

## Preconception Low Dose Aspirin and Time to Pregnancy: Findings From the Effects of Aspirin in Gestation and Reproduction Randomized Trial

Enrique F. Schisterman, Sunni L. Mumford, Karen C. Schliep, Lindsey A. Sjaarda, Joseph B. Stanford, Laurie L. Leshner, Jean Wactawski-Wende, Anne M. Lynch, Janet M. Townsend, Neil J. Perkins, Shvetha M. Zarek, Michael Y. Tsai, Zhen Chen, David Faraggi, Noya Galai, and Robert M. Silver

Epidemiology Branch (E.F.S., S.L.M., K.C.S., L.A.S., N.J.P., S.M.Z.) and Biostatistics and Bioinformatics Branch (Z.C.), Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Bethesda, Maryland 20892; Department of Family and Preventive Medicine (J.B.S.), University of Utah, Salt Lake City, Utah 84112; Department of Obstetrics and Gynecology (L.L.L., R.M.S.), University of Utah and Intermountain Healthcare 84111, Salt Lake City, Utah; Department of Epidemiology and Environmental Health (J.W-W.), University at Buffalo, Buffalo, New York 14260; Department of Obstetrics and Gynecology (A.M.L.), University of Colorado, Denver, Colorado 80045; Department of Family, Community and Rural Health (J.M.T.), Commonwealth Medical College, Scranton, Pennsylvania 18509; Department of Laboratory Medicine and Pathology (M.Y.T.), University of Minnesota Medical School, Minneapolis, Minnesota 55455; and Department of Statistics (D.F., N.G.), University of Haifa, Haifa, Israel 3498838

**Objective:** The objective was to determine the effect of preconception-initiated daily low-dose aspirin (LDA; 81 mg/day) treatment on time to pregnancy in women with a history of pregnancy loss.

**Design:** This was a multicenter, block-randomized, double-blind, placebo-controlled trial. Participants were block-randomized by center and eligibility stratum.

**Setting:** The study was conducted at four U.S.A. medical centers (2007–2012).

**Participants:** Participants women aged 18–40 years actively attempting pregnancy, stratified by eligibility criteria: the “original” stratum, women with one loss <20 weeks’ gestation during the previous year; and the “expanded” stratum, women with one or two previous losses of any gestational age regardless of time since loss.

**Intervention:** Daily LDA was compared with matching placebo for up to six menstrual cycles of attempting pregnancy.

**Main Outcome Measure:** Time to hCG detected pregnancy and clinically confirmed pregnancy, analyzed by intention-to-treat, was measured.

**Results:** Of the 1228 women randomly assigned to LDA (n = 615) or placebo (n = 613), 410 (67%) women receiving LDA achieved pregnancy compared to 382 (63%) receiving placebo, corresponding to a fecundability odds ratio (FOR) of 1.14 (95% CI: 0.97, 1.33). Among women in the original stratum (n = 541), LDA was associated with increased fecundability compared to placebo (FOR: 1.28; 95%CI: 1.02, 1.62).

**Conclusions:** Preconception-initiated LDA treatment resulted in a nonsignificant increase in fecundability of 14% in women with a history of 1–2 pregnancy losses, and a significant increase of 28% in women with a history of only one pregnancy loss of <20 weeks’ gestation in the preceding year. Preconception-initiated LDA may increase fecundability in certain women with a recent early pregnancy loss. (*J Clin Endocrinol Metab* 100: 1785–1791, 2015)

Approximately one in ten women achieving clinical pregnancy will experience a recognized pregnancy loss (1, 2). Moreover, incidence of loss including otherwise unrecognized human chorionic gonadotropin (hCG) detected losses is approximately 20–30% of pregnancies (2, 3). While most women having a pregnancy loss will go on to have one or more successful pregnancies, some will experience recurrent losses. After any pregnancy loss, concern for future fertility and fecundability may be heightened, and recent evidence indicates that time to pregnancy (TTP) following a loss may be lengthened (4). It is plausible that pathophysiological mechanisms which contribute to recurrent pregnancy loss, including a decrease in uterine or ovarian blood flow or inflammation in these organs (5), may also contribute to delayed TTP.

Aspirin is recognized for its effects of decreasing inflammation and increasing blood flow (6) and is generally regarded as safe, widely available, and affordable. Indeed, aspirin improves endometrial vascularization and placentation in women undergoing in vitro fertilization (IVF) (7), and has been shown to improve the chances of reproductive success in pregnancies from assisted reproductive technologies (8). Furthermore, our previous work identified higher live birth rates in women receiving preconception LDA, who had only one early pregnancy loss occurring in the preceding 12 months, but not an effect of LDA on preventing pregnancy loss (9). Therefore, we hypothesized that initiation of LDA preconception in women with a history of pregnancy loss seeking natural conception may improve fecundity. The Effects of Aspirin in Gestation and Reproduction (EAGeR) trial was designed to investigate the impact of LDA initiated preconception on pregnancy and live birth in women with a history of pregnancy loss not undergoing assisted reproduction. The aim of this specific analysis was to investigate the impact of LDA on TTP and cumulative incidence of pregnancy in six cycles of attempting pregnancy, including both hCG detected pregnancy and ultrasound-based clinically confirmed pregnancy.

## Materials and Methods

The EAGeR trial was a multicenter, block-randomized, double-blind, placebo-controlled trial of 1228 women recruited from four medical centers in the U.S. from 2007 to 2012. Institutional Review Board approval was obtained at each of the clinical sites and the data coordinating center. All participants provided written informed consent. A data safety and monitoring board (DSMB) ensured continued patient safety and ongoing monitoring of viability of the trial; adverse events were monitored and recorded throughout the trial by a committee blinded to treatment status and reported to the DSMB. The trial was registered with [ClinicalTrials.gov](http://ClinicalTrials.gov), number NCT00467363. Details of the

study design, methods, and participant characteristics have been previously described (10).

## Study design and population

The effect of LDA on live birth, the primary EAGeR pregnancy outcome, has been reported (9). Planned secondary outcomes were TTP and cumulative incidence of pregnancy, presented here. Women trying to conceive who were 18–40 years old with regular menstrual cycles of 21–42 days in length, no known history of infertility, and confirmation of 1 or 2 prior pregnancy losses were eligible for the study. Participants were block randomized into two strata based on specific eligibility qualifications, as previously discussed (10) (1) original, women with exactly one documented pregnancy loss at <20 weeks' gestation during the preceding 12 months and (2) expanded, women not meeting the entire criteria for the original stratum, and having 1 or 2 prior pregnancy losses of any gestational age or time since the loss occurred.

Recruitment occurred primarily by physician/nurse referral within clinical sites in participating medical centers, and was supplemented with household mailings, local health promotion events, posters, social media, brochures, and local media. Eligible women were scheduled for a randomization visit to coincide with day 2–4 of their next menstrual cycle.

## Treatment regimen and study procedures

Participants were block-randomized using a computerized randomization algorithm by study center and stratum (original/expanded) to receive either the intervention (81 mg LDA plus 400 mcg folic acid daily) ( $n = 615$ ) or an identical looking placebo (also with 400 mcg folic acid) ( $n = 613$ ). Treatment assignment was implemented electronically by the data coordinating center and was concealed from participants, clinicians, and investigators throughout the trial. Study pills were taken daily until completion of six cycles attempting pregnancy or week 36 of gestation for those who became pregnant. Adherence was assessed by self-report and additionally by weighing medication bottles at each study visit.

Participants attended two study visits per menstrual cycle, timed to occur around ovulation and to coincide with day 2–4 of the expected next cycle ("postcycle visit"). Participants also completed daily diaries and daily first-morning urine collection for the first two cycles of study participation. Fertility monitors were used to assist with timing of intercourse and study visits (Clearblue Easy Fertility Monitor; Inverness Medical).

## Outcome measures

The primary outcomes for the present analysis were time to pregnancy and six-month cumulative incidence of pregnancy, with pregnancy defined either as (1) hCG detected pregnancy or (2) clinically confirmed pregnancy. An hCG detected pregnancy was determined from a positive result on a "real-time" urine pregnancy test (Quidel Quickvue, Quidel Corporation), which was sensitive to 25 mIU/mL hCG, conducted each time participants reported missing menses on any postcycle visit; or from batched augmented urine hCG testing that was performed later in the laboratory on the last 10 days of each woman's first cycle of study participation (using daily first-morning urine collected at home) and on spot urine samples collected at all postcycle visits. Free beta hCG was measured in these urine samples to enable a more sensitive detection of very early pregnancy than

possible with conventional urine pregnancy testing. Two laboratory assays for free beta hCG (catalogue No. 4221-16, Diagnostic Automation Inc.; catalogue no. RIS0011R, BioVendor) were sequentially employed to determine, first, “potentially positive” values ( $n = 110$ ), out of which 16 were verified as positive tests for early hCG detected pregnancy.

Clinically confirmed pregnancies were identified by either intra-uterine gestational sac on ultrasound at 6–7 weeks’ gestation, clinical recording of fetal heart tones, or a later-stage confirmation of pregnancy. In cycles with an hCG detected pregnancy, but without evidence of pregnancy on sonogram, the cycle was considered to result in a peri-implantation loss and the participant was continued on the nonpregnancy schedule. In women not achieving a clinically confirmed pregnancy, follow-up ended after six menstrual cycles or after two peri-implantation losses, whichever occurred first.

### Statistical analysis

Fisher’s exact and Student’s  $t$  tests were used to compare demographic and reproductive history characteristics between treatment arms. All analyses followed intent-to-treat principles. Discrete Cox proportional hazard regression models were used to estimate the impact of LDA on TTP, hCG detected or clinically confirmed, by calculating corresponding fecundability odds ratios (FOR) between the LDA and placebo groups. All women were included in the TTP analyses, except for the women who withdrew on the day of randomization ( $n = 14$ ) without any follow up time observed. Women who did not achieve pregnancy were censored at withdrawal cycle or end-of-follow-up without achieving pregnancy (ie, six cycles). The cumulative incidence of hCG detected and clinically confirmed pregnancy over six cycles in the LDA vs placebo groups was compared using a log rank test based on the intent-to-treat principle. All analyses were conducted for the overall study sample, and also according to eligibility strata (original/expanded).

Several sensitivity analyses were conducted to assess the robustness of our findings of the impact of LDA on TTP, including analysis (1) accounting for multiple hCG detected pregnancies during the trial period within the same woman using a conditional Cox model to account for repeated measures (the main analyses included only the first pregnancy occurrence per woman) and (2) including all “potentially positive” hCG detected pregnancies ( $n = 110$ , all “potentially positive” considered positive without regard to two-assay positive verification). Analyses were conducted in SAS version 9.4 (SAS Institute).

### Results

Overall, 1228 women were recruited and randomly assigned to LDA or placebo between June 15, 2007 and July 15, 2011 and followed until August 2012. Fourteen women withdrew on the day of randomization leaving a total of 1214 women (608 LDA, 606 placebo) with observed cycles attempting pregnancy to be included in the analyses (Supplemental Figure 1). Of note, the total included here ( $n = 1214$ ) is greater than our previously published manuscript ( $n = 1078$ ) which was based on an outcome of live birth, and therefore, by the intent to treat

principle for this type of analysis, excluded a greater number lost to follow-up since more women were lost prior to this later pregnancy outcome (9). Specifically, in this analysis we included an additional 19 women who withdrew after a positive pregnancy test (8 of these women withdrew after clinical confirmation of pregnancy) for whom we did not observe their pregnancy outcome, and the additional 16 pregnancies detected by later augmented hCG testing in stored spot/daily urine samples (total of  $n = 792$  hCG pregnancies and  $n = 728$  clinically confirmed pregnancies). Of the hCG detected pregnancies ( $n = 792$ ), 98% ( $n = 776$ ) were detected by positive clinic pregnancy test and 2% ( $n = 16$ ) were detected by the augmented hCG testing in stored samples.

Characteristics of participants, stratified by eligibility criteria and treatment allocation, are shown in Table 1. Participants were predominately white (94.6%), married or living with a partner (91.5%), and high school educated or above (86.2%). Women had a mean age  $\pm$  standard deviation of  $28.7 \pm 4.7$  years and BMI  $26.4 \pm 6.6$  kg/m<sup>2</sup>. Treatment groups were similar with regard to all characteristics. Self-reported adherence to medication assignment was similar among groups; 15.4% of women in the LDA group permanently stopped study medication compared with 13.0% in the placebo group; and 5.6% of women taking LDA temporarily stopped the medication vs 7.9% taking placebo. Similar rates of adherence among groups were also noted based on weighing of medication bottles (data not shown).

The overall median TTP, using life table methods, for hCG detected pregnancy was not significantly different between LDA and placebo groups, with a nonsignificant FOR of 1.14 (95% CI: 0.97, 1.33) (Table 2). Similarly, there was no difference in TTP for clinically confirmed pregnancies in the overall study population. However, the median TTP for hCG detected pregnancy among women in the original stratum receiving LDA vs placebo was shorter, with an FOR of 1.28 (95% CI: 1.02, 1.62). Results for clinically confirmed pregnancy were similar with an FOR of 1.29 (95% CI: 1.02, 1.63) for LDA vs placebo in the original stratum. Conversely, in the expanded stratum, LDA was not associated with any difference in TTP for hCG detected or clinically confirmed pregnancy (Table 2).

Overall, the average rate of hCG detected pregnancy was 12.1% per cycle in the LDA arm and 11.2% in the placebo arm. Pregnancy rates declined across the six cycles of follow-up starting with a first-cycle probability of 23.5% for LDA and 21.3% for placebo. After three cycles of follow-up, 52.6% of women overall receiving LDA achieved hCG detected pregnancy compared to 49.3% receiving placebo, corresponding to 59.1% for LDA in the

**Table 1.** Demographics by Treatment Arm and Original/Expanded Eligibility Strata: Findings From the EAGeR Trial

Characteristics n (%)	Total n = 1228	Overall		Original		Expanded	
		LDA n = 615	Placebo n = 613	LDA n = 275	Placebo n = 274	LDA n = 340	Placebo n = 339
Age, y	28.7 ± 4.8	28.8 ± 4.9	28.7 ± 4.7	28.1 ± 4.9	28.0 ± 4.8	29.4 ± 4.7	29.3 ± 4.6
BMI, kg/m <sup>2</sup>	26.4 ± 6.6	26.3 ± 6.8	26.5 ± 6.4	25.3 ± 5.7	26.2 ± 6.4	27.1 ± 7.5	26.6 ± 6.4
Race							
White	1162 (94.6)	576 (93.7)	586 (95.6)	265 (96.4)	267 (97.5)	311 (91.5)	319 (94.1)
Non-White	66 (5.4)	39 (6.3)	27 (4.4)	10 (3.6)	7 (2.6)	29 (8.5)	20 (5.9)
Marital status							
Married	1124 (91.5)	575 (93.5)	549 (89.6)	264 (96.0)	257 (93.8)	311 (91.5)	292 (86.1)
Living with partner	74 (6.0)	31 (5.0)	43 (7.0)	7 (2.6)	11 (4.0)	24 (7.1)	32 (9.4)
Other	30 (2.4)	9 (1.5)	21 (3.4)	4 (1.5)	6 (2.2)	5 (1.5)	15 (4.4)
> High School Education	1057 (86.2)	526 (85.7)	531 (86.6)	236 (86.1)	256 (93.4)	290 (85.3)	275 (81.1)
Income (annual)							
≥\$100 000	491 (40.0)	241 (39.3)	250 (40.8)	97 (35.4)	104 (38.0)	144 (42.4)	146 (43.1)
\$75 000–\$99 999	149 (12.1)	84 (13.7)	65 (10.6)	41 (15.0)	27 (9.9)	43 (12.7)	38 (11.2)
\$40 000–\$74 999	181 (14.8)	91 (14.8)	90 (14.7)	38 (13.9)	49 (17.9)	53 (15.6)	41 (12.1)
\$20 000–\$39 999	312 (25.4)	147 (24.0)	165 (26.9)	69 (25.2)	76 (27.7)	78 (23.0)	89 (26.3)
≤\$19 999	94 (7.7)	51 (8.3)	43 (7.0)	29 (10.6)	18 (6.6)	22 (6.5)	25 (7.4)
Employed	895 (75.5)	451 (76.1)	444 (75.1)	212 (79.4)	215 (80.5)	239 (73.3)	229 (70.7)
Parity							
Nulliparous	571 (46.5)	283 (46.0)	288 (47.0)	159 (57.8)	166 (60.6)	124 (36.5)	122 (36.0)
Parous (1 or 2 prior live births)	657 (53.5)	332 (54.0)	325 (53.0)	116 (42.2)	108 (39.4)	216 (63.5)	217 (64.0)
Number of previous pregnancy losses							
1	825 (67.2)	422 (68.6)	403 (65.7)	275 (100.0)	274 (100.0)	147 (43.2)	129 (38.1)
2	403 (32.8)	193 (31.4)	210 (34.3)	0 (0.0)	0 (0.0)	193 (56.8)	210 (62.0)
Gestational age of most recent loss, wks	9.2 ± 4.9	9.3 ± 5.2	9.2 ± 4.6	9.0 ± 4.2	9.1 ± 3.0	9.6 ± 5.9	9.2 ± 5.6
Time from last loss to randomization							
≤4 months	651 (53.9)	331 (54.9)	320 (52.8)	175 (65.1)	175 (64.8)	156 (46.7)	145 (43.2)
5–8 months	222 (18.4)	103 (17.1)	119 (19.6)	62 (23.1)	63 (23.3)	41 (12.3)	56 (16.7)
9–12 months	99 (8.2)	50 (8.3)	49 (8.1)	25 (9.3)	27 (10.0)	25 (7.5)	22 (6.6)
>12 months	237 (19.6)	119 (19.8)	118 (19.5)	7 (2.6)	5 (1.9)	112 (33.5)	113 (33.6)

Data are mean ± SD or n (%). Information was missing for income (n = 1), education (n = 2), employment (n = 44), and time from last loss to randomization (n = 19). Women were block randomized into two strata based on eligibility criteria at enrollment: original, women with exactly one prior pregnancy loss <20 weeks' gestation which occurred in the 12 months preceding enrollment; expanded, women not eligible for the original stratum, and with one or two prior pregnancy losses of any gestational age occurring any time prior to enrollment.

original stratum and 49.3% for placebo (Table 3). After complete follow-up, the group with the highest proportion achieving pregnancy (77.1%) was the LDA group within the original stratum, while all other groups within eligibility strata were lower than 70%. These findings were reflected by results for clinically confirmed pregnancies (Table 3).

Several sensitivity analyses were performed to evaluate the robustness of our findings. Because women experiencing pregnancy loss during the study were able to re-enter the preconception observation and become pregnant again, analysis was conducted to account for observation of multiple pregnancies per woman which demonstrated no meaningful change in the results of TTP (Supplemental Table 1). Furthermore, inclusion of all potentially positive pregnancies determined from the first-pass of augmented hCG testing (n = 94 additional pregnancies) revealed stable results (Supplemental Table 1).

Safety symptoms were monitored by a committee as well as the DSMB, and occurred at comparable rates in both treatment arms and strata (9).

## Discussion

Preconception-initiated treatment with LDA resulted in a nonsignificant increase in fecundability of 14% in women with a history of 1–2 pregnancy losses. Furthermore, in women with a single documented pregnancy loss of <20 weeks' gestation occurring with the preceding 12 months, we found a significant increase in fecundability of 28%. The observed effect of LDA on TTP remained whether the outcome was hCG detected or clinically confirmed pregnancy determined by early (6–7 weeks' gestation) ultrasound. This suggests that the impact of LDA is not at the level of maintaining pregnancy after implantation, but rather earlier, potentially influencing ovulation, fertilization, and/or implantation.

Most previous studies of LDA in pregnancy initiated LDA after implantation and in populations with recurrent pregnancy loss precluding the examination of LDA's effects on TTP (11–18). In the current study, LDA signifi-



**Table 2.** Time to Pregnancy for Both hCG Detected and Clinically Confirmed Pregnancy by Treatment Arm and Eligibility Stratum in the EAGeR Trial

	hCG Detected Pregnancy <sup>a</sup>			Clinically Confirmed Pregnancy <sup>b</sup>		
	LDA	Placebo	P Value	LDA	Placebo	P Value
No. Participants Randomized Intention-to-treat population <sup>c</sup> OVERALL	<b>615</b>	<b>613</b>		<b>615</b>	<b>613</b>	
No. of participants	608	606		608	606	
Achieved pregnancy - No. (%)	410 (67)	382 (63)		378 (62)	350 (58)	
Median TTP (95% CI)	3 (3, 4)	4 (3, 4)	.11	4 (3, 4)	4 (4, 4)	.13
Fecundability Odds Ratio (95% CI)	1.14 (0.97, 1.33)			1.13 (0.96, 1.33)		
ORIGINAL						
No. of participants	272	269		272	269	
Achieved pregnancy - No. (%)	196 (72)	173 (64)		183 (67)	160 (59)	
Median TTP (95% CI)	3 (3, 3)	4 (3, 4)	.03	3 (3, 4)	4 (3, 5)	.04
Fecundability Odds Ratio (95% CI)	1.28 (1.02, 1.62)			1.29 (1.02, 1.63)		
EXPANDED						
No. of participants	336	337		336	337	
Achieved pregnancy - No. (%)	214 (64)	209 (62)		195 (58)	190 (56)	
Median TTP (95% CI)	4 (3, 4)	4 (3, 4)	.79	4 (4, 5)	4 (3, 5)	.92
Fecundability Odds Ratio (95% CI)	1.03 (0.83, 1.27)			1.01 (0.81, 1.26)		

Analyses performed using Cox proportional hazards models for discrete survival time. Women not achieving pregnancy within 6 cycles were censored as a TTP = 6, while women who withdrew early from the trial, and who did not achieve pregnancy, were censored at the date of withdrawal. Women were block randomized into two strata based on eligibility criteria at enrollment: original, women with exactly one prior pregnancy loss <20 weeks' gestation, which occurred in the 12 months preceding enrollment; expanded, women not eligible for the original stratum, and with one or two prior pregnancy losses of any gestational age occurring any time prior to enrollment.

<sup>a</sup> Pregnancy identified by hCG positive pregnancy test or from augmented hCG testing of spot and daily urine samples.

<sup>b</sup> Pregnancy identified by 6.5-week ultrasound.

<sup>c</sup> Among 1228 participants, there were 14 withdrawals, whose last visit day was at randomization leaving no observable time "at risk" of pregnancy; thus, these women were excluded from the TTP analyses leaving 1214 women (608 LDA, 606 placebo) included in the TTP analyses.

cantly shortened TTP and influenced pregnancy incidence only among women with one early pregnancy loss in the previous year. In combination with previous findings reported for this trial of no effect on reducing clinically confirmed pregnancy loss (9), these data suggest that any positive effects of LDA occur very early in the process of becoming pregnant, most likely no later than implantation. This is consistent with the observed impact of pre-conception aspirin in the setting of IVF treatment (16–19) where aspirin's anti-inflammatory and vasodilatory properties are hypothesized to improve implantation rates in women undergoing IVF/intracytoplasmic sperm injection (ICSI) (20). Indeed, a recent systematic review evaluated the effect of LDA on pregnancy rate in 17 studies for a total of 6403 patients, reporting that LDA treatment improved probabilities of pregnancy [odds ratio (OR): 1.19; 95% CI: 1.01, 1.39;  $P = .03$ ] but did not impact live birth (OR: 1.08; 95% CI: 0.90, 1.29;  $P = .43$ ) or spontaneous abortion (OR: 1.18; 95% CI: 0.82, 1.68;  $P = .37$ ) (20). Such findings support a potential implantation benefit with pre-conception LDA, in agreement with the present findings. However, aspirin was not associated with IVF outcomes when only seven of the studies deemed to be of "high" quality were evaluated in the aforementioned review (20). Because the studies did not differentiate between different

types of history of pregnancy loss, they are consistent with the nonsignificant difference in fecundability observed here in the general population of women with a history of 1–2 pregnancy losses. Taken together, these observations suggest there is heterogeneity in the extent to which populations of women with pregnancy loss may benefit from preconception LDA.

Aspirin may affect successfully achieving pregnancy through increased blood flow to the ovaries and endometrium. Aspirin inhibits cyclooxygenase (Cox) enzymes Cox-1 and Cox-2, leading to increased vasodilation and decreased platelet aggregation. Even at low doses, as used in this trial, aspirin inhibits platelet production of thromboxane A<sub>2</sub> (TXA<sub>2</sub>), resulting in a net increase in the ratio of prostacyclin I<sub>2</sub> to TXA<sub>2</sub>, thereby decreasing thrombosis and increasing blood flow (6, 21, 22). We speculate that women from the expanded stratum may be a more heterogeneous group that includes women who will go on to receive a future diagnosis of recurrent pregnancy loss where pathologic mechanisms (eg, genetic abnormalities) are not responsive to changes in blood flow or an altered inflammatory environment, both likely targets for LDA's effects on fecundity. In contrast, the most recent loss prior to randomization in the original eligibility stratum, which was limited to the preceding year, may synergistically af-

**Table 3.** Cycle-Specific and Cumulative Fecundability for hCG Detected and Clinically Confirmed Pregnancy (n = 1214 Women)<sup>a</sup>

Cycle	Overall				Original				Expanded			
	LDA n = 608		Placebo n = 606		LDA n = 272		Placebo n = 269		LDA n = 336		Placebo n = 337	
	Cycle-Specific	Cumulative	Cycle-Specific	Cumulative	Cycle-Specific	Cumulative	Cycle-Specific	Cumulative	Cycle-Specific	Cumulative	Cycle-Specific	Cumulative
hCG detected pregnancy (n = 792)												
1	23.5	23.5	21.3	21.3	24.3	24.3	25.3	25.3	22.9	22.9	18.1	18.1
2	15.5	39.0	17.3	38.5	18.5	42.7	14.2	39.5	13.0	35.9	19.7	37.8
3	13.6	52.6	10.8	49.3	16.4	59.1	9.7	49.3	11.2	47.2	11.6	49.5
4	7.7	60.3	9.1	58.4	6.8	65.9	8.4	57.7	8.5	55.7	9.6	59.1
5	6.5	66.9	5.5	63.9	5.3	71.2	5.7	63.5	7.6	63.2	5.2	64.3
6	6.1	73.0	3.2	67.1	5.8	77.1	3.4	66.8	6.2	69.5	3.1	67.5
<i>p</i> <sup>b</sup>				0.11				0.03				0.79
Clinically confirmed pregnancy (n = 728)												
1	20.4	20.4	18.3	18.3	22.1	22.1	22.7	22.7	19.0	19.0	14.8	14.8
2	15.0	35.4	16.4	34.7	18.2	40.2	13.1	35.8	12.4	31.4	19.2	34.0
3	12.7	48.0	10.1	44.9	15.1	55.4	9.0	44.8	10.6	42.0	11.1	45.1
4	7.0	55.0	9.4	54.3	5.1	60.5	8.9	53.7	8.6	50.6	9.8	55.0
5	7.0	62.0	4.45	58.8	6.2	66.7	4.6	58.3	7.6	58.2	4.3	59.3
6	5.5	67.5	3.3	62.1	5.5	72.2	3.8	62.1	5.5	63.8	2.9	62.2
<i>p</i> <sup>b</sup>				0.13				0.04				0.92

Women were block randomized into two strata based on eligibility criteria at enrollment: original, women with exactly one prior pregnancy loss <20 weeks' gestation, which occurred in the 12 months preceding enrollment; expanded, women with one or two prior pregnancy losses of any gestational age occurring any time prior to enrollment.

<sup>a</sup> Among 1228 participants, there were 14 withdrawals whose last visit day was at randomization and thus were excluded from the time to pregnancy analyses.

<sup>b</sup> *P* value represents LDA vs placebo, log rank test of cumulative pregnancy rate.

fect the endometrial vasculature, such that this stratum is more responsive to aspirin's effects of increasing vascular perfusion or altering the inflammatory milieu for implantation to prevent a postloss conception delay in an otherwise fecund woman. Such a mechanism specifically related to the postpregnancy loss milieu would not be possible in women with more historical losses allowed in the expanded stratum. We did find a greater cumulative pregnancy incidence in the study overall, in addition to the original stratum, which begs the question of whether significant differences in TTP and pregnancy incidence may have been observed if the study observed more than six cycles attempting pregnancy. Further work to identify specific mechanisms of action for LDA in women and which women may benefit most from therapy will be essential before any recommendations for LDA usage in women seeking pregnancy can be made. Given the low cost, wide availability, and relative safety of LDA, it is of significant public health value to pursue further research in this area.

This study had numerous strengths. In particular, the size of the study (1214 women and 792 hCG detected pregnancies) and the block-randomized, double-blind, placebo-controlled design lends significant strength to these findings. Time to pregnancy was measured prospectively, avoiding the misclassification and bias that can occur with retrospective

assessment (23, 24). Early pregnancy identification by hCG in urine was systematic for each cycle, with laboratory analysis as an additional procedure to identify all hCG detected pregnancies. Virtually all pregnancies were subsequently verified by early ultrasound (or found to have ended), providing additional information to the stage at which LDA had impact. The differential findings of the effect of LDA on pregnancy incidence and TTP by eligibility strata provides insight into the population of women that may benefit from LDA treatment and aids the interpretation of the previous work in this area, which has been laden with conflicting results.

Our study only observed six cycles of attempting pregnancy, limiting evaluation of TTP among women (or couples) with much lower fecundity. However, sensitivity analyses accounting for multiple pregnancies observed per woman (for cases of women reentering study and achieving pregnancy following a biochemical loss) and the inclusion of early pregnancy losses that were undetected by conventional means (standard urine pregnancy tests) increased the strength of the findings. Also, this study population was predominantly (95%) white, limiting the generalizability of these findings.

Overall, findings from the EAGeR trial indicate that LDA does not significantly shorten TTP in women with any history of pregnancy loss. However, cumulative incidence of

pregnancy within six months was greater in women receiving LDA than placebo, and a significant effect on shortened TTP was observed within women with a history of only one pregnancy loss of < 20 weeks' gestation in the preceding year (a prespecified group of women for the randomization and the analysis). Given that LDA is relatively inexpensive and well-tolerated, it may be reasonable to suggest the preconception use of LDA for women with this specific history for the purposes of a shorter TTP and increased fecundability. Further understanding the effects of LDA on fecundability may help inform our pathophysiologic understanding of subfertility in certain couples and help lead to improved and "personalized" treatment interventions. However, generalized recommendations for preconception LDA use are currently premature, as further work is needed to confirm these findings in other studies examining related health histories, evaluate any additional health risks or added benefits of preconception LDA treatment in women, and better define which women may benefit from treatment through study of the cellular and systemic mechanisms which contribute to the effects of LDA on fecundity.

## Acknowledgments

We would like to especially thank the EAGeR participants for their extraordinary commitment to the study, all of the EAGeR investigators and staff who devoted their time and energy to the success of this trial, and the Data Safety and Monitoring Board members for ongoing oversight, constant support, and advice throughout the trial. Lastly, we thank Aijun Ye, Glotech Inc., for his work on statistical analysis and Lauren Wise, ScD, Boston University, for her comments on previous versions of this paper.

Address all correspondence and requests for reprints to: E. F. Schisterman, PhD, 6100 Executive Blvd., 7B03, Rockville, MD 20854; Tel: 301-435-6893; Fax: 301-402-2084. E-mail: [schistee@mail.nih.gov](mailto:schistee@mail.nih.gov).

This research was supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland (Contract Nos. HHSN267200603423, HHSN267200603424, HHSN267200603426).

Trial Registration: [ClinicalTrials.gov](http://ClinicalTrials.gov), number NCT00467363.

Disclosure Summary: The authors having nothing to disclose.

## References

- Miller JF, Williamson E, Glue J, Gordon YB, Grudzinskas JG, Sykes A. Fetal loss after implantation. A prospective study. *Lancet*. 1980; 2:554–556.
- Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. *N Engl J Med*. 1988;319:189–194.
- Zinaman MJ, Clegg ED, Brown CC, O'Connor J, Selevan SG. Estimates of human fertility and pregnancy loss. *Fertil Steril*. 1996; 65:503–509.
- Sapra KJ, McLain AC, Maisog JM, Sundaram R, Buck Louis GM. Successive time to pregnancy among women experiencing pregnancy loss. *Hum Reprod*. 2014;29:2553–2559.
- Lee RM, Silver RM. Recurrent pregnancy loss: summary and clinical recommendations. *Semin Reprod Med*. 2000;18:433–440.
- Vane JR, Botting RM. The mechanism of action of aspirin. *Thromb Res*. 2003;110:255–258.
- Ruopp MD, Collins TC, Whitcomb BW, Schisterman EF. Evidence of absence or absence of evidence? A reanalysis of the effects of low-dose aspirin in in vitro fertilization. *Fertil Steril*. 2008;90:71–76.
- Dircckx K, Cabri P, Merien A, et al. Does low-dose aspirin improve pregnancy rate in IVF/ICSI? A randomized double-blind placebo controlled trial. *Hum Reprod*. 2009;24:856–860.
- Schisterman EF, Silver RM, Leshner LL, et al. Preconception low-dose aspirin and pregnancy outcomes: results from the EAGeR randomized trial. *Lancet*. 2014;384:29–36.
- Schisterman EF, Silver RM, Perkins NJ, et al. A randomised trial to evaluate the effects of low-dose aspirin in gestation and reproduction: design and baseline characteristics. *Paediatr Perinat Epidemiol*. 2013;27:598–609.
- Di Nisio M, Peters L, Middeldorp S. Anticoagulants for the treatment of recurrent pregnancy loss in women without antiphospholipid syndrome. *Cochrane Database Syst Rev*. 2005;(2):CD004734.
- Empson M, Lassere M, Craig J, Scott J. Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. *Cochrane Database Syst Rev*. 2005:CD002859.
- Empson M, Lassere M, Craig JC, Scott JR. Recurrent pregnancy loss with antiphospholipid antibody: a systematic review of therapeutic trials. *Obstet Gynecol*. 2002;99:135–144.
- Farquharson RG, Quenby S, Greaves M. Antiphospholipid syndrome in pregnancy: a randomized, controlled trial of treatment. *Obstet Gynecol*. 2002;100:408–413.
- Gris JC, Mercier E, Quéré I, et al. Low-molecular-weight heparin versus low-dose aspirin in women with one fetal loss and a constitutional thrombophilic disorder. *Blood*. 2004;103:3695–3699.
- Li DK, Liu L, Odouli R. Exposure to non-steroidal anti-inflammatory drugs during pregnancy and risk of miscarriage: population based cohort study. *BMJ*. 2003;327:368.
- Rai R, Backos M, Baxter N, Chilcott I, Regan L. Recurrent miscarriage—an aspirin a day? *Hum Reprod*. 2000;15:2220–2223.
- Tulppala M, Marttunen M, Söderstrom-Anttila V, Foudila T, Ailus K, Palosuo T, Ylikorkala O. Low-dose aspirin in prevention of miscarriage in women with unexplained or autoimmune related recurrent miscarriage: effect on prostacyclin and thromboxane A2 production. *Hum Reprod*. 1997;12:1567–1572.
- Rubinstein M, Marazzi A, Polak de Fried E. Low-dose aspirin treatment improves ovarian responsiveness, uterine and ovarian blood flow velocity, implantation, and pregnancy rates in patients undergoing in vitro fertilization: a prospective, randomized, double-blind placebo-controlled assay. *Fertil Steril*. 1999;71:825–829.
- Dentali F, Ageno W, Rezoagli E, et al. Low-dose aspirin for in vitro fertilization or intracytoplasmic sperm injection: a systematic review and a meta-analysis of the literature. *J Thromb Haemost*. 2012;10: 2075–2085.
- FitzGerald GA, Oates JA, Hawiger J, et al. Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. *J Clin Invest*. 1983;71:676–688.
- Patrono C, Collier B, Dalen JE, et al. Platelet-active drugs: the relationships among dose, effectiveness, and side effects. *Chest*. 2001; 119:39S–63S.
- Cooney MA, Buck Louis GM, Sundaram R, McGuinness BM, Lynch CD. Validity of self-reported time to pregnancy. *Epidemiology*. 2009;20:56–59.
- Tingen C, Stanford JB, Dunson DB. Methodologic and statistical approaches to studying human fertility and environmental exposure. *Environ Health Perspect*. 2004;112:87–93.