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Low-dose aspirin use in pregnancy and the risk of preterm birth: a Swedish register-based cohort study.

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Condensation: Low-dose aspirin reduces the risk of recurrent preterm birth.

Short title: Low-dose aspirin and recurrent preterm birth

AJOG at a glance

Why was this study conducted?

- Women who have had a previous preterm birth (medically indicated or spontaneous onset) are at increased risk of preterm birth in their subsequent pregnancies.
- Low-dose aspirin reduces the risk of preterm birth among women at risk of developing preeclampsia, but the effect on preterm birth among women with a previous preterm birth is unknown.

B. What are the key findings?

- Low-dose aspirin use was associated with a reduced risk of preterm birth (any onset) and preterm birth with a spontaneous onset.

C. What does this study add to what is already known?

- Low-dose aspirin could be an effective prophylaxis for recurrent preterm birth.
Abstract

Background: Preterm birth is the leading cause of neonatal mortality and morbidity. Women who have had a previous preterm birth are at increased risk of preterm birth in their subsequent pregnancies. Low-dose aspirin reduces the risk of preterm birth among women at risk of developing preeclampsia, however it is unclear whether low-dose aspirin may reduce the risk of recurrent preterm birth.

Objectives: The purpose of this study was to investigate the association between low-dose aspirin and preterm birth among women with a previous preterm birth.

Study Design: We conducted a Swedish register-based cohort study and included women who had a first and second pregnancy between 2006 and 2019, where the first pregnancy resulted in preterm birth (medically indicated or with spontaneous onset <37 weeks of gestation). The association between low-dose aspirin use and preterm birth in the second pregnancy was estimated via logistic regression via standardization and expressed as marginal relative risks (mRR) with 95% confidence interval (CI).

Results: Among the study cohort (N=22,127), 3057 women (14%) were prescribed low-dose aspirin in their second pregnancy and 3703 women (17%) gave birth preterm. Low-dose aspirin use was associated with a reduced risk of preterm birth, (mRR 0.87 95% CI 0.77-0.99). There were no statistically significant associations between low-dose aspirin use and an altered risk of moderate preterm birth, defined as birth 32-36 weeks’ gestation (mRR 0.90 95% CI 0.78-1.03) or very preterm birth, defined as birth <32 weeks’ gestation (mRR 0.75 95% CI 0.54-1.04). Regarding onset of preterm birth, low-dose aspirin use was associated with a reduced risk of spontaneous preterm birth (mRR 0.70 95% CI 0.57-0.86) but no reduced risk of medically indicated preterm birth (mRR 1.09 95% CI 0.91-1.30) was observed.

Conclusion: Among women with a previous preterm birth, low-dose aspirin use was associated with a reduced risk of preterm birth. When investigating preterm birth by onset in the second
pregnancy, low dose aspirin use was associated with a reduced risk of spontaneous preterm birth. Our results suggest that low-dose aspirin may be an effective prophylaxis for recurrent preterm birth.

**Key words**

Aspirin, preterm birth, adverse pregnancy outcome, prevention
Introduction

Preterm birth, defined as birth prior to 37 weeks’ gestation, claims the lives of approximately one million children every year \(^1\). Women who have had a previous preterm birth (medically indicated or with spontaneous onset) are at increased risk of preterm birth in their subsequent pregnancy \(^2-4\).

Low-dose aspirin has been shown to reduce the risk of preeclampsia, a pregnancy condition characterized by hypertension and organ injury. In addition, low-dose aspirin has been shown to protect against preterm birth among women at risk of developing preeclampsia \(^5\). There is also a growing body of evidence suggesting that low-dose aspirin use could be associated with a reduced risk of preterm birth and in particular spontaneous preterm birth in women without major risk factors for preeclampsia \(^6-8\). Still, there is insufficient evidence regarding the use of low-dose aspirin in pregnant women with a previous preterm birth. The APRIL study, a randomized controlled trial with 406 participants, reported a small but non-significant reduction of preterm birth in women using low-dose aspirin with a previous spontaneous preterm birth \(^9\). However, the study was only powered to detect a difference in preterm birth greater than 35% between groups and the included population had a lower-than-expected preterm birth rate. A larger study is needed to investigate whether low-dose aspirin can prevent recurrent preterm birth \(^10\). Thus, we undertook a population-based study, including over 22,000 women with a previous preterm birth, to investigate whether low-dose aspirin was associated with an altered risk of preterm birth occurring before \(<37\) weeks’ gestation, moderate preterm birth \(32-36\) weeks’ gestation, and very preterm birth \(<32\) weeks’ gestation, and the association between low-dose aspirin use and the mode of onset of preterm birth.
Material and Methods

Design and participants

We performed a register-based cohort study using the Swedish Medical Birth Register, the Swedish Prescribed Drug Register, and the education register held by Statistics Sweden. The study was approved by the Ethical Review Board at Uppsala on the 28th of January 2020 (Dnr 2019-04925) and on the 26th of August 2021 (Dnr 8311/2020). The registers were linked using the personal identity number assigned to each Swedish resident at birth or immigration to Sweden. The Swedish Medical Birth Register provides information about pregnancies, labor, and perinatal outcomes from standardized medical records. The data is entered prospectively by health care providers during episodes of prenatal, delivery, and neonatal care. The register covers >98% of all births in Sweden and the information has been validated. The Swedish Prescribed Drug Register started in 2005 and contains patient-level data on all dispensed prescribed drugs in Sweden, using the World Health Organization’s Anatomical Therapeutic Chemical Classification (ATC) codes. The register is complete for the entire Swedish population with <0.3% missing data of all dispensed prescriptions. The education register held by Statistics Sweden holds information about the highest level of education obtained. The data is reported by professional and administrative personnel and the data quality is controlled through regular audits.

The study population consisted of all women with a first and second singleton birth recorded in the Medical Birth Register from 2006 to 2019, and who, in their first pregnancy, gave birth preterm. Preterm birth was defined as a spontaneous or medically indicated birth between 22 weeks + 0 days to 36 weeks + 6 days.
Covariates

Pregnancy variables from the first and second pregnancy were obtained from the Medical Birth Register and included conception via in-vitro fertilization (including intracytoplasmic sperm injection), interpregnancy interval, pregestational disorders (including chronic hypertension, diabetes, chronic kidney disease, systemic lupus erythematosus), preeclampsia, placental abruption, cesarean section, small for gestational age (SGA) birth, and stillbirth. The interpregnancy interval was defined as months from first birth until next conception and categorized into less than six months and six months and above. SGA birth was defined as birthweight below two standard deviations according to Swedish growth charts. Information on conception via in-vitro fertilization, pre-gestational disorders and stillbirth was retrieved from predefined checkboxes in the Medical Birth Register and was self-reported by the women.

Information on maternal demographics during the second pregnancy were obtained from the Medical Birth Register and included maternal age at delivery, height, body mass index (BMI) and smoking (yes/no) and country of birth. In addition, information on the highest obtained education level (university, upper secondary school degree, or <12 years of school attendance) was retrieved from Statistics Sweden.

Exposure

The primary exposure was low-dose aspirin use during the second pregnancy (regardless of dose and duration) based on data obtained from dispensed prescriptions from the Swedish Prescribed Drug Register. Low-dose aspirin use (ATC code B01AC06) was defined as at least one dispensed prescription during pregnancy and included women with a prescription of 75-160 mg aspirin from three months prior to conception. During the study period, there was no national guideline on aspirin prescription in pregnancy and different regions had different
routines. According to the Swedish Society of Obstetrics and Gynecology, most commonly, only women presenting with major risk factors for preeclampsia were prescribed aspirin at a dosage of 75 mg/day. In a few hospitals in the country, the recommended dosage was 160 mg. Low-dose aspirin is only available by prescription in Sweden and aspirin in higher doses that are available over the counter are not recommended during pregnancy.

174 **Outcomes**

The primary outcome was preterm birth in the second pregnancy. Gestational age was based on a first or early second-trimester ultrasound in 93% of the pregnancies. In the remaining pregnancies, gestation age was based on date of the last menstrual period reported at the first antenatal visit, of embryo transfer or a postnatal assessment (<1%)\(^\text{12} \) We further studied preterm birth by severity and onset. Severity was categorized into moderate preterm birth (birth between 36 – 32 weeks’ gestation) and severe preterm birth (birth <32 weeks’ gestation). Preterm birth by onset was categorized into spontaneous and medically indicated. The onset of birth was registered in a standardized manner at the delivery ward by midwives, using checkboxes in the medical record. Spontaneous onset included pre-term labor or preterm premature rupture of the membranes (identified by ICD-code O42). Medically indicated preterm birth included vaginally induced onset of labor and cesarean delivery before onset of labor unless preterm premature rupture of the membranes were present. Information on the gestational age at delivery and the onset of birth was obtained from the Medical Birth Register. Information on the onset of labor was missing for 174 (0.8%) of the preterm births in the first pregnancy and for 24 (0.1%) of the preterm births in the second pregnancy.
**Statistical analysis**

An a-priori statistical analysis plan was agreed upon by all authors. The marginal relative risk (mRR) of preterm birth for women using low-dose aspirin was compared to women not using low-dose aspirin and was computed via standardization from logistic regression models\textsuperscript{17}, adjusted for confounders as outlined below. Standard errors were based on the delta method \textsuperscript{17}. Continuous variables were modelled with natural cubic splines with 3 degrees of freedom in all models to allow a non-linear association with the outcome. R (version 4.2.0) and SPSS Statistics for Windows, version 28.0 (IBM Corp., Armonk, N.Y., USA) was used for all statistical calculations.

**Conceptual model**

A theoretical framework and directed acyclic graphs (DAGs) were used to identify covariates. DAGs serve as a visual representation of the hypothesized relationship between variables and can help to identify the presence of confounding and ways to resolve it\textsuperscript{18}. We included covariates from the first and second pregnancy that could increase the probability of low-dose aspirin prescription (in other words: risk factors for preeclampsia) and the probability of preterm birth. Based on the DAGs the covariates maternal age, BMI, country of birth (divided into Nordic or non-Nordic countries), gestational length of first pregnancy, SGA in the first pregnancy, preeclampsia in the first pregnancy, type of onset of preterm birth in the first pregnancy, pregestational disorders, and date of birth were chosen to estimate the association between low-dose aspirin use and preterm birth regardless of mediation (Supplemental Figure).
218 Subgroup analysis
We analyzed rates of preterm birth in the second pregnancy among women with a previous spontaneous preterm birth and a medically indicated preterm birth separately, adjusting for the same covariates as above, except for onset of labor in the first pregnancy.

232 Results
234 Sample characteristics
The study population consisted of 22,127 women who experienced a preterm birth in their first pregnancy, of which 3,057 women (14%) were prescribed low-dose aspirin in their second pregnancy. In total, 3,703 women (17%) had a recurrent preterm birth and 547 of them (15%) used low-dose aspirin during the second pregnancy. The population is presented in a flow chart (Figure).

The women who used low-dose aspirin were in average 31.5 years old and had a BMI of 25.9, compared to an average age of 30.6 years and an average BMI of 25.0 in the women who did not use aspirin. Pregestational disorders and preeclampsia were common among women using aspirin, 11.1% of the women had at least one pregestational disorder in the second pregnancy and 16.7% were diagnosed with preeclampsia in the second pregnancy (Table 1).

Primary outcome
The incidence of preterm birth in the second pregnancy was 17.9% among women using low-dose aspirin and 16.6% for women not using low-dose aspirin. This resulted in a crude relative risk (RR) of 1.08 (95% CI 1.00-1.17) and a marginal relative risk (mRR) of 0.87 (95% CI 0.77-0.99) for preterm birth for women using low-dose aspirin compared with those not (Table 2).
Preterm birth by severity

Compared with women not using low-dose aspirin, women using low-dose aspirin had an increased crude relative risk of 1.72 (95% CI 1.30-2.27) for preterm birth occurring before 32 weeks’ gestation but not for preterm birth 32-36 weeks’ gestation (RR 0.98 95% CI 0.85-1.12). After adjusting for confounders, low-dose aspirin use was not associated with a reduced risk of preterm birth <32 weeks’ gestation (mRR 0.75 95% CI 0.54-1.04) or 32-36 weeks’ gestation (mRR 0.90 95% CI 0.78-1.03) (Table 2).

Preterm birth by onset

Compared with women not using low-dose aspirin, women using low-dose aspirin had an increased crude relative risk of 3.46 (95% CI 2.92-4.10) for medically indicated preterm birth. However, this risk was reduced and no longer statistically significant after adjusting for confounders (mRR 1.09 95% CI 0.91-1.30). Low-dose aspirin use was associated with a reduced crude relative risk for spontaneous preterm birth of 0.42 (95% CI 0.34-0.53) that remained after adjusting for confounders (mRR 0.70 95% CI 0.57-0.86) (Table 2).

There was no statistically significant association between low-dose aspirin and onset of labor among women with a first medically indicated preterm birth (Supplemental table 1). Among women with a spontaneous preterm birth in their first pregnancy, low-dose aspirin was associated with an increased risk of medically indicated preterm birth (mRR 1.93 95% CI 1.28-2.91) and a decreased risk of spontaneous preterm birth (mRR 0.75 95% CI 0.57-1.00) (Supplemental table 2) that was not statistically significant.
Comment

Principal findings

In this register-based cohort study on women with a previous preterm birth we found an association between low-dose aspirin use and preterm birth occurring <37 weeks’ gestation, but no statistically significant association between low-dose aspirin use and moderate preterm birth 32-36 weeks’ gestation or very preterm birth <32 weeks’ gestation. When separating the risk of spontaneous and medically indicated preterm births in the second pregnancy, we found that low-dose aspirin use was associated with a reduced risk of spontaneous preterm birth <37 weeks’ gestation.

Results in the context of what is known

The incidence of recurrent preterm birth in our study is lower than generally reported\(^2\), but in line with the incidence of recurrent preterm birth in other Nordic countries\(^3,4\).

The ASPIRIN trial investigated low-dose aspirin treatment for the prevention of preterm birth in healthy nulliparous pregnant women and reported that low-dose aspirin reduced the risk of preterm birth <37 weeks’ gestation (aRR 0.89 95% CI: 0.81-0.98) and preterm birth <34 weeks’ gestation (aRR 0.75 95% CI 0.61-0.93)\(^6\). Our point estimate for preterm birth <37 weeks’ gestation is similar although our study population had a higher incidence of preterm birth, which may be attributed to our study population that included women with a previous preterm birth.

We did not find a statistically significant association between low-dose aspirin use and moderate or severe preterm birth, but compared to a randomized controlled trial, it is less certain that women had an actual aspirin intake in our study.

A secondary analysis of a randomized controlled trial has found a reduction of spontaneous preterm birth <34 weeks’ gestation (adjusted odds ratio 0.46 95% CI 0.23-0.89) but not <37
weeks’ gestation in healthy nulliparous pregnant women using low-dose aspirin, whereas a
meta-analysis reported a reduction of spontaneous preterm birth for both <34 weeks’ gestation
(RR 0.86 95% CI 0.76-0.99) and <37 weeks’ gestation (RR 0.93 95% CI 0.86-0.996) for low-
dose aspirin treatment in women at high risk of developing preeclampsia.

Similar to the APRIL study, which found no statistically significant association between low-
dose aspirin and preterm birth among women with a previous spontaneous preterm birth (RR
0.83 95% CI 0.58-1.20), our subgroup analysis of the risk of recurrent spontaneous preterm
birth showed no significant association (mRR 0.75 95% CI 0.57-1.00). However, a small
protective effect cannot be ruled out and we believe that our findings could support the
hypothesis that low-dose aspirin might decrease the risk of recurrent spontaneous preterm birth,
even though we did not reach statistical significance. In our primary analysis, including women
with a first medically indicated or spontaneous preterm birth, low-dose aspirin reduced the risk
of both preterm birth and spontaneous preterm birth in the second pregnancy, which could be
attributed to a larger study population.

**Clinical implications**

Our data suggest that low-dose aspirin may be an effective prophylaxis for recurrent preterm
birth with an effect size similar to the preventative effect of low-dose aspirin on the
development of preeclampsia in women at high risk. Though, low-dose aspirin has potential
side effects and has been associated with an increased risk of intrapartum and postpartum
hemorrhage. This warrants caution and treatment of the right target population at high risk of
developing the condition at the lowest effective dose is important.
Research implications

A larger randomized controlled trial or an individual patient data meta-analysis of smaller randomized controlled trials are warranted to confirm our findings of a protective effect of low-dose aspirin on the risk of recurrent preterm birth. In Sweden the recommended low-dose aspirin dose during pregnancy is 75mg per day and the effect of higher doses, such as 150mg, needs to be further investigated.

Strengths and limitations

The main strengths of our study are the population-based setting that facilitates generalizability to the target population, and the large study sample. We utilized data from national registers that capture data of >98% of the Swedish population and contains information on maternal characteristics, socio-demographic factors and pregnancy variables prospectively collected to the outcome, and available for adjustments in the models.

Our study is limited by the baseline differences between women using low-dose aspirin and those not, which we attempted to overcome in our adjusted modelling by adjusting for gestational length first pregnancy and other covariates that affect the risk of preterm birth. Still, there is a risk that our model might under- or overestimate the preventive effect of low-dose aspirin due to residual confounding. To overcome this, we performed subgroup analyses by spontaneous and medically indicated preterm births in the second pregnancy that showed an association between low-dose aspirin use and a reduced risk of spontaneous preterm birth. However, the low-dose aspirin group had a higher incidence of preeclampsia in the first pregnancy and therefore a higher risk of preeclampsia and medically indicated preterm birth in the second pregnancy. It is also possible that the women in the low-dose aspirin group were more closely monitored and therefore had a medically indicated preterm birth in cases that
otherwise would have resulted in a spontaneous preterm birth. Hence, our model might have overestimated the preventive effect of low-dose aspirin on spontaneous preterm birth. Although our data on low-dose aspirin use is based on dispensed prescriptions, since low-dose aspirin is not available over the counter in Sweden, we do not know the compliance and for which time-period in the pregnancy low-dose aspirin was used.

Conclusion

In this population register-based cohort study, we found that among women with a previous preterm birth, low-dose aspirin use reduced the risk of recurrent preterm birth (any onset) and specifically spontaneous onset. The use of low-dose aspirin in pregnant women with a previous preterm birth could reduce the global burden of preterm birth, but further research is needed to confirm our findings and investigate the optimal low-dose aspirin dosage and timing of use.
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Table 1. Background characteristics in the second pregnancy for women with a preterm birth (PTB) in their first pregnancy, with and without low-dose aspirin prescription in second pregnancy

| Characteristic                        | Missing n= | Total births n=22 127 | No n=19068 | Yes n=3057 |
|---------------------------------------|------------|-----------------------|------------|------------|
| **Second pregnancy**                  |            |                       |            |            |
| Age at delivery (years)               | 2          | 30.7 ± 4.8            | 30.6 ± 4.7 | 31.5 ± 5.1 |
| ≥35                                   |            | 4792 (21.7)           | 3936 (20.6)| 856 (28.0) |
| Height (cm)                           | 231        | 165.5 ± 6.4           | 165.6 ± 6.4| 165.4 ± 6.5|
| Body mass index (kg/m\(^2\))\(a\)    | 1355       | 25.1 ± 5.0            | 25.0 ± 4.9 | 25.9 ± 5.4 |
| Body mass index ≥30                   | 3232       | 2648 (15.6)           | 2648 (14.8)| 584 (20.2) |
| Country of birth                      | 2          |                       |            |            |
| Nordic                                |            | 18135 (82.0)          | 14656 (82.1)| 2479 (81.1)|
| Non-Nordic European\(b\)             | 1017       | 898 (4.7)             | 119 (3.9)  |
| Rest of the world\(†\)               | 2973       | 2512 (13.2)           | 458 (15.0) |
| Smoking first antenatal\(b\) visit   | 1161       | 1011 (4.8)            | 876 (4.8)  | 135 (4.7)  |
| In vitro fertilization                |            | 685 (3.1)             | 554 (2.9)  | 131 (4.3)  |
| Education                             |            | 122                   |            |            |
| University                            | 12886      | 11080 (58.4)          | 1806 (59.4)|            |
| Upper secondary school                | 6148       | 5340 (28.2)           | 808 (26.6) |
|                                      | First pregnancy |            |            |            |
|--------------------------------------|----------------|------------|------------|------------|
| < 12 years of school attendance      |                | 2971 (13.5)| 2543 (13.4)| 428 (14.1) |
| Interpregnancy interval (months)     | 7              | 26.21 ± 18.56 | 26.19 ± 18.4 | 26.35 ± 19.8 |
| <6                                   |                | 1216 (5.5) | 946 (5.0) | 270 (8.8) |
| ≥ 6                                  |                | 20904 (94.5) | 18117 (95.0) | 2787 (91.2) |
| Pre-gestational disorders            |                | 999 (4.5)  | 660 (3.5) | 339 (11.1) |
| Chronic hypertension                 |                | 309 (1.4)  | 152 (0.8) | 157 (5.1) |
| Diabetes                             |                | 528 (2.4)  | 411 (2.2) | 117 (3.8) |
| Chronic kidney disease               |                | 182 (0.8)  | 118 (0.6) | 64 (2.1) |
| Systemic lupus erythematosus         |                | 68 (0.3)   | 21 (0.1)  | 47 (1.5) |
| Pregnancy outcomes                   |                |            |            |            |
| Preeclampsia                         |                | 1136 (5.1) | 625 (3.3) | 511 (16.7) |
| Placental abruption                  |                | 146 (0.7)  | 102 (0.5) | 44 (1.4) |
| Gestational diabetes                 |                | 550 (2.5)  | 444 (2.3) | 106 (3.5) |
| Cesarean section                     | 26             | 4544 (20.6) | 3284 (17.2) | 1260 (41.2) |
| Small for gestational age            | 45             | 621 (2.8)  | 394 (2.1) | 226 (7.4) |
| Stillbirth                           | 2              | 107 (0.5)  | 87 (0.5)  | 20 (0.7) |

**First pregnancy**

|                                      |            |            |            |
|--------------------------------------|------------|------------|------------|
| Gestational age at delivery (weeks)  | 33.8 ± 3.1 | 34.1 ± 2.9 | 31.9 ± 3.7 |
| Moderate preterm birth 32-36 weeks   | 18584 (84.0) | 16682 (87.5) | 1902 (62.2) |
| Outcome                                      | No aspirin use n = 19070 | Aspirin use n= 3057 |
|----------------------------------------------|--------------------------|---------------------|
| Very preterm birth <32 weeks                 | 3543 (16.0)              | 2388 (12.6)         | 1155 (37.8) |
| Medically indicated preterm birth            | 5927 (27.0)              | 3407 (18.0)         | 2520 (83.5) |
| Spontaneous preterm birth                    | 16026 (73.0)             | 15528 (82.0)        | 498 (16.5)  |
| Preeclampsia                                 | 3212 (14.5)              | 1362 (7.1)          | 1850 (60.5) |
| Placental abruption                          | 614 (2.8)                | 427 (2.2)           | 187 (6.1)   |
| Cesarean section                             | 6880 (31.2)              | 4752 (25.0)         | 2131 (70.0) |
| Small for gestational age                    | 2558 (11.7)              | 1147 (6.1)          | 1411 (47.0) |
| Stillbirth                                   | 1040 (4.7)               | 606 (3.2)           | 434 (14.2)  |

Data are presented as n (%) or mean ± SD.

*a* BMI at first antenatal visit.

*b* Combined in the adjusted analysis.

Table 2. Association between low-dose aspirin use and preterm birth in the second pregnancy.
Frequencies (n) and percent (%).

| Outcome                                | n     | N (%) | Odds Ratio (95% CI) | p-value |
|----------------------------------------|-------|-------|---------------------|---------|
| Preterm birth <37 weeks                | 3156  | 16.6  | 1.08 (1.00-1.17)    | 0.77-0.99 |
| Secondary outcomes                     |       |       |                     |         |
| Moderate preterm birth 32 - 36 weeks   | 2717  | 14.2  | 0.98 (0.85-1.12)    | 0.78-1.03 |
| Very preterm birth <32 weeks           | 439   | 2.4   | 1.72 (1.30-2.27)    | 0.54-1.04 |
| Medically indicated preterm birth <37 weeks | 676  | 3.5   | 3.46 (2.92-4.10)    | 0.91-1.30 |
| Spontaneous preterm birth <37 weeks    | 2467  | 12.9  | 0.42 (0.34-0.53)    | 0.57-0.86 |

n=22127 and n=20464 included in the crude and adjusted models, respectively. Primary outcome was estimated in a binomial logistic regression and secondary outcomes in multinomial logistic regression.

Figure. Flow chart describing participant inclusion.
MBR, Medical Birth Register.

Supplemental Figure. Directed acyclic graph.
PTB, Preterm birth; GL1, gestational length first pregnancy; SGA1, small for gestational length first pregnancy; Abruption1, placental abruption first pregnancy; PE1, preeclampsia first pregnancy; Onset1, medically indicated first birth; SGA2, small for gestational length second pregnancy; PE2, preeclampsia second pregnancy; Abruption2, placental abruption second pregnancy; pregest disorder, pregestational disorder first pregnancy; IP, interpregnancy interval; IVF, in vitro fertilization; BMI, body mass index second pregnancy; year, date of birth.

Adjustment sets for estimating the total effect, referring to the set of covariates that closes all biasing paths and leaves all causal paths open, of low-dose aspirin use on preterm birth: age, BMI, country of birth, gestational length first pregnancy, small for gestational length first pregnancy, small for gestational length second pregnancy.
pregnancy, preeclampsia first pregnancy, pregestational disorder second pregnancy, date of birth and medically indicated first birth (www.dagitty.net).
Women with singleton pregnancies registered in MBR with their first and second birth between 2006-2019  
\( n = 396,957 \)

- First birth fullterm  
  \( n = 374,711 \)
- Gestational week at birth missing  
  \( n = 104 \)
  - Misclassified with the first birth before 2006  
    \( n = 15 \)
- First birth preterm < 37 weeks  
  \( n = 22,127 \)
  - Aspirin second pregnancy  
    \( n = 3,057 (14\%) \)
  - No aspirin second pregnancy  
    \( n = 19,070 (86\%) \)
### Appendix

**Supplemental Table 1. Association between low-dose aspirin use and preterm birth in women with a first medically indicated preterm birth.**

| Outcome                          | No aspirin n= 3407 | Aspirin use n= 2520 | Relative risk (95% confidence interval) | Crude | Adjusted |
|----------------------------------|--------------------|---------------------|------------------------------------------|-------|----------|
| **Primary outcome**              |                    |                     |                                          |       |          |
| Preterm birth <37 weeks          | 547 (16.1)         | 433 (17.2)          | 1.07 (0.95-1.20)                         | 0.94  | (0.80-1.10) |
| **Secondary outcomes**           |                    |                     |                                          |       |          |
| Moderate preterm birth 32 - 36 weeks | 452 (13.7)     | 341 (14.0)          | 1.02 (0.85-1.23)                         | 0.98  | (0.83-1.17) |
| Very preterm birth <32 weeks     | 95 (3.2)           | 92 (4.2)            | 1.31 (0.88-1.95)                         | 0.74  | (0.49-1.11) |
| Medically indicated preterm birth <37 weeks | 353 (11.0) | 333 (13.8)          | 1.28 (1.05-1.56)                         | 1.02  | (0.85-1.22) |
| Spontaneous preterm birth <37 weeks | 192 (6.3)       | 96 (4.4)            | 0.68 (0.48-0.95)                         | 0.77  | (0.56-1.07) |

Frequencies (n) and percent (%).
n=5925 and n=5474 included in the crude and adjusted models, respectively. The primary outcome was estimated in a binomial logistic regression and secondary outcomes in multinomial logistic regression.

**Supplemental Table 2. Association between low-dose aspirin use and preterm birth in women with a first spontaneous preterm birth.**

| Outcome                                | No aspirin n= 15528 | Aspirin use n= 498 | Relative risk (95% confidence interval) | Crude | Adjusted |
|----------------------------------------|---------------------|-------------------|----------------------------------------|-------|----------|
| **Primary outcome**                    |                     |                   |                                        |       |          |
| Preterm birth <37 weeks                | 2576 (16.6)         | 111 (22.3)        | 1.34 (1.14-1.59)                       | 0.96  | (0.77-1.18) |
| **Secondary outcomes**                 |                     |                   |                                        |       |          |
| Moderate preterm birth 32 - 36 weeks   | 2235 (14.7)         | 84 (17.8)         | 1.17 (0.88-1.55)                       | 0.95  | (0.75-1.21) |
| Very preterm birth <32 weeks           | 341 (2.6)           | 27 (6.5)          | 2.45 (1.44-4.24)                       | 0.98  | (0.56-1.73) |
| Medically indicated preterm birth <37 weeks | 312 (2.4)        | 39 (9.2)          | 3.90 (2.47-6.13)                       | 1.93  | (1.28-2.91) |
| Spontaneous preterm birth <37 weeks    | 2254 (14.8)         | 72 (15.7)         | 1.00 (0.73-1.35)                       | 0.75  | (0.57-1.00) |
Frequencies (n) and percent (%). n=16022 and n=14990 included in the crude and adjusted models, respectively. The primary outcome was estimated in a binomial logistic regression and secondary outcomes in multinomial logistic regression.