Human Reproduction, Vol.31, No.4 pp. 887–896, 2016
Advanced Access publication on March 2, 2016 doi:10.1093/humrep/dew010

human reproduction

ORIGINAL ARTICLE ***Reproductive epidemiology***

Signs and symptoms associated with early pregnancy loss: findings from a population-based preconception cohort

K.J. Sapra[1,2,*], G.M. Buck Louis[1], R. Sundaram[1], K.S. Joseph[3], L.M. Bates[2], S. Galea[2,4], and C.V. Ananth[2,5]

[1]Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Rockville, MD, USA [2]Department of Epidemiology, *Joseph L. Mailman* School of Public Health, Columbia University, New York, NY, USA [3]Department of Obstetrics and Gynaecology, School of Population and Public Health, University of British Columbia, The Children's and Women's Hospital of British Columbia, Vancouver, Canada [4]School of Public Health, Boston University, Boston, MA, USA [5]Department of Obstetrics and Gynecology, College of Physicians and Surgeons, New York, NY, USA

*Correspondence address. Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, 6100 Executive Blvd, Room 7B05, Rockville, MD 20852, USA. Tel: +1-301-435-6936; Fax: +1-301-402-2084; E-mail: saprakj@mail.nih.gov

Submitted on October 26, 2015; resubmitted on December 29, 2015; accepted on January 11, 2016

STUDY QUESTION: What is the relationship between signs and symptoms of early pregnancy and pregnancy loss <20 weeks' gestation?

SUMMARY ANSWER: Vaginal bleeding is associated with increased incidence of early pregnancy loss, with more severe bleeding and bleeding accompanied by lower abdominal cramping associated with greater incidence of loss; conversely, vomiting is associated with decreased incidence of early pregnancy loss, even in the setting of vaginal bleeding, while nausea alone is not.

WHAT IS KNOWN ALREADY: Two previous cohort studies with preconception enrollment suggested that bleeding is associated with loss while nausea is inversely associated with loss though these studies were limited by small study size and reporting after loss ascertainment. No prior preconception cohort study has examined multiple signs and symptoms in relation to pregnancy loss.

STUDY DESIGN, SIZE, DURATION: Population-based preconception cohort of 501 couples discontinuing contraception to try for pregnancy in 16 counties in Michigan and Texas, USA. Participants were followed daily until positive home pregnancy test or 12 months of trying without an hCG pregnancy; women who became pregnant were followed daily from 2 to 7 weeks post-conception.

PARTICIPANTS, SETTING, METHODS: Three hundred and forty-seven women had a positive home pregnancy test denoting hCG pregnancy. Three hundred and forty-one women remained after excluding ineligible pregnancies. Women recorded daily from 2 to 7 weeks post-conception their signs and symptoms, including vaginal bleeding (none, spotting, light, moderate and heavy), lower abdominal cramping, nausea and vomiting. Pregnancy losses were ascertained by a subsequent negative home pregnancy test, clinical confirmation or onset of menses, depending on gestational age at loss; time-to-loss was measured in days post-conception. Cumulative incidence functions and 95% confidence intervals (CIs) were constructed for each sign or symptom, and hazard ratios (HRs) and 95% CIs for presence compared with absence of signs or symptoms were estimated using Cox proportional hazard models.

MAIN RESULTS AND THE ROLE OF CHANCE: Women experienced lower abdominal cramping (85%), nausea (48%), vomiting (46%) and light/moderate/heavy vaginal bleeding (24%) during early pregnancy. Ninety-five (28%) women experienced a loss. Cumulative incidence of pregnancy loss varied by symptomatology: 19% for vomiting, 27% for lower abdominal cramping, 35% for nausea only, 52% for vaginal bleeding, 81% for vaginal bleeding with lower abdominal cramping. Incidence of pregnancy loss was increased among women with vaginal bleeding (HR: 3.62, 95% CI: 2.29–5.74) and among women with vaginal bleeding and lower abdominal cramping (HR: 5.03, 95% CI: 2.07–12.20). Incidence of pregnancy loss was decreased for women with vomiting (HR: 0.51, 95% CI: 0.30–0.86). In the setting of vaginal bleeding with lower abdominal cramping, vomiting reduced the incidence of pregnancy loss (HR: 0.24, 95% CI: 0.11–0.56).

LIMITATIONS, REASONS FOR CAUTION: There were few losses beyond 14 weeks gestation; thus, the precision of our findings related to losses occurring after the first trimester is limited.

WIDER IMPLICATIONS OF THE FINDINGS: By using sensitive home pregnancy tests, we are able to document and characterize the cumulative incidence of the earliest pregnancy losses, which constitute the majority of losses. The use of daily, prospective capture of signs

and symptoms relative to ascertainment of pregnancy loss minimizes potential biases associated with reporting after rather than before a loss, which could potentially distort the relationship between signs and symptoms and pregnancy loss. The findings of our study suggest that it may be useful to develop prognostic models for pregnancy loss based on signs and symptoms.

STUDY FUNDING/COMPETING INTERESTS: This study was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health (contract numbers N01-HD-3-3355; N01-HD-3-3356; N01-HD-3-3358). The authors have no conflict of interest to declare.

Key words: miscarriage / pregnancy loss / nausea / vomiting / bleeding / cramping / symptoms / signs / threatened abortion / prospective cohort studies

Introduction

Pregnancy loss is common with approximately one-third of conceptions ending in a loss (Wilcox *et al.*, 1988; Wang *et al.*, 2003). However, among spontaneous conceptions, where the endpoints of fertilization and implantation are not readily visualized, loss is often unobserved. This problem contributes to our limited understanding of the earliest stages of pregnancy and human development. In fact, the natural history of pregnancy loss, including signs and symptoms, has yet to be fully described. Valid data on signs and symptoms that portend pregnancy loss would be useful for women and clinicians to prompt medical care and evaluation for women experiencing concerning signs and symptoms. However, valid data can only be obtained from preconception studies, which is the only study design that facilitates the prospective ascertainment of the earliest signs and symptoms prior to any subsequent loss.

Despite this, only two studies with preconception enrollment and prospective ascertainment of signs and symptoms have been conducted. One study evaluated daily vaginal bleeding (Harville *et al.*, 2003) and nonspecific pregnancy symptoms (Sayle *et al.*, 2002), separately, in relation to pregnancy loss but was limited by the small number of losses. Another study evaluated monthly reports of nausea in relation to loss, but reporting often occurred after loss ascertainment (Wen *et al.*, 2001).

As signs and symptoms do not occur in isolation, studies describing multiple signs and symptoms simultaneously in relation to loss are needed to delineate the natural history of pregnancy loss. We identified three such studies, all conducted among women seeking clinical care. Two were pregnancy cohort studies conducted in the 1950s (Speert and Guttmacher, 1954; Medalie, 1957), while the other more recent study only recruited women presenting for evaluation of bleeding during pregnancy (Kouk *et al.*, 2013). Thus, there is a distinct data gap regarding the symptomatology associated with early pregnancy and early pregnancy loss that is particularly acute in the era of home pregnancy testing when many women detect their pregnancies, and even early losses, before presenting for clinical care. Data are needed from non-clinical cohorts that can prospectively and continuously ascertain multiple signs and symptoms early in pregnancy and prior to loss ascertainment.

We, therefore, undertook this study to examine the relationship between multiple signs and symptoms—vaginal bleeding, lower abdominal cramping, nausea and vomiting—and pregnancy loss at <20 weeks gestation in a population-based preconception cohort of women. Our objective was to describe the symptomatology of early pregnancy loss using a unique data set of pregnancies ascertained early using home pregnancy tests with prospective daily collection of multiple signs and symptoms prior to pregnancy loss ascertainment. As the majority of early pregnancy losses occur prior to clinical care (Wilcox *et al.*, 1988; Wang *et al.*, 2003), our study cohort offered a unique opportunity to delineate the signs and symptoms occurring in the first few days of pregnancy before gestational demise.

Materials and Methods

Study population

Details of the recruitment strategy for the Longitudinal Investigation of Fertility and the Environment (LIFE) study have been described elsewhere (Buck Louis *et al.*, 2011). Briefly, the LIFE study was a population-based, prospective, preconception cohort study of couples attempting pregnancy conducted from 2005 to 2009 in 16 counties in Michigan and Texas, USA. Couples who were planning to discontinue contraception to become pregnant and those who had been attempting pregnancy for <2 months were screened for study eligibility. Eligible couples were those who were married or in a committed relationship, in which both partners were able to communicate in English or Spanish, the male partner was aged 18 years or older, and the female partner was aged 18–40 years, had a usual cycle length of 21–42 days, and no hormonal birth control injections in the past year. Couples who had clinically diagnosed infertility and those in which at least one partner was sterilized were excluded. All women had a urine pregnancy test administered at the baseline home interview to ensure they were not already pregnant and still at risk for pregnancy upon study entry.

Study measures

Maternal characteristics

At baseline interview, women reported their age, race/ethnicity, education, income, employment status, current smoking status, reproductive history (past pregnancies, deliveries and losses) and prior diagnosis of gynecological problems (uterine fibroids, endometriosis and polycystic ovarian syndrome). Study personnel measured the women's height and weight using standardized protocols in order to calculate body mass index.

Ascertainment of conception

In the absence of visualization of either ovulation or conception, we used proxy markers of ovulation and conception. At study entry, women were instructed to use the urine-based digital ClearBlue™ Fertility Monitor consistent with the manufacturer's guidance. The monitor records the ratios of estrone-3-glucuronide and luteinizing hormone and stores summary data for up to 6 months. Study personnel downloaded the data every 45 days. The ClearBlue™ Fertility Monitor has been demonstrated to provide an accurate measure of ovulation compared with the gold standard, i.e. ultrasound visualization of ovarian follicles and ovulation (Behre *et al.*, 2000). Day of ovulation was approximated by the peak day of luteinizing hormone as indicated by the fertility monitor. If 2 days of peak luteinizing hormone were indicated, the latter day was taken as the day of ovulation (Behre *et al.*, 2000). For 59 women (17%) who did not have fertility monitor data available for the pregnancy cycle, data from the fertility monitor were available for other

menstrual cycles within the same woman that did not end in pregnancy. The average day of ovulation from the prior cycles was imputed as the day of ovulation for the pregnancy cycle. For an additional 16 women (5%) with no fertility monitor information for any cycles in the study, ovulation was assumed to occur 14 days prior to the positive pregnancy test, consistent with the relatively more stable length of the secretory phase of the menstrual cycle compared with the proliferative phase (Barrett *et al.*, 2013). As previously suggested (Stirnemann *et al.*, 2013), the day of conception was approximated by the day of ovulation in keeping with the short viability of the ovum following ovulation (Royston, 1982).

Ascertainment of pregnancy

Pregnancy was established by one positive urine-based home pregnancy test. All women were provided with ClearBlue™ Digital Pregnancy Test (SPD Development Co., Bedford, UK) kits and multiple urine test sticks for each cycle. The test has an advertised hCG sensitivity of 25 mIU/ml, though independent testing has shown that it can detect even lower concentrations of pure hCG and hyperglycosylated hCG, the predominant form of hCG in early pregnancy (Cole, 2011). Women were instructed to test on the day of expected menses, and, if positive, they were instructed to test again in 1 week as per the manufacturer's guidelines. The digital readout of 'pregnant' and 'not pregnant' removed subjectivity in interpreting the results. Women recorded daily whether they took a pregnancy test and the result of the test. One positive urine pregnancy test denoted an hCG pregnancy.

Ascertainment of signs and symptoms

Signs and symptoms of pregnancy and associated loss were recorded daily for 5 weeks beginning on the day after the positive pregnancy test (~2–7 weeks post-conception). If women experienced a pregnancy loss during that interval, only information on signs and symptoms occurring before the day of event were used. Vaginal bleeding was recorded as none, spotting, light, moderate or heavy using standardized pictographs (Wyatt *et al.*, 2001). Lower abdominal cramping was recorded as present or absent. Nausea and vomiting were recorded as none, nausea only, vomiting only or both nausea and vomiting. Women had the option of completing journals online daily or on hardcopy. If the former, women could not edit previously submitted data unless they notified the data coordinating center they made a mistake. If the latter, women were instructed to not backfill any days they missed; they were instructed to leave those days missing. The hardcopy journals queried daily for 1 week before the postcard with information was returned. Research assistants monitored the web-based data collection system to ensure cards were returned in a timely manner.

Ascertainment of pregnancy loss

The LIFE study allowed women to report pregnancy loss using multiple methods depending on the gestational age at loss. Early pregnancy loss was broadly defined as a loss of an hCG pregnancy <20 weeks post-LMP gestational age. Ectopic pregnancies and pregnancy losses occurring ≥20 weeks gestational age were excluded. Women completed pregnancy loss cards designed to capture the date of the event along with the timing of clinical care sought or receipt of any diagnostic tests (inaudible heartbeat and ultrasound confirmed fetal demise). Losses could also be ascertained from daily journals following a conversion from a positive to negative pregnancy test, or from recorded bleeding patterns consistent with the expulsion of products of conception (Wieringa-de Waard *et al.*, 2003) in the daily journal following a positive pregnancy test.

Statistical analysis

Descriptive analyses. Several descriptive analyses were undertaken to understand the data. Given the multiple methods by which women could ascertain their losses depending upon gestational age, we assessed potential differences in day of positive pregnancy test, day of pregnancy loss ascertainment and maternal characteristics by loss ascertainment method. We also examined the maternal characteristics of women included in the analytic cohort.

Multiple imputation of signs and symptoms. Despite the intensity of daily collection of signs and symptoms, the daily journal data were mostly complete. Seventy-six percent of women were missing <30% of daily bleeding data, and 59% of women were missing <30% of daily cramping, nausea and vomiting data. Any missing daily data on signs and symptoms were imputed using the multiple imputation 'mice' package in the R software (Buuren and Groothuis-Oudshoorn, 2011). One hundred imputed data sets were generated. For each sign or symptom, all available data were used for the imputation, including maternal characteristics, other days of information on the missing sign or symptom and other signs or symptoms. Any imputed data occurring on or after the day of pregnancy loss or loss to the follow-up were set to missing.

Cumulative incidence of signs and symptoms. Cumulative incidence functions (CIFs) of pregnancy loss across gestation were constructed based on the imputed data (Gray *et al.*, 2004). CIF of loss by the presence and absence of the individual signs and symptoms, their severity, combinations and patterns were constructed over time anchored to post-conceptional gestational age. As our definition of pregnancy loss only included losses <20 weeks gestation, any women with births or loss to the follow-up after 20 weeks were censored at 140 days post-last menstrual period (LMP) or 125 days post-conception (assuming conception occurred 15 days post-LMP, which is 14 days prior to median positive pregnancy test at cycle day 29). Rubin's rules were used to combine CIF estimates and 95% confidence intervals (CIs) across imputations. The log–log transformation was applied to 95% CI to ensure the resulting 95% CI of the loss probabilities were in the interval [0, 1].

Signs and symptoms, combinations and patterns of losses. The following categories were created for individual signs and symptoms and their severity: any cramping versus none; any bleeding (spotting, light, moderate and heavy bleeding) versus none; any light, moderate, heavy bleeding versus none/spotting only; any moderate/heavy bleeding versus none/spotting/light bleeding; any nausea and/or vomiting versus none; any vomiting (with or without nausea) versus none/nausea only and any vomiting (with or without nausea) versus nausea alone. 'Any' refers to the presence of the sign or symptom on one or more days during the early pregnancy period.

CIF were also constructed for combinations of three signs and symptoms: bleeding, vomiting and cramping. Combinations that were considered positive for bleeding included women with ≥1 days of light, moderate or heavy bleeding (women with spotting only were considered negative for bleeding). Combinations that were considered positive for vomiting included women with ≥1 days of vomiting (with or without nausea); women with nausea only were considered negative for vomiting. Thus, the five combinations examined in the analysis included the following: no bleeding/ cramping/ vomiting, cramping alone, cramping with vomiting, cramping with bleeding, cramping with vomiting and bleeding. All other combinations were too sparse for stable estimates (<5% of imputations).

We also examined the temporal order of the three signs and symptoms in relation to one another. The five combinations of the three signs and symptoms listed above yielded over two-dozen possible temporal patterns. We only analyzed patterns with sufficient sizes (≥5% of imputations) to allow for stable estimates. These patterns were no bleeding/cramping/vomiting, cramping only, cramping followed by vomiting without bleeding, cramping followed by bleeding without vomiting, cramping followed by vomiting followed by bleeding and cramping followed by bleeding followed by vomiting.

Regression modeling of signs and symptoms and pregnancy loss. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% CIs for individual signs and symptoms, combinations and temporal patterns in relation to pregnancy loss. We examined all maternal characteristics for evidence of confounding; that is, characteristics associated with each sign or symptom and associated with loss among women without each sign or symptom. None of the characteristics met these criteria. We did not conduct any subgroup analyses as our objective was to describe the natural history of signs and symptoms of pregnancy loss among the entire population of women with an hCG pregnancy in this preconception cohort. As with the estimation of CIFs, post-conceptional gestational age was used as the anchor for survival time with censoring at 125 days for women who gave birth or were lost to the follow-up ≥20 weeks. Estimates were combined across imputations using Rubin's rules implemented with PROC MIANALYZE in SAS version 9.4. We tested the proportionality assumption of the Cox proportional hazard model using a time-dependent covariate for the individual signs and symptoms, combinations and patterns. Only moderate/heavy bleeding was time-dependent. On graphically assessing the non-proportionality by comparing the CIF graphs for time-to-loss, we did not detect extreme deviation from the proportionality assumption. As the Cox proportional hazard model is reasonably robust to the proportionality assumption (Lin and Wei, 1989); in this paper, we present the results for the fixed covariate.

Results

Study sample

Of the 501 women enrolled, 347 achieved pregnancy; 341 remained in the study population after exclusions (Fig. 1). Ninety-five (28%) women met the definition of having a pregnancy loss <20 weeks, 203 (60%) pregnancies ended in live birth, 24 (7%) in loss to the follow-up before 20 weeks, and 19 (6%) in loss to the follow-up after 20 weeks gestation.

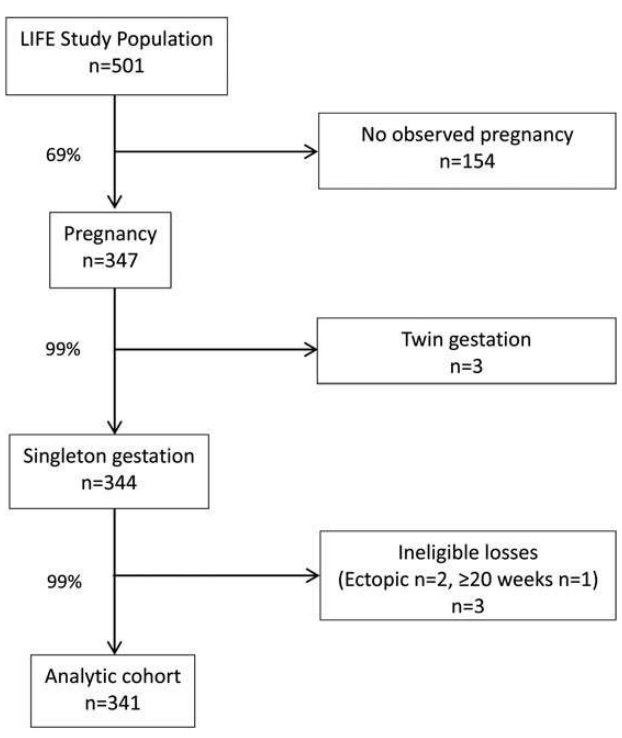

Figure 1 Flowchart for the study population.

The characteristics of 341 women are presented in Table I. Almost half the women were aged 30 years or older (46%), the majority were non-Hispanic white (84%), had attended college (96%), had annual household incomes ≥$50 000 (87%), were employed (80%), were overweight or obese (50%), were recruited from Texas (81%), and did not currently smoke (93%). Thirty-nine percent of women had not been pregnant previously. Of 209 women with a previous pregnancy, 87% had a prior delivery and 33% had a prior pregnancy loss. Ten percent of women reported a gynecological problem.

Median cycle day of the first positive pregnancy test was Day 29; this was the same for women with and without pregnancy loss (data not shown). Among 95 women with losses, there were no differences in maternal characteristics by the method of loss ascertainment (data not shown). As expected, the day of loss ascertainment differed by the ascertainment method: losses at the earliest gestational ages were ascertained by the bleeding pattern and negative pregnancy test, while losses at later gestational ages were ascertained by inaudible heartbeat and ultrasound confirmed fetal demise.

Cumulative incidence of signs and symptoms and relation to loss

Three hundred and thirty-five (98%) women had ≥1 days of signs and symptoms between the positive pregnancy test and pregnancy outcome and were included in modeling. Any nausea and/or vomiting were the most common symptoms (94%); cumulative incidence of vomiting was 46% (Table II). Cramping was also common affecting 85% of women. Cumulative incidence of any bleeding was 43%; cumulative incidence of bleeding exclusive of spotting was 24%. Ninety-nine percent of women had combinations of signs and symptoms that fell into the combinations modeled (Table III). Eighty-seven percent of women had patterns that fell into the six patterns modeled; cramping only (36%), cramping followed by vomiting without bleeding (24%) and no symptoms (10%) were more common than the three patterns with bleeding (5–6% each) (Table IV).

Cumulative incidence functions

The overall CIF of loss for the sample is presented in Fig. 2. The rate increased sharply from 2 (2%) to 3 weeks (10%) post-conception, and continued to rise until 10 weeks post-conception (27%) before plateauing at 14 weeks (28%). The expectation for the incidence of pregnancy loss in the absence of any additional information on signs and symptoms was 28% and could be regarded as a baseline cumulative incidence until 20 weeks post-LMP gestational age.

Figure 3 shows the CIF of pregnancy loss by the presence or absence of any cramping; the incidence of loss is similar among women with and without cramping (~30%). Supplementary Figure S1A shows the CIF of loss for any versus no bleeding; a higher incidence of loss is observed among women with (~40%) than without bleeding (~20%). Figure 4 and Supplementary Figure S1B show the CIF of loss by the severity of bleeding; incidence of loss exceeds 50% among women with more severe bleeding whether measured by light/moderate/heavy bleeding (Fig. 4) or by moderate/heavy bleeding (Supplementary Fig. S1B). The CIFs of loss by any and no nausea/vomiting are shown in Supplementary Fig. S2A with incidence of loss similar among women with and without

Table I Characteristics of women included in the study population (n 5 341)

	n (%)[a]
Age	
18–24 years old	25 (7)
25–29 years old	158 (46)
30–34 years old	114 (33)
35–40 years old	44 (13)
Race/ethnicity	
Non-Hispanic white	283 (84)
Non-Hispanic black	6 (2)
Hispanic	29 (9)
Other	20 (6)
Education	
High school or less	15 (4)
Some college or more	322 (96)
Income	
<$50 000	44 (13)
$50 000–99 999	161 (48)
$100 000+	127 (38)
Employed	
No	68 (20)
Yes	273 (80)
BMI	
<18.5	5 (1)
18.5–24.9	164 (48)
25.0–29.9	88 (26)
30.0+	83 (24)
Site	
Michigan	65 (19)
Texas	276 (81)
Prior pregnancy	
No prior pregnancy	132 (39)
Prior pregnancy	209 (61)
Prior delivery (among those with prior pregnancy)	
No prior delivery	27 (13)
Prior delivery	179 (87)
Prior loss (among those with prior pregnancy)	
No prior loss	139 (67)
Prior loss	68 (33)
Gynecologic problem[b]	
No	307 (90)
Yes	34 (10)
Current smoker	
No	318 (93)
Yes	23 (7)
	Median (IQR)
Cycle day of positive pregnancy test	29 (27, 32)

[a]May not add to total due to missing data.
[b]Gynecological problem includes self-reported physician-diagnosed polycystic ovarian syndrome, endometriosis, uterine fibroid.

Table II Cumulative incidence of individual signs and symptoms and cumulative incidence of pregnancy loss by individual signs and symptoms

	Cumulative incidence of sign or symptom, %	Cumulative incidence of loss, %
Any bleeding		
No	57	18
Yes	43	40
Bleeding severity		
None/spotting only	76	20
Light/moderate/heavy bleeding	24	52
Bleeding severity		
None/spotting/light bleeding	83	21
Moderate/heavy bleeding	17	57
Any cramping		
No	15	31
Yes	85	27
Any nausea and/or vomiting		
No	6	30
Yes	94	27
Nausea and/or vomiting severity		
No nausea or vomiting	6	30
Nausea only	48	35
Any vomiting, with or without nausea	46	19
Any vomiting		
No	54	34
Yes	46	19

nausea/vomiting (~30%). Figure 5 and Supplementary Figure S2B show the CIF of loss by the severity of nausea/vomiting: none, nausea only and any vomiting. Women with any vomiting have a lower incidence of loss (~20%) than women with nausea only (~35%, Supplementary Fig. S2B) and also those without any nausea or vomiting (~30%, Fig. 5).

Figure 6A shows the CIF for the combinations of signs and symptoms. For women experiencing all three symptoms, the cumulative incidence of loss (~35%) is similar to the cumulative incidence of loss among women experiencing none of the three symptoms (~35%). However, when looked at individually or in dual combinations, women with cramping only (~25%) and cramping with vomiting (~10%) have a lower incidence of loss. Women with bleeding and cramping have a markedly higher incidence of loss (~80%).

Figure 6B shows the CIF of loss for various patterns of signs and symptoms. Similar to Fig. 6A, the highest incidence of loss is shown for cramping with bleeding without vomiting (~80%) and the lowest incidence is for cramping with vomiting without bleeding (~10%). Cramping followed by bleeding followed by vomiting and cramping followed by vomiting followed by bleeding have similar incidences of loss (~40%).

Cox proportional hazards models

Results of Cox proportional hazards models with individual signs and symptoms are presented in Table V. Vaginal bleeding is associated with

Table III Cumulative incidence of combinations of signs and symptoms and cumulative incidence of pregnancy loss by combinations of signs and symptoms

	Cumulative incidence of sign or symptom, % [a]	Cumulative incidence of loss, %
No symptoms[b]	10	34
Cramping only	36	23
Cramping and vomiting	26	10
Cramping and bleeding	8	81
Cramping, vomiting, and bleeding	15	36

[a]Does not add to 100% as some combinations too small for stable estimates and not included in modeling.
[b]No symptoms includes women without light/moderate/heavy bleeding, without vomiting and without lower abdominal cramping.

Table IV Cumulative incidence of patterns of signs and symptoms and cumulative incidence of pregnancy loss by patterns of signs and symptoms

	Cumulative incidence of sign or symptom, %[a]	Cumulative incidence of loss, %
No symptoms[b]	10	34
Cramping only	36	23
Cramping followed by vomiting, no bleeding	24	11
Cramping followed by bleeding, no vomiting	5	81
Cramping followed by vomiting followed by bleeding	6	39
Cramping followed by bleeding followed by vomiting	6	38

[a]Does not add to 100% as some combinations too small for stable estimates and not included in modeling.
[b]No symptoms includes women without light/moderate/heavy bleeding, without vomiting, and without lower abdominal cramping.

an increased incidence of pregnancy loss while vomiting is associated with a decreased incidence of pregnancy loss. Specifically, any versus no bleeding is significantly associated with a higher incidence of loss (HR: 2.65, 95% CI: 1.70–4.14). Similarly, light, moderate or heavy bleeding versus none or spotting only (HR: 3.62, 95% CI: 2.29–5.74) and moderate or heavy bleeding versus none, spotting or light bleeding (HR: 4.22, 95% CI: 2.53–6.69) are associated with an increased incidence of loss. Compared with no nausea and/or vomiting, nausea alone is not associated with pregnancy loss. However, any vomiting is inversely associated

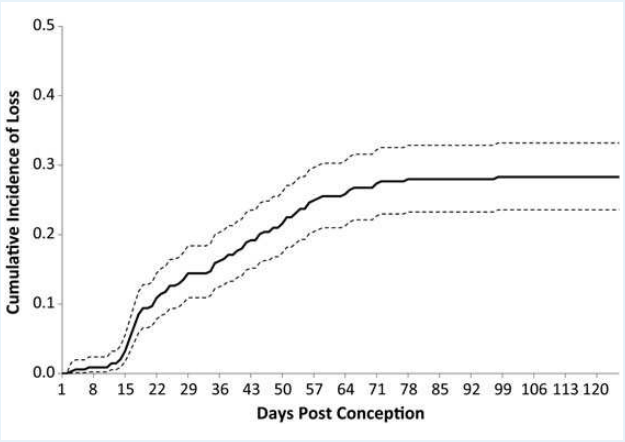

Figure 2 Cumulative incidence of pregnancy loss, estimate and 95% confidence intervals.

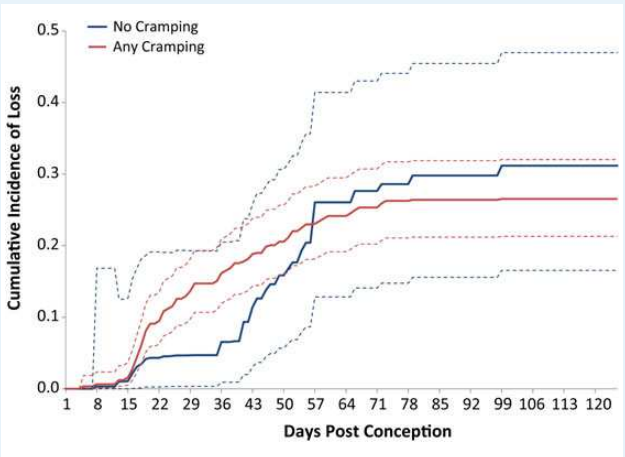

Figure 3 Cumulative incidence of pregnancy loss by cramping status, estimates and 95% confidence intervals.

with loss compared with none/nausea only (HR: 0.51, 95% CI: 0.30–0.86) or compared with nausea alone (HR: 0.50, 95% CI: 0.29–0.85). The presence compared with absence of cramping is not associated with loss.

Results of the Cox proportional hazards models with combinations of signs and symptoms are presented in Table VI and show that signs and symptoms co-occurring with cramping are associated with variations in the incidence of pregnancy loss. Specifically, compared with no symptoms, cramping with vomiting is inversely associated with loss (HR: 0.27, 95% CI: 0.10–0.78), while cramping with bleeding is positively associated with loss (HR: 5.03, 95% CI: 2.07–12.20). Compared with cramping only, cramping with bleeding is positively associated with loss (HR: 7.26, 95% CI: 3.52–14.98). Cramping only, cramping with vomiting, and cramping with vomiting and bleeding are associated with lower incidences of pregnancy loss relative to cramping with bleeding.

Results of Cox proportional hazards models with various patterns of signs and symptoms are presented in Table VII and reflect similar relationships to those seen with combinations of signs and symptoms. Compared

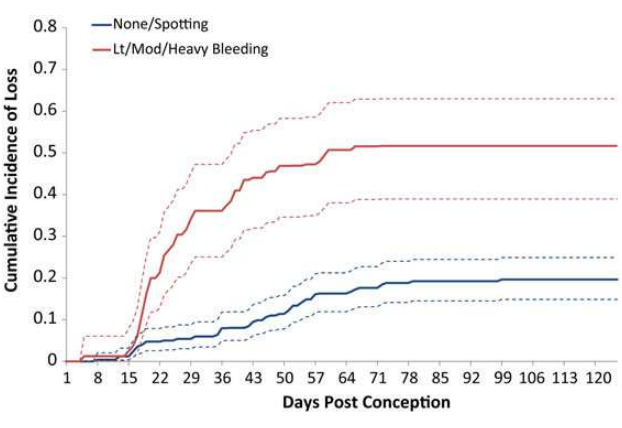

Figure 4 Cumulative incidence of pregnancy loss by intensity of bleeding, estimates and 95% confidence intervals.

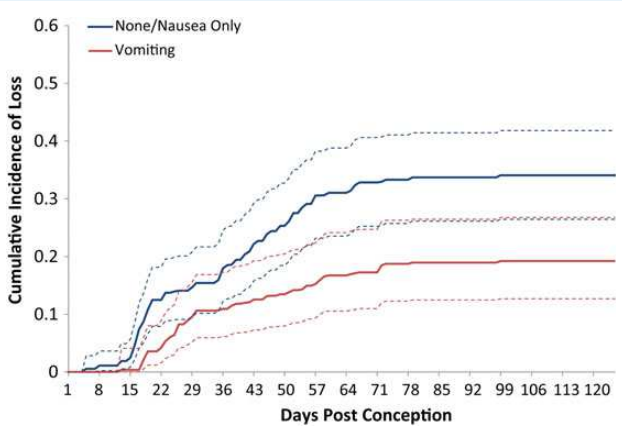

Figure 5 Cumulative incidence of loss by vomiting status, estimates and 95% confidence intervals.

with no symptoms, cramping followed by vomiting without subsequent bleeding is inversely associated with loss (HR: 0.28, 95% CI: 0.10–0.82), while no association is observed for cramping followed by vomiting followed by bleeding. Compared with no symptoms, cramping followed by bleeding without subsequent vomiting is positively associated with loss (HR: 5.01, 95% CI: 1.84–13.67). Compared with cramping with bleeding without subsequent vomiting, cramping followed by bleeding followed by vomiting is inversely associated with loss (HR: 0.29, 95%CI: 0.09–0.96), as are cramping followed by vomiting followed by bleeding, cramping followed by vomiting without subsequent bleeding, and cramping only.

Discussion

Summary of main findings

In this population-based preconception study of women who ascertain their pregnancy status using sensitive home pregnancy tests, multiple signs and symptoms are often reported in early pregnancy (~2–7 weeks post-conception) and differ by pregnancy outcome. Lower abdominal cramping appears to be the norm rather than the exception during early pregnancy, and cramping is not associated with pregnancy loss *per se*. Vaginal bleeding is associated with a higher incidence of pregnancy loss, and more severe bleeding is associated with higher loss rates. Vomiting is

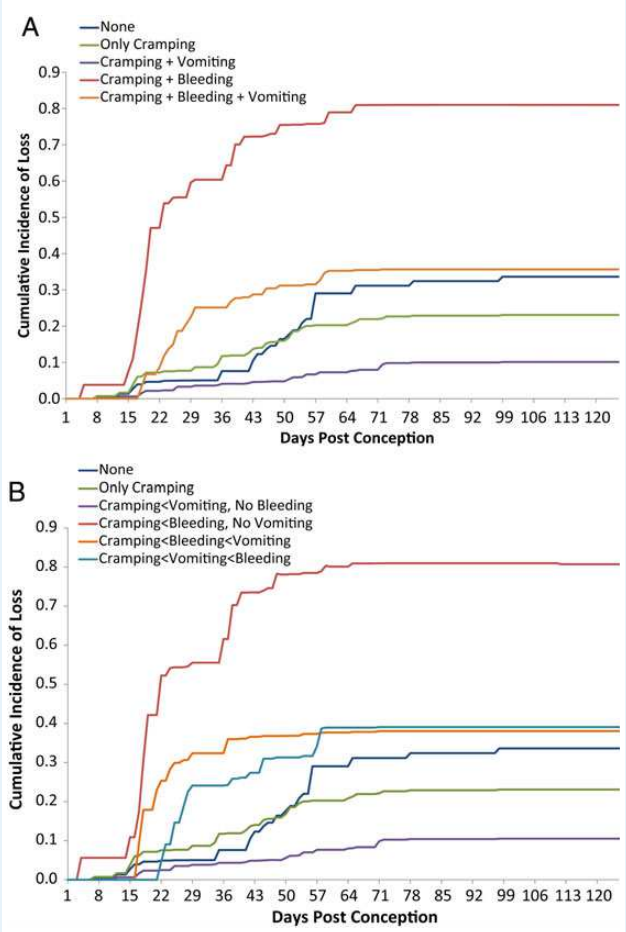

Figure 6 Cumulative incidence of loss by combinations (**A**) and patterns (**B**) of signs and symptoms, estimates.

Table V Cox proportional hazards models showing the association between individual signs and symptoms and pregnancy loss.

	Hazard ratio (95% CI)
Any cramping versus none	0.90 (0.46, 1.77)
Any bleeding versus none	2.65 (1.70, 4.14)
Light/moderate/heavy bleeding versus none/spotting only	3.62 (2.29, 5.74)
Moderate/heavy bleeding versus none/spotting/light bleeding	4.22 (2.53, 6.69)
Any nausea/vomiting versus none	0.93 (0.34, 2.56)
Nausea only versus none	1.26 (0.45, 3.51)
Any vomiting versus none	0.63 (0.22, 1.81)
Any vomiting versus none/nausea only	0.51 (0.30, 0.86)
Any vomiting versus nausea only	0.50 (0.29, 0.85)

Table VI Cox proportional hazards models showing the association between combinations of signs and symptoms and pregnancy loss.

	Hazard ratio (95% CI)
No bleeding, no cramping, no vomiting	Referent
Cramping only	0.69 (0.30, 1.59)
Cramping + vomiting	0.27 (0.10, 0.78)
Cramping + bleeding	5.03 (2.07, 12.20)
Cramping + bleeding + vomiting	1.21 (0.51, 2.89)
Cramping only	Referent
Cramping + vomiting	0.39 (0.15, 1.03)
Cramping + bleeding	7.26 (3.52, 14.98)
Cramping + bleeding + vomiting	1.75 (0.86, 3.56)
Cramping + bleeding	Referent
Cramping only	0.14 (0.07, 0.28)
Cramping + vomiting	0.05 (0.02, 0.15)
Cramping + bleeding + vomiting	0.24 (0.11, 0.56)

Table VII Cox proportional hazards models showing the association between patterns of signs and symptoms and pregnancy loss.

	Hazard ratio (95% CI)
No bleeding, no cramping, no vomiting	Referent
Cramping only	0.69 (0.30, 1.59)
Cramping followed by vomiting, no bleeding	0.28 (0.10, 0.82)
Cramping followed by bleeding, no vomiting	5.01 (1.84, 13.67)
Cramping followed by bleeding followed by vomiting	1.44 (0.46, 4.52)
Cramping followed by vomiting followed by bleeding	1.29 (0.44, 3.78)
Cramping followed by bleeding, no vomiting	Referent
Cramping only	0.14 (0.06, 0.33)
Cramping followed by vomiting, no bleeding	0.06 (0.02, 0.18)
Cramping followed by bleeding followed by vomiting	0.29 (0.09, 0.96)
Cramping followed by vomiting followed by bleeding	0.26 (0.08, 0.79)

associated with lower incidence of pregnancy loss, though nausea alone is not. Compared with cramping alone, cramping accompanied by bleeding is associated with the highest incidence of pregnancy loss; in contrast, cramping with vomiting is associated with the lowest incidence of pregnancy loss. For women with cramping followed by bleeding, incidence of pregnancy loss is lower if vomiting occurs subsequently than if vomiting does not occur.

Strengths and limitations of the study

A notable strength of this work is that it utilizes data from the largest prospective preconception cohort of couples who were followed daily from enrollment through 7 weeks post-conception irrespective of pregnancy outcome (Buck *et al.*, 2004). Secondly, we use highly sensitive home pregnancy tests to ascertain pregnancy status. Urine-based home pregnancy testing is less burdensome on participants than serial urine collection, is less costly, captures more pregnancies than waiting for clinical confirmation, and provides real-time feedback to couples. Thirdly, we are able to minimize the under-ascertainment of losses across all gestational ages by offering women multiple methods by which to record a loss. Importantly, all losses are recognized by the woman; none were 'silent' losses. We are, therefore, able to document and characterize the cumulative incidence of the earliest pregnancy losses, which constitute the majority of losses. Fourthly, the study design allowed for the daily, prospective capture of signs and symptoms relative to ascertainment of pregnancy loss. This minimizes potential biases associated with reporting after rather than before a loss, which could potentially distort the relationship between signs and symptoms and loss. While we cannot be sure that hardcopy journals were completed each day, we do not believe reporting differences varied by pregnancy outcome. Finally, we have a close proxy for the day of conception using fertility monitor data to ascertain the day of ovulation. Thus, we are able to use post-conceptional gestational age, which is a more precise measure of gestational duration than menstrual-based gestational age.

Study limitations include few losses beyond 14 weeks gestation because of the relatively small study size. Thus, the precision of our findings related to losses occurring after the first trimester is limited. Secondly, we have relatively small numbers within each pattern of signs and symptoms given the large numbers of possible patterns. Thirdly, by study design, information on signs and symptoms is only collected daily from 2 to 7 weeks post-conception gestation due to limitations on reporting burden for participants. Fourthly, while women were instructed to test for pregnancy on the day of expected menses, the pregnancy test can detect pregnancies as soon as 8 days after the luteinizing hormone surge (Cole, 2011). There may be some biological variation in timing of positive pregnancy test relative to ovulation; however, measurement error of the proxy day of conception is unlikely to create substantial bias in our results given the small numbers of women impacted in our sample. There is also small (7%) loss to the follow-up prior to 20 weeks gestation in the study; however, this was addressed using a survival analytic censoring approach and available data were included in the study. Finally, we have some missing data on signs and symptoms. We did not find any statistically significant differences in the amount of missing information by the maternal characteristics that were used to inform the multiple imputation models. This provides some reassurance that our data are missing-at-random, which is an assumption challenging to verify in practice. Including several variables in the imputation model, as we did, also makes the missing-at-random assumption more plausible (Schafer and Graham, 2002). Furthermore, we generated 100 imputed data set consistent with the amount of missing data (Graham *et al.*, 2007).

Given the amount of daily data available to us, we carefully considered which days of signs and symptoms to include in the analysis. We chose to include all of the days prior to the day of loss ascertainment for two reasons. First, our understanding of the causes of pregnancy loss and the biologic mechanisms by which a pregnancy is spontaneously terminated is still very much unknown. Thus, we cannot say with any certainty that signs or symptoms occurring prior to a certain period before loss ascertainment are causes of loss while signs or symptoms occurring within a certain period of loss ascertainment are consequences of loss. Another serious concern with excluding data on symptoms is that it would differentially impact early losses and bias the resulting estimates. Thus, we decided to include all data on signs and symptoms up until the day of

loss ascertainment, acknowledging that the day of loss ascertainment is an imperfect proxy for the day of loss.

We did not categorize loss by gestational age in light of no uniformly agreed upon approach, particularly for non-clinical populations for whom most pregnancies and losses are observed only by the woman, and in the absence of a clear understanding of the etiology of pregnancy loss across gestation. The analyses presented here address the average impacts of signs and symptoms in early pregnancy on subsequent pregnancy loss irrespective of timing.

Findings in context with prior literature

Few prior studies have been able to evaluate multiple signs and symptoms in relation to pregnancy loss, and none have explicitly examined lower abdominal cramping. Two pregnancy cohort studies in the 1950s (Speert and Guttmacher, 1954; Medalie, 1957) and a more recent study on threatened abortion (Kouk *et al.*, 2013) reported that in the setting of bleeding, nausea and/or vomiting was protective against pregnancy loss. Our results are similar; however, it is important to note that in our study only vomiting is protective against loss in the setting of bleeding with cramping, while nausea alone is not.

Another pregnancy cohort study reported that the risk of pregnancy loss was greatest in women with heavy bleeding and pain (Hasan *et al.*, 2009). We also find that the combination of bleeding and cramping is associated with the highest incidence of loss when it occurs without vomiting. A new finding in our study is the high prevalence of lower abdominal cramping and the observation that cramping is not associated with increased risk of loss unless accompanied by bleeding (exclusive of spotting).

Evaluating the temporal patterns of signs and symptoms in relation to loss is also novel. For women experiencing cramping and bleeding, subsequent vomiting is a significant prognostic indicator; women without subsequent vomiting are at increased risk of loss while women with subsequent vomiting are not. Collectively, these findings underscore the need to evaluate multiple signs and symptoms simultaneously, reflecting the co-occurrence of these symptoms for many women. For example, in this analysis, 85% of women had one or more symptoms; 40% of these women had two symptoms and 18% had all three symptoms.

Interpretation of findings

Lower abdominal cramping is a common symptom in early pregnancy, and in the absence of other signs and symptoms, is not associated with pregnancy loss. It may be important to distinguish between different types of cramping, if such distinctions exist and can be made. In our study, the daily journals simply inquire about lower belly cramping and did not further qualify such as by severity or typology (e.g. menstrual-like cramps). While uterine quiescence is required for successful implantation of the embryo (Fanchin and Ayoubi, 2009), in the weeks following implantation uterine contractility may be associated with a healthy pregnancy. Potentially, this may be due to the effects of estrogen, which is associated with uterine contractility (Fanchin and Ayoubi, 2009) and with ongoing pregnancy (Lukaszuk *et al.*, 2005). Cramping with bleeding likely reflects the expulsion of products of conception (Wieringa-de Waard *et al.*, 2003). However, it is possible that bleeding is the cause, rather than the consequence, of pregnancy loss. Subchorionic hemorrhage, often associated with vaginal bleeding, may cause oxygen-rich blood to invade the intervillous space prematurely, interfering with trophoblast development (Johns *et al.*, 2006), or causing chronic inflammation, inducing myometrial contractions and expulsion of the gestational sac (Johns *et al.*, 2006).

In contrast to bleeding, vomiting in early pregnancy appears to be protective against pregnancy loss. This is consistent with the hypothesis that caloric restriction consequent to vomiting in early pregnancy causes maternal levels of insulin and insulin growth factor-1 to fall (Huxley, 2000). Maternal anabolic processes are thereby inhibited and nutrient partitioning favors placental development. Nausea may not cause caloric restriction to the same extent as vomiting, and the cascade described above would not be initiated. Alternatively, vomiting may serve as a proxy for high-progesterone levels, which are necessary to maintain a successful pregnancy (Norwitz *et al.*, 2001), and are also associated with nausea and vomiting of pregnancy, potentially through its effects on smooth muscle relaxation and consequent gastric dysrhythmia (Cardwell, 2012).

Visualized endpoints, such as embryo quality among embryos being transplanted in assisted reproductive technology procedures, may be helpful in trying to disentangle some of these relationships between occurrence of signs and symptoms and pregnancy loss.

Conclusions and implications

Though common in early pregnancy, lower abdominal cramping is not associated with pregnancy loss absent other signs and symptoms. Vomiting and nausea are also common in early pregnancy, and vomiting, but not nausea, is associated with a lower incidence of loss. While bleeding is less common in early pregnancy, it is associated with a higher incidence of loss, particularly when accompanied by lower abdominal cramping. The findings of our study suggest that it may be useful to develop prognostic models for pregnancy loss based on signs and symptoms; such models may need to incorporate potentially time-varying effects of signs and symptoms on pregnancy loss. More complete knowledge of the physiologic response of the body to early pregnancy will enhance our understanding of the causes of each sign and symptom and its relation to pregnancy loss.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors' roles

K.J.S. formed the analytic plan, implemented all analyses, and wrote the first draft of the manuscript. G.M.L. designed the LIFE study and assisted in formation of the analytic plan. R.S. provided statistical expertise and assisted in formation of the analytic plan. All authors contributed to the interpretation of the results and revision of the manuscript for important intellectual content and approved the final version of the manuscript. All authors are the guarantors of this work.

Funding

This study was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health (contract numbers N01-HD-3-3355; N01-HD-3-3356 and N01-HD-3-3358).

Conflict of interest

None declared.

References

Barrett E, Thune I, Lipson SF, Furberg A-S, Ellison PT. A factor analysis approach to examining relationships among ovarian steroid concentrations, gonadotrophin concentrations and menstrual cycle length characteristics in healthy, cycling women. *Hum Reprod* 2013;**28**:801–811.

Behre HM, Kuhlage J, Gassner C, Sonntag B, Schem C, Schneider HP, Nieschlag E. Prediction of ovulation by urinary hormone measurements with the home use ClearPlan Fertility Monitor: comparison with transvaginal ultrasound scans and serum hormone measurements. *Hum Reprod* 2000;**15**:2478–2482.

Buck GM, Lynch CD, Stanford JB, Sweeney AM, Schieve LA, Rockett JC, Selevan SG, Schrader SM. Prospective pregnancy study designs for assessing reproductive and developmental toxicants. *Environ Health Perspect* 2004;**112**:79–86.

Buck Louis GM, Schisterman EF, Sweeney AM, Wilcosky TC, Gore-Langton RE, Lynch CD, Boyd Barr D, Schrader SM, Kim S, Chen Z *et al.* Designing prospective cohort studies for assessing reproductive and developmental toxicity during sensitive windows of human reproduction and development—the LIFE Study. *Paediatr Perinat Epidemiol* 2011;**25**:413–424.

Buuren S, Groothuis-Oudshoorn K. Mice: multivariate imputation by chained equations in R. *J Stat Software* 2011;**45**.

Cardwell MS. Pregnancy sickness: a biopsychological perspective. *Obstet Gynecol Surv* 2012;**67**:645–652.

Cole LA. The utility of six over-the-counter (home) pregnancy tests. *Clin Chem Lab Med* 2011;**49**:1317–1322.

Fanchin R, Ayoubi JM. Uterine dynamics: impact on the human reproduction process. *Reprod Biomed Online* 2009;**18**:S57–S62.

Graham JW, Olchowski AE, Gilreath TD. How many imputations are really needed? Some practical clarifications of multiple imputation theory. *Prev Sci* 2007;**8**:206–213.

Gray B, Gray MB, Gray R. The cmprsk package. 2004. The Comprehensive R Archive network. https://cran.r-project.org/web/packages/cmprsk/cmprsk.pdf (28 January 2016, date last accessed).

Harville EW, Wilcox AJ, Baird DD, Weinberg CR. Vaginal bleeding in very early pregnancy. *Hum Reprod* 2003;**18**:1944–1947.

Hasan R, Baird DD, Herring AH, Olshan AF, Jonsson Funk ML, Hartmann KE. Association between first-trimester vaginal bleeding and miscarriage. *Obstet Gynecol* 2009;**114**:860–867.

Huxley RR. Nausea and vomiting in early pregnancy: its role in placental development. *Obstet Gynecol* 2000;**95**:779–782.

Johns J, Jauniaux E, Burton G. Factors affecting the early embryonic environment. *Rev Gynaecol Perinat Pract* 2006;**6**:199–210.

Kouk LJ, Neo GH, Malhotra R, Allen JC, Beh ST, Tan TC, Ostbye T. A prospective study of risk factors for first trimester miscarriage in Asian women with threatened miscarriage. *Singapore Med J* 2013;**54**:425–431.

Lin DY, Wei L-J. The robust inference for the Cox proportional hazards model. *J Am Stat Assoc* 1989;**84**:1074–1078.

Lukaszuk K, Liss J, Lukaszuk M, Maj B. Optimization of estradiol supplementation during the luteal phase improves the pregnancy rate in women undergoing in vitro fertilization–embryo transfer cycles. *Fertil Steril* 2005;**83**:1372–1376.

Medalie JH. Relationship between nausea and/or vomiting in early pregnancy and abortion. *Lancet* 1957;**273**:117–119.

Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. *N Engl J Med* 2001;**345**:1400–1408.

Royston JP. Basal body temperature, ovulation and the risk of conception, with special reference to the lifetimes of sperm and egg. *Biometrics* 1982;**38**:397–406.

Sayle AE, Wilcox AJ, Weinberg CR, Baird DD. A prospective study of the onset of symptoms of pregnancy. *J Clin Epidemiol* 2002;**55**:676–680.

Schafer JL, Graham JW. Missing data: our view of the state of the art. *Psychol Methods* 2002;**7**:147.

Speert H, Guttmacher AF. Frequency and significance of bleeding in early pregnancy. *J Am Med Assoc* 1954;**155**:712–715.

Stirnemann JJ, Samson A, Bernard J-P, Thalabard J-C. Day-specific probabilities of conception in fertile cycles resulting in spontaneous pregnancies. *Hum Reprod* 2013;**28**:1110–1116.

Wang X, Chen C, Wang L, Chen D, Guang W, French J. Conception, early pregnancy loss, and time to clinical pregnancy: a population-based prospective study. *Fertil Steril* 2003;**79**:577–584.

Wen W, Shu XO, Jacobs DR Jr, Brown JE. The associations of maternal caffeine consumption and nausea with spontaneous abortion. *Epidemiology* 2001;**12**:38–42.

Wieringa-de Waard M, Ankum WM, Bonsel GJ, Vos J, Biewenga P, Bindels PJ. The natural course of spontaneous miscarriage: analysis of signs and symptoms in 188 expectantly managed women. *Br J Gen Pract* 2003;**53**:704–708.

Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, Armstrong EG, Nisula BC. Incidence of early loss of pregnancy. *N Engl J Med* 1988;**319**:189–194.

Wyatt KM, Dimmock PW, Walker TJ, O'Brien PS. Determination of total menstrual blood loss. *Fertil Steril* 2001;**76**:125–131.