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Trial of low dose aspirin with an Early Screening Test for preeclampsia and growth restriction TEST Study – A pilot randomised controlled trial

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TITLE: Trial of low dose aspirin with an Early Screening Test for preeclampsia and growth restriction TEST Study – A pilot randomised controlled trial

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ABSTRACT

Objective: Evaluate feasibility and acceptability of routine aspirin in low-risk women, compared to screening-test indicated aspirin for prevention of preeclampsia and fetal growth restriction (FGR) prevention.

Design: Multicentre open-label randomised controlled trial.

Setting: Two tertiary maternity hospitals in Dublin, Ireland.

Participants: 546 low-risk nulliparous women completed the study

Interventions: Women were randomised to; (i) routine aspirin 75mg from 11 until 36 weeks; (ii) no aspirin; and (iii) aspirin based on the Fetal Medicine Foundation screening test.

Primary and secondary outcome measures: (a) proportion agreeing to participate; (b) compliance with protocol; (c) proportion where first trimester uterine artery Doppler was obtainable and; (iv) time taken to issue screening result. Secondary outcomes included rates of preeclampsia and small-for-gestational age fetuses.

Results: 546 were included in the routine aspirin (n=179), no aspirin (n=183) and screen and treat (n=184) groups. 546 of 1054 approached (51.8%), enrolled. Average aspirin adherence was 90%. Uterine artery Doppler was obtained in 98.4% (181/184) and average time to obtain a screening result was 7.6 (0-26) days. Of those taking aspirin, vaginal spotting was greater; n=29 (15.1%), non-aspirin n=143 (7.9%) OR 2.6 (95% CI 1.2-3.6). Post-partum haemorrhage > 500mls was also greater; aspirin n=26 (13.5%), no aspirin n=20 (5.6%) OR 2.6 (95% CI 1.4-4.8). There were no differences in preeclampsia (4.5% vs. 3.8% vs. 3.8% p=0.95) or small-for-gestational-age fetuses (8% vs. 10% vs. 14% p=0.19).

Conclusion: Low-risk nulliparous women are open to taking aspirin in pregnancy and had

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3 high levels of adherence. Aspirin use was associated with greater rates of vaginal bleeding.
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5 An appropriately powered randomized controlled trial is now required to address the efficacy
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7 and safety of universal low dose aspirin in low-risk pregnancy compared to a screening
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9 approach.
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11 **Trial Registration:** www.isrctn.com/ISRCTN15191778
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14 15 16 17 **ARTICLE SUMMARY** 18

19 20 **Strengths and limitations of this study** 21

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23 • Robust multi-centre randomised controlled trial design
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25 • Three methods were used to assess aspirin adherence
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27 • Standardisation of methods
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29 • Potential introduction of reporting bias through open-label design
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INTRODUCTION

Low dose aspirin use prior to 16-weeks can reduce the incidence of preeclampsia in at-risk pregnancies. When commenced at this stage, at a dose of 75mg, its efficacy in low-risk pregnancies is unknown.^{1,2} With the emergence of first trimester screening tests for preeclampsia such as that of the Fetal Medicine Foundation (FMF), one can predict from 11-weeks, the risk of preeclampsia.³ Internationally, there are conflicting consensus statements on screening methods and which women meet criteria for aspirin use.⁴ Application of the FMF screening test and provision of low dose aspirin to screen positive women can significantly reduce the incidence of early-onset preeclampsia (4.3% aspirin vs. 1.6% placebo $p=0.004$), although predictive performance of the algorithm appears to vary between populations.⁵ It has been proposed that performance of the FMF algorithm is superior to the methods recommended by the National Institute of Clinical Excellence and American College of Obstetricians and Gynecologists (ACOG).⁶ It may be more efficacious to prescribe low dose aspirin universally, although there is no evidence to support such a policy as yet.⁷ To determine this, one must first evaluate if low-risk women are willing to take aspirin in pregnancy and if undergoing a comprehensive screening test is realistic in the routine setting. Hence, the primary objective of this multi-center open label randomised controlled trial was to evaluate the acceptability and feasibility of women taking aspirin 75mg from beyond 11-weeks gestation versus screening test-indicated aspirin. Secondary outcomes included rates of; (i) preeclampsia; (ii) small-for-gestational age infants; (iii) pre-term delivery; (iv) admission to neonatal intensive care; (v) placental abruption; (vi) any reported death and; (vii) acceptability of women taking aspirin routinely versus test indicated aspirin, assessed by a questionnaire.

METHODS

Study Design

This open-label multicenter randomised controlled trial (RCT) was performed in two Irish tertiary maternity hospitals with 18,000 deliveries per annum. The aim was to include three centers, however there was a delay in the local ethics committee decision for the third center (subsequently approved), which was excluded in the interests of study schedule. The protocol for this multicenter randomised controlled trial has been published⁸ and was prospectively authorized by the Health Products Regulatory Authority and National Maternity Hospital Central Ethics Committee. The trial was registered with the ISRCTN number 15191778 and was supported by Perinatal Ireland HRB and the HRB Mother and Baby Clinical Trials Network following external peer review for scientific quality. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. An independent Trial Steering Committee and Data Monitoring Committee met quarterly to oversee the safety of the trial participants.

Nulliparous women over 18-years-old between 11 to 13+6 weeks gestation with a viable singleton pregnancy that didn't meet criteria for aspirin commencement based upon major preeclampsia risk factors (chronic kidney disease, autoimmune disease e.g. systemic lupus erythematosus, diabetes mellitus and chronic hypertension) were eligible for inclusion and thus were recruited at antenatal booking clinics selected at random.⁹ In Ireland it is currently not routine obstetric practice to commence aspirin in women that do not have an aforementioned major risk factor for pre-eclampsia as defined by the National Institute of Health and Clinical Excellence.⁹ Exclusion criteria included participants already taking part in a clinical trial, co-existence of fetal congenital anomaly at recruitment or those with aspirin hypersensitivity. All participants provided written informed consent and were recruited by

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3 the research clinician at the first trimester antenatal booking visit.
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6 7 **Randomization**

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9 Participants underwent online computerized randomization based on blocks of six to; (i)
10 aspirin 75mg from 11 to 13+6 weeks once daily until 36-weeks' gestation; (ii) no aspirin and;
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12 (iii) aspirin depending on the result of the FMF screening test. Subjects in non-aspirin taking
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14 groups had routine antenatal care.
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17 18 19 **Intervention**

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21 Enteric coated Nu-Seals Aspirin (Acetylsalicylic Acid) 75mg orally once daily at night from
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23 11 to 36-weeks gestation was provided free of charge from Alliance Pharma®, which were
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25 independent of study protocol and analysis. A dose of 75mg was used as this is currently the
26
27 standard recommended dosage in the UK and Ireland for at-risk women.⁹
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29 Aspirin adherence was assessed subjectively via patient reported diary cards and tablet counts
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31 (checked by research clinician and pharmacist) and objectively via assessment of change in
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33 urinary 11-dehydroxothromboxane-B2 (TxB2). Any reduction in TxB2 between first (pre-
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35 aspirin) and second trimester (post-aspirin) levels was taken to suggest that a subject had
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37 ingested aspirin within the last ten days.¹⁰
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44 **Baseline review and follow-up**

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46 Participants underwent two scheduled study visits, at study recruitment and at 20-22 weeks
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48 with diary cards and aspirin tablets returned to the research team at 36-weeks gestation.
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50 Participants completed an anonymous questionnaire at 20-22 weeks based on acceptability of
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52 taking aspirin in pregnancy.
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3 Study assessments at the time of the recruitment visit included the FMF screening test, the
4 results of which were assessed for those in Group 3 (screen and treat). The FMF screening
5 test was not routine practice within Ireland. Components of the screening test included;
6 maternal history (including ovulation induced conception, race, body mass index, age, mother
7 with preeclampsia); mean arterial blood pressure (MAP); uterine artery Doppler pulsatility
8 index; and pregnancy associated plasma protein-A (PAPP-A) and placental like growth factor
9 (PLGF) multiples of the median. To determine risk of preeclampsia, the FMF algorithm was
10 used and based upon a screen positive rate of 5%, a cut off for preeclampsia prior to 42-
11 weeks at greater than 1:8 was used.³ This cut-off was selected with the aim of capturing the
12 majority of preeclamptics; both pre and post-term and at the time of study commencement
13 this was the optimal screening algorithm for detection of any preeclampsia. Two un-blinded
14 trained clinical research sonographers performed the first trimester uterine artery Doppler
15 waveforms and MAP and interpreted findings. MAP was assessed using an automated blood
16 pressure monitoring device as outlined by the technique stipulated by the FMF.¹¹ Uterine
17 artery Doppler velocimetry was obtained using Viewpoint® Version 5.6.16 GE Healthcare,
18 2012 and Voluson Expert 730®, GE 2012 using the technique outlined from by the
19 International Society of Ultrasound in Obstetrics and Gynecology. The pulsatility index was
20 measured from both uterine arteries and an average value was calculated.¹²

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44 A maternal blood sample was analyzed for PAPP-A and PLGF under standard conditions
45 using a 6000 DELFIA® Xpress, PerkinElmer, 2014 clinical random access screening
46 platform in the hospital clinical biochemistry laboratory. A quantitative immunoturbimetric
47 TxBCardio® immunoassay was used to determine TxB2 levels in urine samples obtained
48 both study visits. These were then standardized as a ratio with creatinine levels and
49 expressed as pg/mg creatinine.
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Outcomes

The primary objective was to evaluate the feasibility and acceptability of low-risk nulliparous women taking aspirin versus test indicated aspirin in pregnancy. Outcome measures included;

- (i) The proportion of eligible women agreeing to participate in a trial where aspirin is prescribed routinely (feasibility);
- (ii) Compliance with study protocol, as measured by the following: (a) adherence to aspirin (acceptability), (b) attendance at study visits (acceptability), (c) satisfactory collection of all endpoints and variables (feasibility), (d) specific study protocol violations (feasibility);
- (iii) The proportion of women in whom it was possible to obtain first trimester trans-abdominal uterine artery Doppler velocimetry examination (feasibility);
- (iv) Proportion of women with a completed screening test who were issued the screening result within one week of having the test performed (feasibility);

Secondary outcomes included rates of; (i) preeclampsia; (ii) small-for-gestational-age (SGA) infants (customised sex-specific birth-weight <10th centile); (iii) pre-term delivery prior to 34-weeks; (iv) admission to neonatal intensive care unit (NICU); (v) placental abruption; (vi) any reported death (stillbirth, neonatal or infant death) and; (vii) acceptability of women taking aspirin routinely versus test indicated aspirin as assessed by an anonymous questionnaire at 20-22 weeks. As part of routine antenatal care women had an appointment with their midwife and or clinician at booking (11-14 weeks), 16-weeks, 18-20 weeks, 25-weeks, 28-weeks, 31-weeks, 34-weeks, 36-weeks, 28-weeks, 40-weeks and 41-weeks gestation in line with hospital protocol. At each visit blood pressure was assessed using mercury sphygmomanometry and a urine dipstick for proteinuria was performed with

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3 symphysio-fundal-height and or fetal biometrical ultrasound assessment as appropriate. Pre-
4 eclampsia was defined based upon the definition from the ISSHP with new onset
5 hypertension (>140mmHg systolic or >90mmHg diastolic) after 20-weeks gestation
6 associated with; (i) proteinuria of at least 1g/L [2+] on urine dipstick testing, and or; (ii)
7 maternal organ dysfunction ; an or fetal growth restriction.¹³ Suspicion of a diagnosis of pre-
8 eclampsia at an antenatal visit prompted further investigation in the fetal assessment unit with
9 clinical examination, blood testing (urea and creatinine, liver function tests and full blood
10 picture), 24-hour urine collection for proteinuria and departmental fetal ultrasound
11 assessment with final diagnosis made by an obstetrician.
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24 Safety data were reported as adverse and serious adverse events and participants discontinued
25 from the study were recorded in addition to the reason for discontinuation and outcome. As
26 an assessment of post-partum haemorrhage, blood loss was weighed at time of delivery.
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33 **Statistical Analysis**

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35 As outlined in the published study protocol, the projected sample size for this study was 500
36 women across two sites with 18,000 deliveries per annum.⁸ To determine preeclampsia as a
37 primary outcome, the anticipated number of patients required is over 15,000 women. As this
38 study aimed to determine the feasibility of such a study, 500 participants were more than
39 adequate as 3% of the number required for a substantive study is required (n=450).¹⁴
40 Accounting for a drop-out rate of 10% (n=45), 500 participants were adequate to obtain the
41 primary outcome. Analysis was performed by a statistician using SAS v.20 on the intention-
42 to-treat (ITT) population, which included all participants randomised, which completed the
43 full second trimester assessment. Measures of variance included standard deviation. Follow-
44 up of serious adverse events continued until 28-days following delivery. Adverse events were
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3 reported as odds ratios (OR). To assess secondary outcome and safety, comparisons of
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5 groups were be performed using two sample t-tests, Wilcoxon Rank-sum tests and Chi-square
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7 tests.
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12 **Patient Involvement**

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15 Although patients were not directly involved in devising the study protocol and design the
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17 burden of the RCT intervention (i.e. taking aspirin and undergoing the FMF screening test)
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19 was assessed by means of an anonymous questionnaire completed at 20-22 weeks gestation.
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21 At the time of study participation subjects were informed that study results could be viewed
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23 following publication on the study website; <http://perinatalireland.ie/research/test/>
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RESULTS

Subjects were recruited between 8th May 2014 to 23rd September 2015. In total 1054 eligible women were approached to take part in the study and of these, 557 underwent randomization [Figure 1]. In the screen and treat population (Group 3) n=184, 13 (7.1%) women had a risk of developing preeclampsia >1:8 and subsequently commenced aspirin until 36-weeks gestation. Eleven women were excluded from the study leaving 546 in the ITT population. In total there were 192 women in the ITT group that were taking aspirin as per randomization and 354 not taking aspirin. There were no significant differences between groups at baseline [Table 1].

| Characteristic | Low Dose Aspirin N=179 | No Aspirin N=183 | Screen & Treat N=184 |
|---|---------------------------|---------------------|-------------------------|
| Age (yr) | 33 (19-44) | 34 (18-43) | 33 (19-44) |
| Race – No. (%) | | | |
| White | 181 (97.9) | 179 (95.7) | 180 (97.3) |
| Black | 1 (0.5) | 2 (1.1) | 0 (0) |
| Asian | 3 (1.6) | 6 (3.2) | 5 (2.7) |
| Other | 0 | 0 (0) | 0 (0) |
| Completed secondary school – No.(%) | 136 (73.5) | 143 (76.4) | 152 (82.2) |
| BMI (kg/m ²) | 25.2 (17.4-39.4) | 22.9 (17.7-41.4) | 23.8 (18.1-45.2) |
| Gestational Age (wks) | 12.9 (11.1-13.9) | 12.9 (11.1-13.9) | 12.9 (11.3-13.9) |
| Smoking – No. (%) | 17(9.2) | 11 (5.9) | 7 (3.8) |
| Subject's mother had preeclampsia - No. (%) | 7 (3.8) | 10 (5.4) | 10 (5.4) |
| Conception – No. (%) | | | |
| IVF | 5 (2.7) | 9 (4.8) | 8 (4.3) |
| ICSI | 3 (1.6) | 4 (2.1) | 3 (1.6) |
| Ovulation induction | 5 (2.7) | 6 (3.2) | 6 (3.2) |
| Spontaneous | 172 (93.0) | 168 (89.9) | 170 (91.9) |
| Previous miscarriage – No. (%) | 20 (10.8) | 31 (16.6) | 31 (16.8) |

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3 Table 1: Baseline characteristics of the study population. Where number (No.) percentage is
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5 not expressed average and range are demonstrated.
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8 9 **Primary outcomes**

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11 (i) The proportion of eligible women agreeing to participate in a trial where aspirin is
12 prescribed routinely (feasibility); 1054 women were approached that were eligible to partake.
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14 497 were subsequently not enrolled as they did not want to take aspirin n=454 or for an
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16 alternative reason n=43 e.g. appointment did not suit. Hence 546/1054 (51.8%) women were
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18 willing to partake in a study where they may have to take aspirin routinely.
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24 (ii) Compliance with study protocol, as measured by the following: (a) adherence to aspirin
25 (acceptability), (b) attendance at study visits (acceptability), (c) satisfactory collection of all
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27 endpoints and variables (feasibility), (d) specific study protocol violations (feasibility);
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33 (a) Of those women included in analysis that were taking aspirin (n=192), the average
34 adherence based upon patient reported diary cards was 96.0% and based upon tablet counts
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36 95.0%. Seven women were non-adherent and 19 (10.0%) poorly compliant (<80%).
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38 Average adherence was 95.0% in both the test indicated aspirin group (3a) and routine aspirin
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40 group (1) [Table 2]. The median first trimester pre-aspirin urine TxB2 level was 8662.2
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42 pg/mg (IQR 2014.5-9931.5) and second trimester (post-aspirin) 2285.1 pg/mg (IQR 591.0-
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44 2300.1). The percentage change in TxB2 was then assessed for all paired samples (n=147)
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46 and found that 124/147 (84.4%) of subjects had a fall in TxB2 levels between the first and
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48 second trimesters versus 23/147 (15.6%) who had an increase p<0.001. The greater the
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50 reduction in urinary TxB2 pre- and post- aspirin dose the greater the degree of aspirin
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3 adherence, as demonstrated in Figure 2. There was no difference between patient groups
4 (routine aspirin and screen positive aspirin) and percentage change in urine TxB2 (p=0.61).
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9 (b) Of those that underwent randomization (n=557), eleven were excluded prior to fulfillment
10 of study participation requirements (attendance at second study visit). Of the eleven, three
11 withdrew consent for participation as they decided that they did not wish to take aspirin
12 following randomization.
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18 (c) Of all 546 subjects collection of outcome measures and variables were obtained for all
19 apart from the questionnaire on patient acceptability, which was completed in 97.1%
20 (530/546).
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28 d) Six protocol violations were recorded (0.01 per 100 participants) including women
29 transferring care to another hospital (n=3), incorrect randomization of women that did not
30 meet inclusion criteria (n=2) and a subject in the non-aspirin group commencing aspirin by
31 their clinician (n=1).
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39 (iii) The proportion of women in whom it was possible to obtain first trimester trans-
40 abdominal uterine artery Doppler velocimetry (feasibility); The FMF screening test was
41 completed in 98.4% (181/184) following successful uterine artery Doppler velocimetry
42 acquisition, of which one was obtained vaginally due to challenges with abdominal
43 acquisition, with an overall sonographer reported ease of acquisition 3.1 (SD +/- 0.91) (score
44 1 (easy) to 5 (unobtainable)) [Table 2].
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(iv) Proportion of women with a completed screening test, issued the result within one week of the test (feasibility); The average time to obtain laboratory analyzed PAPP-A and PLGF so that a screening result could be issued was 7.6 days (0-26) with 78 (42.4%) of women waiting greater than one week and five women being beyond 16-weeks prior to result availability [Table 2].

| Adherence and feasibility parameter | Low-dose Aspirin (N=179) | No Aspirin (N=183) | Screen & Treat (N=184) |
|-------------------------------------|--------------------------|--------------------|------------------------|
| Ease of Doppler acquisition | | | |
| Very easy | | | 8 (4%) |
| Easy | | | 53 (29%) |
| Fair | | | 61 (33%) |
| Difficult | | | 60 (32%) |
| Unobtainable | | | 3 (2%) |
| Days to PLGF/PAPPA visit 1 | | | 7.6 [0 - 26] |
| PLGF/PAPPA result > 16 weeks | | | 5 (3%) |
| Time taken for visit 1 (mins) | 60 [30 – 100] | 60 [25 - 90] | 60 [25 - 90] |
| Median adherence tablet counts | 96% | | 95% (screen positive) |

| | | | |
|------------------------------|--------|--|-----------------------|
| Median adherence diary cards | 94% | | 95% (screen positive) |
| Non-adherent | 7 (4%) | | 0 (0%) |

Table 2: Primary outcomes of feasibility and adherence

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Secondary outcomes

There was no difference between groups in relation to secondary outcomes [Table 3]. For the overall cohort, there were three cases (0.37%) of early onset preeclampsia <34-weeks (0.55%), n=22 (4.03%) any preeclampsia, n=57 (10.44%) SGA infants and 15.02% (n=82) placental disease. Secondary outcomes for groups 3A (screen positive aspirin) and 3B (screen negative no aspirin) are demonstrated in Table S1 [supplementary]. Despite taking aspirin, there remained a significantly greater number with preeclampsia at <37-weeks in the screen positive versus the screen negative group, although numbers were small (n=2 (15.4%) vs. n=2 (1.2%) p=0.02). In terms of taking aspirin in a subsequent pregnancy, the questionnaire revealed that 92.3% (489/530) were willing to take aspirin in a subsequent pregnancy; 92.5% (173/187) of aspirin takers and 91.5% (314/343) of non-aspirin takers.

| Outcome | Low Dose Aspirin (Group 1) N=179 | No Aspirin (Group 2) N=183 | Screen and Treat (Group 3) N=184 | p-value |
|---|-------------------------------------|-------------------------------|-------------------------------------|---------|
| Gestation at delivery (weeks) | 40.2 (1.4) | 39.9 (1.9) | 40.2 (1.5) | 0.13 |
| Birthweight (g) | 3529 (469) | 3478 (493) | 3488 (502) | 0.58 |
| Birthweight <10 th centile No. (%) | 14 (8%) | 18(10%) | 25 (14%) | 0.19 |
| Mode of delivery No. (%) | | | | |
| Spontaneous | 85 (47.5) | 95 (52.0) | 88 (47.8) | 0.64 |
| Instrumental | 56 (31.3) | 47 (25.7) | 51 (27.7) | 0.09 |
| Caesarean | 38 (21.2) | 41 (22.3) | 45 (24.5) | 0.68 |
| Pre-term delivery <34 weeks No. (%) | 1 (0.6) | 3 (1.6) | 2 (1.1) | 0.62 |
| Spontaneous Labor No. (%) | 96 (53.7) | 103 (56.3) | 101 (54.9) | 0.88 |
| Gender No. (%) | | | | |
| Male | 91 (50.8) | 91 (49.7) | 100 (54.3) | 0.65 |
| Female | 88 (49.2) | 92 (50.3) | 84 (45.7) | 0.65 |
| Preeclampsia No. (%) | 8 (4.5) | 7 (3.8) | 7 (3.8) | 0.95 |
| Preeclampsia <34-weeks | 0 (0) | 2 (1.1) | 1 (0.5) | 0.56 |
| Preeclampsia <37-weeks | 2 (1.1) | 2 (1.1) | 2 (1.1) | 0.99 |
| Abruption No. (%) | 1 (0.5) | 0 (0) | 0 (0) | 0.71 |
| NICU admission No. (%) | 9 (5.0) | 7 (3.8) | 9 (4.9) | 0.83 |
| Apgar < 7 No. (%) | 5 (2.8) | 2 (1.6) | 3 (1.6) | 0.46 |
| Cord pH (arterial) | 7.3 (0.1) | 7.3 (0.1) | 7.3 (0.1) | 0.55 |
| Outcome No. (%) | | | | |
| Alive at 6-weeks | 177 (98.9) | 181 (99.0) | 182 (98.9) | 0.99 |
| Stillbirth | 2 (1.1) | 1 (0.5) | 0 (0) | 0.81 |
| Neonatal death | 0 (0) | 1 (0.5) | 2 (1.1) | 0.37 |

Table 3: Secondary outcome measures

(Expressed as average and standard deviation unless otherwise stated)

Safety

There were differences between groups in relation to adverse but not serious adverse events [Tables 4 and S2]. There were six perinatal deaths, all of which underwent postmortem. In the aspirin group there was one placental abruption and one case of intervillous haemorrhage. Perinatal deaths in the non-aspirin groups were due to delayed villous maturation, severe FGR, fetal thrombotic vasculopathy and neonatal septicemia. There was a difference between groups in terms of reported vaginal spotting aspirin 15.1% vs. non-aspirin 7.9% OR 2.1 (CI 1.2-3.6), which was not associated with pregnancy loss. Although, not statistically significant, there was a difference in terms of PPH >1000mls. Although rates of PPH <1000mls were greater in the aspirin-taking group, no differences were noted in terms of blood transfusion or significant hemoglobin drop to <8g/dL.

| Event | | Aspirin n=192 | Non-aspirin n=354 | Odds ratio (95% CI) |
|---------------------------------|---------------------------------|------------------|----------------------|------------------------|
| Adverse Events | | | | |
| Adverse Events Total No. | | 123 | 143 | 2.6 (1.8-3.8) |
| Vaginal spotting* No. (%) | | 29(15.1) | 28 (7.9) | 2.1 (1.2-3.6) |
| Post-partum haemorrhage No. (%) | | | | |
| >500mls* | | 26(13.5) | 20 (5.6) | 2.6 (1.4-4.8) |
| >1000mls | | 7 (3.6) | 5 (1.4) | 2.8 (0.9-9.0) |
| Blood transfusion | | 3 | 4 | 0.5 (0.1-2.7) |
| Hb drop <8g/dL | | 4 | 7 | 0.3 (0.1-1.4) |
| Serious Adverse Event | | | | |
| NICU admission | Sepsis | 3 | 2 | |
| | Hypoglycaemia | 0 | 1 | |
| | Prematurity | 1 | 4 | |
| | Jaundice | 1 | 1 | |
| | Persistently low Apgar | 1 | 3 | |
| | TTN | 1 | 3 | |
| | Meconium aspiration | 1 | 0 | |
| | Hypoxic ischemic encephalopathy | 1 | 1 | |
| | Very low birthweight | 0 | 1 | |
| Total | | 9 | 16 | 1.04 (0.45-2.40) |
| Perinatal Death | | 2 | 4 | |
| Total | | 2 | 4 | 0.92 (0.17-5.10) |
| Maternal admission | Preterm labor | 3 | 2 | |
| | Preeclampsia | 8 | 7 | |
| | Antepartum hemorrhage | 3 | 7 | |
| | PPROM | 0 | 2 | |
| | Fetal compromise | 1 | 2 | |
| | Infection | 2 | 5 | |
| | Other | 4 | 1 | |
| Total | | 21 | 26 | 1.55 (0.85-2.83) |
| Congenital anomaly | Cardiac | 1 | 2 | |
| | Gastrointestinal | 3 | 2 | |
| | Neurological | 0 | 1 | |
| | Renal | 0 | 1 | |
| Total | | 4 | 6 | 1.23 (0.34-4.43) |
| Total serious adverse events | | 36 | 52 | 1.34 (0.84-2.14) |

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5 Table 4: Adverse and serious adverse events in aspirin and non-aspirin taking groups. There
6
7 may be >1 adverse event or serious adverse event per subject * ($p<0.05$) [NICU=Neonatal
8
9 intensive care unit, TTN= transient tachypnea of the newborn, PPOM= preterm premature
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11 rupture of membranes, Very low birthweight = <1500g].
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DISCUSSION

Main Findings

This randomised controlled trial has found that low-risk nulliparous women were open to taking aspirin in pregnancy and were adherent, with a willingness to take it again in a subsequent pregnancy. We can say this as, comparing findings to other RCTs in pregnancy, of which there are few, the uptake in this RCT was much higher as was adherence (e.g. Chiswick, *et al.* 2015; 35% enrolment and 65-67% adherence with metformin use).¹⁵ This is the first trial of its kind, which has assessed the acceptability of women taking aspirin in low-risk pregnancy and the feasibility of an integrated screening test in a routine clinical setting.

Strengths and Limitations

The strengths of this study are the multicenter RCT design with robust protocol and oversight and previously published methodology. Allocation bias was limited by use of a prospective approach and selection bias was limited by randomization. The fact that the same two sonographers and biochemists were responsible for conducting the screening test with use of quality control standards for test completion using the same equipment and technique for all subjects optimized reproducibility. There were a low number of dropouts and almost all patient outcomes were recorded. Although there is currently no validated scientific method of assessing aspirin adherence,¹⁶ a laboratory assessment of change in TxB2 served as a more objective assessment, strengthening reliability. There is currently no accepted test in the literature, which can reliably determine aspirin adherence, hence three different methods were used to optimize reliability.¹⁶ Study weaknesses, were primarily that PAPP-A and PLGF analysis was performed in the laboratory using validated methods with quality assurance, as opposed to the bedside point-of-care tests hence it took longer to obtain a result. In a non-research setting with a greater throughput of patients, one could anticipate a

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3 faster turnaround time. Additionally the open-label nature of the study meant that safety
4 recording was open to reporting bias and, as is often the case with RCTs the uptake of
5 subjects demonstrated dominance for educated women. In RCTs there is always a risk of
6 introducing a Hawthorne effect, whereby subjects act differently in the confines of an RCT as
7 to how they would in a real-life setting, hence adherence rates may have been over-
8 represented.¹⁷ A third trimester visit may have added strength to the study to assess
9 objectively for aspirin adherence and patient satisfaction, however as adherence prior to 16-
10 weeks was deemed the critical time point for preeclampsia prevention, follow-up at 20-22
11 weeks was selected.
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24 **Interpretation**

25
26 A recently published large RCT from the FMF found that, following application of FMF
27 screening and subsequent randomization of women deemed to be at risk of preterm
28 preeclampsia to aspirin 150mg versus placebo, there was a reduction in the incidence of
29 preterm preeclampsia in the aspirin arm.⁵ Our study differs on several counts; (i) routine
30 aspirin arm – use of a third arm assessing provision of routine aspirin assessed the
31 acceptability and feasibility of this policy; (ii) aspirin dosage (150mg vs. 75mg) – in light of
32 limited evidence on dosage and effect, the safest lowest effective dose was selected; (iii)
33 adverse events – rates of PPH and vaginal bleeding were reported. This information would
34 be useful from the FMF study in light of the higher aspirin dosing regime and; (iv) our study
35 was not powered to detect a difference in clinical outcome, with the primary focus feasibility
36 and acceptability.
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52 Few studies have assessed the acceptability of non-routine medications in pregnancy. In the
53 developing world, pregnant women are willing to take calcium, oral iron and
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3 micronutrients.¹⁸⁻²⁰ If instructed about potential side-effects and reminded frequently women
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5 had higher levels of adherence with the greatest barrier being forgetfulness. Average
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7 medication adherence in pregnancy for chronic illness is higher than for non-routine
8
9 medications at 90-95%,²¹ hence it is promising that we have noted a rate as high as this in
10
11 our own study. There was a slight discrepancy in adherence assessed via tablet counts and
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13 diary cards and that more objectively assessed via TxB2. Reasons for this may include the
14
15 potential for aspirin resistance; which although not formally assessed in this study can be
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17 increased when using an enteric-coated preparation.²²
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23 The FMF screening test was feasible in terms of acquiring first trimester uterine artery
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25 Doppler velocimetry measurements, though delays were encountered in obtaining laboratory
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27 analyzed PAPP-A and PLGF. This is relevant as it reflects the practical aspects of such a
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29 screening test in a clinical real life setting. Improved protocols between the clinical and
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31 laboratory staff would be required to allow patients receive results within a reasonable
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33 timeline.
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38 In terms of vaginal spotting and clinically significant PPH with aspirin use, the findings of
39
40 this study are comparable with previous studies although evidence of increased antenatal and
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42 postnatal bleeding, requires further investigation, most notably with use of aspirin at doses
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44 greater than 75mg.²³⁻²⁴ Due to the open-label nature of this study as opposed to placebo
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46 control, there is always a potential of reporting bias of bleeding in the aspirin arms. Although
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48 generally safe in pregnancy, it may be worthwhile considering cessation of aspirin at 32-34
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50 weeks gestation with the aim of reducing the risk of PPH, as opposed to 36-weeks and of
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52 informing women of the unwanted side-effect of increased vaginal spotting.
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Conclusion

It has been proposed that the most cost-effective approach to reducing preeclampsia is the provision of an effective, affordable and safe intervention applied to all mothers without prior testing to assess levels of risk.⁷ A algorithm-based screen-and-treat approach, as proposed by the FMF has can reduce rates of pre-term preeclampsia when doses of 150mg of aspirin are used. Our study was not powered to nor did it detect a difference in rates of preeclampsia between groups, yet has taken the first step to address if low-risk nulliparous women are open to taking aspirin in the first instance and if a screening algorithm is feasible. Moving forward, an RCT is required to address the efficacy of universal low dose aspirin in low-risk pregnancy compared to a screening approach. This will require significant numbers due to the low incidence of early-onset preeclampsia. Although women were open to taking aspirin in pregnancy compared to other RCTs involving medication, almost twice the number enrolled had to be approached to obtain adequate study participants. This must be considered when planning a future trial.

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8
9 Mother and Baby Clinical Trials Network.
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13 **CONTRIBUTION OF AUTHORSHIP:** (i) Conceived and designed the experiments: FM,
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15 CM, PMcP, FB, PD, DM, MC, AS, FC, JM, SD, JH, AC, ET, PD, ZA, FDM, FMcA; (ii)
16
17 Performed the experiments: FM, CM, FC ; (iii) Analyzed the data: FM, PD, ZA, FMcA; (iv)
18
19 Contributed reagents/materials/analysis/tools: PMcP, FB, PD, DM, MC, AS, JM, SD, JH,
20
21 AC, AH, ET, PD, ZA, FDM, FMcA; (v) Wrote the paper: FM, CM, PMcP, FB, PD, DM,
22
23 MC, AS, FC, JM, SD, JH, AC, AH, ET, PD, ZA, FDM, FMcA
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33 **DATA SHARING STATEMENT:** Dataset available from corresponding author on request
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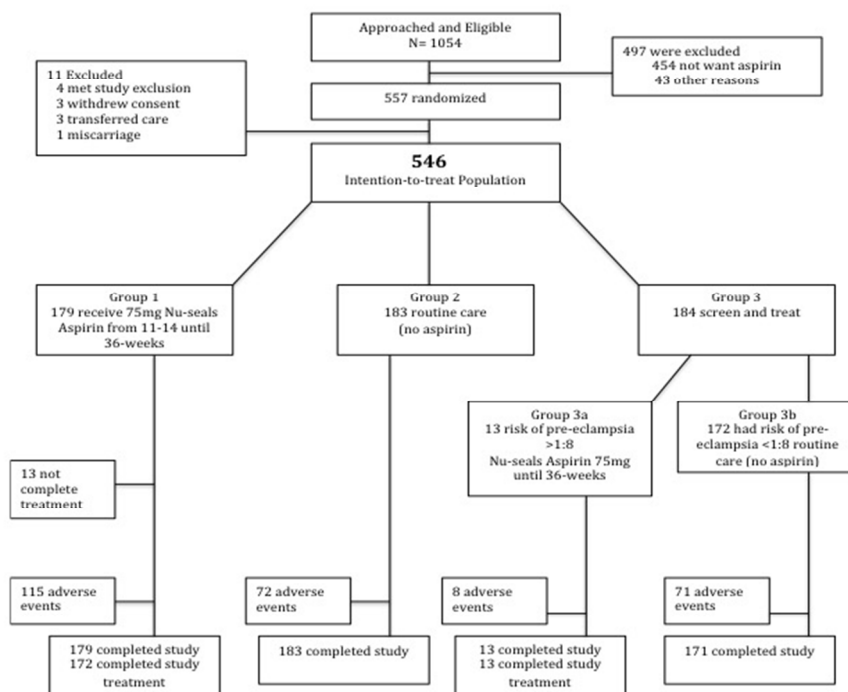
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FIGURE LEGENDS

Figure 1 - Consort diagram

Figure 2 - Histogram demonstrating percentage change in urinary thromboxane-B2 levels pre- and post - aspirin administration (n=147) [TxB2 = urinary-thromboxane level]

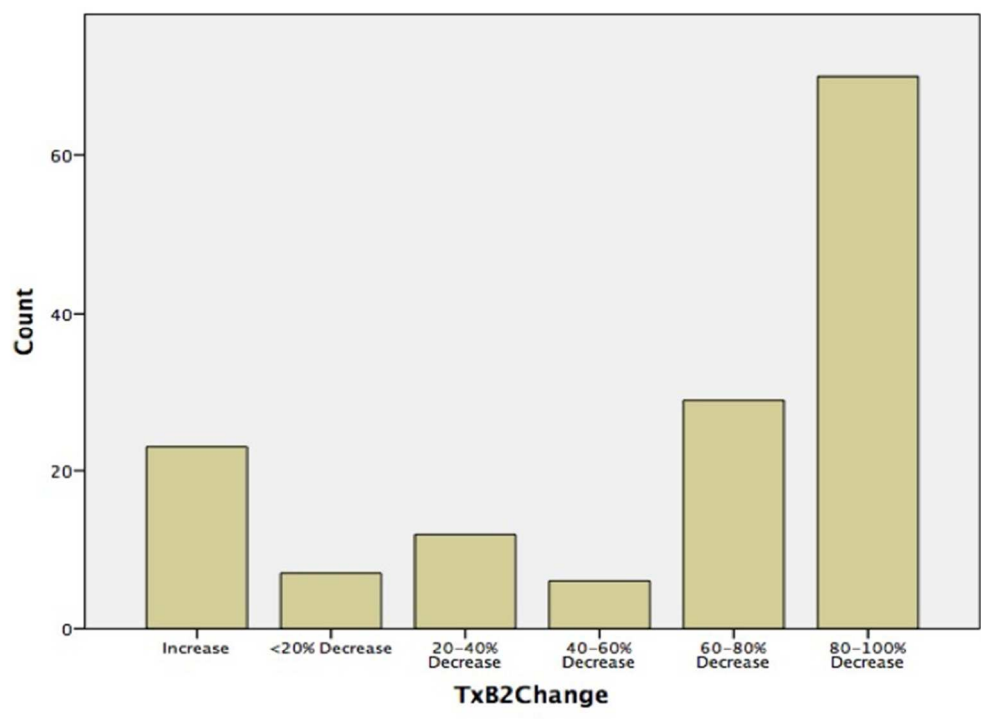
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Consort diagram

254x190mm (72 x 72 DPI)

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Histogram demonstrating percentage change in urinary thromboxane-B2 levels pre- and post - aspirin administration (n=147) [TxB2 = urinary-thromboxane level]

254x190mm (72 x 72 DPI)

www only

| Outcome | Screen positive; Aspirin Group 3A N=13 | Screen negative; No Aspirin Group 3B N=171 | p-value |
|---|---|---|---------|
| Preeclampsia No. (%) | 2 (15.4) | 5 (2.9) | 0.13 |
| Pre-eclampsia <34-weeks | 0 (0) | 2 (1.2) | 0.70 |
| Pre-eclampsia <37-weeks | 2 (15.4) | 2 (1.2) | 0.02 |
| Birthweight <10 th centile No. (%) | 4 (30.7) | 21 (12.3) | 0.15 |
| Pre-term delivery <34 weeks No. (%) | 1 (7.7) | 1 (0.6) | 0.32 |
| NICU admission No. (%) | 0 (0) | 9 (5.3) | 0.86 |
| Outcome No. (%) | | | |
| Alive at 6-weeks | 13 (100) | 169 (98.8) | 0.70 |
| Stillbirth | 0 (0) | 2 (1.2) | 0.70 |
| Neonatal death | 0 (0) | 0 (0) | -- |

Table S1 - Secondary outcome measures in Group 3 (screen and treat)

(Expressed as average and standard deviation unless otherwise stated)

| Adverse/Serious Adverse Event | Low dose Aspirin Group 1 N=179 | No-aspirin Group 2 N=183 | Screen and treat Group 3 N=184 | p-value |
|---------------------------------|--------------------------------------|--------------------------------|--------------------------------------|---------|
| Adverse events | | | | |
| Vaginal spotting No. (%)* | 27 (15.1) | 18 (9.8) | 12 (6.5) | 0.03 |
| Post-partum haemorrhage No. (%) | | | | |
| >500mls* | 25 (13.0) | 9 (4.9) | 12 (6.5) | 0.004 |
| >1000mls | 7 (3.6%) | 1 (0.5) | 4 (2.2) | 0.073 |
| Serious Adverse Events | | | | |
| NICU admission | 9 (5.0) | 7 (3.8) | 9 (4.9) | 0.83 |
| Perinatal Death | 2 (1.1) | 2 (1.1) | 2 (1.1) | 0.99 |
| Maternal admission | 18 (10.1) | 15 (8.2) | 14 (7.6) | 0.69 |
| Congenital anomaly | 3 (1.7) | 4 (2.2) | 3 (1.6) | 0.91 |
| Total serious adverse events | 32 (17.8) | 28 (15.3) | 28 (15.2) | 0.74 |

Table S2 – Adverse and serious adverse events in all three groups. There may be >1 adverse event or serious adverse event per subject * (p<0.05)



CONSORT 2010 checklist of information to include when reporting a randomised trial*

| Section/Topic | Item No | Checklist item | Reported on page No |
|----------------------------------|---------|---|---------------------|
| Title and abstract | | | |
| | 1a | Identification as a randomised trial in the title | 1 |
| | 1b | Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts) | 3 |
| Introduction | | | |
| Background and objectives | 2a | Scientific background and explanation of rationale | 5 |
| | 2b | Specific objectives or hypotheses | 5 |
| Methods | | | |
| Trial design | 3a | Description of trial design (such as parallel, factorial) including allocation ratio | 7 |
| | 3b | Important changes to methods after trial commencement (such as eligibility criteria), with reasons | N/A |
| Participants | 4a | Eligibility criteria for participants | 6 |
| | 4b | Settings and locations where the data were collected | 6 |
| Interventions | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | 7 |
| Outcomes | 6a | Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed | 9 |
| | 6b | Any changes to trial outcomes after the trial commenced, with reasons | N/A |
| Sample size | 7a | How sample size was determined | 10 |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | N/A |
| Randomisation: | | | |
| Sequence generation | 8a | Method used to generate the random allocation sequence | 7 |
| | 8b | Type of randomisation; details of any restriction (such as blocking and block size) | 7 |
| Allocation concealment mechanism | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned | 7 |
| Implementation | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions | 7 |
| Blinding | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those | N/A |

| | | | |
|----|--------------------------|---|------------|
| 1 | | | |
| 2 | | assessing outcomes) and how | |
| 3 | | | |
| 4 | | 11b If relevant, description of the similarity of interventions | N/A |
| 5 | Statistical methods | 12a Statistical methods used to compare groups for primary and secondary outcomes | 10 |
| 6 | | 12b Methods for additional analyses, such as subgroup analyses and adjusted analyses | 10 |
| 7 | | | |
| 8 | Results | | |
| 9 | Participant flow (a | 13a For each group, the numbers of participants who were randomly assigned, received intended treatment, and | 12 & Fig 1 |
| 10 | diagram is strongly | were analysed for the primary outcome | |
| 11 | recommended) | 13b For each group, losses and exclusions after randomisation, together with reasons | Fig 1 |
| 12 | Recruitment | 14a Dates defining the periods of recruitment and follow-up | 12 |
| 13 | | 14b Why the trial ended or was stopped | 12 |
| 14 | Baseline data | 15 A table showing baseline demographic and clinical characteristics for each group | 13 |
| 15 | Numbers analysed | 16 For each group, number of participants (denominator) included in each analysis and whether the analysis was | 12 & Fig 1 |
| 16 | | by original assigned groups | |
| 17 | | | |
| 18 | Outcomes and | 17a For each primary and secondary outcome, results for each group, and the estimated effect size and its | 17 and 19 |
| 19 | estimation | precision (such as 95% confidence interval) | |
| 20 | | 17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended | NA |
| 21 | Ancillary analyses | 18 Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing | NA |
| 22 | | pre-specified from exploratory | |
| 23 | | | |
| 24 | Harms | 19 All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | 18 |
| 25 | | | |
| 26 | Discussion | | |
| 27 | Limitations | 20 Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses | 21 |
| 28 | Generalisability | 21 Generalisability (external validity, applicability) of the trial findings | 20 |
| 29 | Interpretation | 22 Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence | 22 |
| 30 | | | |
| 31 | Other information | | |
| 32 | Registration | 23 Registration number and name of trial registry | 6 |
| 33 | Protocol | 24 Where the full trial protocol can be accessed, if available | 6 |
| 34 | Funding | 25 Sources of funding and other support (such as supply of drugs), role of funders | 6 |
| 35 | | | |

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37 *We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also

38 recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials.

39 Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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BMJ Open

Trial of feasibility and acceptability of routine low dose aspirin versus Early Screening Test indicated aspirin for preeclampsia prevention [TEST Study] – A multicenter randomised controlled trial

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Manuscripts

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TITLE: Trial of feasibility and acceptability of routine low dose aspirin versus Early Screening Test indicated aspirin for preeclampsia prevention [**TEST** Study] – A multicenter randomised controlled trial

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ABSTRACT

Objective: Evaluate feasibility and acceptability of routine aspirin in low-risk women, compared to screening-test indicated aspirin for prevention of preeclampsia and fetal growth restriction (FGR) prevention.

Design: Multicentre open-label feasibility randomised controlled trial.

Setting: Two tertiary maternity hospitals in Dublin, Ireland.

Participants: 546 low-risk nulliparous women completed the study

Interventions: Women underwent computerised randomisation to; Group 1- routine aspirin 75mg from 11 until 36 weeks; Group 2 - no aspirin; and Group 3 - aspirin based on the Fetal Medicine Foundation screening test.

Primary and secondary outcome measures: (a) proportion agreeing to participate; (b) compliance with protocol; (c) proportion where first trimester uterine artery Doppler was obtainable and; (iv) time taken to issue screening result. Secondary outcomes included rates of preeclampsia and small-for-gestational age fetuses.

Results: 546 were included in the routine aspirin (n=179), no aspirin (n=183) and screen and treat (n=184) groups. 546 of 1054 approached (51.8%), enrolled. Average aspirin adherence was 90%. Uterine artery Doppler was obtained in 98.4% (181/184) and average time to obtain a screening result was 7.6 (0-26) days. Of those taking aspirin, vaginal spotting was greater; n=29 (15.1%), non-aspirin n=28 (7.9%) OR 2.1 (95% CI 1.2-3.6). Post-partum haemorrhage > 500mls was also greater; aspirin n=26 (13.5%), no aspirin n=20 (5.6%) OR 2.6 (95% CI 1.4-4.8).

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3 **Conclusion:** Low-risk nulliparous women are open to taking aspirin in pregnancy and had
4 high levels of adherence. Aspirin use was associated with greater rates of vaginal bleeding.
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6 An appropriately powered randomized controlled trial is now required to address the efficacy
7 and safety of universal low dose aspirin in low-risk pregnancy compared to a screening
8 approach.
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13 **Trial Registration:** www.isrctn.com/ISRCTN15191778
14

15 **Funding:** Perinatal Ireland, HRB and the Mother and Baby Clinical Trials Network, HRB
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18 **ARTICLE SUMMARY**

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20 Strengths and limitations of this study
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- 23 • Robust multi-centre randomised controlled trial design
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- 25 • Three methods were used to assess aspirin adherence
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- 27 • Standardisation of methods
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- 29 • Potential introduction of reporting bias through open-label design
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INTRODUCTION

Low dose aspirin use prior to 16-weeks can reduce the incidence of preeclampsia in at-risk pregnancies. When commenced at this stage, at a dose of 75mg, its efficacy in low-risk pregnancies is unknown.^{1,2} With the emergence of first trimester screening tests for preeclampsia such as that of the Fetal Medicine Foundation (FMF), one can predict from 11-weeks, the risk of preeclampsia.³ Internationally, there are conflicting consensus statements on screening methods and which women meet criteria for aspirin use.⁴ Application of the FMF screening test and provision of low dose aspirin to screen positive women can significantly reduce the incidence of early-onset preeclampsia (4.3% aspirin vs. 1.6% placebo $p=0.004$), although predictive performance of the algorithm appears to vary between populations.⁵ It has been proposed that performance of the FMF algorithm is superior to the methods recommended by the National Institute of Clinical Excellence and American College of Obstetricians and Gynecologists (ACOG).⁶ It may be more efficacious to prescribe low dose aspirin universally, although there is no evidence to support such a policy as yet.⁷ To determine this, one must first evaluate if low-risk women are willing to take aspirin in pregnancy and if undergoing a comprehensive screening test is realistic in the routine setting. Hence, the primary objective of this multi-center open label feasibility randomised controlled trial was to evaluate the acceptability and feasibility of women taking aspirin 75mg from beyond 11-weeks gestation versus screening test-indicated aspirin. Secondary outcomes included rates of; (i) preeclampsia; (ii) small-for-gestational age infants; (iii) pre-term delivery; (iv) admission to neonatal intensive care; (v) placental abruption; (vi) any reported death and; (vii) acceptability of women taking aspirin routinely versus test indicated aspirin, assessed by a questionnaire.

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METHODS

Study Design

This open-label feasibility multicenter randomised controlled trial (RCT) was performed in two Irish tertiary maternity hospitals with 18,000 deliveries per annum. The aim was to include three centers, however there was a delay in the local ethics committee decision for the third center (subsequently approved), which was excluded in the interests of study schedule. The protocol for this multicenter randomised controlled trial has been published⁸ and was prospectively authorized by the Health Products Regulatory Authority and National Maternity Hospital Central Ethics Committee. The trial was registered with the ISRCTN number 15191778 and was supported by Perinatal Ireland HRB and the HRB Mother and Baby Clinical Trials Network following external peer review for scientific quality. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript. An independent Trial Steering Committee and Data Monitoring Committee met quarterly to oversee the safety of the trial participants.

Nulliparous women over 18-years-old between 11 to 13+6 weeks gestation with a viable singleton pregnancy that didn't meet criteria for aspirin commencement based upon major preeclampsia risk factors (chronic kidney disease, autoimmune disease e.g. systemic lupus erythematosus, diabetes mellitus and chronic hypertension) were eligible for inclusion and thus were recruited at antenatal booking clinics selected at random.⁹ In Ireland it is currently not routine obstetric practice to commence aspirin in women that do not have an aforementioned major risk factor for pre-eclampsia as defined by the National Institute of Health and Clinical Excellence.⁹ Exclusion criteria included participants already taking part in a clinical trial, co-existence of fetal congenital anomaly at recruitment or those with aspirin hypersensitivity. All participants provided written informed consent and were recruited by

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3 the research clinician at the first trimester antenatal booking visit.
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Randomization

Participants underwent enrollment and online computerized randomization by the study sonographer or clinician based on blocks of six to; Group 1 - aspirin 75mg from 11 to 13+6 weeks once daily until 36-weeks' gestation; Group 2 - no aspirin and; Group 3 - aspirin depending on the result of the FMF screening test. Subjects in non-aspirin taking groups had routine antenatal care. The randomisation sequence was determined prior to study commencement by the off-site statistician and was concealed from assessors, with both the assessor and participant seeing the group allocation at the same time, following online selection.

Intervention

Enteric coated Nu-Seals Aspirin (Acetylsalicylic Acid) 75mg orally once daily at night from 11 to 36-weeks gestation was provided free of charge from Alliance Pharma®, which were independent of study protocol and analysis. A dose of 75mg was used as this is currently the standard recommended dosage in the UK and Ireland for at-risk women.⁹ Aspirin adherence was assessed subjectively via patient reported diary cards and tablet counts (checked by research clinician and pharmacist) and objectively via assessment of change in urinary 11-dehydrothromboxane-B2 (TxB2). Any reduction in TxB2 between first (pre-aspirin) and second trimester (post-aspirin) levels was taken to suggest that a subject had ingested aspirin within the last ten days.¹⁰

Baseline review and follow-up

Participants underwent two scheduled study visits, at study recruitment and at 20-22 weeks (to coincide with their fetal anatomy scan which was performed at the same time) with diary cards and aspirin tablets returned to the research team at 36-weeks gestation. Participants

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3 completed an anonymous questionnaire at 20-22 weeks based on acceptability of taking
4 aspirin in pregnancy.
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7 Study assessments at the time of the recruitment visit included the FMF screening test, the
8 results of which were assessed for those in Group 3 (screen positive and received aspirin (3A)
9 and screen negative no aspirin (3B)). The FMF screening test was not routine practice within
10 Ireland. Components of the screening test included; maternal history (including ovulation
11 induced conception, race, body mass index, age, mother with preeclampsia); mean arterial
12 blood pressure (MAP); uterine artery Doppler pulsatility index; and pregnancy associated
13 plasma protein-A (PAPP-A) and placental like growth factor (PLGF) multiples of the
14 median. To determine risk of preeclampsia, the FMF algorithm was used and based upon a
15 screen positive rate of 5%, a cut off for preeclampsia prior to 42-weeks at greater than 1:8
16 was used.³ This cut-off was selected with the aim of capturing the majority of pre-eclamptics;
17 both pre and post-term and at the time of study commencement this was the optimal
18 screening algorithm for detection of any preeclampsia. Two un-blinded trained clinical
19 research sonographers performed the first trimester uterine artery Doppler waveforms and
20 MAP and interpreted findings. MAP was assessed using an automated blood pressure
21 monitoring device as outlined by the technique stipulated by the FMF.¹¹ Uterine artery
22 Doppler velocimetry was obtained using Viewpoint® Version 5.6.16 GE Healthcare, 2012
23 and Voluson Expert 730®, GE 2012 using the technique outlined from by the International
24 Society of Ultrasound in Obstetrics and Gynecology. The pulsatility index was measured
25 from both uterine arteries and an average value was calculated.¹²
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50 A maternal blood sample was analyzed for PAPP-A and PLGF under standard conditions
51 using a 6000 DELFIA® Xpress, PerkinElmer, 2014 clinical random access screening
52 platform in the hospital clinical biochemistry laboratory. A quantitative immunoturbidimetric
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3 TxBCardio® immunoassay was used to determine TxB2 levels in urine samples obtained
4 both study visits. These were then standardized as a ratio with creatinine levels and
5 expressed as pg/mg creatinine.
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10 11 **Outcomes**

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13 The primary objective of this study was to evaluate the feasibility and acceptability of low-
14 risk nulliparous women taking aspirin versus test indicated aspirin in pregnancy. Outcome
15 measures included:
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20 (i) The proportion of eligible women agreeing to participate in a trial where aspirin is
21 prescribed routinely (feasibility);
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24 (ii) Compliance with study protocol, as measured by the following: (a) adherence to
25 aspirin (acceptability), (b) attendance at study visits (acceptability), (c)
26 satisfactory collection of all endpoints and variables (feasibility), (d) specific
27 study protocol violations (feasibility);
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31 (iii) The proportion of women in whom it was possible to obtain first trimester trans-
32 abdominal uterine artery Doppler velocimetry examination (feasibility);
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36 (iv) Proportion of women with a completed screening test who were issued the
37 screening result within one week of having the test performed (feasibility);
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44 Secondary outcomes included rates of; (i) preeclampsia; (ii) small-for-gestational-age (SGA)
45 infants (customised sex-specific birth-weight <10th centile); (iii) pre-term delivery prior to
46 34-weeks; (iv) admission to neonatal intensive care unit (NICU); (v) placental abruption; (vi)
47 any reported death (stillbirth, neonatal or infant death) and; (vii) acceptability of women
48 taking aspirin routinely versus test indicated aspirin as assessed by an anonymous
49 questionnaire at 20-22 weeks. As part of routine antenatal care women had an appointment
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3 with their midwife and or clinician at booking (11-14 weeks), 16-weeks, 18-20 weeks, 25-
4 weeks, 28-weeks, 31-weeks, 34-weeks, 36-weeks, 28-weeks, 40-weeks and 41-weeks
5 gestation in line with hospital protocol. At each visit blood pressure was assessed using
6 mercury sphygmomanometry and a urine dipstick for proteinuria was performed with
7 symphysio-fundal-height and or fetal biometrical ultrasound assessment as appropriate. -
8 was defined based upon the definition from the ISSHP with new onset hypertension
9 ($>140\text{mmHg}$ systolic or $>90\text{mmHg}$ diastolic) after 20-weeks gestation associated with; (i)
10 proteinuria of at least 1g/L [2+] on urine dipstick testing, and or; (ii) maternal organ
11 dysfunction ; an or fetal growth restriction.¹³ Suspicion of a diagnosis of pre-eclampsia at an
12 antenatal visit prompted further investigation in the fetal assessment unit with clinical
13 examination, blood testing (urea and creatinine, liver function tests and full blood picture),
14 24-hour urine collection for proteinuria and departmental fetal ultrasound assessment with
15 final diagnosis made by an obstetrician.
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33 Safety data were reported as adverse and serious adverse events and participants discontinued
34 from the study were recorded in addition to the reason for discontinuation and outcome. As
35 an assessment of post-partum haemorrhage, blood loss was weighed at time of delivery.
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42 **Statistical Analysis**

43 As outlined in the published study protocol, the projected sample size for this study was 500
44 women across two sites with 18,000 deliveries per annum.⁸ To determine preeclampsia as a
45 primary outcome; the anticipated number of patients required is over 15,000 women. As this
46 study aimed to determine the feasibility of such a study, 500 participants were more than
47 adequate as 3% of the number required for a substantive study is required ($n=450$).¹⁴
48 Accounting for a drop-out rate of 10% ($n=45$), 500 participants were adequate to obtain the
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3 primary outcome. Analysis was performed by a statistician using SAS v.20 on the intention-
4 to-treat (ITT) population, which included all participants randomised, which completed the
5 full second trimester assessment. Measures of variance included standard deviation. Follow-
6 up of serious adverse events continued until 28-days following delivery. Adverse events were
7 reported as odds ratios (OR). To assess secondary outcomes and safety, comparisons of
8 groups were be performed using two sample t-tests, Wilcoxon Rank-sum tests and Chi-square
9 tests.
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Patient Involvement

Although patients were not directly involved in devising the study protocol and design the burden of the RCT intervention (i.e. taking aspirin and undergoing the FMF screening test) was assessed by means of an anonymous questionnaire completed at 20-22 weeks gestation. At the time of study participation subjects were informed that study results could be viewed following publication on the study website; <http://perinatalireland.ie/research/test/>

RESULTS

Subjects were recruited between 8th May 2014 to 23rd September 2015 with follow-up of participants until 11th April 2016, when the study was ended by the steering committee following delivery of the final patient as the target sample size had been achieved. In total 1054 eligible women were approached to take part in the study and of these, 557 underwent randomization [Figure 1]. In the screen and treat population (Group 3) n=184, 13 (7.1%) women had a risk of developing preeclampsia >1:8 and subsequently commenced aspirin until 36-weeks gestation. Eleven women were excluded from the study leaving 546 in the ITT population. In total there were 192 women in the ITT group that were taking aspirin as per randomization and 354 not taking aspirin [Table 1].

| Characteristic | Low Dose Aspirin N=179 | No Aspirin N=183 | Screen & Treat N=184 |
|---|---------------------------|---------------------|-------------------------|
| Age (yr) | 33 (19-44) | 34 (18-43) | 33 (19-44) |
| Race – No. (%) | | | |
| White | 181 (97.9) | 179 (95.7) | 180 (97.3) |
| Black | 1 (0.5) | 2 (1.1) | 0 (0) |
| Asian | 3 (1.6) | 6 (3.2) | 5 (2.7) |
| Other | 0 | 0 (0) | 0 (0) |
| Completed secondary school – No.(%) | 136 (73.5) | 143 (76.4) | 152 (82.2) |
| BMI (kg/m ²) | 25.2 (17.4-39.4) | 22.9 (17.7-41.4) | 23.8 (18.1-45.2) |
| Gestational Age (wks) | 12.9 (11.1-13.9) | 12.9 (11.1-13.9) | 12.9 (11.3-13.9) |
| Smoking – No. (%) | 17(9.2) | 11 (5.9) | 7 (3.8) |
| Subject's mother had preeclampsia - No. (%) | 7 (3.8) | 10 (5.4) | 10 (5.4) |
| Conception – No. (%) | | | |
| IVF | 5 (2.7) | 9 (4.8) | 8 (4.3) |
| ICSI | 3 (1.6) | 4 (2.1) | 3 (1.6) |
| Ovulation induction | 5 (2.7) | 6 (3.2) | 6 (3.2) |
| Spontaneous | 172 (93.0) | 168 (89.9) | 170 (91.9) |
| Previous miscarriage – No. (%) | 20 (10.8) | 31 (16.6) | 31 (16.8) |

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3 Table 1: Baseline characteristics of the study population. Where number (No.) percentage is
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5 not expressed average and range are demonstrated.
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8 9 **Primary outcomes**

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11 (i) The proportion of eligible women agreeing to participate in a trial where aspirin is
12 prescribed routinely (feasibility); 1054 women were approached that were eligible to partake.
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14 497 were subsequently not enrolled as they did not want to take aspirin n=454 or for an
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16 alternative reason n=43 e.g. appointment did not suit. Hence 546/1054 (51.8%) women were
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18 willing to partake in a study where they may have to take aspirin routinely.
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24 (ii) Compliance with study protocol, as measured by the following: (a) adherence to aspirin
25 (acceptability), (b) attendance at study visits (acceptability), (c) satisfactory collection of all
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27 endpoints and variables (feasibility), (d) specific study protocol violations (feasibility);
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33 (a) Of those women included in analysis that were taking aspirin (n=192), the average
34 adherence based upon patient reported diary cards was 96.0% and based upon tablet counts
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36 95.0%. Seven women were non-adherent and 19 (10.0%) poorly compliant (<80%).
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38 Average adherence was 95.0% in both the test indicated aspirin group (3a) and routine aspirin
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40 group (1) [Table 2]. The median first trimester pre-aspirin urine TxB2 level was 8662.2
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42 pg/mg (IQR 2014.5-9931.5) and second trimester (post-aspirin) 2285.1 pg/mg (IQR 591.0-
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44 2300.1). The percentage change in TxB2 was then assessed for all paired samples (n=147)
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46 and found that 124/147 (84.4%) of subjects had a fall in TxB2 levels between the first and
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48 second trimesters versus 23/147 (15.6%) who had an increase p<0.001. The greater the
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50 reduction in urinary TxB2 pre- and post- aspirin dose the greater the degree of aspirin
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3 adherence, as demonstrated in Figure 2. There was no difference between patient groups
4 (routine aspirin and screen positive aspirin) and percentage change in urine TxB2 (p=0.61).
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9 (b) Of those that underwent randomization (n=557), eleven were excluded prior to fulfillment
10 of study participation requirements (attendance at second study visit). Of the eleven, three
11 withdrew consent for participation as they decided that they did not wish to take aspirin
12 following randomization.
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18 (c) Of all 546 subjects collection of outcome measures and variables were obtained for all
19 apart from the questionnaire on patient acceptability, which was completed in 97.1%
20 (530/546).
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28 d) Six protocol violations were recorded (0.01 per 100 participants) including women
29 transferring care to another hospital (n=3), incorrect randomization of women that did not
30 meet inclusion criteria (n=2) and a subject in the non-aspirin group commencing aspirin by
31 their clinician (n=1).
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39 (iii) The proportion of women in whom it was possible to obtain first trimester trans-
40 abdominal uterine artery Doppler velocimetry (feasibility); The FMF screening test was
41 completed in 98.4% (181/184) following successful uterine artery Doppler velocimetry
42 acquisition, of which one was obtained vaginally due to challenges with abdominal
43 acquisition, with an overall sonographer reported ease of acquisition 3.1 (SD +/- 0.91) (score
44 1 (easy) to 5 (unobtainable)) [Table 2].
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(iv) Proportion of women with a completed screening test, issued the result within one week of the test (feasibility); The average time to obtain laboratory analyzed PAPP-A and PLGF so that a screening result could be issued was 7.6 days (0-26) with 78 (42.4%) of women waiting greater than one week and five women being beyond 16-weeks prior to result availability [Table 2].

| Adherence and feasibility parameter | Low-dose Aspirin (N=179) | No Aspirin (N=183) | Screen & Treat (N=184) |
|-------------------------------------|--------------------------|--------------------|------------------------|
| Ease of Doppler acquisition | | | |
| Very easy | | | 8 (4%) |
| Easy | | | 53 (29%) |
| Fair | | | 61 (33%) |
| Difficult | | | 60 (32%) |
| Unobtainable | | | 3 (2%) |
| Days to PLGF/PAPPA visit 1 | | | 7.6 [0 - 26] |
| PLGF/PAPPA result > 16 weeks | | | 5 (3%) |
| Time taken for visit 1 (mins) | 60 [30 – 100] | 60 [25 - 90] | 60 [25 - 90] |
| Median adherence tablet counts | 96% | | 95% (screen positive) |

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| Median adherence diary cards | 94% | | 95% (screen positive) |
| Non-adherent | 7 (4%) | | 0 (0%) |

Table 2: Primary outcomes of feasibility and adherence

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Secondary outcomes

There was no difference between groups in relation to secondary outcomes [Table S1 supplementary]. For the overall cohort, there were three cases (0.37%) of early onset preeclampsia <34-weeks (0.55%), n=22 (4.03%) any preeclampsia, n=57 (10.44%) SGA infants and 15.02% (n=82) placental disease. Secondary outcomes for groups 3A (screen positive aspirin) and 3B (screen negative no aspirin) are demonstrated in Table S2 [supplementary]. Despite taking aspirin, there remained a significantly greater number with preeclampsia at <37-weeks in the screen positive versus the screen negative group, although numbers were small (n=2 (15.4%) vs. n=2 (1.2%) p=0.02). In terms of taking aspirin in a subsequent pregnancy, the questionnaire revealed that 92.3% (489/530) were willing to take aspirin in a subsequent pregnancy; 92.5% (173/187) of aspirin takers and 91.5% (314/343) of non-aspirin takers.

Safety

There were differences between groups in relation to adverse but not serious adverse events [Tables 3 and S3 (supplementary)]. There were six perinatal deaths, all of which underwent postmortem. In the aspirin group there was one placental abruption and one case of intervillous haemorrhage. Perinatal deaths in the non-aspirin groups were due to delayed villous maturation, severe FGR, fetal thrombotic vasculopathy and neonatal septicemia. There was a difference between groups in terms of reported vaginal spotting aspirin 15.1% vs. non-aspirin 7.9% OR 2.1 (CI 1.2-3.6), which was not associated with pregnancy loss. Although, not statistically significant, there was a difference in terms of PPH >1000mls. Although rates of PPH <1000mls were greater in the aspirin-taking group, no differences were noted in terms of blood transfusion or significant hemoglobin drop to <8g/dL.

| Event | | Aspirin n=192 | Non-aspirin n=354 | Odds ratio (95% CI) |
|---------------------------------|---------------------------------|------------------|----------------------|------------------------|
| Adverse Events | | | | |
| Adverse Events Total No. | | 123 | 143 | 2.6 (1.8-3.8) |
| Vaginal spotting* No. (%) | | 29(15.1) | 28 (7.9) | 2.1 (1.2-3.6) |
| Post-partum haemorrhage No. (%) | | | | |
| >500mls* | | 26(13.5) | 20 (5.6) | 2.6 (1.4-4.8) |
| >1000mls | | 7 (3.6) | 5 (1.4) | 2.8 (0.9-9.0) |
| Blood transfusion | | 3 | 4 | 0.5 (0.1-2.7) |
| Hb drop <8g/dL | | 4 | 7 | 0.3 (0.1-1.4) |
| Serious Adverse Event | | | | |
| NICU admission | Sepsis | 3 | 2 | |
| | Hypoglycaemia | 0 | 1 | |
| | Prematurity | 1 | 4 | |
| | Jaundice | 1 | 1 | |
| | Persistently low Apgar | 1 | 3 | |
| | TTN | 1 | 3 | |
| | Meconium aspiration | 1 | 0 | |
| | Hypoxic ischemic encephalopathy | 1 | 1 | |
| | Very low birthweight | 0 | 1 | |
| Total | | 9 | 16 | 1.04 (0.45-2.40) |
| Perinatal Death | | 2 | 4 | |
| Total | | 2 | 4 | 0.92 (0.17-5.10) |
| Maternal admission | Preterm labor | 3 | 2 | |
| | Preeclampsia | 8 | 7 | |
| | Antepartum hemorrhage | 3 | 7 | |
| | PPROM | 0 | 2 | |
| | Fetal compromise | 1 | 2 | |
| | Infection | 2 | 5 | |
| | Other | 4 | 1 | |
| Total | | 21 | 26 | 1.55 (0.85-2.83) |
| Congenital anomaly | Cardiac | 1 | 2 | |
| | Gastrointestinal | 3 | 2 | |
| | Neurological | 0 | 1 | |
| | Renal | 0 | 1 | |
| Total | | 4 | 6 | 1.23 (0.34-4.43) |
| Total serious adverse events | | 36 | 52 | 1.34 (0.84-2.14) |

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5 Table 3: Adverse and serious adverse events in aspirin and non-aspirin taking groups. There
6
7 may be >1 adverse event or serious adverse event per subject * ($p < 0.05$) [NICU=Neonatal
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9 intensive care unit, TTN= transient tachypnea of the newborn, PPOM= preterm premature
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11 rupture of membranes, Very low birthweight = <1500g].
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DISCUSSION

Main Findings

This feasibility randomised controlled trial has found that low-risk nulliparous women were open to taking aspirin in pregnancy and were adherent, with a willingness to take it again in a subsequent pregnancy. We can say this as, comparing findings to other RCTs in pregnancy, of which there are few, the uptake in this RCT was much higher as was adherence (e.g. Chiswick, *et al.* 2015; 35% enrolment and 65-67% adherence with metformin use).¹⁵ This is the first trial of its kind, which has assessed the acceptability of women taking aspirin in low-risk pregnancy and the feasibility of an integrated screening test in a routine clinical setting.

Strengths and Limitations

The strengths of this study are the multicenter RCT design with robust protocol and oversight and previously published methodology. Allocation bias was limited by use of a prospective approach and selection bias was limited by randomization. The fact that the same two sonographers and biochemists were responsible for conducting the screening test with use of quality control standards for test completion using the same equipment and technique for all subjects optimized reproducibility. There were a low number of dropouts and almost all patient outcomes were recorded. Although there is currently no validated scientific method of assessing aspirin adherence,¹⁶ a laboratory assessment of change in TxB2 served as a more objective assessment, strengthening reliability. There is currently no accepted test in the literature, which can reliably determine aspirin adherence, hence three different methods were used to optimize reliability.¹⁶ Study weaknesses, were primarily that PAPP-A and PLGF analysis was performed in the laboratory using validated methods with quality assurance, as opposed to the bedside point-of-care tests hence it took longer to obtain a result. In a non-research setting with a greater throughput of patients, one could anticipate a

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3 faster turnaround time. Additionally the open-label nature of the study meant that safety
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5 recording was open to reporting bias and, as is often the case with RCTs the uptake of
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7 subjects demonstrated dominance for educated women. In RCTs there is always a risk of
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9 introducing a Hawthorne effect, whereby subjects act differently in the confines of an RCT as
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11 to how they would in a real-life setting, hence adherence rates may have been over-
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13 represented.¹⁷ A third trimester visit may have added strength to the study to assess
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15 objectively for aspirin adherence and patient satisfaction, however as adherence prior to 16-
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17 weeks was deemed the critical time point for preeclampsia prevention, follow-up at 20-22
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19 weeks was selected.
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24 **Interpretation**

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26 A recently published large RCT from the FMF found that, following application of FMF
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28 screening and subsequent randomization of women deemed to be at risk of preterm
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30 preeclampsia to aspirin 150mg versus placebo, there was a reduction in the incidence of
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32 preterm preeclampsia in the aspirin arm.⁵ Our study differs on several counts; (i) routine
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34 aspirin arm – use of a third arm assessing provision of routine aspirin assessed the
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36 acceptability and feasibility of this policy; (ii) aspirin dosage (150mg vs. 75mg) – in light of
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38 limited evidence on dosage and effect, the safest lowest effective dose was selected. A recent
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40 meta-analysis, published since completion of this study suggests that there is an aspirin dose-
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42 response effect, with higher doses of aspirin commenced prior to 16-weeks gestation,
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44 associated with a greater reduction in preeclampsia and fetal growth restriction compared to
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46 standard lower doses.¹⁸ When supported by robust safety data when using higher dosing, this
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48 is something to consider in future studies and clinical practice; (iii) adverse events – rates of
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50 PPH and vaginal bleeding were reported. This information would be useful from the FMF
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3 study in light of the higher aspirin dosing regime and; (iv) our study was not powered to
4 detect a difference in clinical outcome, with the primary focus feasibility and acceptability.
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9 Few studies have assessed the acceptability of non-routine medications in pregnancy. In the
10 developing world, pregnant women are willing to take calcium, oral iron and
11 micronutrients.¹⁹⁻²¹ If instructed about potential side-effects and reminded frequently
12 women had higher levels of adherence with the greatest barrier being forgetfulness. Average
13 medication adherence in pregnancy for chronic illness is higher than for non-routine
14 medications at 90-95%,²² hence it is promising that we have noted a rate as high as this in
15 our own study. There was a slight discrepancy in adherence assessed via tablet counts and
16 diary cards and that more objectively assessed via TxB2. Reasons for this may include the
17 potential for aspirin resistance; which although not formally assessed in this study can be
18 increased when using an enteric-coated preparation.²³
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33 The FMF screening test was feasible in terms of acquiring first trimester uterine artery
34 Doppler velocimetry measurements, though delays were encountered in obtaining laboratory
35 analyzed PAPP-A and PLGF. This is relevant as it reflects the practical aspects of such a
36 screening test in a clinical real life setting. Improved protocols between the clinical and
37 laboratory staff would be required to allow patients receive results within a reasonable
38 timeline.
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48 In terms of vaginal spotting and clinically significant PPH with aspirin use, the findings of
49 this study are comparable with previous studies although evidence of increased antenatal and
50 postnatal bleeding, requires further investigation, most notably with use of aspirin at doses
51 greater than 75mg.²⁴⁻²⁵ Due to the open-label nature of this study as opposed to placebo
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3 control, there is always a potential of reporting bias of bleeding in the aspirin arms. Although
4 generally safe in pregnancy, it may be worthwhile considering cessation of aspirin at 32-34
5 weeks gestation with the aim of reducing the risk of PPH, as opposed to 36-weeks and of
6 informing women of the unwanted side-effect of increased vaginal spotting.
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11 12 13 **Conclusion**

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16 It has been proposed that the most cost-effective approach to reducing preeclampsia is the
17 provision of an effective, affordable and safe intervention applied to all mothers without prior
18 testing to assess levels of risk.⁷ A algorithm-based screen-and-treat approach, as proposed by
19 the FMF has can reduce rates of pre-term preeclampsia when doses of 150mg of aspirin are
20 used. This study was not powered to nor did it detect a difference in rates of preeclampsia
21 between groups, yet has taken the first step to address if low-risk nulliparous women are open
22 to taking aspirin in the first instance and if a screening algorithm is feasible. Moving
23 forward, an RCT is required to address the efficacy of universal low dose aspirin in low-risk
24 pregnancy compared to a screening approach. This will require significant numbers due to
25 the low incidence of early-onset preeclampsia. Although women were open to taking aspirin
26 in pregnancy compared to other RCTs involving medication, almost twice the number
27 enrolled had to be approached to obtain adequate study participants. This must be considered
28 when planning a future trial.
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3 **COMPETING INTERESTS STATEMENT:** Authors report no conflict of interest
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9 Mother and Baby Clinical Trials Network.
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15 CM, PMcP, FB, PD, DM, MC, AS, FC, JM, SD, JH, AC, ET, PD, ZA, FDM, FMcA; (ii)
16
17 Performed the experiments: FM, CM, FC ; (iii) Analyzed the data: FM, PD, ZA, FMcA; (iv)
18
19 Contributed reagents/materials/analysis/tools: PMcP, FB, PD, DM, MC, AS, JM, SD, JH,
20
21 AC, AH, ET, PD, ZA, FDM, FMcA; (v) Wrote the paper: FM, CM, PMcP, FB, PD, DM,
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23 MC, AS, FC, JM, SD, JH, AC, AH, ET, PD, ZA, FDM, FMcA
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33 **DATA SHARING STATEMENT:** Dataset available from corresponding author on request
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31 **FIGURE LEGENDS**

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33 Figure 1 - Consort diagram

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35 Figure 2 - Histogram demonstrating percentage change in urinary thromboxane-B2 levels
36 pre- and post - aspirin administration (n=147) [TxB2 = urinary-thromboxane level]
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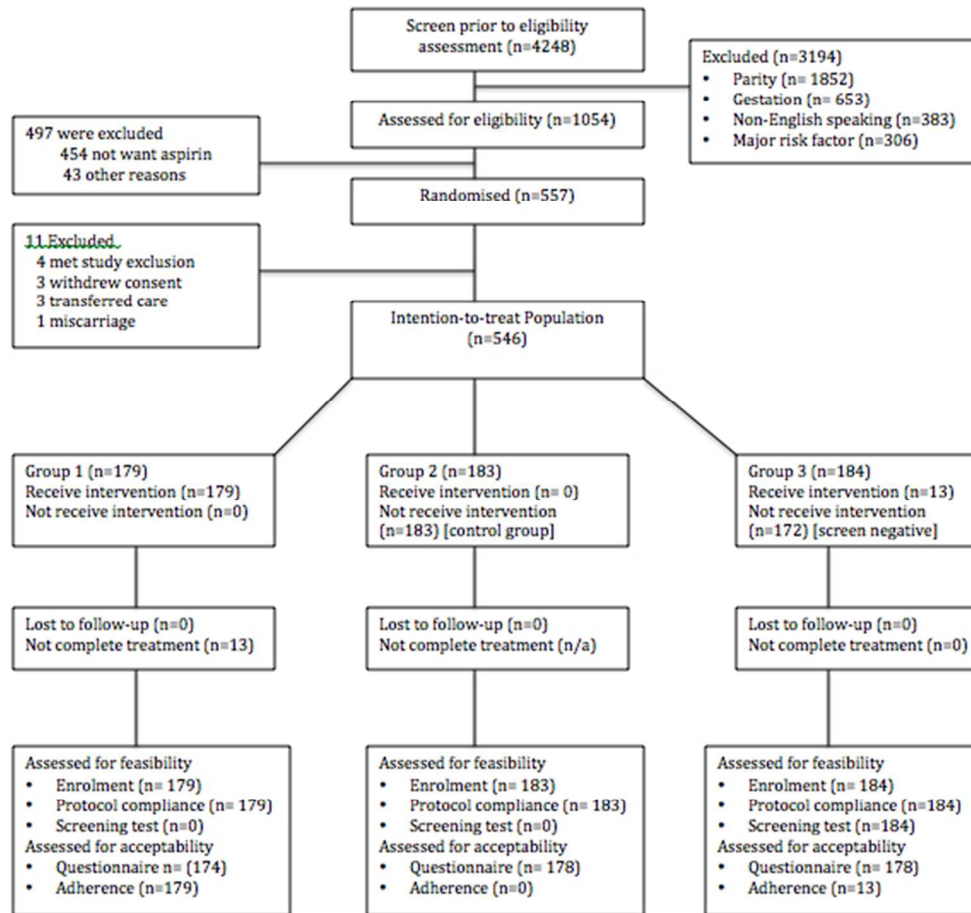
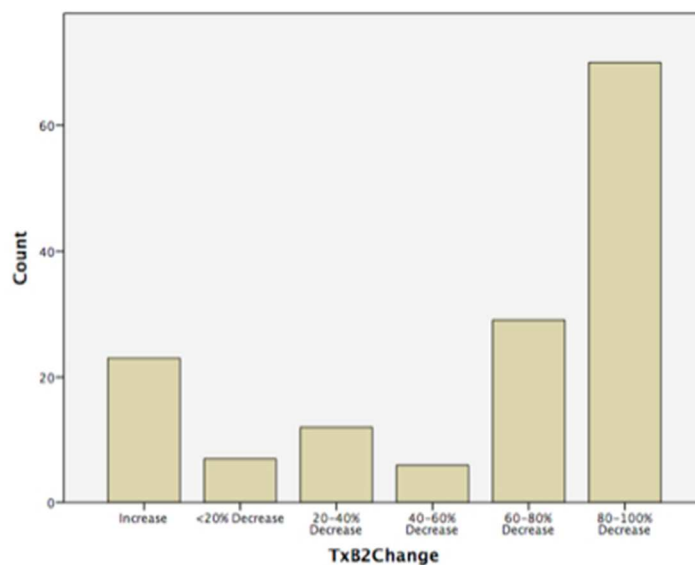


Figure 1 – Consort diagram

Figure 1 - consort diagram

206x206mm (72 x 72 DPI)



[Figure 2 - Histogram demonstrating percentage change in urinary thromboxane-B2 levels pre- and post aspirin administration (n=147) [TxB2 = urinary B2-thromboxane level]

Figure 2 - Histogram demonstrating percentage change in urinary thromboxane-B2 levels pre- and post aspirin administration (n=147) [TxB2 = urinary B2-thromboxane level]

204x180mm (72 x 72 DPI)

| Outcome | Low Dose Aspirin (Group 1) N=179 | No Aspirin (Group 2) N=183 | Screen and Treat (Group 3) N=184 | p-value |
|---|-------------------------------------|-------------------------------|-------------------------------------|---------|
| Gestation at delivery (wks) | 40.2 (1.4) | 39.9 (1.9) | 40.2 (1.5) | 0.13 |
| Birthweight (g) | 3529 (469) | 3478 (493) | 3488 (502) | 0.58 |
| Birthweight <10 th centile No. (%) | 14 (8%) | 18(10%) | 25 (14%) | 0.19 |
| Mode of delivery No. (%) | | | | |
| Spontaneous | 85 (47.5) | 95 (52.0) | 88 (47.8) | 0.64 |
| Instrumental | 56 (31.3) | 47 (25.7) | 51 (27.7) | 0.09 |
| Caesarean | 38 (21.2) | 41 (22.3) | 45 (24.5) | 0.68 |
| Pre-term delivery <34 weeks No. (%) | 1 (0.6) | 3 (1.6) | 2 (1.1) | 0.62 |
| Spontaneous Labor No. (%) | 96 (53.7) | 103 (56.3) | 101 (54.9) | 0.88 |
| Preeclampsia No. (%) | 8 (4.5) | 7 (3.8) | 7 (3.8) | 0.95 |
| Preeclampsia <34-weeks | 0 (0) | 2 (1.1) | 1 (0.5) | 0.56 |
| Preeclampsia <37-weeks | 2 (1.1) | 2 (1.1) | 2 (1.1) | 0.99 |
| Abruption No. (%) | 1 (0.5) | 0 (0) | 0 (0) | 0.71 |
| NICU admission No. (%) | 9 (5.0) | 7 (3.8) | 9 (4.9) | 0.83 |
| Apgar < 7 No. (%) | 5 (2.8) | 2 (1.6) | 3 (1.6) | 0.46 |
| Cord pH (arterial) | 7.3 (0.1) | 7.3 (0.1) | 7.3 (0.1) | 0.55 |
| Outcome No. (%) | | | | |
| Alive at 6-weeks | 177 (98.9) | 181 (99.0) | 182 (98.9) | 0.99 |
| Stillbirth | 2 (1.1) | 1 (0.5) | 0 (0) | 0.81 |
| Neonatal death | 0 (0) | 1 (0.5) | 2 (1.1) | 0.37 |

Table S1: Secondary outcome measures

(Expressed as average and standard deviation unless otherwise stated)

| Outcome | Screen positive; Aspirin Group 3A N=13 | Screen negative; No Aspirin Group 3B N=171 | p-value |
|---|---|---|---------|
| Preeclampsia No. (%) | 2 (15.4) | 5 (2.9) | 0.13 |
| Pre-eclampsia <34-weeks | 0 (0) | 2 (1.2) | 0.70 |
| Pre-eclampsia <37-weeks | 2 (15.4) | 2 (1.2) | 0.02 |
| Birthweight <10 th centile No. (%) | 4 (30.7) | 21 (12.3) | 0.15 |
| Pre-term delivery <34 weeks No. (%) | 1 (7.7) | 1 (0.6) | 0.32 |
| NICU admission No. (%) | 0 (0) | 9 (5.3) | 0.86 |
| Outcome No. (%) | | | |
| Alive at 6-weeks | 13 (100) | 169 (98.8) | 0.70 |
| Stillbirth | 0 (0) | 2 (1.2) | 0.70 |
| Neonatal death | 0 (0) | 0 (0) | -- |

Table S2 - Secondary outcome measures in Group 3 (screen and treat)

(Expressed as average and standard deviation unless otherwise stated)

| Adverse/Serious Adverse Event | Low dose Aspirin Group 1 N=179 | No-aspirin Group 2 N=183 | Screen and treat Group 3 N=184 | p-value |
|--|--------------------------------------|--------------------------------|--------------------------------------|---------|
| Adverse events | | | | |
| Vaginal spotting No. (%) [*] | 27 (15.1) | 18 (9.8) | 12 (6.5) | 0.03 |
| Post-partum haemorrhage No. (%) | | | | |
| >500mls [*] | 25 (13.0) | 9 (4.9) | 12 (6.5) | 0.004 |
| >1000mls | 7 (3.6%) | 1 (0.5) | 4 (2.2) | 0.073 |
| Serious Adverse Events | | | | |
| NICU admission | 9 (5.0) | 7 (3.8) | 9 (4.9) | 0.83 |
| Perinatal Death | 2 (1.1) | 2 (1.1) | 2 (1.1) | 0.99 |
| Maternal admission | 18 (10.1) | 15 (8.2) | 14 (7.6) | 0.69 |
| Congenital anomaly | 3 (1.7) | 4 (2.2) | 3 (1.6) | 0.91 |
| Total serious adverse events | 32 (17.8) | 28 (15.3) | 28 (15.2) | 0.74 |

Table S3 – Adverse and serious adverse events in all three groups. There may be >1 adverse event or serious adverse event per subject * (p<0.05)



CONSORT 2010 checklist of information to include when reporting a pilot or feasibility trial*

| Section/Topic | Item No | Checklist item | Reported on page No |
|----------------------------------|---------|---|---------------------|
| Title and abstract | | | |
| | 1a | Identification as a pilot or feasibility randomised trial in the title | 1-3 |
| | 1b | Structured summary of pilot trial design, methods, results, and conclusions (for specific guidance see CONSORT abstract extension for pilot trials) | 45-72 |
| Introduction | | | |
| Background and objectives | 2a | Scientific background and explanation of rationale for future definitive trial, and reasons for randomised pilot trial | 83-98 |
| | 2b | Specific objectives or research questions for pilot trial | 98-104 |
| Methods | | | |
| Trial design | 3a | Description of pilot trial design (such as parallel, factorial) including allocation ratio | 110, 137-139 |
| | 3b | Important changes to methods after pilot trial commencement (such as eligibility criteria), with reasons | N/A |
| Participants | 4a | Eligibility criteria for participants | 123-133 |
| | 4b | Settings and locations where the data were collected | 110-111 |
| | 4c | How participants were identified and consented | 132-133 |
| Interventions | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | 146-188 |
| Outcomes | 6a | Completely defined prespecified assessments or measurements to address each pilot trial objective specified in 2b, including how and when they were assessed | 190-223 |
| | 6b | Any changes to pilot trial assessments or measurements after the pilot trial commenced, with reasons | n/a |
| | 6c | If applicable, prespecified criteria used to judge whether, or how, to proceed with future definitive trial | 94-98 |
| Sample size | 7a | Rationale for numbers in the pilot trial | 230-236 |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | n/a |
| Randomisation: | | | |
| Sequence generation | 8a | Method used to generate the random allocation sequence | 137-138 |
| | 8b | Type of randomisation(s); details of any restriction (such as blocking and block size) | 138 |
| Allocation concealment mechanism | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned | 141-144 |

| | | | |
|--|-----|---|--------------------|
| Implementation | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions | 137-138, 141-142 |
| Blinding | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how | n/a |
| | 11b | If relevant, description of the similarity of interventions | 138-141 |
| Statistical methods | 12 | Methods used to address each pilot trial objective whether qualitative or quantitative | 236-242 |
| Results | | | |
| Participant flow (a diagram is strongly recommended) | 13a | For each group, the numbers of participants who were approached and/or assessed for eligibility, randomly assigned, received intended treatment, and were assessed for each objective | Figure 1 |
| | 13b | For each group, losses and exclusions after randomisation, together with reasons | Figure 1 |
| Recruitment | 14a | Dates defining the periods of recruitment and follow-up | 253-255 |
| | 14b | Why the pilot trial ended or was stopped | 253-255 |
| Baseline data | 15 | A table showing baseline demographic and clinical characteristics for each group | 262-265 |
| Numbers analysed | 16 | For each objective, number of participants (denominator) included in each analysis. If relevant, these numbers should be by randomised group | Figure 1 |
| Outcomes and estimation | 17 | For each objective, results including expressions of uncertainty (such as 95% confidence interval) for any estimates. If relevant, these results should be by randomised group | 334-365 + Table S2 |
| Ancillary analyses | 18 | Results of any other analyses performed that could be used to inform the future definitive trial | Table S2 |
| Harms | 19 | All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | 346-363 |
| | 19a | If relevant, other important unintended consequences | 346-363 |
| Discussion | | | |
| Limitations | 20 | Pilot trial limitations, addressing sources of potential bias and remaining uncertainty about feasibility | 376-399 |
| Generalisability | 21 | Generalisability (applicability) of pilot trial methods and findings to future definitive trial and other studies | |
| Interpretation | 22 | Interpretation consistent with pilot trial objectives and findings, balancing potential benefits and harms, and considering other relevant evidence | 401-443 |
| | 22a | Implications for progression from pilot to future definitive trial, including any proposed amendments | 454-459 |
| Other information | | | 401-443 |
| Registration | 23 | Registration number for pilot trial and name of trial registry | 116-117 |
| Protocol | 24 | Where the pilot trial protocol can be accessed, if available | Supplementary file |
| Funding | 25 | Sources of funding and other support (such as supply of drugs), role of funders | 116-120 |
| | 26 | Ethical approval or approval by research review committee, confirmed with reference number | 115-116 |

1 Citation: Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. BMJ. 2016;355.
2
3 *We strongly recommend reading this statement in conjunction with the CONSORT 2010, extension to randomised pilot and feasibility trials, Explanation and Elaboration for important
4 clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological
5 treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.
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BMJ Open

Trial of feasibility and acceptability of routine low dose aspirin versus Early Screening Test indicated aspirin for preeclampsia prevention [TEST Study] – A multicenter randomised controlled trial

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Manuscripts

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3 1 **TITLE:** Trial of feasibility and acceptability of routine low dose aspirin versus Early
4 2 Screening Test indicated aspirin for preeclampsia prevention [**TEST** Study] – A multicenter
5 3 randomised controlled trial
6 4

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34 30

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11 45 **ABSTRACT**

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13 46 **Objective:** Evaluate feasibility and acceptability of routine aspirin in low-risk women,
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15 compared to screening-test indicated aspirin for prevention of preeclampsia and fetal growth
16
17 restriction (FGR) prevention.
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19
20 49 **Design:** Multicentre open-label feasibility randomised controlled trial.

21
22 50 **Setting:** Two tertiary maternity hospitals in Dublin, Ireland.

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24 51 **Participants:** 546 low-risk nulliparous women completed the study

25
26 52 **Interventions:** Women underwent computerised randomisation to; Group 1- routine aspirin
27
28 75mg from 11 until 36 weeks; Group 2 - no aspirin; and Group 3 - aspirin based on the Fetal
29
30 Medicine Foundation screening test.
31

32
33 55 **Primary and secondary outcome measures:** (a) proportion agreeing to participate; (b)
34
35 compliance with protocol; (c) proportion where first trimester uterine artery Doppler was
36
37 obtainable and; (iv) time taken to issue screening result. Secondary outcomes included rates
38
39 of preeclampsia and small-for-gestational age fetuses.
40

41
42 59 **Results:** 546 were included in the routine aspirin (n=179), no aspirin (n=183) and screen and
43
44 treat (n=184) groups. 546 of 1054 approached (51.8%), enrolled. Average aspirin
45
46 adherence was 90%. Uterine artery Doppler was obtained in 98.4% (181/184) and average
47
48 time to obtain a screening result was 7.6 (0-26) days. Of those taking aspirin, vaginal
49
50 spotting was greater; n=29 (15.1%), non-aspirin n=28 (7.9%) OR 2.1 (95% CI 1.2-3.6).
51
52 63 Post-partum haemorrhage > 500mls was also greater; aspirin n=26 (13.5%), no aspirin n=20
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54 (5.6%) OR 2.6 (95% CI 1.4-4.8).
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3 66 **Conclusion:** Low-risk nulliparous women are open to taking aspirin in pregnancy and had
4
5 67 high levels of adherence. Aspirin use was associated with greater rates of vaginal bleeding.
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7 68 An appropriately powered randomized controlled trial is now required to address the efficacy
8
9 69 and safety of universal low dose aspirin in low-risk pregnancy compared to a screening
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11 70 approach.

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13 71 **Trial Registration:** www.isrctn.com/ISRCTN15191778

14
15 72 **Funding:** Perinatal Ireland, HRB and the Mother and Baby Clinical Trials Network, HRB

16 17 18 73 **ARTICLE SUMMARY**

19 20 21 74 **Strengths and limitations of this study**

- 22
23 75 • Robust multi-centre randomised controlled trial design
- 24
25 76 • Three methods were used to assess aspirin adherence
- 26
27 77 • Standardisation of methods
- 28
29 78 • Potential introduction of reporting bias through open-label design
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81 INTRODUCTION

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83 Low dose aspirin use prior to 16-weeks can reduce the incidence of preeclampsia in at-risk
84 pregnancies. When commenced at this stage, at a dose of 75mg, its efficacy in low-risk
85 pregnancies is unknown.^{1,2} With the emergence of first trimester screening tests for
86 preeclampsia such as that of the Fetal Medicine Foundation (FMF), one can predict from 11-
87 weeks, the risk of preeclampsia.³ Internationally, there are conflicting consensus statements
88 on screening methods and which women meet criteria for aspirin use.⁴ Application of the
89 FMF screening test and provision of low dose aspirin to screen positive women can
90 significantly reduce the incidence of early-onset preeclampsia (4.3% aspirin vs. 1.6% placebo
91 $p=0.004$), although predictive performance of the algorithm appears to vary between
92 populations.⁵ It has been proposed that performance of the FMF algorithm is superior to the
93 methods recommended by the National Institute of Clinical Excellence and American
94 College of Obstetricians and Gynecologists (ACOG).⁶ It may be more efficacious to
95 prescribe low dose aspirin universally, although there is no evidence to support such a policy
96 as yet.⁷ To determine this, one must first evaluate if low-risk women are willing to take
97 aspirin in pregnancy and if undergoing a comprehensive screening test is realistic in the
98 routine setting. Hence, the primary objective of this multi-center open label feasibility
99 randomised controlled trial was to evaluate the acceptability and feasibility of women taking
100 aspirin 75mg from beyond 11-weeks gestation versus screening test-indicated aspirin.
101 Secondary outcomes included rates of; (i) preeclampsia; (ii) small-for-gestational age infants;
102 (iii) pre-term delivery; (iv) admission to neonatal intensive care; (v) placental abruption; (vi)
103 any reported death and; (vii) acceptability of women taking aspirin routinely versus test
104 indicated aspirin, assessed by a questionnaire.

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3 107 **METHODS**

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6 109 **Study Design**

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8 110 This open-label feasibility multicenter randomised controlled trial (RCT) was performed in
9
10 111 two Irish tertiary maternity hospitals with 18,000 deliveries per annum. The aim was to
11
12 112 include three centers, however there was a delay in the local ethics committee decision for
13
14 113 the third center (subsequently approved), which was excluded in the interests of study
15
16 114 schedule. The protocol for this multicenter randomised controlled trial has been published⁸
17
18 115 and was prospectively authorized by the Health Products Regulatory Authority and National
19
20 116 Maternity Hospital Central Ethics Committee. The trial was registered with the ISRCTN
21
22 117 number 15191778 and was supported by Perinatal Ireland HRB and the HRB Mother and
23
24 118 Baby Clinical Trials Network following external peer review for scientific quality. The
25
26 119 funder of the study had no role in study design, data collection, data analysis, data
27
28 120 interpretation, or writing of the manuscript. An independent Trial Steering Committee and
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30 121 Data Monitoring Committee met quarterly to oversee the safety of the trial participants.
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36 123 Nulliparous women over 18-years-old between 11 to 13+6 weeks gestation with a viable
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38 124 singleton pregnancy that didn't meet criteria for aspirin commencement based upon major
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40 125 preeclampsia risk factors (chronic kidney disease, autoimmune disease e.g. systemic lupus
41
42 126 erythematosus, diabetes mellitus and chronic hypertension) were eligible for inclusion and
43
44 127 thus were recruited at antenatal booking clinics selected at random.⁹ In Ireland it is currently
45
46 128 not routine obstetric practice to commence aspirin in women that do not have an
47
48 129 aforementioned major risk factor for pre-eclampsia as defined by the National Institute of
49
50 130 Health and Clinical Excellence.⁹ Exclusion criteria included participants already taking part
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52 131 in a clinical trial, co-existence of fetal congenital anomaly at recruitment or those with aspirin
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54 132 hypersensitivity. All participants provided written informed consent and were recruited by
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3 133 the research clinician at the first trimester antenatal booking visit.
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136 Randomization

137 Participants underwent enrollment and online computerized randomization by the study
138 sonographer or clinician based on blocks of six to; Group 1 - aspirin 75mg from 11 to 13+6
139 weeks once daily until 36-weeks' gestation; Group 2 - no aspirin and; Group 3 - aspirin
140 depending on the result of the FMF screening test. Subjects in non-aspirin taking groups had
141 routine antenatal care. The randomisation sequence was determined prior to study
142 commencement by the off-site statistician and was concealed from assessors, with both the
143 assessor and participant seeing the group allocation at the same time, following online
144 selection.

145

146 Intervention

147 Enteric coated Nu-Seals Aspirin (Acetylsalicylic Acid) 75mg orally once daily at night from
148 11 to 36-weeks gestation was provided free of charge from Alliance Pharma®, which were
149 independent of study protocol and analysis. A dose of 75mg was used as this is currently the
150 standard recommended dosage in the UK and Ireland for at-risk women.⁹
151 Aspirin adherence was assessed subjectively via patient reported diary cards and tablet counts
152 (checked by research clinician and pharmacist) and objectively via assessment of change in
153 urinary 11-dehydrothromboxane-B2 (TxB2). Any reduction in TxB2 between first (pre-
154 aspirin) and second trimester (post-aspirin) levels was taken to suggest that a subject had
155 ingested aspirin within the last ten days.¹⁰

156

157 Baseline review and follow-up

158 Participants underwent two scheduled study visits, at study recruitment and at 20-22 weeks
159 (to coincide with their fetal anatomy scan which was performed at the same time) with diary
160 cards and aspirin tablets returned to the research team at 36-weeks gestation. Participants

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3 161 completed an anonymous questionnaire at 20-22 weeks based on acceptability of taking
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5 162 aspirin in pregnancy.

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7 163 Study assessments at the time of the recruitment visit included the FMF screening test, the
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9 164 results of which were assessed for those in Group 3 (screen positive and received aspirin (3A)
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11 165 and screen negative no aspirin (3B)). The FMF screening test was not routine practice within
12
13 166 Ireland. Components of the screening test included; maternal history (including ovulation
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15 167 induced conception, race, body mass index, age, mother with preeclampsia); mean arterial
16
17 168 blood pressure (MAP); uterine artery Doppler pulsatility index; and pregnancy associated
18
19 169 plasma protein-A (PAPP-A) and placental like growth factor (PLGF) multiples of the
20
21 170 median. To determine risk of preeclampsia, the FMF algorithm was used and based upon a
22
23 171 screen positive rate of 5%, a cut off for preeclampsia prior to 42-weeks at greater than 1:8
24
25 172 was used.³ This cut-off was selected with the aim of capturing the majority of pre-eclamptics;
26
27 173 both pre and post-term and at the time of study commencement this was the optimal
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29 174 screening algorithm for detection of any preeclampsia. Two un-blinded trained clinical
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31 175 research sonographers performed the first trimester uterine artery Doppler waveforms and
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33 176 MAP and interpreted findings. MAP was assessed using an automated blood pressure
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35 177 monitoring device as outlined by the technique stipulated by the FMF.¹¹ Uterine artery
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37 178 Doppler velocimetry was obtained using Viewpoint® Version 5.6.16 GE Healthcare, 2012
38
39 179 and Voluson Expert 730®, GE 2012 using the technique outlined from by the International
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41 180 Society of Ultrasound in Obstetrics and Gynecology. The pulsatility index was measured
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43 181 from both uterine arteries and an average value was calculated.¹²
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50 183 A maternal blood sample was analyzed for PAPP-A and PLGF under standard conditions
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52 184 using a 6000 DELFIA® Xpress, PerkinElmer, 2014 clinical random access screening
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54 185 platform in the hospital clinical biochemistry laboratory. A quantitative immunoturbimetric
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3 186 TxBCardio® immunoassay was used to determine TxB2 levels in urine samples obtained
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5 187 both study visits. These were then standardized as a ratio with creatinine levels and
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7 188 expressed as pg/mg creatinine.
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10 190 **Outcomes**

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13 191 The primary objective of this study was to evaluate the feasibility and acceptability of low-
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15 192 risk nulliparous women taking aspirin versus test indicated aspirin in pregnancy. Outcome
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17 193 measures included;

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20 194 (i) The proportion of eligible women agreeing to participate in a trial where aspirin is
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22 195 prescribed routinely (feasibility);

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24 196 (ii) Compliance with study protocol, as measured by the following: (a) adherence to
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26 197 aspirin (acceptability), (b) attendance at study visits (acceptability), (c)
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28 198 satisfactory collection of all endpoints and variables (feasibility), (d) specific
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30 199 study protocol violations (feasibility);

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33 200 (iii) The proportion of women in whom it was possible to obtain first trimester trans-
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35 201 abdominal uterine artery Doppler velocimetry examination (feasibility);

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37 202 (iv) Proportion of women with a completed screening test who were issued the
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39 203 screening result within one week of having the test performed (feasibility);
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44 205 Secondary outcomes included rates of; (i) preeclampsia; (ii) small-for-gestational-age (SGA)
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46 206 infants (customised sex-specific birth-weight <10th centile); (iii) pre-term delivery prior to
47
48 207 34-weeks; (iv) admission to neonatal intensive care unit (NICU); (v) placental abruption; (vi)
49
50 208 any reported death (stillbirth, neonatal or infant death) and; (vii) acceptability of women
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52 209 taking aspirin routinely versus test indicated aspirin as assessed by an anonymous
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54 210 questionnaire at 20-22 weeks. As part of routine antenatal care women had an appointment
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3 211 with their midwife and or clinician at booking (11-14 weeks), 16-weeks, 18-20 weeks, 25-
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5 212 weeks, 28-weeks, 31-weeks, 34-weeks, 36-weeks, 28-weeks, 40-weeks and 41-weeks
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7 213 gestation in line with hospital protocol. At each visit blood pressure was assessed using
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9 214 mercury sphygmomanometry and a urine dipstick for proteinuria was performed with
10
11 215 symphysio-fundal-height and or fetal biometrical ultrasound assessment as appropriate. -
12
13 216 was defined based upon the definition from the ISSHP with new onset hypertension
14
15 217 (>140mmHg systolic or >90mmHg diastolic) after 20-weeks gestation associated with; (i)
16
17 218 proteinuria of at least 1g/L [2+] on urine dipstick testing, and or; (ii) maternal organ
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19 219 dysfunction ; an or fetal growth restriction.¹³ Suspicion of a diagnosis of pre-eclampsia at an
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21 220 antenatal visit prompted further investigation in the fetal assessment unit with clinical
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23 221 examination, blood testing (urea and creatinine, liver function tests and full blood picture),
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25 222 24-hour urine collection for proteinuria and departmental fetal ultrasound assessment with
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27 223 final diagnosis made by an obstetrician.
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33 225 Safety data were reported as adverse and serious adverse events and participants discontinued
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35 226 from the study were recorded in addition to the reason for discontinuation and outcome. As
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37 227 an assessment of post-partum haemorrhage, blood loss was weighed at time of delivery.
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42 229 **Statistical Analysis**

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44 230 As outlined in the published study protocol, the projected sample size for this study was 500
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46 231 women across two sites with 18,000 deliveries per annum.⁸ To determine preeclampsia as a
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48 232 primary outcome; the anticipated number of patients required is over 15,000 women. As this
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50 233 study aimed to determine the feasibility of such a study, 500 participants were more than
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52 234 adequate as 3% of the number required for a substantive study is required (n=450).¹⁴
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54 235 Accounting for a drop-out rate of 10% (n=45), 500 participants were adequate to obtain the
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3 236 primary outcome. Analysis was performed by a statistician using SAS v.20 on the intention-
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5 237 to-treat (ITT) population, which included all participants randomised, which completed the
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7 238 full second trimester assessment. Measures of variance included standard deviation. Follow-
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9 239 up of serious adverse events continued until 28-days following delivery. Adverse events were
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11 240 reported as odds ratios (OR) and uncertainty was expressed using 95% confidence intervals.
12
13 241 No hypothesis tests were performed.
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21 247 **Patient Involvement**

22 248 Although patients were not directly involved in devising the study protocol and design the
23 249 burden of the RCT intervention (i.e. taking aspirin and undergoing the FMF screening test)
24 250 was assessed by means of an anonymous questionnaire completed at 20-22 weeks gestation.
25 251 At the time of study participation subjects were informed that study results could be viewed
26 252 following publication on the study website; <http://perinatalireland.ie/research/test/>
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3 255 **RESULTS**

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5 256 Subjects were recruited between 8th May 2014 to 23rd September 2015 with follow-up of
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7 257 participants until 11th April 2016, when the study was ended by the steering committee
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9 258 following delivery of the final patient as the target sample size had been achieved. In total
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11 259 1054 eligible women were approached to take part in the study and of these, 557 underwent
12
13 260 randomization [Figure 1]. In the screen and treat population (Group 3) n=184, 13 (7.1%)
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15 261 women had a risk of developing preeclampsia >1:8 and subsequently commenced aspirin
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17 262 until 36-weeks gestation. Eleven women were excluded from the study leaving 546 in the
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19 263 ITT population. In total there were 192 women in the ITT group that were taking aspirin as
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21 264 per randomization and 354 not taking aspirin. Baseline characteristics were similar and the
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23 265 summaries are presented in Table 1.
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| Characteristic | Low Dose Aspirin N=179 | No Aspirin N=183 | Screen & Treat N=184 |
|---|---------------------------|---------------------|-------------------------|
| Age (yr) | 33 (19-44) | 34 (18-43) | 33 (19-44) |
| Race – No. (%) | | | |
| White | 181 (97.9) | 179 (95.7) | 180 (97.3) |
| Black | 1 (0.5) | 2 (1.1) | 0 (0) |
| Asian | 3 (1.6) | 6 (3.2) | 5 (2.7) |
| Other | 0 | 0 (0) | 0 (0) |
| Completed secondary school – No.(%) | 136 (73.5) | 143 (76.4) | 152 (82.2) |
| BMI (kg/m ²) | 25.2 (17.4-39.4) | 22.9 (17.7-41.4) | 23.8 (18.1-45.2) |
| Gestational Age (wks) | 12.9 (11.1-13.9) | 12.9 (11.1-13.9) | 12.9 (11.3-13.9) |
| Smoking – No. (%) | 17(9.2) | 11 (5.9) | 7 (3.8) |
| Subject's mother had preeclampsia - No. (%) | 7 (3.8) | 10 (5.4) | 10 (5.4) |
| Conception – No. (%) | | | |
| IVF | 5 (2.7) | 9 (4.8) | 8 (4.3) |
| ICSI | 3 (1.6) | 4 (2.1) | 3 (1.6) |
| Ovulation induction | 5 (2.7) | 6 (3.2) | 6 (3.2) |
| Spontaneous | 172 (93.0) | 168 (89.9) | 170 (91.9) |
| Previous miscarriage – No. | 20 (10.8) | 31 (16.6) | 31 (16.8) |

| | | | |
|-----|--|--|--|
| (%) | | | |
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268 Table 1: Baseline characteristics of the study population. Where number (No.) percentage is

269 not expressed average and range are demonstrated.

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272 **Primary outcomes**

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274 (i) The proportion of eligible women agreeing to participate in a trial where aspirin is

275 prescribed routinely (feasibility); 1054 women were approached that were eligible to partake.

276 497 were subsequently not enrolled as they did not want to take aspirin n=454 or for an

277 alternative reason n=43 e.g. appointment did not suit. Hence 546/1054 (51.8%) women were

278 willing to partake in a study where they may have to take aspirin routinely.

279

280 (ii) Compliance with study protocol, as measured by the following: (a) adherence to aspirin

281 (acceptability), (b) attendance at study visits (acceptability), (c) satisfactory collection of all

282 endpoints and variables (feasibility), (d) specific study protocol violations (feasibility);

283

284 (a) Of those women included in analysis that were taking aspirin (n=192), the average

285 adherence based upon patient reported diary cards was 96.0% and based upon tablet counts

286 95.0%. Seven women were non-adherent and 19 (10.0%) poorly compliant (<80%).

287 Average adherence was 95.0% in both the test indicated aspirin group (3a) and routine aspirin

288 group (1) [Table 2]. The median first trimester pre-aspirin urine TxB2 level was 8662.2

289 pg/mg (IQR 2014.5-9931.5) and second trimester (post-aspirin) 2285.1 pg/mg (IQR 591.0-

290 2300.1). The percentage change in TxB2 was then assessed for all paired samples (n=147)

291 and found that 124/147 (84.4%) of subjects had a fall in TxB2 levels between the first and

292 second trimesters versus 23/147 (15.6%) who had an increase. The greater the reduction in

293 urinary TxB2 pre- and post- aspirin dose the greater the degree of aspirin adherence, as

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3 294 demonstrated in Figure 2. Patient groups were similar (routine aspirin and screen positive
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5 295 aspirin) and percentage change in urine TxB2.
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9 297 (b) Of those that underwent randomization (n=557), eleven were excluded prior to fulfillment
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11 298 of study participation requirements (attendance at second study visit). Of the eleven, three
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13 299 withdrew consent for participation as they decided that they did not wish to take aspirin
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15 300 following randomization.
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20 302 (c) Of all 546 subjects collection of outcome measures and variables were obtained for all
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22 303 apart from the questionnaire on patient acceptability, which was completed in 97.1%
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24 304 (530/546).
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29 306 d) Six protocol violations were recorded (0.01 per 100 participants) including women
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31 307 transferring care to another hospital (n=3), incorrect randomization of women that did not
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33 308 meet inclusion criteria (n=2) and a subject in the non-aspirin group commencing aspirin by
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35 309 their clinician (n=1).
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40 311 (iii) The proportion of women in whom it was possible to obtain first trimester trans-
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42 312 abdominal uterine artery Doppler velocimetry (feasibility); The FMF screening test was
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44 313 completed in 98.4% (181/184) following successful uterine artery Doppler velocimetry
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46 314 acquisition, of which one was obtained vaginally due to challenges with abdominal
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48 315 acquisition, with an overall sonographer reported ease of acquisition 3.1 (SD +/- 0.91) (score
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50 316 1 (easy) to 5 (unobtainable)) [Table 2].
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318 (iv) Proportion of women with a completed screening test, issued the result within one week
 319 of the test (feasibility); The average time to obtain laboratory analyzed PAPP-A and PLGF so
 320 that a screening result could be issued was 7.6 days (0-26) with 78 (42.4%) of women
 321 waiting greater than one week and five women being beyond 16-weeks prior to result
 322 availability [Table 2].

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| Adherence and feasibility parameter | Low-dose Aspirin (N=179) | No Aspirin (N=183) | Screen & Treat (N=184) |
|-------------------------------------|--------------------------|--------------------|------------------------|
| Ease of Doppler acquisition | | | |
| Very easy | | | 8 (4%) |
| Easy | | | 53 (29%) |
| Fair | | | 61 (33%) |
| Difficult | | | 60 (32%) |
| Unobtainable | | | 3 (2%) |
| Days to PLGF/PAPPA visit 1 | | | 7.6 [0 - 26] |
| PLGF/PAPPA result > 16 weeks | | | 5 (3%) |
| Time taken for visit 1 (mins) | 60 [30 – 100] | 60 [25 - 90] | 60 [25 - 90] |
| Median adherence tablet counts | 96% | | 95% (screen positive) |

| | | | |
|------------------------------|--------|--|-----------------------|
| Median adherence diary cards | 94% | | 95% (screen positive) |
| Non-adherent | 7 (4%) | | 0 (0%) |

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334 Table 2: Primary outcomes of feasibility and adherence

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3 338 **Secondary outcomes**

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5 339 There was no difference between groups in relation to secondary outcomes [Table S1
6
7 340 supplementary]. For the overall cohort, there were three cases (0.37%) of early onset
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9 341 preeclampsia <34-weeks (0.55%), n=22 (4.03%) any preeclampsia, n=57 (10.44%) SGA
10
11 342 infants and 15.02% (n=82) placental disease. Secondary outcomes for groups 3A (screen
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13 343 positive aspirin) and 3B (screen negative no aspirin) are demonstrated in Table S2
14
15 344 [supplementary]. Despite taking aspirin, there remained a greater number with preeclampsia
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17 345 at <37-weeks in the screen positive versus the screen negative group, although numbers were
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19 346 small (n=2 (15.4%) vs. n=2 (1.2%). In terms of taking aspirin in a subsequent pregnancy, the
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21 347 questionnaire revealed that 92.3% (489/530) were willing to take aspirin in a subsequent
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23 348 pregnancy; 92.5% (173/187) of aspirin takers and 91.5% (314/343) of non-aspirin takers.
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3 350 **Safety**

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5 352 The adverse event profile differed between groups but not the serious adverse event profile

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7 353 [Tables 3 and S3 (supplementary)]. There were six perinatal deaths, all of which underwent

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9 354 postmortem. In the aspirin group there was one placental abruption and one case of

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11 355 intervillous haemorrhage. Perinatal deaths in the non-aspirin groups were due to delayed

12
13 356 villous maturation, severe FGR, fetal thrombotic vasculopathy and neonatal septicemia.

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15 357 There was an observable difference between groups in terms of reported vaginal spotting

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17 358 aspirin 15.1% vs. non-aspirin 7.9% OR 2.1 (CI 1.2-3.6), which was not associated with

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19 359 pregnancy loss. Similarly, the rate of PPH >1000mls was higher in the aspirin group.

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21 360 However, the numbers were small. Rates of blood transfusion or significant hemoglobin

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23 361 drop to <8g/dL were similar.
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| Event | | Aspirin n=192 | Non-aspirin n=354 | Odds ratio (95% CI) |
|---------------------------------|---------------------------------|------------------|----------------------|------------------------|
| Adverse Events | | | | |
| Adverse Events Total No. | | 123 | 143 | 2.6 (1.8-3.8) |
| Vaginal spotting No. (%) | | 29(15.1) | 28 (7.9) | 2.1 (1.2-3.6) |
| Post-partum haemorrhage No. (%) | | | | |
| >500mls | | 26(13.5) | 20 (5.6) | 2.6 (1.4-4.8) |
| >1000mls | | 7 (3.6) | 5 (1.4) | 2.8 (0.9-9.0) |
| Blood transfusion | | 3 | 4 | 0.5 (0.1-2.7) |
| Hb drop <8g/dL | | 4 | 7 | 0.3 (0.1-1.4) |
| Serious Adverse Event | | | | |
| NICU admission | Sepsis | 3 | 2 | |
| | Hypoglycaemia | 0 | 1 | |
| | Prematurity | 1 | 4 | |
| | Jaundice | 1 | 1 | |
| | Persistently low Apgar | 1 | 3 | |
| | TTN | 1 | 3 | |
| | Meconium aspiration | 1 | 0 | |
| | Hypoxic ischemic encephalopathy | 1 | 1 | |
| | Very low birthweight | 0 | 1 | |
| Total | | 9 | 16 | 1.04 (0.45-2.40) |
| Perinatal Death | | 2 | 4 | |
| Total | | 2 | 4 | 0.92 (0.17-5.10) |
| Maternal admission | Preterm labor | 3 | 2 | |
| | Preeclampsia | 8 | 7 | |
| | Antepartum hemorrhage | 3 | 7 | |
| | PPROM | 0 | 2 | |
| | Fetal compromise | 1 | 2 | |
| | Infection | 2 | 5 | |
| | Other | 4 | 1 | |
| Total | | 21 | 26 | 1.55 (0.85-2.83) |
| Congenital anomaly | Cardiac | 1 | 2 | |
| | Gastrointestinal | 3 | 2 | |
| | Neurological | 0 | 1 | |
| | Renal | 0 | 1 | |
| Total | | 4 | 6 | 1.23 (0.34-4.43) |
| Total serious adverse events | | 36 | 52 | 1.34 (0.84-2.14) |

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5 364 Table 3: Adverse and serious adverse events in aspirin and non-aspirin taking groups. There
6
7 365 may be >1 adverse event or serious adverse event per subject [NICU=Neonatal intensive care
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9 366 unit, TTN= transient tachypnea of the newborn, PPOM= preterm premature rupture of
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11 367 membranes, Very low birthweight = <1500g].

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3 369 **DISCUSSION**

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6 371 **Main Findings**

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8 372 This feasibility randomised controlled trial has found that low-risk nulliparous women were
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10 373 open to taking aspirin in pregnancy and were adherent, with a willingness to take it again in a
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12 374 subsequent pregnancy. We can say this as, comparing findings to other RCTs in pregnancy,
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14 375 of which there are few, the uptake in this RCT was much higher as was adherence (e.g.
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16 376 Chiswick, *et al.* 2015; 35% enrolment and 65-67% adherence with metformin use).¹⁵ This is
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18 377 the first trial of its kind, which has assessed the acceptability of women taking aspirin in low-
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20 378 risk pregnancy and the feasibility of an integrated screening test in a routine clinical setting.
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25 380 **Strengths and Limitations**

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27 381 The strengths of this study are the multicenter RCT design with robust protocol and over-
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29 382 sight and previously published methodology. Allocation bias was limited by use of a
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31 383 prospective approach and selection bias was limited by randomization. The fact that the
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33 384 same two sonographers and biochemists were responsible for conducting the screening test
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35 385 with use of quality control standards for test completion using the same equipment and
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37 386 technique for all subjects optimized reproducibility. There were a low number of dropouts
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39 387 and almost all patient outcomes were recorded. Although there is currently no validated
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41 388 scientific method of assessing aspirin adherence,¹⁶ a laboratory assessment of change in
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43 389 TxB2 served as a more objective assessment, strengthening reliability. There is currently no
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45 390 accepted test in the literature, which can reliably determine aspirin adherence, hence three
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47 391 different methods were used to optimize reliability.¹⁶ Study weaknesses, were primarily that
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49 392 PAPP-A and PLGF analysis was performed in the laboratory using validated methods with
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51 393 quality assurance, as opposed to the bedside point-of-care tests hence it took longer to obtain
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53 394 a result. In a non-research setting with a greater throughput of patients, one could anticipate a
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3 395 faster turnaround time. Additionally the open-label nature of the study meant that safety
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5 396 recording was open to reporting bias and, as is often the case with RCTs the uptake of
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7 397 subjects demonstrated dominance for educated women. In RCTs there is always a risk of
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9 398 introducing a Hawthorne effect, whereby subjects act differently in the confines of an RCT as
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11 399 to how they would in a real-life setting, hence adherence rates may have been over-
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13 400 represented.¹⁷ A third trimester visit may have added strength to the study to assess
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15 401 objectively for aspirin adherence and patient satisfaction, however as adherence prior to 16-
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17 402 weeks was deemed the critical time point for preeclampsia prevention, follow-up at 20-22
18
19 403 weeks was selected.
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23 24 405 **Interpretation**

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26 406 A recently published large RCT from the FMF found that, following application of FMF
27
28 407 screening and subsequent randomization of women deemed to be at risk of preterm
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30 408 preeclampsia to aspirin 150mg versus placebo, there was a reduction in the incidence of
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32 409 preterm preeclampsia in the aspirin arm.⁵ Our study differs on several counts; (i) routine
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34 410 aspirin arm – use of a third arm assessing provision of routine aspirin assessed the
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36 411 acceptability and feasibility of this policy; (ii) aspirin dosage (150mg vs. 75mg) – in light of
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38 412 limited evidence on dosage and effect, the safest lowest effective dose was selected. A recent
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40 413 meta-analysis, published since completion of this study suggests that there is an aspirin dose-
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42 414 response effect, with higher doses of aspirin commenced prior to 16-weeks gestation,
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44 415 associated with a greater reduction in preeclampsia and fetal growth restriction compared to
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46 416 standard lower doses.¹⁸ When supported by robust safety data when using higher dosing, this
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48 417 is something to consider in future studies and clinical practice; (iii) adverse events – rates of
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50 418 PPH and vaginal bleeding were reported. This information would be useful from the FMF
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3 419 study in light of the higher aspirin dosing regime and; (iv) our study was not powered to
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5 420 detect a difference in clinical outcome, with the primary focus feasibility and acceptability.

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9 422 Few studies have assessed the acceptability of non-routine medications in pregnancy. In the
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11 423 developing world, pregnant women are willing to take calcium, oral iron and
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13 424 micronutrients.¹⁹⁻²¹ If instructed about potential side-effects and reminded frequently
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15 425 women had higher levels of adherence with the greatest barrier being forgetfulness. Average
16
17 426 medication adherence in pregnancy for chronic illness is higher than for non-routine
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19 427 medications at 90-95%,²² hence it is promising that we have noted a rate as high as this in
20
21 428 our own study. There was a slight discrepancy in adherence assessed via tablet counts and
22
23 429 diary cards and that more objectively assessed via TxB2. Reasons for this may include the
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25 430 potential for aspirin resistance; which although not formally assessed in this study can be
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27 431 increased when using an enteric-coated preparation.²³

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33 433 The FMF screening test was feasible in terms of acquiring first trimester uterine artery
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35 434 Doppler velocimetry measurements, though delays were encountered in obtaining laboratory
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37 435 analyzed PAPP-A and PLGF. This is relevant as it reflects the practical aspects of such a
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39 436 screening test in a clinical real life setting. Improved protocols between the clinical and
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41 437 laboratory staff would be required to allow patients receive results within a reasonable
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43 438 timeline.

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48 440 In terms of vaginal spotting and clinically significant PPH with aspirin use, the findings of
49
50 441 this study are comparable with previous studies although evidence of increased antenatal and
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52 442 postnatal bleeding, requires further investigation, most notably with use of aspirin at doses
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54 443 greater than 75mg.²⁴⁻²⁵ Due to the open-label nature of this study as opposed to placebo

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3 444 control, there is always a potential of reporting bias of bleeding in the aspirin arms. Although
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5 445 generally safe in pregnancy, it may be worthwhile considering cessation of aspirin at 32-34
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7 446 weeks gestation with the aim of reducing the risk of PPH, as opposed to 36-weeks and of
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9 447 informing women of the unwanted side-effect of increased vaginal spotting.
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11 448

13 449 **Conclusion**

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17 451 It has been proposed that the most cost-effective approach to reducing preeclampsia is the
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19 452 provision of an effective, affordable and safe intervention applied to all mothers without prior
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21 453 testing to assess levels of risk.⁷ A algorithm-based screen-and-treat approach, as proposed by
22
23 454 the FMF has can reduce rates of pre-term preeclampsia when doses of 150mg of aspirin are
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25 455 used. This study was not powered to detect a difference in rates of preeclampsia between
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27 456 groups, yet has taken the first step to address if low-risk nulliparous women are open to
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29 457 taking aspirin in the first instance and if a screening algorithm is feasible. Moving forward,
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31 458 an RCT is required to address the efficacy of universal low dose aspirin in low-risk
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33 459 pregnancy compared to a screening approach. This will require significant numbers due to
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35 460 the low incidence of early-onset preeclampsia. Although women were open to taking aspirin
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37 461 in pregnancy compared to other RCTs involving medication, almost twice the number
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39 462 enrolled had to be approached to obtain adequate study participants. This must be considered
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41 463 when planning a future trial.
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3 466 **COMPETING INTERESTS STATEMENT:** Authors report no conflict of interest

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6
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8
9 469 Mother and Baby Clinical Trials Network.

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11 470

12
13 471 **CONTRIBUTION OF AUTHORSHIP:** (i) Conceived and designed the experiments: FM,

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15 472 CM, PMcP, FB, PD, DM, MC, AS, FC, JM, SD, JH, AC, ET, PD, ZA, FDM, FMcA; (ii)

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17 473 Performed the experiments: FM, CM, FC ; (iii) Analyzed the data: FM, PD, ZA, FMcA; (iv)

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19 474 Contributed reagents/materials/analysis/tools: PMcP, FB, PD, DM, MC, AS, JM, SD, JH,

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21 475 AC, AH, ET, PD, ZA, FDM, FMcA; (v) Wrote the paper: FM, CM, PMcP, FB, PD, DM,

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23 476 MC, AS, FC, JM, SD, JH, AC, AH, ET, PD, ZA, FDM, FMcA

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31 480 **DATA SHARING STATEMENT:** Dataset available from corresponding author on request

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31 **FIGURE LEGENDS**

- 32 571
33 572 Figure 1 - Consort diagram
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35 573 Figure 2 - Histogram demonstrating percentage change in urinary thromboxane-B2 levels
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37 574 pre- and post - aspirin administration (n=147) [TxB2 = urinary-thromboxane level]
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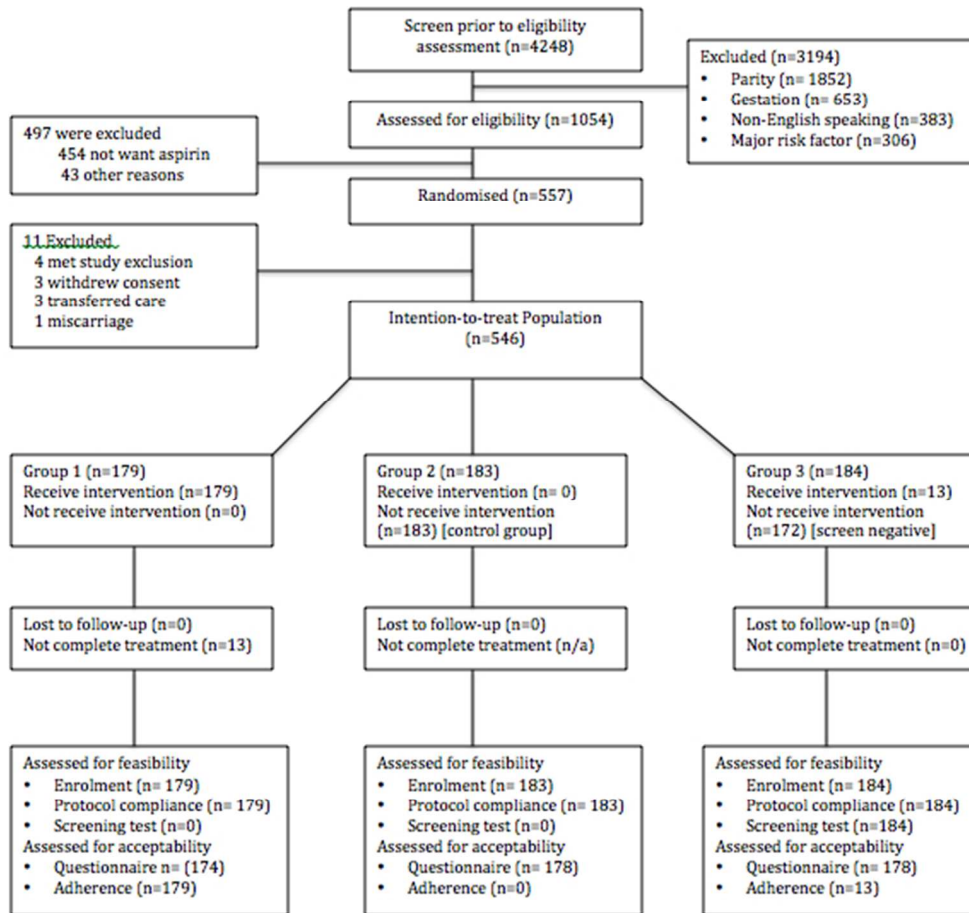


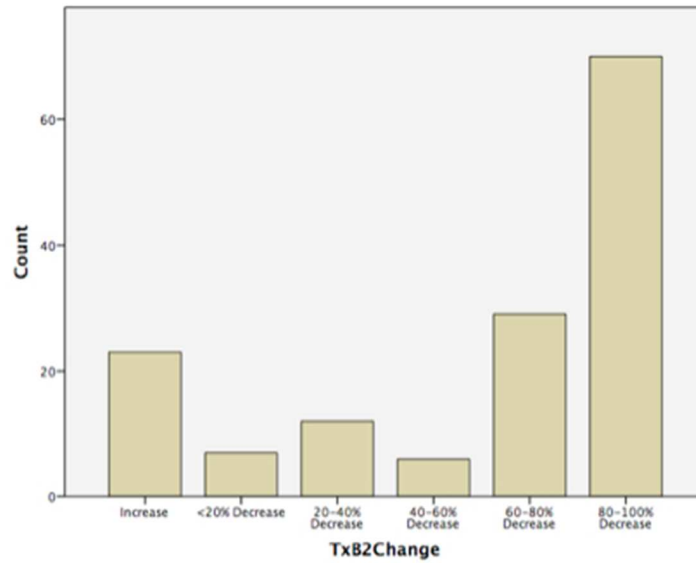
Figure 1 – Consort diagram

Figure 1 - consort diagram

206x206mm (72 x 72 DPI)



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[Figure 2 - Histogram demonstrating percentage change in urinary thromboxane-B2 levels pre- and post aspirin administration (n=147) [TxB2 = urinary B2-thromboxane level]

Figure 2 - Histogram demonstrating percentage change in urinary thromboxane-B2 levels pre- and post aspirin administration (n=147) [TxB2 = urinary B2-thromboxane level]

204x180mm (72 x 72 DPI)

Preprint

| Outcome | Low Dose Aspirin (Group 1) N=179 | No Aspirin (Group 2) N=183 | Screen and Treat (Group 3) N=184 |
|---|--|----------------------------------|---|
| Gestation at delivery (wks) | 40.2 (1.4) | 39.9 (1.9) | 40.2 (1.5) |
| Birthweight (g) | 3529 (469) | 3478 (493) | 3488 (502) |
| Birthweight <10 th centile No. (%) | 14 (8%) | 18(10%) | 25 (14%) |
| Mode of delivery No. (%) | | | |
| Spontaneous | 85 (47.5) | 95 (52.0) | 88 (47.8) |
| Instrumental | 56 (31.3) | 47 (25.7) | 51 (27.7) |
| Caesarean | 38 (21.2) | 41 (22.3) | 45 (24.5) |
| Pre-term delivery <34 weeks No. (%) | 1 (0.6) | 3 (1.6) | 2 (1.1) |
| Spontaneous Labor No. (%) | 96 (53.7) | 103 (56.3) | 101 (54.9) |
| Preeclampsia No. (%) | 8 (4.5) | 7 (3.8) | 7 (3.8) |
| Preeclampsia <34-weeks | 0 (0) | 2 (1.1) | 1 (0.5) |
| Preeclampsia <37-weeks | 2 (1.1) | 2 (1.1) | 2 (1.1) |
| Abruption No. (%) | 1 (0.5) | 0 (0) | 0 (0) |
| NICU admission No. (%) | 9 (5.0) | 7 (3.8) | 9 (4.9) |
| Apgar < 7 No. (%) | 5 (2.8) | 2 (1.6) | 3 (1.6) |
| Cord pH (arterial) | 7.3 (0.1) | 7.3 (0.1) | 7.3 (0.1) |
| Outcome No. (%) | | | |
| Alive at 6-weeks | 177 (98.9) | 181 (99.0) | 182 (98.9) |
| Stillbirth | 2 (1.1) | 1 (0.5) | 0 (0) |
| Neonatal death | 0 (0) | 1 (0.5) | 2 (1.1) |

Table S1: Secondary outcome measures

(Expressed as average and standard deviation unless otherwise stated)

| Outcome | Screen positive; Aspirin Group 3A N=13 | Screen negative; No Aspirin Group 3B N=171 | OR (95% CI) |
|---|---|---|------------------|
| Preeclampsia No. (%) | 2 (15.4) | 6 (3.5) | 5.0 (0.9 – 27.7) |
| Pre-eclampsia <34-weeks | 0 (0) | 2 (1.2) | - |
| Pre-eclampsia <37-weeks | 2 (15.4) | 2 (1.2) | 15.4 (2.0 – 120) |
| Birthweight <10 th centile No. (%) | 4 (30.7) | 21 (12.3) | 3.2 (0.9 – 11.2) |
| Pre-term delivery <34 weeks No. (%) | 1 (7.7) | 1 (0.6) | 14.1 (0.8 – 240) |
| NICU admission No. (%) | 0 (0) | 9 (5.3) | - |
| Outcome No. (%) | | | |
| Alive at 6-weeks | 13 (100) | 169 (98.8) | |
| Stillbirth | 0 (0) | 2 (1.2) | |
| Neonatal death | 0 (0) | 0 (0) | |

Table S2 - Secondary outcome measures in Group 3 (screen and treat)

(Expressed as average and standard deviation unless otherwise stated)

Note: ORs are not presented when number of events is 0 in the Screen –positive group.

| Adverse/Serious Adverse Event | Low dose Aspirin Group 1 N=179 | No-aspirin Group 2 N=183 | Screen and treat Group 3 N=184 |
|---------------------------------|--------------------------------------|--------------------------------|--------------------------------------|
| Adverse events | | | |
| Vaginal spotting No. (%) | 27 (15.1) | 18 (9.8) | 12 (6.5) |
| Post-partum haemorrhage No. (%) | | | |
| >500mls | 25 (13.0) | 9 (4.9) | 12 (6.5) |
| >1000mls | 7 (3.6%) | 1 (0.5) | 4 (2.2) |
| Serious Adverse Events | | | |
| NICU admission | 9 (5.0) | 7 (3.8) | 9 (4.9) |
| Perinatal Death | 2 (1.1) | 2 (1.1) | 2 (1.1) |
| Maternal admission | 18 (10.1) | 15 (8.2) | 14 (7.6) |
| Congenital anomaly | 3 (1.7) | 4 (2.2) | 3 (1.6) |
| Total serious adverse events | 32 (17.8) | 28 (15.3) | 28 (15.2) |

Table S3 – Adverse and serious adverse events in all three groups. There may be >1 adverse event or serious adverse event per subject



CONSORT 2010 checklist of information to include when reporting a pilot or feasibility trial*

| Section/Topic | Item No | Checklist item | Reported on page No |
|----------------------------------|---------|---|---------------------|
| Title and abstract | | | |
| | 1a | Identification as a pilot or feasibility randomised trial in the title | 1-3 |
| | 1b | Structured summary of pilot trial design, methods, results, and conclusions (for specific guidance see CONSORT abstract extension for pilot trials) | 45-72 |
| Introduction | | | |
| Background and objectives | 2a | Scientific background and explanation of rationale for future definitive trial, and reasons for randomised pilot trial | 83-98 |
| | 2b | Specific objectives or research questions for pilot trial | 98-104 |
| Methods | | | |
| Trial design | 3a | Description of pilot trial design (such as parallel, factorial) including allocation ratio | 110, 137-139 |
| | 3b | Important changes to methods after pilot trial commencement (such as eligibility criteria), with reasons | N/A |
| Participants | 4a | Eligibility criteria for participants | 123-133 |
| | 4b | Settings and locations where the data were collected | 110-111 |
| | 4c | How participants were identified and consented | 132-133 |
| Interventions | 5 | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | 146-188 |
| Outcomes | 6a | Completely defined prespecified assessments or measurements to address each pilot trial objective specified in 2b, including how and when they were assessed | 190-223 |
| | 6b | Any changes to pilot trial assessments or measurements after the pilot trial commenced, with reasons | n/a |
| | 6c | If applicable, prespecified criteria used to judge whether, or how, to proceed with future definitive trial | 94-98 |
| Sample size | 7a | Rationale for numbers in the pilot trial | 230-236 |
| | 7b | When applicable, explanation of any interim analyses and stopping guidelines | n/a |
| Randomisation: | | | |
| Sequence generation | 8a | Method used to generate the random allocation sequence | 137-138 |
| | 8b | Type of randomisation(s); details of any restriction (such as blocking and block size) | 138 |
| Allocation concealment mechanism | 9 | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned | 141-144 |

| | | | |
|--|-----|---|--------------------|
| Implementation | 10 | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions | 137-138, 141-142 |
| Blinding | 11a | If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how | n/a |
| | 11b | If relevant, description of the similarity of interventions | 138-141 |
| Statistical methods | 12 | Methods used to address each pilot trial objective whether qualitative or quantitative | 236-242 |
| Results | | | |
| Participant flow (a diagram is strongly recommended) | 13a | For each group, the numbers of participants who were approached and/or assessed for eligibility, randomly assigned, received intended treatment, and were assessed for each objective | Figure 1 |
| | 13b | For each group, losses and exclusions after randomisation, together with reasons | Figure 1 |
| Recruitment | 14a | Dates defining the periods of recruitment and follow-up | 253-255 |
| | 14b | Why the pilot trial ended or was stopped | 253-255 |
| Baseline data | 15 | A table showing baseline demographic and clinical characteristics for each group | 262-265 |
| Numbers analysed | 16 | For each objective, number of participants (denominator) included in each analysis. If relevant, these numbers should be by randomised group | Figure 1 |
| Outcomes and estimation | 17 | For each objective, results including expressions of uncertainty (such as 95% confidence interval) for any estimates. If relevant, these results should be by randomised group | 334-365 + Table S2 |
| Ancillary analyses | 18 | Results of any other analyses performed that could be used to inform the future definitive trial | Table S2 |
| Harms | 19 | All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) | 346-363 |
| | 19a | If relevant, other important unintended consequences | 346-363 |
| Discussion | | | |
| Limitations | 20 | Pilot trial limitations, addressing sources of potential bias and remaining uncertainty about feasibility | 376-399 |
| Generalisability | 21 | Generalisability (applicability) of pilot trial methods and findings to future definitive trial and other studies | |
| Interpretation | 22 | Interpretation consistent with pilot trial objectives and findings, balancing potential benefits and harms, and considering other relevant evidence | 401-443 |
| | 22a | Implications for progression from pilot to future definitive trial, including any proposed amendments | 454-459 |
| Other information | | | 401-443 |
| Registration | 23 | Registration number for pilot trial and name of trial registry | 116-117 |
| Protocol | 24 | Where the pilot trial protocol can be accessed, if available | Supplementary file |
| Funding | 25 | Sources of funding and other support (such as supply of drugs), role of funders | 116-120 |
| | 26 | Ethical approval or approval by research review committee, confirmed with reference number | 115-116 |

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Citation: Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, et al. CONSORT 2010 statement: extension to randomised pilot and feasibility trials. *BMJ*. 2016;355.
*We strongly recommend reading this statement in conjunction with the CONSORT 2010, extension to randomised pilot and feasibility trials, Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

For peer review only