Spontaneous Abortion and a Diet Drug Containing Caffeine and Ephedrine: A Study within the Danish National Birth Cohort

Penelope P. Howards¹*, Irvana Hertz-Picciotto², Bodil H. Bech³, Ellen A. Nohr³, Anne-Marie Nybo Andersen⁴, Charles Poole⁵, Jørn Olsen⁶

¹Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, Georgia, United States of America, ²Division of Environmental and Occupational Health, Department of Public Health Sciences, University of California, Davis, California, United States of America, ³Section for Epidemiology, Department of Public Health, Aarhus University, Aarhus, Denmark, ⁴Section of Epidemiology, Department of Public Health, University of Copenhagen, Copenhagen, Denmark, ⁵Department of Epidemiology, Gillings School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America

Abstract

Background: Medications may be consumed periconceptionally before a woman knows she is pregnant. In this study, the authors evaluate the association of a prescription diet drug (Letigen) containing ephedrine (20 mg) and caffeine (200 mg) with spontaneous abortion (SAB) in the Danish National Birth Cohort.

Methods: Women were recruited during their first prenatal visit from 1996–2002. Pre-conception and early pregnancy medication use was reported on the enrollment form, and pregnancy outcome was determined by linking the mother's Civil Registration Number to the Medical Birth Registry and the National Hospital Discharge Register. Of 97,903 eligible pregnancies, 4,443 ended in SAB between 5 and 20 completed gestational weeks, inclusive. Letigen use was reported for 565 pregnancies. Cox regression models accounting for left truncation were fit to estimate the effect of pre-conception and early pregnancy Letigen use on SAB.

Principal Findings: The estimated maternal age-adjusted hazard ratio for SAB was 1.1 (95% confidence interval 0.8–1.6) for any periconceptional Letigen use compared to no periconceptional use.

Conclusions: Although Letigen has high levels of caffeine (the recommended 3 pills/day are approximately equivalent to caffeine from 6 cups of coffee), periconceptional use does not appear to be associated with an appreciably increased hazard of clinically recognized SAB.

Introduction

Early gestation is a critical time in fetal development when women may be unaware they are pregnant and therefore may use medications, such as diet drugs, that they would not use knowing they were pregnant. Recently, the obesity epidemic in the United States has led the Food and Drug Administration (FDA) to weigh the potential benefits of approving new weight loss drugs against the potential risks, including the risk that reproductive age women may use the drugs before becoming aware of pregnancy. In 2012, the scientific advisory committee to the FDA reversed their 2010 position for one such drug despite concerns that the drug might be associated with an increased risk of oral clefts [1]. In other countries, including Denmark, the prevalence of obesity has also been rising [2], and consequently, reproductive age women may increasingly turn to prescription or over-the-counter weight loss products where available. Letigen (NYCOMED, Denmark) was a prescription diet drug that contained ephedrine and caffeine and was available in Denmark prior to 2002.

There has been little research on the effects of ephedrine on pregnancy, but concern over the potential effect of caffeine on spontaneous abortion (SAB) has resulted in a large but inconclusive literature [3–26]. After considering this literature, the American College of Obstetricians and Gynecologists published a committee opinion in 2010 stating the current evidence did not suggest that consuming less than 200 mg per day of caffeine during pregnancy increased the risk of SAB, but that the evidence for higher doses of caffeine was still unclear [27]. Similarly, the United Kingdom’s Food Standards Agency recommended that pregnant women limit their caffeine consumption to less than...
200 mg per day due to concerns about fetal growth restriction and possibly SAB [28]. A number of methodological issues that are difficult to resolve plague studies of caffeine consumption during pregnancy and SAB making it difficult to draw conclusions about the effect of exposure to high doses of caffeine [29–33]. Although some of these methodological issues do not apply to pre-pregnancy exposure to caffeine, high exposure to pre-pregnancy caffeine has also been inconsistently associated with SAB with modestly elevated effect estimates in some studies [9,12,18,19] but not others [10,20,22]. Most studies of caffeine focused exclusively on caffeine from beverages, including coffee, tea, and cola, that contain other potentially fetotoxic components.

Letigen was a non-beverage source of high doses of caffeine (200–600 mg/day) that some Danish women used pre-conceptionally and inadvertently early in pregnancy. It was banned in Denmark in 2002 and similar products were banned in the United States in 2004 due to reports of adverse cardiovascular events [34,35]. We examine whether preconceptional Letigen use is associated with SAB and a diet drug of caffeine and ephedrine, 1996–2002.

**Materials and Methods**

The Danish National Birth Cohort has been described in detail elsewhere [37]. Briefly, between March 1, 1996 and November 1, 2002, pregnant women across Denmark were provided information about the study during their first prenatal visit. According to a pilot study, approximately 50% of all pregnant women received an invitation to participate, and about 60% of those signed the consent form [37]. The exclusion criteria were not having access to a telephone, not speaking Danish, and not intending to carry the pregnancy to term as of the first prenatal visit. A total of 101,051 pregnancies were enrolled.

The Danish National Birth Cohort was approved by the Danish Scientific Ethics Committee, and this specific study was approved.
Table 1. Summary of counts, fetus-time at risk, and rates per 10,000 for variables stratified on spontaneous abortion (SAB) vs. other pregnancy outcomes for the Danish National Birth Cohort (1996–2002).

|                                | Not SAB       | SAB            | Rate of SAB/10,000 fetus-days |
|--------------------------------|---------------|----------------|------------------------------|
|                                | N  | Fetus-days* | N   | Fetus-days* |                      |
| Letigen use†                   |    |            |     |            |                          |
| None                           | 92,930 | 6,547,875 | 4,408 | 108,617 | 6.6                        |
| Pre-conception only            | 219  | 17,680     | 13   | 274     | 7.2                        |
| Both pre-conception and early pregnancy | 270 | 21,311     | 17   | 346     | 7.8                        |
| Early pregnancy only           | 37   | 2,725      | 5    | 71      | 17.9                       |
| Missing timing                 | 4    | 327        | 0    | 0       |                            |
| Gestational age at entry (completed weeks) |  |            |     |            |                          |
| 5–8                            | 29,102 | 2,766,449 | 2,878 | 76,695 | 10.1                       |
| 9–12                           | 40,260 | 2,886,894 | 1,435 | 30,006 | 4.9                        |
| 13–16                          | 18,680 | 839,147    | 118  | 2,465   | 1.4                        |
| 17–20                          | 5,418  | 97,428     | 12   | 142     | 1.2                        |
| Maternal age (years)           |     |            |     |            |                          |
| 15–19                          | 987   | 64,538     | 40   | 1,120   | 6.1                        |
| 20–24                          | 11,399 | 818,878    | 464  | 11,806  | 5.6                        |
| 25–29                          | 38,814 | 2,797,147  | 1,609 | 40,299  | 5.7                        |
| 30–34                          | 31,581 | 2,183,600  | 1,506 | 37,775  | 6.8                        |
| 35–39                          | 9,756  | 666,161    | 683  | 15,781  | 10.0                       |
| 40–46                          | 923   | 59,594     | 141  | 2,527   | 22.7                       |
| Gravidity                      |     |            |     |            |                          |
| No prior pregnancy             | 30,573 | 2,215,384  | 966  | 25,344  | 4.3                        |
| 1 prior pregnancy              | 57,061 | 3,988,842  | 2,171 | 55,864  | 5.4                        |
| Missing                        | 5,826  | 385,692    | 1,306 | 28,100  |                            |
| Planned pregnancy              |     |            |     |            |                          |
| Planned                        | 66,705 | 4,785,563  | 2,440 | 63,180  | 5.0                        |
| Partially planned              | 11,080 | 765,877    | 371  | 9,349   | 4.8                        |
| Not planned                    | 9,859  | 654,187    | 328  | 8,757   | 4.9                        |
| Missing                        | 5,816  | 384,291    | 1,304 | 28,022  |                            |
| Infertility treatment          |     |            |     |            |                          |
| Yes                            | 5,700  | 386,549    | 167  | 4,581   | 4.3                        |
| No                             | 81,923 | 5,817,343  | 2,972 | 76,705  | 5.0                        |
| Missing                        | 5,837  | 386,026    | 1,304 | 28,022  |                            |
| Prepregnancy body mass index   |     |            |     |            |                          |
| Underweight (<18.5)            | 3,870  | 272,001    | 163  | 4,507   | 5.9                        |
| Normal (18.5–24.9)             | 58,493 | 4,138,880  | 2,076 | 52,949  | 5.0                        |
| Overweight (25.0–29.9)         | 16,707 | 1,182,334  | 613  | 15,805  | 5.1                        |
| Obese (≥ 30.0)                 | 7,160  | 510,755    | 255  | 7,114   | 4.9                        |
| Missing                        | 7,230  | 485,948    | 1,336 | 28,933  |                            |
| Exercise (hours/week)          |     |            |     |            |                          |
| None                           | 55,769 | 3,920,465  | 1,589 | 43,290  | 4.0                        |
| >0 to <2                      | 17,494 | 1,241,710  | 626  | 16,024  | 5.0                        |
| ≥ 2 to <4                     | 9,599  | 697,141    | 543  | 13,291  | 7.6                        |
| ≥ 4 to <7                     | 3,504  | 254,635    | 256  | 5,895   | 9.8                        |
| ≥ 7                            | 1,271  | 91,193     | 124  | 2,774   | 13.2                       |
| Missing                        | 5,823  | 384,774    | 1,305 | 28,034  |                            |
| Smoked during pregnancy (cigarette equivalents/day) | |            |     |            |                          |
| None                           | 67,521 | 4,781,746  | 2,342 | 60,576  | 4.8                        |
| >0 to 10                       | 14,741 | 1,046,138  | 569  | 14,975  | 5.4                        |
by the Danish Data Protection Board. All women participating in the cohort provided written informed consent.

Pregnancy Outcome

Pregnancy outcome was assessed from Danish national registries, which can be linked using the unique identifier, the Civil Registration Number. The Medical Birth Registry and the Civil Registration System were used to obtain data on live and still births. Other pregnancy outcomes were identified in the National Hospital Discharge Register, and emigration prior to the end of pregnancy was determined from the Civil Registration System. Less than one percent of the study pregnancies could not be linked to registry data; in these cases, outcome information from the interviews was used instead [4]. We excluded 34 pregnancies with unknown outcomes and 142 ectopic and molar pregnancies.

We defined SAB as an involuntary intrauterine loss between gestational days 35–146, inclusive (i.e. 5–20 completed weeks) (Figure 1). Primarily, we based gestational age on the National Hospital Discharge Register, which contained an estimate of gestational age that was usually based on a sonogram. However, for 391 pregnancies, we used the last menstrual period (LMP) date reported on the enrollment form because it seemed reasonable and the date from the National Hospital Discharge Register was missing or seemed incorrect. We excluded 124 pregnancies with missing or erroneous gestational ages, 586 pregnancies enrolled after the outcome, 172 pregnancies allegedly enrolled prior to 28 days post-LMP, and 2,090 pregnancies enrolled after 146 days gestation (i.e. no observed time at risk for SAB), yielding a total of 97,903 pregnancies. Eligible multiple births (n = 2,045) were treated as a single event; we treated the one eligible pregnancy with discrepant outcomes as a SAB.

Exposure Assessment

The exposure of interest was periconceptional use of Letigen. Each pill contained 20 mg of ephedrine and 200 mg of caffeine; the recommended dose was three pills per day [38]. Thus, women using Letigen as directed ingested caffeine approximately equivalent to drinking five to six cups of coffee a day [39], as well as ephedrine, and possibly caffeine from other sources. Letigen use was reported on the enrollment form prior to the pregnancy outcome. A pregnancy was defined as exposed if any Letigen use was reported on the enrollment form during the four weeks prior to the woman’s LMP through 13 completed weeks post-LMP (n = 565). Twenty-two pregnancies were classified as unexposed

| Table 1. Cont. |
|----------------|
| | Not SAB | SAB |
| | N | Fetus-days* | N | Fetus-days* | Rate of SAB/10,000 fetus-days |
| >10 | 5,373 | 376,952 | 224 | 5,656 | 5.9 |
| Missing | 5,825 | 385,082 | 1,308 | 28,101 |
| Alcohol during pregnancy (drinks/week) | | | | | |
| No drinks | 48,727 | 3,507,924 | 1,560 | 41,792 | 4.4 |
| >0 to <4 | 37,112 | 2,578,283 | 1,427 | 36,548 | 5.5 |
| ≥ 4 | 1,823 | 120,506 | 152 | 2,946 | 12.3 |
| Missing | 5,798 | 383,205 | 1,304 | 28,022 |
| Coffee (cups/day) | | | | | |
| None | 48,693 | 3,507,238 | 1,477 | 40,674 | 4.2 |
| >0 to <4 | 27,519 | 1,922,961 | 1,016 | 25,636 | 5.2 |
| 4 to <8 | 8,493 | 575,907 | 440 | 10,171 | 7.5 |
| ≥ 8 | 2,932 | 198,957 | 203 | 4,734 | 10.0 |
| Missing | 5,823 | 384,855 | 1,307 | 28,093 |
| Tea (cups/day) | | | | | |
| None | 32,583 | 2,311,375 | 1,200 | 31,184 | 5.1 |
| >0 to <4 | 39,427 | 2,793,264 | 1,334 | 34,381 | 4.7 |
| 4 to <8 | 11,928 | 842,605 | 428 | 11,156 | 5.0 |
| ≥ 8 | 3,699 | 257,799 | 176 | 4,534 | 6.7 |
| Missing | 5,823 | 384,875 | 1,305 | 28,053 |
| Cola (liters/week) | | | | | |
| None | 29,967 | 2,125,366 | 1,136 | 30,021 | 5.3 |
| >0 to <1 | 42,896 | 3,026,527 | 1,310 | 33,935 | 4.3 |
| ≥ 1 | 14,758 | 1,051,921 | 689 | 17,210 | 6.4 |
| Missing | 5,839 | 386,104 | 1,308 | 28,142 |

*Observed fetus days at risk (i.e. time from entry to outcome, censoring, or end of risk period).

1 cigarette = 1 cigarette equivalent, 1 cheroot = 2 cigarettes equivalents, 1 cigar = 2 cigarette equivalents, 1 pipe = 1.5 cigarette equivalents.
despite reported Letigen use because the use predated the periconceptive period. Four women did not report when they used Letigen; we assumed they were exposed. We also defined preconception Letigen use as occurring four weeks prior to LMP through two completed gestational weeks, and early pregnancy use as occurring from three to 13 completed gestational weeks (Figure 1).

Classification of exposure status was complicated by the fact that there were two versions of the enrollment form. On the original form, used for 65% of the pregnancies, medication type and timing of use were reported in text fields for the three months prior to filling out the form (Figure 1). On the revised form, the participant listed medications consumed and marked boxes to indicate weeks when the medication was used. The boxes covered the four weeks prior to the participant’s LMP through enrollment or 13 completed weeks post-LMP, whichever came first (Figure 1).

Four factors affected Letigen exposure assignment. First, women enrolled at different times in their pregnancies and therefore reported Letigen use through different gestational ages (Figure 1). We assumed that no one took Letigen after they knew they were pregnant. Given that all women knew they were pregnant at enrollment, this translated into assuming that no one took Letigen after enrollment. This assumption seems reasonable because almost all women specifically stated that they stopped taking Letigen when they became aware of their pregnancy or clearly reported cessation of use prior to enrollment. This is also consistent with a recent United States study of birth defects and retrospectively-reported weight loss products (including over-the-counter products) that reported exposure dropping from over 1% prior to pregnancy to 0.2% by the third month of pregnancy [40].

| Table 2. Descriptive statistics by Letigen* exposure status for the Danish National Birth Cohort (1996–2002). |
|---------------------------------------------------------------|
| **Non-Letigen** | **Letigen** |
| **N (%)** | **N (%)** |
| Gestational age at entry (completed weeks) | | |
| 5–8 | 31,703 | 32.6 | 277 | 49.0 |
| 9–12 | 41,481 | 42.6 | 214 | 37.9 |
| 13–16 | 18,738 | 19.3 | 60 | 10.6 |
| 17–20 | 5,416 | 5.6 | 14 | 2.5 |
| Maternal age (years) | | |
| 15–19 | 1,023 | 1.1 | 4 | 0.7 |
| 20–24 | 11,764 | 12.1 | 99 | 17.5 |
| 25–29 | 40,183 | 41.3 | 240 | 42.5 |
| 30–34 | 32,911 | 33.8 | 176 | 31.2 |
| 35–39 | 10,399 | 10.7 | 40 | 7.1 |
| 40–46 | 1,058 | 1.1 | 6 | 1.1 |
| Gravidity | | |
| No prior pregnancy | 31,374 | 32.2 | 165 | 29.2 |
| ≥1 prior pregnancy | 58,874 | 60.5 | 358 | 63.4 |
| Missing | 7,090 | 7.3 | 42 | 7.4 |
| Planned Pregnancy | | |
| Planned | 68,859 | 70.7 | 286 | 50.6 |
| Partially planned | 11,336 | 11.6 | 115 | 20.4 |
| Not planned | 10,065 | 10.3 | 122 | 21.6 |
| Missing | 7,078 | 7.3 | 42 | 7.4 |
| Infertility treatment | | |
| Yes | 5,852 | 6.0 | 15 | 2.7 |
| No | 84,388 | 86.7 | 507 | 89.7 |
| Missing | 7,098 | 7.3 | 42 | 7.4 |
| Prepregnancy body mass index | | |
| Underweight (<18.5) | 4,032 | 4.1 | 1 | 0.2 |
| Normal (18.5–24.9) | 60,408 | 62.1 | 161 | 28.5 |
| Overweight (25.0–29.9) | 17,109 | 17.6 | 211 | 37.3 |
| Obese (≥30.0) | 7,272 | 7.5 | 143 | 25.3 |
| Missing | 8,517 | 8.7 | 49 | 8.7 |
| Exercise (hours/week) | | |
| None | 57,010 | 58.6 | 348 | 61.6 |
| >0 to <2 | 18,035 | 18.5 | 85 | 15.0 |
| ≥2 to <4 | 10,086 | 10.4 | 56 | 9.9 |
| ≥4 to <7 | 3,739 | 3.8 | 21 | 3.7 |
| ≥7 | 1,382 | 1.4 | 13 | 2.3 |
| Missing | 7,086 | 7.3 | 42 | 7.4 |
| Smoked during pregnancy (cigarette equivalents/day1) | | |
| None | 69,511 | 71.4 | 352 | 62.3 |
| >0 to 10 | 15,190 | 15.6 | 120 | 21.2 |
| >10 | 5,546 | 5.7 | 51 | 9.0 |
| Missing | 7,091 | 7.3 | 42 | 7.4 |
| Alcohol during pregnancy (drinks/week) | | |
| No drinks | 49,980 | 51.3 | 307 | 54.3 |
| >0 to <4 | 38,336 | 39.4 | 203 | 35.9 |
| ≥4 | 1,962 | 2.0 | 13 | 2.3 |

*Letigen is composed of 20 mg ephedrine and 200 mg caffeine.
1 cigarette = 1 cigarette equivalent, 1 cheroot = 2 cigarettes equivalents, 1 cigar = 2 cigarette equivalents, 1 pipe = 1.5 cigarette equivalents.

Table 2. Cont.

| Non-Letigen* | **Letigen** |
|---------------|-------------|
| **N (%)** | **N (%)** |
| Missing | 7,060 | 7.3 | 42 | 7.4 |
| Coffee (cups/day) | | |
| None | 49,842 | 51.2 | 328 | 58.1 |
| >0 to <4 | 28,410 | 29.2 | 125 | 22.1 |
| 4 to <8 | 8,883 | 9.1 | 50 | 8.8 |
| ≥8 | 3,115 | 3.2 | 20 | 3.5 |
| Missing | 7,088 | 7.3 | 42 | 7.4 |
| Tea (cups/day) | | |
| None | 33,567 | 34.5 | 216 | 38.2 |
| >0 to <4 | 40,532 | 41.6 | 229 | 40.5 |
| 4 to <8 | 12,300 | 12.6 | 56 | 9.9 |
| ≥8 | 3,853 | 4.0 | 22 | 3.9 |
| Missing | 7,086 | 7.3 | 42 | 7.4 |
| Cola (liters/week) | | |
| None | 30,976 | 31.8 | 127 | 22.5 |
| >0 to <1 | 43,981 | 45.2 | 225 | 39.8 |
| >1 | 15,276 | 15.7 | 171 | 30.3 |
| Missing | 7,105 | 7.3 | 42 | 7.4 |

*Letigen is composed of 20 mg ephedrine and 200 mg caffeine.
1 cigarette = 1 cigarette equivalent, 1 cheroot = 2 cigarettes equivalents, 1 cigar = 2 cigarette equivalents, 1 pipe = 1.5 cigarette equivalents.

[doi:10.1371/journal.pone.0050372.t002]
For each version of the enrollment form, the mean gestational age at entry was ten (standard deviation (SD) three) weeks. However, the two forms referred to different timeframes (Figure 1). The exposure period of interest was covered by the checkbox enrollment form and the text-based form for women enrolling by eight weeks. However, women who used the text-based form and enrolled after eight completed weeks were asked only about part of this timeframe. In practice, Letigen users who completed the text-based form and enrolled late tended to report medication usage covering the entire timeframe of interest (i.e. usage more than three months prior to enrollment). However, women who used the text-based enrollment form and did not report Letigen use might be mistakenly classified as unexposed if they used Letigen preconceptionally and enrolled late. We examined this issue analytically by performing sub-analyses where we excluded late enrollers or stratified on the version of the form used.

The third issue was that the timing of Letigen use was often reported vaguely on the text-based form. We reviewed each text field individually and developed rules regarding the timing of exposure for commonly used phrases (e.g., six days in early September was assumed to be the middle six days in the first ten days of the month). Because we assessed broad exposure windows, most women were clearly exposed or unexposed periconceptionally, despite nonspecific responses.

The final challenge was that women reported Letigen use relative to self-reported LMP, which was not always consistent with the estimated LMP based on the National Hospital Discharge Register. For 87% of the women, the dates were within a week of each other (30% were identical) so reports pertained to the relevant timeframe despite minor date discrepancies. Of the 1,452 pregnancies where LMP dates differed by more than four completed weeks and the National Hospital Discharge Register date was determined to be the best estimate, 11 women reported using Letigen. Their exposure status did not change regardless of the gestational age definition used. Some of the 1,441 women who were classified as unexposed might have been exposed if they had reported on the relevant timeframe only, but the number is likely to be small. We addressed this uncertainty through a sensitivity analysis excluding pregnancies for which gestational age was based on the National Hospital Discharge Register and for which the self-reported and Register-based LMPs differed by more than four completed weeks.

### Other Variables

Maternal age at LMP was calculated using birth date from the Civil Registration System. Information on all other covariates was collected through telephone interviews. Of the 97,903 pregnancies in this study, 7,094 were never interviewed. Of the women

### Table 3. Summary statistics for the number of weeks that Letigen* was used at least once among women in the Danish National Birth Cohort (1996–2002).

| Timeframe of use† | Weeks | N     | Mean | SD  | Minimum | Maximum |
|-------------------|-------|-------|------|-----|---------|---------|
| Total eligible time | 561   | 6.3   | 3.3  | 1   | 16      |
| Pre-conception only | 232  | 4.1   | 1.9  | 1   | 7       |
| Both pre-conception and early pregnancy | 287  | 8.7   | 2.5  | 2   | 16      |
| Pre-conception time | 6.0   | 1.9  | 1   | 7 |
| Early pregnancy time | 2.7   | 1.5  | 1   | 9       |
| Early pregnancy only | 42  | 2.3   | 1.5  | 1   | 8       |

*Letigen is composed of 20 mg ephedrine and 200 mg caffeine.
†Total eligible time includes the 4 weeks prior to last menstrual period through gestational age 13 completed weeks, pre-conception includes the 4 weeks prior to last menstrual period through gestational age 2 completed weeks, early pregnancy includes gestational age 3–13 completed weeks.

### Table 4. Unadjusted and age-adjusted hazard ratios and 95% confidence intervals for the association between Letigen* use and spontaneous abortion (SAB) in the Danish National Birth Cohort (1996–2002).

| Timeframe of exposure§ | SAB | Fetus-days$ | Unadjusted | Age adjusted† |
|------------------------|-----|-------------|------------|---------------|
| None                   | 4,408 | 6,656,492 | 1.0 ref | 1.0 ref |
| Pre-conception only    | 13   | 17,954     | 1.0 0.6; 1.6 | 1.0 0.6; 1.7 |
| Both pre-conception and early pregnancy | 17 | 21,657 | 1.0 0.6; 1.7 | 1.1 0.7; 1.7 |
| Early pregnancy only   | 5    | 2,796      | 2.6 1.1; 6.5 | 2.7 1.1; 6.6 |

*Letigen is composed of 20 mg ephedrine and 200 mg caffeine.
†Adjusted for maternal age using the following categories: 15–19, 20–24, 30–34, 35–39, 40–46 with 25–29 as the referent.
§Pre-conception includes the 4 weeks prior to last menstrual period through gestational age 2 completed weeks; early pregnancy includes gestational age 3–13 completed weeks.
$Observed fetus days at risk (i.e. time from entry to outcome, censoring, or end of risk period).
Abbreviations: HR, hazard ratio; CI, confidence interval.

doi:10.1371/journal.pone.0050372.t003
Results

Statistical Analyses

We fit Cox regression models accounting for left truncation to estimate the effect of Letigen on SAB. We used gestational age in days with entry into observation defined as enrollment in the study. We employed the robust Lin and Wei confidence interval option in SAS 9.2 (Cary, North Carolina) to account for the fact that some women (n = 8,099) contributed more than one pregnancy to the study. Pregnancies that ended in an induced abortion (n = 425) and where the mother died (n = 3) or emigrated (n = 40) prior to 20 completed weeks gestation were censored.

We considered the following potential confounders: maternal age, pre-pregnancy body mass index (BMI), exercise, smoking, alcohol consumption, and consumption of caffeine from beverages. Although these factors could conceivably be confounders or proxies for confounders, none substantially changed the effect-measure estimates for Letigen separately or as a group (≤3% change in estimate). Given that a higher proportion of women with SABs did not have interviews compared with women with other pregnancy outcomes (29% vs. 6%) and the fact that none of the other covariates appeared to confound the effect-measure estimate considerably, we opted to report the results for the model adjusted for maternal age only. This allowed us to include all eligible pregnancies.

Discussion

Periconceptional Letigen use is not appreciably associated with SAB in this study. Although pre-pregnancy Letigen use showed little or no association with SAB, early pregnancy use was associated with an elevated HR. However, the elevated HR was based on only five exposed SABs. If Letigen was causally related to SAB when taken in early pregnancy, it would be expected to be harmful for any exposed pregnancy, whether or not the woman also took it prior to conception. However, women exposed during both periods showed no elevated risk. Further, the effect measure estimate for Letigen use during early pregnancy only may be confounded. Although adjusting for measured potential confounders did not change the estimate meaningfully, it is possible that women who began using Letigen after conception may have been more likely to participate in unmeasured behaviors that increase the hazard of SAB.

Some assumptions were made to determine exposure status during the timeframe of interest; to the degree that we could test these assumptions, the overall effect-measure estimates appeared robust. We accounted for left truncation analytically. However, this approach assumes that the pregnancies under observation at a given gestational age are representative of all pregnancies at that gestational age. Bias could be introduced if this was not the case, especially if entry was associated with the exposure [41]. Further, gestational age may be less accurate for SABs because live births are more likely to have a sonogram. However, previous work suggests bias due to differential accuracy in gestational age by outcome is likely to be small given realistic assumptions about the magnitude of these differences [42].

Letigen was composed of ephedrine and caffeine. To our knowledge, no prior studies looked at weight loss products in relation to SAB. A National Birth Defects Prevention Study reported that periconceptional use of weight loss products containing ephedra (a botanical source of the alkaloid ephedrine) was associated with increased risk for some birth defects although the effect-measure estimates were imprecise [40]. While the relation between diet drugs and SAB has not been studied, an extensive literature addresses caffeine and SAB [3–26]. Opinions published in both the United States and the United Kingdom could not reach consensus regarding the relation between consumption of high doses of caffeine during pregnancy and SAB, but both recommended women limit their consumption to less than 200 mg per day [27,28].

High coffee consumption (greater than 4 cups per day) during pregnancy is associated with late SAB in the Danish National Birth Cohort [4], which contrasts with the results for periconceptional Letigen consumption. The results may differ for several reasons. First, the study of coffee and SAB only included late losses because pregnancies interviewed after the outcome were excluded to avoid recall bias. In contrast, earlier losses were included for periconceptional Letigen use, which was reported at enrollment, precluding recall bias. Second, although both coffee and Letigen contain caffeine, coffee also contains other substances that may affect SAB. Additionally, caffeine levels from coffee were likely precluding recall bias. In contrast, caffeine levels from coffee were likely more variable and more likely to be misclassified.

SAB and a Diet Drug of Caffeine and Ephedrine
of use during early pregnancy may have been brief or sporadic. Letigen use was reported for an average of less than three weeks during the exposure period of interest. It is possible that regular exposure to high doses of caffeine affects SAB but sporadic exposure to high doses does not.

One concern in studies of caffeine consumption during pregnancy is that pregnancy induced nausea can lead to food and beverage aversions, while lack of nausea is strongly associated with an increased risk of SAB. As a result, observed associations between caffeine and SAB could be spurious \([10,30,32,33]\). An advantage of assessing pre-pregnancy caffeine consumption is that the exposure predates the onset of nausea so any observed association is not a result of nausea’s effect on caffeine consumption. However, pre-conception caffeine exposure cannot be generalized to caffeine exposure during pregnancy because effects may differ during different developmental periods. In addition, pre-conception caffeine exposure may have an effect on early pregnancy loss (<5 completed weeks), but not on clinically recognized SAB.

Women tended to cease using Letigen once they became aware of their pregnancy. Therefore, there was limited early pregnancy exposure in this study, but Letigen users were exposed to caffeine for longer periods prior to conception. Researchers examining pre-pregnancy (beverage) caffeine consumption and SAB \([9,10,12,14,18–20,22]\) reported inconsistent results even for high levels of caffeine. However, the effect-measure estimates for high caffeine intake prior to pregnancy are smaller than estimates for high caffeine intake during pregnancy in studies that looked at both periods. We also found a stronger association between SAB and Letigen use during early pregnancy compared to pre-conceptional use although relatively few women consumed Letigen in early pregnancy exclusively. The association between SAB and Letigen use during both periods was essentially null.

We did not have adequate data to evaluate whether caffeine from other medications might confound the association between Letigen and SAB. Some painkillers and migraine medications that contained caffeine were available during the study period. These types of drugs were likely used sporadically and had much lower caffeine content \((50–100 \text{ mg/pill})\) than Letigen \((200 \text{ mg/pill})\). Therefore, it seems likely that few study participants were regularly exposed to high doses of caffeine from other medications.

We evaluated whether caffeine from beverages could confound this study. Although women were asked about coffee, tea, and cola consumption, the questions were not anchored to a specific time period. We considered whether reported caffeine intake from beverages could approximate periconceptional caffeine consumption as well as whether caffeine from beverages might be an intermediate between Letigen and SAB. If the latter were true, adjustment for beverage caffeine would be inappropriate. Only cola consumption appeared to be associated with Letigen use in our data. High prior cola consumption could contribute to obesity, which could lead to Letigen use, or alternatively, obesity could lead to both Letigen use and diet cola consumption. In both of these scenarios cola could be a confounder if caffeine from cola caused SAB. Adjustment for cola consumption did not change the results appreciably \((1.0; 0.7–1.5)\) although residual confounding due to imprecise measurement of cola consumption is possible.

In the Danish National Birth Cohort, periconceptional use of a diet pill containing ephedrine and caffeine does not appear to be appreciably associated with clinically recognized SAB.

**Acknowledgments**

The authors thank Gitte Nielsen for help with the translations; Antonio Sylvester Vethanayagam for technical support; and Dr. Ivanka Orozova-Bekkevold, Inge Eiseeure, and Lone Fredshlund Møller for data extraction. The authors thank the Helen Riaboff Whiteley Center for providing a work environment conducive to writing this manuscript.

**Author Contributions**

Conceived and designed the experiments: PPH IHP BHB EAN AMNA CP JO. Analyzed the data: PPH. Contributed reagents/materials/analysis tools: JO. Wrote the paper: PPH IHP EAN AMNA CP JO.

**References**

1. U.S. Food and Drug Administration (2012) Summary Minutes of the Endocrinologic and Metabolic Drugs Advisory Committee Meeting February 22, 2012.
2. Due P, Heimann BL, Sorensen TI (2007) Prevalence of obesity in Denmark. Obes Rev 8: 187–189.
3. Armstrong BG, McDonald AD, Sloan M (1992) Cigarette, alcohol, and coffee consumption and spontaneous abortion. Am J Public Health 82: 85–87.
4. Bech BH, Nohr EA, Vaeth M, Henriksen TB, Olsen J (2003) Caffeine and fetal death: a cohort study with prospective data. Am J Epidemiol 162: 983–990.
5. Cnattingius S, Signorollo LB, Anneren G, Clausson B, Ekblom A, et al. (2000) Caffeine intake and the risk of first-trimester spontaneous abortion. N Engl J Med 343: 1839–1845.
6. Dlugosz L, Belanger K, Hellenbrand K, Holford TR, Leaderer B, et al. (1996) Maternal caffeine consumption and spontaneous abortion: a prospective cohort study. Epidemiology 7: 250–255.
7. Dominguez-Rojas V, de-Juarez-Pardo JR, Astacio-Ariza P, Ortega-Molina P, Gordillo-Flores R (1994) Spontaneous abortion in a hospital population: are tobacco and coffee intake risk factors? Eur J Epidemiol 10: 663–668.
8. Fenster L, Eskenazi B, Windham GC, Swan SH (1991) Caffeine consumption during pregnancy and spontaneous abortion. Epidemiology 2: 168–174.
9. Fenster L, Hubbard AE, Swan SH, Windham GC, Waller K, et al. (1997) Caffeinated beverages, decaffeinated coffee, and spontaneous abortion. Epidemiology 8: 515–523.
10. Giannelli M, Doyle P, Roman E, Munk M, Simonsen L (2005) Caffeine and alcohol intake before pregnancy predict the occurrence of spontaneous abortion. Hum Reprod 18: 2704–2710.
11. Greenwood DC, Alwan N, Boylan S, Cade JE, Charvill J, et al. (2010) Caffeine and smoke: how does it affect pregnancy outcomes? BJOG 117: 107–108.
12. Kline J, Levitov S, Silverman JS, Kinney AM, Stein Z, et al. (1997) Caffeine and spontaneous abortion of known karyotype. Epidemiology 8: 409–417.
13. Klebanoff MA, Levine RJ, DerSimonian R, Clemens JD, Wilkins DG (1999) Caffeine use and the risk of spontaneous abortion. N Engl J Med 341: 1639–1644.
14. Kline J, Levin B, Silverman J, Kinney A, Stein Z, et al. (1997) Caffeine and spontaneous abortion of known karyotype. Epidemiology 2: 409–417.
15. Macnoche N, Doyle P, Prior S, Siminos R (2007) Risk factors for first trimester miscarriage–results from a UK-population-based case-control study. BJOG 114: 170–186.
16. Milhui JI, Holmes LB, Arrows JM, Simpson JL, Brown ZA, et al. (1993) Moderate caffeine use and the risk of spontaneous abortion and intrauterine growth retardation. JAMA 269: 593–597.
17. Parazzini F, Bocciolone L, Federle I, Negri E, La Vecchia C, et al. (1993) Risk factors for spontaneous abortion. Int J Epidemiol 20: 157–161.
18. Parazzini F, Chatenoud L, Di Cintio E, Mezzopane R, Surace M, et al. (1998) Caffeine consumption and risk of hospitalized miscarriage before 12 weeks of gestation. Hum Reprod 13: 2286–2291.
19. Parazzini F, Chatenoud L, La Vecchia C (1994) Fetal loss and caffeine intake. JAMA 272: 20–29.
20. Pollack AZ, Buck Louis GM, Sundaram R, Lum KY (2010) Caffeine consumption and miscarriage: a prospective cohort study. Fertility and Sterility 93: 304–306.
21. Rasch V (2005) Cigarette, alcohol, and caffeine consumption: risk factors for spontaneous abortion. Acta Obstet Gynecol Scand 84: 152–188.
22. Savitz DA, Chau RN, Herring AH, Howards PP, Hartmann KE (2008) Caffeine and miscarriage risk. Epidemiology 19: 53–62.
23. Tompkins JS, Kaiser SK, Munk C, Madsen LB, Otsen B, et al. (2003) Does caffeine and alcohol intake before pregnancy predict the occurrence of spontaneous abortion? Hum Reprod 18: 2704–2710.
24. Wathersbee PS, Olsen LK, Lodge JR (1977) Caffeine and pregnancy. A retrospective survey. Postgrad Med 62: 64–69.
25. Wen W, Shu XO, Jacobs DR Jr., Brown JE (2001) The associations of maternal caffeine consumption and nausea with spontaneous abortion. Epidemiology 12: 31–42.
26. Weng X, Oduoli R, Li DK (2008) Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. Am J Obstet Gynecol 198: 279.e271–278.
27. (2010) ACOG Committee Opinion No. 462: Moderate caffeine consumption during pregnancy. Obstetrics and Gynecology 116: 467–468.
28. Food Standards Agency (2008) Food Standards Agency publishes new caffeine advice for pregnant women. Food Standards Agency, United Kingdom.
29. Dlugosz L, Bracken MB (1992) Reproductive effects of caffeine: a review and theoretical analysis. Epidemiol Rev 14: 83–100.
30. Feinstreit L, Windham GC, Swan SH, Ekenozzi B (1991) Miscarriage, caffeine, and the epiphenomena of pregnancy: the causal model. Epidemiology 2: 313.
31. Kline J, Levin B, Kinney A, Stein Z, Suss M, et al. (1994) Fetal loss and caffeine intake. JAMA 272: 27–28, author reply 28–29.
32. Sguerillo LB, McLaughlin JK (2004) Maternal caffeine consumption and spontaneous abortion: a review of the epidemiologic evidence. Epidemiology 15: 229–239.
33. Stein Z, Suss M (1991) Miscarriage, caffeine, and the epiphenomena of pregnancy: the causal model. Epidemiology 2: 163–167.
34. Danish Medicines Agency (2003) Company Account 2002. In: Agency DM, editor.
35. U.S. Food and Drug Administration (2004) FDA Announces Rule Prohibiting Sale of Dietary Supplements Containing Ephedrine Alkaloids Effective April 12. U.S. Food and Drug Administration.
36. Bracken MB, Triche E, Grosso L, Hellenbrand K, Belanger K, et al. (2002) Heterogeneity in assessing self-reports of caffeine exposure: implications for studies of health effects. Epidemiology 13: 165–171.
37. Olsen J, Melbye M, Olsen SF, Sorensen TI, Aaby P, et al. (2001) The Danish National Birth Cohort—its background, structure and aim. Scand J Public Health 29: 300–307.
38. (2002) Lægemiddelkataloget 2002. København, Denmark: Dansk Lægemiddel Information A/S.
39. Barone JJ, Roberts HR (1996) Caffeine consumption. Food Chem Toxicol 34: 119–129.
40. Bitsko RH, Reefbuis J, Louie C, Weeler M, Feldkamp ML, et al. (2008) Periconceptional use of weight loss products including ephedra and the association with birth defects. Birth Defects Res A Clin Mol Teratol 82: 553–562.
41. Howards PP, Hertz-Picciotto I, Poole C (2007) Conditions for bias from differential left truncation. Am J Epidemiol 165: 444–452.
42. Howards PP, Hertz-Picciotto I, Weinberg CR, Poole C (2006) Misclassification of gestational age in the study of spontaneous abortion. Am J Epidemiol 164: 1126–1136.