VAGINAL BLEEDING IN EARLY PREGNANCY: PATTERNS, PREDICTORS, AND ASSOCIATION WITH MISCARRIAGE

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ABSTRACT
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Vaginal bleeding in early pregnancy:
Patterns, predictors, and association with miscarriage
(Under the direction of Donna Baird and Andrew Olshan)

First-trimester vaginal bleeding is common in pregnancy; however, few data have described the distribution, characteristics, and predictors of early bleeding episodes. The relationship between bleeding and miscarriage is not well understood.

Data from *Right From the Start* (RFTS), a prospective, community-based pregnancy cohort were used for all analyses. We used descriptive statistics to characterize first-trimester bleeding episodes and logistic regression to identify predictors of bleeding. Bleeding characteristics (such as heaviness, duration, timing, and color) predictive of miscarriage were identified using classification and regression trees. The relationship between bleeding and miscarriage was modeled using discrete-time hazard models. We compared retrospectively collected bleeding reports from the first trimester interview with prospective data from a daily diary to obtain sensitivity, specificity, and kappa statistics. Log-linear models were used to identify predictors of agreement. In all analyses, we removed bleeding episodes that ended within four days of miscarriage.

Approximately one-fourth of participants reported bleeding in early pregnancy, mostly spotting or light bleeding episodes. Most episodes lasted fewer than 3 days, and most occurred between gestational weeks 5 and 8. Heavy episodes, reported by about 2% of women, were more likely to be painful, of longer duration, and red in

color. Predictors of bleeding were age (particularly between 28 and 34), increasing education, nulliparity, and menstrual cycle length less than 27 days or greater than 33 days. Maternal conditions (diabetes, fibroids), prior pregnancy outcomes (miscarriage, induced abortion), reproductive tract infections, smoking, and alcohol intake were also predictive of bleeding. Women who reported heavy bleeding had nearly three times the risk of miscarriage compared to women without bleeding (OR 3.0, 95% CI 1.9, 4.6). Spotting and light bleeding episodes were not related to miscarriage. Bleeding episodes and characteristics were reported with high levels of agreement in the diary and interview. No predictors of agreement were identified in this analysis.

To summarize, we found that vaginal bleeding was a common first-trimester symptom. However, the majority of episodes were spotting or light bleeding episodes, which did not confer an increased risk for miscarriage. Although few women reported heavy bleeding, heavy bleeding was more strongly related to pregnancy loss.

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AIC	Akaike's information criterion
BMI	Body mass index
CART	Classification and regression tree
CATI	Computer-assisted telephone interview
CI	Confidence interval
ICE	Imputation using chained equations
LMP	Last menstrual period
NC	North Carolina
OR	Odds ratio
RFTS	*Right From the Start*
RR	Risk ratio
SCH	Subchorionic hematoma
TN	Tennessee
TX	Texas

CHAPTER 1 : INTRODUCTION AND SPECIFIC AIMS

A. INTRODUCTION

Miscarriage is a common, and poorly understood, adverse pregnancy outcome. Understanding the nature of common biologic processes, symptoms, and behavioral changes that occur during early pregnancy may contribute to increased knowledge of miscarriage risk factors. The relationship between vaginal bleeding, occurring in an estimated 20% of all pregnancies, and miscarriage remains unclear. Although vaginal bleeding occurs commonly in early pregnancy and may mark a miscarriage event, it is not always associated with imminent pregnancy loss. Characterization of the timing and frequency of bleeding in early pregnancy will provide useful information that will increase understanding of the role of this symptom in pregnancy. It may also give some insight into the extent of errors in gestational age dating, which may occur when early pregnancy bleeding is confused for the last menstrual period.

The goal of this project was to better understand vaginal bleeding symptoms occurring in early pregnancy and its association with miscarriage. The timing, duration, heaviness, and pain associated with bleeding episodes was described. Maternal characteristics that predict the presence of bleeding were investigated. This was followed by an analysis of the association between bleeding and miscarriage. Prospectively collected bleeding data from a small subset of women

were used to validate bleeding episode information obtained from the first trimester interview.

Data from *Right From the Start* (RFTS), a prospective pregnancy cohort, were used to meet the aims of this project. RFTS enrollment is community-based, and occurs in early pregnancy. Data from this cohort will answer critical questions related to early pregnancy symptoms, an important topic from the perspective of patients, clinicians, and researchers.

B. SPECIFIC AIMS

Vaginal bleeding is a common and alarming symptom during early pregnancy. It has been estimated to affect 7 to 24% of all pregnancies.[1-3] Several reports suggest that bleeding is associated with an increased risk of various adverse pregnancy outcomes, including preterm birth and low birth weight.[4-6] Many of these studies are limited by their choice of study population and bleeding assessment. Furthermore, the relationship between episodes of bleeding and miscarriage is not well characterized. Although vaginal bleeding often occurs as a result of miscarriage, we focus on other prior bleeding episodes that do not immediately result in miscarriage. There are no large-scale descriptions of the usual patterns of bleeding in a pregnant population to provide a context in which to consider bleeding symptoms in early pregnancy. It is not clear whether a bleeding episode in itself, or a host of other factors, are important in predicting later miscarriage. It is also unclear what mechanism operates to relate bleeding to adverse pregnancy outcomes.

One reason for the paucity of information in this area of research relates to the difficulty of studying early pregnancy. The outcome of interest in this analysis, miscarriage, occurs prior to the completion of 20 weeks of pregnancy, and frequently occurs prior to clinical recognition of pregnancy. Studies of this early pregnancy period are limited by issues related to early/pre-pregnant recruitment, incomplete case ascertainment, recall bias, and generalizability. In order to ideally examine factors related to miscarriage, a representative group of women recruited before pregnancy or during early pregnancy should compose the study population, and information about exposures and symptoms during pregnancy should be collected

soon after enrollment. Such a population, with some limitations, is present in the *Right From the Start* (RFTS) cohort. Some attractive characteristics of this cohort include 1) comprehensive bleeding episode assessment; 2) longitudinal data obtained on >4000 pregnancies over an 8 year period (2000-2008); and 3) ascertainment of early pregnancy outcomes, including over 500 miscarriages. A comprehensive first trimester interview is conducted for all RFTS participants, which collects bleeding episode information. A subset of women completes prospective daily diaries, providing information about pregnancy symptoms and exposures prior to pregnancy and throughout the first trimester. Bleeding information collected at the first trimester interview will be compared to the diary reports for this subset.

This cohort is well designed to answer a broad range of hypotheses related to miscarriage risk factors. This analysis will focus on the relationship between first trimester bleeding and miscarriage. In undertaking this analysis, we hope to learn more about bleeding patterns in early pregnancy, as well as determine the association between various maternal characteristics and bleeding. We hypothesize that sporadic, light bleeding is a common occurrence of early pregnancy. Heavy bleeding associated with reports of pain or cramping will be more strongly associated with miscarriage. Finally, we hypothesize that retrospective reports of bleeding will contain fewer reports of light spotting and bleeding compared to the prospective diary. These hypotheses will be explored under the following specific aims:

1. Determine the usual patterns and characteristics of vaginal bleeding in pregnancy, including details regarding timing, frequency, heaviness, color, and pain associated with bleeding. Identify the maternal characteristics that predict bleeding in early pregnancy, including maternal age, maternal comorbidities, prior birth outcomes, and cycle characteristics.

2. Evaluate the association between patterns of bleeding in early pregnancy and the occurrence of miscarriage.

3. Evaluate the extent of agreement between bleeding episodes from retrospective first trimester interviews and prospectively collected data from daily diaries.

Descriptive analysis, logistic regression, discrete-time hazard models, and log-linear models will be used to answer these study aims. The results of these analyses will be useful to patients, providers, and researchers who wish to understand the context in which to evaluate early pregnancy bleeding symptoms.

CHAPTER 2 : BACKGROUND AND SIGNIFICANCE

A. MISCARRIAGE

Because miscarriage is difficult to study, few reports describing its causes and preventive factors exist. A review of the existing literature describing the current state of knowledge related to miscarriage, including epidemiology and risk factors, follows.

Epidemiology

Pregnancy loss is the most common adverse pregnancy outcome. Approximately one-third of implanted embryos are lost prior to live birth.[7,8] Between 20% and 40% of losses are pre-clinical losses, occurring prior to the first missed menstrual period.[8,9] Miscarriage is defined as the loss of a clinically detected pregnancy prior to twenty completed weeks of gestation. These losses, accounting for about 15% of all clinically recognized pregnancies,[10] can be categorized as early or late losses, depending on whether the loss occurs in the first or second trimester, respectively.[11] In addition to the pre-clinical and clinically recognized pregnancy losses discussed herein, it should also be noted that an unknown number of occult losses occur, comprised of conceptions that fail to implant. These conceptuses, which do not survive more than a few days after conception, are undetectable due to the absence of an easily accessible, specific marker for the pre-implantation

embryo.[7] The total number of conceptions that are spontaneously lost before twenty weeks gestation may be as high as 70%.[7]

Embryonic and endometrial characteristics associated with miscarriage

The causes of miscarriage remain unclear. About half of early losses are thought to be due to fetal chromosomal abnormalities leading to non-viability.[12,13] Chromosomal abnormalities result from nondisjunction in gamete formation, resulting in early errors in zygote cell division and subsequent complications with blastocyst differentiation.[11] Genetic abnormalities also stem directly from the maternal or paternal genotype, as in the case of unbalanced translocations that are passed on from the sperm or egg.[11,14] Genetic factors may lead to structural or developmental aberrations in the embryo, slowing growth and progress towards subsequent stages in development, such as implantation. Because the endometrial environment is dynamically changing throughout the cycle, if processes such as implantation are delayed substantially, the endometrial environment may not adequately support the embryo. A larger proportion of embryos that undergo late implantation (more than nine days after ovulation) are subsequently lost.[15] Furthermore, a chromosomally abnormal embryo that implants successfully may not express necessary factors or respond to signaling molecules at appropriate times during development, eventually leading to fetal demise.

An interplay between the chromosomal makeup of the embryo and the endometrial environment contributes to the occurrence of pregnancy loss. Factors affecting endometrial receptivity, particularly the uterine environment around the time of implantation, account for a proportion of those pregnancy losses that cannot be

directly related to genetic abnormalities.[13] Defects in the early processes of implantation, invasion into the myometrium, or access to the uterine vasculature may contribute to pregnancy loss weeks or months after the event.[13] Later complications, such as pre-eclampsia, may also result from similar early insults and abnormalities in placental growth and differentiation.[16] The site of implantation in the uterus also plays a role in pregnancy viability, with implantations occurring in the middle and lower regions of the uterus more likely to miscarry.[17]

To summarize, the events and environment of very early pregnancy influence the eventual outcome of the pregnancy. From an epidemiologic and clinical perspective, it is difficult to assess the extent to which these early, often unobservable, events predispose to an outcome like miscarriage. Because of this, the majority of epidemiologic studies of the causes of miscarriage have focused on maternal characteristics that confer an increased risk of miscarriage, rather than assessments of embryonic genetic makeup or markers of endometrial receptivity.

Maternal characteristics associated with miscarriage

Studies of maternal factors have uncovered a variety of characteristics associated with miscarriage. In general, miscarriage risk increases with increasing maternal age and number of prior miscarriages.[18-20] These trends may be the result of an increased frequency of age-related errors in DNA replication, other aspects of oocyte and embryo quality, or a uterine environment that is less amenable to the development of the embryo.[19] Other maternal factors thought to affect the risk of miscarriage include structural uterine anomalies, such as bicornuate uterus, or benign tumors, such as fibroids. These structural malformations physically interfere

with the ability of the conceptus to implant or grow in the uterus due to their space-occupying effect.[11,18,21,22]

Maternal comorbidities have also been investigated, particularly in populations of women with recurrent miscarriage. Women with thyroid disturbances, autoimmune diseases, thrombophilic defects, and other systemic disorders such as polycystic ovarian syndrome have an increased risk of miscarriage and decreased fertility.[11,23,24] Similarly, maternal obesity and poorly controlled diabetes have also been linked to miscarriage.[25-28] Other hormone alterations may also be related to miscarriage,[29,30] including luteal phase defects. This condition is characterized by low progesterone production by the corpus luteum, resulting in miscarriage or reduced fertility due to an inability to maintain pregnancy.[31-33] These factors contribute to a suboptimal uterine environment and decreased endometrial receptivity.[33]

Menstrual cycle length and regularity may be related to pregnancy loss. Specifically, long cycles have been associated with miscarriage.[34-37] Short cycles and irregular cycles have also been associated with miscarriage.[34,35] These relationships may be modified by other systemic factors, such as obesity.[38]

Maternal infection may also play a role in miscarriage, although this has not been investigated extensively. Asymptomatic bacterial vaginosis may be associated with second trimester miscarriage,[39,40] and some evidence also indicates that oral infections and placental inflammation may be related to late miscarriage.[41,42]

In addition to medical conditions that may be related to miscarriage risk, maternal behaviors and occupational factors have also been suggested to increase

the risk of miscarriage. Work schedule, particularly working at night or working overtime during the first trimester, has been associated with increased risk of miscarriage.[43] Work-related stress and stress due to acute or chronic stressors have also been found to be related to a higher risk of miscarriage.[44-48]

Both active smoking and exposure to environmental tobacco smoke have been associated with miscarriage.[49,50] This may result from both reduced maternal fertility and altered endometrial receptivity; in a population of women undergoing in-vitro fertilization, heavy smokers were less likely to achieve pregnancy.[51] Maternal dietary exposures, including alcohol and caffeine exposure, have also been associated with increased risk of miscarriage, although some of the evidence is equivocal.[52-56] Additionally, some studies found an increased risk of miscarriage for caffeine exposure that occurred prior to pregnancy, regardless of consumption during pregnancy.[57] Certain medication exposures have also been thought to increase the risk of miscarriage, including non-steroidal anti-inflammatory drugs[58,59] and some classes of anti-depressants.[60,61]

Paternal characteristics associated with miscarriage

A smaller literature outlines the relationship between paternal characteristics and miscarriage. Because the sperm contributes half of the genetic make-up of the embryo, a substantial proportion of genetic abnormalities related to miscarriage likely derive from paternal factors. Paternal factors may affect chromosomal and structural abnormalities in the sperm.[14] Additionally, some investigators have found a link between paternal age and miscarriage, likely mediated by sperm quality.[62] Paternal environmental exposures and behaviors are also thought to play a role.[56,63]

B. VAGINAL BLEEDING

Epidemiology

Large, systematic, population-based descriptions of bleeding symptoms in early pregnancy that are not restricted to women having a live birth have not been undertaken. More studies focusing on later pregnancy bleeding, associated with placental abruption or placenta previa, exist.[64-70] However, anecdotal knowledge suggests that bleeding is relatively common in early pregnancy, and some evidence indicates that if it occurs, it increases the risk of adverse pregnancy outcomes.[4] Studies which attempt to link early pregnancy bleeding with pregnancy outcomes have reported a range of prevalence estimates for bleeding in early pregnancy, ranging from 7 to 24%.[1-3]

Previous studies

Three reports attempt to describe early pregnancy bleeding patterns in a pregnant population, by Yang et al,[1] Harville et al,[71] and Axelsen et al.[3] Most participants were enrolled in conjunction with clinical care.[1,3] These studies are limited by retrospective data collection,[1,3] second-trimester recruitment from prenatal clinics,[1,3] and small sample size.[71] Although these studies contribute important knowledge, there are no reports describing the bleeding patterns in a large, population-based study that is not limited by a long time to recall or inadequate follow-up time.

The Yang analysis was based on a clinic-based population of pregnant women (n=2800) who reported their early pregnancy bleeding patterns at the end of the second trimester (26 to 30 weeks of pregnancy).[1] This study found that 25% of

women reported vaginal bleeding during pregnancy, with peak incidence during the first completed month of pregnancy. Because bleeding assessment occurred in a telephone interview conducted around the 28th week of gestation, the extent to which these results are affected by recall error is unclear. The timing of bleeding was not assessed in detail, with episodes reported in monthly intervals. Additionally, only those women whose pregnancies continued to the mid/late second trimester (20 to 26 weeks) were included, eliminating all women who had a miscarriage. This study focused on later pregnancy outcomes such as preterm birth.

The Harville analysis focused on the reports of 14 women (9% of total n = 151) who prospectively reported bleeding symptoms during the first eight weeks of pregnancy.[71] Twelve of the fourteen women with bleeding continued to live birth; bleeding was not associated with miscarriage in this study. This study also found no evidence for the presence of implantation bleeding. Although the details obtained from this study are useful, this study is limited by the small numbers of participants and data collection only through the eighth week of gestation. This study is the only prospective, longitudinal description of daily bleeding patterns in very early pregnancy.

The Axelsen study analyzed a group of Danish women in prenatal care.[3] About 20% of participants (n=1091) reported bleeding in a 16 week questionnaire. The median week of first occurrence of bleeding was eight weeks, and two-thirds of all women did not report pain in association with their bleeding symptoms. Although this study is population-based (97% of women in their area receive care at the prenatal clinics) and has ~6800 participants, the analysis only includes data from

women whose pregnancies progress to live birth, lacking a complete ascertainment of all pregnancies in the population (such as those ending in miscarriage or fetal death). The focus of this study is primarily on the relationship between recalled bleeding and later outcomes such as preterm birth.

Two additional studies published over 30 years ago provide some descriptive information about the incidence and patterns of vaginal bleeding in early pregnancy.[72,73] These studies describe the timing of bleeding and maternal characteristics associated with increased bleeding occurrence. One of the studies found that approximately 27% of pregnancies with vaginal bleeding result in miscarriage.[73] Unfortunately, neither of these publications contains a complete methods section; no details regarding data collection procedures or sample recruitment are provided, making it difficult to assess the validity of their results.

Based on this review of the identified early pregnancy bleeding literature, it is clear that little data exists that would be relevant for miscarriage as an outcome. The prevalence of bleeding reported by these studies is wide (7-25%).[1-3] This basic information needs to be clarified before undertaking additional analyses of the relationship between bleeding symptoms and pregnancy outcomes such as miscarriage or preterm birth.

Predictors of bleeding

Few publications have outlined the maternal and pregnancy characteristics associated with bleeding, most of which have evaluated predictors using unadjusted analyses. Only one previous study has systematically investigated the maternal predictors of bleeding in a general obstetric population.[1] This research found that

women of advanced maternal age, with passive smoking exposure, prior preterm birth, multiple prior elective terminations and prior miscarriages were more likely to experience intense vaginal bleeding, as measured by several characteristics including heaviness, duration, and index of total blood loss.

Another recent study of emergency department visits for vaginal bleeding found that Hispanic and younger (ages 20-29) women had higher rates of Emergency Department visits than other subpopulations studied. This analysis was based on a national database of Emergency Department visits, and may reflect national patterns in access to care.[74]

Other studies have also reported unadjusted associations with increasing maternal age, minority race/ethnicity, prior obstetric outcomes (induced abortion, miscarriage, stillbirth, preterm delivery), or use of assisted reproductive technologies.[5,75,76]

Sources of vaginal bleeding in early pregnancy

A bleeding episode in pregnancy may have several sources. Most superficially, bleeding may result from vaginal or cervical pathology. This could be due to a local lesion, inflammation, or a polyp.[77,78] Bleeding may also be related to a uterine fibroid.[79] Very early bleeding may also be related to physiological changes associated with implantation,[80] or with usual cycles of menses.[81]

Bleeding may also occur due to low levels of progesterone. Presence of sufficient levels of progesterone during pregnancy is required for pregnancy maintenance.[82] Decreasing progesterone levels are the trigger for the onset of menses during the usual menstrual cycle. If progesterone levels decrease during

pregnancy, it is conceivable that bleeding episodes may occur, by a mechanism similar to what triggers the onset of menses.[82] More details about the potential role of progesterone can be found in “Biologic Mechanisms.”

Bleeding may also occur in areas where the placenta and fetal membranes detach from the uterine wall, similar to the process underlying placental abruption in later pregnancy. Potential mechanisms underlying the detachment are outlined in “Biologic Mechanisms”. This separation can result in subchorionic bleeding, which may be observable on ultrasound as a subchorionic hematoma (SCH). SCH are found in approximately 20% of patients who present to a hospital with vaginal bleeding,[80,83,84] and are rare in general obstetric populations.[85] SCH have been associated with alterations in serum markers of fetal well-being.[86] It has been hypothesized that clinically recognized vaginal bleeding is the result of subchorionic bleeding that escapes into the cervical canal.[87]

Studies have found associations between the presence of a SCH and adverse obstetric outcomes, such as miscarriage, preterm birth, and fetal growth restriction.[88-90] The interplay between SCH, vaginal bleeding, and miscarriage has also been investigated, with some findings that presence of vaginal bleeding alone (without evidence of SCH) is an independent risk factor for miscarriage.[85,91] On the other hand, some studies have found that the presence of a SCH does not adversely affect obstetric outcome, particularly for SCH detected in the first trimester among women with bleeding.[83,92-95] Some studies concluded that SCH are common and insignificant sonographic findings in women with vaginal bleeding.[83,94]

Specific characteristics of SCH, such as size, location, and gestational age at formation, have also been associated with certain outcomes. Specifically, large-volume SCH have been associated with poor obstetric outcome,[91,96] while other reports suggest that the location of SCH is more important in determining pregnancy outcome.[90] Studies of the size of SCH must be interpreted with caution, however, as the size of a SCH is determined by the amount of bleeding in the subchorionic space, as well as the amount of external vaginal bleeding that has occurred.[85] The time at which the ultrasound is conducted in relation to symptoms may bias SCH size.

Although SCH are found in a substantial proportion of women who present with vaginal bleeding, SCH are not found in all women who experience vaginal bleeding, likely due to the fact that the blood has not accumulated internally for visualization.[83] It is possible that the origins of these cases of vaginal bleeding are similar to the physiologic processes underlying the development of SCH.

C. BLEEDING AND PREGNANCY OUTCOMES

Although the underlying source of bleeding episodes remains unclear, the relationship between bleeding symptoms in pregnancy and various pregnancy outcomes has been investigated.[4,85] Studies of adverse outcomes in both early and late pregnancy are briefly reviewed.

Miscarriage

Women who present to the clinic or emergency department with early pregnancy bleeding are usually considered to have a 'threatened abortion'. Approximately 35 - 66% of women hospitalized with threatened abortion proceed to miscarriage.[76,97-99] Women with threatened abortion and ultrasound-detected fetal cardiac activity have a lower risk of miscarriage, ranging from 5 to 23%.[100-103] These reports of the risk of miscarriage are based on clinical populations whose symptoms and outcomes are collected retrospectively in obstetric clinics or emergency departments.

Nine previous studies have been identified that examined the relationship between vaginal bleeding and miscarriage.[5,76,98,99,104-107] These are summarized in Table 2.1. Most studies have reported some relationship between early pregnancy bleeding and miscarriage. Gracia and colleagues found that a complaint of bleeding was associated with miscarriage in their study population recruited in an urban emergency department (OR 7.4, 95% CI 5.7, 9.4).[104] Weiss et al. conducted a similar analysis among women presenting for prenatal care in several sites throughout the country and reported an OR of 2.5 (95% CI 1.5, 4.3) for the relationship between light bleeding and miscarriage and an OR of 4.2 (1.6, 10.9) for

heavy bleeding and miscarriage.[5] A less stable estimate of the OR was also provided by Chung et al., who reported that vaginal bleeding similar to menses was associated with miscarriage (OR of 10.5; 95% CI 1.5, 74.4) when compared to light bleeding.[98] This study was conducted among 1000 consecutive bleeding cases presenting to a university hospital. Tongsong et al. reported a risk ratio (RR) of 2.9 (95% CI 1.1, 8.0) for the relationship between first trimester threatened abortion and miscarriage.[105] Strobino and Pantel-Silverman published a report showing that moderate or heavy bleeding was related to pregnancy loss of both a normal (OR 3.6; 95% CI 2.1, 6.2) and abnormal karyotype (OR 4.9; 95% CI 2.1, 11.6).[76] However, slight bleeding was only associated with a miscarriage of a normal karyotype (OR 2.7; 95% CI 2.0, 3.6).[76] Bennett et al. found that the risk of miscarriage more than doubled when bleeding occurred in the first eight weeks of pregnancy, compared to later episodes of bleeding.[91] The remaining studies describing the relationship between bleeding and miscarriage used unadjusted tabular analyses to report the risk of miscarriage among those who reported bleeding and those who did not.[99,106,107] Additional details about these studies are listed in Table 2.1.

However, these studies have important limitations. No uniform definition of bleeding has been used in the literature; some studies focused on bleeding quantified by number of pads used and other studies included light spotting in their bleeding definition. Most studies were prenatal clinic- or hospital/emergency department-based studies of pregnant women seeking care. Recruitment only from prenatal clinics is especially difficult for studies of miscarriage because many miscarriages occur before entry to prenatal care. Additionally, recruitment in a

hospital setting only captures the most serious episodes of bleeding that occur as a direct consequence to miscarriage. Thus, to have complete ascertainment of all women experiencing bleeding during pregnancy, a community- or population-based recruitment design is preferred, permitting enrollment of participants very early in pregnancy, before entry to prenatal care.

Another drawback of the published literature is that presence of vaginal bleeding was an eligibility criterion in almost all of these studies. Because only women with bleeding were assessed, the conclusions that can be drawn from these studies are limited. These studies do not have an appropriate comparison group to which the risk of miscarriage can be compared. Many miscarriages are not associated with any symptoms of bleeding. Some of the studies categorized different 'types' of bleeding (such as light, heavy, etc.) in order to create different groups for comparison. The study by Weiss and colleagues was the only analysis that used a general clinic-based population of pregnant women, rather than focusing only on those with bleeding symptoms.[5] This study enrolled participants between 10 and 14 weeks of pregnancy, not accounting for pregnancy losses occurring prior to that time. The reported results are for the relationship between bleeding in the month prior to enrollment and second trimester miscarriage and the overall focus of the study is primarily on the effect of bleeding and later pregnancy outcomes. A case-control study by Strobino and colleagues likewise only reports on the relationship between first-trimester bleeding and second-trimester fetal loss (defined as loss occurring up to 28 weeks of gestation).[76]

Later pregnancy outcomes

Vaginal bleeding in early pregnancy has also been related to a variety of outcomes that occur later during pregnancy. Studies have focused on preterm birth,[2,108-110] small-for-gestational age births,[109,111] low birthweight,[2,75,112] placental abruption[113] and rate of Caesarean section.[114,115] This literature has been systematically reviewed.[4] It is clear that vaginal bleeding is of interest not only with regards to early pregnancy outcomes, but also for later outcomes. Proper characterization of this symptom is essential as an initial step towards understanding its role in pregnancy.

D. BIOLOGIC MECHANISMS

A bleeding episode in pregnancy may be associated with a variety of pregnancy outcomes, including miscarriage. Evidence from the basic science literature provides some insight into biologic mechanisms that may underlie the association between bleeding and miscarriage.

Miscarriage as a disorder of placentation

A hormonally functional placenta begins to produce sufficient amounts of progesterone to support the pregnancy around the 7th week of gestation.[82,116] Progesterone plays a vital role in maintaining pregnancy, by preventing uterine contractility, maintaining the endometrium, and altering the maternal immune response to prevent rejection of the embryo.[117] If sufficient amounts of progesterone are not produced, miscarriage may result.[82]

Some evidence also suggests that during the first ten weeks of gestation, the fetus develops in a largely hypoxic environment.[118,119] The gestational sac serves as a barrier to prevent oxygen transfer to the fetus, whose metabolism is largely anaerobic during this time.[120] Additionally, extravillous trophoblastic cells of the fetus migrate to the edge of the intervillous space during most of the first trimester to plug the spiral arteries and seal off the intervillous space. This creates a trophoblastic shell that protects the fetus from the maternal blood supply.[121] Furthermore, at this time, the spiral arteries are narrow, high-resistance vessels that inhibit blood flow.[122] These barriers between the maternal and fetal circulation create a physiologically hypoxic environment during early pregnancy. Early onset of maternal-fetal circulation may expose the fetus to high levels of oxidative stress. Specifically, free oxygen

radicals interact with lipids, proteins, and DNA, to destroy membranes and contribute to cellular dysfunction and cell death. Overall, oxidative stress damages fetal tissues, disrupts organogenesis, and affects other developmental processes during this critical period of pregnancy.[120,123]

At about ten weeks of gestation, the plugs located at the periphery of the placenta begin to disintegrate, and maternal-fetal circulation begins in the intervillous space.[123] The spiral arteries of the placenta transform into low-resistance vessels to accommodate increased blood volume. By fourteen weeks of pregnancy, maternal blood flows freely into the placenta, permitting the exchange of nutrients and other essential factors.[16] By this time, fetal antioxidant enzymes are functional, providing the fetus with additional defense mechanisms to maintain the balance of oxidative factors.[124]

A proportion of miscarriages may result from premature onset of maternal blood flow and fetal exposure to oxidative stress.[16,118] Due to defective placentation, the trophoblastic shell may be fragmented and inadequately prevent the entry of maternal blood into the intervillous space.[121,125] Premature onset of circulation exposes the fetus to the damaging effects of free oxygen radicals. Markers of oxidative stress were increased in miscarriage tissues compared to controls.[126] Bleeding into the intervillous space may also lead to subchorionic bleeding, which may be clinically observed as vaginal bleeding or observed on ultrasound (see “Sources of vaginal bleeding in pregnancy”). Subchorionic bleeding has been associated with increased production of free oxygen radicals, and may exert a mechanical space-occupying effect that interferes with fetal presence in the

uterus.[127] Furthermore, subchorionic bleeding may cause a chronic inflammatory reaction and uterine contractions that directly lead to miscarriage.[127] Extra-cellular matrix degradation may also destabilize and weaken fetal membranes, increasing the likelihood of pregnancy loss.[125] These defects in placentation probably originate very early in gestation; the processes described may relate to anomalies of implantation or early fetal cell organization.[121]

Miscarriage and bleeding associated with other physiological changes

Bleeding could also lead to a cascade of other events that may be involved in the pathophysiology of miscarriage. A hematoma may result in an inflammatory reaction, leading to uterine contractions and loss of pregnancy.[127] Some studies have described links between cytokine imbalances and bleeding and miscarriage,[128,129] while this has been disputed by other authors.[130] Little is known about the role of the immune response predisposing to early pregnancy loss. Previous work suggested that a Th2-biased immune response may be characteristic of women with miscarriage or threatened abortion;[128] however, this paradigm has recently become controversial due to new data describing the role of previously uninvestigated cytokines and other immune cells in early pregnancy loss.[131] Overall, immune and inflammatory mediators may be altered during threatened abortion, although no definitive conclusions exist.

Infection may mediate the relationship between bleeding and miscarriage.[132] Infection during pregnancy has been implicated as a factor underlying a variety of adverse outcomes, including preterm birth,[133] and may predispose to some of the previously mentioned immune alterations. Investigations of the role of infection in the

manifestation of bleeding symptoms have concluded that bleeding in pregnancy may be the only symptom related to a concurrent underlying infection of the reproductive tract.[104,134] On the other hand, early pregnancy bleeding may also result in infection by opening access to areas of the reproductive tract that were previously inaccessible to pathogens.

Endocrinologic changes occurring in early pregnancy may also be associated with bleeding and loss. Alterations of levels of hormones and metabolic factors among women with miscarriage compared to women whose pregnancy continues have been found in studies of women with vaginal bleeding. The central role of progesterone in maintaining pregnancy has been previously discussed.[117] Differences in the levels of human chorionic gonadotrophin,[104,135,136] thyroid hormones,[136] and endocannabinoids[137] have been reported for women with miscarriage compared to women with continuing pregnancy. Although these differences may simply be representative of the overall health of the pregnancy, it is noteworthy that different hormone levels have been documented by miscarriage status among women with bleeding. Some studies also contain an external control group of women who did not experience any vaginal bleeding. In these comparisons, lower levels of human chorionic gonadotrophin[135] and higher levels of thyroid hormone[136] were found among women with bleeding. There may also be a relationship between alterations of thyroid hormone levels and immune function.[136] All in all, it is clear that endocrinologic changes occur within the maternal system in response to miscarriage and also in response to an episode of bleeding. Whether these events are causally related remains unclear.

E. CONCEPTUAL FRAMEWORK

This project seeks to increase understanding of the implications of a bleeding episode in early pregnancy. We hypothesize that a bleeding episode during early pregnancy may indicate one of the following processes:

1) a marker of pregnancy loss (e.g., bleeding may be related to infection, or abnormal placentation, which is the actual causative agent);

2) the result of pregnancy loss (e.g., fetal demise may have occurred days or weeks prior to the onset of bleeding, where bleeding is merely a symptom of an event that has already occurred);

3) a physiological phenomenon of early pregnancy (e.g. implantation bleeding).

Figure 2.1 summarizes some of the mechanisms underlying the relationship between vaginal bleeding and miscarriage, which were discussed in the previous section. In this analysis, we eliminate episodes that immediately precede pregnancy loss, so as to focus our efforts on understanding (1) and (3).

Although this model depicts potential mechanisms by which some pregnancy losses occur, it does not explain why so many women with symptoms of vaginal bleeding do not have a miscarriage. Variations in bleeding intensity or timing may be related to differences in outcomes. Many of the processes depicted in the figure are unmeasured in this study. As a beginning step, characterization of the patterns of bleeding and related maternal characteristics will provide a foundation for future studies in this area.

F. PUBLIC HEALTH SIGNIFICANCE

Although vaginal bleeding may be associated anecdotally with pregnancy loss (perhaps due to the fact that bleeding is often a primary symptom of loss), understanding the characteristics and distribution of first trimester bleeding will clarify its role in predicting adverse outcomes. This project will evaluate the relationship between vaginal bleeding experienced by some women in early pregnancy and miscarriage. Based on the review of the identified literature on this topic to date, it is evident that there is little solid data in this area and that an analysis of RFTS data can contribute a great deal of knowledge to this field. This research will inform future studies of early pregnancy, as the timing, frequency, or intensity of symptoms may provide clues of the gestational/developmental stages at which bleeding may be most relevant. Clinically, any research that gives insight to the processes and mechanisms operating during early pregnancy is useful. This time period in pregnancy is not well understood, despite the fact that the most common adverse outcome of pregnancy, miscarriage, frequently occurs during this time.

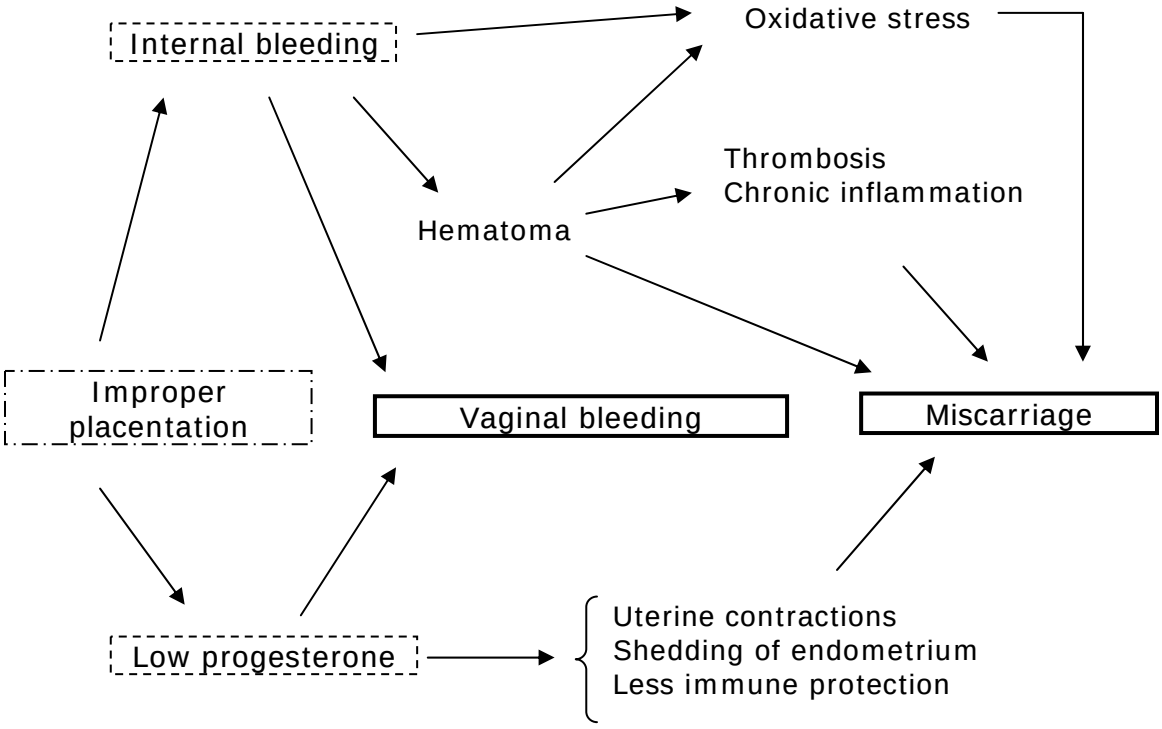
Table 2.1. Previous studies of vaginal bleeding and miscarriage.

Author (year)	n	Setting	Eligibility	Bleeding ascertainment	Relationship examined	Effect (95% CI)
Gracia et al. (2005)[104]	2,026	Case-control study in an Emergency Department Philadelphia, PA	Patients presenting with pelvic pain or vaginal bleeding in the first trimester, before definitive diagnosis	Information obtained at time of presentation	Any bleeding and miscarriage versus no bleeding	OR 7.4 (5.7, 9.4)
Weiss et al. (2004)[5]	16,506	Prospective, multicenter cohort, objective is to examine first- and second-trimester serum and ultrasound markers to assess Down syndrome risk, 14 centers in USA	Unselected obstetric population	Interview at 10-14 weeks gestation, bleeding in 4 weeks prior to enrollment only	Light bleeding and 2nd trimester miscarriage versus no bleeding; heavy bleeding and 2nd trimester miscarriage versus no bleeding	Heavy bleeding: OR 4.2 (1.6, 10.9) Light bleeding: OR 2.5 (1.5, 4.3)
Chung et al. (1999)[98]	739	Cohort study of 1000 consecutive women in a university teaching hospital Hong Kong	Pregnant patients presenting with a history of bleeding in the past 24 hours	All symptomatic, bleeding assessed at time of presentation	Heavy bleeding and miscarriage versus light bleeding; increasing bleeding and miscarriage versus decreasing bleeding	Heavy bleeding: OR 10.5 (1.5, 74.4) Increasing bleeding: 2.3 (1.3, 4.1)
Everett (1997)[99]	626	Rural community study United Kingdom	Pregnant women with hospital discharge summaries indicating bleeding, or who complained of bleeding in a visit to the ultrasound, antenatal, or midwife clinic	Charts and discharge summaries reviewed for bleeding before 20 weeks of gestation	Descriptive analysis	Bleeding occurred in 21% of pregnancies. Miscarriage occurred in 12% of pregnancies
Bennett et al. (1996)[91]	516	Retrospective chart review of clinic patients with bleeding Boston, MA	Patients with vaginal bleeding, a subchorionic hematoma, a single gestational sac with identifiable, regular heart beat on ultrasound	All symptomatic; bleeding occurring at 6-13 weeks of gestation	Compared miscarriage among early bleeding (≤8 weeks) versus later bleeding (>8 weeks)	Bleeding ≤8 weeks: 13.7% risk of miscarriage Bleeding >8 weeks: 5.9% risk of miscarriage

Tongsong et al. (1995)[105]	255	Case-control study, patients seeking care for threatened abortion in a hospital Thailand	Threatened abortion with visible heart beat	All cases were symptomatic, controls were not asked about bleeding episodes	Comparison group was 265 uncomplicated pregnancies undergoing ultrasound for size measurements	Study group had a miscarriage rate of 5.5% compared to miscarriage rate of 1.9% in comparison group
Strobino et al. (1987)[76]	889	Case-control study, 670 chromosomally normal, 219 chromosomally abnormal losses compared to 3089 controls from a general obstetric population New York City, NY	Women with fetal losses after 14 weeks of gestation composed the case groups.	Interview at time of loss (cases), during 2nd trimester (controls); bleeding in first trimester, info on number of episodes and date of onset/severity of the first episode only	Bleeding in the first trimester was compared between cases and controls, who were selected from a general obstetric population	Heavy bleeding associated with both chromosomally normal (OR 3.6 [2.1-6.2]) and abnormal (OR 4.9 [2.1, 11.6]) loss. Light bleeding: OR 2.7 (2.0, 3.6) for chromosomally normal loss, but not with chromosomally abnormal loss (OR 1.1, [0.7, 1.9])
Evans et al. (1970)[107]	3082	Prospective cohort of prenatal clinic patients Australia	All pregnant prenatal patients were asked about bleeding	Interview at first prenatal visit, less than 13 weeks of gestation, and follow-up questions at every subsequent visit	Descriptive	Incidence of miscarriage was 5.6%, two-thirds of cases not preceded by bleeding
Johannsen (1970)[106]	266	Prospective cohort of a hospitalized population with bleeding Denmark	Bleeding episode requiring admission to hospital	All symptomatic; bleeding occurring prior to 20 weeks gestation	Descriptive	50.8% of patients miscarried

*OR: odds ratio; CI: confidence interval

Figure 2.1. Conceptual model outlining the relationship between bleeding and miscarriage, *Right From the Start*.



CHAPTER 3 : METHODS

A. RIGHT FROM THE START

Right From the Start (RFTS) is a multi-phase and multi-center cohort study of early pregnancy. The study is referred to colloquially by participants and the community as *Right From the Start*; however, different grants provide funding to allow different phases of the study to focus on unique exposures occurring in early pregnancy. The different phases of the study are referred to by the research team, and in this proposal, by the order in which the phase was funded (RFTS 1, 2, 3). The phases of the study are united by similar study activities/protocols, recruitment methods, and questionnaires, with slight modifications in the timing of some study activities. Data from all phases of the study will be used for this project.

RFTS Background and Eligibility

RFTS 1 began recruitment in 2000 in three areas of the United States: the Raleigh/Durham region of North Carolina, Galveston, Texas, and Memphis, Tennessee. This first phase was funded by the American Water Works Association Research Foundation to study the effects of water disinfection byproducts on birth outcomes in three cities of the United States. In 2004, RFTS 1 ended, and recruitment began for RFTS 2, continuing only in North Carolina. RFTS 2 focused on the presence of fibroids and change in fibroid size during

pregnancy. In Spring 2007, RFTS 3 began recruitment. RFTS 3 and RFTS 2 recruitment occurred simultaneously (see Table 3.1 for differences in eligibility criteria). RFTS 3 obtained a detailed, prospective assessment of non-prescription medication use and other pregnancy symptoms in early pregnancy. RFTS 2/3 expanded to Nashville, TN in the summer of 2007, leading to a brief period of overlap in recruitment between the North Carolina and Tennessee sites. In late 2007, recruitment stopped in North Carolina, and active recruitment continued only in Tennessee. RFTS 2 and 3 are funded by the National Institute of Child and Human Development. Figure 3.1 highlights the main events occurring on the timeline of RFTS study activities.

Despite a shift in focus with each phase of the study, the bulk of participant questionnaires and participant activities remained the same. All questions required for this project have remained the same throughout the eight year period under study. Eligibility criteria for the study are listed in Table 3.1.

Notable changes in eligibility criteria between the phases of the study are highlighted in Table 3.2, which also includes other unique characteristics of each study phase. The main alterations in eligibility are summarized in these two points:

1. RFTS 1 allowed recruitment up to the twelfth week of pregnancy by self-reported last menstrual period (LMP) at time of enrollment, while RFTS 2 and 3 required women to be less than 9 weeks pregnant by self-reported LMP.

2. RFTS 3 had the additional eligibility criteria of only enrolling women prior to pregnancy and requiring participants to have daily access to the Internet. However, since RFTS 2 and 3 were enrolling participants concurrently and shared the same protocol, those participants without Internet access or who were already pregnant were enrolled in RFTS 2, lessening the concern for selection bias into this study phase. Other eligibility criteria and study activities remained the same for the two phases of the study.

Beyond these differences, the study activities and procedures for each phase of the study remained virtually the same, as described in 'RFTS protocol.'

RFTS Recruitment

The RFTS study team builds on nearly ten years experience recruiting and enrolling participants during early pregnancy. Recruitment has always occurred in close collaboration with community organizations, including private obstetric clinics, university obstetric groups, health departments, and through the use of other recruitment methods such as bus advertisements, mailings to new homeowners, mass emails, flyers and information placed in pharmacies and other local points of interest. Although the study is not a random population-based sample, the goal of RFTS recruitment strategies has been to enroll a group of women that is more representative than a standard prenatal clinic-based sample. A strictly clinic-based sample has been previously shown to yield biased participant characteristics, potentially affecting results.[138] Additionally, when studying miscarriage, basing a

study on a clinic-based population could lead to lower ascertainment of miscarriage since many women do not enter prenatal care until later in the first trimester. Table 3.3 depicts the similarities and differences between characteristics of the RFTS 1 Raleigh cohort and characteristics of all births in the Raleigh enrollment area, as obtained from 2001 Vital Statistics data. Maternal characteristics in the RFTS 1 cohort and the general population were similar, with the exceptions that RFTS 1 participants were, on average, more highly educated and less likely to be Latina. Strong efforts to remediate some of these dissimilarities have been undertaken in subsequent phases of RFTS, with a focus on minority recruitment.

Table 3.4 summarizes the average gestational age at various stages of the study; women enroll in the study, on average, at a very early stage of pregnancy.

Women who are not yet pregnant, but have been trying to become pregnant for no more than six months (RFTS 2) or three months (RFTS 3) are eligible to pre-enroll in RFTS. Formal enrollment occurs when they become pregnant. All RFTS 3 women are pre-enrolled; these women are also required to provide daily information about common symptoms and exposures via a web-based diary during the pre-pregnancy period and during the first trimester. Based on current data, about 56% of pre-pregnant enrollees become pregnant within 6 months and formally enroll in the study.

By the end of November 2007, RFTS has screened 8026 women for eligibility to participate in the study. Sixty-one percent (n=4916) of these women were eligible and agreed to participate in the study. After enrollment, some women (1.9%, n=95) formally withdrew their consent to participate in the study. Reasons for withdrawal

include time constraints, health status changes, concern about pregnancy, too many study activities/demands, and an unwillingness to share personal/medical information.

Surveys among RFTS participants suggest that many reasons motivate women to participate in RFTS.[139] The most common reasons include the free ultrasound and pregnancy test kits (for pre-enrolled participants). Many women also comment on their desire to contribute to knowledge about pregnancy complications and a sense of altruism.

RFTS Participants

Because of the multi-site nature of the study and some differences in recruitment strategies employed by each site, the study population differs slightly at each site (Table 3.5). For instance, the Galveston site successfully recruited many women from its local health department. As a result, the study population in Galveston has a greater proportion of minority and low-income women. Additionally, almost 30% of participants from the Memphis and Galveston sites were obese, a higher proportion compared to women recruited in North Carolina. The North Carolina population is highly educated and more white, reflecting the demographics of the cities where many participants enrolled. This population also has a higher consumption of alcohol during pregnancy. Table 3.5 summarizes the differences in study populations by site for variables of interest to pregnancy studies. Minority enrollment has also been a priority for RFTS. In an effort to enroll women with a wide range of characteristics, the study devoted special efforts to recruitment of

African-American and Latina women. Up to August 2007, 16.1% of subjects enrolled in RFTS 2 and 3 were African-American and 8.3% were Latina.

RFTS Protocol

Women are eligible to enroll in the study once they report a positive pregnancy test. During the enrollment process, a brief intake interview is conducted and an ultrasound is scheduled. Signed, informed consent is obtained from participants prior to the ultrasound.

Differences among RFTS phases were previously highlighted. Figure 3.2 outlines participant activities. Recruitment of a diverse, representative study population has been a focus of RFTS since its inception (see 'RFTS Recruitment' for details). Collaboration with public and private obstetric clinics, community coalitions, and direct mailings were used to achieve a representative sample of the source population. A toll-free study telephone number and website (www.mom2be.org) is provided on all recruitment materials, facilitating communication and dissemination of information about the study. Recruitment is not targeted to participants with specific concerns about miscarriage or specific exposures associated with miscarriage, but rather more generally towards all pregnant women, in order to increase the representativeness of the RFTS study sample.

Study activities are concentrated in the first trimester. At enrollment, participants complete a brief baseline intake interview, focusing on demographic information and symptoms and behaviors of early pregnancy, such as nausea and vomiting, alcohol intake, and cigarette smoke exposure.

An ultrasound is scheduled as soon as possible after the fifth week of pregnancy. The ultrasound serves as an incentive for participants to join the study and assesses fetal viability and gestational age. With the inclusion of ultrasounds into the study protocol, RFTS gestational age estimation is of highest possible quality. All study sonographers have at least five years experience prior to joining the study, specialize in early pregnancy ultrasounds, and are trained to conduct study ultrasounds. A previous validation substudy of ongoing pregnancies in RFTS 1 found that self-reported LMP, on average, estimates gestational age to be 0.8 days (SD 8.0, median 0) longer than ultrasound-based estimates for live births in this cohort.[140] Proportions of births classified as preterm based on ultrasound and LMP-based methods were similar, and the overall conclusion was that self-reported LMP is a reliable indicator of gestational age in RFTS.[140] In a miscarriage analysis, reliance on self-reported LMP estimates is necessary because restricted early fetal growth among miscarriages may systematically affect ultrasound-based dating of the pregnancy.[141,142] Our use of LMP-based estimates is further justified by the high quality LMP dates provided by our participants.

A comprehensive telephone interview takes place at the end of the first trimester (no later than 16 6/7 weeks of pregnancy) for RFTS 2 and 3. In RFTS 1, an initial interview was conducted during the first trimester, and a follow-up interview was completed around 20 weeks of pregnancy. Similar questions were asked during both interviews. This computer-assisted telephone interview (CATI) obtains detailed information about a broad range of information about important covariates, including details about demographic characteristics (race, education, income level), comorbid

conditions (diabetes, hypertension, thyroid dysfunction), maternal behaviors (caffeine intake, smoking status, alcohol use, nutritional exposures), and other maternal characteristics (height, weight, reproductive history). This interview also collects a great deal of information regarding the early pregnancy period, from which bleeding data and associated symptoms will be obtained.

The rich amount of information collected from RFTS participants at this early stage of pregnancy makes it an ideal dataset for exploring questions related to exposures and symptoms of the early pregnancy period. The average gestational age of completion for each of these components of the study is displayed in Table 3.4. An overview of the type and extent of data collected in RFTS is found in Table 3.6. First trimester CATIs are conducted by staff at the Batelle Memorial Institute, a research organization which is subcontracted by RFTS. This organization maintains rigorous training and quality control activities for its interviewers and has been working with the study since its inception.

Women who are not yet pregnant are eligible to pre-enroll in the study. Pre-enrolled participants receive free monthly pregnancy test kits to encourage early pregnancy recognition. Pre-enrolled women must have been attempting pregnancy for fewer than six months (RFTS 1 and 2) or fewer than three months (RFTS 3) to be eligible, in order to avoid bias associated with enrollment of a study population of infertile or sub-fertile women.

Pre-enrolled participants in RFTS 3 complete an initial interview and submit daily web-based diaries during the pre-pregnant period and throughout the first trimester. The diary is designed to take less than two minutes to complete and

queries common symptoms and exposures of the early pregnancy period. The diary format for collection of data is preferred in this situation not only because of the ease with which data can be collected and compiled on a daily basis, but also because questions of a sensitive nature (sexual intercourse, vaginal bleeding, use of alcohol while pregnant) may be more accurately and feasibly collected using a diary format.[143,144] Additional questions documenting presence of spotting/bleeding episodes are included in the diary for the validation component of the study.

The web-based interface is available in English to individuals with a valid username and password, and all information submitted via diary is time-stamped and transmitted in an encrypted format. Study staff send reminders to participants who do not complete their diary entry within 48 hours of availability.

A 2005 pilot study of 40 RFTS women overwhelmingly suggested that a web-based diary is a feasible and acceptable data collection tool. More than 95% of eligible diary entries were completed in this pilot study. The diary captured more reports of symptoms and medication use compared to a telephone interview several weeks later. The daily diary has now been in use for at least twelve months in the RFTS study population. More information about exposure assessment and validation will be provided in the following sections of this chapter.

Once pre-enrolled women report a positive pregnancy test, participants formally enroll in the study. After formal enrollment, the pre-enrolled sub-group of women continues to provide daily diary information throughout the first trimester. Beyond these activities unique to the RFTS 3 cohort, this group completes all other study activities in the same manner as other RFTS participants.

RFTS Data Management

A rigorous system for managing data and maintaining quality control has been in place since the start of RFTS. The data management system contains many internal checks to assure the integrity of the data. The database draws on data obtained from several sources (Battelle first-trimester CATIs, diary data, intake interviews, ultrasound and medical records information) and does not allow entry of implausible values or values that do not fulfill logic check parameters. Regular checks of vital variables (such as gestational age at end pregnancy) are conducted to ensure that all values are within an acceptable range. Reports of relevant metrics (recruitment, enrollment, outcomes) are regularly distributed to the study team and data management issues and inconsistencies are discussed and resolved at investigators meetings. Error detection, correction, and tracking procedures are monitored by study staff. In addition to these built-in data checks, careful cleaning of the data is conducted prior to beginning any analyses. Any implausible or unrealistic data are discussed within the study team to determine the most efficient and appropriate way to move forward.

B. EXPOSURE ASSESSMENT

Data on vaginal bleeding and spotting were obtained in the first trimester interview. Specific questions regarding vaginal bleeding are listed in Appendix 1. Appendix 2 highlights additional questions asked specifically of women with a loss, available for RFTS 2 and 3. The wording of these questions is slightly modified for women who have had a miscarriage (for example, instead of “since you got pregnant,” the interviewer asks, “during your recent pregnancy,”). Detailed information, including timing, duration, heaviness, color, and pain associated with each episode, was obtained for all participants.

C. OUTCOME ASSESSMENT

Participants are asked to complete a pregnancy outcome form when a live birth, miscarriage, or other pregnancy outcome occurs. For participants who do not self-report an outcome, several alternate mechanisms exist to obtain this information. Early losses are detected in Section A of the first trimester interview, which is completed by all participants who have not yet submitted a pregnancy outcome form. Additionally, linkage to North Carolina birth certificates and fetal death certificates captures outcome information about live births and fetal deaths occurring after 20 weeks gestation. This data is up-to-date through 2008. Medical records are requested for all participants.

RFTS consent includes a HIPAA Authorization form and consent for release of medical records. All records pertaining to the RFTS pregnancy are abstracted, including records from the prenatal care site, records from emergency department or hospital visits, as well as discharge summaries from time of delivery. Trained study staff supervise and conduct all abstraction activities.

D. COVARIATE ASSESSMENT

Data on other variables used in these analyses are self-reported by participants during the first trimester interview, with the exception of fibroid status. The presence of fibroids is assessed using the early pregnancy ultrasound. Variable specification was based on substantive considerations and the distribution of observations in relation to the outcome, with the objective being to specify each variable parsimoniously while retaining the ability to distinguish important subgroups of the population.

E. HYPOTHESES AND ANALYTIC APPROACH

Overall Approach and Variable Definitions

Univariate and bivariate analyses were initially used to describe the distribution of covariates. Stratified analyses were conducted to increase familiarity with all possible categorizations of the data prior to multivariate modeling. These steps are essential to ensure that the summarizations and smoothing produced by the modeling process are valid and that assumptions are upheld.

Covariates and outcomes were stratified by bleeding status for descriptive analysis of the data. Exploratory analyses of continuous variables were undertaken in order to determine the most appropriate way of modeling continuous variables. Continuous variables were modeled as splines, and, if appropriate, as categorical variables, with category cutpoints determined by *a priori* knowledge and informed by the patterns observed in the data, with the intent of achieving a precise and stable estimate for the relationship between each category and the outcome. Similar strategies guided collapsing of multi-category variables into fewer categories.

Potential covariates were included in models based on *a priori* relationships identified on a directed acyclic graph, strength of association in stratified analyses, or based on substantive area knowledge. Inclusion of covariates in predictive models was determined by a likelihood ratio test comparing nested models, with an alpha of 0.15.

The full dataset was restricted to observations that are not missing or uncertain for essential values such as last menstrual period and gestational age at

outcome or loss to follow-up. Because some women enroll in RFTS more than once, only the first pregnancy for which data exists was used for this analysis. Ectopic pregnancies were excluded from the analysis. Women who had induced abortions were included in the analysis and censored at the time of induced abortion. Participants who did not complete the first trimester interview were not included in the analysis. Additionally, women for whom the last menstrual period occurred less than twenty weeks since the creation of the dataset were not included in the analysis.

Bleeding was analyzed according to several definitions. A dichotomous variable was created based on the response to whether any bleeding occurred during pregnancy (Appendix 1, question H8a). As appropriate, a multi-category variable was also used in some analyses, with categories of no bleeding, spotting only, light bleeding, or heavy bleeding, based on responses to question H10a (Appendix 1). The duration of an episode, color, and associated pain, were also incorporated into the definition or used as further stratification variables (Appendix 1, questions H9, H10, H11). Episodes of bleeding that terminate within four days of a reported date of miscarriage were not included in the bleeding definition, as these episodes may overlap with symptoms of miscarriage. Sensitivity analyses were conducted as part of this project to estimate the impact of varying the termination cutpoint to seven days.

The outcome, miscarriage, was coded as a binary variable. The time at which a miscarriage occurs was also incorporated into survival models for Aim 2.

Covariates of interest and their potential categorization schemes are outlined in Table 3.7. Continuous variables, such as age, body mass index, and percent

poverty threshold, were evaluated as continuous variables initially to determine the best way to model the covariate-outcome association. Variables were specified to include quadratic or cubic terms or splines. The specification that best fit the observed relationship was used to model the variables. If appropriate, continuous variables were categorized. Sample category cutpoints are listed in the table. Categories for body mass index (BMI) were defined according to the criteria of the National Heart, Lung, and Blood Institute (Underweight <18.5; Healthy weight 18.5-24.9; Overweight 25.0-29.9; Obese ≥30.0). Percent poverty threshold was calculated according to the 2008 Poverty Guidelines, as determined by the Department of Health and Human Services, accounting for the size of family unit. These guidelines are used in determining financial eligibility for federal programs, and are informally referred to as the “federal poverty level.” Data for all other covariates were based on self-reported information obtained from the intake and the first trimester interview, or from the first trimester ultrasound.

Aim 1

Determine the usual patterns and characteristics of vaginal bleeding and spotting in pregnancy. Identify the maternal characteristics that predict vaginal bleeding and spotting in early pregnancy.

Description

This aim had two components: a descriptive analysis (Sub-aim 1.1) and identification of maternal predictors of bleeding (Sub-aim 1.2). These components are discussed separately.

Sub-aim 1.1: To begin the process of analyzing the data, a descriptive analysis of first trimester bleeding, was undertaken. This characterization was essential for subsequent analysis steps. Bleeding data was obtained from the first trimester interview questions and follow-up interview (RFTS 1 only) (Appendix 1 and 2).

Sub-aim 1.2: This component assessed which maternal characteristics predicted the presence of one or more bleeding episodes. The maternal characteristics that predicted the heaviness of a bleeding episode were also assessed. Data for all covariates and bleeding were obtained from the baseline and first trimester interviews and follow-up interview (RFTS 1 only).

Hypotheses

Sub-aim 1.1: Because this is a descriptive analysis, no hypotheses were associated with this specific aim.

Sub-aim 1.2: Based on only one study of maternal predictors of bleeding in pregnancy, we hypothesized that advanced maternal age, passive smoking

exposure, prior preterm birth, and multiple prior miscarriages and elective terminations were associated with more intense bleeding episodes.[1] Based on substantive area knowledge, we also hypothesized that overweight or obese status, maternal history of diabetes, menstrual cycle function (measured by the length of menstrual cycles), and current or previous smoking were associated with occurrence and heaviness of bleeding in pregnancy.

Analytic plan

Sub-aim 1.1: All bleeding characteristics were fully explored (including all components of the questionnaire, such as timing, duration, associated pain, color, and heaviness) and summarized. Bleeding characteristics were stratified by gestational age periods and heaviness of bleeding. Crude descriptions of the association between bleeding, covariates, and pregnancy outcomes were also completed.

Time periods of interest included very early reports of spotting/bleeding, coinciding with the time of implantation and the time of expected menstrual period. These times were explored, accounting for the length of each woman's menstrual cycle. Additionally, reports of bleeding between 8 and 12 weeks of pregnancy were of interest because this is the time that maternal-fetal circulation begins to develop. Cyclic bleeding patterns that coincide with the expected timing of the usual menstrual cycle were also assessed.

For miscarriage, careful attention was given to bleeding occurring immediately prior a reported pregnancy loss. These episodes of bleeding did not contribute to the main definition of bleeding in pregnancy, as this bleeding is likely

specifically related to passing of fetal products of conception. Bleeding episodes that terminate fewer than four days before the miscarriage were excluded from consideration, with sensitivity analyses conducted in later components of the project to determine the impact of using the four-day cutpoint.

Sub-aim 1.2: All covariates listed in Table 3.7 were assessed. Several analytic steps were followed. These steps sought to evaluate the relationship between maternal characteristics and occurrence of bleeding, and the association between maternal characteristics and heaviness of bleeding.

First, a logistic regression model was used to evaluate these predictors in relation to a dichotomous outcome (bleeding versus no bleeding). The contribution of each predictor was evaluated on the basis of a likelihood ratio test, testing whether each predictor contributed substantially to the model. Decisions will be made based on an alpha level of 0.15, considering predictors whose likelihood ratio test p-values are less than 0.15 to be substantial contributors to the model, and thus, important predictors of the outcome. This proposed model is mathematically represented by Equation 3.1.

Equation 3.1

$$\log\left[\frac{\Pr(Y_i = 1 \mid x_i)}{\Pr(Y_i = 0 \mid x_i)}\right] = \beta_0 + \sum_{k=1}^{K} \beta_k x_{ik}$$

Y is the outcome, denoted by presence (Y=1) or absence (Y=0) of bleeding symptoms for each woman, indexed by i. The x_k represents all the $k = 1,\ldots, K$ predictor variables that will be included in the model, which are depicted in Table 3.7.

The β_k represent the coefficients of these predictor variables, and the β_0 represents the baseline log odds of the outcome in all reference categories.

To evaluate the association between maternal characteristics and severity of bleeding, additional analyses were completed. Severity of bleeding was coded as a four-level ordinal variable (no bleeding, spotting, light bleeding, heavy bleeding). The definitions for the categories of this variable come from question H10a (Appendix 1), and are outlined in Table 3.8. All covariates included in the first part of this analysis were similarly assessed for their predictive ability.

A multinomial logistic model was used to evaluate the relationship between maternal characteristics and bleeding heaviness. This model permits estimation of individual odds ratios for each ordinal threshold of outcome. For this analysis, each woman's most severe episode was used as the outcome to avoid non-independence of outcomes.

A predictive modeling strategy was followed. The contribution of each covariate was assessed using a likelihood ratio test, with an alpha of 0.15.

The proposed model is mathematically represented by Equation 3.2.

Equation 3.2

$$\log\left[\frac{\Pr(Y_i = y_j \mid x_i)}{\Pr(Y_i = y_0 \mid x_i)}\right] = \beta_{0j} + \sum_{k=1}^{K} \beta_k x_{ik}$$

Y is the outcome, denoted by the 4 levels depicted in Table 9 (j = 0, 1, 2, 3) for each woman, indexed by *i*. The *x* represents the *k = 1, …, K* predictor variables that will be included in the model, listed in Table 3.7. The β_k are the coefficients of these predictor variables, representing the log odds of the outcome. The β_{0j} represents the

threshold-specific baseline log odds of the outcome for covariates in all reference categories. This model provides odds ratios that compare predictors of higher severity bleeding ($Y=y_j$) to the baseline level of no bleeding ($Y=y_0$) for each incremental change in predictor variable category.

Equation 3.2, as described, is a constrained form of the multinomial logistic model. This model assumes the coefficients for each variable to be the same across all threshold-specific comparisons made in the analysis. Separate coefficients for each threshold may be required, to identify relationships between variables and each level of bleeding intensity. To do this, an unconstrained version of the model was fit (estimating β_{kj}), resulting in threshold-specific coefficients and odds ratios. Because the constrained model was nested within the unconstrained model, we conducted a homogeneity test of equivalence of coefficients. This test uses the difference in log-likelihoods of the two models, which is a chi-square statistic with degrees of freedom equal to the difference in number of parameters between the models. A p-value threshold of 0.15 was used to determine whether the separate estimates for each threshold were necessary. If the p-value is greater than 0.15, we concluded that one coefficient, describing the combined effect for several thresholds, was sufficient.

Missing values for all observations were imputed using Stata's Imputation using Chained Equations (ICE) command.[145] ICE imputes missing values for all variables in the specified model using values of beta coefficients and standard errors from a posterior distribution that is based on a regression of the non-missing values of a variable on the other predictors in the model. All predictors present in the final

model were included in creating the imputed dataset. Two datasets were created, one for the model assessing predictors of presence or absence of bleeding, and one for the model assessing predictors of the heaviness of bleeding. Five imputation cycles were used in creating the final, complete dataset of imputed observations. Subsequent analyses were completed across all replicates of the dataset, combining point estimates using simple arithmetic means and standard errors that account for both within- and between-imputation variation. This combining procedure was done by the *micombine* command in Stata.[145]

Limitations of approach

Because the data are collected from the first trimester interview, differential recall may bias the bleeding reports of women with and without a miscarriage. However, the validity of first trimester reports compared to prospectively collected data were examined in Aim 3. The results obtained in this aim can be interpreted in light of the results from Aim 3. In the analysis of predictors of heaviness of bleeding, few women reported heavy bleeding, which resulted in less stable estimates.

Sample size considerations

Sub-aim 1.1: This component focused on a descriptive analysis of bleeding patterns in the early pregnancy period; no hypothesis testing was required to fulfill the objectives of this component. Thus, no power analysis was necessary.

Sub-aim 1.2: This component focused on maternal characteristics associated with vaginal bleeding. We were interested in identifying predictors of bleeding, within our current dataset. Because we are not seeking to determine differences in estimates by the status of any other variable, no sample size calculation is warranted.

Aim 2

Evaluate the association between vaginal bleeding and spotting in early pregnancy and the occurrence of miscarriage.

Description

This aim sought to determine whether an association existed between episodes of bleeding in early pregnancy and miscarriage. Although some bleeding episodes in early pregnancy are the direct consequence of miscarriage, many miscarriages occur without any symptoms of bleeding and many pregnancies that result in live birth have bleeding episodes during early pregnancy. This analysis sought to describe the association between episodes of bleeding and miscarriage, and to uncover whether a bleeding episode was associated with subsequent miscarriage after accounting for other miscarriage predictors. The conceptual framework for this aim was that bleeding is a marker of an underlying condition or a physiologic change that may be related to miscarriage.

Special care was taken to only consider episodes of bleeding not thought to be a direct consequence of miscarriage. Bleeding episodes that immediately precede miscarriage were removed from the analysis. Bleeding and covariate information were obtained from the first trimester interview. Outcome information was obtained from the RFTS pregnancy outcome form. Subanalyses were considered that utilize ultrasound data to identify pregnancies that are known to have slowed or arrested growth early in gestation. Additional stratification by time of interview with relation to miscarriage was also incorporated into the analysis. The

analysis was also restricted to women in their first pregnancy to account for the potential effect of prior pregnancy outcomes on reporting of bleeding.

Hypotheses

Based on prior studies, we hypothesized that bleeding episodes that occur during pregnancy were associated with the occurrence of miscarriage. Specifically, we hypothesized that heavier episodes were associated with pregnancy loss. The relationship between the main exposure and outcome was considered independent of the effect of other known predictors of miscarriage, including maternal age, prior miscarriage, or smoking status.

Analytic plan

As a preliminary step, classification and regression tree (CART) analysis was used to identify important characteristics of bleeding episodes that cluster together in units that predict miscarriage.[146] CART is a data-driven analysis approach that splits the data into sub-groups that differentially predict an outcome (in this case, miscarriage). This method categorizes the data to minimize the misclassification of the outcome within each group, so that each group can be categorized as associated or not associated with the outcome. Our CART analysis evaluated the relationship between miscarriage and the following characteristics of first trimester bleeding: heaviness, duration, color, timing, and associated pain. CART is implemented in four automated steps: (1) a splitting process, which maximizes the homogeneity of the outcome within each category and builds the best and most elaborate tree for the full dataset (the reference tree); (2) class assignment, which assigns an outcome to each category by minimizing misclassification of the

outcome; (3) cross-validation, which partitions the data into 90% samples of the total data and builds trees appropriate for each 90% subset. The remaining 10% data sample for each subset is used to calculate each tree's outcome classification error rate, and this process is repeated for each 90% sample of the data. Finally, (4) a pruning process identifies the tree with the smallest outcome classification error rate, and prunes the reference tree to this optimal tree size. This method was used as an initial, exploratory step to identify which bleeding characteristics (heaviness, duration, pain, and color) are important in relation to miscarriage in the population of women who report bleeding.

A discrete-time hazard model was used to evaluate the association between bleeding and miscarriage. Each week in pregnancy was considered the conditional time unit in the analysis. This modeling strategy has the advantage of accounting for left truncation and right censoring. It is also able to incorporate time-dependent covariates. Participants were censored at twenty completed weeks of pregnancy, on the gestational age of miscarriage, or gestational age at loss to follow-up or termination. Only pregnancy weeks at risk of miscarriage were included in the analysis. Pregnancy weeks that took place prior to enrollment, after miscarriage, or after censoring were not included in the weekly risk set for analysis. In other words, the experience of a woman with miscarriage that occurs during the seventh week of pregnancy will only be compared to other pregnancies at risk of the outcome during the seventh week of pregnancy.

In order to conduct this analysis, a generalized linear model was used with a logit link, conditioning for each week under study. Risk sets began at week 5 of

pregnancy and ended at week 20. Due to sparse data, weeks 18-20 were constrained to have the same coefficients. Weeks of pregnancy were calculated beginning with the date of the last menstrual period, with the first 6 days labeled as week 0, the next 7 days considered week 1, the next 7 days considered week 2, and so forth. Odds ratios describing the association between bleeding and miscarriage, given that the fetus has survived up to the week in which miscarriage occurs, were reported. Both the presence and heaviness of bleeding were assessed. Heaviness categories were defined as in Table 3.8.

Miscarriage predictors such as maternal age, prior miscarriage, and smoking status were included in adjusted analyses. Time interactions were evaluated, with important time interactions identified using an alpha of 0.05. Specifically, time-dependent effects were carefully examined for vaginal bleeding, to identify time-sensitive effects of this symptom. Effect measure modification by pain and cramping associated with the bleeding episode was also evaluated.

The proposed model is mathematically presented as Equation 3.3.

Equation 3.3

$$\log it[\Pr((Y_i = y_t \mid Y_i \geq y_t) \mid x_i)] = \beta_{0t} + \beta_{es} x_e + \sum_{k=1}^{K} \beta_k x_{ik}$$

Y is each woman's time to the outcome, denoted by *t = 1, … , 20* weeks of pregnancy in which a miscarriage can occur. Each woman is indexed by *i*. The *x* represent the main exposure variable, bleeding (x_e), and the *k = 1, …, K* predictor variables that were included in the model, which are outlined in Figure 3.4. Bleeding was incorporated into the model as a dichotomous time-dependent variable and as

an episode-specific four-level indicator categorical variable, representing levels of heaviness depicted in Table 3.8. The β_e and β_k are the coefficients of the bleeding and predictor variables, respectively, representing the log odds of the outcome for each variable, conditional on survival up to that point in pregnancy. The β_{0t} represent the baseline, week-specific log odds of the outcome in all reference categories of predictor variables. Time interactions that are included in the final model take the form of an interaction term between bleeding and the time period of interest, *s*. This term identifies specific time periods of pregnancy that are of interest.

Based on the level of misclassification detected in Aim 3, we conducted sensitivity analyses to identify how much deviation exists between the results obtained in this aim and those expected under ideal exposure assessment conditions.

Limitations of approach

As with the other aims of this analysis, the data are limited by retrospective recall of bleeding reports, which potentially contributed to a bias in effect estimate. This analysis was also limited by the fact that no data exists in RFTS to examine the physiologic changes that may underlie any causal relationships between bleeding (that does not immediately result in miscarriage) and miscarriage. However, this project did not seek to detect a causal relationship. We sought to identify an association between previous bleeding during pregnancy and miscarriage, and to make our estimates relevant to obstetric patients and providers who want to assess the overall risk of miscarriage after a bleeding episode occurs.

Sample size considerations

This aim focused on the relationship between bleeding symptoms and miscarriage. For this aim, a dual-approach power analysis was conducted. Assuming a dichotomous exposure (bleeding versus no bleeding) and a dichotomous outcome (miscarriage versus live birth), a two-group continuity-corrected chi-square test of equal proportions was conducted. This analysis is based on 3285 participants reporting no episode of bleeding (11.6% who have a miscarriage), and 1204 participants reporting at least one episode of bleeding, values that were based on the numbers of women reporting bleeding in our study. The results based on this approach show that, with a two-sided alpha of 0.05, there is over 90% power to detect an OR of 1.4.

To supplement this approach, we also conducted a sample size calculation based on logistic regression with a binary covariate. Using the same values as in the previous paragraph to describe our population, we found that we had 90% power to detect an OR of 1.4. The consistency of these results is reassuring and suggests that there will be an adequate sample size to detect a relatively small difference in effect.

Aim 3

Evaluate the extent of misclassification of retrospective reports of bleeding exposure compared with prospectively collected data from daily diaries.

Description

A validation of the bleeding assessment was conducted as part of this aim. For this aim, the reports of presence, heaviness, and timing of bleeding symptoms in the first trimester interview were compared against the prospectively collected data from the daily diary. The diary contains two questions related to bleeding, assessing the presence and severity of bleeding. Because the diary is completed on a daily basis and time-stamped when submitted, the timing of the bleeding is inherently incorporated into the daily diary assessment.

This aim is essential for the overall analysis as it helps to quantify the accuracy of bleeding reports in the first trimester interview, and provides some insight into the extent of recall bias present in the data.

Hypotheses

In this aim, we hypothesized that some episodes of early pregnancy spotting captured by the daily diary were not reported in the first trimester interview. The extent of misclassification is hypothesized to decrease with increasing severity of the bleeding episode, and the most severe episodes will be accurately recalled. Additionally, we hypothesized that episodes of bleeding that occur later in the first trimester are more likely to be recalled accurately.

Analytic plan

As a first step, descriptive analyses similar to those of Aim 1 were conducted using the daily diary data. These analyses served as the comparison point for the validation of the results from the first trimester interview. A comparison of maternal characteristics of women who provided diary data to those who did not was also undertaken.

The validity of reports of presence of bleeding was assessed by calculating sensitivity (Se), specificity (Sp), and kappa (κ) statistics. Data obtained from the daily diary was considered the reference standard.

Sensitivity and specificity were calculated using standard formulas. Kappas were calculated using StatXact. Kappas were also used to compare the severity of reported bleeding using the most severe episode for each woman as the episode for comparison. Weighted kappa was used for non-binary variables.

A log-linear model was used to evaluate the patterns of association between data obtained at recall and in the diary. This model allowed comparison of the level of agreement beyond chance within categories of relevant covariates. This model was used to examine the extent of agreement and the presence, timing, and severity of an episode.

This model is mathematically represented as Equation 3.4.

Equation 3.4

$$\log \mu_{ij} = \lambda + \lambda_i^D + \lambda_j^R + \lambda_k^Z + \delta_{ij} I(i = j)$$

μ_{ij} represents the expected frequency of counts in cell *ij* of an *I* * *J* table cross-classifying the diary data (*D*) and interview-based recalled response (*R*) of the *n*

subjects for whom both sources of data is available. λ is the overall mean of the expected counts. λ_i^D are the diary effects (column effects). λ_j^R are the recalled effects (row effects). Both of these terms represent chance agreement. λ_k^Z represent k levels of other covariates of interest in predicting agreement. The addition of these terms and their interactions allows agreement to be evaluated within strata of the covariates. δ_{ij} accounts for the agreement beyond what is expected by chance. I equals 1 if $i = j$, and I equals 0 if $i \neq j$. Thus, if $\delta_{ij} > 0$, more agreement occurs than is expected by chance, and the interaction between this variable and other covariates indicates whether categories of the covariate is predictive of better agreement.

Agreement was be examined for several variables of interest, including bleeding severity (none, spotting, bleeding) and timing (by the week in which bleeding occurred, from the fourth week of pregnancy to the thirteenth, inclusive). Other covariates of interest, such as outcome (miscarriage or live birth), prior miscarriage, and maternal age, were considered for their predictive ability.

Limitations of approach

In this validation study, the data obtained from the daily diary was considered the gold standard for bleeding reports. The diary may not be a true gold standard in that the data are not truly collected prospectively and may still not accurately represent all bleeding episodes. However, there is no superior method of collecting this information. Medical records and hospital reports will only report the most serious of the bleeding episodes; thus, the data obtained from the daily diary is an appropriate reference standard in this analysis.

The generalizability of women enrolling in the diary component of the study is also a limitation of this aim. The women enrolled in the diary component of the study provide email addresses on enrollment and have consistent Internet access. These women differ from the general RFTS population in several ways, including the fact that they are planning their pregnancy. They are also highly motivated women who agree to provide daily documentation of their pregnancy-related symptoms.

Sample size considerations

The first part of the analysis sought to describe the sensitivity, specificity, and kappa statistic associated with two different methods of obtaining bleeding symptom data in pregnancy. No sample size calculation was required for this component. The log-linear component of the analysis sought to identify predictors of agreement. As with Aim 1, since our goal was not to obtain an effect estimate beyond a certain threshold, no sample size calculation was required.

F. SOFTWARE AND APPROVALS

Data analysis was conducted primarily in STATA, version 9.2. StatXact (version 6) was used for components of Aim 3. DTREG was used in classification and regression tree exploratory analyses for Aim 2.

The Institutional Review Board (IRB) at the University of North Carolina and the National Institutes of Health reviewed this project. IRB approval was obtained from the National Institutes of Health IRB, and a determination that IRB review was not necessary for this project was obtained from the University of North Carolina IRB.

Figure 3.1. Timeline of *Right From the Start* phases and activities.

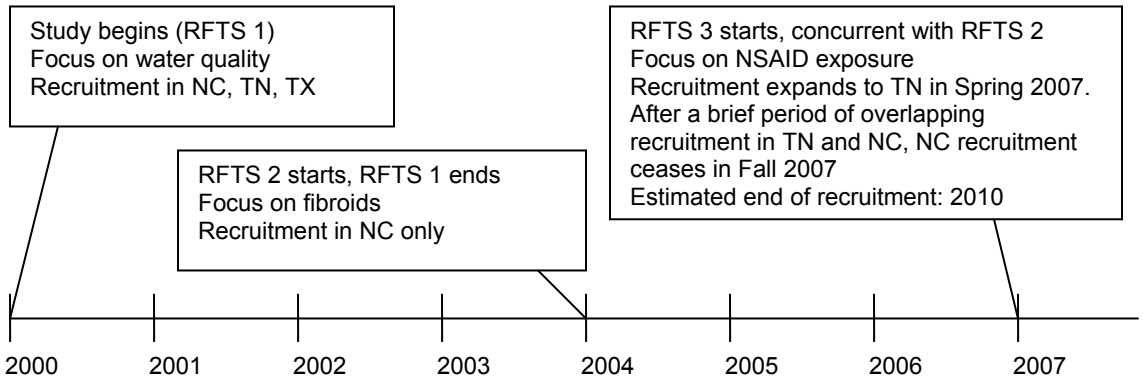


Figure 3.2. Overview of *Right From the Start* study activities.

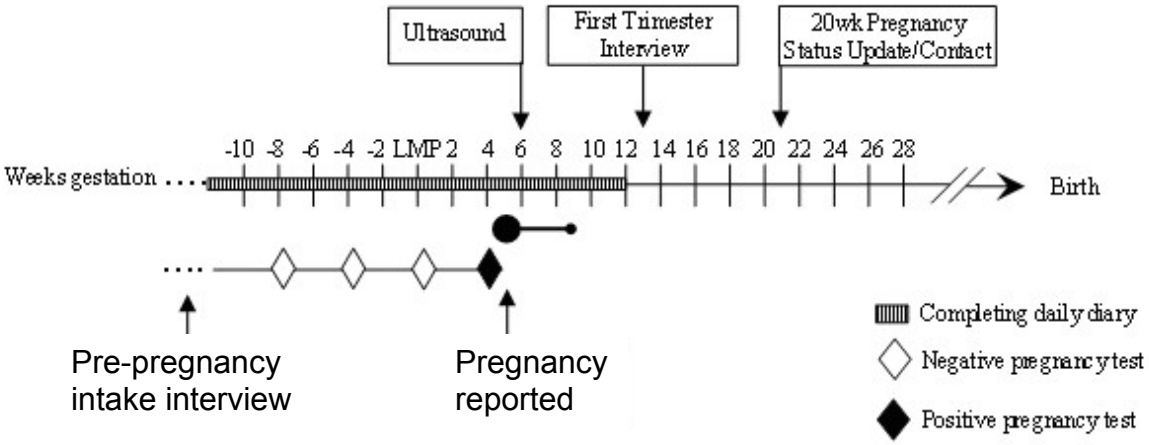


Table 3.1. Common eligibility criteria across all phases of *Right From the Start*.

Criterion	Details
Age	≥18 years at enrollment
Language	English/Spanish-speaking
Fertility	No use of assisted reproductive technologies, pre-enrolled women must have been attempting to conceive for ≤6 months
Other	Intent to carry pregnancy to term, intent to stay in the area for the next 18 months, willingness to have an ultrasound, access to a telephone

Table 3.2. Unique characteristics of each phase of *Right From the Start*.

	RFTS1	RFTS2	RFTS3
Time period	2000-2004	2004-present	2007-present
Gestational age eligibility	≤12 6/7 weeks	≤9 6/7 weeks	pre-pregnant
Internet access required?	No	No	Yes
Special characteristics of study	Water samples collected from various areas of water distribution system in study site	Fibroid sub-cohort followed in greater detail with additional post-partum ultrasounds and questionnaires	Daily web-based diary detailing symptoms (nausea, fatigue) and exposures (pain medications) of early pregnancy completed from time of enrollment up to the end of the first trimester or time of pregnancy loss

Table 3.3. Characteristics of *Right From the Start* 1 participants and births in the recruitment area.

	RFTS1 n = 1112	Births in Area* n = 5172
Maternal Age (yr ± sd)	29.7 ± 5.5	28.1 ± 6.0
Primigravid	334 (30%)	1758 (34%)
Race/ethnicity		
White	745 (67%)	2482 (48%)
Black	278 (25%)	1500 (29%)
Hispanic	33 (3%)	931 (18%)
Other	56 (5%)	259 (5%)
Education		
≤ High School	189 (17%)	2017 (39%)
Some College	200 (18%)	931 (18%)
≥ College	723 (65%)	2224 (43%)

* NC Vital Statistics 2001; Raleigh Births

Table 3.4. Median gestational age in days (interquartile range) of completion of study activities, stratified by phases of *Right From the Start*.

	RFTS 1 n=2332	RFTS 2 n=2078	RFTS 3 n=129
Enrollment	55 (45-66)	43 (36-53)	35 (31-39)
Ultrasound	66 (58-78)	52 (46-62)	47.5 (44-51)
First Trimester Interview	65 (54-78)	98 (95-104)	97 (94-103)

Table 3.5. Characteristics of *Right From the Start* participants, stratified by study site.

		Galveston RFTS 1 n=408	Memphis RFTS 1 n=854	Raleigh RFTS 1 n=1070	Triangle RFTS 2 / 3 n=2022	Nashville RFTS 2 / 3 n=185
		n (%)	n (%)	n (%)	n (%)	n (%)
Age	18-27	250 (63.5)	446 (52.2)	418 (39.1)	598 (29.8)	71 (38.4)
	28-34	116 (28.4)	305 (35.7)	503 (47.0)	1060 (52.4)	99 (53.5)
	35-45	33 (8.1)	103 (12.1)	149 (13.9)	364 (18.0)	14 (8.1)
Race	White/non-Hispanic	151 (37.1)	475 (55.8)	710 (66.4)	1537 (76.2)	147 (79.5)
	Black/non-Hispanic	95 (23.3)	336 (39.4)	277 (25.9)	241 (11.9)	18 (9.7)
	Hispanic	152 (37.4)	19 (2.2)	31 (2.9)	130 (6.4)	9 (4.9)
	Native American/Asian/Other	9 (2.2)	22 (2.6)	52 (4.9)	110 (5.5)	11 (6.0)
	Missing	1	2	0	4	4
Education	≤ High School	222 (54.4)	261 (30.6)	191 (17.9)	203 (10.0)	22 (11.9)
	Some college	103 (25.3)	195 (22.8)	197 (18.4)	302 (14.9)	25 (13.5)
	≥ 4 years college	83 (20.3)	398 (46.6)	682 (63.7)	1516 (75.0)	138 (74.6)
	Missing	0	0	0	1	0
Marital Status	Married/cohabiting	307 (75.3)	685 (80.2)	917 (85.7)	1905 (94.2)	178 (96.2)
	Single/divorced/not living with partner	101 (24.8)	169 (19.8)	153 (14.3)	117 (5.8)	7 (3.8)
Household Income	≤500% poverty level	372 (96.4)	736 (90.3)	873 (85.0)	1623 (83.3)	137 (76.1)
	>500% poverty level	14 (3.6)	79 (9.7)	154 (15.0)	326 (16.7)	43 (23.9)
	Missing	22	39	43	73	5
Parity	Nulliparous	151 (39.3)	359 (43.9)	545 (52.0)	955 (47.3)	103 (56.3)
	1+ live births	233 (60.7)	459 (56.1)	503 (48.0)	1063 (52.7)	80 (43.7)
	Missing	24	36	22	4	2
BMI	Underweight	21 (5.4)	73 (8.6)	100 (9.4)	158 (8.0)	18 (10.3)
	Normal weight	172 (42.3)	392 (46.3)	580 (54.7)	1192 (60.4)	91 (52.3)
	Overweight	71 (18.3)	132 (15.6)	149 (14.1)	248 (12.6)	24 (13.8)
	Obese	124 (32.0)	250 (29.5)	231 (21.8)	375 (19.0)	41 (23.6)
	Missing	20	7	10	49	11
Smoking	No	316 (77.6)	709 (83.3)	908 (84.9)	1845 (91.7)	170 (92.9)
	Yes	91 (22.4)	142 (16.7)	162 (15.1)	168 (8.4)	13 (7.1)
	Missing	1	3	0	9	2

Alcohol	No	181 (44.5)	374 (44.0)	408 (38.2)	951 (47.2)	89 (48.6)
	Yes	226 (55.5)	477 (56.1)	659 (61.8)	1062 (52.8)	94 (51.4)
	Missing	1	3	3	9	2
Caffeine	No	97 (23.8)	227 (26.7)	340 (31.8)	661 (32.8)	58 (31.5)
	Yes	310 (76.2)	624 (73.3)	730 (68.2)	1354 (67.2)	126 (68.5)
	Missing	1	3	0	7	1
Outcome	Miscarriage	48 (12.5)	87 (10.9)	122 (11.9)	272 (13.9)	15 (13.9)
	Live birth	335 (87.5)	712 (89.1)	903 (88.1)	1687 (86.1)	93 (86.1)
	Missing	25	55	45	63	77

Table 3.6. Overview of data sources and type of information obtained in *Right From the Start*.

	Demographic characteristics	Symptom severity and location	Medication use (name, dose, freq)	Vaginal bleeding	Current pregnancy (details/symptoms)	Reproductive and medical history	Diet, and behaviors including tobacco, alcohol, caffeine	Fetal development; uterine / adnexal anatomy	Other medication, herbals, supplements	Pregnancy status and care sites through 20 weeks
Intake Interview	✓	✓	✓	---	---	basic*	basic*	---	✓	---
Diary (Pre-preg to 12 weeks)	---	✓	✓	✓	✓	---	✓	---	✓	---
Ultrasound (6 weeks, as late as 9 6/7)	---	---	---	---	---	---	---	✓	---	---
First Trimester Interview (13 to 17 weeks)	✓	✓	✓	✓	✓	detailed**	detailed**	---	✓	---
Follow-up call (20 to 24 weeks)	---	---	---	---	---	---	---	---	---	✓
Vital & medical records	✓	---	✓	---	---	detailed**	---	✓	---	✓

*basic information includes single questions relating to the number of previous pregnancies, fibroid status, and medication use, etc.

**detailed information includes follow-up questions that provide more comprehensive data related to participant's obstetric and reproductive history, dietary and supplement exposures, intake of caffeine or alcohol, and smoking history.

Table 3.7. Covariates of interest for this project, *Right From the Start*.

Socioeconomic status	**Maternal behaviors**	**Maternal characteristics**
Race	Smoking status	Body mass index
White, non-Hispanic	No exposure	Underweight
Black, non-Hispanic	Exposed pre-pregnant	Healthy weight
Hispanic	Exposed in pregnancy	Overweight
Asian, Native Am, Oth		Obese
Education	Passive smoking	Gravidity
< High school	No exposure	Primigravida
High school	Exposed in pregnancy	Multigravida
> High school		
Percent poverty threshold	Alcohol use	Parity
≤ 500%	No exposure	Nulliparous
> 500%	Exposed pre-pregnant	Primiparous
Continuous	Exposed in pregnancy	2+ prior pregnancies
Marital status	Caffeine	Age
Married/cohabiting	Yes	<35 years old
Other	No	≥35 years old
	Continuous (amount)	continuous
Current pregnancy	**Prior obstetric outcomes**	Cycle length
Prenatal vitamins	Prior miscarriage*	27-33 days
Yes	Yes	<27 days
No	No	>33 days
Infection	Prior preterm birth*	History of diabetes
Yes	Yes	Yes
No	No	No
Progesterone use	Prior elective termination*	
Yes	Yes	
No	No	
Fibroids		
Yes		
No		

*will also examine relationships with multiple occurrences of these events, as noted in the aims

Table 3.8. Categorization of bleeding severity, *Right From the Start*.

Model	Interview
No bleeding (j=0)	No bleeding
Spotting (j=1)	Light
Light bleeding (j=2)	Lighter than heavy flow
Heavy bleeding (j=3)	Like heavy flow
Heavy bleeding (j=3)	More than heavy flow

CHAPTER 4 : GENERAL RESULTS

This chapter outlines general results that do not belong in any manuscript, focusing on general characteristics of the study population, the overall hazard of miscarriage, and some descriptions of missing data.

A. HAZARD OF MISCARRIAGE

The hazard of miscarriage, by week of pregnancy, is depicted in Figure 4.1. This figure shows a general pattern that is similar to other week-specific risk of miscarriage estimates in large populations.[147] The sample used for this figure includes all participants contributing to the analysis for Aim 2. Table 4.1 shows the raw data used for this figure.

B. SAMPLE COMPOSITION

Aim 1 and Aim 2

The original dataset contained 5017 participants. Exclusion criteria, and numbers of participants excluded for each criterion to obtain the final sample size, are listed in Table 4.2. A total of 4539 participants contributed to these analyses. However, for Aim 2, the analysis required fetal survival to at least week 5 of pregnancy and to the beginning of the week following enrollment for inclusion in risk sets for analysis. Twenty-nine participants were excluded due to this requirement, leaving 4510 total participants for the Aim 2 analysis.

Aim 3

Participants eligible for this analysis were required to have completed the daily diary throughout the first trimester as well as complete the first trimester interview. A total of 153 participants contributed to this analysis.

C. PARTICIPANT CHARACTERISTICS

Overall participant characteristics are outlined in Table 3.5 (Chapter 3). Overall, RFTS women are highly educated, married, and about slightly less than half are nulliparous. Comparison of the overall study population used in this analysis with two interesting sub-groups is outlined in Table 4.3 (Primigravida women), and Table 4.4 (Comparison of women enrolling prior to pregnancy with women enrolling during early pregnancy).

Women in their first pregnancy are generally more highly educated and younger than women with a pregnancy history. Women who enrolled prior to pregnancy were characterized by being, on average, older, non-smokers, white, and highly educated.

D. MISSING DATA

Vaginal bleeding

Vaginal bleeding data was missing for 149 women who did not complete the first trimester interview. An additional 21 women who completed the interview did not provide information on bleeding. A comparison of general characteristics of women who did not provide data on bleeding (n=170) with the rest of the study population is found in Table 4.5. In general, women with missing data on vaginal bleeding were more likely to be younger, less educated, and not living with their partners.

Miscarriage

Pregnancy outcome information is missing on 5.8% of women in the study (n=269). Sixty-nine of these women had LMPs in 2008 and were not known to have delivered by the time the data for this analysis was obtained. A comparison of women with missing outcome information and women without missing outcome is found in Table 4.6. In general, women with missing outcomes were more likely to be younger, less educated, and non-white.

Figure 4.1. Conditional probability (95% CI) of miscarriage by week of pregnancy, *Right From the Start* (n=517 miscarriages, n=4510 total population), 2000-2008.

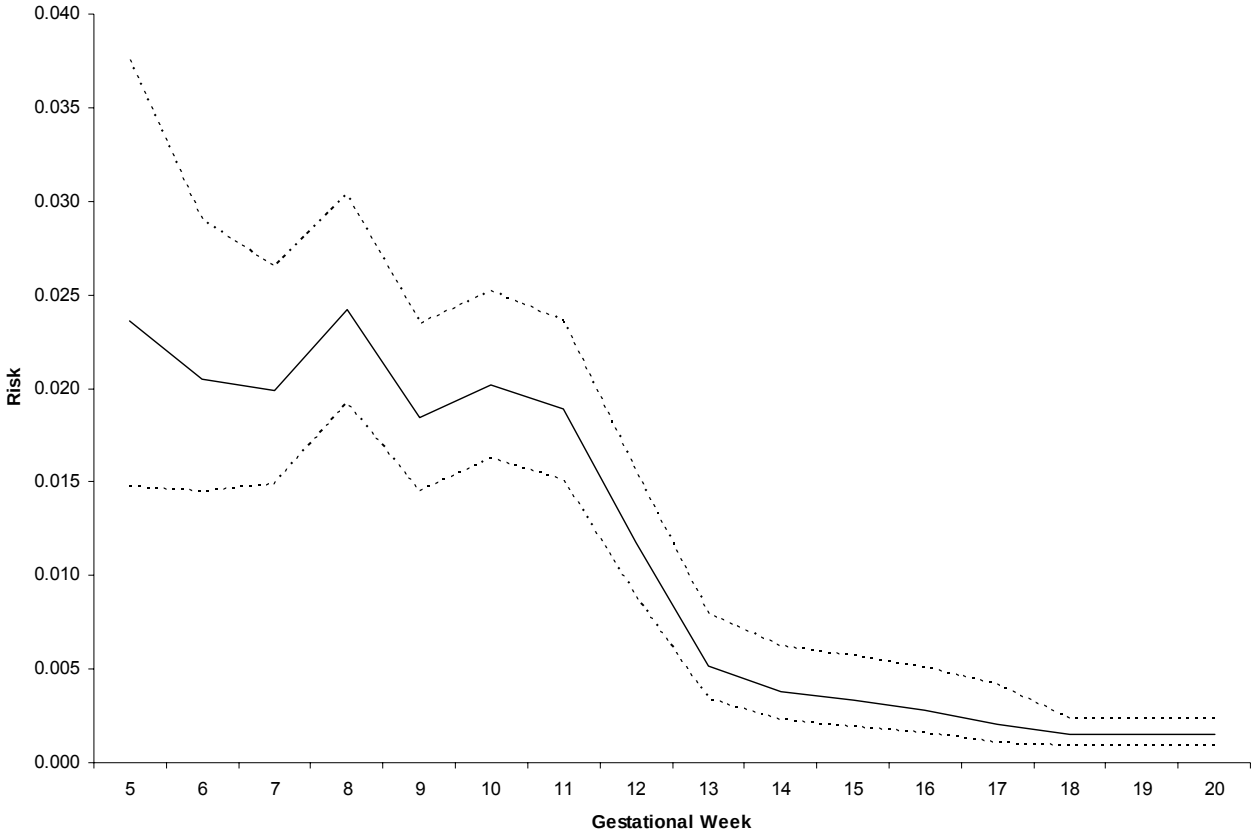


Table 4.1. Conditional probability of miscarriage in the first 20 weeks of pregnancy, *Right From the Start*, 2000-2008.

Week	Conditional probability	95% CI
5	0.024	0.015, 0.038
6	0.021	0.014, 0.029
7	0.020	0.015, 0.026
8	0.024	0.019, 0.030
9	0.018	0.014, 0.023
10	0.020	0.016, 0.025
11	0.019	0.015, 0.024
12	0.012	0.009, 0.016
13	0.005	0.003, 0.008
14	0.004	0.002, 0.006
15	0.003	0.002, 0.006
16	0.003	0.002, 0.005
17	0.002	0.001, 0.004
18	0.001	0.001, 0.002
19	0.001	0.001, 0.002
20	0.001	0.001, 0.002

Table 4.2. Aim 1 and Aim 2 basic exclusion criteria, *Right From the Start*, 2000-2008 (total n=4539).

Criterion	n excluded	Total n
Original dataset		5017
LMP after 14 July 2008	57	4960
No first trimester interview	170	4790
No LMP	2	4788
Outcome reported on or before day of enrollment	6	4782
Ectopic pregnancy	5	4777
2nd or greater enrollment by same woman	238	4539

Table 4.3. Comparison of participants in their first pregnancy with participants in subsequent pregnancy, *Right From the Start* (n=4539), 2000-2008.

	Primigravida (n=1527) Freq (%)	Multigravida (n=3000) Freq (%)
Age		
18-27 years	742 (48.6)	1042 (34.7)
28-34 years	687 (45.0)	1393 (46.4)
35-45 years	98 (6.4)	565 (18.8)
Race/ethnicity		
White, non-Hispanic	1084 (71.1)	1930 (64.4)
Black, non-Hispanic	259 (17.0)	702 (23.4)
Hispanic	94 (6.2)	247 (8.3)
Other	88 (5.8)	116 (3.9)
Missing	2	5
Marital status		
Married/cohabiting	1323 (86.6)	2663 (88.8)
Other	204 (13.4)	337 (11.2)
Education		
High School or less	240 (15.7)	654 (21.8)
Some college	206 (13.5)	615 (20.5)
College or more	1080 (70.8)	1731 (57.7)
Missing	1	0
Smoking status		
No	1344 (88.4)	2601 (86.7)
Yes	177 (11.6)	399 (13.3)
Missing	5	0

Table 4.4. Comparison of participants enrolled prior to pregnancy with women enrolled during pregnancy, *Right From the Start* (n=4539), 2000-2008.

	Enrolled prior to pregnancy (n=958) Freq (%)	Enrolled during pregnancy (n=3581) Freq (%)
Age		
18-27 years	257 (26.8)	1535 (42.9)
28-34 years	548 (57.2)	1535 (42.9)
35-45 years	153 (16.0)	511 (14.3)
Race/ethnicity		
White, non-Hispanic	764 (79.8)	2256 (63.1)
Black, non-Hispanic	96 (10.0)	871 (24.4)
Hispanic	51 (5.3)	290 (8.1)
Other	46 (4.8)	158 (4.4)
Missing	1	6
Marital status		
Married/cohabiting	932 (97.3)	3060 (85.5)
Other	26 (2.7)	521 (14.6)
Education		
High School or less	68 (7.1)	831 (23.2)
Some college	97 (10.1)	725 (20.3)
College or more	793 (82.8)	2024 (56.5)
Missing	0	1
Parity		
Nulliparous	496 (52.0)	1617 (46.2)
Parous	458 (48.0)	1880 (53.8)
Missing	4	84
Prior miscarriage		
None	741 (77.7)	2716 (77.7)
One	172 (18.0)	623 (17.8)
Two or more	41 (4.3)	158 (4.5)
Missing	4	84
Smoking status		
No	913 (95.5)	3035 (85.1)
Yes	43 (4.5)	533 (14.9)
Missing	1	13

Table 4.5. Women missing information on vaginal bleeding compared to women who are not missing data on vaginal bleeding, *Right From the Start* (n=4688), 2000-2008.

	No missing data (n=4518) Freq (%)	Missing data (n=170)* Freq (%)
Age		
18-27 years	1780 (39.4)	101 (50.4)
28-34 years	2077 (46.0)	55 (32.4)
35-45 years	661 (14.6)	14 (8.2)
Race/ethnicity		
White, non-Hispanic	3007 (66.7)	101 (60.1)
Black, non-Hispanic	961 (21.3)	40 (23.8)
Hispanic	339 (7.5)	19 (11.3)
Other	204 (4.5)	8 (4.8)
Missing	7	2
Marital status		
Married/cohabiting	3980 (88.1)	94 (54.3)
Other	538 (11.9)	76 (44.7)
Education		
High School or less	892 (19.8)	63 (37.1)
Some college	819 (18.2)	42 (24.7)
College or more	2806 (62.1)	65 (38.2)
Missing	1	0
Parity		
Nulliparous	2104 (47.4)	9 (100.0)
Parous	2338 (52.6)	0 (0)
Missing	76	161
Prior miscarriage		
None	3448 (77.6)	9 (100.0)
One	795 (17.9)	0 (0)
Two or more	199 (4.5)	0 (0)
Missing	76	161
Smoking status		
No	3942 (87.3)	6 (100.0)
Yes	576 (12.8)	0 (0)
Missing	0	164

*after all other exclusions of the data

Table 4.6. Women missing information on pregnancy outcome compared to women who are not missing data on pregnancy outcome, *Right From the Start* (n=4539), 2000-2008.

	No missing data (n=4274) Freq (%)	Missing data (n=265) Freq (%)
Age		
18-27 years	1634 (38.2)	158 (59.6)
28-34 years	1988 (46.5)	95 (35.9)
35-45 years	652 (15.3)	12 (4.5)
Race/ethnicity		
White, non-Hispanic	2887 (67.7)	133 (50.2)
Black, non-Hispanic	887 (20.8)	80 (30.2)
Hispanic	306 (7.2)	35 (13.2)
Other	187 (4.4)	17 (6.4)
Missing	7	0
Marital status		
Married/cohabiting	3784 (88.5)	208 (78.5)
Other	490 (11.5)	57 (21.5)
Education		
High School or less	806 (18.9)	93 (35.1)
Some college	759 (17.8)	63 (23.8)
College or more	2708 (63.4)	109 (41.1)
Missing	1	0
Parity		
Nulliparous	1982 (47.1)	131 (54.1)
Parous	2227 (52.9)	111 (45.9)
Missing	65	23
Prior miscarriage		
None	3266 (77.6)	191 (78.9)
One	758 (18.0)	37 (15.3)
Two or more	185 (4.4)	14 (5.8)
Missing	65	23
Smoking status		
No	3738 (87.7)	210 (80.5)
Yes	525 (12.3)	51 (19.5)
Missing	11	4

CHAPTER 5 : PATTERNS AND PREDICTORS OF VAGINAL BLEEDING IN THE FIRST TRIMESTER OF PREGNANCY

A. ABSTRACT

Background

Although first-trimester vaginal bleeding is commonly considered a marker of an at-risk pregnancy, few studies have investigated the prevalence and predictors of early bleeding. This study characterizes early pregnancy bleeding and identifies maternal characteristics associated with bleeding.

Methods

Participants (n=4539) were women ages 18-45 enrolled in *Right From the Start*, a community-based pregnancy study conducted in three states (2000-2008). Bleeding information included timing, heaviness, duration, color, and associated pain. Life table analyses were used to describe gestational timing of bleeding. Predictors of bleeding were investigated using multiple logistic regression, and multiple imputation was used for missing data.

Results

Approximately one-fourth of participants (n=1207) reported bleeding (n=1656 episodes), but only 8% of participants reported heavy bleeding. Of the episodes with only spotting or light bleeding (n=1555), 28% were associated with pain. Among heavy episodes, 54% were associated with pain. Most episodes lasted less than three days, and most occurred between gestational weeks 5-8. Twelve percent of

women with bleeding and 13% of those without experienced miscarriage. Predictors of bleeding were age (particularly between 28 and 34), increasing education, nulliparity, and either short or long menstrual cycle length. Maternal conditions (diabetes, fibroids), prior pregnancy outcomes (miscarriage, induced abortion), reproductive tract infections, smoking, and alcohol intake were also predictive of bleeding.

Conclusions

Consistent with the hypothesis that bleeding is a marker for placental dysfunction, bleeding is most likely to be seen around the time of the luteal-placental shift. Further analyses will examine the association between bleeding and adverse pregnancy outcomes.

B. INTRODUCTION

Although first-trimester vaginal bleeding is commonly considered a marker of a pregnancy at risk for adverse outcomes,[4,5,75,109] few studies have rigorously investigated the prevalence and predictors of bleeding. Estimates of bleeding prevalence in early pregnancy are imprecise and range from 7 to 24%.[1-3,114,132]

Only three reports have attempted to systematically describe early pregnancy bleeding patterns.[1,3,71] One collected data only through the eighth gestational week on a small sample.[71] The other two studies recruited participants and collected data during the second trimester, focusing on bleeding that occurs among women who deliver after twenty weeks,[1] or among women with live births.[3] These studies exclude pregnant women whose pregnancies result in miscarriage.

One previous study has investigated the maternal predictors of vaginal bleeding; however, it focused on preterm birth and excluded all losses prior to 20 weeks.[1] This study found that women of advanced maternal age, with passive smoking exposure, prior preterm birth, multiple prior elective terminations or with prior miscarriages were more likely to experience “intense” vaginal bleeding, measured by heaviness, duration, and an index of total blood loss. Unadjusted analyses from other studies suggest associations with increasing maternal age, minority race/ethnicity, prior obstetric outcomes (induced abortion, miscarriage, stillbirth, preterm delivery), or use of assisted reproductive technologies.[5,75,76]

To better characterize and understand the patterns and predictors of early pregnancy bleeding, we conducted this analysis using data from *Right From the Start*, a community-based early pregnancy cohort. Participants are recruited early in

pregnancy and provide detailed information about first-trimester bleeding. We describe the timing and heaviness of bleeding episodes in the first trimester of pregnancy, excluding bleeding immediately prior to a miscarriage event. We also report maternal characteristics associated with the occurrence and heaviness of bleeding episodes.

C. METHODS

Study Population

Right From the Start (RFTS) is an ongoing cohort that began enrollment of pregnant women in 2000. Over time, the study has included three phases (RFTS 1, 2, and 3) and has been active in Galveston, TX, Memphis and Nashville, TN, and the Triangle region (including Raleigh, Durham, and Chapel Hill), NC, USA. Participants were at least 18 years old, spoke English or Spanish, had not used assisted reproductive technologies to conceive, and intended to carry the pregnancy to term. Women who were not yet pregnant but attempting to conceive could pre-enroll prior to pregnancy and were considered enrolled once they reported a positive pregnancy test. Pre-enrolled women must have been attempting pregnancy for fewer than six months (RFTS 1 and 2) or fewer than three months (RFTS 3) to be eligible. Women entered the study prior to twelve completed weeks of gestation (RFTS 1), prior to nine completed weeks of gestation (RFTS 2), or only pre-enrolled (RFTS 3). Formal enrollment occurred, on average, at 53 days of gestation for women who enrolled while pregnant (n=3581), and at 38 days of gestation for women who pre-enrolled in the study (n=958). Informed, signed consent was obtained from each study subject in compliance with all Institutional Review Board procedures.

Participants had an early pregnancy ultrasound to assess fetal viability and document the gestational age of the fetus. Gestational age was calculated using self-reported last menstrual period (LMP). If self-reported LMP was unavailable,

ultrasound-based LMP was used (n=15). Seventy-five percent of ultrasounds were completed by the end of the ninth week of gestation.

Participants completed a short intake interview. Additional telephone interviews were conducted to collect more detailed information about the first trimester, including information about personal medical history, reproductive history, and pregnancy-related behaviors. All women, regardless of pregnancy outcome, provided this detailed information. In the first phase of the study (RFTS 1), two additional interviews were conducted: one shortly after enrollment during the first trimester, followed by a second interview around 20 weeks of pregnancy. Data from both of these interviews were compiled to reflect all first trimester events and conditions occurring during the entire first trimester. Later phases of RFTS (RFTS 2, RFTS 3) included only one additional interview, conducted at the end of the first trimester, no later than the 16^{th} week of pregnancy. Average time of completion of this interview was during the fourteenth week of pregnancy. If miscarriage occurred before the scheduled interview, the interview occurred as soon as possible after pregnancy loss. We refer to these interviews as the 'first trimester interview'.

Women who had their last menstrual period before July 14, 2008 were included in this analysis. Exclusions from the analysis sample include: women who did not complete the first trimester interview (n=170), participants missing both LMP and ultrasound (n=2), women with inconsistent enrollment or pregnancy end dates (n=6), and women with ectopic pregnancies (n=5). Women could enroll during more than one pregnancy, but only the first was included (n=238 subsequent pregnancies excluded). A total of 4539 pregnancies contributed to this analysis.

A separate sub-analysis was conducted among women for whom bleeding data was available for at least two enrollments in the study. Using this restricted sample, we calculated the relative risk of bleeding in a subsequent pregnancy, given that the woman reported bleeding in a prior pregnancy.

Variable definitions

Bleeding information

Bleeding was self-reported by each participant in the first trimester interview (Appendix 1). Participants reported the total number of episodes experienced during the first trimester and detailed information about the timing, heaviness, color, duration, and pain associated with the first three episodes. If bleeding stopped for at least two days and then started again, this was considered two separate episodes of bleeding. Participants provided the exact date on which an episode began; if this was unavailable, the week and month in which the episode occurred was recorded. The duration of the episode was reported in days. The heaviness of each episode was defined according to the heaviest flow in an episode, and was compared to a participant's usual flow during a menstrual period. A 'spotting' episode was one that was only noticed when wiping, a 'light bleeding' episode was defined as having the heaviest day(s) of flow being lighter than the heavy flow of a usual menstrual period, and a 'heavy bleeding' episode was defined as having the heaviest day(s) of flow as heavy or heavier than the heavy flow of a usual menstrual period. Participants could describe the color of each episode as 'pink,' 'red,' or 'brown.' If participants reported bleeding-associated pain, they were asked to characterize the pain as mild, moderate, or severe.

This analysis focuses on bleeding episodes that occur during the first trimester, regardless of whether a miscarriage occurs. To exclude bleeding that occurs at the time of miscarriage, we do not include any bleeding episodes that ended within 4 days of a miscarriage. This cutpoint was chosen after exploring several meaningful cutpoints based on the distribution of episodes in the data. We chose 4 days to maximize our use of the data and varied this cutpoint in later sensitivity analyses to identify whether the choice of cutpoint affected results.

Other characteristics

Data collected at the first trimester interview included demographic factors (age, race/ethnicity, education, marital status, percent of poverty level [according to the 2008 poverty guidelines, accounting for number of persons in household]), pre-pregnancy weight and height from which we calculated body mass index, usual menstrual cycle length in days, maternal morbidities (reproductive tract infections during pregnancy and diabetes), maternal behaviors (active and passive smoking, prenatal vitamin use, alcohol intake, caffeine intake), and prior obstetric history (parity, gravidity, history of miscarriage, induced abortion, or preterm birth). The early pregnancy ultrasound included systematic screening for uterine fibroids.[148] We created a dichotomous variable for presence or absence of fibroids.

Women were classified according to whether or not they were treated for one or more of the following infections at any time from LMP to the end of the first trimester: yeast infection, urinary tract infection, bacterial vaginosis, pelvic inflammatory disease, chlamydia, trichomonas, gonorrhea, syphilis, genital warts, or outbreaks of genital herpes. Women with pre-existing diabetes or who had

gestational diabetes in a previous pregnancy were classified as having diabetes. Women who smoked cigarettes at any point during pregnancy were identified as smokers. Passive smoking was defined according to whether an individual in the participant's household was a regular smoker. Women who reported drinking any alcoholic beverages during the pregnancy were classified as being exposed to alcohol.

Coding and categorization decisions for covariates were based on evaluation of the association between the presence of bleeding and various covariate specifications. Where appropriate, different coding schemes were compared using Akaike's information criterion (AIC) to choose the best variable specification. Categorical, indicator specification was favored due to ease of interpretation of results. Categories showing similar magnitude of association with bleeding were combined. Age, percent poverty level, caffeine intake, and cycle length were originally continuous variables that were categorized, with cutpoints chosen based on knots identified from use of smoothing splines. Gravidity, parity, and the other prior obstetric history variables were initially assessed as ordinal variables and categorized based on sparse data in higher-order categories and similarity of estimates in combined categories. Prior induced abortion was specified as a three-level categorical indicator variable when the outcome was presence of any bleeding and as a dichotomous variable when the heaviness of bleeding was modeled due to sparse data. Active smoking, prenatal vitamin use, and alcohol intake were converted from nominal categorical variables to dichotomous variables representing

any or no intake because of sparse data and similarities of category-specific estimates.

Data Analysis

All analyses were conducted in Stata, version 9.2 (College Station, TX).

Descriptive analyses

Episodes were categorized into weeks of pregnancy based on the day in which an episode began. Weeks of pregnancy were defined with the first 6 days after LMP labeled as week 0, the next 7 days considered week 1, the next 7 days considered week 2, and so forth. We used life table analyses to calculate the percent of pregnancies with bleeding for each week of gestation during the first trimester. The same was repeated for heavy bleeding only. Participants were censored at time of miscarriage (n=464), induced abortion (n=14), or interview when it occurred before the end of the first trimester (n=114). Miscarriage and induced abortion dates were based on participant self-report. The distribution of episodes by heaviness, duration, color, and associated pain were examined descriptively for all bleeding episodes. The same analyses were repeated using only the woman's heaviest episode (based on heaviest bleed), but patterns were substantially unchanged; thus, only analyses based on all episodes are shown.

Predictive modeling

Maternal characteristics predicting the presence/absence of bleeding were evaluated using a logistic model. All covariates were included in the model and the least important (highest p-value) was sequentially removed if the p-value from the

likelihood ratio test was greater than 0.15. The final reduced model consisted of only variables with an associated p-value less than 0.15.

Predictors of the heaviness of bleeding (based on each woman's heaviest episode) were evaluated using a multinomial logistic model, with a reference group of "no bleeding" and index categories of "spotting," "light bleeding," and "heavy bleeding." We followed the same strategy previously described to identify variables predicting the heaviness of bleeding, while requiring the previously identified predictors of any bleeding to remain in the model. For the multinomial logistic model, likelihood ratio tests were conducted to determine whether categories of heaviness could be combined. If heaviness levels were not different from each other at an *a priori* p-value of 0.15, the categories were combined.

Sensitivity analyses

Multiple imputation procedures (Stata, Imputation with Chained Equations[145]) were used so that participants with missing covariate data could be included. This method imputes missing values of a variable from a posterior distribution based on a regression of the non-missing values of the variable on all other predictors in the model. Five imputation cycles were used. Most variables were missing for less than 3% of the sample, but poverty level, fibroid status, and cycle length were missing for 4.0%, 6.9% and 16.9% of the sample, respectively. A sensitivity analysis was conducted that dropped women with missing data on any covariates in the final model (n=1082). A second sensitivity analysis was conducted, restricting the sample to primigravidae (n=1527), to eliminate potential bias associated with prior pregnancy outcomes.

D. RESULTS

The 4539 women in this study ranged in age from 18 to 45. Most self-identified as white, black, or Hispanic and were generally of high educational attainment (Table 5.1). About half of all women were nulliparous. In total, about two-thirds of women with miscarriage reported some bleeding during pregnancy. After excluding bleeding episodes that occurred within 4 days of miscarriage, 24.6% of women with miscarriage reported at least one episode of bleeding during the first trimester, similar to the proportion of women without miscarriage who reported bleeding during pregnancy (26.8%). Of those reporting bleeding, 70.9% reported only one episode (n=856); 20.0% reported two episodes (n=241); and 9.1%, three or more (n=110).

Bleeding episodes occurred during all weeks of the first trimester, peaking around the sixth and seventh week of pregnancy (Figure 5.1). Heavy episodes showed a similar pattern, and the peak extended throughout a longer period of the first trimester.

Most episodes were characterized as ‘spotting only’ (75.6%), and the majority were painless (70.7%) (Table 5.2). Heavy episodes comprised fewer than 10% of reported episodes. Half of all episodes persisted for only one day, 30% were of two or three days duration, and approximately 20% of episodes continued for more than 3 days. Heavy episodes were more likely to be painful, of longer duration, and red in color (Table 5.2).

Of the women who reported bleeding, about 15% reported an episode that occurred around the time of their expected menstrual period. Women who reported

more than one episode (n=351) had variable intervals between episodes. More than half of all multiple episodes occurred less than two weeks apart. Fewer than 3% of women (n=10) with multiple episodes reported episode intervals consistent with the timing and length of their usual menstrual cycles, and none of these had heaviness similar to usual menses.

Women who enrolled in the study more than once and reported bleeding in their first enrollment were 1.9 (95% CI 1.2, 2.9) times as likely to report bleeding in a subsequent pregnancy, compared to women who did not report bleeding in the prior pregnancy. We were unable to assess whether the heaviness of prior pregnancy bleeding was associated with heaviness in a subsequent pregnancy due to sparse data.

Table 5.3 shows the factors predictive of bleeding. Maternal age (particularly between 28 and 34 years), more years of education, long (≥34 days) and short (<27 days) cycle length, fibroids, infection, pre-existing or prior gestational diabetes, nulliparity, history of prior miscarriage, and history of induced abortion were strong predictors of bleeding. Reproductive tract infection during pregnancy was found to be a predictor, but had only a weakly elevated association with bleeding. The strongest predictor based on strength of association was history of miscarriage. Body mass index, race/ethnicity, marital status, percent poverty level, active or passive smoking, prenatal vitamin use, alcohol intake, caffeine intake, gravidity, and prior preterm birth, were not strong predictors. Estimates from the fully adjusted model are found in Appendix 3.

When assessing heaviness of bleeding, likelihood ratio tests indicated that the categories of spotting and light bleeding could be constrained to have the same coefficients (X^2=14.7, df=13, p=0.32), whereas the coefficients for the light and heavy bleeding categories were substantially different and could not be constrained to be the same (X^2=25.6, df=13, p=0.02). Because of this, the spotting and light bleeding categories were combined for the multinomial analysis.

Table 5.4 shows the important predictors of bleeding heaviness: maternal age, long/short menstrual cycle length, fibroids, infection, pre-existing or prior gestational diabetes, smoking, alcohol intake, nulliparity, and history of miscarriage and induced abortion. Estimates for some categories are highly imprecise due to the small number of observations within some strata of heavy bleeding. Most factors showed a stronger relationship with heavy bleeding compared with light bleeding, but education, parity, and induced abortion history appeared to be associated primarily with light bleeding. Of note, active smoking in pregnancy was inversely associated with spotting/light bleeding (OR 0.84, 95% CI 0.66, 1.05) and associated with heavy bleeding (OR 1.42, 95% CI 0.81, 2.47). Alcohol exposure was predictive of heavy bleeding only (OR 1.60, 95% CI 1.03, 2.48). These variables were not important predictors of 'any' bleeding. Estimates from the models including all tested covariates can be found in Appendix 3.

Comparing the results of the model with multiple imputation to the model with no missing data for any covariates, the results did not change substantially, though confidence intervals around estimates were wider for the model without missing data, which was expected given the reduced sample size. Restriction of the population to

participants without prior pregnancy history (n=1527) and removal of pregnancy history variables did not meaningfully change the estimates of the variables remaining in the model. Stratification of the main model by participant education provided some evidence that women with more years of education were more likely to report spotting and light bleeding episodes (Appendix 4).

E. DISCUSSION

We provide new evidence that bleeding episodes occur throughout the first trimester, peaking during the sixth and seventh weeks. Different characteristics of bleeding tend to cluster together. Heavy bleeding episodes (similar or heavier than those of a woman's normal menses) are more likely to be associated with pain, longer duration, bright red color, and presence of multiple episodes, while spotting episodes are more likely to occur in isolation and be of shorter duration and without pain. This suggests that heavy bleeding may arise from different underlying biologic events than spotting.

Though the causes of bleeding in later pregnancy have been investigated (due to placenta previa, abruption, or infection), there has been little investigation of first trimester bleeding.[132,134,149] It is interesting that the peak in bleeding episodes coincides with the development of a hormonally functional placenta. In very early pregnancy, the corpus luteum produces progesterone. The shift to placental production of progesterone occurs by the 7th week of pregnancy.[82] If the decrease in progesterone during this transition period is substantial enough to mimic the progesterone drop at the end of the luteal phase of the menstrual cycle, this might trigger an episode of bleeding. Such an episode may suggest that the early placenta is not performing its functions adequately.

The maternal spiral arteries are blocked by a trophoblastic shell during most of the first trimester, maintaining a low oxygen environment for fetal development.[121] The onset of maternal-fetal circulation usually begins in the periphery of the placenta around the ninth or tenth week of gestation.[16,121] Vaginal bleeding around this time

may be the external expression of internal uterine bleeding that occurs during the premature onset of maternal-fetal circulation or abnormal formation of placental membranes.[121] The peak in vaginal bleeding episodes observed in the middle of the first trimester may serve as an important marker of the developing placenta's function. It has been suggested that improper placentation may play a causal role in later adverse pregnancy outcomes.[16] Our future analyses will focus on the relationship between bleeding and pregnancy outcomes, including miscarriage.

Our results show that early pregnancy bleeding rarely mimics the bleeding of menses. Although some women reported bleeding at the time of expected menses, this bleeding was light, of short duration, and did not resemble bleeding of a usual menstrual cycle. Gestational age dating was verified by ultrasound, and a validation substudy of ongoing pregnancies in RFTS 1 found that the average difference between gestational age based on self-reported LMP and ultrasound was less than one day.[150] Based on these results, it is unlikely that many women misdate their pregnancy by mistaking early pregnancy bleeding for their LMP, consistent with the results of a previous study.[71] We found little evidence that cyclic, menstrual-like bleeding occurs during the first trimester. Few women reported intervals between episodes consistent with menstrual cycling, and none of these reported heaviness similar to their menses.

Several maternal characteristics emerged as important predictors of the presence of bleeding, including maternal age (especially women 28-34), higher education level, nulliparity, prior miscarriage or induced abortion, the presence of fibroids, pre-existing diabetes or history of gestational diabetes, reproductive tract

infection in pregnancy, and either long or short menstrual cycles. Education, nulliparity, and previous induced abortion may be important as measures of reporting sensitivity because they were associated with increased reporting of spotting or light bleeding but not heavy bleeding.

History of prior miscarriage was an important independent predictor of bleeding, consistent with the results of a previous study.[1] Prior obstetric outcomes that involve vaginal bleeding may make a woman more concerned and more likely to remember the bleeding episodes of a current pregnancy. Women who miscarried in the past may also have recurring problems that cause bleeding and/or loss.

Maternal conditions such as diabetes and reproductive tract infections may be associated with bleeding due to related biological processes, such as inflammation or placental infarction and hemorrhage. Fibroids have been associated with abnormal bleeding outside of pregnancy, and the same mechanisms may increase risk of bleeding during pregnancy, but those mechanisms are not yet understood.[151]

Maternal behaviors, such as active smoking and alcohol intake during pregnancy, were important predictors of heavy, but not light, bleeding. It is important to investigate what mechanisms underlie these relationships. Smoking was also inversely associated with light bleeding, likely related to decreased reporting of spotting and light bleeding episodes among smokers.

We acknowledge several important limitations. Our results are based on retrospective reporting of bleeding. Participants were asked to define an entire episode as spotting, light, or heavy; we did not have data on the number of days within an episode that were associated with heavy bleeding. Our study is also limited

by small sample size for some predictors, such as diabetes. However, unlike other studies, our results are based on a pregnant population recruited from the community that does not exclude participants whose pregnancies terminated prior to twenty weeks of gestation. Timing of gestational age was verified with ultrasound, which gave us confidence in our gestational age dating and also provided unique information about uterine fibroids not available to other perinatal researchers. Our study is also strengthened by detailed assessment of bleeding characteristics, including data on the timing, heaviness, duration, color, and overall number of episodes.

In conclusion, spotting and light bleeding are common symptoms of early pregnancy. Heavy bleeding is much less common. Whether both light and heavy bleeding arise from the same mechanisms or have different etiologies is an important question for future research. Future steps include investigating the relationship between bleeding episodes, early pregnancy biology, placental pathophysiology, and pregnancy outcomes such as miscarriage and preterm birth.

Table 5.1. Participants of *Right From the Start*, 2000-2008 (n=4539).

	Frequency	(%)
Age		
18-27 years	1792	(39.5)
28-34 years	2083	(45.9)
35-45 years	664	(14.6)
Missing	0	
Race/ethnicity		
White, non-Hispanic	3020	(66.6)
Black, non-Hispanic	967	(21.3)
Hispanic ethnicity	341	(7.5)
Other	204	(4.5)
Missing	7	
Education		
High school or less	899	(19.8)
Some college	822	(18.1)
College or more	2817	(62.1)
Missing	1	
Smoking		
No	3948	(87.3)
Yes	576	(12.7)
Missing	15	
Parity		
Nulliparous	2113	(47.5)
Primiparous	1549	(34.8)
Multiparous	789	(17.7)
Missing	88	
Bleeding		
None	3311	(73.3)
Any bleeding	1207	(26.7)
Missing	21	

Figure 5.1. Percent with bleeding among all pregnancies that reach each gestational week through the first trimester, *Right From the Start*, 2000-2008 (n=4539).
All bleeding: 1656 episodes of spotting, light, or heavy bleeding reported by 1207 women.
Heavy bleeding: 100 heavy bleeding episodes reported by 97 women.

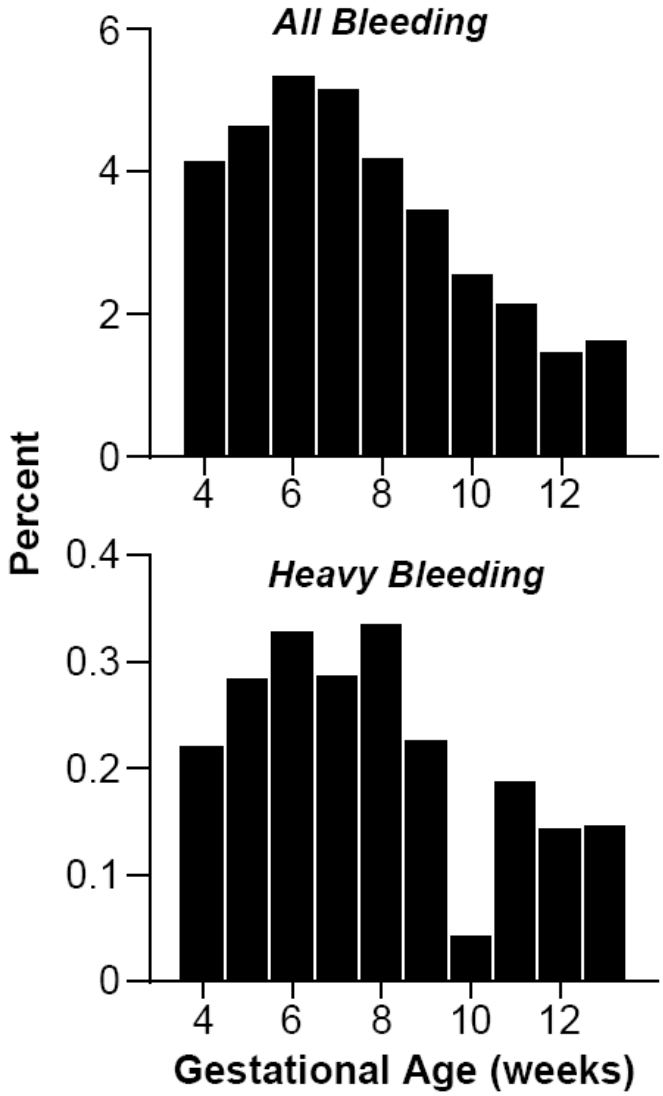


Table 5.2. Descriptive characterization of all bleeding episodes (n=1656 episodes from 1207 women) and episodes of heavy bleeding (n=100 episodes from 97 women) of participants in *Right From the Start*, 2000-2008.

Bleeding characteristic	All episodes (n=1656)		Heavy bleeding episodes (n=100)	
Heaviness	Freq	(%)	Freq	(%)
Spotting	1,251	(75.6)	--	--
Light bleeding	304	(18.4)	--	--
Heavy bleeding	100	(6.1)	--	--
Missing	1			
Color				
Pink	511	(31.0)	4	(4.0)
Red	419	(25.4)	84	(84.0)
Brown	717	(43.5)	12	(12.0)
Missing	9			
Pain				
None	1,168	(70.7)	46	(46.0)
Mild	350	(21.1)	19	(19.0)
Moderate	100	(6.0)	19	(19.0)
Severe	35	(2.1)	16	(16.0)
Missing	3			
Duration				
1 day	852	(51.5)	38	(38.0)
2 days	285	(17.2)	16	(16.0)
3 days	195	(11.8)	7	(7.0)
4-6 days	161	(9.7)	14	(14.0)
7+ days	160	(9.7)	25	(25.0)
Missing	3			

Table 5.3. Predictors of the occurrence of bleeding, *Right From the Start*, (2000-2008).

	n	%	Unadjusted OR	95% CI	p	Adjusted OR*	95% CI	p
Age					<0.01			<0.01
18-27 years	1792	39.5	1.00			1.00		
28-34 years	2083	45.9	1.39	(1.20, 1.61)		1.34	(1.14, 1.58)	
35-45 years	664	14.6	1.23	(1.00, 1.51)		1.13	(0.90, 1.42)	
Education					<0.01			0.11
≤High school	899	19.8	1.00			1.00		
Some college	822	18.1	1.27	(1.02, 1.59)		1.19	(0.94, 1.49)	
College or more	2817	62.1	1.37	(1.15, 1.64)		1.24	(1.01, 1.52)	
Cycle length					<0.01			<0.01
<27 days	667	17.7	1.33	(1.12, 1.57)		1.35	(1.14, 1.61)	
27-33 days	2793	74.1	1.00			1.00		
≥34 days	312	8.3	1.27	(0.99, 1.63)		1.27	(0.98, 1.64)	
Infection					0.11			0.06
No	3442	76.2	1.00			1.00		
Yes	1076	23.8	1.13	(0.97, 1.32)		1.17	(1.00, 1.36)	
Fibroids					<0.01			0.01
No	3753	88.8	1.00			1.00		
Yes	472	11.2	1.42	(1.15, 1.76)		1.29	(1.04, 1.61)	
Diabetes					0.15			0.07
None	4381	97.0	1.00			1.00		
Pre-existing/prior	137	3.0	1.32	(0.91, 1.90)		1.43	(0.98, 2.07)	
Parity					<0.01			<0.01
≥1 live birth	2338	52.5	1.00			1.00		
Nulliparous	2113	47.5	1.23	(1.08, 1.40)		1.33	(1.16, 1.54)	
Miscarriage history					0.03			0.01
None	3457	77.7	1.00			1.00		
One	795	17.9	1.16	(0.97, 1.37)		1.20	(1.01, 1.43)	
Multiple	199	4.5	1.41	(1.04, 1.91)		1.50	(1.09, 2.05)	

Induced abortion history				<0.01			<0.01
None	3729	83.7	1.00		1.00		
One	546	12.3	1.35	(1.11, 1.63)	1.34	(1.10, 1.64)	
Multiple	179	4.0	1.37	(1.00, 1.88)	1.39	(1.01, 1.92)	

*Factors are adjusted for all other variables in the table.

Table 5.4. Predictors of bleeding heaviness, *Right from the Start*, (2000-2008).

			Unadjusted model							Adjusted model*				
	No bleeding		Spotting/Light Bleeding			Heavy Bleeding				Spotting/Light Bleeding		Heavy Bleeding		
	n	OR	n	OR	95% CI	n	OR	95% CI	p	OR	95% CI	OR	95% CI	p
Age									<0.01					0.01
18-27 years	1367	1.00	372	1.00		40	1.00			1.00		1.00		
28-34 years	1462	1.00	570	1.43	(1.23, 1.67)	45	1.05	(0.68, 1.62)		1.35	(1.14, 1.61)	1.14	(0.69, 1.88)	
35-45 years	482	1.00	167	1.28	(1.04, 1.58)	12	0.86	(0.45, 1.66)		1.16	(0.91, 1.47)	0.80	(0.37, 1.62)	
Education									<0.01					0.20
≤High school	693	1.00	173	1.00		25	1.00			1.00		1.00		
Some college	601	1.00	197	1.32	(1.05, 1.66)	21	0.97	(0.54, 1.75)		1.21	(0.95, 1.53)	0.96	(0.52, 1.75)	
College or more	2016	1.00	739	1.47	(1.22, 1.78)	51	0.70	(0.43, 1.14)		1.27	(1.02, 1.57)	0.77	(0.42, 1.38)	
Cycle length									<0.01					<0.01
<27 days	457	1.00	195	1.34	(1.11, 1.62)	15	1.32	(0.78, 2.22)		1.38	(1.14, 1.67)	1.30	(0.77, 2.22)	
27-33 days	2085	1.00	651	1.00		55	1.00			1.00		1.00		
≥34 days	217	1.00	89	1.34	(1.02, 1.76)	5	1.09	(0.44, 2.73)		1.34	(1.01, 1.77)	1.18	(0.47, 2.98)	
Infection									0.24					0.18
No	2542	1.00	831	1.00		69	1.00			1.00		1.00		
Yes	769	1.00	278	1.10	(0.94, 1.29)	28	1.34	(0.86, 2.10)		1.15	(0.99, 1.36)	1.22	(0.77, 1.93)	
Fibroids									<0.01					0.07
No	2765	1.00	895	1.00		73	1.00			1.00		1.00		
Yes	316	1.00	143	1.39	(1.13, 1.72)	13	1.46	(0.80, 2.69)		1.24	(1.00, 1.54)	1.54	(0.83, 2.88)	
Diabetes									0.27					0.19
None	3218	1.00	1070	1.00		92	1.00			1.00		1.00		
Prior diabetes	93	1.00	39	1.26	(0.86, 1.84)	5	1.88	(0.75, 4.73)		1.37	(0.93, 2.02)	1.79	(0.70, 4.59)	
Parity									<0.01					<0.01
≥1 live birth	1752	1.00	530	1.00		56	1.00			1.00		1.00		
Nulliparous	1496	1.00	567	1.26	(1.10, 1.45)	40	0.85	(0.56, 1.29)		1.37	(1.18, 1.59)	0.92	(0.59, 1.43)	
Miscarriage history									0.02					0.01
None	2552	1.00	831	1.00		64	1.00			1.00		1.00		
One	564	1.00	207	1.12	(0.94, 1.33)	24	1.68	(1.04, 2.73)		1.17	(0.98, 1.40)	1.64	(1.00, 2.68)	
Two or more	132	1.00	59	1.39	(1.02, 1.90)	8	2.46	(1.16, 5.23)		1.48	(1.02, 2.04)	2.45	(1.13, 5.35)	

Induced abortion history								<0.01				<0.01
None	2757	1.00	880	1.00		79	1.00		1.00		1.00	
One or more	491	1.00	217	1.38	(1.16, 1.65)	17	1.21 (0.71, 2.06)		1.42	(1.18, 1.71)	1.02 (0.59, 1.77)	
Smoking								0.01				0.12
No	2876	1.00	989	1.00		77	1.00		1.00		1.00	
Yes	435	1.00	120	0.80	(0.64, 0.99)	20	1.70 (1.03, 2.80)		0.84	(0.66, 1.05)	1.42 (0.81, 2.47)	
Alcohol exposure								0.14				0.09
No	1483	1.00	483	1.00		34	1.00		1.00		1.00	
Yes	1826	1.00	625	1.05	(0.92, 1.21)	63	1.49 (0.98, 2.27)		0.98	(0.85, 1.13)	1.60 (1.03, 2.48)	

*Factors are adjusted for all other variables in the table.

CHAPTER 6 : THE ASSOCIATION BETWEEN FIRST TRIMESTER VAGINAL BLEEDING AND MISCARRIAGE

A. ABSTRACT

Background

Miscarriage is often recognized by vaginal bleeding, but many women experience first trimester bleeding that does not immediately precede miscarriage. Though this is a common, potentially alarming symptom, no previous study has estimated the risk of miscarriage in these women.

Objective

To evaluate the association between miscarriage and first trimester bleeding that is temporally remote from the loss.

Methods

Women enrolled before pregnancy or during early gestation in *Right from the Start*, a community-based pregnancy study designed to identify miscarriage risk factors. Detailed bleeding data for the first trimester were collected by telephone interview. Bleeding episodes proximal to miscarriage (within 4 days) were excluded. We used discrete-time hazard models to evaluate the association between gestational-age specific bleeding and miscarriage. The effect of the presence and heaviness of bleeding was modeled, both with and without adjustment for covariates (maternal age, prior miscarriage, and smoking). Exploratory regression tree analysis was used

to evaluate the relative importance of other bleeding characteristics (duration, associated pain, color, gestational timing).

Results

Of the 4510 participants, 1204 (26.8%) reported first-trimester vaginal bleeding or spotting, and 517 miscarriages were observed. Eight percent of participants reported heavy bleeding episodes. When we evaluated any bleeding, including episodes of only spotting, the unadjusted relative odds (OR) of miscarriage for women with bleeding was 1.1 (95% confidence interval [CI] 0.9, 1.3). However, women who reported heavy bleeding (as heavy or heavier than heaviest flow during menses) had nearly three times the risk of miscarriage compared to women without bleeding during the first trimester (OR 3.0, 95% CI 1.9, 4.6). Adjustment for covariates did not change estimates. Exploratory analyses suggested that women with heavy bleeding accompanied by pain were the group accounting for most of the elevated risk.

Conclusion

Heavy bleeding in the first trimester, particularly when accompanied by pain, is associated with higher risk of miscarriage. Spotting and light episodes, especially if only lasting 1-2 days, do not predict pregnancy loss.

B. INTRODUCTION

Vaginal bleeding is a common first trimester complication, often considered to be a sign of a problem in pregnancy. Bleeding has been related to preterm birth, low birthweight, and small-for-gestational age infants.[4,75,108,109] Inconsistent results have also been found relating bleeding and congenital malformations.[112,114,152] These studies are limited by focusing on bleeding episodes that come to clinical attention or bleeding episodes that are reported later in pregnancy or after delivery. Such methodologic differences result in widely varying baseline bleeding prevalences in these studies (7 to 24%), making it difficult to compare their results.

Studies that have looked specifically at the relationship between bleeding and miscarriage are usually conducted in populations recruited from hospital clinics or emergency departments.[91,98,101,104,105] Many of these bleeding episodes that require immediate medical attention mark the actual miscarriage event; thus, these studies do not provide useful information about the risk of miscarriage for women who experience bleeding that does not directly precede miscarriage. Only two studies have evaluated bleeding that is temporally separated from miscarriage. Both studies evaluated first-trimester bleeding in relation to second-trimester miscarriage, and both reported increased risk of late loss, especially for heavy bleeding (odds ratios for heavy bleeding were 3.6 and 4.9).[5,76]

However, most miscarriage occurs during the first trimester, and study of this outcome requires enrollment early in pregnancy so that early miscarriages can be identified. We collected detailed data about the timing and characteristics of first trimester bleeding from a large, community-based study that enrolled early in

pregnancy or prior to pregnancy in order to examine the association between bleeding and miscarriage, including first-trimester miscarriage.

C. METHODS

Study Population

Right From the Start (RFTS) is an ongoing pregnancy cohort that began enrollment of pregnant women in 2000. Over time, the study has included three phases (RFTS 1, 2, and 3) and has been active in Galveston, TX, Memphis and Nashville, TN, and the Triangle region of NC (including Raleigh, Durham, and Chapel Hill, NC). Participants were at least 18 years old, spoke English or Spanish, had not used assisted reproductive technologies to conceive, and intended to carry the pregnancy to term. Women who were not yet pregnant but attempting to conceive could pre-enroll prior to pregnancy and were followed until formal enrollment at the time of a positive pregnancy test. Pre-enrolled women must have been attempting pregnancy for fewer than six months (RFTS 1 and 2) or fewer than three months (RFTS 3). Women entered the study prior to twelve completed weeks of gestation (RFTS 1), prior to nine completed weeks of gestation (RFTS 2), or only pre-enrolled (RFTS 3). Formal enrollment occurred, on average, at 53 days of gestation for women who enrolled while pregnant (n=3581), and at 38 days of gestation for women who pre-enrolled in the study (n=958). Informed, signed consent was obtained from each study participant in compliance with all Institutional Review Board procedures.

Participants had an early pregnancy ultrasound to assess fetal viability and document the gestational age of the fetus. Gestational age was calculated based on self-reported last menstrual period (LMP). If self-reported LMP was unavailable, ultrasound-based LMP was used (n=15). Seventy-five percent of ultrasounds were

completed by the end of the ninth week of gestation, and the average difference between LMP- and ultrasound-based gestational age for ongoing pregnancies was less than one day in a RFTS 1 validation substudy.[140]

Participants completed an intake interview. Additional interviews were conducted to collect more detailed information about each participant, including demographic information such as race/ethnicity and education, reproductive history, and pregnancy-related behaviors such as smoking and symptoms, including bleeding. Women who smoked cigarettes at any point during pregnancy were identified as smokers. All women, regardless of outcome, provided this detailed information. In the first phase of the study, two interviews were conducted after intake: one occurred shortly after enrollment during the first trimester, followed by a second interview around 20 weeks of pregnancy. Data from both of these interviews were compiled to obtain an assessment of events and conditions occurring during the entire first trimester. Later phases of RFTS included only one interview after intake, conducted at the end of the first trimester, no later than the sixteenth week of pregnancy. Average time of completion of this interview was during the fourteenth week of pregnancy. Participants who experienced pregnancy loss before the scheduled interview were interviewed as soon as possible after miscarriage. We refer to the interviews that provide our data as the ‘first trimester interview’.

Women who had their last menstrual period before July 14, 2008 were included in this analysis. Exclusions from the analysis sample include: women who did not complete the first trimester interview (n=170), participants missing both LMP and ultrasound (n=2), women with inconsistent enrollment or pregnancy end dates

(n=6), and women with ectopic pregnancies (n=5). Women could enroll during more than one pregnancy, but only the first was included (n=238 subsequent pregnancies excluded). An additional 26 women were excluded from this analysis because they had immediate losses or were lost to follow-up prior to the beginning of the gestational week following their enrollment. A total of 4510 pregnancies contributed to this analysis.

Bleeding information

Bleeding was self-reported by each participant in the first trimester interview. Participants reported the total number of episodes experienced during the first trimester, and detailed information was collected about the timing, heaviness, color, duration, and pain associated with the first three reported episodes. If bleeding stopped for at least two days and then started again, this was considered two separate episodes of bleeding. Participants provided the exact date on which an episode began; if this was unavailable, the week and month in which the episode occurred was recorded. The duration of the episode was reported in days of bleeding. The heaviness of each episode was defined according to the heaviest flow in an episode. A ‘spotting’ episode was one that was only noticed when wiping, a ‘light bleeding’ episode was defined as being lighter than the heavy flow of a usual menstrual period, and a ‘heavy bleeding’ episode had at least one day when flow was as heavy or heavier than the heavy flow of a usual menstrual period. Participants could describe the color of each episode as ‘red,’ ‘brown,’ or ‘pink.’ Participants were also asked if bleeding was associated with pain, and if so, to characterize the pain as mild, moderate, or severe.

This analysis focused on bleeding episodes that occurred during the first trimester, regardless of pregnancy outcome. To exclude bleeding that occured at the time of miscarriage, we did not include any episodes that terminated less than 4 days before a miscarriage, and conducted a sensitivity analysis in which this cutpoint was extended to 7 days prior to miscarriage. These cutpoints were chosen after exploring several meaningful cutpoints based on the distribution of episodes in the data.

Pregnancy outcomes

Pregnancy was verified by ultrasound or pregnancy test. Miscarriage was defined as loss of a recognized pregnancy prior to twenty completed weeks of gestation. Outcomes were self-reported by participants, and prenatal records were obtained to verify the outcome. The date of a miscarriage was self-reported as the date of dilatation and evacuation or as the day of most severe bleeding. Women with induced abortions (n=14) were censored at the time of the induced abortion.

Statistical Analysis

All analyses were conducted in Stata, version 9.2 (College Station, TX) and DTREG (Brentwood, TN). We used discrete-time hazard models to evaluate the relationship between first trimester bleeding episodes and miscarriage and calculated week-specific odds ratios for the probability of having a miscarriage in a given gestational week, conditional on a woman still being pregnant at the beginning of that week. Due to the rarity of week-specific miscarriage in our sample, the

conditional odds ratios obtained from this model closely approximate the risk ratio. Thus, we refer to our results using 'risk' terminology.

Weeks of pregnancy were calculated beginning with the date of the last menstrual period, with the first 6 days labeled as week 0, the next 7 days considered week 1, the next 7 days considered week 2, and so forth. Analysis began at gestational week 5, and women entered analysis at the gestational week following their enrollment (e.g. a woman who entered on day 2 of gestational week 5 would enter analysis at gestational week 6). Participants contributed to analysis risk sets until an outcome occurred or loss to follow-up. All participants were censored at week 20 if an outcome or loss to follow-up had not yet occurred.

Because bleeding episodes are considered a marker of a pregnancy at risk, the effect of a bleeding episode was considered to extend indefinitely during the pregnancy (e.g., if bleeding occurred at week 5, a woman was entered as having bleeding in all subsequent weeks).

We conducted both unadjusted and adjusted analyses. In adjusted analyses, we controlled for maternal age, prior miscarriage, and maternal smoking status, prior predictors of miscarriage. Estimates for any bleeding and heaviness of bleeding (none, spotting, light, heavy) were calculated. In the hazard model, the heaviest episode prior to each analysis week was used to define heaviness (e.g., if a woman had light bleeding in week 6, heavy bleeding in week 8, and light bleeding in week 10, she would be initially coded none, then light, then heavy which would remain despite the subsequent light bleed).

We evaluated whether the effects of any bleeding or bleeding heaviness differed across weeks of pregnancy and assessed the proportional hazards assumption using linear and categorical time interactions and separate estimates for bleeding per week.

Other characteristics of bleeding episodes, such as duration, color, and associated pain, were evaluated using a two-phase approach that involved preliminary descriptive assessment of characteristics associated with miscarriage using classification and regression tree (CART) analysis, followed by an analysis of interactions with bleeding characteristics in the main model.[146] CART is a data-driven analysis approach that splits the data into sub-groups that differentially predict an outcome (in this case, miscarriage). This method categorizes the data to minimize the misclassification of the outcome within each group, so that each group can be categorized as associated or not associated with the outcome. Our CART analysis evaluated the relationship between miscarriage and the following characteristics of first trimester bleeding: heaviness, duration, color, timing, and associated pain. We restricted this analysis to women who experienced any bleeding. CART is implemented in four automated steps: (1) a splitting process, which maximizes the homogeneity of the outcome within each category and builds the best and most elaborate tree for the full dataset (the reference tree); (2) class assignment, which assigns an outcome to each category by minimizing misclassification of the outcome; (3) cross-validation, which partitions the data into 10 subsets and builds 10 trees appropriate for 10 different 90% samples of the data (9 of the subsets) and uses the 10% remaining sample (1 subset) to calculate each

tree's outcome classification error rate, and (4) a pruning process, which identifies the tree with the smallest outcome classification error rate, and prunes the reference tree to this optimal tree size.

After using CART to identify bleeding episode characteristics that were important predictors of miscarriage, we conducted a conditional logistic analysis of interactions between these characteristics and the main effect of bleeding. Several specifications of each variable were considered, informed by patterns observed in the CART analysis. Heaviness was specified as either a binary variable (heavy or not heavy) or as a three-level variable (spotting, light, or heavy bleeding). Pain was coded as a binary variable (present, absent) or as a four-level variable including pain severity (none, mild, moderate, severe pain). Duration was coded as a binary (<3 days, 3+ days) and as a three-level variable (1 day, 2 days, 3+ days). The contribution of interaction terms for episode characteristics to the main models was evaluated using Akaike's information criterion and likelihood ratio tests for nested models ($p=0.10$).

Because previous studies have evaluated the relationship between bleeding and second trimester miscarriage, we used logistic regression models to replicate these analyses, obtaining estimates for any bleeding and heaviness of bleeding.

Sensitivity analyses were conducted to assess the consistency of our results under various scenarios. (1) We re-analyzed our data using a 7-day rather than 4-day cutpoint for eliminating bleeding episodes that are proximate in time to the miscarriage. (2) We restricted our study population to participants for whom gestational age by ultrasound and by last menstrual period differed by no more than

3 or 7 days. This eliminates women whose reporting of early pregnancy events and symptoms may be inaccurate (potentially confusing an episode of early bleeding with the last menstrual period) and fetuses which may display early signs of abnormal development and growth. (3) We stratified our analysis by whether participants completed their interview before or after the time of miscarriage. (4) We restricted our analyses to women in their first pregnancy to eliminate the potential effect of prior pregnancy outcomes on reporting. (5) Finally, we applied the estimates of sensitivity and specificity obtained from a validation sub-study comparing recalled interview data and daily diary data to our results using both a deterministic and probabilistic framework to evaluate the potential effect of recall error on bleeding episode reporting. Sensitivities and specificities were drawn from a trapezoidal distribution for the probabilistic analysis.[153]

D. RESULTS

The 4510 women in this study ranged in age from 18 to 45. Most were white, but substantial numbers of blacks and Hispanics also participated. Eighty percent had more than a high school education (Table 6.1). About half were nulliparous. Twenty-seven percent reported at least one episode of bleeding during the first trimester. Of those reporting bleeding, 70.9% reported only one episode (n=854); 20.0% reported two episodes (n=241); and 9.1%, three or more (n=109). About eight percent of women with bleeding reported heavy episodes.

The association between bleeding and miscarriage is shown in Table 6.2. Overall, twelve percent of participants experienced a miscarriage, and bleeding in pregnancy was not associated with a significantly increased risk of miscarriage (OR 1.10, 95% CI: 0.90, 1.34). However, 24% of women with heavy bleeding experienced miscarriage (n=23) and this represented a significantly elevated risk (OR 2.97, 95% CI: 1.93, 4.56). Adjustment of our results for age, prior miscarriage history, and smoking status had little effect on the estimates.

Figure 6.1 shows the probability of miscarriage, conditional on survival to that week of pregnancy, for women who had experienced different levels of bleeding heaviness. Given the small number of losses in any given week by heaviness of bleeding, confidence intervals are broad (Appendix 5). Data were not sufficient to estimate miscarriage risk for some weeks where outcomes occurred only among those with bleeding (light bleeding: weeks 11, 16, 18-20; heavy bleeding: weeks 5, 13, 15, 17). However, women who experienced heavy bleeding were at increased risk of both first and second trimester miscarriage, while the risk for women with less

severe bleeding (spotting, light bleeding) was similar to those for women who did not bleed.

Because previous studies of bleeding and miscarriage looked only at second trimester miscarriage, we also formally examined that outcome. The risk of second-trimester miscarriage (n=61) for women reporting first trimester bleeding compared to those who reported none was 1.6 (95% CI: 1.0, 2.8), while the risk among women reporting heavy bleeding was 7.1 (95% CI: 3.1, 16.5); these values were similar range to previous results.[5,76]

To assure that bleeding episodes for women who miscarried were not all clustered near the time of loss, we examined the time from bleeding episodes to time of miscarriage for both heavy and spotting/light episodes. For heavy episodes, the median time from the end of the index episode to the time of miscarriage was 13 days (interquartile range (IQR) 6, 46), and for spotting and light episodes, the median time for the end of the index episode to time of miscarriage was 20 days (IQR 10, 33).

Of the characteristics of bleeding evaluated in the exploratory CART analysis, heaviness and pain associated with bleeding appeared to be the two most important characteristics predicting miscarriage, followed by duration (data not shown). The total number of episodes and color of bleeding appeared to have little importance. Because of these results, we evaluated the risk of miscarriage associated with combinations of heaviness, pain, and duration by including subgroups of these characteristics in the overall unadjusted hazard model. Figure 6.2 shows the relationship between specific types of bleeding episodes and miscarriage. Women

with heavy bleeding and pain had the highest risk of miscarriage (OR 4.79, 95% CI: 2.97, 7.73).

The finding that miscarriage was associated with heavy bleeding but not less severe bleeding was robust to sensitivity tests, including changes in our definition of bleeding episodes (Appendix 6). In all cases the risk of miscarriage was low for spotting and light bleeding (unadjusted odds ratios all below 1.5) but moderate to high for heavy bleeding (unadjusted odds ratios varied from 2.1 to 4.5). Furthermore, because the specificity of bleeding reporting was 100% in our validation sub-study, and sensitivity was non-differential by outcome near 0.8, there was no substantial change in the estimates after accounting for low sensitivity in both deterministic and probabilistic sensitivity analyses.

E. DISCUSSION

Vaginal bleeding is a common, and potentially alarming, symptom in early pregnancy. Yet, its relationship with miscarriage has not been carefully studied. We found that heavy bleeding (similar or greater than that seen during a woman's normal menses) was strongly associated with miscarriage, associated with three times the risk compared to women without bleeding. Further exploratory analyses suggested that women who had heavy bleeding that was accompanied by pain were at the greatest risk. Women with spotting or light bleeding that was associated with pain and continued for several days may also be at increased risk, though confidence intervals were wide.

Adjustment for maternal age, prior miscarriage, and smoking status did not affect our results, suggesting that bleeding is not merely a mediator of adverse effects on pregnancy reflected in these factors. We emphasize the unadjusted results because they are applicable to clinical care. If a pregnant woman informs her obstetrician of a prior episode of bleeding or spotting during a prenatal visit and inquires about the potential impact of such an episode on the health of her pregnancy, our results provide risk estimates.

Our main results showed no substantial differences when subjected to several sensitivity analyses. Results were similar when analysis was restricted to women in their first pregnancy, to women whose gestational dating by LMP was consistent with ultrasound, and to women whose time of interview occurred either before or after the loss. Little changed when we excluded bleeding episodes within 7 days of a loss instead of within 4 days as in the main analysis. A diary sub-study

provided estimates of the quality of the bleeding data and adjusting for misclassification did not change the results.

Many previous studies estimated the risk of miscarriage for women presenting to emergency or hospital care.[98,104,105] Many in such a sample are presenting with a current miscarriage, and such a study excludes many pregnant women, including those who experience bleeding but do not seek emergency care. Two population-based previous studies examined the relationship between first-trimester bleeding and second-trimester miscarriage.[5,76] We replicated these analyses, and obtained similar results.

In the *Right From the Start* sample, bleeding prevalence is highest around gestational weeks 5-8 (Chapter 5). The timing of this peak coincides with the timing of important phases of placental development. A hormonally functional placenta is required for the luteal to placental shift in progesterone production that occurs around gestational week 7.[82] Additionally, around the 10th week of pregnancy, the trophoblast blockage of the spiral arteries breaks down, remodeling of the arteries occurs, and the resulting blood flow to the developing placenta dramatically increases the oxygen tension.[121] Premature onset of maternal-fetal circulation may expose the placenta and fetus to harmful levels of oxidative stress. Heavy bleeding during this time in pregnancy may be indicative of an underlying defect in placental development. Early placental insufficiency has been implicated in several adverse pregnancy outcomes, including miscarriage.[16] Additionally, our observation that the most substantial increase in risk occurs for heavy, painful bleeding episodes suggests the presence of uterine contractions, which may occur due to low

progesterone levels. Presence of contractions may facilitate the transfer of pathogens from the vagina to the uterus,[154] further jeopardizing pregnancy. A pregnancy study that prospectively monitors hormone levels and placental blood flow could provide valuable insights on potential mechanisms.

Importantly, our study provides evidence that spotting or light bleeding episodes, especially those without pain and lasting only a day or two, do not increase the risk of miscarriage. Pregnant patients reporting these symptoms can be reassured that their risk of miscarriage is not higher than the general population. Most previous studies have been unable to assess the effect of light bleeding or spotting because most were conducted in hospital or clinic-based populations, or based entirely on medical records, and such episodes come to clinical attention less frequently.[91,98,99,104,105] To our knowledge, this is the first study to rigorously evaluate the relationship between early pregnancy bleeding and both first and second-trimester miscarriage in a community-based early pregnancy study.

Our study is also strengthened by several factors. The incorporation of early pregnancy ultrasound in our study protocol allows assessment of fetal viability and verification of gestational dating early during pregnancy. Our participants are a highly motivated group of women who have demonstrated their ability to provide accurate data on the presence and timing of early pregnancy events, such as timing of last menses.[140]

Limitations of this analysis include our inability to know the exact time of fetal demise. Although we have removed those bleeding episodes that directly result in miscarriage from our analysis, we may be including some loss-specific episodes in

our bleeding definition, especially if miscarriage does not result within 4 days of the end of the episode. Additionally, despite our relatively large study population, few women reported heavy bleeding episodes, yielding imprecise estimates.

To conclude, we found that painful bleeding episodes with heaviness similar to or greater than usual menses were associated with risk of miscarriage. Although only about 2% of pregnant women in our sample report heavy bleeding, reports of such episodes warrant greater concern for the health of the pregnancy. Among intrauterine pregnancies, light bleeding or spotting of short duration does not increase the risk of miscarriage.

Table 6.1. Participants of *Right From the Start*, 2000-2008 (n=4510).

	Frequency	(%)
Age		
18-28 years	1783	(39.5)
28-34 years	2068	(45.9)
34-45 years	659	(14.6)
Missing	0	
Race/ethnicity		
White, non-Hispanic	3002	(66.7)
Black, non-Hispanic	960	(21.3)
Hispanic ethnicity	340	(7.6)
Other	201	(4.5)
Missing	7	
Education		
High school or less	895	(19.9)
Some college	819	(18.2)
College or more	2795	(62.0)
Missing	1	
Smoking		
No	3921	(87.2)
Yes	574	(12.8)
Missing	15	
Parity		
Nulliparous	2095	(47.4)
Primiparous	1542	(34.9)
Multiparous	786	(17.8)
Missing	87	
Bleeding		
None	3285	(73.2)
Any bleeding	1204	(26.8)
Missing	21	
Heaviness of bleeding		
No bleeding	3285	(73.2)
Spotting	866	(19.3)
Light	240	(5.4)
Heavy	97	(2.2)
Missing	22	
Outcome		
Miscarriage	517	(11.5)
Live birth	3690	(81.8)
Other	40	(0.9)
Missing*	263	(5.8)

*Missing includes 69 women who are known to be beyond 20 weeks of pregnancy but had not reported deliveries at the time of this analysis. The remaining missing observations were censored at the last time of contact with the study.

Table 6.2. Association between bleeding and miscarriage, *Right From the Start*, 2000-2008 (n=4510).

	Total n (%)	Miscarriage (% of total)	Unadjusted OR	95% CI	Adjusted OR*	95% CI
Presence of bleeding						
None	3285 (73.2)	381 (11.6)	1.00		1.00	
Any	1204 (26.8)	131 (10.9)	1.10	0.90, 1.34	1.10	0.90, 1.35
Heaviness of bleeding						
None	3285 (73.2)	381 (11.6)	1.00		1.00	
Spotting	866 (19.3)	80 (9.2)	0.91	0.72, 1.17	0.93	0.73, 1.19
Light	240 (5.3)	28 (11.7)	1.18	0.80, 1.74	1.16	0.78, 1.71
Heavy	97 (2.2)	23 (23.7)	2.97	1.93, 4.56	2.84	1.82, 4.43

*Adjusted for maternal age, smoking, prior miscarriage

Figure 6.1. Week-specific probability of miscarriage by bleeding status, *Right From the Start*, 2000-2008 (n=4510).

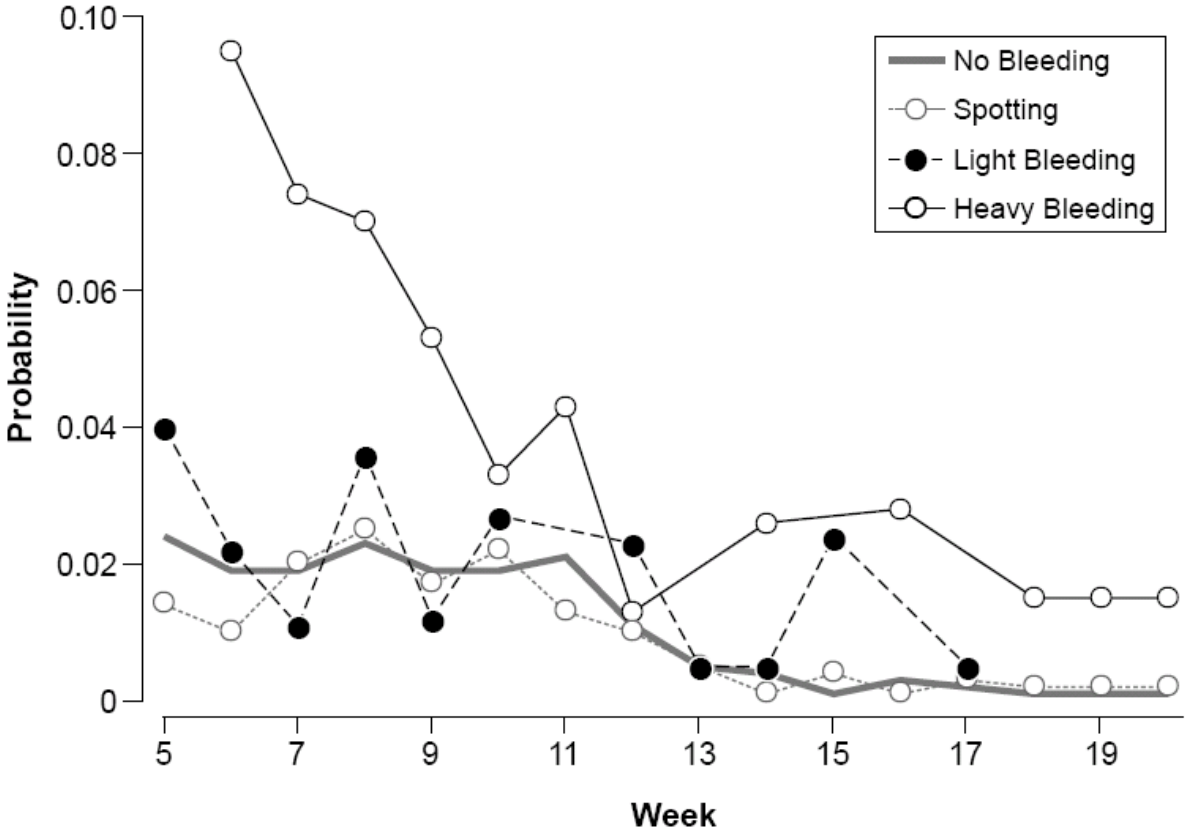
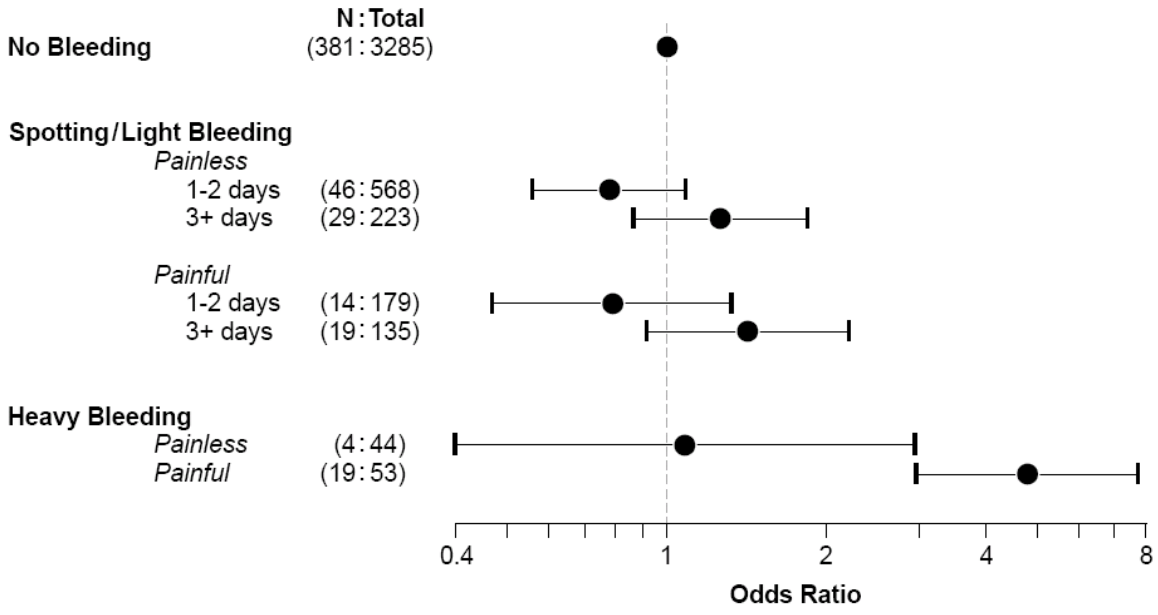


Figure 6.2. Relationship between bleeding episodes, characterized by levels of heaviness, pain, and duration, and miscarriage, *Right From the Start*, 2000-2008 (Total n=4510). Heavy bleeding categories could not be subdivided by duration because of small numbers. N refers to the number of miscarriages in each category.



CHAPTER 7 : ACCURACY OF INTERVIEW REPORTING OF BLEEDING DURING CURRENT PREGNANCY: COMPARISON WITH FIRST TRIMESTER DAILY DIARY

A. ABSTRACT

Background/Significance

Vaginal bleeding during pregnancy has been considered a marker of an at-risk pregnancy, but the accuracy of reported bleeding has not been assessed.

Objective

To evaluate the agreement in vaginal bleeding reports based on prospective daily diary and retrospective recall at first trimester interview and to investigate predictors of reporting accuracy.

Methods

Participants recruited prior to pregnancy for a community-based pregnancy cohort (n=153) completed web-based daily diaries beginning prior to pregnancy up through the end of the first trimester. A comprehensive first-trimester interview was conducted, and the bleeding data from diary and interview were compared. Kappa statistics were used to quantify agreement, and agreement was examined visually, using individual bleeding patterns reported in the diary and interview. Log-linear models were used to investigate maternal age, prior miscarriage, and current pregnancy outcome as potential predictors of agreement.

Results

Bleeding episodes and characteristics from the diary and interview were reported with high levels of agreement, with kappas ranging from 0.77-0.84. Sensitivity of any bleeding reports was 0.80, and specificity was 1.0. No important predictors of agreement were identified in this analysis, but the sample is small.

Conclusion

The presence of vaginal bleeding, a common and potentially alarming symptom of early pregnancy, may be assessed retrospectively with reasonable accuracy in a pregnancy study.

B. INTRODUCTION

Vaginal bleeding is common in early pregnancy, and has been previously associated with adverse pregnancy outcomes.[4,5,75,108] Many studies of vaginal bleeding rely on maternal self-report during late pregnancy or after delivery. Prior studies have found that recalled data may be influenced by outcome and other events in pregnancy.[53,155] No studies have evaluated the accuracy of reports of vaginal bleeding in pregnancy.

This analysis was undertaken to compare retrospective bleeding data collected from interview with prospective data obtained from daily, web-based diaries in a population of pregnant women in their first trimester. We assessed the extent of agreement of vaginal bleeding reports between interview and diary. We also investigated characteristics predictive of increased agreement.

C. METHODS

Study Population

Right From the Start (RFTS) is an ongoing community-based pregnancy cohort that began enrollment of pregnant women in several states of the United States (US) in 2000.[156] Briefly, participants were at least 18 years old, English- or Spanish-speaking, had not used assisted reproductive technologies to conceive, and intended to carry the pregnancy to term. Women who were not yet pregnant but attempting to conceive could pre-enroll prior to pregnancy and were followed until formal enrollment at the time of a positive pregnancy test. Formal enrollment occurred, on average, at 36 days of gestation for women who pre-enrolled in the study. Starting with the third phase of the study, pre-enrolled women completed a web-based daily diary during the pre-enrolled period and throughout the first trimester. The diary included information about common symptoms and signs of pregnancy, including vaginal bleeding and spotting. A comprehensive telephone interview was completed (median gestational week 13) which collected detailed information about the first trimester, including information about personal medical history, reproductive history, and pregnancy-related behaviors. If miscarriage occurred before the scheduled interview, the interview occurred as soon as possible after pregnancy loss. Informed, signed consent was obtained from each study participant in compliance with all Institutional Review Board procedures.

This analysis focuses on the 153 participants enrolled in RFTS who completed the daily diary during the first trimester and completed the first trimester interview. Although all participants included in this analysis provided diary data and

completed the first trimester interview, some participants included in the analysis sample had not yet reported a pregnancy outcome at the time of the analysis.

Bleeding Episodes

This analysis focuses on bleeding episodes that occurred during the first trimester, regardless of pregnancy outcome. We do not seek to describe the patterns or characteristics of bleeding episodes that occur at the time of a miscarriage event. Because of this, bleeding episodes that terminate within four days of miscarriage are not included in this analysis.

Bleeding episodes from interview

Bleeding was self-reported by each participant in the first trimester interview. Participants reported the total number of episodes experienced during the first trimester, and detailed information was collected about the timing, heaviness, color, duration, and pain associated with the first three reported episodes. If bleeding stopped for at least two days and then started again, this was considered two separate episodes of bleeding. Participants provided the exact date on which an episode began; if this was unavailable, the week and month in which the episode occurred was recorded. Episode duration was reported in days of bleeding. The heaviness of each episode was defined according to the heaviest flow in an episode. A ‘spotting’ episode was one that was only noticed when wiping; a ‘bleeding’ episode included at least one day of light or heavy bleeding.

Bleeding episodes from diary

In the daily diary, bleeding and spotting were queried separately. From these daily data, episodes were defined in a manner similar to that in the interview, i.e., episodes were separated by at least two days without any spotting or bleeding. All episodes were classified according to their timing (date began), duration (number of days with any bleeding or spotting), and heaviness (bleeding or spotting).

Statistical Analysis

Episodes of bleeding and spotting from the diary and the interview were compared. We first visually examined the individual bleeding patterns (including the timing, heaviness, and duration of bleeding episodes) for all women reporting bleeding in this study. This was followed by a quantitative comparison of the reports of the occurrence of any episode, the total number of episodes (no report, 1 episode, or 2+ episodes), the total duration of all episodes combined (no report, 1 day, or 2+ days), the timing of the first episode (no report, before 7 weeks gestation, 7-9 weeks gestation, 10+ weeks gestation), and the heaviness of the heaviest episode (no report, spotting only, bleeding) for all women. Sensitivity, specificity, and kappa for presence of any episode were calculated, and a weighted kappa statistic was calculated for all other comparisons. For the weighted kappa, the default in StatXact was used so that adjacent categories were given greater weight. Sensitivity and specificity were also calculated for episodes reported in the diary and the interview, in one- and two-week intervals of the first trimester.

Log-linear models were used to determine predictors of agreement for the number of episodes reported and heaviness of bleeding. This method models the

distribution of observations in a contingency table, accounting for agreement due to chance and beyond-chance agreement in the data.[157-159] Further cross-classification by predictor variables of interest (maternal age, prior miscarriage, current pregnancy outcome) was evaluated by including each variable and its interactions with other variables in the model to assess whether it predicted agreement in the diary and interview. Potential predictors were evaluated separately from each other due to small sample size. The coefficient for the interaction of the predictor with the beyond-chance agreement term indicates which group has better agreement. A positive coefficient indicates that agreement is greater than expected due to chance, and a negative coefficient indicates that agreement is less than expected due to chance in the index category. Although education level would be an interesting variable to examine due to its association with reporting accuracy in other studies,[160,161] examination of this variable as a predictor was limited by the homogeneity of our sample.

Stata (version 9.2) and StatXact (version 6) were used for all analyses.

D. RESULTS

The majority of participants were white, married, and had at least a college education (Table 7.1). About half of all women with prior pregnancies reported having had a miscarriage, consistent with other women enrolled in this phase of the study. Of the women whose current pregnancy resulted in a miscarriage (n=19), 16% reported bleeding at some point during their pregnancy (n=3). Sixty-five women (42%) reported at least one episode of bleeding or spotting in the diary; fifty-two of these women reported episodes in the first-trimester interview (sensitivity=0.80). No participants reported episodes in the interview without reporting some episodes in the diary (specificity=1.0). More spotting episodes were reported in the diary compared to interview. The thirteen women who had diary episodes but did not report any episodes in interview all reported only spotting episodes in the diary. All participants who reported bleeding episodes in the diary reported at least one episode (spotting or bleeding) in the interview, although some misclassification in heaviness was present (Table 7.2).

Figure 7.1a shows the distribution of women reporting episodes in the diary and the corresponding reports of bleeding in the interview, by week of pregnancy. Specificity remained high throughout the first trimester (≥0.94 for all weeks), while sensitivity was lower and more variable. Sensitivity increased when we examined sensitivity within two-week intervals (Figure 7.1b).

The extent of agreement between information reported in the diary and interview was evaluated by calculating Cohen's kappa and weighted kappa statistics. The kappa for agreement of overall bleeding reports (not including bleeding

characteristics) was 0.82 (95% CI 0.73, 0.91). Results from the diary and interview were also reported with high levels of agreement for specific bleeding characteristics, with all kappas greater than 0.75 (Table 7.2).

None of the factors that we examined as potential predictors of agreement (maternal age, prior miscarriage, or miscarriage in current pregnancy) were important predictors of agreement. Estimates were imprecise with wide confidence intervals, due to the small sample size (Table 7.3).

Visual comparisons of diary and interview reports found that bleeding episode information obtained from the diary was more detailed compared to interview data. The number, duration, and heaviness of episodes reported in the diary were often attenuated when reported in the interview (Appendix 7).

E. DISCUSSION

Overall, more episodes were reported in the diary compared to the interview, but these were only spotting episodes, suggesting that spotting is more easily forgotten than bleeding. Despite these errors in recall, our overall measures of agreement were high. Kappas suggested that recall of bleeding episodes and characteristics is accurate at the end of the first trimester. Although some of the bleeding characteristics were analyzed in broad categories (e.g., categories of total duration defined by no report, one day, two or more days), similar kappas were obtained when more detailed categories were used to classify variables. Both the presence and characteristics of bleeding episodes were similarly reported in the diary and interview. The high level of agreement between diary-based collection of data and retrospective interview data supports the use of recalled data from the first trimester interview, though exact timing is not well reported. The increase in sensitivity of episode reports based on two-week intervals compared to one-week intervals suggests if precise timing of bleeding is required, more frequent or prospective data collection may provide more accurate information than recalled data.

The major limitation of this analysis is the homogeneity of our study population. Participants were highly educated women who not only planned their pregnancy but also enrolled in a community-based pregnancy cohort study that was not directly affiliated with their prenatal care provider. A third had had a prior miscarriage. These participants are likely to be highly aware of their pregnancy-related symptoms and accurately report bleeding episodes, both in the daily diary

and in the first trimester interview. The proportion of women reporting any bleeding in interview (approximately 34%) was higher in this analysis compared to a related analysis of the entire cohort, in which approximately 26% of participants reported any bleeding. The act of filling out the diary may result in higher levels of reporting in the interview for this subgroup of women. Thus, our results may be viewed as a best-case scenario, based on a select population of women whose recall may be better than the general population.

To conclude, we found a relatively high level of agreement for reports of vaginal bleeding episodes obtained from daily diary and recalled interview. No important predictors of agreement were identified, although our results are limited by small sample size.

Table 7.1. Participants who completed the daily diary and first trimester interview (n=153), *Right From the Start* (2000-2008).

	Freq	(%)
Maternal age		
≤ 30	95	(62.1)
>30	58	(37.9)
Race		
White	132	(86.8)
Other	20	(13.2)
Missing	1	
Education		
Less than college	16	(10.4)
College or more	137	(89.5)
Marital status		
Married, cohabiting	152	(99.3)
Single	1	(0.7)
Smoking in pregnancy		
No	148	(96.7)
Yes	5	(3.3)
Gravidity		
Primigravida	54	(35.3)
1 or more prior pregnancy	99	(64.7)
History of miscarriage*		
No	50	(50.5)
Yes	49	(49.4)
History of induced abortion*		
No	85	(85.8)
Yes	14	(14.1)
Outcome		
Miscarriage	19	(15.4)
Live birth/stillbirth	104	(84.6)
Missing	30	

*among women with previous pregnancies

Table 7.2. Comparison of bleeding characteristics reported in diary and interview (n=153), *Right From the Start*, (2000-2008).

	Interview	Diary					Percent agreement	Kappa (95% CI)
Heaviness of heaviest episode		None	Spot	Bleed	Total			
	None	88	13	0	101		0.84	0.82 (0.75, 0.88)
	Spot	0	33	5	38			
	Bleed	0	6	8	14			
	Total	88	52	13	153			
Number of episodes		None	1	2+	Total		0.84	0.83 (0.76, 0.91)
	None	88	11	2	101			
	1	0	20	9	29			
	2+	0	3	20	23			
	Total	88	34	31	153			
Total duration of all episodes (days)		None	1	2+	Total		0.86	0.84 (0.76, 0.92)
	None	88	8	5	101			
	1	0	9	7	16			
	2+	0	2	34	36			
	Total	88	20	46	153			
Timing of first episode (weeks)		None	≤6	7-9	10+	Total	0.84	0.77 (0.65, 0.89)
	None	88	7	4	2	101		
	≤6	0	26	3	2	31		
	7-9	0	4	7	0	11		
	10+	0	0	3	7	10		
	Total	88	37	17	11	153		

Figure 7.1a. Number of participants reporting at least one episode in a given gestational week via diary (black); among participants reporting episodes via diary, number also reporting episodes during that week during interview (white). Numbers above bars represent the week-specific sensitivity, *Right From the Start* (n=153) (2000-2008).

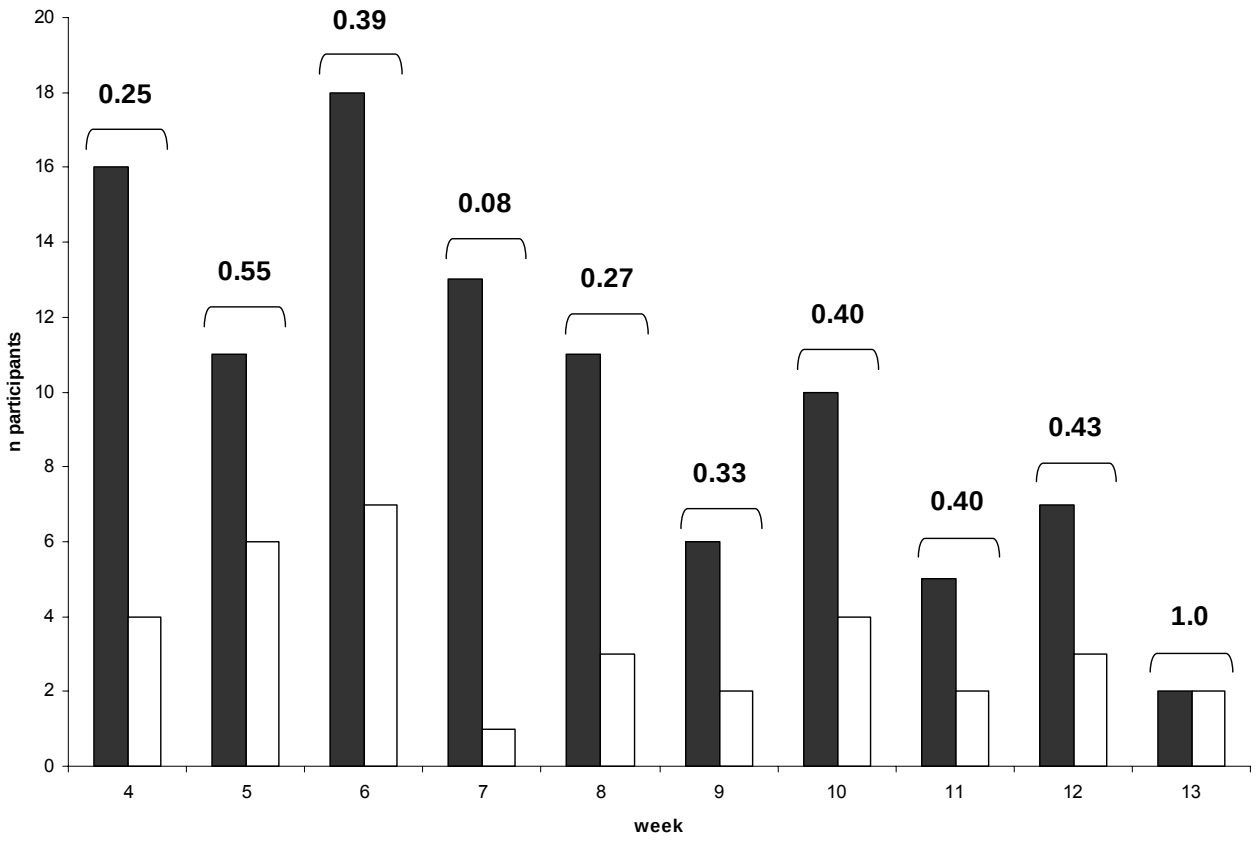


Figure 7.1b. Number of participants reporting at least one episode in a given two-week interval via diary (black); among participants reporting episodes via diary, number also reporting episodes during those two weeks during interview (white). Numbers above bars represent the category-specific sensitivity, *Right From the Start* (n=153) (2000-2008).

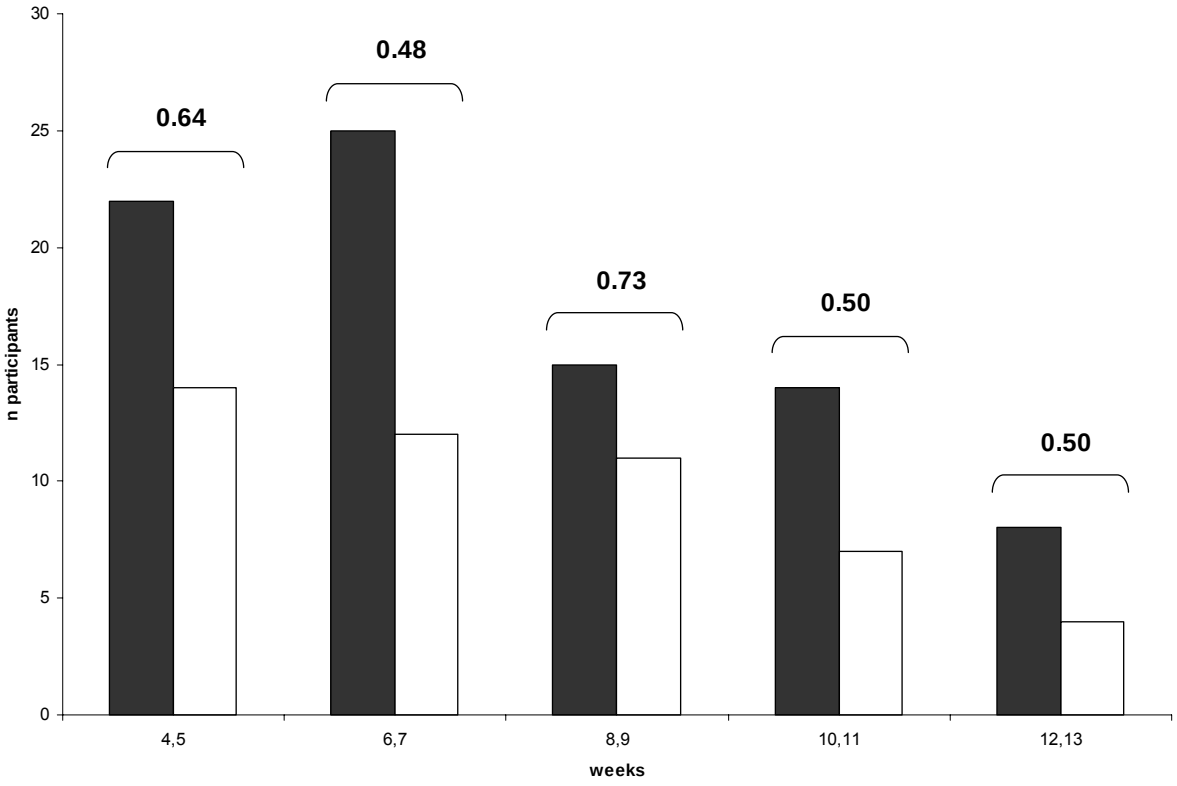


Table 7.3. Predictors of agreement in the number and heaviness of episodes, *Right From the Start* (2000-2008).

	Coefficient	95% CI	p
Number of episodes			
Prior miscarriage	0.52	(-0.60, 1.65)	0.35
Current miscarriage	0.31	(-1.85, 2.46)	0.77
Age ≥30	-0.001	(-1.06, 1.06)	0.99
Heaviness of episodes			
Prior miscarriage	-0.07	(-1.06, 0.91)	0.89
Current miscarriage	0.55	(-1.54, 2.65)	0.58
Age ≥30	-0.73	(-1.70, 0.25)	0.14

CHAPTER 8 : DISCUSSION

Pregnancy loss is a common and poorly understood pregnancy outcome. Few modifiable miscarriage risk factors are known and the two most common risk factors – advanced maternal age and prior miscarriage – do not clarify biological mechanisms to explain why miscarriage occurs so commonly, even among young women or those without a history of miscarriage. Because miscarriage occurs early in pregnancy, its study has been limited by the challenges of recruiting a study sample in early pregnancy whose results apply to a general obstetric population. As a beginning step, characterizing and understanding common early pregnancy symptoms and their association with miscarriage may provide clues about biological mechanisms that underlie miscarriage.

Few studies of a large, generalizable population have examined the course of early pregnancy symptoms and their association with early pregnancy outcomes. This project focused on vaginal bleeding, a common and potentially alarming symptom of early pregnancy. We described the patterns of bleeding, identified predictors of bleeding, and evaluated the association between bleeding and miscarriage. To determine the extent of misclassification associated with the assessment of bleeding in this study, we compared bleeding reports in the standard interview questionnaire with reports obtained from a daily, web-based diary. In all analyses, we exclude bleeding episodes that immediately precede miscarriage.

A. SUMMARY OF FINDINGS

About one-fourth of participants reported bleeding in the first trimester. This proportion was similar whether or not participants ultimately had a miscarriage. We found that over 90% of bleeding episodes were spotting or light bleeding episodes. The small number of heavy episodes were more often characterized as painful, of longer duration, and red in color, compared to spotting or light episodes. The distribution of bleeding in the first trimester had a peak in bleeding episodes in the mid-first trimester, around the time of the luteal-placental shift.

Identification of predictors of first trimester bleeding included maternal characteristics such as fibroids, diabetes, reproductive tract infections, long and short cycle length, maternal age (primarily between 28 and 34), and prior miscarriage or induced abortion. Increasing education level and nulliparity also predicted bleeding, and we believe that these covariates likely served as reporting variables, due to their association with spotting and light bleeding episodes. More detailed analysis of predictors of bleeding heaviness found that the strength of association between predictors for spotting episodes and light bleeding episodes were similar, so they were collapsed in the analysis. This additional analysis revealed that alcohol intake and smoking were also predictors of heavy bleeding.

We conducted an analysis to compare the agreement of bleeding reports in the first trimester interview with episodes reported in a prospective, web-based daily diary (gold standard). We found high kappas for agreement for reports of various episode characteristics, including total duration and heaviness. However, the use of kappa necessarily required categorization of episodes. When examining the week-

specific sensitivity and specificity of episode reports, we found that specificity remained high, while sensitivity was lower and more variable during the first trimester. Sensitivity increased when broader categories were used, and an overall analysis of the sensitivity and specificity of any episode reports found that overall sensitivity was 0.8 and specificity was 1.0. These results were applied to the evaluation of the relationship between bleeding and miscarriage.

We evaluated the association between bleeding and miscarriage and found that heavy episodes were associated with a three-fold increased risk of miscarriage. An exploratory analysis further identified that heavy bleeding episodes associated with pain accounted for the majority of this increased risk of pregnancy loss. The week-specific risk of miscarriage for spotting and light bleeding episodes closely mirrored the baseline risk of miscarriage for women without bleeding, providing further evidence that these most-common episodes do not confer increased risk for early pregnancy loss. Under the conditions of several sensitivity analyses, including application of the results of the sensitivity and specificity obtained previously, we obtained consistent results.

B. STRENGTHS AND LIMITATIONS

This project has several strengths. *Right From the Start* (RFTS) is a rigorous early pregnancy study whose participants have demonstrated their ability to provide accurate information, both in the timing of their last menstrual period and in the presence or absence of bleeding episodes and their characteristics. The first trimester interview collects detailed information on first trimester bleeding episodes. After eight years of recruiting participants, RFTS has accrued a large sample size. RFTS is a community-based study, allowing recruitment during very early pregnancy, often prior to the initiation of prenatal care. Some participants enroll prior to pregnancy. Because of this, the study ascertains a large number of miscarriages.

Our analytic approach is also a strength of this project. The evaluation of the relationship between bleeding and miscarriage used survival analysis, allowing incorporation of information about participant time of entry into the study and loss to follow-up. We were also able to precisely specify the week in which bleeding episodes were first reported and account for the changes in heaviness of episodes reported during the first trimester if multiple episodes were reported.
We also subjected our results to a wide range of sensitivity analyses. Our results were consistent under many restrictions of the data.

Several limitations of this project should also be noted. Miscarriage was defined based on participant self-report, 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, this was not a proxy for exact time of fetal demise. We were unable to know the exact time of fetal

demise from the available data. We attempted to minimize the possibility of misclassification of miscarriage-related bleeding from other bleeding episodes that occur during pregnancy by removing episodes that ended immediately before miscarriage (within four or seven days). However, fetal demise may have occurred several weeks before a miscarriage becomes symptomatic or detected at ultrasound, and bleeding episodes reported earlier during pregnancy may actually be a direct result. Such episodes should not be included in an analysis of the relationship between bleeding and miscarriage because the bleeding episodes would be differentially reported by women with symptomatic miscarriage. However, our results are applicable to women who experience pregnancy bleeding that does not result in immediate miscarriage, regardless of whether that episode is known to be symptom or direct component of miscarriage.

Although our sample size was sufficient to conduct most of the analyses in this project, some sub-analyses were limited by small sample size. Because heavy episodes were infrequent compared to spotting or light episodes, our analysis did not permit more detailed evaluation of heavy bleeding stratified by multiple characteristics, such as duration or associated pain. Few miscarriages were reported among women in some categories, resulting in imprecise estimates. Few participants reported some covariates, such as diabetes.

C. PUBLIC HEALTH IMPLICATIONS

Clinically, any research that gives insight to the processes and mechanisms operating during early pregnancy is useful. This time period in pregnancy is not well understood, despite the fact that the most common adverse outcome of pregnancy, miscarriage, frequently occurs during this time. Our results provide reassurance to many pregnant women who experience spotting or light bleeding episode during pregnancy. Our results also suggest that more careful, prolonged follow-up of women with heavy bleeding may be warranted, as a heavy bleeding episode may suggest an underlying problem with fetal or placental development. Mechanisms involving early abnormalities in placental development are hypothesized to underlie various later pregnancy outcomes, including pre-eclampsia.

Our epidemiologic data also provide supporting details that may be important for clarification of biologic processes occurring in early pregnancy. The peak of bleeding episode reports occurs during the mid-first trimester, around the same time as the luteal to placental shift in production of progesterone. Progesterone plays a vital role in the preservation of early pregnancy, promoting maintenance of the endometrium, inhibiting uterine contractions and altering maternal immunity to prevent rejection of the fetus. If the placenta is not sufficiently developed to produce adequate amounts of progesterone to maintain pregnancy when the corpus luteum regresses, bleeding may occur through mechanisms involving decreased progesterone levels, similar to those which promote the onset of menses. Heavier bleeding may be suggestive of greater

placental dysfunction, associated with a greater decrease in progesterone, leading to uterine contractions and pain. As a marker of placental dysfunction, this may explain why women with heavy episodes and pain have the greatest risk of miscarriage, since such episodes are presumably associated with a greater drop in progesterone and uterine contractions.

Presence of early pregnancy bleeding may also increase risk to fetal well-being through a mechanism that reflects the premature onset of maternal-fetal circulation. Evidence suggests that the early maternal spiral arteries are blocked until the last few weeks of the first trimester, allowing the fetus to develop in an environment of low oxygen tension. Factors associated with the onset of circulation prior to the development of defense mechanisms against excess oxidative stress have been associated with pregnancies that continue to miscarriage. Further studies that include collection of biologic samples are necessary to increase our understanding of these early pregnancy processes in relation to miscarriage.

D. CONCLUSIONS

To summarize, our analysis found that early pregnancy bleeding was a relatively common occurrence in pregnancy. Over one-fourth of participants in our study reported some bleeding during the first trimester, and the majority of episodes were spotting or light bleeding episodes. Women with such bleeding episodes can be reassured that there is little evidence to suggest that these episodes are associated with miscarriage.

Our results do suggest that the minority of women who report heavy episodes may be at higher risk of miscarriage. The hypothesized mechanisms that underlie these relationships need to be confirmed in a pregnancy study that collects longitudinal data on progesterone levels and other early pregnancy factors, monitors placental blood flow, and obtains products of conception, when available, to identify associations between reported symptoms and biologic markers that are related to early pregnancy maintenance and loss. Clarification of these biologic mechanisms will increase our knowledge of the pathophysiology of miscarriage and allow for identification of at-risk pregnancies. Such information is also essential for eventual development of appropriate and effective interventions that may help prevent miscarriage.

Appendix 1: Bleeding questionnaire in the first trimester interview, *Right From the Start*.

H8a. Since you got pregnant, have you had any bleeding or spotting with blood?

H8b. Did the bleeding or spotting start at the time you expected your menstrual period?

H9a. As best as you can remember when did you start to bleed or spot for the first/2nd/3rd time? (month/day/year)

H9b. (if H9a 'don't know') Do you remember what week that was (1st, 2nd, 3rd, 4th, etc.)

H10a. Compare this spotting/bleeding to amount of bleeding you usually have: on the day of your heaviest spotting or bleeding in the 1st/2nd/3rd episode, would you describe the bleeding as light, lighter than heavy flow, like heavy flow, more than heavy flow

H10b. What color was the blood, was it generally red, pink, or brown?

H10c. How many days did it last? If it stopped for at least 2 days and started again, consider this a separate episode.

H11a. Did you have any pain during the time you had spotting or bleeding?

H11b. Overall, would you describe the pain as mild, moderate, or severe?

H12. Did you have a 2nd/3rd time when you had spotting or bleeding? (start back at H9a)

Appendix 2: Additional questions about bleeding asked of women who report a miscarriage (*Right From the Start* 2 and 3 only).

A10f. Did you first suspect you might be having a miscarriage because
You noticed symptoms such as bleeding or pain?
Your health care provider found a problem during a physical exam?
Your health care provider found a problem during an ultrasound?
Or something else? _____

A10g. What, if any, symptoms or problems did you notice?
Bleeding or spotting? __yes __no
Pain? __yes __no
Something else? Specify _____

A10h. What was the first day that you noticed any of those symptoms?
Month, Day, Year

A10i. (if don't know for 10h) Do you remember what week that was?
Week, Month

Appendix 3: All predictors included in Aim 1 models.

Table 1. All potential predictors of bleeding in the first trimester, *Right From the Start*, 2000-2008 (n=4539).

	n	Unadjusted OR	95% CI	Adjusted OR	95% CI
Age					
18-28 years	1792	1.00		1.00	
28-34 years	2083	1.39	(1.20, 1.61)	1.33	(1.12, 1.57)
34-45 years	664	1.23	(1.00, 1.51)	1.12	(0.88, 1.42)
Missing	0				
Race/ethnicity					
White	3020	1.00		1.00	
Black	967	1.01	(0.86, 1.20)	1.08	(0.88, 1.32)
Hispanic	341	1.17	(0.91, 1.49)	1.38	(1.06, 1.80)
Other	204	1.09	(0.80, 1.50)	1.03	(0.74, 1.42)
Missing	7				
Education					
High school or less	899	1.00		1.00	
Some college	822	1.27	(1.02, 1.59)	1.23	(0.98, 1.56)
College or more	2817	1.37	(1.15, 1.64)	1.28	(1.01, 1.61)
Missing	1				
Marital status					
Married/cohabiting	3992	1.00		1.00	
Other	547	0.95	(0.77, 1.17)	1.01	(0.79, 1.29)
Missing	0				
Percent poverty level					
≤500%	3741	1.00		1.00	
>500%	616	1.31	(1.09, 1.57)	1.09	(0.88, 1.36)
Missing	182				
Body mass index					
Underweight	370	0.99	(0.77, 1.27)	1.05	(0.82, 1.36)
Healthy weight	2427	1.00		1.00	
Overweight	624	0.91	(0.75, 1.11)	0.89	(0.73, 1.09)
Obese	1021	0.89	(0.76, 1.06)	0.91	(0.75, 1.09)
Missing	97				

Cycle length					
<27 days	667	1.33	(1.12, 1.57)	1.35	(1.13, 1.61)
27-33 days	2793	1.00		1.00	
>33 days	312	1.27	(0.99, 1.63)	1.27	(0.98, 1.65)
Missing	767				
Infection					
No	3442	1.00		1.00	
Yes	1076	1.13	(0.97, 1.32)	1.17	(1.00, 1.38)
Missing	21				
Fibroid					
No	3753	1.00		1.00	
Yes	472	1.42	(1.15, 1.76)	1.30	(1.04, 1.63)
Missing	314				
Diabetes					
No	4381	1.00		1.00	
Prior diabetes*	137	1.32	(0.91, 1.90)	1.46	(1.00, 2.12)
Missing	21				
Vitamin use					
Yes	4083	1.00		1.00	
No	373	1.00	(0.79, 1.27)	1.10	(0.86, 1.41)
Missing	83				
Alcohol intake					
No	2003	1.00		1.00	
Yes	2518	1.08	(0.95, 1.24)	1.01	(0.88, 1.17)
Missing	18				
Caffeine intake					
None	1383	1.00		1.00	
1st quintile (<76.7 mgs)	429	0.85	(0.66, 1.09)	0.85	(0.65, 1.09)
2nd quintile (76.8-207.4 mgs)	830	0.99	(0.82, 1.20)	1.03	(0.84, 1.26)
3rd quintile (207.5-386.3 mgs)	618	0.98	(0.79, 1.22)	0.99	(0.79, 1.23)
4th quintile (386.4-698.9 mgs)	638	1.03	(0.83, 1.27)	1.03	(0.83, 1.29)
5th quintile (>699.0 mgs)	629	1.04	(0.84, 1.29)	1.07	(0.85, 1.33)
Missing	12				

Active smoking					
No	3948	1.00		1.00	
Yes	576	0.88	(0.72, 1.07)	0.91	(0.72, 1.15)
Missing	15				
Passive smoking					
No	4011	1.00		1.00	
Yes	512	0.88	(0.71, 1.10)	0.98	(0.77, 1.26)
Missing	16				
Gravidity					
First pregnancy	1527	1.00		1.00	
Second pregnancy	1424	0.87	(0.74, 1.03)	0.95	(0.73, 1.22)
Third or greater	1576	1.00	(0.85, 1.17)	0.97	(0.67, 1.41)
Missing	12				
Parity					
One or more previous birth	2338	1.00		1.00	
Nulliparous	2113	1.23	(1.08, 1.40)	1.28	(0.99, 1.65)
Missing	88				
Miscarriage history					
None	3457	1.00		1.00	
One	795	1.16	(0.97, 1.37)	1.21	(0.98, 1.51)
Multiple	199	1.41	(1.04, 1.91)	1.49	(1.05, 2.12)
Missing	88				
Induced abortion history					
None	3726	1.00		1.00	
One	546	1.35	(1.11, 1.63)	1.37	(1.08, 1.74)
Multiple	179	1.37	(1.00, 1.88)	1.40	(1.96, 2.04)
Missing	88				
Preterm birth history					
None	4070	1.00		1.00	
One or more	381	0.93	(0.74, 1.18)	1.03	(0.80, 1.33)
Missing	88				

*pre-existing diabetes, or gestational diabetes in a previous pregnancy

Table 2. All potential predictors of light and heavy bleeding in the first trimester, *Right From the Start*, 2000-2008 (n=4524).

	Spotting or light bleeding				Heavy bleeding			
	Unadjusted OR	95% CI	Adjusted OR	95% CI	Unadjusted OR	95% CI	Adjusted OR	95% CI
Age								
18-28 years	1.00		1.00		1.00		1.00	
28-34 years	1.43	(1.23, 1.67)	1.36	(1.14, 1.62)	1.05	(0.68, 1.62)	1.03	(0.61, 1.73)
34-45 years	1.28	(1.04, 1.58)	1.17	(0.92, 1.49)	0.86	(0.45, 1.66)	0.72	(0.33, 1.53)
Race/ethnicity								
White	1.00		1.00		1.00		1.00	
Black	1.00	(0.85, 1.19)	1.09	(0.88, 1.35)	1.05	(0.63, 1.75)	0.87	(0.46, 1.63)
Hispanic	1.09	(0.84, 1.41)	1.30	(0.99, 1.72)	1.99	(1.07, 3.67)	2.03	(1.04, 3.95)
Other	1.09	(0.79, 1.51)	1.01	(0.73, 1.41)	1.01	(0.36, 2.81)	1.08	(0.38, 3.05)
Education								
High school or less	1.00		1.00		1.00		1.00	
Some college	1.32	(1.05, 1.66)	1.25	(0.98, 1.59)	0.97	(0.54, 1.75)	1.10	(0.59, 2.06)
College or more	1.47	(1.22, 1.78)	1.30	(1.02, 1.66)	0.70	(0.43, 1.14)	0.96	(0.49, 1.89)
Marital status								
Married/cohabiting	1.00		1.00		1.00		1.00	
Other	0.90	(0.73, 1.11)	1.00	(0.77, 1.28)	1.35	(0.77, 2.36)	1.17	(0.60, 2.27)
Percent poverty level								
≤500%	1.00		1.00		1.00		1.00	
>500%	1.33	(1.10, 1.61)	1.06	(0.85, 1.33)	1.14	(0.64, 2.04)	1.73	(0.85, 3.54)
Body mass index								
Underweight	0.98	(0.76, 1.26)	1.04	(0.81, 1.34)	1.10	(0.52, 2.36)	1.23	(0.57, 2.66)
Healthy weight	1.00		1.00		1.00		1.00	
Overweight	0.93	(0.76, 1.14)	0.91	(0.73, 1.12)	0.82	(0.41, 1.63)	0.78	(0.38, 1.58)
Obese	0.85	(0.71, 1.01)	0.87	(0.72, 1.05)	1.43	(0.90, 2.28)	1.30	(0.78, 2.17)
Cycle length								
<27 days	1.34	(1.11, 1.62)	1.37	(1.13, 1.66)	1.32	(0.78, 2.22)	1.28	(0.74, 2.21)
27-33 days	1.00		1.00		1.00		1.00	
>33 days	1.34	(1.02, 1.76)	1.34	(1.02, 1.78)	1.09	(0.44, 2.73)	1.21	(0.48, 3.05)
Infection								
No	1.00		1.00		1.00		1.00	
Yes	1.10	(0.94, 1.29)	1.16	(0.98, 1.36)	1.34	(0.86, 2.10)	1.26	(0.79, 2.00)

Fibroid								
No	1.00		1.00		1.00		1.00	
Yes	1.39	(1.13, 1.72)	1.25	(1.00, 1.57)	1.46	(0.80, 2.69)	1.55	(0.82, 2.98)
Diabetes								
No	1.00		1.00		1.00		1.00	
Prior diabetes*	1.26	(0.86, 1.84)	1.41	(0.95, 2.10)	1.88	(0.75, 4.73)	1.71	(0.65, 4.48)
Vitamin use								
Yes	1.00		1.00		1.00		1.00	
No	0.98	(0.77, 1.26)	1.11	(0.85, 1.43)	1.29	(0.66, 2.51)	1.22	(0.61, 2.46)
Alcohol intake								
No	1.00		1.00		1.00		1.00	
Yes	1.05	(0.92, 1.21)	0.98	(0.84, 1.13)	1.49	(0.98, 2.27)	1.46	(0.93, 2.29)
Caffeine intake								
None	1.00		1.00		1.00		1.00	
1st quintile (<76.7 mgs)	0.86	(0.67, 1.12)	0.86	(0.66, 1.12)	0.63	(0.24, 1.67)	0.60	(0.23, 1.60)
2nd quintile (76.8-207.4 mgs)	1.00	(0.82, 1.22)	1.05	(0.85, 1.29)	0.89	(0.45, 1.75)	0.79	(0.40, 1.59)
3rd quintile (207.5-386.3 mgs)	0.93	(0.75, 1.17)	0.96	(0.76, 1.20)	1.64	(0.88, 3.05)	1.46	(0.77, 2.75)
4th quintile (386.4-698.9 mgs)	0.99	(0.79, 1.23)	1.00	(0.80, 1.26)	1.52	(0.81, 2.85)	1.33	(0.70, 2.55)
5th quintile (>699.0 mgs)	0.99	(0.79, 1.23)	1.03	(0.82, 1.30)	1.82	(1.00, 3.33)	1.58	(0.83, 3.00)
Active smoking								
No	1.00		1.00		1.00		1.00	
Yes	0.80	(0.64, 0.99)	0.85	(0.67, 1.08)	1.70	(1.03, 2.80)	1.21	(0.67, 2.19)
Passive smoking								
No	1.00		1.00		1.00		1.00	
Yes	0.82	(0.65, 1.03)	0.95	(0.73, 1.23)	1.62	(0.95, 2.77)	1.33	(0.71, 2.51)
Gravidity								
First pregnancy	1.00		1.00		1.00		1.00	
Second pregnancy	0.85	(0.72, 1.01)	0.92	(0.69, 1.23)	1.22	(0.73, 2.05)	0.71	(0.31, 1.64)
Third or greater	0.97	(0.83, 1.15)	0.95	(0.62, 1.45)	1.36	(0.83, 2.25)	0.43	(0.12, 1.50)
Parity								
One or more previous birt	1.00		1.00		1.00		1.00	
Nulliparous	1.26	(1.10, 1.45)	1.31	(0.98, 1.75)	0.85	(0.56, 1.29)	0.50	(0.21, 1.19)
Miscarriage history								
None	1.00		1.00		1.00		1.00	
One	1.12	(0.94, 1.33)	1.19	(0.94, 1.50)	1.68	(1.04, 2.73)	2.28	(1.16, 4.47)
Multiple	1.39	(1.02, 1.90)	1.48	(1.03, 2.13)	2.46	(1.16, 5.23)	3.44	(1.34, 8.79)

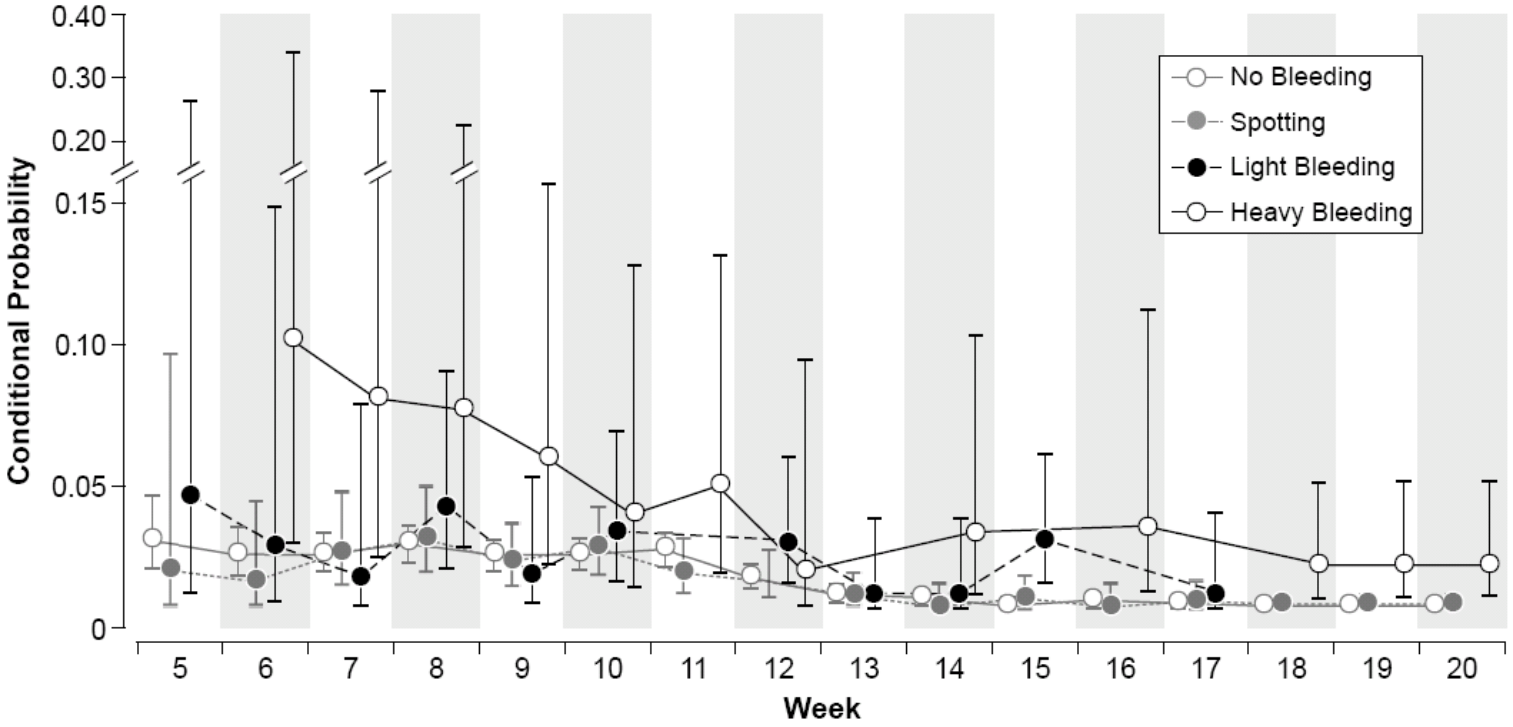
Induced abortion history								
None	1.00		1.00		1.00		1.00	
One or more	1.38	(1.16, 1.65)	1.45	(1.13, 1.86)	1.21	(0.71, 2.06)	1.41	(0.72, 2.77)
Preterm birth history								
None	1.00		1.00		1.00		1.00	
One or more	0.91	(0.71, 1.17)	1.02	(0.78, 1.34)	1.24	(0.63, 2.43)	1.06	(0.51, 2.20)

*pre-existing diabetes, or gestational diabetes in a previous pregnancy

Appendix 4: Stratification of Aim 1 results by education level.

	Less than college		College or more	
	OR	95% CI	OR	95% CI
Age				
18-28 years	1.00		1.00	
28-34 years	1.49	(1.14, 1.95)	1.29	(1.04, 1.59)
35-45 years	1.11	(0.72, 1.71)	1.15	(0.86, 1.53)
Cycle length				
<27 days	1.32	(1.00, 1.75)	1.39	(1.10, 1.75)
27-33 days	1.00		1.00	
≥34 days	1.47	(0.89, 1.75)	1.20	(0.90, 1.60)
Infection				
No	1.00		1.00	
Yes	1.29	(1.02, 1.64)	1.06	(0.87, 1.32)
Fibroids				
No	1.00		1.00	
Yes	1.16	(0.78, 1.73)	1.36	(1.04, 1.77)
Diabetes				
None	1.00		1.00	
Pre-existing/prior	1.40	(0.81, 2.41)	1.44	(0.86, 2.41)
Parity				
≥1 live birth	1.00		1.00	
Nulliparous	1.31	(1.10, 1.57)	1.35	(1.06, 1.72)
Miscarriage history				
None	1.00		1.00	
One	1.85	(1.40, 2.44)	0.88	(0.70, 1.11)
Multiple	1.92	(1.20, 3.06)	1.24	(0.81, 1.89)
Induced abortion history				
None	1.00		1.00	
One	1.62	(1.21, 2.19)	1.16	(0.89, 1.51)
Multiple	1.16	(0.73, 1.84)	1.70	(1.06, 2.72)

Appendix 5: Risk of miscarriage by week of pregnancy, including error bars around estimates.



Appendix 6: Further analyses and restrictions to evaluate the association between bleeding and miscarriage, *Right From the Start* (2000-2008).

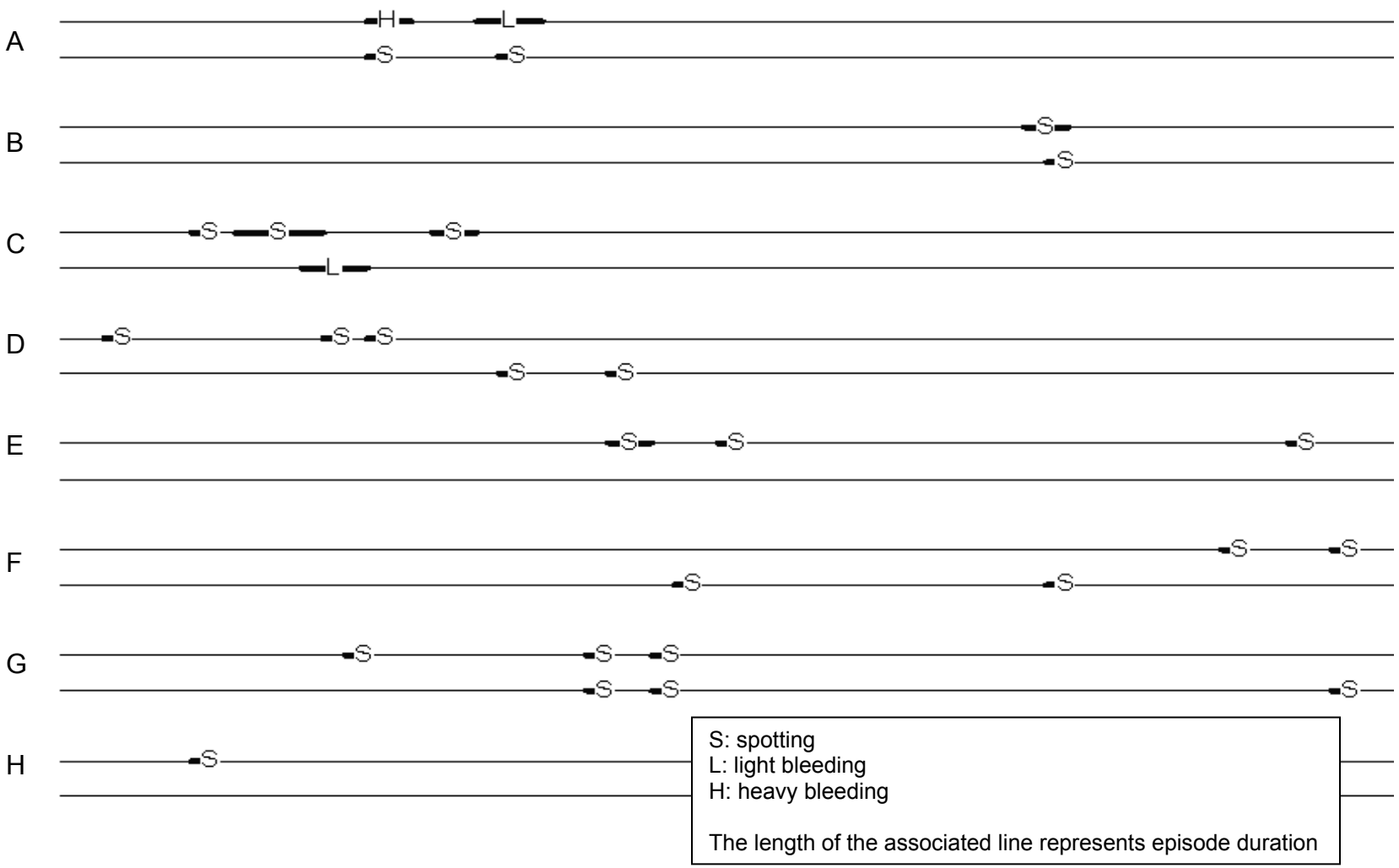
	No bleeding			Any bleeding			Spotting/Light			Heavy		
	n	n SAB***	OR	n	n SAB***	OR* (95% CI)	n	n SAB***	OR* (95% CI)	n	n SAB***	OR* (95% CI)
Changing episode definition												
4 days (overall analysis results)	3285	381	1.0	1204	131	1.1 (0.9, 1.3)	1106	108	1.0 (0.8, 1.2)	97	23	3.0 (1.9, 4.6)
7 days	3306	402	1.0	1183	110	0.9 (0.7, 1.1)	1091	93	0.8 (0.6, 1.0)	91	17	2.1 (1.3, 3.5)
Gestational age**												
3 days	1408	53	1.0	532	21	1.2 (0.7, 2.0)	495	18	1.1 (0.6, 1.8)	37	3	2.5 (0.8, 8.2)
7 days	2124	82	1.0	797	33	1.2 (0.8, 1.8)	743	29	1.1 (0.7, 1.7)	54	4	2.4 (0.9, 6.5)
Loss and interview timing												
Loss before interview	2904	244	1.0	1073	61	0.9 (0.6, 1.1)	998	52	0.8 (0.6, 1.0)	74	9	2.1 (1.1, 4.1)
Interview before loss	2904	135	1.0	1073	67	1.5 (1.1, 2.0)	998	54	1.3 (0.9, 1.8)	74	13	4.5 (2.5, 8.0)
Restriction												
Women in first pregnancy	1087	98	1.0	419	46	1.4 (1.0, 2.0)	391	41	1.4 (0.9, 2.0)	27	5	2.7 (1.1, 6.8)

*unadjusted odds ratio

**only including pregnancies whose gestational age calculated by last menstrual period and by ultrasound agree within the specified amount (3 days or 7 days)

***SAB: spontaneous abortion, or miscarriage

Appendix 7: Comparison of bleeding patterns reported in the diary and in the interview for a select number of participants (labeled A-H). The top line represents episodes reported in the diary, the bottom line represents episodes reported in the interview, gestational days 20-76, Right From the Start (2000-2008).



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