TIMING OF GESTATIONAL ARREST PRIOR TO MISCARRIAGE

By

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To my family,
Sankar, Swapna and Sucharita Mukherjee,
And to my grandfather,
Dr. Kali Sankar Banerjee,
Who have each given me love, support and unwavering faith to pursue my dreams

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LIST OF ABBREVIATIONS

aHR	Adjusted hazard ratio
BMI	Body mass index
BPD	Biparietal diameter
BPM	Beats per minute for fetal heart rate
CRL	Crown rump length
CI	Confidence interval
DAG	Directed acyclic graph
EGA	Estimated gestational age based on self-reported last menstrual period
FHR	Fetal heart rate
FTI	First-trimester interview
GAAD	Gestational age at arrest of development
hCG	Human chorionic gonadotropin
HR	Hazard ratio
IVF	*In vitro* fertilization
LMP	Last menstrual period
MSD	Mean sac diameter
NC	North Carolina

NFGS	National family growth survey
OR	Odds ratio
RFTS	Right from the Start
RR	Risk ratio
SAB	Spontaneous abortion (i.e. miscarriage)
SD	Standard deviation
TN	Tennessee
TAUS	Transabdominal ultrasound
TVUS	Transvaginal ultrasound
TX	Texas

ABSTRACT

Risk of miscarriage (i.e. a pregnancy loss before 20 completed weeks of gestation) is known to differ by race but timing of loss is not well established in the literature. The gap between biological pregnancy loss identified by ultrasound and clinical manifestation of that loss may bias effect estimates for early-pregnancy exposures associated with miscarriage. *Right from the Start* (RFTS) is a unique and diverse prospective pregnancy cohort that captures uniform early first-trimester ultrasound information and pregnancy-related behaviors from first-trimester interviews in order to study the distribution of this gap.

Nearly 13% of women in this cohort experienced a pregnancy loss (n=697), the majority of whom have ultrasound data available (73%, n=509). Ultrasounds were conducted between 40 and 95 days gestation from last menstrual period (LMP) for this cohort. Gestational arrest prior to miscarriage was observed in 38.7% of losses (n=197). The mean gap between LMP and estimated gestational age at arrested development (GAAD) was 19.3 ± 15.0 days (median GAAD gap was 19 days). The GAAD gap did not differ by race or pregnancy intention.

In order to determine if failing to account for this gap influences effect estimates we assessed exposures commonly associated with pregnancy loss. We compared models that estimated gestational age based on self-reported LMP and models that incorporated gestational age at time of arrested development (GAAD). We used bootstrap methods to determine the magnitude of bias for both models. Smoking during pregnancy was not modified by race and was not associated with miscarriage risk within this cohort for either current or former smokers compared to never smokers in either model. Stratified by race and adjusted for confounding, the protective effect of vitamin use on miscarriage risk was stronger among White women than

Black women when using the LMP models (Whites aHR=0.34, 95% CI [0.21, 0.54]; Blacks aHR=0.53, 95% CI [0.33, 0.84]), while no substantial difference by race was observed with the GAAD models (Whites aHR=0.43, 95% CI [0.24, 0.76]; Blacks aHR=0.44, 95% CI [0.26, 0.74]).

Models that use self-reported LMP to estimate gestational age underestimate the true value of first-trimester smoking exposure on miscarriage risk by as much as 15% for current smokers and 5% of former smokers when compared to models that use GAAD (the bootstrap bias ratio between models for current smokers ratio=0.85, 95% CI [0.75, 0.94]; for former smokers ratio=0.95, 95% CI [0.92, 0.97]). When stratified by race, the bias was nearly 20% for both Whites and Blacks for miscarriage risk associated with early pregnancy vitamin exposure (Whites bias ratio= 0.79, 95% CI [0.62, 0.87]; Blacks bias ratio=1.19, 95% CI [1.13, 1.45]). These results suggest that early-pregnancy exposures associated with miscarriage risk are influenced by proper classification of gestational arrest prior to loss, and that the magnitude and direction of bias differs by race. By more accurately identifying which insults have occurred prior to pregnancy arrest and differentiating them from exposures that occur after developmental arrest but before the onset of bleeding, we have a more optimal method to assess miscarriage risk by not mis-assigning exposure time.

CHAPTER I

INTRODUCTION AND SPECIFIC AIMS

Between 10-15% of all clinically recognized pregnancies end in miscarriage (gestation < 20 completed weeks). However, more than a fifth of all conceptions may result in early pregnancy loss, between implantation and the anticipated time of menses when detecting losses by human chorionic gonadotropin (hCG) levels from daily urine samples.[1] Pregnancy loss can be determined by laboratory tests, symptoms such as bleeding or cramping, and ultrasound confirmation. Traditionally, pregnancy and the timing of loss is dated from the first day of a woman's last reported menstrual period (LMP), and is often referred to as gestational age.

However, embryologic development may stop days to weeks prior to the onset of clinical recognition of miscarriage. Basing timing of miscarriage on the time from LMP to the clinical recognition of loss alone ignores the developmental state of the embryo prior to the loss. This is potentially problematic if a pregnancy had, as is common, arrested earlier. For example a pregnancy loss based on self-reported LMP dates at 11 weeks may be a loss that is 11 weeks along in gestational development when assessed immediately prior to the loss by ultrasound. It is also possible that a loss at 11 weeks may in fact be a pregnancy that arrested at five weeks based on early embryonic development verified on ultrasound. Alternatively, it is also possible that a self-reported 11 week loss may be a loss that is only nine weeks along in gestation when confirmed by ultrasound in part due to inaccurate dating of LMP. In reproductive epidemiology, and particularly studies of miscarriage, early exposures during pregnancy are treated the same in terms of exposure time (i.e. 11 weeks), regardless of which of the scenarios described above truly occurred. Misattributing exposure time may result in overestimation of

certain risk factors and lead to biased estimation of the effect of exposures associated with miscarriage.

Further, we know that timing of loss differs between Blacks and Whites. Blacks are more likely to have a clinical loss later in gestation compared to Whites. Black women are overall more likely to experience pregnancy loss compared to White women (adjusted hazard ratio (aHR) 1.57, 95% confidence interval (CI) 1.27, 1.93), with a nearly two-fold greater risk of clinically recognized loss during gestational weeks 10-20 (aHR 1.93, 95% CI 1.48, 2.51).[2] Currently, little is known regarding the relationship between the actual timing of arrest in fetal development and the clinical recognition of miscarriage and whether this timing may explain differences observed in pregnancy loss by race. Furthermore, key embryologic markers of growth may differ by race during early pregnancy and may influence timing and recognition of a clinical loss.

A better understanding of embryologic and fetal development in relation to miscarriage timing, including differences by race, are important in epidemiologic studies when studying factors in early-pregnancy that may cause or prevent pregnancy loss. My objective is to gain further insight into the patterns of timing of loss and determine if mean differences between ultrasound developmental stage at arrest and clinical onset of symptoms for miscarriage exists between Blacks and Whites during early fetal development. Such research has potential to advance overall knowledge about causes of pregnancy loss and help to identify risks that may be preventable.

Right from the start (RFTS) is a unique and diverse prospective cohort of women recruited in early pregnancy. Beginning in 2000, RFTS enrolled women who were either pregnant or were trying to become pregnant using community-based recruitment from nine

metropolitan sites in three states. [3] RFTS captures uniform early first-trimester transvaginal ultrasound (TVUS) data, a baseline interview at enrollment, a detailed first-trimester interview (FTI), including reproductive and medical history and pregnancy-related behaviors on all study participants. TVUS information is available for nearly three-quarters (74%) of women who experienced a miscarriage in RFTS. For remaining women who reported a pregnancy loss, miscarriage occurred prior to ultrasound (26%). RFTS is well suited to address timing of miscarriage risk.

Using data from RFTS, I aim to accomplish the following objectives:

1. To determine the variation and distribution in the number of days between ultrasound estimated developmental stage at arrest and clinical onset of symptoms of miscarriage among women who experience a pregnancy loss

Miscarriages are classified based on developmental stage at loss. Developmental stage at loss (also referred to as fetal demise or embryologic loss) is characterized initially in the following way: women who are considered to have normal fetal development (i.e. fetal pole with normal heart rate) and women who are considered to have abnormal or arrested development (i.e. either fetal pole with abnormal or no heart rate, or anembryonic gestation) at time of transvaginal ultrasound. I estimate gestational age at arrest based on developmental stage on ultrasound. For each woman with loss I assign developmental stage at arrest, estimated in days gestation based on a pre-specified nomogram calculated from ultrasound measures. This is referred to as the gestational age at arrest of development (GAAD). I then determine the mean difference in days between estimated gestational age at clinical loss based on LMP and estimated GAAD based on ultrasound for all women who had a loss. This difference will be

referred to as the GAAD gap. I report and describe the distribution of the GAAD gap within this cohort. Given that the known prevalence of anembryonic arrest is 40% in RFTS,[2] I hypothesize that the GAAD gap will be greater than 10 days for women with a pregnancy loss in this cohort. Furthermore I investigate if predictors of long than median vs. shorter than median GAAD gap differ within our cohort.

2. To investigate if GAAD influences estimates of common putative factors (e.g. smoking and prenatal vitamin use) associated with miscarriage

Because embryologic development may stop days or weeks prior to the onset of clinical symptoms or diagnostic recognition of miscarriage, I hypothesize that the proper classification of gestational age at arrest will result in attenuated risk estimates of miscarriage associated with either smoking or vitamin use when compared to models that use estimated gestational age based on LMP alone. These exposures were chosen to compare the two models since they have been established in the literature as factors that may either cause (e.g. smoking) or prevent (e.g. vitamin use) miscarriage and RFTS captures in detail both these early pregnancy exposures. I use Cox proportional hazard models to test the association between smoking and vitamin use with miscarriage risk controlling for potential confounders. Because risk of miscarriage declines with increasing gestational age, women who enter the study later will have less opportunity for miscarriage to be observed, I left truncate the gestational age at study enrollment for each woman. I compare hazard ratios from models that use GAAD with models that use traditional self-reported LMP for gestational age. I determine the extent of overestimation and potential bias in our prior reported estimates for both models using bootstrap methods.

3. To determine if the GAAD gap differs by race and if risk of loss associated with common early pregnancy exposures are modified by race

Blacks and Whites have different gestational ages at clinical loss, with Black women having greater risk between weeks 10 and 20 in gestation.[2] In order to determine if the observed later losses in Blacks are due to difference in developmental stage at loss, I compare the GAAD gap between Blacks and Whites. I hypothesize that the GAAD gap will be greater in Blacks compared to Whites. Furthermore, I use Cox proportional hazard models to determine if risk of loss associated with common early pregnancy exposures are modified by race. I compare models that use GAAD with models that use traditional self-reported LMP to estimate gestational age. If effect modification by race is found, I report stratified estimates in both models and determine the extent of potential bias in our prior reported estimates and in the literature.

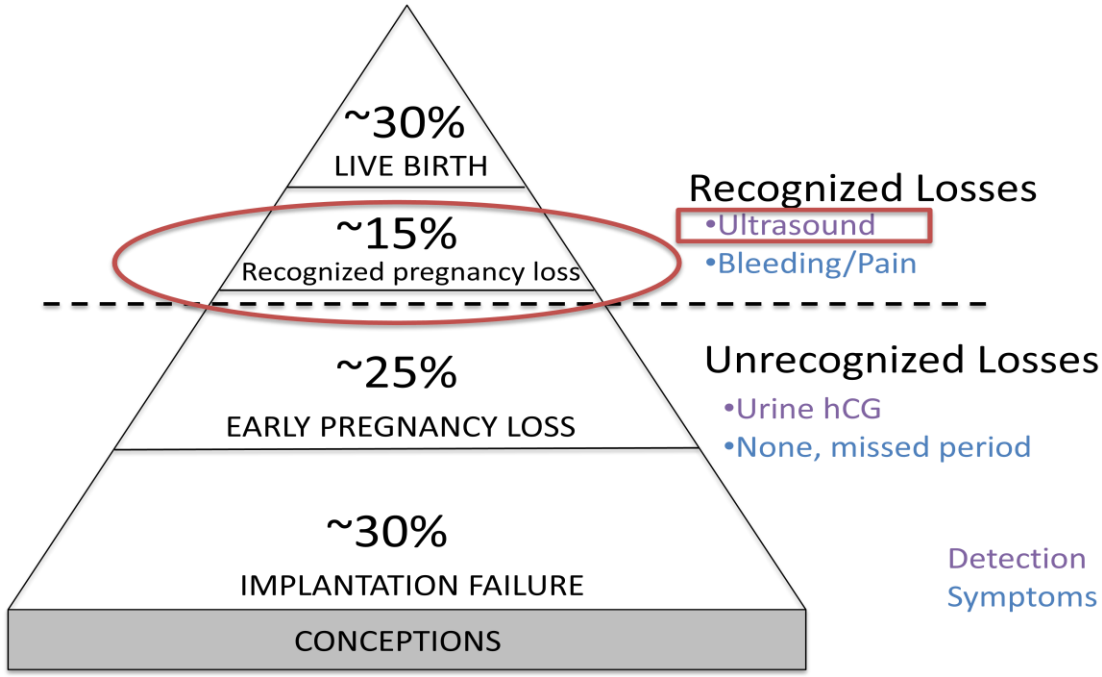
Finally, as a secondary analysis I plan to assess the impact of pregnancy intention on the GAAD gap and its interaction with race within this cohort. Pregnancy intention varies by race[4-6] in the U.S. general population and women with unintended pregnancies may be less sure of their LMP dates and may have greater variability in their GAAD estimates based on developmental stage at ultrasound when compared to women who are planning a pregnancy. I hypothesize that Black women will have more unintended pregnancies than White women and that GAAD gap will be greater for unintended pregnancies than intended pregnancies among these women.

CHAPTER II

BACKGROUND

Miscarriage is a complex biological process. Approximately 10 to 15% of recognized pregnancies end in miscarriage (also sometimes referred to as spontaneous abortion) and defined as a pregnancy loss before completion of 20 weeks of gestation.[1,7,8] Up to 70% of all conceptions are lost prior to live birth. [9] As many as 25% of all conceptions may end in early pregnancy loss when taking into account unrecognized losses (**Figure 2-1**).[1] The majority of these losses occur prior to the time of missed menstrual period and are usually unrecognized losses. Pregnancy loss can be detected by both biomarkers (urine hCG) and ultrasound characteristics.

Figure 2-1. Explaining pregnancy loss (modified from Macklon et al., 2002)



Challenges to study miscarriage

Epidemiologic studies of the causes of miscarriage are challenging, because of the difficulty in identifying large numbers of women before or very early in pregnancy. Delayed maternal recognition of pregnancy increases the incidence of undetected losses and decreases the incidence of clinically recognized loss. The identification of the onset of pregnancy is subject to uncertainty by the woman herself, and over half of women who conceive do not actively plan the pregnancy.[7] There is no consistent timing for enrollment in prenatal care in the United States, which poses a practical challenge for researchers to identify large numbers of women very early in pregnancy. Additionally, the clinical challenge to observe the course of early pregnancy is limited, with the timing of losses often not clear without special diagnostic efforts. The study of miscarriage requires careful assessment of gestational time at study entry because women who enter a study later will have less opportunity for a miscarriage to be observed. Recruiting women who are planning a pregnancy provides a larger window of time for enrollment without losing information about either early pregnancy exposure or losses.

During pregnancy, fetal development is traditionally dated from the first day of a woman's last reported menstrual period. This method of dating is often referred to in the literature as gestational age. Additionally, gestational age can be further verified in part based on key embryologic developmental markers during ultrasound examination. These markers can be used to determine fetal (or embryologic) stages of growth. Fetal growth is important both clinically and in epidemiologic studies relating to reproductive outcomes, such as miscarriage, preterm birth and low birth weight. Basing timing miscarriages solely the LMP ignores the developmental state of the fetus prior to the loss.[10] Gestational timing based on ultrasound is

traditionally not used for miscarriage studies since most pregnancy losses occur very early in gestation, and recruiting women prior to pregnancy may be difficult.

Embryologic markers and miscarriage risk

Researchers have conducted studies to determine if key embryologic markers (ex. abnormal gestational sac diameters or crown rump lengths) measured during early ultrasounds are indicative of early pregnancy loss. The biologic timing of "loss", that is the time at which a pregnancy that has arrested and is no longer developing, is not the same as the clinical recognition or experienced (i.e. symptomatic) timing of loss. Many pregnancies may arrest days or weeks prior to clinical recognition.

Abnormal development, including slower growth in the first-trimester may be indicative of a failed pregnancy.[10-15] Past research indicates that shorter than expected gestational sac diameters[14] or fetal pole lengths [13] may be associated with increased risk of pregnancy loss. Additionally, crown rump length (CRL) of these fetuses was smaller than expected based on gestational age. Some evidence suggests that fetuses that eventually miscarried had on average smaller CRL than those that did not.[13] Embryos that were relatively slow growing in the first-trimester were at a greater risk for pregnancy loss.[10] Other factors like missing fetal yolk sac and slow fetal heart rate have also been associated with risk of miscarriage.[15] One study found that 60% of fetuses with slow heart rate (< 90 beats per minute) at <7 weeks gestation ended in miscarriage (n=188 of 310 pregnancies).[12] Another study found that 94% of embryos with small gestational sac (defined as difference between mean gestational sac diameter and CRL < 5mm) resulted in pregnancy loss regardless of normal fetal heart rate (n=15 of 16 pregnancies).[11] Maternal factors are also known to influence first-trimester growth. A study by Bottomley *et al.*

found that the rate of increase in CRL was greater in fetuses of Black versus White women and increased with advancing maternal age.[16]

In 2011 Jeve *et al.* conducted a systematic review to assess the accuracy of first-trimester ultrasound for diagnosis of early embryonic demise. They reported sensitivity and specificity of key fetal markers including fetal heart rate, gestational sac and yolk sac among women who experience miscarriage.[17] Among eight identified articles for review, an empty gestational sac with mean diameter ≥ 25 mm and absent yolk sac with mean gestational sac diameter ≥ 20mm were the thresholds with the highest and most precise estimates of specificity for diagnosing early embryonic demise (specificity 1.0, 95% CI 0.93, 1.0 for both).[17] They conclude there is scarce high-quality prospective data on which to base guidelines for the accurate diagnosis of early pregnancy loss. However no detailed search criteria for the review were provided and rationale for why 97% of the studies (n=697 of 720 studies searched) did not meet eligibility criteria was lacking. Furthermore all studies that were included (n=8 studies) dated prior to 1992.

Changes in ultrasound technology since the early nineties have resulted in more precise imaging and more advanced assessment of fetal viability. The transvaginal ultrasound provides the most accurate information in early pregnancy, given that the gestational sac and fetal pole are still developing at this point and a vaginal ultrasound can get closer to the developing pregnancy and provide a more precise measurement and therefore an improved assessment of viability.[18,19] Finally, the review by Jeve *et al.,* failed to differentiate women who were symptomatic of miscarriage (i.e. bleeding, cramps) compared to women who were asymptomatic.[17] Women who are symptomatic of miscarriage may present a different etiology than women who are asymptomatic and undergoing a first-trimester ultrasound, since bleeding

is a predictor that strengthens the diagnostic test properties. This could be one explanation for the heterogeneity seen across studies.

Race and miscarriage risk

Maternal factors are known to influence first-trimester growth, including race.[16,17] Studies incorporating race into multivariable models of miscarriage risk have not specifically focused on evaluating the presence or magnitude of disparity. One study reported race as a confounder when assessing putative risk factors associated with miscarriage, suggesting that Blacks have twice the risk of miscarriage compared with other racial groups,[20] and others report no association between race and miscarriage risk.[21-23] A summary of results addressing race and miscarriage risk can be found in **Table 2-1**.

Table 2-1. Studies addressing race and miscarriage risk

Author	Study type Location	Study Period	Population	Definition of Loss	No. of Losses	Race	No. (%)	Effect estimate (95% CI)	Confounders in model
Risch et al., 1988[22]	Case-control, United States and Canada	1974-1981	Matched controls from a study on either cervical, ovarian or colorectal cancer registries	Not defined	805	Whites Non-Whites	Not provided	RR=1.0 RR=0.8 (0.52-1.23)	None
Goldhaber et al., 1991[21]	Prospective, California, United States	1981-1982	Single HMO covered plan	<28 weeks gestation	833	Whites Black Other	7,154 (79.0) 570 (6.3) 1,331 (14.7)	0.11[a] 0.10[a] 0.12[a]	NA
Zhang et al., 1996[20]	Prospective, Connecticut Unites States	1988-1991	11 private clinics and 2 HMO plans	Not defined	628	Whites Blacks and Others	2,568 (93.5) 178 (6.5)	1.0 RR=2.57 (1.54-4.30)	Maternal age (dichotomous >35 years)
Wen et al., 2001[23]	Prospective, Minnesota, United States	1989-1992	Single HMO covered plan	Not defined	75	Whites Other	624 (96.3) 24 (3.7)	RR=1.0 RR=1.1 (0.40-3.20)	None
Mukherjee et al. 2013[2]	Prospective Southeastern Unites States	2000-2012	Clinic and community based recruitment	<20 weeks gestation	537	Whites Blacks	3,138 (77.1) 932 (22.9)	1.0 HR=1.57 (1.27-1.93)	Maternal age (continuous), alcohol use

No=number; CI= confidence interval; HR=hazard ratio; RR=relative risk; NA=not applicable [a]Estimated incidence within cohort RR and 95% CI not available.

While there is limited research on racial disparities in risk of miscarriage, other adverse pregnancy outcomes, including spontaneous preterm birth and fetal growth restriction have been shown to differ significantly by race and may have their origins in event as early as placentation. Studies have shown that the overrepresentation of preterm births in non-Hispanic Black women is observed independently of conventional maternal medical and socioeconomic factors captured in epidemiologic research and that complex causal pathways may link the social construct of race to the biological outcome of preterm birth.[24-26] Furthermore, the study of miscarriage requires careful assessment of gestational time at study entry because women who enter a study later will have less opportunity for a miscarriage to be observed than women who enter very early in pregnancy. A recent review found only four prior studies that collected adequate data to estimate miscarriage risk by week of gestation from early in pregnancy, and none evaluated these differences by race.[27]

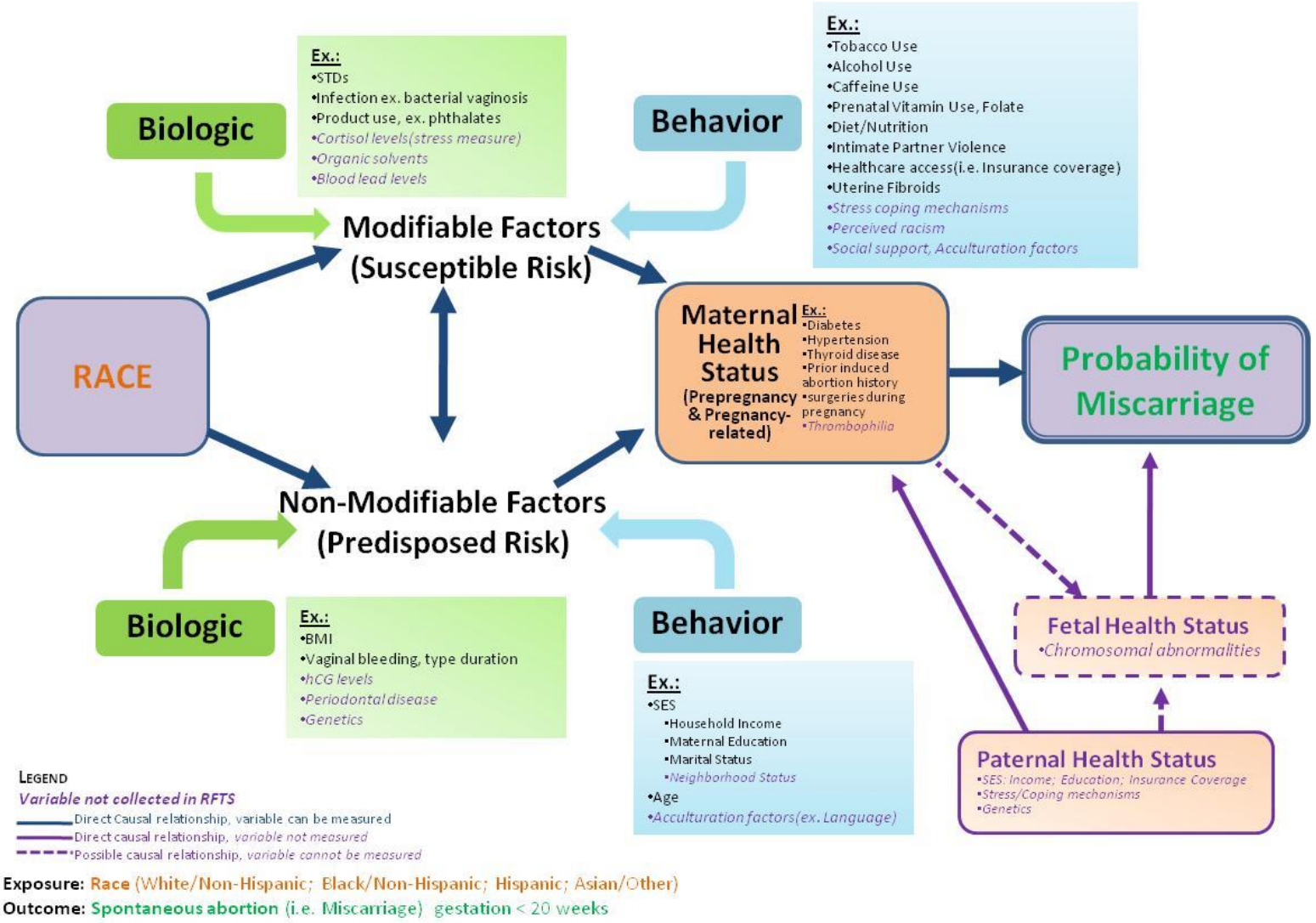
Our study within the RFTS cohort found that the overall risk of miscarriage remains elevated for Blacks compared to Whites (aHR= 1.57, 95% CI 1.27, 1.93). Our primary finding indicated Black women have a nearly two-fold higher risk of miscarriage compared with white women during gestational weeks 10–20 (loss ≥ 10 weeks, aHR= 1.93, 95% CI 1.48, 2.51), while there was no apparent difference in the risk of earlier miscarriage (loss < 10 weeks aHR=1.15, 95% CI 0.82, 1.62).[2] There is a higher risk of loss early in pregnancy that declines with increasing gestational age. The finding of increased risk for Blacks during weeks 10–20 is less vulnerable to bias because most women have recognized their pregnancy by the tenth week of gestation. We used ultrasound examination data to evaluate fetal viability among study participants and to assess developmental stage prior to pregnancy loss. Ultrasound information was available for the majority of women who experienced a loss in our cohort, and we observed

similar patterns of early embryological arrest in Blacks and Whites. This also suggests that the increased risk of later loss among Black women may reflect events during fetal development after initial organogenesis is complete (rather than early embryologic insults).

Heterogeneity in miscarriage risk

One approach to coping with etiologic heterogeneity in miscarriage is to consider miscarriages of different gestational ages as different outcomes.[28] Silver and colleagues suggest that early pregnancy losses as losses prior to ten weeks gestation, and fetal deaths as losses occurring between 10 and 20 weeks gestation. The suggested nomenclature is based on developmental periods in gestation which may share similar pathophysiology to other adverse birth outcomes including preterm birth and stillbirth along the pregnancy spectrum. Since many of these pregnancy outcomes are known to differ by race, by addressing the potential heterogeneity in outcomes such as miscarriage, researchers may be better equipped to identify putative risk factors and frame plausible biological causal pathways that may bar by race and influence early fetal well-being.[2] A causal framework assessing both biologic and behavioral factors for race and risk of miscarriage can be found below (**Figure 2-2**). Factors include both modifiable and non-modifiable risk factors. The factors assessed in this causal framework can be used to establish measureable associations between independent variables, race and the risk of miscarriage. Isolating specific variables within study framework and investigating these causal relationships through statistical analysis can give further insight into risk factors associated with miscarriage and the relationships between these factors. The greater the heterogeneity in risk, the more likely it is that we can discover causal factors associated with that risk by separating etiologic distinct outcomes that may otherwise appear similar.

Figure 2-2. Causal model for race and miscarriage risk



Classifying types of miscarriage

The existing classification of pregnancy loss ignores both developmental biology and clinical manifestation. This is problematic for grouping women who may have different pathophysiology and thus different recurrence risk as the same condition. This further limits research and confounds epidemiologic data collection and assessment for reproductive outcomes. A potentially more useful way to catalog pregnancy loss may be by developmental periods in gestation.[28] Early pregnancy losses are losses that occur before ten completed weeks of gestation. They can be further classified as peri-implatational losses, which are losses before five weeks gestation with no gestational sac visible on ultrasound; pre-embryonic losses occurring between five and six weeks gestation, in which gestational sac, yolk sac or both may be visible on ultrasound but no visible embryo and; embryonic losses occurring between six and ten weeks gestation in which an embryo is visible with CRL < 10mm with no fetal heart tones (**Table 2-2**). Fetal deaths can be classified as losses occurring between 10 and 20 weeks gestation with a CRL measuring at least 30 mm. Recurrent miscarriage is defined as three or more pregnancies that end in loss, and occurs in about 1% of women who miscarry. A summary of common reproductive and ultrasound terminology can be found in the Appendix.

Table 2-2. Defining pregnancy loss by gestational age and ultrasound (modified from Silver et al.)

Type of Loss	Definition	Gestational weeks	Ultrasound characteristics
Early Pregnancy Loss	Loss prior to 10 weeks gestation	< 10	
Peri-implatational loss	Loss before 5 weeks gestation	< 5	No gestational sac
Pre-embryonic loss	Loss between 5 and 6 weeks gestation	5 to <6	Gestational sac or yolk sac or both, no visible embryo
Embryonic loss	Loss between 6 and 10 weeks gestation	6 to <10	Embryo with CRL<10mm and no FHR
Fetal Death	Loss between 10 and 20 weeks gestation	10 to <20	Either 1. Passage of a conceptus with CRL ≥30mm 2. dead conceptus in utero with CRL ≥30mm or 3. loss of a conceptus after documented FHR at or beyond 10 weeks of gestation
Early fetal death	Loss between 10 and 16 weeks gestation	10 to <16	
Late fetal death	Loss between 16 and 20 weeks gestation	16 to <20	
Recurrent miscarriage	Three or more pregnancies that end in loss	< 20 three or more times	NA

CRL=crown-rump length; FHR=fetal heart rate; NA=not applicable

Importance of estimating gestational age

Accurate knowledge of gestational age is arguably the most important piece of information for pregnancy management. The estimation of pregnancy dates is important, not only for the expectant mother but also when considering therapy and interpreting diagnostic tests. Gestational age (GA) can be determined using the first day of the mother's last period and can also be referred to as the menstrual age. Comparatively, the true fetal age is the conceptional age (CA), which refers to the pregnancy length from the time of conception. A study by Savitz *et*

al. found that using LMP alone is subject to systematic tendency to overestimate the duration of gestation when compared to ultrasound based gestational age, by assigning a gestation 2.8 days longer on average than with ultrasound scanning and predict delivery among term birth less accurately.[29] Having an accurate GA is crucial for different clinical aspects, such as the timing of chorionic villous sampling in the 1st trimester, genetic amniocentesis in the 2nd trimester, dating of the fetus, and determination of fetal size.[30] Precise GA may also be important when evaluating a potential miscarriage, since timing of miscarriage occurrence may result in differences in clinical management decisions, such as expectant care, medical or surgical interventions.

Clinical approaches to estimate gestational age

The traditional ways to estimate GA include: known coital history, accurate menstrual history with a known LMP date, thorough clinical examination, ultrasonography and serial beta-hCG levels. Dating by ultrasound is more accurate than by assessing menstrual dates alone. Using LMP alone to estimate GA assumes that conception occurs on day 14 of a 28 day menstrual cycle, when in fact ovulation varies greatly both from cycle to cycle and between individuals.[31] Many women are uncertain of their exact cycle dates[32], and even if the menstrual history is known to be correct, individual variations for the time of ovulation can alter the length of the cycle.[18] Up to 30% of women may be uncertain of their LMP dates or have irregular cycles.[31,33] And even among women who are certain of their LMP dates, the discrepancy between menstrual age and ultrasound GA can be as high as 45% among pregnancies that are induced post-term (>294 days), probably due to the variability in length of the follicular phase the menstrual cycle.[34] Furthermore, a number of clinical signs, such as uterine size and quickening (i.e. perceived fetal movement by the patient) may be helpful but are unreliable in solely determining GA.[35] Therefore there has been a strong move towards scanning all obstetric

patients as a way to verify GA. **Table 2-3** is a summary of the early studies used to estimate gestational age using ultrasongraphy technology. These seminal studies provide the framework used to estimate gestational age.

Table 2-3. Summary of previous studies estimating gestational age

Author	Year	Location	No. of Subjects	GA at Scan (days)	IVF	Ultrasound Type	CRL?	Other[a]	Equation to estimate GA
Robinson[36]	1975	Scotland	334	44-98	No	TA	Yes		$t=8.052(CRL)^{1/2}+23.73$ $t=((0.374+((0.374)^2+4x0.012(CRL)^{1/2}))/(2 X 0.012)$
Drumm[37]	1976	Ireland	253	47-101	No	TA	Yes		$t=15.583+(242.84+((83.333x CRL))^{1/2})$[b]
Nelson[38]	1981	North Carolina, USA	83	Not provided[c]	No	TA	Yes		$t=51.0008+0.6(CRL)$
Pedersen[39]	1982	Denmark	105	49-98	No	TA	Yes		$t=6.40-0.266(CRL)+0.0116(CRL)^2$
Hadlock [40]	1984	Texas, USA	361	84-294	No	TA		Yes	$t=10.85+0.060(HC)(FL)+0.6700(BPD)+0.1680(AC)$
MacGregor[41]	1987	Chicago, USA	72	49-93	Yes	TA	Yes		$t=45.96+8.49(CRL)-0.2223(CRL)^2$
Vollebergh[42]	1989	Netherlands	47	42-91	Yes	TA	Yes		$t=7.23(CRL)^{1/2}+31.7$
Tezuka[43]	1991	Japan	143	35-56	No	TV		Yes	$t=(54.64+FHR)/3.850$
Klustermann[44]	1992	Italy	183	42-108	No	TV	Yes		$t=-3.98-0.308(CRL)+0.0117(CRL)^2$

Author	Year	Location	No. of Subjects	GA at Scan (days)	IVF	Ultrasound Type	CRL?	Other[a]	Equation to estimate GA
Hadlock[45]	1992	Texas, USA	416	35-138	No	TV, TA	Yes		$t=\exp[1.685+0.316(CRL)-0.049(CRL)^2+0.004(CRL)^3-0.0001(CRL)^4] \times 7$
Daya[46]	1993	Canada	94	43-99	Yes	TV, TA	Yes		$t=40.447+1.125(CRL)+0.0058(CRL)^2$
Goldstein[47]	1994	New York, USA	143	44-67	No	TV		Yes	$t=L+42$
Wisser[48]	1994	Germany	160	35-98	Yes	TV		Yes	$t=35.72+1.082(L)^{1/2}+1.472L-0.09749L^{3/2}$
Joshi[30]	2009	Nepal	123	49-98	No	TA	Yes		$t=8.26+0.08(CRL)$

IVF=in vitro fertilization; GA=gestational age where *t* refers to the estimated gestational age (in days); TA=transabdominal; TV=transvaginal; CRL=crown-rump length; L=longest embryonic length; [a]Other may include: HC=head circumference; AC=abdominal circumference; FL=femur length BPD=fetal biparietal diameter or FHR=fetal heart rate. [b]transformed formula based on Nelson[38] [c]scans were conducted 1 to 2 weeks after first visit.

Early studies estimating gestational age from crown-rump length

The first-trimester is the period during which the most accurate assessment of GA is possible. During this period, growth rate is most rapid and consistent and variation in size between fetuses of the same age is at its smallest. Crown-rump length (CRL), which is the length of a human embryo from the top of the head (crown) to the bottom of the buttocks (rump), often can be determined using ultrasonography images. The first report of ultrasound measurement of the fetal CRL was done by Robinson and Fleming, where they found that measuring the CRL, which can be done as early as the 7^{th} week, can be used as an accurate determination of GA.[36] Using fetal CRL measurements as a way to determine gestational age, Robinson and Fleming reported normal values for CRL growth from 6-14 weeks.[36] These values were derived from 334 measurements and a weight nonlinear analysis was performed. A "point estimate" of the GA was given by the equation:

$$t = 8.052 \times CRL^{1/2} + 27.73$$

where *t* refers to the time as gestational age in number of days. The accuracy of using CRL to determine GA is ± 4.7 days with only a single measurement and ± 2.7 days with three independent measures.[36] Based upon this, it is evident that the CRL determined sonography in the first-trimester provides an accurate estimation of GA for measures of early embryologic development during early pregnancy. In another study, Drumm found that for any given CRL, it was within a range of three days of the GA, as determined by menstruation history.[37] This was among a population of singleton births among women with known LMP dates, a menstrual cycle between 26 and 31 days, no uterine bleeding prior to ultrasound and no evidence of maternal conditions such as diabetes mellitus, renal disease or hypertension that may affect fetal growth.

The normal curves provided by Drumm *et al.* vary from Robinson *et al*. by no more than 2mm at any stage throughout the first-trimester.[37] It was found that the most precise estimate of fetal age was during a GA of 10-11 weeks, which was equivalent to CRL measurements of 31-40mm.[39]

Potential measurement errors in crown-rump length assessment

Many studies have shown that the CRL is the most consistent measurement for determining gestational age in early development.[36,37,45,46] Prior to 10 weeks, the small CRL dimensions are more susceptible to measurement and operator inaccuracies.[42] There are two types of errors: *random* and *systematic*. Random includes errors of operator judgment and the measurement process from the ultrasound. When the embryo is first visualized on ultrasound, the measurement is based upon the "greatest length" since it is fairly straight and there is no curvature to the image.[49] As the fetus continues to develop, it assumes a C-shaped structure, and further unfolding of the head and regression of the tail allows accurate measurement at about 18 mm.[49] Most clinicians do not allow for fetal flexion and instead just take the longest direct distance from the crown of the head to the fetal rump. Early measurement of CRL can be more difficult, since measurement error is proportionally greater in small rather than large CRL values, leading to underestimation of CRL, which subsequently leads to an underestimation of GA.[42]

In another study, CRL from menstrually-derived pregnancies underestimated the true GA when compared to ovulation-timed pregnancies.[41] The discrepancy in timing was 3.2-3.5 days, which corresponded to 5.0-5.7% underestimate in gestational age[41] when compared to studies by Robinson and Flemming[36] or Drumm *et al*.[37] These findings demonstrate that menstrual histories are not always accurate when determining GA, suggesting that among populations which use assisted reproductive technologies to conceive, ovulation dates should be used instead.[41] It is important to carefully examine CRL in patients who conceived through IVF, since issues of

recurrent pregnancy loss can be seen if early fetal growth retardation is observed prior to fetal death. Among populations using IVF, an alternate equation for CRL and GA has been developed in which the date of conception is known.[46]

Variations in crown-rump length measurements

A study focusing on the Nepalese population observed that CRL measurements were a reliable method for estimating GA, and corresponded to the Robinson nomogram up to 9 weeks GA. It was noted that there were slight differences between weeks 10-12, which might have been due to ethnic differences of fetal development.[30] There have also been some variations noted in CRL, where the measurement is smaller than expected. Examples of factors associated with smaller CRL values include female fetuses, a fetus in early diabetic pregnancy and fetuses in threatened abortions.[39] The difference in sizes between the sexes had a genetic rather than a hormonal mechanism. It is possible that if there exists a discrepancy between the CRL measurement and the menstrual age, it may be indicative of a threatened abortion or maternal diabetes.[39] This discrepancy between CRL and menstrual age may have some prognostic value in identifying at risk fetuses. Differences between nomograms used to estimate GA based on CRL can be noted because of differences in sample size, selected population, advancements in ultrasound technology, and ethnic population variation.[30]

Crown-rump length measurement in threatened miscarriages

In a study focusing on threatened abortions (n=255), it was found that the CRL of fetuses were smaller than expected based upon their menstrual age.[13] Of the 8 fetal losses in this series (7 abortions and 1 intrauterine death) all except for one had a CRL measurement below the mean for their calculated GA.[13] This phenomenon suggests that there was early fetal growth delay, which can be considered a marker of impending loss in threatened abortions. Additionally in this

study, Mantoni and Pederson found that in those who had been bleeding for more than three days, the increased discrepancy in size was a further two days. [13] However, it should be noted that second and third-trimester growth rates were all normal.

Alternative fetal measurements to estimate gestational age

Alternative fetal measurements have been proposed as measurement parameters for ultrasound assessment of GA.[38,40] After 11 weeks, the variation of fetal flexion makes accurate CRL measurement more difficult. Therefore the real limitation of CRL is that towards the end of the first-trimester, the fetus tends to assume a curled position, rendering the CRL measurement more unreliable. During late first-trimester and in the later stages of pregnancy, using other parameters such as biparietal diameters results in more accuracy. Biparietal diameter (BPD) is the measurement of the fetal head from one parietal eminence to the other. The optimum time to determine BPD is between 18-26 weeks and there is no significant difference whether the BPD is obtained using B-mode ultrasound equipment or real-time ultrasound.[38] However it is important to keep in mind that overall CRL is a more accurate method of predicting delivery date when compared to BPD in the first-trimester.[38]

Other alternative fetal measurement includes head circumference, abdominal circumference and femur length. The fetal trunk circumference is measured at a point just caudal to the cardiac pulsation. It is also important to note that the femur length is more difficult to visualize and thus to measure in the early weeks, with a practical cutoff at 12-13 weeks gestation. One study examined GA prediction using multiple fetal ultrasonographic measurements (biparietal diameter, head circumference, abdominal circumference and femur length) in a racially mixed population.[50] The use of multiple parameters to estimate fetal age proved to be more accurate than the use of any single measure during a later gestational window (84-294

days) but may not necessarily apply to early first-trimester.

Other common fetal measurements to estimate gestational age include fetal heart rate and mean sac diameter. During the first-trimester, fetal heart rates vary with gestational age (refer to **Table 2-4**). Estimated fetal heart rate before six weeks gestation is between 100-115 beats per minute (BPM). After which, FHR increases rapidly (ex. at eight weeks gestation: 144-159 BPM; nine weeks gestation 137-144 BPM).[51] A summary of estimated MSD and FHR by gestational week can be found in **Table 2-4**.

Table 2-4. Expected first-trimester ultrasound characteristics by gestational week

Gestational age [weeks]	Mean sac diameter (MSD)[a] [mm ± SD]	Mean Fetal heart rate (FHR)[b] [bpm ± 1 SD]
5-5.95	3.0 ± 0.8	101.2 ± 8.7
6-6.95	3.0 ± 0.7	124.5 ± 12.1
7-7.95	4.0 ± 0.9	128.0 ± 11.7
8-8.95	4.7 ± 0.6	144.3 ± 19.5
9-9.95	5.2 ± 0.6	138.7 ± 12.4
10-10.95	5.9 ± 0.6	136.9 ± 10.9
11-11.95	5.4 ± 0.9	139.8 ± 18.9
12-12.95	4.4 ± 0.6	137.3 ± 12.9

bpm=beats per minute; mm=millimeters; SD=standard deviation; modified from [a]Jauniaux *et al*. (1991)[52] and [b]Hertzberg *et al.* (1988)[51]

Changes in ultrasound technology to estimate gestational age

Transabdominal ultrasound scanning for early pregnancy was the accepted method until the mid-1980s. Transabdominal ultrasound rarely permits anatomic description of internal organs in the early embryo, and a full urinary bladder displaces the pelvic organs away from the probe.

Transabdominal ultrasound imaging can also be difficult in obese patients. The challenge within this patient population is that in pathologic pregnancies, a transabdominal ultrasound often is not enough. Transvaginal sonography (TVUS) can help fill in the gaps and provide relevant information not otherwise shown by a transabdominal ultrasound.

Transvaginal vs. transabdominal ultrasounds

Additionally transvaginal ultrasounds allow for a better understanding of embryonic anatomic stages.[47] When compared to Robinson's study, Goldstein and colleagues found that CRL measurement was approximately 2 to 3 mm shorter for equal values of GA when using endovaginal ultrasonography.[47] Robinson's study was published prior to the sonographic description of the yolk sac and his early embryonic measurements may have inadvertently included a portion of the yolk sac in its reporting. With use of transvaginal ultrasound the distinction between yolk sac and adjacent embryonic structures is more readily observable. Furthermore endovaginal ultrasounds allow for sufficient magnification to see anatomic detail including cardiac activity that may not be as readily visible in transabdominal ultrasounds. Goldstein and colleagues used information from endovagianl ultrasounds to establish a nomogram for GA by measuring an embryo prior to the development of a "crown" or "rump". [47] Other ways to use the transvaginal ultrasound examination would be to inspect the embryonic structure and measure the greatest embryonic length. Accordingly, the estimation of GA [48](t) according to measurement of embryonic length (L) was found to be:

$$t = 35.72 + 1.082L^{1/2} + 1.472L - 0.09749L^{3/2}$$

Another study employed the use of transvaginal sonography to establish reference ranges according to cephalic, abdominal and limb measurements.[44] The mean values derived in the

study was nearly identical to those previously obtained by Robinson and Fleming using the transabdominal static scanning.[44] With the rapid growth of a fetus during the first-trimester, fetal measurement during this period provides an accurate assessment of gestational age and development. Kustermann and colleagues highlight the importance of both systematic and instrumental errors that are inherent to the equipment that is used in measurement. Despite this, the rapid growth rate of the fetus allows errors of several millimeters to be of minor clinical significance.

Rationale for transvaginal ultrasounds in assessing miscarriage risk

There have been progressive changes in ultrasound technology though the introduction of transvaginal probes which are better able to assess gestational age in early first-trimester.[15] Furthermore, accurate embryologic and gestational dating to determine fetal viability prior to loss is essential if we want to better understand the causes of early pregnancy loss. There is scarcity of high-quality, prospective data on which to base guidelines for the accurate diagnosis of early pregnancy demise.[27] Studies of miscarriage risk are limited by early enrollment of pregnant women, the small number of miscarriage cases observed, inclusion of symptomatic together with asymptomatic women, and variable reference standards for confirming diagnosis of early pregnancy demise. An appropriately powered study using current ultrasound technology (i.e. a transvaginal approach) and an explicit reference standard for pregnancy success or loss may be required before setting future standards for the accurate diagnosis of early embryonic demise. A consensus about an appropriate methodological approach to assess gestational age prior to arrest should be reached before evaluating exposure to risk factors during early pregnancy.[15]

Exposures and miscarriage risk

Research that gives insight to the biologic mechanisms of exposures operating during early pregnancy is useful, especially since this time period in pregnancy is not well understood. Despite also the fact that miscarriage is a common adverse pregnancy outcome that frequently occurs during this time period. However, a primary challenge in reproductive epidemiologic research is the accurate and early exposure assessment during early pregnancy. By more precisely identifying which insults have occurred prior to pregnancy loss and assessing exposures that occur after developmental arrest but before the onset of clinical symptoms we have more optimal method to assess miscarriage risk by not misattributing exposure time.

Knowledge about risk factors influencing early pregnancy period is sparse and often times contradictory. This may be because the risk of early-pregnancy exposures on pregnancy loss remains unclear due to the challenges associated with prospectively recruiting women very early in pregnancy. It may also be due to the heterogeneity in classifying different types of loss which may have very different etiologies, given that 40% of losses arrest earlier when assessed by early first-trimester ultrasound.

In specific aim 2 I investigate if proper classification of gestational age at arrest influences estimates of common putative risk factors (ex. smoking and prenatal vitamin use) associated with miscarriage. I assess both self-reported smoking and vitamin use in first-trimester with risk of miscarriage in my analyses. These risk factors were chosen because they have been associated with first-trimester miscarriage risk in some studies and have been established to influence fetal well-being and may be episodic during early pregnancy. One is a protective factor (i.e. vitamin use) and one is a risk factor (i.e. smoking) associated with miscarriage. By assessing a better methodological approach to classify timing of gestational

arrest prior to loss we can determine the extent of potential overestimation of certain factors that may lead to biased estimates for factors associated with early pregnancy loss.

Smoking

Tobacco use remains one of the most commonly abused substances among pregnant women. Of women who smoke, 60% stop as soon as they find out they are pregnant. [53] Based on results from the National Natality Survey, smoking prevalence among US pregnant women was approximately 12%. [53] Some studies found an increase in risk of miscarriage among smokers,[54-61] while others have reported no association or only a weak relationship.[8,62-64] Two studies reported a clear dose-response relationship between smoking and spontaneous abortion.[54,55] In their study population, Armstrong and colleagues estimated that cigarette smoking accounted for 11% of all miscarriages and could explain up to 40% of losses among women smoking 20 or more cigarettes per day.[54] Ness and colleagues reported the risk of loss related to smoking habits is probably underestimated when using self-report (RR~1.4) compared to biomarker detection of cotinine levels from hair samples (RR~1.9).[65] However, because smoking is generally not associated with fetal anomaly, it is suspected that the smoking-related risk of loss may occur predominantly among miscarriages with normal fetal karyotype (i.e., a fetus with normal chromosomes).[53]

The association between early miscarriage and smoking has been inconsistent.[54-56,62,63,65-68] The inconsistencies may in part be dues to limitation in sample size, inadequate control for confounders, and differences in recall bias of smoking status among subjects. A synopsis of the major studies published since 1975 that have reported risk ratios between smoking and miscarriage are summarized in **Table 2-5**.

In summary, although many studies have found a positive modest association between maternal cigarette smoking and risk of miscarriage, dose-response associations have been reported only by some studies, and other studies have reported no association between smoking and risk of pregnancy loss. The mechanisms underlying the possible association between smoking and risk of miscarriage may involve toxic effects of nicotine, carbon monoxide and other constituents of tobacco smoke.[53] In reproductive epidemiology, the timing of these exposures may be distinct or cumulative in their overall risk for loss. The contradictory findings may be further confounded by the heterogeneity in the type of loss assessed (e.g. early pregnancy loss vs. fetal death). A better assessment in the timing of loss is warranted so that we can understand biologically meaningful causal associations between smoking and miscarriage risk.

Table 2-5. Studies on miscarriage risk and smoking

Author, Study type, Location	Population	Study period	Smoking status	Relative risk (95% CI)	Adjustment factors
Kline et al. 1977[56] Case-control United States	574 losses < 28 weeks gestation , 320 births	1974-1976	Nonsmokers Smokers	1.0 1.80 (1.30-2.50)	Age at last menses, history of abortion and live births
Ericson and Källén 1986[66] Case-control Sweden	219 losses, 1,032 births without major malformation	1980-1981	Nonsmokers Smokers	1.0 1.00 (0.60-1.50)	Video screen use, stress
Sandahl 1989[62] Case-control Sweden	610 losses, 1,337 births	1980-1985	Nonsmokers Smokers Any smoking > 10 cigaretts/day	1.0 0.90 (0.80-1.00) 0.90 (0.70-1.00)	Maternal age parity
Armstrong et al. 1992[54] Cohort Canada	10, 191 losses, 47,146 pregnant women	1982-1984	Nonsmokers Smokers 1-9 cigarettes/day 10-19 cigarettes/day ≥ 20 cigarettes/day	1.0 1.10 (1.00-1.20) 1.20 (1.10-1.30) 1.70 (1.60-1.80)	Maternal age, education, ethnicity, employment during pregnancy

Author, Study type, Location	Population	Study period	Smoking status	Relative risk (95% CI)	Adjustment factors
Windham et al. 1992[63] Case-control United States	626 losses < 20 weeks' gestation 1,300 births	1986-1987	Nonsmokers Smokers 1-10 cigarettes/day > 10 cigarettes/day	1.0 0.90 (0.70-1.20) 1.10 (0.80-1.60)	Maternal age, previous fetal loss, marital status, insurance, alcohol intake, intake of bottled water
Dominguez-Rojas et al. 1994[55] Cohort Spain	169 losses, 711 women with > 1 pregnancy	1989-1991	Nonsmokers Smokers 1-10 cigarettes/day >11 cigarettes/day	1.0 1.00 (0.60-1.50) 3.40 (1.70-6.90)	Maternal age, age at menarche, previous spontaneous abortion, marital status
Chatenoud et al. 1998[67] Case-control Italy	782 losses < 12 weeks' gestation 1,543 births	1990-1997	Nonsmokers Former smokers Smokers before pregnancy Smokers before and during pregnancy	1.0 0.90 (0.70-1.20) 0.70 (0.50-1.00) 1.30 (1.00-1.60)	Maternal age, education, marital status, history of spontaneous abortion or miscarriage, nausea, alcohol or coffee intake in first trimester
Ness et al. 1999[65] Case-control United States	400 losses <22 weeks' gestation 570 births	1995-1997	Nonsmokers Former smokers Current smokers	1.0 0.90 (0.60-1.30) 1.40 (1.00-1.90)	None

Author, Study type, Location	Population	Study period	Smoking status	Relative risk (95% CI)	Adjustment factors
Windham et al. 1999[68] Cohort United States	499 losses, 5,342 pregnant women	1990-1991	Nonsmokers Smokers 1-4 cigarettes/day 5 cigarettes/day	1.0 0.90 (0.60-1.50) 1.30 (0.90-1.90)	Maternal age, prior fetal loss, alcohol intake, caffeine intake, gestational age at interview
Maconochie et al. 2007[8] Population-based Case-control England	603 losses < 13 weeks' gestation 6,116 births > 13 weeks gestation	2000-2001	Nonsmoker Smokers < 5 cigarettes/day 5-10 cigarettes/day 11-20 cigarettes/day >20 cigarettes/day	1.0 0.68 (0.43–1.07)[a] 1.03 (0.71–1.50)[a] 1.13 (0.88–1.44)[a] 1.19 (0.86–1.66)[a]	Year of conception, maternal age, previous miscarriage and previous live birth.

CI=confidence interval; [a]adjusted odds ratio

Vitamins

Multi-vitamin supplementation is commonly recommended for all women who are pregnant or planning a pregnancy. Prenatal vitamin supplementation during early pregnancy is related to lower risk of neural tube defects and is associated with decreased risk of adverse pregnancy outcomes including preterm birth, pre-eclampsia and low-birth weight.[69-71] A summary of studies that have investigated the relationship between vitamin exposure, including folic acid supplementation and risk for miscarriage can be found in **Table 2-6.**. Investigators have reported both increased and decreased risk of miscarriage associated with vitamin use during early pregnancy.[8,61,72-76] A Cochrane review assessing nearly 100,000 pregnancies from 28 clinical trials found no significant differences between women taking any vitamins compared with controls for total fetal loss (relative risk (RR) 1.04, 95% confidence interval (CI) 0.95, 1.14) or early or late miscarriage (RR 1.09, 95% CI 0.95, 1.25).[75] In another study, taking vitamins reduced the odds of miscarriage by 50% (OR=0.46, 95% CI 0.38, 0.55),[8] the effect was most marked among those taking folic acid, iron or multivitamins. A study within the RFTS population, vitamin use was prospectively reported by 95% of study participants.[73] Odds of miscarriage was 60% lower for women exposed to vitamin supplementation during early pregnancy compared to those who were not (adjusted odds ratio= 0.43, 95% CI 0.30,0.60).[73] Differences observed in effect estimates across studies may be a result of exposure ascertainment, including biomarker detection through plasma levels, exposure definitions, and model adjustments. Vitamin use may be a proxy measure of other health-conscious behaviors associated with pregnancy health such as alcohol intake and physical activity during pregnancy.[73] The importance of vitamin supplementation may also be related to other lifestyle and behavioral factors including pregnancy intendedness. A woman who is intending a

pregnancy may be more health conscious which may include positive behaviors towards the intended pregnancy, such as taking vitamin supplementation. Conversely, a woman who has an unintended or unwanted pregnancy may not be as likely to take positive health measures, like vitamin supplementation.

Table 2-6. Studies on miscarriage risk and vitamin use

Author, Study type, Location	Population	Study period	Vitamin use	Odds Ratio (95% confidence interval)	Adjustment factors
Hook and Czeizet 1997[74] Randomized control trial Hungary	363 losses 2,787 births	1984-1992	No Folate acid supplement Folate acid supplement	1.0 1.16 (1.01-1.3)[a,b]	None
Windham et al. 2000[76] Cohort United States	499 losses, 4,645 pregnancies	1990-1991	No Folate acid supplement Folate acid supplement	1.0 1.14 (0.96-1.35)[a,b]	None
Gindler et al. 2001[72] Population-based Case-control China	2,155 losses, 23,806 births	1993-1995	No Folate acid supplement Folate acid supplement	1.0 0.97 (0.84-1.12)[a]	education
George et al. 2006[61] Case-control Sweden	562 losses, 1,037 births	1996-1998	No Folic acid supplement Folic acid supplement Plasma folate (nmol/L) ≤4.9 5.0–8.9 9.0–13.9 ≥14.0	1.0 3.10 (1.4-6.60)[b] 0.80 (0.4-1.90) 1.0 2.30 (1.10-4.60) 2.20 (1.00- 4.90)	maternal age, previous pregnancy history, induced abortions, myoma, time to conceive, marital status, smoking, caffeine and alcohol intake

Author, Study type, Location	Population	Study period	Vitamin use	Odds Ratio (95% confidence interval)	Adjustment factors
Maconochie et al. 2007[8] Population-based Case-control England	603 losses < 13 weeks' gestation 6,116 births > 13 weeks	2000-2001	No Vitamins Any Vitamins Folic acid Iron Zinc Vitamin C Other multivitamin Other vitamins	1.0 0.46 (0.38–0.55) 0.46 (0.37–0.56) 0.25 (0.16–0.37) 0.50 (0.20–1.23) 0.55 (0.30–1.01) 0.59 (0.39–0.88) 0.52 (0.27–0.97)	Year of conception, maternal age, previous miscarriage and previous live birth.
Hasan et al. 2009[73] Cohort United States	524 losses, 3,659 pregnancies >20 weeks' gestation	2000-2008	No Vitamins Vitamin use	1.0 0.43 (0.30-0.60)	maternal age, gravidity, progesterone use in early pregnancy, smoking, race/ethnicity, education, marital status, and study site
Rumbold et al. 2011[75] Meta-analysis	28 clinical trials, with 11,723 losses and 98,267 pregnancies	Cochrane Pregnancy and Childbirth Group Trial 2010	No Vitamins Vitamin use	1.0 1.04 (0.95-1.14)[a,c]	

[a] Risk ratio; [b]Unadjusted estimates [c]Meta-analysis estimates

CHAPTER III

METHODS

Methods overview

In order to better understand timing and disparities in miscarriage risk I use data from *Right from the Start,* ,a large prospective pregnancy cohort. In this section. I describe in more detail the cohort, data collection methods and proposed statistical analysis for each of my specific aims. Below is a summary table that describes an overview of the purpose of each specific aim, lists the exposure and outcome of interest to be studied, which portion of the study population used, including total number of subjects and overview of proposed statistical analyses (**Table 3-7**).

Briefly, in specific aim 1 I establish an alternate method to estimate gestational age using early ultrasound data in order to have a better outcome measurement of gestational arrest prior to miscarriage. I use a sub-cohort of women who experienced miscarriage and have ultrasound data available. In specific aim 2 I assess this new method within the full cohort by comparing models that use self-reported LMP to estimate gestational age with models that use the new measurement for gestational age at arrest. I assess overall bias in these models by comparing risk estimates for common putative factors associated with miscarriage risk. Finally in aim 3 I further describe differences in gestational age at arrest between Blacks and Whites within this cohort, including women with intended pregnancies.

Table 3-7. Overview of specific aims, study population, and statistical analyses

Specific Aim	Purpose	Exposure	Outcome	RFTS Study Population	Analyses
Aim 1	Establish and describe GAAD, GAAD gap	N/A	Miscarriage	Sub-cohort: Women with miscarriage **and** Ultrasounds only (n=504)	• Descriptive statistics (Chi-square, t-test) • Sensitivity analyses[a]
Aim 2	Compare models with different estimates for gestational age [i.e. GAAD vs. LMP]	Smoking **or** vitamin use	Miscarriage	Full cohort: Women with term birth **or** miscarriage (n=5,513)	• Cox PH models (overall and patterns of use) • Bootstrap analyses (overall use)
Aim 3	Race(Black/White) and GAAD gap, model comparison	Race*pregnancy intention interaction with Smoking **or** vitamin use	Miscarriage	Sub-cohort: Women(Black/White) with miscarriage **and** Ultrasounds only (n=447) **and** Full-cohort: Women (Black/White) with term birth **or** miscarriage (n=4,903)	• Descriptive statistics (Chi-square, t-test) • Cox PH models (overall and patterns of use) • Bootstrap analyses (overall use)

RFTS=*Right from the Start*; GAAD=gestational age at arrest of development; LMP=last menstrual period; N/A=not applicable; PH=proportional hazard models. [a]Sensitivity analyses include women with pregnancy losses before or after first-trimester interviews; women with early(<10 weeks) vs. late loss(≥10weeks); nulliparous women only; women who pre-enrolled; women without prior history of loss; women who had losses >3 days of study enrollment.

RFTS Study population

Right from the Start (RFTS) is an ongoing prospective community-based pregnancy cohort study that began enrollment in 2000. Over time, the study has included three phases (RFTS 1, 2, and 3). Women, either pregnant or planning a pregnancy, enrolled from nine areas in three states (North Carolina, Texas and Tennessee). Participants were between 18 and 45 years of age, spoke English or Spanish, intended to carry the pregnancy to term and had not used assisted reproductive technologies to conceive.[3] The study was designed to recruit women from a variety of clinic and community-based settings.[3]

Women who were not yet pregnant but trying to conceive could pre-enroll before pregnancy and were followed until a positive pregnancy test. To avoid over-enrollment of sub-fertile women, non-pregnant participants in the study must have been attempting to get pregnant for fewer than six months (RFTS 1 and 2) or fewer than three months (RFTS 3). Women were eligible for up to 12 months of pre-enrollment. Women entered the study before 12 completed weeks of gestation (RFTS 1), before nine completed weeks of gestation (RFTS 2), or only prior to pregnancy (RFTS 3). Informed, written consent was obtained from each study participant in compliance with institutional review board procedures. A summary of RFTS eligibility and research activities by study phase can be found in **Table 3-8.**

Table 3-8. Right from the Start study phase and eligibility criteria

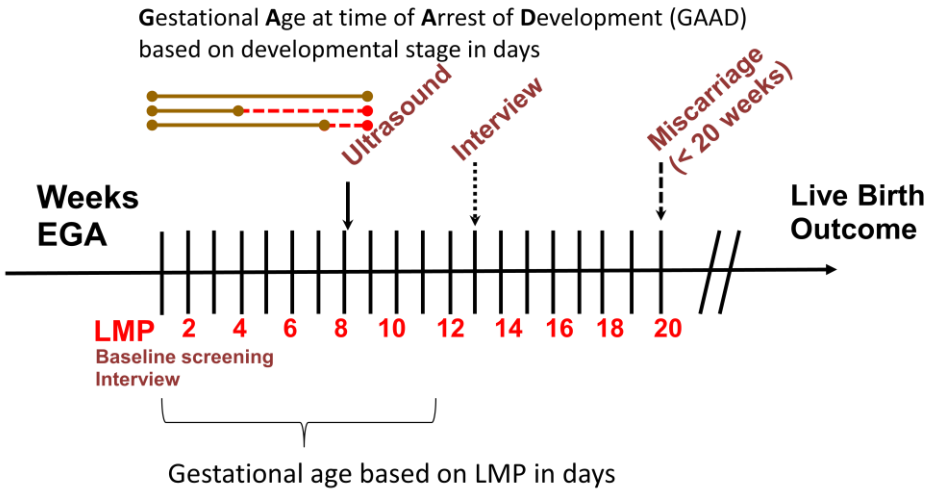
RFTS Study features and activities	RFTS 1	RFTS 2	RFTS 3
Active period of enrollment	12/200-07/04	08/04-11/08	05/06-01/12
Number of participants	2,481	3,034	590
Pre-pregnant participants	232	843	589
Web and phone based daily diary	No	No	Yes
Enrollment age 18-45 years	Yes	Yes	Yes
States	TX, NC, TN	NC, TN	TN
Reproductive technology to conceive	No	No	No
Informed consent	Yes	Yes	Yes
Language: English or Spanish	Yes	Yes	Yes
Attempting to get pregnant	< 6 months	< 6 months	< 3 months
Gestational age at enrollment	< 12 weeks	< 9 weeks	Pre-enrolled
Median age of ultrasound (weeks)?	9.29	7.29	6.86
Median age of ultrasound if miscarriage occurred (weeks)?	8.86	7.14	6.86

TX=Texas; NC=North Carolina; TN=Tennessee

Women who had their LMP before May 5, 2012 were included in this dataset (n=6,105). Participants had an early-pregnancy ultrasound for assessment of embryologic viability, documentation of stage of development, and confirmation of gestational dating. Research ultrasounds were conducted at a time in gestation in which all normal pregnancies would be expected to have a fetal pole and heart rate identifiable. The transvaginal ultrasound (TVUS) used in RFTS provides the most accurate information in early pregnancy, given that the early developing gestational sac and fetal pole are still developing at this point and a vaginal ultrasound can get closer to the developing pregnancy and provide a more accurate assessment of

gestational dating.[19] Participants completed a baseline interview at the time of enrollment and comprehensive computer assisted telephone interview in the first-trimester. In the interview, information collected included reproductive and medical history, socio-demographic characteristics, and health behaviors around the time of conception or during pregnancy. Participants who experienced pregnancy loss before the scheduled interview were interviewed as soon as possible after miscarriage. Pregnancy outcomes were self-reported by study participants and verified by medical records. A timeline of RFTS study activities can be found in **Figure 3-3**.

Figure 3-3. Timeline of Right from the Start activities and estimating GAAD



EGA=Estimated Gestational age based on last menstrual period (LMP)

Estimating GAAD based on ultrasound for RFTS cohort:

- For women with loss before ultrasound GAAD cannot be calculated
- For women with anembryonic gestation GAAD= GA at time of arrested development
- For women with abnormal or normal gestation GAAD=GA at time of ultrasound + midpoint between ultrasound and date of loss

Exclusions

Women were allowed to enroll more than once in RFTS. To allow for independent observations, subsequent pregnancies are excluded for women who enrolled more than once (n=333). Furthermore, women who had induced abortions (n=17), and women who had ectopic/molar pregnancies (n=11) were also excluded. Women with these rare birth outcomes

may have a different etiology than women who miscarry and therefore they were excluded from our analysis. Women whose pregnancy outcome was not known at the time of analysis were also excluded (n=231, 3.8%). We compared women who experience a miscarriage(<20 weeks gestation) with women who have term pregnancy(≥20 weeks gestation). We documented miscarriage in 697 women (12.6%) during the study period. Pregnancy was verified by ultrasound or repeat pregnancy tests. Participants who had birth outcomes at a gestational age later than 20 weeks served as a comparison group. The comparison group (n=4,816) consisted of women who had live births (n=4,787) or stillbirths (n=29).

Estimating gestational age from ultrasound

Research ultrasounds were conducted at a time in gestation (> 5 $\frac{4}{7}$ weeks after LMP) in which normal pregnancies would be expected to have a fetal pole and heart rate. Traditionally gestational age is based on a women's self-reported LMP, referred to here as the estimated gestational age (EGA). Accuracy of self-reported LMP dating in this cohort is excellent within +/- 1 day compared to ultrasound estimates (0.8 days longer than ultrasound estimates).[77] However, since developing pregnancies can end days or weeks before clinical onset of symptoms for miscarriage, we estimate gestational age based on characteristics at the time of ultrasound for all women within our cohort. Women were classified based on developmental stage at loss (i.e. gestational age at time of arrested development). This new estimation for gestational age variable is referred to as GAAD (**Table 3-9**).

Characterizing developmental stage at ultrasound

Initially we characterized the developmental stage at ultrasound in the following way based on the following characteristics, women who have normal fetal development, women who have abnormal fetal development and women who have anembryonic development at time of first-

trimester transvaginal ultrasound (**Table 3-9**). Women who are considered to have normal fetal development include women with a fetal pole and normal FHR at ultrasound. Women who are considered to have abnormal fetal development included women with a visible fetal pole and either abnormal or no FHR visible on ultrasound. Women who are considered to have anembryonic development include women with an empty uterus, gestational sac only, or gestational and yolk sac visible on ultrasound but no fetal pole or FHR visible.

Table 3-9. Estimating gestational age at arrest (GAAD) in Right from the Start

Ultrasound Developmental Stage[a] (n)	**Ultrasound characteristics (n)**	**Estimating GAAD variable (days)**
Loss before Ultrasound (188)	n/a	Initially exclude all losses before ultrasound, subsequent sensitivity analysis
Fetal Pole (312)		
	Normal FHR (180)**	Gestational age based on formula A + Midpoint from ultrasound date to date of loss
	Abnormal FHR (32)	Gestational age based on formula A + Midpoint from ultrasound date to date of loss
	No FHR (100)	Gestational age based on formula A + Midpoint from ultrasound date to date of loss
Anembryonic gestation (160)		
	Gestational sac only (83)	Gestational age based on formula B
	Gestational and yolk sac (77)	Gestational age based on formula B
Empty uterus (37)		If ≤ 4.5 weeks: Self-reported LMP to date of ultrasound If > 4.5weeks: Assign 4.5 weeks (32 days) for all women

[a]Developmental stage among women who experienced miscarriage only (n=697). GAAD= gestational age at arrested development; FHR=fetal heart rate; Formula (A)[45]: $t=\exp[1.685+0.316(CRL)-0.049(CRL)^2+0.004(CRL)^3-0.0001(CRL)^4]x7$; Formula (B)[47]: $t=L+42$ where *t* refers to estimated gestational age in number of days and L refers to measurement of length of the mean gestational sac diameter.

Estimating GAAD and the GAAD gap in RFTS

Estimating gestational age at arrest based on ultrasound information within this cohort was done in the following way: For women who had a loss before their transvaginal ultrasound GAAD cannot be calculated. I conducted a complete case analysis removing these women from our analysis. For women with anembryonic gestation, the GAAD was the gestational age at time of arrested development using the criteria outlined in **Table 3-9**. For women with anembryonic development in which gestational sac or gestational and yolk sac are visible GAAD was estimated based on the formula by Goldstein and colleagues.[47] A special case of anembryonic gestation is women with empty uterus. For women with an empty uterus in which neither the yolk sac or gestational sac are visible, GAAD is the EGA based on self-reported LMP to the date of the ultrasound if less than 4.5 weeks (i.e. 32 days). If greater than 4.5 weeks, I assigned 32 days gestation for these women (**Table 3-9**). This designation is based on early developmental structures visible on ultrasound. In a normal pregnancy, the first structure visible on TVUS is a gestational sac by week 5 (**Table 2-2**), For anembryonic gestation of empty uterus, no gestational sac is visible on ultrasound, and therefore a conservative estimate 4.5 weeks (i.e. 32 days) gestation is assigned.

For women with normal or abnormal ultrasounds I estimated gestational age based on a nomogram for CRL plus the midpoint from date of ultrasound to date of clinical loss. We have information on embryologic development from a single time point from their first-trimester TVUS. For women with normal or abnormal fetal development who subsequently go on to miscarry, we know the date of loss happened sometime between the date of ultrasound and the reported date of loss. Embryologic development may have stopped at any time between the date of ultrasound and when they reported a loss. Since we do not have daily ultrasounds for these

women to determine the exact date their development stopped, I estimated GAAD based on the formula assessing CRL by Hadlock and colleagues[45] plus the midpoint from ultrasound date to the date of loss. Since we do not know the exact date that development stopped for women with normal and abnormal ultrasounds from a single ultrasound measurement, we simplifed by choosing the midpoint between date of ultrasound and date of reported loss for these women.

Based on the data available from early first-trimester ultrasounds, we used the following formulas to estimate GAAD in days gestation (Hadlock et al.[45] (**Equation 1**) and Goldstein et al.[47] (**Equation 2**)):

Equation 1: $t=\exp[1.685+0.316(CRL)-0.049(CRL)^2+0.004(CRL)^3-0.0001(CRL)^4]x7$

Equation 2: $t=L+42$

where *t* refers to the gestational age in number of days and L the measurement of length of the mean gestational sac diameter in millimeters (mm). Many studies have shown that the CRL is the most consistent measurement for determining gestational age in early development.[36,37,45,46] However, if a woman is missing a CRL measurement from their first-trimester ultrasound, I estimated GAAD based on formula B and the longest embryonic length. The Hadlock formula estimates gestational age in weeks gestation and with CRL measured in centimeters (cm). The Hadlock formula was multiplied by 7 in order to estimate GAAD in days gestation.

I also determined the GAAD gap for each woman. The GAAD gap is defined as the difference in days gestation between self-reported LMP date of loss and the newly estimated GAAD based on **Table 3-9**.

Rationale for chosen nomograms

The formulas to estimate GAAD were chosen based on the two largest prior studies conducted in the United States to estimate gestational age from ultrasound characteristics (**Table 2-3**). Both studies used a single early first-trimester transvaginal ultrasound visit to estimate gestaional age within a study population that had not used in vitro fertilization in order to conceive. Ultrasound measurements were made among singleton births in women with no diseases known to adversely affect fetal growth(e.g. diabetes mellitus) (Hadlock et al.[45] n=416; Goldstein et al.[47] n=143). Both studies consisted of subjects with a known history of LMP. Furthermore, study ultrasounds were performed by physicians and or registered sonographers.

Additionally, these formulas allow us to estimate GAAD for women in our cohort as a continuous variable in days gestation. Instead of grouping all women by ultrasound characteristic and assigning the appropriate gestational week based on development, (for example, assigning $5\frac{4}{7}$ weeks gestation for all women with a gestational and yolk sac only visible on ultrasound) our estimates for days gestation was a continuous variable. Categorizing a continuous variable can result in loss of information and the statistical power to detect an association between a predictor variable and an outcome.[78] It would also conceal any non-linearity in the relation between the variable and outcome. Furthermore, it underestimate the extent of variation in an outcome between groups, such as the risk of miscarriage. There may be considerable variability in risk of miscarriage within each group by developmental stage at ultrasound (e.g. among normals and abnormals). For these reasons, the above nomograms are well suited to estimate GAAD within the RFTS study population.

Specific aim 1: Determining GAAD gap, exploratory analysis

For specific aim 1 investigate the variation and distribution of the GAAD gap for RFTS women who have ultrasound data. I defined the GAAD gap as the difference in days gestation between estimated gestational age based on self-reported LMP and estimated gestational age based on developmental stage at arrest (i.e. GAAD gap= (self-reported LMP – GAAD)). I expect GAAD gap to be positive for most women in this cohort. This would indicate that self-reported LMP is greater than newly estimated GAAD, which would mean that for most women pregnancies had arrested earlier than when they self-reported a clinical loss. If the GAAD gap was negative, it would indicate that these women may have been unaware of their LMP at time of ultrasound.

I describe and report the distribution of the GAAD gap by developmental stage at ultrasound within the cohort. I conduct initial exploratory and descriptive analyses to assess the GAAD gap (e.g. mean, median and mode of GAAD gap). I assess GAAD gap differences by assessing both predictors for longer than median vs. shorter than median GAAD gap within our cohort. I test associations with predictors of miscarriage risk such as first-trimester bleeding[79] as well as established factors in the literature associated with pregnancy loss (e.g. age, parity, pregnancy intention, prior history of loss). I use non-parametric methods to assess the GAAD gap when comparing groups. Non-parametric tests imply that there is no assumption of a specific distribution for the population. I used the Mann-Whitney U-test to compare sample medians. Mann-Whitney U-test assumes a non-normal distribution in the GAAD gap, which is especially important if data is skewed. The p-value for a two-tailed test has a null hypothesis that assumes the medians are equal between groups.

Specific aim 1: Hypothesis

In specific aim #1 I *hypothesized that the GAAD gap was greater than 10 days within this cohort.* That is among women with a pregnancy loss who have ultrasound information, the number of days between self-reported LMP and GAAD will be greater than 10 days.

Sensitivity analyses for Specific aim 1:

I conducted the following sensitivity analyses to specific aim 1 to assess the robustness of estimating GAAD within this cohort.

Women with prior or recurrent miscarriage history

Prior miscarriage history is a strong predictor for future miscarriage, however we did not use prior miscarriage history as a potential cofounder in our analysis since it may be an intermediate covariate for future risk. Adjusting for prior loss could lead to biased estimates for the risk of smoking on miscarriage due to residual confounding in our model (i.e. since we would be conditioning on a collider pathway).[80,81] I did a sensitivity analysis removing women who have ever experienced a miscarriage or who have ever experienced recurrent miscarriage (i.e. 3 or more prior losses) in order to determine if GAAD gap changes appreciably. Recurrent miscarriage in our cohort was defined either, as at least 3 prior pregnancy losses or as 2 prior losses and current birth outcome as a miscarriage.

Miscarriage pre and post-interview

In order to determine if recall bias is present within our study I assessed GAAD gap based on time of the first-trimester interview. Women who experience loss prior to the interview may be more unsure of their LMP than women who experience loss after the interview. For a majority of women in our study, the ascertainment of behavioral factors during pregnancy

including smoking status and vitamin use was collected after the loss (n=445, 68.2%).[2] Overall, the mean interval between loss and first-trimester interview was less than 3.5 weeks (24.8 (standard deviation, 17.5) days), and does not differ significantly by race. Furthermore, questions on exposure status in the FTI clearly asked about behaviors during that pregnancy and not after the loss occurred. Therefore I believe that recall bias was minimal in our cohort. I report overall analysis and analyses stratified pre and post interview to determine if our results change appreciably.

Miscarriage within 3 days of enrollment

The hazard for miscarriage tends to be higher in prospective cohorts for the first few days of study enrollment. Elimination of the first three days of follow-up (which means excluding from study losses that happened in these three days) seems to eliminate this form of bias.[21] Women who experience a loss within three days of enrollment may already be experiencing a threatened miscarriage, especially if associated with heavy bleeding. I did a sensitivity analysis removing women who experienced a pregnancy loss within three days of study enrollment (n=49, 7.0%) to determine if our results change.

Specific aim 2: Comparing GAAD models with LMP models

For specific aim 2 I investigate if incorporating proper classification of gestational age at arrest influences estimates of common putative factors associated with pregnancy loss. The study strength of the RFTS study is that we capture a wide variety of pregnancy-related health behaviors in early first trimester in our first-trimester interview. I assess both a protective factor (e.g. vitamin use) as well as risk factor (e.g. smoking) associated with miscarriage. These factors were chosen as examples to test the new GAAD method and assess the impact of bias on our estimates for miscarriage risk. The analyses were useful to determine if GAAD can explain in

part some of the differences reported in the literature for miscarriage risk associated with either smoking or vitamin use. Misclassifying time at loss in studies of miscarriage can lead to biased estimates of the effect of that exposure on pregnancy loss.

Specific aim 2: Hypotheses

Using time to event analyses, I compare models that use GAAD with models that use traditional self-reported LMP for gestational age. I determine the extent of potential bias in our reported estimates for risk of miscarriage associated with either smoking or vitamin use in the first-trimester. When comparing LMP models to GAAD models for smoking exposure, *I hypothesize that models that use the classified GAAD will attenuate the risk associated with miscarriage compared to models that use LMP for gestational age.* For vitamin use, a protective factor, *I also hypothesize that GAAD models will attenuate the association with miscarriage compared to models that use LMP*. A separate DAG for each exposure was assessed to determine potential confounders.

Rationale for Cox Models

Descriptive summary statistics on maternal characteristics during pregnancy between women who experience miscarriage and women who did not experience miscarriage were compared using log-rank test for equality for survival functions. Cox regression were used to estimate hazard ratios (HR) for the effect of each exposure on risk of miscarriage. Models were left truncated to include gestational age at enrollment. Miscarriage studies that recruit pregnant women are left truncated because women enter the study at different gestational ages and an unknown proportion of the source population experiences losses prior to enrollment.[82] In RFTS participants are followed from the time of enrollment in the study and contribute to the analysis

until an outcome or loss to follow-up occurs. Thus, Cox models address actual time "at risk" and "in view" for each woman by accounting for variable gestational age at study entry.

Cox models were used to screen candidate confounders. Cox model is preferred over the logistic model when survival time information is available and data requires censoring. The Cox model uses more information (i.e. the survival times) than logistic regression models, which considers a binary outcome and ignores survival times, censoring and truncation. [83] Additionally, simulation studies suggest that bias in the odds ratio exceed 20% when average gestational age at entry for the exposed versus the unexposed differs by 10 days or more, which has been observed when assessing various socioeconomic factors, such as education and ethnicity in miscarriage risk. Cox regression can correct for left truncation and is no more difficult to perform than logistic regression.[82] Therefore Cox models with left truncation to include gestational age at enrollment were preferred over logistic models in our analyses.

Cox models were used to compare overall risk for miscarriage as well as early (<10 weeks gestation) versus late miscarriage (≥ 10 weeks gestation). All analyses were conducted using Stata SE/12 software (StataCorp LP, College Station, TX).

Exposures

In specific aim 2 I investigate if proper classification of gestational age at arrested development influences the estimates of common putative risk factors associated with miscarriage. I assess the following exposures, smoking and vitamin use and their association with risk for miscarriage in our RFTS cohort. I assessed these risk factors with miscarriage risk by comparing models with improved classification of gestational age at arrest development with traditional models that use self-reported LMP to estimate gestational age.

Smoking

Smoking exposure during early pregnancy is a self-reported measure obtained during the first-trimester interview. Briefly, smoking status was assessed based on the following items from the first-trimester interview:

1. While you were pregnant this most recent time, did you smoke cigarettes regularly, I mean one or more cigarettes every day?
2. Have you ever smoked cigarettes regularly, and by regularly I mean one or more cigarettes every day for at least a month?
3. When did you stop smoking? (Month, Day, Year)
4. Before you stopped, how many cigarettes did you usually smoke a day?
5. In the past 4 months, has your smoking changed in any way?
6. When did your smoking change? (Month, Day, Year)

Smoking status was assessed in the following way: Women were classified as unexposed if indicated they have never smoked. If women indicate that they currently smoke during their pregnancy they were classified as exposed in pregnancy. For women who quit smoking within four months prior to the interview, (meaning exposures in the pregnancy and/or periconception window) or women who quit ≥ four months from interview were classified as exposed pre-pregnant (i.e. former smokers).

Vitamin use

Vitamin exposure in RFTS was assessed in a similar way to the study by Hassan and colleagues.[73] Vitamin use is a self-reported measure during the FTI. Briefly, vitamin use was assessed based on the following items from the first-trimester interview:

1. Do you now take prescription or nonprescription prenatal vitamins?
2. In the past four months, did you take prescription or nonprescription prenatal vitamins?
3. Did you start taking prescription or nonprescription prenatal vitamins more than four months ago?
4. Do you now take multivitamins other than prenatal vitamins?
5. In the past four months, did you take multivitamins other than prenatal vitamins?
6. Did you start taking multivitamins other than prenatal vitamins more than four months ago?

Women who reported any use of prenatal or multivitamin use during pregnancy was considered among the exposed group. All others were considered unexposed.

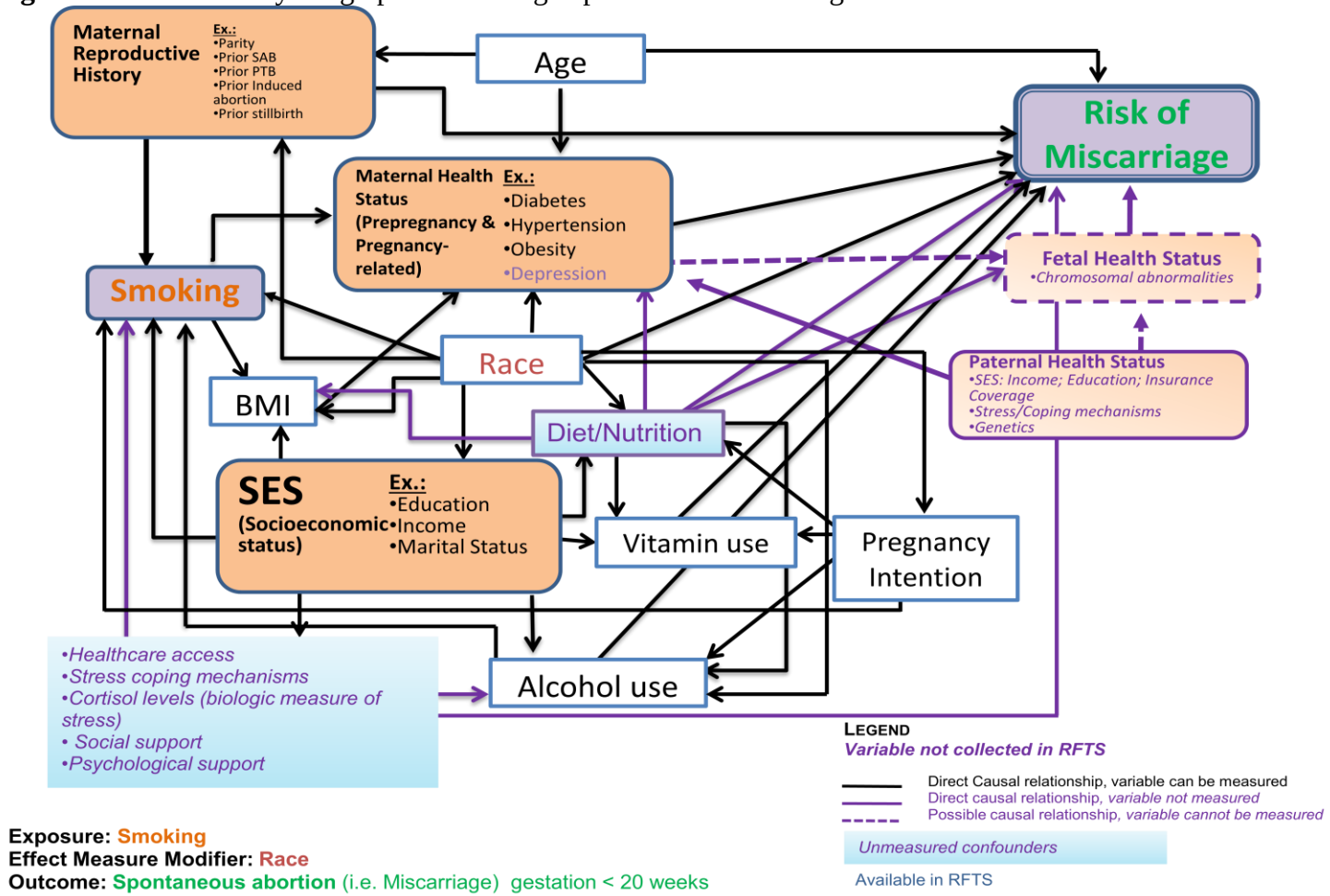
Outcome

In our cohort, pregnancy was verified by ultrasound examination or repeat pregnancy tests. Miscarriage was defined as loss of a recognized pregnancy prior to 20 completed weeks of gestation. Outcomes are self-reported and verified by medical records. Miscarriage events are self-reported in both the pregnancy outcome form as well as the first-trimester interview. If dates for self-reported miscarriage do not match, medical records were abstracted for those records. Participants who had a birth outcome at a gestational age later than 20 weeks served as the comparison group. Because of the etiologic heterogeneity in miscarriage[28] we considered subsequent sensitivity analyses by grouping miscarriages of different gestational ages as different outcomes. Based on conceptualization by Silver and colleagues we defined early pregnancy losses as losses occurring up to 10 weeks gestation, and the median in our data, and fetal death as losses occurring between 10 and 20 weeks gestation.[28]

Confounders

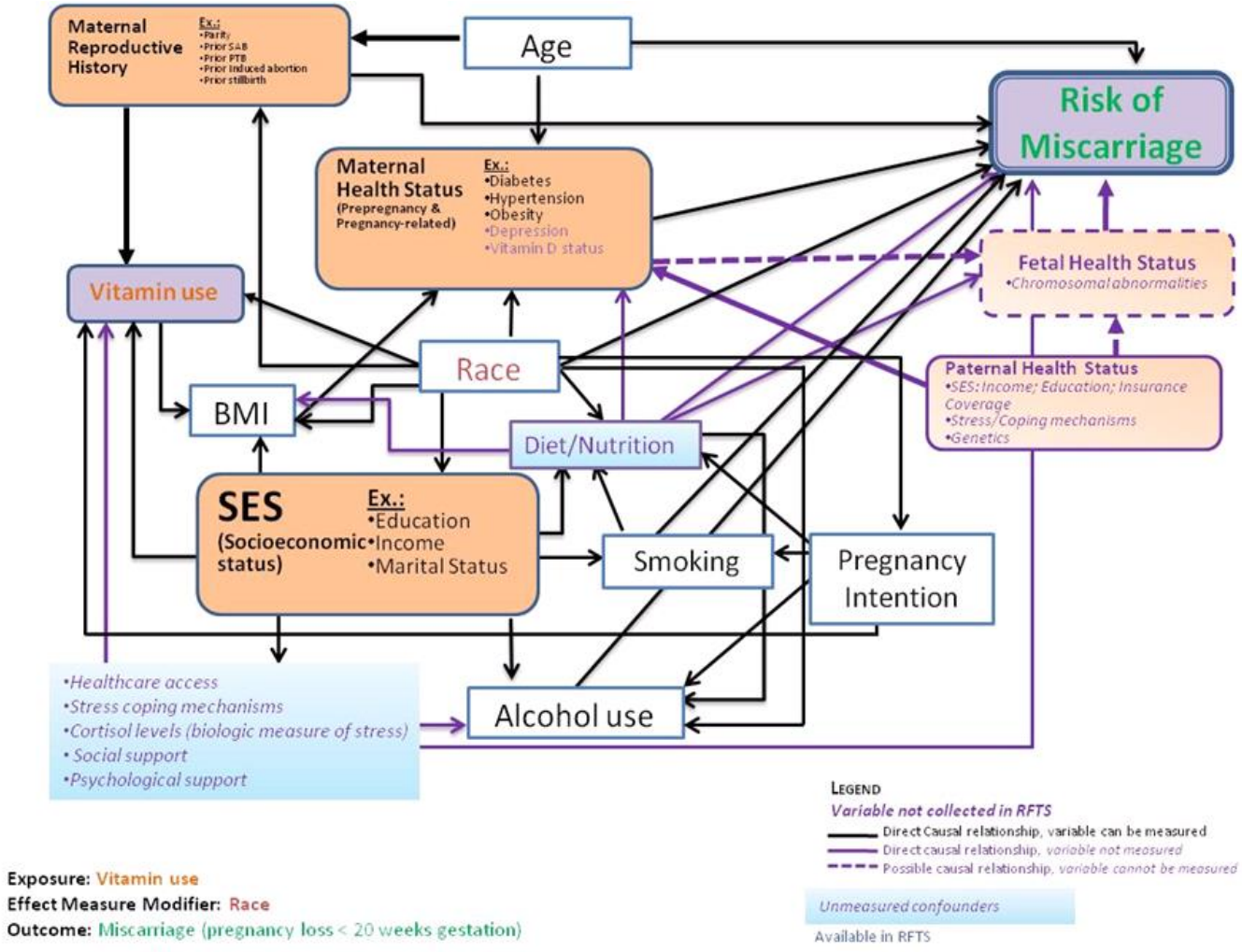
Potential confounders examined from baseline and the first-trimester interviews included factors known to be associated with both spontaneous abortion and the exposure of choice have been chosen *a priori* based on current literature using directed acyclic graphs (DAG). DAGs are causal diagrams that incorporate qualitative, *a priori* subject matter knowledge because statistical criteria are insufficient to characterize either confounding or selection bias, I have also used causal diagrams to incorporate qualitative *a priori* subject matter knowledge.[84] **Figure 3-4** is a DAG diagram for risk of miscarriage with smoking as a risk factor. A similar DAG diagram for risk of miscarriage with vitamin exposure was also be assessed **Figure 3-5**. A summary of other covariates of interest can be found in **Table 3-10**.

Figure 3-4. Directed acyclic graph of smoking exposure and miscarriage risk



Exposure: Smoking
Effect Measure Modifier: Race
Outcome: Spontaneous abortion (i.e. Miscarriage) gestation < 20 weeks

Figure 3-5. Directed acyclic graph of vitamin use and miscarriage risk



I report categorical breakdowns for maternal age and body mass index (continuous, and categories[BMI <19.8; 19.8 to ≤26.0 [referent]; 26.1 to ≤ 29.0; > 29 kg/m^2]) based on meaningful clinical cut-points for ease of interpretability in our summary of descriptive statistics. However, only maternal age (years, continuous and categories) was assessed as a potential confounder in our analyses based on the DAG. Age was assessed initially as a continuous variable in our multivariate modeling. Dichotomizing a continuous predictor variables may result in biased estimation, in Cox proportional hazard regression[78,85,86] it may result in inflation of the type-I error of the risk factor.[78,87] I tested the linearity assumption of the relationship between continuous variables and the log-hazard of miscarriage risk. The linear relationship will be assessed by the use of restricted cubic splines. A Wald chi-square test will be used to determine if a linear relationship between continuous predictors and the log-hazard of miscarriage exists. If linearity assumptions were not met, quadratic terms for continuous variables were used in our modeling and reported adjusted hazard ratios.

Other demographic factors assessed as cofounders in analysis included household income (≤$40,000; $40,001 to $80,000; >$80,000 [referent]), maternal education (≤ high school; some college; ≥ 4 years college [referent]), marital status (married/living as married [referent]; other). In addition I assessed potential confounders related to maternal reproductive history and health behaviors during pregnancy, namely parity (yes/no), previous preterm birth (yes/no), previous induced abortion (yes/no), diabetes status (yes/no), pregnancy intention (yes/no), prenatal vitamin use (yes/no), alcohol use (unexposed(referent), exposed in pregnancy, exposed pre-pregnant) and smoking status (unexposed (referent), exposed in pregnancy, exposed pre-pregnant) (**Table 3-10**). The variables that are assessed as confounders were chosen *a priori* based on my DAG. These were variables that were believed to be (1) associated with the

outcome (time to miscarriage) among unexposed women (i.e. non-smokers and non-vitamin users); (2) associated with the exposure within the entire source population and; (3) not within the causal pathway between exposure and miscarriage risk. Confounders were assessed one at a time. For the association between smoking and miscarriage risk the following variables were assessed as potential confounders based on my DAG, age, race, maternal education, marital status, household income, parity, alcohol use and pregnancy intention (**Figure 3-4**). For the association between vitamin use and miscarriage risk the following variables were assessed as potential confounders based on my DAG, age, race, maternal education, marital status, household income, parity, smoking, alcohol use and pregnancy intention (**Figure 3-5**). Confounding was defined as a 10% change from the crude hazard ratio (HR) for miscarriage risk between those exposed to smoking in the first-trimester compared to unexposed(referent), or between those exposed to vitamin use (referent) compared to those unexposed to vitamin use. Confounding was assessed using Cox proportional hazard models with gestational age estimated based on self-reported LMP for either exposure and miscarriage risk. If a 10% change is observed from the crude hazard ratio, the variable was retained in the final models. Final model adjustments for smoking and vitamin use, and overall model assessment are discussed in Chapter 5.

Table 3-10. Covariates for analysis

Socioeconomic Status	Maternal behaviors	Maternal characteristics	Current pregnancy	Prior obstetric outcomes
Race	Smoking	Body mass index (kg/m^2)	Prenatal vitamin	Previous miscarriage
Non-Hispanic White	Never	(continuous)	use	No
Non-Hispanic Black	Current	Body mass index	No	Yes
Hispanic	Former	Underweight (<18.5)	Yes	Recurrent Miscarriage (3
Other	Alcohol Use	Normal weight (18.5–24.9)	Fibroids	or more losses)
Household income	Never	Overweight (25.0–29.9)	No	No
≤$40,000	Current	Obese (≥30.0)	Yes	Yes
$40,001–$80,000	Former	Body mass index, (restricted cubic	Pregnancy Intention	Previous induced abortion
>$80,000		splines)	No	No
Missing		Maternal age, years (continuous)	Yes	Yes
Maternal education		Maternal age, years		Prior preterm birth
High school or less		<20		No
Some college		20–24		Yes
College (≥4 years)		25–29		Prior stillbirth
Missing		30–34		No
Marital status		≥35		Yes
Married, living as		Maternal age, years (restricted		
married, single		cubic splines)		
Other		Parity		
Missing		Nulliparous		
		Primaparous (1)		
		2+ prior pregnancies		
		History of diabetes		
		No		
		Yes		

Pregnancy intention

I am also interested in considering pregnancy intention as a potential confounder for risk of miscarriage. Unwanted or unintended pregnancies may be associated with modifiable behavioral factors in early first-trimester such as smoking or vitamin use. For example, women may be less likely to smoke, or more likely to use vitamins for intended pregnancies compared to unintended pregnancies. Furthermore the influence of pregnancy intention is known to differ by race for outcomes such as preterm birth, low birth weight and small for gestational age,[4-6] and may be reasonable to assume that it may differ for miscarriage outcomes as well. Additionally, women who are not planning a pregnancy may be less sure of their LMP dates. Therefore I hypothesize that *I would expect the GAAD gap to be greater for women not planning a pregnancy (i.e. an unintended pregnancy) compared to women with planned pregnancies(i.e. intended pregnancy).*

Pregnancy intention will be coded based on previous work reported in the National Family Growth Survey (NFGS) for unplanned pregnancies.[88] Questions in RFTS first-trimester interview regarding pregnancy intention were based on criteria of the NFGS. Pregnancy intention was coded as a yes/no variable for these analyses. Unintended pregnancies include both mistimed and unwanted pregnancies. Intended pregnancies include pregnancies that were planned. Pregnancy intention is based on the following questions from the first-trimester interview: FTG7(contraception and planning at time of conception) or FTE4(a)(contraception use (yes/no)) as well as FTG13(pregnancy timing) or FTG12(wanted pregnancy? (yes/no)). The full set of these questions and their respective responses can be found in the appendices. Women who did not answer, refused to answer or listed did not know to any of the above questions in the

FTI were not be assessed for pregnancy intention based on the criteria listed below (n=633). A summary of how pregnancy intention was coded can be found in **Table 3-11**.

Briefly, a pregnancy was considered intended if any of the following criteria are true:

Ftg7=1 **and** Ftg13=Either later, right time, or didn't care when or,

Fte4(a)=no contraception **and** Ftg13=Either later, right time, or didn't care when

An unintended pregnancy was coded as mistimed if any of the following criteria are true:

Ftg7=2 **and** Ftg13=too soon or,

Ftg7=3 **and** Ftg13=too soon or,

Fte4(a)=no contraception **and** Ftg13=too soon or,

Fte4(a)=yes contraception **and** Ftg13=too soon **and** Ftg12=yes (wanted baby in future)

And finally an unintended pregnancy was coded as unwanted if any of the following criteria are met:

Ftg7=4 **and** Ftg12=no (did not want baby in future) or,

Fte4(a)=yes contraception **and** Ftg12= no (did not want baby in future).

Table 3-11. Coding pregnancy intention in Right from the Start

RFTS Variable name	**FTG7**	**FTE4(a)**	**FTG13**	**FTG12**
Variable Description	Contraception and planning	Contraception use (yes/no)	Pregnancy timing	Wanted pregnancy (yes/no)
Variable Question	Which of the following best describes your situation around the time you got pregnant?	What are all the birth control methods you used in the 12 months before you got pregnant?	Did you get pregnant this most recent time, sooner than you wanted, later than you wanted, or at about the right time?	At the time you got pregnant this most recent time, did you want to have another baby at some time in your life?
Pregnancy Intention (Yes)				
	1=stopped using contraception because you wanted to get pregnant		Either Later, right time or didn't care when	
		No contraception	Either Later, right time or didn't care when	
Pregnancy Intention (No)				
Mistimed	2= not using contraception and were not really trying to get pregnant		Too soon	
	3= got pregnant during a change or gap in using contraception and you were not trying to get pregnant		Too soon	
		No contraception	Too soon	

RFTS Variable name	**FTG7**	**FTE4(a)**	**FTG13**	**FTG12**
Variable Description	Contraception and planning	Contraception use (yes/no)	Pregnancy timing	Wanted pregnancy (yes/no)
		Yes (any type)	Too soon	Yes
Unwanted	4= got pregnant while using contraception every time and you were not trying to get pregnant	--	--	No
		Yes (any type)		No

Bootstrapping to compare models (GAAD vs. LMP) for miscarriage risk

In specific aim 2 I report and compare hazard estimates for both methods (e.g. GAAD vs. self-reported LMP) with common early pregnancy exposures associated with miscarriage (i.e. smoking and vitamin use). For each exposure I use bootstrap methods to estimate the ratio of effect sizes and 95% confidence interval between either model (i.e. GAAD model vs. LMP model). I defined the bias ratio as the adjusted hazard ratio of LMP model divided by the adjusted hazard ratio of GAAD model (i.e. bias ratio= $\frac{\text{adjusted HR ratio of LMP model}}{\text{adjusted HR ratio of GAAD model}}$).

Bootstrapping approaches use resampled data to make adjustments for statistical biases as well as random error.[89] Furthermore it can be used as a way to assess internal study validity. To bootstrap the sampling distribution of the specific effects within a model and to assess bias, a sample of size n cases with replacement from the original sample is used. In other words, a given case can be selected as part of a bootstrap sample not at all, once, twice, or even multiple times in resampled data.[90] The bootstrap approach is used mainly for the estimation of parameters and their variability in a given model and can be applied to Cox regression analyses.[91] I used 1,000 bootstrap replications with our data, since this has been shown to improve model stability and precision of estimates.[91,92]

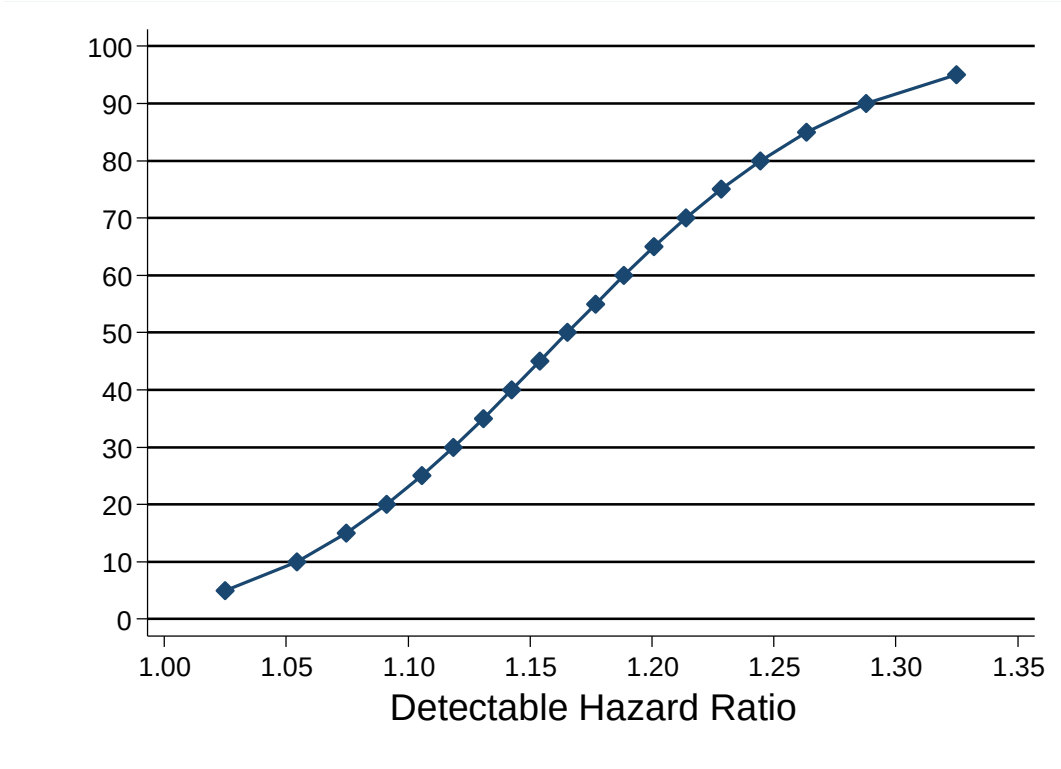
Power calculations for chosen exposures

Smoking

For this analysis I have determined that 697 women had miscarriages, and 4,816 comparison subjects had term pregnancies. The incidence of pregnancy loss among never smokers is 12.5%. With 80% power and alpha=0.05, I am able to detect true hazard risk of 1.24

in smokers compared to never smokers. **Figure 3-6** shows power and detectable hazard ratio for miscarriage risk in smokers compared to never smokers within RFTS.

Figure 3-6. Power and detectable hazard ratio for miscarriage risk in smokers compared to never smokers in Right from the Start



Vitamin use

For our vitamin analysis we have determined that 205 women reported not using vitamins in the first trimester and nearly ninety seven percent of women reported using vitamins (n=5,033). The incidence of miscarriage among unexposed women is 12.5%. The median survival time among controls was 39.7 gestational weeks from LMP (i.e. women who did have vitamin exposure). With 80% power and a type I error probability of 0.05, I am able to detect true hazard ratios of failure (i.e. miscarriage) for vitamin exposed subjects relative to vitamin

unexposed subjects of 0.55. The Type I error probability associated with this test of the null hypothesis is that the vitamin exposed and vitamin unexposed survival curves are equal. Power analyses were conducted using Cox proportional hazard power calculator in Stata (StataCorp LP, College Station, Texas).

Sensitivity analyses for specific aim 2:

I conducted the following sensitivity analyses for specific aim 2 in order to assess the robustness of our results.

Estimating GAAD for women with loss prior to ultrasound

Initially in aim 1 I did a complete case analysis: women with a loss prior to ultrasound were excluded. I did a subsequent sub-analyses in which I estimate GAAD for women who have a loss prior to their ultrasound and compare results to the complete case scenario. I estimate GAAD for these women by assigning estimated gestational age based on self-reported LMP for all women with losses prior to ultrasound. I report the hazard estimates for risk of miscarriage associated with either smoking or vitamin use and compare results to complete case scenario.

Specific aim 3: Comparing GAAD gap by race and if risk of loss associated with common early pregnancy exposures are modified by race

For specific aim 3 I assess if the GAAD gap differs by race. I further assess Cox proportional hazard models to determine if risk of loss for common factors (i.e. smoking and vitamin use) associated with miscarriage risk is modified by race. I compare models that use GAAD with models that use traditional self-reported LMP to estimate gestational age for each exposure. If effect modification by race is found, I report stratified estimates in both models and determine the extent of potential bias in our reported estimates.

Race is a self-reported measure obtained during baseline interview at enrollment. For the purpose of this analysis race were grouped into the following categories non-Hispanic white (referred to throughout as "White") and non-Hispanic black (referred to throughout as "Black"). Women with missing information for race or who declined to self-identify their race were excluded from this analysis (n=9). Women who self-identified as Hispanic regardless of White or Black racial self-identification (n=361) or as other races(n=240), which include Native Americans and Asians, were also be excluded. Interaction terms were generated by strata of each exposure status and either Black or White race. Interactions were assessed on the multiplicative scale, using the likelihood ratio test to compare two nested regression models: the "full" model, containing the main effects and the interaction term, and the reduced model, containing only the main effects. If the likelihood ratio test has a p,0.10, the interaction term was retained in the final model.

Race and pregnancy intention interaction

In specific aim 2 I investigate the risk of miscarriage associated with either first-trimester smoking or vitamin use. Because pregnancy intendedness varies by race [4-6] in the U.S. general population and may influence behavior factors such as smoking and vitamin use I include an interaction for race and pregnancy intendedness in our models. The interaction term was generated as the multiplication of the pregnancy intendedness (Yes or No) times race (Black or White). Interactions were assessed using the likelihood ratio test to compare two nested regression models: the "full" model, containing the main effects and the interaction term, and the reduced model, containing only the main effects. If the likelihood ratio test has $p<0.10$, the interaction term was retained in our final adjusted models.

Specific aim 3: Hypothesis

For specific aim 3 *I hypothesize that the GAAD gap will be greater in Black women compared to White women.* Because Black women experience later losses, and since they are more likely to enroll later than White women in RFTS,[2] they are further along in pregnancy and less likely to have gestational arrest at time of ultrasound. Furthermore in time to event analyses *I hypothesize that within our cohort putative risk factors associated with miscarriage risk will not be modified by race.* Finally, for our secondary analyses *I hypothesize that Black women will have more unintended pregnancies compared to White women in this cohort, that the GAAD gap will be greater for unintended pregnancies than intended pregnancies among Black and White women and that that interaction between race and pregnancy intention will be significant in our models.*

Handling missing data and correlated data in analysis

All variables were checked to determine missingness. If missing data was found, ultrasound records were reviewed to assure whether data entered into the dataset were indeed missing. Only five women with miscarriages and ultrasound data had missing CRL or MSD measurements. For data from the first-trimester interview, if data were determined to be missing, I assessed how the missing observations were coded in the dataset. If the missing data was coded as a number, it was set to "." using Stata. If data are coded as 997 "Refused" or 998 "Don't know" the values were set to missing. No assumptions was made about the gestational age of individuals who were not available during a specific period of time. Correlations between variables were assessed with Spearman's or Pearson's correlation coefficients, where appropriate. Covariates with a correlation higher than 70% were not included simultaneously in a multivariate model, to avoid instability of regression coefficients and inflation of standard errors.

Potential considerations in analysis

I have outlined potential considerations and alternative approaches that could be used for this analysis below.

Early ultrasounds for abnormal development

Because of early first-trimester ultrasounds, RFTS may identify women who have a loss earlier than when they would have experienced that loss had they not enrolled in this study. This may be particularly important for women who have abnormal ultrasounds, and subsequently go on to miscarry. The mean gestational age at loss is slightly earlier for women who have abnormal or anembryonic gestation compared to women with normal appearing ultrasounds within our cohort, although with similar confidence bounds.[2] These women may be more likely to be conscious of a potentially failed pregnancy before the miscarriage is clinically symptomatic. This bias is less likely for women who have anembryonic gestations because developmentally their pregnancies had already arrested regardless of an early first-trimester ultrasound. Furthermore, prior to the 10 weeks gestation, the small CRL dimensions are more susceptible to measurement and operator inaccuracies.[42] There is the potential for these early pregnancies to be identified as abnormal due to the measurement process on ultrasound. To account for this potential bias for women with abnormal ultrasounds, GAAD estimates have incorporated developmental stage at ultrasound plus variability based on midpoint between ultrasound and reported date of loss in specific aim 1.

Early ultrasounds *after* reported date of loss

Furthermore, there is a small subset of women who had an ultrasound done after their reported date of loss (n=19). These women might be in the process of a threatened miscarriage at

the time of ultrasound in which the full conceptus had not evacuated the uterus and heterogeneous tissues such as the gestational sac may distort the endometrial echo on ultrasound.[93] I first determined the date of pregnancy loss based on medical abstraction for these women. If the date of loss based on medical records is in fact before their ultrasound date, I did a sensitivity analysis removing these women to determine if risk estimates for miscarriage change appreciably in specific aims 1 and 2. Both the overall analyses and analyses with women who have date of loss before ultrasound are reported.

Follicular phase length for scans ≥45 days from self-reported LMP

Another potential limitation in determining timing of loss is the uncertainty in pregnancy dating. After the occurrence of a positive pregnancy test, time of conception is traditionally dated as two weeks after the LMP. However, dating may be imprecise due to differences in follicular phase length and time between menses and ovulation.[29] I can do a sensitivity analysis restricting women who are scanned ≥45 days from self-reported LMP, a time in which fetal viability should be present by ultrasound detection for all women to determine if risk estimates for miscarriage change appreciably for specific aim 2. Both the overall analyses and analyses with only women with scans ≥45 days from self-reported LMP were reported for specific aim 1.

Differences in fetal growth by race for chosen nomograms

The nomograms chosen to estimate GAAD were based on results from populations very early in pregnancy. The racial distributions for these populations are not provided in these studies. [45,47] Early first-trimester fetal growth varies by race, however these differences are very small. Blacks have a greater increase in CRL compared to Whites, equivalent to an extra increase in CRL of 0.019 mm per day of gestation.[16] This increase overall accounts for 0.81 mm

difference in CRL at six weeks gestation compared to White women.[16] Since differences in fetal growth very early in pregnancy are minute, these do not necessarily result in clinically meaningful differences. Therefore I do not believe that the racial distribution of the populations used for our chosen nomgrams will significantly alter the conclusions of this analysis.

CHAPTER IV

ESTIMATING THE GAP BETWEEN TIME OF GESTATIONAL ARREST AND CLINICAL SYMPTOMS OF MISCARRIAGE

Abstract

Approximately 15% of recognized pregnancies end in miscarriage before 20 weeks. Conventionally, gestational age at loss is estimated from the first day of a woman's last menstrual period (LMP) until self-reported loss based on symptoms or care is sought. However, embryologic development may stop days to weeks prior to onset of clinical symptoms of miscarriage. Women from the southeastern United States (North Carolina, Texas and Tennessee) were enrolled from 2000-2012 in *Right from the Start*, a prospective community-based pregnancy cohort. RFTS conducts standardized early first-trimester transvaginal ultrasounds, as well as detailed interviews for all participants. Developmental stage of arrest prior to loss was estimated from ultrasound and used to calculate the mean gap between embryologic arrest and clinical loss. Ultrasounds, conducted between 40 and 95 days gestation from LMP, were available for nearly three-quarters of women who experienced miscarriage (n=509, 73.0%). Mean gestational age at clinical loss based on LMP was 71.7±22.2 days. Gestational arrest prior to miscarriage was observed in 38.7% of losses (n=197). Estimated mean gestational age at arrest of development (GAAD) was 58.1±16.1 days. The mean GAAD gap was nearly three weeks (GAAD gap 19.3±15.0days). The mean gap between LMP and estimated gestational age at arrest did not differ by race or pregnancy intention. Basing timing of miscarriage from LMP to clinical recognition of loss ignores the developmental state of the embryo prior to the loss in cases in which the pregnancy arrested earlier. In models that estimate risk of time-varying

exposures in early pregnancy, this gap has the potential to bias effect estimates by over-estimating or mis-assigning exposure time.

Running Head: Gestational arrest prior to miscarriage

Key Words: Miscarriage, gestational arrest, ultrasound, reproductive epidemiology, prospective cohort

Introduction

Approximately 10 to 15% of clinically recognized pregnancies end in miscarriage, defined as a pregnancy loss before completion of 20 weeks of gestation.[1,8,94,95] More than 30% of all conceptions may end in loss when taking into account unrecognized pregnancies. [1] Conventionally, pregnancy is dated from the first day of a woman's last reported menstrual period (LMP) and time from LMP is referred to as gestational age. In studies of miscarriage, LMP is often used to estimate gestational age at loss; however, embryologic development may stop days to weeks prior to the onset of clinical recognition of miscarriage. Basing timing of miscarriage on the time from LMP to the clinical recognition of loss alone ignores the developmental state of the embryo prior to the loss. This is potentially problematic if a pregnancy had, as is clinically known to be common, in fact arrested earlier.[2,93,96-101]

Prior work from our group indicates nearly forty percent of losses in this cohort have anembryonic gestation or abnormal development of the embryo as assessed by absent fetal heart rate or fetal pole development inconsistent with dates.[2] Ultrasound can be used to estimate when development stopped prior to clinically recognized miscarriage. In reproductive epidemiology, misattributing exposure time may in fact result in overestimation of certain risk factors and lead to biased estimation of the effect of exposures associated with miscarriage.

A better understanding of the patterns in timing of loss and the differences between ultrasound developmental stage at arrest and clinical onset of symptoms for miscarriage are important for epidemiologic studies. We aim to describe the variation and distribution in the number of days between ultrasound estimated developmental stage at arrest and clinical onset of symptoms of miscarriage among women who experience a pregnancy loss within a prospective pregnancy cohort.

Materials and Methods

Study population and data collection

Right from the Start (RFTS) is a prospective community-based pregnancy cohort study that began enrollment in 2000. Women, either pregnant or planning a pregnancy, enrolled from metropolitan and suburban areas in south-eastern United States in three states (Texas, Tennessee and North Carolina). The study sites included Galveston, Texas; Chattanooga, Knoxville, Memphis and Nashville, Tennessee and the Greater Triangle region of North Carolina(including Raleigh, Durham and Chapel Hill). Participants were between 18 and 45 years of age, spoke English or Spanish, intended to carry the pregnancy to term, and had not used assisted reproductive technologies to conceive. [3,73,79] Over time, the study has included three phases (RFTS 1, 2, and 3).

Women who were not yet pregnant but trying to conceive could pre-enroll before pregnancy and were followed until a positive pregnancy test. To avoid over-enrollment of sub-fertile women, pre-enrolled women in the study must have been attempting to get pregnant for fewer than six months (RFTS 1 and 2) or fewer than three months (RFTS 3). Women were provided pregnancy test kits for up to 12 months of pre-enrollment. Women formally enrolled in the study before 12 completed weeks of gestation (RFTS 1), before nine completed weeks of gestation (RFTS 2), or at time of positive pregnancy test after pre-enrollment prior to pregnancy (RFTS 3). [79] Informed, written consent was obtained from each study participant in compliance with institutional review board procedures.

Women who had their LMP before May 5, 2012 were included in this dataset (n=6,105). Participants had an early-pregnancy ultrasound for assessment of embryologic viability, documentation of stage of development, and confirmation of gestational dating for ongoing

pregnancies. Accuracy of self-reported LMP dating in this cohort is excellent, with a mean difference of less than one day, and has been previously described. [77] Research ultrasounds were conducted at a time in gestation (> 5 4/7 weeks after LMP) in which normal pregnancies would be expected to have a fetal pole and heart rate identifiable. Participants completed a baseline interview at the time of enrollment and comprehensive computer assisted telephone interview in the first-trimester. Information collected from the interview included reproductive and medical history, socio-demographic characteristics, and health behaviors around the time of conception or during pregnancy. Participants who experienced pregnancy loss before the scheduled interview were interviewed as soon as possible after the loss.

Pregnancy outcomes were self-reported by study participants and verified by medical records. Exclusions from the analysis include women who enrolled during more than one pregnancy (n=333, only the first pregnancy was included), women who had induced abortions (n=17), women who had a missing pregnancy outcome status at the time of analysis (n=231), and women who had ectopic/molar pregnancies (n=11).

Variable definitions GAAD

Miscarriage was defined as loss of a recognized pregnancy prior to 20 completed weeks of gestation using LMP dating. Pregnancy was verified by ultrasound or repeat pregnancy test. We documented 697 miscarriages during the study period. Of the women who experienced loss nearly one-third experienced their loss prior to ultrasound (n=188).

Women with ultrasounds were classified based on developmental stage at loss (i.e. gestational age at time of arrested development). This new estimation for gestational age at arrest of development is referred to as GAAD. GAAD was estimated using prespecified established nomograms[45,47] to estimate gestational age using key characteristics from ultrasound (Table 1).

Developmental stage at ultrasound was initially categorized based on the following characteristics, normal fetal development, abnormal fetal development, and anembryonic development at time of first-trimester transvaginal ultrasound **(Table 4-12).**

Women considered to have normal fetal development included those with a fetal pole and normal fetal heart rate (FHR) at ultrasound. Women considered to have abnormal fetal development included women with a visible fetal pole and either abnormal or no FHR visible on ultrasound. Women who are considered to have anembryonic development include women with an empty uterus (and no subsequent diagnosis of ectopic gestation), gestational sac only, or gestational and yolk sac visible on ultrasound but no fetal pole or FHR visible.

Estimating GAAD within RFTS was done in the following way: GAAD was not calculated for women who had a loss before their transvaginal ultrasound. Women with anembryonic gestation had a GAAD equal to the gestational age at time of arrested development using the criteria outlined in Table 1. The earliest structure in development that can be visualized on transvaginal ultrasound is the gestational sac between 4.5 to 5 weeks from LMP. Among women with anembryonic development in which gestational sac or gestational and yolk sac are visible, GAAD was estimated based on the formula by Goldstein and colleagues. [47] A special case of anembryonic gestation is women with empty uterus. Among women with an empty uterus in which neither the yolk sac or gestational sac are visible, GAAD was assigned based on the estimated gestational age based on self-reported LMP to the date of the ultrasound if less than 4.5 weeks (i.e. 32 days). If greater than 4.5 weeks, 32 days gestation for these women was assigned.

For women who had normal or abnormal fetal development on ultrasound in which a fetal pole could be visualized we estimated GAAD based on a nomogram for CRL by Hadlock

and colleagues [45] plus the midpoint from date of ultrasound to date of loss. Since we do not know the exact date that embryologic development stopped for women with normal and abnormal ultrasounds from a single ultrasound measurement, we simplified estimating GAAD by choosing the midpoint between date of ultrasound and date of reported loss for these women.

Based on the data available from early first-trimester ultrasounds, we used the following formulas to estimate GAAD in days gestation for women in this cohort (Hadlock et al. [45] (Formula A) and Goldstein et al. [47] (Formula B)):

A. $t=\exp[1.685+0.316(CRL)-0.049(CRL)^2+0.004(CRL)^3-0.0001(CRL)^4]x7$

B. $t=L+42$

where *t* refers to the gestational age in number of days and L the measurement of length of the mean gestational sac diameter (MSD) in millimeters (mm). Many studies have shown that the crown-rump length (CRL) is the most consistent measurement for determining gestational age in early development.[36,37,45,46] The Hadlock formula estimates gestational age in weeks gestation and with CRL measured in centimeters (cm). The Hadlock formula was multiplied by seven in order to estimate GAAD in days gestation. GAAD was rounded up to the nearest whole integer in days gestation. Women who had ultrasounds but were missing either CRL or MSD measurements were excluded when estimating GAAD (n=4). We were able to estimate GAAD for 504 women who experienced a loss and had ultrasound measurements. We also determined the GAAD gap for these women. The GAAD gap was defined as the difference in days gestation between self-reported LMP date of loss and the newly estimated GAAD. A study subject flow chart for specific aim 1 can be found in **Figure 4-7**.

Statistical analysis

We report the overall distribution of estimated gestational age based on LMP and GAAD based on developmental state of the embryo for women who experienced loss and had ultrasounds in RFTS. In order to better understand characteristics of GAAD, we conducted initial exploratory and descriptive analyses to assess the GAAD gap. Chi-square testing was used to compare categorical variables. For categories with fewer than five individuals, Fisher's exact test was used. Continuous variables were compared using t-test. Gestations were further categorized based on the median GAAD gap within this cohort (median GAAD gap 19 days). We tested GAAD gap for associations with other established predictors of miscarriage risk (e.g. age, parity, pregnancy intention, prior history of loss) to determine if these factors differ among those women with longer than median (> 19 days) vs. shorter than median GAAD gap (≤ 19 days). Both overall results and results with GAAD gap dichotomized at the median are presented. We used a sign-rank test to compare the observed versus hypothesized GAAD gap of 10 days.

We also conducted sensitivity analyses to determine the robustness of our GAAD distribution within this cohort. These include the following scenarios: 1. To address potential recall bias, we stratified our analysis by whether participants completed their interviews before or after their loss, 2. to investigate timing of loss based on key embryologic development, we dichotomized loss at 10 weeks, the median gestational age at time of loss based on LMP for our cohort. We grouped losses into early loss (prior to 10 weeks gestation) and late loss (≥ 10 weeks gestation). We further restricted women who had ultrasounds conducted ≥45 days from LMP, a time in which fetal viability determined by fetal pole and visible heart rate should be present, 3. to eliminate the potential for prior pregnancy outcomes to have an influence on the quality of reporting we restricted our analysis to women in their first pregnancies, with intended

pregnancies and women who were not pregnant prior to enrollment, 4. to address potential for recurring reasons for loss, we also restricted our analysis to exclude those women with prior loss, recurrent loss (3 or more reported losses), and with a loss within 3 days of study enrollment. Analyses were conducted using Stata SE/12.1 software (StataCorp LP, College Station, TX).

Results

Six-hundred ninety-seven miscarriages were documented. Ultrasound data was available for nearly three-quarters of women with a pregnancy loss (n=509, 73.0%). Among women who experienced a pregnancy loss key participant characteristics did not differ significantly by ultrasound status (**Table 4-13.**). Compared to women with ultrasounds, women without ultrasounds were more likely to be slightly older, have a lower BMI (< 30kg/m^2), income > $80,000, have a college degree, White, be married, be parous (> 1+), no prior loss and to abstain from tobacco products. Additionally, women without ultrasounds were less likely than women with ultrasounds to be pregnant at time of enrollment and have a prior history of pregnancy loss. Mean gestational age at enrollment was similar among women with ultrasounds (6.5 weeks) and those without ultrasounds (6.6 weeks) (**Table 4-13**).

The mean gestational age at loss based on LMP for women without ultrasounds was 58.6 (±18.3) days (**Table 4-14**). Mean gestational age at clinical loss based on LMP was 71.7 (±22.2) days. Anembryonic gestation was observed in 38.7% of losses (n=197). Estimated mean GAAD was 58.1 (±16.1) days among women who had losses with ultrasound data available (**Table 4-14**). The median difference between GAAD and a clinically symptomatic loss based on LMP was 19 days(**Table 4-14**). The median midpoint between ultrasound and symptomatic loss for women with fetal poles was smallest among women with no heart rate (median midpoint no heart rate, abnormal heart rate, normal heart rate: 4, 7 and 14 days respectively) (results not shown). The observed GAAD gap differed significantly from the hypothesized GAAD gap of 10 days (sign rank test p-value=0.0000).

When dichotomized by the median GAAD gap within this cohort (i.e. 19 days) key participant characteristics did not differ significantly between those with longer vs, shorter than

median GAAD gap (**Table 4-15**). Women with a longer than median GAAD gap (> 19 days) however were more likely to enroll in Texas, have a slightly later gestational age at time of study enrollment, and less likely to report having recurrent miscarriage (3 or more losses) than women with shorter than median GAAD gap (≤ 19 days). (**Table 4-15**). Furthermore only twenty seven women had a GAAD gap tht fell within 3 days of their self-reported LMP (5.4%), suggesting most losses have observable delays in development. Of the 504 women with estimated GAAD, 39 did not complete the first-trimester interview [FTI] (7.7%), and 308 completed the interview after their loss (61.1%). In order to further evaluate the distribution of GAAD and the GAAD gap within this cohort, we conducted several sensitivity tests (Table 5). These included restriction by timing of loss, consideration of time of interview in relation to loss, restricting by parity, pregnancy intention, prior history of loss and loss within 3 days of enrollment. Across scenarios, the median estimates for GAAD varied from 49 to 65 days, and median GAAD gap varied between 11 and 23 days (**Table 4-16**).

Discussion

We used ultrasound data to evaluate fetal viability among study participants and assess developmental stage prior to pregnancy loss. We demonstrate that the mean GAAD gap between LMP and estimated gestational age at arrested development for women with miscarriage in this cohort is nearly three weeks (mean GAAD gap 19.3 ± 15.0days, median 19 days). Gestational arrest is observed in a majority of pregnancy losses and misclassifying timing of loss by as much as three weeks may result in biased risk estimates for the effect of early pregnancy exposures associated with loss. This is potentially problematic in studies of reproductive epidemiology in which data is often sparse and contradictory for factors influencing early pregnancy. Because of early first-trimester ultrasounds, RFTS likely identifies women will have a loss earlier than when developmental arrest would have been identified had they not enrolled in this study. The mean gestational age at clinical loss is slightly earlier for women who have abnormal or anembryonic gestation compared to women with normal appearing ultrasounds within our cohort, although with similar confidence bounds.[2] Women with abnormal ultrasounds may be more likely to be conscious of a potentially failed pregnancy before the miscarriage occurs. This bias is less likely for women who have anembryonic gestations because developmentally their pregnancies had already arrested regardless of an early first-trimester ultrasound. Furthermore, prior to 10 weeks gestation, the small CRL dimensions are more susceptible to measurement and operator inaccuracies, though such error is typically measured in days.[42] Nonetheless this introduces the potential for these early pregnancies to be identified as abnormal due to the ultrasound measurement process alone.

One potential limitation within this cohort is that gestational age was estimated based on measurements from a single ultrasound visit. However by taking cross-sectional ultrasound

information we have the ability to estimate probable developmental state prior to miscarriage and have a better developmental outcome measurement for loss than self-report. The formulas to estimate GAAD were chosen based on the two largest prior studies conducted in the United States to estimate gestational age from ultrasound characteristics. Both studies used a single early first-trimester transvaginal ultrasound visit to estimate GA within a study population that had not used in vitro fertilization in order to conceive. [45,47] Study ultrasounds used to develop the established nomograms were performed by physicians and or registered sonographers. Ultrasound measurements were made among singleton births in women with no diseases known to adversely affect fetal growth (e.g. diabetes mellitus) and consisted of subjects with a known LMP. Additionally, the formulas to estimate GAAD for women in our cohort remain a continuous variable in days gestation. Our work suggests it is possible for researchers to more accurately identify the timing of insults prior to pregnancy loss and to set aside exposures that occur after developmental arrest but before the onset of symptoms such as bleeding. This would prevent misattribution of exposure time.

Timing of miscarriage, as is typical in the literature, was defined in our study based on participant self-report, either based on the day of dilatation and evacuation or the day of heaviest bleeding for each woman. As in all studies without sequential ultrasound or high sensitivity human chorionic gonadotropin levels, we are unable to know the exact time of embryonic demise for participants who subsequently miscarry. For simplicity in this application we estimated GAAD from a previously developed nomogram and chose the mid-point between ultrasound date and date of loss for women with normal or abnormal ultrasounds. An ideal study would have repeated ultrasound measures on each subject with a known time of initiation until date of loss was clinically detectable. However, factors including cost and participant burden make such

studies difficult and unlikely to be conducted. Future studies that determine developmental state and measures developmental progress variables at one time point could use our analysis approach to assess exposure-time misclassification and influence on miscarriage risk for risk factors associated with pregnancy loss.

Another potential limitation of our approach is the generalizability of our findings may be limited by the enrollment of subjects. In order to elaborate differences in the timing of miscarriage we used ultrasound data from a diverse prospective community-based cohort, however we cannot exactly define the population base of women that was theoretically eligible to be in the study within these communities. RFTS emphasizes community-based recruitment among women who are planning a pregnancy and is likely less biased than recruitment from populations like academic medical centers. However, women who choose to enroll in a study of early pregnancy health may be different than the general population of women trying to get pregnant, potentially contributing to selection bias into the study. Participants in RFTS tend to be better educated, more health conscious with lifestyle factors related to pregnancy planning and have access to care that may restrict generalizablity.[73]

Analyses of clinical populations often overestimate the larger population occurrence of adverse birth outcomes because they potentially include women who may be at higher risk initially for adverse birth outcomes in addition to those women who can seek out prenatal care.[73,79] RFTS captures a greater proportion of actual pregnancy losses compared to clinic-based recruitment by enrolling women prior to the typical onset of prenatal care and by enrolling a proportion of women as they begin to plan conceptions.[3] Women do not alter prenatal care

choices to enroll, and therefore it is unlikely that enrollment procedures and study activities influenced outcomes within this population.

We conducted several sensitivity analyses in which to assess GAAD estimation within this cohort. Our overall results remain robust. Previous history of loss is an established predictor for future miscarriage.[1,8,57,102] We did a simple sensitivity analysis, removing women who had ever had a prior miscarriage (n=122) from the analysis. Estimates for GAAD did not change appreciably when these women were removed (median GAAD 54 days, median GAAD gap 19 days). One potential limitation in determining timing of loss is the uncertainty in pregnancy dating. After the occurrence of a positive pregnancy test, time of conception is traditionally dated as two weeks after the LMP. However, dating may be imprecise due to differences in follicular phase length and time between menses and ovulation.[29] Sensitivity analysis restricting women who are scanned ≥45 days from self-reported LMP, a time in which fetal viability should be present by ultrasound detection for all women did not change GAAD distribution appreciably (median GAAD 56 days, median GAAD gap 19 days). Finally we assessed if predictors of miscarriage differed significantly by median length of GAAD gap for women in this cohort. When we stratified by median GAAD gap (i.e. ≤19 days vs. >19 days) our results did not change appreciably (**Table 4-15**). Since distribution of the GAAD gap has not been the focus of prior research, we hypothesized that women with smaller GAAD gaps may indicate a subset of women who are more confident in their LMP dating, and by proxy other pregnancy-related behaviors may differ than women with larger GAAD gaps. However only a small subset of women (n=27) had a GAAD gap that fell within 3 days of their self-reported LMP and no notable differences in demographic characteristics were observed when these women were removed from analysis (data not shown).

Early fetal growth is important both in epidemiologic studies and clinical settings when related to reproductive outcomes such as miscarriage. Development may stop days to weeks prior to the onset of clinical recognition of miscarriage. This research proposes a novel method for assessment of gestational arrest prior to loss. We are not aware of prior studies that have addressed timing of loss in the context of gestational arrest using early first-trimester ultrasound information. Research that gives insight to the mechanisms operating during early pregnancy is useful, especially since this time period in pregnancy is not well understood. The timing of embryologic of fetal insult can help to differentiate distinct mechanisms of loss.[89,103] For example, chromosomal abnormalities have been observed in at least half of all pregnancy losses occurring in the first trimester, but represent a higher fraction of early losses than of later losses.[57] Basing pregnancy loss on the time from LMP to clinical recognition of loss ignores the developmental state of the fetus prior to the loss. Nearly 40% of pregnancy losses have arrest in gestational development when assessed by early first-trimester ultrasound.[2] This results in inappropriate overestimation of exposure time for common risk factors in early pregnancy. A better understanding of embryologic and fetal development in relation to miscarriage timing is important in epidemiologic studies when studying factors in early-pregnancy that may cause or prevent pregnancy loss by their presence in specific windows of embryologic development.

Table 4-12. Estimating Gestational Arrest at Development (GAAD) for women with miscarriage within *Right from the Start*, 2000-2012

Ultrasound Developmental Stage	Ultrasound Characteristics	No. of losses	Estimating gestational age at arrested development (GAAD)
Loss before ultrasound Anembryonic gestation	N/A	188	Cannot be estimated
	Empty Uterus	37	≤ 4.5 weeks: Self-reported LMP to date of ultrasound > 4.5 weeks: assign 32 days gestation
	Gestational sac only	83	Gestational age based on mean gestational sac diameter[47]
	Gestational and yolk sac	77	Gestational age based on mean gestational sac diameter[47]
Fetal pole present	No fetal heart rate	100	Gestational age based on crown rump length[45] + midpoint from date of ultrasound to date of loss
	Abnormal fetal heart rate	32	Gestational age based on crown rump length[45] + midpoint from date of ultrasound to date of loss
	Normal fetal heart rate	180	Gestational age based on crown rump length[45] + midpoint from date of ultrasound to date of loss

Table 4-13. Characteristics of participants who experienced miscarriage within *Right from the Start*, 2000-2012

	Miscarriage among RFTS Study Participants					
	With Ultrasounds N=509 (73.0%)			Without Ultrasound N=188 (27.0%)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Maternal age, years	30.6 (5.8)			30.6(5.5)		
Maternal age, years						
<20		17	3.3		3	1.6
20–24		65	12.8		21	11.2
25–29		134	26.3		51	27.1
30–34		163	32.0		70	37.2
≥35		130	25.5		43	22.9
Missing		0			0	
Body mass index[a]	26.4 (6.6)			25.3(5.5)		
Body mass index						
Underweight (<18.5)		13	2.6		5	2.9
Normal weight (18.5–24.9)		247	49.2		97	56.1
Overweight (25.0–29.9)		127	25.3		37	21.4
Obese (≥30.0)		115	22.9		34	19.7
Missing		7			15	
Household income						
≤$40,000		130	28.5		48	26.8
$40,001–$80,000		164	36.0		57	31.8
>$80,000		162	35.5		74	41.3
Missing		51			9	
Maternal education						
High school or less		86	16.9		26	13.8
Some college		89	17.5		28	14.9
College (≥4 years)		333	65.6		134	71.3
Missing		1			0	
Marital status						
Married, living as married, single		443	87.0		170	90.4
Other		66	13.0		18	9.6
Missing		0			0	
Race						
Non-Hispanic White		348	68.4		140	74.5
Non-Hispanic Black		104	20.4		32	17.0
Hispanic		32	6.3		10	5.3
Other		25	4.9		6	3.2
Missing		0			0	
Pregnancy Intention						
No		108	26.5		36	25.2

	Miscarriage among RFTS Study Participants					
	With Ultrasounds N=509 (73.0%)			Without Ultrasound N=188 (27.0%)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Yes		299	73.5		107	74.8
Missing		102			45	
Gestational age at enrollment, weeks	6.5 (1.6)	6.3[b]		6.6 (2.0)	6.2[b]	
Pregnant at time of recruitment						
No		158	31.0		68	36.2
Yes		351	69.0		120	63.8
Missing		0			0	
Parity						
Nulliparous		210	45.5		77	42.1
1		162	35.1		74	40.4
≥2		90	19.5		32	17.5
Missing		47			5	
Previous miscarriage						
No		340	73.6		129	70.5
Yes		122	26.4		54	29.5
Missing		47			5	
Recurrent Miscarriage(3 or more)[c]						
No		86	70.5		44	81.5
Yes		36	29.5		10	18.5
Missing		0			0	
Previous induced abortion						
No		380	82.3		158	86.3
Yes		82	17.7		25	16.7
Missing		47			5	
Diabetes						
No		448	95.5		175	96.7
Type 1		1	0.2		1	0.6
Type 2		4	0.9		1	0.6
Gestational diabetes						
Nulliparous		1	0.2		0	0.0
1		5	1.1		0	0.0
≥2		8	1.7		3	1.7
Missing		42			8	
Hypertension						
No		312	95.4		122	91.0
Yes		15	4.6		12	9.0
Missing		182			54	
Nausea						
No		156	33.3		69	38.1

	Miscarriage among RFTS Study Participants					
	With Ultrasounds N=509 (73.0%)			Without Ultrasound N=188 (27.0%)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Yes		313	66.7		112	61.9
Missing		40			7	
Site						
North Carolina		294	57.8		111	59.0
Tennessee		178	35.0		63	33.5
Texas		37	7.3		14	7.5
Prenatal vitamin use						
No		71	15.1		28	15.6
Yes		398	84.9		151	84.4
Missing		40			9	
Smoking						
Never		342	73.1		137	75.7
Current		23	4.9		4	2.2
Former		103	22.0		40	22.1
Missing		41			7	
Alcohol Use						
Never		57	12.2		17	9.4
Current		82	17.5		46	25.6
Former		330	70.4		117	65.0
Missing		40			8	

Abbreviations: SD, standard deviation; No., number.

[a] Body mass index: weight (kg)/height $(m)^2$.

[b] Median for gestational age at enrollment.

[c] Only among women who experienced a prior miscarriage 122 with ultrasound and 54 without ultrasound

Table 4-14. Estimating gestational age in pregnancies with miscarriage based on ultrasound characteristics for self-reported LMP and GAAD within *Right from the Start*, 2000-2012

Ultrasound Developmental Stage	Ultrasound Characteristics	RFTS Study Participants with Miscarriage					
		LMP (days)		GAAD (days)		GAAD gap (days)	
		Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
Loss before Ultrasound	N/A	58.6 (18.3)	57	N/A	N/A	N/A	N/A
Anembryonic gestation							
	Empty Uterus	60.9 (21.4)	55	32.4 (2.3)	32	28.5 (22.3)	23
	Gestational sac only	69.3 (15.3)	69	55.8 (11.0)	52	14.3 (15.5)	13
	Gestational and yolk sac	70.0 (16.7)	69	55.8 (10.6)	53	15.2 (18.4)	13
Fetal Pole							
	No fetal heart rate	74.0 (11.5)	73	53.2 (8.5)	51	21.8 (9.9)	22
	Abnormal fetal heart rate	72.8 (14.4)	72.5	52.9 (6.8)	52	20.9 (9.3)	18.5
	Normal fetal heart rate	88.0 (25.7)	84	69.3 (17.4)	65	19.7 (13.4)	19
Overall		**71.7 (22.2)**	**70**	**58.1 (16.1)**	**55.5**	**19.3 (15.0)**	**19**

LMP=Last menstrual period; GAAD=gestational age at arrest of development; SD=standard deviation; N/A=not applicable

Table 4-15. Characteristics of participants by median gestational age at arrest of development (GAAD) gap in *Right from the Start*, 2000-2012

	RFTS Study Participants with Miscarriage					
	GAAD gap >19 days N=246			GAAD gap ≤ 19 days N=258		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Maternal age, years	30.3 (5.9)			30.8 (5.8)		
Maternal age, years						
<20		10	4.1		7	2.7
20–24		37	15.0		28	10.9
25–29		60	24.4		72	27.9
30–34		79	32.1		82	31.8
≥35		60	24.4		69	26.7
Missing		0			0	
Body mass index[a]	27.2 (7.3)			25.7 (5.7)		
Body mass index						
Underweight (<18.5)		4	1.7		9	3.5
Normal weight (18.5–24.9)		114	47.1		129	50.6
Overweight (25.0–29.9)		59	24.4		68	26.7
Obese (≥30.0)		65	26.9		49	19.2
Missing		4			3	
Household income						
≤$40,000		64	28.4		65	28.8
$40,001–$80,000		81	36.0		51	35.8
>$80,000		80	35.6		80	35.4
Missing		21			32	
Maternal education						
High school or less		46	18.7		39	15.2
Some college		43	17.5		46	17.9
College (≥4 years)		157	63.8		172	66.9
Missing		0			1	
Marital status						
Married, living as married, single		212	86.2		227	88.0
Other		34	13.8		31	12.0
Missing		0			0	
Race						
Non-Hispanic White		163	66.3		181	70.2
Non-Hispanic Black		56	22.8		47	18.2
Hispanic		14	5.7		18	7.0
Other		13	5.3		12	4.6
Missing		0			0	
Pregnancy Intention						
No		61	28.8		49	22.8

	RFTS Study Participants with Miscarriage					
	GAAD gap >19 days N=246			GAAD gap ≤ 19 days N=258		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Yes		151	71.2		166	77.2
Missing		34			43	
Gestational age at enrollment, weeks	6.8 (1.7)	6.6[b]		6.2 (1.5)	6.0[b]	
Pregnant at time of recruitment						
No		69	28.0		85	33.0
Yes		177	72.0		173	67.0
Missing		0			0	
Parity						
Nulliparous		98	44.1		107	45.5
1		79	35.6		83	35.3
≥2		45	20.3		45	19.2
Missing		24			23	
Previous miscarriage						
No		166	74.8		169	71.9
Yes		56	25.2		66	28.1
Missing		24			23	
Recurrent Miscarriage(3 or more)[c]						
No		46	82.1		40	60.6
Yes		10	17.9		26	39.4
Missing		0			0	
Previous induced abortion						
No		184	82.9		191	81.3
Yes		38	17.1		44	18.7
Missing		24			23	
Diabetes						
No		218	94.8		225	96.2
Type 1		1	0.4		0	0.0
Type 2		2	0.9		2	0.9
Gestational diabetes						
Nulliparous		1	0.4		0	0.0
1		0	0.0		5	2.1
≥2		6	2.6		2	0.9
Missing		16			24	
Hypertension						
No		143	94.7		165	95.9
Yes		8	5.3		7	4.1
Missing		95			86	

	RFTS Study Participants with Miscarriage					
	GAAD gap >19 days N=246			GAAD gap ≤ 19 days N=258		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Fibroids						
No		214	87.0		221	85.7
Yes		32	13.0		37	14.3
Missing		0			0	
Age of menarche, years						
≤ 10		19	8.3		17	7.3
11–13		151	65.9		165	70.8
>13		59	25.8		51	21.9
Missing		17			25	
Nausea						
No		69	30.0		87	37.2
Yes		161	70.0		147	62.8
Missing		16			24	
Bleeding						
No		106	46.1		103	44.0
Yes		124	53.9		131	56.0
Missing		16			24	
Site						
North Carolina		132	53.7		161	62.4
Tennessee		89	36.2		85	33.0
Texas		25	10.1		12	4.6
Prenatal vitamin use						
No		37	16.1		32	13.7
Yes		193	83.9		202	86.3
Missing		16			24	
Smoking						
Never		168	73.0		169	72.5
Current		12	5.2		11	4.7
Former		50	21.7		53	22.8
Missing		16			25	
Alcohol Use						
Never		29	12.6		27	11.5
Current		34	14.8		46	19.7
Former		167	72.6		161	68.8
Missing		16			24	

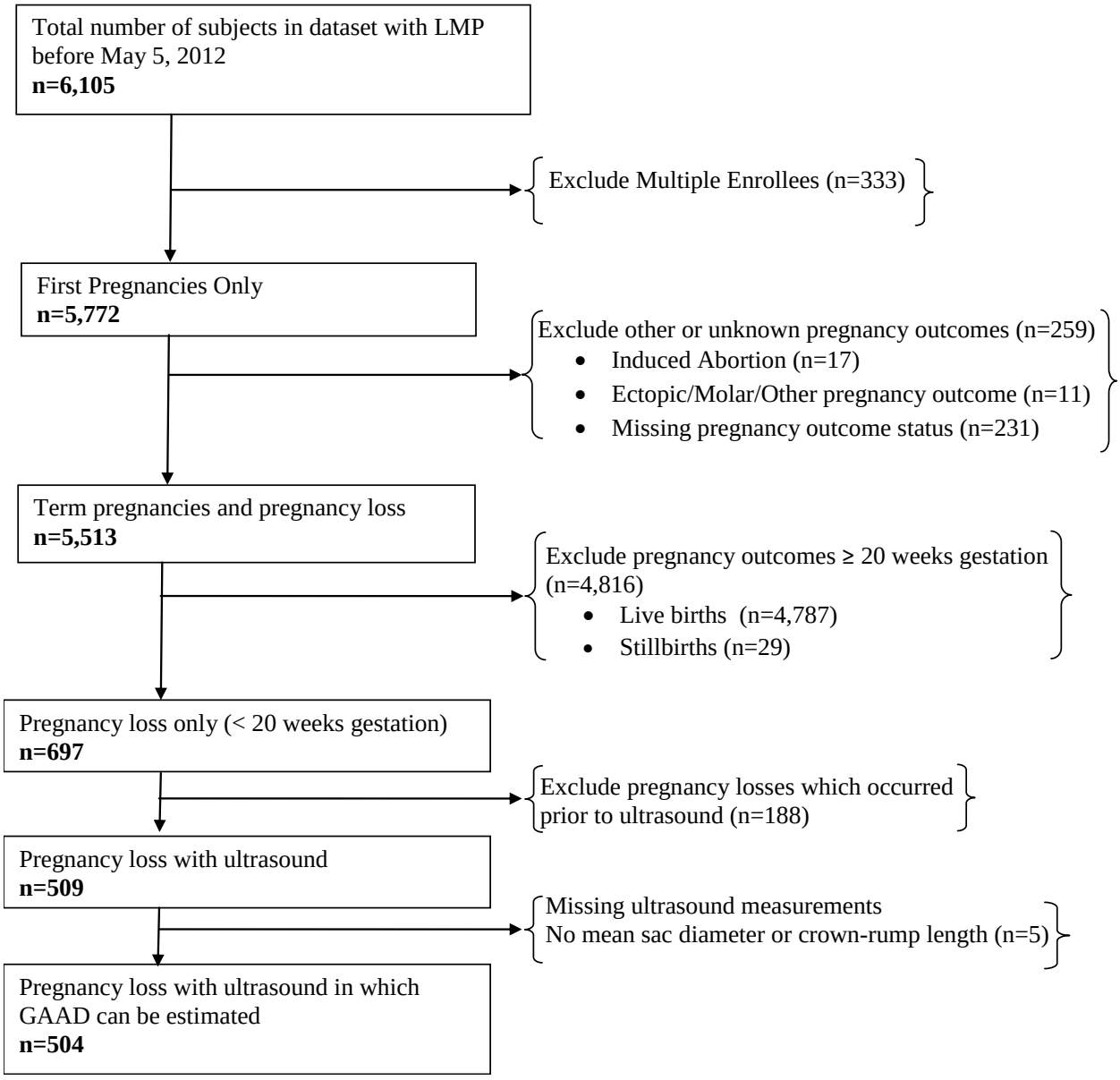
Abbreviations: SD, standard deviation; No., number. [a] Body mass index: weight (kg)/height $(m)^2$. [b] Median gestational age at enrollment.[c] Only among women who had prior miscarriage

Table 4-16. Further analyses and restrictions to evaluate gestational age at arrest of development (GAAD) in *Right from the Start*, 2000–2012

	RFTS Study Participants with Miscarriage					
			GAAD (days)		GAAD gap (days)	
	No. of Losses	% of Loss	Mean (SD)	Median	Mean (SD)	Median
Time of Miscarriage						
No Interview available	39	7.7	56.5 (21.4)	51	20.8 (21.4)	17
Interviewed before loss	157	31.2	67.4 (19.6)	65	23.5 (17.4)	23
Interviewed after loss	308	61.1	53.7 (10.6)	52	16.9 (12.0)	17
Early loss (<10 weeks) from LMP	201	39.9	48.4 (8.6)	49	10.6 (9.8)	11
Late loss (≥10 weeks) from LMP	303	60.1	64.7 (16.7)	62	25.0 (15.2)	23
Restrictions						
Nulliparous women only	205	40.7	57.1 (16.0)	55	20.0 (14.3)	19
Women with intended pregnancies only	295	58.5	55.8 (12.9)	53	18.8 (12.2)	19
Women not pregnant at time of enrollment	154	30.6	54.2 (14.0)	52	16.6 (13.6)	17.5
Ultrasound scan ≥ 45 days from LMP	446	88.5	58.5(15.8)	56	19.8(14.0)	19
Exclusions						
Women with prior loss	122	24.2	56.9 (15.8)	54	19.6 (15.1)	19
Women with recurrent loss (3 or more losses)[a]	36	7.1	58.0 (16.4)	56	19.6 (15.2)	19
Women with a loss within 3 days of enrollment	11	2.2	58.5 (16.1)	56	19.5(15.0)	19

Abbreviations: SD, standard deviation; No., number. [a]Includes women with 3 or more prior losses (n=13) as well as women with 2 prior losses with current outcome also a miscarriage (n=23).

Figure 4-7. Aim 1: Study subject inclusion criteria



CHAPTER V

COMPARING MODELS FOR TIMING OF MISCARRIAGE USING COMMON EARLY PREGNANCY EXPOSURES

Abstract

In miscarriage studies (pregnancy loss at <20 weeks), gestational age at time of loss is estimated from using the first day of a woman's last menstrual period (LMP) to the onset of symptoms. Conventionally embryologic development may stop weeks prior to onset of clinical symptoms. In models that estimate risk of time-varying exposures in early pregnancy, this gap between arrest of development and symptoms has the potential to bias effect estimates by over-estimating exposure time. To determine if failing to account for this gap influences effect estimates, we chose to assess both a risk and protective factor associated with miscarriage. Women were enrolled in Right from the Start (RFTS), a prospective pregnancy cohort, from 2000-2012. Participants completed study ultrasounds as well as detailed first-trimester interviews. We compared models that estimated gestational age based on self-reported LMP and models that incorporated gestational age at time of arrested development (GAAD). We used bootstrap methods to determine the magnitude of potential bias for both models. There were 697 observed miscarriages among 5,513 women. The risk of miscarriage was reduced among those who took vitamins for either models (adjusted hazard ratio (aHR) 0.41 95% confidence interval (CI) [0.30, 0.55] LMP model; aHR=0.43 95% CI [0.27, 0.69] GAAD model). Smoking during pregnancy was not associated with miscarriage (current smokers compared to never smokers aHR=0.93 95% CI [0.61, 1.41] LMP model; aHR=1.09 95% CI [0.64, 1.88] GAAD model). The bias ratio using bootstrap analysis was significant for smoking use (current smokers ratio=0.85,

95% CI [0.75, 0.94]) but not vitamin use (ratio=0.93, 95% CI [0.86, 1.02]). Misattributing exposure-time that results from arrest of development can bias risk estimates in studies of miscarriage.

Running head: LMP vs. GAAD Model comparison and magnitude of bias

Key Words: Miscarriage, gestational arrest, reproductive epidemiology, prospective cohort, early pregnancy exposures, misclassified exposure-time, bias

Introduction

Approximately 10 to 15% of clinically recognized pregnancies end in miscarriage, a pregnancy loss before completion of 20 weeks of gestation. [1,8,94] However, the existing classification of pregnancy loss ignores the developmental biology of a pregnancy loss, and focuses solely on clinical manifestation of a pregnancy loss. This is problematic for grouping women who may have different pathophysiology and thus different recurrence risk as the same condition. It further limits research and confounds epidemiologic data collection and assessment for reproductive outcomes. A potentially more useful way to classify pregnancy loss may be by developmental periods in gestation.[28] By more precisely identifying which insults have occurred prior to pregnancy loss and assessing exposures that occur after developmental arrest but before the onset of clinical symptoms researcher's will have more optimal method to assess miscarriage risk by not misattributing exposure time. Our group has demonstrated that the mean gap between an arrested pregnancy and the clinical manifestation of that loss in a diverse prospective community-based pregnancy cohort is on average approximately 19 days (or 2.7 weeks) but can range anywhere between 11 and 23 days (*work in preparation, Mukherjee et. al*). Our work suggests it is possible for researchers to more accurately identify the timing of insults prior to pregnancy loss and to set aside exposures that occur after developmental arrest but before the onset of symptoms such as bleeding.

By assessing a better methodological approach to classify timing of gestational arrest prior to loss we can determine the extent of potential overestimation of certain factors that may lead to biased estimates for their association with early pregnancy loss. Knowledge about risk factors influencing early pregnancy period is sparse and often contradictory. This may be due to

the heterogeneity in classifying different types of loss which may have very different etiologies, given that up to 40% of losses arrest earlier than onset of clinical symptoms when assessed by early first-trimester ultrasound.[2]

We wanted to study if the gap in timing of loss influences effect estimates for exposures associated with loss. Tobacco use remains one of the most commonly abused substances among pregnant women. Based on results from the National Natality Survey, smoking prevalence among US pregnant women was approximately 12%,[53] and of women who smoke, 60% stop as soon as they find out they are pregnant. [53] The association between early miscarriage and smoking has been inconsistent.[54-56,62,63,65-68] Some studies found an increase in risk of miscarriage among smokers,[54-61] while others have reported no association or only a weak relationship.[8,62-64] The inconsistencies may in part be dues to limitation in sample size, inadequate control for confounders, and differences in recall bias of smoking status among subjects. Alternatively, multi-vitamin supplementation is commonly recommended for all women who are pregnant or planning a pregnancy. Prenatal vitamin supplementation during early pregnancy is related to lower risk of neural tube defects and is associated with decreased risk of adverse pregnancy outcomes including preterm birth, pre-eclampsia and low-birth weight,[69-71] however the magnitude of association with miscarriage risk has remained imprecise.[8,61,72-76]

In reproductive epidemiology, the timing of exposures like these examples may be distinct or cumulative in their overall risk for loss and the contradictory findings may be further confounded by the heterogeneity in the type of loss assessed (i.e. early pregnancy loss vs. fetal death). A better assessment in the timing of loss that takes into account developmental stage at

arrest is warranted so that we can understand biologically meaningful causal associations between these candidate factors and miscarriage risk. We chose exposures that have been established in the literature as factors that influence fetal well-being, may be episodic during early pregnancy, and may either cause (e.g. smoking) or prevent (e.g. vitamin use) first-trimester miscarriage risk. We aim to compare models that take into account gestational age at arrest of development with those that use self-reported LMP and determine the magnitude of bias present in the latter estimates.

Materials and Methods

Study population and data collection

Right from the Start is an ongoing prospective community-based pregnancy cohort study that began enrollment in 2000. Over time, the study has included 3 phases designated "RFTS 1," "RFTS 2," and "RFTS 3." Women, either pregnant or planning a pregnancy, enrolled from 9 areas in 3 states (North Carolina, Texas, and Tennessee). Participants were between 18 and 45 years of age, spoke English or Spanish, intended to carry the pregnancy to term, and had not used assisted reproductive technologies to conceive.[3,73,79] The study was designed to recruit women from a variety of clinic- and community-based settings and has been described in detail elsewhere.[3] Informed, written consent was obtained from each study participant in compliance with institutional review board procedures and approvals.

Women who had their last menstrual period before May 5, 2012, were included in this data set (n=6,105). Participants had an early pregnancy ultrasound examination for assessment of embryological viability, documentation of stage of development, and confirmation of gestational dating. The accuracy of self-reported last menstrual period dating in this cohort is excellent and has been described.[77] Research ultrasound examinations were conducted at a time in gestation (>5 4/7 weeks from the LMP) in which normal pregnancies would be expected to have a fetal pole and heart rate. Participants completed a baseline interview at the time of enrollment and a comprehensive computer-assisted telephone interview in the first trimester. In the interview, information collected included reproductive and medical history,

sociodemographic characteristics, and health behaviors around the time of conception or during pregnancy. Participants who experienced pregnancy loss before the scheduled interview were interviewed as soon as possible after the loss.

Pregnancy outcomes were self-reported and verified by medical records. Exclusions from the analysis include women who enrolled during more than one pregnancy (n=333, only the first pregnancy was included), women who had induced abortions (n=17), women who had a missing pregnancy status at the time of analysis (n=231), and women who had ectopic/molar pregnancies (n=11).

Variable definitions

The primary exposures of interest in this analysis are smoking and vitamin use. Exposure status is a self-reported measure acquired during the first-trimester interview. For this analysis, smoking was grouped into the following categories: never smokers (referent), current smokers, and former smokers (within 4 months prior to the interview, meaning exposures in the pregnancy and/or periconception window, or ≥4 months from interview). Information about the frequency of use was also obtained. Questions were asked separately for prenatal vitamins and multivitamins (see Appendix); in order to remain consistent with previously published work within this cohort both categories of supplements were combined because of potential misclassification by participants. Both types of supplements were referred to simply as ‘‘vitamins.’’ Participants were asked whether they were currently taking vitamins or, in the case of a miscarriage, whether they had taken vitamins during pregnancy. Information about the frequency and timing of

vitamin use in an average week was also obtained. Participants who reported any use of vitamins during pregnancy were considered exposed.

Miscarriage was defined as loss of a recognized pregnancy prior to 20 completed weeks of gestation from LMP. We documented miscarriage in 697 women (12.6%) during the study period. Pregnancy was verified by ultrasound examination. Participants who had birth outcomes at a gestational age later than 20 weeks served as a comparison group. The comparison group (n=4,816) consisted of women who had livebirths (n=4,787) or stillbirths (n=29). **Figure 5-8** summarizes subject inclusion for specific aim 2.

Statistical analysis

Confounders

Potential confounders examined from baseline and the first-trimester interviews included factors recognized to be associated with both miscarriage and the exposure of choice. Candidate confounders related to sociodemographic factors included age (years); household income (≤$40,000, $40,001–$80,000, >$80,000 (referent)); maternal education (high school or less, some college, 4 or more years of college (referent)); marital status (married/living as married (referent), other); and maternal race (non-Hispanic White (referred to throughout as "White") (referent), non-Hispanic Black (referred to throughout as "Black"), Hispanic regardless of white or black racial self-identification, and other races which include Native Americans and Asians). In addition, we assessed potential confounders related to maternal reproductive history and health

behaviors during pregnancy, namely, parity (yes/no), previous induced abortion (yes/no), diabetes status (yes/no), prenatal vitamin use (yes/no), alcohol use (never, current, former, within 4 months prior to the interview (meaning exposures in the pregnancy and/or periconception window) or ≥4 months from interview), smoking status (never, current, former), and study site (Galveston, Texas; Raleigh and Research Triangle Park, North Carolina (referent); and Chattanooga, Knoxville, Memphis and Nashville, Tennessee). We did not consider prior history of miscarriage as a confounder in our data because we would be potentially overadjusting when a factor that caused a previous miscarriage may also be a causal factor in the current pregnancy.[80]

Timing of pregnancy loss and regression analysis

Cox regression was used to estimate hazard ratios for the association between either smoking or vitamin use and risk of miscarriage. Participants were followed from the time of enrollment in the study and contributed to analysis until an outcome or loss to follow-up occurred. Cox models accounted for variable gestational age at study entry and were used to screen candidate confounders. Confounding was defined as a greater than 10% change from the crude hazard ratio for miscarriage risk for current or former smokers compared to never smokers (referent) or those exposed to vitamin use (referent) compared to those unexposed to vitamin use. If a 10% change was observed from the crude hazard ratio, the variable was retained in the final models.

Gestational age at the time of loss was calculated from the first day of the LMP for the index pregnancy to the end of that gestation. In addition, we used ultrasound examination

findings by grouping losses into developmental stage documented on ultrasound examination prior to pregnancy loss and estimated a gestational age at arrested development (GAAD). Separate Cox models were used to compare overall risk for miscarriage with early pregnancy exposures using gestational age based on either self-reported LMP (i.e. LMP models) or estimated GAAD (i.e. GAAD models). LMP models include all women with losses, including women without ultrasounds. GAAD models include women with losses and ultrasound information. We restricted the analysis to those with complete covariate information. Final hazard models for smoking exposure included adjustment for maternal age and alcohol use and for vitamin use included adjustment for age only. To optimize fit, maternal age was specified by the inclusion of linear and quadratic terms in the model.

Bias ratio and bootstrap analysis

Finally in order to assess the potential magnitude of bias in our risk estimates for miscarriage risk we used bootstrap analysis to estimate the ratio of effect sizes between models. Bootstrapping approaches use resampled data to make adjustments for statistical biases as well as random error. The bias ratio was defined as the ratio between the two models (i.e. bias ratio= $\frac{\text{adjusted HR ratio of LMP model}}{\text{adjusted HR ratio of GAAD model}}$) for either exposure and miscarriage risk. We conducted a 1000 bootstrap replications to estimate the bias ratio and 95% confidence interval between models. All analyses were conducted using Stata SE/12.1 software (StataCorp LP, College Station, Texas).

Results

Nearly 13% of women experienced a miscarriage in this cohort (n=697, 12.6%). Ultrasound data was available for most women who had term births (n=4,694, 97.5%). Of women who reported a pregnancy loss, 73% had ultrasound data (n=509). Most key participant characteristics did not differ significantly by pregnancy outcome (Table 1). However, women who had miscarriages tended to be slightly older, have a higher household income (>$80,000), have a prior history of miscarriage and indicated having used alcohol compared to women who had term births (Table 1). Mean gestational age at the time of enrollment was earlier for women with miscarriage (6.5 weeks) compared to women who had term births (7.0 weeks) (**Table 5-17**). Twenty-seven percent of the women in our cohort were recruited prior to pregnancy (n=1,468) (**Table 5-17**).

Self-reported smoking and amount of smoking (i.e. number of cigarettes per day) did not differ significantly by pregnancy outcome (**Table 5-18**). The majority of women in our cohort reported being never smokers (n=3,863; 70.1%). Women who had miscarriages were less likely to report early first-trimester vitamin use (n=601; 92.8%) compared to women who had term births (n=4,432; 96.6%) (**Table 5-18**). The majority of women who indicated exposure to vitamins during the first-trimester reported taking them five or more times per week (n=4,523; 89.9%). There were less than five percent missing values among self-reported early pregnancy exposure status from the first-trimester interview (missing smoking status n=260, 4.7%; missing vitamin use n=275, 4.9%) (**Table 5-18**).

Risk of miscarriage was significantly reduced with exposure to early pregnancy vitamin use when adjusted for maternal age in either LMP or GAAD based models (adjusted hazard ratio

(aHR) = 0.41 95%, confidence interval (CI) [0.30, 0.55] LMP model; aHR=0.43, 95% CI [0.27, 0.69] GAAD model) (**Table 5-19**). When we assessed frequency of vitamin exposure during early first-trimester the risk of miscarriage was still significantly reduced (**Table 5-19**). This protective effect was most evident among women who reported taking vitamins less than five times per week compared to women who did not report any vitamin use during early pregnancy (aHR=0.28, 95% CI [0.18, 0.43] LMP model; aHR=0.28 95% CI [0.15, 0.52] GAAD model) (**Table 5-19**).

Adjusted for maternal age and alcohol use, smoking during early pregnancy was not associated with miscarriage risk in either models in this cohort (current smokers compared to never smokers aHR=0.93, 95% CI [0.61, 1.41] LMP model; aHR=1.09, 95% CI [0.64, 1.88] GAAD model; former smokers compared to never smokers aHR=0.88, 95% CI [0.73, 1.07] LMP model; aHR=0.93, 95% CI [0.72, 1.20] GAAD model) (**Table 5-19**). When assessing frequency of smoking associated with miscarriage, though no significant effect was observed, the number of cigarettes per day among current smokers compared to never smokers occurred in opposite directions for the GAAD model compared to the LMP model (< 10 cigarettes per day compared to never smokers aHR=0.88, 95% CI [0.52, 1.51] LMP model; aHR=1.03, 95% CI [0.53, 2.00] GAAD model; (≥ 10 cigarettes per day compared to never smokers aHR=0.93, 95% CI [0.49, 1.77] LMP model; aHR=1.07, 95% CI [0.43, 2.66] GAAD model) (**Table 5-19**). The amount of smoking among former smokers compared to never smokers was similar in both LMP and GAAD models (**Table 5-19**).

We conducted bootstrap analysis of our two models using a thousand replications to compare the ratio of effect sizes (i.e. bias ratio= $\frac{\text{adjusted HR ratio of LMP model}}{\text{adjusted HR ratio of GAAD model}}$). For early pregnancy smoking, the bias ratio comparing the two models was 15% for current smokers and 5% for former smokers (current smokers ratio=0.85, 95% CI [0.75, 0.94]; former smokers ratio=0.95, 95% CI[0.92, 0.97]) (**Table 5-20**). The bias ratio was not significant for early pregnancy vitamin use in this cohort (ratio=0.93, 95% CI [0.86, 1.02]) (**Table 5-20**). Similar results were observed for consistency of early-pregnancy exposure (**Table 5-20**). This suggests that misclassification in timing of loss influences risk estimates for exposures associated with loss. It further implies that strong effects associated with loss may be less variable and prone to bias when gestational arrest prior to loss is properly classified.

Discussion

Our analysis demonstrates that proper classification of gestational age at arrest may bias the risk estimates of miscarriage associated with early-pregnancy smoking when compared to models that use gestational age estimated by self-reported LMP alone. Models that use self-reported LMP to estimate gestational age differ by as much as 15% for current smokers and 5% of former smokers when compared to models that use GAAD to estimate gestational age. Although, a similar magnitude of bias was observed with early pregnancy vitamin use (7%), the small number of women reporting no vitamin use may have affected precision estimates, but still likely indicates some evidence of bias present. These results suggest early-pregnancy exposures associated with miscarriage risk are influenced by proper classification of gestational arrest prior to loss.

We demonstrate that misclassifying time at loss in studies of miscarriage results in biased risk estimates. Embryologic development may stop weeks prior to the onset of clinical symptoms or diagnostic recognition of miscarriage.[2,93,96-101] We used ultrasound data to evaluate fetal viability among study participants and assess developmental stage prior to pregnancy loss. Because of early first-trimester ultrasounds, RFTS likely identifies women who will have a pregnancy loss earlier than when developmental arrest would have been identified had they not enrolled in this study. We are able to identify gestational arrest prior to clinically recognized loss in nearly 40% of all losses. Our aim was to assess a better methodological approach to classify timing of gestational arrest prior to loss in order to determine the extent of potential overestimation of putative factors that may lead to biased estimates for their association with early pregnancy loss.

The early-pregnancy exposures that were chosen were a means to assess this new methodological approach within a diverse prospective pregnancy cohort. These factors have been associated with first-trimester miscarriage in the literature, have been established to influence fetal well being and may be episodic during early pregnancy. We wanted to compare this new approach to what is traditionally used in miscarriage studies to estimate gestational age, namely self-reported LMP. Bias was assessed with bootstrap analysis. Bootstrap methods were used to estimate and compare effect size between models with different outcome measurement (i.e. that is miscarriage outcome based on GAAD vs. miscarriage outcome based on LMP) and to assess the robustness of our findings. Bootstrapping allows for resampling within the data and can be used as a way to assess internal study validity. Bootstrapping approaches use resampled data to make adjustments for statistical biases as well as random error, [89] are used to estimate parameters and their variability in a given model and can be applied to Cox regression analyses.[91]

One potential limitation within this cohort is that gestational age was estimated based on measurements from a single ultrasound. However by taking cross-sectional ultrasound information we have the ability to estimate probable developmental state prior to miscarriage and have a better developmental outcome measurement for loss than self-reported LMP. Another limitation may include collection of data in first-trimester interview in relation to pregnancy loss. Of the women who had miscarriages, 5% did not complete the first-trimester interview (n=38) and for approximately 64% of women (n=445), ascertainment of behavioral factors during pregnancy including alcohol consumption, smoking, and vitamin use, was collected after the loss. Among women who experienced a loss prior to their interview, the mean interval between

loss and first-trimester interview was less than 3.5 weeks (24.8 sd (17.5) days), and the questions in the interview were clearly asking about behaviors during their recent pregnancy. In addition, less than five percent of data was missing for these women, suggesting that RFTS does a thorough assessment of collecting data for many early-pregnancy behaviors and characteristics.

The primary strength of our study is our ability to follow a large diverse sample of women recruited from the community prospectively through their pregnancies, many of whom were enrolled prior to pregnancy.[3] In addition, we were able to evaluate numerous potential confounders and analyze the data with hazard models that account for variation in gestational age at study entry. A study of miscarriage requires careful assessment of gestational time at study entry because women who enter a study later will have less opportunity for a miscarriage to be observed than women who enter very early in pregnancy. Furthermore, RFTS avoids over selection of women who may be subtly symptomatic or at high risk by advertising as a study about pregnancy health. Women do not alter prenatal care choices to enroll, and therefore it is unlikely that enrollment procedures and study activities influenced behaviors or outcomes within this population.

Research that gives insight to the biologic mechanisms of exposures operating during early pregnancy is useful, especially since this time period in pregnancy is not well understood. A primary challenge in reproductive epidemiologic research is the accurate and early exposure assessment during early pregnancy. An analysis that takes into account gestational arrest prior to clinical loss would prevent misattribution of exposure time in epidemiologic studies of early-pregnancy exposures and miscarriage risk. Estimates of miscarriage risk very early in pregnancy are in the time period most vulnerable to this bias, so further studies with early ascertainment of

pregnancy and careful longitudinal follow-up are needed. Next steps in this type of analysis could include assessing the influence of time-varying exposures such as over-the counter medication use,[104] or anti-depressant use in early first-trimester[105-107] both of which have been associated with increased miscarriage risk. By more accurately identifying which insults have occurred prior to pregnancy loss and assessing exposures that occur after developmental arrest but before the onset of bleeding we will have more optimal method to assess miscarriage risk by not misattributing exposure time.

Table 5-17. Comparing common characteristics by pregnancy outcome among *Right from the Start*, 2000–2012

	RFTS Study Participants					
	Miscarriage N=697 (12.6%)			Term Births N=4,816 (87.4%)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Maternal age, years	30.6 (5.7)			28.7 (5.0)		
Maternal age, years						
<20		20	2.9		166	3.5
20–24		86	12.3		789	16.4
25–29		185	26.5		1,713	35.6
30–34		233	33.4		1,540	32.0
≥35		173	24.8		605	12.6
Missing		0			3	
Body mass index[a]	26.1 (6.4)			25.8 (6.2)		
Body mass index						
Underweight (<18.5)		18	2.7		121	2.6
Normal weight (18.5–24.9)		344	51.0		2,552	53.9
Overweight (25.0–29.9)		164	24.3		1,121	23.7
Obese (≥30.0)		149	22.1		938	19.8
Missing		22			84	
Household income						
≤$40,000		178	28.0		1,376	30.9
$40,001–$80,000		221	34.8		1,693	38.0
>$80,000		236	37.2		1,385	31.1
Missing		60			343	
Maternal education						
High school or less		112	16.1		850	17.7
Some college		117	16.8		872	18.1
College (≥4 years)		467	67.1		3,094	64.2
Missing		1			0	
Marital status						
Married, living as married, single		613	88.0		4,286	89.0
Other		84	12.0		530	11.0
Missing		0			0	
Race						
Non-Hispanic White		488	70.0		3,409	70.9
Non-Hispanic Black		136	19.5		870	18.1
Hispanic		42	6.0		3.19	6.6
Other		31	4.5		209	4.4
Missing		0			9	

	RFTS Study Participants					
	Miscarriage N=697 (12.6%)			Term Births N=4,816 (87.4%)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Pregnancy Intention						
No		144	26.2		1,151	29.5
Yes		406	73.8		2,751	70.5
Missing		537			96	
Gestational age at enrollment, weeks	6.5 (1.7)			7.0 (2.0)		
Pregnant at time of recruitment						
No		226	32.4		1,242	25.8
Yes		471	67.6		3,574	74.2
Missing		0			0	
Parity						
Nulliparous		287	44.5		2,196	48.2
1		236	36.6		1,576	34.6
≥2		122	18.9		785	17.2
Missing		52			259	
Previous miscarriage						
No		469	72.7		3,560	78.1
Yes		176	27.3		997	21.9
Missing		52			259	
Alcohol Use						
Never		74	11.4		656	14.3
Current		128	19.7		158	3.4
Former		447	68.9		3,788	82.3
Missing		48			214	
Has study ultrasound						
No		188	27.0		122	2.5
Yes		509	73.0		4,694	97.5
Missing		0			0	
Site						
North Carolina		405	58.1		2,687	55.8
Tennessee		241	34.6		1,779	36.9
Texas		51	7.3		350	7.3

Abbreviations: RFTS, “Right from the Start”; SD, standard deviation.

[a] Body mass index: weight (kg)/height $(m)^2$.

[b] Median for gestational age at enrollment.

Table 5-18. Common early pregnancy exposures and consistency of use in *Right from the Start*, 2000-2012

	Miscarriage		Term Births	
	N	%	N	%
Smoking overall				
Never	479	73.8	3,384	73.5
Current	27	4.2	157	3.4
Former	143	22.0	1,063	23.1
Missing	48		212	
Consistency of smoking overall				
Non-smoker	479	73.9	3,384	73.7
<10 cigarettes per day	102	15.7	709	15.5
≥10 cigarettes per day	67	10.3	497	10.8
Missing	49		226	
Consistency of use among current smokers				
<10 cigarettes per day	17	63.0	104	66.7
≥10 cigarettes per day	10	37.0	52	33.3
Missing	0		1	
Consistency of use among former smokers				
<10 cigarettes per day	85	59.9	605	57.6
≥10 cigarettes per day	57	40.1	445	42.4
Missing	1		13	
Vitamin use overall				
No	47	7.3	158	3.4
Yes	601	92.8	4,432	96.6
Missing	49		226	
Consistency of vitamin use[a]				
<5 times per week	39	6.1	465	10.1
≥ 5 times per week	558	86.6	3,965	86.4
Frequency per week unknown	4		2	

[a]Only among women who indicated vitamin use 601 who had miscarriages and 4,432 who had term births

Table 5-19. Models for miscarriage risk associated with common early pregnancy exposures using gestational age based on self-reported LMP or GAAD among *Right from the Start*, 2000–2012

	Unadjusted Model: LMP		Adjusted Model 1: LMP		Adjusted Model 2: GAAD	
	HR	95% CI	aHR	95% CI	aHR	95% CI
Smoking Overall[a]						
Never	1.0	Referent	1.0	Referent	1.0	Referent
Current	1.24	0.83, 1.86	0.93	0.61, 1.41	1.09	0.64, 1.88
Former	0.99	0.82, 1.19	0.88	0.73, 1.07	0.93	0.72, 1.20
Consistency of use among Current Smokers[a]						
Non-smoker	1.0	Referent	1.0	Referent	1.0	Referent
<10 cigarettes per day	1.12	0.67, 1.87	0.88	0.52, 1.51	1.03	0.53, 2.00
≥10 cigarettes per day	1.54	0.82, 2.89	0.93	0.49, 1.77	1.07	0.43, 2.66
Consistency of use among Former Smokers[a]						
Non-smoker	1.0	Referent	1.0	Referent	1.0	Referent
<10 cigarettes per day	1.01	0.80, 1.28	0.86	0.68, 1.08	0.99	0.73, 1.34
≥10 cigarettes per day	0.97	0.74, 1.27	0.91	0.69, 1.20	0.84	0.57, 1.24
Vitamin use overall[b]						
No	1.0	Referent	1.0	Referent	1.0	Referent
Yes	0.43	0.32, 0.58	0.41	0.30, 0.55	0.43	0.27, 0.69
Consistency of vitamin use[b]						
No vitamin use	1.0	Referent	1.0	Referent	1.0	Referent
<5 times per week	0.29	0.19, 0.45	0.28	0.18, 0.43	0.28	0.15, 0.52
≥ 5 times per week	0.44	0.33, 0.60	0.42	0.31, 0.57	0.45	0.28, 0.71

Gestational age based on self-reported last menstrual period (LMP) or gestational age at arrest of development (GAAD)

Abbreviations: LMP, last menstrual period; GAAD, gestational age at arrest of development; HR, unadjusted hazard ratio; aHR, adjusted hazard ratio; CI, confidence interval.

[a] Models for smoking, adjusted for age(quadratic) and alcohol use

[b] Models for vitamin use, adjusted for age(quadratic)

Table 5-20. Bootstrap analysis bias ratio for risk of miscarriage associated with common early pregnancy exposures in *Right from the Start*, 2000–2012

	Model comparison Magnitude of bias	
	Bias Ratio[c] $\frac{\text{aHR LMP model}}{\text{aHR GAAD model}}$	95% CI
Smoking[a]		
Current	0.85	0.75, 0.94
Former	0.95	0.92, 0.97
Consistency of use among Current Smokers[a]		
<10 cigarettes per day	0.87	0.74, 0.99
≥10 cigarettes per day	0.87	0.71, 1.09
Consistency of use among Former Smokers[a]		
<10 cigarettes per day	0.86	0.83, 0.90
≥10 cigarettes per day	1.08	1.05, 1.12
Vitamin use[b]		
Yes	0.93	0.86, 1.02
Consistency of Vitamin use[b]		
<5 times per week	1.01	0.93, 1.08
≥ 5 times per week	0.94	0.87, 1.01

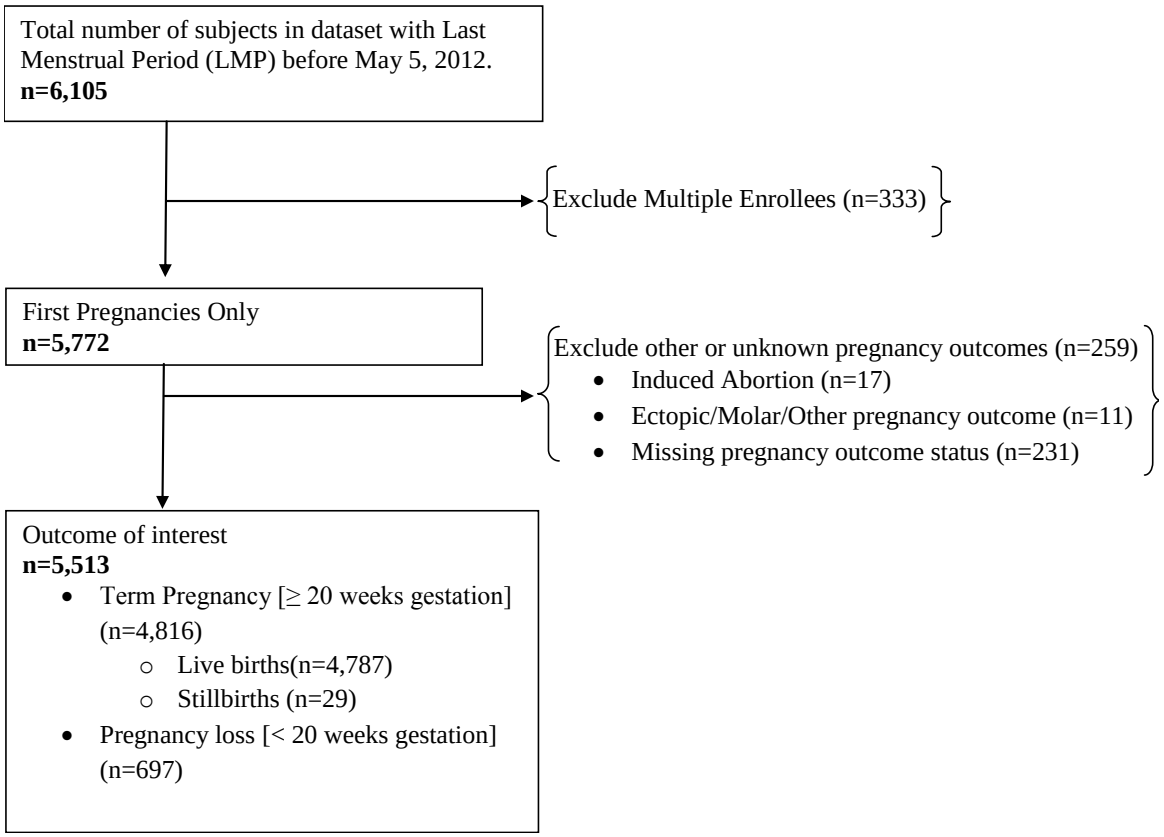
Abbreviations: CI, confidence interval

[a] Models for smoking, adjusted for age (quadratic) and alcohol use

[b] Models for vitamin use, adjusted for age (quadratic)

[c] Bias Ratio: $\frac{\text{aHR LMP model}}{\text{aHR GAAD model}}$

Figure 5-8. Aim 2: Study subject inclusion criteria



CHAPTER VI

RACIAL DIFFERENCES IN RISK OF MISCARRIAGE ASSOCIATED WITH COMMON EARLY PREGNANCY EXPOSURES

Abstract

Racial disparities for factors associated with miscarriage risk are sparse and may be influenced by differences in pregnancy intention and timing of loss. We examined if GAAD gap differs between Blacks and Whites and whether early pregnancy exposures associated with loss are modified by race. Women were enrolled in *Right from the Start*, a diverse community-based cohort (2000-2012). Study participants completed study ultrasounds and detailed first-trimester interviews. We compared models that estimated gestational age based on self-reported LMP and models that incorporated gestational age at time of arrested development (GAAD). We used bootstrap analysis to determine the magnitude of bias in our risk estimates. Over twenty percent of women self-identified as Black (n=1,006). There were 624 observed miscarriages among 4,903 women. The median GAAD gap was longer for Blacks compared to Whites (median GAAD gap 21 days vs. 19 days, p-value=0.04). Using the LMP models unadjusted for confounders we did not observe effect modification by race for the relationship between smoking and miscarriage risk (likelihood ratio test p=0.34), but did for the relationship between vitamin use and miscarriage risk (likelihood ratio test p=0.06). Adjusted for confounding, the protective effect of vitamin use on miscarriage risk was stronger among White women than Black women when using the LMP method (Whites aHR=0.34, 95% CI [0.21, 0.54]; Blacks aHR=0.53, 95% CI [0.33, 0.84], race interaction p-value=0.18), while no substantial difference by race was observed with the GAAD method (Whites aHR=0.43, 95% CI [0.24, 0.76]; Blacks aHR=0.44, 95% CI [0.26, 0.74], race interaction p-value=0.93). The magnitude of bias in our reported

estimates was roughly twenty percent for both races (Whites bias ratio=0.79, 95% CI [0.62, 0.87]; Blacks bias ratio=1.19, 95% CI [1.13, 1.45]). These results suggest that the protective effect of vitamin use on miscarriage risk from the LMP model may be overestimated for Whites and underestimated for Blacks within this pregnancy cohort. Early-pregnancy vitamin use associated with miscarriage risk is influenced by proper classification of gestational arrest prior to loss, and the magnitude and direction of bias differs by race.

Running head: Race stratified estimates for miscarriage and magnitude of bias

Key Words: Miscarriage, gestational arrest, race, vitamin use, pregnancy intention, bias

Introduction

Miscarriage (a pregnancy loss < 20 weeks gestation) is a common clinical outcome. A better understanding of embryologic and fetal development in relation to timing of miscarriage, including differences by race, is important in epidemiologic studies when studying factors in early-pregnancy that may cause or prevent pregnancy loss. In studies of miscarriage, last menstrual period (LMP) is often used to estimate gestational age at loss; however, embryologic development may stop days to weeks prior to the onset of clinical symptoms of miscarriage. Basing timing of miscarriage on the time from LMP to the clinical recognition of loss alone ignores the developmental state of the embryo prior to the loss. This is potentially problematic if a pregnancy had, as is clinically known to be common, in fact arrested earlier.[2,93,96-101]

Research on racial differences in miscarriage risk are sparse and may be influenced by differences in pregnancy intention. Prior work within RFTS has indicated that Blacks and Whites have different gestational ages at clinical loss, with Black women having greater risk for miscarriage between weeks 10 and 20 in gestation (aHR=1.93, 95% CI [1.48, 2.51]).[2] In order to determine if the observed later losses in Blacks are due to difference in developmental stage at loss, we aim to assess gestational arrest prior to loss and compare the GAAD gap by race. Additionally, pregnancy intention varies by race [4-6] in the U.S. general population and may influence behavioral factors such as smoking and vitamin use during early pregnancy associated with pregnancy loss. Women with unintended pregnancies may be less certain of their LMP dates and may have greater variability in their GAAD estimates based on developmental stage at ultrasound when compared to women who are planning a pregnancy.

In this study, we build upon our prior work and use data from the RFTS pregnancy cohort to examine the racial differences in the GAAD gap and if early pregnancy exposures as it relates to timing of miscarriage risk are modified by race. The aims of this study are 1. to test whether GAAD gap differs by race or pregnancy intention; 2. to assess if timing of gestational arrest associated with early pregnancy exposures is modified by race by comparing models that use GAAD or self-reported LMP to estimate gestational age and; 3. to determine the magnitude of bias in our reported estimates when comparing these models.

Materials and Methods

Study population and data collection

Data was available from the *Right from the Start* pregnancy cohort. Women who had their last menstrual period before May 5, 2012, were included in this data set (n=6,105). Participants completed a baseline interview at the time of enrollment and a comprehensive computer-assisted telephone interview in the first trimester. In the interview, information collected included reproductive and medical history, sociodemographic characteristics, and health behaviors around the time of conception or during pregnancy. Participants who experienced pregnancy loss before the scheduled interview were interviewed as soon as possible after the loss. In addition, in order to enroll in the study women also consented to early first-trimester transvaginal ultrasounds. Research ultrasound examinations were conducted at a time in gestation (>5 4/7 weeks from the LMP) in which normal pregnancies would be expected to have a fetal pole and heart rate. Study ultrasounds were conducted for assessment of embryological viability, documentation of stage of development, and confirmation of gestational dating. Additionally, pregnancy outcomes were self-reported by study participants and verified by medical records.

For women who enrolled in the study for more than one pregnancy, we limited our study population to their first enrolled pregnancy in RFTS (n=333 subsequent pregnancies excluded). We further excluded women who had induced abortions (n=17), women who had a missing pregnancy status at the time of analysis (n=231), and women who had ectopic/molar pregnancies (n=11). Additionally, in order to study effect modification by race we restricted our analyses to women who self-identified as non-Hispanic White (referred to throughout as White) or non-

Hispanic Black (referred to through as Black). Women with missing information for race (n=7) or who decline to self-identify their race (n=2) were excluded from this analysis. Women who self-identified as Hispanic regardless of White or Black racial self-identification or as other races, which include Native Americans and Asians, were also excluded from this analysis (n=361 and n=240, respectively). A total of 4,903 women contributed to these analyses. Study subjects included in analysis for specific aim 3 can be found in **Figure 6-9.**

Outcome definitions

Pregnancy was verified by ultrasound or repeat pregnancy test. Miscarriage was defined as loss of a recognized pregnancy prior to 20 completed weeks of gestation using self-reported LMP dating. This was referred to as the LMP method to estimate gestational age at loss. We documented 624 miscarriages during the study period. Of the women who experienced loss nearly one-third experienced their loss prior to ultrasound (n=172). Women with ultrasounds were classified based on developmental stage at loss (i.e. gestational age at time of arrested development or GAAD). GAAD was estimated using prespecified established nomograms[45,47] to estimate gestational age using key characteristics from ultrasound and described in detail in Aim 1. GAAD was estimated among losses with complete ultrasound data (four White women and one Black woman had ultrasounds, but were missing both mean sac diameter and crown-rump length measurements, and were therefore excluded from our GAAD estimates). The GAAD gap was defined as the difference in days gestation between self-reported LMP date of loss and the newly estimated GAAD. GAAD and the GAAD gap were estimated for 344 White and 103 Black losses.

Exposure definitions

The primary exposures of interest in this analysis are smoking and vitamin use. Exposure status is a self-reported measure acquired during the first-trimester interview. For this analysis, smoking was grouped into the following categories: never smokers (referent), current smokers, and former smokers (within four months prior to the interview (meaning exposures in the pregnancy and/or periconception window) or ≥4 months from interview)). Information about the frequency of use was also obtained among women reporting current or former smokers. Questions were asked separately for prenatal vitamins and multivitamins (see Appendix). In order to remain consistent with previously published work within this cohort both categories of supplements were combined because of potential misclassification by participants. Both types of supplements are referred to as ‘‘vitamins.’’ Participants were asked whether they were currently taking vitamins or, in the case of a miscarriage, whether they had taken vitamins during pregnancy. Information about the frequency and timing of vitamin use in an average week was also obtained. Participants who reported any use of vitamins during pregnancy were considered exposed.

Variable definition for pregnancy intention

We were also interested in the potential interaction between race and pregnancy intention. Pregnancy intention was defined based on criteria from the National Family Growth Survey (NFGS) for unplanned pregnancies.[88] Pregnancy intention was coded as a yes/no variable for these analyses. Intended pregnancies include pregnancies that were planned. Unintended pregnancies include both mistimed and unwanted pregnancies. Pregnancy intention was based on the following questions from the first-trimester interview: FTG7 (contraception and planning at

time of conception), FTE4(a)(contraception use (yes/no)), FTG13(pregnancy timing) and FTG12(wanted pregnancy (yes/no)). A list of pregnancy intention questions from the first-trimester interview and their respective responses can be found in the appendices (Appendix 3.3). Women who did not answer, refused to answer or listed did not know to any of the above questions in the first-trimester interview were not assessed for pregnancy intention based on the criteria listed below (n=552). Briefly, a pregnancy was considered intended if the woman stopped using contraception or had not used contraception because she wanted to become pregnant and the pregnancy occurred at about the right time, later or didn't care when in her life (FTG13= either later, right time or didn't care when). A pregnancy was considered mistimed if the woman stopped using contraception or had a gap in contraception use and she became pregnant too soon (FTG13=too soon) or if the woman was still using contraception and became pregnant too soon but wanted to have another baby eventually (FTG12= yes). A pregnancy was classified as unwanted if the woman became pregnant while using contraception and did not want to have another baby at any time in the future (FTG12=no). A more detailed description of the pregnancy intention variable and its classification can be found in chapter 3 (**Table 3-11**). Among women with pregnancy losses, GAAD and the GAAD gap were estimated for 285 intended and 96 unintended pregnancies.

Statistical analysis

Descriptive statistics and subsequent analyses were generated using SE/12.1 software (StataCorp LP, College Station, Texas). Descriptive statistics were expressed as frequencies and proportions for categorical variables and means and standard deviations for continuous variables, stratified by race. We further examined developmental stage at ultrasound by race and report the

overall distribution of GAAD gap by race and pregnancy intention. A Wilcoxon rank-sum test was used to compare the medians between groups.

We used Cox proportional hazard survival models with variable gestational age at study entry to estimate the risk of miscarriage associated with early pregnancy exposures (i.e. smoking (current or former vs. never); and vitamin use(any vs. none)). Participants were followed from the time of enrollment in the study and contributed to the analysis until an outcome of either miscarriage, birth or stillbirth occurred or loss to follow-up happened. Gestational age at the time of loss was calculated from the first day of a woman's self-reported LMP for the index pregnancy to the end of that gestation (herein referred to as the LMP method to estimate gestational age at loss). Cox models using LMP method were used to screen for candidate confounders and to test for effect modification by race. For each level of the exposure status, we tested effect modification by race unadjusted for confounders using a likelihood ratio test using the LMP method. Race stratified analyses were only presented if these tests suggested heterogeneity present ($p<0.10$). Additionally, we used a likelihood ratio test to test the contribution of a race and pregnancy intention interaction within our model. If the likelihood ratio test had a p-value<0.10, the race-pregnancy intention interaction term was retained in our final models. Schoenfeld residuals were also tested to assess the proportionality of hazards for the final Cox model (results not shown). The Breslow method was used to handle ties.

Potential confounders examined from baseline and the first-trimester interviews included factors known to be associated with both miscarriage and exposure of choice. Race was not considered as a confounder in stratified models, but was assessed as a potential confounder in the non-stratified models. A change in estimate of at least 10% was used to classify a variable as a

confounder in non-stratified models. Similarly, in race stratified models if a 10% change in estimate was observed in either Blacks or Whites, the variable was considered a confounder. Candidate confounders for smoking and miscarriage relationship included maternal age, household income, maternal education, marital status, maternal race, parity, alcohol use and pregnancy intention. Candidate confounders for vitamin use and miscarriage relationship included the above factors with the addition of smoking. If a 10% change was observed from the crude hazard ratio, the variable was retained in the final models. To optimize fit, maternal age was specified by the inclusion of linear and quadratic terms in the model. We did not consider prior history of miscarriage as a confounder in our data because we would be potentially overadjusting by including a causal factor for current risk.[80] Analysis was restricted to those with complete covariate information. We used similar adjusted Cox regression models using gestational age estimated based on GAAD (herein referred to as the GAAD method) for overall risk for miscarriage with early pregnancy exposures.

Finally in order to assess the potential magnitude of bias in our risk estimates for miscarriage we conducted 1000 bootstrap replications to estimate the bias ratio and 95% confidence interval between either model. The bias ratio was defined as the ratio between the two models (i.e. bias ratio= $\frac{\text{adjusted HR ratio of LMP model}}{\text{adjusted HR ratio of GAAD model}}$) for either exposure and miscarriage risk and described previously (refer to Chapter 5).

Results

Nearly 21% of participants self-identified as Black (n=1,006) (**Table 6-21**). Twenty-seven percent of the women in our cohort were recruited prior to pregnancy (n=1,341) and the majority had ultrasound information. Compared with White women, Black women were more likely to be younger, to have a higher body mass index (≥30 kg/m^2), to have income <$80,000, not to have a college degree, to be unmarried, to be parous (>1). Additionally, Blacks were more likely than Whites to self-report an unintended pregnancy and to have had prior history of adverse pregnancy outcomes, including prior history of stillbirths, preterm births and induced abortions. In terms of behaviors during pregnancy, Blacks were more likely to abstain from alcohol and tobacco products during pregnancy, but were more likely to not use vitamins compared to Whites. Mean gestational age at the time of enrollment was later for Blacks (7.8 weeks) than for Whites (6.1weeks) (**Table 6-21**).

There were 624 miscarriages observed. Among women with miscarriages, over seventy percent had ultrasound data available (Whites 71%, Blacks 76%). Ultrasound characteristics did not differ by race (**Table 6-22**). Anembryonic gestation was observed in over a third of all miscarriages with recorded ultrasound information (39.7% Whites, 35.6% Blacks). The median gestational age at loss based on self-reported LMP was nearly a week and half earlier for Whites than for Blacks (median LMP Whites 68 days, Blacks 79.5 days; p=0.0000), and was earlier for women with intended pregnancies than women with unintended pregnancies (median LMP intended pregnancies 69 days, unintended pregnancies 76 days; p=0.0001) (**Table 6-23**). The median estimated GAAD for women with pregnancy loss and ultrasound information was also earlier for Whites compared to Blacks (median GAAD Whites 54 days, Blacks 60 days;

p=0.0002) and for women with intended pregnancies compared to unintended pregnancies (median GAAD intended pregnancies 54 days, unintended pregnancies 59 days; p=0.0023). The median GAAD gap was similar by race (Whites 19 days, Blacks 21 days; p=0.04) and pregnancy intention (intended pregnancies 18 days, unintended pregnancies 22 days; p=0.08) (**Table 6-23**). We did not find evidence of effect modification by maternal race for the association between smoking and risk of miscarriage (LR test race p=0.34 from LMP method). Additionally, the interaction term between race and pregnancy intention did not contribute substantially to the model for smoking and miscarriage risk (LR test race and pregnancy intention p=0.31 from LMP method). After assessing for potential confounders for inclusion, the final model was adjusted for maternal age and alcohol use. Overall results for smoking-miscarriage risk relationship, including assessing the magnitude of bias have been previously presented and are not reported here (refer to Chapter 5, **Table 5-19**). Proportional hazards test suggest that proportional hazard assumptions for risk of miscarriage associated with smoking are not violated using either method (adjusted [global] model LMP method p=0.22, GAAD method p=0.09) (results not shown).

We observed effect modification by race for the effect of vitamin use on miscarriage risk (LR test for race p=0.06 from LMP method), however the interaction between race and pregnancy intention did not contribute substantially to the model (LR test for race and pregnancy intention p=0.16 from LMP method) and was not included (**Table 6-24**). For our stratified models, race was not considered as a confounder. Final models for the relationship between vitamin use and miscarriage risk were stratified by race and adjusted for age. The protective effect of overall vitamin use on miscarriage risk was stronger among White women than Black women when using the LMP method (Whites aHR=0.34, 95% CI [0.21, 0.54]; Blacks

aHR=0.53, 95% CI [0.33, 0.84]), while no substantial difference by race was observed using the GAAD method (Whites aHR=0.43, 95% CI [0.24, 0.76]; Blacks aHR=0.44, 95% CI [0.26, 0.74]) (**Table 6-24**).

We conducted a thousand replication bootstrap analyses of our two models to compare the ratio of effect sizes (i.e. the bias ratio). The overall magnitude of bias was nearly twenty percent for both Whites and Blacks (Whites bias ratio=0.79, 95% CI [0.62, 0.87]; Blacks bias ratio=1.19, 95% CI [1.13, 1.45]), although it occurred in different directions (**Table 6-25**). Compared to GAAD models, which present effect estimates based on improved classification of gestational age at loss, the LMP model overestimated the protective effect of vitamin use on miscarriage risk for Whites, and underestimated the protective effect for Blacks. Similar magnitude of bias was demonstrated with consistency of vitamin use for women taking vitamins ≥5 times per week (Whites bias ratio=0.78, 95% CI [0.62, 0.88]; Blacks bias ratio=1.15, 95% CI [1.07, 1.45]) (**Table 6-25**).

Discussion

We have demonstrated that risk of miscarriage associated with vitamin use differs by race when self-reported LMP is used to estimate gestational age. However by properly classifying gestational age at loss, we observed no difference in the effect of vitamin use between Whites and Blacks. We observed no such differences in miscarriage risk by race for early pregnancy smoking exposure. No differences in developmental stage on ultrasound by race or influence of pregnancy intention by race were observed. For both Blacks and Whites, vitamin use significantly protects against miscarriage risk for women in this cohort using either model to estimate gestational age (i.e. LMP or GAAD), with the strongest effects seen in women taking vitamins <5 times per week. However, we further demonstrate that the magnitude of bias introduced in these estimates is nearly twenty percent and occur in opposite directions for Blacks and Whites. These results suggest that the protective effect of vitamin use on miscarriage risk may be overestimated for Whites and underestimated for Blacks within this prospective pregnancy cohort. The patterns of bias (i.e. overestimating the protective effect of vitamin use for Whites and underestimating the protective effect for Blacks) remain when accessing frequency of vitamin use. These results suggest that the effect of early-pregnancy vitamin use associated with miscarriage risk is influenced by proper classification of gestational arrest prior to loss, and that the direction of bias differs by race while the magnitude is similar.

Of note, we found important demographic differences between participants who self-identified as Black compared to women who self-identified as White, suggesting that the results shown here may partly represent a collection of lifestyle factors related to preconception access to care, pregnancy planning, and self-selection into our study. For example, pregnancy intention

is an important indicator of a woman's readiness to bear a child, her mental and physical health, and her sociodemographic context.[5] While Black women were more likely to indicate unintended pregnancies in our cohort compared to White women, the interaction between race and pregnancy intention was not significant in our models. We further illustrate that the GAAD gap was greater for women with unintended pregnancies compared to women with intended pregnancies. This suggests that women with unintended pregnancies have greater variability in their GAAD estimates based on developmental stage at ultrasound and may be less sure of their LMP dates when compared to women with intended pregnancies. Although preventing unintended pregnancies remains an important public health concern, understanding underlying contributors to unwanted and mistimed pregnancies may help explain concurrent risk factors associated with adverse pregnancy outcomes.[5,6]

This study examined the overall relationship between vitamin use and miscarriage risk, not the effect of specific supplement components, such as folic acid.[8,61,72,74,76] We may be measuring a proxy for other health-conscious or preventative behaviors that are related to vitamin supplementation during pregnancy.[73] Future studies would benefit from the inclusion of both biologic and self-reported information on vitamin use. Finally, more consistent vitamin use was associated with a hazard ratio slightly closer to the null compared to less frequent use for both Blacks and Whites. This appears counterintuitive, but it could occur if women who are more vigilant about daily vitamin supplementation are at higher risk of miscarriage than women who are not as attentive to taking their daily vitamin. These results remain consistent with previously published work on vitamin use within RFTS cohort.[73]

One potential limitation within this cohort is that gestational age was estimated based on measurements from a single ultrasound. However by taking cross-sectional ultrasound information we have the ability to estimate probable developmental stage prior to miscarriage and have a better way to classify time at loss than self-reported LMP. Other limitations may include collection of data in first-trimester interview in relation to pregnancy loss. However, on average the interval between loss and first-trimester interview was less than 3.5 weeks, and the questions in the interview were clearly asking about behaviors during their recent pregnancy.

The primary strength of our study is our ability to follow a large diverse sample of women recruited from the community prospectively through their pregnancies, many of whom were enrolled prior to pregnancy.[3] RFTS is a community-based pregnancy cohort. Clinic-based studies may be demographically different from population-based studies, and may overestimate the occurrence of adverse outcomes.[108] We believe our results can be informative for pregnant women, and women planning pregnancies in the United States. In addition, we were able to evaluate potential confounders and analyze the data with hazard models that account for variation in gestational age at study entry. Furthermore, women do not alter prenatal care choices in order to enroll in RFTS, and therefore it is unlikely that enrollment procedures and study activities influenced behaviors or outcomes within this population.

Although we have documented no overall effect for miscarriage risk due to smoking exposure by race, we show evidence for racial differences in vitamin use during pregnancy when self-reported LMP is used to estimate gestational age. We observed that Blacks were less likely to take vitamins compared with Whites, and there were significant racial differences in miscarriage risk due to vitamin exposure. We have demonstrated that in this cohort traditional

models that use self-reported LMP to estimate gestational age, race modifies that association between vitamin use and miscarriage risk. However no modification by race is observed when gestational age was estimated using GAAD for risk of miscarriage with vitamin use. We illustrate that misclassifying time at loss in studies of miscarriage results in biased risk estimates when stratified by race in direction of effect but not magnitude of effect. In other words, by misclassifying time at loss, we have artificially introduced effect modification by race for risk of miscarriage associated with vitamin use. These data may help to explain the inconsistent findings across studies of miscarriage risk associated with vitamin use. By properly classifying gestational arrest prior to loss, effect modification by race disappears for the vitamin use miscarriage association. Further investigation is warranted to examine if the protective effect of vitamin use on miscarriage risk remains consistent when assessing self-reported information on vitamin use with biologic assessment of vitamin supplementation.

Table 6-21. Characteristics by race of *Right from the Start* participants, 2000–2012

	RFTS Study Participants					
	Whites N=3,897 (79.5%)			Blacks N=1,006 (20.5%)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Maternal age, years	29.7 (4.6)			26.6 (5.7)		
Maternal age, years						
<20		60	1.5		92	9.2
20–24		404	10.4		331	32.9
25–29		1,420	36.5		280	27.8
30–34		1,411	36.2		202	20.1
≥35		601	15.4		101	10.0
Missing		1			0	
Body mass index[a]	25.0 (5.5)			29.3 (7.9)		
Body mass index						
Underweight (<18.5)		109	2.8		19	1.9
Normal weight (18.5–24.9)		2,284	59.4		306	31.0
Overweight (25.0–29.9)		861	22.4		274	27.7
Obese (≥30.0)		594	15.4		389	39.4
Missing		49			18	
Household income						
≤$40,000		720	19.8		558	62.4
$40,001–$80,000		1,528	41.9		226	25.3
>$80,000		1,395	38.3		110	12.3
Missing		254			112	
Maternal education						
High school or less		386	9.9		388	38.6
Some college		587	15.1		282	28.0
College (≥4 years)		2,923	75.0		336	33.4
Missing		1			0	
Marital status						
Married, living as married, single		3,714	95.3		649	64.5
Other		183	4.7		357	35.5
Missing		0			0	
Pregnancy Intention						
No		788	22.5		439	51.9
Yes		2,718	77.5		407	48.1
Missing		392			160	
Gestational age at enrollment, weeks	6.6 (1.8)	6.3[b]		7.8 (2.1)	7.7[b]	

	RFTS Study Participants					
	Whites N=3,897 (79.5%)			Blacks N=1,006 (20.5%)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Pregnant at time of recruitment						
No		1,217	31.2		124	12.3
Yes		2,680	68.8		882	87.7
Missing		0			0	
Parity						
Nulliparous		1,785	48.2		423	45.3
1		1,328	35.9		284	30.4
≥2		589	15.9		226	24.2
Missing		195			73	
Previous miscarriage						
No		2,894	78.2		700	75.0
Yes		808	21.8		233	25.0
Missing		195			73	
Recurrent miscarriage[c]						
No		725	89.7		212	1.0
Yes		83	10.3		21	9.0
Missing		0			0	
Previous preterm birth						
No		3,430	92.7		820	87.9
Yes		272	7.3		113	12.1
Missing		195			73	
Previous stillbirth						
No		3,670	99.1		909	97.4
Yes		32	0.9		24	2.6
Missing		195			73	
Previous induced abortion						
No		3,273	88.4		676	72.5
Yes		429	11.6		257	27.5
Missing		195			73	
Vitamin use						
No		61	1.7		113	11.7
Yes		3,639	98.4		851	88.3
Missing		197			42	
Smoking						
Never		2,645	71.3		763	79.0
Current		128	3.4		45	4.6
Former		938	25.3		158	16.4
Missing		186			40	

	RFTS Study Participants					
	Whites N=3,897 (79.5%)			Blacks N=1,006 (20.5%)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Alcohol use						
Never		381	10.3		217	22.5
Current		248	6.7		11	1.1
Former		3,081	83.0		737	76.4
Missing		187			41	
Age of menarche, years	12.7 (1.5)			12.4 (1.9)		
Age of menarche, years						
≤ 10		182	5.0		137	14.3
11-13		2,568	70.0		589	61.4
>13		921	25.0		234	24.3
Missing		226			46	
Current outcome miscarriage						
No		3,409	87.5		870	86.5
Yes		488	12.5		136	13.5
Missing		0			0	
Has study ultrasound						
No		190	4.9		76	7.6
Yes		3,707	95.1		930	92.4
Missing		0			0	
Site						
North Carolina		2,253	57.8		519	51.6
Tennessee		1,492	38.3		397	39.5
Texas		152	3.9		90	8.9

Abbreviations: No., number; SD, standard deviation.

[a] Body mass index: weight (kg)/height $(m)^2$.

[b] Median for gestational age at enrollment.

[c] Only among women who experienced a prior miscarriage 808 Whites and 233 Blacks.

Table 6-22. Ultrasound characteristics by race for women who experienced miscarriage within *Right from the Start*, 2000-2012

		RFTS Study Participants with Miscarriage			
		Whites (n=488)		Blacks (n=136)	
Ultrasound Developmental Stage	Ultrasound Characteristics	No. of Losses	% of Loss	No. of Losses	% of Loss
Loss before Ultrasound	N/A	140	N/A	32	N/A
Anembryonic gestation					
	Empty uterus	23	6.6	11	10.6
	Gestational sac only	57	16.4	12	11.5
	Gestational and yolk sac	58	16.7	14	13.5
Fetal pole present					
	No fetal heart rate	68	19.5	22	21.2
	Abnormal fetal heart rate	21	6.0	7	6.7
	Normal fetal heart rate	121	34.8	38	36.5

Abbreviations: No., number; N/A., not applicable

Table 6-23. Estimating gestational age of pregnancy loss based on self-reported LMP and GAAD by race and pregnancy intention among women who experienced miscarriage within *Right from the Start*, 2000-2012

	RFTS Study Participants with Miscarriage					
	LMP (days)		GAAD (days) [a]		GAAD gap (days) [a]	
Estimated gestational age at Loss	Mean (sd)	Median	Mean (sd)	Median	Mean (sd)	Median
Race						
Whites	68.8 (20.6)	68	56.0 (13.8)	54	18.6 (13.4)	19
Blacks	81.5 (26.1)	79.5	65.2 (22.2)	60	21.7 (19.4)	21
Pregnancy Intention[b]						
No	79.1 (24.5)	76	63.5 (19.0)	59	21.5 (16.2)	22
Yes	68.9 (19.7)	69	56.3 (14.0)	54	18.7 (14.3)	18

Abbreviations: LMP, last menstrual period; GAAD, gestational age at arrest of development; sd, standard deviation.
[a]GAAD estimated among losses with complete ultrasound data, 344 White and 103 Black losses (4 White women and 1 Black woman had ultrasounds, but were missing both mean sac diameter and crown-rump length measurements);
[b]Among women who self-identified as either White or Black and had complete data on pregnancy intention (96 Unintended pregnancy losses and 285 Intended pregnancy losses).

Table 6-24. Models for miscarriage risk associated with vitamin exposure stratified by race, within *Right from the Start*, 2000-2012

	No.	Adjusted Model 1: LMP method[a]		Adjusted Model 2: GAAD method[a]		P[b]
Vitamin use exposure		aHR	95% CI	aHR	95% CI	
Race x vitamin use interaction						0.060
Race x pregnancy interaction						0.160
Whites						
Vitamin use						
No	61	1.0	Referent	1.0	Referent	
Yes	3,639	0.34	0.21, 0.54	0.43	0.24, 0.76	
Consistency of vitamin use[c]						
No vitamin use		1.0	Referent	1.0	Referent	
<5 times per week	327	0.26	0.14, 0.46	0.35	0.17, 0.71	
≥ 5 times per week	3,311	0.34	0.21, 0.55	0.43	0.24, 0.77	
Blacks						
Vitamin use						
No	113	1.0	Referent	1.0	Referent	
Yes	851	0.53	0.33, 0.84	0.44	0.26, 0.74	
Consistency of vitamin use[c]						
No vitamin use		1.0	Referent	1.0	Referent	
<5 times per week	111	0.27	0.11, 0.63	0.11	0.03, 0.40	
≥ 5 times per week	736	0.55	0.35, 0.89	0.48	0.29, 0.81	

Abbreviations: No., number; LMP, last menstrual period; GAAD, gestational age at arrest of development; aHR, adjusted hazard ratio; CI, confidence interval.

[a]Models for vitamin use adjusted for age(quadratic).

[b]P values from likelihood ratio test of unadjusted models using LMP method to assess for effect modification by race.

[c]Vitamins per week missing for 5 women (Whites 1, Blacks 4).

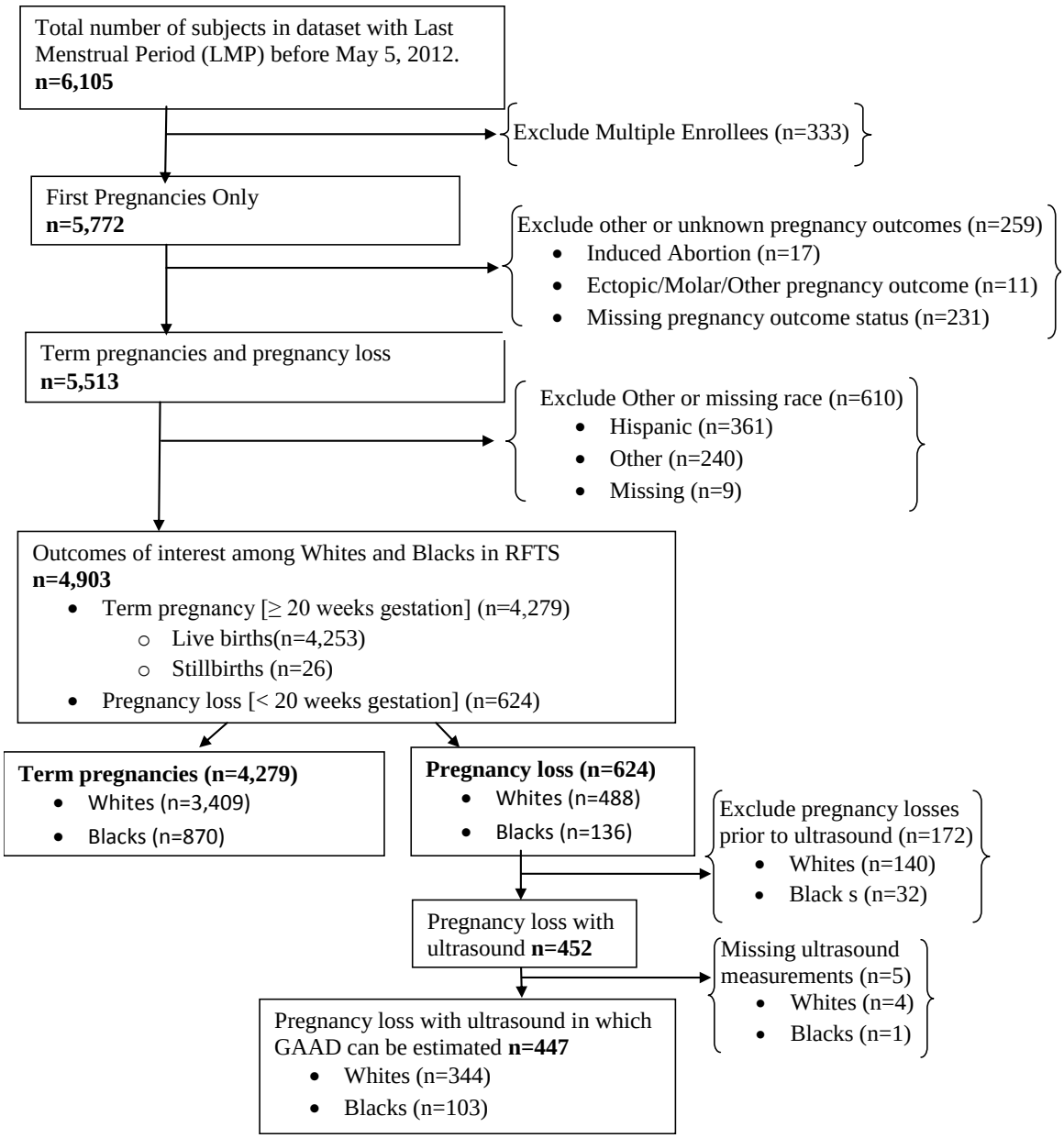
Table 6-25. Bootstrap analysis bias ratio for risk of miscarriage associated with vitamin exposure, stratified by race in *Right from the Start*, 2000–2012

	Model comparison Magnitude of bias	
Vitamin exposure	Bias Ratio [a] aHR (LMP model) / aHR (GAAD model)	95% CI
Whites		
Vitamin use		
Yes	0.79	0.62, 0.87
Consistency of vitamin use		
<5 times per week	0.73	0.63, 0.74
≥ 5 times per week	0.78	0.62, 0.88
Blacks		
Vitamin use		
Yes	1.19	1.13, 1.45
Consistency of vitamin use		
<5 times per week	2.25	0.93, 2.56
≥ 5 times per week	1.15	1.07, 1.45

Abbreviations: aHR, adjusted hazard ratio; CI, confidence interval

[a] Bias Ratio: $\frac{\text{aHR LMP model}}{\text{aHR GAAD model}}$

Figure 6-9. Aim 3: Study subject inclusion criteria



CHAPTER VII

CONCLUSIONS AND FUTURE DIRECTIONS

Overall study conclusions

We were able to establish that there is on average nearly a three week gap between gestational arrest and clinical manifestation of loss based on self-reported LMP within a large diverse community-based pregnancy cohort. Both Blacks and Whites within RFTS exhibited similar trends of developmental stage at loss observed on ultrasound. Nearly 40% of losses had anembryonic development at time of ultrasound (i.e. empty uterus, gestation sac only, or gestational and yolk sac only; n= 197). The GAAD gap did not differ by most key characteristics between those with longer vs. shorter than median GAAD gaps. We were able to demonstrate that the gap was greater for Blacks compared to Whites (median GAAD gap 21 and 19 days respectively). Overall this gap biased risk estimates up to 15% for current smokers and 5% of former smokers when compared to LMP models. When stratified by race, the bias was nearly 20% for both Whites and Blacks for miscarriage risk associated with early pregnancy vitamin exposure but occurred in opposite directions. This implies that the protective effect of vitamin use on miscarriage risk may be overestimated for Whites while a similar protective effect may be underestimated for Blacks. We illustrate that effect modification by race disappears for vitamin use miscarriage relationship when gestational arrest is properly classified. These results suggest that early-pregnancy exposures associated with miscarriage risk are influenced by proper classification of gestational arrest prior to loss, and that the direction of bias differs by race, while the magnitude was similar.

We successfully demonstrate that it is possible for researchers to more accurately identify timing of miscarriage prior to clinical pregnancy loss which traditionally use self-reported LMP to estimate gestational age and to set aside exposures that occur after developmental arrest but before clinical loss and which would not contribute exposure-time when assessing miscarriage risk. Next steps for this analysis include assessing time-varying exposures on miscarriage risk using GAAD models, and also assessing if the GAAD gap is as large in other pregnancy cohorts which have robust miscarriage data and early first-trimester ultrasound data available.

Study strengths

Our study has several strengths. RFTS has prospective data collection to assess miscarriage risk and improve documentation of exposures and gestational dating. Because participants enroll in RFTS very early in pregnancy, we are able to observe a greater proportion of pregnancy losses in our study population than if prenatal clinic-based recruitment occurred. Community-based recruitment which encompassed women who ultimately sought care in a full range of settings, many of whom were not yet engaged in prenatal care at the time of enrollment, providing a study population potentially more representative of the general population of women trying to conceive than clinic based populations. Women who seek care at academic tertiary care centers may be less representative than the general population of women trying to conceive, and onset of care is typically later than our enrollment criterion required. Early recruitment also provided earlier capture of both covariates and outcomes for use in multivariable models. Prior work has shown that clinic-based samples may be demographically different from population-based samples.[108] Analyses based on clinical samples may overestimate the occurrence of adverse outcomes. Furthermore RFTS avoids overselection of women who may be subtly

symptomatic or at high risk by advertising as a study about pregnancy health. RFTS participants did not have to alter prenatal care choices for entry into the study; thus, I believe it is unlikely that our enrollment procedures and study activities will influence our results.

RFTS participants have routine study ultrasounds conducted by trained study sonographers early in pregnancy; thus, gestational age assignment is accurate in early pregnancy and fetal viability can be confirmed. Ultrasound information is available on over 70% of women who have miscarriage as an outcome. Additionally, a high proportion of pre-pregnant women and those in early pregnancy were enrolled in RFTS. Twenty-six percent of women in our cohort were recruited prior to pregnancy (n=1,468). All women in our cohort entered before the end of the first-trimester with the median at 10 weeks estimated gestational age based on self-reported LMP.

Furthermore our analysis will provide models that properly reflect total time at risk and in view. A study on miscarriage requires careful assessment of gestational time at study entry because women who enter a study later will have less opportunity for a pregnancy loss to be observed. Only the first phase of RFTS enrollment allowed women to enter the study later than the ninth week. Another strength of RFTS is that we can accurately time the occurrence of events and exposures in pregnancy in models. Detecting pregnancy loss depends markedly on timing of pregnancy recognition (i.e. the earlier it is recognized, the higher the proportion that will result in miscarriage). With each recruitment phase, there were increasing proportions of women recruited prior to pregnancy because the gestational age at study entry was lower. In RFTS, the proportion of miscarriage was greater for women recruited before pregnancy compared to women already

pregnant at enrollment (15.4% vs. 11.6%). This allows us to observe more losses, including identifying gestational arrest prior to miscarriage.

Potential limitations of study

This dissertation aims to better understand gestational arrest prior to loss and the impact of bias this may have on risk for miscarriage. This research is the first step in addressing different outcomes for miscarriage in the context of gestational arrest (i.e. biological arrest compared to clinical loss based on LMP). An ideal study would address both time-varying nature of the outcome (e.g. gestational arrest vs. clinical loss) as well as time-varying nature of exposure status during the early pregnancy period.

This first-step provides a better measurement tool to estimate timing of miscarriage outcome. Both a protective factor (e.g. vitamin use) and risk factor (e.g. smoking) were chosen to assess this new method for estimating GAAD. These putative factors have been well established in literature, described in detail within this cohort, and are associated with miscarriage risk. These factors serve as a tool to assess our method and the impact of bias these may have on effect estimates associated with pregnancy loss. It is possible that other time-varying exposures, for example time-varying exposures that have a high (e.g. alcohol use) or low (e.g. illicit drug use) cumulative risk in early pregnancy, or a time-varying exposure that is established in literature and known not to affect miscarriage risk (e.g. caffeine use) could have served as alternative factors to assess our GAAD method. But my objective was to choose exposures in which an effect was established and significant and that were well captured well within RFTS to determine if effect estimates change appreciably with new GAAD method.

One potential limitation is that gestational age will be estimated based on measurements from a single ultrasound visit. However by taking cross-sectional ultrasound information with the ability to estimate growth provides an opportunity to estimate probable developmental state prior to miscarriage. This can help researchers to more accurately identify which insults have occurred prior to pregnancy loss and assess exposures that occur after developmental arrest but before the onset of bleeding by not attributing inaccurate exposure time for these exposures.

Miscarriage was defined based on participant self-report, either based on the day of dilatation and evacuation or the day of heaviest bleeding for each woman. Although this was the best measure for time of miscarriage available for this project when assessing estimated gestational age based on self-reported LMP, this was not a proxy for exact time of embryonic demise. We were unable to know the exact time of embryonic demise from the available data for women who have normal or abnormal ultrasounds and subsequently go on to miscarry. An ideal study would have repeated ultrasound measures on each subject with a known time of initiation until date of loss is detected by ultrasound. However, factors including cost and participant burden make such studies difficult and expensive to conduct. A similar study that determines developmental state and measures developmental progress variables at one time point could use our analysis approach to assess exposure-time misclassification and influence on miscarriage risk for risk factors associated with pregnancy loss.

Another potential limitation is the generalizibility of our findings may be limited by the enrollment of subjects. While this study population includes a prospective cohort of women in order to study risk factors associated with miscarriage, limitations include the inability to define the exact population base of women that is theoretically eligible to be in the study within these

communities. RFTS emphasizes community-based recruitment among women who are planning a pregnancy. However, women who choose to enroll in a study for early pregnancy health may be different than the general population of women trying to get pregnant, potentially leading to selection bias into the study. Participants in RFTS tend to be better educated, more health conscious with lifestyle factors related to pregnancy planning and have access to care before pregnancy.[73] Given that nearly a third of Black women in RFTS have at least four years of college, our sample might be expected to be at a lower risk of miscarriage than Blacks in the general population. However, despite this possibility which would attenuate affects, we have observed a clear elevation in risk for miscarriages occurring after the tenth week for Blacks in our cohort,[2] but not a difference in timing of gestational arrest. Furthermore, we demonstrated that race-stratified estimates in risk of loss associated with vitamin use have a magnitude of bias of nearly twenty percent for both Whites and Blacks. However, the bias occurs in opposite directions, suggesting that the protective effect of vitamin use may be overestimated for Whites while a similar protective effect may be underestimated for Blacks. With proper classification of gestational arrest prior to loss, effect modification by race disappears for the vitamin use miscarriage association. This suggests that by misclassifying time at loss we can artificially introduce effect modification by race, which does not exist if gestational arrest is properly classified.

Study implications

This research proposes a novel methods assessment for gestational arrest prior to loss. I am aware of no prior studies that have addressed timing of loss in the context of gestational arrest using early first-trimester ultrasound information. Research that gives insight to the

mechanisms operating during early pregnancy is useful, especially since this time period in pregnancy is not well understood. Miscarriage is a common adverse pregnancy outcome occurring frequently in early pregnancy. The timing of loss can help to differentiate distinct mechanisms of loss.[89] For example, chromosomal abnormalities have been observed in at least half of all pregnancy losses occurring in the first trimester, but represent a higher fraction of early losses than of later losses.[57]

Early fetal growth is important both in epidemiologic studies and clinical settings when related to reproductive outcomes such as miscarriage. Development may stop days to weeks prior to the onset of clinical recognition of miscarriage. Basing pregnancy loss on the time from LMP to clinical recognition of loss ignores the developmental state of the fetus prior to the loss. Nearly 40% of pregnancy losses have anembryonic gestation when assessed by early first-trimester ultrasound.[2] This results in inappropriate exposure time for common risk factors in early pregnancy. This research provides insight into our foundational understanding of the timing of miscarriage and in particular the challenge of accurate and early exposure assessment during early pregnancy.

Furthermore, I have elaborated differences in the timing of miscarriage between Blacks and Whites by using ultrasound data from a diverse prospective community-based cohort. Retrospective data (from medical records or self-report) used to assess miscarriage risk is often times incomplete, due to under-ascertainment of early pregnancy loss. Prospective data can provide the most accurate estimates of miscarriage risk, especially if women are enrolled early in their pregnancy, since we may be able to identify pregnancies that arrest prior to symptoms of miscarriage. There are significant racial and ethnic disparities observed in other adverse

pregnancy outcomes that may have origins in early pregnancy, such as events like placentation. Such research has potential to advance overall knowledge about causes of pregnancy loss and to help to identify risks that may be preventable when differentially distributed by race.

Future directions

Next steps in analysis of GAAD and timing of loss could include assessing the influence of time-varying exposures such as over-the counter medication use, or anti-depressant use in early first-trimester both of which have been associated with increased miscarriage risk. By more accurately identifying which insults have occurred prior to pregnancy loss and assessing exposures that occur after developmental arrest but before the onset of bleeding we will have more optimal method to assess miscarriage risk by not misattributing exposure time. Additional steps may also include estimating GAAD and the GAAD gap in other cohorts that have early ultrasound data available and include women who experience miscarriage. Since the risk of miscarriage diminishes with increasing gestational age, more prospective studies that accurately date gestational age are needed, even in cases where LMP dates are certain and when early ultrasound assessment is not always feasible.

Furthermore, we know that first-trimester fetal growth is not uniform. Variation in fetal growth in the early first-trimester may be a result of may many maternal factors, such as race, age, smoking history, BMI or vaginal bleeding.[18] The determinants of growth of the early embryo need to be explored to assess whether potentially modifiable maternal factors, such as obesity and smoking, affect growth and subsequent pregnancy outcomes. If high risk pregnancies and associated factors can be identified with high sensitivity and specificity, by assessing very

early pregnancy growth, then early interventions, such as lifestyle changes, including before conception, may potentially influence the course of the pregnancy.

Additionally, further research is needed for determining biologically plausible disparities in miscarriage risk. The elevated risk in fetal loss in Blacks compared to Whites may involve a variety of plausible causal pathways including differences in environmental or product exposures that accrue over weeks across a pregnancy, risk of insult from health vulnerabilities such as anemia or insulin resistance that vary by race and exert greater influences on fetal rather than embryological viability or genetic mechanisms such as inflammatory or immunological pathways that may vary by race and influence fetal well-being. Such research has the potential to advance overall knowledge on the causes of pregnancy loss and help identify risks that may be preventable.

APPENDIX

Appendix 1. Defining common reproductive terminology

Term	Definition
Miscarriage terms	
Blighted ovum	anembryonic gestation characterized by a normal-appearing gestational sac but absence of an embryo
Spontaneous abortion	miscarriage, loss of pregnancy before 20 completed weeks of gestation
Threatened abortion	first-trimester pregnancy that demonstrates uterine bleeding and/or cramping
Recurrent miscarriage	occurrence of three or more pregnancies that end in miscarriage
Early pregnancy loss	pregnancy loss before 10 completed weeks of gestation
Early fetal death	pregnancy loss between 10 and 16 weeks of gestation
Late fetal death	pregnancy loss between 16 and 20 weeks of gestation
Reproductive terms	
Conceptual age	pregnancy length from time of conception; "true" fetal age
Gestational age	traditionally determined from the first day of the mother's last menstrual period, can be estimated from ultrasound findings
Human chorionic gonadotropin(hCG)	hormone produced during pregnancy made by the developing placenta commonly detected through urine or blood tests

Continued…

Term	Definition
Ultrasound terms	
Crown rump length	Longest measurement of developing human fetus. Identified by ultrasound and used to estimate gestational age.
Fetal heart rate	the number of heartbeats in the fetus that occur in a given unit of time(e.g. beats per minute)
Fetal pole	thickening on the margin of the yolk sac of the fetus during pregnancy
Gestational sac	first definitive structure identified in early pregnancy by ultrasound
Mean sac diameter	measurement of the gestational sac used to date early first-trimester pregnancy
Yolk Sac	first anatomic structure identified within the gestational sac

Appendix 2. *Right from the Start* ultrasound form

Right from the Start 2

Study ID# T RSID

Sonographer: __ ULTSONOGRAPHER ____ ***NO SHOW*** ☐ (check, fax page 1 toRFTS)

Sonography Site: ULTUSSITE ***Print Requested*** ☐ (check)

FIRST TRIMESTER ULTRASOUND REPORT (ULTUSTYPE)

Date of US M M D D Y Y ULTUSDATE

DOB M M D D Y Y ULTDOB

Participant Initials ULTPPT INIT

Weight __ULTWEIGHT _____ **lbs** **Height** ULTHEIGHTFT _ftULTHEIGHTIN

Multiple Gestations? (circle)
ULTMULTIPLEGEST
NO YES → How many?
ULTMULTIPLEGESTCOUNT

At today's ultrasound you will be assessing gestation and looking for uterine fibroids. Please conduct a thorough examination of the entire myometrium using transvaginal and transvesical/abdominal approaches as required. Complete the entire form.

1. Measure weight and height - round to the nearest pound for weight and to the nearest ½ inch for height. For ½ pounds, round up to nearest pound (150.5 = 151 pounds); for ¼ inch, round up to nearest ½ inch (5' 5 ¼" = 5' 5 ½").
2. Provide a **digital image of the fetal pole** with caliper markings. (If a fetal pole is not seen, provide a digital image and measurements of most clearly identified intrauterine/gestational structure (i.e. gestational sac with diameters, decidual reaction without gestational sac, etc.).
3. If you have any technical comments related to performing the ultrasound, please make a note. DO NOT record comments related to pregnancy well being or fetal anatomy. A comment section has been provided under questions 3,4,5,6 and 7.
4. Provide a still photo or digital image on CD of the ultrasound to the participant. (Add a RFTS disclaimer label on the back of the photo or CD).
5. Prepare a **digital cine loop**. Record a full slow transverse sweep of the uterus moving from the woman's right to left side and a longitudinal sweep of the uterus from the anterior to posterior portion of the uterus.
6. Store all digital images on a CD labeled with the participant's study number (RSID) and date and type of the ultrasound (first trimester).
7. **If fibroids are present**, for each fibroid, record the following (If an indeterminate area is noted complete as much information as you can about the area):
   - Note all characteristics of each fibroid – check all that apply.
   - Provide three perpendicular diameters of each fibroid (or indeterminate area) (length, width and height). Please take 3 independent measurements and record the average mm value on page 3 of this form.
   - Use the Addendum form if more than six fibroids are identified.
   - Record on a CD a **digital image of each fibroid or indeterminate area** with caliper marks. Label each fibroid image with the fibroid number that corresponds to the measurements recorded on page 3 of this form. **If a fibroid is pedunculated, provide a digital image of the fibroid and stalk.**
   - Draw and label each fibroid or indeterminate area seen today on the uterine diagrams on page 4.
   - (UNC/WHI ONLY) Inform the participant about the follow-up ultrasounds, schedule 15-week US appointment.

     15 week US appointment: Date: _____ Time: _____ Site: _____
8. (UNC/WHI ONLY) **If a woman does not show for her appointment,** attempt to call and reschedule by the end of the day. If unable to reach, rescheduling will be handled by RFTS staff in main office.**rescheduled US appointment: Date: _____Time:_____ Site: _____**

Right from the Start 2

Study ID# T RSID

ULTUSDATE ULTDOB

Date of US M M D D Y Y | DOB M M D D Y Y

1. **Uterine Length** (Longitudinal Axis) ULT1 **REQUIRED** ___ mm

2. **Transverse Uterine Diameter** ULT2 **REQUIRED** ___ mm

3. **Gestational sac** visualized? ULT3A (ULT3A_2 for baby 2 & ULT3A_3 for baby 3)

Q 0 No

Q 2 Indeterminate **Comments:** ULT3ACOM (ULT3ACOM_2 for baby 2 & ULT3ACOM_3 for baby 3)

Q 1 Yes → Mean Sac Diameter: **REQUIRED** ULT3B ___ **mm** (3 diameter average)

Q 4 NA if EGA greater than 9 weeks (ULT3B_2 for baby 2 & ULT3B_3 baby 3)

4. **Yolk sac** visualized? ULT4A (ULT4A_2 for baby 2 & ULT4A_3 for baby 3)

Q 0 No

Q 1 Yes → Maximum Diameter: **REQUIRED** ULT4B ___ **mm** (inner to inner)

Q 2 NA if EGA greater than 9 weeks (ULT4B_2 for baby 2 & ULT4B_3 baby 3)

5. **Fetal pole** visualized? ULT5A (ULT5A_2 for baby 2 & ULT5A_3 for baby 3)

Q 0 No

Q 2 Indeterminate **Comments:** ULT5ACOM (ULT5ACOM_2 for baby 2 & ULT5ACOM_3 for baby 3)

Q 1 Yes → Crown-rump Length**: **REQUIRED** ULT5B_2 baby 2, ULT5B_3 baby 3 ULT5B ___ **mm**

** Q If CRL < 5mm (6w0d), then use MSD to calculate weeks EGA and EDC. (mark box if used)
New Variable Names
** Q If CRL > 53.7mm (12w0d), then measure both femur length (FL) and bi-parietal diameter (BPD) to calculate weeks EGA and EDC. (mark box if used)

New Variable Names

Femur Length: ___ **mm**

Bi-parietal Diameter: ___ **mm**

= _ ULT5CWEEKS & ULT5CDAYS_ **weeks EGA** = ___ ULT5D _____ **EDC** (ULT5D_2 & ULT5D_3 for babies2/3)

EGA COMMENTS: ULTCOMMENT

6. **Cardiac motion** present? ULT6A (ULT6A_2 for baby 2 & ULT6A_3 for baby 3)

Q 0 No

Q 2 Indeterminate **Comments:** ULT6ACOM, ULT6ACOM_2, ULT6ACOM_3

Q 1 Yes → Fetal Heart Rate: **REQUIRED** ULT6B ULT6B_2 ULT6B_3 ___ **Beats/minute**

Appendix 3. Selected questions from first-trimester interview

3.1 Prenatal and multivitamin use

L1a. Do you now take prescription or non-prescription prenatal vitamins?

L1b. In the past 4 months have you taken prescription or non-prescription prenatal vitamins?

L1d. Did you start taking prescription or non-prescription prenatal vitamins more than 4 months ago?

L1e. When did you start taking prescription or non-prescription prenatal vitamins? (Month, Day, Year)

L2a. Do you now take multivitamins other than prenatal vitamins?

L2b. In the past 4 months have you taken multivitamins other than prenatal vitamins?

L2d. Did you start taking multivitamins other than prenatal vitamins more than 4 months ago?

L2e. When did you start taking multivitamins other than prenatal vitamins? (Month, Day, Year)

3.2 Smoking

C35. Have you ever smoked cigarettes regularly, and by regularly I mean one or more cigarettes every day for at least a month?

C36. How old were you when you started smoking at least one cigarette a day?

C37. At this time, are you smoking cigarettes regularly, I mean one or more cigarettes every day?

C38. Do you usually smoke menthol or non-menthol cigarettes?

C39. At this time, how many cigarettes do you usually smoke a day?

C40a. When did you stop smoking? (Month, Day, Year)

C41. Before you stopped, how many cigarettes did you usually smoke a day?

C42. In the past 4 months, has your smoking changed in any way?

C43a. When did your smoking change? (Month, Day, Year)

3.3 Pregnancy intention

E4 (4a). We are interested in all birth control methods you used in the 12 months before your most recent pregnancy, including methods like natural family planning, condoms or rubbers, and hormonal methods like birth control pills. What are all of the methods you used in the 12 months before you got pregnant?

G7. Which of the following best describes your situation around the time you got pregnant?

- ❑ You stopped using protection or contraception or weren't using any because you wanted to get pregnant
- ❑ You were not using protection or contraception and you were not really trying to get pregnant
- ❑ You got pregnant during a change or gap in using protection or contraception and you were not trying to get pregnant *[A change could be anytime a woman goes from one type of contraception to another; a gap could mean that she missed a few pills or had sex with out a condom one time, etc.]*
- ❑ You got pregnant while you were using protection or contraception every time you had sexual intercourse and you were not trying to get pregnant
- ❑ Don't know
- ❑ Refused

G12. At the time you got pregnant this most recent time, did you want to have a/another *[if already has children]* baby at some time in your life?

- ❑ No → *skip to Section H.*
- ❑ Yes
- ❑ Don't know
- ❑ Refused

G13. Did you get pregnant this most recent time, sooner than you wanted, later than you wanted, or at about the right time?

- ❑ Sooner
- ❑ Later
- ❑ Right time
- ❑ Didn't care when
- ❑ Don't know
- ❑ Refused

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