Examining Complex Relationships Between Alcohol Exposure and Miscarriage

By

Alexandra C. Sundermann

Dissertation

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSPHY

in

Epidemiology

May 10, 2019

Nashville, Tennessee

Approved:

Katherine E. Hartmann, MD, PhD

Digna R. Velez Edwards, PhD, MS

James C. Slaughter, DrPH

Pingsheng Wu, PhD

ACKNOWLEDGEMENTS

This research was made possible by the support of the National Institute of Health (grants R01HD043883, R01HD049675, F30HD094345, T32GM07347, UL1TR000445) and the American Water Works Association Research Foundation (grants 2579). I especially want to thank the Eunice Kennedy Shriver National Institute of Child and Human Development for funding my individual predoctoral fellowship. I am indebted to the women who participated in *Right from the Start* for their willingness to share personal information throughout their pregnancies to advance understanding of human health.

A deepest thank you to my dissertation committee: Drs. Katherine Hartmann, Digna Velez Edwards, Christopher Slaughter, and Pingsheng Wu. Your combined expertise has elevated this work and your thoughtful mentorship has shaped who I am as scientist. Dr. Hartmann, I admire the drive, passion, and thoughtfulness you exemplify in both the personal and professional aspects of your life and appreciate having you as an example during this formative stage of my training. Thank you for providing me many opportunities to grow as an investigator and equipping me with the necessary tools for success. Dr. Velez Edwards, thank you for being such a reliable source of helpful insight and encouragement. I've have greatly benefited from your thoughtful mentorship. Thank you to Dr. Slaughter for teaching me how to question assumptions and test uncertainties for myself. I have appreciated your patient instruction. Thank you, Dr. Wu, for your investment in finding the best solutions to challenging problems, your commitment to excellence, and your sincere enthusiasm for discussing my work.

Thank you to the Medical Scientist Training Program, in which I found support and community that made Vanderbilt feel like home. Thank you to the program's leadership team, who made my training at Vanderbilt possible by paving the way for a dual-degree in epidemiology.

Thank you to my lab members (Ayush, Jackie, Michael, Kathy, and Brian) for your comradery and encouragement through the successes and disappointments of academia. To Eric and Sarah, thank you for prioritizing my questions whenever I needed help and for providing valuable insight into the study. Many thanks to Helen and Adrienne whose administrative prowess made my life much easier and whose conversation always brightened my day.

Thank you to Drs. Baker and Abell for the potential you saw in me, which emboldened me to pursue this career path. You taught me that scholarship is a great privilege and I hope I steward my opportunities in a way that makes you proud.

Thank you to the people who make up my community in Nashville. I'm humbled and blessed by the friendships forged here.

Thank you to my parents and sister for loving and supporting me in every stage in life. I've learned the value of perseverance and faith from your example. Thank you to Bill, Gretchen, and Evan for always being enthusiastic to learn more about my work.

To my Emery, you have filled this part of my life with a special lightness and joy. I deeply desire to leverage my abilities and opportunities to positively change the world. I hope you do the same. I can't wait to see what the impact you will have on this earth.

To my husband, Carter, you have been my greatest friend in this endeavor. You have made it your mission to provide me every opportunity to prosper and have done so with great kindness and grace. I am forever grateful for your love and constancy and count it my life's greatest blessing to be your wife and partner.

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15-PGDH	15-hydroxyprostoglandin dehydrogenase
ALDH	aldehyde dehydrogenase
ADH1C	alcohol dehydrogenase 1C
BMI	body mass index
BPM	beats per minute
CATI	computer-assisted telephone interview
CDC	Centers for Disease Control and Prevention
CI	confidence interval
cm	centimeter
D&C	dilation and curettage
DAG	directed acyclic graph
DNA	deoxyribonucleic acid
DNBC	Danish National Birth Cohort
EGA	estimated gestational age
FASD	fetal alcohol spectrum disorder
FHR	fetal heart rate
GA	gestational age
GAAD	gestational age at arrest of development
LMP	last menstrual period
HR	hazard ratio
IQR	inter-quartile range
KPNC	Kaiser Permanente Northern California
MOOSE	Meta-Analysis of Observational Studies in Epidemiology

mm	millimeter
NC	North Carolina
OR	odds ratio
PECOTS	population, exposure, comparison, outcome, timing, study design
PLA2	phospholipase A2
PRISMA	Preferred Reporting Item for Systematic Reviews and Meta-Analyses
REDCap	Research Electronic Data Capture
RFTS	*Right from the Start*
RMSE	root mean squared error
RR	risk ratio
SAB	spontaneous abortion
SD	standard deviation
SE	standard error
SNP	single nucleotide polymorphism
TN	Tennessee
TX	Texas
wk	week

I. SPECIFIC AIMS

More than half of women use alcohol in the first trimester of pregnancy though the Centers for Disease Control and Prevention (CDC) recommends women who intend to or could become pregnant to abstain.[1-3] While 70 to 85% of women refrain from alcohol consumption once pregnancy is confirmed, exposure prior to pregnancy is common regardless of pregnancy intention.[4-8] Understanding effects of alcohol exposure early in gestation is critical.

Miscarriage occurs in approximately one in six recognized pregnancies and evidence about early pregnancy alcohol exposure and miscarriage is limited. Research on alcohol use and miscarriage risk is hindered by selection bias (different recruitment methods by pregnancy outcome), recall bias (assessing exposure after pregnancy loss), and simplified modeling of exposure (treating consumption as a fixed average).[9-33] While some studies assess whether a change in alcohol use occurred in early pregnancy, impact of presence and timing of a change in use on miscarriage has not been well assessed. A systematic review and meta-analysis of alcohol use in pregnancy and miscarriage provides a summary of how the association has been measured and highlights need for more sophisticated analytical methods.

Right from the Start: A Study of Early Pregnancy Health (RFTS) is a prospective pregnancy cohort that is well-suited to overcome common challenges in the literature. Women who were pregnant or planning a pregnancy were recruited into RFTS between 2000 and 2012 in North Carolina, Texas, and Tennessee.[34] Participants were queried about alcohol consumption in the first trimester of pregnancy, amount of alcohol consumed, and changes in alcohol consumption during early pregnancy. As a result, gestational age-specific alcohol exposure was available for 5,424 participants; fifty percent of whom were exposed to alcohol at last menstrual period. These data enable me to incorporate information about pattern of alcohol use in early pregnancy in more nuanced measures of miscarriage risk.

Alcohol dehydrogenase 1C (*ADH1C*) haplotype is associated with different rates of alcohol metabolism.[35,36] Individuals with the *ADH1C* variant related to slower alcohol metabolism have higher blood alcohol concentration for longer duration compared to individuals without the variant for similar levels of alcohol use. *ADH1C* haplotype may modify the relationship between alcohol use in pregnancy and miscarriage. Genetic data from 987 RFTS participants allows me to assess if maternal alcohol metabolism profile, as indicated by *ADH1C* haplotype, modifies the relationship between alcohol exposure and miscarriage risk.[37]

The following aims were devised to better understand the relationship between alcohol use in the first trimester of pregnancy and miscarriage risk:

Specific Aim 1: To conduct a systematic review and meta-analysis of the relationship between alcohol exposure during pregnancy and miscarriage risk among women with recognized pregnancies.

a. Synthesize a summary estimate for the association between alcohol exposure and miscarriage risk and gauge heterogeneity attributable to study design and outcome definition.
b. Dissect limitations in participant recruitment, exposure ascertainment, and statistical modeling.

Specific Aim 2: To determine the relationship between self-reported alcohol consumption and miscarriage risk, defined as pregnancy loss before 20 completed weeks' gestation, in the RFTS cohort. **Hypothesis:** The relationship between alcohol consumption in pregnancy and risk of miscarriage is dependent on timing of exposure.

a. Determine the most robust model for quantifying the association between alcohol consumption and miscarriage by testing performance of different approaches on datasets

simulated to reflect plausible relationships between pattern of exposure and risk of outcome.

b. Use the models identified in Aim 2A to estimate the association between alcohol exposure in pregnancy and risk of miscarriage in the RFTS cohort.

Specific Aim 3: To assess the role of alcohol metabolism, as indicated by *ADH1C* haplotype, in modifying the relationship between alcohol exposure in the first trimester and miscarriage risk in the RFTS pregnancy cohort. **Hypothesis:** Women with the haplotype signifying slower alcohol metabolism will have increased risk of miscarriage compared with women with the haplotype related to faster enzyme activity for similar levels of exposure.

a. Characterize pattern of alcohol exposure by *ADH1C* haplotype.
b. Test for association between haplotype and miscarriage risk.
c. Determine if haplotype modulates the association between alcohol consumption and miscarriage risk.

In the United States, more than a million women a year experience a miscarriage. Many have related healthcare needs, psychological distress, and concerns about what caused the event. Loss of a pregnancy provokes a drastic shift in a family's perceived future, which can result in substantial emotional impact regardless of whether pregnancy was planned.[38-41] The objective of this work is to provide more specific information about how alcohol consumption pattern, duration of use, and amount consumed relate to miscarriage risk to empower women to make the best choices for their pregnancies.

II. BACKGROUND

Alcohol exposure in pregnancy

In the United States, more than half of women consume alcohol in early pregnancy whether or not pregnancy was intended.[1,4,42] Exposure burden is heaviest around time of conception and during early development before pregnancy is confirmed.[3,4,7,8,43,44] Fewer than half of women detect pregnancy by four weeks of gestation, the time of missed expected menses. More than forty percent of women have not yet recognized pregnancy at six weeks.[43] While most women stop using alcohol after a pregnancy is detected, approximately 10 to 27% continue to use alcohol throughout pregnancy.[4,42,45-51] This proportion is comparable to those observed in other developed countries.[2,7,52] Among women who continue to use alcohol during pregnancy, 80% reduce consumption after pregnancy recogniztion.[4]

Prevalence of risky drinking among women is on the rise. Twelve-month prevalence of alcohol use disorder as defined by DSM-VI has risen 83% among women between 2002 and 2013.[48] The gap between men and women is closing for both regular consumption and patterns suggestive of problematic use.[53] Binge drinking has increased by 17.5% between 2005 and 2012 among women, but only 4.9% among men.[53] In the United States, 10.2% of pregnant women and 53.6% of nonpregnant women aged 18-44 years old report alcohol use within the last 30 days. Eighteen percent of nonpregnant women reported binge drinking, or consuming four or more drinks on any occasion, within the past 30 days as compared with 3.1% of pregnant women.[42] Prevalence of exposure to risky drinking in very early pregnancy is likely closer to what is observed on nonpregnant populations since many women do not change behaviors until a positive pregnancy test.[49]

As the result of public health education efforts,[54] 92% of women consider regular alcohol consumption during pregnancy harmful.[55] Fetal alcohol syndrome was first described in 1971,

and in 1977, the National Institute on Alcohol Abuse and Alcoholism issued the first health advisory on risks of alcohol during pregnancy.[54,56] The labels of alcohol-containing beverages are required to include a warning about potential teratogenic effects of alcohol. However, labeling of alcoholic beverages resulted in only a slight reduction of alcohol use in pregnancy.[57,58] Knowledge of potential harms of alcohol use during pregnancy only weakly correlates with behavior.[59] Most women consider alcohol use during pregnancy as "irresponsible," but plan to use until pregnancy is confirmed.[60] A third of women state they would use alcohol while trying to conceive because they believe low levels of alcohol consumption do not significantly increase risk to the fetus.[61,62] Others have been exposed to alcohol in prior pregnancies without having an adverse outcome and therefore feel more comfortable with exposure during the next pregnancy.[61,62] Women also report diminished motivation to change their consumption patterns if prenatal care providers do not emphasize abstinence.[61] Recent estimated prevalence of fetal alcohol spectrum disorders (FASD) are much higher than previously thought (31.1–50.0 cases per 1,000 children[63] compared with 10 per 1,000 children),[64,65] suggesting that dangerous patterns of alcohol exposure throughout pregnancy are not uncommon in the United States.

Women who are 35 or older are almost twice as likely to consume alcohol during pregnancy than women who are 25 or younger.[4,42] Women who have a college degree or more are 1.5 to 2.0 times more likely to consume alcohol during pregnancy than women who are not college-educated.[4,42,43,46,49,66,67] This contrasts with the populations typically targeted for health education interventions. While some studies suggest women exposed to alcohol in pregnancy are also more likely to be married than women who abstain,[4,68] others report the opposite.[42,43,69] Women who continue to smoke during pregnancy are twice as likely to be alcohol exposed than non-smokers.[4,45,68,69] Women with unintended pregnancies are 31% more likely to use alcohol

before pregnancy detection[4] and twice as likely to continue use after pregnancy recognition.[7,68] White women are more likely to report alcohol consumption during pregnancy than those of other races.[4,43,70,71] The relationship between alcohol use during pregnancy and parity is inconsistent. Several studies suggest exposure is most common among primiparous women,[4,72] while others indicate women are more likely to be exposed with each additional pregnancy.[68]

The epidemiology of miscarriage

Approximately one in six recognized pregnancies end in miscarriage, defined as pregnancy loss before 20 completed weeks' gestation.[73,74] When including pregnancies ending prior to detection, the proportion of pregnancies to end in miscarriage reaches 30%.[75] Loss is most likely to occur prior to 13 weeks' gestation with risk peaking between seven and eleven weeks, and then rapidly declining as gestational age increases.[74]

Despite the frequency of this adverse pregnancy outcome, few determinants are conclusively proved. Maternal age is the most established risk factors for miscarriage with risk increasing gradually until age 35 and then more rapidly thereafter.[76,77] Elevated risk of miscarriage in older mothers may be driven by deterioration of oocyte quality over time,[78] leading to a higher proportion of chromosomally abnormal conceptuses (1 in 5 pregnancies ending in loss among mothers older than 35 have a trisomy compared to 1 in 20 among younger mothers).[77] Alternatively, increasing risk may be secondary to diminishing uterine adaptibility and hormonal function.[76] Paternal age is also related to miscarriage risk. Pregnancies for which the fathers is older than 40 have 60% increased risk of miscarriage compared to those for which the father is 25 to 29 years old.[79] Risk of miscarriage also varies by ethnicity with black women having a 57% greater risk of loss than white women after accounting for key confounders.[80]

Obstetric history is closely related to probability of next pregnancy's success. Women with a history of consistently successful pregnancies have 40% reduced risk of loss than women

with a history of miscarriage.[26] Women with recurrent miscarriage, defined as two or more consecutive losses, have almost four times the risk of loss as women who have never had a miscarriage (adjusted odds ratio [OR] 3.86, 95% confidence intervals [CI] 2.29, 6.54).[26] Isolating causal effects of obstetric history on risk in next pregnancy is difficult since factors influencing past pregnancy success, such as maternal environment, lifestyle exposures, and uterine structure, are often similar across pregnancies.[81,82]

Smoking is the most well-characterized modifiable risk factor for miscarriage,[9,83,84] and increases risk of pregnancy loss in chromosomally normal conceptuses.[85] Caffeine consumption was previously thought to be associated with miscarriage risk, but it is likely studies that measured an association between caffeine and miscarriage were affected by reporting bias.[86] The lack of conclusive evidence concerning other proposed risk factors is likely due to challenges such as logistic difficulties in enrolling women early in pregnancy, obstacles in properly measuring exposure and outcome timing, and methodological limitations in modeling the risk-relationship.

Mechanisms by which alcohol may harm normal pregnancy

Alcohol exposure can affect pregnancy at different stages of development and through various mechanisms.[87-90] Alcohol use can alter bio-availability of important nutrients such as glucose[89,91,92] and triglycerides[93] and affects regulation of hormones including estrogen,[94] progesterone,[95] and luteinizing hormone.[96] It can reach the fallopian tubes and uterine cavity to act on the conceptus prior to implantation.[97] Alcohol negatively impacts placentation by inhibiting differentiation and function of trophoblast stem cells[98] and impairs placental perfusion via dose-dependent vasoconstriction.[99,100] Once the maternal-fetal circulation is established, alcohol readily crosses the placenta and can act directly on the developing embryo.[90,101] Periconceptional alcohol use is associated with decreased placental weight which indicates

impaired placental development and function.[102-104] Exposure during the week of ovulation has also been shown to hinder fertilization[105] and implantion.[89] Since the primary interest of my work is loss of recognized pregnancy, I will focus on plausible mechanisms by which alcohol exposure could increase risk of miscarriage after implantation:

Oxidative stress during placentation

Alcohol exacerbates oxidative stress and excessive oxidative stress is a common mechanism for loss.[106,107] Oxidative stress may be present at baseline secondary to inadequate plugging of spiral arteries by invading trophoblast.[107-110] Prior to pregnancy, spiral capillaries are narrow, reactive vessels supplying the endometrium. During pregnancy, they become distended and are capable of accommodating more than one hundred times the rate of blood flow typical of the nonpregnant state.[111] Approximately six days after fertilization, the blastocyst adheres to the uterine wall.[112] The leading edge of the fetal tissue is lined with trophoblast cells. These cells integrate with the endometrium, forming the precursor to the placenta.[113] Endovascular trophoblasts migrate into the endometrium and occlude spiral arteries.[111,114,115] This invasion limits maternal blood flow into the space the embryo is developing, which is instead filled with maternal plasma filtrate and uterine gland secretions.[116,117] Adequate spiral artery plugging keeps the partial pressure of oxygen in the placenta low (20 mmHg during embryogenesis compared with greater than 50 mmHg during fetal development).[118]

Maintaining a low oxygen environment in early pregnancy is critical for embryogenesis. In normal development, trophoblast plugs begin to break down around eight weeks of gestation to allow the mature intraplacental circulation to develop by week twelve. When this barrier breaks down, the embryo's antioxidant defense mechanisms mature to combat the oxidative stress caused by the rise in oxygen-content.[109] Abnormally early onset of maternal blood flow may lead to miscarriage by overwhelming immature antioxidant pathways.[119 107-109] Two thirds

of detected miscarriages show signs of inadequate placentation characterized by impaired trophoblast migration and reduced spiral artery plugging.[120] Unchecked presence of reactive oxygen species results in DNA damage, protein dysfunction, and lipid peroxidation causing disruption of cell membrane, and, ultimately, cell death.[107] This process may be worsened by alcohol exposure.

Ethanol can increase oxidative stress directly by forming free radicals and indirectly by reducing the concentration of endogenous antioxidants and inhibiting the mitochondrial respiratory chain.[119,121,122] Tissue from normal human placenta display markers of oxidative stress two hours after perfusion with ethanol concentrations comparable to those observed with moderate alcohol use.[123,124] Alcohol exposure during pregnancy could increase risk of pregnancy loss by exacerbating oxidative stress in the presence of inadequate spiral artery plugging early in the first trimester or by enhancing physiological oxidative burst that occurs between ten to twelve weeks of pregnancy, such that fetal antioxidant efforts are overpowered (Figure 1). Depending on stage of development at exposure, increased oxidative stress secondary to alcohol use can endanger pregnancy by acting on the developing placenta or embryo.[119]

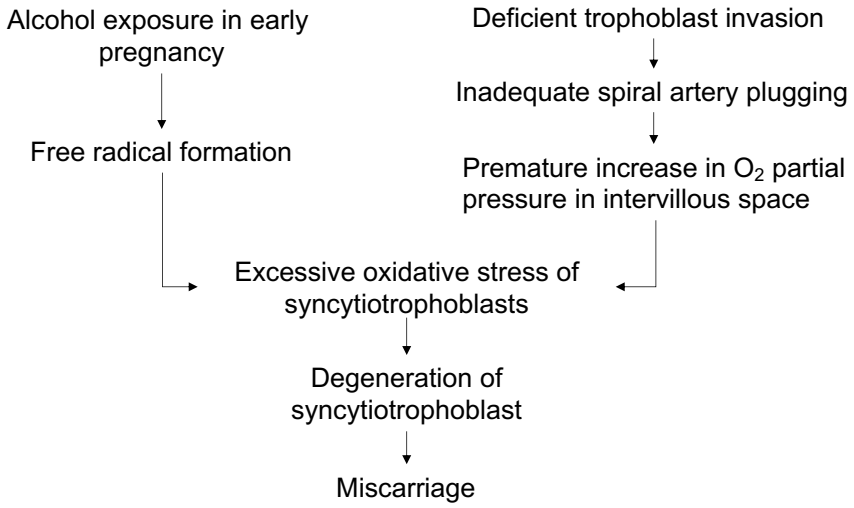

Figure 1. Alcohol exposure in early pregnancy exacerbates oxidative stress from inadequate spiral artery plugging leading to pregnancy loss. Adapted from Jauniaux et al., 2000.

Disruption of prostaglandin levels

Since some prostaglandins are capable of causing cervical softening and uterine contractions, disruption of prostaglandin homeostasis is another proposed mechanism of pregnancy loss.[125,126] Prostaglandin levels in the decidua are lower near time of implantation than at any other point in the menstrual cycle.[112,126] The rate-limiting step of prostaglandin synthesis is release of unesterified arachidonic acid from the plasma membrane catalyzed by phospholipase A2 (PLA2). Maternal secretory component and gravidin, secreted by fetal tissue, inhibit PLA2 which leads to suppression of prostaglandin synthesis.[126,127] Progesterone further decreases uterine prostaglandin levels both through uptake of arachidonic acid[128] and promotion of prostaglandin synthesis inhibitors.[126]

Alcohol exposure increases PGE_2, $PGF_{2\alpha}$, and thromboxane levels.[121,129-131] PGE_2, $PGF_{2\alpha}$, and thromboxane are vasoactive compounds that cause constriction of uterine vessels. Thromboxane also causes platelet aggregation and $PGF_{2\alpha}$ increases contractility of the

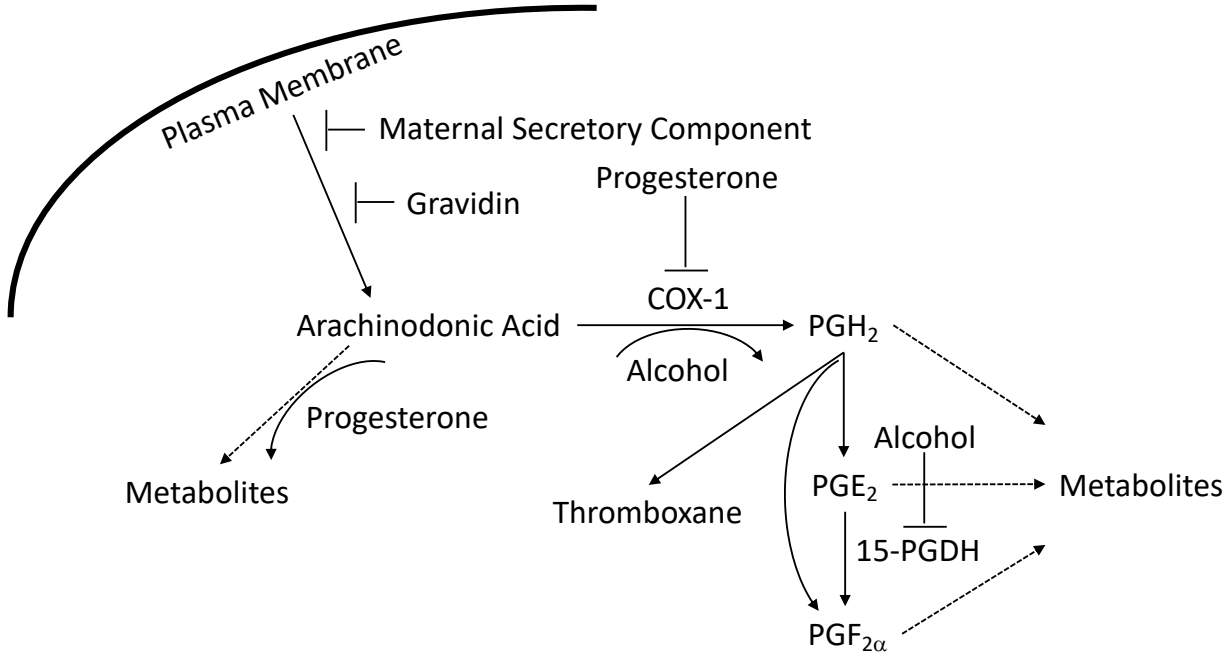

Figure 2. Factors impacting prostaglandin homeostasis in pregnancy.

myometrium. Ethanol stimulates synthesis of PGE_2, $PGF_{2\alpha}$, and thromboxane through upregulation of COX-1.[132,133] Ethanol also inhibits 15-hydroxyprostoglandin dehydrogenase (15-PGDH), an important enzyme on the main pathway for prostaglandin metabolism. Repeated exposure to alcohol inhibits 15-PGDH and leads to impaired clearance of PGE_2 and $PGF_{2\alpha}$ (Figure 2).[134,135] Infusion of ethanol over an one-hour period to achieve a blood alcohol concentration consistent with a moderate drinking episode led to a 45% increase in placental PGE_2 secretion in sheep[136] and prenatal alcohol exposure disrupts prostaglandin homeostasis in rats.[137-139] Animals exposed to alcohol in utero at consumption levels consistent with maternal binge drinking (four or more drinks per sitting) have higher levels of malformation and fetal death.[132,140] Increased risk observed in alcohol-exposed animals are reversed with concurrent administration of indomethacin or aspirin, which block prostaglandin synthesis, providing further evidence these outcomes are prostaglandin-mediated.[141-144] Since exogenous prostaglandin administration can induce abortion at any point in pregnancy, disruption of prostaglandin homeostasis by alcohol may endanger pregnancy at any point in gestation.[145]

Impairment of retinoic acid synthesis

Secretion of retinoic acid is upregulated in the uterus during pregnancy[146] and leads to increased uterine vascularization in preparation for implantation.[147] Retinoic acid facilitates embryonic cell differentiation[148] and apoptosis in early development.[149] Further, retinoid signaling promotes trophoblast differentiation and human chorionic gonadotropin secretion.[150] Retinoic acid synthesis depends on the same pathway as alcohol metabolism and ethanol substrate is the preferred substrate for the enzymes involved (Figure 3).[151,152] Consistent exposure to alcohol may lead to chronic inhibition of retinol oxidation to retinoic acid, leading to reduction of retinoic acid.[153] Since precise levels of retinoic acid are important for normal

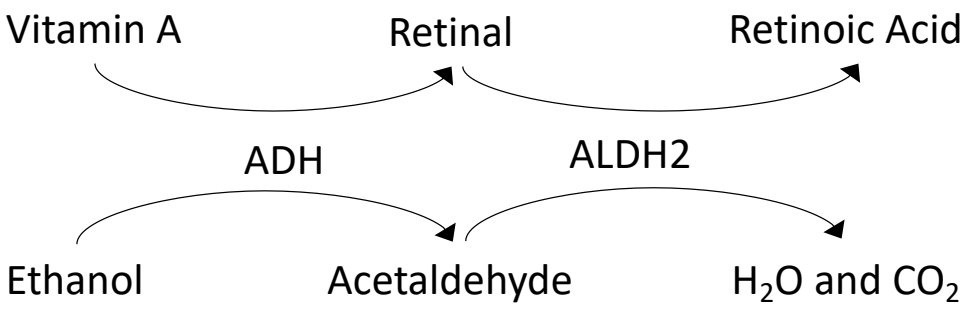

Figure 3. Shared enzymatic pathway for alcohol metabolism and retinoic acid synthesis. Abbreviations: ADH, alcohol dehydrogenase, ALDH, aldehyde dehydrogenase 2. Adapted from Gray et al., 2012.

pregnancy development,[154] ethanol mediated disruption of retinoic acid homeostasis may contribute to risk of loss.

Mechanism final note

Several hypotheses about how alcohol may endanger a pregnancy exist. Alcohol acts through multiple pathways and the degree to which individual mechanisms contribute to miscarriage risk likely depends on gestational age at exposure.

Alcohol metabolism

The primary enzymes responsible for alcohol metabolism are alcohol dehydrogenase (ADH) and aldehyde dehydrogenase 2 (ALDH2). Oxidative metabolism is the main method of alcohol clearance and produces reactive oxygen species. ADH converts ethanol to acetaldehyde in the cytosol. ALDH2 then converts acetaldehyde to acetate in the mitochondria (Figure 4).[155] CYP2E1 and catalase can also participate in oxidative metabolism of alcohol when alcohol is present in large amounts.[156] The oxidative pathway requires reduction of cytosol NAD^+, which impedes the cell's ability to neutralize damaging byproducts of metabolism. Though minimal, alcohol can be metabolized through non-oxidative pathways either by reacting with fatty acids to produce fatty acid ethyl esters or through a reaction catalyzed by phospholipase D. Ninety-five

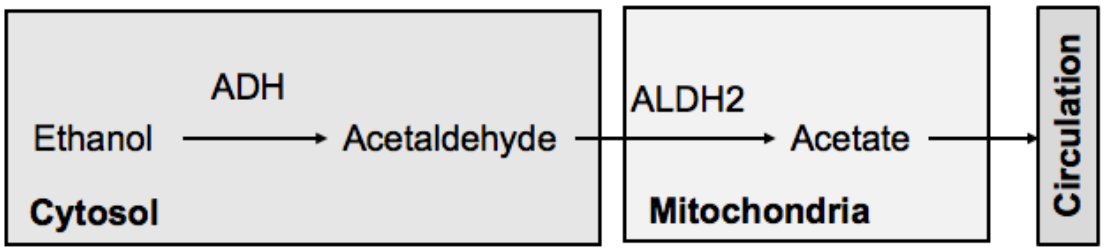

Figure 4. Oxidative alcohol metabolism. Abbreviations: ADH, alcohol dehydrogenase, ALDH2, aldehyde dehydrogenase 2. Adapted from Zakhari et al., 2006.

percent of alcohol metabolism takes place in the liver, but alcohol dehydrogenase is also present in the lung, stomach, and placenta and alcohol can be excrete through urine and sweat.[155,157] ADH constitutes an enzyme family that has five known classes. *ADH1C* codes for enzymes in the γ family of ADH. Two haplotypes of the *ADH1C* gene (*ADH1C*1* and *ADH1C*2*) code for proteins (γ_1 and γ_2, respectively) with different rates of alcohol metabolism.[35-37] Individuals homozygous for γ_1 metabolize ethanol at more than twice the rate of individuals homozygous for γ_2.[35] *ADH1C*2* contains two nonsynonymous single nucleotide polymorphisms (SNPs) (rs698 and rs1693482) in perfect linkage-disequilibrium among populations of European decent (r^2=1.0, D'=1.0, minor allele frequency 47.5%).[158] Individuals homozygous for *ADH1C*2* do not have severe alcohol intolerance as seen in the mutation of *ALDH2* prevalent in Asian populations (minor allele frequency 17.4%), but instead have increased concentration and duration of alcohol in circulation for similar exposure compared with individuals homozygous for *ADH1C*1*.[37] *ADH1C* haplotype modifies the association between alcohol exposure and risk of oral cleft defects and may be important for determining risk of other adverse pregnancy outcomes.[159]

Other factors influencing blood alcohol concentration

Blood alcohol concentration depends on rate of absorption and clearance. Absorption is determined by concentration of alcohol in the beverage, rate of ingestion, and type of food in the stomach. Rate of alcohol clearance depends on metabolism rate including genetic determinants

of enzyme activity, body mass index, nutritional state, time of day, age, liver function, and presence of drugs that interact with alcohol metabolism pathways.[156]

Challenges in studying the association between alcohol and miscarriage

I identified twenty-four studies reporting about the association between alcohol use in pregnancy and miscarriage.[9-33] Please see Chapter III for a systematic review and meta-analysis of the literature. I detail some common challenges to studying this association below:

Recruitment in early pregnancy

Recruiting women early enough in pregnancy to capture all miscarriage events is difficult.[160] Many miscarriages occur prior to pregnancy detection and cannot be studied without the use of serial biomarker assays.[75] Therefore, my work focuses on miscarriage risk of recognized pregnancies. To optimally study miscarriage, women must detect pregnancy early and be promptly enrolled into a cohort. Studies of women recruited at the first prenatal visit miss losses occurring early in pregnancy and early pregnancy time must be truncated. Since women initiate care at different points in gestation, time under observation in studies that recruit at the first prenatal visit depend on when a woman comes in for care, which may be associated with other factors related to risk of loss. For example, while 77.1% of women in the United States initiate prenatal care in the first trimester, mothers who are in their thirties initiate care earlier than younger mothers and women with a parity of greater than three are less likely to initiate care in the first trimester than women with lower birth-order births.[161] Proportion of mothers to initiate prenatal care in the first trimester increases with increasing maternal education.[161,162] Also, approximately half of pregnancies are unplanned**,** which can lead to delayed pregnancy detection and prenatal care.[163]

Exposure assessment

Data about alcohol use are generally collected through self-report since no biomarker for exposure is readily available. Some biomarkers present in blood, urine, hair, and fingernails can confirm reports of alcohol use, but are not sufficiently sensitive or specific for exposure classification.[164-166] For the added financial and logistical barriers of obtaining biomarker measures (i.e., systematic collection of specimens, uniform processing of samples), the quality of information they can provide is limited. These tests are often used for dichotomous exposure classification (use versus no use), as frequency and quantity of use cannot be specified with current biomarker measurements.[165] Additionally, quantity of alcohol consumed and pattern of consumption (e.g., five drinks in one day versus one drink per day for five days) and timing of testing in relation to use will influence whether a biomarker tests positive. As a result, exposure classification in studies of alcohol use in pregnancy typically rely on maternal self-report.

Ascertainment of exposure status around time of conception and early pregnancy is logistically difficult. Exposure information is frequently collected later in pregnancy, after miscarriage occurs, even in prospective cohort studies. Therefore, recall bias often hinders studies of miscarriage.[167,168] Differential recall of exposure between women with loss compared with women with surviving pregnancies can lead to spurious associations,[30] especially when exposure is thought to be teratogenic.[167]

Validity of self-reported alcohol exposure is likely influenced by timing and method of assessment.[168] Generally, women who have experienced an adverse pregnancy outcome are more likely to report an exposure.[169] At the same time, participants' responses about alcohol exposure in pregnancy may be impacted by social desirability bias. Since alcohol consumption in pregnancy is publicized as a risky behavior, a stigma is attached to use and may lead women to underreport exposure.[170] In a study that assessed maternal alcohol consumption during and after

pregnancy, women who experienced adverse outcomes recalled significantly lower levels of consumption then they originally reported.[167] Another study did not find differences in prospective versus retrospective reporting of alcohol use for mid-pregnancy exposure.[171] Degree of social desirability bias depends on mode of data collection and sense of anonymity, with bias being stronger for in-person interviews than questionnaires administered over the phone.[172] Therefore, careful consideration of assessment tool development and delivery is critical for accurate disclosure. This involves nonjudgmental wording of questionnaire items, provision of specific options for reporting frequency of use and amount consumed, and assurance of confidentiality. Since recall and social desirability bias may act in opposing directions, predicting their effect on maternal reporting is difficult and may vary from woman to woman.[173]

Another challenge in alcohol exposure assessment is collecting accurate information about amount consumed. Many studies measure dose as number of drinks per day or week. This metric is limited since it is subject to an individual's interpretation of a single "drink" and since alcohol content varies by beverage type.[174,175] Some researchers calculate standardized number of drinks (equal to 0.5 ounces of absolute alcohol) or absolute alcohol equivalents using information about number of drinks consumed and expected alcohol content of beverage type. However, these measures may give a false sense of precision since they still rely on the quality of participants' responses.

Exposure operationalization

Alcohol is implicated as a potential risk factor for miscarriage and mechanism by which use endangers pregnancy likely depends on timing and pattern of exposure and timing in pregnancy.[88,89,176] Consequently, defining "risky drinking" is difficult. While one might expect risk associated with alcohol use to be dose-dependent, other aspects of use such as timing, frequency, maximum episodic dose, and baseline alcohol metabolism likely play a role. This

challenge is mirrored in studies of alcohol use and FASD.[177] While neurodevelopmental outcomes and impaired fetal growth are more commonly linked with binge drinking during pregnancy,[178-181] other features of FASD including craniofacial defects and developmental delays are observed with relatively low levels of alcohol use (less than 0.5 drinks per day).[182-186] Dose-dependent effects are not a rule in pregnancy outcomes associated with alcohol use, and the impact of exposure on pregnancy is likely the product of frequency of consumption, episodic dose, and timing of exposure relative to stage of fetal development.[177,187,188]

At least two distinct exposure patterns exist for many women during pregnancy: behavior before and after pregnancy recognition.[49] Women planning pregnancies do not necessarily abstain from alcohol consumption, but 88% cease or reduce alcohol use once pregnancy is detected.[4] *Right from the Start* participants reported alcohol consumption from the periconception period through the first trimester, including change in pattern of alcohol consumption. Date of alcohol use cessation or reduction aligns with date of first positive pregnancy test in one in every three women, regardless of pregnancy intention (Figure 5).[4]

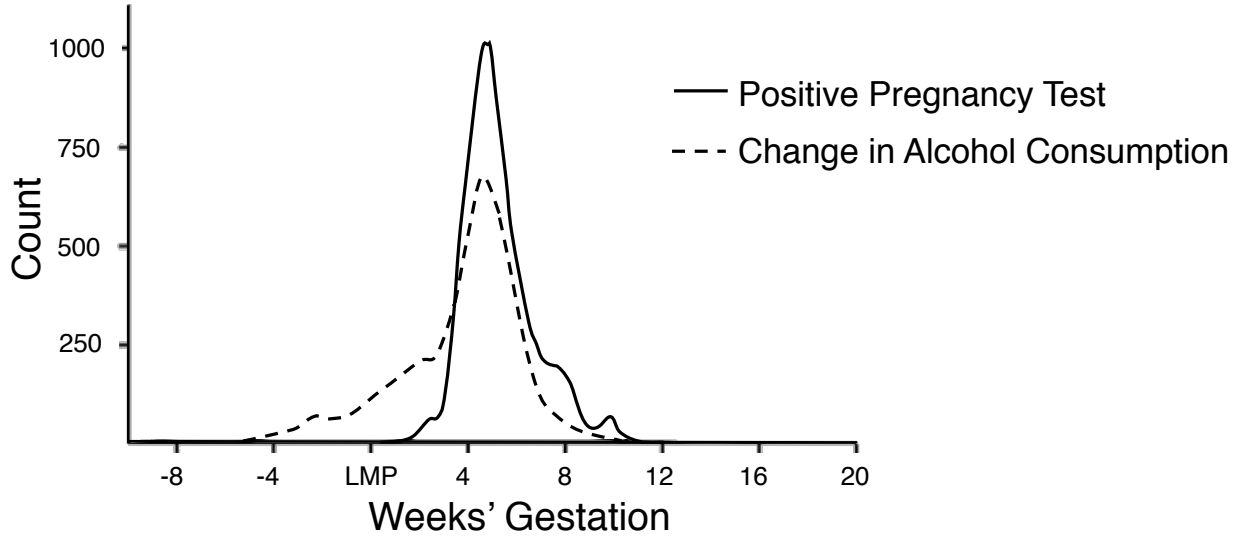

Figure 5. Timing of alcohol consumption change and pregnancy detection for participants in the Right from the Start cohort.

Alcohol use is susceptible to flawed measurement, operationalization, and modeling by not accounting for changes in exposure during pregnancy. Of twenty-four studies identified in the systematic review and meta-analysis presented in Chapter III, half assessed change in alcohol use in early pregnancy, but no studies incorporated timing of change into measures of risk. Instead, alcohol exposure was operationalized as an across-pregnancy average or consumption after change in use. Modeling risk associated with a woman's self-reported, average intake after pregnancy recognition misses potentially important details about frequency of exposure and episodic exposure dose and neglects risk associated with intake prior to pregnancy recognition. Quantifying the risk relationship is limited by lack of specificity and uniformity in characterizing alcohol exposure in early pregnancy.

Designation of outcome time

Conventionally, gestational age is measured from the first day of a woman's last menstrual period (LMP) and studies of miscarriage risk often use gestational age at start or peak of miscarriage symptoms to assign pregnancy endpoint. However, pregnancy arrest, the time when the prengnacy is no longer viable, can occur days to weeks prior to when bleeding starts and contents of the uterus are expelled.[189,190] Exposures occurring after development stops, when loss has already become inevitable, do not likely influence the pregnancy's fate. In studies of miscarriage, using gestational age at miscarriage to determine pregnancy endpoint allows time to accrue during the window in which the pregnancy is nonviable, potentially leading to miscalssification of exposure status or overestimation of exposure duration. Accurately specifying time at risk may be particularly important when estimating risk associated with time-varying exposures and failure to do so may contribute to the scarcity of well-defined and consistently characterized modifiable risk factors for miscarriage.

Ultrasound measures such as gestational sac diameter[191,192] and crown rump length[193-196] are reliable and precise for gestational dating. Discrepancies between observed and expected features on ultrasound prior to a loss can be leveraged to estimate gestational age at arrest of development.[189,190,197] Please see Chapter V "Gestational age at arrest of development" for more information about how I attempted to account for time between arrest of development and miscarriage in Aim II and Aim III analyses.

Clinical relevance

Alcohol exposure in the first trimester occurs in 50% of pregnancies[2] regardless of whether a pregnancy was intended.[4] In the United States, three in four women wanting to conceive "as soon as possible" report ongoing alcohol use, putting them at risk of having an alcohol-exposed pregnancy.[1] Though miscarriage has modest influence on overall reproductive potential, loss often has substantial emotional impact.[38-41] Most women who experience a loss report grief and limitation of daily function regardless of whether pregnancy was planned.[40] Better understanding the relationship between alcohol consumption and risk of loss is pertinent to identifying detrimental patterns of exposure. For example, if risk increases for every week exposed, interventions targeting early detection of pregnancy would be important for minimizing risk. Alcohol consumption in pregnancy is directly related to information provided about potential risk.[61] More detailed information about how alcohol exposure can endanger pregnancy could empower women to make the best choices about alcohol use near conception.[198]

Abstinence in early pregnancy is not the norm although clinical guidelines maintain no level of alcohol exposure in pregnancy is safe and the CDC and Surgeon General recommend that women who are or could become pregnant do not use alcohol.[1,199] The sooner the relationship between alcohol exposure pattern and miscarriage risk is understood, more targeted action can be taken towards mitigating risk attributable to alcohol exposure.

III. FIRST AIM: SYSTEMATIC REVIEW AND META-ANALYSIS OF THE ASSOCIATION BETWEEN ALCOHOL EXPOSURE AND MISCARRIAGE RISK

Abstract

Background: More than 50% of women report exposure to alcohol during pregnancy with most use occurring in early gestation and declining after pregnancy detection. Evidence about the impact of alcohol use on miscarriage is inconclusive. Our objective was to systematically review and critically evaluate studies about alcohol exposure during pregnancy and miscarriage.

Methods: We searched five databases for relevant publications and selected studies of alcohol exposure during pregnancy and miscarriage. I conducted a random-effects meta-analysis of the association between alcohol exposure and miscarriage and quantified a dose-response relationship using generalized least squares regression with and without restricted cubic spline terms for dose.

Results: Of 2,136 articles identified, 24 were eligible for inclusion. Meta-analysis of data from 231,808 pregnant women found those exposed to alcohol during pregnancy have a greater risk of miscarriage compared with those who abstained (odds ratio [OR] 1.19; 95% confidence intervals [CI] 1.12, 1.28). In a subgroup analysis of twelve studies presenting dose-specific effects, each additional drink per week was associated with a six percent increase in risk (OR 1.06, 95% CI 1.01, 1.10). The literature is limited by imperfect capture of early miscarriages and oversimplified methods for modeling alcohol exposure.

Conclusions: Alcohol use during pregnancy is associated with a dose-mediated increase in miscarriage risk. This meta-analysis aligns with guidance that no known amount of alcohol exposure during pregnancy is safe. Future studies evaluating change in alcohol use in pregnancy are needed to provide insight into how alcohol consumption across all stages of gestations impacts risk.

Overview

Miscarriage occurs in up to one in six recognized pregnancies,[74,75,200] and may result in substantial emotional impact regardless of whether pregnancy was planned.[38,201] Though miscarriage is common, few modifiable determinants of miscarriage are known. More than half of women consume alcohol in the first trimester of pregnancy with most use occurring in early gestation prior to pregnancy detection and then rapidly tapering.[2,7,52] While alcohol use in pregnancy has been repeatedly linked to adverse outcomes,[185,202,203] estimates of alcohol's effect on miscarriage range from protective to a 3.8-fold increase in risk.[9-33] A previous systematic review of the literature up to 2005 of low-to-moderate alcohol consumption in pregnancy reported five of eight studies suggested use increased miscarriage risk.[204] This aim extends previous work by providing an updated evaluation of the literature and a meta-analysis of the association between amount of alcohol consumed and miscarriage.

The goal of this aim was to calculate a summary estimate for the relationship between alcohol exposure during pregnancy and miscarriage risk from a systematic review of the literature. I quantified risk related to alcohol use and amount consumed with the hypothesis that higher levels of alcohol use in pregnancy associate with higher risk of miscarriage.

Alcohol use and miscarriage is challenging to study because participants must be recruited before typical onset of care, exposure cannot be directly measured and relies on self-report, and miscarriage events can be missed. Therefore, my secondary objective was to evaluate risk of bias in past studies and to identify common limitations.

Methods

The literature search, study selection, coding plan, and meta-analysis adhere to the Preferred Reporting Item for Systematic Reviews and Meta-Analyses (PRISMA) statement (Appendix 1: PRISMA Checklist) and the MOOSE guidelines for reporting systematic reviews

and meta-analysis of observation studies (Appendix 2: MOOSE Checklist for Meta-analyses of Observational Studies).[205,206] Studies that evaluate the relationship between alcohol exposure during pregnancy and risk of miscarriage, have a reference group of women who are abstainers or minimally exposed to alcohol, and assess miscarriage as an independent outcome were eligible for this analysis (Table 1).

Table 1. PECOTS for systematic review

Population	Women with recognized pregnancies
Exposure	Self-reported alcohol consumption in early pregnancy
Comparator	Women who abstained from or were minimally exposed to alcohol in pregnancy
Outcome	Miscarriage as defined by study
Timing	Followed sufficiently to observe a miscarriage event
Study Design	All study types

Search

Relevant studies were identified through searches of electronic databases (PubMed, EMBASE, PsycINFO, ProQuest, and ClinicalTrials.gov) in September 2018 using the following terms: ('spontaneous abortion' or 'miscarriage' or 'pregnancy loss' or 'abortion') and ('alcohol' or 'ethanol') (Appendix 3: Systematic Review Full Search Strategy). To ensure capture of relevant studies, I conducted backward and forward citation searches of included studies. Only studies published after January 1, 1970 and available in English were included.

Eligibility

Human studies evaluating the association between alcohol exposure during pregnancy and miscarriage risk were eligible. Randomized controlled trials, cohort studies, and case-control studies were eligible for inclusion. Exposure was alcohol use during pregnancy and outcome was miscarriage. Studies that only evaluated pre-conception alcohol use were excluded. Because gestational age threshold for miscarriage varied, studies were not excluded based on miscarriage definition, but instead I performed a sensitivity analyses conditioned on outcome definition.

Table 2. Meta-analysis inclusion and exclusion criteria

Inclusion Criteria	Exclusion criteria
• Original research • Among pregnant women • Systematically quantifies alcohol exposure in the first trimester • Assesses miscarriage as an outcome	• Study considers alcohol exposure outside of gestation as main exposure • Study does not provide sufficient information to calculate effect estimate

I completed abstract screening (Appendix 4: REDCap Abstract Screening Tool) and full-text review (Appendix 4: REDCap Abstract Screening Tool) validated by one other investigator. Inclusion and exclusion criteria are listed above (Table 2). If a study was not excluded by both reviewers at the abstract screening stage, we conducted a full text review. A full text review and eligibility decision was made independently by another reviewer and me. Discrepancies were adjudicated by a third reviewer, who was masked to prior decisions.

Data collection

Another reviewer and I used standardized forms in the Research Electronic Data Capture (REDCap) to double-enter data from included articles.[207] A third party resolved differences in coding through a conversation with reviewers about entry rationale. Data abstraction elements included study design, study years, country, counts of study participants by exposure status and pregnancy outcome, recruitment setting, exposure window, reference group definition, exposure definition and operationalization, miscarriage definition, outcome comparator, crude and adjusted effect estimates and confidence intervals, and covariates included in adjusted models. If a dose-response analysis was performed, crude and adjusted effect estimates were collected for all dose categories (Appendix 6: REDCap Data Extraction Tool). I practiced aspirational coding (i.e., attempting to collect all pieces of desired information from every study) during data extraction and contacted study authors for missing information. This allowed for comprehensive data collection and revealed when reporting of critical study information was absent.

Two reviewers assessed study quality using the Newcastle-Ottawa scale, which focuses on vulnerabilities for bias.[208] Each reviewer collected information about participant inclusion (comparing methods for recruitment of exposed and unexposed in cohort studies and case and control identification for case-control studies), loss to follow-up/non-participation rates, average gestational age at recruitment, timing of alcohol exposure assessment (before or after pregnancy outcome), exposure assessment method (self-administered questionnaire or interviewer-conducted survey), assessment of alcohol consumption change during pregnancy, alcohol exposure operationalization, statistical modeling, and covariates included in the adjusted analysis (Table 3).

Table 3. Newcastle-Ottawa scale quality domains

Recruitment
Equitable recruitment of exposed and unexposed (cohort studies)
Equitable recruitment of cases and controls (case-control studies)
Recruitment allows for selection of participants representative of general population
Minimal loss to follow-up (< 20% loss or < 5% non-participation rate)
More than 80% of participants recruited prior to 10 weeks' gestation
Outcome Ascertainment
Appropriate comparator group (pregnancies surviving past 20 weeks' gestation)
Exposure Ascertainment
Exposure assessed prior to pregnancy outcome to minimize recall bias (cohort studies)
Exposure assessed through self-administered questionnaires to minimize reporting bias
Study queried change in consumption during pregnancy
Statistical Modeling
Alcohol modeled as a time-varying exposure
Adjusted for maternal age +/- other confounders
Use of time-to event analysis

Statistical analysis

I evaluated the association between alcohol exposure and miscarriage risk by modeling alcohol use as a dichotomous (exposed versus unexposed) and a continuous variable (number of drinks per week). Analyses were performed in Stata (Version 14.2, StataCorp, College Station, TX). I used the "metan" package to estimate aggregate odds ratios (ORs) and 95% confidence

intervals (CIs) and the "glst" package to estimate the dose-response effect. I used a random-effects model for all pooled estimates since I expected the true effect estimate to vary across populations and contexts.[209] Analyses included adjusted estimates when available and sensitivity analyses were performed excluding studies without adjusted estimates. When effect estimates were not reported, odds ratios were calculated using counts provided in the text. I assessed study heterogeneity with the I^2 and τ^2 statistics. I^2 is the estimated proportion of heterogeneity attributable to true between-study differences.[210] The τ^2 statistic estimates variance of the true effect or the variability of true effect sizes in different populations.[211]

I evaluated publication bias using funnel plots, a trim-and-fill analysis, and Egger's test. Funnel plots are scatterplots of study effect size versus estimate precision. If bias is not present, the effect estimates should be symmetrically distributed around the summary estimate, with precise studies clustering near the top of the graph and imprecise studies evenly distributed at the bottom of the graph. Asymmetry in a funnel plot indicates the presence of publication bias. Most commonly, publication bias results from the absence of published imprecise or null studies.[212] Egger's test is a regression of the effect estimate and study precision.[212]Trim-and-fill analysis also assumes a symmetrical distribution of studies around the summary estimates with variance in the estimate increasing as study precision decreases. This sensitivity analysis predicts studies missing due to publication bias and adjusts the summary estimates accordingly.

I included studies reporting dose-specific effect estimates in a meta-analysis for amount of alcohol consumed. I converted alcohol exposure categories to average number of drinks per week using the midpoint of each study-specific exposure category. For open-ended categories, I divided the interval of the next highest category by two and added that value to the lower boundary of the highest category (e.g., if categories were 0, 1–4, 5–8, and ≥9, amounts used in the model would be 0, 2.5, 6.5, and 10.5). To standardize estimates, I converted dose categories

to number of drinks per week. I used generalized least squares regression models to estimate a log-linear trend between number of drinks per week and miscarriage risk. This method accounts for non-independence between effect estimates using the same reference category (i.e., effect estimates for multiple doses in a single study) by estimating a variance-covariance matrix of the beta coefficients.[213] I evaluated the possibility of a non-linear relationship between amount of alcohol consumed and miscarriage risk using restricted cubic splines with three knots and compared model fit to the fit of the log-linear relationship using the Wald test.[214] Since studies differed in miscarriage definitions, I conducted subgroup analyses of studies limited to first trimester losses and studies of miscarriage at any time. I performed subgroup analyses restricted to studies presenting adjusted estimates and evaluated pooled estimates for observational studies by study type (case-control versus cohort). I analyzed studies reporting dose-effects in terms of hazard ratios (HR) separately as to not combine estimates that incorporate survival data with those that do not.

Results

After duplicates were removed, 2,136 articles were identified through the search strategies described. Twenty-four studies were eligible for analysis including 231,808 pregnant women (Figure 6).[9-32] If data from the same study sample was present in multiple reports,[33,215-218] the report with the most complete information was used. Fourteen were cohort studies and ten were case-control (Table 4). The United States contributed the largest proportion of studies (38%), followed by Denmark (13%) and the United Kingdom (13%). Included studies were published between 1980 and 2016 and sample size ranged from 161 to 89,339 participants.

Studies varied in method for assessing alcohol use in pregnancy. Participants in thirteen studies were asked to report the average number of drinks they consumed in a typical week or day, while six studies classified alcohol as a dichotomous exposure. Other studies collected more

granular information about alcohol use whether that be daily use reported in a self-administered questionnaire,[13] daily use in the past two weeks reported at each prenatal visit,[15] or total number and type of drinks consumed since LMP.[10] Although 50% of studies queried whether a change from pre-pregnancy alcohol use had occurred, none incorporated information about timing of change into effect estimates. Instead, studies classified alcohol as an average dose or as consumption after a change in use.

Synthesis of results

Twelve of the twenty studies reporting an effect estimate found some level of alcohol exposure was associated with increased risk of miscarriage (Table 5). In this meta-analysis of the association between alcohol use and miscarriage, exposed pregnancies where 19% more likely to end in miscarriage (OR 1.19, 95% CI 1.12, 1.28; τ^2 0.004; Figure 7). We observed significantly less between-study heterogeneity among cohort studies compared with case-control studies (I^2 12.3% [low heterogeneity] versus 69.1% [moderately high heterogeneity]). However, only three studies reported an adjusted risk estimate for the effect of alcohol operationalized as a dichotomous exposure,[11,12,24] so the dose-specific meta-analysis is a more informative aggregate estimate.

Seventeen studies reported dose-specific effects of alcohol on miscarriage risk. In the random effects meta-analysis of the twelve studies using non-survival data, a dose-response relationship between alcohol use and miscarriage risk is apparent (Figure 8; spline model). Each additional drink per week in pregnancy was associated with a 6% increase in miscarriage risk (OR 1.06, 95% CI 1.01, 1.10; log-linear model). Estimates were similar when restricting analysis to adjusted studies and when comparing results from cohort and case-control studies (Table 6). The pooled effect was lower among studies limited to first trimester miscarriage (OR 1.02, 95%

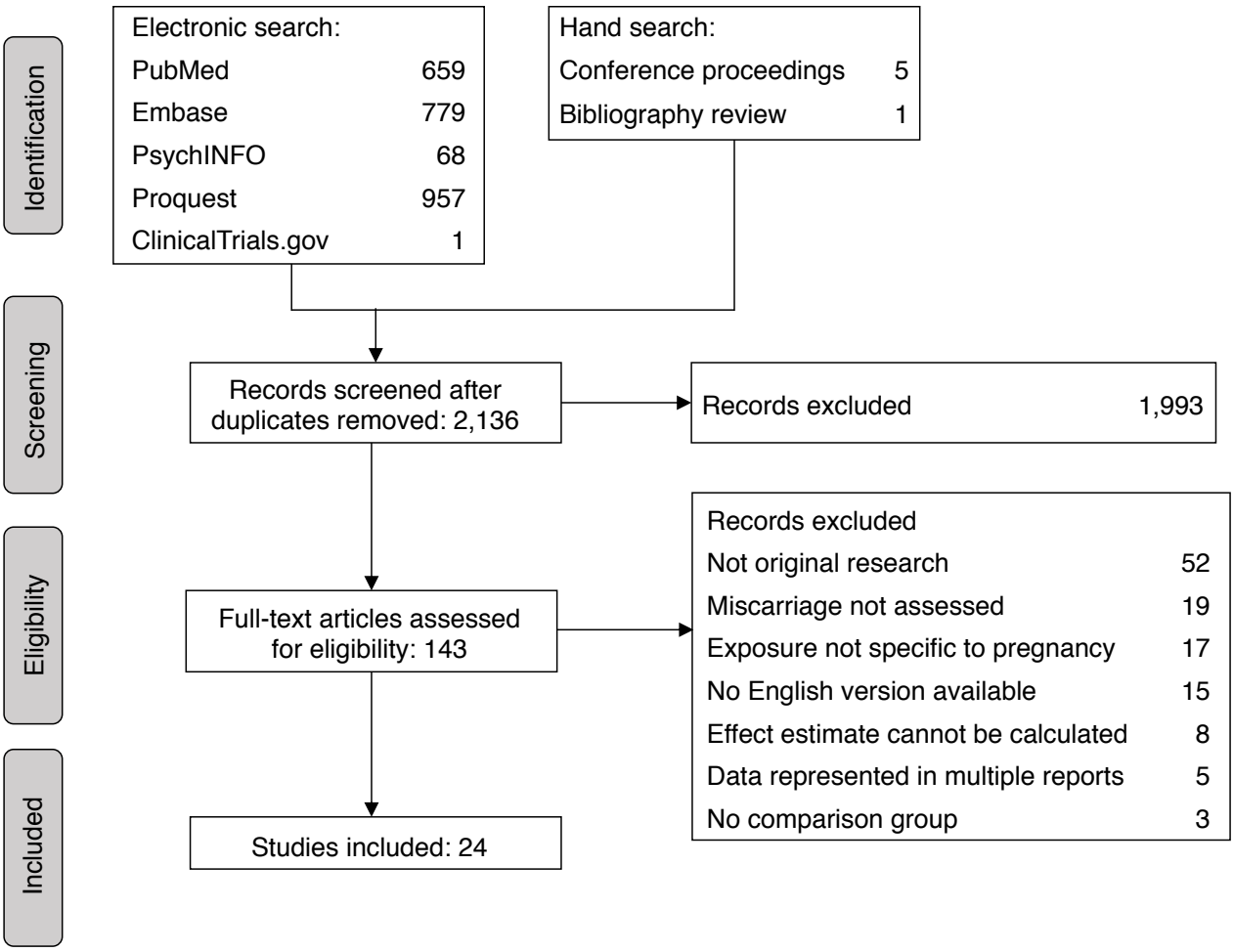

Figure 6. Flow diagram of studies identified in the systematic review.

Table 4. Characteristics of studies in systematic review

Author, Year	Study Design	Country	Study Years	n (SAB/no SAB)	Recruited population	Exposure Ascertainment*	SAB Cutoff (GA)	Comparator
Armstrong, 1992	Cohort	Canada	1982–1984	47,146 (10,191/36,955)	Women delivering or receiving care for SAB across 11 hospitals	In-person interview for first trimester exposure in index and prior pregnancies	<28	Births
Avalos, 2014	Cohort	USA	1996–1998	1,061 (172/889)	KPNC members with record of a positive pregnancy test prior to 10 weeks' gestation	In-person interview prior to 15 weeks' gestation	≤20	Pregnancies surviving past 20 weeks
Borges, 1997	Cohort	Mexico	1988	4,634 (197/4,437)	Women with a prior pregnancy randomly surveyed in urban areas of Mexico	In-person interview for alcohol consumption in most recent pregnancy	—	Pregnancies not ending in SAB
Boyles, 2000	Case-Control	USA	1995–1997	970 (400/570)	Women presenting to the emergency department before 22 weeks' gestation	In-person interview during emergency department visit	≤22	Pregnancies surviving past 22 weeks
Buck Louis, 2016	Cohort	USA	2005–2009	344 (98/246)	Couples discontinuing contraception with the intention of becoming pregnant	Daily lifestyle journals pre-conception through seven weeks post-conception	≤22	Pregnancies surviving past 22 weeks
Cavallo, 1995	Cohort	Italy	—	527 (55/472)	Women at first blood test during pregnancy	In-person interview during hospital visit	—	Live births
Chiodo, 2012	Cohort	USA	1999–2001	302 (23/279)	Women initiating prenatal care before 28 weeks' gestation at urban clinics	In-person interview repeated at each prenatal visit	≤20	Pregnancies surviving past 20 weeks
Conde-Ferraez, 2013	Case-Control	Mexico	2008–2009	281 (143/138)	Women receiving curettage for SAB (cases) or delivering at term (controls)	In-person interview during hospitalization	≤20	Live, term births
Davis, 1982	Cohort	UK	1980	973 (22/951)	Women at booking prenatal visit at study hospital	Self-administered questionnaire at booking visit	—	Stillbirths and live births
Dlugosz, 1996	Cohort	USA	1988–1992	2,839 (135/2,704)	Women initiating prenatal care before 16 weeks' gestation	At-home interview before 17 weeks' gestation about exposure in first month	<28	Live births
Feodor Nilsson, 2014	Cohort	Denmark	1996–2002	89,339 (3,018/86,321)	DNBC women initiating prenatal care before 22 weeks' gestation	CATI targeted for 12 weeks' gestation	≤22	Pregnancies surviving past 22 weeks
Halmesmaki, 1989	Case-Control	Finland	—	161 (80/81)	Women presenting to hospital for SAB (cases) or prenatal ultrasound (controls, gestational age-matched)	In-person interview at hospitalization	—	Live, term births
Han, 2012	Cohort	South Korea	—	3,507 (254/3,253)	Women participating in the Korean Motherisk Program	Self-administered questionnaire repeated at each prenatal visit	—	Pregnancies surviving past SAB cutoff

Table 4. Continued

Author, Year	Study Design	Country	Study Years	n (SAB/no SAB)	Recruited population	Exposure Ascertainment*	SAB cutoff (GA)	Comparator
Harlap, 1980	Cohort	USA	1974–1977	32,019 (1,503/30,516)	KPNC members initiating prenatal care before 28 weeks' gestation	Self-administered questionnaire during prenatal care	<28	Pregnancies surviving past 28 weeks
Kesmodel, 2002	Cohort	Denmark	1989–1996	24,663 (321/24,342)	Women initiating prenatal care before 8 weeks' gestation at participating hospital	Self-administered mailed questionnaire (median GA 14.7 weeks)	≤28	Pregnancies surviving past 28 weeks
Kline, 1980	Case-Control	USA	1974–1978	1,248 (616/632)	Women presenting to hospital for SAB (cases) or for non-SAB pregnancy outcome (controls, age- and hospital-matched)	Interview at pregnancy outcome	—	Pregnancies surviving past 28 weeks
Long, 1994	Case-Control	UK	—	3,443 (95/3,348)	Consecutive women presenting with SAB or singleton live births past 28 weeks' gestation (controls)	Interview at admission for SAB (cases) or at first prenatal clinic visit (controls)	<13	Live births occurring past 28 weeks
Maconochie, 2007	Case-Control	UK	1980–2001	6,458 (569/5,889)	Women responding to a postal survey indicating their most recent pregnancy ended in first trimester SAB (cases) or survived past 13 weeks (controls)	Self-administered postal survey in 2001 (pregnancies since 1980 included)	<13	Pregnancies surviving past 13 weeks
Parazzini, 1994	Case-Control	Italy	1990–1993	1,276 (462/814)	Women presenting to hospital for SAB (cases) or delivery (controls, hospital-matched)	In-person interview during hospitalization for pregnancy outcome	<13	Live, term births (normal weight and Apgar score)
Paszkowski, 2016	Cohort	Poland	2001–2004	242 (105/137)	Women hospitalized for threatened abortion	Self-administered questionnaire	—	Live, term births
Rasch, 2003	Case-Control	Denmark	1994–1996	1,454 (320/1,134)	Women hospitalized for a D&C for SAB (cases) or women initiating prenatal care and between 6-16 weeks of gestation (controls)	Self-administered questionnaire during hospitalization (cases) or during first prenatal visit (controls)	6-16	Pregnancies surviving past 16 weeks
Windham, 1992	Case-Control	USA	1986–1987	1,919 (623/1,296)	Women presenting to hospital for SAB (cases) or delivery (controls, hospital- and LMP-matched)	CATI after pregnancy outcome	<20	Live births
Windham, 1997	Cohort	USA	1990–1991	5,142 (500/4,642)	KPNC member initiating prenatal care before 12 weeks' gestation	Telephone interviews within two weeks of scheduling first prenatal visit	≤20	Pregnancies surviving past 20 weeks
Xu, 2014	Case-Control	China	2009–2012	1,860 (620/1,240)	Women presenting to hospital for SAB (cases) or attending prenatal care past 13 weeks' gestation (controls, age-matched)	In-person interview within week of loss (cases) or during gestation (controls)	<13	Pregnancies surviving to 13 weeks

Abbreviations: SAB, spontaneous abortion; KPNC, Kaiser Permanente Northern California; DNBC, Danish National Birth Cohort; D&C, dilation and curettage; LMP, last menstrual period; CATI, computer-assisted telephone interview; GA, gestational age; — represents missing data.

Table 5. Methods and findings of studies about alcohol use and miscarriage.

Study	Exposure Measure*	GA†	Findings	Analysis comments
Armstrong, 1992 N=47,146	Type and number of drinks per week during the first trimester of index and prior pregnancies; in-person interview after delivery; converted to average number of drinks per week	<28	Alcohol (drinks/week) aOR 95% CI None 1.00 Referent 1–2 1.11 1.05, 1.18 3–6 1.23 1.13, 1.34 7–20 1.47 1.31, 1.65 21≤ 1.82 1.21, 2.34	Adjusted for maternal age, education level, ethnic group, employment during pregnancy, prior live births, prior miscarriages, cigarette smoking, and coffee consumption
Avalos, 2014 N=1,061	Total number and type of drinks consumed since LMP; in-person interview before 15 weeks' gestation; converted to average number of drinks per week	≤20	Alcohol (drinks/week) aHR 95% CI None 1.00 Referent <4 1.12 0.81, 1.55 ≥4 2.65 1.38, 5.10	Adjusted for maternal age, caffeine consumption, vitamins, marital status, and pregnancy intention
Borges, 1997 N=4,634	Any alcohol use during past pregnancy; in-person interview occurring up to many years after pregnancy	—	Alcohol use during pregnancy aOR 95% CI No 1.00 Referent Yes 1.33 0.86, 2.02	Adjusted for maternal age, tobacco use, and geographical region
Boyles, 2000 N=970	Any alcohol use during pregnancy or in the preconception period; in-person interview at emergency department visit	≤22	Alcohol use during pregnancy aOR 95% CI No 1.0 Referent Yes 1.0 0.8, 1.4	Adjusted for tobacco use, cocaine use, prenatal care, stressful life events, and living with the father
Buck Louis, 2016 N=344	Number of drinks consumed; daily self-administered questionnaire from pre-conception through 7 weeks' gestation; converted to average number of drinks per day in early pregnancy (conception to 7 weeks' gestation)	≤22	Alcohol (drinks/day) aHR 95% CI Cont. 1.65 0.77, 3.54	Adjusted for maternal age, difference in partners' ages, prior pregnancy loss, intercourse frequency, paternal alcohol use, maternal and paternal BMI, cigarette smoking, caffeine consumption, vitamin use
Cavallo, 1995 N=527	Average daily number of drinks during pregnancy; in-person interview at hospital visit	—	Alcohol (drinks/day) aHR 95% CI 0 1.00 Referent 1 0.86 0.42, 1.77 ≥2 1.16 0.53, 2.55	Adjusted for maternal age, marital status, occupation, parity, prior miscarriage, coffee consumption, and cigarette smoking
Chiodo, 2012 N=302	Daily alcohol use in the past two weeks; in-person interview a at each prenatal clinic; converted to absolute alcohol equivalents per day	≤20	Alcohol (absolute alcohol equivalent/day) aOR 95% CI Cont. 2.37 1.25, 4.48	Adjusted for maternal age, education, socioeconomic classification, marital status, smoking status
Conde-Ferraez, 2013 N=281	Any alcohol use during past pregnancy; in-person interview after pregnancy outcome	≤20	Alcohol use during pregnancy OR 95% CI No 1.00 Referent Yes 1.19 0.60, 2.38	Not adjusted for confounders
Davis, 1982 N=973	Alcohol consumption as mL of absolute alcohol equivalent during pregnancy; self-administered questionnaire during prenatal care	—	Alcohol (mL/day) N Miscarriage 0 479 2% 1–10 359 2% 11–20 107 4% ≥21 28 4%	No measure of association calculated

Study	Exposure Measure*	GA[a]	Findings	Analysis comments
Dlugosz, 1996 N=2,839	Alcohol consumption during weeks 3–6 of gestation; in-person interview before 17 weeks' gestation; converted to average number of ounces per day	<28	Alcohol (ounces/day) aOR 95% CI 0 1.00 Referent 0.01–0.10 1.16 0.76, 1.78 0.11–0.50 1.14 0.70, 1.84 >0.50 1.37 0.67, 2.80	Adjusted for maternal age, gestational age at interview, caffeine consumption, and cigarette smoking
Feodor Nilsson, 2014 N=89,339	Average number of drinks per week during pregnancy; computer assisted telephone interview targeted for 12 weeks' gestation	<12	Alcohol (drinks/week) aHR 95% CI 0 1.00 Referent 0.5–1.5 1.05 0.93, 1.18 2–3.5 1.56 1.32, 1.81 ≥4 2.81 2.25, 3.50	Adjusted for maternal age, exercise, coffee consumption, heavy lifting, parity, occupation, cigarette smoking, weight, work schedule, and genital disease; hazards calculated separately for first and second trimester miscarriages
		12–22	aHR 95% CI 0 1.0 Referent 0.5–1.5 1.13 1.00, 1.26 2–3.5 1.34 1.13, 1.58 ≥4 1.64 1.23, 2.19	
Halmesmaki, 1989 N=161	Any alcohol consumption during pregnancy; in-person interview after miscarriage (cases) or at prenatal ultrasound (controls)	—	Any alcohol use N Exposed Case 80 58% Control 81 58%	No measure of association calculated
Han, 2012 N=3,507	Alcohol use during pregnancy; self-administered questionnaire at prenatal visits	—	Any alcohol use N Miscarriage Yes 1,667 7.5% No 1,840 7.0%	No effect estimate calculated; chi-squared p-value=0.5
Harlap, 1980 N=32,019	Average number of drinks per day in the first three months of pregnancy; self-administered questionnaire at first prenatal visit	<15	Alcohol (drinks/day): aRR 95% CI 0 1.00 Referent <1 1.12 0.59, 2.13 1–2 1.15 0.57, 2.30 ≥3 1.16 0.58, 2.30	Adjusted for maternal age and gestational age at study entry; adjusted relative risks calculated separately for first and second trimester miscarriages
		15–27	aRR 95% CI 0 1.00 Referent <1 1.03 0.57, 1.86 1–2 1.98 1.04, 3.77 ≥3 3.53 1.77, 7.01	
Kesmodel, 2002 N=24,663	Current number of drinks per week; self-administered mailed questionnaires (median GA 14.7 weeks)	7–11	Alcohol (drinks/week): aHR 95% CI <1 1.0 Referent 1–2 1.3 0.8, 2.0 3–4 0.8 0.4, 1.7 ≥5 3.7 2.0, 6.8	Adjusted for maternal age, smoking, caffeine use, pre-pregnant body mass index, marital status, occupational status, education, and parity; adjusted hazard ratios calculated separately for first and second trimester miscarriages
		12–28	aHR 95% CI <1 1.0 Referent 1–2 1.2 0.9, 1.7 3–4 1.1 0.7, 1.9 ≥5 0.6 0.2, 1.9	
Kline, 1980 N=1,248	Type, frequency, and amount of alcohol use during and before pregnancy; interview after pregnancy outcome (cases) or during pregnancy (controls)	—	Alcohol (frequency) aOR 95% CI Never 1.00 Referent ≤ 2/mo 0.78 0.56, 1.08 <2/wk 1.02 0.62, 1.68 2–6/wk 2.33 1.33, 4.08 Daily 2.58 0.93, 7.14	Adjusted for maternal age, week of gestation, and alcohol use before pregnancy

Study	Exposure Measure*	GA†	Findings			Analysis comments
Long, 1994 N=3,443	Average number of drinks per week during pregnancy; interview after pregnancy outcome (cases) or first prenatal visit (controls)	<13	Alcohol (drinks/week)			Not adjusted for confounders
				RR	95% CI	
			0	1.00	Referent	
			1–10	3.79	1.18, 12.17	
			11–14	8.36	2.52, 27.69	
			>15	5.08	1.18, 21.84	
Maconochie, 2007 N=6,458	Frequency of alcohol consumption and average amount of alcohol consumed per week; self-administered questionnaire completed up to 20 years after pregnancy	<13	Alcohol (drinks/week)			Adjusted for maternal age, year of conception, prior miscarriages, and prior live births
				aOR	95% CI	
			None	1.00	Referent	
			<1	0.99	0.77, 1.26	
			1–7	1.29	1.05, 1.60	
			>7–14	1.23	0.86, 1.77	
			>14	1.64	1.09, 2.47	
Parazzini, 1994 N=1,276	Number of drinking days per week, number of drinks per drinking day, and type of alcohol consumed during the first trimester; in-person interview after pregnancy outcome; converted to average drinks per week	<13	Alcohol (drinks/week)			Adjusted for maternal age, education, prior live births, prior miscarriages, coffee consumption, smoking status
				aRR	95% CI	
			0	1.0	Referent	
			1–7	1.1	0.8, 1.4	
			>7	0.8	0.5, 1.1	
Paszkowski, 2016 N=242	Any alcohol use during pregnancy; self-administered questionnaire during hospitalization for threatened abortion	—	Any alcohol use			No effect estimate calculated; chi-squared p-value=0.84
				N	Exposed	
			Miscarriage	105	20.0%	
			Birth	137	19.0%	
Rasch, 2003 N=1,454	Average number of drinks per week and type of drinks; self-administered questionnaire after dilation and curettage (cases) or during prenatal care (controls); converted to alcohol units per week	6–16	Alcohol (units/week)			Adjusted for maternal age, parity, occupation, cigarette use, and caffeine consumption
				aOR	95% CI	
			0	1.00	Referent	
			1–4	1.00	0.74, 1.34	
			5	4.84	2.87, 8.16	
Windham, 1992 N=1,919	Any change in alcohol consumption during first trimester, amount before change, and amount after change; computer assisted telephone interview after pregnancy outcome; converted to average drinks per week during first trimester	<20	Alcohol (drinks/week)			Adjusted for maternal age, smoking, passive smoking, and nausea
				aOR	95% CI	
			<1/2	1.00	Referent	
			1–3	1.2	0.92, 1.5	
			4–6	1.2	0.81, 1.9	
			≥7	1.7	0.95, 3.1	
Windham, 1997 N=5,142	Number of drinking days and number of drinks per drinking day prior to pregnancy and during the week prior to interview occurring within two weeks of initiating prenatal care and time of consumption change; converted to average drinks per week	≤20	Alcohol (drinks/week)			Adjusted for maternal age, prior miscarriage, cigarette smoking, and caffeine consumption; left truncation prior to interview
				aHR	95% CI	
			0	1.00	Referent	
			0.5	1.8	1.0, 3.3	
			1–3	1.0	0.72, 1.5	
			>3	2.2	1.2, 4.0	
Xu, 2014 N=1,860	Frequency of alcohol consumption during pregnancy; in-person interview within week of loss (cases) or during prenatal care (controls)	<13	Alcohol use (times/week)			Adjusted for prior miscarriage and induced abortion, vitamin use, night shifts, staying up late, physical exercise and smoking
				aOR	95% CI	
			<1	1.00	Referent	
			1–3	0.87	0.65, 1.19	
			≥4	1.04	0.79, 1.27	

Abbreviations: aHR, adjusted hazard ratio; aRR, adjusted relative risk; aOR, adjusted odds ratio; CI, confidence interval; Cont., continuous; GA, gestational age; mo, month; OR, odds ratio; wk, week

*All measures based on self-report; † Miscarriage definition in terms of gestational age

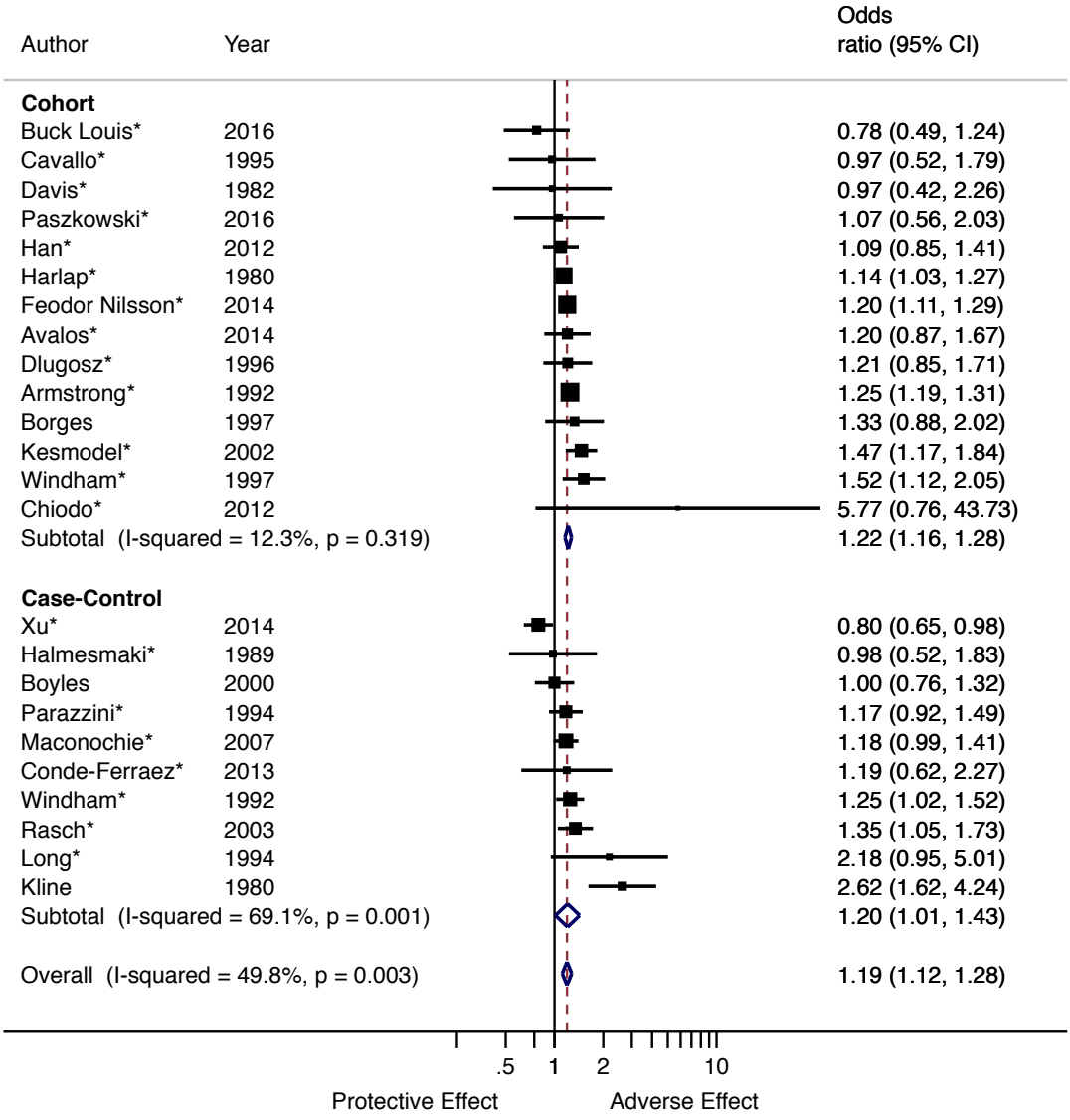

*Figure 7. Forest plot for association between alcohol exposure in pregnancy and risk of miscarriage with subgroup estimates by study design. Weights are from random effects analysis. Abbreviations: CI, confidence interval. *Crude estimate.*

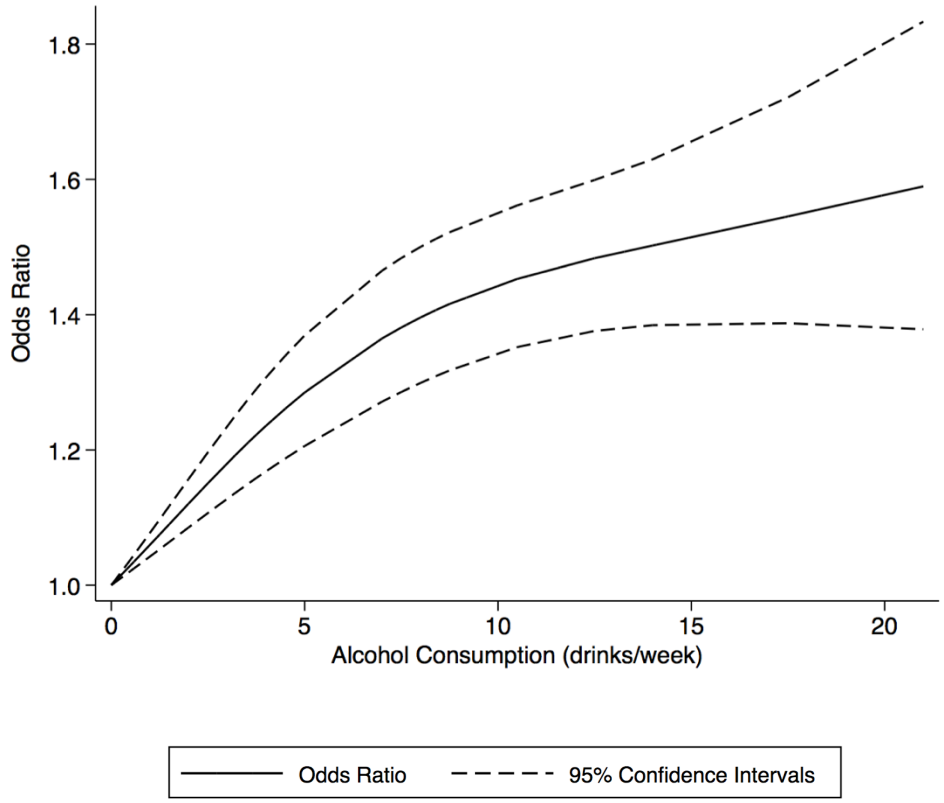

Figure 8. Meta-analysis of dose-response trend between average number of alcoholic drinks per week during pregnancy and miscarriage risk from studies not using survival data, spline model

Table 6. Risk of miscarriage for each additional drink per week in pregnancy from studies not using survival data, linear model

Analysis	n*	OR	95% CI	τ^2
All eligible studies †	12	1.06	1.01, 1.10	0.004
Studies with adjusted estimates	9	1.05	1.00, 1.11	0.005
Cohort studies	6	1.03	1.02, 1.03	<0.001
Case-control studies	6	1.09	0.96, 1.23	0.023
Studies of first trimester miscarriage	4	1.02	1.00, 1.04	<0.001
Excluding studies limited to first trimester miscarriage	8	1.07	1.02, 1.13	0.005

Abbreviations: OR, odds ratio; CI, confidence interval
* n is number of included studies
† Armstrong, 1992; Cavallo, 1995; Chiodo, 2012; Davis, 1982; Dlugosz, 1996; Harlap, 1980; Kline, 1980; Long 1994; Maconochie, 2007; Parazzini, 1994; Rasch, 2003; Windham, 1992

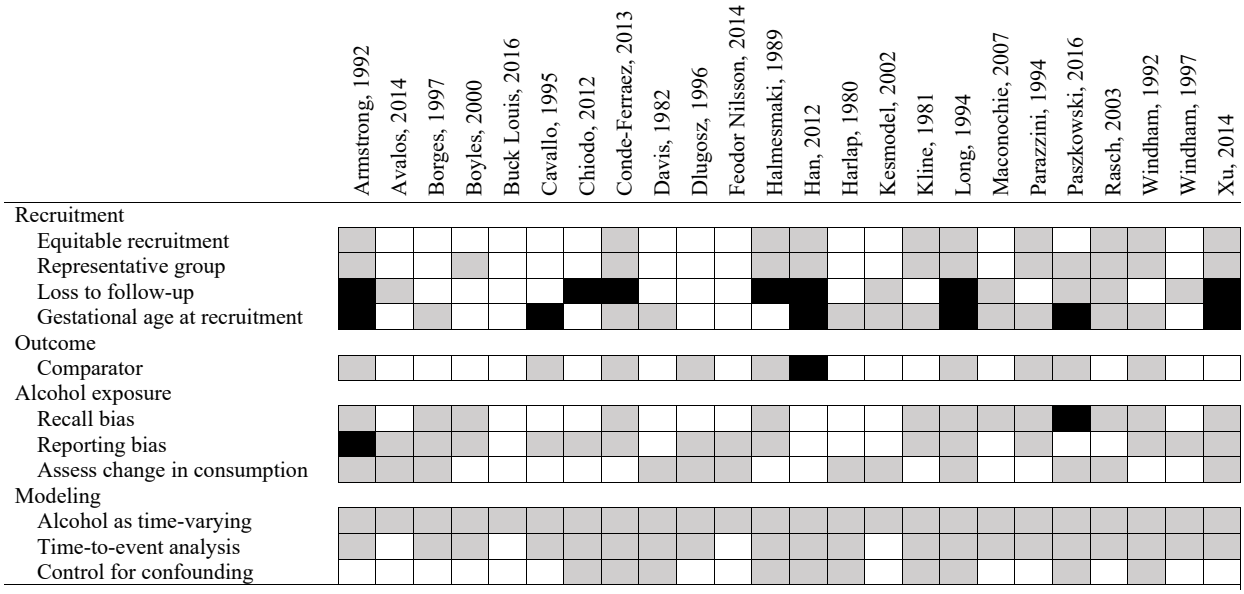

Figure 9. Summary of risk of bias for eleven quality metrics (white-fulfilled; gray-unfulfilled; black-did not report).

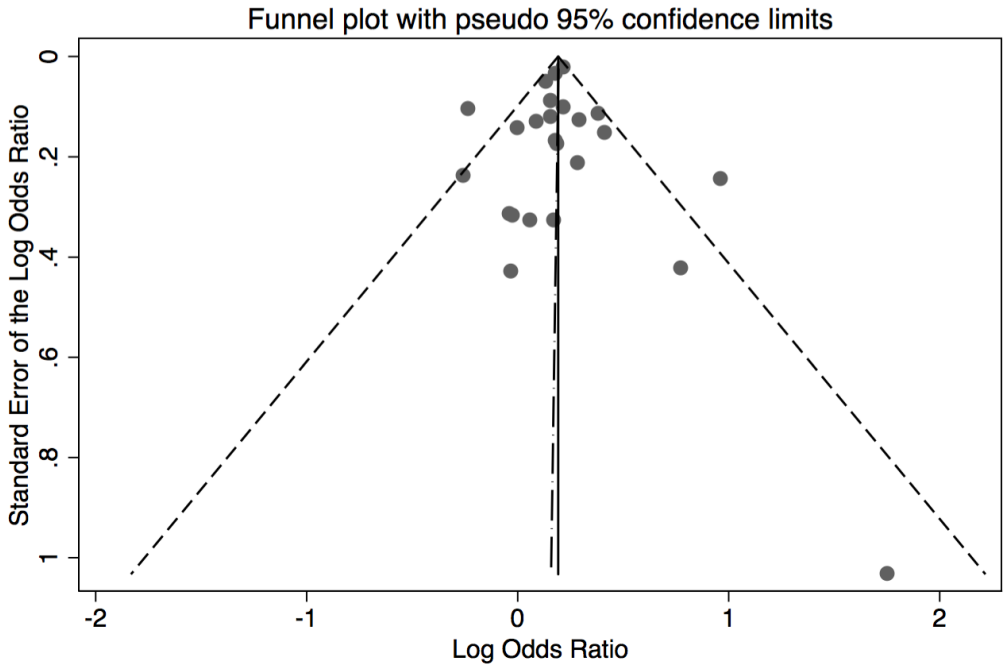

Figure 10. Funnel plot of estimates from included studies with Egger's linear regression, not suggestive of publication bias (p-value 0.96).

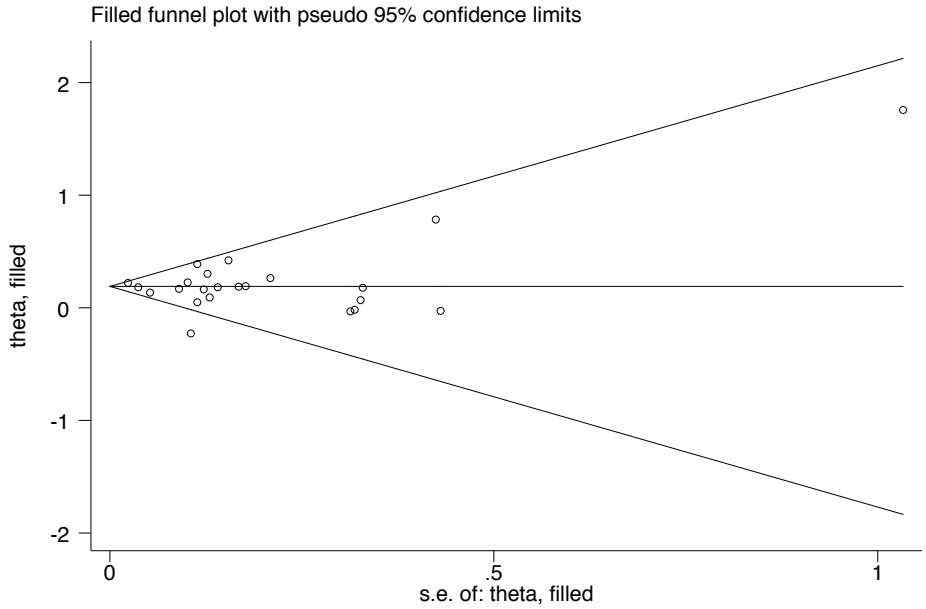

Figure 11. Trim-and-fill plot of included studies does not predict missing studies. Abbreviations: s.e., standard error.

CI 1.00, 1.04; four studies). When aggregating the five studies using survival data, each additional drink per week in pregnancy was associated with a 13% increase in miscarriage hazard (HR 1.13, 95% CI 1.04, 1.22). Subgroup analyses by miscarriage definition could not be carried out for the survival data estimates due to the limited number of studies.

Risk of bias

Included studies scored between two and eight out of nine on the New Castle Ottawa Scale, with higher scores reflecting better study quality (Figure 9). Some of the deducted quality domains may have been met, but many publications lacked sufficient information about study recruitment and follow-up for scoring. Fifty percent of studies assessed alcohol exposure after pregnancy outcome. Sixty-three percent collected information about alcohol exposure through interviews while the remainder used self-administered questionnaires. Forty-three percent of cohorts recruited most participants in the first trimester or pre-conception and 80% of case-control studies, cases were recruited when receiving emergency care and controls were recruited at birth. A funnel plot was not suggestive of publication bias (Figure 10; Egger's test p-value 0.96). Trim-and-fill analysis did not predict missing studies, so the effect estimate remained unchanged (Figure 11).

Comments

Main findings

In this systematic review of alcohol use during pregnancy and miscarriage, alcohol exposure is associated with a dose-dependent increase in risk. Study design and methods varied considerably across included studies. The most common limitations in the literature included imperfect capture of pregnancies ending in miscarriage and oversimplification of methods for assessing and classifying alcohol use during pregnancy. Public health entities recommend complete abstinence for women who are or could become pregnant,[1,199] yet 8 to 20% of women

drink alcohol throughout pregnancy and more than half are exposed in early gestation.[6,45,47] Despite limitations, this review affirms previous guidance that no amount of alcohol exposure is known to be safe[1,199] and provides information about incremental risk for each additional drink per week consumed.

Comparison with other reviews

I aimed to capture literature with data about the relationship between alcohol and miscarriage in this review. A past systematic review described significantly increased risk among women with low-to-moderate alcohol use in five of eight identified studies.[204] Similarly, in this review including an additional sixteen studies, alcohol use was significantly associated with miscarriage in more than half of reports, though individual effects varied in magnitude. The aggregate risk estimate was attenuated compared with a smaller meta-analysis of three studies conducted in 1998 (OR 1.35 versus 1.19; total N 3,156 versus 231,808).[219] Unlike this prior meta-analysis, we required included studies to evaluate miscarriage as an outcome independent of stillbirth and estimated the dose-response risk-relationship.

Considerations

Since most miscarriages occur in early pregnancy,[74] enrolling women soon after pregnancy detection is critical for capturing a representative sample of miscarriages. Six of the fourteen cohort studies in this review failed to either recruit most participants within the first trimester or did not report average gestational age at enrollment. This limits generalizability of these findings for very early losses. Recruitment was also limited in case-control studies. Eight of the ten depended on hospital-based recruitment of miscarriages, which misses women who received out-patient care or no medical care for loss and women with losses that occurred very early in gestation (up to 75% of women opt for expectant management of miscarriage and never receive emergency or inpatient care).[220] Finally, we are unable to comment on the relationship

between alcohol and the estimated one in five pregnancies to end prior to detection[1] since this meta-analysis only included studies of recognized pregnancies.

Since miscarriage definitions varied between studies, I performed subgroup analyses to assess the impact of gestational age cutoff. Four studies restricted to first trimester miscarriages were included in the dose-response meta-analysis and their combined effect was lower than that observed in studies of both first and second trimester miscarriages (OR for each additional drink per week: 1.02 versus 1.07). One explanation is miscarriages with chromosomal abnormalities tend to occur in early pregnancy[221] and the fate of these pregnancies are unlikely impacted by external exposures, such as alcohol use. Another possible explanation is the four studies restricted to first trimester miscarriages were of case-control design. Alcohol use during pregnancy is stigmatized and desirability bias, or the tendency to respond in a way viewed favorably by others, may impact reporting to varying degrees based on level of anonymity in exposure assessment. These sources of bias have strongest influence on results from case-control studies since pregnancy outcome is known when information about exposure is collected.

Modeling of alcohol use in estimates of miscarriage risk did not reflect true patterns of consumption. More than half of women consume alcohol during pregnancy, but most quit or sharply decrease their consumption upon pregnancy detection.[4,7] While 50% of studies assessed whether there was a change from pre-pregnancy alcohol use, none included change in models of risk. Instead, alcohol was classified as consumption after a change in use or an across-pregnancy average. These approaches are limited since the first neglects effect of early alcohol exposure and the second disregards that most use is in early gestation and then rapidly tapers after pregnancy detection. My work in Aim 2 improves on prior studies by evaluating impact of changes in alcohol consumption across gestation.

Methods for determining amount of alcohol consumed varied across studies and did not uniformly account for alcohol content by liquor type and drink size. Further, both pregnant women and women in the general population tend to overestimate the size of a standard drink.[174,175] On average, alcohol content of a drink as judged by women in the general population is 43% more than a standard drink.[175] As a result, categories used in the dose-response analysis approximate true exposure to varying degrees. Imprecision in determining amount of alcohol used would diminish the ability to precisely estimate a dose-response relationship. Additionally, three of seventeen studies with information about dose-specific effects were not adjusted for potential confounders. Nonetheless, subgroup analysis of studies with adjusted estimates did not differ from the estimate including all dose-specific effects (OR 1.05 versus 1.06).

Since only two studies reported miscarriage risk by alcohol type,[12, 29] I could not analyze how this characteristic was associated with risk. One study indicated women who drank only spirits during pregnancy had a greater than two-fold risk of miscarriage compared with abstainers, while drinking only wine, only beer, or a combination of alcohol types was not associated with increased miscarriage risk.[12] The other study did not detect an association between number of glasses of wine or total alcoholic beverages per week and miscarriage risk.[28]

Conclusion

Every additional drink per week in pregnancy increases miscarriage risk. While most women reduce or quit consuming alcohol after pregnancy detection, few studies collected information about change in consumption or assess impact of early alcohol exposure. In Aim 2, I extend past work by leveraging data from a cohort study that enrolled patients early in pregnancy and evaluated timing of change in alcohol use during the first trimester. I use these data to assess the effect of gestational age-specific alcohol exposure to determine the implications of timing of pregnancy detection and alcohol use modification.

IV. SECOND AIM PART A: METHODS FOR MEASURING THE RELATIONSHIP BETWEEN A TIME-VARYING EXPOSURE IN PREGNANCY AND MISCARRIAGE

Abstract

Background: When estimating the association between a time-varying exposure and miscarriage, specifying a statistical model that accurately captures the relationship can be difficult. Overly simplistic modeling likely contributes to the scarcity of known modifiable risk factors for loss. In this simulation study, I sought to determine how assumptions implicit in five modeling approaches influence effect estimates for different risk relationships involving a time-varying exposure.

Methods: I implemented five modeling approaches in distinct, simulated relationships between a time-varying exposure and miscarriage. I use alcohol as an example of an exposure that changes in early pregnancy. Data about consumption patterns from more than 5,000 women in the *Right from the Start* cohort informed prevalence of alcohol exposure before and after pregnancy recognition, distribution in gestational age at alcohol use cessation, and likelihood of miscarriage by week of gestation. I then compared bias and precision of effect estimates and power from different modeling approaches in each simulation context.

Results: Accuracy and precision of effect estimates from each modeling approach heavily depended on how model assumptions aligned with the simulated relationship. Approaches that incorporated data about pattern of exposure were more powerful and less biased than simpler models when risk depended on timing or duration of exposure.

Conclusions: Models contain implicit assumptions about the relationship between outcome and exposure. Ability to accurately quantify a risk-association depends on how well the model reflects the measured relationship, underlining the importance of carefully defining hypotheses about the casual model of risk prior to analysis design.

Overview

Epidemiologists often seek to quantify associations for which the biological relationship between exposure and outcome is unknown. Determining the most appropriate model and method for operationalizing exposure can be difficult since the best model for estimating an association depends on the nature of the relationship. Modeling decisions are especially important when measuring relationships in early pregnancy since exposures occur amid a distinct developmental timeline.[222]

One in six pregnancies end in loss,[75] yet few modifiable determinants of miscarriage are known. One challenge in identifying risk factors for pregnancy loss may rest in limitations of methods used for modeling risk-associations for time-varying behaviors. Both maternal behaviors and a pregnancy's susceptibility to exposures evolve during early pregnancy. Many studies of miscarriage assign exposure based on a woman's status after pregnancy recognition, even though most women alter health behaviors, such as alcohol use, at time of a positive pregnancy test.[4,6,7] This approach fails to reflect exposure history and neglects effects of behaviors in early pregnancy. My objective in Aim 2 is to quantify the relationship between alcohol consumption in the first trimester and miscarriage risk. Accounting for the time-varying aspect of alcohol use during analysis may uncover a clearer relationship between exposure and miscarriage risk. However, alcohol use as a time-varying exposure can be modeled in several ways and the correct approach depends on biological assumptions about how timing of exposure dictates risk.

Based on my understanding of the literature, alcohol may relate to miscarriage in several ways (Table 7). In this simulation study, I test the performance of five approaches for modeling the association between alcohol use and miscarriage in data simulated to reflect different relationships between pattern of exposure and risk of outcome. My primary aim was to assess

how assumptions implicit to different approaches impact the ability to detect and measure an effect in distinct contexts.

Table 7. Potential mechanisms by which alcohol may relate to miscarriage risk

Mechanism	Assumption	Comment
No relationship	Alcohol does not increase risk of miscarriage	Biologically plausible if alcohol exposure does not threaten normal pregnancy development.
Any exposure	Any alcohol exposure (yes/no) during the first trimester increases risk independent of amount used or duration of use	This mechanism does not seem biologically likely, but is the framework on which most literature relies.
Exposure in a critical window	Pregnancy is particularly vulnerable during a specific time in gestation	Biologically plausible since developmental windows exist when a pregnancy is particularly vulnerable to insult.[223,224] For example, oxidative stress from exposure between 5–8 weeks' gestation (when normal pregnancy develops in an anaerobic state) may be more detrimental than alcohol exposure after maternal-fetal circulation is fully established.
Cumulative exposure	Total amount of alcohol consumed while pregnant impacts risk of miscarriage in a dose-response fashion	Biologically plausible since alcoholic beverages contain congeners that may accumulate in placental tissue and be toxic to pregnancy development.[10]
Steady exposure	Regular exposure to alcohol in pregnancy increases risk at all points in gestation	Biologically plausible since alcohol can increase oxidative stress, alter placental profusion, and reduce retinoic acid signaling at any point in the first trimester.[88]
Critical dose	Alcohol increases risk of miscarriage if it reaches a threshold dose in a single episode	Biologically plausible since other teratogens are known to be required to be present at a specific level to have an effect. Since this proposal is focused on regular alcohol consumption not binge episodes, evaluating this mechanism is beyond the scope of this work.

Methods

Empirical data

Right from the Start (RFTS) is a community-based, prospective cohort of women from North Carolina, Tennessee, and Texas who were pregnant between 2000 and 2012.[34] To be eligible, women had to be at least 18 years of age, English- or Spanish-speaking, and not using reproductive technologies to conceive. In the simulation study, I used observations from 5,424

pregnancies to inform assignment of outcome timing, prevalence of alcohol use in early and late pregnancy, and gestational age at change in alcohol consumption. For more information about study recruitment and data collection, please see Chapter V.

Briefly, during the first trimester, participants were asked about current alcohol consumption and whether a change in alcohol use had occurred in the past four months. If a participant reported a change in alcohol use, she was asked about timing of change and alcohol consumption prior to change. If a participant had already experienced a loss prior to interview, she received an interview with modified language that acknowledge the pregnancy had ended and asked about behavior prior to loss. Alcohol use near conception and in early gestation was common in both women with intended and unintended pregnancies (>50%) and 91% of participants who used alcohol modified their behavior during the first trimester (median gestational age at change: 30 days, IQR: 21–36 days).[4] Pregnancy outcome was obtained through maternal self-report and validated by medical or vital records.

Relationships modeled

I modeled five hypothetical relationships between exposure and miscarriage risk in simulated datasets:

Relationship 1. Exposure not related to miscarriage risk. In this scenario, alcohol exposure does not influence risk of miscarriage, allowing us to assess model performance under the null.

Relationship 2. Any exposure uniformly increases risk of loss. In this scenario, the presence of alcohol exposure at any point during pregnancy independent of timing or duration impacts risk of miscarriage.

Relationship 3. Exposure in gestational week five increases risk. In this relationship, exposure in a critical window of development is linked to risk.

Relationship 4. Cumulative exposure associates with risk. This relationship represents the scenario where duration of exposure during pregnancy impacts risk of miscarriage in a dose-response fashion.

Relationship 5. Exposure increases risk during the following week. To represent an exposure that elevates risk of outcome for a limited period of time, I modeled risk to be increased for seven days following exposure.

Simulation parameters

I conducted a series of simulation studies to investigate performance of five regression approaches for quantifying effect of a time-varying exposure following one of the five above risk-relationships. Each scenario was replicated 1,000 times in datasets of 1,500 individuals.

In simulated data, I assumed 55% of subjects were exposed at baseline (t=0). Among those exposed at baseline, 6% continued exposure through 140 days (t=140). Distribution of timing of alcohol cessation was based on observations from RFTS. I used binomial distributions to assign exposure status. Exposure status only changed from exposed to unexposed since I did not observe any instances of participants who initiated alcohol use during pregnancy.

Simulation parameters were designed so the expected proportion of pregnancies to end in miscarriage in the population was 12% for all scenarios to reflect prevalence of outcome observed in RFTS. Distribution of outcome timing for pregnancies ending in miscarriage reflected the distribution of gestational age at loss observed in RFTS. Subjects without pregnancies ending in miscarriage were censored at 140 days' gestation (Table 8).

Modeling approaches

Approach 1. Simple Cox proportional hazard model. In this approach, exposure enters the model as a dichotomous variable (exposed yes/no), where X_1 is constant and β_1 is the log-hazard attributable to any exposure during pregnancy.

$$\lambda(t) = \lambda_0(t)\exp(\beta_1 X_1)$$

Approach 2. Cox proportional hazard model with lag term. This model incorporates information about timing of exposure cessation where X_1 is exposure status at t and β_1 is the log-hazard of exposure status at $(t - x)$. In the simulation, I set x to seven days to indicate exposure anytime in the past week could influence hazard at time t.

$$\lambda(t) = \lambda_0(t)\exp\left(\beta_1 X_1(t - 7)\right)$$

Approach 3. Sequential logistic model. This approach quantifies risk associated with exposure in each week of gestation in separate models, where $X_{1,n}$ is exposure status in week n and $\beta_{n,1}$ is the log-odds of miscarriage given alcohol exposure in week n. Individuals who had not had an event by the first day of week n were included in equation for week n.

$$\text{logit}(p_1) = \beta_0 + \beta_{1,1}X_{1,1}$$
$$\text{logit}(p_2) = \beta_0 + \beta_{1,2}X_{1,2}$$
$$\ldots$$
$$\text{logit}(p_n) = \beta_0 + \beta_{1,n}X_{1,n}$$

Approach 4. Poisson regression with time interaction. In this model, time t in days' gestation is modeled using a fractional polynomial determined by the simulated data and t', and t'' are the first and second fractional polynomial terms, respectively. X_1 denotes exposure status at time t. Terms for the interaction between exposure and time allow the effect of alcohol exposure to be time-dependent in this model.

$$\log(u) = \beta_0 + \beta_1 X_1 + \beta_2 t' + \beta_3 t'' + \beta_4 X_1 \cdot t' + \beta_5 X_1 \cdot t'' + \log(t)$$

Approach 5. Cumulative Cox regression. In this model, $X_1(t)$ denotes cumulative number of days a participant was exposed to alcohol at time t and β_1 represents incremental risk associated with each additional day of exposure.

$$\lambda(t) = \lambda_0(t)\exp\left(\beta_1 X_1(t)\right)$$

Table 8. Parameter assignment in simulation studies

Parameter	Assignment Rule	Comments
Same across all simulations		
Exposure status at time t=0	$X \sim B(1, 0.5500)$	
Exposure status at time t=140	$X \sim B(1, 0.0900)$	Given an individual was exposed at time t=0
Timing of change in exposure (days' gestation)	$\big(X \sim \text{Beta}(7, 5)\big) \cdot 100 - 30$	Negative values denote a change that occurred prior to last menstrual period.
Outcome timing (days' gestation)	$(X \sim \text{Beta}(4.5, 8.6) \cdot 126 + 14$	Given pregnancy ends in miscarriage
Specific to Relationship 1. Exposure not related to miscarriage risk		
Miscarriage risk	$X \sim B(1, 0.1235)$	
Outcome timing (days' gestation)	$(X \sim \text{Beta}(4.5, 8.6) \cdot 126 + 14$	Given pregnancy ends in miscarriage
Specific to Relationship 2. Any exposure increases risk		
Miscarriage risk given exposed in pregnancy	$X \sim B(1, 0.0969)$	
Miscarriage risk given unexposed in pregnancy	$X \sim B(1, 0.1453)$	
Specific to Relationship 3. Exposure in week five increases risk		
Miscarriage risk given exposed in week five of pregnancy	$X \sim B(1, 0.1698)$	
Miscarriage risk given unexposed in week five of pregnancy	$X \sim B(1, 0.1132)$	Includes individuals who were unexposed and those whose exposure status change prior to week five
Specific to Relationship 4. Cumulative exposure associates with risk		
Miscarriage risk given exposed in pregnancy	$X \sim B(1, 0.1060 \cdot 1.0137^{t-14})$	*t* equals outcome time if exposure change occurs after outcome or *t* equals time of exposure change if exposure change occurs before outcome
Miscarriage risk given unexposed in pregnancy	$X \sim B(1, 0.1060)$	Includes individuals who were unexposed throughout pregnancy and whose exposure status change prior to day 14

Parameter	Assignment Rule	Comments
Specific to Relationship 5. Exposure increases risk during the following week		
Miscarriage risk given exposure within seven days of outcome	$X \sim B(1, 0.1960)$	
Miscarriage risk given no exposure within seven days of outcome	$X \sim B(1, 0.1130)$	

In survival model approaches (1, 2, 4, and 5), women accrued time in the model until gestational age at pregnancy loss or 140 days (20 weeks), whichever comes first.

Performance measures

I evaluated performance of the five modeling approaches under each simulated relationship. For each iteration, I collected point estimate, standard error, and significance of effect estimates resulting from each approach (β_1 for Approaches 1 and 2, $\beta_{1,n}$ for Approach 3, and a linear combination of β_1, β_4, and β_5 for Approach 4). For modeling approaches that allow effect estimates to vary with time (Approaches 2 and 4), estimates were stored for the first day of weeks four through eight. I report mean and bias of simulated log-effect estimates and root mean squared error (RMSE) of the effect estimate calculated as the square root of the average squared standard errors. I report coverage as the proportion of simulations in which the 95% confidence intervals for the effect estimate include the true effect and power as the proportion of confidence intervals not including the null.

Results

All models performed well under the null simulation setting in terms of bias, nominal confidence interval coverage rate, and type I error (Figure 10; Table 9).For Relationship 2, where any exposure uniformly increased risk of miscarriage, the simple Cox regression model performed best in terms of bias, coverage, and power (Table 10, Table 11). The sequential logistic model and the Poisson regression with time as a fractional polynomial consistently

underestimated strength of the association (Table 12). Estimates from the sequential model attenuated towards the null as the number of exposed individuals to experience outcome decreased with increasing gestational age whereas for the Poisson model, estimates in earlier weeks of gestation were more biased (Figure 13A). The Cox model with lag term underestimated risk associated with exposure, had low nominal coverage (88%), and was underpowered compared with the simple Cox model (17% versus 83%).

In Relationship 3, where exposure in gestational week five increased risk, the sequential logistic approach was the only model to correctly identify week five as the critical exposure window (Figure 13B). At week five in the sequential logistic approach, bias was minimal (-0.010), nominal confidence interval coverage was satisfactory (95%), but power was low (57%). While week five was the only window designed to associate with risk, I observed effects in adjacent weeks due to correlation in exposure status between weeks (i.e., the tendency for a woman exposed in week n to also be exposed in week $n-1$ and $n+1$). The Poisson model misspecified weeks six and seven as critical weeks of exposure and had less precise estimates throughout compared with the sequential model. The simple Cox model and the Cox model with the lag term could not capture interaction between exposure and gestational age and underestimated the association.

For Relationship 4, where cumulative duration of exposure was associated with risk, the cumulative Cox modeled performed best. The sequential and Poisson models inappropriately measured a decrease in risk for exposure in later weeks of gestation (Figure 13C; Table 12). In simulations of Relationship 5 (exposure increases risk during the following week), the Cox model with lag term provided the most accurate estimate and other approaches underestimated the true association (Figure 13D; Table 10).

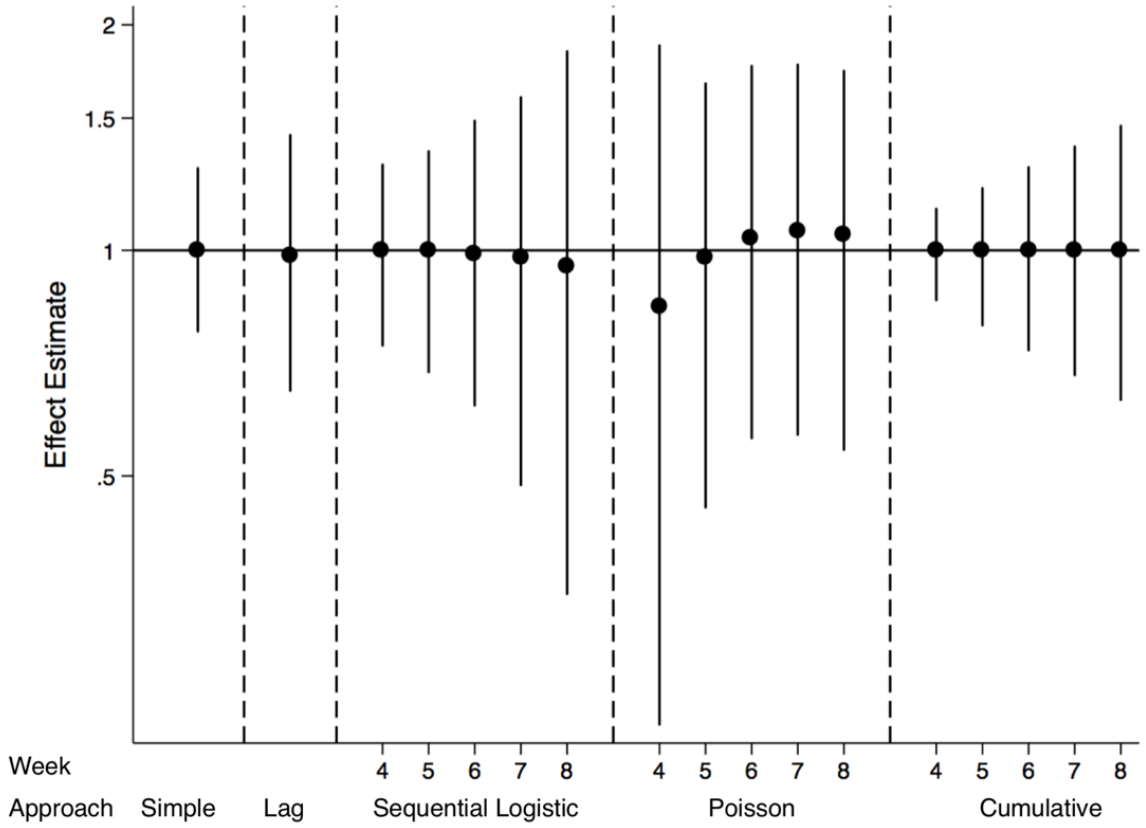

Figure 12. Distribution of effect estimates from five modeling approaches in 1,000 simulation trials under Relationship 1 (exposure is not related to miscarriage risk). Black circle is 50th percentile and intervals span the 5th to 95th percentile of simulated effect estimates. Solid line indicates true effect in dataset.

Table 9. Performance of five models when exposure is not related to miscarriage risk (Relationship 1)

	Simple	Lag	Sequential					Poisson					Cumulative				
Week	N/A	N/A	4	5	6	7	8	4	5	6	7	8	4	5	6	7	8
True effect*†	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean effect*†	0.00	-0.02	0.00	-0.01	-0.02	-0.06	-0.12	-0.26	-0.07	0.01	0.02	-0.04	0.00	0.00	-0.01	-0.01	-0.01
Bias‡	0.00	-0.02	0.00	-0.01	-0.02	-0.06	-0.12	-0.26	-0.07	0.01	0.02	-0.04	0.00	0.00	-0.01	-0.01	-0.01
RMSE	0.15	0.24	0.17	0.20	0.26	0.37	0.54	0.67	0.39	0.34	0.49	0.88	0.08	0.13	0.17	0.21	0.25
Coverage	0.94	0.96	0.96	0.95	0.95	0.96	0.97	0.99	0.96	0.93	0.94	0.96	0.93	0.93	0.93	0.93	0.93
Power§	0.06	0.04	0.04	0.05	0.05	0.04	0.03	0.02	0.04	0.07	0.06	0.04	0.07	0.07	0.07	0.07	0.07

Abbreviations: RMSE, root mean squared error

* On natural log scale

† Effect estimate is β_1 for simple, lag, and sequential models and a linear combination for the Poisson and cumulative models

‡ Mean ln(effect estimate) – ln(effect estimate)

§ Proportion of confidence intervals not including the null

Table 10. Comparison of the bias[*] of effect estimates for gestational weeks' 4–8 from five modeling approaches and model power[†] across four simulated relationships between exposure and outcome

Scenario	*True Effect*	Simple	Lag	Sequential	Poisson	Cumulative
			Bias[*]			
Relationship 2-Any exposure increases risk						
Week 4	*0.405*	0.027	-0.205	-0.120	-0.384	-0.247
Week 5	*0.405*	0.027	-0.205	-0.161	-0.237	-0.168
Week 6	*0.405*	0.027	-0.205	-0.198	-0.167	-0.089
Week 7	*0.405*	0.027	-0.205	-0.228	-0.150	-0.010
Week 8	*0.405*	0.027	-0.205	-0.290	-0.177	0.069
Relationship 3-Exposure in week five increases risk						
Week 4	*0*	0.165	0.231	0.292	0.095	0.164
Week 5	*0.405*	-0.240	-0.174	-0.010	-0.190	-0.159
Week 6	*0*	0.165	0.231	0.293	0.262	0.329
Week 7	*0*	0.165	0.231	0.175	0.254	0.411
Week 8	*0*	0.165	0.231	0.047	0.198	0.493
Relationship 4-Cumulative exposure associates with risk						
Week 4	*0.190*	0.036	0.025	0.129	0.251	-0.013
Week 5	*0.286*	-0.060	-0.071	0.041	-0.153	-0.020
Week 6	*0.381*	-0.155	-0.166	-0.066	-0.156	-0.027
Week 7	*0.476*	-0.250	-0.261	-0.242	-0.231	-0.033
Week 8	*0.572*	-0.346	-0.357	-0.489	-0.365	-0.040
Relationship 5-Exposure increases risk during the following week						
Week 4	*0.405*	-0.282	-0.034	-0.222	-0.153	-0.294
Week 5	*0.405*	-0.282	-0.034	-0.212	-0.046	-0.239
Week 6	*0.405*	-0.282	-0.034	-0.234	-0.035	-0.183
Week 7	*0.405*	-0.282	-0.034	-0.326	-0.073	-0.128
Week 8	*0.405*	-0.282	-0.034	-0.468	-0.184	-0.072
			Power[†]			
Relationship 2-Any exposure increases risk						
Week 4		0.825	0.172	0.409	0.030	0.544
Week 5		0.825	0.172	0.258	0.089	0.544
Week 6		0.825	0.172	0.174	0.151	0.544
Week 7		0.825	0.172	0.106	0.158	0.544
Week 8		0.825	0.172	0.080	0.136	0.544
Relationship 3-Exposure in week five increases risk						
Week 4		0.182	0.236	0.437	0.057	0.558
Week 5		0.182	0.236	0.572	0.131	0.558
Week 6		0.182	0.236	0.281	0.181	0.558
Week 7		0.182	0.236	0.118	0.172	0.558
Week 8		0.182	0.236	0.051	0.119	0.558

Scenario	*True Effect*	Simple	Lag	Sequential	Poisson	Cumulative
Relationship 4-Cumulative exposure associates with risk						
Week 4		0.312	0.195	0.488	0.038	0.615
Week 5		0.312	0.195	0.422	0.091	0.615
Week 6		0.312	0.195	0.305	0.151	0.615
Week 7		0.312	0.195	0.165	0.160	0.615
Week 8		0.312	0.195	0.091	0.133	0.615
Relationship 5-Exposure increases risk during the following week						
Week 4		0.125	0.461	0.209	0.098	0.314
Week 5		0.125	0.461	0.174	0.252	0.314
Week 6		0.125	0.461	0.128	0.312	0.314
Week 7		0.125	0.461	0.065	0.312	0.314
Week 8		0.125	0.461	0.047	0.135	0.314

* Mean ln(effect estimate) – ln(effect estimate)
† Proportion of confidence intervals including the null

Overall, the simple Cox model performed well when the simulated relationship between exposure and outcome was not time-dependent (Relationship 1 and 2). In other scenarios, it tended to underestimate the true association and it could not detect time-varying relationships if present (Relationships 3–5). The Cox model with a lag term had poor nominal confidence interval coverage and power throughout, but provided the best estimate in Relationship 5. Likewise, this approach could not detect or characterize complex interactions between exposure and gestational age. The sequential logistic approach accurately identified a critical window of exposure (Relationship 3), but precision of its estimates suffered across simulation scenarios in later weeks of gestation (>7 weeks) where there were few individuals in the population who were both exposed and went on to experience the outcome. The Poisson regression approach detected exposure-time interactions, but did not correctly approximate the shape of the relationship across weeks 4–8 in most scenarios. Parameter estimates were less precise compared with the sequential logistic model throughout and was underpowered compared to other approaches. The cumulative model forced a dose-response effect by week of gestation in all scenarios, but was the only model to correctly approximate the scenario where duration of exposure related to risk (Relationship 4).

Table 11. Comparison of confidence interval coverage[*] of estimates for gestational weeks' 4–8 from five modeling approaches across four simulated relationships between exposure and outcome

Scenario	Simple	Lag	Sequential	Poisson	Cumulative
Relationship 2-Any exposure increases risk					
Week 4	0.965	0.876	0.886	0.972	0.091
Week 5	0.965	0.876	0.870	0.934	0.696
Week 6	0.965	0.876	0.896	0.930	0.907
Week 7	0.965	0.876	0.933	0.954	0.934
Week 8	0.965	0.876	0.972	0.953	0.926
Relationship 3-Exposure in week five increases risk					
Week 4	0.817	0.764	0.563	0.943	0.442
Week 5	0.626	0.897	0.954	0.936	0.716
Week 6	0.817	0.764	0.719	0.819	0.442
Week 7	0.817	0.764	0.881	0.828	0.442
Week 8	0.817	0.764	0.949	0.881	0.442
Relationship 4-Cumulative exposure associates with risk					
Week 4	0.943	0.947	0.887	0.975	0.919
Week 5	0.916	0.955	0.941	0.957	0.919
Week 6	0.808	0.904	0.943	0.943	0.919
Week 7	0.621	0.809	0.939	0.920	0.919
Week 8	0.400	0.657	0.927	0.849	0.919
Relationship 5-Exposure increases risk during the following week					
Week 4	0.543	0.965	0.760	0.987	0.022
Week 5	0.543	0.965	0.837	0.965	0.492
Week 6	0.543	0.965	0.865	0.954	0.794
Week 7	0.543	0.965	0.906	0.964	0.902
Week 8	0.543	0.965	0.965	0.960	0.931

* Coverage defined as the proportion of simulations where the confidence intervals for the effect estimates include the true effect

Table 12. Comparison of the mean effect[*] and root mean squared error[†] of effect estimates for gestational weeks' 4–8 from five modeling approaches across four simulated relationships between exposure and outcome

Scenario	*True Effect*	Simple Mean Effect (RMSE)	Lag Mean Effect (RMSE)	Sequential Mean Effect (RMSE)	Poisson Mean Effect (RMSE)	Cumulative Mean Effect (RMSE)
Relationship 2-Any exposure increases risk						
Week 4	*0.405*	0.432 (0.154)	0.201 (0.222)	0.285 (0.166)	0.022 (0.598)	0.158 (0.080)
Week 5	*0.405*	0.432 (0.154)	0.201 (0.222)	0.244 (0.189)	0.168 (0.353)	0.237 (0.120)
Week 6	*0.405*	0.432 (0.154)	0.201 (0.222)	0.207 (0.243)	0.239 (0.300)	0.316 (0.160)
Week 7	*0.405*	0.432 (0.154)	0.201 (0.222)	0.177 (0.340)	0.255 (0.308)	0.396 (0.200)
Week 8	*0.405*	0.432 (0.154)	0.201 (0.222)	0.115 (0.486)	0.228 (0.325)	0.475 (0.2400)
Relationship 3 Exposure in week five increases risk						
Week 4	*0*	0.165 (0.150)	0.231 (0.220)	0.292 (0.166)	0.095 (0.592)	0.164 (0.080)
Week 5	*0.405*	0.165 (0.150)	0.231 (0.220)	0.396 (0.185)	0.216 (0.350)	0.246 (0.120)
Week 6	*0*	0.165 (0.150)	0.231 (0.220)	0.293 (0.237)	0.262 (0.300)	0.329 (0.160)
Week 7	*0*	0.165 (0.150)	0.231 (0.220)	0.175 (0.340)	0.254 (0.311)	0.411 (0.200)
Week 8	*0*	0.165 (0.150)	0.231 (0.220)	0.047 (0.500)	0.198 (0.335)	0.493 (0.240)
Relationship 4-Cumulative exposure associates with risk						
Week 4	*0.190*	0.226 (0.154)	0.215 (0.225)	0.320 (0.168)	0.060 (0.650)	0.177 (0.081)
Week 5	*0.286*	0.226 (0.154)	0.215 (0.225)	0.327 (0.190)	0.133 (0.374)	0.266 (0.121)
Week 6	*0.381*	0.226 (0.154)	0.215 (0.225)	0.315 (0.239)	0.225 (0.310)	0.354 (0.162)
Week 7	*0.476*	0.226 (0.154)	0.215 (0.225)	0.234 (0.337)	0.245 (0.318)	0.443 (0.202)
Week 8	*0.572*	0.226 (0.154)	0.215 (0.225)	0.082 (0.501)	0.206 (0.340)	0.532 (0.243)
Relationship 5- Exposure increases risk during the following week						
Week 4	*0.405*	0.123 (0.152)	0.371 (0.210)	0.183 (0.170)	0.252 (0.519)	0.111 (0.082)
Week 5	*0.405*	0.123 (0.152)	0.371 (0.210)	0.193 (0.196)	0.360 (0.320)	0.167 (0.123)
Week 6	*0.405*	0.123 (0.152)	0.371 (0.210)	0.172 (0.252)	0.380 (0.290)	0.222 (0.165)
Week 7	*0.405*	0.123 (0.152)	0.371 (0.210)	0.080 (0.365)	0.332 (0.303)	0.278 (0.206)
Week 8	*0.405*	0.123 (0.152)	0.371 (0.210)	-0.062 (0.551)	0.221 (0.339)	0.333 (0.247)

Abbreviations: RMSE, root mean squared error.
* Natural log of the mean estimated effect
† Root mean squared error calculated as the square root of the average squared standard errors

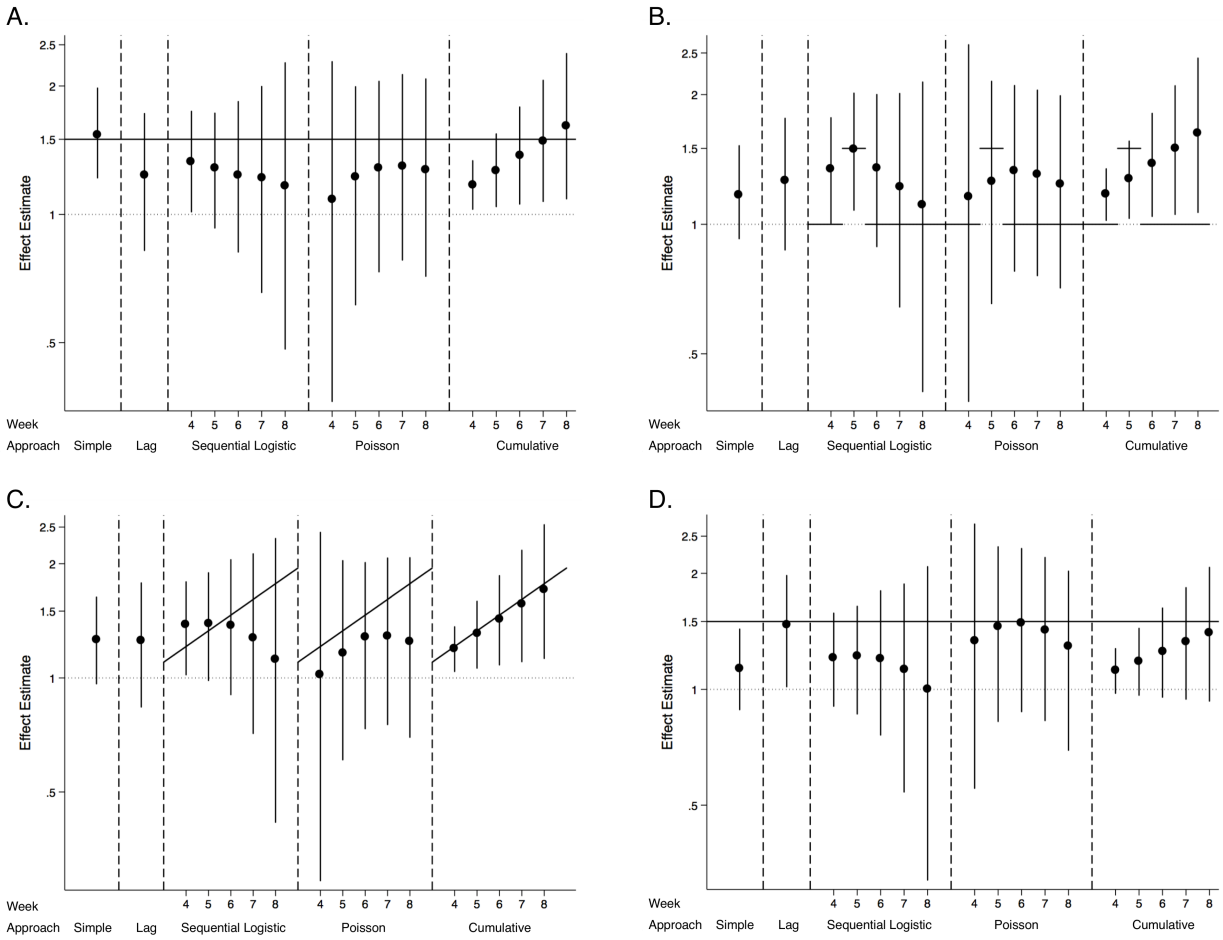

Figure 13. Distribution of effect estimates from five modeling approaches in 1,000 simulation trials under Relationship 2 (any exposure during pregnancy increases risk; Panel A), Relationship 3 (exposure in gestational week five increases risk; Panel B), Relationship 4 (duration of exposure has a dose-response effect on risk; Panel C), and Relationship 5 (exposure increases risk during the following week; Panel D). Black circle is 50th percentile and intervals span the 5th to 95th percentile of simulated effect estimates. Solid line indicates true effect in dataset.

Comments

In this simulation study based on observations from the *Right from the Start* pregnancy cohort, performance of modeling approaches for measuring the effect of alcohol as a time-varying exposure depended on the relationship applied in the underlying, simulated dataset. Conventional methods for estimating risk associated with behaviors in pregnancy often involve a gross simplification of temporal pattern of exposure. Many studies use self-reported behavior after pregnancy recognition to assign exposure status even though many women alter habits after pregnancy detection.[4,6,7] As a result, effects of behaviors occurring early in pregnancy frequently go unmeasured. Leveraging longitudinal data for time-varying exposures captures more information than using simpler methods, but selecting the best modeling approach depends on assumptions about how exposure, outcome, and timing interrelate. The degree to which an approach's estimates approximated true effect varied considerably across simulated relationships, underlining the importance of defining beliefs about mechanism of effect when developing an analytical plan.

During pregnancy, exposures occur in the context of gestational age and behavioral exposures tend to change near time of pregnancy recognition. More than 50% of women used alcohol near conception regardless of pregnancy intention in the RFTS pregnancy cohort and 6% reported continued use through the first trimester. In a review of twenty studies that measure the relationship between alcohol exposure and miscarriage, 45% assess only alcohol use after pregnancy recognizition.[9-11,19,22,23,25,28,29] This approach misses information about early pregnancy alcohol use and misrepresents exposure status for 90% of women who consume alcohol during pregnancy. Only using data about behaviors after pregnancy detection assumes exposure in very early gestation do not influences miscarriage risk. Risk unlikely operates in this way biologically since critical milestones in development occur in the first weeks of gestation,

when women who use alcohol tend to be exposed. Also, duration of alcohol exposure varies between women. While some studies assess change in alcohol use during the first trimester, none model alcohol use as a time-varying exposure.[12-15,20,21,24,26,27,30,31] Instead, alcohol exposure before and after pregnancy were included in separate models or operationalized as an across-pregnancy average dose, even though exposure was ubiquitously heavier and more prevalent in early gestation.

I designed this simulation study to assess how assumptions about the relationship between exposure timing and outcome inherent to different modeling approaches influence estimates of association. Prevalence of exposure and the gestational age distributions for alcohol cessation and miscarriage were the same in all simulated datasets. Despite these constants, altering how risk of outcome was conditioned on exposure pattern drastically altered performance of the models. For example, when exposure status was set to increase miscarriage risk in the following week, the Cox model with a lag term performed the best (Modeling Approach 2). However, this approach cannot accurately specify scenarios in which risk varies across gestational age (i.e., when exposure in a given gestational week or duration of exposure determines risk). Similarly, when exposure in week five of gestation was set to drive risk, the sequential logistic modeling approach (Approach 3) accurately identified the critical window of exposure and effect magnitude. Yet when risk was not tied to exposure in a specific week of gestation, this approach systematically underestimated risk. Modeling approach performance heavily relied on the underlying simulated relationship. These findings emphasize arriving at accurate estimates requires correctly specified assumptions. Difficulty remains when little biological evidence exists to inform modeling decisions.

Considerations

Observations from more than 5,000 pregnancies informed assignment of exposure prevalence and cessation timing in the simulation. I only allowed non-reversible cessation of alcohol use because we only observed participants who ceased or continued use. We did not observe any instances of participants who were unexposed to alcohol at the beginning of pregnancy and initiated use during pregnancy. We cannot comment on how these approaches would perform for exposures with more complex temporal patterns. While approaches 2–5 incorporate more data about exposure pattern than conventional models, limiting exposure characterization to gestational age-specific status still simplifies true behaviors and likely discounts crucial information about determinants of risk. Aspects of an exposure apart from temporal pattern often contribute to risk biologically. For example, interaction between alcohol dose and consumption timing may have a much stronger effect on risk than either characteristic individually.

Conclusion

All models are wrong, but some are useful.[225] This simulation study highlights how a model's usefulness is fettered to its alignment with nuances of the relationship it measures. Epidemiologists must carefully consider the biological mechanism by which an exposure is thought to increase risk to determine which characteristics of the exposure to measure and model. When no definitive mechanism exists, designing several analytical plans based on hypotheses of plausible biological mechanisms is recommended. In studies of pregnancy, timing of exposure is of particular significance since behavioral changes occur in the context of a distinct developmental timeline. Careful consideration of how to best model exposure timing in studies of pregnancy health may unmask risk factors and provide insight into which

characteristics of exposure dictate risk. The results of this simulation study inform the statistical analysis in Aim 2 Part B.

V. SECOND AIM PART B: TO UNCOVER THE RELATIONSHIP BETWEEN ALCOHOL AND MISCARRIAGE RISK IN A PROSPECTIVE PREGNANCY COHORT

Abstract

Background: Most women stop using alcohol once pregnancy is detected, but 50% of pregnancies are exposed during the first weeks of gestation. Data about alcohol use prior to pregnancy recognition are sparse. This work incorporates information about timing of alcohol use cessation into measures of miscarriage risk.

Methods*:* Participants in the *Right from the Start* prospective pregnancy cohort (2000–2012) provided information about timing of change in alcohol use in the first trimester and characteristics of use before and after change. I estimated how gestational week-specific alcohol exposure and duration of exposure associated with miscarriage risk. Risk associated with exposure during each week of gestation was measured using logistic regression. Risk associated with duration of exposure was measured using extended Cox survival models. I also assessed whether alcohol type or drinks per week modified risk. Models were adjusted for maternal age, race/ethnicity, parity, smoking status, education, and pregnancy intention.

Results: Among 5,424 participants, 50% reported using alcohol during early pregnancy and 12% experienced miscarriage. Median gestational age at change in alcohol use was 29 days (inter-quartile range 15–35). Exposure during gestational week five through ten was associated with increased miscarriage risk after accounting for multiple testing (adjusted odds ratios: 1.42–4.85). Each additional week of alcohol exposure during pregnancy was associated with an 8% increase in miscarriage risk relative to those who were unexposed (hazard ratio 1.08, 95% confidence interval 1.04, 1.12). Risk was not related to beverage type or number of drinks per week.

Conclusions: Approximately half of pregnancies are unplanned and exposure up until pregnancy detection is common regardless of pregnancy intention. Alcohol use during week five of

gestation, after the point pregnancy is detectable through a home test, through week ten is linked with increased risk of miscarriage. Early pregnancy recognition and alcohol use cessation could curtail the risk of miscarriage attributable to alcohol use during pregnancy.

Overview

Although 50% of women report alcohol exposure around the conception,[1,3,42] the relationship between alcohol use during pregnancy and miscarriage remains poorly established. Biological rationale behind concern about alcohol use during early gestation includes alcohol's ability to induce oxidative stress,[121-123] hinder retinoic acid synthesis,[151,153] alter maternal hormone levels,[98] and impair placental development and perfusion.[99,102,103] Weekly consumption of three or more alcoholic beverages has been reported to increase risk of miscarriage up to 3-fold, yet other studies report no association between moderate levels of consumption and pregnancy loss.[9-33] Most studies of this association recruit participants during prenatal care, meaning enrollment takes place later in gestation than many miscarriages occur. Others have cases limited to women seeking emergency care for symptoms of loss. Most importantly, past analyses neglect the time-varying nature of alcohol consumption during pregnancy, treating use as a constant exposure.

While alcohol use is common in early pregnancy regardless of pregnancy intention, 90% of those exposed alter consumption once they are aware of their pregnancy.[4,8] Given there are two distinct patterns of alcohol exposure during pregnancy for most women, analyses of risk should acknowledge both behaviors.

Information about how timing of change in alcohol use impacts miscarriage risk is scarce. To advance understanding of the influence of alcohol exposure on pregnancy, I sought to incorporate timing of change in alcohol use into measures of miscarriage risk. In this cohort, participants were recruited to reflect the general obstetric population, were enrolled during early

pregnancy or while planning a pregnancy, and reported alcohol use before and after a change in behavior. I evaluated how gestational age-specific alcohol use and duration of exposure related to miscarriage risk. I also assessed whether amount consumed or beverage type modified risk.

Methods

Right from the Start

RFTS is a community-based, prospective pregnancy cohort conducted between 2000 and 2012 across three phases (RFTS1, RFTS2, RFTS3). Participants were recruited from three states (North Carolina, Tennessee, and Texas). While study focus varied slightly between phases (Table 13), phases shared similar study events, recruitment methods, and data collection forms.

Table 13. Characteristics of Right from the Start phases

Phase Trait	RFTS1	RFTS2	RFTS3
Time	2000–2004	2004–2012	2007–2012
Gestational age at enrollment	< 13 weeks' gestation	≤ 10 weeks' gestation	Prior to conception
Exposure focus	Water disinfection	Uterine fibroids	Early pregnancy symptoms and events
N	2,322	2,631	479
States	NC, TN, TX	NC, TN	TN
Additional study activities	Collection of water samples throughout study catchment area	Nested substudy involving serial ultrasounds throughout pregnancy	Daily diary throughout the pre-pregnancy period and first trimester

Abbreviations: NC, North Carolina; RFTS, *Right from the Start*; TN, Tennessee; TX, Texas

Recruitment

Recruitment strategies for RFTS maximized enrollment of participants representative of the underlying population. Clinic-based studies may be biased by underrepresentation of women who do not seek prenatal care early in pregnancy or who are at higher risk for adverse outcomes.[160] RFTS investigators endeavored to enroll a sample more representative of the general population than those accrued from academic clinical care sites alone by boosting study visibility for reproductive-aged women in the community. The study was advertised in the

community through letters to new homeowners, emails, bus ads, and flyers. RFTS also partnered with private obstetrics clinics, hospital obstetrics and gynecology departments, health departments, and other prenatal care providers such as Planned Parenthood. These partners posted information about the study in their offices, offered brochures, and provided additional information to patients who showed interest in participating.

Eligibility

To be eligible, participants were at least 18 years of age at LMP, English- or Spanish-speaking, not using assisted reproductive technologies to conceive, and intending to carry pregnancy to term. Maximum gestational age permitted at time of enrollment varied with study phase (Table 13) and all participants were enrolled by twelve weeks of gestation. Women participating in RFTS3 had to have internet access to complete web-based daily diaries to be eligible. Participants could enroll for multiple pregnancies.

Women intending to become pregnant who were between the ages of 18 and 45 could enter the study if they had been trying to conceive for fewer than six months (RFTS1, RFTS2) or fewer than three months (RFTS3). Permitted trying-time was limited to prevent over-selection of women who were sub-fertile or infertile. Women were provided free pregnancy tests and instructed to test for pregnancy on the first day of expected menses and alert study personnel of first positive test. A subset of women who entered the study prior to conception participated in web-based daily diaries documenting lifestyle and medication exposures, symptoms, and intercourse patterns.

Study events

At enrollment, eligible women were informed of the study events and requirements (Figure 14). A brief intake interview was conducted to collect contact information and data about

maternal demographics, date of LMP, symptoms of early pregnancy, and lifestyle behaviors (e.g., cigarette use, caffeine intake, vitamin supplementation).

Participants underwent a transvaginal research ultrasound to document presence of developmental features, to confirm gestational dating, and to characterize uterine fibroid size, type, and location. Ultrasounds were performed by study-trained sonographers with five or more years of clinical obstetric experience. Ultrasounds were targeted for the sixth week of gestation and took place no later than the twelfth week of gestation. At this time, maternal anthropometric measurements were taken (weight and height [RFTS2, RFTS3]) and signed informed consent forms were obtained from participants (Appendix 7: *Right from the Start* Informed Consent). Sonographers did not discuss findings of research ultrasound with participants.

Participants completed a computer-assisted telephone interview (CATI) at the end of the first trimester (targeted for 13 weeks' gestation [RFTS 2, 3] and occurring no later than 16 completed weeks' gestation). This interview collected information including maternal medical conditions, reproductive history, cigarette use, and alcohol consumption.

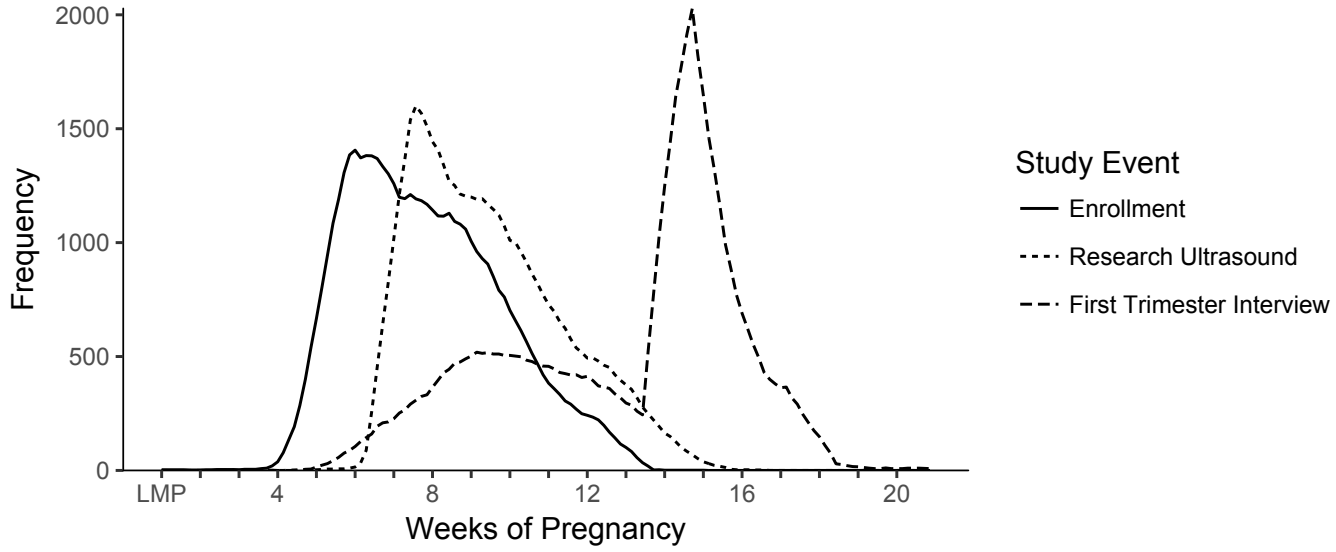

Figure 14. Distribution of gestational age at which Right from the Start study events occurred.

Exposure assessment

During the first trimester interview, participants provided detailed information about alcohol consumed within the past four months (Appendix 8: First Trimester Interview Questions About Alcohol Consumption). This window was selected to capture alcohol exposure immediately prior to pregnancy and throughout the first trimester. Participants reported whether they altered alcohol use during this period, date of change in use, and frequency, amount, and type of alcohol consumed before and after change. Participants were also asked about the presence and number of binge episodes in the same window, defined as more than four drinks on any one occasion. Survey items about alcohol exposure included:

- At this time, do you drink any alcoholic beverages, like beer, wine, or liquor including gin, whiskey, rum, or mixed drinks?
- How often do you drink an alcoholic beverage?
- On the occasion you drink alcoholic beverages, how many drinks do you usually have?
- What type(s) of alcohol do you usually drink?
- In the past four months, have you changed how often and/or how many alcoholic beverages you drink?
- When did this change occur?
- Before this change how often did you drink?
- Before this change, how many drinks did you usually have on each occasion?
- Before the change, what type(s) of alcohol did you drink?

Over 80% of women who endorsed altering their alcohol use provided the exact date of change (2,375/2,962). Of the 587 participants who did not know the exact date of change, 97.4% provided the week and month change occurred. For these participants, date of change was assigned as the midpoint of the week.[226] For the fifteen participants who only provided the month

of change in use, the midpoint of the month was assigned as the date of change. Gestational age at change in alcohol use was calculated as the difference between date of reported change and LMP. Knowing when a change occurred relative to LMP allowed me to assign gestational age-specific exposure status and number of drinks per week.

A four-level categorical variable was constructed to describe pattern of alcohol use: never drinkers, stopped alcohol consumption before LMP, stopped alcohol consumption during first trimester, and exposed through first trimester (included women who did not report change in alcohol use or who altered frequency or amount of use but continued to consume alcohol). Average number of drinks per week was calculated for before and after change based on reported frequency of drinking and average number of drinks per drinking episode. When converting responses to drinks per week, I assumed 4.35 weeks are in one month. If a woman reported consuming less than one drink per month, she was assigned an exposure of 0.12 drinks per week (equivalent to 0.5 drinks per month). I categorized drinks per week based on the distribution of exposure at LMP (unexposed, less than or equal to one drink per week, more than one to two drinks per week, more than two to four drinks per week, more than four drinks per week). To assess alcohol type, I determined participants exposure to wine, beer, and/or liquor (spirits consumed alone or in mixed drinks). These categories are not mutually exclusive. Women could report using multiple beverage types.

Outcome assessment

Participants provided pregnancy status and information about intended birth hospital during a follow-up telephone interview or through a paper form at 20–25 weeks of pregnancy. Self-reported pregnancy outcome was corroborated by information abstracted by trained study personnel from vital records, birth certificates, fetal death certificates, and medical records. Miscarriage was defined as loss of pregnancy prior to 20 completed weeks of gestation. The

comparison group included women with pregnancies surviving past 20 weeks' gestation (live births and stillbirths) and participants with unknown pregnancy outcome censored at date of last study contact. I used self-reported LMP to determine gestational dating for pregnancy events. Ultrasound-based dating is subject to systematic misclassification for pregnancies ending in loss since normal pregnancy development may have already arrested at time of ultrasound, impacting features used to determine gestational age.[227] Self-reported LMP is validated in the RFTS cohort (average difference of 0.8 days between LMP versus ultrasound dating).[228]

Studies that fail to account for time between arrest of normal pregnancy development and miscarriage overestimate time at risk (Figure 15). Correctly identifying when a pregnancy departs from its normal trajectory is especially important when modeling the relationship between miscarriage and an exposure that varies with time. Since most women change their alcohol consumption once pregnancy is detected, carefully defining time at risk is critical. I modeled outcome timing in two ways: gestational age at miscarriage (traditional method) and gestational age at arrest of development (GAAD) based on features observed during research ultrasound.[229]

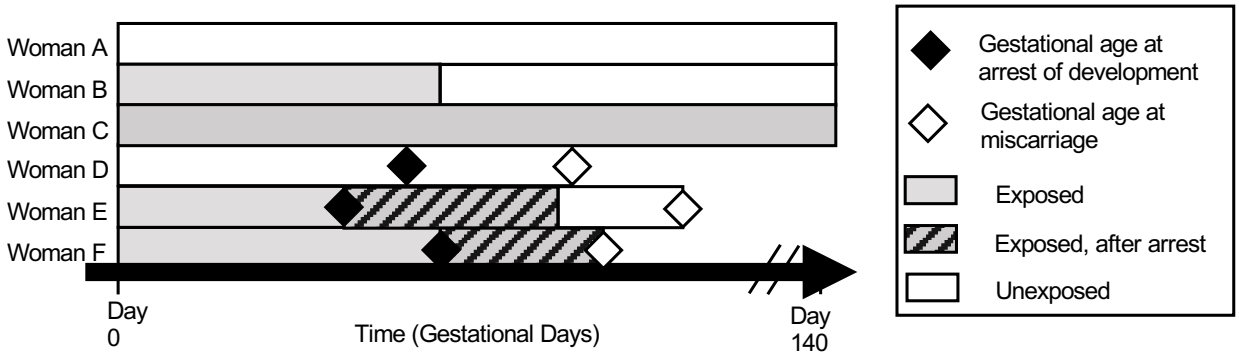

Figure 15. Illustration of how gestational age at miscarriage may lead to misclassification of time at risk, exposure duration, and exposure status at outcome.

Gestational age at arrest of development

I used structural features observed during ultrasound to classify five stages of pregnancy development: empty uterus, only gestational sac present, only gestational sac and yolk sac present, fetal pole without fetal cardiac activity, and fetal pole with fetal cardiac activity. I used well-established formulas specific to features observed during ultrasound to calculate gestational age at arrest of development (GAAD; Table 14). For anembryonic pregnancies (no detectable embryo) with a gestational sac present, I used mean gestational sac diameter to estimate GAAD.[191] For pregnancies with a fetal pole present (detectable embryo), I used crown-rump length to estimate GAAD.[193] If cardiac activity was observed during ultrasound, indicating a pregnancy had not yet arrested, I calculated gestational age at ultrasound using crown-rump length and added the midpoint between date of ultrasound and miscarriage to estimate GAAD. GAAD was calculated for women with losses who had complete ultrasound information and known LMP and date of loss (n=462).

Seventy-one percent of participants with pregnancies ending in loss had sufficient data for estimating GAAD (462/649). For women with loss who were missing a research ultrasound, I assigned the time between gestational age at arrest and miscarriage symptoms by randomly sampling observed gaps between GAAD and miscarriage among participants with miscarriage occurring during the same gestational week. If a woman missing a research ultrasound had a loss prior to 32 days of gestation, I assigned GAAD as gestational age at miscarriage since morphologic features would not be observable during ultrasound prior to this point.

Covariates and variables of interest

I used a directed acyclic graph (DAG) to depict the relationship between candidate confounders for the association between alcohol use and miscarriage (Figure 16). To confound an association, a variable must be related to miscarriage risk among women who abstain from

Table 14. Stage of pregnancy development at time of ultrasound and method for calculating gestational age at arrest of development for women with pregnancies ending in miscarriage

Developmental stage at ultrasound	No. of losses (n=698)	No. used in GAAD gap estimation (n=500) *	Calculation of estimated GAAD †
Loss before ultrasound	176	0	Cannot be estimated
Anembryonic gestation			
Empty uterus	38	36	If US < 31 days: GAAD=GA at ultrasound If GA at US ≥ 32 days: GAAD=32 days
Gestational sac only	83	82	GAAD=0.882(MSD)+33.117
Gestational and yolk sac	77	74	GAAD=0.882(MSD)+33.117
Fetal pole present			
No FHR	102	101	$GAAD = 7 \cdot \exp[1.685 + 0.316(CRL) - 0.049(CRL)^2 + 0.004(CRL)^3 - 0.0001(CRL)^4]$
FHR Present	215	207	$GAAD = (7 \cdot \exp[1.685 + 0.316(CRL) - 0.049(CRL)^2 + 0.004(CRL)^3 - 0.0001(CRL)^4])$ + midpoint between day of ultrasound and day of miscarriage
No measures possible at ultrasound	7	0	Cannot be estimated

Abbreviations: CRL, crown rump length (cm); FHR, fetal heart rate; GA gestational age; GAAD, gestational age at arrest of development (days); MSD, mean gestational sac diameter (mm); No., number; US, ultrasound.

* Differences between columns for women with ultrasound data denote women without a self-reported last menstrual period for whom GAAD could not be calculated

† GAAD calculation based on gestational sac diameter from Daya et al., 1991 and based on crown rump length from Hadlock et al., 1992

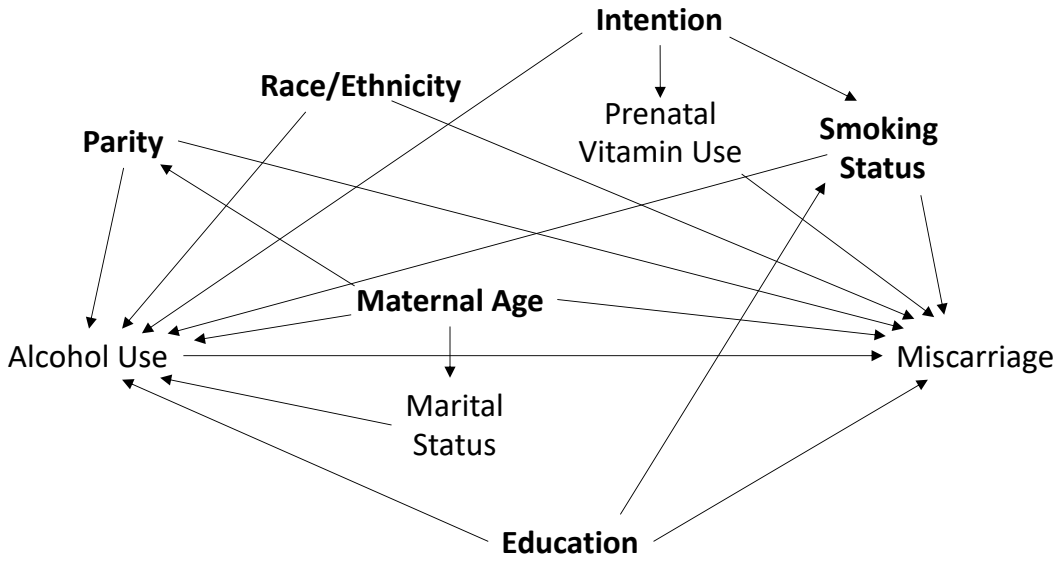

Figure 16. Directed acyclic graph for the alcohol-miscarriage risk-association. Variables included in the adjusted model bolded.

alcohol during pregnancy and with alcohol exposure among all participants. If the DAG is correctly specified, adjusting for maternal age, education, cigarette use, race/ethnicity, pregnancy intention, and parity would result in an unbiased estimate of the association between alcohol use and miscarriage risk. I decided *a priori* to include this covariate set in adjusted models. History of miscarriage was not considered as a confounder since participant attributes associated with past losses are likely to be associated with risk of loss in the study pregnancy.[230]

Most variables were collected through self-report during the intake interview at enrollment or the first trimester interview. Interviews were conducted using standardized questionnaires. If a woman had a loss before the first trimester interview, study personnel used a questionnaire modified to reflect the pregnancy of interest was no longer ongoing. Questions had "don't know" and "refused" response options. For this analysis, these responses were treated as missing. Table 15 describes the source and format in which covariates were collected and how they were operationalized in this analysis.

Table 15. Operationalization of covariates

Characteristic	Format of Collected Data	Operationalization for Analyses	Source
Maternal age	Continuous years, calculated as time between self-reported birthdate and LMP	Continuous in models: Using restricted cubic splines, four knots	Intake interview
Race/ethnicity	Non-Hispanic, White Non-Hispanic, Black Hispanic Native American Asian Other Refused Don't Know	Categorical: Non-Hispanic, White (referent) Non-Hispanic, Black Other	Intake interview
Education	Highest level of education completed, years	Categorical High school or less Some college College or more	Intake interview
Parity	Total number of prior deliveries after 20 weeks' gestation	Categorical 0 (nulliparous) 1 prior birth ≥ 2 prior births	First trimester interview
Smoking status	Participants asked if ever-smoker, smoking status at time of interview, and quit date	Categorical Never smoker/distant quit Quit within the past four months Current smoker	First trimester interview
Pregnancy intention	Participants provided context around conception including whether they were actively trying to conceive, using contraception use at time of conception, wanting another child, and preference for pregnancy timing	Categorical: Intended (trying) Unintended (not trying) Based on definition used by CDC[163]	First trimester interview

Abbreviations: CDC, Centers for Disease Control

Population selection and characteristics

A total of 6,105 pregnancies were enrolled in RFTS. If a woman participated in RFTS for multiple pregnancies (n=325), only the first pregnancy was included. I excluded women with molar or ectopic pregnancies (n=11) or pregnancies ending in induced abortions (n=17). I also excluded women who did not have a first trimester interview (n=297) or sufficient information to classify pattern of alcohol exposure during pregnancy (n=31). Data from 5,424 pregnancies were eligible for analysis (Figure 17). Median gestational age at enrollment was 47 days (IQR 38–83 days) and 25.8% of women entered the study prior to conception (1,401/5,424).

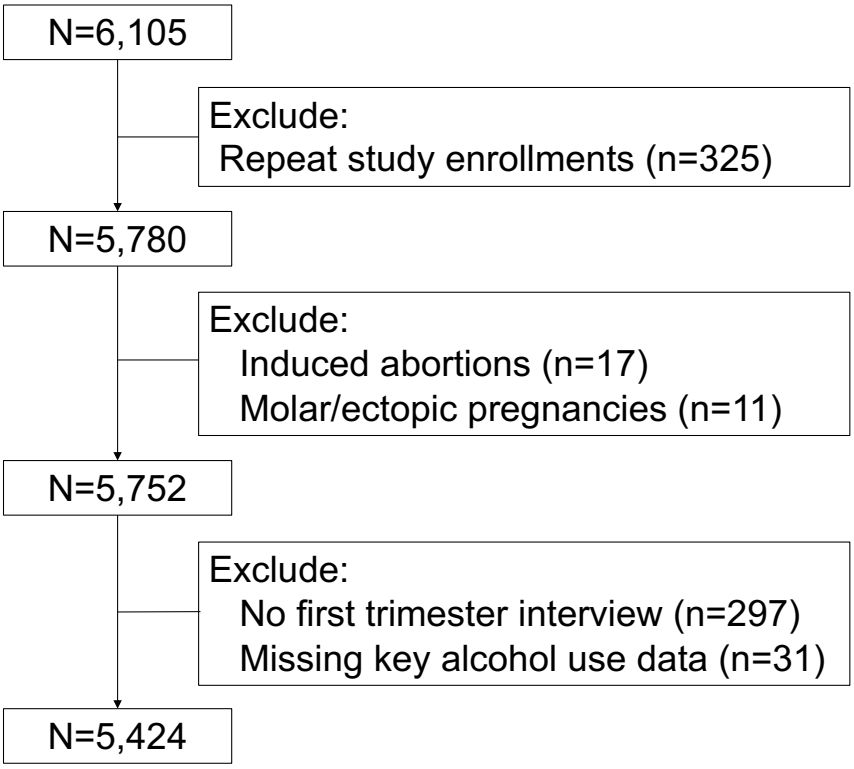

Figure 17. Flow diagram showing inclusion and exclusion criteria for study sample.

Analysis in *Right from the Start*

I used two main modeling approaches to quantify risk associated with alcohol use in pregnancy since timing of alcohol exposure may influence risk in multiple ways (See simulation study in Chapter IV). First, I assessed how exposure in each week of gestation relates to

miscarriage. Second, I evaluated how duration of alcohol exposure relates to miscarriage. These approaches incorporate information about presence and timing of alcohol exposure during early pregnancy, improving on typical methods which treat alcohol as a constant exposure.

Gestational age-specific exposure

Timing of exposure during pregnancy maps to embryologic development and thus informs risk, so I examined gestational week-specific effects of alcohol use. To assess week-specific associations between alcohol use and miscarriage, I performed separate logistic regressions to estimate crude and adjusted odds ratios for alcohol exposure (yes/no) during each gestational week of the first trimester and miscarriage. Participants who did not use alcohol during pregnancy were counted as unexposed and participants who did not change consumption or who only altered amount consumed were considered exposed for all weeks. Participants who stopped using alcohol during the first trimester were classified as exposed in weeks prior to reported change and unexposed thereafter. Participants were included in the week-specific model if they had not yet had a loss or been censored by the beginning of the week.

To evaluate the role of gestational age-specific amount consumed, I quantified the association between miscarriage risk and number of drinks per week in four developmental windows in the first trimester: peri-implantation (gestational weeks one through four), early embryonic (gestational weeks five through seven), late embryonic (gestational weeks eight through ten), and fetal (gestational weeks eleven and twelve). These periods were selected since they mirror developmental windows during which teratogens are expected to confer risk through different mechanisms.[223] I performed separate logistic regressions for amount consumed and miscarriage risk for each window. Participants were included in the regression if they had not yet had a loss or been censored by the beginning of the window.

Duration of exposure

I also considered duration of alcohol use during pregnancy may drive risk. To quantify the association between duration of alcohol exposure and miscarriage, I used extended Cox survival models classifying alcohol use as a time-varying exposure (duration of use at gestational day *t*). I calculated crude and adjusted hazard ratios and 95% confidence intervals for duration of alcohol use defined as number of days between LMP and time *t* or gestational age at cessation of alcohol use, whichever came first. If a participant did not quit using alcohol during the first trimester, duration accumulated until date of first trimester interview. I present results as hazard ratios associated with each additional week of use.

I also calculated crude and adjusted hazard ratios associated with each additional week of use by amount consumed at LMP (continuous and categorical) and beverage type. Since women could be exposed to more than one alcohol type, I estimated the effect associated with each type in separate models. In each analysis for beverage type, exposure was modeled as a three-level variable (unexposed to alcohol [referent], exposed to given alcohol type, exposed to other alcohol types only).

Any exposure

Alcohol use during pregnancy is often treated as an unvarying exposure, which does not account for changes in use that occur in most women who are exposed. To assess how results compare with analyses where exposure is simplified in this way, I used Cox proportional hazards survival models to calculate crude and adjusted HRs for presence compared with absence of alcohol use after LMP.

Commonalities between approaches

In survival analyses, participants contributed time in the model from day of enrollment through 20 weeks' gestation (140 days), arrest of development, or loss to follow up, whichever

came first. Pregnancies with unknown outcomes (n=145) were censored at gestational age of last study contact. Left truncation (censoring time before cohort entry) based on gestational age at enrollment accounts for a subject not having a loss prior to cohort entry.[231]

I performed a set of sensitivity analyses in which I defined pregnancy endpoint for losses as gestational age at miscarriage (as opposed to GAAD). I also performed sensitivity analyses excluding participants with losses who did not have a research ultrasound. A final sensitivity analysis assessed robustness of results to the inclusion of binge drinking in the models (evaluated as a dichotomous [yes/no] and continuous [number of binge episodes] variable).

I used two-sided tests with a significance level of 0.05. To minimize type I errors due to multiple testing, I used a threshold for significance Bonferroni-corrected by a factor equal to the number of tests performed in the hypothesis. For example, when testing whether gestational week-specific alcohol exposure was associated with miscarriage, the threshold for significance was Bonferroni-corrected with a factor of twelve because we examined gestational age specific effects for weeks one through twelve. Analyses were performed using Stata (Version 14.2, StataCorp, College Station, TX).

Assessment of confounding

Adjusted models included a covariate set selected *a priori* based on known or suspected relationships with alcohol consumption and miscarriage risk. Adjusted models include maternal age (years), race/ethnicity (non-Hispanic, white/non-Hispanic, black/Other), education (high school or less/some college/college or more), cigarette use (never smoker or distant quit/quit within four months of first trimester interview or current smoker), pregnancy intention (intended/unintended), and parity (nulliparous/one prior birth/two or more prior births). Restricted cubic splines with four knots were used to allow for a flexible relationship between maternal age and miscarriage risk (placement of knots determined by data).

Missing data

Selection criteria mandated complete information to assign pattern of alcohol use throughout the first trimester. For the analysis including a dose interaction, I excluded six women missing information on exposure dose. Seventy-one women (1.3%) were missing data for one or more variables in the covariate set. The covariates with the highest rates of missingness were parity (1.1%) and pregnancy intention (0.2%). For crude and adjusted measures of association, complete cases were used (n=5,353). Maternal demographic and exposure characteristics were compared for participants with and without complete covariate data (Table 16).

Power calculation

For a Cox proportional hazard model including alcohol exposure as a dichotomous variable, I have 80% power to detect a true hazard ratio of 1.11 when alpha is set to 5% and n=5,000 (Figure 18). Power calculations and curves were created using PS: Power and Sample Size Calculation (version 3.1.2, 2014) for the smallest detectable alternative for survival studies.[232]

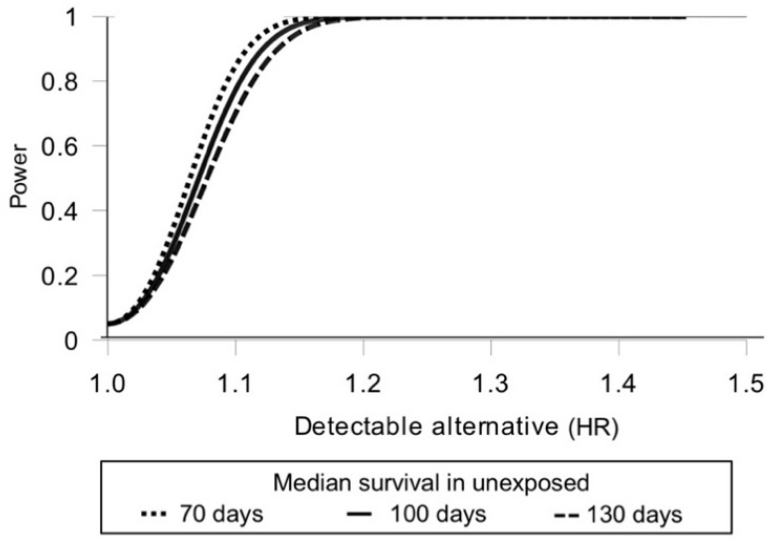

Figure 18. Power curves for simple hazard model.

Table 16. Comparison of participants with complete covariate data to those missing data for one or more covariates (n=5,424)

Characteristic	Complete Case (n=5,353)		Missing Covariates (n=71)		P-value*
Maternal age, median, IQR, years	29	26–32	25	21–30	<0.01
Race/ethnicity, No., %					<0.01
White, non-Hispanic	3,775	70.5	16	22.5	
Black, non-Hispanic	987	18.4	39	54.9	
Other	591	11.0	13	18.3	
Refused	0	0.0	3	4.2	
Education, No., %					<0.01
High school or less	926	17.3	42	59.2	
Some college	962	18.0	14	19.7	
College or more	3,465	64.7	14	19.7	
Missing	0		1	1.4	
Marital status, No., %					<0.01
Married or cohabitating	4,796	89.6	43	60.6	
Other	557	10.4	28	39.4	
Parity, No., %					0.01
Nulliparous	2,563	47.9	6	8.5	
1 prior delivery	1,853	34.6	2	2.8	
2+ prior deliveries	937	17.5	5	7.0	
Missing	0		58	81.7	
Smoking status, No., %†					<0.01
Never or distant quit	4,720	88.2	53	74.6	
Current or recent quit	633	11.8	18	25.4	
Pregnancy Intention, No., %					<0.01
Intended	3,923	73.3	23	32.4	
Not intended	1,430	26.7	37	52.1	
Missing	0		11	15.5	
Alcohol Use, No., %‡					0.23
Yes	2,662	49.7	30	42.3	
No	2,691	50.3	41	57.7	
Gestational age at change, median, IQR, days	29	15–35	22	6–35	0.10
Dose at LMP, median, IQR, drinks/week	2.0	1.0–4.0	2.0	0.3–3.0	0.27
Pregnancy Outcome, No., %					0.14
Miscarriage	645	12.0	4	5.6	
No miscarriage	4,708	88.0	67	93.4	

Abbreviations: IQR, inter-quartile range; No., number.

* P-value calculated using Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables (missing not included)

† Quitting within the four months prior to the end of first trimester interview is considered a recent quit; quitting before that time is considered a distant quit

‡ Alcohol use defined as use past last menstrual period

Results

Of 5,424 women eligible for this analysis, 49.6% used alcohol during pregnancy (2,692/5,424) and 12.0% experienced a miscarriage (649/5,424). Fourteen percent of participants reported no alcohol use (769/5,424), 36.2% quit prior to LMP (1,963/5,424), 44.3% quit after LMP (2,401/5,424), and 5.4% continued use (291/5,424; Figure 19). Of 2,962 women who reported a change in alcohol exposure within the month prior to conception or during the first trimester, 91.2% quit using alcohol (2,702/2,962), 8.0% reduced use (236/2,962), and 0.5% increased use (15/2,962). Median gestational age at change was 29 days (IQR 15–35 days) and 41.0% of participants who reported a change altered use within three days of a positive pregnancy test (1,213/2,962). Forty women did not change frequency or amount of alcohol used during the first trimester.

Higher maternal age, household income, and level of education were associated with alcohol exposure during pregnancy (Table 17). White, non-Hispanic women, nulliparous women, and smokers were more likely to be exposed to alcohol during pregnancy than their counterparts. Median amount of alcohol consumed at the onset of pregnancy was two drinks per week (IQR 1–4 drinks per week). Among participants who continued to use alcohol up until the first trimester interview, 67.7% consumed less than one drink per week (197/291) and 9.3% consumed four or more drinks per week (27/291).

Gestational age-specific exposure

When considering exposure in each week of gestation, alcohol use was associated with miscarriage in weeks five through ten after adjusting for multiple comparisons (adjusted ORs range 1.42–4.85; Figure 20). Risk peaked for exposure in week nine of gestation (adjusted OR 4.85, 95% CI 3.03, 7.13). Estimates for later weeks in the first trimester were less precise, as

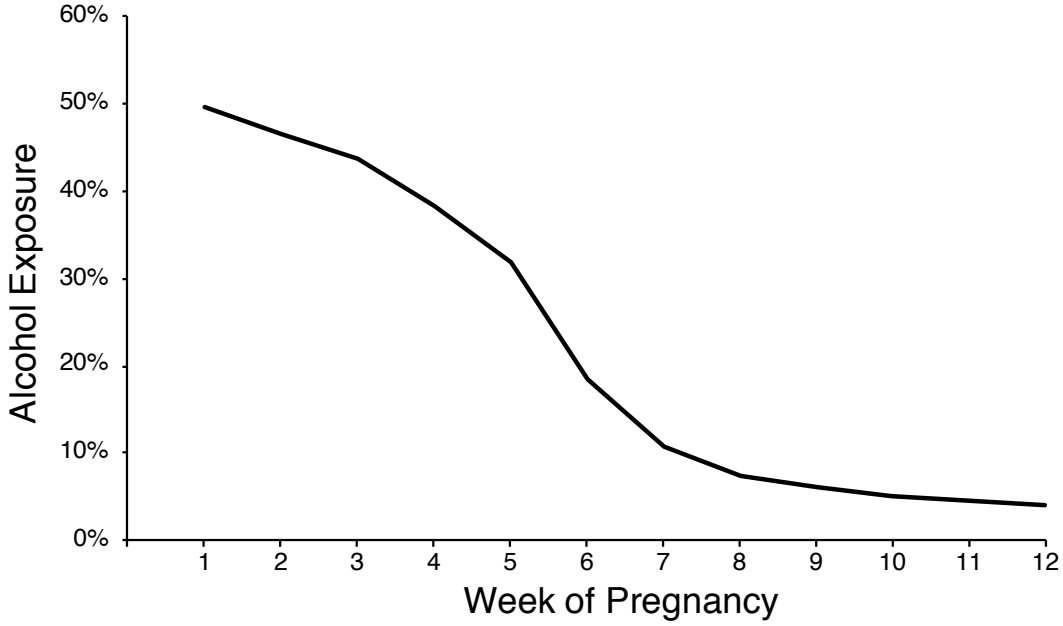

Figure 19. Proportion of Right from the Start participants exposed to alcohol by week of pregnancy (n=5,424)

*Table 17. Participant characteristics by alcohol use during pregnancy (n=5,424)**

Characteristic	No Alcohol Use (n=2,732)		Alcohol Use (n=2,692)		Unadjusted OR	95% CI
	N	%	N	%		
Maternal age, years						
<25	646	23.6	430	16.0	1.00	Referent
25–29	969	35.5	890	33.1	1.38	1.19, 1.61
30–34	789	28.9	940	34.9	1.79	1.53, 2.09
≥35	328	12.0	432	16.0	1.98	1.64, 2.39
Race/ethnicity						
White, non-Hispanic	1,726	63.2	2,065	76.7	1.00	Referent
Black, non-Hispanic	663	24.3	363	13.5	0.46	0.40, 0.53
Other	341	12.5	263	9.8	0.64	0.54, 0.77
Refused	2	0.1	1	0.0		
Education						
High school or less	614	22.5	354	13.2	1.00	Referent
Some college	526	19.3	450	16.7	1.48	1.24, 1.78
College or more	1,592	58.3	1,887	70.1	2.06	1.78, 2.38
Missing	0	0.0	1	0.0		
Household income, $						
≤ 40,000	995	36.4	659	24.5	1.00	Referent
40,001 to 80,000	976	35.7	971	36.1	1.50	1.32, 1.72
> 80,000	648	23.7	989	36.7	2.30	2.00, 2.65
Missing	113	4.1	73	2.7		
Marital status						
Married or cohabitating	2,416	88.4	2,423	90.0	1.00	Referent
Other	316	11.6	269	10.0	0.85	0.71, 1.01
Parity						
Nulliparous	1,152	42.2	1,417	52.6	1.00	Referent
1 prior delivery	985	36.1	870	32.3	0.72	0.64, 0.81
2+ prior deliveries	561	20.5	381	14.2	0.55	0.47, 0.63
Missing	34	1.2	24	0.9		
Past miscarriage						
0	2,026	74.2	2,120	78.8	1.00	Referent
1	519	19.0	444	16.5	0.82	0.71, 0.94
≥2	153	5.6	104	3.9	0.65	0.50, 0.84
Missing	34	1.2	24	0.9		
BMI, kg/m^2						
<18.5	68	2.5	67	2.5	0.87	0.62, 1.23
18.5–24.9	1,345	49.2	1,520	56.5	1.00	Referent
25–29.9	653	23.9	616	22.9	0.83	0.73, 0.95
≥30	626	22.9	466	17.3	0.66	0.57, 0.76
Missing	40	1.5	23	0.9		
Smoking status†						
Never or distant quit	2,485	91.0	2,288	85.0	1.00	Referent
Current or recent quit	247	9.0	404	15.0	1.78	1.50, 2.10
Pregnancy Intention						
Intended	1,994	73.0	1,952	72.5	1.00	Referent
Not intended	732	26.8	735	27.3	1.03	0.91, 1.16
Missing	6	0.2	5	0.2		

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

* Alcohol use defined as exposure past last menstrual period

† Quitting within the four months prior to the end of first trimester interview is considered a recent quit; quitting before that time is considered a distant quit

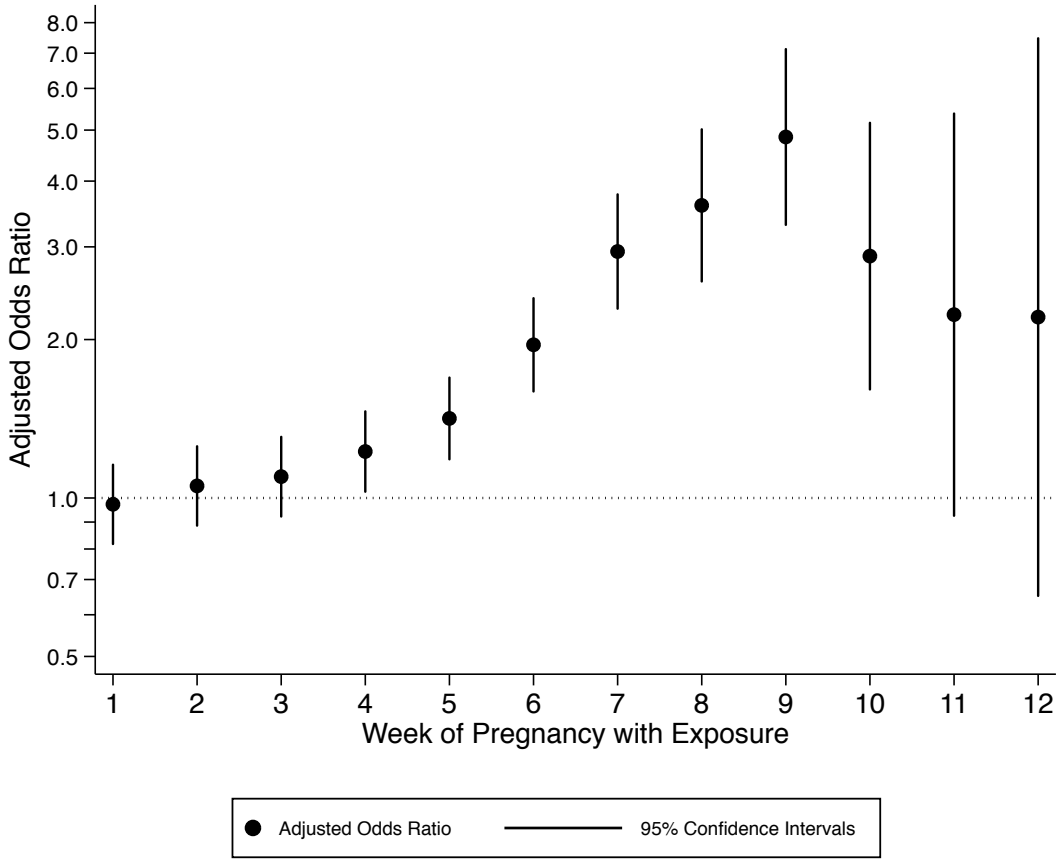

Figure 20. Risk of miscarriage associated with each week of alcohol exposure in pregnancy, gestational age at arrest of development used as pregnancy endpoint. Estimates are adjusted for maternal age, race/ethnicity, education, parity, smoking status, and pregnancy intention. Participants with complete data for adjusted analysis are included (n=5,353). Weeks five through ten are significant after adjusting for multiple comparisons (Bonferroni-corrected with a factor of 12).

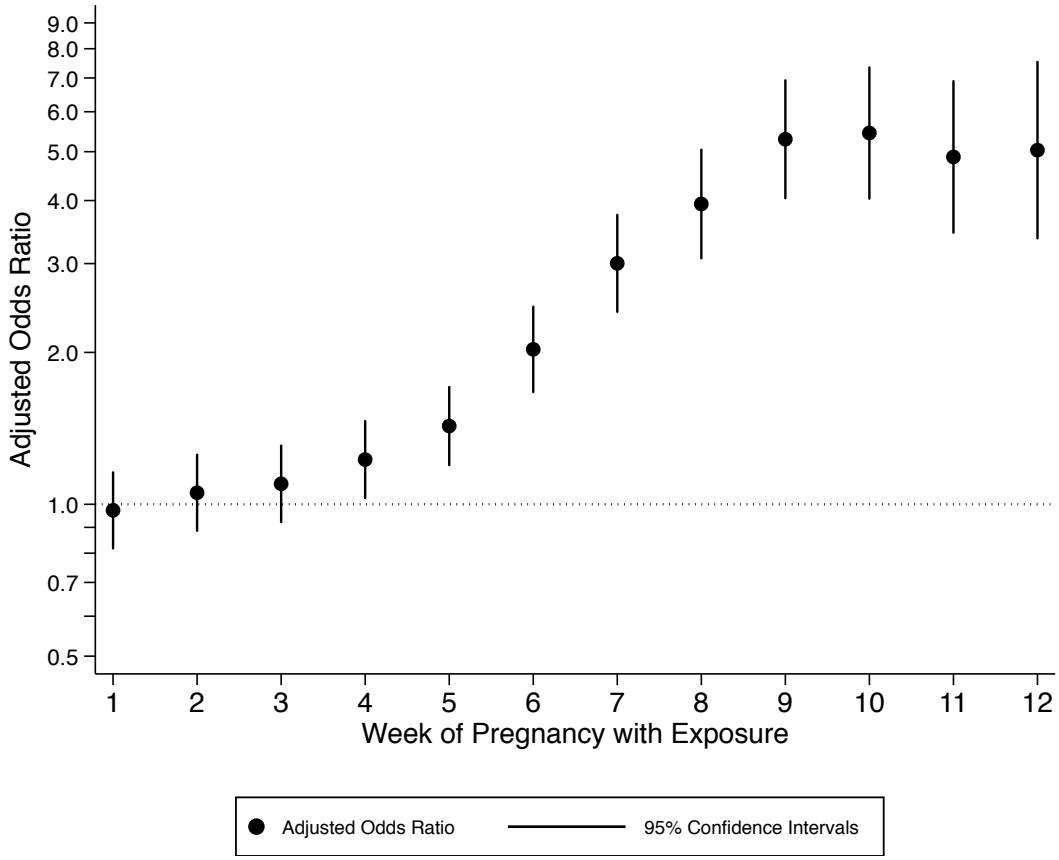

Figure 21. Risk of miscarriage associated with each week of alcohol exposure in pregnancy, pregnancy endpoint for losses defined as gestational age at miscarriage as opposed to gestational age at arrest of development. Estimates are adjusted for maternal age, race/ethnicity, education, parity, smoking status, and pregnancy intention. Participants with complete data for adjusted analysis are included (n=5,353). Weeks five through twelve are significant after adjusting for multiple comparisons (Bonferroni-corrected with a factor of 12).

Table 18. Risk of miscarriage associated with number of drinks per week in four developmental windows

Alcohol Use Characteristic	Births		Miscarriages		Crude OR	95% CI	Adjusted OR†	95% CI
	N*	%	N*	%				
Weeks 1–4	4,708		645					
Unexposed	2,367	50.3	324	50.2	1.00	Referent	1.00	Referent
≤ 1 drink/week	931	19.8	120	18.6	0.94	0.75, 1.18	0.91	0.73, 1.14
1.01–2 drinks/week	449	9.5	67	10.4	1.09	0.82, 1.44	1.11	0.83, 1.48
2.01–4 drinks/week	440	9.3	60	9.3	1.00	0.74, 1.34	0.98	0.72, 1.33
> 4 drinks/week	521	11.1	74	11.5	1.04	0.79, 1.36	0.97	0.73, 1.29
Weeks 5–7	4,708		642					
Unexposed	3,240	68.8	393	61.2	1.00	Referent	1.00	Referent
≤ 1 drink/week‡	537	11.4	96	15.0	1.47	1.16, 1.88	1.44	1.12, 1.84
1.01–2 drinks/week	279	5.9	49	7.6	1.45	1.05, 1.99	1.52	1.09, 2.11
2.01–4 drinks/week	282	6.0	45	7.0	1.32	0.94, 1.83	1.38	0.98, 1.94
> 4 drinks/week	370	7.9	59	9.2	1.31	0.98, 1.76	1.32	0.97, 1.80
Weeks 8–10	4,708		379					
Unexposed	4,434	94.2	223	79.9	1.00	Referent	1.00	Referent
≤ 1 drink/week‡	181	3.8	41	14.7	4.50	3.13, 6.49	3.97	2.71, 5.83
1.01–2 drinks/week	37	0.8	6	2.2	3.22	1.35, 7.72	2.98	1.21, 7.37
2.01–4 drinks/week	32	0.7	4	1.4	2.49	0.87, 7.09	2.29	0.79, 6.64
> 4 drinks/week	24	0.5	5	1.8	4.14	1.57, 10.96	3.42	1.25, 9.35

Abbreviations: CI, confidence interval; OR, odds ratio.

* Counts reflect participants that contributed to analysis for each developmental window. Participants were included if they had complete data for adjusted analysis and had not had a miscarriage or been censored by the beginning of the week.

† Adjusted for maternal age (continuous, spline), race/ethnicity, education, parity, smoking status, and pregnancy intention.

‡ Significant after adjustment for multiple comparisons (Bonferroni-corrected with a factor of 12).

number of participants exposed to alcohol decreased with increasing gestational age. Findings of elevated risk in weeks five through ten of pregnancy remained consistent when conducting the analysis with pregnancy endpoint for losses defined as gestational age at miscarriage as opposed to GAAD (Figure 21).

Point estimates for all levels of exposure were greater in later weeks of gestation. A dose-response trend was not detected in any developmental stage (Table 18). Exposure to the lowest exposure category (one drink or less per week) was associated with elevated risk of miscarriage in gestational week five and beyond (adjusted ORs range: 1.44–3.97). I was not able to estimate dose-specific effects for exposure in the fetal window (weeks 11–12) because high levels of alcohol exposure late in the first trimester were rare.

Duration of exposure

Total for all participants and average follow-up time for individuals were 702,652 and 129 days, respectively (follow-up time truncated at 140 days). Each additional week of alcohol exposure during pregnancy was associated with an 8% relative increase in risk of miscarriage compared with risk among women who were unexposed (adjusted HR 1.08, 95% CI 1.04, 1.12; Table 19). In other words, quitting alcohol use at day 29 of pregnancy (median gestational age at change in the cohort) is associated with a 37% increase in risk of miscarriage relative to those who were unexposed (adjusted HR 1.37, 95% CI 1.18, 1.60). If use in this cohort is comparable to the larger population, this would translate to alcohol use in pregnancy being responsible for 15 miscarriages out of every 1,000 pregnancies.

A dose-response trend was not detected when evaluating risk associated with each additional week of alcohol exposure (adjusted HR for interaction between duration of use and drinks per week [continuous] 1.00, 95% CI 0.99, 1.01; [categorical] adjusted HRs range 1.00-1.06; Table 19). Estimates did not vary by alcohol type (p-value 0.99, Wald test). Estimates did

*Table 19. Hazard for miscarriage associated with each additional week of alcohol use by use characteristic, all participants**

Alcohol Use Characteristic	Births (n=4,708)†		Miscarriages (n=645)†		Crude HR	95% CI	Adjusted HR‡	95% CI
	N	%	N	%	Per additional week		Per additional week	
Any Use								
No	2,367	50.3	324	50.2	1.00	Referent	1.00	Referent
Yes§	2,341	49.7	321	49.8	1.09	1.05, 1.13	1.08	1.04, 1.12
Amount at LMP‖								
Unexposed	2,367	50.3	324	50.2	1.00	Referent	1.00	Referent
≤ 1 drink/week§	931	19.8	120	18.6	1.09	1.05, 1.14	1.08	1,04, 1.13
1.01–2 drinks/week	449	9.5	67	10.4	1.06	1.00, 1.12	1.06	1.00, 1.12
2.01–4 drinks/week	440	9.3	60	9.3	1.05	1.00, 1.10	1.05	1.00, 1.10
> 4 drinks/week	521	11.1	74	11.5	1.02	0.97, 1.07	1.00	0.96, 1.05
Alcohol Type¶								
Wine§	1,545	32.8	201	31.2	1.07	1.03, 1.11	1.07	1.02, 1.11
Beer§	1,089	23.1	138	21.4	1.07	1.02, 1.12	1.07	1.02, 1.12
Liquor	858	18.2	106	16.4	1.03	0.97, 1.09	1.04	0.98, 1.10

Abbreviations: CI, confidence interval; HR, hazard ratio.

* Alcohol modeled as a time-varying exposure for duration of use, left truncation based on gestational age at enrollment.

† Participants with complete data for adjusted analysis are included in this table (n=5,353).

‡ Adjusted for maternal age (continuous, spline), race/ethnicity, education, parity, smoking status, and pregnancy intention.

§ Significant after adjustment for multiple comparisons (Bonferroni-corrected with a factor of four for alcohol amount and three for alcohol type).

‖ Categories reflect level of alcohol consumption prior to change in use, duration defined as pre-change use.

¶ Alcohol type categories are not mutually exclusive. Women who reported alcohol exposure in pregnancy but did not provide alcohol type are excluded from this analysis (n=30). Referent group is women unexposed to alcohol.

*Table 20. Hazard for miscarriage associated with each additional week of alcohol use by use characteristic, cases restricted to participants with a research ultrasound for estimating gestational age at arrest of development**

Alcohol Use Characteristic	Births (n=4,708)†		Miscarriages (n=462)†		Crude HR	95% CI	Adjusted HR‡	95% CI
	N	%	N	%	Per additional week		Per additional week	
Any Use								
No	2,367	50.3	239	51.7	1.00	Referent	1.00	Referent
Yes§	2,341	49.7	223	48.3	1.08	1.04, 1.13	1.07	1.03, 1.12
Amount at LMP‖								
Unexposed	2,367	50.3	239	51.7	1.00	Referent	1.00	Referent
≤ 1 drink/week	931	19.8	74	16.0	1.07	1.02, 1.13	1.06	1.01, 1.12
1.01–2 drinks/week	449	9.5	49	10.6	1.07	1.00, 1.13	1.07	1.00, 1.13
2.01–4 drinks/week	440	9.3	45	9.7	1.05	0.99, 1.11	1.05	0.99, 1.11
> 4 drinks/week	521	11.1	55	11.9	1.03	0.98, 1.09	1.01	0.96, 1.07
Alcohol Type¶								
Wine§	1,545	32.8	138	29.9	1.07	1.02, 1.12	1.06	1.01, 1.11
Beer	1,089	23.1	96	20.8	1.07	1.02, 1.13	1.07	1.01, 1.12
Liquor	858	18.2	79	17.1	1.03	0.96, 1.10	1.03	0.96, 1.10

Abbreviations: CI, confidence interval; HR, hazard ratio.

* Alcohol modeled as a time-varying exposure for duration of use, left truncation based on gestational age at enrollment.

† Participants with complete data for adjusted analysis and research ultrasound for estimation of gestational age at arrest of development are included in this table (n=5,170).

‡ Adjusted for maternal age (continuous, spline), race/ethnicity, education, parity, smoking status, and pregnancy intention.

§ Significant after adjustment for multiple comparisons (Bonferroni-corrected with a factor of four for alcohol amount and three for alcohol type).

‖ Categories reflect level of alcohol consumption prior to change in use, duration defined as pre-change use.

¶ Alcohol type categories are not mutually exclusive. Women who reported alcohol exposure in pregnancy but did not provide alcohol type are excluded from this analysis (n=30). Referent group is women unexposed to alcohol.

*Table 21. Hazard for miscarriage associated with each additional week of alcohol use by use characteristic, using gestational age at miscarriage to define pregnancy endpoint for participants with losses**

Alcohol Use Characteristic	Births (n=4,708)†		Miscarriages (n=645)†		Crude HR	95% CI	Adjusted HR‡	95% CI
	N	%	N	%	Per additional week		Per additional week	
Any Use								
No	2,367	50.3	324	50.2	1.00	Referent	1.00	Referent
Yes§	2,341	49.7	321	49.8	1.11	1.08, 1.13	1.10	1.08, 1.13
Amount at LMP‖								
Unexposed	2,367	50.3	324	50.2	1.00	Referent	1.00	Referent
≤ 1 drink/week§	931	19.8	120	18.6	1.09	1.06, 1.12	1.07	1.04, 1.11
1.01–2 drinks/week§	449	9.5	67	10.4	1.08	1.05, 1.12	1.08	1.04, 1.12
2.01–4 drinks/week	440	9.3	60	9.3	1.06	1.02, 1.10	1.06	1.02, 1.10
> 4 drinks/week	521	11.1	74	11.5	1.04	1.01, 1.08	1.03	1.00, 1.07
Alcohol Type¶								
Wine§	1,545	32.8	201	31.2	1.08	1.06, 1.11	1.08	1.05, 1.11
Beer§	1,089	23.1	138	21.4	1.09	1.05, 1.12	1.09	1.15, 1.12
Liquor§	858	18.2	106	16.4	1.07	1.03, 1.11	1.08	1.04, 1.12

Abbreviations: CI, confidence interval; HR, hazard ratio.

* Alcohol modeled as a time-varying exposure for duration of use, left truncation based on gestational age at enrollment.

† Participants with complete data for adjusted analysis are included in this table (n=5,353).

‡ Adjusted for maternal age (continuous, spline), race/ethnicity, education, parity, smoking status, and pregnancy intention.

§ Significant after adjustment for multiple comparisons (Bonferroni-corrected with a factor of four for alcohol amount and three for alcohol type).

‖ Categories reflect level of alcohol consumption prior to change in use, duration defined as pre-change use.

¶ Alcohol type categories are not mutually exclusive. Women who reported alcohol exposure in pregnancy but did not provide alcohol type are excluded from this analysis (n=30). Referent group is women unexposed to alcohol.

not differ when excluding cases with imputed GAAD (Table 20) or when defining pregnancy endpoint as gestational age at miscarriage (Table 21).

Any exposure

The Cox model that classified alcohol use as a uniform exposure (unchanging with time) suggested no association between alcohol use and miscarriage risk (adjusted HR 0.87, 95% CI 0.71, 1.07). This approach neglects that 97% of participants exposed at LMP altered alcohol use during the first trimester (2,609/2,692).

Binge drinking

Eleven percent of women reported a binge episode during the periconception period or first trimester (599/5,420; n=4 missing data for presence of binge episodes). Median number of binge episodes was two (IQR 1–4) and 10.7% of participants who binged reported ten or more episodes (63/591; n=8 missing data for number of binge episodes). Reporting binge episodes was not associated with miscarriage (adjusted HR 0.81, 95% CI 0.56, 1.12).

Comments

In this prospective, community-recruited cohort, timing of alcohol use cessation during pregnancy was a key determinant of miscarriage risk. Alcohol exposure occurred in half of pregnancies with many participants not altering use until a positive pregnancy test. Each additional week of alcohol use in the first trimester was associated with increased risk of miscarriage and risk was most strongly related to exposure in weeks five through ten of pregnancy.

The prevalence of alcohol use at the onset of pregnancy observed in this cohort aligns with national data about exposure among nonpregnant, reproductive-aged women.[42] Forty percent of women who were exposed reported a change in alcohol use within three days of a positive pregnancy test. Timing of change was similar between participants with intended and

unintended pregnancies,[4] indicating planned pregnancies do not necessarily involve preparatory changes in alcohol use.[1,233] Women who were older than 35, white, college-educated, and from high-income households were more likely to use alcohol than their counterparts. Although these demographics are consistently linked with alcohol use during pregnancy, [1,42,43,49] this population is not generally flagged for high-risk behaviors and may be a group more commonly overlooked for risk counseling.

Most studies of the association between alcohol exposure and miscarriage risk are limited by methods for ascertaining and modeling exposure (see Literature Review in Chapter III).[9-33] Many define exposure as alcohol use after pregnancy recognition. In RFTS, this definition misclassifies 44.3% of participants as unexposed. Others calculate an across-pregnancy average dose or describe pre-pregnancy alcohol use and its associated risk separately.[10,12-16,20,21,24,26,27,30,31] An across-pregnancy average dose neglects that exposure is disproportionately concentrated in early pregnancy. Evaluating "pre-pregnancy" exposure separately without considering how long this behavior persists into the first trimester disregards that risk associated with alcohol use may be tied to the gestational age at which it occurs. These analyses do not incorporate data about timing of change in alcohol use in measures of miscarriage risk.

Alcohol use typically occurs prior to pregnancy detection and rapidly tapers thereafter. As a result, the bulk of exposure occurs during early pregnancy concurrently with the first steps of embryo development. My results suggest miscarriage risk associated with alcohol use is related to timing of exposure. In line with these findings, a study that evaluated rates of miscarriage by week of pregnancy with alcohol use documented exposure to more than three drinks per week during gestational weeks seven through ten were associated with elevated risk, with exposure in week nine having the highest risk.[31] This is consistent with the pattern of risk I observed.

Considerations

Before considering the implications of these findings, I will audit the level of confidence we should have in the results. We relied on self-report to determine alcohol use since no sufficiently sensitive and specific biomarker for longitudinal alcohol exposure exists.[166] Social desirability bias, or responding in a way deemed favorable by others, may lead women to underreport alcohol use during pregnancy.[168,170] We attempted to minimize this bias by conducting interviews in a nonclinical and confidential setting using questionnaires with alcohol-related survey items crafted to be nonjudgmental. Prevalence of exposure at pregnancy onset in this cohort is consistent with the proportion observed among nonpregnant, reproductive-aged women,[42] which provides reassurance social desirability bias did not unduly influence reporting about presence of alcohol exposure. Acknowledgement of alcohol use at the onset of pregnancy in half of participants bolsters confidence in the validity of reporting about timing in change of use.

Assessment of alcohol exposure followed loss for 67.2% of miscarriages (436/649), allowing potential for recall bias.[167,169] The proportion of women with losses who reported alcohol exposure during pregnancy did not differ by interview timing (50.7% who were interviewed before loss reported exposure compared to 49.5% who were interviewed after loss; chi-squared p-value 0.78) and gestational age at change in alcohol consumption was similar between the groups (median gestational age at change 31 days for those interviewed before loss compared to 32 days for those interviewed after loss; Wilcoxon rank-sum p-value 0.36).

I did not observe a dose-response relationship between alcohol exposure and risk. The absence of this finding could be explained by the following. While many biological relationships operate on a dose-dependent gradient, timing of alcohol use may drive miscarriage risk with a threshold effect observed at low levels of exposure. Alternatively, imprecision or bias in

reporting of amount consumed may obscure a dose-dependent effect. Since alcohol use during pregnancy is stigmatized, information about amount consumed may be more vulnerable to reporting biases than responses about the mere presence or absence of exposure. The lowest level of exposure (≤ 1 drink/week) was consistently associated with increased risk of miscarriage. This category may signify regular alcohol consumption on a weekly basis instead of actual dose. Additionally, misconceptions about size and alcohol content of a standard drink may lead to error in earnest reporting of alcohol dose.[174] We expect this to cause unintentional underreporting of dose that is non-differential by outcome.[175]

Eleven percent of participants reported binge drinking during pregnancy, which is consistent with levels observed among nonpregnant women.[234] Neither binge drinking nor number of binge episodes were associated with miscarriage risk. The association between weekly alcohol use and miscarriage risk was not confounded by binge drinking.

RFTS prioritized early recruitment of pregnancies to capture as many miscarriage events as possible: 25.8% of participants entered the study prior to conceiving (1,401/5,424) and 71.6% were enrolled prior to 7 competed weeks' gestation (3,884/5,242). Advertising the study in the community and contacting potential participants prior to conception or initiation of prenatal care allowed enrollment earlier in gestation than clinic-based studies. While this is an improvement over many studies of miscarriage, losses occurring very early in gestation are inevitably underrepresented in this sample. I truncated time prior to study enrollment in survival analyses, thus eliminating immortal time. This accounts for the fact that a participant had to have an ongoing pregnancy at enrollment to be observed. Risk associated with alcohol use in the first weeks of pregnancy may be higher than estimated if losses that were unobserved because they occurred before a woman could be enrolled were highly associated with alcohol exposure.

I used both gestational age at miscarriage and GAAD derived from features observed during ultrasound prior to loss to assign time at risk. Estimates of risk associated with duration of use did not differ by method of assigning time at risk. In the analysis of gestational week-specific exposure, estimates were similar between methods for weeks one through nine. Risk for weeks ten through twelve were lower when using GAAD to determine pregnancy endpoint compared with gestational age of miscarriage. This is because arrest occurs before loss and therefore cases are excluded from models earlier in gestation when using GAAD to determine pregnancy endpoint and these cases are more likely to be exposed.

There may be concern the measured association is a product of reverse causality. Nausea is a sign of a thriving pregnancy.[235-237] Women who experience nausea may abstain from alcohol use due to aversion or because symptoms led to pregnancy detection earlier in gestation than asymptomatic women. Relatively lower levels of alcohol consumption in women with robust pregnancies due to nausea may lead to a spurious association between alcohol use and miscarriage. However, I did not detect a difference in alcohol exposure or timing of change by reports of nausea, which was systematically queried. Further adjustment of the association between alcohol use and miscarriage for nausea did not alter estimates.

A few points should be noted when generalizing these findings. The women in this study may be more likely to practice healthy behaviors since RFTS enrolled women who were either planning a pregnancy or volunteered for the study early in pregnancy. Though we cannot dismiss the possibility alcohol exposure might be related to participation, prevalence of exposure in early pregnancy was in line with national data.[42] A higher proportion of pregnancies in this cohort were intended than the national average (69% compared with 51%).[163] The proportion of participants exposed to alcohol did not differ by pregnancy intention, though women with intended pregnancies changed alcohol use four days earlier than women with unintended

pregnancies. A higher proportion of women in this study were white compared with the national average and race/ethnicity was associated with both alcohol exposure and risk of miscarriage. Measures of association were adjusted for race/ethnicity, but estimates of absolute attributable risk may differ in a population with different demographics.

Conclusion

Optimally, exposure to alcohol during pregnancy could be prevented, but half of pregnancies are unintended and abstaining from use when planning a pregnancy is not the norm. In this prospective cohort study of early pregnancy health, timing of alcohol use cessation is a key driver of miscarriage. Each additional week of alcohol use in the first trimester is linked with increased miscarriage risk and exposure during weeks five through ten of gestation are most concerning for miscarriage. These findings imply early detection of pregnancy and accompanying lifestyle changes could curtail the risk of miscarriage related to alcohol use.

VI. THIRD AIM: TO DETERMINE THE ROLE OF ALCOHOL METABOLISM IN MODULATING MISCARRIAGE RISK

Abstract

Background: Alcohol exposure in pregnancy is associated with increased risk of miscarriage. This risk may be modified by genetic variants related to alcohol metabolism.

Methods: Participants in *Right from the Start,* a community-based, prospective pregnancy cohort, provided information about gestational age-specific alcohol exposure during pregnancy. DNA samples were genotyped for two common SNPs in the alcohol dehydrogenase 1C (*ADH1C*) gene, which results in slower alcohol metabolism. *ADH1C* haplotype data were used to classify participants as fast, moderate, or slow metabolizers. I used logistic regression to assess whether alcohol use during pregnancy differed by *ADH1C* haplotype and whether haplotype independently associated with increased risk of miscarriage. Extended Cox survival models were used to test whether the association between duration of alcohol use in pregnancy and miscarriage was modified by *ADH1C* haplotype. Analyses were limited to women of white race.

Results: Among 987 participants, 52% reported alcohol exposure during pregnancy and 20% experienced a miscarriage. As indicated by haplotype, 16% of women were slow metabolizers and 50% and 34% were moderate and fast metabolizers, respectively. Alcohol use during pregnancy did not differ by *ADH1C* haplotype and haplotype was not associated with risk of miscarriage (fast activity [referent]; moderate activity adjusted hazard ratio [HR] 0.75, 95% confidence intervals [CI] 0.52, 1.09; slow activity adjusted-HR 0.78, 95% CI 0.46, 1.30). Each additional week of alcohol use in the first trimester was associated with an increased risk of miscarriage (adjusted-HR 1.07, 95% CI 1.00, 1.14). I did not find evidence that *ADH1C*

haplotype modifies the association between duration of alcohol use during pregnancy and miscarriage.

Conclusions*:* Increased risk of miscarriage related to duration of alcohol use during pregnancy does not depend on alcohol metabolism profile as indicated by *ADH1C* haplotype. Other factors related to blood alcohol concentration and duration of exposure may be more important for determining risk related to alcohol use in pregnancy.

Overview

In Aim 2, I established alcohol exposure during pregnancy increases miscarriage risk. Teratogenic effects of alcohol use in pregnancy may not be the same in all maternal-fetal pairs. Since alcohol metabolism influences concentration and duration of alcohol circulating in the blood, genetic variants related to metabolism efficiency may modify the association between alcohol exposure and miscarriage.

Alcohol metabolism involves two steps. First alcohol is oxidized by alcohol dehydrogenase to form acetaldehyde, which is converted to acetate by aldehyde dehydrogenase 2.[155] *ADH1C* encodes γ proteins in the alcohol dehydrogenase family[155] and a common variant of this gene is associated with slower alcohol metabolism.[35,37] Individuals who are homozygous for the variant metabolize alcohol at half the rate of individuals without the variant and therefore have prolonged exposure to circulating alcohol for similar levels of consumption.

In this aim, I sought to determine if the association between duration of alcohol use (in weeks) during the first trimester and miscarriage risk is modified by *ADH1C* variant. I hypothesized alcohol use among women with the *ADH1C* variant signifying slower alcohol metabolism would be associated with higher risk of miscarriage compared to women without the variant.

Methods

Please see Chapter V for details about the *Right from the Start* parent study.

Right from the Start genetic substudy

RFTS participants were recontacted between 2010 and 2015 and invited to participate in a genetic substudy. RFTS maintained contact with participants after pregnancy through regular mailed newsletters about study updates and findings. Participants' social security numbers, current addresses, telephone numbers, and information for a secondary contact were collected at enrollment to help locate participants in the future. People-finding search tools available online (Spokeo, Whitepages, and city-specific Property Assessor searches)[238,239] were used to determine new addresses of participants who had relocated. Participants could opt out of being contacted by the study at any time. Individuals who agreed to participate in the genetic substudy were mailed consent forms (Appendix 9: *Right from the Start* DNA Repository Informed Consent) and Oragene ® DNA self-collection kits (DNA Genotek, Inc., Ontario, Canada). Contact, collection, and use of DNA samples was approved by Vanderbilt's IRB (100396).

Sample collection and storage

Each participant was mailed Oragene collection kits and instructions for sample collection. Participants collected loose cells in their mouth by providing a saliva specimen.[240] Saliva samples are stored by the Vanderbilt University Medical Center Vanderbilt Technologies for Advanced Genomics (VANTAGE) Resources Core. Study unique identifiers are used to track samples received and processed by VANTAGE. Samples are stored at -20°C as directed by the manufacturer. These procedures are conservative since DNA in saliva samples collected in Oragene kits can be stored at room temperature for up to five years without compromising sample integrity.[241]

Genotyping candidate SNPs

Two distinct haplotypes for *ADH1C* are differentially related to alcohol metabolism rate.[35,37] *ADH1C*1* encodes wildtype protein (γ_1) and *ADH1C*2* encodes γ_2. A pair of nonsynonymous single nucleotide polymorphisms (SNPs) (rs1693482 and rs698) are specific to *ADH1C*2* and result in amino acid changes (Arg272 to Gln272 and Ile350 toVal350, respectively).[35] These SNPs are in perfect linkage-disequilibrium among populations of European decent (r2=1.0, D'=1.0) (Figure 22).[158] Individuals who are *ADH1C*1* homozygotes have alcohol dehydrogenase enzymes that covert 90 ethanol molecules to acetaldehyde per minute at saturating ethanol concentrations, compared with individuals homozygous for *ADH1C*2* who covert 40 ethanol molecules to acetaldehyde per minute. As a result, *ADH1C*2* homozygotes have increased blood alcohol concentration and duration of exposure compared with *ADH1C*1* homozygotes for similar levels of alcohol use. I classified participants as having fast, moderate, or slow activity based on haplotype (homozygous *ADH1C*1*, heterozygous, and homozygous *ADH1C*2*, respectively).[242]

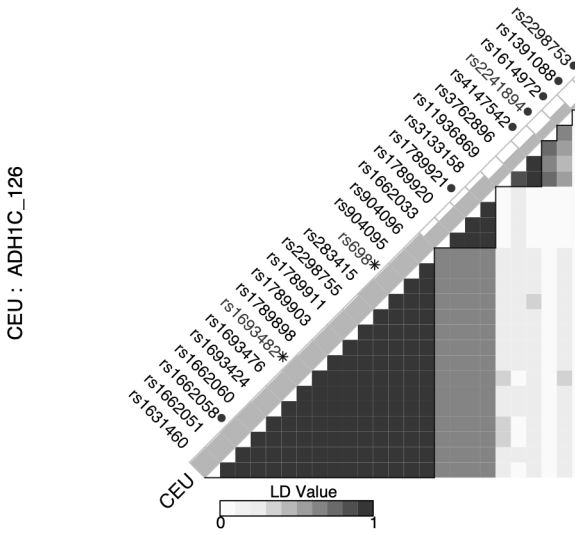

Figure 22. HapMap of ADH1C in CEU reference data. Shading represents linkage disequilibrium between SNPs with darker shading indicating a higher likelihood of SNPs being inherited together. Plot created using Haploview 3.2.

DNA was isolated from saliva samples and genotyped using allelic discrimination. An allelic discrimination assay is a multiplexed end-point assay that detects variants of a single nucleic acid sequence. These assays classify samples as homozygote (having only allele 1 or allele 2) or heterozygote (samples having both allele 1 and allele 2). Genotyping assessed two SNPs resulting in amino acid changes in *ADH1C* (rs698 and rs1693482).[35] Genotyping was performed using the 7900HT Fast Real-Time PCR System (Applied Biosystems Life Technologies)[243-245] using pre-made and verified probes from ThermoFisher Scientific Inc. Laboratory personnel were masked to pregnancy outcome.

To ensure high quality genotyping data, I observed precautions recommended for candidate gene studies.[246] To assess assay precision, CEPH duplicates were included on each plate. These are standardized samples of individuals with known genetic sequencing. Samples from women with and without pregnancies ending in miscarriage were plated randomly to protect against false discovery due to batch effect. VANTAGE ensures batches are sequenced consecutively within the same day to minimize between-batch variation. Hardy-Weinberg equilibrium was assessed among controls and did not show any significant deviations (p=0.58)

Alcohol exposure

Participants were classified as exposed to alcohol during pregnancy if they reported alcohol use after LMP. I used responses about alcohol exposure and date of change in alcohol consumption to determine duration of alcohol use and to categorize use into four patterns: never exposed, stopped using alcohol before LMP, stopped using alcohol during the first trimester, and exposed throughout the first trimester (includes women who did not change their alcohol use behavior or who reduced amount consumed, but continued to use alcohol through time of interview). I calculated weekly dose of alcohol exposure in drinks per week at LMP as a continuous and categorical measure (unexposed, less than or equal to one drink per week, more

than one and less than four drinks per week, and four or more drinks per week). Since risk may depend on maximum blood alcohol concentration instead of weekly exposure, I also assessed risk by number of drinks per sitting. Amount per sitting was classified as less than four drinks per sitting or greater than or equal to four drinks per sitting, which is consistent with the definition of binge drinking for women. Please see Chapter V for more information about exposure assessment and operationalization.

Outcome

Pregnancy outcome was assessed through self-report and validated by vital record, birth certificate, fetal death certificates, or medical records. Miscarriage was defined as pregnancy loss prior to 20 completed weeks' gestation.

Eligibility for analysis

Of the RFTS participants, 1,326 provided genetic samples. If a woman participated in RFTS for multiple pregnancies (n=61), only the first was included in this analysis. Pregnancies ending in induced abortion (n=1) and molar or ectopic pregnancies (n=3) were excluded. Participants missing the first trimester interview (n=84) or without sufficient information to classify alcohol use in pregnancy (n=6) were excluded. Analysis is limited to non-Hispanic, white women since *ADH1C*2* is prevalent in individuals of European ancestry (minor allele frequency 47%), but not individuals of African ancestry (minor allele frequency 1.0%; n=175 excluded). Four participants had insufficient samples for DNA extraction and low call rates for both SNPs resulting in the inability to determine haplotype for an additional five samples. Data from 987 pregnancies were included in this analysis (Figure 23).

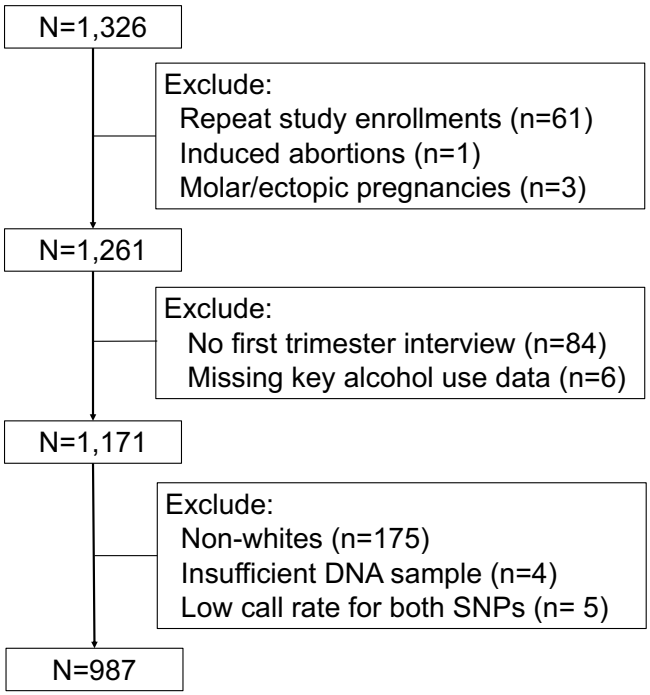

Figure 23. Flow diagram for genetic analysis population.

Power calculations

DNA data are available for 987 women, 194 of whom had pregnancies ending in miscarriage. For a minor allele frequencies of 47%,[247,248] this sample provides 80% power to detect an independent haplotype effect with a HR of 1.31 or greater (Figure 24).

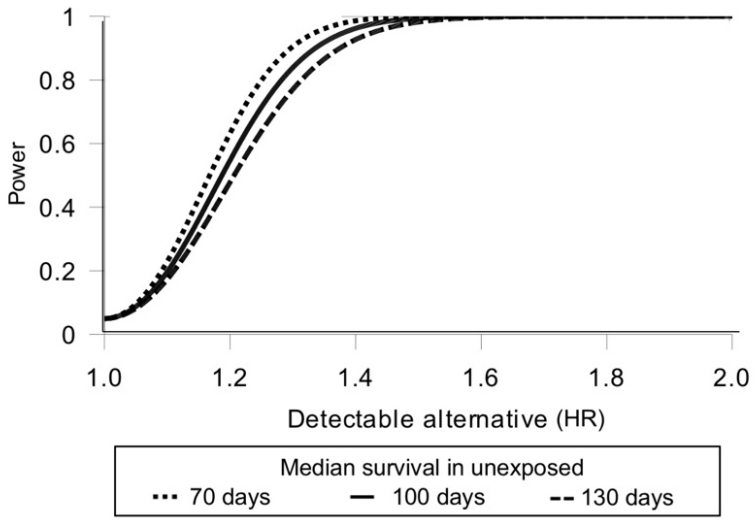

Figure 24. Power curves for independent haplotype effect.

This sample provides 80% power to detect an interaction between haplotype and alcohol exposure with an OR of 1.92 or greater assuming a significance level of 0.05, a prevalence of early pregnancy alcohol exposure of 50%, a marginal alcohol effect of OR=1.3, a marginal effect of haplotype of OR=1.0, and a log-additive relationship for the risk alleles (Quanto 2009, University of Southern California) (Figure 25).

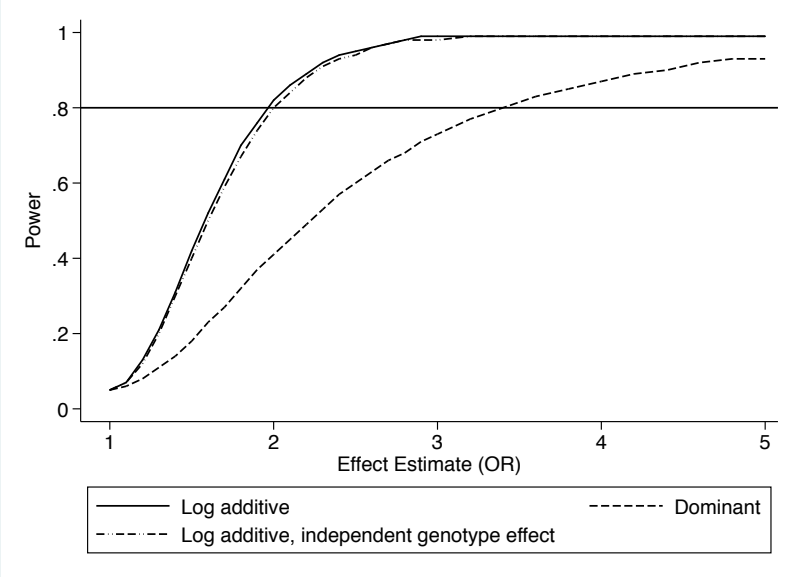

Figure 25. Power curve for gene-exposure interaction.

Statistical analysis

Analyses were performed in Stata (Version 14.2, StataCorp, College Station, TX) using two-sided tests with a significance level of 0.05. In survival analyses, participants contribute time in the model from day of enrollment to gestational age at arrest of development, censorship, or twenty completed weeks' gestation, whichever came first. Left truncation based on timing of enrollment accounts for a subject not having a loss prior to cohort entry.[231]

Haplotype-exposure association

Genes tied to alcohol metabolism are often associated with alcohol use behaviors,[249] so I assessed whether alcohol exposure, number of drinks per week, and number of drinks per sitting differed by *ADH1C* haplotype using Pearson's χ^2 test.

Haplotype-miscarriage association

ADH's primary function is converting ethanol to acetaldehyde. It also metabolizes retinol, glycol ethers, and methanol. Since miscarriage risk may be related to slower metabolism of these other compounds, I tested whether haplotype was independently associated with miscarriage risk using Cox proportional hazards models. Exposure is operationalized as number of *ADH1C*2* alleles (signifying slower enzyme activity) present. Since genetic allele is theoretically unaffected by other exposures, no other covariates are included in the model since they are not expected to bias the association.

Haplotype-exposure interaction relating to miscarriage risk

I used extended Cox survival models to estimate the association between duration of alcohol use as a time-varying exposure and miscarriage for the total sample and stratified by maternal *ADH1C* haplotype. Duration of use is defined as number of days between LMP and time *t* or gestational age at cessation of alcohol use, whichever came first. In the models for alcohol amount per week and per sitting, duration of alcohol use was defined as number of days between LMP and time *t* or gestational age at change in alcohol use, whichever came first. Participants who were unexposed during pregnancy served as the referent group. Results are presented as hazard ratios and 95% confidence intervals associated with each additional week of use. I tested for interaction by *ADH1C* haplotype on a multiplicative scale using the likelihood ratio test to compare models with and without an interaction term for haplotype and alcohol use.

Measures of association between alcohol exposure and miscarriage were adjusted for the following covariates selected *a priori* based on known and suspected relationships with alcohol use and miscarriage risk: maternal age (years, continuous, restricted cubic splines with four knots), education (high school or less, some college, college or more), cigarette use (never smoker or distant smoker, quit within four months of first trimester interview or current smoker),

pregnancy intention (intended, unintended), and parity (nulliparous, one prior birth, two or more prior births). Please see Chapter V for more information about data collection and covariate operationalization. All participants had complete covariate data.

Results

Of participants in RFTS, 987 were eligible for this analysis and had available genetic samples. Fifty-two percent were exposed to alcohol during pregnancy and 19.7% experienced a miscarriage. Median gestational age at change in alcohol use was 29 days' gestation (inter-quartile range [IQR]: 17–35 days). Among participants who were exposed at LMP, the median amount of alcohol consumed was two drinks per week before change in use (IQR: 1–4 drinks per week). Women who were older than thirty and from high income households were more likely to be exposed to alcohol during pregnancy compared with their counterparts (Table 22). Eleven percent of women reported at least one binge episode in the first trimester and 2.5% of women reported consuming four or more drinks per sitting at least once a week.

As indicated by haplotype, 33.7% of participants had fast *ADH1C* activity compared with 50.1% and 16.2% of participants that had moderate and slow activity, respectively. These proportions are consistent with persons of European ancestry in North America.[247,248,250] No differences in alcohol use behaviors by *ADH1C* haplotype were detected (Table 23; Figure 26). Independent from alcohol consumption, *ADH1C* haplotype and miscarriage risk were not associated (fast activity [referent]; moderate activity adjusted HR 0.75, 95% CI 0.52, 1.09; slow activity adjusted HR 0.78, 95% CI 0.46, 1.30).

As reported in Aim 2, each additional week of alcohol exposure led to increased risk of miscarriage (adjusted HR 1.07, 95% CI 1.00, 1.14). I did not find a dose-response trend for average number of drinks per week or number of drinks per drinking episode. I did not observe

effect modification by *ADH1C* haplotype for the association between miscarriage and alcohol exposure, number of drinks per week, or number of drinks per sitting (Table 24).

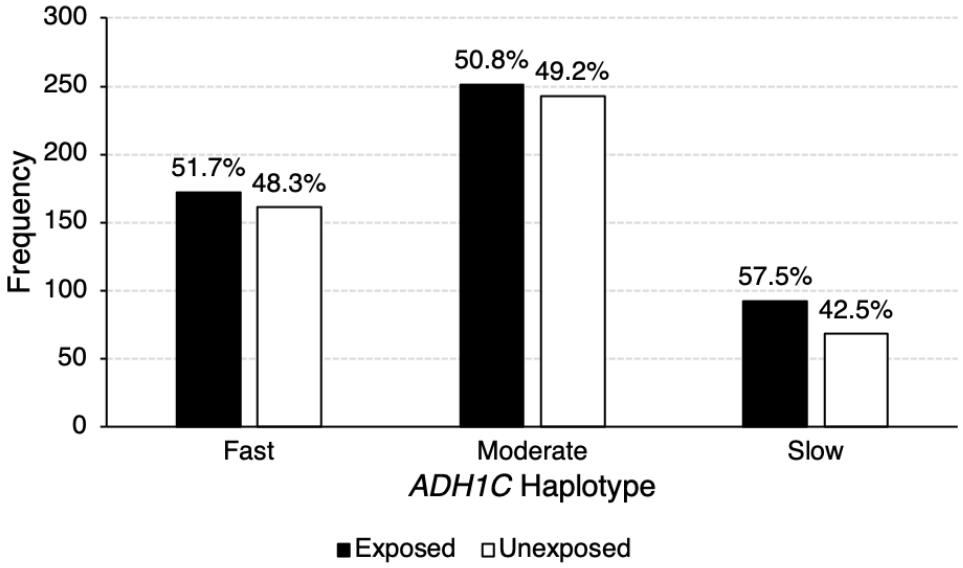

Figure 26. Alcohol exposure during pregnancy by ADH1C *haplotype.*

Table 22. Participant characteristics by alcohol use during pregnancy

Maternal Characteristics	No Alcohol Use* (n=472) N	%	Alcohol Use* (n=515) N	%	Unadjusted OR	95% CI
Maternal age, years						
<25	53	11.2	32	6.2	1.00	Referent
25–29	191	40.5	167	32.4	1.45	0.98, 2.35
30–34	171	36.2	204	39.6	1.98	1.22, 3.20
≥35	57	12.1	112	21.7	3.25	1.89, 5.60
BMI, kg/m^2						
<18.5	11	2.4	17	3.3	1.37	0.63, 2.98
18.5–24.9	266	56.8	300	58.7	1.00	Referent
25–29.9	117	25.0	120	23.5	0.91	0.67, 1.23
≥30	74	15.8	74	14.5	0.89	0.62, 1.27
Missing	4		4			
Education						
High school or less	33	7.0	26	5.0	1.00	Referent
Some college	71	15.0	54	10.5	0.97	0.62, 1.80
College or more	368	78.0	435	84.5	1.50	0.88, 2.55
Household income, $						
≤ 40,000	82	17.6	68	13.3	1.00	Referent
40,001 to 80,000	213	45.8	208	40.8	1.18	0.81, 1.71
> 80,000	170	36.6	234	45.9	1.66	1.14, 2.42
Declined/missing	7		5			
Marital status						
Married or cohabitating	465	98.5	504	97.9	1.00	Referent
Other	7	1.5	11	2.1	1.45	0.56, 3.77
Parity						
Nulliparous	216	45.8	295	57.3	1.00	Referent
1 prior delivery	169	35.8	150	29.1	0.65	0.49, 0.86
2+ prior deliveries	87	18.4	70	13.6	0.59	0.41, 0.84
Past miscarriage						
0	366	77.5	414	80.4	1.00	Referent
1	78	16.5	77	14.9	0.87	0.62, 1.23
≥2	28	5.9	24	4.7	0.76	0.43, 1.33
Smoking status†						
Never/distant quit	449	95.1	476	92.4	1.00	Referent
Current/recent quit	23	4.9	39	7.6	1.60	0.94, 2.72
Pregnancy Intention						
Intended	429	90.9	445	86.4	1.00	Referent
Not intended	43	9.1	70	13.6	1.57	1.04, 2.34
ADH1C activity						
Fast	161	34.1	172	33.4	1.00	Referent
Moderate	243	51.5	251	48.7	0.97	0.73, 1.28
Slow	68	14.4	92	17.9	1.27	0.87, 1.85

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.
* Alcohol use defined as exposure past last menstrual period.
† Quitting within the four months prior to the end of first trimester interview is considered a recent quit. Quitting before that time is considered a distant quit.

Table 23. Characteristics of alcohol exposure during pregnancy by ADH1C activity

Exposure Characteristics	Alcohol Dehydrogenase 1C Activity*						P-value†
	Fast (n=333)		Moderate (n=494)		Slow (n=160)		
	N	%	N	%	N	%	
Alcohol exposure‡							0.33
None	161	48.3	243	49.1	68	42.5	
Any	172	51.7	251	50.8	92	57.5	
Pattern of exposure							0.29
Never exposed	45	13.5	71	14.4	18	11.3	
Stopped before LMP	116	34.8	172	34.8	50	31.2	
Stopped during first trimester	150	45.0	212	42.9	71	44.4	
Exposed throughout first trimester	22	6.6	39	7.9	21	13.2	
Drinks/wk, before change							0.55
Unexposed	161	48.3	243	49.2	68	42.5	
Tertile 1 (≤1 drink/wk)	65	19.5	92	18.6	34	21.3	
Tertile 2(1.01–3.99 drinks/wk)	45	13.5	78	15.8	22	13.8	
Tertile 3 (≥4 drinks/wk)	62	18.6	81	16.4	36	22.5	
Drinks/sitting, before change							0.65
Unexposed	161	48.3	243	49.1	68	42.5	
<4 drinks/sitting	164	49.2	240	48.6	86	53.8	
≥4 drinks/sitting	8	2.4	11	2.2	6	3.8	
Binge episodes							0.68
None	302	90.7	443	89.7	136	85.0	
Any	31	9.3	51	10.3	24	15.0	

Abbreviations: *ADH1C*, alcohol dehydrogenase 1C; LMP, last menstrual period.
* Fast: homozygous *ADH1C*1*; moderate: heterozygous; slow: homozygous *ADH1C*2.*
† P-values derived from chi-square test.
‡ Alcohol use defined as exposure past last menstrual period.

Table 24. Association between alcohol use and miscarriage by ADH1C activity

Alcohol use characteristics	Alcohol Dehydrogenase 1C Activity*						P-value‡
	Fast (n=333)		Moderate (n=494)		Slow (n=160)		
	HR†	95% CI	HR†	95% CI	HR†	95% CI	
	Per week		Per week		Per week		
Alcohol Use							0.12
None	1.00	Referent	1.00	Referent	1.00	Referent	
Any	1.09	0.99, 1.21	1.11	1.01, 1.23	0.92	0.77, 1.10	
Drinks per Week, Before Change[d]							0.16
Unexposed	1.00	Referent	1.00	Referent	1.00	Referent	
≤1 drink/week	1.08	0.95, 1.24	1.08	0.98, 1.19	0.92	0.71, 1.19	
1.01–3.99 drinks/week	1.13	1.04, 1.23	1.06	0.91, 1.23	1.02	0.83, 1.26	
≥4 drinks/week	0.82	0.67, 1.02	1.07	0.98, 1.16	0.87	0.71, 1.06	
Drinks per Sitting, Before Change§							0.27
Unexposed	1.00	Referent	1.00	Referent	1.00	Referent	
<4 drinks/sitting	1.04	0.97, 1.13	1.07	0.99, 1.15	0.91	0.78, 1.06	
≥4 drinks/sitting	1.15	0.89, 1.47	1.07	0.93, 1.25	0.98	0.74, 1.30	

Abbreviations: *ADH1C*, alcohol dehydrogenase 1 C; CI confidence interval; HR, hazard ratio.

* Fast: homozygous *ADH1C*1*; moderate: heterozygous; slow: homozygous *ADH1C*2.*

† Alcohol modeled as a time-varying exposure for duration of use, left truncation based on gestational age at enrollment; adjusted for maternal age (continuous, spline), education, parity, smoking status, and pregnancy intention.

‡ P-value from likelihood ratio test comparing models with and without interaction term.

§ Categories reflect level of alcohol consumption prior to change in use, duration defined as pre-change use.

Comments

Slower alcohol metabolism as indicated by *ADH1C* did not modify the association between duration of alcohol use in pregnancy and miscarriage in this prospective pregnancy cohort. The rate alcohol is metabolized varies by *ADH1C* haplotype, meaning individuals with the variant related to slower metabolism have relatively higher concentration of and prolonged exposure to circulating alcohol. Number of weeks of alcohol exposure in the first trimester was related to miscarriage risk and this association was not dependent on maternal *ADH1C* haplotype.

ADH1C modifies the association between alcohol use and squamous carcinoma of the head and neck,[251] colorectal cancer,[252] and breast cancer.[253] Evidence of whether *ADH1C* modifies the relationship between alcohol use and coronary heart disease is mixed.[254-256] In the context of pregnancy, *ADH1C* haplotype amplifies the association between maternal alcohol use and oral cleft defects. However, effect modification was only observed if both mother and infant had the haplotype signifying slower metabolism and if the levels of alcohol consumption was high (five or more alcoholic drinks per drinking episode).[159]

An interaction between *ADH1C* and alcohol use may not have been detected in this analysis for several reasons. In Aim 2, I established duration of alcohol use in the first trimester was a determinant of miscarriage risk. I did not find a dose-response trend in terms of average number of drinks consumed per week or per sitting. It is possible a threshold effect for alcohol use occurs at low levels of exposure. If this is true, the degree by which slower alcohol metabolism affects blood alcohol concentration may only marginally impact risk.

If concentration and duration of circulating alcohol does influence risk, effect modification by *ADH1C* would be expected. Effect modification may have gone undetected if features of alcohol use relevant to this interaction were imprecisely measured or rare. Amount of

alcohol consumed is the primary determinant of blood alcohol concentration. As discussed in Chapter V, reporting about number of drinks consumed may be imprecise due to variations in participants' notion of a standard drink or may be vulnerable to reporting biases.[167,169,174,175] Recall bias may lead to differential reporting by outcome for cases interviewed after a loss,[168,170] but reporting would not likely be differential by maternal haplotype. Imprecision or bias in measures of alcohol dose would inhibit detection of an interaction with *ADH1C* haplotype.

In a prior study, effect modification by *ADH1C* haplotype of alcohol use in pregnancy and oral cleft defects was only present for those who consumed five or more drinks per sitting.[159] Exposure at this high of a dose was rare in the *Right from the Start* cohort. While 11% of participants endorsed at least one binge episode (defined as drinking four or more drinks per episode), only 3% reported binging regularly. Scarcity of high levels of alcohol use may contribute to the absence of effect modification by *ADH1C* in this cohort.

An interaction between *ADH1C* and alcohol use may be obscured by other factors influencing blood alcohol concentration. For example, consuming alcohol with a meal alters absorption and clearance. Presence of food in the stomach slows gastric emptying which decreases the alcohol gradient for absorption.[140,156] Clearance is also more rapid when alcohol is consumed with food since ADH concentration is higher in the fed nutritional state.[257,258] On the other hand, alcohol use with medications that inhibit ADH or competitively bind with ADH impairs alcohol clearance.[156] While I would have liked to have more information about context of alcohol use, I cannot comment on how intake with food or medication may have influenced measures of effect. Body weight also effects blood alcohol concentration. Individuals with low body weight have higher blood alcohol concentration compared with individuals with higher body weight for the same exposure.[156] Results did not change when the models were further adjusted for maternal BMI.

Other genetic variants are implicated in alcohol metabolism. Approximately 240 SNPs have been identified within the seven genes encoding ADH.[35] Most occur in non-coding regions and only a handful are related to alcohol metabolism. In this analysis, I focused on *ADH1C* since SNPs in this gene are highly prevalent in individuals of European descent (47% compared with 3% for the second most prevalent variant [*ADH1B*]).[247] The second enzyme in the pathway for alcohol clearance, aldehyde dehydrogenase (ALDH), metabolizes acetaldehyde produced from ethanol oxidation. Several SNPs in genes coding for this enzyme impact alcohol metabolism, the most common being a variant in *ALDH2* that produces a flushing reaction. This variant is common in people of Chinese and Japanese descent, but is rare in individuals with predominantly European ancestry and was therefore not assessed in this cohort.[35]

In a prior study, modification by *ADH1C* of teratogenic effects of alcohol use in pregnancy was only observed when considering both maternal and fetal haplotype.[159] Since *ADH1C* is expressed in the placenta,[157] fetal haplotype may play an important role in modifying risk. I did not have genetic data for conceptuses of pregnancies ending in loss, so I was not able to assess whether a joint effect between maternal and fetal haplotype modified the association between alcohol use and miscarriage.

In this prospective cohort study of pregnancy health, the increase in miscarriage risk associated with each additional week of alcohol use in pregnancy was not modified by *ADH1C* haplotype. Exposure to alcohol in early pregnancy is an important driver of miscarriage risk for all women and genetic propensity for fast alcohol metabolism does not confer protection against regular alcohol exposure. Early pregnancy testing and cessation of alcohol use should be encouraged to mitigate risk of miscarriage associated with alcohol use in pregnancy.

VII. CONCLUSION AND FUTURE DIRECTIONS

Summary

In this dissertation, I established the relationship between alcohol use during pregnancy and miscarriage risk with the central hypothesis the association depends on timing of exposure. Studies of alcohol use during pregnancy are common, but information about how changes in alcohol use in early pregnancy relate to miscarriage risk is scarce. By incorporating data about pattern and timing of alcohol exposure into measures of miscarriage risk, I discovered timing of cessation of alcohol use during pregnancy is a key determinant of miscarriage.

My first aim was to conduct a systematic review and meta-analysis of the literature about alcohol exposure during pregnancy and miscarriage. Twenty-four studies reported on alcohol use and risk of miscarriage. Twelve out of the twenty providing a measure of association observed increased risk among women who use alcohol. Limitations in recruitment methods were widespread. Often studies enrolled women later in gestation than most miscarriages occur or required contact with the healthcare system for case identification. Methods for assessing and operationalizing alcohol exposure during pregnancy were heterogeneous. Most studies used alcohol behavior post pregnancy recognition as the primary exposure. Some studies queried pre-pregnancy alcohol use and its association with miscarriage risk, but information about how long "pre-pregnancy" use continued into the first trimester was scarce. Studies used simple methods for modeling risk (only four used survival analyses) and neglected the time-varying nature of behavior. This audit of past studies informed my analytical approach in my second aim.

In Aim 2, I established the relationship between alcohol consumption and miscarriage in the RFTS pregnancy cohort. To determine which modeling strategy to deploy using RFTS data, I first conducted a simulation study to critically evaluate how timing of exposure may relate to risk and how that dynamic might impact model performance. I demonstrated how a model's ability to

detect and accurately measure an association closely relates to how well its assumptions align with the interplay between time, exposure, and outcome in the underlying relationship. For example, models treating an exposure with time-dependent effects as uniform often fail to detect risk relationships. This work confirmed that traditional methods of operationalizing exposure in the literature (i.e., treating exposure as constant or separately modeling exposure before and after a change regardless of timing) are inadequate for assessing risk related to alcohol use during pregnancy.

Using what I learned in the simulation study, I quantified the association between alcohol use and miscarriage in RFTS using valid and more sophisticated statistical methods. Since RFTS collected data about alcohol consumption before and after a change in use and timing of that change, I was able to determine gestational age-specific exposure. When considering exposure in each week of gestation, alcohol use in weeks five through ten was associated with miscarriage when adjusting for multiple comparisons and exposure during week nine was associated with the highest risk. When examining duration of alcohol use, each additional week of exposure during pregnancy was associated with an 8% relative increase in risk of miscarriage compared with risk among women who were unexposed. This is notable considering the median time of change in consumption was 29 days' gestation, which corresponds with a 37% increase in risk of miscarriage relative to women who abstain from use. If what I observed is true in the greater population, this translates to alcohol use in pregnancy attributing to 15 miscarriage out of 1,000 pregnancies. Amount consumed and alcohol type were not linked with risk of miscarriage in this cohort.

In Aim 3, I assessed how genetic predisposition for alcohol metabolism as indicated by *ADH1C* haplotype modified the relationship between alcohol exposure during the first trimester and miscarriage. *ADH1C* haplotype was not associated with type or amount of alcohol consumed

during pregnancy. I expected to see elevated risk among participants who were homogenous for the haplotype signifying slower alcohol metabolism. However, haplotype was not independently associated with miscarriage and did not modify risk associated with alcohol use. This implies other characteristics of exposure or factors influencing blood alcohol concentration are more important for driving risk.

Implications

This work provides evidence that when a woman stops using alcohol in pregnancy is an important determinant of miscarriage risk. Recommendations from national advisory bodies that women who are or could be pregnant should abstain from alcohol use evoked backlash as paternalistic and impractical, which undermined an important message about risk of alcohol use during early pregnancy. Findings in Aim 2 offer more specific information about how timing and duration of alcohol consumption during pregnancy influence risk of loss. Preventing exposure to alcohol during pregnancy would be ideal, but pregnancy planning is not necessarily associated with reduction or cessation of alcohol use.[1,259] Encouraging early testing around anticipated menses may be an effective strategy for decreasing miscarriage risk related to alcohol use since most women change behavior at the time of a positive pregnancy test. Since cost and inconvenience may prevent women from testing early and frequently, increasing access to pregnancy tests would likely lead to prompt reduction in alcohol exposure during pregnancy.

Since information about miscarriage risk and alcohol consumption is only useful and actionable prior to initiation of prenatal care, public health efforts should focus on reaching reproductive age women before conception. The CDC encourages healthcare providers to educate their patients about risks of alcohol exposure during pregnancy.[260] Brief motivational interventions such as counseling about risky drinking and contraception use reduces the number of pregnancies at risk for alcohol exposure.[261] Discussions about early pregnancy testing and

change in alcohol use need to occur during routine care visits for reproductive age women before pregnancy to be effective.[224] Other methods for promoting the importance of quitting alcohol use early in pregnancy, such as warnings on alcohol labels or in establishments serving alcohol, should be considered since every week alcohol use in pregnancy is prevented corresponds with a decreased risk of miscarriage.

Future Directions

Additional research about harms of alcohol use during pregnancy is warranted. Many hypotheses about how alcohol may affect a developing pregnancy exist. A major challenge of this work was determining how to model the association between alcohol exposure and miscarriage to best reflect leading theories about mechanisms of risk. Better understanding about how alcohol may endanger pregnancy at different stages of gestation may help elucidate which drinking behaviors are riskiest. This would also require development and adoption of more sophisticated statistical methods for modeling specific hypotheses about the relationship between gestational age, exposure, and outcome.

I did not detect a dose-response relationship between amount of alcohol consumed and miscarriage risk. This may be due to limitations in methods for ascertaining amount consumed. Most individuals overestimate volume and alcohol content of a standard drink,[174,175] which leads to unintentional underreporting of number of drinks consumed. Picture references of a standard drink by beverage type may increase precision and accuracy of dose reports. Better information about alcohol consumed per sitting may uncover clearer relationships between episodic dose and frequency of drinking episodes. If a dose-response relationship is present, it may have also been masked by the limited range of alcohol consumption reported in this cohort. Median alcohol consumption before and after change was modest (two drinks per week and less than one drink

per week, respectively). A broader range of exposure levels may be necessary to determine if higher levels of alcohol exposure are associated with increased risk.

Although it is reasonable to assume factors impacting alcohol clearance would influence risk, I did not observe effect modification by *ADH1C* haplotype. A study of alcohol use and risk of oral cleft defects by *ADH1C* haplotype measured an increase in risk only when both mother and infant had the haplotype associated with slower metabolism.[159] For pregnancies ending in loss, I did not have genetic data for conceptuses. This data is challenging to obtain systematically, but may become more readily available as methods advance for obtaining fetal free DNA from maternal serum. Effect modification by *ADH1C* may be observed when accounting for haplotype of both mother and baby. Other factors affecting alcohol absorption and clearance exist and their influence on risk is unknown.

Finally, this work is restricted to evaluating miscarriage risk. Alcohol exposure at pregnancy onset concurs with foundational steps of embryo development and may affect risk of outcomes occurring later in pregnancy or childhood. For example, craniofacial anomalies characteristic of FASD, can be traced to impairment in neural crest migration occurring in the first weeks of pregnancy.[186] Other neurodevelopmental and behavioral features of FASD can be observed with alcohol use limited to the first trimester.[183,184,187] A closer look into how timing and duration of alcohol use in early gestation impacts risk of other outcomes is necessary to truly understand risk associated with alcohol use during pregnancy.

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IX. APPENDICES

Appendix 1: PRISMA Checklist

Section/topic	#	Checklist item	On page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	20
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	20
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	21
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	21
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	22
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	22
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	22
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	22, A3
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	22-23
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	23-24
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	23-24, A6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	24-25
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	24-26
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	24-26
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	26
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	25-26

RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	F6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	T4
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	F9
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	T5
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	F7, F8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	F10, F11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	T6
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	38-39
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	39-41
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	41
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	ii

Appendix 2: MOOSE Checklist for Meta-analyses of Observational Studies

Item No	Recommendation	Reported on Page No
Reporting of background should include		
1	Problem definition	21
2	Hypothesis statement	21
3	Description of study outcome(s)	21
4	Type of exposure or intervention (or exposure) used	21
5	Type of study designs used	21
6	Study population	22
Reporting of search strategy should include		
7	Qualifications of searchers (e.g., librarians and investigators)	22
8	Search strategy, including time period included in the synthesis and key words	22, A3
9	Effort to include all available studies, including contact with authors	22
10	Databases and registries searched	22
11	Search software used, name and version, including special features used (e.g., explosion)	A3
12	Use of hand searching (e.g., reference lists of obtained articles)	22
13	List of citations located and those excluded, including justification	Upon Request
14	Method of addressing articles published in languages other than English	22
15	Method of handling abstracts and unpublished studies	22
16	Description of any contact with authors	23
Reporting of methods should include		
17	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	22
18	Rationale for the selection and coding of data (e.g., sound clinical principles or convenience)	23
19	Documentation of how data were classified and coded (e.g., multiple raters, blinding and interrater reliability)	23
20	Assessment of confounding (e.g., comparability of cases and controls in studies where appropriate)	23
21	Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results	23-24
22	Assessment of heterogeneity	25
23	Description of statistical methods (e.g., complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated	24-26
24	Provision of appropriate tables and graphics	34-37
Reporting of results should include		
25	Graphic summarizing individual study estimates and overall estimate	34
26	Table giving descriptive information for each study included	29-33
27	Results of sensitivity testing (e.g., subgroup analysis)	35

28	Indication of statistical uncertainty of findings	34-35
Reporting of discussion should include		
29	Quantitative assessment of bias (e.g., publication bias)	37
30	Justification for exclusion (e.g., exclusion of non-English language citations)	28
31	Assessment of quality of included studies	36
Reporting of conclusions should include		
32	Consideration of alternative explanations for observed results	39-41
33	Generalization of the conclusions (i.e., appropriate for the data presented and within the domain of the literature review)	41
34	Guidelines for future research	41
35	Disclosure of funding source	ii
Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: A proposal for reporting. JAMA 2000;283(15):2008-12.		

Appendix 3: Systematic Review Full Search Strategy

MEDLINE

("Abortion, Spontaneous"[Mesh] OR "Miscarriage"[tiab] OR "Pregnancy loss"[tiab] OR "abortion"[tiab]) AND ("Alcohol Drinking"[Mesh] OR "Ethanol"[Mesh] OR "alcohol"[tiab])

ProQuest PsycINFO and ProQuest Dissertations and Theses Global

(miscarriage OR "spontaneous abortion" OR ti,ab(pregnancy loss)) AND (ti,ab(alcohol OR drink* OR ethanol) OR SU.EXACT(Drinking Behavior) OR SU.EXACT(Alcohol Drinking Patterns))

EMBASE

1 exp alcohol consumption/ OR exp alcohol abstinence/ OR exp alcohol/ OR alcohol.mp. OR exp alcohol abuse/

2 miscarriage.mp. OR exp spontaneous abortion/

3 pregnancy loss.mp.

4 2 OR 3

5 1 AND 4

ClinicalTrials.gov

Advanced Search, no filters:

(miscarriage OR spontaneous abortion OR pregnancy loss) AND (alcohol OR drinking)

Appendix 4: REDCap Abstract Screening Tool

Confidential

Initial_Abstract_Screen

Record ID _____

In this meta-analysis, we are evaluating the relationship between alcohol exposure during pregnancy and risk of spontaneous abortion (miscarriage). At the abstract screening level, our goal is to remove articles that are clearly irrelevant. This includes studies done among animals, commentaries or letters that do not contain any primary data, and primary studies that do not pregnancy outcomes.

For the field "Should this article move onto the full text review?":

1. Select "No" if an article is clearly irrelevant or does not include any primary data.

2. Select "Yes" if an article is clearly applicable or of undetermined relevance

Study author: _____

Publication year: _____

Publication Type
◯ Peer-Reviewed
◯ Grey Literature

Journal: _____

Article title:

_____

Abstract:

_____

Reviewer 2:
◯ Kathy Zhao
◯ Chantay Young

Should this article move on to the full-text review?
◯ Yes
◯ No

Appendix 5: REDCap Full Text Screening Tool

Confidential

Full_Text_Screen

Record ID _____

In this meta-analysis, we are evaluating the relationship between alcohol exposure during pregnancy and risk of spontaneous abortion (miscarriage). At the full text screening level, our goal is to identify articles that are eligible for inclusion in the review and to indicate reason for exclusion of ineligible articles.

Eligibility Criteria

Does this article present original research?	◯ Yes ◯ No
Study available in English?	◯ Yes ◯ No
Does the study focus on pregnant women and pregnancy outcomes?	◯ Yes ◯ No
Does the study systematically assess and report miscarriage as an outcome?	◯ Yes ◯ No
Was alcohol consumption assessed as an exposure?	◯ Yes ◯ No
Was information about alcohol exposure specific to pregnancy?	◯ Yes ◯ No
Was there an adequate reference group of women who were unexposed to alcohol?	◯ Yes ◯ No
Was the study limited to women using assisted reproductive technologies to conceive?	◯ Yes ◯ No

Systematic Review Eligibility

Is this study eligible fore the systematic review?	◯ Yes ◯ No (Answer yes if all eligibility criteria are met.)
Does the study provide enough information to quantify an effect estimate for the relationship between alcohol exposure and miscarriage risk?	◯ Yes ◯ No

Meta-Analysis Eligibility

Is the study eligible for the meta-analysis?	◯ Yes ◯ No (Answer yes if eligible for systematic review and effect estimate information present.)

Appendix 6: REDCap Data Extraction Tool

Confidential

Data_Extraction_Form

Record ID _____

This data extraction form is to be performed by two reviewers for each article that is eligible for the systematic review.

First author: _____

Year published: _____

Journal: _____

Study design:
◯ Prospective Cohort
◯ Retrospective Cohort
◯ Case-Control
◯ Cross-sectional
◯ Other

Study design if other: _____

Years of study: _____

Study setting: _____

Study population: _____
(Describe the population from which individuals were recruited into the study.)

Recruitment strategy: _____
(Describe how individuals were recruited into the study.)

Study inclusion criteria: _____
(List the study's inclusion criteria.)

Exclusion criteria: _____
(List study's exclusion criteria.)

How was alcohol consumption assessed (content)? _____
(e.g., number of drinks since LMP, average number of drinks per week, etc.)

How was alcohol consumption assessed (mode)? _____
(e.g., self-administered questionnaire, in-person interview, etc.)

How did the study define the exposure window? _____

How was alcohol exposure operationalized? _____
(i.e., drinks/week, mL/day, etc.)

Did the study ask if there was a change in alcohol consumption patterns during pregnancy?
◯ Yes
◯ No

Did the study model change in alcohol consumption pattern in analysis?
◯ Yes
◯ No

At what time point was alcohol exposure assessed? _____

How was spontaneous abortion defined by the study? _____

How was pregnancy outcome assessed in the study? _____

Who served as the comparison group for pregnancy outcome? _____
(e.g., all pregnancies surviving past 20 weeks' gestation, all live births, etc.)

Was alcohol the primary exposure studied?
◯ Yes
◯ No

How many women were in the study? _____

How many women were exposed to alcohol in pregnancy? _____

How many women were unexposed to alcohol in pregnancy? _____

How many women who were exposed to alcohol have a miscarriage? _____

How many women who were exposed to alcohol did not have a miscarriage? _____

How many women who were unexposed have a miscarriage? _____

How many women who were unexposed did not have a miscarriage? _____

What was the unadjusted effect estimate for the relationship between any alcohol exposure and miscarriage risk? _____

What was the confidence interval for the unadjusted effect estimate for the relationship between any alcohol exposure and miscarriage risk? _____

What was the adjusted effect estimate for the relationship between any alcohol exposure and miscarriage risk? _____

What was the adjusted confidence interval for the effect estimate for the relationship between any alcohol exposure and miscarriage risk? _____

What covariates were included in the adjusted model? _____

What type of effect estimate was calculated? _____
(e.g., hazard ratios, risk ratios, odds ratios, etc.)

Was alcohol exposure modeled in any other ways?
◯ Yes
◯ No
(e.g., continuous, categorical, etc.)

In what other ways was alcohol exposure modeled? _____

What were the corresponding unadjusted effect estimates and confidence intervals? _____

What were the corresponding adjusted effect estimates and confidence intervals? _____

Aim 1: REDCap risk of bias assessment *

Confidential

Risk of Bias Estimator-Case Control

Abstract Number _____
(Unique assigned abstract number)

Selection

Case definition is adequate
- ◯ Yes, with independent validation (one star)
- ◯ Yes, based on symptoms and self-report
- ◯ No description

Representativeness of cases
- ◯ A consecutive or obviously representative series of cases (one star)
- ◯ Potential for selection bias, not described

Selection of controls:
- ◯ Community controls (one star)
- ◯ Hospital controls
- ◯ No description

Definition of controls:
- ◯ Confirmed surviving pregnancy at 20 weeks' gestation (end point) (one star)
- ◯ No description of source

Exposure

Ascertainment of exposure:
- ◯ Self-reported alcohol exposure in pregnancy, self-administered questionnaire (one star)
- ◯ Self-reported alcohol exposure in pregnancy, in-person interview (one star)
- ◯ No description
- ◯ Other

Non-response rate
- ◯ same for both groups (one star)
- ◯ different be described
- ◯ not described

(Same means similar within 10%)

Same method of ascertainment for cases and controls
- ◯ Yes
- ◯ No

Comparability

Comparability of cohorts on the basis of the design or analysis controlled for confounders:
- ◯ The study controls for age (one star)
- ◯ Study controls for other factors (list) _____(one star)
- ◯ Cases and controls are not comparable on the basis of the design or analysis controlled for confounders'

What other factors does the analysis control for? _____

Outcome

Assessment of outcome:

- ◯ Independent blind assessment (one star)
- ◯ Record linkage (one star)
- ◯ Self report
- ◯ No description
- ◯ Other

Risk of Bias Estimator-Cohort

Abstract Number _____
(Unique assigned abstract number)

Selection

Representativeness of the exposed cohort
- ◯ Truly representative (one star)
- ◯ Somewhat representative of the general population of reproductive-age women (one star)
- ◯ Selected group
- ◯ No description of the derivation of the cohort

Selection of the non-exposed cohort:
- ◯ Drawn from the same community as the exposed cohort (one star)
- ◯ Drawn from a different source
- ◯ No description of the derivation of the non-exposed cohort

Exposure

Ascertainment of exposure
- ◯ Self-reported alcohol exposure in pregnancy, self-administered questionnaire (one star)
- ◯ Self-reported alcohol exposure in pregnancy, in-person interview (one star)
- ◯ No description
- ◯ Other

Demonstration that outcome of interest was not present at start of study:
- ◯ Yes
- ◯ No

Comparability

Comparability of cohorts on the basis of the design or analysis controlled for confounders:
- ◯ The study controls for age (one star)
- ◯ Study controls for other factors (gestational age at enrollment) (one star)
- ◯ Cohorts are not comparable on the basis of the design or analysis controlled for confounders

What other factors did the study control for? _____

Outcome

Assessment of outcome:
- ◯ Independent blind assessment (one star)
- ◯ Record linkage (one star)
- ◯ Self report
- ◯ No description
- ◯ Other

Was follow-up long enough for outcomes to occur:
- ◯ Yes
- ◯ No

Indicate the median duration of follow-up and a brief rationale for the assessment above: _____

Adequacy of follow-up of cohorts:

- ◯ Complete follow up- all subject accounted for (one star)
- ◯ Subjects lost to follow up unlikely to introduce bias- number lost less than or equal to 20% or description of those lost suggested no different from those followed. (one star)
- ◯ Follow up rate greater than 80% and no description of those lost
- ◯ No statement

*Wells GA, Shea B, O'Connell D. The Newcastle-Ottawa Scale for assessing the quality of non-randomised studies in meta-analyses, http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp; [accessed May 5, 2016 2016].

Appendix 7: *Right from the Start* Informed Consent

Vanderbilt University Institutional Review Board
Informed Consent Document for Research

Principal Investigator: Katherine E. Hartmann, MD, PhD **Revision Date: April 17, 2009**
Study Title: Right From the Start: A Study of Early Pregnancy Health
Institution/Hospital: Vanderbilt University Medical Center

This informed consent applies to healthy female volunteers

Name of participant: _____ Age: _____

The following is given to you to tell you about this research study. Please read this form with care and ask any questions you may have about this study. Your questions will be answered. Also, you will be given a copy of this consent form.

1. What is the purpose of this study?

This is a Vanderbilt University Medical Center research study conducted by Dr. Katherine Hartmann. We are doing this study to find out how women's own health, the things they do, and the things they come in contact with affect pregnancy health. Examples are type of work, tobacco use, medications, exercise intensity, and if women have uterine fibroids. Our motivation is to build knowledge that may help prevent miscarriage and other pregnancy complications. Your being in this study is your choice. You do not have to do it. You can refuse to answer questions and still be part of this study. You may also choose to be in some parts of the study and not in others.

2. What will happen and how long will you be in the study?

a. If you are currently completing the daily and weekly online diary, you will continue through 12 weeks of pregnancy.
b. You will have a first trimester ultrasound, which is for research data collection and not clinical care. The study sonographer, one individual you choose, and potentially a female chaperone will be present. The trained sonographer will do the ultrasound. As most early pregnancy ultrasounds, the ultrasound for this study will be done using a probe about the size of a tampon put in the vagina. The probe will get a picture of the growing fetus. There is no charge for this research ultrasound. Study staff will schedule the appointment for you at the best time for you. It is very important that this ultrasound be done as close to the 6th week of pregnancy as possible. We will not do the ultrasound later than the 12th week.
c. We will ask the small group of women who have a pregnancy loss before the scheduled ultrasound to have an ultrasound preferably within 2 to 4 weeks after their loss. We will not do the ultrasound later than 3 months after a loss. The ultrasound should take about 20 minutes.
d. Research study staff will call you at a phone number you give us to do a phone interview. We will call you when you are about 13 weeks pregnant. This interview will last about an hour. During the interview, we will ask about your job and your lifestyle as well as questions about your normal diet and exercise. We will ask questions about physical or sexual abuse and drug abuse. We will also ask questions about medication use and medical history. You may refuse to answer any questions that you do not want to answer. We will not do the interview later than 16 weeks of pregnancy. At your ultrasound visit we will give you a paper diary for use if you are not participating in the online diary. The paper diary will help you keep track of information related to your pregnancy and may help with answering the interview questions. You can still do the interview even if you don't complete the paper diary.
e. If you have a pregnancy loss before the interview, we will hope to do the interview within two weeks of the pregnancy loss or as soon as you are able. We will ask to do the interview no later than what would have been the 16th week of pregnancy or no later than 2 months after a loss, whichever date is later. This interview will be about one hour. During the interview, we will about jobs, and lifestyle, usual diet and exercise. We will ask questions about physical or sexual abuse and drug abuse. We will also ask questions about medication use. You may refuse to answer any questions that you do not want to answer.
f. In your 7th month of pregnancy, you will mail us a one-page update form with information about where you plan to have your baby. We will also ask you for updated contact and prenatal care provider information. The form should take about 5 minutes. You will mail the updated information to us in a postage-paid envelope. Study staff may call you to follow-up.
g. At the end of your pregnancy we ask that you mail the study office the "Notice of Pregnancy Outcome" form in a postage-paid envelope with information about your pregnancy. The form asks for simple information like confirming

your care provider, if you had insurance, and what date your pregnancy ended. The form should take about 5 minutes.

h. At the end of your pregnancy we will review your and your baby's medical records for this pregnancy only. We will get these records at the doctor's office, hospital, urgent care center or emergency department where you got care. The information we will get from your medical records includes lab results, clinic visit information, vital signs, ultrasound information, hospital admissions, progress notes, operative or procedure notes, any pathology reports, consultation notes, birth details and discharge summaries. We will ask you to sign a medical record release form for this reason. You may refuse to do this and still be in the study.

You will be in this study for up to 6 months after your pregnancy ends. Study staff may review medical records about this pregnancy and your baby's hospital records after those six months. We expect to record reviews within a year, but will not access records later than 2011. At some later time we may ask you and/or your partner through you to be in other studies but you do not have to accept.

3. Costs to you if you take part in this study:
It will not cost you any money to be in this study.

4. Side effects and risks that you can expect if you take part in this study:

This study might involve the following risks and/or discomforts to you.

- The surveys ask personal questions about menstrual cycles, sexual intercourse, physical and sexual abuse, prior lost pregnancies, and drug and alcohol use that may make you uncomfortable.
- If, as part of the study, you test for pregnancy earlier than you would have otherwise, it may make it more likely that a very early loss or "chemical pregnancy" is recognized. This is not harmful to your health but can be stressful.
- The ultrasounds use a small probe that is placed in the vagina. This may cause a sense of pressure or discomfort.
- The ultrasounds done for this study are very limited and are not as complete as ultrasounds done for medical care. It could be harmful to you if you are falsely reassured by having the study ultrasound and do not seek medical care. **It is very important that you seek medical care if you have symptoms that worry you.**

5. Information the study ultrasounds will and will not give you:
Study sonographers are not providing medical care. It will probably take several weeks before a research doctor (Dr. Hartmann or a colleague) will look at the ultrasound report for research data only. Some times we may ask you to discuss your ultrasound report with your doctor. We may also ask you to speak with the study doctor about your ultrasound.

The ultrasounds done for this study are for *research only*. The first ultrasound is done to confirm your pregnancy. It will look for the fetal heart rate. It will also estimate your due date for study records. Ultrasounds also will measure fibroids (if present).

These are not diagnostic ultrasounds and cannot be part of your medical care. We will send a copy of the report to the prenatal care giver you choose as a courtesy to your prenatal care team. The ultrasound is ***not*** being done to find conditions that could hurt you, such as ectopic (tubal) pregnancy. It is also ***not*** being done to find problems with the pregnancy, or the uterus and ovaries after pregnancy.

6. Risks that are not known:
There may be risks that are not known.

7. Payment in case you are injured while in this study:

We do not foresee ways in which being in the study could cause injury. If you are hurt from being in this study, immediate and necessary care for injury will be done at Vanderbilt without charge. There are no plans for Vanderbilt to pay for further treatment beyond this care or provide money for such injury.

8. Good effects that might result from this study:

The benefits to science and humankind that might result from this study are: Participants will contribute to medical knowledge about early pregnancy health with hopes that in the future we may be able to help women and their care providers prevent some miscarriages or other pregnancy problems.

The benefits you might get from being in this study are:

- If you have a first trimester ultrasound, you will get an estimate of due date to discuss with your care provider.
- We may be able to provide a photograph at ultrasound(s). The photograph will be labeled for "research purposes only". (We must emphasize that the picture in no way assesses the well being of the baby).
- Your ultrasound findings will be provided as a courtesy to the care provider designated by you – we do not put results in any medical records because they are not for medical care. However, your healthcare provider may opt to add them to your medical records
- You will have access to the study website.
- On the website, you will have access to news about new study findings and publications.

9. Other treatments you could get if you decide not to be in this study:

You do not have to be in this research study. You may choose not to be in this study. This study **does not include any medical care** or require any changes in your regular care. Whether or not you participate does not in any way influence your healthcare, services, or other rights.

10. Payments for your time spent taking part in this study or expenses:

If you pre-enrolled in the study while you were trying to get pregnant and called us when you were less than 45 days pregnant you will receive $25.

All women in the study will get:

- $10 for doing the 13-week phone interview. You will get $10 if you have a pregnancy loss and do a modified version of the first survey.
- $5 cash or gift, such as magnet picture frame, key chain, and jar opener, for doing and mailing the 7-month update form in a postage-paid envelope.
- $5 cash or gift, such as diaper wipe holder and baby bib, for doing and mailing Notice of Pregnancy Outcome form in a postage-paid envelope.
- a bonus gift for telling an eligible woman who enrolls about the study (up to two gifts, all bonus gifts will be of about the same value).

If you are doing at least 6 of the 7 daily diary entries each week and also doing the weekly diary, you get:

- $5 gift cards for each week for the completed diaries. These gift cards are mailed once every month and you will let us know you received it by clicking on the web site.

If you have a pregnancy loss before the first trimester ultrasound:

- $25 for doing an early loss ultrasound

11. Reasons why you may be taken out of this study:
You may be taken out of the study if: you decline the first trimester ultrasound, terminate your pregnancy before completing the first trimester ultrasound, move from the study area prior to becoming pregnant or having the first ultrasound. You may be taken out of the study if you begin infertility treatment with medications or surgery.

12. What will happen if you decide to stop being in this study?
Your being in this study is your choice. You do not have to do it. You can decide not to be in this study at any time without penalty and without losing benefits you would otherwise be getting. You may refuse to answer questions and still be part of this study. You may also choose to be in some parts of the study and not in others. Your decision about being in this study will not, in any way, affect your health care. If you decide not to continue to participate or wish to drop out of the study, information collected up to that point will be retained unless you specifically request that all your information be removed. To drop out of the study, or if you have any questions, call study staff free at 1- 866-346-2684.

13. Who to call for any questions or in case you are injured:
Today you can ask all your questions about this study. If you have more questions you can call for free at 1-866-346-2684. You can also call the Project Coordinator, Sarah Jones, collect, at (615) 936-3976. You can write her at the Institute for Medicine and Public Health. The address is Vanderbilt University Medical Center, Sixth Floor, Suite 600, 2525 West End, Nashville, TN 37203-1738.

For additional information about giving consent or your rights as a person in this study, please feel free to call the Vanderbilt University Institutional Review Board Office at (615) 322-2918 or toll free at (866) 224-8273.

14. Confidentiality:
All efforts, within reason, will be made to keep your research data private. As part of the study, Dr. Katherine Hartmann and her study team may share the results of your study ultrasound with the care **provider you designate** to receive your ultrasound reports. Other information is not available in a form that we can share with you or with care providers. Data from many women will be combined to study how certain factors relate to risk of pregnancy problems. Data used by the research team for data analysis does not include information that can be traced back to you like name or address. Such contact information is only available to study staff to help with study activities. Your answers to interviews are linked only to a study ID number. The research team preparing study results will only use data that has study IDs and no identifying information.

We do not plan to share any study information with official groups, however there is a risk that study data can be requested by groups that include the Federal Government Office for Human Research Protections, the Vanderbilt University Institutional Review Board or National Institutes of Health. Federal privacy rules may not apply to these groups; they have their own rules and codes to assure that all efforts, within reason, will be made to keep your data private. The sponsor, Vanderbilt, Dr. Hartmann and her staff will keep your data in strict confidence, and will comply with any and all laws regarding the privacy of such information.

Study data will be kept as part of research records for at least six years after the study is finished. No study results will be entered in your medical records unless your own care provider chooses to place your study ultrasound in your care

record. Unless told otherwise, your consent to use your data does not expire. If you change your mind, we ask that you contact the Principal Investigator, Dr. Katherine Hartmann in writing and let her know that you withdraw your consent.

Her mailing address is:

Institute for Medicine and Public Health
Vanderbilt University Medical Center
Sixth Floor, Suite 600
2525 West End Avenue
Nashville, TN 37203-1738

At that time, we will stop getting any more data about you. But, the data we stored before you withdrew your consent may still be used for reporting and for assuring research quality.

If you decide not to take part in this research study, it will not affect your treatment, payment or enrollment in any health plans or affect your ability to get benefits. You will get a copy of this form after it is signed.

☐ **I would like to be contacted about future studies.**

STATEMENT BY PERSON AGREEING TO BE IN THIS STUDY

I have read this consent form and the research study has been explained to me verbally. All my questions have been answered, and I freely and voluntarily choose to take part in this study.

_____ _____
Date Signature of patient/volunteer

Consent obtained by:

_____ _____
Date Signature

_____
Printed Name and Title

Appendix 8: First Trimester Interview Questions About Alcohol Consumption

Interviewer: Some women drink alcoholic beverages while they are pregnant, while others do not.

C49. Have you <u>ever</u> had alcoholic beverages, like beer, wine, or liquor including gin, whiskey, rum, or mixed drinks?

Yes — No → *Skip to C63a.*

Don't know → *Skip to C63a.* — Refused → *Skip to C63a.*

C50. At this time, do you drink any alcoholic beverages, like beer, wine, or liquor including gin, whiskey, rum, or mixed drinks?

Yes — No → *Skip to C54.*

Don't know → *Skip to C54.* — Refused → *Skip to C54.*

C51. How often do you drink an alcoholic beverage, by that I mean at least one beer, one glass of wine, one mixed drink, or one shot of liquor? *[circle day, week or month]*

_____ # times a day / a week / a month

_____ < 1x month → *Skip to C55.*

Don't know

Refused

C52. On those occasions that you drink alcoholic beverages, how many drinks do you usually have?

_____ # per occasion

Don't know

Refused

C53. At this time, what type(s) of alcohol do you usually drink? To make it easier for you to respond, I'm going to read you a list of options and you can simply say "stop" or "OK" when you hear the answer you want to choose. Do you drink _____?

[Read list, mark all that apply. If she says "stop" at "other" and hesitates then interviewer should try to provide suggestions so that respondent does not put herself at risk.]

- ❑ Beer
- ❑ Wine
- ❑ Mixed drinks
- ❑ Shot of liquor
- ❑ Other alcohol _____
- ❑ Don't know
- ❑ Refused

→ → *After answering C53. skip to C55.*

C54. Did you stop drinking alcoholic beverages in the past 4 months or more than 4 months ago?

_____ Within past 4 months → *Skip to C56a.*

_____ More than 4 months ago → *Skip to C63a.*

_____ Don't know→ *Skip to C56a.*

_____ Refused → *Skip to C56a.*

C55. In the past 4 months, have you changed how often and/or how many alcoholic beverages you drink?

Yes No → *Skip to C60.*

Don't know → *Skip to C60.* Refused → *Skip to C60.*

C56a. When did this change occur? *[If she changed more than once, ask for date of most recent change.]*

Month: ____ Day: _____ *[If doesn't remember day ask C56b.]* Year: _____ →*C57*

- ❑ Don't know →*C57*
- ❑ Refused →*C57*

C56b. Do you remember what week in [month] that was, the first, second, third, fourth, or fifth?

___1st ____2nd ___ 3rd ____ 4th ___ 5th

- ❑ Don't know
- ❑ Refused

C57. Before this change, how often did you drink?

_____ # times a day / a week / a month

_____ < 1x month → *Skip to C60.*

None → *Skip to C60*.

Don't know

Refused

C58. Before this change, on those occasions when you drank alcoholic beverages, how many drinks did you usually have on each occasion?

_____ # per occasion

Don't know

Refused

C59. What type(s) of alcohol did you usually drink? Did you drink _____? *[Read list, mark all that apply]*

- ❑ Beer
- ❑ Wine
- ❑ Mixed drinks
- ❑ Shot of liquor
- ❑ Other alcohol _____
- ❑ Don't know
- ❑ Refused

C60. In the past 4 months, have you had more than 4 drinks on any one occasion? *[at any one given time]*

Yes No → *Skip to C63a*.

Don't know → *Skip to C63a*. Refused → *Skip to C63a*.

C61. How many times in the past 4 months have you had more than 4 drinks on any occasion?

____ # times

Don't know

Refused

C62. On those occasions when you had more than 4 drinks, what type(s) of alcohol did you usually drink? Did you drink _____? *[Read list, mark all that apply]*

- ❑ Beer
- ❑ Wine
- ❑ Mixed drinks
- ❑ Shot of liquor
- ❑ Other alcohol _____
- ❑ Don't know
- ❑ Refused

Appendix 9: *Right from the Start* DNA Repository Informed Consent

Vanderbilt University Institutional Review Board
Informed Consent Document for Research

Principal Investigator: Digna R Velez Edwards and Katherine Hartmann **Revision Date: 11-17-2010**
Study Title: Right From The Start Pregnancy Cohort DNA Repository
Institution/Hospital: Vanderbilt University

This informed consent applies to healthy female volunteers.

Name of participant: _____ Age: _____

The following is given to you to tell you about this research study. Please read this form with care and ask any questions you may have about this study. Your questions will be answered. Also, you will be given a copy of this consent form.

You do not have to be in this research study. You may choose not to be in this study and get other treatments without changing your healthcare, services or other rights. You can stop being in this study at any time. If we learn something new that may affect the risks or benefits of this study, you will be told so that you can decide whether or not you still want to be in this study.

1. **What is the purpose of this study?**

   This is a Vanderbilt University Medical Center research study conducted by Dr. Digna R. Velez Edwards and Dr. Katherine Hartmann. You have been asked to participate in this study because of your previous participation in Right from the Start. The purpose of this study is to look at genes (DNA) and how they contribute to pregnancy health, pregnancy outcomes, and long-term development. Genes are the instruction manual for your body. The genes you get from your parents decide what you look like and how your body behaves. They can also tell us a person's risk for certain diseases and how they will respond to treatment. The information collected may be combined with other research to answer questions such as: "What genetic indicators predict risk for pregnancy outcomes?" Our motivation is to build knowledge that may help prevent miscarriage, spontaneous preterm birth, low birth weight, and other complications that afflict women and/or infants during pregnancy.

2. **What will happen and how long will you be in the study?**

   You are being asked to give a saliva sample for genetic research. You will provide the sample using the Oragene ® (DNA Genotek, Inc., Ontario, Canada) DNA kit. A kit and instructions have been sent to you in the mail. Your saliva sample will be collected by spitting directly into the plastic container provided. It is important to follow the directions provided with the collection kit. You will return your sample, and this signed consent, to Vanderbilt in the postage-paid envelope provided. The Vanderbilt Center for Human Genetics DNA Resources Core will store the saliva sample and extract genetic material. What we learn about you from this sample will not be put in your health record. No one else (like a relative, boss, or insurance company) will be given results from tests run using your DNA. Your sample will only be used for research at Vanderbilt University and will not be sold. Health insurance companies and group health plans may not request your genetic information that comes from this research. Your de-identified samples and data may be shared with others to use for research. Your name and personal information are not attached to the samples and data that you provide. Your sample will be used to make DNA that will be kept for an unknown length of time for future research. The sample will be destroyed when it is not longer needed. It should take around 10-15 minutes to provide the sample and seal with the consent document in the postage paid envelope included.

3. **Costs to you if you take part in this study:**

   It will not cost you any money to be in this study.

4. **Possible discomforts, inconveniences and / or risks you can expect if you take part in this study:**

   There are no known risks or lasting side effects to providing a saliva sample.
   You may experience the following mild discomforts:
   - Uneasiness about providing the saliva sample
   - Temporary dry mouth

5. Risks that are not known:

One risk of giving samples for this research may be the release of your name that could link you to the stored samples and/or the results of the tests run on your samples. This may be a concern due to problems with insurance or getting a job. To prevent this, these samples will be given a code. Only the study staff will know the code. The name that belongs to the code will be kept in a locked file or in a computer with a password. Only Drs. Velez Edwards and Hartmann will have access to your name. There may be risks that we do not know about at this time.

6. Good effects that might result from this study:

You will not receive benefits as a result of the tests done on your samples. These tests may help us learn more about the causes, risks, treatments, or how to prevent this and other health problems. The benefits to science and humankind that might result from this study are: Participants will contribute to medical knowledge about early pregnancy health with hopes that in the future we may be able to help women and their care providers prevent some miscarriages or other pregnancy problems.

7. Other treatments you could get if you decide not to be in this study:

You do not have to be in this research study. You may choose not to be in this study. This study **does not include any medical care** or require any changes in your regular care. Whether or not you participate does not in any way influence your healthcare, services, or other rights.

8. Payments for your time spent taking part in this study or expenses:

There will be no costs to you for any of the tests done on your samples. After we receive your saliva sample, you will receive a $20 gift card to Target or Wal-Mart. The gift card will be mailed within 2 weeks of your return of the saliva sample. You must provide your social security number in order to receive monetary compensation. If you do not provide your social security number, we will mail you a small gift, such as a coffee mug or water bottle. After we receive notice that you have delivered, we will send a kit for the baby's saliva sample. If you choose to allow your child to participate, and send in your child's saliva sample, you will receive an additional $10 gift card. You may choose not to provide your child's saliva sample.

9. Reasons why the study doctor may take you out of this study:

You may be removed from this study if you do not return your saliva sample within a reasonable amount of time.

10. What will happen if you decide to stop being in this study?

We will not be able to destroy research data that has already been gathered using your sample. If you decide not to provide a saliva sample at this time, please return the unused DNA kit in the postage paid envelope provided. If you change your mind about being in this study after you have provided your saliva sample, you may ask to have your sample destroyed. At any time, you may ask to have your sample destroyed at any time without penalty. To request that your sample be destroyed and withdraw your permission for your sample to be used, please send a written request to Drs. Digna R Velez Edwards and Katherine Hartmann at the address below:

Vanderbilt Institute for Medicine and Public Health
Sixth Floor, Suite 600
2525 West End

Nashville, TN 37203-1738.

12. Who to call for any questions or in case you are injured:

We want you to ask all your questions about this study. If you have questions you can call for free at 1-866-346-2684. You can also call Sarah Jones, collect, at (615) 936-3976. You can write her at the Institute for Medicine and Public Health. The address is Vanderbilt Institute for Medicine and Public Health, Sixth Floor, Suite 600, 2525 West End, Nashville, TN 37203-1738.

For additional information about giving consent or your rights as a person in this study, please feel free to call the Vanderbilt University Institutional Review Board Office at (615) 322-2918 or toll free at (866) 224-8273.

13. Confidentiality:

All efforts, within reason, will be made to keep your research data private. What we learn about you from this sample will not be put in your health record. Your sample will only be used for research at Vanderbilt University and will not be sold. Health insurance companies and group health plans may not request your genetic information that comes from this research.

Your saliva sample and genetic data will be linked to a study ID number. Access to your data will be monitored by Drs. Velez Edwards and Hartmann and will only be granted to approved researchers. Data used by the research team does not include information that can be traced back to you like name or address. Such contact information is only available to study staff to help with study activities. Data from many participants will be combined to study how certain factors relate to pregnancy health and outcomes. Unless you request it, the permission to use your sample/data does not have an expiration date.

We do not plan to share any study information with official groups; however there is a risk that study data can be requested by groups that include the Federal Government Office for Human Research Protections, and the Vanderbilt University Institutional Review Board. Federal privacy rules may not apply to these groups; they have their own rules and codes to assure that all efforts, within reason, will be made to keep your data private. The sponsor, Vanderbilt, Drs. Velez Edwards and Hartmann and their staff will keep your data in strict confidence, and will comply with any and all laws regarding the privacy of such information.

Please check Yes or No to the questions below:

My de-identified saliva sample may be used for gene research.

☐ Yes ☐ No

My de-identified saliva sample may be stored/shared for future gene research.

☐ Yes ☐ No

My de-identified saliva sample may be stored/shared for future gene research for other health problems (such as preterm birth, miscarriage, etc).

☐ Yes ☐ No

Principal Investigator: Digna R Velez Edwards and Katherine Hartmann **Revision Date: 11-17-2010**
Study Title: Right From The Start Pregnancy Cohort DNA Repository
Institution/Hospital: Vanderbilt University

If you decide not to take part in this research study, it will not affect your treatment, payment or enrollment in any health plans or affect your ability to get benefits. You will keep a copy of this form after it is signed.

Please check one box below:

☐ **I would like to be contacted about future studies.**
☐ **I would not like to be contacted about future studies.**

STATEMENT BY PERSON AGREEING TO BE IN THIS STUDY

I have read this consent form and the research study has been explained to me verbally. All my questions have been answered, and I freely and voluntarily choose to take part in this study.

Date	Signature of patient/volunteer

Consent obtained by:

Date	Signature
	Printed Name and Title