ORIGINAL ARTICLE: ENVIRONMENT AND EPIDEMIOLOGY

Lifestyle and pregnancy loss in a contemporary cohort of women recruited before conception: The LIFE Study

Germaine M. Buck Louis, Ph.D., M.S.,[a] Katherine J. Sapra, M.Phil., M.P.H.,[a] Enrique F. Schisterman, Ph.D., M.A.,[b] Courtney D. Lynch, Ph.D., M.P.H.,[c] José M. Maisog, M.D., M.S.,[d] Katherine L. Grantz, M.D., M.S.,[b] and Rajeshwari Sundaram, Ph.D., M.S.[e]

[a] Office of the Director and [b] Epidemiology Branch, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Rockville, Maryland; [c] Department of Obstetrics and Gynecology, The Ohio State University College of Medicine, Columbus, Ohio; [d] Glotech, Inc.; and [e] Biostatistics and Bioinformatics Branch, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Rockville, Maryland

Objective: To estimate pregnancy loss incidence in a contemporary cohort of couples whose lifestyles were measured during sensitive windows of reproduction to identify factors associated with pregnancy loss for the continual refinement of preconception guidance.
Design: Prospective cohort with preconception enrollment.
Setting: Sixteen counties in Michigan and Texas.
Patient(s): Three hundred forty-four couples with a singleton pregnancy followed daily through 7 postconception weeks of gestation.
Intervention(s): None. Couples daily recorded use of cigarettes, caffeinated and alcoholic beverages, and multivitamins. Women used fertility monitors for ovulation detection and digital pregnancy tests. Pregnancy loss was denoted by conversion to a negative pregnancy test, onset of menses, or clinical confirmation depending upon gestation. Using proportional hazards regression and accounting for right censoring, we estimated adjusted hazard ratios and 95% confidence intervals (aHR, 95% CI) for couples' lifestyles (cigarette smoking, alcoholic and caffeinated drinks, multivitamins) during three sensitive windows: preconception, early pregnancy, and periconception.
Main Outcome Measure(s): Incidence and risk factors for pregnancy loss.
Result(s): Ninety-eight of 344 (28%) women with a singleton pregnancy experienced an observed pregnancy loss. In the preconception window, loss was associated with female age $\geq$35 years (1.96, 1.13–3.38) accounting for couples' ages, women's and men's consumption of $>$2 daily caffeinated beverages (1.74, 1.07–2.81; and 1.73, 1.10–2.72, respectively), and women's vitamin adherence (0.45, 0.25–0.80). The findings were similar for lifestyle during the early pregnancy and periconception windows.
Conclusion(s): Couples' preconception lifestyle factors were associated with pregnancy loss, although women's multivitamin adherence dramatically reduced risk. The findings support continual refinement and implementation of preconception guidance. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Caffeine, lifestyle, miscarriage, pregnancy loss, multivitamins

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/louisg-lifestyle-pregnancy-loss/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Received October 26, 2015; revised March 3, 2016; accepted March 4, 2016.
G.M.B.L. has nothing to disclose. K.J.S. has nothing to disclose. E.F.S. has nothing to disclose. C.D.L. has nothing to disclose. J.M.M. has nothing to disclose. K.L.G. has nothing to disclose. R.S. has nothing to disclose.
This study was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (contract nos. N01-HD-3-3355, N01-HD-3-3356, and N01-HD-3-3358).
Reprint requests: Germaine M. Buck Louis, Ph.D., M.S., Office of the Director, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, 6100 Executive Boulevard, Room 7B03, Rockville, Maryland 20852 (E-mail: louisg@mail.nih.gov).

Fertility and Sterility® Vol. ■, No. ■, ■ 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.03.009

Over the past few decades, the detection of pregnancy for many women not receiving infertility treatment has shifted from clinical settings to the privacy of the home. This change is largely attributed to the development of a hCG beta-subunit radioimmunoassay (1), leading to the availability of the first early pregnancy test in 1976. Currently, there are ≈60 home pregnancy test kits available in the United States, accounting for

approximately $228 million in annual sales (2, 3). Home pregnancy tests now generate more revenue than any other over-the-counter home diagnostic test, including those for blood pressure or glucose testing (4), and are used by approximately 85%–90% of women for identifying pregnancy (5).

The shift in pregnancy detection from the clinical setting to the home means that women may now recognize more pregnancies than they did when clinical identification was the norm, usually occurring after one or two missed periods. To date, only a few prospective cohort studies with preconception enrollment of women or couples have ever been conducted worldwide to estimate the incidence of pregnancy loss. Overall, loss rates range from a low of 8% among 92 pregnant women in Newcastle, United Kingdom (6), to a high of 52% among 118 pregnant women in Southampton, United Kingdom (7). Recent data from the U.S. National Survey of Family Growth noted an increasing pattern of self-reported miscarriage between 1970 and 2000, with much of the increase being for losses in the first 7 weeks of gestation, possibly reflecting women's use of home pregnancy testing (8). While various risk factors have been identified from past research ranging from health and pregnancy history to lifestyle, recent guidance from the American College of Obstetricians and Gynecologists notes the absence of any known interventions to prevent pregnancy loss except for anti-phospholipid syndrome and P supplementation for recurrent losses (9).

Another notable societal change in pregnancy is the shift in thinking from pregnant females to pregnant couples, as evident by colloquial terms such as "we are pregnant" along with the early and sustained involvement of male partners during prenatal care and delivery in many cases. Despite such changes, research has traditionally focused on characteristics of either the female or male partner relative to pregnancy loss but not both in light of few couples-based cohorts.

We sought to estimate the incidence of pregnancy loss and lifestyle risk factors in a contemporary cohort of couples desiring pregnancy who were recruited before conception and followed throughout trying to conceive and pregnancy. This design facilitated the capture of early pregnancy and losses consistent with the time period in which most losses occur. Pregnancy loss is an endpoint indicative of reproductive and/or developmental toxicity that may be associated with a variety of environmental exposures. Another unique aspect of this design is the ability to assess potential male-mediated risk factors for pregnancy loss such as age, employment, and use of cigarettes, alcohol, and caffeine in the context of women's lifestyle (10–13). As such, this approach goes beyond most of the available research conducted to date that focuses exclusively on female determinants and offers insight for preconception guidance that is increasingly focusing on both men and women.

MATERIAL AND METHODS

Study Design and Population

The study cohort comprised 347/501 (69%) women who had an observed pregnancy while participating in the Longitudinal Investigation of Fertility and the Environment (LIFE) Study, which was designed and powered to examine the association between environmental and lifestyle factors and fecundity impairments among couples, including pregnancy loss. We excluded three couples with twin pregnancies, resulting in a cohort comprising 344 couples with singleton pregnancies. The LIFE Study used population-based sampling frameworks to recruit couples discontinuing contraception for purposes of becoming pregnant from 16 counties in Michigan and Texas. By design, eligibility criteria were minimal and included [1] being in a committed relationship; [2] ability to communicate in English or Spanish; [3] women ages 18–40 and men ages $\geq$18 years; [4] womens' menstrual cycles between 21 and 42 days as required by the fertility monitors; [5] no history of injectable hormonal contraception in the past year or currently breastfeeding for 6+ months; [6] no clinically diagnosed infertility in either partner; and [7] off contraception $<$2 months. Before enrollment, female partners' urine was tested to ensure they were not pregnant. Human subjects approval was obtained from participating institutions, and all men and women gave written informed consent before data collection. Complete details about the population-based sampling framework used along with response rates have been published elsewhere (14).

Data Collection and Follow-up

Couples were interviewed individually upon enrollment to ascertain sociodemographic, lifestyle, and medical history information, followed by the measurement of height and weight to calculate body mass index (BMI). The couple was then instructed in the completion of daily journals to record their lifestyle behaviors in a manner consistent with how people think about such exposures (e.g., number of cigarettes smoked per day, number of alcoholic and caffeinated beverages consumed per day, taking daily multivitamins). Females also reported sexual intercourse, menses, and home pregnancy test results. Couples completed journals daily until a positive home pregnancy test or up to 12 months of trying. Pregnant women continued journals daily through 7 postconception weeks of gestation and then monthly journals until a loss or delivery. Journal entries were purposefully designed to be short to minimize participant burden and encourage daily adherence.

Standardized prompts were provided in the daily journals regarding lifestyle. Specifically, one alcoholic drink equaled one can or bottle of beer, a glass of wine, a shot of liquor, or a mixed drink. A caffeinated drink equaled a cup of coffee or tea or a can of soda (excluding decaffeinated or caffeine-free beverages). Vitamins included over-the-counter multivitamins and prescription prenatal vitamins and were dichotomized relative to daily usage (yes/no). Couples reported the daily number of cigarettes smoked and alcoholic and/or caffeinated beverages consumed and were instructed to record a zero if none was used. Couples had the option of reporting data via the web or of returning weekly journal cards consistent with prospective measurement.

Home Fertility and Pregnancy Testing

Women were trained in the use of the Clearblue fertility monitor (Inverness Medical Innovations), a urinary test kit

that tracks estrone-3-glucuronide and LH to predict the day of ovulation and help couples time intercourse to maximize their chances of conceiving. In comparison to the gold standard of ultrasound for the detection of ovulation, the monitor is reported to accurately detect the LH surge (99%) (15). Women also were trained in the use of the Clearblue digital pregnancy test, which has demonstrated sensitivity and reliability for detecting 25 mIU/mL of hCG and is interpreted accurately by women (16). Women tested their urine for pregnancy on the day they expected menstruation, consistent with the manufacturer's guidance. Pregnancy loss was defined as conversion to a negative pregnancy test, clinical confirmation, or onset of menstruation depending on gestational dating and included two women with ectopic pregnancies. Three twin pregnancies were excluded from analysis.

Statistical Analysis

The cohort was first characterized by pregnancy outcome to identify factors associated with loss, with significance based on either the χ^2 or the Kruskal-Wallis tests. We estimated the cumulative incidence of pregnancy loss after conception using Kaplan-Meier techniques, while accounting for right censoring (withdrawals) and the time required to become pregnant. For pregnancy cycles with missing fertility monitor data required for estimating the day of ovulation (n = 59; 17%), we assumed ovulation to be 14 days before the first positive pregnancy test. This assumption is consistent with the secretory phase being less variable than the proliferative phase of the cycle (17). We defined time to pregnancy loss as the number of observed days between ovulation or the monitor's peak fertility day (LH surge) and the date of the reported loss. The peak (LH) monitor day was assumed to be the day of conception, given the ovum's short (≈24 hours) interval for fertilization.

A priori, we defined three sensitive windows for ascertainment of couples' lifestyles and time to pregnancy loss: [1] preconception window, the time from enrollment to estimated conception; [2] early pregnancy window, the day after conception (or peak LH) through 7 postconception weeks; and [3] periconception window, inclusive of the two windows as an overall summary window. Thereafter, women converted to monthly reporting for the remainder of pregnancy through loss or delivery.

To ensure proper model specification, we thoroughly inspected spaghetti plots for each lifestyle by partner during each window and modeled lifestyles as daily averages over the observed time in each window. Since only women completed early pregnancy journals, we assumed male partners' preconception lifestyle remained the same during early pregnancy. To maintain a couples-based analytic framework, we included the male partner in the estimation of hazard ratios (HRs) for early pregnancy and periconception along with the prospectively measured female exposures.

We first modeled each lifestyle behavior separately during each of the sensitive windows and then included potential confounders that were identified a priori based on a review of the literature: female age (18), couples' ages modeled as the difference (years) given their known correlation and reports of male age being associated with loss (10), couples' BMI (continuous) (19, 20), sexual frequency (21), and history of loss conditional on gravidity (22). Using Cox's proportional hazards model (23), we modeled time to loss (postconception days) to estimate HRs and 95% confidence intervals (CIs) after first assessing potential nonlinear relationships using splines. Despite assessing nonlinear relationships for all behaviors and characteristics (e.g., age, alcohol, BMI) with pregnancy loss, we observed threshold effects only for female age (≥35 years) and male and female caffeinated beverage consumption at ≈2 daily cups depending upon the sensitive window. We modeled these risk factors accordingly. We estimated the average number of daily cigarettes smoked and alcoholic drinks consumed to fully use all daily information in keeping with pregnancy being cycle based. Also, averages were thought to be amenable to guidance rather than to simply model totals during the windows. Multivitamin usage was modeled in terms of adherence or the proportion of days during each sensitive window in which the female or male partner reported taking vitamins, ranging from 0 (no vitamins taken) to 1 (vitamins taken daily). Intercourse frequency was modeled as the average number of acts during the pregnancy cycle. Separate models were run for each of the three time intervals in anticipation that couples may change their behaviors or lifestyles when becoming pregnant. We empirically assessed such lifestyle changes using paired *t*-tests.

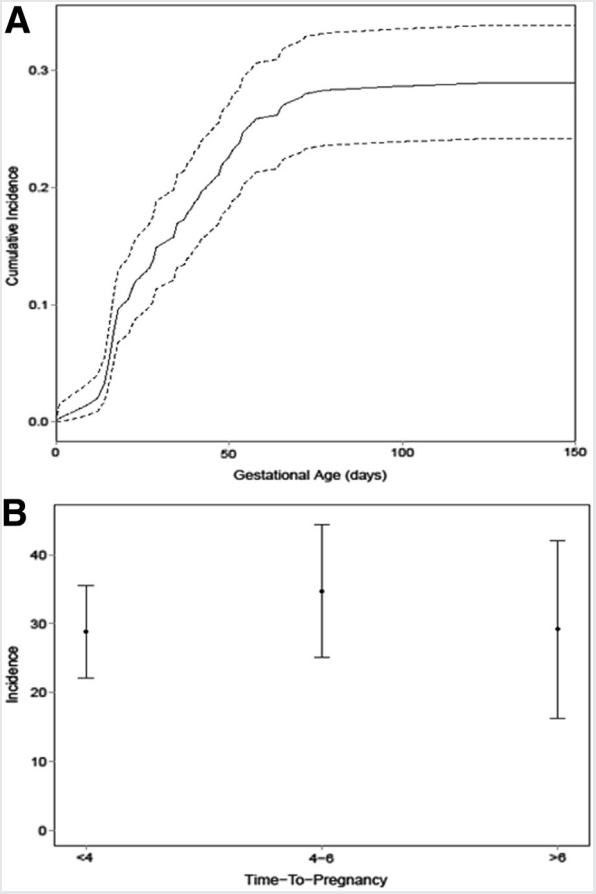
RESULTS

Ninety-eight (28%) of the 344 couples who became pregnant during the study experienced a pregnancy loss. Among women becoming pregnant, the cumulative incidence of pregnancy loss after the estimated day of conception increased during early pregnancy and then plateaued (≈72 days), with 1% of losses occurring in the first 10 days and increasing to 15% by 30 days and 28% by 126 days. All losses occurred before 22 weeks of gestation. Notably, time to pregnancy as prospectively ascertained was not associated with pregnancy loss (Fig. 1A and B, respectively; $P=.97$).

Few significant differences emerged regarding baseline characteristics and pregnancy loss status among couples achieving an observed pregnancy with the exception of maternal age ≥35 years after accounting for the difference in couples' ages given their correlated nature ($r = 0.70$) and paternal education (≤high school versus higher; Table 1).

Certain lifestyles were significantly ($P<.05$) associated with pregnancy loss across various sensitive windows (Fig. 2). During the preconception window, male but not female partner's caffeinated beverage consumption was positively (62% and 41%; $P<.001$) associated with pregnancy loss, while female vitamin adherence was negatively associated (59% and 69%; $P=.02$). During the early pregnancy and periconception windows, partners' caffeine consumption was positively associated with pregnancy loss, while female partner's vitamin adherence was negatively associated ($P<.001$).

FIGURE 1



Cumulative incidence and 95% CIs of pregnancy loss by gestation (**A**) and the incidence and 95% CIs of pregnancy loss by time to pregnancy in observed menstrual cycles (**B**).

Buck Louis. Couples' lifestyle and pregnancy loss. Fertil Steril 2016.

Several significant adjusted HRs (aHRs) emerged for lifestyle in each sensitive window along with female age ≥35 years accounting for the couples' ages (Table 2). Most notably, female caffeine consumption >2 daily caffeinated drinks remained significantly associated with pregnancy loss (preconception [aHR = 1.74, 1.07–2.81], early pregnancy [aHR = 3.05, 1.75–5.34], and periconception [aHR = 2.58, 1.56–4.27]). Similarly, male partners' caffeinated beverage consumption was also significantly associated with loss (preconception [aHR = 1.73, 1.10–2.72], early pregnancy [aHR = 1.88, 1.21–2.93], and periconception [aHR = 1.75, 1.12–2.74]). Female vitamin adherence was associated with a reduced risk of loss in every sensitive window (preconception [aHR = 0.45, 0.25–0.80], early pregnancy [aHR = 0.21, 0.10–0.43], and periconception [aHR = 0.22, 0.11–0.44]). None of the other lifestyle characteristics were significantly associated with pregnancy loss in any of the final adjusted models.

DISCUSSION

In our couples-based prospective cohort that was enrolled before conception, the incidence of pregnancy loss among couples who achieved a recognized pregnancy during the study was 28%, which is comparable (25%–31%) to earlier estimates from prior preconception cohort studies that used urinary hormonal profiles to define pregnancy loss (24–26). However, our incidence is notably higher than that reported for a prospective occupational cohort of couples recruited before conception who were followed for 6 months (17%, 12%–22%) (27). Time to pregnancy was not associated with pregnancy loss in our study, affirming recent data from a large pregnancy cohort study (28). Our incidence estimate relative to earlier cohorts does not support an increased incidence of pregnancy loss as suggested by survey data (8). Still, the relatively high incidence underscores the inefficiency of human reproduction (29).

While female age has long been associated with pregnancy loss, we observed a threshold effect at ≥35 years even when accounting for the difference in the couples' ages. The extent to which this finding reflects aged gametes or cumulative environmental exposures inclusive of lifestyle requires further investigation. Couples' lifestyle behaviors were associated with pregnancy loss, most notably consumption of >2 daily caffeinated beverages. The relation between consumption of caffeinated beverages and pregnancy loss has received considerable attention in the literature, with much of the available evidence relying upon retrospective ascertainment of caffeine consumption including after pregnancy loss that may induce reporting bias (30). Interpreting our findings within the context of available literature that used prospective cohort designs with preconception enrollment is challenging in light of how few of such studies there are. However, a recent preconception cohort study that recruited women trying for pregnancy via the Internet reported a positive association between women's caffeine consumption during early pregnancy but not before conception and pregnancy loss (31). However, there are noteworthy differences between the methods used in that study and ours that may impact findings, including a lower incidence of pregnancy loss in the Internet-based cohort study in comparison with ours (14% and 28%, respectively), reliance on last menstrual period for gestational dating versus capture of the peak LH day as an estimate of conception in our study, and reliance on a single measurement of recalled caffeine intake before and during pregnancy in the Internet-based study in comparison with our capture of daily consumption before and during early pregnancy. Pregnancy-based cohorts also have reported affirming other studies focusing on caffeine consumption during early pregnancy (32, 33), but contrasting with a negative finding in another preconception cohort study (34). Our observation that preconception caffeine consumption >2 daily beverages is also associated with pregnancy loss fails to support reverse causation as a possible explanation, where one assumes that women with a healthy pregnancy may avoid caffeine consumption due to the nausea/vomiting or food aversions that are believed to be associated with a healthy pregnancy.

With regard to alcohol consumption, whose prevalence was much higher than that for cigarette smoking in our cohort, earlier authors reported that a couple's alcohol consumption (≥10 weekly drinks) in the conception cycle was

TABLE 1

Comparison of couples' sociodemographic characteristics by observed pregnancy loss, the LIFE Study (n = 344).

Sociodemographic characteristic	Pregnancy loss (n = 98)	No pregnancy loss (n = 222)	Lost to follow-up (n = 24)
Female partner			
Age*			
≤29	48 (49)	118 (53)	18 (75)
30–34	31 (32)	82 (37)	3 (13)
≥35	19 (19)	22 (10)	3 (13)
Mean (±SD)	30.3 (±4.1)	29.6 (±3.8)	29.3 (±3.7)
Race/ethnicity			
White, non-Hispanic	82 (85)	180 (82)	23 (96)
Black, non-Hispanic	3 (3)	3 (1)	0 (0)
Hispanic	7 (7)	21 (10)	1 (4)
Other, non-Hispanic	5 (5)	16 (7)	0 (0)
Education			
≤High school	6 (6)	9 (4)	0 (0)
Some/college graduate	91 (94)	211 (96)	23 (100)
Health insurance			
No	6 (6)	6 (3)	3 (13)
Yes	91 (94)	214 (97)	21 (88)
Household income, $			
<50,000	11 (12)	30 (14)	3 (12)
50,000–99,999	53 (56)	96 (44)	14 (58)
≥100,000	30 (32)	91 (42)	7 (29)
Employed			
No	22 (24)	44 (20)	4 (17)
Yes	76 (78)	178 (80)	20 (83)
BMI			
Under/healthy ≤24.9	44 (45)	111 (50)	15 (63)
Overweight 25.0–29.9	23 (24)	61 (28)	4 (17)
Obese ≥30	31 (32)	49 (22)	5 (21)
Mean (±SD)	27.8 (±6.8)	26.6 (±6.6)	26. (±7.3)
Prior history of pregnancy loss			
Nulligravid	37 (38)	86 (39)	10 (42)
Gravid, with no prior history	36 (37)	93 (42)	12 (50)
Gravid, with prior history	24 (25)	42 (19)	2 (8)
Male partner			
Age			
≤29	28 (29)	86 (39)	9 (38)
30–34	40 (41)	84 (38)	9 (38)
≥35	30 (31)	52 (23)	6 (25)
Mean (±SD)	32.3 (±4.7)	31.4 (±4.6)	31.0 (±3.7)
Race/ethnicity			
White, non-Hispanic	84 (87)	182 (82)	19 (79)
Black, non-Hispanic	2 (2)	5 (2)	0 (0)
Hispanic	4 (4)	20 (9)	3 (13)
Other, non-Hispanic	7 (7)	14 (6)	2 (8)
Education**			
≤High school	7 (7)	5 (2)	6 (25)
Some/college graduate	90 (93)	215 (98)	18 (75)
Health insurance			
No	8 (8)	10 (5)	3 (13)
Yes	89 (92)	211 (96)	21 (88)
Household income, $			
<50,000	9 (10)	26 (12)	2 (8)
50,000–99,999	50 (54)	102 (46)	13 (54)
≥100,000	34 (37)	92 (42)	9 (38)
Employed			
No	3 (3)	6 (3)	0 (0)
Yes	95 (97)	216 (97)	24 (100)
BMI			
Under/healthy ≤24.9	14 (15)	46 (21)	3 (13)
Overweight 25.0–29.9	43 (45)	85 (39)	9 (39)
Obese ≥30	38 (40)	86 (40)	11 (48)
Mean (±SD)	29.8 (±5.3)	29.1 (±4.8)	30.2 (±4.9)
Previously fathered a pregnancy			
No	39 (40)	86 (39)	12 (50)
Yes	59 (60)	136 (61)	12 (50)

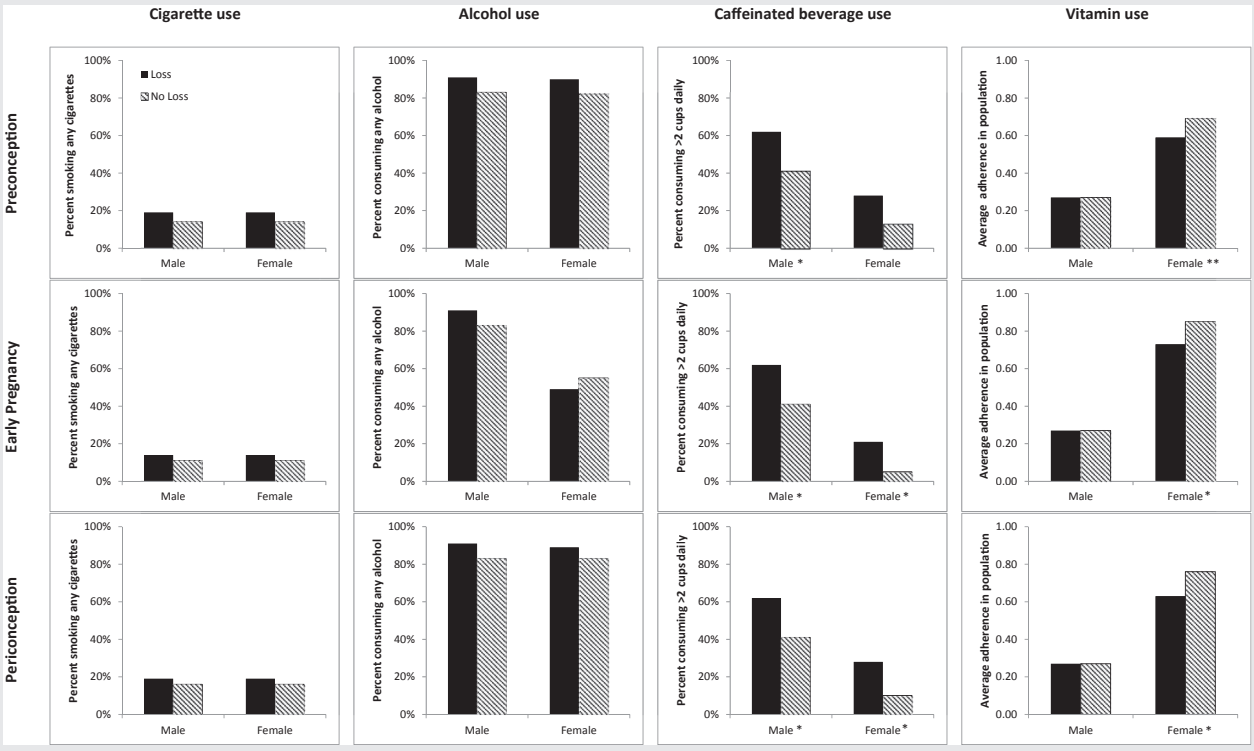
Note: Data are n (%) unless otherwise specified. Three twin pregnancies were uniformly excluded from all analyses. Pregnancy losses all occurred before 21 weeks. Categorical measures were tested with χ^2-test, and continuous measures with the Kruskal-Wallis test. All *P*-values are two-sided.

* $P < .05$.

** $P < .0001$.

Buck Louis. Couples' lifestyle and pregnancy loss. Fertil Steril 2016.

FIGURE 2



Comparison of male and female partner's lifestyle by pregnancy loss and sensitive window (preconception, early pregnancy, periconception). * $P<.001$; ** $P=.02$.

Buck Louis. Couples' lifestyle and pregnancy loss. Fertil Steril 2016.

associated with a higher risk of spontaneous abortion (12), as was consuming ≥ 4 weekly drinks in a cohort of pregnant women (35). We did not observe alcohol consumption to be associated with pregnancy loss after covariate adjustment, which may reflect differences in the sampling framework (e.g., recruitment of occupational and pregnant women vs. population-based sampling), differences in the baseline prevalence of alcohol consumption or its timing and frequency during sensitive windows, model specification that includes multivitamin usage, or residual confounding by other lifestyle factors such as smoking or caffeine consumption.

An important and previously unreported lifestyle behavior is the tremendous reduction in pregnancy loss associated with vitamin adherence irrespective of sensitive window. For example, a 55% reduction in pregnancy loss was found for women taking vitamins daily (100% adherence) in the preconception periods versus for those who did not (0% adherence), increasing to a 79% reduction for adherence during early pregnancy. In an extended analysis, we assessed whether preconception multivitamin usage is independent of early pregnancy usage and vice versa, with the important caveat that women's behaviors were correlated over windows, resulting in very inefficient estimators for effect decomposition and findings that may be susceptible to collider bias in the presence of unmeasured confounders. The unadjusted and adjusted HRs for daily preconception multivitamin usage controlling for similar usage in the early pregnancy window reflected the importance of the preconception window (HR = 0.58, 95% CI, 0.34, 0.96; and HR = 0.83, 95%, CI 0.34, 2.04, respectively). Similarly when modeling early pregnancy vitamin adherence controlling for similar preconception usage, a reduced risk of loss was observed in the unadjusted and adjusted analyses (HR = 0.25, 95% CI, 0.13, 0.49; and 0.23, 95% CI, 0.07, 0.73), respectively. These data may support usage in both sensitive windows.

This strong protective effect for preconception multivitamin usage is somewhat consistent with findings from a preconception cohort of female Chinese textile workers, where women in the highest versus lowest quartile of vitamin B_6 had a lower odds of pregnancy loss (adjusted odds ratio = 0.5; 95% CI, 0.3–1.0) (36). Related support stems from previous studies that involved self-reported vitamin or folate usage and pregnancy loss. Pregnant women were queried about the combined use of multi- and prenatal vitamins, which were associated with significantly reduced odds of loss and self-reported pregnancy loss corroborated by medical records (37). In a cohort of women with prospectively collected folate intake and self-reported pregnancy loss, women in the highest versus lowest quintile of folate intake had a 19% reduction in risk of loss (38). Collectively, these data are supportive of a beneficial association between vitamin usage and pregnancy loss.

Our study has many strengths, including its unique population-based couple design that captured both partners' lifestyles in advance of and during early pregnancy or within time intervals that are typically missed when relying on

TABLE 2

Lifestyle during sensitive windows of human reproduction and development and pregnancy loss—proportional HRs: The LIFE Study (n = 344).

	Preconception		Early pregnancy		Periconception (preconception and early pregnancy)	
Characteristic	**Unadjusted**	**Adjusted**	**Unadjusted**	**Adjusted**	**Unadjusted**	**Adjusted**
Female age (≥35 vs. <35 y)	1.81 (1.09, 3.01)	1.96 (1.13, 3.38)	1.81 (1.09, 3.01)	1.85 (1.10, 3.13)	1.81 (1.09, 3.01)	1.95 (1.13, 3.35)
Difference in partners' ages	1.02 (0.96, 1.08)	1.02 (0.96, 1.08)	1.02 (0.96, 1.08)	1.02 (0.96, 1.07)	1.02 (0.96, 1.08)	1.03 (0.97, 1.08)
Prior pregnancy, no prior loss[a]	0.92 (0.58, 1.45)	0.78 (0.48, 1.26)	0.92 (0.58, 1.45)	0.68 (0.42, 1.11)	0.92 (0.58, 1.45)	0.69 (0.43, 1.13)
Prior pregnancy, prior loss[b]	1.31 (0.79, 2.20)	1.15 (0.66, 2.00)	1.31 (0.79, 2.20)	1.05 (0.61, 1.81)	1.31 (0.79, 2.20)	1.03 (0.59, 1.80)
Female BMI	1.02 (0.99, 1.05)	1.01 (0.98, 1.04)	1.02 (0.99, 1.05)	1.00 (0.97, 1.03)	1.02 (0.99, 1.05)	1.01 (0.98, 1.04)
Male BMI	1.02 (0.98, 1.06)	1.01 (0.97, 1.06)	1.02 (0.98, 1.06)	1.01 (0.97, 1.06)	1.02 (0.98, 1.06)	1.02 (0.98, 1.06)
Female average cigarette smoking[c]	1.06 (0.99, 1.14)	1.01 (0.92, 1.10)	1.11 (1.03, 1.19)	1.02 (0.92, 1.13)	1.09 (1.01, 1.16)	1.01 (0.91, 1.11)
Male average cigarette smoking[c]	1.03 (0.99, 1.07)	1.01 (0.95, 1.07)	1.03 (0.99, 1.07)	1.00 (0.94, 1.07)	1.03 (0.99, 1.07)	0.99 (0.93, 1.06)
Female average alcohol consumption[c]	1.10 (0.79, 1.53)	1.12 (0.70, 1.79)	2.16 (1.16, 4.02)	1.65 (0.77, 3.54)	1.35 (0.86, 2.12)	1.19 (0.64, 2.23)
Male average alcohol consumption[c]	1.01 (0.82, 1.24)	0.97 (0.73, 1.28)	1.01 (0.82, 1.24)	0.95 (0.76, 1.20)	1.01 (0.82, 1.24)	0.97 (0.73, 1.27)
Female caffeinated beverage consumption (>2 vs. ≤2 daily cups)	2.25 (1.44, 3.53)	1.74 (1.07, 2.81)	3.58 (2.18, 5.86)	3.05 (1.75, 5.34)	2.79 (1.79, 4.36)	2.58 (1.56, 4.27)
Male caffeinated beverage consumption (>2 vs. ≤2 daily cups)	2.02 (1.34, 3.05)	1.73 (1.10, 2.72)	2.02 (1.34, 3.05)	1.88 (1.21, 2.93)	2.02 (1.34, 3.05)	1.75 (1.12, 2.74)
Female vitamin adherence[d]	0.58 (0.34, 0.96)	0.45 (0.25, 0.80)	0.25 (0.13, 0.49)	0.21 (0.10, 0.43)	0.35 (0.19, 0.63)	0.22 (0.11, 0.44)
Male vitamin adherence[d]	0.99 (0.57, 1.71)	1.27 (0.70, 2.30)	0.99 (0.57, 1.71)	1.15 (0.65, 2.03)	0.99 (0.57, 1.71)	1.36 (0.75, 2.47)
Average intercourse frequency[e]	1.00 (0.96, 1.05)	1.01 (0.96, 1.05)	1.00 (0.96, 1.05)	0.99 (0.95, 1.04)	1.00 (0.96, 1.05)	0.99 (0.95, 1.04)

Note: Data are HR (95% CI). Models adjust for all characteristics simultaneously. Restricted to women with singleton pregnancies; three twin pregnancies were excluded from analysis. Men's lifestyles for the early pregnancy and periconception windows were modeled based on reported usage in the preconception window (as men did not record lifestyle during early pregnancy).

[a] No prior pregnancy loss versus no prior pregnancy.

[b] Prior pregnancy loss versus no prior pregnancy.

[c] Average daily number of cigarettes smokes or alcoholic drinks consumed.

[d] Adherence denotes the proportion of days vitamins taken, ranging from 0 (none) to 1 (daily), during each sensitive window.

[e] Average number of intercourse acts during the pregnancy cycle.

Buck Louis. Couples' lifestyle and pregnancy loss. Fertil Steril 2016.

pregnancy cohorts. Our use of fertility monitors enabled us to accurately estimate conception using ovulation as its proxy, and we used digital pregnancy tests with demonstrated validity and user accuracy (16). We also were able to quantify small but highly significant ($P \leq .001$) reductions in women's daily mean number of cigarettes smoked (–0.15) or alcoholic (–0.34) and caffeinated (–0.51) beverages consumed between the preconception and early pregnancy sensitive windows, underscoring the need for continual guidance aimed at promoting healthy behaviors.

Still, important limitations need to be considered when interpreting the findings. We chose to assess pregnancy loss without further categorization relative to gestational age in light of no established standard endocrine criteria for defining loss (39). We recognize that we may have missed some losses if hCG concentrations were below analytical sensitivity limits or given the molecular heterogeneity of hCG and its variants. For instance, despite our standardized protocol for testing for pregnancy, it is possible that some women tested too late and thereby missed an hCG rise and fall. We also did not have information on the specific types of beverages consumed or specific vitamins taken that would be helpful to delve into potential biological mechanisms underlying the association between multivitamin behavior and pregnancy loss, nor did we have information on decaffeinated or caffeine free beverages or other aspects of lifestyle such as physical activity, diet, recreational drugs, or sleep habits. As such, we cannot rule out the potential for residual confounding.

CONCLUSIONS

In summary, our data suggest that couples' lifestyles are associated with the risk of pregnancy loss, including during the preconception period, emphasizing the need for continued efforts to promote healthy lifestyles before pregnancy begins, that is, preconception guidance. Our findings underscore the feasibility and utility of prospective cohort designs with preconception recruitment of couples for assessing exposures associated with reproductive and developmental toxicants in support of an earlier conclusion (40). Also, couples might continue to be advised that advanced age increases the risk of pregnancy loss and to limit caffeinated intake to fewer than 3 daily beverages irrespective of source and that women should continue to take daily multivitamins before and during pregnancy consistent with clinical guidance. Our findings are not intended to suggest substituting decaffeinated or caffeine-free beverages as a safer alternative, as we did not query couples on these products. Collectively, our findings are supportive of the need for ongoing efforts to ensure couples receive preconception guidance, and our findings await corroboration from other preconception couples-based cohorts to ensure that preconception guidance issued for the U.S. population (http://www.cdc.gov/preconception) is responsive to discoveries to the extent possible.

In conclusion, our findings are highly relevant for contemporary cohorts of couples at risk for or planning pregnancy who might want to adopt or maintain lifestyles that minimize the risk of pregnancy loss. All such lifestyles are amenable to change, and such guidance has been shown to influence healthy behaviors (41).

REFERENCES

1. Vaitukaitis JL, Braunstein GD, Ross GT. A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. Am J Obstet Gynecol 1972;113:751–8.
2. Braunstein GD. The long gestation of the modern pregnancy. Clin Chem 2014;60:18–21.
3. http://www.bloomberg.com/bw/articles/2013-11-27/clearblue-wins-over-the-pregnancy-test-market. Accessed September 1, 2015.
4. http://www.statista.com/statistics/296137/top-ten-us-over-the-counter-brands-for-diagnostics/. Accessed September 1, 2015.
5. http://www.clearblueeasy.com/healthcare/heritage-of-clearblue.php. Accessed September 1, 2015.
6. Whittaker PG, Taylor A, Lind T. Unsuspected pregnancy loss in healthy women. Lancet 1983;1:1126–7.
7. Edmonds DK, Lindsay KS, Miller JF, Williamson E, Wood PJ. Early embryonic mortality in women. Fertil Steril 1982;38:447–53.
8. Lang K, Nuevo-Chiquero A. Trends in self-reported spontaneous abortions: 1970–2000. Demography 2012;49:989–1009.
9. American College of Obstetrics and Gynecology. Early pregnancy loss. Practice Bulletin 150. Obstet Gynecol 2015;125:1258–67.
10. Slama R, Bouyer J, Windham G, Fenster L, Werwatz A, Swan SH. Influence of paternal age on the risk of spontaneous abortion. Am J Epidemiol 2005;161:816–23.
11. Ford JH, MacCormac L, Hiller J. PALS (pregnancy and lifestyle study): associations between occupational and environmental exposure to chemicals and reproductive outcome. Mutat Res 1994;313:153–64.
12. Henriksen TB, Hjollund NH, Jensen TK, Bonde JP, Andersson A-M, Kolstad H, et al. Alcohol consumption at the time of conception and spontaneous abortion. Am J Epidemiol 2004;160:661–7.
13. Venners SA, Wang X, Chen C, Wang L, Chen D, Guang W, et al. Paternal smoking and pregnancy loss: a prospective study using a biomarker of pregnancy. Am J Epidemiol 2004;159:993–1001.
14. Buck Louis GM, Schisterman EF, Sweeney AM, Wilcosky TC, Gore-Langton R, Lynch CD, 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;25:413–24.
15. Behre HM, Kuhlage J, Gahner C, Sonntag B, Schem C, Schneider HPG, et al. 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;15:2478–82.
16. Johnson S, Cushion M, Bond S, Godbert S, Pike J. Comparison of analytical sensitivity and women's interpretation of home pregnancy tests. Clin Chem Lab Med 2015;53:391–402.
17. Barrett ES, Thune I, Lipson SF, Furberg AS, 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;28:801–11.
18. Andersen A-MN, Wohlfahrt J, Christens P, Olsen J, Melbye M. Maternal age and fetal loss: population based register linkage study. BMJ 2000;320:1708–12.
19. Hahn KA, Hatch EE, Rothman KJ, Mikkelsen EM, Brogly SB, Sørensen HT, et al. Body size and risk of spontaneous abortion among Danish pregnancy planners. Paediatr Perinat Epidemiol 2014;28:412–23.
20. Nohr EA, Bech BH, Davies MJ, Frydenberg M, Henriksen TB, Olsen J. Prepregnancy obesity and fetal death: a study within the Danish National Birth Cohort. Obstet Gynecol 2005;106:250–9.
21. Steiner AZ, Pritchard DA, Young SL, Herring AH. Peri-implantation intercourse lowers fecundability. Fertil Steril 2014;102:178–82.
22. Regan L, Braude PR, Trembath PL. Influence of past reproductive performance on risk of spontaneous abortion. Br Med J 1989;299:541–5.

23. Cox DR. Regression models and life-tables. JRSS, Series B (Methodological) 1972:187–200.
24. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, et al. Incidence of early loss of pregnancy. N Engl J Med 1988;319:189–94.
25. Zinamen MJ, Clegg ED, Brown CC, O'Connor J, Selevan SG. Estimates of human fertility and pregnancy loss. Fertil Steril 1996;65:503–9.
26. 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;79:577–84.
27. Bonde JP, Hjollund NH, Jensen TK, Ernst E, Kolstad H, Henriksen TB, et al. A follow-up study of environmental and biologic determinants of fertility among 430 Danish first-pregnancy planners: design and methods. Reprod Toxicol 1988;12:19–27.
28. Nilsson SF, Andersen PK, Strandberg-Larsen K, Andersen A-MN. Risk factors for miscarriage from a prevention perspective: a nationwide follow-up study. Br J Obstet Gynaecol 2014;121:1375–85.
29. Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. N Engl J Med 2001;345:1400–8.
30. Savitz DA, Chan RL, Herring AH, Howards PP, Hartmann KE. Caffeine and miscarriage risk. Epidemiology 2008;19:55–62.
31. Hahn KA, Wise LA, Rothman KJ, Mikkelsen EM, Brogly SB, Sorensen HT, et al. Caffeine and caffeinated beverage consumption and risk of spontaneous abortion. Hum Reprod 2015;30:1246–55.
32. Weng X, Odouli R, De-Kun L. Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. Am J Obstet Gynecol 2008;198:279.e1–8.
33. Greenwood DC, Alwan N, Boylan S, Cade JE, Charvill J, Chipps KC, et al. Caffeine intake during pregnancy, late miscarriage and stillbirth. Eur J Epidemiol 2010;25:275–80.
34. Wilcox AJ, Weinberg CR, Baird DD. Risk factors for early pregnancy loss. Epidemiology 1990;1:382–5.
35. Avalos LA, Roberts SC, Kaskutas LA, Block G, Li DK. Volume and type of alcohol during early pregnancy and the risk of miscarriage. Subst Use Misuse 2014;49:1437–45.
36. Ronnenberg AG, Venners SA, Xu X, Chen C, Wang L, Guang W, et al. Preconception B-vitamin and homocysteine status, conception, and early pregnancy loss. Am J Epidemiol 2007;166:304–12.
37. Hasan R, Olshan AF, Herring AH, Savitz DA, Siega-Riz AM, Hartmann KE. Self-reported vitamin supplementation in early pregnancy and risk of miscarriage. Am J Epidemiol 2009;169:1312–8.
38. Gaskins AJ, Rich-Edwards JW, Hauser R, Williams PL, Gillman MW, Ginsburg ES, et al. Maternal prepregnancy folate intake and risk of spontaneous abortion and stillbirth. Obstet Gynecol 2014;124:23–31.
39. Lohstroh PN, Overstreet JW, Stewart DR, Nakajima ST, Cragun JR, Boyers SP, et al. Secretion and excretion of human chorionic gonadotropin during early pregnancy. Fertil Steril 2005;83:1000–11.
40. Buck GM, Lynch CD, Stanford JB, Sweeney AM, Schieve LA, Rockett JC, et al. Prospective pregnancy study designs for assessing reproductive and developmental toxicants. Environ Health Perspect 2004;112:79–86.
41. Williams L, Zapata LB, D'Angelo DV, Harrison L, Morrow B. Associations between preconception counseling and maternal behaviors before and during pregnancy. Matern Child Health 2012;16:854–61.