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ORIGINAL ARTICLE *Early pregnancy*

First-trimester bleeding characteristics associate with increased risk of preterm birth: data from a prospective pregnancy cohort

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BACKGROUND: Prior evidence linking first-trimester bleeding with preterm birth (PTB, <37 weeks gestation) risk has been inconsistent and may be biased by subject selection and/or incomplete documentation of bleeding episodes for all participants. Prior studies have not carefully examined the role of bleeding characteristics in PTB risk. In the present study, we estimate the association between first-trimester bleeding and PTB in a non-clinical prospective cohort and test whether bleeding characteristics better predict risk.

METHODS: Women were enrolled in Right from the Start (2000–2009), a prospective pregnancy cohort. Data about bleeding and bleeding characteristics were examined with logistic regression to assess association with PTB.

RESULTS: Among 3978 pregnancies 344 were PTB and 3634 term. Bleeding was reported by 986 (26%) participants. After screening candidate confounders, only multiple gestations remained in the model. Bleeding associated with PTB [odds ratio $(OR)_{adjusted} = 1.40$, 95% confidence interval (CI) 1.09–1.80]. Risk did not vary by race/ethnicity. Compared with non-bleeders, PTB risk was higher for bleeding with red color ($OR_{adjusted} = 1.92$, 95% CI, 1.32–2.82), for heavy episodes ($OR_{adjusted} = 2.40$, 95% CI 1.18–4.88) and long duration ($OR_{adjusted} = 1.67$, 95% CI 1.17–2.38).

CONCLUSIONS: Bleeding associated with PTB was not confounded by common risk factors for bleeding or PTB. PTB risk was greatest for women with heavy bleeding episodes with long duration and red color and would suggest that combining women with different bleeding characteristics may affect the accuracy of risk assessment. These data suggest a candidate etiologic pathway for PTB and warrant further investigation of the biologic mechanisms.

Key words: epidemiology / first-trimester / pregnancy / preterm birth / vaginal bleeding

Introduction

Birth before 37 weeks gestation is the predominant predictor of perinatal mortality in the USA (Weinberg *et al.*, 1992) consuming a large portion of funds spent for perinatal health care (Berkowitz and Papiernik, 1993; Institute of Medicine, 1993). In the USA, preterm birth (PTB) rates have been slowly increasing over the last decade from 9.7% in 1990 to 12.7% in 2005.(Martin *et al.*, 2002; Hamilton *et al.*, 2007) This trend has also been demonstrated outside of the USA (Langhoff-Roos *et al.*, 2006). Efforts to predict impending PTB and delay its occurrence have experienced limited success (Shiono and Klebanoff, 1993; Hauth *et al.*, 1995; Burton *et al.*, 1989) with the strongest predictors of PTB being a prior PTB and multifetal gestations (Berkowitz and Papiernik, 1993; Savitz and Pastore, 1999). Other factors associated with risk include maternal age, minority status, genitourinary tract infection, smoking and low pre-pregnancy weight, with smoking cessation counseling and progestogen injections being the only interventions to demonstrate risk reduction among pregnant women (Ernest *et al.*, 1988; Mueller-Heubach and Guzick, 1989; Savitz *et al.*, 1991; Schieve *et al.*, 2000; Shah and Bracken, 2000; Ovalle and Levancini, 2001; FDA US Food and Drug Administration, 2011).

Observational studies have shown that first-trimester bleeding occurs in 7–24% of pregnancies and may associate with PTB risk (Batzofin *et al.*, 1984; Sipila *et al.*, 1992; Axelsen *et al.*, 1995; French *et al.*, 1999; Yang *et al.*, 2005; Hasan *et al.*, 2010a,b). The imprecision in these estimates of bleeding is due, in great part, to the lack of adequately powered studies to examine prevalence rates, as well as use of recalled data and biased sampling (Batzofin *et al.*, 1984; Sipila *et al.*, 1992; Axelsen *et al.*, 1995; French *et al.*, 1999; Yang *et al.*, 2005). Prior studies have not carefully examined bleeding characteristics despite the fact that specific bleeding characteristics may have distinct etiology. For example, light bleeding may be normal and directly related to specific embryological events that occur during the first-trimester. However, stronger, longer and heavier episodes may be indicative of more serious complications and should therefore be examined separately from light bleeding. Several biological mechanisms during pregnancy that may lead to bleeding and PTB have been proposed, including aberrations in the coagulation cascade and platelet activation (Lockwood, 2006). However, it is difficult to document patterns of bleeding, particularly in early pregnancy (Axelsen *et al.*, 1995; Harville *et al.*, 2003; Yang *et al.*, 2005) and as a result the causes of first-trimester bleeding that result in a PTB rather than pregnancy loss are few (Salafia *et al.*, 1995; Chen *et al.*, 1996; Yang *et al.*, 2004).

Prior evidence linking first-trimester vaginal bleeding with risk for PTB may be potentially biased by subject selection (e.g. tertiary care databases) and/or incomplete documentation of bleeding episodes for all participants (e.g. case–control studies). We used data from the Right from the Start (RFTS) study (2000–2009), a non-clinical, prospective community-based pregnancy cohort, to examine bleeding patterns among women who experienced a PTB or term birth. We evaluated bleeding episodes and tested whether individual bleeding characteristics are better predictors of PTB risk than bleeding alone. We included analyses of the heaviness, color and duration for each bleeding episode. We also examined associations stratified by race/ethnicity.

Materials and Methods

Study population and data collection protocol

The analyses presented are of a prospective study of first-trimester vaginal bleeding and PTB risk. RFTS is an ongoing community-based cohort that began enrolling study participants in 2000. Women enrolled are either trying to get pregnant or are currently pregnant. Over time, RFTS has been funded through three major phases with distinctive research questions (RFTS 1, 2, 3). These studies enrolled participants in Galveston, TX; Memphis, Nashville, Knoxville and Chattanooga, TN; and the Research Triangle region (Raleigh, Durham and Chapel Hill) in NC. RFTS participants were 18 years or older and had not used assisted reproductive technologies to conceive. Consent was obtained to review all records pertaining to the study pregnancy. Participants were actively recruited and followed from pre-conception or very early pregnancy through the end of pregnancy. Follow-up was conducted to document outcomes. Participants completed an intake computer-assisted telephone interview (CATI) at approximately 13 weeks gestation, providing information on history of bleeding, medication use and exposure to potential confounders in the time since last menstrual period (LMP). At enrollment, a study transvaginal ultrasound was scheduled at a participating ultrasound site to assess embryonic development, to systematically examine the uterus for presence of fibroids, gestational sac, yolk sac and fetal pole and to measure fetal heart rate, uterine length and uterine width. The institutional review boards of Vanderbilt University, Nashville, TN and the University of North Carolina, Chapel Hill, NC approved this study.

Pregnancy outcomes were self-reported and abstraction of prenatal records was used to verify outcomes. Live births were linked to state vital records to assist in verifying the pregnancy outcomes. A PTB was defined as a live birth with delivery at <37 weeks gestation and a term birth was defined as delivery at ≥ 37 weeks gestation. Women with spontaneous abortions (<20 weeks gestation) ($n = 572$) and ectopic pregnancies ($n = 6$) were excluded. We excluded spontaneous abortions because, although they may have experienced first-trimester bleeding, they are a separate phenotype and are regulated by distinct biological mechanisms. The gestational age was estimated from LMP and adjusted for the first-trimester ultrasound. If the self-reported LMP was ≥ 7 days from the calculated ultrasound LMP, then the ultrasound was used to assign gestational age. Women could enroll in RFTS during more than one pregnancy, but only the first enrollment was included ($n = 206$ subsequent pregnancies excluded).

Variable definitions

Participants reported up to three bleeding events during their first-trimester interview. First-trimester bleeding data have been collected across RFTS all phases (1, 2 and 3) and was collected with planned secondary analyses to examined risk for adverse pregnancy outcomes. Data collected included the timing of the bleeding event, heaviness, color and duration experienced for each bleeding event. The duration of a bleeding event was reported in days. Heaviness was assessed by comparing bleeding during each episode to bleeding experienced in a normal menstrual period. 'Spotting' was documented if it was only noticed by wiping; 'light bleeding' was defined as lighter than heavy flow during their normal menstrual period, 'heavy bleeding' was defined as similar to the heavy flow of a menstrual period or more than the heavy flow of a menstrual period. The color of the blood for each bleeding episode was also reported and included 'brown', 'pink' and 'red'. Finally, the duration of the bleeding episode was documented in days and we grouped them into 1, 2, 3, 4–6 and 7 or more days.

Maternal characteristics and previous obstetric history were also examined. These included: maternal age, PTB history (more than one), race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic and other), multiple gestation, gravidity, spontaneous abortion history, induced abortion history and smoking status (current or not current smokers). Maternal characteristics are obtained either in person during first-trimester ultrasounds or from the first-trimester CATI.

Statistical analysis

All statistical analyses were generated and conducted using STATA statistical software version 11.0 (StataCorp LP, College Station, TX, USA).

Logistic regression was used to test for association between demographic variables and PTB outcome. Logistic regression was also used to estimate the odds ratio (OR) for the relationship of bleeding (yes/no) and PTB risk both adjusted and unadjusted for risk factors for PTB and bleeding. These candidate confounders included maternal age, PTB history, Black race, multiple gestation, spontaneous abortion history, induced abortion history, fibroids and pregnancy smoking status. Covariates that resulted in a change in the effect size between bleeding and PTB risk by 5% or more were retained in the model. This resulted in the inclusion of multiple gestations in all subsequent adjusted models. We did not adjust for a previous history of PTB due to the fact that adjusting for a previous history of PTB may reduce our precision and ability to

Table I Study characteristics of preterm and term births of participants in the RFTS study, 2000–2009.

Variable	n	Preterm birth (n = 344)	Term birth (n = 3634)
Maternal age (mean years ± SD)	3978	28.3 ± 5.7	28.8 ± 5.0
Gravidity (mean ± SD)	3870	2.5 ± 1.74	2.24 ± 1.34
Missing	108	—	—
Parity (%)	3826		
0	1839	48.3	48.0
1	1324	26.9	35.3
≥2	663	24.8	16.7
Missing	152	—	—
Maternal race/ethnicity (%)			
Non-Hispanic White	2714	55.6	69.7
Non-Hispanic Black	791	34.1	18.6
Hispanic	286	7.9	7.1
Other	178	2.9	4.6
Missing	4	—	—
Household income (%)			
≤$40 000	1180	44.7	30.3
$40 001–$80 000	1397	28.3	38.1
>$80 000	1171	27.0	31.6
Missing	230	—	—
Marital status (%)			
Married	3519	80.2	89.3
Missing	1	—	—
Gestational age delivery (days ± SD)	3978	241.4 ± 21.2	278.9 ± 9.4
Fibroids			
None (%)	3636	94.5	93.8
Any (%)	245	5.5	6.2
Missing		—	—
History of preterm birth (%)			
0	3511	79.6	92.8
≥1	316	20.4	7.2
Missing	151	—	—
History of spontaneous abortions (%)			
0	3001	77.1	78.5
≥1	826	22.9	21.5
Missing	151	—	—
History of induced abortions (%)			
0	3230	81.8	84.6
≥1	598	18.2	15.4
Missing	151	—	—
Multiple gestations (%)			
No	3939	94.6	99.5
Yes	37	5.4	0.5
Missing	2	—	—
Smoking status (%)			
Not current smokers	3731	93.0	96.8
Current	138	7.0	3.2
Missing	109	—	—

Continued

see an effect due to over-adjusting for factors that may be intermediates on the causal pathway between the exposure and the disease outcome (Schisterman *et al.*, 2009; Weinberg, 1993). However, we do present analyses stratified by previous PTB history. We also tested for effect modification by race/ethnicity, first creating a binary variable for each race (Non-Hispanic Black and Hispanic) comparing them with Non-Hispanic Whites as the referent group and then testing these variables for interactions with bleeding. We also performed race/ethnicity-stratified analyses to further examine the relationship between first-trimester bleeding and PTB risk among Non-Hispanic White, Non-Hispanic Black and Hispanic women both unadjusted and adjusted for covariates. Finally, we performed subanalyses to assess whether risk varied by early or late PTB (early PTB <34 weeks gestation; late PTB ≥34 weeks gestation) and by women with and without a history of PTB (≥1).

Bleeding characteristics were examined with logistic regression (no bleeding as referent) and included heaviness, color and duration of the bleeding episodes. Individuals were classified according to the most severe classification across the three episodes for each bleeding characteristic (e.g. heaviest bleed, longest duration or reddest color). For these analyses, we classified women as follows for each characteristic: heaviness was stratified by women with 'spotting or light' and 'heavy' bleeding, color by 'brown or pink' and 'red' color, and duration by 'short' duration (<3 days) and 'long' duration (≥3 days). Each bleeding characteristic was analyzed independently of the others both unadjusted and adjusted for covariates.

Results

Table I presents the maternal characteristics of the RFTS study population in relation to PTB status. We included a 344 preterm and 3634 term pregnancies in these analyses. A total of 3978 women were enrolled, 97.2% reported on first-trimester bleeding. Among these, 32.6% of women who experienced a PTB reported a bleeding episode while 24.8% of women with term pregnancies reported a bleeding episode. When we analyzed predictors of PTB for their association with PTB risk, we observed significant increase in risk for women with multiple gestations [OR = 11.78, 95% confidence interval (CI) 6.12–22.66], a history of PTB (OR = 3.32, 95% CI 2.46–4.49), Black race (Non-Hispanic White referent, OR = 2.30, 95% CI 1.79–2.94) and were current smokers (OR = 2.24, 95% CI 1.41–3.56).

A total of 986 women experienced at least one bleeding episode, with a total of 1991 bleeding episodes across all reported episodes (Table II). Any first-trimester bleeding was associated with PTB with a similar effect size for adjusted (for multiple gestation) and unadjusted analyses ($OR_{adjusted}$ = 1.40, 95% CI 1.09–1.80, $P = 0.008$). Among PTB cases, 25% (<34 weeks gestation) were early PTBs and 75% were late PTBs (≥34 weeks gestation). Further analyses to assess whether PTB risk associated with first-trimester bleeding differed

Table II Adjusted and unadjusted analyses of first-trimester bleeding for association risk of preterm birth in RFTS, 2000–2009.

First-trimester vaginal bleeding	*n*	Preterm birth %	Term birth %	Unadjusted OR (95% CI)	*P*-value	Adjusted[a] OR (95% CI)	*P*-value
Overall					**0.002**		**0.008**
None	2880	67.4	75.2	1.00		1.00	
Any bleeding	986	32.6	24.8	1.46 (1.15–1.87)		1.40 (1.09–1.80)	
Early PTB (<34 weeks gestation)					0.508		0.994
None	2718	72.0	75.2	1.00		1.00	
Any bleeding	902	28.0	24.8	1.18 (0.72–1.92)		1.00 (0.60–1.67)	
Late PTB (≥34 weeks gestation)					**0.001**		**0.003**
None	2821	65.9	75.2	1.00		1.00	
Any bleeding	963	34.2	24.8	1.56 (1.19–2.06)		1.52 (1.16–2.01)	
Previous PTB (≥1)					**0.040**		**0.042**
None	272	66.7	78.2	1.00		1.00	
Any bleeding	87	33.3	21.8	1.79 (1.03–3.12)		1.78 (1.02–3.11)	
No previous PTB					**0.007**		**0.005**
None	2608	65.6	74.9	1.00		1.00	
Any	899	34.4	25.1	1.81 (1.18–2.77)		1.85 (1.20–2.86)	
Non-Hispanic White-only					**0.019**		0.063
None	1980	67.4	75.3	1.00		1.00	
Any bleeding	670	32.6	24.8	1.46 (1.06–2.04)		1.37 (0.98–1.91)	
Non-Hispanic Black-only					0.090		0.084
None	575	68.8	76.3	1.00		1.00	
Any bleeding	190	31.3	23.7	1.47 (0.95–2.30)		1.51 (0.96–2.36)	
Hispanic-only					0.174		0.163
None	196	60.0	73.0	1.00		1.00	
Any bleeding	77	40.0	27.0	1.80 (0.77–4.20)		1.83 (0.78–4.27)	

[a]Adjusted for multiple gestation.
In bold are statistically significant associations ($P < 0.05$).

between early and late PTBs showed an association among later PTBs ($OR_{adjusted} = 1.52$, 95% CI 1.16–2.01, $P = 0.003$) but not early PTBs ($OR_{adjusted} = 1.00$, 95% CI 0.60–1.67, $P = 0.994$). Despite our reduced power for stratified analyses, these data would indicate that there is a null effect for early PTBs given the narrow CIs.

Analyses stratified by women with and without a history of PTB (only included women with one or more previous pregnancies) showed that women with bleeding were at increased risk of PTB whether they did or did not have a history of PTB (Table II). There was a slightly higher risk for PTB among women with bleeding and a previous history of PTB ($OR_{adjusted} = 1.78$, 95% CI 1.02–1.79, $P = 0.042$) relative those without a history of PTB ($OR_{adjusted} = 1.85$, 95% CI 1.20–2.85, $P = 0.005$). However, the differences in effect size may be due to differences in power, there were only 87 women with bleeding and a history of PTB. In addition, the effect sizes overlap with the CIs between those with and without a history of PTB, suggesting that the risk is not different between these two groups.

When analyses for any first-trimester bleeding were further examined for effect modification by race/ethnicity, there was no evidence for an interaction with race ($P > 0.05$) (Table II). Despite the decrease in precision for race/ethnicity-stratified analyses, analyses showed a statistically significant association among Non-Hispanic Whites but not Blacks or Hispanics for unadjusted analyses (Non-Hispanic Whites $OR_{unadjusted} = 1.47$, 95% CI 1.06–2.04, $P = 0.019$). Blacks but not Hispanics showed a trend toward statistical significance (Blacks $OR_{unadjusted} = 1.48$, 95% CI 0.96–2.30, $P = 0.090$). The effect size was very similar between racial/ethnic groups and all effect sizes were within the 95% CI for each racial/ethnic group, suggesting that the lack of association among Blacks is due to reduced sample size.

Examination of the bleeding characteristics (Tables III and IV) indicated that a greater increase in PTB risk was associated with bleeding episodes with a longer duration ($OR_{adjusted} = 1.67$, 95% CI 1.17–2.38, $P = 0.005$), bleeding that involved red color ($OR_{adjusted} = 1.92$, 95% CI 1.32–2.82, $P = 0.001$) and heavy bleeding ($OR_{adjusted} = 2.40$, 95% CI 1.18–4.88, $P = 0.015$). Spotting and light bleeding ($OR_{adjusted} = 1.34$, 95% CI 1.04–1.74, $P = 0.024$) also resulted in a smaller but still significant increase in risk for PTB compared with no bleeding. However, it is of note that bleeding with pink or brown color and bleeding of short duration were not statistically significantly associated with an increased risk for PTB, suggesting that minor bleeding is not a significant risk factor for PTB.

Discussion

In this study, we observed an increased risk for PTB for those with first-trimester bleeding, with an effect size ($OR_{adjusted}$ = 1.40, 95% CI 1.09–1.80) consistent with some previous studies, but inconsistent with two previous meta-analyses (Ananth and Savitz, 1994; Saraswat *et al.*, 2010) that indicated a much higher risk [Ananth and Savitz, 1994; relative risk (RR) = 2.2, 95% CI 2.1–2.40; Saraswat *et al.*, 2010; OR = 2.05, 95% CI 1.76–2.4] (Ananth and Savitz, 1994; Rochon, 1998; Yang *et al.*, 2004; Lockwood, 2006; Hossain *et al.*, 2007; Lykke *et al.*, 2010). Our data also indicated a higher incidence of first-trimester bleeding (32.5% in PTB and 24.9% in term) relative to other studies that have observed bleeding to occur in 24.4% of all pregnancies (Yang *et al.*, 2004). In our cohort, the early enrollment, timing of the interview and prospective collection of the data may facilitate better recall for bleeding episodes, particularly minor bleeding episodes that may not have been collected in other studies. We believe that having study interviews conducted immediately after the first-trimester reduces recall bias compared with other comparable studies that rely on second and third trimester study interviews. The study being prospective and the study interview conducted prior to pregnancy outcome ensures that recall is not biased by pregnancy outcome, as it may be for studies of pregnancy losses where losses happen in the first-trimester. In addition, our ability to include these more minor episodes may have resulted in a lower relative risk than studies based on recall, which likely are only capturing more significant amounts of bleeding. In addition, we observed an increased risk for late (OR = 1.52) but not early PTB among women who had experienced first-trimester bleeding, which has not been previously reported.

We observed that women with specific bleeding characteristics, that include episodes with heavy bleeding, red color, and long duration of bleeding, were at increased risk for PTB. Bleeding characersitics have not been examined extensively for association with PTB in previous studies, which may be due to the lack of detailed questionnaires regarding bleeding characteristics and the limited sample sizes for previous studies. Our data would indicate that these individual bleeding characteristics are stronger predictors of PTB than bleeding alone. The findings from examining bleeding characteristics are particularly informative from a clinical perspective because they would indicate that some light bleeding is normal in the first-trimester and does not put a woman at increased risk for PTB, with 24% of term births also experiencing a bleeding episode. However, if the bleeding episode was heavy and lasted for a long period of time then a woman may require further surveillance for potential risk of PTB.

Table III Descriptive characteristics across heaviest/severest bleeding reported from one to three episodes for preterm and term births in RFTS, 2000–2009.

Variable	$n_{heaviest/severest}$	Heaviest/severest reported	
		Preterm birth %	Term birth %
Heaviness			
Spotting	733	71.0	74.9
Light bleeding	191	18.7	19.5
Heavy bleeding	60	10.3	5.6
Color			
Brown	432	32.7	45.3
Pink	284	31.8	28.5
Red	268	35.5	26.2
Duration			
1 Day	488	47.7	49.8
2 Days	159	10.3	16.9
3 Days	114	11.2	11.6
4–6 Days	118	12.1	12.0
≥7 days	105	18.7	9.7

Table IV Adjusted and unadjusted first-trimester bleeding characteristics analyses for risk of preterm birth in RFTS, 2000–2009.

First-trimester bleeding	Unadjusted OR (95% CI)	*P*-value	Adjusted[a] OR (95% CI)	*P*-value
Heaviness				
None	1.00		1.00	
Spotting and light bleeding (n = 925)	1.39 (1.08–1.79)	**0.010**	1.34 (1.04–1.74)	**0.024**
Heavy bleeding (n = 60)	2.70 (1.38–5.27)	**0.004**	2.40 (1.18–4.88)	**0.015**
Color				
No bleeding	1.00		1.00	
Pink or brown (n = 717)	1.28 (0.96–1.70)	0.087	1.21 (0.91–1.63)	0.182
Red (n = 268)	1.99 (1.37–2.88)	**<0.001**	1.92 (1.32–2.82)	**0.001**
Duration				
No bleeding	1.00		1.00	
Short duration (n = 646)	1.27 (0.95–1.72)	0.105	1.27 (0.94–1.71)	0.120
Long duration (≥3 days) (n = 338)	1.85 (1.31–2.60)	**<0.001**	1.67 (1.17–2.38)	**0.005**

[a]Adjusted for multiple gestation.
In bold are statistically significant associations ($P < 0.05$).

Our data also indicate that combining women with light and heavy bleeding episodes may dilute the magnitude of the risk for PTB associated with first-trimester bleeding, with a higher risk among heavy bleeders (OR = 2.40) relative to when all bleeders were combined (OR = 1.40). Further detailed analyses of bleeding and bleeding characteristic are necessary to assess whether risk is modified by the gestaional age of the bleeding episode.

The association observed between bleeding and PTB among Non-Hispanic Whites in race stratified analyses is consistent with two previous studies that observed a significant increase in risk for PTB and first-trimester bleeding among Non-Hispanic Whites (Yang and Savitz, 2001; Yang *et al.*, 2004). Our results, however, are consistent with the pregnancy, infection and nutrition study, which observed an RR = 1.40, 95% CI 1.10–1.90 (Yang *et al.*, 2004). The OR for bleeding and PTB risk in our study were similar across racial/ethnic groups for race/ethnicity-stratified analyses, supporting that there is no strong disparity in bleeding and risk for PTB despite the known disparity in PTB risk.

Bleeding during the first-trimester of pregnancy can be a powerful indicator of a pregnancy complication. Bleeding may result from intrauterine blood collecting in a variety of positions relative to the developing sac and placenta. This intrauterine blood may track away from the placenta, often elevating the attached chorionic membrane, dissecting it and the decidua and surrounding the gestational sac. The bleeding resulting from this hematoma will become apparent through vaginal bleeding as the blood begins to collect in the endometrial cavity. Although the causes of later pregnancy bleeding have been more extensively investigated and are commonly attributable to placental previa, abruption or infection, the causes of first-trimester bleeding are less well understood and results in general have been inconsistent (French *et al.*, 1999; Gomez *et al.*, 2005). These inconsistencies may result from the location rather than the size of the hematoma; for example, a small retroplacental hematoma may have a more adverse effect on pregnancy than a large hematoma located within the endometrial cavity, away from the placenta. Such details have not been captured in previous early pregnancy studies. The risk for PTB attributable to first-trimester bleeding has not been examined extensively in published research; although bleeding has been hypothesized to have a role through decidual hemorrhage, retroplacental hematomas and retrochorionic hematomas (Salafia *et al.*, 1995; Nagy *et al.*, 2005; Lockwood, 2006). Studies often overlook decidual bleeding as a risk factor for PTB due to the fact that traditional methods rely on pathological examination of the placenta for a diagnosis. As a result, establishing decidual hemorrhage as the cause of PTB becomes a retrospective diagnosis unless vaginal bleeding or spotting is observed during pregnancy or there are ultrasonographic signs indicating an abruption (Salafia *et al.*, 1995) Although we did not have data on the presence of haematomas in our cohort, our study findings do support a relationship between first-trimester vaginal bleeding and PTB that may be due to the presence of subchorionic/retrochorionic haematomas.

The certainty of our results is limited by the quality of self-reported bleeding episodes (Hasan *et al.*, 2010b). The dates and estimates of the 'heaviness', 'color' and 'duration' for each bleeding episode during the first-trimester of pregnancy are subjective and thus vulnerable to potential recall bias. To limit recall bias, we performed the first-trimester interview as soon as possible after the completion of the first-trimester. Most previous studies have conducted interviews later in pregnancy. We also limited our study to only bleeding episodes in the first-trimester of pregnancy rather than examining bleeding episodes over the course of the pregnancy. This was due to the fact that few published studies have comprehensively examined first-trimester vaginal bleeding episodes in the context of PTB risk. RFTS is unique in that the majority of study participants were recruited either when they were trying to conceive or very early in the first-trimester (<10 weeks), and although we also rely on recalled data we have well-timed collection and documented exposure data for the first few weeks of pregnancy, which is not common among other studies. We performed sensitivity analyses including and excluding multiple gestations (5% of cohort) and did not observe a significant change in point estimates for the association between PTB and bleeding for models excluding multiple gestations. Finally, we had a PTB rate in our cohort of ~8%; as a result, we had reduced power to perform the analyses stratified by race/ethnicity particularly among Hispanics in our cohort. We were also underpowered to detect an association when stratifying by early and late PTB, which may explain why we only observed an association with late PTB but not early PTB as would be expected. In future studies as our sample size grows we may be able to perform more detailed stratified analyses.

In conclusion, we observed a statistically significant increase in risk for PTB among those who experienced first-trimester bleeding, had bleeding episodes that lasted >3 days, was red in color, and heavy bleeding. Although some prior evidence has linked first-trimester vaginal bleeding with risk for PTB, these studies were potentially biased by subject selection and/or incomplete documentation of bleeding episodes for all participants and limited number of PTB subjects for analysis. We had the advantage of a well characterized nonclinical prospective cohort with ultrasound data to estimate gestational age for all participants, a large sample size, well documented covariates and carefully captured bleeding episodes and characteristics. This study not only demonstrates that first-trimester bleeding is associated with PTB, but also that the individual characteristics of the bleeding episode may be better predictors of risk than bleeding alone. These data would indicate that women with light bleeding were at less risk of PTB than women with heavier bleeding episodes as defined by heaviness, color and duration. Further understanding of the implications and underlying basis for this association can only come from more biologically refined research.

Authors' roles

D.R.V.E. drafted the first draft of the manuscript and performed the statistical analyses; D.D.B. assisted in drafting the manuscript, consulted on statistical analyses and was involved in the original data collection for data included in this manuscript; D.S. assisted in drafting the manuscript, consulted on statistical analyses and was involved in the original data collection for data included in this manuscript; R.H. assisted in drafting the manuscript and consulted on statistical analysis; K.E.H. assisted in drafting the manuscript, consulted on statistical analysis and was P.I. for data collected for this manuscript.

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