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A randomised trial to evaluate the Effects of low dose Aspirin in Gestation and Reproduction (EAGeR): Design and baseline characteristics

Enrique F. Schisterman[1], **Robert M. Silver**[2], **Neil J. Perkins**[1], **Sunni L. Mumford**[1], **Brian W. Whitcomb**[3], **Joseph B. Stanford**[2,4], **Laurie L. Lesher**[2], **David Faraggi**[5], **Jean Wactawski-Wende**[6], **Richard W. Browne**[7], **Janet M. Townsend**[8], **Mark White**[8], **Anne M. Lynch**[9], and **Noya Galai**[5]

[1]Epidemiology Branch, Division of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, MD, United States.

[2]Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, UT, United States.

[3]Division of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts at Amherst, Amherst, MA, United States.

[4]Department of Family and Preventive Medicine, University of Utah, Salt Lake City, UT, United States.

[5]Department of Statistics, University of Haifa, Haifa, Israel

[6]Department of Social and Preventive Medicine, University at Buffalo, Buffalo, NY, United States.

[7]Department of Biotechnical And Clinical Laboratory Sciences, University at Buffalo, Buffalo, NY, United States.

[8]Department of Family, Community and Rural Health, Commonwealth Medical College, Scranton, PA, United States.

[9]Department of Obstetrics and Gynecology, University of Colorado, Denver, CO, United States.

Abstract

Background—Low dose aspirin (LDA) has been proposed to improve pregnancy outcomes in couples experiencing recurrent pregnancy loss. However, results from studies of LDA on pregnancy outcomes have been inconsistent, perhaps because most studies evaluated LDA-initiated post-conception. The purpose of the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial was to determine whether preconception-initiated LDA improves live-birth rates in women with 1–2 prior losses.

Methods—We performed a multicenter, block randomised, double-blind, placebo-controlled trial. Study participants were recruited using community-based advertisements and physician referral to four university medical centers in the US (2006–12). Eligible women were aged 18–40 years actively trying to conceive with 1–2 prior losses. Participants were randomised to receive daily LDA (81 mg/day) or a matching placebo, and all were provided with daily 400 mcg folic acid. Follow-up continued for ≤six menstrual cycles while attempting to conceive. For those that conceived, treatment was continued until 36 weeks gestation. The primary outcome was the cumulative live birth rate over the trial period.

Corresponding Author: Enrique F. Schisterman, Eunice Kennedy Shriver National Institute of Child Health and Human Development; 6100 Executive Blvd., 7B03; Rockville, MD 20854, Tel: 301-435-6893; Fax: 301-402-2084; schistee@mail.nih.gov.

Results—1228 women were randomised (615 LDA, 613 placebo). Participants had a mean age of 28.7, were mostly white (95%), well educated (86% >high school education), and employed (75%) with a household income >$100,000 annually (40%). Characteristics of those in the treatment and placebo arms were well-balanced.

Conclusions—We describe the study design, recruitment, data collection, and baseline characteristics of participants enrolled in EAGeR, which aimed to determine the effect of LDA on live birth and other pregnancy outcomes in these women.

Keywords

Low-dose aspirin; conception; pregnancy; miscarriage; sub-fertility

Introduction

Factors that cause infertility, implantation failure, miscarriage, stillbirth, and pregnancy complications are often poorly understood. A unifying feature of these conditions is a decrease in uterine, ovarian, and placental blood flow.[1] Aspirin, though commonly recognized for its analgesic properties, also exerts anti-inflammatory, antipyretic, and anti-thrombotic effects, which may thus prevent decreased blood flow or may increase blood flow in multiple organ systems, including the uterus and ovaries.[2–4] Given the substantial impact abnormal vascular flow can have on reproduction, aspirin has the potential to improve vascularization to reproductive organs, prevent placental thrombosis, and positively affect live birth.

Use of low dose aspirin (LDA) post-conception has been extensively investigated with respect to early pregnancy loss and is a commonly prescribed treatment, despite unproven efficacy.[5–14] Preconception use of LDA appears to improve endometrial vascularization and placentation in women undergoing *in vitro* fertilization (IVF).[2] Accordingly, initiation of LDA preconception has potential to influence critical windows in reproduction such as ovulation, implantation, and placentation, with the possibility of positive downstream effects. Moreover, LDA has not been evaluated among women with only 1 to 2 prior losses.

The Effects of Aspirin on Gestation and Reproduction (EAGeR) trial was therefore designed as a prospective, double-blind, placebo-controlled, block randomised trial to evaluate the impact of daily preconceptional LDA treatment among women who have experienced one or two pregnancy losses compared to placebo. The purpose of this paper is to describe the design of the EAGeR trial, including recruitment and methodology, as well as present baseline characteristics of the enrolled participants.

Methods

Study objectives

The goal of EAGeR was to evaluate the effects of daily LDA therapy begun prior to conception on live birth and other pregnancy outcomes among healthy women ages 18 to 40 with a history of 1–2 prior pregnancy losses but with no known diagnosis of infertility. The primary objective was to determine the effect of LDA relative to placebo, in combination with folic acid, on the cumulative live birth rate over the trial period. The secondary objectives were to determine the effect of LDA relative to placebo, in combination with folic acid, on (i) the occurrence of a pregnancy (hCG detected and/or clinically recognized by a 6.5 weeks ultrasound); (ii) the incidence of early and late pregnancy loss; and (iii) specific pregnancy outcomes, including gestational age at birth, preterm birth, birth weight, major neonatal complications, length of hospital stay for infant, and preeclampsia. Additional

objectives of the trial were to evaluate safety of LDA in the participants and fetuses. A biological specimen repository was established.

Design and Target Population

The EAGeR trial was a multicenter, double blind, block randomised placebo-controlled trial. Eligible women were randomised and assigned to LDA or placebo prior to conception. Follow-up continued for up to six menstrual cycles. For women becoming pregnant during these six cycles, follow-up extended through the remainder of that pregnancy. EAGeR sites obtained Institutional Review Board approvals at each clinical center and the DCC. Participants provided written informed consent. The trial was registered on clinicaltrials.gov, #NCT00467363. An independent Data Safety and Monitoring Board (DSMB) ensured continued patient safety and ongoing monitoring of the trial.

Eligibility Criteria—Initial eligibility was restricted to healthy women ages 18 to 40 years trying to conceive who: (i) experienced exactly one documented prior pregnancy loss <20 weeks gestation within the past year; (ii) ≤ one prior live birth; (iii) ≤one elective termination or ectopic pregnancy; and (iv) regular menstrual cycles of 21–42 days in the preceding year. As a precaution, the trial was designed to allow for a second randomisation stratum, expanding the eligibility. After two months of recruitment and enrolling only 9 women, eligibility were expanded to allow women with one or two prior pregnancy losses, losses ≥20 weeks gestation, or losses occurring >one year prior, and up to two prior live births. Women meeting these criteria but not initially eligible were assigned to the "expanded" stratum, while women meeting the initial eligibility were assigned to the "original" stratum. Participants were independently randomised within each of the original and expanded strata, thereby maintaining the initial trial design within the original eligibility criteria. The original stratum intended to capture effects of LDA on implantation and placentation. The expanded stratum allowed for evaluation of effects on a more pathologically heterogeneous population, which more fully approximates the clinical population of women without known subfertility who have had a pregnancy loss and are seeking to achieve a live birth. Full details regarding inclusion and exclusion criteria are outlined in Table 1.

Recruitment of Subjects—The University of Utah in Salt Lake City, Utah, and the University at Buffalo in Buffalo, New York were the initial clinical sites. The Utah site included four hospitals: The University of Utah Health Sciences Center (UUHSC), McKay-Dee hospital (MKD), LDS hospital (LDS), and Utah Valley Regional Medical Center (UVRMC). The Buffalo site was a free-standing women's health research center at the University of Buffalo. After two years, Moses Taylor Hospital at Scranton, Pennsylvania and the University of Colorado, Denver, Colorado, were included in an attempt to enhance recruitment. A data coordinating center (DCC) at Haifa University, Israel, was responsible for developing a computerized remote data capture system, data management, and reporting to the DSMB.

Participants were enrolled over four years through clinical and community-based recruitment to reach a diverse study group. An average of 25.4 women were screened per week (median 25 per week), with an average rate of randomisation of 5.8 women per week (median 6 per week) over the four years of recruitment in all clinical sites combined.

Study Intervention—Participants were instructed to take the study medication daily as randomized, 81 mg aspirin (LDA) or a placebo tablet, throughout six cycles or if pregnant until week 36 of pregnancy. Placebo tablets were manufactured to match on size, color, taste, and weight. The first batch of medication, capsules manufactured by Fisher

(Rockville, MD) were difficult to swallow and over-coated tablets were produced for the second, third, and fourth batches by UPM pharmaceutical, Baltimore, MD. All women also received daily 400 mcg folic acid (generic). All received fertility monitors to assist in timing of intercourse (ClearBlue).

Baseline visit—Women completed a baseline visit prior to randomisation. Blood (in multiple anticoagulants) and spot urine specimens were collected, processed, and multiple replicate aliquots were stored at −80° C. Vaginal swabs were collected, plated, and stored. Participants completed questionnaires on the following topics: demographic background, occupation, lifestyle habits, medical and reproductive history, family medical history, and side effects. Physical measurements were obtained, including height, weight, and blood pressure.

Randomisation—Women whose eligibility was confirmed, were not pregnant, remained interested in participating, and who completed a baseline visit were randomised into one of the treatment arms during day 2–4 of their next menstrual cycle. Women were allowed to complete the baseline and randomisation visits on the same day. The randomisation algorithm was a permuted block design with blocks of size 6 or 8 in random order. Randomisation was stratified by study center (7 hospitals/clinics) and eligibility (original/expanded). Participant randomisation assignment was obtained automatically and blinded in the clinic (based on the computer algorithm developed by the DCC). Women received two-study medication bottles, with additional bottles given during follow-up ensuring that participants were never left with fewer than 30 tablets in the event of a missed visit. Participants, trial staff, and investigators remained blinded to the treatment assignment throughout.

Follow-up after randomisation—After randomisation, participants were followed for up to six menstrual cycles or until becoming pregnant. Participants becoming pregnant were followed throughout pregnancy. A detailed schedule of the follow-up visits, data and specimen collection are shown in Figures 1 and 2 for non-pregnancy and pregnancy follow-up, respectively. The first two menstrual cycles of follow up were called “active follow up” and the following four were called “passive follow up” as seen in Figure 1. Arrows on Figure 1 show clinic visits were scheduled around the time of ovulation during active follow-up and an end cycle visit on day 2–4 of the next menstrual cycle for all cycles. Women used fertility monitors to assist with timing of intercourse and ovulation so mid-cycle visits could be scheduled accordingly. Active follow-up included at home collection of daily diary information and daily first-morning urine collected in a vial and stored in home freezers. Daily diaries were used to record adherence to study medication, other medication/herbs used, fertility monitor reading, pregnancy test results, bleeding, intercourse, nausea and vomiting, pelvic pain/cramping, alcohol, caffeine and tobacco consumption, stress level, and symptoms or side effects. Blood and urine was obtained at clinic visits, processed, and frozen. Questionnaires were completed at each clinic visit in Figure 1, which included safety and adherence. Questionnaire and biospecimen collection was standardized across all study centers. If a woman did not become pregnant within six cycles, follow-up was considered complete.

Participants reporting missing menses on any end-cycle visit received an in clinic urine pregnancy test. A positive spot urine pregnancy test was followed by an obstetric ultrasound between 6 to 7 weeks of gestation to confirm a viable pregnancy, initiating pregnancy follow-up. If pregnancy was not confirmed by ultrasound, a peri-conception loss was recorded and the participant continued her non-pregnancy follow-up where she left off. Women with two peri-conception losses during follow-up concluded their participation in

the trial. For the purposes of scheduling follow-ups and ultrasound, the time of the positive clinic pregnancy test was considered to be 4 weeks of gestation.

Pregnancy follow-up included clinic or telephone visits every 4 weeks up to week 36 of gestation (Figure 2), and daily diary and daily first-morning urine collection for one month after the initial positive pregnancy test (i.e., approximately during weeks 4–8 of pregnancy by gestational age). Safety and adherence questionnaires were completed at all visits along with clinic visit blood and spot urine samples. Participants continued daily LDA or placebo as randomised until week 36 of pregnancy. Participants contacted the study staff when they went into labor in Utah and Colorado and after delivery at all other sites. Maternal blood and fetal specimens including cord blood and placental tissue were collected at the Utah and Colorado sites as well as products of conception with pregnancy losses. All specimens were processed and stored for future investigation.

A post-partum phone visit was conducted 6–8 weeks after delivery or pregnancy loss. A medical chart abstraction was completed on each participant in the study that included prenatal care, labor and delivery, and birth information. Participants who withdrew early from follow-up were contacted a year after their withdrawal and asked if they had become pregnant and about pregnancy outcomes following the study.

Safety and Monitoring

Participant safety was closely monitored and all adverse events were reported using standardized case report forms. An internal Adverse Events Committee routinely blindly evaluated adverse events, as did an external DSMB using summary information. The DSMB reviewed reports every 6 to 12 months, which were unblinded to treatment assignment if requested. Routine safety questionnaires were administered at every clinic and telephone visit on a bi-weekly (non-pregnancy) or monthly (pregnancy) basis regarding gastrointestinal discomfort (nausea, vomiting, other), bleeding (unusual, vaginal, other), allergic reactions, rashes, swelling and others. The DSMB also monitored efficacy and futility of the trial.

Adherence to study medication (LDA/placebo and folic acid) was evaluated by both self-reported adherence questionnaires and weighing the medication container at every visit. On average, self-reported adherence was high (>90%) declining slightly over time, and was similar in both treatment arms (average differences per visit ranging from −.005 to −0.063 pills/day). A total of 172 (14.2%) women reported permanently stopping study medication; 15.5% (93/606) in the LDA and 13.0% (79/606) in the placebo arms (P=0.25). The average time of medication stoppage was 122.5 days on follow-up. An additional 82 (6.8%) women reported stopping their study medication temporarily for limited time periods averaging 49.3 days.

Outcome Measures

The primary outcome of interest for the EAGeR trial was live birth. Several secondary outcomes also were defined. Clinical outcomes were determined based on medical record abstraction. The EAGeR trial endpoints and brief operational definitions are listed in Table 2.

Statistical Analysis Plan and Power Calculations

The statistical analysis plan called for an “intent to treat” (ITT) approach, where participant adherence with study protocol did not affect treatment allocation in analyses. Analysis of the primary endpoint (live birth) and stated secondary endpoints used ITT. The difference between the proportions of live births for the two treatment groups was tested via two-sided

χ^2 test for independence, with permutation tests performed to relax any distributional assumptions regarding the asymptotic properties. Time from randomisation to event is of interest for outcomes such as pregnancy (hCG or clinically confirmed), where survival analysis methods were applied with the log-rank test comparing the two treatment groups. Parametric t-test, or non-parametric Mann-Whitney-Wilcoxon, methods were used as appropriate for continuous outcomes such as birth weight. In order to evaluate LDA effects within groups defined by the eligibility criteria (original or expanded), stratified analysis for all trial outcomes was also performed.

Random effect models were considered in order to account for the stratification by clinical center and the permuted block randomisation scheme. Sensitivity analyses were performed in order to address some degree of missing endpoint information related to drop-out. This analysis involved investigating the potential impact the150 participants that withdrew could have had on the on the observed live birth rates had they completed the EAGeR trial by assigning each a hypothetical outcome (live birth or no live birth) and combining with the observed outcomes.?This was performed repeatedly after assigning every possible combination of outcomes to the 150 withdrawals, 80 and 70 in the LDA and placebo groups, respectively.

A priori power calculations for the EAGeR trial were based on assessing the effects of LDA on the primary outcome (live birth). Assuming 75% of participant pregnancies assigned to placebo achieve live births over 6 months, the study was powered to detect a 10% absolute increase for LDA versus placebo with 80% power and a 5% type I error rate. Assuming a cumulative 40% pregnancy rate for trial participants attempting conception over 6 months, leads to a necessary sample size of 1,254. Accounting for a potential 20% loss to follow-up, 1,600 was the recruitment target.

The interim analysis plan was based on the alpha spending function with O'Brien Fleming boundaries[24] considering the primary endpoint of live birth. Power was calculated for 0–2 interim looks assuming: (a) a total of n=1600 participants equally randomised between LDA and placebo; (b) a two-sided alpha = 0.05; (c) live birth rate of 0.48 in the placebo arm and an RR=1.20 considering six menstrual cycles of attempting pregnancy (based on conservative estimates of 17.5% chance of conception per cycle and a 70% likelihood of live birth given pregnancy); and (d) Chi-square test for comparison. Calculations were done using PASS software,[25] and power ranged from 49% for an interim look after 45% trial completion, 72% for an interim look after 60% completion, and 97% after 100% completion.

Results

Recruitment

Trial recruitment started on June 15, 2007, and the last participant was randomised on July 15, 2011. Figure 3 shows the stages of screening and recruitment with counts at each step. EAGeR randomised 1228 women, or 76.8% of the initial target of 1600. The withdrawal rate was 12.2% (150/1228). Sixteen women withdrew immediately after randomisation and contributed no time to follow-up. Nine withdrew after a positive pregnancy test. The rates of withdrawal were similar between study arms: 13.0% in the LDA group and 11.4% in the placebo group (P=0.43).

During 49 months of recruitment, 5409 women were screened of whom 22.7% were randomised and participated in the trial (Figure 3). Over half (52.3%) of the women screened were not eligible, and of the 2,247 (41.5%) who were initially eligible, 1,577 (70.2%) had a baseline visit. Out of the 1,397 women with confirmed eligibility, consented,

and expressed initial interest in the study, 60 (4.3%) choose to discontinue the enrollment process. An additional 109 (7.8%) tested positive for pregnancy at the baseline visit or became pregnant before their randomisation visit. The average time between the baseline and randomisation visits was 14.1 days (5^{th} – 95^{th} % range: 0 – 47 days).

Description of EAGeR Cohort at Baseline

A description of baseline characteristics of women participating in EAGeR by treatment arm and eligibility strata is depicted in Table 3. Randomised women had a mean age of 28.7, were mostly white (95%), well educated (86% > high school education), employed (75%), and had a household income above $100,000 annually (40%). There were no differences observed between the active treatment and placebo arms with respect to demographic characteristics or any other factor either overall or by eligibility strata.

Table 4 shows the reproductive history at baseline for randomised EAGeR participants, stratified by treatment arm and eligibility strata. Overall, the median lag-time from the last pregnancy loss to randomisation was 3.6 months and the median number of previous pregnancies was 2. About a third of the women had 2 previous pregnancy losses while two thirds had one previous loss. No important differences were observed between the two treatment arms with regard to reproductive history of the participants (overall or stratified by eligibility).

In Table 5, physical measurements and lifestyle variables at baseline are shown for randomised EAGeR participants, stratified by treatment arm and eligibility strata. In this cohort of reportedly healthy women, blood pressure values were in the normal range. Less than a quarter of the group reported lifetime smoking of more than 100 cigarettes, and only 12.3% reported any smoking during the last year prior to trial enrollment. Alcohol consumption was also low. While average BMI was 26.4, 24.2% were obese with a BMI above 30. There were no differences in physical measurements and lifestyle habits between the two treatment arms overall or by eligibility strata.

Selected Characteristics by Clinical Center—The distribution of the original and expanded strata by center is illustrated in Figure 4. A greater proportion (55.4%) of participants was recruited in the expanded stratum at all sites. Proportions of the participants recruited under the expanded criteria by site were: Scranton, 58.4%; Buffalo, 62.3%; Utah, 54.1%; and Colorado, 61.6%. Since randomisation was stratified both by center and eligibility criteria, a balanced treatment assignment in these groups was demonstrated.

Comparison of Eligibility Strata—A comparison between the two eligibility strata for selected characteristics (without regard to treatment assignment) is demonstrated in Table 6. Women in the original stratum were relatively younger, less likely to have had no previous pregnancies excluding losses, more likely to be Caucasian, had a shorter interval from the last pregnancy loss, a higher education level, a lower proportion with BMI≥30, and a slight non-significantly lower withdrawal rate than those in the expanded stratum.

Summary of Follow-up and Trial Endpoints

In total, 16,171 visits were performed. Of these, 2,456 were baseline and randomisation visits, 8,273 were visits in the non-pregnancy follow-up (6,250 clinic visits, 1,960 phone visits, and 63 unscheduled clinic visits) and 5,442 visits were during pregnancy (3,605 clinic visits, 1,129 phone visits, and 708 post-partum visits among 728 women who became pregnant). The total follow-up time for randomised participants was 790.3 person-years: 362.9 for non-pregnancy and 427.4 for pregnancy follow-up. A total of 509,207 maternal and fetal biological specimens have been collected, which included 360,247 urine, 143,782

aliquots of blood, 1528 tissue specimens, 2202 cord blood specimens, and 1448 vaginal swab slides.

Comment

LDA may improve uterine perfusion, and thereby favorably impact aspects of reproduction. The EAGeR trial is a multi-center, double-blind, placebo-controlled, block randomised trial that was designed to determine the effect of daily LDA taken prior to conception on the live birth rate, as well as other pregnancy outcomes, among women with a history of one or two prior pregnancy losses, and without any known history of subfertility. This design is based on the presumption that women without known subfertility but with a modest history of reproductive failure may most likely benefit from a low-cost and widely available over the counter treatment like LDA. By design, the results of the EAGeR trial will not be applicable to women with no history of any pregnancy loss, with documented subfertility, or those with recurrent pregnancy loss (three or more documented pregnancy losses).

Thus far we have demonstrated the feasibility of enrolling and randomising a very large prospective cohort of women prior to conception. As the preconception and peri-conception windows are critical times in fecundity, early embryogenesis, and placentation, preconception studies are needed to tease apart effects at these early stages of development. The detailed follow-up and daily urine collection will yield unique data to better understand the earliest stages of human development. Analysis of these data in the context of a clinical trial is forthcoming and will help us to better understand not only the potential of LDA to improve pregnancy outcomes in this group of women, but also affords opportunity to investigate other effects of exposures during the peri-conception time period on pregnancy outcomes.

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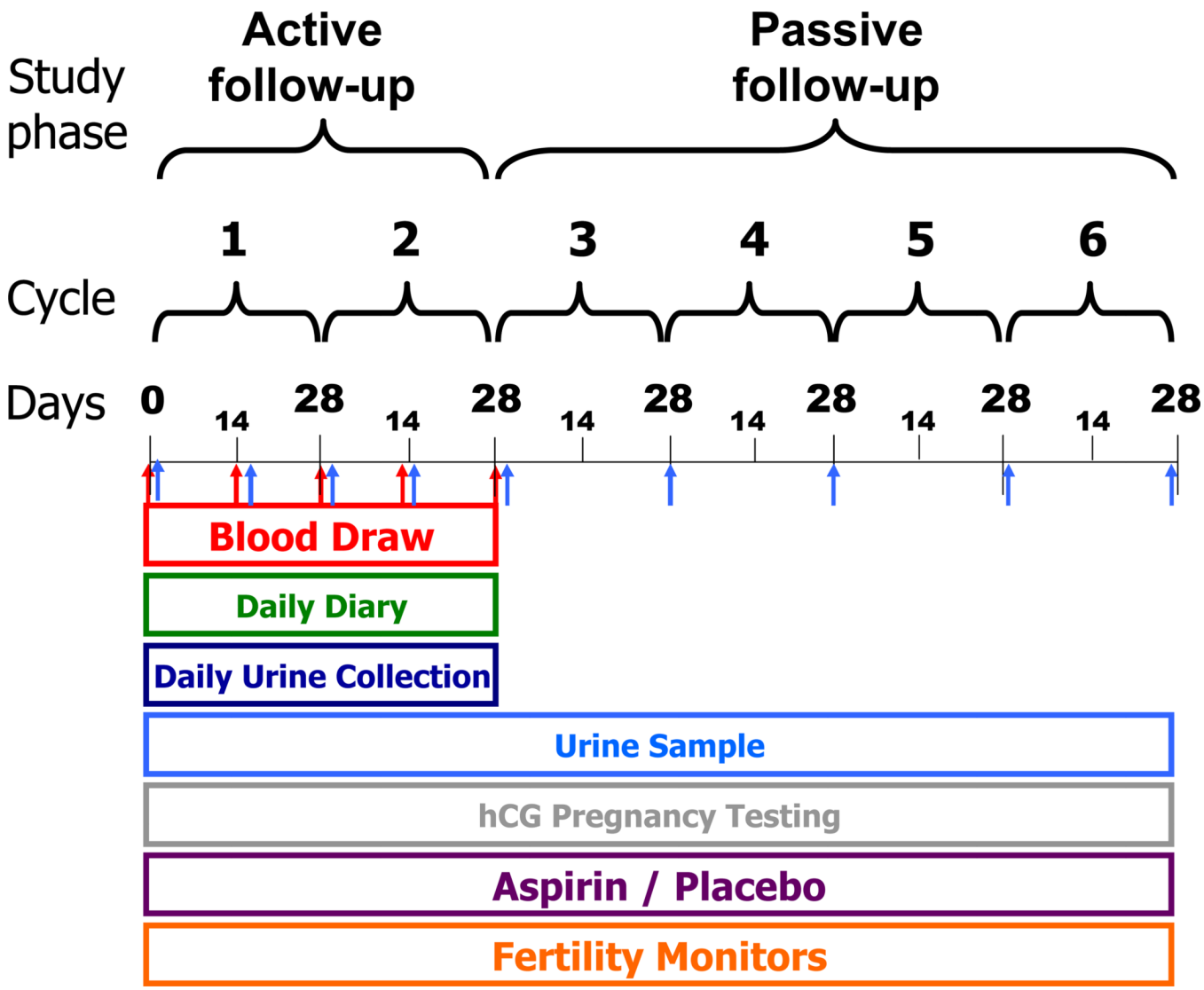

Figure 1.
Timeline for participant follow-up while attempting to conceive *
* Note: We use cycles of length 28 days solely for illustrative purposes with day 14 indicating an idealized day of ovulation.

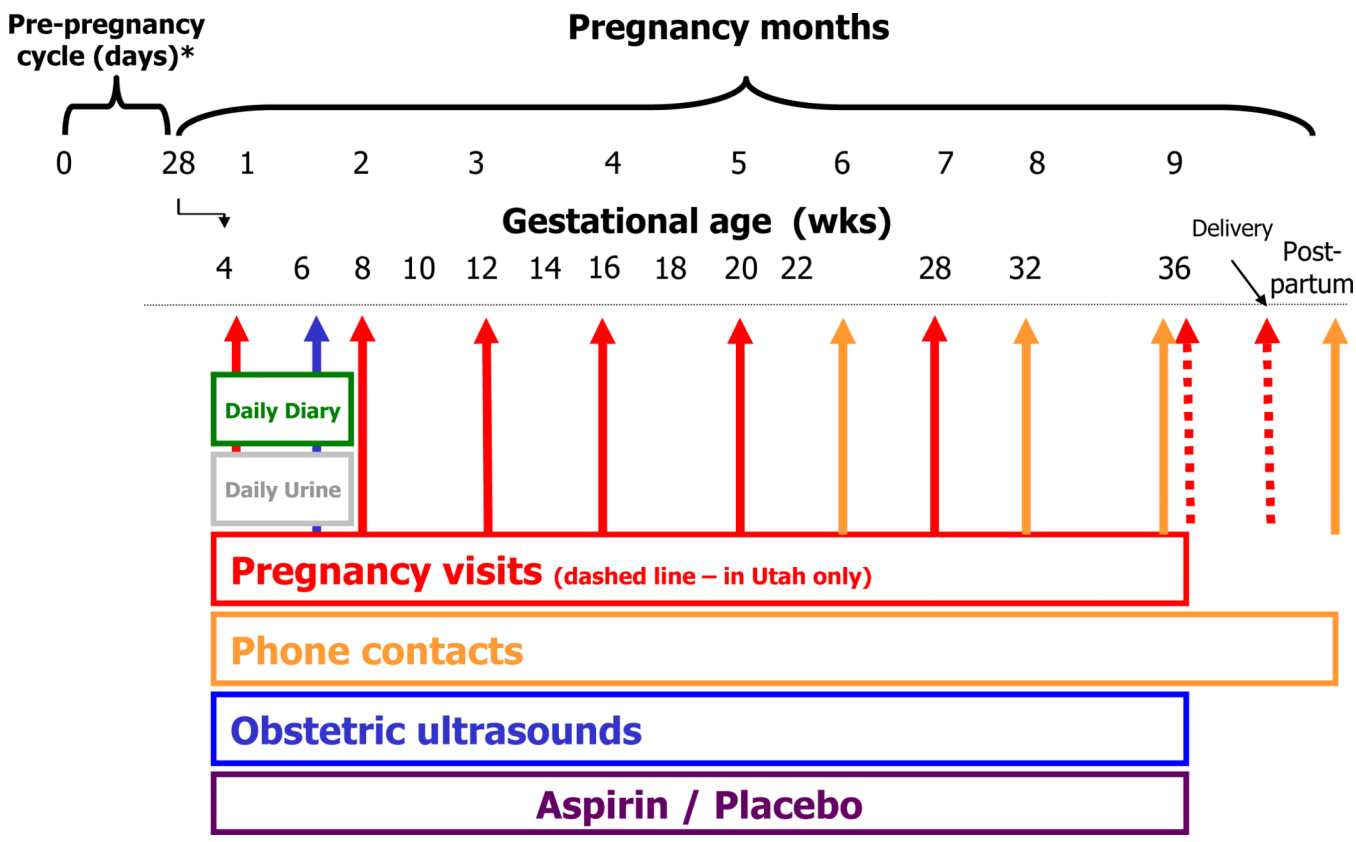

Figure 2.
Timeline for participant follow-up during pregnancy.

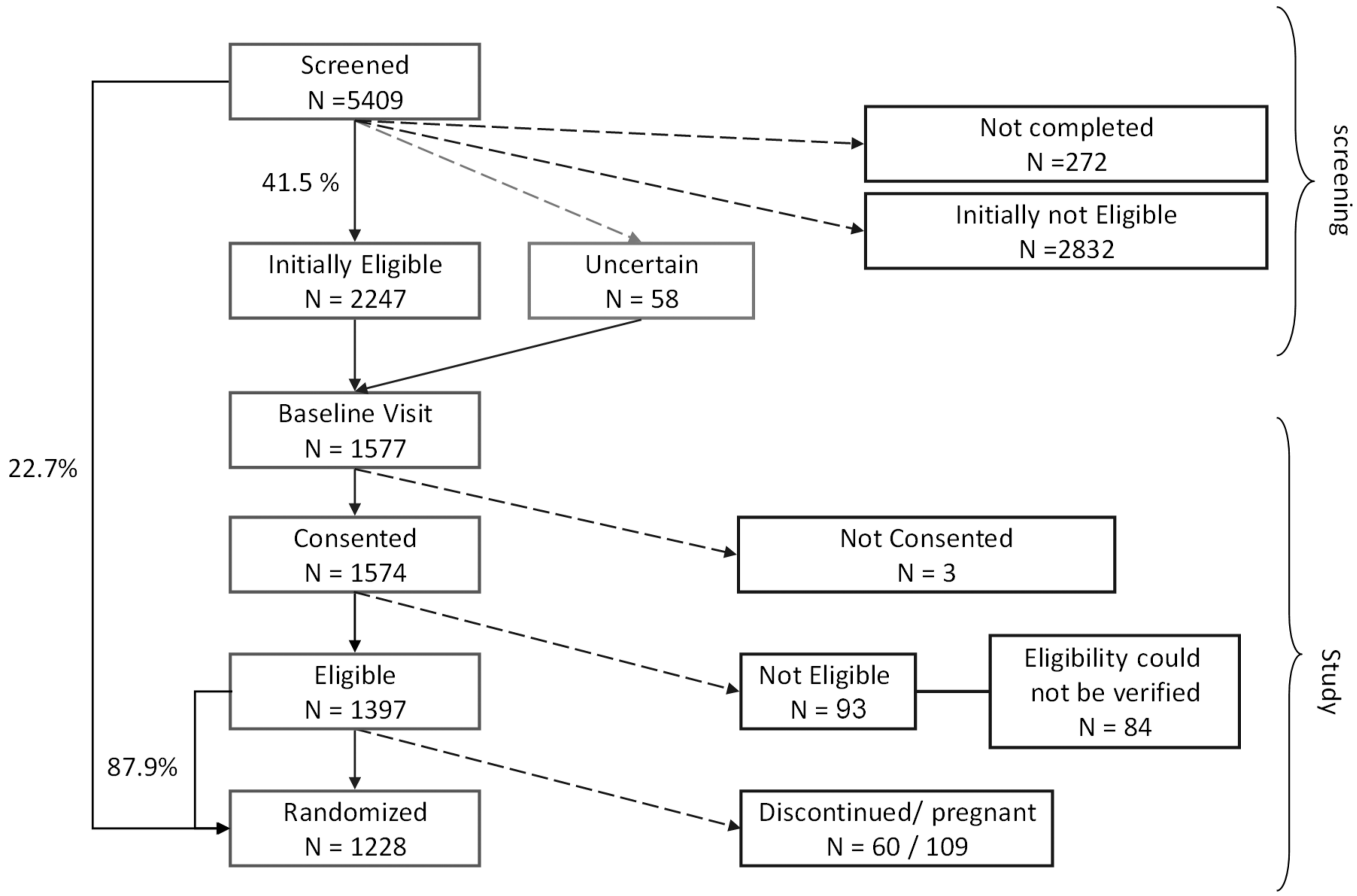

Figure 3.
EAGeR Recruitment Stages: Number of women who were screened, eligible, and completed baseline and randomisation visits.

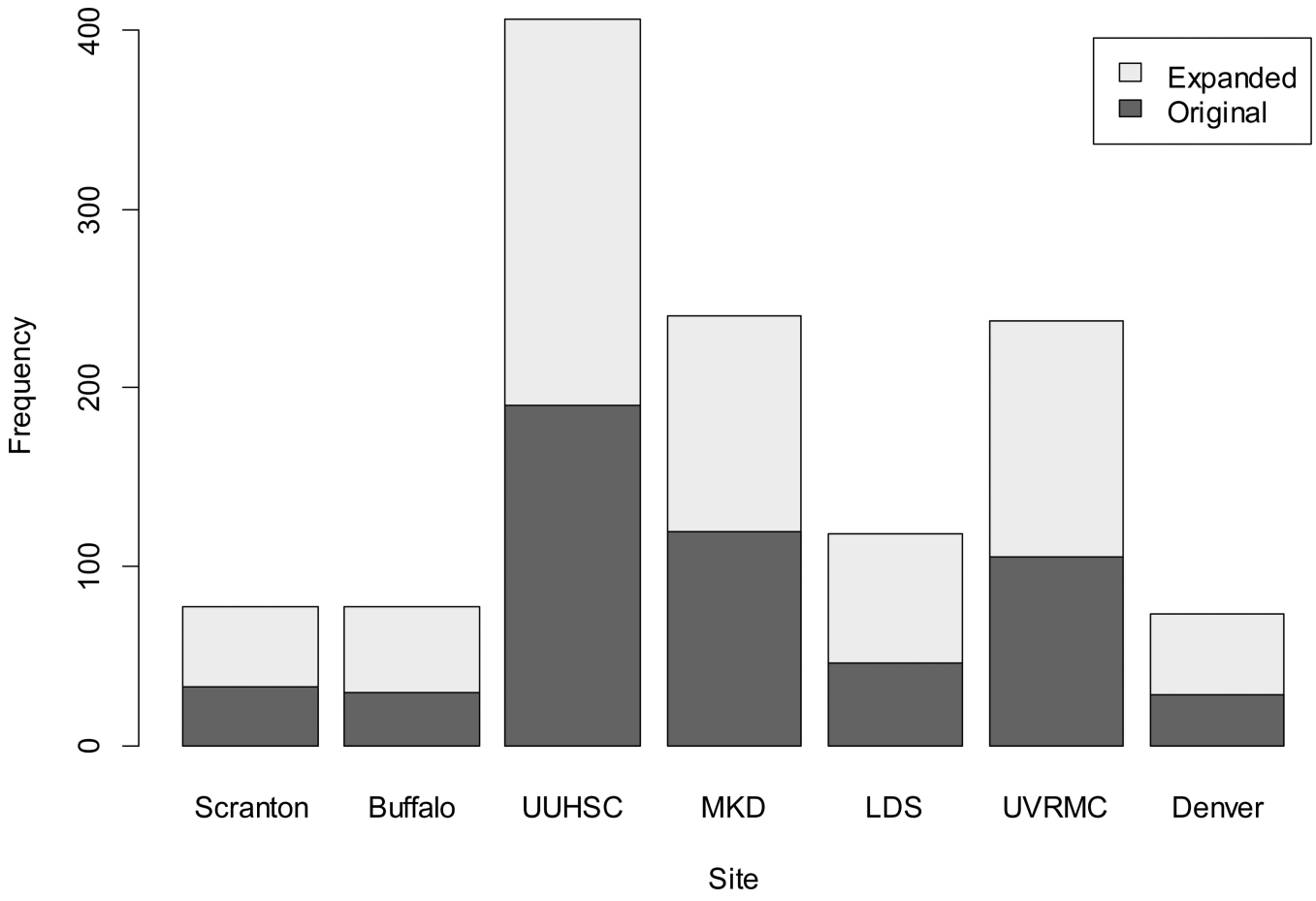

Figure 4.
Distribution of eligibility criteria strata for randomised EAGeR participants by clinical center.a

[a] Site abbreviations—UUHSC: The University of Utah Health Sciences Center, Utah; MKD: McKay-Dee hospital, Utah; LDS: LDS hospital, Utah; UVRMC: Utah Valley Regional Medical Center, Utah; Buffalo: University of Buffalo, New York; Scranton: Moses Taylor Hospital, Pennsylvania; Denver: University of Colorado, Colorado.

Table 1

Inclusion and exclusion criteria for the EAGeR trial.

Inclusion Criteria *

- "**Original Stratum**": (a) Women with one well documented pregnancy loss in the past 12 months that was up to 20 weeks of gestation; (b) 1 to 3 pregnancies in total including the pregnancy losses, and up to one prior pregnancies that did not end in a loss.
- "**Expanded Stratum**": (a) Women with one or two documented pregnancy losses in the past at any gestational age; (b) 1 to 5 pregnancies in total including the pregnancy losses, and up to two prior pregnancies that did not end in a loss; c) and who do not meet the criteria for pregnancy history "original stratum."
- Presence of intact tubes (both), ovaries (both), and uterus
- Between 18 and 40 years of age at baseline, and actively trying to conceive
- Regular menstrual cycles, 21–42 days in length, and no more than one missed menses in the past 12 months
- Not pregnant at the baseline or randomisation visits (i.e., negative urine pregnancy test at both visits)
- Willingness to be randomised and comply with the study protocol
- Within first four days of menstrual flow at the randomisation visit

Exclusion Criteria

- Known allergies to aspirin or non-steroidal anti-inflammatory agents (NSAID)
- Clinical indication for anticoagulant therapy, including prior or current thrombosis, antiphospholipid syndrome (APS) or known major thrombophilia
- Clinical indication for chronic use of NSAIDs, such as rheumatoid arthritis
- Indication for additional folic acid supplementation, such as prior infant with neural tube defect (NTD), or taking medication for seizure disorder
- Medical contraindication to aspirin therapy, including uncontrolled asthma, nasal polyps, bleeding disorders, or history of gastrointestinal ulcer
- Presence of major medical disorders (regardless of severity)
- Currently undergoing or planning use of medical fertility therapies during trial (including clomiphene intra-uterine insemination, or in vitro fertilization)
- History of infertility or sub-fertility
- Presence of unstable mental disorder, including bipolar illness, schizophrenia, uncontrolled depression, uncontrolled anxiety disorder
- Known current or recent alcohol abuse or illicit drug use
- Current diagnosis of sexually transmitted infection (temporary exclusion)

* Except as noted specifically by stratum, all inclusion and exclusion criteria are identical for both strata

Table 2

Operational definitions of primary and secondary outcomes in the EAGeR trial.

Outcome	Operational Definition
hCG recognized pregnancy	A positive spot urine in the clinic (Quidel Quickview™) was considered evidence of implantation.
Clinically recognized pregnancy	Clinical recognition of pregnancy was determined by documentation of the gestational sac from an ultrasound scan at about 6 to 7 weeks (goal of 6.5 weeks) (or alternatively when no ultrasound confirmation of pregnancy was available, clinically detected fetal heart tones at a medical visit, serum Hcg levels or histologic confirmation of gestational tissue resulting from pregnancy loss).
Pregnancy loss	A clinically recognized pregnancy loss was verified by chart abstraction and classified into one of several types: (a) Embryonic demise in the case of any visible embryo with no heart beat (CRL < 30 mm); (b) Fetal demise in the case of any visible fetus with no heart beat (CRL > 30 mm); (c) Pre-embryonic demise in the case of a mean gestational sac diameter > 10 mm without a yolk sac or mean gestational sac diameter > 18 mm without an embryo; (d) Completed miscarriage in the case where no gestational sac is identified on sonogram after prior sonogram with gestational sac present; (e) Still birth for any pregnancy loss occurring at or after 20 weeks of gestation.
Peri-implantation loss	A loss of pregnancy following implantation and prior to detection of pregnancy by ultrasound or fetal heart tones.
Ectopic Pregnancy	Based on medical record abstraction or diagnosis during the ultrasound.
Live birth	Live delivered infant as indicated from medical records.
Gestational Age	Calculated using the estimated weeks and days of gestation from the early pregnancy ultrasound and the difference, in days, from the date of the early ultrasound to the end of the pregnancy. Alternatively, if an early ultrasound was not performed, gestational age was calculated using the last menstrual period (LMP) estimated from the fertility monitor log (using date of LH surge +14 days or date of LMP). If fertility monitor log was not available, then gestational age was determined from review of delivery medical records.
Birth weight	Obtained from hospital medical records.
Molar Pregnancy	Based on medical record abstraction, including confirmation by pathology.
Preeclampsia	Indicated from medical record diagnosis and based on the criteria listed below. **Mild preeclampsia**: **1** Systolic pressure ≥140 mmHg and/or diastolic pressure ≥90 mmHg that does not antedate the pregnancy and presents after 20th weeks on ≥ 2 occasions at least 6 hours apart and no more than 1 week apart AND **2** Proteinuria ≥0.3 grams in a 24-hour urine specimen or 1+ on dipstick on two occasions at least 4 hours apart **Severe preeclampsia:** Meeting diagnostic criteria for mild preeclampsia plus at least one of the following: **1** Systolic pressure ≥160 mmHg and/or diastolic pressure ≥110 mmHg that does not antedate the pregnancy and presents after 20th weeks on ≥2 occasions at least 6 hours apart and no more than 1 week apart OR **2** Proteinuria ≥5 grams in a 24 hour urine specimen or 3+ on dipstick on two occasions at least 4 hours apart OR **3** IUGR <10% OR **4** Thrombocytopenia ≤100,000 cells/mm^3 OR **5** Impaired liver function (≥2 times normal limit) OR **6** Oligouria ≤ 500cc/24 hours or creatinine >1.2 mg/dL OR **7** Neurologic symptoms (eg persistent headache, blurred vision, or scotomata) OR **8** Persistent epigastric or right upper quadrant pain OR **9** Pulmonary edema or cyanosis **HELLP Syndrome**: **1** Hemolysis:

Outcome	Operational Definition
	a. abnormal peripheral smear OR
	b. LDH > 600 IU/L OR
	c. total bilirubin ≥1.2 mg/dL AND
	2 Impaired liver function (≥2 times normal limit) AND
	3 Thrombocytopenia ≤ 100,000 cells/mm3
SGA infant	Birth weight ≤ 10th percentile for gestational age, as defined by the standards of Kramer et al.[23]
Preterm birth	Delivery of a live baby (or babies) prior to 37 weeks' completed gestation, defined using the calculated gestational age at delivery.
Abnormal fetal testing	Based on medical record abstraction.
Fetal intolerance of labor	Based on medical record abstraction.
Abruption or vaginal bleeding	Based on medical record abstraction.
Length of hospital stay for the infant	Based on medical record abstraction.

Table 3

Demographic characteristics at baseline for randomised EAGeR participants, stratified by treatment arm and eligibility strata. [Entries for discrete variables are frequencies and percentages; for continuous variables mean (standard deviation)].[a]

Characteristics	Total	LDA	Placebo	Original LDA	Original Placebo	Expanded LDA	Expanded Placebo
N (%)	N=1228	N=615	N=613	N=275	N=274	N=340	N=349
Age, y: Mean ± SD	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
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 (23.9)	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)

[a]Data on covariates were missing for income (n=1), smoking (n=11), alcohol (n=6), education (n=2), and employment (n=44).

Table 4

Reproductive history at baseline for randomised EAGeR participants, stratified by treatment arm and eligibility strata.

Characteristics	Total	LDA	Placebo	Original LDA	Original Placebo	Expanded LDA	Expanded Placebo
N (%)	**N=1228**	**N=615**	**N=613**	**N=275**	**N=274**	**N=340**	**N=349**
Time from last loss to randomisation [a]							
≤ 4 Months	331 (54.9)	320 (52.8)	651 (53.9)	175 (65.1)	175 (64.8)	156 (46.7)	145 (43.2)
5–8 Months	103 (17.1)	119 (19.6)	222 (18.4)	62 (23.1)	63 (23.3)	41 (12.3)	56 (16.7)
9–12 Months	50 (8.3)	49 (8.1)	99 (8.2)	25 (9.3)	27 (10.0)	25 (7.5)	22 (6.6)
>12 Months	119 (19.8)	118 (19.5)	237 (19.6)	7 (2.6)	5 (1.9)	112 (33.5)	113 (33.6)
Number of previous live births							
0	577 (47.0)	287 (46.7)	290 (47.3)	161 (58.6)	167 (61.0)	126 (37.1)	123 (36.3)
1	438 (35.7)	218 (35.5)	220 (35.9)	111 (40.4)	101 (36.9)	107 (31.5)	119 (35.1)
2	213 (17.4)	110 (17.9)	103 (16.8)	3 (1.1)	6 (2.2)	107 (31.5)	97 (28.6)
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)
Months trying to become pregnancy (prior to randomisation) , Median (Q1, Q3)	3 (1, 7)	3 (1, 7)	3 (1, 6)	3 (1, 7)	3 (1, 6)	3 (1, 7)	3 (1, 7)

[a] Data on covariates were missing for time from last loss to randomisation (n=19 participants).

Table 5

Physical measurements and life habits at baseline for randomised EAGeR participants, stratified by treatment arm and eligibility strata. [Entries for discrete variables are frequencies and percents, for continuous variables mean (standard deviation)].

Characteristics	Total (N = 1228)	LDA N=615	Placebo N=613	Original LDA N=275	Original Placebo N=274	Expanded LDA N=340	Expanded Placebo N=349
BMI							
Mean (SD)	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)
Diastolic blood pressure[1]							
Mean (SD)	72.6 (9.3)	72.9 (9.1)	72.4 (9.4)	73.2 (8.9)	72.6 (9.4)	72.6 (9.3)	72.2 (9.4)
Systolic blood pressure[a]							
Mean (SD)	111.6 (12.1)	111.7 (12.2)	111.4 (12.1)	111.9 (12.0)	111.8 (12.3)	111.6 (12.3)	111.2 (12.0)
Smoking in past year							
Never	1067 (86.7)	529 (87.0)	538 (88.3)	243 (89.3)	250 (91.6)	286 (85.1)	288 (85.7)
Sometimes (<6 times/week)	87 (7.2)	41 (6.7)	46 (7.6)	16 (5.9)	16 (5.9)	25 (7.4)	25 (7.4)
Daily	63 (5.2)	38 (6.3)	25 (4.1)	13 (4.8)	7 (2.6)	25 (7.4)	18 (5.4)
Ever smoked at least 100 cigarettes							
Yes	280 (23.3)	142 (23.7)	138 (22.9)	54 (20.3)	52 (19.2)	88 (26.4)	86 (25.8)
No	924 (76.7)	458 (76.3)	466 (77.1)	212 (79.7)	219 (80.8)	246 (73.6)	247 (74.2)
Exercise per week							
High	405 (33.0)	203 (33.1)	202 (33.0)	91 (33.1)	83 (30.3)	112 (33.0)	119 (35.1)
Moderate	500 (40.8)	256 (41.7)	244 (39.8)	116 (42.2)	127 (46.4)	140 (41.3)	117 (34.5)
Low	322 (26.2)	155 (25.2)	167 (27.2)	68 (24.7)	64 (23.4)	87 (25.7)	103 (30.4)
Alcohol consumption in past year							
Often	26 (2.1)	18 (3.0)	8 (1.3)	8 (2.9)	6 (2.2)	10 (3.0)	2 (0.6)
Sometimes	380 (31.4)	187 (31.0)	193 (31.7)	81 (29.7)	79 (28.8)	106 (31.5)	114 (33.7)
Never	806 (66.5)	398 (66.0)	408 (67.0)	184 (67.4)	189 (69.0)	221 (65.6)	222 (65.7)

[a]Average of 1–3 measurements per person.

Table 6

Comparison of baseline characteristics in the Original and Expanded eligibility strata for randomised EAGeR participants.[a]

Characteristics N (%)	Total N=1228	Original N=549	Expanded N=679	P-value
Age, y: Mean ± SD	28.7 ± 4.8	28.0 ± 4.9	29.3 ± 4.7	<0.0001[b]
Race				0.0014
White	1162 (94.6)	532 (96.9)	630 (92.8)	
Non-White	66 (5.4)	17 (3.1)	49 (7.2)	
Marital status				0.0004
Married	1124 (91.5)	521 (94.9)	603 (88.8)	
Living with partner	74 (6.0)	18 (3.3)	56 (8.3)	
Other	30 (2.4)	10 (1.8)	20 (3.0)	
> High School Education	1057 (86.2)	492 (89.8)	565 (83.2)	0.0009
Annual income (US $)				0.2682
≥ $100,000	491 (40.0)	201 (36.7)	290 (42.7)	
$75,000–$99,999	149 (12.1)	68 (12.4)	81 (11.9)	
$40,000–$74,999	181 (14.8)	87 (15.9)	94 (13.8)	
$20,000–$39,999	312 (25.4)	145 (26.5)	167 (24.6)	
≤ $19,999	94 (7.7)	47 (8.6)	47 (6.9)	
Employed	895 (75.5)	427 (78.0)	468 (72.0)	0.0023
Time from last loss to randomisation (months)				<0.0001
≤ 4 Months	651 (53.9)	350 (64.9)	301 (44.9)	
5–8 Months	222 (18.4)	125 (23.2)	97 (14.5)	
9–12 Months	99 (8.2)	52 (9.7)	47 (7.0)	
>12 Months	237 (19.6)	12 (2.2)	225 (33.6)	

Demographics by Treatment Arm and Recent Sporadic/Expanded Eligibility Criteria: The EAGeR Trial.[a]

Characteristics N (%)	Total N=1228	Original N=549	Expanded N=679	P-value
Number of previous live births				NA[c]
0	577 (47.0)	328 (59.7)	249 (36.7)	
1	438 (35.7)	212 (38.6)	226 (33.3)	
2	213 (17.4)	9 (1.6)	204 (30.0)	
Number of previous pregnancy losses				NA
1	825 (67.2)	549 (100.0)	276 (40.7)	
2	403 (32.8)	0 (0.0)	403 (59.3)	
BMI, kg/m^2, Mean ± SD	26.4 ± 6.6	25.7 ± 6.1	26.9 ± 7.0	0.0136[d]
Smoking in past year				0.0233
Never	1067 (87.7)	493 (90.5)	574 (85.4)	
Sometimes (<6 times/week)	87 (7.2)	32 (5.9)	55 (8.2)	
Daily	63 (5.2)	20 (3.7)	43 (6.4)	
Alcohol consumption in past year				0.3132

Demographics by Treatment Arm and Recent Sporadic/Expanded Eligibility Criteria: The EAGeR Trial.[a]

Characteristics N (%)	Total N=1228	Original N=549	Expanded N=679	P-value
Often	26 (2.1)	14 (2.6)	12 (1.8)	
Sometimes	380 (31.1)	160 (29.5)	220 (32.9)	
Never	816 (66.8)	369 (68.0)	437 (65.3)	
Months trying to become pregnancy (prior to randomisation) Median (Q1, Q3)	3 (1, 7)	3 (1, 6)	3 (1, 7)	0.4313[e]

[a] Data on covariates were missing for income (n=1), time from last loss to randomisation (n=19), smoking (n=11), alcohol (n=6), education (n=2), and employment (n=44). P-values are based on Fisher exact test for proportions.

[b] These criteria were part of the definition of the two eligibility strata and therefore cannot be tested for statistical significance

[c] T-test.

[d] Wilcoxon-Mann-Whitney test.

[e] Median test.