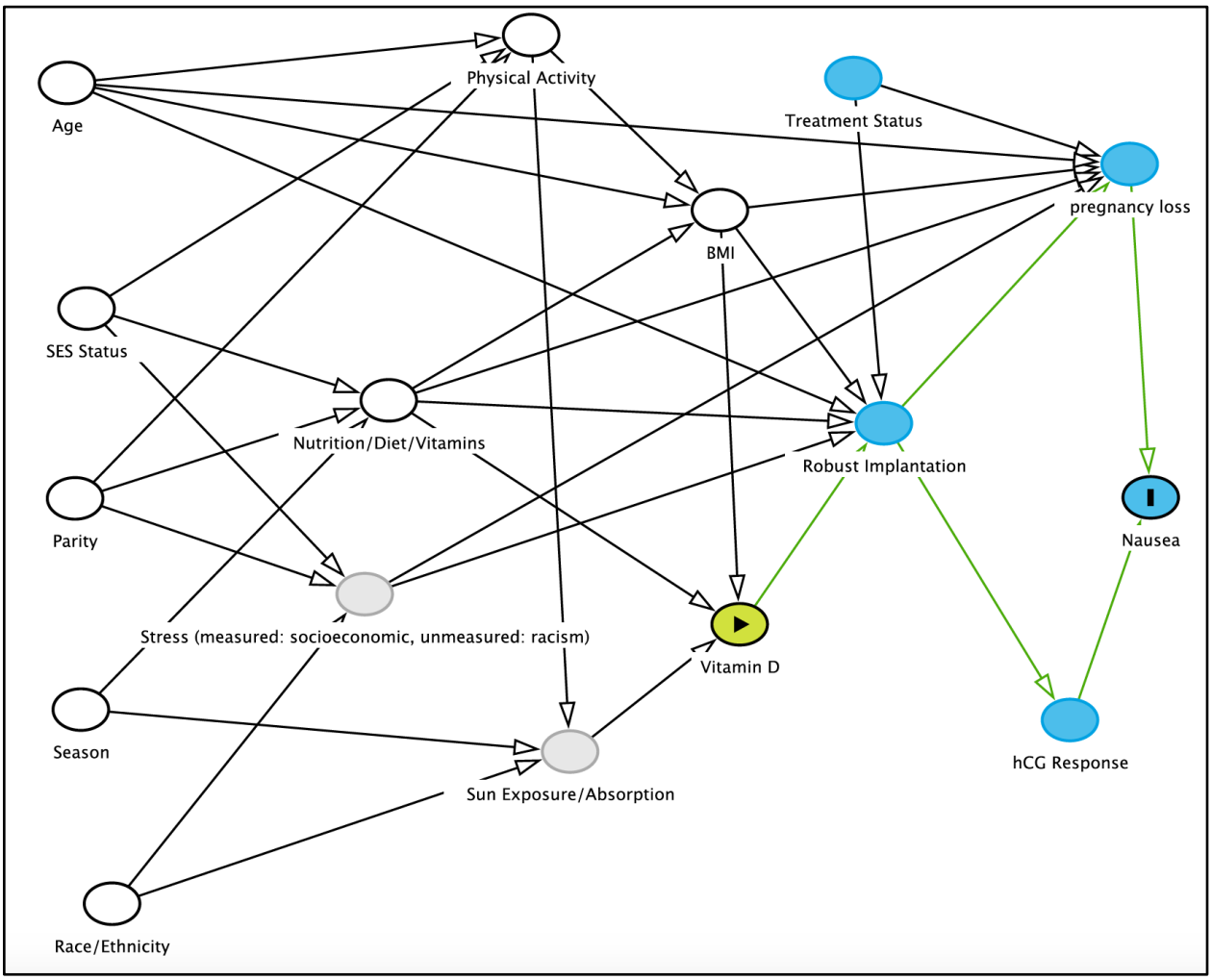

Figure 2.4. Directed Acyclic Graph (DAG) of confounders conceptualization for Aim 2 assessing maternal serum vitamin D at preconception versus 8-week gestation and nausea.

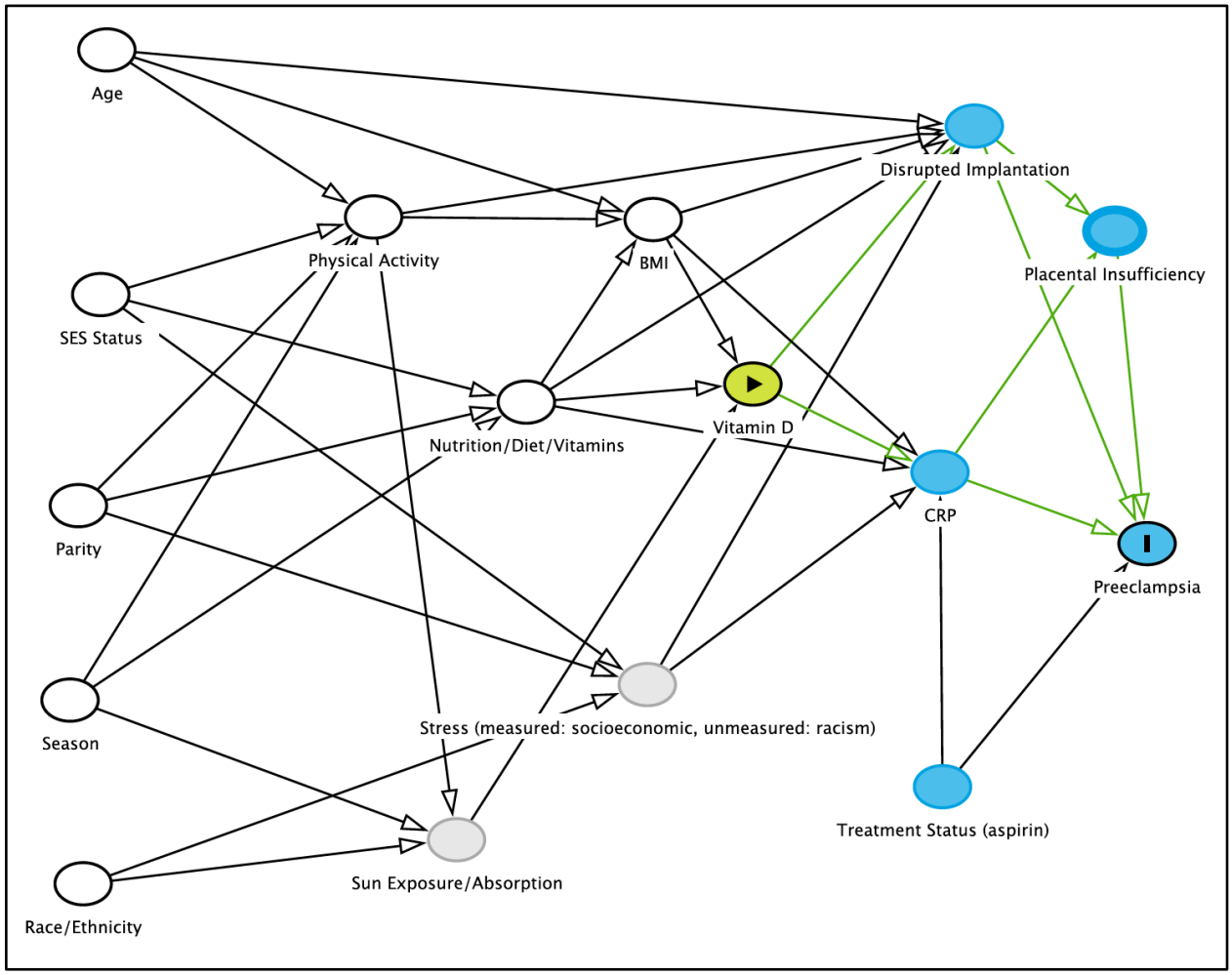

Figure 2.5. Directed Acyclic Graph (DAG) of confounders conceptualization for Aim 3 assessing maternal serum vitamin D levels at preconception and 8-week gestation on preeclampsia.

Chapter 3: METHODS

3.1. Data Sources and Population

This research used data from the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial (2007-2011), which is a multisite, prospective, double-blind, block-randomized, placebo-controlled clinical trial designed to evaluate the effect of low-dose aspirin (LDA) on live-birth in healthy women with regular menstrual cycles and 1-2 prior pregnancy losses.[48] The EAGeR trial enrolled 1,228 women between 18 and 40 years of age who were attempting pregnancy after 1-2 prior pregnancy losses, of which 597 had a live birth. Women enrolled in the trial could not have received fertility treatments prior or during their enrollment in the EAGeR trial or have a prior diagnosis of infertility. The institutional review boards at each study site and the data coordinating center approved the protocol for the trial. All participants provided their written consent prior to enrolling in the study. The trial was registered with ClinicalTrials.gov (#NCT00467363). Secondary analysis of the EAGeR trial were used to assess the proposed research aims.

3.2. Analytic Sample

The analytic sample was restricted to women in the EAGeR trial for whom there is measured serum 25(OH)D levels at preconception or 8-weeks' gestation, had a live birth, and were not missing data on the outcomes of interest for each Aim. Restriction to a live birth was used to examine the effect of vitamin D on clinical outcomes independent of those factors that may lead to a pregnancy loss. This restriction is especially important because preeclampsia is a condition that develops later in pregnancy, after the period many pregnancy losses may have already occurred. I accounted for this potential selection effect in the analyses and examined this restriction further in sensitivity analyses described below. Pregnancy status was determined via positive urine hCG pregnancy tests (Quidel Quickvue, Quidel Corporation), conducted at home or in the clinic at the time of expected menses. The institutional review boards at each study site (Salt Lake City, Utah; Denver,

Colorado; Buffalo, New York; Scranton, Pennsylvania) and the data coordinating center approved the protocol for the trial. Access to this data source requires approval from *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), which I have acquired through a data user agreement (DUA).

3.3. Measurement and Operationalization of Variables

3.3.1. Exposure Measurement: Vitamin D

3.3.1.1. Laboratory Assessment

Serum 25(OH)D samples were collected at baseline prior to randomization to LDA and at 8-week gestation post conception. The serum vitamin D samples were stored at −80°C until used for analysis.[48] Combined concentrations of 25-hydroxyvitamins D2 and D3 (25(OH)D) were measured using the 25(OH)D ELISA solid phase sandwich enzyme immunoassay (BioVendor R&D, Ashville, NC, USA). The ELISA solid phase sandwich enzyme immunoassay has been validated.[195] Although it is suggested that liquid chromatography-tandem mass spectrometry is the gold standard for vitamin D measurement, previous studies conducted have found vitamin D measurement results to be similar in immunoassays through the Vitamin D External Quality Assessment Scheme (DEQAS).[195–197] Therefore, the ELISA solid phase sandwich enzyme immunoassay is a precise and valid measurement for vitamin D concentrations.

3.3.2. Outcome Measurement: – Clinical markers of implantation and preeclampsia

3.3.2.1. Self-reported data from daily dairies

Vaginal Bleeding and Nausea. Vaginal bleeding or nausea/vomiting symptoms were recorded by participants using the preconception daily diaries, which were provided for their first 2 menstrual cycles. If conception occurred, the participants recorded the conception in their pregnancy daily diaries, which began once they received a positive pregnancy test (PPT). On average, 72% of

participants completed their daily diaries for everyday in a week, 83% completed 5 out of 7 days in a week, and 91.2% completed 3 out of 7 days in a week. Previous studies have validated the outcomes of vaginal bleeding and nausea self-report and have found reasonable accuracy between the self-reported data and clinical presentations.[198,199] Daily bleeding was recorded in the daily diaries based on standardized pictographs as either none, spotting and/or very light, light, moderate, heavy, or very heavy (see Appendix II). Daily nausea was recorded as none, nausea, vomiting once per day, or vomiting more than once per day (see Appendix II). The first day of the last mensural period (LMP) was used to assess symptom timing. Ascertainment of symptoms was assessed between 3-8 weeks post LMP, which would be the clinical approximation between date of ovulation to potential pregnancy.[150]

3.3.2.2. Questionnaire data or medical record

Subchorionic Hemorrhage. Diagnoses of subchorionic hemorrhage in pregnancy were obtained prospectively by maternal report on questionnaires and/or abstracted from participant delivery records by trained research staff.[50]

Vaginal Bleeding. Diagnoses of vaginal bleeding in pregnancy were obtained prospectively by maternal report on questionnaires and/or abstracted from participant delivery records by trained research staff.[50]

Preeclampsia. Diagnoses of preeclampsia in pregnancy were obtained prospectively by maternal report on questionnaires and/or abstracted from participant delivery records by trained research staff after 12 weeks of gestation.[50]

3.3.3. Operationalization of Measures for Analysis

The research aims examined serum 25(OH)D levels (exposure) on markers of implantation (vaginal bleeding or subchorionic hemorrhage, nausea) and adverse pregnancy outcomes

(preeclampsia) among women who achieved pregnancy. Specifically, I aimed to examine: **1)** the association between pre-pregnancy and 8-week gestation serum 25(OH)D levels on risk of vaginal bleeding/subchorionic hemorrhage; **2)** the association between pre-pregnancy and 8-week gestation serum 25(OH)D levels on the likelihood of nausea/vomiting*;* **3)** the association between pre-pregnancy and 8-week gestation serum 25(OH)D levels on risk of preeclampsia.

3.3.3.1. Exposure

The vitamin D cutoffs that were used in these analyses are based on levels designated by the Endocrine Society (<30 ng/mL equivalent to 75 nmol/L).[9] Women were classified as vitamin D 25(OH)D deficient (≤20 ng/mL), insufficient (21-29 ng/mL), or sufficient (≥30 ng/mL) at preconception and 8-week gestation.[9] Initially, I looked at vitamin D as a categorical variable to aid in clinical interpretation and comparison with other studies conducted, based on the Endocrine Society's vitamin D cut off recommendations. The Endocrine Society's cut offs were developed originally for bone health and not based on reproductive health.[9] As such, I examined continuous models for vitamin D. To inform additional cut offs for vitamin D, including continuous models, I ran exploratory analyses of lowess regression models of continuous vitamin D on the binary indicators of the outcome measures to determine any threshold effects. If threshold effects were found, I modeled the continuous vitamin D measure using linear splines or categories defined at these cut-points.

3.3.3.2. Outcome measures

Subchorionic Hemorrhage. Categorized as (yes/no) and obtained from medical records.

Vaginal Bleeding. Categorized as (yes/no) and obtained from medical records.

Preeclampsia. Clinical diagnosis as (yes/no) at any point after 12 weeks of gestation from medical records.

Vaginal bleeding and Nausea/Vomiting (Daily Diaries)

Daily diaries data will be summarized biweekly from 3 weeks and 0 days to 4 weeks and 6 days, 5 weeks and 0 days to 6 weeks and 6 days, and 7 weeks and 0 days to 8 weeks and 0 days. Any vaginal bleeding or nausea/vomiting reported within a 2-week period will be defined as vaginal bleeding or nausea/vomiting within that period. For both vaginal bleeding and nausea/vomiting, these categories were defined at biweekly intervals between 3-4, 5-6, and 7-8 weeks of gestation. Subchorionic hemorrhage and vaginal bleeding (medical records). Vaginal bleeding was categorized in three ways: 1) women who had any vaginal bleeding versus none 2) women who had light bleeding versus none or 3) women who had any moderate to heavy bleeding versus none. Similarly, nausea and vomiting were categorized in three ways: 1) women having reported any nausea/vomiting versus none or 2) women having reported nausea only versus none or 3) women having reported vomiting once per day or more than once per day versus none.

3.3.3.3. Confounders

Sociodemographic and other health characteristics were available on all the women within the EAGeR dataset, including age, race/ethnicity, education, employment, income, BMI, parity, season, physical activity, alcohol intensity, and multivitamin use. Models were informed by Directed Cyclical Graph (DAG) developed for the relationship between the covariates (see Chapter 2). Variables were selected based on their relationship to exposure to different sources of vitamin D, including sun exposure and diet or nutrition, and the outcome or preceding factors that might affect the outcome. Season was defined as the season of baseline during which blood was drawn for the sample of serum 25(OH)D measured. Physical activity was assessed using the International Physical Activity Questionnaire and defined as low, moderate, or high.[200] Alcohol intensity was defined as the amount of alcohol consumed in the past year and was measured as never, sometimes,

and often. Multivitamin use was measured as the type of vitamins that were consumed by the women prior to the study and were defined as not taking any vitamins or folic acid, taking vitamins with no folic acid, and taking vitamins with folic acid.

Due to aspirin's blood thinning effects, women who use aspirin during pregnancy may be at a higher risk of vaginal bleeding.[184] However, previous studies have shown that aspirin has been associated with a decreased risk of pregnancy loss and disrupted implantation.[185,186] Treatment status is not a confounder in these analyses, but is an antecedent of the outcome. Adjustment for antecedents of the outcome can, in some cases, increase precision of covariate estimates.[187] Finally, aspirin has been shown to increase the incidence of robust implantation and therefore decrease the risk of pregnancy loss.[185,186] Finally, previous studies have shown that aspirin has been associated with a decreased risk of inflammation and preeclampsia.[184–186,188,189]

3.4. Analytic Plan

3.4.1. Descriptive Analyses

Relationships between baseline characteristics and vitamin D levels by deficient versus insufficient versus sufficient levels (preconception, 8-week gestation) and pregnancy outcomes (vaginal bleeding/subchorionic hemorrhage and nausea/vomiting at bi-weekly intervals and preeclampsia after 12 week gestation) were examined using chi-square tests or ANOVA for comparing categorical or continuous variables, respectively.

3.4.2. Aims 1 & 2

The odds ratio between preconception serum 25(OH)D levels on vaginal bleeding/subchorionic hemorrhage and nausea/vomiting at biweekly intervals in the first 8 weeks of pregnancy (3 time points) were examined using generalized estimating equations (link: log, family: binomial) and an unstructured correlation matrix. Odds ratios evaluating associations between serum 25(OH)D at

8-week gestation and vaginal bleeding/subchorionic hemorrhage and nausea/vomiting (6-8 weeks' gestation) were estimated using multinomial logistic regression models with robust standard errors. Models were adjusted for relevant confounders as determined by DAGs (see Chapter 2).

For Aims 1 and 2, my outcomes are conditional on becoming pregnant, therefore I included inverse probability weights to control for potential selection bias introduction by restricting to women who became pregnant. Inverse probability weights were determined from models that include covariates associated with the probability of pregnancy, such as age, BMI, race, number of prior losses, physical activity, parity, treatment assignment, and preconception 25(OH)D concentrations.

3.4.3. Aim 3

Risk ratios evaluating associations between preconception and 8-weeks' gestation serum 25(OH)D levels and preeclampsia were estimated using log binomial regression models with robust standard errors. Models were adjusted for relevant confounders as determined by DAGs (see Chapter 2). I ran separate models for preconception vitamin D and 8-weeks' gestation vitamin D levels. For Aim 3, my outcome is conditional on becoming pregnant and continuing to a live birth, I also included inverse probability weights to control for potential selection bias introduced by restricting to women with a live birth given that vitamin D may also influence the probability of achieving and maintaining a pregnancy. This approach is often applied in perinatal epidemiology to account for the conditional nature of human reproduction (i.e. becoming pregnant and remaining pregnant). Because several processes must occur for successful reproduction, a portion of those trying to conceive will achieve pregnancy, and a lower percentage of those will successfully have a live birth.[201] Bias occurs when preconception exposure could affect the chance of pregnancy, or the survival of the pregnancy, and the analysis is restricted to those with a pregnancy or a live birth.[201]

Weights were determined from models that include covariates associated with the probability of pregnancy, such as age, BMI, race, number of prior losses, physical activity, parity, treatment assignment, and preconception 25(OH)D concentrations.

3.4.4. Sensitivity Analyses

In addition to restriction to live births in Aims 1 and 2, I examined associations of vitamin D on each of the outcomes stratified by treatment assignment (placebo vs. low dose aspirin) for all analyses. Assessment of vitamin D in all three aims was stratified by treatment assignment of aspirin/placebo, as previously assessed in a study of vitamin D and pregnancy loss using this data.[11] Previous evidence has shown that an increased risk of vaginal bleeding has been seen with low-dose aspirin (LDA) due to its ability to thin the blood to reduce or prevent blood clots from forming.[150,188] In addition to thinning the blood, aspirin has been shown to reduce inflammation by blocking the production of prostaglandins, which help regulate pain and inflammation within the body.[188,202]

Chapter 4: Aim 1- The Association between Preconception and 8-week Gestation Serum 25(OH)D Levels on the Risk of Vaginal Bleeding and Subchorionic Hemorrhage

4.1. Introduction

The periconception and early pregnancy period marks a critical time for establishing a healthy pregnancy.[18] Successful implantation and placentation involve complex processes that rely on optimal endometrial receptivity and a host of hormonal and immunologic signaling events.[18] Disruptions to this process may be indicated by early clinical markers, such as early vaginal bleeding and subchorionic hemorrhage.[19,130,131,150] However, It has been postulated that maternal nutrient stores may play a critical role in this process.[18] Vaginal bleeding during the first trimester is one of the most common pregnancy complications and is experienced by 16-25% of women.[130] One cause of vaginal bleeding during pregnancy is subchorionic hemorrhage, which occurs when the chorion membranes connected to the uterus partially detach and cause abnormal bleeding during pregnancy.[19] Roughly 11% of women experience subchorionic hemorrhage during pregnancy and it is considered to be the most frequent cause of vaginal bleeding between 10-20 weeks' gestation.[19] First trimester vaginal bleeding and subchorionic hemorrhage are considered potential clinical markers of disruption to implantation, placentation, or pregnancy loss;[130] although, little is known about risk factors that may contribute to experiences of vaginal bleeding early in pregnancy.

One potential nutrient that has been linked to other adverse pregnancy outcomes is maternal 25-hydroxyvitamin D (25(OH)D), with up to 69% of the US pregnant population having 25(OH)D levels of <30 ng/mL.[9,10] Although no clinical cut off for reproductive and perinatal health outcomes has been established, the Endocrine Society has recommended that a minimum level of 30ng/mL of circulating vitamin D is critical to support bone health.[1] Vitamin D may have an impact in the

early critical periods of implantation and the health trajectory of the pregnancy.[29,107,110,146,147] Specifically, vitamin D deficiency may disrupt functioning of reproductive organs, leading to adverse pregnancy outcomes.[37,112,116,118] In particular, low vitamin D may result in the disruption of hormones that help to maintain the pregnancy (e.g., estrogen and progesterone) and may result in local and systemic immune functioning, which facilitate endometrial receptivity and implantation.[37,112,116,118] With successful implantation, human chorionic gonadotropin (hCG) is produced, which facilitates further development of the placenta and maintenance of the pregnancy.[132–134,150] Vitamin D receptors on trophoblasts, which are located on the outer layer of a blastocyst and form the placenta, can also facilitate the maintenance of pregnancy in the presence of sufficient vitamin D levels.[18,22,112] Additionally, sufficient vitamin D may provide anti-inflammatory effects in the uterus and placenta, which could mitigate disruptions during implantation.[29] Vitamin D may operate more systemically to modulate maternal immune tolerance, which may also influence endometrial receptivity and implantation.[150,203–207]

Prior epidemiologic studies have linked lower vitamin D to adverse reproductive outcomes, including pregnancy loss and preeclampsia, both of which may be associated with disrupted implantation and placentation.[14,130,131,150,207,208] However, few studies have examined other clinical markers of disrupted implantation, such as vaginal bleeding, which may occur with or without a pregnancy loss, and can be a source of considerable stress and anxiety for both women and obstetric care providers. [19,130,131,148,149] Prior studies have linked vaginal bleeding, particularly later in pregnancy, to placental dysfunction, such as placenta previa, placental abruption, or infection.[209,209–211] One previous study suggested that vaginal bleeding, particularly light bleeding, between 5-8 weeks of gestation could be due to a luteal-placenta shift occurring, which is a normal process that results in a drop in progesterone as the placenta takes over hormone production from

the corpus luteum.[212] However, in the instance of placental insufficiency, this may result in more sustained drop in progesterone which may result in more frequent or severe vaginal bleeding.[212]

I aim to assess the relationship between preconception and early pregnancy circulating maternal serum vitamin D levels and vaginal bleeding and subchorionic hemorrhage via both daily diaries and medical reports, respectively, among a cohort of healthy women with a history of 1-2 prior pregnancy losses.

4.2. Methods

4.2.1. Data Source

The Effects of Aspirin in Gestation and Reproduction (EAGeR) trial was used to conduct this analysis. The EAGeR trial enrolled 1,228 healthy women and was a multisite, prospective, double-blind, block-randomized, placebo-controlled clinical trial designed to evaluate the effect of low-dose aspirin (LDA) on live-birth in healthy women between the ages of 18 and 40, with regular menstrual cycles and 1-2 prior pregnancy losses.[48] Women enrolled in the trial could not have a prior diagnosis of infertility or used any fertility treatment. The institutional review boards at each study site (Salt Lake City, Utah; Denver, Colorado; Buffalo, New York; Scranton, Pennsylvania) and the data coordinating center approved the protocol for the trial. All participants provided their written consent prior to enrolling in the study. The trial was registered with ClinicalTrials.gov (#NCT00467363).

4.2.2. Analytic Sample

The analytic sample consisted of women in the EAGeR trial who became pregnant and for whom there is measured serum 25(OH)D levels at preconception or 8-weeks' gestation, and available data on the outcomes of interest, vaginal bleeding or subchorionic hemorrhage. Measurements of vitamin D were taken at baseline which could be from 1-6 months during the enrollment period.

Women were then followed for 1-6 months and on average around 3 months to conceive. Restriction to pregnancy status allows the capture of women who became pregnant and therefore could have subchorionic hemorrhage or vaginal bleeding occur during their pregnancy. Sensitivity analyses were conducted to restrict the analysis to live birth to allow the assessment of subchorionic hemorrhage and vaginal bleeding independent of any factors that may lead to a pregnancy loss for a sensitivity analysis. Restriction to a live birth could result in a potential selection bias given that 25(OH)D has been associated with a lower risk of pregnancy loss. Therefore, analytic inverse probability weights were used to account for any selection biases that could result from this restriction has been used using methods described previously.[201,213] Pregnancy status was determined via positive urine hCG pregnancy tests (Quidel Quickvue, Quidel Corporation), conducted at home or in the clinic at the time of expected menses. Access to this data source requires approval from NICHD, which I acquired through a data use agreement (DUA).

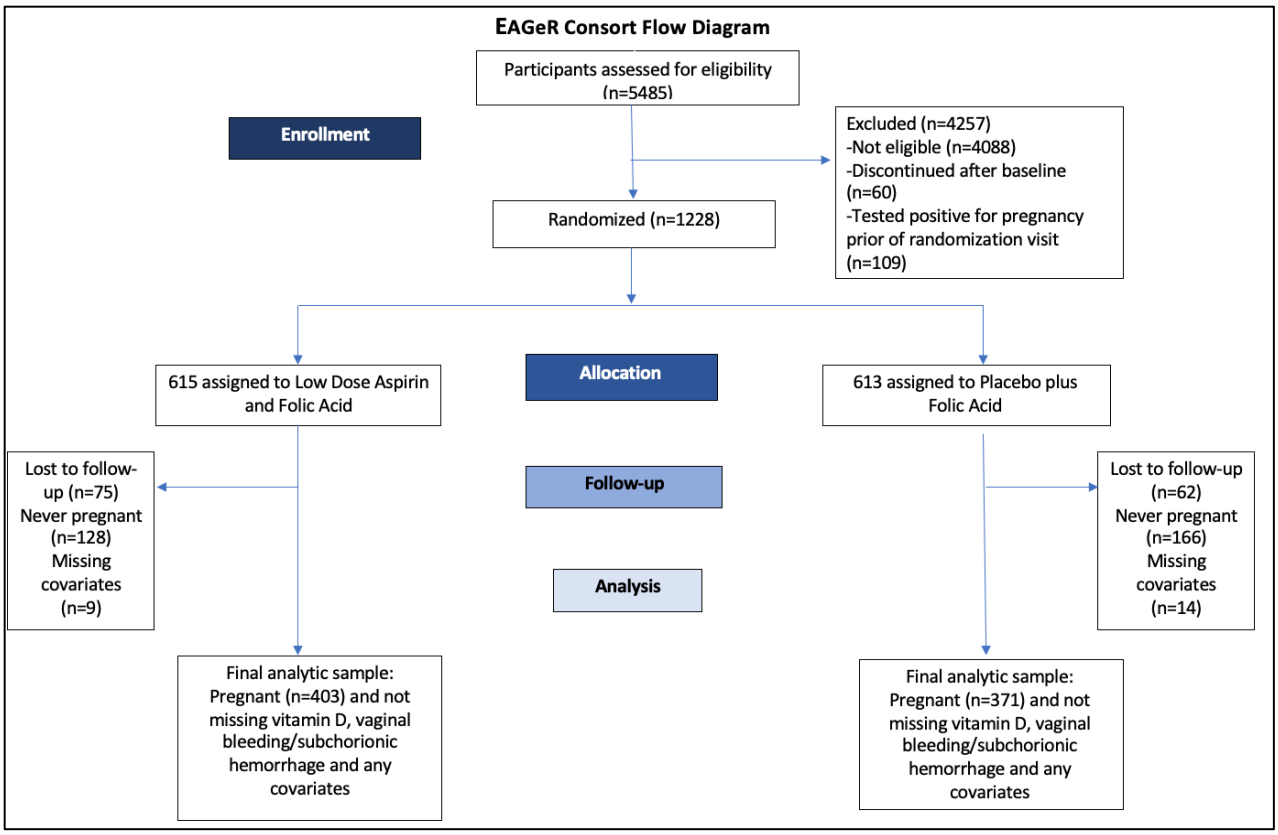

Figure 4.1. EAGeR Trial Consolidated Standards of Reporting Trials (CONSORT) Flow Diagram for final analytic sample used (n=797) (Adapted from Mumford et al.).[207]

4.2.3. Measures

4.2.3.1. Exposure Measure

The exposure variable in this analysis was maternal serum vitamin D levels.[9] Serum samples were collected at baseline prior to randomization to LDA and at 8-week gestation if they conceived. The serum samples were stored at −80°C until used for analysis.[48] Combined concentrations of 25-hydroxyvitamins D2 and D3 (25(OH)D) were measured in stored serum samples at baseline and 8-week gestation using the 25(OH)D ELISA solid phase sandwich enzyme immunoassay (BioVendor R&D, Ashville, NC, USA), which has been validated previously.[195]

The vitamin D cutoffs that are used in this analysis are based on levels designated by the Endocrine Society, which help to inform clinical interpretation and comparison with other studies using these recommended designations.[9] Women were classified as vitamin D 25(OH)D deficient

(≤20 ng/mL), insufficient (21-29 ng/mL), or sufficient (≥30 ng/mL) at preconception and 8-week gestation.[9] Change in vitamin D status between preconception and 8-week gestation was assessed by combining deficient and insufficient vitamin D together and categorizing change as: deficient/insufficient to sufficient, sufficient to deficient/insufficient, no change: deficient/insufficient, and no change: sufficient. A continuous measure of vitamin D (ng/mL) was also examined in supplemental analyses. In addition, lowess models were used to inform the relationship for continuous vitamin D and vaginal bleeding in supplementary analyses (Supplemental Figures 1, 2 and 3).

4.2.3.2. Outcome Measures

4.2.3.2.1. Vaginal Bleeding/ Subchorionic Hemorrhage Chart Abstractions

Vaginal bleeding and subchorionic hemorrhage was assessed using medical chart abstractions, which were check-box questions on the medical chart abstraction (yes/no).[184] Medical chart abstractions were recorded during ultrasounds, pregnancy loss visits, hospitalization visits, emergency care visits, and delivery visits.[184] Following study completion, case report forms and open-ended questions completed through questionnaires and medical records were independently reviewed by two board-certified reproductive endocrinologists as well as a perinatal epidemiologist.[184] For analysis, this information was categorized as vaginal bleeding only, subchorionic hemorrhage (with or without vaginal bleeding), or no documented vaginal bleeding or subchorionic hemorrhage.

4.2.3.2.2. Vaginal Bleeding Daily Diaries

Information was collected daily on vaginal bleeding using daily diaries. Daily bleeding in the daily diaries was recorded based on standardized pictographs as either none, spotting and/or very light, light, moderate, heavy, or very heavy (see Appendix II) and based on self-report. Daily diary

questions assessing vaginal bleeding were: “Please tell us if you had any bleeding or spotting. Refer to the ‘Bleeding and spotting chart’ to help you assess the degree of bleeding. If none, please enter ‘0.’” Response options included: 0=none, 1=spotting and/or very light, 2=light, 3=moderate, 4=heavy, 5=very heavy. Previous studies have validated the outcomes of self-reported vaginal bleeding and have found reasonable accuracy between the self-reported data and clinical presentations.[198] Additionally, information was collected on the date of the beginning of each week of the daily diary entry, which was used in combination with the first day of the last menstrual period (LMP) to assess timing of vaginal bleeding relative to weeks of gestation. This was further grouped into 2-week windows: 3-4 weeks, 5-6 weeks, and 7-8 weeks from LMP date.[150]

Vaginal bleeding categories were defined at biweekly intervals of 3-4, 5-6, and 7-8 weeks of gestation and coded based on the highest level of bleeding during that interval. These groups were further categorized for analysis in three ways: 1) women who had any vaginal bleeding versus none or 2) women who had light vaginal bleeding versus none or 3) women who had any moderate to heavy bleeding versus none.

4.2.3.3. Confounders

Demographic information was captured in questionnaires at the first study visit.[184] A baseline questionnaire was used to assess pregnancy history information.[184] Baseline health characteristics such as body mass index (BMI) were calculated using height and weight measurements completed by trained study staff.[184] The covariates considered included sociodemographic characteristics, including age, exercise, income, race, education, parity, employment, and season, and lifestyle characteristics, including physical activity, smoking, alcohol intensity, multivitamin use, aspirin, and BMI (kg/m^2). Season was defined as the season during which the baseline sample of blood was drawn for serum 25(OH)D assessment. Physical activity was assessed using the International

Physical Activity Questionnaire and defined as low, moderate, or high.[200] Alcohol intensity was defined as the amount of alcohol consumed in the past year and was categorized as never, sometimes, and often. Multivitamin use was measured as the type of vitamins that were consumed by the women prior to the study and were defined as not taking any vitamins or folic acid, taking vitamins with no folic acid, not taking any multivitamins but taking folic acid, and taking vitamins with folic acid. Additionally, aspirin/placebo, the assigned treatment in this trial, will be considered as a confounder in this analysis, as previously applied in a study of vitamin D and pregnancy loss using this data.[11] Evidence has shown an increased risk of vaginal bleeding with LDA.[150,202]

4.3. Analysis

4.3.1. Descriptive Analyses

Differences in the prevalence of vaginal bleeding and subchorionic hemorrhage across different baseline characteristics and bivariate associations of covariates with vitamin D levels (preconception and 8-week gestation) were examined using chi-square tests or F-statistics for comparing categorical or continuous variables, respectively.

4.3.2. Multinomial Logistic Regression Models

Odds ratios between the change in preconception and 8-week gestation serum 25(OH)D levels and vaginal bleeding and subchorionic hemorrhage were estimated using multinomial logistic regression models with robust standard errors with inverse probability weights to account for selection bias that may occur by only including women who have had a pregnancy (i.e., excludes women who did not become pregnant during the study period, which may also be related to the exposure under study). Vitamin D was assessed at preconception and 8 week gestation (if a loss did not occur prior to that point). The inverse probability weights used to account for selection biases that could result from this restriction use methods described previously and include

covariates associated with the probability of being pregnant such as age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamin D, vitamins, and BMI.[201,213] Unadjusted and adjusted multinomial logistic regression models examined associations with vaginal bleeding only (no subchorionic hemorrhage) or subchorionic hemorrhage (with or without vaginal bleeding) versus no bleeding or subchorionic hemorrhage. Models were adjusted for relevant confounders as determined by DAGs (see Chapter 1), which included: a model adjusted for all sociodemographic covariates which includes age, exercise, income, race, education, parity, employment, and season (Model 1), a model adjusted for all sociodemographic and lifestyle covariates including physical activity, smoking, alcohol intensity, multivitamin use, aspirin (excluding BMI) (Model 2), and a model adjusted for all sociodemographic and lifestyle covariates, including BMI (Model 3).

4.3.3. Generalized Estimating Equations (GEE) Regression Models

The odds ratio between preconception serum 25(OH)D levels on any vaginal bleeding at biweekly intervals in the first 8 weeks of pregnancy (3 time points) were examined using generalized estimating equations (link: logit, family: binomial) with an unstructured correlation matrix. Vitamin D levels at preconception applied to the first interval (3-4 weeks) and vitamin D levels measured at 8 weeks were applied to the last time interval (7-8 weeks gestation). For weeks 5-6, an average of the preconception and 8-week vitamin D level was imputed. Models were adjusted for the same set of baseline covariates applied above. Additionally, separate regression models were used to examine vaginal bleeding classified as moderate to heavy bleeding compared to none and light bleeding compared to none. Inverse probability weights were used to account for potential selection bias that may result from restricting to women who became pregnant as described above.

4.3.4. Sensitivity Analyses

For both multinomial logistic regression and GEE regression models, I examined models restricted to only pregnancies resulting in live birth. Restriction to a live birth was used to examine the effects of vitamin D on vaginal bleeding and subchorionic hemorrhage independent of a pregnancy loss. Restriction to a live birth could result in a potential selection bias given that deficient 25(OH)D has been associated with a higher risk of pregnancy loss.[11] Therefore, analytic inverse probability weights were used to account for any selection biases that could result from this restriction using methods described previously.[201,213]Additional inverse probability weights were estimated to account for selection of pregnancy and live birth using age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamin D, vitamins, and BMI.[201,213] Additionally, I replaced categorical vitamin D with the continuous vitamin D (per 1 ng/mL) in GEE models. Potential interactions between vitamin D groups and low-dose aspirin treatment assignment were examined. Stratification by low dose aspirin or placebo was conducted since aspirin has been associated with vaginal bleeding.[202] Analyses were performed using STATA version 17.0.

4.4. Results

4.4.1. Descriptive Analyses

747 participants were pregnant and had a measured preconception 25(OH)D serum level, with 50% sufficient, 37% insufficient, and 13% deficient (Table 1). Those in the deficient 25(OH)D category had a mean BMI of 30.5 compared with 26.9 among insufficient and 24.5 among sufficient (p-value <0.0001). Those in the deficient 25(OH)D group were also more likely to be white (83.7%), have >high school education (80.4%), have a fall season of blood draw (36.9%), have moderate exercise level (41.3%), have never consumed alcohol in the past year (73.9%), and

have a low CRP at baseline (35.2%) compared with women in the insufficient or sufficient vitamin D groups. Age, employment, vitamin use, smoking, number of previous pregnancy losses, and treatment assignment were not associated with preconception 25(OH)D.

605 participants were pregnant and had a measured 8-week 25(OH)D serum level with 56% sufficient, 38% insufficient, and 5% deficient. Those in the deficient 25(OH)D category had a mean BMI of 30.5 compared with 26.3 among insufficient and 24.2 among sufficient (p-value <0.001). Women in the deficient 25(OH)D group were also more likely to be white (81.3%), have >high school education (81.3%), take folic acid and vitamins (90.6%), have a fall season of blood draw (46.9%), have low exercise level (56.3%), have never consumed alcohol in the past year (73.9%), and have a moderately high CRP level at baseline (40.3%) compared with women in the insufficient and sufficient 25(OH)D groups. Age, smoking, number of previous pregnancy losses, alcohol consumption, and treatment assignment were not associated with 8-week 25(OH)D.

Bivariate associations between the prevalence of vaginal bleeding and subchorionic hemorrhage and preconception and 8-week 25(OH)D serum levels and sociodemographic and lifestyle characteristics are presented in Table 3. Although not associated in bivariate analyses, the prevalence of vaginal bleeding was comparable among women with insufficient (35.9% for preconception; 40.9% 8 week gestation) and deficient (35.9% for preconception; 40.6% for 8 week gestation) 25(OH)D levels compared to sufficient vitamin D levels (34.5% for preconception; 38.5% for 8 week gestation). Additionally, the prevalence of vaginal bleeding/subchorionic hemorrhage was higher for younger age (39.5%), those who are obese (37.9%), non-white (36.0%), educated ≤ high school (41.6%), having an annual household income of ≤ $19,999 (41.2%), not employed (37.3%), not taking folic acid or vitamins (49.6%), smoking daily in the past year (37.9%), have a fall season of blood draw (42.0%), low or high exercise (35.4%), often

consumed alcohol in the past year (36.8%), and taking low dose aspirin (37.3%) compared to other categories within each respective variable.

4.4.2. Multinomial Logistic Regression Results

In the multinomial logistic regression models assessing vaginal bleeding and subchorionic hemorrhage documented in medical records (Table 4), women who were deficient/insufficient at preconception and remained deficient/insufficient at 8-week gestation had 1.91 (95% CI: 1.06, 3.44) times higher odds of having a subchorionic hemorrhage in the unadjusted model and 2.18 times higher (95% CI: 1.13, 4.20) after adjustment compared to those who were persistently sufficient across both time periods. Although precision was limited, odds ratios for vaginal bleeding (OR: 1.59, 95% CI: 0.86, 2.92) and subchorionic hemorrhage (OR: 1.48, 95% CI: 0.61, 3.62) were higher if a woman changed from sufficient to insufficient/deficient compared to persistently sufficient 25(OH)D group after adjustment. Odds ratios for other groups were attenuated and closer to the null. When restricting to live births in sensitivity analyses, associations were further attenuated; however, the magnitude of association for women who persisted with deficient/insufficient 25(OH)D at preconception and 8-week gestation were higher in magnitude, but the estimates were imprecise (OR: 1.75 95% CI: 0.86, 3.55) (Supplemental Table S4.1).

4.4.3. Generalized Estimating Equations (GEE) Results

In longitudinal analyses of daily diary data using GEE (Table 5), deficient 25(OH)D status slightly increased the odds of any bleeding during pregnancy in fully adjusted models (OR: 1.27, 95% CI: 0.74, 2.20) compared to women with sufficient 25(OH)D, but the estimates were imprecise. Partitioning this into moderate/heavy or light bleeding versus none showed an elevated odds of moderate to heavy bleeding (OR: 3.02, 95% CI: 1.13, 8.13) and close to a null association for light vaginal bleeding (OR: 1.07, 95% CI: 0.58, 2.00) for women with deficient 25(OH)D

compared to sufficient 25(OH)D levels. Patterns were consistent when restricted to live births in supplemental analyses, although less precise (Supplemental Table S4.2).

Among those with a pregnancy loss and vaginal bleeding, missing information on 25(OH)D increased over time with 0.71% in weeks 3-4 and 52.9% in weeks 7-8. Among those without a loss, missing information on vaginal bleeding was less with 0.19% at 3-4 weeks and 18.6% in weeks 7-8 (Supplemental Table S4.3).

4.4.4. Sensitivity Analyses

Consistent with categorical classifications of 25(OH)D, GEE regression models based on continuous 25(OH)D levels showed that the risk of vaginal bleeding, particularly moderate/heavy, was reduced with increasing levels of 25(OH)D when restricted to pregnancy (OR: 0.98, 95% CI: 0.94, 1.02), though not precise (Supplemental Table S4.4). This reduction in the odds of moderate/heavy vaginal bleeding was even further reduced after restriction to live births (OR: 0.93, 95% CI: 0.88, 0.99) (Supplemental Table S4.5).

Furthermore, I examined associations of 25(OH)D on vaginal bleeding and subchorionic hemorrhage stratified by treatment assignment (placebo vs. low dose aspirin) and found no association. Furthermore, all interaction coefficients between deficient 25(OH)D change groups and aspirin were not significant for either vaginal bleeding (p-value= 0.51) nor subchorionic hemorrhage (p-value 0.96). In the GEE analyses, of the association between 25(OH)D and vaginal bleeding stratified by treatment assignment (placebo vs. low dose aspirin) showed the association between deficient 25(OH)D and moderate/heavy vaginal bleeding was attenuated in the aspirin group, but still indicative of higher odds compared with sufficient 25(OH)D levels (OR: 3.12; 95% CI: 0.59, 16.5). In the placebo group, the magnitude was larger (OR: 7.63; 95% CI: 1.75, 33.31).

However, the interaction between deficient 25(OH)D (p-value=0.78) and aspirin and insufficient 25(OH)D (p-value=0.19) that corresponds to that and aspirin was imprecise.

4.5. Discussion

Among a cohort of healthy women with a history of 1-2 prior pregnancy losses and no known diagnosis of infertility, those with persistently low vitamin D (deficient/insufficient) between preconception and 8-week were more likely to have subchorionic hemorrhage. Given that subchorionic hemorrhage is often associated with more extensive vaginal bleeding[215], these results were consistent with the GEE analysis using the daily diary data of vaginal bleeding in early pregnancy, which showed deficient levels of 25(OH)D were strongly associated with moderate to heavy bleeding, but not light bleeding, relative to sufficient vitamin D levels. Although the estimates were less precise, the associations were consistent when restricted to only live births, suggesting that this relationship may hold even when vaginal bleeding may occur independent of pregnancy loss. Taken together, these findings may indicate a potential pathway between deficient vitamin D levels and early implantation/placentation processes as indicated by vaginal bleeding or subchorionic hemorrhage.

There is limited evidence on the relationship between preconception and early gestation maternal 25(OH)D levels and vaginal bleeding and subchorionic hemorrhage among a cohort of healthy women. Our study is consistent with previous studies that have noted deficient vitamin D levels increase the risk of adverse pregnancy outcomes.[11,14,31,42,208] In particular, one study by Mumford et. al found an association between preconception deficient (<20 ng/mL) maternal serum vitamin D levels and risk of pregnancy loss among a cohort of healthy women with 1-2 prior pregnancy losses.[11] In addition, a more recent study assessing the preconception maternal serum 25(OH)D levels on successful implantation and pregnancy found that deficient (<20 ng/mL)

maternal serum levels of 25(OH)D prior to conception lowered the rate of a successful pregnancy.[38] Therefore, the preconception period could be an important period for reducing risk of placental insufficiency or dysfunction that may lead to vaginal bleeding; however, few studies have examined risk factors in this critical window.

Pregnancy loss during the study may be one reason for findings associated with more moderate/heavy bleeding. However, when analyses were restricted to those with a live birth, the magnitude of this association remained, but was less precise given the smaller sample size. Compared to those with persistently sufficient vitamin D, we found that persistently deficient vitamin D was associated with subchorionic hemorrhage, which is indicative of disruptions in placentation. Further studies with larger samples are needed to examine the mechanisms for this association and to distinguish bleeding due to pregnancy loss from those related to disruptions in placentation, such as subchorionic hemorrhage. While these relationships held for pregnancies that survived to a live birth, findings were less precise.

Previous studies have assessed vaginal bleeding episodes during the early pregnancy and gestation period and have found that spotting/light bleeding tend to have different characteristics than heavy bleeding episodes.[212] This is most likely to arise from different biological mechanisms, such as subchorionic hemorrhage, placenta previa, abruption, or infection, in comparison to spotting/light bleeding episodes that may be connected to implantation bleeding, which is thought to occur as part of a normal process of implantation.[149,209,210,212,216] In particular, spotting/light bleeding may occur during the early pregnancy period when there is a shift in the production of progesterone to maintain the pregnancy from the corpus luteum to the fully functioning placenta.[29,116,118] Clinical distinctions in the type of bleeding and development of subchorionic

hemorrhage may illuminate differences in biological mechanisms that could explain our findings and the role of early windows of exposure (preconception through early pregnancy).[148,212,217,218]

Although estimates were imprecise, our findings also suggested potential attenuation of these effects in the presence of low dose aspirin. Previous evidence has shown that an increased risk of vaginal bleeding has been seen with LDA due to blood thinning that occurs systemically. Leading to a reduction or prevention of blood clot formation.[150,188] In addition to thinning the blood, aspirin has been shown to reduce inflammation by blocking the production of prostaglandins, which help regulate pain and inflammation within the body.[188,202] I was unable to explore this relationship further due to smaller sample size, but the attenuation of findings may be explained by either: 1) experiences of more vaginal bleeding in the aspirin group overall, regardless of vitamin D status, or 2) potential for reduced inflammation that may afford some level of protection against moderate/heavy bleeding or subchorionic hemorrhage even in the presence of deficient vitamin D status. Future studies are needed to assess the association between vitamin D and vaginal bleeding and subchorionic hemorrhage and the potential role of aspirin in mitigating some of these adverse associations in combination with early vitamin D supplementation.

4.5.1. Strengths and Limitations

This study has several limitations worth noting. One limitation of this data is its limited diversity of the cohort. However, I examined bleeding behaviors using both medical records in cross-sectional analyses and daily diary data in longitudinal analyses to discern effects and findings were consistent. Another limitation in the longitudinal analysis was having to infer vitamin D levels for the 5-6 week gestation period as the average between preconception and 8-week gestational ages, rather than a direct measure and variation in time from measurement of preconception vitamin D to the first 3-4 weeks of pregnancy. Medical records often capture later pregnancy-related events;

however, daily dairy data allowed me to capture information early in pregnancy from those with and without a pregnancy loss. Although I did not have large samples to fully differentiate the effects of bleeding due to loss from vaginal bleeding for other reasons, restricting to live births showed the magnitude of associations were similarly large, although less precise, suggesting these relationships hold independent of pregnancy loss. Unmeasured confounding may still be a contributor in the analysis.[219,220] To address this, I calculated e-values to assess the extent to which unmeasured confounding may explain the associations found (Supplemental Tables S4.6 and S4.7).[219,220] Calculated e-values for deficient or persistently deficient vitamin D for subchorionic hemorrhage and moderate to heavy bleeding ranged between 2.87-2.90. While other potential unmeasured confounders may be associated with the outcomes and exposures assessed in this analysis, the associations of the unmeasured confounders would need to be fairly strong to fully explain the associations found in this analysis. Unmeasured factors that could contribute to increased risk of vaginal bleeding or subchorionic hemorrhage include both maternal and paternal exposure to toxic chemicals, and environmental influences such as pollution. Measured exposures and paternal nutrition would also be important to assess, as preconception paternal health, in particularly through epigenetics and sperm, plays a key role in placentation and pregnancy outcomes.[221–226] Finally, the study did not use gold-standard liquid chromatography-tandem mass spectrometry for vitamin D measurement; however, previous studies conducted have found vitamin D measurement results to be similar in immunoassays through the Vitamin D External Quality Assessment Scheme (DEQAS).[195–197] Therefore, the ELISA solid phase sandwich enzyme immunoassay is a precise and valid measurement for vitamin D concentrations.[195] Finally, missingness of measurement of serum 25(OH)D over time is due to the design of the trial (due to potential pregnancy loss), rather than missing from respondent not filling out the questionnaire.

There are many strengths of the data to highlight. For one, preconception and early gestation are critical time points in which an intervention may be most likely to have an effect and few studies have data to evaluate these measures prior to and early in pregnancy. Furthermore, the longitudinal assessment is another strength to highlight as the women used daily diaries to record their symptoms prospectively and allowed differentiation of light vs. more moderate or heavy bleeding, which may be clinically different and indicate different biologic mechanisms. I was also able to compare these findings with medical record information on subchorionic hemorrhage, which is one of the leading causes of vaginal bleeding in the first half of pregnancy.[19] As such, I was able to isolate a clinical condition (that may lead to vaginal bleeding) and which has been shown to be indicative of disruptions to placentation[215]; thus, allowing for more information on potential processes by which vitamin D may affect early pregnancy complications. Finally, the use of daily diaries has been shown to provide more thorough assessment of indicators that may change frequently with time.[150]

4.5.2. Conclusion

While the biologic pathways regarding the effects of preconception and early gestation maternal serum vitamin D on adverse pregnancy outcomes, such as vaginal bleeding and subchorionic hemorrhage, are multifactorial, this study is suggestive of the importance of sufficient maternal vitamin D nutrient stores prior to conception and within the early pregnancy period may improve the outcomes of pregnancy. In particular, the early programming model posits that exposures, including nutrition, during sensitive and critical periods of fetal development may alter or change the development of the fetus and affect their short and long term health, potentially leading to adverse pregnancy outcomes, such as preeclampsia.[143] Further research is needed to understand mechanisms by which preconception and early pregnancy vitamin D may enhance implantation

and placentation, leading to healthier pregnancies.

Tables

Table 4.1. Descriptive characteristics of women in the EAGeR Trial who became pregnant by preconception maternal serum vitamin D status (n=747).

EAGeR- Preconception Vitamin D Descriptive Analyses				
	Vitamin D Sufficient (≥ 30 ng/mL)	**Vitamin D Insufficient (≥20 ng/mL & <30 ng/mL)**	**Vitamin D Deficient (<20 ng/mL)**	**P-value**
N	377	278	97	
Age, years				
Mean ± SD	28.7 ± 4.4	28.7 ± 4.6	28.5 ± 5.3	0.37
18-24.9	91 (24.1)	59 (21.2)	22 (23.9)	0.55
25-29.9	144 (38.2)	121 (43.5)	40 (44.5)	
30-34.9	94 (24.9)	74 (26.6)	21 (22.8)	
35-40.9	48 (12.7)	24 (8.6)	9 (9.8)	
***BMI, kg/m^2**				
Mean ± SD	24.5 ± 5.1	26.9 ± 6.3	30.5 ± 8.6	<0.0001
Underweight <18.5	15 (3.9)	12 (4.3)	3 (3.3)	<0.0001
Normal ≥18.5 & <25	242 (64.2)	129 (46.4)	28 (30.4)	
Overweight ≥25 & <30	76 (20.2)	85 (30.6)	17 (18.5)	
Obese ≥30	44 (11.7)	52 (18.7)	44 (47.8)	
***Race**				
White	373 (98.9)	271 (97.8)	77 (83.7)	<0.0001
Non-White	4 (1.1)	6 (2.2)	15 (16.3)	
Education				
≤ High School	37 (9.8)	22 (7.9)	18 (19.6)	0.01
> High School	340 (90.2)	256 (92.1)	74 (80.4)	
Annual Household Income				
≥ $100,000	143 (37.9)	127 (45.7)	33 (35.9)	0.03
$75,000-$99,999	63 (16.7)	39 (14.0)	6 (6.5)	
$40,000-$74,999	62 (16.5)	33 (11.9)	13 (14.1)	
$20,000-$39,999	83 (22.0)	64 (23.0)	30 (32.6)	
≤ $19,999	26 (6.9)	15 (5.4)	10 (10.9)	
Employed				
Yes	289 (76.7)	200 (71.9)	65 (70.7)	0.30
No	88 (23.3)	78 (28.1)	27 (29.4)	
Vitamin Use				

No Folic Acid-No Vitamins	29 (7.7)	12 (4.3)	7 (7.6)	0.19
No Folic Acid- Yes Vitamins	61 (16.2)	41 (14.8)	7 (7.6)	
Yes Folic Acid- Yes Vitamins	287 (76.1)	225 (80.9)	78 (85)	
Smoking in the past year				
Never	334 (88.6)	253 (91.0)	77 (83.7)	0.10
<6 times/ week	31 (8.2)	14 (5.0)	9 (9.8)	
Daily	12 (3.2)	11 (3.9)	6 (6.5)	
Season of blood draw				
Fall (Sep-Nov)	103 (27.3)	70 (25.2)	34 (36.9)	0.02
Winter (Dec-Feb)	76 (20.2)	64 (23.0)	25 (27.2)	
Spring (Mar-May)	198 (25.9)	83 (29.9)	24 (26.1)	
Summer (Jun-Aug)	100 (26.5)	61 (21.9)	9 (9.8)	
***Exercise**				
Low	87 (23.1)	73 (26.3)	35 (38.0)	0.02
Moderate	164 (43.5)	107 (38.5)	38 (41.3)	
High	126 (33.4)	98 (35.3)	19 (20.7)	
Number of previous pregnancy losses				
1	220 (58.4)	172 (61.9)	60 (65.2)	0.41
2	157 (41.6)	106 (38.1)	32 (34.8)	
Alcohol consumption in the past year				
Never	231 (61.3)	207 (74.5)	68 (73.9)	0.001
Sometimes	137 (36.3)	61 (21.9)	24 (26.1)	
Often	9 (2.4)	10 (3.6)	0 (0)	
***Baseline CRP µg/mL**				
Mean ± SD	2.3 ± 5.1	2.8 ± 3.7	4.8 ± 7.9	0.007
Low <1	198 (52.5)	134 (48.2)	32 (35.2)	0.001
Borderline ≥1 & <3	116 (30.8)	73 (26.3)	24 (26.4)	
Moderately High ≥3 & <10	54 (14.3)	56 (20.1)	28 (30.8)	
High Concentrations ≥10	9 (2.4)	15 (5.4)	7 (7.7)	
Treatment assignment				
Placebo	173 (45.9)	140 (50.4)	45 (48.9)	0.52
Low Dose Aspirin	204 (54.1)	138 (49.6)	47 (51.1)	

*Non-white participants include American Indian/Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, Black or African American, more than one Race, Unknown or Not Reported *BMI- Body Mass Index, CRP- C-Reactive Protein

*Exercise level was measured through the International Physical Activity Questionnaire and assessed the level of physical activity as low, moderate, and high

Table 4.2. Descriptive characteristics of pregnant women in the EAGeR Trial by 8-week maternal serum vitamin D status (n= 605)

EAGeR- 8-week Vitamin D Descriptive Analyses				
	Vitamin D Sufficient (≥ 30 ng/mL)	**Vitamin D Insufficient (<30 ng/mL & ≥20 ng/mL)**	**Vitamin D Deficient (<20 ng/mL)**	**P-value**
N	341	232	32	
Age, years				
Mean ± SD	28.7 ± 4.6	28.4 ± 4.4	28.7 ± 5.0	0.33
18-24.9	79 (23.2)	55 (23.7)	6 (18.8)	0.27
25-29.9	131 (38.4)	110 (47.4)	16 (50.0)	
30-34.9	91 (6.7)	45 (19.4)	6 (18.8)	
35-40.9	40 (11.7)	22 (9.5)	4 (12.5)	
***BMI, kg/m^2**				
Mean ± SD	24.2 ± 4.7	26.3 ± 6.2	30.5 ± 8.9	<0.001
Underweight <18.5	11 (3.2)	7 (3.0)	0 (0.0)	<0.001
Normal ≥18.5 & <25	211 (61.9)	110 (47.4)	10 (31.3)	
Overweight ≥25 & <30	80 (23.5)	60 (25.9)	9 (28.1)	
Obese ≥30	39 (11.4)	55 (23.7)	13 (40.6)	
***Race**				
White	336 (98.5)	225 (96.9)	26 (81.3)	<0.001
Non-White	5 (1.5)	7 (3.0)	6 (18.8)	
Education				
≤ High School	35 (10.3)	15 (6.5)	9 (28.1)	<0.001
> High School	306 (89.7)	217 (93.5)	23 (71.9)	
Annual Household Income				
≥ $100,000	144 (42.2)	97 (41.8)	10 (31.3)	0.003
$75,000-$99,999	59 (17.3)	26 (11.2)	1 (3.1)	
$40,000-$74,999	47 (13.8)	38 (16.4)	3 (9.4)	
$20,000-$39,999	70 (20.5)	61 (26.3)	12 (37.5)	
≤ $19,999	21 (6.2)	10 (4.3)	6 (18.8)	
Employed				
Yes	255 (74.8)	173 (74.6)	17 (53.1)	0.03
No	86 (25.2)	59 (25.4)	15 (46.9)	

Vitamin Use				
No Folic Acid-No Vitamins	17 (4.9)	17 (7.3)	1 (3.1)	0.04
No Folic Acid- Yes Vitamins	57 (16.7)	21 (9.1)	2 (6.3)	
Yes Folic Acid- Yes Vitamins	267 (78.3)	194 (83.6)	29 (90.6)	
Smoking in the past year				
Never	307 (90.0)	212 (91.4)	27 (84.4)	0.62
<6 times/ week	22 (6.5)	10 (4.3)	3 (9.4)	
Daily	12 (3.5)	10 (4.3)	2 (6.3)	
Season				
Fall (Sep-Nov)	81 (23.8)	71 (30.6)	15 (46.9)	0.01
Winter (Dec-Feb)	65 (19.1)	59 (25.4)	5 (15.6)	
Spring (Mar-May)	105 (30.8)	58 (25.0)	6 (18.8)	
Summer (Jun-Aug)	90 (26.4)	44 (18.9)	6 (18.8)	
***Exercise**				
Low	79 (23.2)	68 (29.3)	18 (56.3)	0.002
Moderate	147 (43.1)	96 (41.4)	7 (21.9)	
High	115 (33.7)	68 (29.3)	7 (21.9)	
Number of previous pregnancy losses				
1	210 (61.6)	146 (62.9)	18 (56.3)	0.76
2	131 (38.4)	86 (37.1)	14 (43.8)	
Alcohol consumption in the past year				
Never	223 (65.4)	166 (71.6)	22 (68.8)	0.48
Sometimes	107 (31.4)	61 (26.3)	10 (31.3)	
Often	11 (3.2)	5 (2.2)	0 (0.0)	
***Baseline CRP µg/mL**				
Mean ± SD	2.2 ± 4.69	2.4 ± 2.95	3.9 ± 4.46	0.09
Low <1	185 (54.3)	102 (44.2)	11 (34.4)	0.002
Borderline ≥1 & <3	92 (26.9)	76 (32.9)	5 (15.6)	
Moderately High ≥3 & <10	53 (15.5)	42 (18.2)	13 (40.6)	
High Concentrations ≥10	11 (3.2)	11 (4.8)	3 (9.4)	
Treatment assignment				
Placebo	147 (43.1)	123 (53.0)	16 (50.0)	0.06
Low Dose Aspirin	194 (56.9)	109 (46.9)	16 (50.0)	

*Non-white participants include American Indian/Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, Black or African American, more than one Race, Unknown or Not Reported *BMI- Body Mass Index, CRP- C-Reactive Protein

*Exercise level was measured through the International Physical Activity Questionnaire and assessed the level of physical activity as low, moderate, and high.
Table 4.3. EAGeR Covariates and Prevalence of Vaginal Bleeding/Subchorionic Hemorrhage: EAGeR Trial n=747

EAGeR Covariates and Prevalence of Vaginal Bleeding/Subchorionic Hemorrhage			
Covariates	**N**	**Prevalence of Vaginal Bleeding/Subchorionic Hemorrhage N (%)**	**P-value**
Preconception Vitamin D			0.92
Sufficient ≥30 ng/mL	377	130 (34.5)	
Insufficient ≥20 & <30 ng/mL	278	100 (35.9)	
Deficient <20 ng/mL	92	33 (35.9)	
8-week Vitamin D			0.82
Sufficient ≥30 ng/mL	341	131 (38.5)	
Insufficient ≥20 & <30 ng/mL	232	95 (40.9)	
Deficient <20 ng/mL	32	13 (40.6)	
Demographics			
Age, years			0.26
18-24.9	172	68 (39.5)	
25-29.9	305	106 (34.7)	
30-34.9	189	61 (32.3)	
35-40.9	81	28 (34.6)	
***BMI, kg/m^2**			0.75
Underweight <18.5	30	10 (33.3)	
Normal ≥18.5 & <25	399	134 (33.6)	
Overweight ≥25 & <30	178	66 (37.1)	
Obese ≥30	140	53 (37.9)	
***Race**			0.93
White	722	254 (35.2)	
Non-White	25	9 (36.0)	
Education			0.22
≤ High School	77	32 (41.6)	
> High School	670	231 (34.5)	
Annual Household Income			0.73

≥ $100,000	303	110 (36.3)	
$75,000-$99,999	108	37 (34.3)	
$40,000-$74,999	108	33 (30.6)	
$20,000-$39,999	177	62 (35.0)	
≤ $19,999	51	21 (41.2)	
Employed			0.48
Yes	554	191 (34.5)	
No	193	72 (37.3)	
Vitamin Use			0.55
No Folic Acid-No Vitamins	48	19 (49.6)	
No Folic Acid- Yes Vitamins	109	34 (31.2)	
Yes Folic Acid- Yes Vitamins	590	210 (35.6)	
Smoking in the past year			0.75
Never	664	234 (35.2)	
<6 times/ week	54	18 (33.3)	
Daily	29	11 (37.9)	
Season of blood draw			0.29
Fall (Sep-Nov)	207	87 (42.0)	
Winter (Dec-Feb)	165	56 (33.9)	
Spring (Mar-May)	205	66 (32.2)	
Summer (Jun-Aug)	170	54 (31.7)	
***Exercise**			0.99
Low	195	69 (35.4)	
Moderate	309	108 (34.9)	
High	243	86 (35.4)	
Alcohol consumption in the past year			0.98
Never	506	177 (34.9)	
Sometimes	222	79 (35.6)	
Often	19	7 (36.8)	
Treatment Assignment			0.22
Placebo	358	118 (32.9)	
Low Dose Aspirin	389	145 (37.3)	

*8 week vitamin D missing 142 women *P-values based on Fisher's Exact Test *BMI- Body Mass Index
*Non-white participants include American Indian/Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, Black or African American, more than one Race, Unknown or Not Reported
*Exercise level was measured through the International Physical Activity Questionnaire and assessed the level of physical activity as low, moderate, and high.

Table 4.4. Association between the change in preconception and 8-week categorical 25(OH)D and Vaginal Bleeding and Subchorionic Hemorrhage among women who became pregnant in the EAGeR Trial (n=747)

EAGeR Multinomial Logit Regression Models from Preconception to 8 Weeks 25(OH)D and Subchorionic Hemorrhage and Vaginal Bleeding						
	Vaginal Bleeding only			**Subchorionic Hemorrhage (with or without bleeding)**		
	N=181	Unadjusted[1] OR (95% CI)	Adjusted[3] OR (95% CI)	N=82	Unadjusted[1] OR (95% CI)	Adjusted[3] OR (95% CI)
Change in 25(OH)D from Preconception to 8-Week Gestation						
Deficient/Insufficient to Sufficient	41 (22.7)	0.91 (0.58, 1.44)	0.89 (0.55, 1.45)	17 (20.7)	0.90 (0.47, 1.75)	0.91 (0.44, 1.89)
Sufficient to Deficient/Insufficient	24 (13.3)	1.57 (0.88, 2.82)	1.59 (0.86, 2.92)	9 (10.9)	1.53 (0.66, 3.54)	1.48 (0.61, 3.62)
No Change: Deficient/Insufficient	45 (24.9)	1.26 (0.79, 2.00)	1.17 (0.73, 1.88)	30 (36.6)	**1.91 (1.06, 3.44)**	**2.18 (1.13, 4.20)**
No Change: Sufficient	71 (39.2)	ref	ref	26 (31.7)	ref	ref

*OR-Odds Ratio

[1]Unadjusted for covariates and weighted to control for potential selection bias introduced by restricting to a sample of pregnancy.

[3]Adjusted for all sociodemographic and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamins, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of pregnancy.

Table 4.5. Maternal Serum 25(OH)D Levels and Vaginal Bleeding Episodes from 3-8 weeks gestation, Odds Ratio and 95% Confidence Intervals: EAGeR Trial

EAGeR Generalized Estimating Equations Regression Models for 25(OH)D and Vaginal Bleeding Episodes				
		Unadjusted- M1[1] OR (95% CI)	Adjusted- M2[2] OR (95% CI)	Adjusted- M3[3] OR (95% CI)
Any Bleeding (vs. None)	N=1,742			
Deficient		1.14 (0.70, 1.84)	1.31 (0.78, 2.22)	1.27 (0.74, 2.20)
Insufficient		1.10 (0.81, 1.47)	1.15 (0.85, 1.60)	1.11 (0.81, 1.51)
Sufficient		ref	ref	ref
Light Bleeding (vs. None)	N=1,701			
Deficient		0.94 (0.54, 1.64)	1.10 (0.60, 2.00)	1.07 (0.58, 2.00)
Insufficient		1.16 (0.84, 1.59)	1.23 (0.89, 1.70)	1.17 (0.84, 1.63)
Sufficient		ref	ref	ref
Moderate/Heavy Bleeding (vs. None)	N=1,496			
Deficient		**2.41 (1.06, 5.44)**	**3.00 (1.12, 7.58)**	**3.02 (1.13, 8.13)**
Insufficient		0.77 (0.37, 1.60)	0.80 (0.39, 7.58)	0.83 (0.39, 1.80)
Sufficient		ref	ref	ref

*OR-Odds Ratio, N corresponds to longitudinal observations (not women)
*Time Varying Vitamin D- 3-4 weeks accounts for preconception vitamin D, 5-6 weeks accounts for average between preconception and 8 week vitamin D, 7-8 weeks accounts for 8-week vitamin D.
[1]Unadjusted model and weighted to control for potential selection bias introduced by restricting to a sample of Pregnancy.
[2]Adjusted for all sociodemographic covariates which include age, exercise, income, race, education, parity, employment, and season and weighted to control for potential selection bias introduced by restricting to a sample of Pregnancy.
[3]Adjusted for all sociodemographic and lifestyle covariates which included age, exercise, income, race, education, parity, employment, season, smoking, alcohol, vitamins, aspirin, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of Pregnancy.

Supplemental Tables

Supplemental Table S4.1. Association between the change in preconception and 8-week categorical 25(OH)D and Vaginal Bleeding and Subchorionic Hemorrhage among women who had a live birth in the EAGeR Trial (n=557)

EAGeR Multinomial Logit Regression Models from Preconception to 8 Weeks 25(OH)D and Subchorionic Hemorrhage and Vaginal Bleeding						
	Vaginal Bleeding only			Subchorionic Hemorrhage (with or without bleeding)		
	N=140	Unadjusted[1] OR (95% CI)	Adjusted[3] OR (95% CI)	N=72	Unadjusted[1] OR (95% CI)	Adjusted[3] OR (95% CI)
Change in 25(OH)D from Preconception to 8-Week Gestation						
Deficient/Insufficient to Sufficient	25 (23.6)	0.86 (0.49, 1.51)	0.97 (0.52, 1.80)	14 (13.2)	1.08 (0.52, 2.27)	1.00 (0.44, 2.27)
Sufficient to Deficient/Insufficient	19 (13.2)	1.17 (0.61, 2.25)	1.20 (0.61, 2.39)	8 (11.9)	1.17 (0.48, 2.86)	1.05 (0.40, 2.71)
No Change: Deficient/Insufficient	38 (23.0)	1.01 (0.61, 1.70)	0.96 (0.57, 1.62)	28 (17.0)	1.63 (0.87, 3.05)	1.75 (0.86, 3.55)
No Change: Sufficient	58 (26.5)	ref	ref	22 (10.1)	ref	ref

*OR-Odds Ratio

[1]Unadjusted for covariates and weighted to control for potential selection bias introduced by restricting to a sample of pregnancy.

[3]Adjusted for all sociodemographic and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamins, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of pregnancy.

Supplemental Table S4.2. Odds Ratio and 95% CI for maternal serum 25(OH)D level and vaginal bleeding episodes from 3-8 weeks gestation restricted to live birth: EAGeR Data (Time Varying Vitamin D)

EAGeR Generalized Estimating Equations Regression Models for 25(OH)D and Vaginal Bleeding				
		Unadjusted- Model 1[1] OR (95% CI)	Adjusted- Model 2[2] OR (95% CI)	Adjusted- Model 3[3] OR (95% CI)
Any Bleeding (vs. None)	N=1,486			
Deficient		0.84 (0.45, 1.55)	0.98 (0.51, 1.89)	1.10 (0.57, 2.15)
Insufficient		1.00 (0.71, 1.40)	1.04 (0.73, 1.49)	1.04 (0.72, 1.49)
Sufficient		ref	ref	ref
Light Bleeding (vs. None)	N=1,467			
Deficient		0.70 (0.35, 1.40)	0.80 (0.38, 1.67)	0.92 (0.43, 1.94)
Insufficient		1.07 (0.75, 1.53)	1.14 (0.79, 1.64)	1.13 (0.78, 1.64)
Sufficient		ref	ref	ref
Moderate/Heavy Bleeding (vs. None)	N=1,285			
Deficient		1.91 (0.60, 6.07)	2.97 (0.91, 9.70)	2.85 (0.81, 10.01)
Insufficient		0.46 (0.15, 1.41)	0.64 (0.26, 1.60)	0.76 (0.35, 1.62)
Sufficient		ref	ref	ref

[*]OR-Odds Ratio, N corresponds to longitudinal observations (not women)

*Time Varying Vitamin D- 3-4 weeks accounts for preconception vitamin D, 5-6 weeks accounts for average between preconception and 8 week vitamin D, 7-8 weeks accounts for 8-week vitamin D.

[1]Unadjusted model and weighted to control for potential selection bias introduced by restricting to a sample of Live Birth.

[2]Adjusted for all sociodemographic covariates which include age, exercise, income, race, education, parity, employment, and season and weighted to control for potential selection bias introduced by restricting to a sample of Live Birth.

[3]Adjusted for all sociodemographic and lifestyle covariates which included age, exercise, income, race, education, parity, employment, alcohol, vitamins, aspirin, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of Live Birth.

Supplemental Table S4.3. Distribution of vitamin D and vaginal bleeding via daily diaries for women who had a pregnancy loss versus did not have a pregnancy loss over gestational weeks 3-4, 5-6, 7-8 weeks gestation: EAGeR Data

	Had a pregnancy loss			**Did not have a pregnancy loss**		
	3-4 Weeks N=140	**5-6 Weeks N=140**	**7-8 Weeks N=140**	**3-4 Weeks N=581**	**5-6 Weeks N=581**	**7-8 Weeks N=581**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Vaginal bleeding						
None	95 (67.9)	70 (50.0)	56 (40.0)	490 (84.3)	459 (79.0)	430 (74.0)
Light	23 (16.4)	31 (22.1)	10 (7.1)	83 (14.3)	99 (17.0)	40 (6.9)
Moderate/ Heavy	21 (15.0)	15 (10.7)	--	7 (1.2)	11 (1.9)	3 (0.5)
Missing	1 (0.71)	24 (17.1)	71 (52.9)	1 (0.19)	12 (2.07)	108 (18.6)

Supplemental Table S4.4. Odds Ratio and 95% CI between continuous maternal serum 25(OH)D level and vaginal bleeding episodes from 3-8 weeks gestation restricted to women who became pregnant: EAGeR Trial

EAGeR Generalized Estimating Equations Regression Models for 25(OH)D and Vaginal Bleeding Episodes Between 3-8 Weeks Gestation				
		Unadjusted- M1[1] OR (95% CI)	Adjusted- M2[2] OR (95% CI)	Adjusted- M3[3] OR (95% CI)
Any Bleeding (vs. None)	N= 1,742			
Continuous 25 (OH)D (per 1 ng/mL)		0.99 (0.98, 1.01)	0.99 (0.98, 1.01)	1.00 (0.98, 1.01)
Light Bleeding (vs. None)	N= 1,701			
Continuous 25 (OH)D (per 1 ng/mL)		1.01 (0.99, 1.02)	1.00 (0.98, 1.01)	1.00 (0.98, 1.01)
Moderate/Heavy Bleeding (vs. None)	N=1,496			
Continuous 25 (OH)D (per 1 ng/mL)		0.99 (0.95, 1.02)	0.98 (0.95, 1.02)	0.98 (0.94, 1.02)

*OR-Odds Ratio, N corresponds to longitudinal observations (not women)
[1]Unadjusted model and weighted to control for potential selection bias introduced by restricting to a sample of Pregnancy.
[2]Adjusted for all sociodemographic covariates which include age, exercise, income, race, education, parity, employment, and season and weighted to control for potential selection bias introduced by restricting to a sample of Pregnancy.
[3]Adjusted for all sociodemographic and lifestyle covariates which included age, exercise, income, race, education, parity, employment, alcohol, vitamins, aspirin, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of Pregnancy.

Supplemental Table S4.5. Odds Ratio and 95% CI between continuous maternal serum 25(OH)D level and vaginal bleeding episodes from 3-8 weeks gestation restricted to women who had a live birth: EAGeR Trial

EAGeR Generalized Estimating Equations Regression Models for 25(OH)D and Vaginal Bleeding Episodes Between 3-8 Weeks Gestation				
		Unadjusted- M1[1] OR (95% CI)	Adjusted- M2[2] OR (95% CI)	Adjusted- M3[3] OR (95% CI)
Any Bleeding (vs. None)	N= 1,486			
Continuous 25 (OH)D (per unit ng/mL)		1.01 (0.96, 1.02)	0.99 (0.98, 1.01)	0.99 (0.98, 1.01)
Light Bleeding (vs. None)	N= 1,467			
Continuous 25 (OH)D (per unit ng/mL)		1.03 (0.99, 1.02)	0.99 (0.98, 1.02)	0.99 (0.98, 1.02)
Moderate/Heavy Bleeding (vs. None)	N=1,286			
Continuous 25 (OH)D (per unit ng/mL)		0.99 (0.94, 1.03)	0.97 (0.92, 1.02)	**0.93 (0.88, 0.99)**

*OR-Odds Ratio, N corresponds to longitudinal observations (not women)
[1]Unadjusted model and weighted to control for potential selection bias introduced by restricting to a sample of Live Birth.
[2]Adjusted for all sociodemographic covariates which include age, exercise, income, race, education, parity, employment, and season and weighted to control for potential selection bias introduced by restricting to a sample of Live Birth.
[3]Adjusted for all sociodemographic and lifestyle covariates which included age, exercise, income, race, education, parity, employment, alcohol, vitamins, aspirin, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of Live Birth.

Supplemental Table S4.6. Assessment of unmeasured confounding in the associations between preconception and 8 week 25(OH)D and risk of Vaginal Bleeding and Subchorionic Hemorrhage

Categorical 25(OH)D on risk of Vaginal Bleeding/Subchorionic Hemorrhage				
	VB Adjusted- M3[3] RR (95% CI)	E-Value Risk Ratio	SH Adjusted- M3[3] RR (95% CI)	E-Value Risk Ratio
Deficient/Insufficient to Sufficient	0.97 (0.52, 1.80)	1.21	1.00 (0.44, 2.27)	1.00
Sufficient to Deficient/Insufficient	1.20 (0.61, 2.39)	1.69	1.05 (0.40, 2.71)	1.28
No Change: Deficient/Insufficient	0.96 (0.57, 1.62)	1.25	1.75 (0.86, 3.55)	2.90
No Change: Sufficient	ref	ref	ref	ref

[3]Adjusted for all sociodemographic and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamins, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of live births.

Supplemental Table S4.7. Assessment of unmeasured confounding in the associations between 25(OH)D and episodes of vaginal bleeding: Longitudinal analysis.

	Adjusted- M3[3] OR (95% CI)	E-Value Observed Odds Ratio
Categorical 25(OH)D on risk of Vaginal Bleeding Episodes 3-8 Weeks		
Any Bleeding (vs. None)		
Deficient	1.27 (0.74, 2.20)	1.51
Insufficient	1.11 (0.81, 1.51)	1.29
Sufficient	ref	ref
Light Bleeding (vs. None)		
Deficient	1.07 (0.58, 2.00)	1.22
Insufficient	1.17 (0.84, 1.63)	1.38
Sufficient	ref	ref
Moderate/Heavy Bleeding (vs. None)		
Deficient	**3.02 (1.13, 8.13)**	2.87
Insufficient	0.83 (0.39, 1.80)	1.43
Sufficient	ref	ref
Continuous 25(OH)D on risk of Vaginal Bleeding Episodes 3-8 Weeks		
Any Bleeding (vs. None)		
Continuous 25 (OH)D (per 10 ng/mL)	1.00 (0.98, 1.01)	1.00
Light Bleeding (vs. None)		
Continuous 25 (OH)D (per 10 ng/mL)	1.00 (0.98, 1.01)	1.00
Moderate/Heavy Bleeding (vs. None)		
Continuous 25 (OH)D (per 10 ng/mL)	0.98 (0.94, 1.02)	1.11

[3]Adjusted for all sociodemographic and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamins, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of live births.

Figures

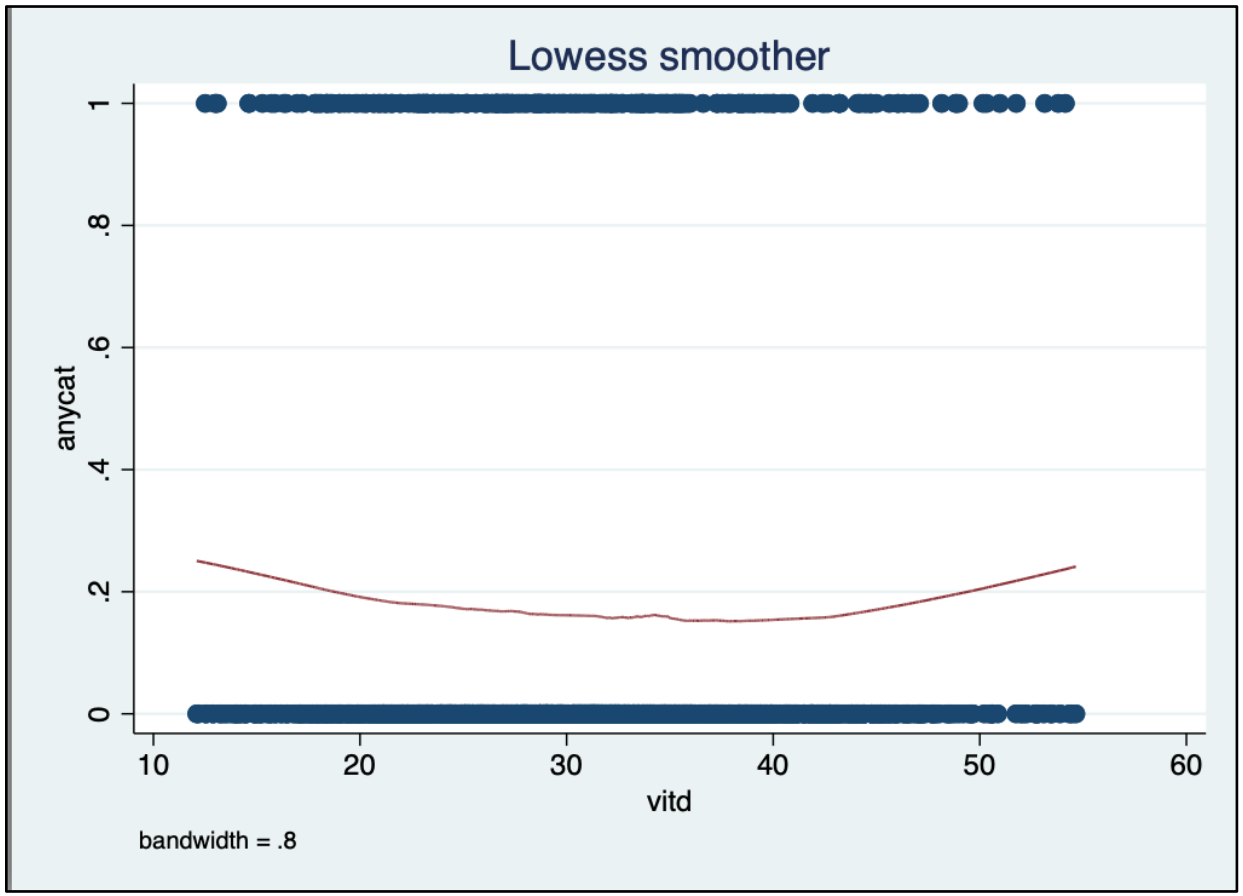

Figure 4.2. Non-parametric Lowess Curve for continuous 25(OH)D and any bleeding to express the best fitting for a smooth curve in connection to the data points presented between ≥12 ng/mL and ≤ 55 ng/mL to remove outliers, EAGeR Data (Total Sample N=1,743) *N= corresponds to longitudinal observations (not women)

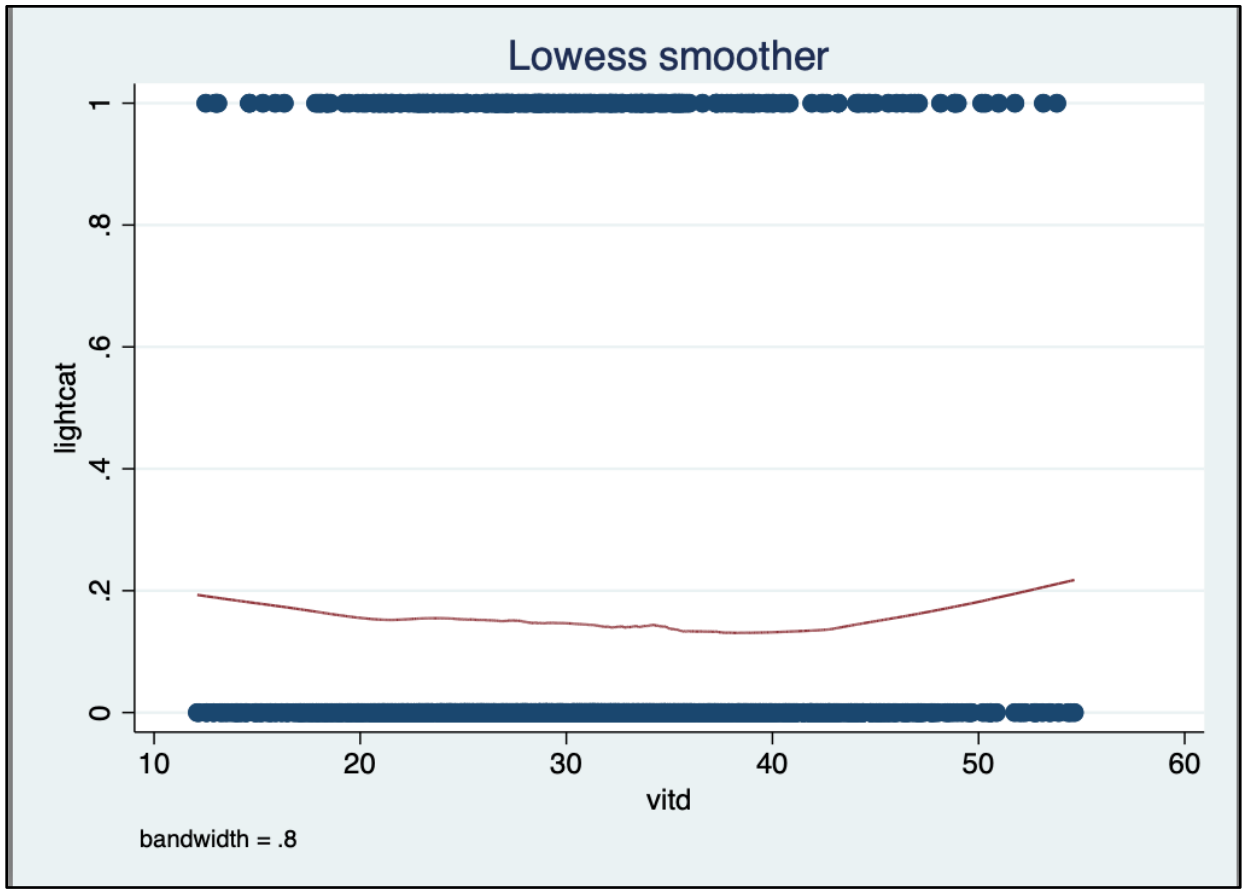

Figure 4.3. Non-parametric Lowess Curve for continuous 25(OH)D and light bleeding to express the best fitting for a smooth curve in connection to the data points presented between ≥12 ng/mL and ≤ 55 ng/mL to remove outliers, EAGeR Data (Total Sample N=1,702) *N= corresponds to longitudinal observations (not women)

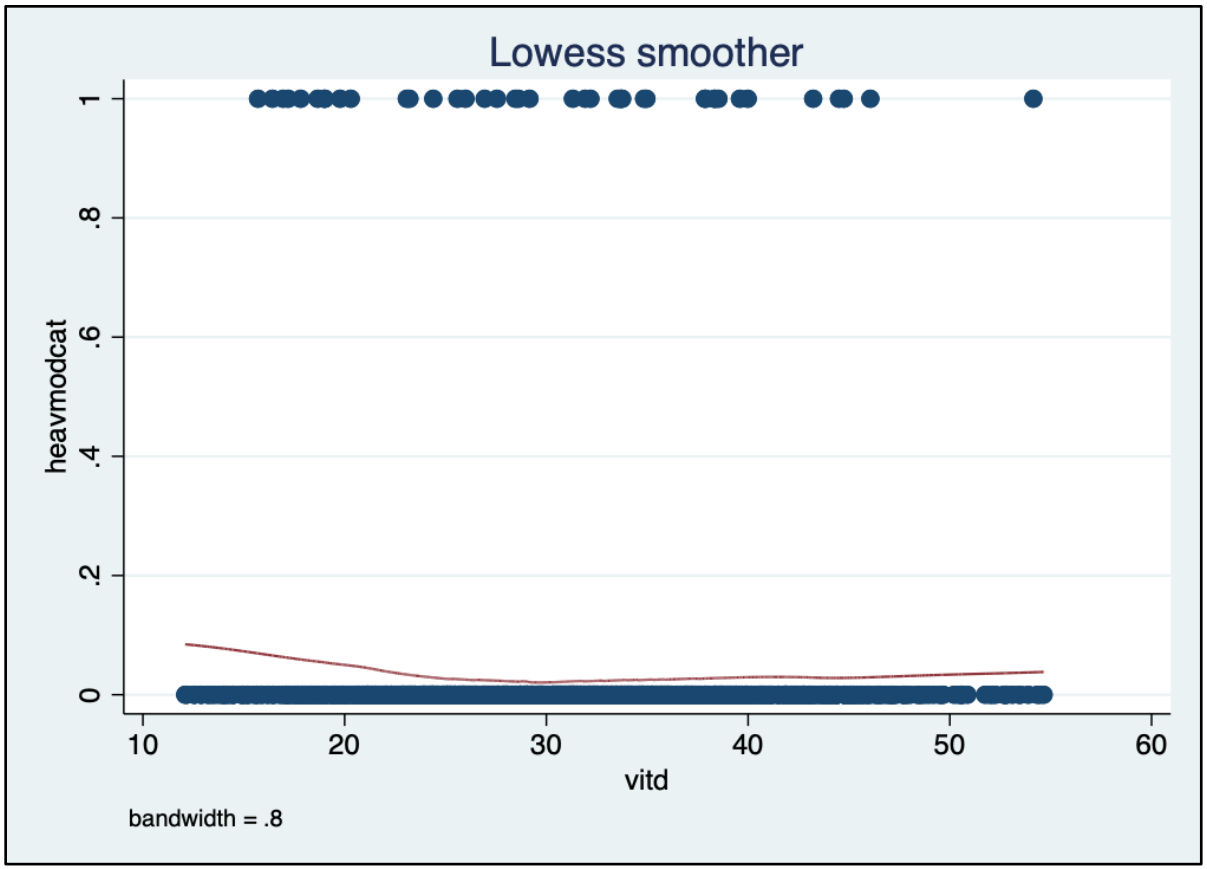

Figure 4.4. Non-parametric Lowess Curve for continuous 25(OH)D and heavy/moderate bleeding to express the best fitting for a smooth curve in connection to the data points presented between ≥12 ng/mL and ≤ 55 ng/mL to remove outliers, EAGeR Data (Total Sample N=1,495)

*N= corresponds to longitudinal observations (not women)

Chapter 5: Aim 2- The Association between Preconception and 8-week Gestation Serum 25(OH)D Levels and Nausea or Vomiting

5.1. Introduction

Nausea is a very common pregnancy symptom which affects between 50-70% of pregnant women.[132] Nausea is commonly reported in early pregnancy with the onset of symptoms occurring between 2-4 weeks of gestation.[132] It has been suggested that successful implantation and healthy placental function result in higher secretion of Human Chorionic Gonadotropin (hCG), which may manifest clinically as a more heightened nausea response.[132] It has been previously established that nausea during early pregnancy is a clinical marker for successful implantation and placental function.[133] Previous studies have acknowledged that nausea during early pregnancy was associated with a lower risk of miscarriage, preterm birth, low birth weight (LBW), and perinatal death.[134,227] However, the severity of symptoms for nausea and or vomiting may be different across pregnant women and across multiple pregnancies.

Although nausea is thought to be indicative of a more robust implantation response, mechanisms for why nausea and emesis (i.e., vomiting) may vary across pregnancies remains unclear. In some cases, extreme nausea or vomiting may be associated with nutrient depletion; however, this is difficult to discern as nutrient depletion may be caused by emesis or inability to take in nutrients from food sources due to extreme nausea.[228,229] Extreme nausea and vomiting in pregnancy may lead to a diagnosis of hyperemesis gravidarum (HG), which affects roughly 0.3-3% of pregnant women.[230,231] HG may be detrimental to the health and well-being of both the mother and baby and is the leading cause of hospitalization during pregnancy in the first trimester.[232,233] HG has been associated with maternal complications, such as severe dehydration, malnutrition, death, permanent disability (due to encephalopathy, caused by B1 vitamin deficiency), hemorrhage (due to vitamin K deficiency), and higher rates of depression and anxiety

during pregnancy.[230,232–238] HG has also been associated with higher fetal complications such as low birthweight and preterm birth, although the literature has been inconsistent.[239–241] HG may also create an onset of electrolyte abnormalities, also known as hypokalemia, that can lead to perinatal morbidity and mortality.[242,243] While the biological mechanisms leading to HG are still unclear, several hormonal factors have been identified in women with incidence of HG.[230] One hormonal pathway includes the Growth/Differentiation Factor 15 (GDF15), which is a hormone that is produced by the placenta through trophoblasts and is expressed early on during pregnancy.[244,245] One factor leading to an increase of GDF15 expression is nutrient depletion, specifically in a low-fat diet, which points to a potential nutritional pathway that may lead to more extreme cases of nausea/vomiting.[244,245] In addition, studies have shown depletion of vitamins K and B1 have been associated with increased incidence of HG.[246] However, the data are inconsistent and previous research has pointed to potentially higher levels of hCG in pregnancies that are affected by HG.[231,247,248] As such, there may be differing mechanisms for the role of nutrition on clinical experiences of nausea and emesis based on severity in early pregnancy.

In particular, vitamin D has been postulated to have an impact in the early critical periods of implantation, and may lead to adverse pregnancy outcomes if not above recommended sufficiency levels (>30ng/mL) by the Endocrine Society.[1,29,107,110,146,147] If sufficient levels of vitamin D are present in trophoblast cells and help form the placenta successfully, this may lead to a robust implantation process, increasing levels of hCG, which is associated with a higher likelihood of nausea in pregnancy.[132,242] In contrast, vitamin D deficiency may lead to placental dysfunction due to the disruption of hormones that help maintain a pregnancy and support endometrial receptivity and implantation of the uterus, such as that of estrogen and progesterone.[37,112,116,118] Specifically, trophoblasts are located in the outer layers of endometrial

cells and help the embryo implant successfully and then form the placenta.[28] These trophoblasts then support endometrial receptivity via vitamin D receptors in the uterus by providing an anti-inflammatory environment for successful implantation and placentation. [37,112,116,118] If any disruptions occur during this time, placental dysfunction may occur, which could lead to adverse pregnancy outcomes such as HG.[240,243,245,247–249]

The objective of this study was to evaluate whether preconception and/or 8 weeks gestation of maternal serum 25-hydroxyvitamin D (25(OH)D) concentrations may increase the risk of nausea or vomiting in women with proven fecundity.

5.2. Methods

5.2.1. Data Source

The proposed research uses data from the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial. The EAGeR trial was a multisite, prospective, double-blind, block-randomized, placebo-controlled clinical trial designed to evaluate the effect of low-dose aspirin (LDA) on live-birth in healthy women with regular menstrual cycles and 1-2 prior pregnancy losses.[48] 1,228 healthy women between 18 and 40 years of age who were attempting pregnancy after 1-2 prior pregnancy losses were enrolled. They could not have received fertility treatments prior to or during their enrollment or have a prior diagnosis of infertility. The institutional review boards at each study site (Salt Lake City, Utah; Denver, Colorado; Buffalo, New York; Scranton, Pennsylvania) and the data coordinating center approved the protocol for the trial. All participants provided their written consent prior to enrolling in the study. The trial was registered with ClinicalTrials.gov (#NCT00467363).

5.2.2. Analytic Sample

The analytic sample will be restricted to pregnant women in the EAGeR trial for whom there is measured serum 25(OH)D levels at preconception or 8-weeks' gestation, and no missing data on nausea or vomiting. Measurements of vitamin D were taken at baseline which could be from 1-6 months during the enrollment period. Women were then followed for 1-6 months and on average around 3 months to conceive. The analytic sample was restricted to women who became pregnant. In sensitivity analyses, further analyses were restricted to live birth to assess whether associations remained independent of survival of the pregnancy, since nausea may be absent due to a pregnancy loss.[133,228] Inverse probability weights were applied to account for any selection biases resulting in restriction to only those who became pregnant or who had a live birth.[201,213] Inverse probability weights were used and included covariates associated with the probability of becoming pregnant and having a live birth such as age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamin D, vitamins and BMI. Pregnancy status was determined via positive urine hCG pregnancy tests (Quidel Quickvue, Quidel Corporation), conducted at home or in the clinic at the time of expected menses. Access to this data source required approval from NICHD, which I have acquired through a data use agreement (DUA).

5.2.3. Measures

5.2.3.1 Exposure Measure

The exposure variable in this analysis was maternal serum vitamin D levels.[9] Serum samples were collected at baseline prior to randomization to low-dose aspirin (LDA) or placebo and at 8 weeks' gestation if they conceived. The serum samples were stored at −80°C until used for measurement of 25(OH)D.[48] Combined concentrations of 25-hydroxyvitamins D2 and D3 (25(OH)D) were

measured using the 25(OH)D ELISA solid phase sandwich enzyme immunoassay (BioVendor R&D, Ashville, NC, USA), which has been validated previously.[195]

The Endocrine Society has established vitamin D cutoffs that have been used in this analysis to inform clinical interpretation.[9] Vitamin D categories were classified as 25(OH)D deficient (≤20 ng/mL), insufficient (21-29 ng/mL), or sufficient (≥30 ng/mL) at preconception and 8 weeks' gestation.[9] Continuous vitamin D (ng/mL) was examined in supplemental analyses. In addition, change in vitamin D status between preconception and 8 weeks gestation was assessed by combining deficient and insufficient vitamin D together and categorizing change as: deficient/insufficient to sufficient, sufficient to deficient/insufficient, no change: deficient/insufficient, and no change: sufficient.

5.2.3.2. Outcome Measures

5.2.3.2.1 Nausea/Emesis Chart Abstractions

Nausea/Emesis was assessed using check-box questions via medical chart abstractions. Nausea/Emesis was a check-box question (yes/no) using the medical chart abstraction. Medical chart abstractions were used to capture adverse events using case/incident report forms which were filled out by study staff. In addition,, systematic safety interviews were conducted by study staff, and, during pregnancy, other questionnaires were completed by study participants. [184] Medical chart abstractions were recorded during ultrasounds, pregnancy loss visits, hospitalization visits, emergency care visits, and delivery visits. [184] Medical chart abstractions were completed using checkboxes or open-ended questions. [184] Following study completion, case report forms and open-ended questions completed through questionnaires and medical records were independently reviewed by two board-certified reproductive endocrinologists as well as a perinatal epidemiologist.[184]

5.2.3.2.2. Nausea/Vomiting Daily Diaries

Time-varying outcomes of nausea and vomiting information were available through daily diaries. Nausea and vomiting information were collected daily and completed by women in the trial. Daily diary questions assessing nausea and vomiting were: "Please report any nausea or vomiting that you have experienced today. Record these symptoms regardless of the reason. If none, please enter 0." Responses were categorized as follows: 0=none, 1=nausea, 2=vomiting once per day/vomiting more than once per day." Daily nausea and vomiting categories were recorded based on questionnaires (see Appendix II). Previous studies have validated the outcomes of nausea self-report and have found reasonable accuracy between the self-reported data and clinical presentations.[199]

Additionally, information was collected on the date of the beginning of each week of the daily diary entry, which was used in combination with the first day of the last menstrual period (LMP) to assess timing of nausea and vomiting relative to weeks of gestation. Nausea and vomiting categories were then defined at biweekly intervals between 3-4 weeks, 5-6 weeks, and 7-8 weeks of gestation. These groups were further categorized for analysis in three ways: 1) women having reported any nausea/vomiting versus none or 2) women having reported nausea only versus none or 3) women having reported vomiting once per day or more than once per day versus none.

5.2.3.3. Confounders

Demographic information for baseline characteristics were captured in questionnaires at the first study visit.[184] A reproduction baseline questionnaire was used to assess pregnancy history information. [184] During the study, medical record abstractions were used for women who became pregnant. Baseline health characteristics such as height and weight were measured by trained study staff and used to calculate body mass index (BMI).[184] The covariates considered in the models

were sociodemographic and other health characteristics which include age, race/ethnicity, education, employment, income, BMI kg/m^2, parity, season, physical activity, alcohol intensity, and multivitamin use. Season was defined as the season of baseline during which blood was drawn for the sample of preconception serum 25(OH)D. Physical activity was assessed using the International Physical Activity Questionnaire and defined as low, moderate, or high.[200] Alcohol intensity was defined as the amount of alcohol consumed in the past year and was measured as never, sometimes, and often. Multivitamin use was measured as the type of vitamins that were consumed by the women prior to the study and were defined as not taking any vitamins or folic acid, taking vitamins with no folic acid, and taking vitamins with folic acid. Additionally, aspirin/placebo, the assigned treatment in this trial, will be considered as a confounder in this analysis, as previously applied in a study of vitamin D and pregnancy loss using this data.[11]

5.3 Analysis

5.3.1. Descriptive Analyses

Differences in the prevalence of nausea/vomiting across different baseline characteristics and bivariate associations of covariates with vitamin D levels (preconception and 8 weeks gestation) are examined using chi-square tests or F-statistics for comparing categorical or continuous variables, respectively.

5.3.2. Multinomial Logistic Regression Models

Odds ratios between change in preconception and 8-weeks' gestation serum 25(OH)D levels and nausea/vomiting were estimated using multinomial logistic regression models with robust standard errors and inverse probability weights to account for selection that may occur by only including women who have had a pregnancy (i.e., excludes women who did not become pregnant during the study period, which may also be related to the exposure under study). Due to nausea/vomiting

being a common outcome during pregnancy, odds ratios are poor approximations for the relative risk of nausea/vomiting. The inverse probability weights used to account for selection bias that could result from restriction to pregnancy were derived from models that included covariates associated with the probability of being pregnant such as age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamin D, vitamins, and BMI.[201,213]

An unadjusted log binomial regression model was used to examine associations with nausea/vomiting. Models were then adjusted for relevant confounders, as determined by DAGs (see Chapter 1), which included all sociodemographic: age, season, income, race, education, parity, employment; and lifestyle: smoking, exercise, alcohol, aspirin, vitamin D, vitamins covariates, including BMI.

5.3.3. Generalized Estimating Equations (GEE) Regression Models

In longitudinal analyses, the odds ratio between serum 25(OH)D levels on any nausea or vomiting at biweekly intervals in the first 8 weeks of pregnancy (3 time points) were examined using generalized estimating equations (link: log, family: binomial). Vitamin D levels at preconception applied to the first interval (3-4 weeks) and vitamin D levels measured at 8 weeks were applied to the last time interval (7-8 weeks gestation). For weeks 5-6, an average of the preconception and 8 week vitamin D level was imputed. Models were adjusted for the same set of covariates applied above. Additionally, separate regression models were used to examine vomiting classified as once per day or more than once per day (vs. none), nausea only (vs. none) and any nausea/vomiting (vs. none). Similarly, inverse probability weights to account for potential selection bias of becoming pregnant were included as described above.

5.3.4. Sensitivity Analyses

To account for nausea/vomiting tending to be associated with a reduced risk of pregnancy loss, I also compared results to models restricted to women who achieved a live birth (Supplemental Table S5.1). Given selection bias may result from restricting to pregnancy that survive to a live birth, inverse probability weights were applied to account for any potential selection biases.[201,213] Furthermore, models to derive the inverse probability weights accounted for age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamin D, vitamin use and BMI.[201,213] Restriction to a live birth allows the assessment of the robustness of my findings.[11] In addition, interactions between low-dose aspirin treatment and placebo was examined. All analyses were performed using STATA version 17.0.

5.4. Results

5.4.1. Descriptive Analyses

At baseline, 747 participants were pregnant and had a measured preconception 25(OH)D serum level with 50% sufficient, 37% insufficient, and 13% deficient (Table 1). Those in the deficient 25(OH)D category had a mean BMI of 30.5 compared with 26.9 among insufficient and 24.5 among sufficient (p-value <0.0001). Women in the deficient 25(OH)D group were also more likely to be white (83.7%), have >high school education (80.4%), have a fall season of blood draw (36.9%), have moderate exercise level (41.3%), have never consumed alcohol in the past year (73.9%), and have a low CRP baseline (35.2%), compared with women in the insufficient or sufficient 25(OH)D groups. Age, employment, vitamin use, smoking, number of previous pregnancy losses, and treatment assignment were not associated with preconception 25(OH)D.

605 participants were pregnant and had a measured 8 week 25(OH)D serum level with 56% sufficient, 38% insufficient, and 5% deficient. Those in the deficient 25(OH)D category had a mean BMI of 30.5 compared with 26.3 among insufficient and 24.2 among sufficient (p-value

<0.001). Women in the deficient 25(OH)D group were also more likely to be white (81.3%), have >high school education (81.3%), take folic acid and vitamins (90.6%), have a fall season of blood draw (46.9%), have low exercise level (56.3%), have never consumed alcohol in the past year (73.9%), and have a moderately high CRP baseline (40.3%) compared with those in the insufficient and sufficient 25(OH)D groups. Age, smoking, number of previous pregnancy losses, alcohol consumption, and treatment assignment were not associated with 8 week 25(OH)D.

Bivariate associations between the prevalence of nausea (emesis) and preconception and 8-week 25(OH)D serum levels and sociodemographic and lifestyle characteristics were not statistically significant, with the exception of age, which showed a decrease in nausea (emesis) with increasing age (p=0.01). Although not significant, the prevalence of nausea (emesis) was highest among women with insufficient (72.3% for preconception; 86.6% 8 weeks gestation) and deficient (69.6% for preconception; 75.0% for 8 week gestation) 25(OH)D levels, those who were overweight (74.2%), white (72.0%), educated ≤ High School (72.7%), had an annual household income of ≤ $19,999 (74.5%), not employed (76.7%), took folic acid and vitamins (72.0%), never smoked in the past year (73.4%), had a summer season of blood draw (74.1%), reported low exercise (73.9%), often consumed alcohol in the past year (78.9%), and were in the low dose aspirin treatment assignment group (71.9%).

5.4.2. Multinomial Logistic Regression Results

In Table 4 assessing nausea (emesis) documented in medical records, women who were deficient/insufficient at preconception and then became sufficient at 8 weeks gestation had 1.69 (95% CI: 1.14, 2.50) times higher odds of having nausea (emesis) in the unadjusted model and 1.71 times higher odds (95% CI: 1.12, 2.61) after adjustment compared to those who were persistently sufficient across both time periods. Additionally, the odds of nausea/emesis were

lower for women who changed from sufficient to deficient/insufficient (OR: 0.44, 95% CI; 0.22, 0.87) or who remained persistently deficient/insufficient (OR: 0.34, 95% CI: 0.20, 0.60). When restricting to live births, these associations were no longer significant and, in some cases, the magnitude of association differed between live births and all pregnancies (Table 4). However, those who were persistently deficient/insufficient consistently had lower odds of nausea/emesis (OR: 0.64, 95% CI: 0.25, 1.63) (Table 4).

5.4.3. Generalized Estimating Equations (GEE) Results

In the longitudinal analyses of daily diary data (Table 5), deficient vitamin D status was associated with lower odds of any nausea or vomiting during pregnancy in the fully adjusted models (OR 0.65; 95% CI 0.40, 1.06) compared to women with sufficient vitamin D, but this was imprecise. Partitioning this further into nausea only or vomiting once per day or more than once per day vs. none, showed decreased odds of vomiting once per day or more than once per day (OR 0.54; 95% CI: 0.28, 1.04) vs. none with deficient vitamin D status, and this association was similarly seen for nausea only (OR 0.84 95% CI: 0.63, 1.12) vs. none. The odds of experiencing any nausea (OR 0.86; 95% CI: (0.65, 1.15), vs none, nausea only (OR 0.84; 95% CI: (0.63, 1.12) vs. none, or vomiting once per day or more than once per day (OR 1.12; 95% CI: 0.80, 1.56) vs. none, were increased among women with insufficient vitamin D levels compared to sufficient levels of 25(OH)D. These patterns were consistent when restricted to live births in supplemental analyses (Supplemental Table S5.1).

5.4.4. Longitudinal Patterns of Missingness

Among those with a pregnancy loss, missing information on nausea/vomiting increased over time with 1.4% in weeks 3-4 and 50.7% in weeks 7-8. Among those without a pregnancy loss, missing

information on nausea/vomiting was less with 1.7% at 3-4 weeks and 19.6% in weeks 7-8 (Supplemental Table S5.2).

5.5. Discussion

In Table 4, multinomial logistic regression models examining medical records, those with persistently deficient/insufficient 25 (OH)D at preconception and 8 weeks gestation were less likely to have nausea/emesis among a cohort of healthy women with a history of 1-2 prior pregnancy losses and no known history of infertility. In our GEE findings, daily diary data of nausea and vomiting intensity showed 25(OH)D deficiency was also associated with lower odds of both nausea and vomiting compared to sufficient 25(OH)D levels. In addition, when restricting to live births, these associations were no longer significant and, in some cases, the magnitude of association differed between live births and all pregnancies (Supplemental Table S5.1). These findings may present a potential pathway between 25(OH)D levels and early implantation/placentation processes as indicated by nausea and/or vomiting. As such, these findings suggest that higher levels of 25(OH)D are biologically consistent with increased robustness of implantation and placentation, which would increase the incidence of nausea and vomiting in pregnancy.[132,133]

There is limited information on the relationship between preconception and early gestation maternal vitamin D and clinical experiences of nausea and vomiting in early pregnancy. Our study, however, is consistent with a previous study which measured preconception vitamin D status and found sufficient preconception vitamin D may increase live birth, which may correlate with robust implantation.[11] In addition, a previous IVF study measured preconception vitamin D status and found sufficient preconception vitamin D increased the incidence of pregnancy, also suggesting a robustness in implantation and placentation.[38]

The biological pathways for the role of preconception and early pregnancy period and the role of vitamin D in increasing the risk of nausea and vomiting are likely multifactorial. This study is suggestive of the importance of sufficient maternal vitamin D stores prior to conception to improve robust implantation which may increases the production of hCG, which aids in maintaining the pregnancy and has been associated with increased incidence of nausea and vomiting.[133,150,228] Future studies with larger sample sizes are needed to assess the association between preconception vitamin D on severe nausea and vomiting (HG) during pregnancy.

5.5.1. Strengths and Limitations

This study has several limitations. The sample size was limited for examining more detailed groupings or interactions. However, I was able to examine nausea and vomiting through as reported by medical records and self-reported daily diaries, the latter of which enabled time-varying longitudinal analyses. The consistency of findings across different types of data collection strategies, each with different potential biases, provides further support for these relationships. The lack of diversity within the cohort may limit generalizability of the results to the general population, which is important given the high incidence of deficient and insufficient vitamin D levels in the pregnant population. Another limitation in the longitudinal analysis was having to infer vitamin D levels for the 5-6 week gestation period as the average between preconception and 8 weeks gestational ages, rather than a direct measure and variation in time from measurement of preconception vitamin D to the first 3-4 weeks of pregnancy. Additionally, pregnancy loss among study participants could have resulted in missing data, which may be associated with outcomes under study. However, results were consistent (i.e., similar magnitude of association) after restricting to live births for the persistently deficient/sufficient 25(OH)D group in multinomial logistic regression analyses and the deficient group in GEE analyses levels, suggesting these

relationships likely hold independent of pregnancy loss. Unmeasured confounding may still be a concern, resulting in biased estimates.[219,220] To address this, I calculated e-values to assess the extent to which unmeasured confounding may explain the associations found (Supplemental Tables S5.3 and S5.4), which varied based on the magnitude of the association.[219,220] Unmeasured confounders that may have been important to adjust for are both maternal and paternal exposures to environmental exposures, such as exposure to toxic chemicals or air pollutants, which have been associated with fertility and adverse pregnancy outcomes.[221,222,224–226] In addition, paternal nutrition and health factors are important to assess due to epigenetics and sperm playing a key role in both placentation and pregnancy health.[221–226] Calculated e-values for deficient or persistently deficient vitamin D ranged between 2.06-5.33. While other potential unmeasured confounders may be associated with the outcomes and exposures assessed in this analysis, the associations of the unmeasured confounders would need to be fairly strong to fully explain the associations found in this analysis. Finally, although the gold standard for serum vitamin D measurement is the liquid chromatography-tandem mass spectrometry, previous studies have found immunoassays through the Vitamin D External Quality Assessment Scheme (DEQAS) to be similar.[195–197] Therefore, it is still a valid measurement for vitamin D concentrations through the use of the ELISA solid phase sandwich enzyme immunoassay.[195] Finally, missingness of measurement of serum 25(OH)D over time is due to the design of the trial (due to potential pregnancy loss), rather than missing from respondent not filling out the questionnaire.

Having preconception and early gestation serum 25(OH)D measurements is a strength of the study, because this may be a critical time period that influences implantation and placentation processes and, potentially, later pregnancy outcomes.[11,38] In addition, the daily diary data allowed assessment of nausea and vomiting as early as 3-4 weeks into pregnancy, which captures a time

point prior to many women finding out they are pregnant. Furthermore, the detailed diary data allowed the assessment of nausea and vomiting to be recorded prospectively through longitudinal assessment. Thus, to assess indicators that may change with time, the use of daily diaries provides a more precise assessment of the onset of symptoms in pregnant women.[150]

5.5.2. Conclusion

The biologic pathways regarding the effects of preconception and early gestation maternal serum 25(OH)D on nausea and vomiting are most likely multifactorial, and this study is suggestive of deficient maternal 25(OH)D nutrient stores prior to conception being associated with reduced odds of experiencing nausea or vomiting. Sufficient 25(OH)D nutrient stores during the preconception and early gestation period are important and may be an indicator of a more robust implantation/placentation and therefore a healthy pregnancy. Future studies are needed with larger sample sizes to assess the association between 25(OH)D and extreme nausea and vomiting (HG) in the preconception and early gestation period in more diverse populations.

Tables

Table 5.1. Descriptive Analyses of preconception maternal serum levels of vitamin D in the EAGeR Trial among women with a Pregnancy (n=774).

EAGeR- Preconception Vitamin D Descriptive Analyses				
	Vitamin D Sufficient (≥ 30 ng/mL)	**Vitamin D Insufficient (<30 ng/mL & ≥20 ng/mL)**	**Vitamin D Deficient (<20 ng/mL)**	**P-value**
N	392	285	97	
Age, years				0.37
Mean ± SD	28.7 ± 4.4	28.7 ± 4.6	28.5 ± 5.3	0.83
18-24.9	95 (24)	59 (21)	22 (23)	
25-29.9	148 (38)	126 (44)	41 (42)	
30-34.9	97 (25)	76 (27)	23 (24)	
35-40.9	52 (13)	24 (8)	11 (11)	
***BMI, kg/m^2**				<0.0001
Mean ± SD	24.5 ± 5.1	26.9 ± 6.3	30.5 ± 8.6	<0.0001
Underweight <18.5	15 (4)	12 (4)	3 (3)	
Normal ≥18.5 & <25	247 (64)	132 (46)	30 (31)	
Overweight ≥25 & <30	81 (21)	88 (31)	17 (18)	
Obese ≥30	45 (12)	52 (18)	46 (48)	
***Race**				<0.0001
White	387 (99)	278 (98)	81 (84)	
Non-White	5 (1)	7 (2)	16 (16)	
Education				0.01
≤ High School	40 (11)	23 (8)	18 (19)	
> High School	352 (90)	262 (92)	79 (81)	
Income, n				0.03
≥ $100,000	151 (39)	130 (46)	33 (34)	
$75,000-$99,999	64 (16)	40 (14)	8 (8)	
$40,000-$74,999	65 (17)	34 (12)	13 (13)	
$20,000-$39,999	86 (22)	64 (22)	32 (33)	
≤ $19,999	26 (7)	17 (6)	11 (11)	
Employment				0.30
Yes	92 (24)	79 (28)	28 (30)	
No	295 (76)	203 (72)	65 (70)	
Vitamin Use				0.19

No Folic Acid-No Vitamins	29 (7)	12 (4)	7 (7)	
No Folic Acid- Yes Vitamins	66 (17)	41 (14)	10 (10)	
Yes Folic Acid- Yes Vitamins	297 (76)	232 (81)	80 (82)	
Smoking				0.29
Never	345 (89)	190 (90.5)	60 (88.2)	
<6 times/week	32 (8)	12 (5.7)	6 (8.8)	
Daily	12 (3)	8 (3.8)	2 (2.9)	
Season				0.005
Fall (Sep-Nov)	108 (28)	71 (25)	37 (38)	
Winter (Dec-Feb)	76 (19)	65 (23)	27 (28)	
Spring (Mar-May)	105 (28)	86 (30)	24 (25)	
Summer (Jun-Aug)	103 (26)	63 (22)	9 (9)	
***Exercise**				0.003
Low	89 (23)	74 (26)	39 (40)	
Moderate	173 (44)	110 (39)	39 (40)	
High	130 (33)	101 (35)	19 (20)	
Number of previous pregnancy losses				0.27
0				
1	229 (59)	176 (62)	65 (67)	
2	163 (42)	109 (38)	32 (33)	
Alcohol Intensity				0.001
Never	238 (61)	210 (75)	69 (71)	
Sometimes	141 (36)	61 (22)	28 (29)	
Often	9 (2)	10 (4)	0 (0)	
***Baseline CRP**				0.001
Mean ± SD	2.3 ± 5.1	2.8 ± 3.7	4.8 ± 7.9	0.007
Low <1	203 (52)	136 (48)	34 (35)	
Borderline ≥1 & <3	123 (31)	75 (26)	25 (26)	
Moderately High ≥3 & <10	54 (14)	59 (21)	29 (30)	
High Concentrations ≥10	12 (3)	15 (5)	8 (8)	
Treatment assignment				0.43
Placebo	179 (46)	144 (51)	48 (49)	
Low Dose Aspirin	213 (54)	141 (49)	49 (51)	

*Non-white participants include American Indian/Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, Black or African American, more than one Race, Unknown or Not Reported *BMI- Body Mass Index, CRP- C-Reactive Protein

*Exercise level was measured through the International Physical Activity Questionnaire and assessed the level of physical activity as low, moderate, and high

Table 5.2. Descriptive Analyses of 8-week maternal serum levels of vitamin D in the EAGeR Trial among women with a Pregnancy (n= 641)

EAGeR- 8-week Vitamin D Descriptive Analyses				
	Vitamin D Sufficient (≥ 30 ng/mL)	**Vitamin D Insufficient (<30 ng/mL & ≥20 ng/mL)**	**Vitamin D Deficient (<20 ng/mL)**	**P-value**
N	**361**	**246**	**34**	
Age, years				0.32
Mean ± SD	28.7 ± 4.6	28.4 ± 4.4	28.7 ± 5.0	0.33
18-24.9	81 (22)	57 (23)	6 (18)	
25-29.9	142 (39)	117 (48)	17 (50)	
30-34.9	96 (27)	47 (20)	7 (21)	
35-40.9	42 (12)	25 (10)	4 (12)	
***BMI, kg/m^2**				<0.001
Mean ± SD	24.2 ± 4.7	26.3 ± 6.2	30.5 ± 8.9	<0.001
Underweight <18.5	11 (3)	7 (3)	0 (0.0)	
Normal ≥18.5 & <25	223 (62)	115 (47)	10 (29)	
Overweight ≥25 & <30	85 (24)	62 (26)	9 (26)	
Obese ≥30	39 (11)	59 (24)	15 (44)	
***Race**				<0.001
White	355 (98)	239 (97)	28 (82)	
Non-White	6 (2)	7 (3)	6 (18)	
Education				<0.001
<= High School	40 (11)	19 (8)	11 (32)	
> High School	321 (89)	227 (92)	23 (68)	
Income, n				0.002
≥ $100,000	156 (43)	102 (41)	11 (32)	
$75,000-$99,999	61 (17)	28 (11)	1 (3)	
$40,000-$74,999	52 (14)	40 (16)	3 (9)	
$20,000-$39,999	70 (19)	66 (27)	13 (38)	
≤ $19,999	22 (6)	10 (4)	6 (18)	
Employed				0.08
Yes	265 (74)	177 (73)	19 (56)	
No	93 (26)	66 (27)	15 (44)	
Vitamin Use				0.05
No Folic Acid-No Vitamins	20 (6)	18 (7)	1 (3)	

No Folic Acid- Yes Vitamins	61 (17)	24 (10)	2 (6)	
Yes Folic Acid- Yes Vitamins	280 (78)	204 (83)	31 (91)	
Smoking				0.69
Never	324 (90)	225 (91)	29 (85)	
<6 times/week	23 (6)	11 (5)	3 (9)	
Daily	12 (3)	10 (4)	2 (6)	
Season				0.009
Fall (Sep-Nov)	86 (24)	74 (30)	16 (47)	
Winter (Dec-Feb)	67 (19)	62 (25)	6 (18)	
Spring (Mar-May)	115 (32)	61 (25)	6 (18)	
Summer (Jun-Aug)	93 (26)	49 (20)	46(18)	
Exercise				0.001
Low	81 (22)	71 (29)	19 (56)	
Moderate	156 (43)	100 (41)	7 (21)	
High	124 (34)	75 (30)	8 (24)	
Number of previous pregnancy losses				0.69
0				
1	223 (62)	156 (63)	19 (56)	
2	138 (38)	90 (37)	15 (44)	
Alcohol Intensity				0.51
Never	234 (66)	173 (71)	24 (71)	
Sometimes	112 (31)	65 (27)	10 (29)	
Often	11 (3)	5 (2)	0 (0.0)	
***Baseline CRP**				0.002
Mean ± SD	2.2 ± 4.69	2.4 ± 2.95	3.9 ± 4.46	0.09
Low <1	190 (53)	106 (44)	11 (34)	
Borderline ≥1 & <3	101 (28)	80 (33)	5 (16)	
Moderately High ≥3 & <10	54 (15)	46 (19)	13 (41)	
High Concentrations ≥10	14 (4)	11 (5)	3 (9)	
Treatment assignment				0.17
Placebo	160 (44)	127 (52)	18 (53)	
Low Dose Aspirin	201 (56)	119 (48)	16 (47)	

*Non-white participants include American Indian/Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, Black or African American, more than one Race, Unknown or Not Reported *BMI- Body Mass Index, CRP- C-Reactive Protein

*Exercise level was measured through the International Physical Activity Questionnaire and assessed the level of physical activity as low, moderate, and high.

Table 5.3. Prevalence of Nausea/Vomiting among women in the EAGER Trial by Vitamin D Status and Other Covariates (N=774)

EAGeR Covariates and Prevalence of Nausea/Vomiting			
Covariates	**N**	**Prevalence of Nausea/Vomiting (%)**	**P-value**
Preconception Vitamin D			
Sufficient ≥30 ng/mL	377	272 (72.2)	0.86
Insufficient ≥20 & <30 ng/mL	278	201 (72.3)	
Deficient <20 ng/mL	92	64 (69.6)	
8-week Vitamin D			
Sufficient ≥30 ng/mL	341	292 (85.6)	0.21
Insufficient ≥20 & <30 ng/mL	232	201 (86.6)	
Deficient <20 ng/mL	32	24 (75.0)	
Demographics			
Age, years			
18-24.9	172	132 (76.7)	0.01
25-29.9	305	228 (74.8)	
30-34.9	189	129 (68.3)	
35-40.9	81	48 (59.3)	
***BMI, kg/m^2**			
Underweight <18.5	30	17 (56.7)	0.11
Normal ≥18.5 & <25	399	294 (73.7)	
Overweight ≥25 & <30	178	132 (74.2)	
Obese ≥30	140	94 (67.1)	
***Race**			
White	722	520 (72.0)	0.65
Non-White	25	17 (68.0)	
Education			
≤ High School	77	50 (64.9)	0.18
> High School	670	487 (72.7)	
Income			
≥ $100,000	303	223 (73.6)	0.79
$75,000-$99,999	108	73 (67.6)	

$40,000-$74,999	108	76 (70.4)	
$20,000-$39,999	177	127 (71.8)	
≤ $19,999	51	38 (74.5)	
Employed			
Yes	554	389 (70.2)	0.09
No	193	148 (76.7)	
Vitamin Use			
No Folic Acid-No Vitamins	48	34 (70.8)	0.96
No Folic Acid- Yes Vitamins	109	78 (71.6)	
Yes Folic Acid- Yes Vitamins	590	425 (72.0)	
Smoking in the past year			
Never	664	485 (73.4)	0.12
Fewer	54	33 (61.1)	
Daily	29	19 (65.5)	
Season of blood draw			
Fall (Sep-Nov)	207	150 (72.5)	0.70
Winter (Dec-Feb)	165	113 (68.5)	
Spring (Mar-May)	205	148 (72.2)	
Summer (Jun-Aug)	170	126 (74.1)	
***Exercise**			
Low	195	144 (73.9)	0.77
Moderate	309	219 (70.9)	
High	243	174 (72.6)	
Alcohol consumption in the past year			
Never	506	371 (73.3)	0.28
Sometimes	222	151 (68.0)	
Often	19	15 (78.9)	
Treatment assignment			
Placebo	358	257 (71.8)	1.00
Low Dose Aspirin	389	280 (71.9)	

*P-values based on Fisher's Exact Test

*Non-white participants include American Indian/Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, Black or African American, more than one Race, Unknown or Not Reported
*Exercise level was measured through the International Physical Activity Questionnaire and assessed the level of physical activity as low, moderate, and high.

Table 5.4. Association between the change in preconception and 8-week 25(OH)D status and nausea/emesis restricted to pregnancy and live birth: EAGeR Trial

EAGeR Multinomial Logit Regression Models for Preconception Change to 8 Weeks 25(OH)D and Nausea/Emesis						
	Restricted to Pregnancy			Restricted to Live Birth		
	N= 747	Unadjusted[1] OR (95% CI)	Adjusted[3] OR (95% CI)	N= 557	Unadjusted[1] OR (95% CI)	Adjusted[3] OR (95% CI)
Change in 25(OH)D from Preconception to 8 Weeks Gestation						
Deficient/Insufficient to Sufficient	105 (19.6)	**1.69 (1.14, 2.50)**	**1.71 (1.12, 2.61)**	99 (19.1)	0.88 (0.34, 2.24)	0.82 (0.32, 2.07)
Sufficient to Deficient/Insufficient	65 (12.1)	**0.44 (0.23, 0.84)**	**0.44 (0.22, 0.87)**	61 (11.8)	1.15 (0.43, 3.06)	1.01 (0.33, 3.08)
No Change: Deficient/Insufficient	160 (29.8)	**0.40 (0.24, 0.66)**	**0.34 (0.20, 0.60)**	156 (30.1)	0.77 (0.32, 1.82)	0.64 (0.25, 1.63)
No Change: Sufficient	207 (38.6)	ref	ref	202 (39.0)	ref	ref

*OR-Odds Ratio

[1]Unadjusted for covariates and weighted to control for potential selection bias introduced by restricting to a sample of pregnancy.

[3]Adjusted for all sociodemographic and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamins, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of pregnancy

Table 5.5. Odds Ratio and 95% CI between time-varying maternal serum 25(OH)D level and Nausea/Vomiting episodes from 3-8 weeks gestation Restricted to Pregnancy: EAGeR Trial

EAGeR Generalized Estimating Equations Regression Models for Preconception and 8-Week Gestation 25(OH)D and Nausea/Vomiting				
		Unadjusted- Model 1[1] OR (95% CI)	Adjusted- Model 2[2] OR (95% CI)	Adjusted- Model 3[3] OR (95% CI)
Any Nausea (vs. None)	N=1,723			
Deficient		0.70 (0.46, 1.07)	0.77 (0.49, 1.23)	0.65 (0.40, 1.06)
Insufficient		0.92 (0.70, 1.20)	0.91 (0.69, 1.20)	0.86 (0.65, 1.15)
Sufficient		ref	ref	ref
Nausea Only (vs. None)	N=1,562			
Deficient		0.67 (0.43, 1.03)	0.76 (0.47, 1.21)	0.65 (0.40, 1.07)
Insufficient		0.88 (0.67, 1.16)	0.88 (0.66, 1.17)	0.84 (0.63, 1.12)
Sufficient		ref	ref	ref
Vomiting once per day or more than once per day (vs. None)	N=498			
Deficient		0.87 (0.48, 1.60)	0.69 (0.35, 1.35)	0.54 (0.28, 1.04)
Insufficient		1.29 (0.92, 1.82)	1.19 (0.84, 1.69)	1.12 (0.80, 1.56)
Sufficient		ref	ref	ref

*OR-Odds Ratio

[1]Unadjusted model and weighted to control for potential selection bias introduced by restricting to a sample of Pregnancy.

[2]Adjusted for all sociodemographic covariates which include age, exercise, income, race, education, parity, employment, and season and weighted to control for potential selection bias introduced by restricting to a sample of Pregnancy.

[3]Adjusted for all sociodemographic and lifestyle covariates which included age, exercise, income, race, education, parity, employment, alcohol, vitamins, aspirin, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of Pregnancy.

Supplemental Tables

Supplemental Table S5.1. Odds Ratio and 95% CI between Time-Varying Maternal Serum 25(OH)D level and Nausea/Vomiting Episodes from 3-8 weeks Gestation Restricted to Live Birth: EAGeR Trial

EAGeR Generalized Estimating Equations Regression Models for 25(OH)D and Nausea/Vomiting				
		Unadjusted- Model 1[1] OR (95% CI)	Adjusted- Model 2[2] OR (95% CI)	Adjusted- Model 3[3] OR (95% CI)
Any Nausea (vs. None)	N=1,468			
Deficient		0.68 (0.42, 1.10)	0.73 (0.43, 1.24)	0.60 (0.35, 1.05)
Insufficient		0.94 (0.69, 1.27)	0.93 (0.68, 1.27)	0.89 (0.65, 1.23)
Sufficient		ref	ref	ref
Nausea Only (vs. None)	N=1,323			
Deficient		0.62 (0.38, 1.01)	0.69 (0.40, 1.18)	0.58 (0.33, 1.02)
Insufficient		0.89 (0.65, 1.21)	0.89 (0.65, 1.22)	0.86 (0.62, 1.19)
Sufficient		ref	ref	ref
Vomiting once per day or more than once per day (vs. None)	N=402			
Deficient		1.08 (0.57, 2.06)	0.88 (0.44, 1.78)	0.64 (0.33, 1.26)
Insufficient		1.35 (0.95, 1.94)	1.21 (0.85, 1.71)	1.12 (0.80, 1.57)
Sufficient		ref	ref	ref

*OR-Odds Ratio

*Time Varying Vitamin D- 3-4 weeks accounts for preconception vitamin D, 5-6 weeks accounts for average between preconception and 8 week vitamin D, 7-8 weeks accounts for 8 weeks vitamin D.

[1]Unadjusted model and weighted to control for potential selection bias introduced by restricting to a sample of Live Birth.

[2]Adjusted for all sociodemographic covariates which include age, exercise, income, race, education, parity, employment, and season and weighted to control for potential selection bias introduced by restricting to a sample of Live Birth.

[3]Adjusted for all sociodemographic and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamins, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of Live Birth.

Supplemental Table S5.2. Distribution of vitamin D and Nausea/Vomiting via daily diaries for women who had a pregnancy loss versus did not have a pregnancy loss over gestational weeks 3-4, 5-6, 7-8 weeks gestation: EAGeR Trial

	Had a pregnancy loss			Did not have a pregnancy loss		
	3-4 Weeks N=140	5-6 Weeks N=140	7-8 Weeks N=140	3-4 Weeks N=581	5-6 Weeks N=581	7-8 Weeks N=581
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Nausea and Vomiting						
None	52 (37.1)	33 (23.6)	27 (19.3)	168 (29.0)	46 (7.9)	70 (12.0)
Nausea Only	81 (57.9)	74 (53.0)	39.5 (27.9)	375 (64.5)	428 (73.7)	346 (59.6)
Vomiting once per day or more than once per day	5 (3.6)	9 (6.4)	3 (2.1)	28 (4.8)	86 (14.8)	51 (8.8)
Missing	2 (1.4)	24 (17.1)	71 (50.7)	10 (1.7)	21 (3.6)	114 (19.6)

Supplemental Table S5.3. Assessment of unmeasured confounding in the associations between 25(OH)D and episodes of Nausea/Vomiting Restricted to Pregnancy: Longitudinal analysis

Categorical 25(OH)D on risk of Nausea/Vomiting Episodes 3-8 Weeks		
	Adjusted- M3[3] OR (95% CI)	E-Value Observed Odds Ratio
Any Nausea (vs. None)		
Deficient	0.65 (0.40, 1.06)	1.79
Insufficient	0.86 (0.65, 1.15)	1.37
Sufficient	ref	ref
Nausea Only (vs. None)		
Deficient	0.65 (0.40, 1.07)	1.79
Insufficient	0.84 (0.63, 1.12)	1.41
Sufficient	ref	ref
Vomiting once per day or more than once per day (vs. None)		
Deficient	0.54 (0.28, 1.04)	2.06
Insufficient	1.12 (0.80, 1.56)	1.31
Sufficient	ref	ref

[3]Adjusted for all sociodemographic and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamins, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of pregnancy.

Supplemental Table S5.4. Assessment of unmeasured confounding in the associations between preconception and 8 week 25(OH)D and risk of Nausea/Vomiting Restricted to Pregnancy: EAGeR Trial

Categorical 25(OH)D on risk of Nausea/Vomiting		
	Adjusted- M3[3] RR (95% CI)	E-Value Risk Ratio
Deficient/Insufficient to Sufficient	1.71 (1.12, 2.61)	2.81
Sufficient to Deficient/Insufficient	0.44 (0.22, 0.87)	3.97
No Change: Deficient/Insufficient	0.34 (0.20, 0.60)	5.33
No Change: Sufficient	ref	ref

[3]Adjusted for all sociodemographic and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamins, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of pregnancy.

Chapter 6: Aim 3- The Association Between Preconception and 8-week Gestation Maternal Serum 25(OH)D Levels and Risk of Preeclampsia

6.1. Introduction

The periconception and early pregnancy period marks a critical time for establishing a healthy pregnancy.[18] Successful implantation and placentation involve a complex process that relies on optimal endometrial receptivity and a host of hormonal and immunologic signaling events.[18] Disruptions to this process may be indicated by later adverse outcomes such as preeclampsia, which has been linked to disruptions in early placentation.[20,21] It is postulated that maternal nutrient stores, such as that of vitamin D, may play a critical role in this process.[18] Several in vitro studies using mouse and human cells have examined the role of vitamin D in implantation and placentation.[22–24] It is suggested that vitamin D may influence endometrial receptivity through the expression of homeobox gene HOXA10 in endometrial stroma cells, which are essential for endometrial development and uterine receptivity for implantation.[25] Additionally, vitamin D has been shown to exert immunosuppressive components in the early stages of pregnancy and suppress cytokines, which may lower inflammation and further support successful implantation.[26,27]

The complex processes of successful implantation and placentation include trophoblasts, which are cells that form the outer layer of a blastocyst, giving rise to a large portion of the placenta.[28] Placental trophoblasts support the production of growth factors and hormone secretion, cellular proliferation and modulation of maternal immune responses and vascularization of the placenta during pregnancy.[28] Studies using both mouse and human cells have found high levels of vitamin D receptors in trophoblastic cells in the placenta that are hypothesized to provide anti-inflammatory effects that can support a successful pregnancy.[29,30] This suggests that vitamin D may modulate inflammatory processes through vitamin D receptors that are located in key reproductive organs.[29,30] Accordingly, vitamin D hypovitaminosis may increase the maternal risk

of inflammatory pregnancy disorders, such as preeclampsia, which poses an increased health risk for both the mother and baby.[12,21,31–33] Binding of vitamin D to these receptors are hypothesized to modulate inflammatory processes and support a successful pregnancy.[29,30] However, a previous study has found that women with higher BMI have higher levels of inflammation which decrease the success of implantation and live birth rates in women undergoing in vitro fertilization.[250] This further postulates that higher inflammation in the months or weeks preceding conception or in early pregnancy around the time of implantation may influence the success of healthy implantation and placentation.[250]

Preeclampsia is a leading cause of maternal morbidity, mortality, and preterm birth.[33,135] Preeclampsia is a maternal hypertensive disorder and previous studies have shown significantly lower 25(OH)D levels in women who are diagnosed.[12,13,31,34,136] Early onset and severe preeclampsia have been shown to be associated with placental insufficiency, which is characterized by impaired placentation and decreased trophoblast invasion, which facilitates oxygenation of the placenta.[20,28] These implantation disruptions include human tissue that are expressed in trophoblasts, which are located in the outer thin layer of cells to help an embryo implant to the uterus successfully.[28] These cells then help form the placenta.[28] Since high levels of vitamin D receptors are found in trophoblastic cells within the placenta, vitamin D is hypothesized to provide anti-inflammatory effects in the uterus and placenta that may mitigate some of this disruption.[29]

Preeclampsia has been associated with increased inflammatory cytokines, which further promotes an inflammatory state.[21] These particular inflammatory cytokines, called CD4+ T cells, are seen in women with preeclampsia and are associated with placental ischemia, which is a vascular disorder that results in poor placental circulation.[21] The imbalance of pro-inflammatory

cytokine excretion further leads to placental inflammation and increases in the maternal risk of pregnancy complications.[21] Additionally, women who develop preeclampsia have impaired interactions between trophoblasts within the cells that become the placenta and endometrial lining which contributes to abnormal placentation.[3] Hypothesized mechanisms through inflammatory pathway via vitamin D receptors and trophoblast and endometrial cells have been acknowledged in previous studies that have shown a 5-fold increased risk of preeclampsia in women who had vitamin D levels <15ng/mL.[32] Previous studies have seen similar associations of low maternal vitamin D, defined as <20 ng/mL (<50 nmol/liter), and increased risk of preeclampsia.[9,14,32,137] Studies have largely examined vitamin D in pregnancy, rather than prior to pregnancy, which may influence vitamin D measurement due to biologic changes during pregnancy.[13,14,109,123]

Therefore, the objective of this study was to evaluate the associations between maternal preconception and 8-week gestation levels of serum 25(OH)D concentrations and risk of preeclampsia in healthy women with 1-2 prior pregnancy losses.

6.2. Methods

6.2.1. Data Source

The Effects of Aspirin in Gestation and Reproduction (EAGeR) trial is a multisite, prospective, double-blind, block-randomized, placebo-controlled clinical trial designed to evaluate the effect of low-dose aspirin (LDA) on live births in 1,228 healthy women with regular menstrual cycles and 1-2 prior pregnancy losses.[48,48] Women between 18 and 40 years of age were enrolled in the trial, of which 597 had a live birth. Women enrolled in the trial could not have received fertility treatments prior or during their enrollment in the EAGeR trial or have a prior diagnosis of infertility. The institutional review boards at each study site (Salt Lake City, Utah; Denver, Colorado; Buffalo, New York; Scranton, Pennsylvania) and the data coordinating center approved

the protocol for the trial. All participants provided their written consent prior to enrolling in the study. The trial was registered with ClinicalTrials.gov (#NCT00467363).

6.2.2. Analytic Sample

The analytic sample included women in the EAGeR trial for whom there is measured serum 25(OH)D levels at preconception or 8-week gestation, had a live birth, and were not missing data on the outcome, preeclampsia, or covariates assessed (n=552). Measurements of vitamin D were taken at baseline which could be from 1-6 months during the enrollment period. Women were then followed for 1-6 months and on average around 3 months to conceive. Restriction to a live birth allows parceling the effect of 25(OH)D on clinical outcomes independent of factors that may lead to a pregnancy loss, especially because preeclampsia is a condition that develops later in pregnancy (after 20 week of gestation). However, this restriction could result in a potential selection bias given that deficient (<20 ng/mL) vitamin D has been associated with pregnancy loss.[11] Therefore, I employ analytic weights to account for selection biases that could result from this restriction using methods described previously.[201,213] Pregnancy status was determined via positive urine Human Chorionic Gonadotropin (hCG) pregnancy tests (Quidel Quickvue, Quidel Corporation), conducted at home or in the clinic at the time of expected menses. The final analytic sample included women in the EAGeR trial for whom there were measured serum 25(OH)D levels at preconception (n=552) or 8-week gestation (n=530), had a live birth, and were not missing data on preeclampsia or covariates assessed (Figure 1). Restriction to a live birth will be used to examine the effect of 25(OH)D on clinical outcomes independent of those factors that may lead to a pregnancy loss, especially because preeclampsia is a condition that develops later in pregnancy (after 20 week of gestation). However, this restriction could result in a potential selection bias given that vitamin D has been associated with pregnancy loss.[11] Therefore, inverse probability

weights were used to account for selection biases that could result from this restriction using methods described below.[201,213] Pregnancy status was determined via positive urine hCG pregnancy tests (Quidel Quickvue, Quidel Corporation), conducted at home or in the clinic at the time of expected menses. Pregnancy status was determined via positive urine hCG pregnancy tests (Quidel Quickvue, Quidel Corporation), conducted at home or in the clinic at the time of expected menses.[48]

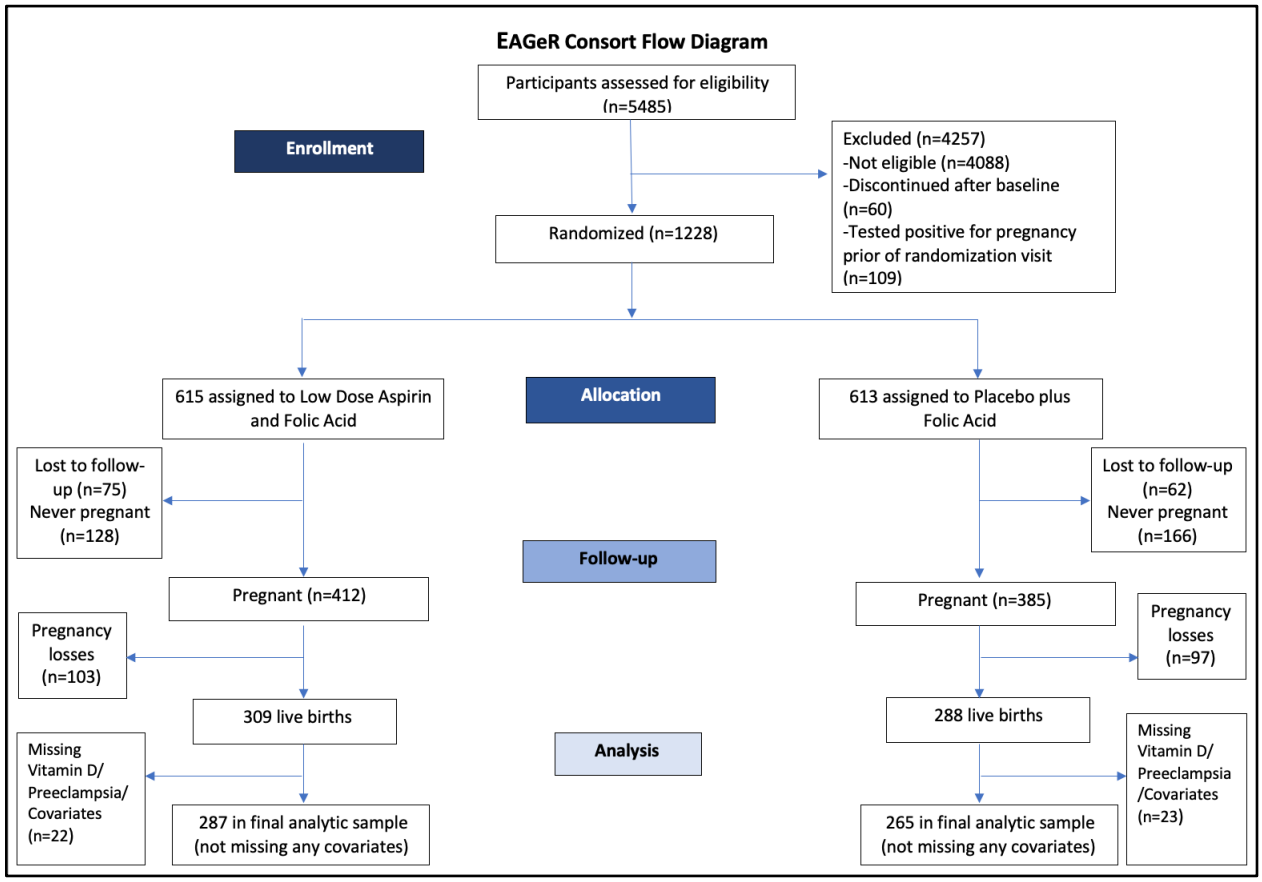

Figure 6.1. EAGeR trial Consolidated Standards of Reporting Trials (CONSORT) Flow Diagram for final analytic sample used for this paper. (n=552) (Adapted from Mumford et al.).[207]

6.2.3. Measures

6.2.3.1. Exposure Measure

Serum 25(OH)D samples were collected at baseline prior to randomization to LDA and at 8-week gestation post conception. The serum vitamin D samples were stored at −80°C until used for analysis.[48] Combined concentrations of 25-hydroxyvitamins D2 and D3 (25(OH)D) were measured using the 25(OH)D ELISA solid phase sandwich enzyme immunoassay (BioVendor R&D, Ashville, NC, USA). The ELISA solid phase sandwich enzyme immunoassay and has been validated.[195] Although it is suggested that liquid chromatography-tandem mass spectrometry to be the gold standard for vitamin D measurement, previous studies conducted have found vitamin D measurement results to be similar in immunoassays through the Vitamin D External Quality Assessment Scheme (DEQAS).[195–197] Therefore, the ELISA solid phase sandwich enzyme immunoassay is a precise and valid measurement for vitamin D concentrations.

The 25-hydroxyvitamin D (25(OH)D) cutoffs that are used in this analysis are based on levels designated by the Endocrine Society (<30 ng/mL equivalent to 75 nmol/L).[9,9] Women are classified as 25(OH)D deficient (≤20 ng/mL), insufficient (21-29 ng/mL), or sufficient (≥30 ng/mL) at preconception and 8-week gestation.[9,9] Initially, vitamin D was assessed using these Endocrine Society categories to aid in clinical interpretation and comparison with other studies conducted. However, because the Endocrine Society's cut offs were developed originally for bone health, and not based on reproductive health, continuous models for 25(OH)D were examined using linear spline terms to evaluate other cut points.[9,9]

To inform additional cut offs for 25(OH)D, exploratory analyses of lowess-smoothed regression models were used to examine the relationship between continuous 25(OH)D levels and preeclampsia. Models were restricted to 25(OH)D levels between 12 ng/mL (n=9 for

preconception and n=3 for 8-week gestation) and 55 ng/mL (n=25 for preconception and n=11 for 8-week gestation) to remove the effects of outliers on the smoothing function. Based on lowess curves (Figures 2 and 3), a knot between the 40 and 45 ng/mL was selected as the cutoff point. To determine which knot in that interval best fit the data, I fit a series of models with knots at 40 to 45 were run separately and estimated for each model using Akaike Information Criterion (AIC) and Bayes Information Criterion (BIC) statistics to determine which knot provided the best fit for the model (i.e., the model with the lowest AIC and BIC). This was determined to be 43 and a linear spline model with a knot at 43 was fit for both preconception and 8-week 25(OH)D levels.

6.2.3.2. Outcome Measure

Preeclampsia is categorized as a clinical diagnosis (yes/no) at any point after 12 weeks of gestation. This information was extracted from medical records filled out by medical providers during the EAGeR trial.

6.2.3.2.1. Laboratory Assessment

Blood samples to measure C-Reactive Protein (CRP) were obtained at the baseline preconception study visit and at 4, 8, 12, and 20, 36 weeks of gestation. Preconception samples were collected on day 2-3 of the menstrual cycle and prior to initiation of low dose aspirin or placebo. A validated measure was used for an immunoturbidimetric assay using a Roche COBAS 6000 autoanalyzer (Roche Diagnostics, Indianapolis, IN) to measure CRP.[202] CRP was categorized according to clinical recommendations that included low <1mg/L, borderline 1-3mg/L, moderately-high 3.01-10mg/L, and markedly high >10mg/L concentrations.[251,252]

6.2.3.3. Confounders

Sociodemographic and other health characteristics are available on all the women within the EAGeR dataset, including age, race/ethnicity, education, employment, income, BMI, parity,

season, physical activity, alcohol intensity, and multivitamin use. Season was defined as the season of baseline during which blood was drawn for the sample of serum 25(OH)D measured. Physical activity was assessed using the International Physical Activity Questionnaire and defined as low, moderate, or high.[200] Alcohol intensity was defined as the amount of alcohol consumed in the past year and was measured as never, sometimes, and often. Multivitamin use was measured as the type of vitamins that were consumed by the women prior to the study and were defined as not taking any vitamins or folic acid, taking vitamins with no folic acid, and taking vitamins with folic acid.

Additionally, aspirin/placebo, the assigned treatment in this trial, was considered as a confounder in this analysis, as previously applied in a study of 25(OH)D and pregnancy loss using this data as previous evidence has shown an increased risk of reducing inflammation.[11,202] In addition, inflammation has also been shown to increase risk of preeclampsia and studies have linked CRP, a biomarker of inflammation, to the prediction of preeclampsia.[138] Given vitamin D's immunomodulatory effects, it has been hypothesized that sufficient vitamin D may reduce inflammation and, consequently, risk for preeclampsia, but few studies have examined this pathway.[139,140] Therefore, we wanted to assess inflammation as measured by CRP in our models.

Sociodemographic and other health characteristics are available on all the women within the EAGeR dataset. I included age, race/ethnicity, education, employment, income, BMI, parity, season, physical activity, alcohol intensity, and multivitamin use in the models. Models were informed by Directed Cyclical Graph (DAG) developed for the relationship between the covariates (see chapter 1). Variables were selected based on their relationship to exposure to different sources of vitamin D, including sun exposure and diet or nutrition, and the outcome or preceding factors that might affect the outcome. Season was defined as the season of which blood was drawn for the sample of serum 25(OH)D measured. Physical activity was defined as the level of exercise daily

and was recorded as low, moderate, or high intensity based on the International Physical Activity Questionnaire.[200] Alcohol consumed in the past year and was categorized as never drinking alcohol in the past year, sometimes drinking alcohol in the past year, and often drinking alcohol in the past year. Multivitamin use was measured as the type of vitamins that were consumed prior to the study and were defined as not taking any vitamins, taking vitamins without folic acid, taking vitamins with folic acid. Additionally, aspirin/placebo, the assigned treatment in this trial, was considered as a confounder in this analysis, as previously applied in a study of 25(OH)D and pregnancy loss using this data as previous evidence has shown an increased risk of reducing inflammation.[11,202]

6.3. Analysis

6.3.1. Descriptive Analyses

The prevalence of preeclampsia across different baseline characteristics and vitamin D levels defined as deficient, insufficient, and sufficient vitamin D levels (preconception, 8-week gestation) is examined using chi-square tests or ANOVA for comparing categorical or continuous variables, respectively (Tables 1 and 2).

6.3.2. Log Binomial Regression Models

Risk ratios between preconception and 8-week gestation serum 25(OH)D levels and preeclampsia were estimated using log binomial regression models with robust standard errors with inverse probability weights to account for selection bias. In particular, I used log-binomial models because they provide risk ratios as opposed to logistic regressions which provide odds ratios.[214] The inverse probability weights used to account for selection biases that could result from this restriction using methods described previously include covariates associated with the probability of being pregnant such as age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamin D, vitamins, and BMI. [201,213]

Models evaluated preconception and 8-week 25(OH)D status (sufficient vs. insufficient vs. deficient) to support clinical interpretations of the results. 25(OH)D was further assessed through continuous models with additional cut points, including splines. Models were adjusted for relevant confounders as determined by DAGs (see Appendix I). Log binomial regression models for preconception and 8-week gestation 25(OH)D and preeclampsia were analyzed using four models to assess associations between preconception 25(OH)D and preeclampsia weighted to account for selection on live births. Our four models included: an unadjusted model (Model 1), a model adjusted for all sociodemographic covariates (Model 2: age, exercise, income, race, education, parity, employment, season), a model adjusted for all sociodemographic and lifestyle covariates except for BMI (Model 3: Model 2 + smoking, exercise, alcohol, treatment assignment, vitamin use), and a model adjusted for all sociodemographic and lifestyle covariates, including BMI (Model 4: Model 3 + BMI). BMI was shown to be a strong confounder in the association of 25(OH)D and preeclampsia, and therefore we examined this separately. Separate models were run Sociodemographic covariates included age, exercise, income, race, education, parity, employment, and season. Model 2 adjusted included sociodemographic plus lifestyle factors which included smoking, season, alcohol, aspirin, and vitamins. Adjusted model 3 included all previous covariates plus BMI. I ran separate models for preconception 25(OH)D and 8-week gestation 25(OH)D levels. Inverse probability weights were included to control for potential selection bias introduced by restricting to women who had a live birth. Weights were determined from models that include covariates associated with the probability of pregnancy, which included age, smoking, employment, vitamins, BMI, race, physical activity, parity, treatment assignment, and preconception 25(OH)D concentrations.

Potential interactions between vitamin D and treatment assignment (aspirin vs. placebo) were considered and no significant interactions were observed. Analyses were performed using STATA version 17.0.

6.4. Results

6.4.1. Descriptive Analyses

Descriptive analyses were assessed for the EAGeR analytic study sample comparing both the preconception and 8-week gestation maternal serum vitamin D with covariates within our analytic sample of livebirths. The median age women were 28 years old, with the highest BMI in the vitamin D deficient group (30.50 ± 8.63). Most women were of white race 280 (99%) and were vitamin D sufficient or insufficient (228 vs. 205). Over 90% of women who were vitamin D sufficient or insufficient were educated beyond high school, and around 80% of women who were vitamin D deficient were educated beyond high school. Over 30% of women in the vitamin D sufficient and insufficient categories had income equal or over $100,000 per year. Around 70% of women in all three vitamin D categories were employed. Over 75% of women in all three categories used vitamins that include folic acid in them. Over 60% of women in all three vitamin D categories never smoked. An increase in vitamin D deficiency was seen in the fall season 25 (38%). Between 59-71% of women had 1 prior pregnancy loss before they were enrolled in the EAGeR Trial. Between 63-75% of women never consume alcohol. The baseline CRP of women is seen in dose relationship as vitamin D decreases (vitamin D sufficient 2.26 ± 5.14 vs. vitamin D insufficient 2.75 ± 3.72 vs. vitamin D deficient 4.77 ± 7.90). About half (50%) of participants were in either the placebo or aspirin group and there were no significant differences between either group or vitamin D levels measured.

For the preconception maternal serum vitamin D group, BMI was highly associated with preconception maternal serum vitamin D (p-value <0.0001), as was race (p-value <0.0001), and income, (p-value 0.02), exercise (p-value 0.003), alcohol intensity (p-value 0.005), and baseline CRP (p-value 0.002).

Descriptive analyses were assessed for the EAGeR analytic study sample using the 8-week maternal serum vitamin D on covariates. The median age women were 28 years old, with the highest BMI in the vitamin D deficient group (30.50 ± 8.63). Most women were of white race 296 (98%) and were vitamin D sufficient or insufficient (301 vs. 207). Over 90% of women who were vitamin D sufficient or insufficient were educated beyond high school, and around 70% of women who were vitamin D deficient were educated beyond high school. Over 30% of women in the vitamin D sufficient and insufficient categories had income equal or over $100,000 per year, while 27% vitamin D deficient category had income equal or over $100,000 per year. Around 70% of women in the sufficient and insufficient vitamin D categories were employed, and 45% of women in the deficient vitamin D category were employed.

Over 75% of women in all three categories used vitamins that include folic acid in them. Over 80% of women in all three vitamin D categories never smoked. An increase in vitamin D deficiency was seen in the fall season (59%). Around 60% of women had 1 prior pregnancy loss before they were enrolled in the EAGeR Trial. Over 60% of women in all vitamin D categories had never consumed alcohol. The baseline CRP of women is seen in dose relationship as vitamin D decreases (vitamin D sufficient 2.26 ± 5.14 vs. vitamin D insufficient 2.75 ± 3.72 vs. vitamin D deficient 4.77 ± 7.90). For the 8-week gestation maternal serum vitamin D group, BMI was highly associated with maternal serum 8-week vitamin D (p-value <0.001), race (p-value <0.001),

education (p-value 0.009), income (p-value 0.002), employment (p-value 0.01), season (0.001), baseline CRP (p-value 0.02).

While there were no significant associations seen between preconception 25(OH)D and 8-week 25(OH)D serum levels and the prevalence of preeclampsia, the trends were striking and majority of women who had prevalence of preeclampsia were in the vitamin D deficiency category. Overall, within the prevalence of preeclampsia in preconception 25(OH)D maternal serum levels, majority of women had vitamin D deficiency <20 ng/mL (16.92%), followed by vitamin D sufficiency (9.96%), and vitamin D insufficiency (8.29%). For 8-week gestation 25(OH)D maternal serum levels, and prevalence of preeclampsia, majority of women were vitamin D deficient (13.64%), then insufficient (12.08%), and sufficient (8.97%). There was an increased risk of preeclampsia with increasing BMI: underweight 5.6%, normal weight 4.9%, overweight 12.9%, and obese 22.5%. Women who engaged in moderate exercise had an increased prevalence of preeclampsia 12.4% vs. low exercise 4.1%. There was a dose-relationship seen between CRP and preeclampsia in both the baseline CRP and 8-week CRP groups. At baseline 4.7% of women with preeclampsia had low levels vs. 14.1% borderline vs. 15.2% moderately high vs. 23.8% high concentrations. At 8-week CRP concentrations were 3.8% of women had low levels vs. 8.6% borderline vs. 11.8% moderately high vs. 22.2% high.

6.4.2. Log-Binomial Regression Results

Using log-binomial regression models, I examined the association of preconception and 8-week gestation maternal serum levels of 25(OH)D on risk of preeclampsia (Table 2). In particular, I used log-binomial models because they provide risk ratios as opposed to logistic regressions which provide odds ratios. After adjustment for both sociodemographic and lifestyle (excluding BMI) for Model 3, the precision of the estimate was reduced, Deficient (RR: 2.32, 95% CI: 1.09, 4.95).

However, after inclusion of BMI (Model 4), the risk ratios for insufficient preconception maternal serum 25(OH)D levels on risk of preeclampsia (RR: 0.80, 95% CI: 0.44, 1.47) and for deficient (RR: 1.45, 95% CI: 0.64, 3.29) found similar patterns but were attenuated and no longer significant.

For 8-week gestation, there is a suggestion of similar increased risk to preconception models, however, the confidence intervals were too wide and there is a large attenuation of effect when adjusting for BMI. After adjustment for both sociodemographic and lifestyle (excluding BMI) for Model 3, insufficient 25(OH)D showed a slight association (RR: 1.37, 95% CI: 0.84, 2.23) and for deficient (RR: 2.70, 95% CI: 0.73, 10.02). However, for adjusted Model 4, the risk ratios for insufficient 8-week gestation maternal serum 25(OH)D levels on risk of preeclampsia (RR: 1.11, 95% CI: 0.66, 1.86) and for deficient (RR: 1.42, 95% CI: 0.38, 5.32) were also attenuated.

6.4.3. Linear Spline Results

For the linear spline models, 43 was selected as the knot with the best fit based on AIC and BIC statistics (i.e., the model with the lowest AIC and BIC). In Table 3, I modeled 25(OH)D at preconception versus 8-week gestation was modeled with a linear spline model at the 43 ng/mL measurement. Before the 43 ng/mL 25(OH)D serum level, the risk of 25(OH)D on preeclampsia in the preconception Model 4 is (RR: 0.97; 95% CI: 0.93, 1.00]. At 43 ng/mL per 1ng/mL unit increase of 25(OH)D, there is a slight change in risk of preeclampsia in the preconception Model 4 (RR: 1.03; 95% CI: 1.00, 1.06). These results are still significant after adjustment. For 8-week gestation, before the 43 ng/mL 25(OH)D serum level, the risk of 25(OH)D on preeclampsia in the 8-week gestation Model 4 is (RR: 0.97; 95% CI: 0.93, 1.02). At 43 ng/mL per 1ng/mL unit increase of 25(OH)D, there is a slight change in risk of preeclampsia in the 8-week gestation Model 4 (RR:

1.02; 95% CI: 0.93, 1.12). In addition, the prevalence of preeclampsia in both preconception and 8-week gestation were 9.3% and 5.2% respectively.

6.5. Discussion

Among a sample of healthy women with 1-2 prior pregnancy losses, the unadjusted analyses accounting for sociodemographic and some lifestyle factors, both maternal serum 25(OH)D levels at preconception, and potentially 8-week gestation, based on Endocrine Society cut points were associated with the risk of preeclampsia have shown an increased risk of preeclampsia, though using Endocrine Society's standard cut-offs based on the categorical models used in this analysis. However, once BMI was included in the adjusted models, the relationship was no longer significant. Although the association was attenuated after adjustment for BMI. However, the between maternal serum 25(OH)D levels and risk of preeclampsia is not significant, the magnitude of the association is suggestive of an increased risk of preeclampsia, within the deficient preconception 25(OH)D group, respectively. The linear spline models suggest that reductions in preeclampsia associated with preconception maternal serum 25(OH)D. For each 1 unit increase in 25(OH)D up to 43 ng/mL, women had a reduced risk of preeclampsia. This association was significant even after accounting for BMI. These results highlight the importance of the preconception window on the risk of preeclampsia and the significant role of BMI (Table 3). Future research is needed to assess The Endocrine Society's cut offs for sufficient vitamin D levels ($\geq$ 30 ng/mL) on reproductive health outcomes.

This study is consistent with previous research that has found an association between maternal vitamin D and risk of preeclampsia, although our study addresses a critical gap in timing of vitamin D serum measurement.[12,13,34,136] Previous epidemiologic studies have shown maternal serum vitamin D levels <15ng/mL had a 5-fold increased risk of preeclampsia.[12] In addition,

previous studies have found that supplementation during later pregnancy was ineffective, and one reasoning could be that beginning vitamin D supplementation during pregnancy was too delayed to reduce the risk of preeclampsia later during pregnancy.[39,42] Although, one study found that early vitamin D supplementation within the first trimester of pregnancy aids in reducing the reoccurrence of preeclampsia in previous preeclamptic women.[31] In addition, previous studies have assessed the preconception period of vitamin D with pregnancy loss and pregnancy success, though not with other adverse pregnancy outcomes.[11,38] Specifically, a previous study using the EAGeR data by Mumford et al. found an increased risk of pregnancy loss with lower preconception vitamin D versus at 8-week gestation.[11] Finally, a recent study assessed preconception vitamin D levels on the success of in vitro fertilization (IVF) and found that women who had vitamin D levels above ≥ 20 ng/mL had a significantly higher likelihood of pregnancy success than women who were ≤20 ng/mL.[38] Our work importantly extends these findings highlighting the importance of the preconception period for later adverse pregnancy outcomes, such as preeclampsia, as well.

Previously, the Endocrine Society's clinical guidelines for vitamin D cutoffs have been based on supporting bone health and fall prevention, and currently no clinical recommendations for optimal levels for reproductive health is provided. Our study suggests that for each 1 unit increase in maternal serum 25(OH)D levels there is a reduction on the risk of preeclampsia up until the 43 ng/mL measurement. There was also some suggestion that risk may not be decreased after this point; however, this should be investigated in further studies as higher serum 25(OH)D values were less common in this cohort and the data cannot discern how precise this may be. This study is consistent with previous research that has found an association between maternal vitamin D and risk of preeclampsia, although our study addresses a critical gap in timing of vitamin D serum measurement.[12,13,34,136] A previous epidemiologic study has shown maternal serum vitamin D

levels <15ng/mL had a 5-fold increased risk of preeclampsia.[12] However, one study found that supplementation during the third trimester pregnancy was not significant in reducing risk of preeclampsia.[253] One rationale for a lack of an effect could be that vitamin D supplementation was initiated later in pregnancy, after the period in which implantation and placentation may have already occurred. [253] Prior studies assessing the role of vitamin D in the preconception period on risk of pregnancy outcomes. In particular, a study by Mumford et al. using EAGeR data found an increased risk of pregnancy loss with lower preconception vitamin D and higher pregnancy success with increased vitamin D.[11] A recent study assessed preconception vitamin D levels on the success of in vitro fertilization (IVF) and found that women who had vitamin D levels above ≥ 20 ng/mL had a significantly higher likelihood of pregnancy success than women who were ≤20 ng/mL.[38] Thus, findings from this study and other studies on pregnancy outcomes suggest that preconception and early pregnancy vitamin D levels may be important to promote healthy and successful implantation and placentation and reduce the risk of adverse outcomes, such as preeclampsia. The Endocrine Society's cutoffs for vitamin D are used to inform and support bone health, currently no clinical recommendations for optimal levels for reproductive health are available. My study suggests that while 25(OH)D level cut offs of deficiency (≤ 20ng/mL), insufficiency (21-29 ng/mL), and sufficiency (≥ 30 ng/mL) are within the optimal levels to reduce the risk of preeclampsia up to the 43 ng/mL mark. There was also some suggestion that risk may increase after the 43 ng/mL 25(OH)D level. However, this possibility should be further studied as higher serum 25(OH)D values >43 ng/mL were less prevalent in this cohort (Preconception: 9.3% and 8-week: 5.2%) and the data cannot discern how precise this may be.

The findings with BMI being highly correlated with preeclampsia and attenuating our results is relatively consistent with previous literature.[254–256] Previous research has found that

maternal obesity increases a mother's risk by three-four times of developing preeclampsia compared to maternal normal weight. [255,257,258] These findings suggest that potential inflammatory pathways introduced by higher obesity levels may attenuate the anti-inflammatory effects of vitamin D on the risk of preeclampsia.[259–261] As previous findings, including in our study, we found that as a woman's BMI increases her risk of preeclampsia increases as well.[257,258,262]

Previous literature highlights the importance of assessing the critical period prior to implantation and the role of vitamin D in supporting this process. This is consistent with our findings regarding preconception and early gestation maternal serum vitamin D levels and in particular critical timings of vitamin D exposure on the potential of reducing the risk of adverse pregnancy outcomes, such as preeclampsia. This study further suggests that additional studies are needed to identify optimal levels of circulating 25(OH)D for reproductive and perinatal health. Previous literature highlights the role that vitamin D may play in supporting reduction in inflammation, specifically in the uterus.[26,27] Our findings suggest that there may be a critical period prior to conception in which vitamin D levels may affect this process and, ultimately, affect the development of pregnancy complications related to impaired placentation.[18,25,29,34,190] However, further research is needed to identify specific mechanisms and optimal levels of circulating 25(OH)D for reproductive and perinatal health.

6.5.1. Strengths and Limitations

This sample is not representative of the United States population. However, this research may provide the first step in assessing preconception 25(OH)D on preeclampsia and may serve as preliminary data for future studies. The main challenge with preconception and early pregnancy data is the potential for pregnancy loss. Specifically, when assessing the preconception versus 8-week gestation time points, some women experience pregnancy loss prior to the 8-week gestation

measurement. I have applied inverse probability weighting to account for potential selection bias factors related to surviving to pregnancy loss or not becoming pregnant that may have occurred by restricting to a live birth.

These approaches have been successfully applied in prior work to account for potential selection bias introduced when evaluating preconception factors and post conception outcomes - a scenario that is very common in reproductive and perinatal epidemiology.[263,264] However, it may not account for all potential selection factors. In addition, our small sample size limits precision and our ability to assess interactions within our models. Specifically, BMI was significantly associated with both vitamin D and preeclampsia, and strongly attenuated our models. Small samples of deficient vitamin D and obesity precluded examination of the interaction between these variables on the risk of preeclampsia. In addition, this is a secondary analysis of a trial which was not designed to examine vitamin D and pregnancy outcomes. Finally, an addition limitation is unmeasured confounding, which would have to be at a certain point estimate with a lower confidence interval value to explain the association as explained in Table S6.1 (Supplemental Tables).[265]

There are strengths of the data to highlight. Preconception serum 25(OH)D assessment on the risk of preeclampsia has not been evaluated previously in the literature. This may be a critical time point in which an intervention may be most likely to have an effect and elucidate important mechanisms that lead to preeclampsia. In addition, the use of the EAGeR data is novel in that it allows for the examination of this association using prospective data with a well-defined, closely monitored cohort and exposures that temporally proceed the outcomes of interest. This allows me the ability to compare both the preconception and early pregnancy serum vitamin D levels to assess these longitudinal relationships. Finally, this data is uniquely positioned to address gaps in the

literature by focusing on vitamin D during the early critical windows of development, including preconception and early pregnancy.

6.5.2. Conclusion

This study shows that there is an increased risk of preeclampsia in women with deficient serum 25(OH)D at preconception, although the risk is attenuated after adjusting for BMI. In the linear spline model, there is suggestion of an effect between deficient preconception and early pregnancy 25(OH)D levels on the risk of preeclampsia, even after adjustment for BMI. In addition, this study suggests that risk of preeclampsia continues to decline at 25(OH)D levels beyond insufficient status (>30 up to 40-45 ng/mL). However, further studies with larger sample sizes are needed to assess the variation in optimal vitamin D levels for pregnancy-related outcomes that may be different from bone health and examine potential interactions between BMI and vitamin D. This information can be used to further understand the relationship between critical preconception and implantation period and their effect on risk of adverse pregnancy outcomes, such as preeclampsia, particularly in more diverse populations. In addition, future studies may use this information to potentially modify the Endocrine Society's Guidelines or obstetric practices based on when vitamin D supplementation may be started during the reproductive period as well as how much IU's per day are taken.

Tables

Table 6.1. Descriptive Analyses of preconception maternal serum levels of vitamin D in the EAGeR Trial Restricted to Live Births (n=552)

EAGeR- Preconception Vitamin D Descriptive Analyses				
	Vitamin D Sufficient (≥ 30 ng/mL)	**Vitamin D Insufficient (<30 ng/mL & ≥20 ng/mL)**	**Vitamin D Deficient (<20 ng/mL)**	**P-value**
N	282	205	65	
Age, years				
Mean ± SD	28.73 ± 4.5	28.67 ± 4.6	28.50 ± 5.3	0.91
18-24.9	71 (25.2)	44 (21.5)	15 (23.1)	0.56
25-29.9	111 (39.4)	93 (45.4)	32 (49.2)	
30-34.9	78 (24.1)	52 (25.4)	13 (20.0)	
35-40.9	32 (11.4)	16 (7.8)	5 (7.7)	
***BMI, kg/m^2**				
Mean ± SD	24.47 ± 5.1	26.85 ± 6.3	30.50 ± 8.6	0.031
Underweight <18.5	9 (3.2)	7 (3.4)	2 (3.1)	<0.0001
Normal ≥18.5 & <25	185 (65.6)	100 (48.8)	19 (29.2)	
Overweight ≥25 & <30	59 (20.9)	59 (28.8)	14 (21.5)	
Obese ≥30	29 (10.3)	39 (19.0)	30 (46.2)	
***Race**				
White	280 (99.3)	200 (97.6)	55 (84.6)	<0.0001
Non-White	2 (0.7)	5 (2.4)	10 (15.4)	
Education				
≤ High School, n (%)	25 (8.9)	13 (6.3)	13 (20.0)	0.004
> High School, n (%)	257 (91.1)	192 (93.7)	52 (80.0)	
Income, n				
≥ $100,000	103 (36.5)	98 (47.8)	22 (33.9)	0.02
$75,000-$99,999	47 (16.7)	28 (13.7)	4 (6.2)	
$40,000-$74,999	47 (16.7)	23 (11.2)	10 (15.4)	
$20,000-$39,999	63 (22.3)	47 (22.9)	22 (33.9)	
≤ $19,999	22 (7.8)	9 (4.4)	7 (10.8)	
Employment				0.29
Yes	214 (75.9)	144 (70.2)	45 (69.2)	
No	68 (24.1)	61 (29.8)	20 (30.8)	
Multivitamin Use				0.28

No Folic Acid-No Vitamins	20 (7.1)	9 (4.4)	5 (7.7)	
No Folic Acid- Yes Vitamins	45 (15.9)	27 (13.2)	5 (7.7)	
Yes Folic Acid- Yes Vitamins	217 (76.9)	169 (82.4)	55 (84.6)	
Smoking				0.83
Never	256 (90.8)	186 (90.7)	57 (87.7)	
<6 per day	17 (6.0)	11 (5.4)	6 (9.2)	
Daily	9 (3.2)	8 (3.9)	2 (3.1)	
Season				0.17
Fall (Sep-Nov)	80 (28.4)	50 (24.4)	25 (38.5)	
Winter (Dec-Feb)	55 (19.5)	46 (22.4)	16 (24.6)	
Spring (Mar-May)	73 (25.9)	58 (28.3)	16 (24.6)	
Summer (Jun-Aug)	74 (26.2)	51 (24.9)	8 (12.3)	
***Exercise Level**				0.003
Low	62 (21.9)	56 (27.3)	29 (44.6)	
Moderate	136 (45.4)	79 (37.1)	24 (33.9)	
High	96 (32.6)	76 (35.6)	14 (21.5)	
Number of previous pregnancy losses, n				0.17
0				
1	167 (59.2)	132 (64.4)	46 (70.8)	
2	115 (40.8)	73 (35.6)	19 (29.2)	
Alcohol Intensity				
Never	177 (62.8)	151 (73.7)	49 (75.4)	0.005
Sometimes	98 (34.8)	44 (21.5)	16 (24.6)	
Often	7 (2.5)	10 (4.9)	0 (0.0)	
Baseline CRP				
Mean ± SD	2.26 ± 5.1	2.75 ± 3.7	4.77 ± 7.9	0.031
Low <1	156 (55.3)	99 (48.3)	20 (31.3)	0.002
Borderline ≥1 & <3	80 (28.4)	56 (27.3)	20 (31.3)	
Moderately High ≥3 & <10	41 (14.5)	38 (18.5)	20 (31.3)	
High Concentrations ≥10	5 (1.8)	12 (5.9)	4 (6.3)	
Aspirin Use				0.74
Placebo	131 (46.5)	101 (49.3)	33 (50.8)	
Low Dose Aspirin	151 (54.6)	104 (50.7)	32 (49.2)	

*Non-white participants include American Indian/Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, Black or African American, more than one Race, Unknown or Not Reported

*BMI- Body Mass Index, CRP- C-Reactive Protein *Exercise level is defined as low, moderate, and high

Table 6.2. Descriptive Analyses of 8-week maternal serum levels of vitamin D in the EAGeR Trial Restricted to Live Births (N=530)

EAGeR- 8-week Vitamin D Descriptive Analyses				
	Vitamin D Sufficient (≥ 30 ng/mL)	**Vitamin D Insufficient (<30 ng/mL & ≥20 ng/mL)**	**Vitamin D Deficient (<20 ng/mL)**	**P-value**
N	301	207	22	
Age, years				
Mean ± SD	28.7 ± 4.6	28.4 ± 4.4	28.7 ± 5.0	0.297
18-24.9	68 (22.6)	51 (24.6)	4 (18.2)	0.19
25-29.9	118 (39.2)	196 (46.4)	13 (59.1)	
30-34.9	81 (26.9)	43 (20.8)	5 (22.7)	
35-40.9	34 (11.3)	17 (8.2)	0 (0.0)	
***BMI, kg/m^2**				
Mean ± SD	24.2 ± 4.7	26.3 ± 6.2	30.5 ± 8.9	<0.001
Underweight <18.5	8 (2.7)	7 (3.4)	0 (0.0)	<0.001
Normal ≥18.5 & <25	188 (62.5)	98 (47.3)	8 (36.4)	
Overweight ≥25 & <30	73 (24.3)	51 (24.6)	5 (22.7)	
Obese ≥30	32 (10.6)	51 (24.6)	9 (40.9)	
***Race**				
White	296 (98.3)	200 (96.6)	18 (81.8)	<0.001
Non-White	5 (1.7)	7 (3.4)	4 (18.2)	
Education				
≤ High School, n (%)	28 (9.3)	15 (7.3)	6 (27.3)	0.009
> High School, n (%)	273 (90.7)	192 (92.8)	16 (72.7)	
Income, n				
≥ $100,000	123 (40.9)	85 (41.1)	6 (22.7)	0.002
$75,000-$99,999	53 (17.6)	25 (12.1)	0 (0.0)	
$40,000-$74,999	46 (15.3)	31 (14.9)	2 (9.1)	
$20,000-$39,999	60 (19.9)	57 (27.5)	9 (40.9)	
≤ $19,999	19 (6.3)	9 (4.4)	5 (22.7)	
Employment				
Yes	224 (74.4)	155 (74.9)	10 (45.5)	0.01
No	77 (25.6)	52 (25.1)	12 (54.6)	
Multivitamin Use				
No Folic Acid-No Vitamins	17 (5.7)	15 (7.3)	1 (4.5)	0.05

No Folic Acid- Yes Vitamins	53 (17.6)	19 (9.2)	1 (4.5)	
Yes Folic Acid- Yes Vitamins	231 (76.7)	173 (83.6)	20 (90.9)	
Smoking				
Never	271 (90.0)	190 (91.8)	19 (86.4)	0.38
<6 per day	20 (6.6)	9 (4.4)	3 (13.6)	
Daily	10 (3.3)	8 (3.9)	0 (0.0)	
Season				
Fall (Sep-Nov)	70 (23.3)	66 (31.9)	13 (59.1)	0.001
Winter (Dec-Feb)	58 (19.3)	52 (25.1)	1 (4.6)	
Spring (Mar-May)	90 (29.9)	49 (22.7)	4 (18.2)	
Summer (Jun-Aug)	83 (27.6)	40 (19.3)	4 (18.2)	
***Exercise Level**				
Low	71 (23.6)	57 (27.5)	15 (68.2)	0.98
Moderate	129 (42.9)	89 (43.0)	2 (9.1)	
High	101 (33.6)	61 (29.5)	5 (22.7)	
Number of previous pregnancy losses, n				
0				0.40
1	187 (62.1)	130 (62.8)	14 (63.6)	
2	114 (37.9)	77 (37.2)	8 (36.4)	
Alcohol Intensity				
Never	198 (65.8)	145 (70.1)	15 (68.2)	0.72
Sometimes	92 (30.6)	57 (27.5)	7 (31.8)	
Often	11 (3.7)	5 (2.4)	0 (0.0)	
Baseline CRP				
Mean ± SD	2.24 ± 4.7	2.37 ± 2.9	3.89 ± 4.5	0.280
Low <1	167 (55.5)	91 (44.2)	7 (31.8)	0.004
Borderline ≥1 & <3	79 (26.3)	67 (32.5)	4 (18.2)	
Moderately High ≥3 & <10	46 (15.3)	38 (18.5)	10 (45.5)	
High Concentrations ≥10	9 (2.9)	10 (4.9)	1 (4.6)	
Aspirin Use				
Placebo	127 (42.2)	113 (54.6)	11 (50.0)	0.02
Low Dose Aspirin	174 (57.8)	94 (45.4)	11 (50.0)	

*Non-white participants include American Indian/Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, Black or African American, more than one Race, Unknown or Not Reported

*BMI- Body Mass Index, CRP- C-Reactive Protein

*Exercise level is defined as low, moderate, and high

Table 6.3. Prevalence of Preeclampsia by 25(OH)D status at preconception and 8-week gestation and sociodemographic and health characteristics among participants that achieved a live birth in the EAGeR trial (n=552)

EAGeR Covariates and Prevalence of Preeclampsia			
Covariates	**N=55**	**Prevalence of Preeclampsia (%)**	**P-value**
Preconception 25(OH)D			0.14
Sufficient ≥30 ng/mL	282	27 (9.57)	
Insufficient ≥20 & <30 ng/mL	205	17 (8.29)	
Deficient <20 ng/mL	65	11 (16.92)	
8-week 25(OH)D			0.42
Sufficient ≥30 ng/mL	301	27 (8.9)	
Insufficient ≥20 & <30 ng/mL	207	25 (12.1)	
Deficient <20 ng/mL	22	3 (13.6)	
Demographics			
Age, years			0.20
18-24.9	130	14 (10.8)	
25-29.9	236	17 (7.2)	
30-34.9	133	16 (12.0)	
35-40.9	53	8 (15.1)	
***BMI, kg/m^2**			<0.01
Underweight <18.5	18	1 (5.6)	
Normal ≥18.5 & <25	304	15 (4.9)	
Overweight ≥25 & <30	132	17 (12.9)	
Obese ≥30	98	22 (22.5)	
***Race**			0.68
White	535	53 (9.9)	
Non-White	17	2 (11.8)	
Education			0.81
≤ High School	51	4 (7.8)	
> High School	501	51 (10.2)	
Annual Household Income			0.29
≥ $100,000	223	29 (13.0)	

$75,000-$99,999	79	5 (6.3)	
$40,000-$74,999	80	9 (11.3)	
$20,000-$39,999	132	9 (6.8)	
≤ $19,999	38	3 (7.9)	
Employment			0.15
Yes	403	45 (11.2)	
No	149	10 (6.7)	
Multivitamin Use			0.93
No Folic Acid-No Vitamins	34	4 (11.8)	
No Folic Acid- Yes Vitamins	77	7 (9.1)	
Yes Folic Acid- Yes Vitamins	441	44 (9.9)	
Smoking in past year			0.22
Never	499	50 (10.0)	
<6 per day	34	5 (14.7)	
Daily	19	0 (0.0)	
Season of blood draw			0.68
Fall (Sep-Nov)	155	19 (12.3)	
Winter (Dec-Feb)	117	12 (10.3)	
Spring (Mar-May)	147	13 (8.8)	
Summer (Jun-Aug)	133	11 (8.3)	
***Exercise Level**			0.01
Low	147	6 (4.1)	
Moderate	226	28 (12.4)	
High	179	21 (11.7)	
Alcohol consumption in the past year			0.85
Never	377	36 (9.5)	
Sometimes	158	18 (11.4)	
Often	17	1 (5.9)	
Baseline CRP			<0.01
Low <1	275	13 (4.7)	
Borderline ≥1 & <3	156	22 (14.1)	
Moderately High ≥3 & <10	99	15 (15.2)	

High Concentrations ≥10	21	5 (23.8)	
8-Week CRP			<0.01
Low <1	106	4 (3.8)	
Borderline ≥1 & <3	175	15 (8.6)	
Moderately High ≥3 & <10	186	22 (11.8)	
High Concentrations ≥10	14	14 (22.2)	
Treatment Assignment			1.00
Placebo	265	26 (9.8)	
Low Dose Aspirin	287	29 (10.1)	

*Non-white participants include American Indian/Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, Black or African American, more than one Race, Unknown or Not Reported
*P-values based on Fisher's Exact Test
*22 women were missing for 8-week 25(OH)D measurement
*BMI- Body Mass Index, CRP- C-Reactive Protein
*Exercise level is defined as low, moderate, and high.

Table 6.4. Association between preconception and 8-week categorical 25(OH)D and Risk Ratio (RR) of preeclampsia: EAGeR Trial

EAGeR Binomial Regression Models for Preconception and 8-week Gestation 25(OH)D (categorical) and Preeclampsia					
	Preeclampsia N (%)	Unadjusted– M1[1] RR (95% CI)	Adjusted – M2[2] RR (95% CI)	Adjusted – M3[3] RR (95% CI)	Adjusted – M4[4] RR (95% CI)
Categorical 25(OH)D (Based on Endocrine Society's Guidelines)					
Preconception 25(OH)D N=552					
Deficient	11 (20)	1.85 (0.99, 3.45)	2.59 (1.24, 5.39)	2.32 (1.09, 4.95)	1.45 (0.64, 3.29)
Insufficient	17 (31)	0.90 (0.51, 1.58)	0.99 (0.55, 1.76)	0.96 (0.54, 1.72)	0.80 (0.44, 1.47)
Sufficient	27 (49)	Ref	Ref	Ref	Ref
8-Week 25(OH)D N=530					
Deficient	3 (13)	1.49 (0.49, 4.54)	2.73 (0.73, 10.20)	2.70 (0.73, 10.02)	1.42 (0.38, 5.32)
Insufficient	25 (46)	1.34 (0.81, 2.23)	1.41 (0.86, 2.32)	1.37 (0.84, 2.23)	1.11 (0.66, 1.86)
Sufficient	27 (49)	Ref	Ref	Ref	Ref

*RR- Risk Ratio
[1]Unadjusted
[2]Adjusted for all sociodemographic covariates which include age, exercise, income, race, education, parity, employment, and season and weighted to control for potential selection bias introduced by restricting to a sample of live births.
[3]Adjusted for all sociodemographic covariates and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, and vitamins except for BMI and weighted to control for potential selection bias introduced by restricting to a sample of live births.
[4]Adjusted for all sociodemographic and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamins, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of live births.

Table 6.5. Association between preconception and 8-week using continuous linear splines of 25(OH)D and Risk Ratio (RR) of preeclampsia: EAGeR Trial

EAGeR Binomial Regression Models for Preconception and 8-week Gestation 25(OH)D (continuous) and Preeclampsia					
	Preeclampsia N	Unadjusted– M1[1] RR (95% CI)	Adjusted – M2[2] RR (95% CI)	Adjusted – M3[3] RR (95% CI)	Adjusted – M4[4] RR (95% CI)
Continuous 25(OH)D with Splines (per 1 ng/mL)					
Preconception 25(OH)D N=552					
<43 ng/mL	48	0.97 (0.94, 1.00)	0.95 (0.92, 0.99)	0.95 (0.92, 0.99)	0.97 (0.93, 1.00)
≥43 ng/mL	7	1.02 (1.00, 1.05)	1.03 (1.00, 1.07)	1.03 (1.00, 1.07)	1.03 (1.00, 1.06)
8-Week 25(OH)D N=530					
<43 ng/mL	51	0.97 (0.94, 1.01)	0.96 (0.92, 1.00)	0.96 (0.92, 1.00)	0.97 (0.93, 1.02)
≥43 ng/mL	4	1.02 (0.93, 1.12)	1.02 (0.92, 1.13)	1.03 (0.93, 1.13)	1.02 (0.93, 1.12)

[*]RR- Risk Ratio
[1]Unadjusted
[2]Adjusted for all sociodemographic covariates which include age, exercise, income, race, education, parity, employment, and season and weighted to control for potential selection bias introduced by restricting to a sample of live births.
[3]Adjusted for all sociodemographic covariates and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, and vitamins except for BMI and weighted to control for potential selection bias introduced by restricting to a sample of live births.
[4]Adjusted for all sociodemographic and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamins, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of live births.

Supplemental Tables

Supplemental Tables 6.1. Assessment of unmeasured confounding in the associations between preconception and 8-week 25(OH)D and risk of preeclampsia

Categorical 25(OH)D on risk of Preeclampsia		
	Adjusted- M3[3] RR (95% CI)	E-Value Observed Risk Ratio
Preconception 25(OH)D		
Deficient	1.45 (0.64, 3.29)	2.26
Insufficient	0.80 (0.44, 1.47)	1.81
Sufficient	Ref	Ref
8-Week 25(OH)D		
Deficient	1.42 (0.38, 5.32)	2.19
Insufficient	1.11 (0.66, 1.86)	1.46
Sufficient	Ref	Ref
Continuous 25(OH)D on risk of Preeclampsia		
	Adjusted- M3[3] RR (95% CI)	E-Value Observed Risk Ratio
Preconception 25(OH)D		
<43 ng/mL	0.97 (0.94, 1.00)	1.32
≥43 ng/mL	1.02 (1.00, 1.05)	1.25
8-Week 25(OH)D		
<43 ng/mL	0.97 (0.94, 1.01)	1.32
≥43 ng/mL	1.02 (0.93, 1.12)	1.25

[3]Adjusted for all sociodemographic and lifestyle covariates which included age, smoking, season, exercise, income, race, education, alcohol, parity, aspirin, employment, vitamins, and BMI and weighted to control for potential selection bias introduced by restricting to a sample of live births.

Figures

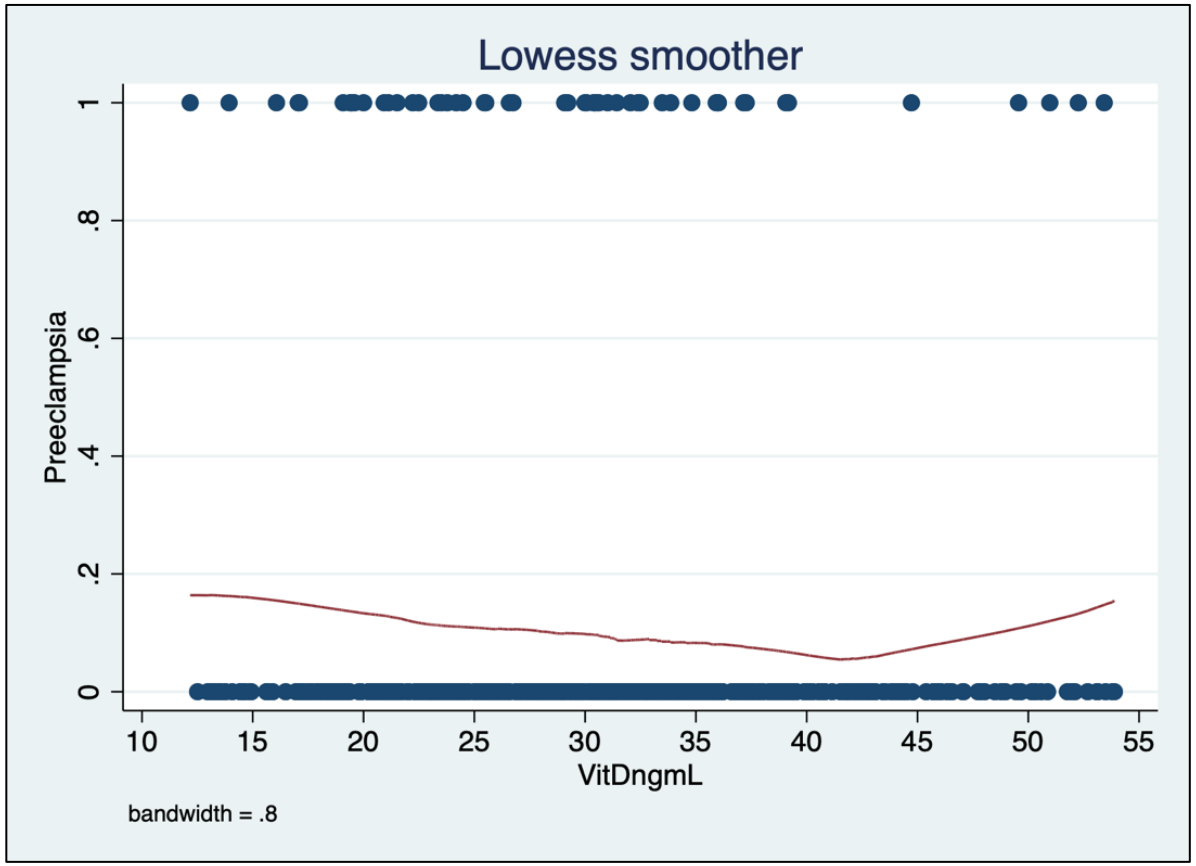

Figure 6.2. Non-parametric Lowess Curve for preconception 25(OH)D to express the best fitting for a smooth curve in connection to the data points presented between ≥12 ng/mL and ≤ 55 ng/mL to remove outliers, EAGeR Data (Total sample N=520; N=32 missing due to being outliers)

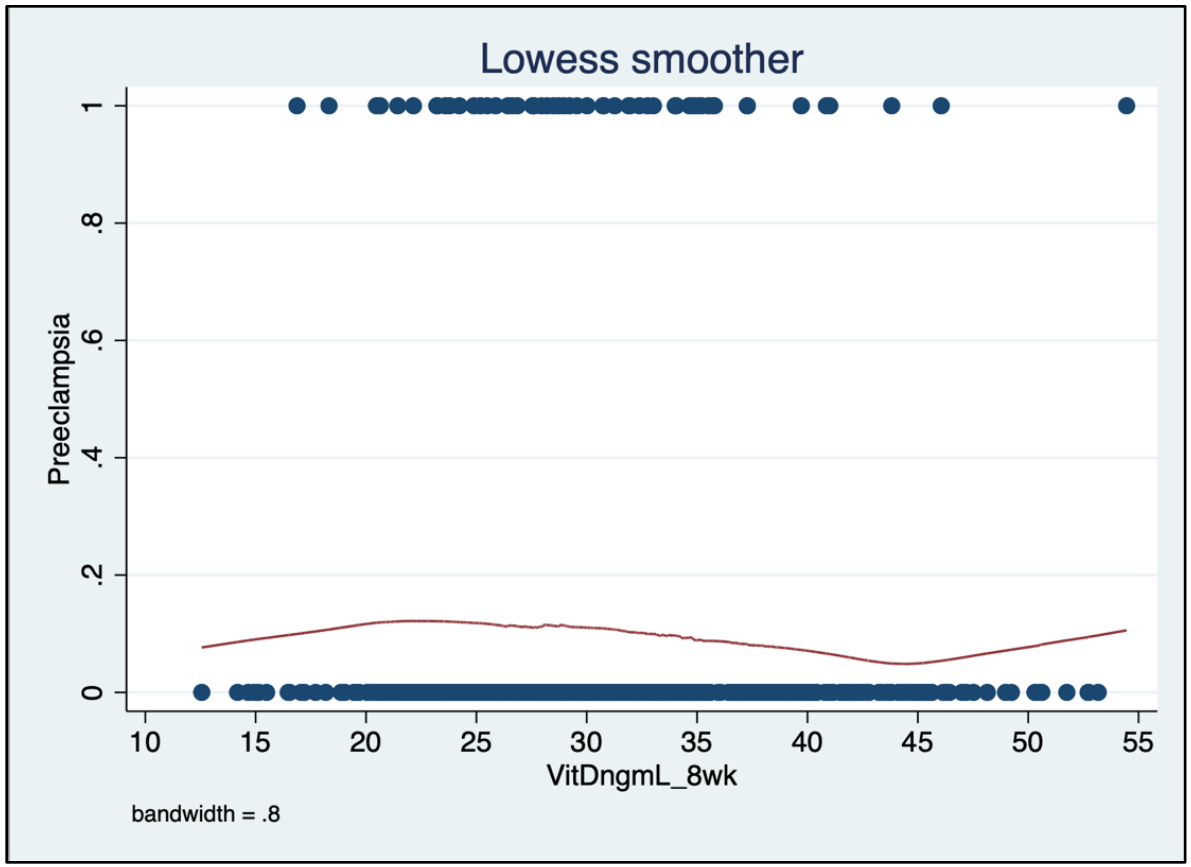

Figure 6.3. Non-parametric Lowess Curve for 8-week 25(OH)D to express the best fitting for a smooth curve in connection to the data points presented between ≥12 ng/mL and ≤ 55 ng/mL to remove outliers, EAGeR Data (Total Sample N=516; N=14 missing due to being outliers)

Chapter 7: Concluding Remarks

7.1 Summary of Major Findings

In summary, findings from these 3 studies suggest that critical early periods of implantation and placentation during the preconception and early gestation period, may impact adverse pregnancy outcomes, such as subchorionic hemorrhage and preeclampsia, and clinical markers of a healthy pregnancy, such as nausea or the absence of vaginal bleeding. In this dissertation, I was able to directly assess 25(OH)D levels marked by the preconception and early gestation period, which may support the development of a healthy pregnancy. In addition, an assessment of continuous 25(OH)D levels using linear spline models are suggestive of a potential of higher threshold of 25(OH)D which may reduce the risk of preeclampsia; however, additional research is needed to understand the optimal 25(OH)D level that may support reproductive and perinatal outcomes.

In Aim 1, the primary purpose was to identify whether change in preconception and 8-week gestation maternal serum 25(OH)D levels were associated with higher odds of vaginal bleeding/subchorionic hemorrhage. Analyses using both medical records and self-reported daily diaries of vaginal bleeding were consistent. Taken together, findings suggest that deficient vitamin D, or persistently deficient vitamin D, was associated with an increased risk of moderate to heavy vaginal bleeding or subchorionic hemorrhage, the latter of which is associated with higher levels of vaginal bleeding. Additionally, subchorionic hemorrhage is a clinically confirmed marker of disrupted placentation. These findings were consistent even when restricted to pregnancies that resulted in a live birth, suggesting that vaginal bleeding may be indicative of disruptions in placentation independent of a pregnancy loss. In contrast, I did not find associations between deficient vitamin D and lighter vaginal bleeding.

This study suggests that the maintenance of sufficient preconception and early gestation

25(OH)D levels may be important during the early implantation/placentation process as indicated by early clinical markers of vaginal bleeding and development of subchorionic hemorrhage. However, there may be differentiating biologic pathways. In these analyses, the extent of vaginal bleeding was distinguished as light compared to moderate/heavy bleeding. Light vaginal bleeding during early pregnancy may be indicative of implantation bleeding or the normal transition in the production of progesterone by the corpus luteum to the placenta and a corresponding temporary drop in progesterone levels, which may lead to some spotting or light vaginal bleeding. In contrast, moderate to heavy bleeding during the early conception period may be indicative of disruptions to the implantation or placentation process, and the development of subchorionic hemorrhage, which occurs when the chorion membranes that connect to the uterus partially detach, causing abnormal bleeding during pregnancy.[19]

In Aim 2, the primary purpose was to identify whether preconception versus 8-week gestation maternal serum 25(OH)D levels were associated with higher odds of nausea or vomiting. This study suggests that 25(OH)D levels at baseline (prior to conception) and early in pregnancy (8 weeks gestation) are associated with reduced odds of experiencing nausea or vomiting. Overall, the models showed that women who were persistently deficient or insufficient using medical records and self-reported daily diaries had a decreased odds of nausea and/or vomiting. Restrictions to live birth found consistent results for persistent vitamin D deficiency and insufficiency, although some results for other categories changed and require further analyses among a larger sample of pregnancies that survive to a live birth.

This study suggests that sufficient 25(OH)D levels prior to conception and during early gestation are important as they may be an indicator of more robust implantation and placentation by enhancing the production of hCG which increases the odds of experiencing nausea or vomiting.

Our results align with previous studies that have found nausea being a robust marker of implantation and, therefore, a sign of a healthy pregnancy.

In Aim 3, the primary purpose was to identify whether preconception and 8-week gestation maternal serum 25(OH)D levels were associated with higher risk of preeclampsia. Overall, preconception and 8 week gestation maternal serum 25(OH)D deficient levels increased the risk of preeclampsia but was less precise. Linear splines showed reduced preeclampsia risk with increasing vitamin D up to a threshold of 40-45 ng/ml. Additionally, an unexpected finding of increased risk of preeclampsia was found after the threshold of 40-45 ng/mL, but the sample size was limited at higher thresholds. Accordingly, this study is suggestive of potential lower risk of preeclampsia with higher vitamin D; however, this requires further evaluation from larger studies.

7.2. Strengths and Limitations

Specific strengths and limitations have been discussed for each study aim in Chapters 4-6; however, there are general strengths and limitations that pertain to all 3 study aims. Overall, there are many strengths of the data to highlight. First, this is one of the few studies that assesses 25(OH)D prior to and after conception to evaluate the effects on early clinical markers of pregnancy and preeclampsia. In addition, a detailed biomarker measurement of 25(OH)D and daily diary information on vaginal bleeding and nausea/vomiting is provided in this study. The use of daily diaries has been shown to provide more thorough assessment of indicators that may change frequently with time.[150] Daily diary information enabled an examination of longitudinal patterns of 25(OH)D. The unique nature of the detailed data during both the preconception and early pregnancy period using both medical records and daily diary information allows for a more robust capture of the critical windows of development, which is generally hard to capture. In addition, this study has captured many important confounders through the detailed data provided to allow

for more accurate assessment of our exposure and outcome. And finally, while this study includes women with a history of 1-2 prior pregnancy losses, this research may still apply to a large proportion of the population of reproductive aged women.

There are several limitations to note. First, when assessing the preconception versus 8-week gestation time point, some women will experience pregnancy loss prior to the 8-week gestation measurement. Although I used inverse probability weighting to account for some bias of never becoming pregnant or reaching a live birth, it may not have been able to account for all potential selection biases introduced when evaluating preconception factors and post conception outcomes. Furthermore, there may still be an issue of unmeasured confounding. To address this, I have calculated e-values to assess the extent to which unmeasured confounding may explain the associations found.[219,220] Potential unmeasured confounders include maternal and paternal exposures to pollution and toxic chemicals, which may impact adverse pregnancy outcomes.[221,223,226] In addition, paternal nutrition and sperm health were unmeasured, and could be potential confounders within these analyses.[221,223,226] Another general limitation across each of the studies was sample size limitations to explore more detailed categorizations of change in 25(OH)D or interactions with other variables, such as BMI and low-dose aspirin. Additionally, preeclampsia and subchorionic hemorrhage are less prevalent resulting in imprecision due to small numbers. Finally, the sample was limited in the diversity of the sample, as majority of women were of white race. Therefore, our studies may not be representative of a more general population, which may not have prior pregnancy losses or is more demographically diverse and have different levels of 25(OH)D absorption and metabolism.

7.3. Public Health Implications & Future Research

The life course model posits that the early pregnancy period is one that includes life altering processes that may predict one's trajectory of health and disease.[144] It is postulated that nutritional exposures during sensitive and critical time windows may alter the health and wellbeing of a pregnancy and thus, potentially leading to adverse pregnancy outcomes. Folate deficiency is the most widely-accepted example of the critical importance of preconception nutrient levels on the formation of the neural tube and growth of the fetus. [223] The findings from this dissertation also provide support for a potential role of vitamin D in maintaining a healthy pregnancy and its potential role in implantation and placentation.[22,29,266] Accordingly, this may translate to pregnancy complications, such as pregnancy loss, vaginal bleeding, or subchorionic hemorrhage; and later life health due to preeclampsia and preterm birth.

The findings from previous studies on vitamin D have implications that can inform multiple levels of the socio-ecological framework. The socio-ecological model framework includes the individual, interpersonal, organizational, environmental/community, and public policy implications.[267,268] This framework emphasizes the idea that these levels interact in various ways to shape the health of individuals and populations.[267,268] This framework begins by acknowledging how individuals would be directly affected by vitamin D and its effect on pregnancy outcomes, while taking into consideration physical and cognitive characteristics. In addition, communities and social networks surrounding the individuals would have implications of vitamin D on pregnancy outcomes. Furthermore, implications of vitamin D on pregnancy outcomes would impact the structured community and those within larger units on the organizational level, such as institutions and cultural/physical environments. And finally, public policy level changes may be made to address the effects of vitamin D on pregnancy outcomes. Using this framework, can help

to understand the public health implications of this research and areas that could be explored in future research.

At the **individual level**, these findings can provide more information to people with the capacity for pregnancy on potential health implications of deficient 25(OH)D and whether they may want to consider testing if they are deficient in 25(OH)D prior to conceiving. Given recommendations to supplement with 25(OH)D if deficient, based on clinical cut-offs used in these analyses, an individual may then pursue supplementation to ensure optimal 25(OH)D levels when entering pregnancy. Additionally, acquiring knowledge and skills to individuals will provide them self-esteem and self-confidence for effective prevention strategies.[267,268] At the **interpersonal level**, this knowledge may extend to social networks, including other friends or family intending to conceive or who may want to conceive in the future.

At the **organizational/community level**, these findings may inform obstetric and gynecologic practices. Currently, screening for maternal 25(OH)D may occur within the primary care wellness visit, but not at a gynecologic visit. Gynecologists may query patients on their 25(OH)D status or recommend screening at their primary care physicians, particularly if the patients are wanting to conceive. Gynecologists may also recommend 25(OH)D supplementation for patients if they are deficient, in line with current recommendations. In addition, institutions may influence exposure to outdoors and sunlight or increased intake of 25(OH)D through food sources. In addition, those who practice religious and cultural behaviors that may limit 25(OH)D exposure, such as religious headdress or covering, may be encouraged to test for 25(OH)D deficiency prior to conceiving. Furthermore, there may be variation in screening for 25(OH)D deficiency and recommendations for supplementation by health care professionals for vulnerable

populations, such as pregnant and non-Hispanic Black people who may be more susceptible to vitamin D deficiency.[154,269,270]

At the **societal/policy level**, currently up to 69% of the pregnancy population are deficient in 25(OH)D.[266,269] Increasing guidance on 25(OH)D screening and supplementation and its potential benefits for pregnancy may reduce the prevalence of deficient 25(OH)D in the pregnant population. If these findings are confirmed in other studies, it may also lead to interventions or policies that could reduce the risks for adverse pregnancy outcomes, such as preeclampsia, which is a leading cause of maternal morbidity and mortality in the United States.[33] Recently, the Pregnancy Worker Fairness Act was passed on June 27th, 2023, which allows for reasonable accommodations for pregnant workers by providing flexible hours, receiving additional break time to use the bathroom, eat, and rest, and ability to drink more water and take leave or time off following childbirth.[271] These policies are vital as the accommodations may allow pregnant workers to improve the health of their pregnancy by potentially using the time to take more breaks outside, thus increasing their levels of vitamin D, attend prenatal care visits, and focus on their overall nutrition status. With additional research, the United States may also consider more specific policy guidance on optimal levels for pregnant people independent of current Endocrine Society Guidelines, which has been designated for optimal bone health.[9] Therefore, policies to support research on the adequate levels of 25(OH)D level cut offs to be recommended for reproductive health is needed.[9,16] Recently, public health policies have begun to focus on increasing the Dietary Reference Intake guidelines on 25(OH)D supplementation, some of which have led to changes in prenatal vitamin levels or recommendations to begin supplementation prior to conception, yet recommended levels in prenatal vitamins remain quite low compared to what has been documented based on previous studies to increase sufficient 25(OH)D levels.[17,41,42] In addition, policies may

aid in guidance on reduction of food deserts through public policy implementation to ensure sufficient 25(OH)D exposure through food and supplement sources are available to reduce disparities and adverse pregnancy outcomes that may be linked to vitamin D deficiency.[59,60,92]

Future Research

Future studies are needed to better understand the critical preconception and early pregnancy period and its impact on adverse pregnancy outcomes. While previous research has assessed the time-period of serum 25(OH)D during the late term pregnancy period, very few studies have assessed the critical preconception period. While these studies fill this gap, more information is needed to understand mechanisms by which vitamin D may influence pregnancy health among women. In addition, future studies are needed to assess nutritional deficiencies in male partners due to their key role in placental health and development.[221,222,226]

In addition, future research should examine BMI as an interaction between vitamin D and adverse pregnancy outcomes in more diverse and larger populations, as BMI has been highly associated with vitamin D and preeclampsia (Chapter 6). Based on our findings, BMI was highly associated with vitamin D and preeclampsia, and therefore associations between vitamin D and preeclampsia were attenuated (Chapter 6).[254–256] Moderation of these effects by BMI could not be evaluated due to small numbers in this study. In addition, future research should examine aspirin as an interaction between vitamin D and adverse pregnancy outcomes, such as preeclampsia and subchorionic hemorrhage, and more robust pregnancy outcomes, such as nausea (Chapters 4, 5, and 6). Our findings suggest that aspirin may mitigate inflammation and reduce the risk of adverse pregnancy outcomes, such as preeclampsia, but may increase bleeding in subchorionic hemorrhage if it has already occurred due to preconception deficient 25(OH)D levels (Chapters 4 and 6). In addition, our findings may suggest taking an aspirin supplement to increase robustness in

implantation by reducing inflammation, and therefore increase the incidence of nausea (emesis) (Chapter 5). Although the change in magnitude of these associations by aspirin status differed, interactions were not significant, which may have been due to small samples.

Additionally, these findings should be evaluated further with a more representative population. In particularly, Black women are important to include in future research as the highest rates of preeclampsia and adverse pregnancy outcomes are experienced within that population.[272–275] Furthermore, Black women are more likely to be 25(OH)D deficient, as their absorption and metabolism of 25(OH)D is differs due to changes in allele's that help metabolize 25(OH)D.[90,154,269,276] Therefore, future research may also assess how these associations may differ within and between race/ethnicity groups.

In examining vitamin D levels as a continuous variable, rather than clinically defined cut-offs, I found that risk of preeclampsia varied based on a different threshold than what is often defined clinically. Therefore, additional assessment of optimal levels of vitamin D needed within the pregnant population to improve reproductive and pregnancy health outcomes. Based on our findings, there may be different vitamin D cut off recommendations for adverse pregnancy outcomes, such as preeclampsia (Chapter 6). Additionally, studies may also evaluate preconception vitamin D supplementation to assess the effects on reproductive pregnancy outcomes. This may aid in guidance of the clinical recommendations provided by the Dietary Reference Intake for supplementation prior to pregnancy, in particularly within more vulnerable populations.

Finally, future research is needed to directly assess additional biomarkers that may be vital in better understanding the relationship between preconception 25(OH)D on perinatal outcomes. Examining changes in hCG levels during the early pregnancy period may be important in better

understanding the role of 25(OH)D in supporting more robust implantation, which may lead to an increase in nausea or vomiting. In addition, examining the effects of vitamin D on hormone level changes, such as progesterone or estrogen, or markers of inflammation (e.g., C-reactive protein (CRP), which may guide a better understanding of the specific biological pathways between 25(OH)D metabolism during the preconception and implantation period on the risk of adverse pregnancy outcomes. This research could potentially lead to low-cost and safe treatment such as additional 25(OH)D supplementation prior to the preconception period to support a healthy pregnancy and reduce the risk of adverse pregnancy outcomes.

7.4 Conclusion

In keeping with a life course framework, this research may indicate sensitive and critical periods on the effect of 25(OH)D on adverse pregnancy outcomes. Critical windows during the preconception and early gestation period may alter proper implantation and placentation, thus leading to adverse pregnancy outcomes such as subchorionic hemorrhage and preeclampsia. In addition, sufficient maternal 25(OH)D levels during the early pregnancy period may support placental maintenance of the pregnancy and facilitating a greater nausea (emesis) response, which is a marker of robust implantation and healthy placentation. Due to vitamin D's source of anti-inflammatory support for the uterus and placenta during critical period of implantation and placentation, having sufficient vitamin D in both preconception and early gestation may be a critical component for a healthy pregnancy.[29,107,110,146,147] Additionally, this research can inform future research on potential mechanisms or clinical studies evaluating supplementation and the role of vitamin D for improving health before, during, and after pregnancy.

APPENDIX I: Tables and Figures

Table A1. Summary of key variables to be used in the analysis		
Concept	**Measure**	**Variable Description**
Exposure Variables		
Vitamin D ng/mL	Level of serum 25(OH)D of the woman at baseline of the EAGeR trial	Categorized as deficient (21-29 ng/mL), insufficient (≤ 20 ng/mL-30ng/mL) or sufficient (≥30 ng/mL) of 25(OH)D. Continuous levels of 25(OH)D will be assessed by increasing levels of 1ng/mL
Vitamin D ng/mL at 8-week gestation	Level of serum 25(OH)D of the woman at 8-week gestation of the EAGeR trial	Categorized as deficient (21-29 ng/mL), insufficient (≤ 20 ng/mL-30ng/mL) or sufficient (≥30 ng/mL) of 25(OH)D. Continuous levels of 25(OH)D will be assessed by increasing levels of 1ng/mL
Outcome Variables		
Nausea	If the woman had nausea during pregnancy within the EAGeR trial. Measured biweekly at weeks 4, 6 8, and 10 of gestation.	0=No 1=Yes
Vaginal Bleeding	If the woman had vaginal bleeding during pregnancy within the EAGeR trial. Measured biweekly at weeks 4, 6 8, and 10 of gestation.	0=No 1=Yes
Subchorionic Hemorrhage	If the woman had subchorionic hemorrhage during pregnancy within the EAGeR trial.	0=No 1=Yes
Preeclampsia	If the woman had preeclampsia during pregnancy within the EAGeR trial.	0=No 1=Yes
Covariates- Maternal Characteristics		
Age	Age of women at baseline of EAGeR trial	Initial categories of 5-year age groups (Younger than 20, 20-24, 25-29, 30-34, 35-40), may be collapsed depending on cell sizes
Race/Ethnicity	Race/Ethnicity of the woman at baseline of EAGeR trial	Categorized as 1=white and 0=non-white due to limited race and ethnic groups in trial

BMI	BMI of the woman at baseline of the EAGeR trial	Categorized as: Underweight <18.5 Normal ≥18.5 & <25 Overweight ≥25 & <30 Obese ≥30
Education	Highest education the woman has received at baseline of the EAGeR trial	Categorized based on highest degree attained (less than high school, high school, Bachelor's degree or higher).
Employment	Employment status of the woman at baseline of the EAGeR trial	0= Unemployed 1= Employed
Income	Income of the woman at baseline of the EAGeR trial	Income is categorized as 1=less than $19,999 2=$20,000-$39,999 3=$40,000-$74,999 4=$75,000-$99,999 5=$100,000 or over
Number of Losses	Number of prior losses the woman has had at baseline of the EAGeR trial	0=0 losses 1=1 losses 2=2 losses
Nulliparity	Number of prior live births among women in the EAGeR trial at baseline	0=0 1=1 2=2
Season	The season at baseline of the EAGeR trial	Measurement using standard months for each of the seasons: Spring Summer Fall Winter
Exercise	The level of exercise or physical activity of the woman at baseline of the EAGeR trial based on the IPAQ questionnaire	Low Moderate High
Alcohol Intensity	The level of alcohol intake of the woman at baseline of the EAGeR trial	1= Never 2= Sometimes 3= Often
Assigned Treatment	The assigned treatment of the woman at baseline of the EAGeR trial	0= Placebo 1= Low dose aspirin
Multivitamin Use	The use of multivitamins of the woman at baseline of the EAGeR trial	Yes(a)= With folic acid Yes(b)=No folic acid (take vitamins) No= No folic acid (no vitamins)
C-Reactive Protein (CRP)	If the woman had CRP measured at during the EAGeR trial. Measured at weeks 4 and 8 of gestation.	The continuous measured level of CRP.

Pregnancy	If the woman became pregnant during the EAGeR trial	0=No 1=Yes
Live Birth	If the woman had a live birth during the EAGeR trial	0=No 1=Yes

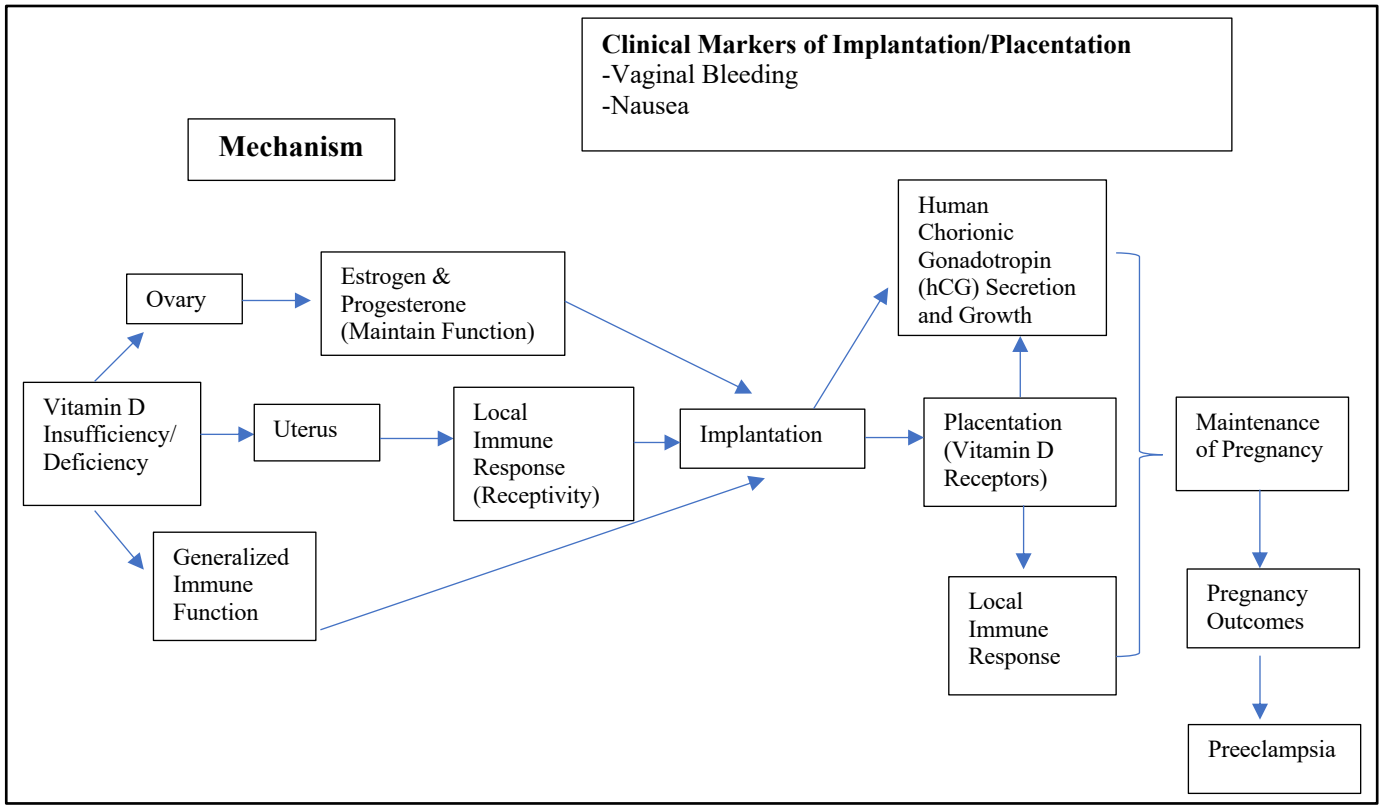

Figure A1. Mechanisms for implantation and placentation on pregnancy outcomes.

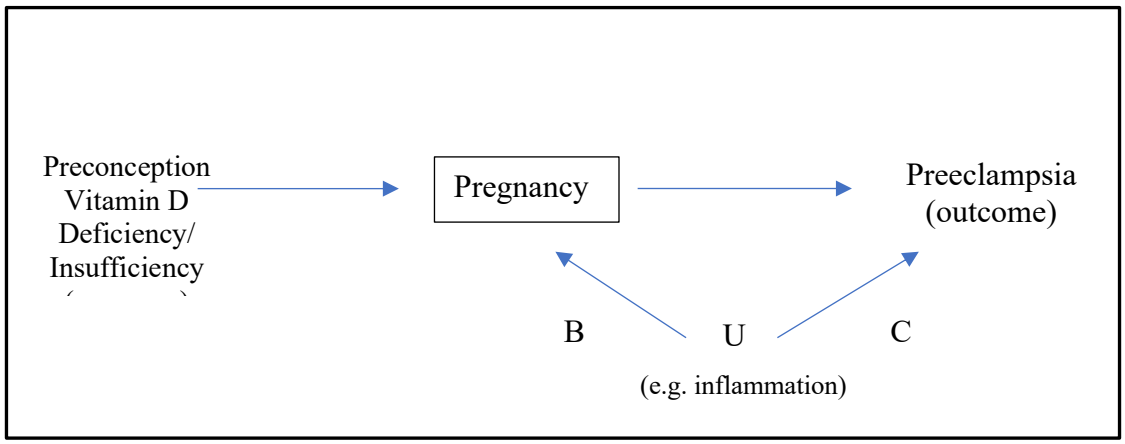

Figure A2. This causal diagram assesses the relationships between preconception vitamin D deficiency/insufficiency (exposure), through arrow A to Pregnancy, through arrow to Preeclampsia (outcome) with arrows B and C, which are other factors that may affect this relationship such as age, lifestyle, and inflammation. The box around pregnancy indicates that selection into the population is conditional on pregnancy. If we only evaluate a preconception exposure among women who successfully conceived and are not able to account for all factors identified by U (e.g., inflammation), then selection bias is in action. Adapted from: Flannagan, K., & Mumford, S. L. (2020). Preconception exposures and postconception outcomes: selection bias in action. *Fertility and sterility*, *114*(6), 1172–1173. https://doi.org/10.1016/j.fertnstert.2020.10.057

APPENDIX II: Data Collection Instruments

EAGeR Questionnaire- Chart Abstractions

EAGeR The Effects of Aspirin in Gestation & Reproduction	*Chart Abstraction Instruments* C.3. DELIVERY HOSPITALIZATION CAC3 v 2.1 Page 1 of 5 Aug/ 23 / 09	Study I.D

Instructions: Complete the Delivery Hospitalization on all women enrolled into the study using the maternal chart at the hospital where the baby was delivered. INFORMATION PERTAINS TO THE ENTIRE MATERNAL HOSPITALIZATION FOR DELIVERY. If the response is not documented in the chart, enter '- 9' for permanently missing and '- 8' for not applicable.

Institution _____
(*data source*)

If first filled on paper, enter date _____ **and initials** _____ **Department** _____
MM/ DD/ Y Y Y Y First / Last (*data source*)

Delivery Hospitalization

Hospital name (scroll)…….. |_____|
Other: _____

1. Admission Date: __ __ /__ __ /__ __ __ __ **Discharge Date:** __ __ /__ __ /__ __ __ __
mm dd yyyy *mm dd yyyy*

2. Presenting complaints of the mother / complaints elicited on history……

☐ Check if none noted

01=Yes, CHIEF complaint, 02=Yes, elicited on history / noted in chart as present, 03=No

a. fetal movement decreased or absent	……..……..	|__|__|
b. sent for induction / delivery of IUFD	……..……..	|__|__|
c. sent for induction / delivery of baby other than an IUFD	……..……..	|__|__|
d. sent for evaluation / admission	……..……..	|__|__|
e. vaginal bleeding	……..……..	|__|__|
f. contractions	……..……..	|__|__|
g. leakage of fluid	……..……..	|__|__|
h. abdominal pain	……..……..	|__|__|
i. fever / infection	……..……..	|__|__|
j. nausea / vomiting	……..……..	|__|__|
k. trauma / motor vehicle accident	……..……..	|__|__|
l. other, specify _____	……..……..	|__|__|

3. Presenting complaints of the mother / complaints elicited on history..... ☐ Check if none noted
01=Yes, CHIEF complaint, 02=Yes, elicited on history / noted in chart as present, 03=Not noted in chart

a. fetal movement decreased or absent..... |__|__|
b. sent for evaluation / admission ..... |__|__|
c. vaginal bleeding ..... |__|__|
d. contractions..... |__|__|
e. leakage of fluid ..... |__|__|
f. abdominal pain..... |__|__|
g. fever / infection..... |__|__|
h. nausea / vomiting ..... |__|__|
i. trauma / motor vehicle accident ..... |__|__|
j. other, specify _____ ..... |__|__|

4. If sent for evaluation / antepartum admission indicate primary reason ..... |__|__|
01=fetal monitoring 02=size-date discrepancy 03=hypertension
04=diabetes / glucose control 05=diagnosed with fetal demise 06=other

If other specify:_____

5. Fetal heart tones / cardiac activity found during visit ..... |__|__|
01=Present for all babies 02=Absent for any baby

6. Cervical dilation....... |__|__| cm

7. Objective evidence of contractions by monitor ..... *01=Yes, 02=No* ..... |__|__|

8. Vaginal bleeding on examination..... *01=Yes, 02=No* ..... |__|__|

Instructions: Complete this form using hospital records for women in the study who were had Hospitalizations / L&D Triage / ER Visits during her pregnancy on study. Complete one of these forms for each visit to the facility.

NOTE: THIS FORM IS NOT USED FOR THE DELIVERY VISIT. Use the Delivery Hospitalization form to record information on the delivery hospitalization. If the response is not documented in the chart, enter '- 9' for permanently missing and '- 8' for not applicable.

Institution _____
(*data source*)

If first filled on paper, enter date _____ **and initials** _____ **Department** _____
MM/ DD/ Y Y Y Y First / Last (*data source*)

C.2.1. Hospitalizations / L&D Triage / ER Visits ☐ Check if not done

1. **Hospital Name (Scroll)**..... _____

2. **Number of times the woman visited this hospital during her pregnancy** ..... |__|__|

3. **Visit number (to this hospital)**..... |__|__|

C.2.2. Visit Details

1. **Presentation Date:** ..... __ __ /__ __ /__ __ __ __
mm dd yyyy

2. **Resulting in:** ..... |__|__|
01=hospitalization 02=L&D triage 03=ER visit

If Hospitalization a) **Admission Date:** ..... __ __ /__ __ /__ __ __ __
mm / dd / yyyy

b) **Discharge Date:** ..... __ __ /__ __ /__ __ __ __
mm / dd / yyyy

9. Ultrasound performed..... *01=Yes, 02=No* ..... |__|__|
If yes, enter results on PRENATAL ULTRASOUND DIAGNOSIS FORM

10. Lab tests performed ..... *01=Yes, 02=No* ..... |__|__|
If yes, enter results on PRENATAL LABS FORM

11. Highest temperature recorded ..... a. |__|__| . |__| ° C ***OR*** b. |__|__|__| . |__| ° F

12. Highest systolic blood pressure recorded .....Date: __ __ /__ __ /__ __ __ __ |__|__|__| mm Hg
mm / dd / yyyy

13. Highest diastolic blood pressure recorded ……..... Date: __ __ /__ __ /__ __ __ __ |__|__|__| mm Hg
mm / dd / yyyy

C.2.3. Diagnoses

1. Diagnoses specifically noted in the chart (indicate all that apply)..... ☐ Check if none noted

01=Yes 02=No 03= not noted in chart

a. premature rupture of membranes (PROM)…….. |__|__|
b. preterm labor ……..... |__|__|
c. cervical incompetence ……..... |__|__|
d. gestational hypertension / preeclampsia ……..... |__|__|
e. placenta previa ……..... |__|__|
f. placental abruption ……..... |__|__|
g. pyelonephritis ……..... |__|__|
h. appendicitis ……..... |__|__|
i. cholecystitis / cholelithiasis ……..... |__|__|
j. impaired glucose tolerance /gestational diabetes……. |__|__|
k. other, specify _____. |__|__|

17. Conditions specifically noted in the chart..... ☐ **Check if none noted**

01=Yes *02=No*

a. premature rupture of membranes (PROM) ..... |__|__|
b. preterm labor ..... |__|__|
c. cervical incompetence ..... |__|__|
d. chorioamnionitis ..... |__|__|
e. preeclampsia/gestational hypertension ..... |__|__|
If yes → *01=mild/mod,* *02=severe,* *03=superimposed* ..... |__|__|
f. placenta previa ..... |__|__|
g. placental abruption ..... |__|__|
h. Non-reassuring fetal heart rate tracing ..... |__|__|
i. endometritis ..... |__|__|
j. other systemic infection, specify _____ ..... |__|__|
k. other, specify _____ ..... |__|__|

18. Type of Delivery Onset..... |__|__|

01 = Spontaneous Labor *02 = Spontaneous Rupture of Membranes* *03 = Induction*
04 = Cesarean without labor

19. Highest systolic blood pressure recorded .....Date: __ __ /__ __ /__ __ __ __ |__|__|__| mm Hg
mm / dd / yyyy

Highest diastolic blood pressure recorded …….... Date: __ __ /__ __ /__ __ __ __ |__|__|__| mm Hg
mm / dd / yyyy

20. Headache..... |__|__|

01 = Yes *02 = No*

21. Right upper quadrant abdominal pain..... |__|__|

01 = Yes *02 = No*

EAGeR Questionnaire- Daily Diaries

DAILY PREGNANCY DIARY CHART

Every day, please answer the following questions. Do not leave blanks.

	DAY
DID YOU TAKE YOUR STUDY MEDICATION TODAY? 0 = No, 1 = Yes, 2=Aspirin/Placebo only, 3=Folic Acid only	1
PREGNANCY TEST RESULTS? 0 = Did not test, 1 = pregnant, 2 = Not pregnant	2
BLEEDING OR SPOTTING 0 = None, 1 = Spotting/ Very light, 2 = Light, 3 = Moderate, 4 = Heavy, 5 = Very heavy	3
NAUSEA AND VOMITING 0 = None, 1 = Nausea, 2 = Vomiting once per day, 3 = Vomiting more than once per day	4
PELVIC PAIN OR CRAMPING 0 = None, 1 = Mild, 2 = Moderate, 3 = Severe	5
NUMBER OF ALCOHOLIC DRINKS CONSUMED Please enter number of drinks; 0 = None	6
ANY TOBACCO EXPOSURE? 0 = No, 1 = Yes, 2 = Passive for over ten minutes	7
NUMBER OF CAFFEINATED DRINKS CONSUMED Please enter number of drinks; 0 = None	8
STRESS LEVEL TODAY 0 = No stress, 1 = Little sress, 2 = Moderate stress, 3 = A lot of stress	9
MEDICATIONS OR HERBS USED 0 = None , 1 = Yes (Fill in Medication Chart)	10
ILLNESS, SIDE EFFECTS OR SPECIAL CIRCUMSTANCES 0 = No , 1 = Yes (Fill in Illness Chart)	11

INSTRUCTIONS

Please complete the pregnancy daily diary each day during the four weeks after your initial positive pregnancy test in the EAGeR study clinic. The Pregnancy Daily Diary contains a card for each week and a column for each day. Please answer all the questions every day, considering that each day ends at midnight. It is best to complete the card the same time every day.

***Remember,** bring your medication bottles, pill-box, samples, and the pregnancy daily diary to your next study appointment. If you cannot make an appointment, please call in advance to make other arrangements.*

Below are explanations for some questions.

DID YOU TAKE YOUR STUDY MEDICATION TODAY?

The question refers to both study treatment (Aspirin or Placebo) and the folic acid.

PREGNANCY TEST RESULTS

Did you perform a home pregnancy test today? If you did not test or cannot get a result, please enter "0". Enter "1" for positive (pregnant), or "2" for negative (not pregnant).

BLEEDING OR SPOTTING

Please tell us if you had any bleeding or spotting. Refer to the "Bleeding and Spotting Chart" to help you assess the degree of bleeding. If none, please enter "0". If you experience any blood clots please also record the details as an event in the "Illness Chart".

NAUSEA AND VOMITING

Please report any nausea or vomiting that you have experienced today. Record these symptoms regardless of the reason. If none, please enter "0".

PELVIC PAIN OR CRAMPING

Please report any nausea or vomiting that you have experienced today. Record these symptoms regardless of the cause. Please note the most severe symptom if you have more than one. If none, please enter "0".

ALCOHOL INTAKE

Please report the number of alcoholic drinks you consumed today. One drink is equivalent to one can of beer, one glass of wine, or one shot of liquor.

TOBACCO EXPOSURE

Please report any exposure to tobacco including smoking cigarettes or being passively exposed to smoking indoors (for more than 10 minutes) where other people smoked.

If you have smoked yourself and also spent time with other people smoking, enter "1". If you did not smoke but were exposed to others smoking, please enter "2".

NUMBER OF CAFFEINATED DRINKS CONSUMED

Please enter the total number of cups of caffeinated drinks you have consumed today. Caffeinated drinks include coffee (not decaf), tea, and/or caffeinated soda. Enter "0" if none. One drink is a 12 oz cup of coffee or tea or a 12 oz can of caffeinated soda. If your cup is larger than 12 oz, please estimate how many 12 oz cans it is equal to.

Please do not include decaffeinated soda pops (such as ginger ale, Sprite, 7-Up, etc.).

STRESS LEVEL TODAY

Please rate your average level of stress today.

MEDICATIONS OR HERBS USED

Have you used ANY prescription, over-the-counter medications, vitamins, or herbal supplements other than study medication today? If yes, please enter "1" and complete the "Medication Chart" printed on the back. If you need more room please use the additional chart pages at the end of the pregnancy daily diary.

ILLNESS, SIDE EFFECTS OR SPECIAL CIRCUMSTANCES

Have you suffered from illness, medication side effects or other special circumstances today? If yes, please enter "1" and complete the "Illness Chart" printed on the back. If you need more room please use the additional chart pages at the end of the pregnancy daily diary.

REMINDERS

1) Please collect and store your morning urine sample.
2) Take your study medication daily.
3) Call your study coordinator with questions.

DURING PREGNANCY

It is best to avoid tobacco and reduce alcohol consumption.

DURING PREGNANCY

It is common to have some spotting during pregnancy. However, if you experience moderate or heavy bleeding or if you pelvic pain or cramping an concerns please call your OB cl

PREGNANCY TEST INSTRU

If the result of a home pregna please call the study coordinat schedule a clinic visit.

TISSUE COLLECTION (IN U

If you do experience bleed much of the tissue as you ca container the research nurse the container in the refrigerat the research nurse as soon as

CALL THE STUDY COORDI IMMEDIATELY IF:

1. The result of a home pregr negative.
2. You started menstruating.
3. You experience severe pel cramping.

Please use the following chart to help you estimate the amount of bleeding you experience. For each day of bleeding please enter in the pregnancy daily diary the code that best describes the amount of bleeding.

Sanitary napkin/pad		Tampon
	1 = Spotting/ Very light	
	2 = Light	
	3 = Moderate	
	4 = Heavy	
	5 = Very heavy	

ginning __/__/____ Week No.____ Study ID_____

ıositive pregnancy test __/__/____

ın	Mon	Tues	Wed	Thur	Fri	Sat

BIBLIOGRAPHY

1. Lerchbaum E, Obermayer-Pietsch B. Vitamin D and fertility: a systematic review. *Eur J Endocrinol*. 2012;166(5):765-778. doi:10.1530/EJE-11-0984

2. Raia-Barjat T, Sarkis C, Rancon F, et al. Vitamin D deficiency during late pregnancy mediates placenta-associated complications. *Sci Rep*. 2021;11(1):20708. doi:10.1038/s41598-021-00250-5

3. Schröder-Heurich B, Springer CJP, von Versen-Höynck F. Vitamin D Effects on the Immune System from Periconception through Pregnancy. *Nutrients*. 2020;12(5). doi:10.3390/nu12051432

4. Cyprian F, Lefkou E, Varoudi K, Girardi G. Immunomodulatory Effects of Vitamin D in Pregnancy and Beyond. *Front Immunol*. 2019;10. doi:10.3389/fimmu.2019.02739

5. Kuhr DL, Sjaarda LA, Alkhalaf Z, et al. Vitamin D is associated with bioavailability of androgens in eumenorrheic women with prior pregnancy loss. *Am J Obstet Gynecol*. 2018;218(6):608.e1-608.e6. doi:10.1016/j.ajog.2018.03.012

6. Dominguez LJ, Farruggia M, Veronese N, Barbagallo M. Vitamin D Sources, Metabolism, and Deficiency: Available Compounds and Guidelines for Its Treatment. *Metabolites*. 2021;11(4):255. doi:10.3390/metabo11040255

7. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis | The American Journal of Clinical Nutrition | Oxford Academic. Accessed March 29, 2022. https://academic.oup.com/ajcn/article/79/3/362/4690120?login=true

8. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr*. 2008;87(4):1080S-6S. doi:10.1093/ajcn/87.4.1080S

9. Holick M, Binkley N, Heike BF, et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline | The Journal of Clinical Endocrinology & Metabolism | Oxford Academic. Published 2011. Accessed February 26, 2021. https://academic.oup.com/jcem/article/96/7/1911/2833671

10. Ginde AA, Sullivan AF, Mansbach JM, Camargo CA. Vitamin D insufficiency in pregnant and nonpregnant women of childbearing age in the United States. *Am J Obstet Gynecol*. 2010;202(5):436.e1-436.e8. doi:10.1016/j.ajog.2009.11.036

11. Mumford SL, Garbose RA, Kim K, et al. Preconception serum 25 hydroxyvitamin D levels are associated with increased live birth and reduced pregnancy loss: a prospective cohort study. *Lancet Diabetes Endocrinol*. 2018;6(9):725-732. doi:10.1016/S2213-8587(18)30153-0

12. Bakacak M, Serin S, Ercan O, et al. Comparison of Vitamin D levels in cases with preeclampsia, eclampsia and healthy pregnant women. *Int J Clin Exp Med*. 2015;8(9):16280-16286.

13. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab*. 2007;92(9):3517-3522. doi:10.1210/jc.2007-0718

14. Akbari S, Khodadadi B, Ahmadi SAY, Abbaszadeh S, Shahsavar F. Association of vitamin D level and vitamin D deficiency with risk of preeclampsia: A systematic review and updated meta-analysis. *Taiwan J Obstet Gynecol*. 2018;57(2):241-247. doi:10.1016/j.tjog.2018.02.013

15. Vitamin D: Screening and Supplementation During Pregnancy. Accessed May 13, 2021. https://www.acog.org/en/clinical/clinical-guidance/committee-opinion/articles/2011/07/vitamin-d-screening-and-supplementation-during-pregnancy

16. Office of Dietary Supplements - Vitamin D. Published 2021. Accessed March 9, 2021. https://ods.od.nih.gov/factsheets/VitaminD-health%20Professional/

17. Dawodu A, Saadi HF, Bekdache G, Javed Y, Altaye M, Hollis BW. Randomized Controlled Trial (RCT) of Vitamin D Supplementation in Pregnancy in a Population With Endemic Vitamin D Deficiency. *J Clin Endocrinol Metab*. 2013;98(6):2337-2346. doi:10.1210/jc.2013-1154

18. Ganguly A, Tamblyn JA, Finn-Sell S, et al. Vitamin D, the placenta and early pregnancy: effects on trophoblast function. *J Endocrinol*. 2018;236(2):R93-R103. doi:10.1530/JOE-17-0491

19. Bondick CP, M Das J, Fertel H. Subchorionic Hemorrhage. In: *StatPearls*. StatPearls Publishing; 2021. Accessed September 15, 2021. http://www.ncbi.nlm.nih.gov/books/NBK559017/

20. Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. *Biol Reprod*. 2003;69(1):1-7. doi:10.1095/biolreprod.102.014977

21. Harmon AC, Cornelius DC, Amaral LM, et al. The role of inflammation in the pathology of preeclampsia. *Clin Sci Lond Engl 1979*. 2016;130(6):409-419. doi:10.1042/CS20150702

22. Chan SY, Susarla R, Canovas D, et al. Vitamin D promotes human extravillous trophoblast invasion in vitro. *Placenta*. 2015;36(4):403-409. doi:10.1016/j.placenta.2014.12.021

23. Liu NQ, Ouyang Y, Bulut Y, et al. Dietary Vitamin D Restriction in Pregnant Female Mice Is Associated With Maternal Hypertension and Altered Placental and Fetal Development. *Endocrinology*. 2013;154(7):2270-2280. doi:10.1210/en.2012-2270

24. Ma SL, Tian XY, Wang YQ, Zhang HF, Zhang L. Vitamin D Supplementation Prevents Placental Ischemia Induced Endothelial Dysfunction by Downregulating Placental Soluble FMS-Like Tyrosine Kinase-1. *DNA Cell Biol*. 2017;36(12):1134-1141. doi:10.1089/dna.2017.3817

25. Bagot CN, Troy PJ, Taylor HS. Alteration of maternal Hoxa10 expression by in vivo gene transfection affects implantation. *Gene Ther*. 2000;7(16):1378-1384. doi:10.1038/sj.gt.3301245

26. Evans KN, Nguyen L, Chan J, et al. Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells. *Biol Reprod*. 2006;75(6):816-822. doi:10.1095/biolreprod.106.054056

27. Evans KN, Bulmer JN, Kilby MD, Hewison M. Vitamin D and placental-decidual function. *J Soc Gynecol Investig*. 2004;11(5):263-271. doi:10.1016/j.jsgi.2004.02.002

28. Role of Placental VDR Expression and Function in Common Late Pregnancy Disorders. Accessed April 27, 2021. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5713309/

29. Liu NQ, Kaplan AT, Lagishetty V, et al. Vitamin D and the regulation of placental inflammation. *J Immunol Baltim Md 1950*. 2011;186(10):5968-5974. doi:10.4049/jimmunol.1003332

30. Park H, Wood MR, Malysheva OV, et al. Placental vitamin D metabolism and its associations with circulating vitamin D metabolites in pregnant women. *Am J Clin Nutr*. 2017;106(6):1439-1448. doi:10.3945/ajcn.117.153429

31. Behjat Sasan S, Zandvakili F, Soufizadeh N, Baybordi E. The Effects of Vitamin D Supplement on Prevention of Recurrence of Preeclampsia in Pregnant Women with a History of Preeclampsia. *Obstet Gynecol Int*. 2017;2017. doi:10.1155/2017/8249264

32. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab*. 2007;92(9):3517-3522. doi:10.1210/jc.2007-0718

33. Ghulmiyyah L, Sibai B. Maternal mortality from preeclampsia/eclampsia. *Semin Perinatol*. 2012;36(1):56-59. doi:10.1053/j.semperi.2011.09.011

34. Fischer D, Schroer A, Lüdders D, et al. Metabolism of vitamin D3 in the placental tissue of normal and preeclampsia complicated pregnancies and premature births. *Clin Exp Obstet Gynecol*. 2007;34(2):80-84.

35. Bodnar LM, Platt RW, Simhan HN. Early-Pregnancy Vitamin D Deficiency and Risk of Preterm Birth Subtypes. *Obstet Gynecol*. 2015;125(2):439-447. doi:10.1097/AOG.0000000000000621

36. Bhupornvivat N, Phupong V. Serum 25-hydroxyvitamin D in pregnant women during preterm labor. *Asia Pac J Clin Nutr*. 2017;26(2):287-290. doi:10.6133/apjcn.112015.11

37. Sandovici I, Hoelle K, Angiolini E, Constância M. Placental adaptations to the maternal-fetal environment: implications for fetal growth and developmental programming. *Reprod Biomed Online*. 2012;25(1):68-89. doi:10.1016/j.rbmo.2012.03.017

38. Hasan HA, Barber TM, Cheaib S, Coussa A. Preconception Vitamin D Level and In Vitro Fertilization: Pregnancy Outcome. *Endocr Pract*. Published online January 2023:S1530891X23000162. doi:10.1016/j.eprac.2023.01.005

39. Ali AM, Alobaid A, Malhis TN, Khattab AF. Effect of vitamin D3 supplementation in pregnancy on risk of pre-eclampsia - Randomized controlled trial. *Clin Nutr Edinb Scotl*. 2019;38(2):557-563. doi:10.1016/j.clnu.2018.02.023

40. Anderson CM, Gillespie SL, Thiele DK, Ralph JL, Ohm JE. Effects of Maternal Vitamin D Supplementation on the Maternal and Infant Epigenome. *Breastfeed Med*. 2018;13(5):371-380. doi:10.1089/bfm.2017.0231

41. Eggemoen ÅR, Falk RS, Knutsen KV, et al. Vitamin D deficiency and supplementation in pregnancy in a multiethnic population-based cohort. *BMC Pregnancy Childbirth*. 2016;16(1):7. doi:10.1186/s12884-016-0796-0

42. Fogacci S, Fogacci F, Banach M, et al. Vitamin D supplementation and incident preeclampsia: A systematic review and meta-analysis of randomized clinical trials. *Clin Nutr Edinb Scotl*. 2020;39(6):1742-1752. doi:10.1016/j.clnu.2019.08.015

43. Lo TH, Wu TY, Li PC, Ding DC. Effect of Vitamin D supplementation during pregnancy on maternal and perinatal outcomes. *Ci Ji Yi Xue Za Zhi Tzu-Chi Med J*. 2019;31(4):201-206. doi:10.4103/tcmj.tcmj_32_19

44. Meng X, Zhang J, Wan Q, et al. Influence of Vitamin D supplementation on reproductive outcomes of infertile patients: a systematic review and meta-analysis. *Reprod Biol Endocrinol RBE*. 2023;21:17. doi:10.1186/s12958-023-01068-8

45. Shahgheibi S, Farhadifar F, Pouya B. The effect of vitamin D supplementation on gestational diabetes in high-risk women: Results from a randomized placebo-controlled trial. *J Res Med Sci Off J Isfahan Univ Med Sci*. 2016;21. doi:10.4103/1735-1995.175148

46. Zhang Q, Cheng Y, He M, Li T, Ma Z, Cheng H. Effect of various doses of vitamin D supplementation on pregnant women with gestational diabetes mellitus: A randomized controlled trial. *Exp Ther Med*. 2016;12(3):1889-1895. doi:10.3892/etm.2016.3515

47. Vitamin D and risk of preterm birth: Up-to-date meta-analysis of randomized controlled trials and observational studies - PubMed. Accessed June 4, 2021. https://pubmed.ncbi.nlm.nih.gov/28150405/

48. EB Research: Effects of Aspirin in Gestation and Reproduction (EAGeR) Study. https://www.nichd.nih.gov/. Accessed October 17, 2021. https://www.nichd.nih.gov/about/org/dir/dph/officebranch/eb/effects-aspirin

49. Schisterman EF, Silver RM, Perkins NJ, et al. A randomised trial to evaluate the effects of low-dose aspirin in gestation and reproduction: design and baseline characteristics. *Paediatr Perinat Epidemiol*. 2013;27(6):598-609. doi:10.1111/ppe.12088

50. Schisterman EF, Silver RM, Lesher LL, et al. Preconception low-dose aspirin and pregnancy outcomes: results from the EAGeR randomised trial. *Lancet Lond Engl*. 2014;384(9937):29-36. doi:10.1016/S0140-6736(14)60157-4

51. Alkhalaf Z, Kim K, Kuhr DL, et al. Markers of vitamin D metabolism and premenstrual symptoms in healthy women with regular cycles. *Hum Reprod Oxf Engl*. 2021;36(7):1808-1820. doi:10.1093/humrep/deab089

52. Levine LD, Kim K, Purdue-Smithe A, et al. Urinary Phytoestrogens and Relationship to Menstrual Cycle Length and Variability Among Healthy, Eumenorrheic Women. *J Endocr Soc*. 2020;4(2):bvz003. doi:10.1210/jendso/bvz003

53. Mumford SL, Flannagan KS, Radoc JG, et al. Cannabis use while trying to conceive: a prospective cohort study evaluating associations with fecundability, live birth and pregnancy loss. *Hum Reprod Oxf Engl*. 2021;36(5):1405-1415. doi:10.1093/humrep/deaa355

54. Pilz S, Zittermann A, Trummer C, et al. Vitamin D testing and treatment: a narrative review of current evidence. *Endocr Connect*. 2019;8(2):R27-R43. doi:10.1530/EC-18-0432

55. Chang SW, Lee HC. Vitamin D and health - The missing vitamin in humans. *Pediatr Neonatol*. 2019;60(3):237-244. doi:10.1016/j.pedneo.2019.04.007

56. Vitamin D- American Academy of Dermatology Association. Accessed May 3, 2022. https://www.aad.org/media/stats-vitamin-d

57. Holick MF. VITAMIN D STATUS: MEASUREMENT, INTERPRETATION AND CLINICAL APPLICATION. *Ann Epidemiol*. 2009;19(2):73-78. doi:10.1016/j.annepidem.2007.12.001

58. Bouillon R. Comparative analysis of nutritional guidelines for vitamin D. *Nat Rev Endocrinol*. 2017;13(8):466-479. doi:10.1038/nrendo.2017.31

59. Pilz S, März W, Cashman KD, et al. Rationale and Plan for Vitamin D Food Fortification: A Review and Guidance Paper. *Front Endocrinol*. 2018;9:373. doi:10.3389/fendo.2018.00373

60. Cashman KD. Vitamin D: dietary requirements and food fortification as a means of helping achieve adequate vitamin D status. *J Steroid Biochem Mol Biol*. 2015;148:19-26. doi:10.1016/j.jsbmb.2015.01.023

61. Macdonald HM, Mavroeidi A, Fraser WD, et al. Sunlight and dietary contributions to the seasonal vitamin D status of cohorts of healthy postmenopausal women living at northerly latitudes: a major cause for concern? *Osteoporos Int*. 2011;22(9):2461-2472. doi:10.1007/s00198-010-1467-z

62. Bouillon R. Genetic and Racial Differences in the Vitamin D Endocrine System. *Endocrinol Metab Clin North Am*. 2017;46(4):1119-1135. doi:10.1016/j.ecl.2017.07.014

63. Bhattoa HP, Bettembuk P, Ganacharya S, Balogh A. Prevalence and seasonal variation of hypovitaminosis D and its relationship to bone metabolism in community dwelling postmenopausal Hungarian women. *Osteoporos Int*. 2004;15(6):447-451. doi:10.1007/s00198-003-1566-1

64. Kull M, Kallikorm R, Tamm A, Lember M. Seasonal variance of 25-(OH) vitamin D in the general population of Estonia, a Northern European country. *BMC Public Health*. 2009;9(1):22. doi:10.1186/1471-2458-9-22

65. van Schoor NM, Knol DL, Deeg DJH, Peters FPAMN, Heijboer AC, Lips P. Longitudinal changes and seasonal variations in serum 25-hydroxyvitamin D levels in different age groups: results of the Longitudinal Aging Study Amsterdam. *Osteoporos Int*. 2014;25(5):1483-1491. doi:10.1007/s00198-014-2651-3

66. Snellman G, Melhus H, Gedeborg R, et al. Seasonal Genetic Influence on Serum 25-Hydroxyvitamin D Levels: A Twin Study. *PLOS ONE*. 2009;4(11):e7747. doi:10.1371/journal.pone.0007747

67. Medeiros JFP, de Oliveira Borges MV, Soares AA, et al. The impact of vitamin D supplementation on VDR gene expression and body composition in monozygotic twins: randomized controlled trial. *Sci Rep*. 2020;10(1):11943. doi:10.1038/s41598-020-69128-2

68. Webb AR, Kazantzidis A, Kift RC, Farrar MD, Wilkinson J, Rhodes LE. Colour Counts: Sunlight and Skin Type as Drivers of Vitamin D Deficiency at UK Latitudes. *Nutrients*. 2018;10(4):457. doi:10.3390/nu10040457

69. Roomi MA, Farooq A, Ullah E, Lone KP. Hypovitaminosis D and its association with lifestyle factors. *Pak J Med Sci*. 2015;31(5):1236-1240. doi:10.12669/pjms.315.7196

70. Tønnesen R, Hovind PH, Jensen LT, Schwarz P. Determinants of vitamin D status in young adults: influence of lifestyle, sociodemographic and anthropometric factors. *BMC Public Health*. 2016;16(1):385. doi:10.1186/s12889-016-3042-9

71. Lips P, Cashman KD, Lamberg-Allardt C, et al. Current vitamin D status in European and Middle East countries and strategies to prevent vitamin D deficiency: a position statement of the European Calcified Tissue Society. *Eur J Endocrinol*. 2019;180(4):P23-P54. doi:10.1530/EJE-18-0736

72. Fiscaletti M, Stewart P, Munns C. The importance of vitamin D in maternal and child health: a global perspective. *Public Health Rev*. 2017;38:19. doi:10.1186/s40985-017-0066-3

73. Lips P, Cashman KD, Lamberg-Allardt C, et al. Current vitamin D status in European and Middle East countries and strategies to prevent vitamin D deficiency: a position statement of the European Calcified Tissue Society. *Eur J Endocrinol*. 2019;180(4):P23-P54. doi:10.1530/EJE-18-0736

74. El-Hajj Fuleihan G. Vitamin D Deficiency in the Middle East and its Health Consequences. *Clin Rev Bone Miner Metab*. 2009;7:77-93. doi:10.1007/s12018-009-9027-9

75. Naeem Z. Vitamin D Deficiency- An Ignored Epidemic. *Int J Health Sci*. 2010;4(1):V-VI.

76. Full article: Hypovitaminosis D in the Middle East and North Africa. Accessed April 3, 2022. https://www.tandfonline.com/doi/full/10.4161/derm.25111

77. Ardawi MSM, Sibiany AM, Bakhsh TM, Qari MH, Maimani AA. High prevalence of vitamin D deficiency among healthy Saudi Arabian men: relationship to bone mineral density, parathyroid hormone, bone turnover markers, and lifestyle factors. *Osteoporos Int*. 2012;23(2):675-686. doi:10.1007/s00198-011-1606-1

78. Al Shaikh AM, Abaalkhail B, Soliman A, et al. Prevalence of Vitamin D Deficiency and Calcium Homeostasis in Saudi Children. *J Clin Res Pediatr Endocrinol*. 2016;8(4):461-467. doi:10.4274/jcrpe.3301

79. Pludowski P, Grant WB, Bhattoa HP, et al. Vitamin D Status in Central Europe. *Int J Endocrinol*. 2014;2014:e589587. doi:10.1155/2014/589587

80. van der Wielen RPJ, de Groot LCPGM, van Staveren WA, et al. Serum vitamin D concentrations among elderly people in Europe. *The Lancet*. 1995;346(8969):207-210. doi:10.1016/S0140-6736(95)91266-5

81. Burnand B, Sloutskis D, Gianoli F, et al. Serum 25-hydroxyvitamin D: distribution and determinants in the Swiss population. *Am J Clin Nutr*. 1992;56(3):537-542. doi:10.1093/ajcn/56.3.537

82. Souberbielle JC, Massart C, Brailly-Tabard S, Cavalier E, Chanson P. Prevalence and determinants of vitamin D deficiency in healthy French adults: the VARIETE study. *Endocrine*. 2016;53(2):543-550. doi:10.1007/s12020-016-0960-3

83. Buchebner D, McGuigan F, Gerdhem P, Malm J, Ridderstråle M, Åkesson K. Vitamin D insufficiency over 5 years is associated with increased fracture risk—an observational cohort study of elderly women. *Osteoporos Int*. 2014;25(12):2767-2775. doi:10.1007/s00198-014-2823-1

84. Plasma 25-Hydroxyvitamin D Levels and Fracture Risk in a Community-Based Cohort of Elderly Men in Sweden | The Journal of Clinical Endocrinology & Metabolism | Oxford Academic. Accessed April 3, 2022. https://academic.oup.com/jcem/article/95/6/2637/2598180?login=true

85. Cashman KD, Dowling KG, Škrabáková Z, et al. Standardizing serum 25-hydroxyvitamin D data from four Nordic population samples using the Vitamin D Standardization Program protocols: Shedding new light on vitamin D status in Nordic individuals. *Scand J Clin Lab Invest*. 2015;75(7):549-561. doi:10.3109/00365513.2015.1057898

86. Dovnik A, Mujezinović F, Treiber M, et al. Determinants of maternal vitamin D concentrations in Slovenia : A prospective observational study. *Wien Klin Wochenschr*. 2017;129(1-2):21-28. doi:10.1007/s00508-016-1142-2

87. Léger-Guist'hau J, Domingues-Faria C, Miolanne M, et al. Low socio-economic status is a newly identified independent risk factor for poor vitamin D status in severely obese adults. *J Hum Nutr Diet*. 2017;30(2):203-215. doi:10.1111/jhn.12405

88. Honório OS, Pessoa MC, Gratão LHA, et al. Social inequalities in the surrounding areas of food deserts and food swamps in a Brazilian metropolis. *Int J Equity Health*. 2021;20(1):168. doi:10.1186/s12939-021-01501-7

89. Harris SS, Soteriades E, Coolidge JA, Mudgal S, Dawson-Hughes B. Vitamin D insufficiency and hyperparathyroidism in a low income, multiracial, elderly population. *J Clin Endocrinol Metab*. 2000;85(11):4125-4130. doi:10.1210/jcem.85.11.6962

90. Harris SS, Dawson-Hughes B. Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. *Am J Clin Nutr*. 1998;67(6):1232-1236. doi:10.1093/ajcn/67.6.1232

91. Harris SS. Vitamin D and African Americans. *J Nutr*. 2006;136(4):1126-1129. doi:10.1093/jn/136.4.1126

92. Cooksey Stowers K, Jiang Q, Atoloye AT, Lucan S, Gans K. Racial Differences in Perceived Food Swamp and Food Desert Exposure and Disparities in Self-Reported Dietary Habits. *Int J Environ Res Public Health*. 2020;17(19):7143. doi:10.3390/ijerph17197143

93. Dubowitz T, Dastidar MG, Troxel WM, et al. Food Insecurity in a Low-Income, Predominantly African American Cohort Following the COVID-19 Pandemic. *Am J Public Health*. 2021;111(3):494-497. doi:10.2105/AJPH.2020.306041

94. Vitamin D binding protein polymorphisms significantly impact vitamin D status in children | Pediatric Research. Accessed February 24, 2022. https://www.nature.com/articles/s41390-019-0322-y

95. Polymorphisms | IntechOpen. Accessed May 8, 2022. https://www.intechopen.com/chapters/61204

96. Hameed A, Akhtar N. The Skin Melanin: An Inhibitor of Vitamin-D3 Biosynthesis: With Special Emphasis with Structure of Skin. A Mini Review. *Dermatol Case Rep*. 2019;04(01). doi:10.35248/2684-124X.19.4.149

97. Özdemir AA, Ercan Gündemir Y, Küçük M, et al. Vitamin D Deficiency in Pregnant Women and Their Infants. *J Clin Res Pediatr Endocrinol*. 2018;10(1):44-50. doi:10.4274/jcrpe.4706

98. Díaz-López A, Jardí C, Villalobos M, Serrat N, Basora J, Arija V. Prevalence and risk factors of hypovitaminosis D in pregnant Spanish women. *Sci Rep*. 2020;10(1):15757. doi:10.1038/s41598-020-71980-1

99. Yang C, Jing W, Ge S, Sun W. Vitamin D status and vitamin D deficiency risk factors among pregnancy of Shanghai in China. *BMC Pregnancy Childbirth*. 2021;21(1):431. doi:10.1186/s12884-021-03889-0

100. Wagner CL, Hollis BW. The Implications of Vitamin D Status During Pregnancy on Mother and her Developing Child. *Front Endocrinol*. 2018;9. doi:10.3389/fendo.2018.00500

101. Mulligan ML, Felton SK, Riek AE, Bernal-Mizrachi C. Implications of vitamin D deficiency in pregnancy and lactation. *Am J Obstet Gynecol*. 2010;202(5):429.e1-429.e9. doi:10.1016/j.ajog.2009.09.002

102. Rodrigues MRK, Lima SAM, Mazeto GMF da S, et al. Efficacy of vitamin D supplementation in gestational diabetes mellitus: Systematic review and meta-analysis of randomized trials. *PloS One*. 2019;14(3):e0213006. doi:10.1371/journal.pone.0213006

103. Christesen HT, Falkenberg T, Lamont RF, Jørgensen JS. The impact of vitamin D on pregnancy: a systematic review. *Acta Obstet Gynecol Scand*. 2012;91(12):1357-1367. doi:10.1111/aogs.12000

104. Knabl J, Vattai A, Ye Y, et al. Role of Placental VDR Expression and Function in Common Late Pregnancy Disorders. *International Journal of Molecular Sciences*. Published online November 2017. doi:10.3390/ijms18112340

105. Knabl J, Hüttenbrenner R, Hutter S, et al. Gestational Diabetes Mellitus Upregulates Vitamin D Receptor in Extravillous Trophoblasts and Fetoplacental Endothelial Cells. *Reprod Sci*. 2015;22(3):358-366. doi:10.1177/1933719114542020

106. Morelli SS, Mandal M, Goldsmith LT, Kashani BN, Ponzio NM. The maternal immune system during pregnancy and its influence on fetal development. *Res Rep Biol*. 2015;6:171-189. doi:10.2147/RRB.S80652

107. Chen L, Deng H, Cui H, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. 2017;9(6):7204-7218. doi:10.18632/oncotarget.23208

108. Zhang Q, Chen H, Wang Y, et al. Severe vitamin D deficiency in the first trimester is associated with placental inflammation in high-risk singleton pregnancy. *Clin Nutr*. 2019;38(4):1921-1926. doi:10.1016/j.clnu.2018.06.978

109. Bakacak M, Serin S, Ercan O, et al. Comparison of Vitamin D levels in cases with preeclampsia, eclampsia and healthy pregnant women. *Int J Clin Exp Med*. 2015;8(9):16280-16286.

110. Jabbour HN, Sales KJ, Catalano RD, Norman JE. Inflammatory pathways in female reproductive health and disease. *Reproduction*. 2009;138(6):903-919. doi:10.1530/REP-09-0247

111. Kany S, Vollrath JT, Relja B. Cytokines in Inflammatory Disease. *Int J Mol Sci*. 2019;20(23). doi:10.3390/ijms20236008

112. Schumacher A, Zenclussen AC. Human Chorionic Gonadotropin-Mediated Immune Responses That Facilitate Embryo Implantation and Placentation. *Front Immunol*. 2019;10:2896. doi:10.3389/fimmu.2019.02896

113. Guo J, Liu S, Wang P, Ren H, Li Y. Characterization of VDR and CYP27B1 expression in the endometrium during the menstrual cycle before embryo transfer: implications for endometrial receptivity. *Reprod Biol Endocrinol*. 2020;18(1):24. doi:10.1186/s12958-020-00579-y

114. Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin. *J Immunol Baltim Md 1950*. 2007;179(4):2060-2063. doi:10.4049/jimmunol.179.4.2060

115. Shah NM, Lai PF, Imami N, Johnson MR. Progesterone-Related Immune Modulation of Pregnancy and Labor. *Front Endocrinol*. 2019;10:198. doi:10.3389/fendo.2019.00198

116. Raghupathy R, Szekeres-Bartho J. Progesterone: A Unique Hormone with Immunomodulatory Roles in Pregnancy. *Int J Mol Sci*. 2022;23(3):1333. doi:10.3390/ijms23031333

117. Robinson DP, Klein SL. Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. *Horm Behav*. 2012;62(3):263-271. doi:10.1016/j.yhbeh.2012.02.023

118. Deng W, Sun R, Du J, et al. Prediction of miscarriage in first trimester by serum estradiol, progesterone and β-human chorionic gonadotropin within 9 weeks of gestation. *BMC Pregnancy Childbirth*. 2022;22(1):112. doi:10.1186/s12884-021-04158-w

119. Zhang W, Tian Y, Xie D, Miao Y, Liu J, Wang X. The impact of peak estradiol during controlled ovarian stimulation on the cumulative live birth rate of IVF/ICSI in non-PCOS patients. *J Assist Reprod Genet*. 2019;36(11):2333-2344. doi:10.1007/s10815-019-01568-w

120. Betz D, Fane K. Human Chorionic Gonadotropin. In: *StatPearls*. StatPearls Publishing; 2022. Accessed April 4, 2022. http://www.ncbi.nlm.nih.gov/books/NBK532950/

121. Sandovici I, Hoelle K, Angiolini E, Constância M. Placental adaptations to the maternal-fetal environment: implications for fetal growth and developmental programming. *Reprod Biomed Online*. 2012;25(1):68-89. doi:10.1016/j.rbmo.2012.03.017

122. Epigenetic processes during preeclampsia and effects on fetal development and chronic health | Clinical Science | Portland Press. Accessed May 13, 2022. https://portlandpress.com/clinsci/article-abstract/135/19/2307/229932/Epigenetic-processes-during-preeclampsia-and?redirectedFrom=fulltext

123. Robinson CJ, Alanis MC, Wagner CL, Hollis BW, Johnson DD. Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. *Am J Obstet Gynecol*. 2010;203(4):366.e1-6. doi:10.1016/j.ajog.2010.06.036

124. Kim SM, Kim JS. A Review of Mechanisms of Implantation. *Dev Reprod*. 2017;21(4):351-359. doi:10.12717/DR.2017.21.4.351

125. Ma W ge, Song H, Das SK, Paria BC, Dey SK. Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. *Proc Natl Acad Sci*. 2003;100(5):2963-2968. doi:10.1073/pnas.0530162100

126. Psychoyos A. Uterine Receptivity for Nidationa. *Ann N Y Acad Sci*. 1986;476(1):36-42. doi:10.1111/j.1749-6632.1986.tb20920.x

127. Burton GJ, Jauniaux E. Development of the Human Placenta and Fetal Heart: Synergic or Independent? *Front Physiol*. 2018;9:373. doi:10.3389/fphys.2018.00373

128. Trophoblastic Oxidative Stress in Relation to Temporal and Regional Differences in Maternal Placental Blood Flow in Normal and Abnormal Early Pregnancies - ScienceDirect. Accessed June 8, 2022. https://www.sciencedirect.com/science/article/pii/S0002944010638035

129. Benirschke K, Kaufmann P, Baergen R. *Pathology of the Human Placenta, 5th Edition*. Accessed June 26, 2022. https://link.springer.com/book/10.1007/b137920

130. Lykke JA, Dideriksen KL, Lidegaard Ø, Langhoff-Roos J. First-Trimester Vaginal Bleeding and Complications Later in Pregnancy. *Obstet Gynecol*. 2010;115(5):935-944. doi:10.1097/AOG.0b013e3181da8d38

131. Harville EW, Wilcox AJ, Baird DD, Weinberg CR. Vaginal bleeding in very early pregnancy. *Hum Reprod Oxf Engl*. 2003;18(9):1944-1947. doi:10.1093/humrep/deg379

132. Huxley RR. Nausea and vomiting in early pregnancy: its role in placental development. *Obstet Gynecol*. 2000;95(5):779-782. doi:10.1016/S0029-7844(99)00662-6

133. Hinkle SN, Mumford SL, Grantz KL, et al. Association of Nausea and Vomiting During Pregnancy With Pregnancy Loss. *JAMA Intern Med*. 2016;176(11):1621-1627. doi:10.1001/jamainternmed.2016.5641

134. Petitti DB. Nausea and Pregnancy Outcome. *Birth*. 1986;13(4):223-226. doi:10.1111/j.1523-536X.1986.tb01052.x

135. MacDonald EJ, Lepine S, Pledger M, Geller SE, Lawton B, Stone P. Pre-eclampsia causing severe maternal morbidity – A national retrospective review of preventability and opportunities for improved care. *Aust N Z J Obstet Gynaecol*. 2019;59(6):825-830. doi:10.1111/ajo.12971

136. Baker AM, Haeri S, Camargo CA, Espinola JA, Stuebe AM. A nested case-control study of midgestation vitamin D deficiency and risk of severe preeclampsia. *J Clin Endocrinol Metab*. 2010;95(11):5105-5109. doi:10.1210/jc.2010-0996

137. Tabesh M, Salehi-Abargouei A, Tabesh M, Esmaillzadeh A. Maternal vitamin D status and risk of pre-eclampsia: a systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2013;98(8):3165-3173. doi:10.1210/jc.2013-1257

138. Najat Nakishbandy BM, Barawi SAM. Level of C - reactive protein as an indicator for prognosis of premature uterine contractions. *J Prenat Med*. 2014;8(1-2):25-30.

139. Vitamin D status affects the relationship between lipid profile and high-sensitivity C-reactive protein | Nutrition & Metabolism | Full Text. Accessed November 29, 2021. https://nutritionandmetabolism.biomedcentral.com/articles/10.1186/s12986-020-00455-x

140. Azizieh F, Alyahya KO, Raghupathy R. Association between levels of vitamin D and inflammatory markers in healthy women. *J Inflamm Res*. 2016;9:51-57. doi:10.2147/JIR.S103298

141. Barker DJ, Osmond C. Diet and coronary heart disease in England and Wales during and after the second world war. *J Epidemiol Community Health*. Published online 1986. doi:10.1136/jech.40.1.37

142. Halfon N, Larson K, Lu M, Tullis E, Russ S. Lifecourse Health Development: Past, Present and Future. *Matern Child Health J*. 2014;18(2):344-365. doi:10.1007/s10995-013-1346-2

143. Shapira N. Prenatal Nutrition: a Critical Window of Opportunity for Mother and Child. *Womens Health*. 2008;4(6):639-656. doi:10.2217/17455057.4.6.639

144. Barker hypothesis. Oxford Reference. doi:10.1093/oi/authority.20110803095447459

145. Imbard A, Benoist JF, Blom HJ. Neural Tube Defects, Folic Acid and Methylation. *Int J Environ Res Public Health*. 2013;10(9):4352-4389. doi:10.3390/ijerph10094352

146. Dudley DJ. Immunoendocrinology of preterm labor: the link between corticotropin-releasing hormone and inflammation. *Am J Obstet Gynecol*. 1999;180(1 Pt 3):S251-256. doi:10.1016/s0002-9378(99)70711-8

147. Wagner CL, Hollis BW. The Implications of Vitamin D Status During Pregnancy on Mother and her Developing Child. *Front Endocrinol*. 2018;9:500. doi:10.3389/fendo.2018.00500

148. Aki M, Katsumata M, Yamanoi K, et al. The significance of clinical symptoms of subchorionic hematomas, “bleeding first”, to stratify the high-risk subgroup of very early preterm delivery. *Taiwan J Obstet Gynecol*. 2022;61(2):243-248. doi:10.1016/j.tjog.2022.02.011

149. Axelsen SM, Henriksen TB, Hedegaard M, Secher NJ. Characteristics of vaginal bleeding during pregnancy. *Eur J Obstet Gynecol Reprod Biol*. 1995;63(2):131-134. doi:10.1016/0301-2115(95)02236-8

150. DeVilbiss EA, Naimi AI, Mumford SL, et al. Vaginal bleeding and nausea in early pregnancy as predictors of clinical pregnancy loss. *Am J Obstet Gynecol*. 2020;223(4):570.e1-570.e14. doi:10.1016/j.ajog.2020.04.002

151. Halfon N, Forrest CB. The Emerging Theoretical Framework of Life Course Health Development. In: Halfon N, Forrest CB, Lerner RM, Faustman EM, eds. *Handbook of Life Course Health Development*. Springer; 2018. Accessed February 24, 2022. http://www.ncbi.nlm.nih.gov/books/NBK543722/

152. Lu MC, Halfon N. Racial and ethnic disparities in birth outcomes: a life-course perspective. *Matern Child Health J*. 2003;7(1):13-30. doi:10.1023/a:1022537516969

153. Geronimus AT. Black/white differences in the relationship of maternal age to birthweight: A population-based test of the weathering hypothesis. *Soc Sci Med*. 1996;42(4):589-597. doi:10.1016/0277-9536(95)00159-X

154. Bodnar LM, Simhan HN. Vitamin D may be a link to black-white disparities in adverse birth outcomes. *Obstet Gynecol Surv*. 2010;65(4):273-284. doi:10.1097/OGX.0b013e3181dbc55b

155. Vitamin D in the Middle East and North Africa. Accessed November 14, 2021. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6020111/

156. Hajizadeh S, Rankin Shary J, Gayle Reed S, Lynn Wagner C. The prevalence of hypovitaminosis D and its risk factors in pregnant women and their newborns in the Middle East: A systematic review. *Int J Reprod Biomed*. 2019;17(10):685-708. doi:10.18502/ijrm.v17i10.5284

157. Hamedanian L, Badehnoosh B, Razavi-Khorasani N, Mohammadpour Z, Mozaffari-Khosravi H. Evaluation of vitamin D status, parathyroid hormone, and calcium among Iranian pregnant women with preeclampsia: A case-control study. *Int J Reprod Biomed*. 2019;17(11):831-840. doi:10.18502/ijrm.v17i10.5494

158. DAGitty v3.0. Accessed April 24, 2023. http://www.dagitty.net/dags.html

159. Thamattoor A. Race/ethnicity differences in vitamin D levels and impact on cardiovascular disease, bone health, and oral health. Published online January 4, 2021:2021.01.02.21249149. doi:10.1101/2021.01.02.21249149

160. Williams DR, Priest N, Anderson N. Understanding Associations between Race, Socioeconomic Status and Health: Patterns and Prospects. *Health Psychol Off J Div Health Psychol Am Psychol Assoc*. 2016;35(4):407-411. doi:10.1037/hea0000242

161. Grant WB. Vitamin D Status May Help Explain Maternal Race and Ethnic Factors in Primary Cesarean Section Delivery. *Am J Perinatol*. Published online April 24, 2020. doi:10.1055/s-0040-1709494

162. Kenny LC, Lavender T, McNamee R, O'Neill SM, Mills T, Khashan AS. Advanced Maternal Age and Adverse Pregnancy Outcome: Evidence from a Large Contemporary Cohort. *PLoS ONE*. 2013;8(2):e56583. doi:10.1371/journal.pone.0056583

163. Singh J, Hariharan C, Bhaumik D. Role of vitamin D in reducing the risk of preterm labour. *Int J Reprod Contracept Obstet Gynecol*. 2017;4(1):86-93.

164. Dieke AC, Zhang Y, Kissin DM, Barfield WD, Boulet SL. Disparities in Assisted Reproductive Technology Utilization by Race and Ethnicity, United States, 2014: A Commentary. *J Womens Health 2002*. 2017;26(6):605-608. doi:10.1089/jwh.2017.6467

165. Ahmed F, Khosravi-Boroujeni H, Khan MR, Roy AK, Raqib R. Prevalence and Predictors of Vitamin D Deficiency and Insufficiency among Pregnant Rural Women in Bangladesh. *Nutrients*. 2021;13(2):449. doi:10.3390/nu13020449

166. BURKUŠ J, KAČMAROVÁ M, KUBANDOVÁ J, et al. Stress exposure during the preimplantation period affects blastocyst lineages and offspring development. *J Reprod Dev*. 2015;61(4):325-331. doi:10.1262/jrd.2015-012

167. Rebholz SL, Jones T, Burke KT, et al. Multiparity leads to obesity and inflammation in mothers and obesity in male offspring. *Am J Physiol - Endocrinol Metab*. 2012;302(4):E449-E457. doi:10.1152/ajpendo.00487.2011

168. Rosenberg N, Daviglus ML, DeVon HA, Park CG, Eldeirawi K. The Association between Parity and Inflammation among Mexican-American Women of Reproductive Age Varies by Acculturation Level: Results of the National Health and Nutrition Examination Survey (1999–2006). *Womens Health Issues Off Publ Jacobs Inst Womens Health*. 2017;27(4):485-492. doi:10.1016/j.whi.2017.03.002

169. Hasan HA, Barber TM, Cheaib S, Coussa A. Preconception Vitamin D Level and In Vitro Fertilization: Pregnancy Outcome. *Endocr Pract Off J Am Coll Endocrinol Am Assoc Clin Endocrinol*. Published online January 12, 2023:S1530-891X(23)00016-2. doi:10.1016/j.eprac.2023.01.005

170. Al-Ajlan A, Al-Musharaf S, Fouda MA, et al. Lower vitamin D levels in Saudi pregnant women are associated with higher risk of developing GDM. *BMC Pregnancy Childbirth*. 2018;18(1):86. doi:10.1186/s12884-018-1723-3

171. Imai K. Parity-based assessment of anemia and iron deficiency in pregnant women. *Taiwan J Obstet Gynecol*. 2020;59(6):838-841. doi:10.1016/j.tjog.2020.09.010

172. Parity related changes in obesity and some antioxidant vitam... : Nigerian Journal of Clinical Practice. Accessed April 25, 2023. https://journals.lww.com/njcp/Fulltext/2012/15040/Parity_related_changes_in_obesity_and_some.2.aspx

173. Walle BM, Adekunle AO, Arowojolu AO, Dugul TT, Mebiratie AL. Micronutrients Deficiency and Their Associations with Pregnancy Outcomes: A Review . *Nutr Diet Suppl*. 2020;12:237-254. doi:10.2147/NDS.S274646

174. Nascimento SL, Surita FG, Godoy AC, Kasawara KT, Morais SS. Physical Activity Patterns and Factors Related to Exercise during Pregnancy: A Cross Sectional Study. *PLoS ONE*. 2015;10(6):e0128953. doi:10.1371/journal.pone.0128953

175. Orces C. The Association between Body Mass Index and Vitamin D Supplement Use among Adults in the United States. *Cureus*. 11(9). doi:10.7759/cureus.5721

176. Wang J, Geng L. Effects of Socioeconomic Status on Physical and Psychological Health: Lifestyle as a Mediator. *Int J Environ Res Public Health*. 2019;16(2):281. doi:10.3390/ijerph16020281

177. Akpene Amenya PC, Annan RA, Apprey C, Agbley EN. The relationship between nutrition and physical activity knowledge and body mass index-for-age of school-aged children in selected schools in Ghana. *Heliyon*. 2021;7(11):e08298. doi:10.1016/j.heliyon.2021.e08298

178. Zhang J, Cao ZB. Exercise: A Possibly Effective Way to Improve Vitamin D Nutritional Status. *Nutrients*. 2022;14(13):2652. doi:10.3390/nu14132652

179. Cárdenas Fuentes G, Bawaked RA, Martínez González MÁ, et al. Association of physical activity with body mass index, waist circumference and incidence of obesity in older adults. *Eur J Public Health*. 2018;28(5):944-950. doi:10.1093/eurpub/cky030

180. Gladwell VF, Brown DK, Wood C, Sandercock GR, Barton JL. The great outdoors: how a green exercise environment can benefit all. *Extreme Physiol Med*. 2013;2:3. doi:10.1186/2046-7648-2-3

181. Timeva T, Shterev A, Kyurkchiev S. Recurrent Implantation Failure: The Role of the Endometrium. *J Reprod Infertil*. 2014;15(4):173-183.

182. Suryadinata RV, Wirjatmadi B, Adriani M, Lorensia A. Effect of age and weight on physical activity. *J Public Health Res*. 2020;9(2):1840. doi:10.4081/jphr.2020.1840

183. Winter JE, MacInnis RJ, Nowson CA. The Influence of Age the BMI and All-Cause Mortality Association: A Meta-Analysis. *J Nutr Health Aging*. 2017;21(10):1254-1258. doi:10.1007/s12603-016-0837-4

184. Ahrens K, Silver RM, Mumford SL, et al. Complications and Safety of Preconception Low-Dose Aspirin Among Women With Prior Pregnancy Losses. *Obstet Gynecol*. 2016;127(4):689-698. doi:10.1097/AOG.0000000000001301

185. Naimi AI, Perkins NJ, Sjaarda LA, et al. The effect of preconception-initiated low-dose aspirin on hCG pregnancy, pregnancy loss, and live birth: per-protocol analysis of a randomized trial. *Ann Intern Med*. 2021;174(5):595-601. doi:10.7326/M20-0469

186. Connell MT, Sjaarda LA, Radin RG, et al. The Effects of Aspirin in Gestation and Reproduction (EAGeR) Trial: A Story of Discovery. *Semin Reprod Med*. 2017;35(4):344-352. doi:10.1055/s-0037-1606384

187. Robinson LD, Jewell NP. Some Surprising Results about Covariate Adjustment in Logistic Regression Models. *Int Stat Rev Rev Int Stat*. 1991;59(2):227-240. doi:10.2307/1403444

188. Cadavid AP. Aspirin: The Mechanism of Action Revisited in the Context of Pregnancy Complications. *Front Immunol*. 2017;8. Accessed June 8, 2022. https://www.frontiersin.org/article/10.3389/fimmu.2017.00261

189. Van Doorn R, Mukhtarova N, Flyke IP, et al. Dose of aspirin to prevent preterm preeclampsia in women with moderate or high-risk factors: A systematic review and meta-analysis. *PLoS ONE*. 2021;16(3):e0247782. doi:10.1371/journal.pone.0247782

190. Gupta T, Singh S, Gupta S, Gupta N. Normal Implantation, Placentation, and Fetal Development. In: Mehta S, Gupta B, eds. *Recurrent Pregnancy Loss*. Springer; 2018:13-40. doi:10.1007/978-981-10-7338-0_2

191. Li Y, Wang E, Huang S, et al. Autoantibodies in association with subchorionic haematoma in early pregnancy. *Ann Med*. 2021;53(1):841-847. doi:10.1080/07853890.2021.1936150

192. C-Reactive Protein (CRP) | Lab Tests Online. Accessed May 25, 2021. https://labtestsonline.org/tests/c-reactive-protein-crp

193. Gandevani S. Association of high-sensitivity C-reactive protein serum levels in early pregnancy with the severity of preeclampsia and fetal birth weight. Accessed April 13, 2022. https://pubmed.ncbi.nlm.nih.gov/22700647/

194. Haram K, Mortensen JH, Myking O, Roald B, Magann EF, Morrison JC. Early development of the human placenta and pregnancy complications. *J Matern Fetal Neonatal Med*. 2020;33(20):3538-3545. doi:10.1080/14767058.2019.1578745

195. Freeman J, Wilson K, Spears R, Shalhoub V, Sibley P. Performance evaluation of four 25-hydroxyvitamin D assays to measure 25-hydroxyvitamin D2. *Clin Biochem*. 2015;48(16-17):1097-1104. doi:10.1016/j.clinbiochem.2015.05.021

196. Fraser WD, Milan AM. Vitamin D assays: past and present debates, difficulties, and developments. *Calcif Tissue Int*. 2013;92(2):118-127. doi:10.1007/s00223-012-9693-3

197. Whitcomb BW, Perkins NJ, Albert PS, Schisterman EF. Treatment of batch in the detection, calibration, and quantification of immunoassays in large-scale epidemiologic studies. *Epidemiol Camb Mass*. 2010;21 Suppl 4:S44-50. doi:10.1097/EDE.0b013e3181dceac2

198. Hasan R, Jonsson Funk ML, Herring AH, Olshan AF, Hartmann KE, Baird DD. Accuracy of Reporting Bleeding During Pregnancy. *Paediatr Perinat Epidemiol*. 2010;24(1):31-34. doi:10.1111/j.1365-3016.2009.01089.x

199. Wikström L, Nilsson M, Broström A, Eriksson K. Patients' self-reported nausea: Validation of the Numerical Rating Scale and of a daily summary of repeated Numerical Rating Scale scores. *J Clin Nurs*. Published online 2019. doi:10.1111/jocn.14705

200. Craig CL, Marshall AL, Sjöström M, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;35(8):1381-1395. doi:10.1249/01.MSS.0000078924.61453.FB

201. Flannagan KSJ, Mumford SL. Preconception exposures and postconception outcomes: selection bias in action. *Fertil Steril*. 2020;114(6):1172-1173. doi:10.1016/j.fertnstert.2020.10.057

202. Sjaarda LA, Radin RG, Silver RM, et al. Preconception Low-Dose Aspirin Restores Diminished Pregnancy and Live Birth Rates in Women With Low-Grade Inflammation: A Secondary Analysis of a Randomized Trial. *J Clin Endocrinol Metab*. 2017;102(5):1495-1504. doi:10.1210/jc.2016-2917

203. Hewison M. Vitamin D and immune function: autocrine, paracrine or endocrine? *Scand J Clin Lab Investig Suppl*. 2012;243:92-102. doi:10.3109/00365513.2012.682862

204. Lagishetty V, Liu NQ, Hewison M. Vitamin D metabolism and innate immunity. *Mol Cell Endocrinol*. 2011;347(1-2):97-105. doi:10.1016/j.mce.2011.04.015

205. Ozkan S, Jindal S, Greenseid K, et al. Replete vitamin D stores predict reproductive success following in vitro fertilization. *Fertil Steril*. 2010;94(4):1314-1319. doi:10.1016/j.fertnstert.2009.05.019

206. Farzadi L, Khayatzadeh Bidgoli H, Ghojazadeh M, et al. Correlation between follicular fluid 25-OH vitamin D and assisted reproductive outcomes. *Iran J Reprod Med*. 2015;13(6):361-366.

207. Mumford SL, Garbose RA, Kim K, et al. Preconception serum 25 hydroxyvitamin D levels are associated with increased live birth and reduced pregnancy loss: a prospective cohort study. *Lancet Diabetes Endocrinol*. 2018;6(9):725-732. doi:10.1016/S2213-8587(18)30153-0

208. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab*. 2007;92(9):3517-3522. doi:10.1210/jc.2007-0718

209. French JI, McGregor JA, Draper D, Parker R, McFee J. Gestational bleeding, bacterial vaginosis, and common reproductive tract infections: risk for preterm birth and benefit of treatment. *Obstet Gynecol*. 1999;93(5 Pt 1):715-724. doi:10.1016/s0029-7844(98)00557-2

210. Williams MA, Hickok DE, Zingheim RW, Mittendorf R, Kimelman J, Mahony BS. Low birth weight and preterm delivery in relation to early-gestation vaginal bleeding and elevated maternal serum alpha-fetoprotein. *Obstet Gynecol*. 1992;80(5):745-749.

211. Gómez R, Romero R, Nien JK, et al. Idiopathic vaginal bleeding during pregnancy as the only clinical manifestation of intrauterine infection. *J Matern-Fetal Neonatal Med Off J Eur Assoc Perinat Med Fed Asia Ocean Perinat Soc Int Soc Perinat Obstet*. 2005;18(1):31-37. doi:10.1080/14767050500217863

212. Hasan R, Baird DD, Herring AH, Olshan AF, Jonsson Funk ML, Hartmann KE. Patterns and predictors of vaginal bleeding in the first trimester of pregnancy. *Ann Epidemiol*. 2010;20(7):524-531. doi:10.1016/j.annepidem.2010.02.006

213. Kahan BC, Rehal S, Cro S. Risk of selection bias in randomised trials. *Trials*. 2015;16. doi:10.1186/s13063-015-0920-x

214. Estimating the Relative Risk in Cohort Studies and Clinical Trials of Common Outcomes | American Journal of Epidemiology | Oxford Academic. Accessed April 18, 2023. https://academic.oup.com/aje/article/157/10/940/290159

215. So S, Mochizuki O, Yamaguchi W, Murabayashi N, Miyano N, Tawara F. Impact of subchorionic hematoma in early pregnancy on obstetric complications: A retrospective cohort study in women who had live births after frozen-thawed embryo transfer. *Reprod Med Biol*. 2020;19(4):398-403. doi:10.1002/rmb2.12343

216. Csapo AI, Pulkkinen M. Indispensability of the human corpus luteum in the maintenance of early pregnancy. Luteectomy evidence. *Obstet Gynecol Surv*. 1978;33(2):69-81. doi:10.1097/00006254-197802000-00001

217. Inman ER, Miranian DC, Stevenson MJ, Kobernik EK, Moravek MB, Schon SB. Outcomes of subchorionic hematoma-affected pregnancies in the infertile population. *Int J Gynecol Obstet*. 2022;159(3):743-750. doi:10.1002/ijgo.14162

218. Naert MN, Khadraoui H, Muniz Rodriguez A, Naqvi M, Fox NS. Association Between First-Trimester Subchorionic Hematomas and Pregnancy Loss in Singleton Pregnancies. *Obstet Gynecol*. 2019;134(2):276. doi:10.1097/AOG.0000000000003360

219. Linden A, Mathur MB, VanderWeele TJ. Conducting sensitivity analysis for unmeasured confounding in observational studies using E-values: The evalue package. *Stata J*. 2020;20(1):162-175. doi:10.1177/1536867X20909696

220. VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. *Ann Intern Med*. 2017;167(4):268. doi:10.7326/M16-2607

221. Bhadsavle SS, Golding MC. Paternal epigenetic influences on placental health and their impacts on offspring development and disease. *Front Genet*. 2022;13:1068408. doi:10.3389/fgene.2022.1068408

222. Ding T, Mokshagundam S, Rinaudo PF, Osteen KG, Bruner-Tran KL. Paternal developmental toxicant exposure is associated with epigenetic modulation of sperm and placental Pgr and Igf2 in a mouse model†. *Biol Reprod*. 2018;99(4):864-876. doi:10.1093/biolre/ioy111

223. Hoek J, Steegers-Theunissen RPM, Willemsen SP, Schoenmakers S. Paternal Folate Status and Sperm Quality, Pregnancy Outcomes, and Epigenetics: A Systematic Review and Meta-Analysis. *Mol Nutr Food Res*. 2020;64(9):1900696. doi:10.1002/mnfr.201900696

224. Khoshkerdar A, Eryasar E, Morgan HL, Watkins AJ. REPRODUCTIVE TOXICOLOGY: Impacts of paternal environment and lifestyle on maternal health during pregnancy. *Reproduction*. 2021;162(5):F101-F109. doi:10.1530/REP-20-0605

225. Murugappan G, Li S, Leonard SA, Winn VD, Druzin ML, Eisenberg ML. Association of preconception paternal health and adverse maternal outcomes among healthy mothers. *Am J Obstet Gynecol MFM*. 2021;3(5):100384. doi:10.1016/j.ajogmf.2021.100384

226. Stuppia L, Franzago M, Ballerini P, Gatta V, Antonucci I. Epigenetics and male reproduction: the consequences of paternal lifestyle on fertility, embryo development, and children lifetime health. *Clin Epigenetics*. 2015;7(1):120. doi:10.1186/s13148-015-0155-4

227. Mitsuda N, Eitoku M, Yamasaki K, et al. Nausea and vomiting during pregnancy associated with lower incidence of preterm births: the Japan Environment and Children's Study (JECS). *BMC Pregnancy Childbirth*. 2018;18(1):268. doi:10.1186/s12884-018-1911-1

228. Chan RL, Olshan AF, Savitz DA, et al. Severity and duration of nausea and vomiting symptoms in pregnancy and spontaneous abortion. *Hum Reprod Oxf Engl*. 2010;25(11):2907-2912. doi:10.1093/humrep/deq260

229. Lacroix R, Eason E, Melzack R. Nausea and vomiting during pregnancy: A prospective study of its frequency, intensity, and patterns of change. *Am J Obstet Gynecol*. 2000;182(4):931-937. doi:10.1016/s0002-9378(00)70349-8

230. Jennings LK, Mahdy H. Hyperemesis Gravidarum. In: *StatPearls*. StatPearls Publishing; 2023. Accessed April 26, 2023. http://www.ncbi.nlm.nih.gov/books/NBK532917/

231. Lacasse A, Rey E, Ferreira E, Morin C, Bérard A. Epidemiology of nausea and vomiting of pregnancy: prevalence, severity, determinants, and the importance of race/ethnicity. *BMC Pregnancy Childbirth*. 2009;9:26. doi:10.1186/1471-2393-9-26

232. Gazmararian JA, Petersen R, Jamieson DJ, et al. Hospitalizations during pregnancy among managed care enrollees. *Obstet Gynecol*. 2002;100(1):94-100. doi:10.1016/s0029-7844(02)02024-0

233. Trovik J, Vikanes Å. Hyperemesis Gravidarum is associated with substantial economic burden in addition to severe physical and psychological suffering. *Isr J Health Policy Res*. 2016;5:43. doi:10.1186/s13584-016-0099-y

234. Mitchell-Jones N, Gallos I, Farren J, Tobias A, Bottomley C, Bourne T. Psychological morbidity associated with hyperemesis gravidarum: a systematic review and meta-analysis. *BJOG Int J Obstet Gynaecol*. 2017;124(1):20-30. doi:10.1111/1471-0528.14180

235. Spruill SC, Kuller JA. Hyperemesis gravidarum complicated by Wernicke's encephalopathy. *Obstet Gynecol*. 2002;99(5 Pt 2):875-877. doi:10.1016/s0029-7844(01)01603-9

236. Togay-Işikay C, Yiğit A, Mutluer N. Wernicke's encephalopathy due to hyperemesis gravidarum: an under-recognised condition. *Aust N Z J Obstet Gynaecol*. 2001;41(4):453-456. doi:10.1111/j.1479-828x.2001.tb01330.x

237. Popa SL, Barsan M, Caziuc A, et al. Life-threatening complications of hyperemesis gravidarum. *Exp Ther Med*. 2021;21(6):642. doi:10.3892/etm.2021.10074

238. Shahrook S, Ota E, Hanada N, Sawada K, Mori R. Vitamin K supplementation during pregnancy for improving outcomes: a systematic review and meta-analysis. *Sci Rep*. 2018;8:11459. doi:10.1038/s41598-018-29616-y

239. Vikanes ÅV, Støer NC, Magnus P, Grjibovski AM. Hyperemesis gravidarum and pregnancy outcomes in the Norwegian mother and child cohort – a cohort study. *BMC Pregnancy Childbirth*. 2013;13(1):169. doi:10.1186/1471-2393-13-169

240. Moberg T, Veeken LV der, Persad E, Hansson SR, Bruschettini M. Placenta-associated adverse pregnancy outcomes in women experiencing mild or severe hyperemesis gravidarum – a systematic review and meta-analysis. Published online November 11, 2022:2022.11.09.22281870. doi:10.1101/2022.11.09.22281870

241. Varela P, Deltsidou A. Hyperemesis gravidarum and neonatal outcomes: A systematic review of observational studies. *Taiwan J Obstet Gynecol*. 2021;60(3):422-432. doi:10.1016/j.tjog.2021.03.007

242. Fairweather DV. Nausea and vomiting in pregnancy. *Am J Obstet Gynecol*. 1968;102(1):135-175. doi:10.1016/0002-9378(68)90445-6

243. Walch A, Duke M, Auty T, Wong A. Profound Hypokalaemia Resulting in Maternal Cardiac Arrest: A Catastrophic Complication of Hyperemesis Gravidarum? *Case Rep Obstet Gynecol*. 2018;2018:4687587. doi:10.1155/2018/4687587

244. Patel S, Alvarez-Guaita A, Melvin A, et al. GDF15 Provides an Endocrine Signal of Nutritional Stress in Mice and Humans. *Cell Metab*. 2019;29(3):707-718.e8. doi:10.1016/j.cmet.2018.12.016

245. Fejzo MS, Trovik J, Grooten IJ, et al. Nausea and vomiting of pregnancy and hyperemesis gravidarum. *Nat Rev Dis Primer*. 2019;5(1):1-17. doi:10.1038/s41572-019-0110-3

246. Nijsten K, van der Minnen L, Wiegers HMG, et al. Hyperemesis gravidarum and vitamin K deficiency: a systematic review. *Br J Nutr*. 128(1):30-42. doi:10.1017/S0007114521002865

247. Panesar NS, Li CY, Rogers MS. Are thyroid hormones or hCG responsible for hyperemesis gravidarum? A matched paired study in pregnant Chinese women. *Acta Obstet Gynecol Scand*. 2001;80(6):519-524. doi:10.1034/j.1600-0412.2001.080006519.x

248. yıldız gazi. Hyperemesis gravidarum and its relationships with placental thickness, PAPP-A and free beta-HCG: a case control study. *South Clin Istanb Eurasia*. Published online January 1, 2021. doi:10.14744/scie.2021.93546

249. Bolin M, Åkerud H, Cnattingius S, Stephansson O, Wikström A. Hyperemesis gravidarum and risks of placental dysfunction disorders: a population-based cohort study. *Bjog*. 2013;120(5):541-547. doi:10.1111/1471-0528.12132

250. García-Ferreyra J, Carpio J, Zambrano M, Valdivieso-Mejía P, Valdivieso-Rivera P. Overweight and obesity significantly reduce pregnancy, implantation, and live birth rates in women undergoing In Vitro Fertilization procedures. *JBRA Assist Reprod*. 2021;25(3):394-402. doi:10.5935/1518-0557.20200105

251. Ridker PM, Cook N. Clinical usefulness of very high and very low levels of C-reactive protein across the full range of Framingham Risk Scores. *Circulation*. 2004;109(16):1955-1959. doi:10.1161/01.CIR.0000125690.80303.A8

252. Pearson TA, Mensah GA, Alexander RW, et al. Markers of Inflammation and Cardiovascular Disease. *Circulation*. 2003;107(3):499-511. doi:10.1161/01.CIR.0000052939.59093.45

253. Effect of vitamin D supplementation in the reduce risk of preeclampsia in nulliparous women - PMC. Accessed April 19, 2023. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4770602/

254. Lisonkova S, Joseph KS. Incidence of preeclampsia: risk factors and outcomes associated with early- versus late-onset disease. *Am J Obstet Gynecol*. 2013;209(6):544.e1-544.e12. doi:10.1016/j.ajog.2013.08.019

255. Rasmussen S, Irgens LM, Espinoza J. Maternal obesity and excess of fetal growth in pre-eclampsia. *BJOG Int J Obstet Gynaecol*. 2014;121(11):1351-1357. doi:10.1111/1471-0528.12677

256. Shao Y, Qiu J, Huang H, et al. Pre-pregnancy BMI, gestational weight gain and risk of preeclampsia: a birth cohort study in Lanzhou, China. *BMC Pregnancy Childbirth*. 2017;17(1):400. doi:10.1186/s12884-017-1567-2

257. Bodnar LM, Catov JM, Klebanoff MA, Ness RB, Roberts JM. Prepregnancy body mass index and the occurrence of severe hypertensive disorders of pregnancy. *Epidemiol Camb Mass*. 2007;18(2):234-239. doi:10.1097/01.ede.0000254119.99660.e7

258. Bodnar LM, Ness RB, Markovic N, Roberts JM. The risk of preeclampsia rises with increasing prepregnancy body mass index. *Ann Epidemiol*. 2005;15(7):475-482. doi:10.1016/j.annepidem.2004.12.008

259. Artemniak-Wojtowicz D, Kucharska AM, Pyrżak B. Obesity and chronic inflammation crosslinking. *Cent-Eur J Immunol*. 2020;45(4):461-468. doi:10.5114/ceji.2020.103418

260. de Oliveira LF, de Azevedo LG, da Mota Santana J, de Sales LPC, Pereira-Santos M. Obesity and overweight decreases the effect of vitamin D supplementation in adults: systematic review and meta-analysis of randomized controlled trials. *Rev Endocr Metab Disord*. 2020;21(1):67-76. doi:10.1007/s11154-019-09527-7

261. Denison FC, Roberts KA, Barr SM, Norman JE. Obesity, pregnancy, inflammation, and vascular function. *Reproduction*. 2010;140(3):373-385. doi:10.1530/REP-10-0074

262. Mbah AK, Kornosky JL, Kristensen S, et al. Super-obesity and risk for early and late pre-eclampsia. *BJOG Int J Obstet Gynaecol*. 2010;117(8):997-1004. doi:10.1111/j.1471-0528.2010.02593.x

263. Exploring the Subtleties of Inverse Probability Weighting and Marginal Structural Models - PubMed. Accessed April 18, 2023. https://pubmed.ncbi.nlm.nih.gov/29384789/

264. Cole SR, Hernán MA. Constructing inverse probability weights for marginal structural models. *Am J Epidemiol*. 2008;168(6):656-664. doi:10.1093/aje/kwn164

265. Name Y. E-value calculator. E-value calculator. Accessed April 18, 2023. https://www.evalue-calculator.com//evalue/

266. Ates S, Sevket O, Ozcan P, Ozkal F, Kaya MO, Dane B. Vitamin D status in the first-trimester: effects of Vitamin D deficiency on pregnancy outcomes. *Afr Health Sci*. 2016;16(1):36-43. doi:10.4314/ahs.v16i1.5

267. Timmermans YEG, van de Kant KDG, Krumeich JSM, et al. Socio-ecological determinants of lifestyle behavior of women with overweight or obesity before, during and after pregnancy: qualitative interview analysis in the Netherlands. *BMC Pregnancy Childbirth*. 2020;20. doi:10.1186/s12884-020-2786-5

268. Okafor UB, Goon DT. Applying the Ecological Model to understand pregnant women's perspectives on the modifiable constraints to physical activity during pregnancy. *Medicine (Baltimore)*. 2020;99(49):e23431. doi:10.1097/MD.0000000000023431

269. Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. *J Nutr*. 2007;137(2):447-452. doi:10.1093/jn/137.2.447

270. Powe CE, Evans MK, Wenger J, et al. Vitamin D–Binding Protein and Vitamin D Status of Black Americans and White Americans. *N Engl J Med*. 2013;369(21):1991-2000. doi:10.1056/NEJMoa1306357

271. What You Should Know About the Pregnant Workers Fairness Act. US EEOC. Accessed July 2, 2023. https://www.eeoc.gov/wysk/what-you-should-know-about-pregnant-workers-fairness-act

272. Thoma ME, Drew LB, Hirai AH, Kim TY, Fenelon A, Shenassa ED. Black-White Disparities in Preterm Birth: Geographic, Social, and Health Determinants. *Am J Prev Med*. 2019;57(5):675-686. doi:10.1016/j.amepre.2019.07.007

273. Black pregnant people are less likely to be recommended aspirin prophylaxis for preeclampsia - American Journal of Obstetrics & Gynecology. Accessed June 16, 2023. https://www.ajog.org/article/S0002-9378(21)02047-0/fulltext

274. Burris HH, Lorch SA, Kirpalani H, Pursley DM, Elovitz MA, Clougherty JE. Racial disparities in preterm birth in USA: a biosensor of physical and social environmental exposures. *Arch Dis Child*. 2019;104(10):931-935. doi:10.1136/archdischild-2018-316486

275. Mohamed SA, Thota C, Browne PC, Diamond MP, Al-Hendy A. Why is Preterm Birth Stubbornly Higher in African-Americans? *Obstet Gynecol Int J*. 2014;1(3):00019. doi:10.15406/ogij.2014.01.00019

276. Vitamin D–Binding Protein and Vitamin D Status of Black Americans and White Americans | NEJM. Accessed June 22, 2021. https://www.nejm.org/doi/full/10.1056/NEJMoa1306357