BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-022056
Article Type:	Research
Date Submitted by the Author:	30-Jan-2018
Keywords:	preeclampsia, screening, aspirin, feasibility, low risk

Complete List of Authors:

Mone, Fionnuala; UCD Perinatal Research Centre, Obstetrics and Gynaecology, School of Medicine, University College Dublin,
Mulcahy, Cecilia; UCD Perinatal Research Centre, Obstetrics and Gynaecology, School of Medicine, University College Dublin
McParland, Peter; UCD Perinatal Research Centre, Obstetrics and Gynaecology, School of Medicine, University College Dublin
Brethnach, FIONNUALA; Royal College of Surgeons in Ireland
Downey, Paul; Department of Pathology and Laboratory Medicine, National Maternity Hospital
McCormack, Dorothy; Department of Pharmacy, National Maternity Hospital
Culliton, Marie; Department of Pathology and Laboratory Medicine, National Maternity Hospital
Staunton, Alice; Royal College of Surgeons in Ireland
Cody, Fiona; Royal College of Surgeons in Ireland
Morrison, John; National University of Ireland Galway, Obstetrics and Gynaecology
Daly, Sean; Coombe Women and Infants University Hospital, Obstetrics and Gynaecology
Higgins, John; Department of Obstetrics and Gynaecology, University College Cork
Cotter, Amanda; Department of Obstetrics and Gynaecology, Graduate Entry Medical School, University of Limerick
Hunter, Alyson; Royal Maternity Hospital, Grosvenor Road, BT12 6BB
Tully, Elizabeth; Royal College of Surgeons in Ireland, Perinatal Ireland
Dicker, Patrick; Perinatal Ireland
Alfirevic, Zarko; University of Liverpool, Department of Women's and Children's Health
Malone, Fergal; Royal College of Surgeons in Ireland, Obstetrics and Gynaecology
McAuliffe, Fionnuala; University College Dublin, School of Medicine; National Maternity Hospital, Obstetrics and Gynaecology

TITLE: **T**rial of low dose aspirin with an **E**arly **S**creening **T**est for preeclampsia and growth restriction **TEST** Study – A pilot randomised controlled trial

AUTHORS: Fionnuala MONE[1], Ms. Cecilia MULCAHY[1], Peter McPARLAND[1], Fionnuala BREATHNACH[2], Paul DOWNEY[3], Ms. Dorothy MCCORMACK[4], Ms. Marie CULLITON[3], Alice STANTON[5], Ms. Fiona CODY[2], John MORRISON[6], Sean DALY[7], John HIGGINS[8], Amanda COTTER[9], Alyson HUNTER[10], Elizabeth TULLY[2], Patrick DICKER[2], Zarko ALFIREVIC[11], Fergal D MALONE [2], Fionnuala M McAULIFFE[1]*

AFFILIATIONS:
1.UCD Perinatal Research Centre, School of Medicine, University College Dublin, National Maternity Hospital, Dublin, Ireland. 2. Department of Obstetrics and Gynaecology, Royal College of Surgeons in Ireland, Rotunda Hospital, Parnell Square, Dublin 1, Ireland. 3. Department of Pathology and Laboratory Medicine, National Maternity Hospital, Holles St, Dublin 2, Ireland. 4. Department of Pharmacy, National Maternity Hospital, Holles St, Dublin 2, Ireland. 5. Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin 2, Ireland. 6. Department of Obstetrics and Gynaecology, National University of Ireland, Galway, Ireland. 7. Department of Obstetrics and Gynaecology, Coombe Women's and Infant's University Hospital, Dublin 8, Ireland. 8. Department of Obstetrics and Gynaecology, University College Cork, Cork, Ireland. 9. Department of Obstetrics and Gynaecology, Graduate Entry Medical School, University of Limerick, Limerick, Ireland. 10. Royal Jubilee Maternity Hospital, Belfast, UK. 11. Department of women's and Children's Health, Institute of Translational Medicine, University of Liverpool, Liverpool, UK

*Corresponding author: Prof FM McAuliffe. UCD Perinatal Research Centre, School of Medicine, University College Dublin, National Maternity Hospital, Dublin, Ireland. T:+353 1 6373216 E: Fionnuala.mcauliffe@ucd.ie

WORD Count: 3, 423 words

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

high levels of adherence. Aspirin use was associated with greater rates of vaginal bleeding. An appropriately powered randomized controlled trial is now required to address the efficacy and safety of universal low dose aspirin in low-risk pregnancy compared to a screening approach.

Trial Registration: www.isrctn.com/ISRCTN15191778

ARTICLE SUMMARY

Strengths and limitations of this study

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

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 published[8] 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 erythematosis, 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

the research clinician at the first trimester antenatal booking visit.

Randomization

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

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-dehydroxo-thromboxane-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 with diary cards and aspirin tablets returned to the research team at 36-weeks gestation. Participants completed an anonymous questionnaire at 20-22 weeks based on acceptability of taking aspirin in pregnancy.

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

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

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 $<10^{th}$ 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

symphysio-fundal-height and or fetal biometrical ultrasound assessment as appropriate. Pre-eclampsia was defined based upon the definition from the ISSHP with new onset hypertension (>140mmHg systolic or >90mmHg diastolic) after 20-weeks gestation associated with; (i) proteinuria of at least 1g/L [2+] on urine dipstick testing, and or; (ii) maternal organ dysfunction ; an or fetal growth restriction.[13] Suspicion of a diagnosis of pre-eclampsia at an antenatal visit prompted further investigation in the fetal assessment unit with clinical examination, blood testing (urea and creatinine, liver function tests and full blood picture), 24-hour urine collection for proteinuria and departmental fetal ultrasound assessment with final diagnosis made by an obstetrician.

Safety data were reported as adverse and serious adverse events and participants discontinued from the study were recorded in addition to the reason for discontinuation and outcome. As an assessment of post-partum haemorrhage, blood loss was weighed at time of delivery.

Statistical Analysis

As outlined in the published study protocol, the projected sample size for this study was 500 women across two sites with 18,000 deliveries per annum.[8] To determine preeclampsia as a primary outcome, the anticipated number of patients required is over 15,000 women. As this study aimed to determine the feasibility of such a study, 500 participants were more than adequate as 3% of the number required for a substantive study is required (n=450).[14] Accounting for a drop-out rate of 10% (n=45), 500 participants were adequate to obtain the primary outcome. Analysis was performed by a statistician using SAS v.20 on the intention-to-treat (ITT) population, which included all participants randomised, which completed the full second trimester assessment. Measures of variance included standard deviation. Follow-up of serious adverse events continued until 28-days following delivery. Adverse events were

reported as odds ratios (OR). To assess secondary outcome and safety, comparisons of groups were be performed using two sample t-tests, Wilcoxon Rank-sum tests and Chi-square tests.

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. 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/m2)	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)

Table 1: Baseline characteristics of the study population. Where number (No.) percentage is not expressed average and range are demonstrated.

Primary outcomes

(i) The proportion of eligible women agreeing to participate in a trial where aspirin is prescribed routinely (feasibility); 1054 women were approached that were eligible to partake. 497 were subsequently not enrolled as they did not want to take aspirin n=454 or for an alternative reason n=43 e.g. appointment did not suit. Hence 546/1054 (51.8%) women were willing to partake in a study where they may have to take aspirin routinely.

(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);

(a) Of those women included in analysis that were taking aspirin (n=192), the average adherence based upon patient reported diary cards was 96.0% and based upon tablet counts 95.0%. Seven women were non-adherent and 19 (10.0%) poorly compliant (<80%). Average adherence was 95.0% in both the test indicated aspirin group (3a) and routine aspirin group (1) [Table 2]. The median first trimester pre-aspirin urine TxB2 level was 8662.2 pg/mg (IQR 2014.5-9931.5) and second trimester (post-aspirin) 2285.1 pg/mg (IQR 591.0-2300.1). The percentage change in TxB2 was then assessed for all paired samples (n=147) and found that 124/147 (84.4%) of subjects had a fall in TxB2 levels between the first and second trimesters versus 23/147 (15.6%) who had an increase p<0.001. The greater the reduction in urinary TxB2 pre- and post- aspirin dose the greater the degree of aspirin

adherence, as demonstrated in Figure 2. There was no difference between patient groups (routine aspirin and screen positive aspirin) and percentage change in urine TxB2 (p=0.61).

(b) Of those that underwent randomization (n=557), eleven were excluded prior to fulfillment of study participation requirements (attendance at second study visit). Of the eleven, three withdrew consent for participation as they decided that they did not wish to take aspirin following randomization.

(c) Of all 546 subjects collection of outcome measures and variables were obtained for all apart from the questionnaire on patient acceptability, which was completed in 97.1% (530/546).

d) Six protocol violations were recorded (0.01 per 100 participants) including women transferring care to another hospital (n=3), incorrect randomization of women that did not meet inclusion criteria (n=2) and a subject in the non-aspirin group commencing aspirin by their clinician (n=1).

(iii) The proportion of women in whom it was possible to obtain first trimester trans-abdominal uterine artery Doppler velocimetry (feasibility); The FMF screening test was completed in 98.4% (181/184) following successful uterine artery Doppler velocimetry acquisition, of which one was obtained vaginally due to challenges with abdominal acquisition, with an overall sonographer reported ease of acquisition 3.1 (SD +/- 0.91) (score 1 (easy) to 5 (unobtainable)) [Table 2].

(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

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 <10th 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* >1000mls Blood transfusion Hb drop <8g/dL		 26(13.5) 7 (3.6) 3 4	 20 (5.6) 5 (1.4) 4 7	 2.6 (1.4-4.8) 2.8 (0.9-9.0) 0.5 (0.1-2.7) 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)

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

DISCUSSION

Main Findings

This randomised controlled trial has found that low-risk nilliparous 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 over-sight 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

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

Interpretation

A recently published large RCT from the FMF found that, following application of FMF screening and subsequent randomization of women deemed to be at risk of preterm preeclampsia to aspirin 150mg versus placebo, there was a reduction in the incidence of preterm preeclampsia in the aspirin arm.[5] Our study differs on several counts; (i) routine aspirin arm – use of a third arm assessing provision of routine aspirin assessed the acceptability and feasibility of this policy; (ii) aspirin dosage (150mg vs. 75mg) – in light of limited evidence on dosage and effect, the safest lowest effective dose was selected; (iii) adverse events – rates of PPH and vaginal bleeding were reported. This information would be useful from the FMF study in light of the higher aspirin dosing regime and; (iv) our study was not powered to detect a difference in clinical outcome, with the primary focus feasibility and acceptability.

Few studies have assessed the acceptability of non-routine medications in pregnancy. In the developing world, pregnant women are willing to take calcium, oral iron and

micronutrients.[18-20] If instructed about potential side-effects and reminded frequently women had higher levels of adherence with the greatest barrier being forgetfulness. Average medication adherence in pregnancy for chronic illness is higher than for non-routine medications at 90-95%,[21] hence it its promising that we have noted a rate as high as this in our own study. There was a slight discrepancy in adherence assessed via tablet counts and diary cards and that more objectively assessed via TxB2. Reasons for this may include the potential for aspirin resistance; which although not formally assessed in this study can be increased when using an enteric-coated preparation.[22]

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

In terms of vaginal spotting and clinically significant PPH with aspirin use, the findings of this study are comparable with previous studies although evidence of increased antenatal and postnatal bleeding, requires further investigation, most notably with use of aspirin at doses greater than 75mg.[23-24] Due to the open-label nature of this study as opposed to placebo control, there is always a potential of reporting bias of bleeding in the aspirin arms. Although generally safe in pregnancy, it may be worthwhile considering cessation of aspirin at 32-34 weeks gestation with the aim of reducing the risk of PPH, as opposed to 36-weeks and of informing women of the unwanted side-effect of increased vaginal spotting.

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.

COMPETING INTERESTS STATEMENT: Authors report no conflict of interest

FUNDING STATEMENT: This work was supported by Perinatal Ireland, HRB and HRB Mother and Baby Clinical Trials Network.

CONTRIBUTION OF AUTHORSHIP: (i) Conceived and designed the experiments: FM, CM, PMcP, FB, PD, DM, MC, AS, FC, JM, SD, JH, AC, ET, PD, ZA, FDM, FMcA; (ii) Performed the experiments: FM, CM, FC ; (iii) Analyzed the data: FM, PD, ZA, FMcA; (iv) Contributed reagents/materials/analysis/tools: PMcP, FB, PD, DM, MC, AS, JM, SD, JH, AC, AH, ET, PD, ZA, FDM, FMcA; (v) Wrote the paper: FM, CM, PMcP, FB, PD, DM, MC, AS, FC, JM, SD, JH, AC, AH, ET, PD, ZA, FDM, FMcA

ACKNOWLEDGEMENTS: The women that took part in the study

DATA SHARING STATEMENT: Dataset available from corresponding author on request

REFERENCES

1. Henderson JT, Whitlock EP, O'Connor E, Senger CA, Thompson GH, Rowland MG. Low-dose aspirin for prevention of morbidity and mortality from preeclampsia: A systematic evidence review from the U.S. Preventative Services Task Force. *Ann Intern Med.* 2014; 160: 695-703

2. Sibai BM, Caritis SN, Thom E, Klebanoff M, McNellis D, Rocco L, *et al*. Prevention of preeclampsia with low-dose aspirin in healthy, nulliparous pregnant women. The National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. *N Eng J Med* 1993; 329(17): 1213-8

3. Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. *Fetal Diagn Ther.* 2013;33(1): 8-15

4. Mone F, Mulcahy C, McParland, McAuliffe FM. Should we recommend universal aspirin for all pregnant women. *Am J Obstet Gynecol.* 2017; 216(2): 141.e1-141.e5

5. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallaa C, *et al*. Aspirin versus placebo in pregnancies at high risk for preterm preeclampsia. *N Eng J Med* 2017; 377: 613-22

6. O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, de Alvarado M, *et al*. Multicenter screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks' gestation: comparison to NICE guidelines and ACOG recommendations. *Ultrasound Obstet Gynecol* 2017; 49(6):756-760

7. Meads CA, Cnossen JS, Meher S, Juarez-Garcia A, ter Riet G, Duley L, *et al.* Methods of prediction and prevention of pre-eclampsia: systematic reviews of accuracy and effectiveness literature with economic modelling. *Health Technol Assess.* 2008;12(6).

8. Mone F, Mulcahy C, McParland P, *et al.* An open-labeled controlled trial of low dose aspirin with an early screening test for pre-eclampsia and growth restriction (TEST): Trial Protocol. *Contemp Clin Trials* 2016; 49: 143-8

9. NICE. Guideline CG107 : Hypertension in Pregnancy : The management of hypertensive disorders during pregnancy. National Institute for Health and Clinical Excellence 2010. (Accessed Sept 5, 2016, at https://www.nice.org.uk/guidance/cg107)

10. Perneby C, Vahter M, Akesson A, Bremme K, Hjemdahl P. Thromboxane metabolite excretion during pregnancy – influence of preeclampsia and aspirin treatment. *Thromb Res* 2011;127(6):605-6

11. Poon LCY, Zymery NA, Zamprakou A, Syngelaki A, Nicolaides KH. Protocol for measurement of mean arterial pressure at 11-13 weeks' gestation. *Fetal Diagn Ther.* 2012;31(1):42-48

12. Bhide A, Acharya G, Bilardo CM, Brezinka C, Cafici D, Hernandez-Andrade E, *et al.* ISUOG Practice guidelines: use of Doppler ultrasonography in obstetrics. *Ultrasound Obstet Gynecol.* 2013; 41(2), 233-239

13. International society for the study of hypertension in pregnancy. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health* 2014;4:97-104

14. Billingham SAM, Whitehead AL, Julious SA. An audit of sample sizes for pilot and feasibility trials being undertaken in the United Kingdom registered in the United Kingdom Clinical Research Network database. *BMC Med Res Methodol* 2013;13:104

15. Chiswick C, Reynolds RM, Denison F, Drake AJ, Forbes S, Newby DE, *et al*. Effect of metformin on maternal and fetal outcomes in obese pregnant women (EMPOWaR): a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol 2015;3(10):778-786

16. Navaratnam K, Alfirevic A, Alfirevic Z. Low dose aspirin and pregnancy: how important is aspirin resistance? *BJOG* 2016;123(9):1481-7

17. McCambridge J, Witton J, Elbourne DR. Systematic review of the Hawthorne effect: New concepts are needed to study research participation effects. *J Clin Epidemiol* 2014; 67(3): 267-77

18. Thapa K, Sanghvi H, Rawlins B, Karki YB, Regmi K, Arval S, *et al.* Coverage, compliance, acceptability and feasibility of a program to prevent pre-eclampsia and eclampsia through calcium supplementation for pregnant women: an operations research study in one district of Nepal. *BMC Pregnancy Childbirth*. 2015; 16: 241

19. Kulkarni B, Christian P, LeClerq SC, Khatry SK. Determinants of compliance to antenatal micronutrient supplementation and women's perceptions of supplement use in rural Nepal. *Public Health Nutr.* 2010; 13(1): 82-90

20. Seck BC and Jackson RT. Determinants of compliance with iron supplementation among pregnant women in Sengal. *Public Health Nutr.* 2008; 11(6): 596-605

21. De Jonge L, de Walle HEK, de Jong-van den Berg LTW, van Langen IM, Bakker MK. Actual Use of Medications Prescribed During Pregnancy: A Cross-Sectional Study Using Data from a Population-Based Congenital Anomaly Registry. *Drug Saf.* 2015; 38: 737-47

22. Caron N, Rivard GE, Michon N, Morin F, Pilon D, Moutquin JM, Rey E. Low dose ASA response using the PFA-100 in women with high risk pregnancy. *J Obstet Gynaecol Can*. 2009; 31: 1022-7

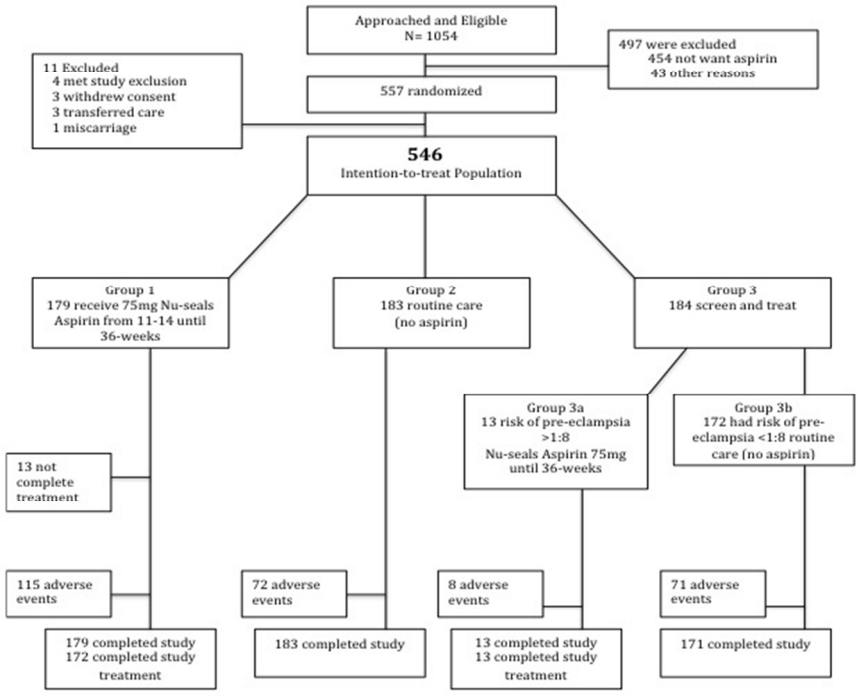
23. Schisterman EF, Silver RM, Lesher LL, Faraggi D, Wactawski-Wende J, Townsend JM, *et al.* Preconception low-dose aspirin and pregnancy outcomes: results from the EAGeR randomised trial. *Lancet.* 2014; 384: 29-36

24. Golding J. A randomised trial of low dose aspirin for primiparae in pregnancy: The Jamaica Low Dose Aspirin Study Group. *BJOG*. 1998; 105(3): 293-9

FIGURE LEGENDS

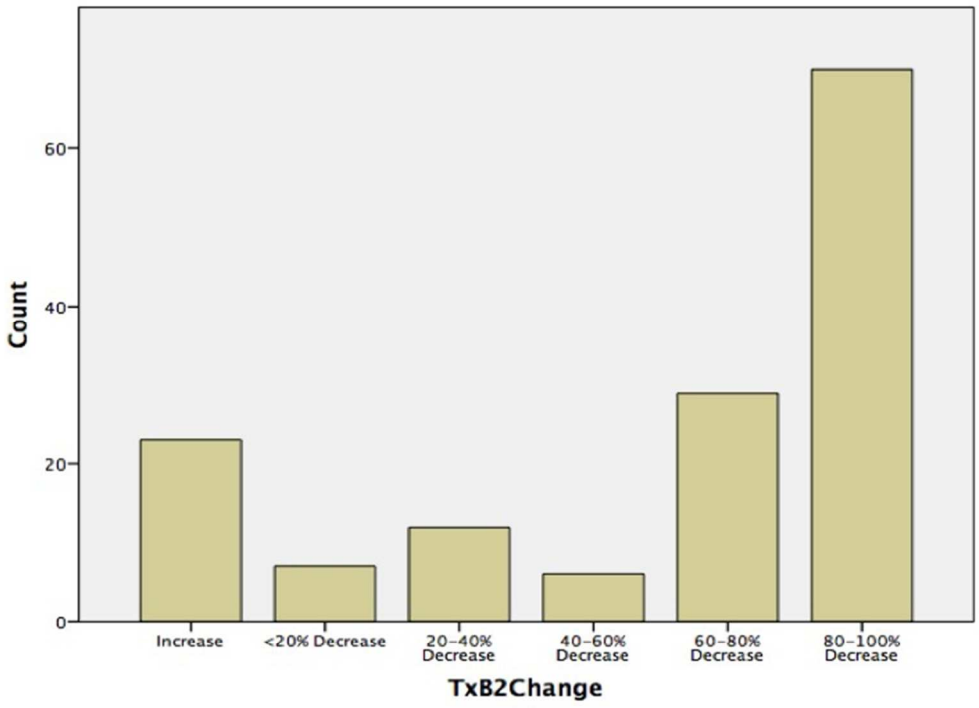
Figure 1 - Consort diagram

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



Consort diagram

254x190mm (72 x 72 DPI)



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)

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 <10th 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* >1000mls	 25 (13.0) 7 (3.6%)	 9 (4.9) 1 (0.5)	 12 (6.5) 4 (2.2)	 0.004 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

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

*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 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.

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-022056.R1
Article Type:	Research
Date Submitted by the Author:	29-Mar-2018
Complete List of Authors:	Mone, Fionnuala; UCD Perinatal Research Centre, Obstetrics and Gynaecology, School of Medicine, University College Dublin, Mulcahy, Cecilia; UCD Perinatal Research Centre, Obstetrics and Gynaecology, School of Medicine, University College Dublin McParland, Peter; UCD Perinatal Research Centre, Obstetrics and Gynaecology, School of Medicine, University College Dublin Brethnach, FIONNUALA; Royal College of Surgeons in Ireland Downey, Paul; Department of Pathology and Laboratory Medicine, National Maternity Hospital McCormack, Dorothy; Department of Pharmacy, National Maternity Hospital Culliton, Marie; Department of Pathology and Laboratory Medicine, National Maternity Hospital Staunton, Alice; Royal College of Surgeons in Ireland Cody, Fiona; Royal College of Surgeons in Ireland Morrison, John; National University of Ireland Galway, Obstetrics and Gynaecology Daly, Sean; Coombe Women and Infants University Hospital, Obstetrics and Gynaecology Higgins, John; Department of Obstetrics and Gynaecology, University College Cork Cotter, Amanda; Department of Obstetrics and Gynaecology, Graduate Entry Medical School, University of Limerick Hunter, Alyson; Royal Maternity Hospital, Grosvenor Road, BT12 6BB Tully, Elizabeth; Royal College of Surgeons in Ireland, Perinatal Ireland Dicker, Patrick; Perinatal Ireland Alfirevic, Zarko; University of Liverpool, Department of Women's and Children's Health Malone, Fergal; Royal College of Surgeons in Ireland, Obstetrics and Gynaecology McAuliffe, Fionnuala; University College Dublin, School of Medicine; National Maternity Hospital, Obstetrics and Gynaecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Health policy
Keywords:	preeclampsia, screening, aspirin, feasibility, low risk

TITLE: **T**rial of feasibility and acceptability of routine low dose aspirin versus **E**arly **S**creening **T**est indicated aspirin for preeclampsia prevention [**TEST** Study] – A multicenter randomised controlled trial

AUTHORS: Fionnuala MONE[1], Ms. Cecilia MULCAHY[1], Peter McPARLAND[1], Fionnuala BREATHNACH[2], Paul DOWNEY[3], Ms. Dorothy MCCORMACK[4], Ms. Marie CULLITON[3], Alice STANTON[5], Ms. Fiona CODY[2], John MORRISON[6], Sean DALY[7], John HIGGINS[8], Amanda COTTER[9], Alyson HUNTER[10], Elizabeth TULLY[2], Patrick DICKER[2], Zarko ALFIREVIC[11], Fergal D MALONE [2], Fionnuala M McAULIFFE[1]*

AFFILIATIONS:
1.UCD Perinatal Research Centre, School of Medicine, University College Dublin, National Maternity Hospital, Dublin, Ireland. 2. Department of Obstetrics and Gynaecology, Royal College of Surgeons in Ireland, Rotunda Hospital, Parnell Square, Dublin 1, Ireland. 3. Department of Pathology and Laboratory Medicine, National Maternity Hospital, Holles St, Dublin 2, Ireland. 4. Department of Pharmacy, National Maternity Hospital, Holles St, Dublin 2, Ireland. 5. Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin 2, Ireland. 6. Department of Obstetrics and Gynaecology, National University of Ireland, Galway, Ireland. 7. Department of Obstetrics and Gynaecology, Coombe Women's and Infant's University Hospital, Dublin 8, Ireland. 8. Department of Obstetrics and Gynaecology, University College Cork, Cork, Ireland. 9. Department of Obstetrics and Gynaecology, Graduate Entry Medical School, University of Limerick, Limerick, Ireland. 10. Royal Jubilee Maternity Hospital, Belfast, UK. 11. Department of women's and Children's Health, Institute of Translational Medicine, University of Liverpool, Liverpool, UK

*Corresponding author: Prof FM McAuliffe. UCD Perinatal Research Centre, School of Medicine, University College Dublin, National Maternity Hospital, Dublin, Ireland. T:+353 1 6373216 E: Fionnuala.mcauliffe@ucd.ie

WORD Count: 3, 927 words

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).

Conclusion: Low-risk nulliparous women are open to taking aspirin in pregnancy and had high levels of adherence. Aspirin use was associated with greater rates of vaginal bleeding. An appropriately powered randomized controlled trial is now required to address the efficacy and safety of universal low dose aspirin in low-risk pregnancy compared to a screening approach.

Trial Registration: www.isrctn.com/ISRCTN15191778

Funding: Perinatal Ireland, HRB and the Mother and Baby Clinical Trials Network, HRB

ARTICLE SUMMARY

Strengths and limitations of this study

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

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.

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 published[8] 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 erythematosis, 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

the research clinician at the first trimester antenatal booking visit.

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-dehydroxo-thromboxane-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

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

Study assessments at the time of the recruitment visit included the FMF screening test, the results of which were assessed for those in Group 3 (screen positive and received aspirin (3A) and screen negative no aspirin (3B)). The FMF screening test was not routine practice within Ireland. Components of the screening test included; maternal history (including ovulation induced conception, race, body mass index, age, mother with preeclampsia); mean arterial blood pressure (MAP); uterine artery Doppler pulsatility index; and pregnancy associated plasma protein-A (PAPP-A) and placental like growth factor (PLGF) multiples of the median. To determine risk of preeclampsia, the FMF algorithm was used and based upon a screen positive rate of 5%, a cut off for preeclampsia prior to 42-weeks at greater than 1:8 was used.[3] This cut-off was selected with the aim of capturing the majority of pre-eclamptics; both pre and post-term and at the time of study commencement this was the optimal screening algorithm for detection of any preeclampsia. Two un-blinded trained clinical research sonographers performed the first trimester uterine artery Doppler waveforms and MAP and interpreted findings. MAP was assessed using an automated blood pressure monitoring device as outlined by the technique stipulated by the FMF.[11] Uterine artery Doppler velocimetry was obtained using Viewpoint® Version 5.6.16 GE Healthcare, 2012 and Voluson Expert 730®, GE 2012 using the technique outlined from by the International Society of Ultrasound in Obstetrics and Gynecology. The pulsatility index was measured from both uterine arteries and an average value was calculated.[12]

A maternal blood sample was analyzed for PAPP-A and PLGF under standard conditions using a 6000 DELFIA® Xpress, PerkinElmer, 2014 clinical random access screening platform in the hospital clinical biochemistry laboratory. A quantitative immunoturbimetric

TxBCardio® immunoassay was used to determine TxB2 levels in urine samples obtained both study visits. These were then standardized as a ratio with creatinine levels and expressed as pg/mg creatinine.

Outcomes

The primary objective of this study 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 $<10^{th}$ 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 symphysio-fundal-height and or fetal biometrical ultrasound assessment as appropriate. - was defined based upon the definition from the ISSHP with new onset hypertension (>140mmHg systolic or >90mmHg diastolic) after 20-weeks gestation associated with; (i) proteinuria of at least 1g/L [2+] on urine dipstick testing, and or; (ii) maternal organ dysfunction ; an or fetal growth restriction.[13] Suspicion of a diagnosis of pre-eclampsia at an antenatal visit prompted further investigation in the fetal assessment unit with clinical examination, blood testing (urea and creatinine, liver function tests and full blood picture), 24-hour urine collection for proteinuria and departmental fetal ultrasound assessment with final diagnosis made by an obstetrician.

Safety data were reported as adverse and serious adverse events and participants discontinued from the study were recorded in addition to the reason for discontinuation and outcome. As an assessment of post-partum haemorrhage, blood loss was weighed at time of delivery.

Statistical Analysis

As outlined in the published study protocol, the projected sample size for this study was 500 women across two sites with 18,000 deliveries per annum.[8] To determine preeclampsia as a primary outcome; the anticipated number of patients required is over 15,000 women. As this study aimed to determine the feasibility of such a study, 500 participants were more than adequate as 3% of the number required for a substantive study is required (n=450).[14] Accounting for a drop-out rate of 10% (n=45), 500 participants were adequate to obtain the

primary outcome. Analysis was performed by a statistician using SAS v.20 on the intention-to-treat (ITT) population, which included all participants randomised, which completed the full second trimester assessment. Measures of variance included standard deviation. Follow-up of serious adverse events continued until 28-days following delivery. Adverse events were reported as odds ratios (OR). To assess secondary outcomes and safety, comparisons of groups were be performed using two sample t-tests, Wilcoxon Rank-sum tests and Chi-square tests.

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/m2)	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)

Table 1: Baseline characteristics of the study population. Where number (No.) percentage is not expressed average and range are demonstrated.

Primary outcomes

(i) The proportion of eligible women agreeing to participate in a trial where aspirin is prescribed routinely (feasibility); 1054 women were approached that were eligible to partake. 497 were subsequently not enrolled as they did not want to take aspirin n=454 or for an alternative reason n=43 e.g. appointment did not suit. Hence 546/1054 (51.8%) women were willing to partake in a study where they may have to take aspirin routinely.

(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);

(a) Of those women included in analysis that were taking aspirin (n=192), the average adherence based upon patient reported diary cards was 96.0% and based upon tablet counts 95.0%. Seven women were non-adherent and 19 (10.0%) poorly compliant (<80%). Average adherence was 95.0% in both the test indicated aspirin group (3a) and routine aspirin group (1) [Table 2]. The median first trimester pre-aspirin urine TxB2 level was 8662.2 pg/mg (IQR 2014.5-9931.5) and second trimester (post-aspirin) 2285.1 pg/mg (IQR 591.0-2300.1). The percentage change in TxB2 was then assessed for all paired samples (n=147) and found that 124/147 (84.4%) of subjects had a fall in TxB2 levels between the first and second trimesters versus 23/147 (15.6%) who had an increase p<0.001. The greater the reduction in urinary TxB2 pre- and post- aspirin dose the greater the degree of aspirin

adherence, as demonstrated in Figure 2. There was no difference between patient groups (routine aspirin and screen positive aspirin) and percentage change in urine TxB2 (p=0.61).

(b) Of those that underwent randomization (n=557), eleven were excluded prior to fulfillment of study participation requirements (attendance at second study visit). Of the eleven, three withdrew consent for participation as they decided that they did not wish to take aspirin following randomization.

(c) Of all 546 subjects collection of outcome measures and variables were obtained for all apart from the questionnaire on patient acceptability, which was completed in 97.1% (530/546).

d) Six protocol violations were recorded (0.01 per 100 participants) including women transferring care to another hospital (n=3), incorrect randomization of women that did not meet inclusion criteria (n=2) and a subject in the non-aspirin group commencing aspirin by their clinician (n=1).

(iii) The proportion of women in whom it was possible to obtain first trimester trans-abdominal uterine artery Doppler velocimetry (feasibility); The FMF screening test was completed in 98.4% (181/184) following successful uterine artery Doppler velocimetry acquisition, of which one was obtained vaginally due to challenges with abdominal acquisition, with an overall sonographer reported ease of acquisition 3.1 (SD +/- 0.91) (score 1 (easy) to 5 (unobtainable)) [Table 2].

(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

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* >1000mls Blood transfusion Hb drop <8g/dL		 26(13.5) 7 (3.6) 3 4	 20 (5.6) 5 (1.4) 4 7	 2.6 (1.4-4.8) 2.8 (0.9-9.0) 0.5 (0.1-2.7) 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)

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

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 over-sight 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

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

Interpretation

A recently published large RCT from the FMF found that, following application of FMF screening and subsequent randomization of women deemed to be at risk of preterm preeclampsia to aspirin 150mg versus placebo, there was a reduction in the incidence of preterm preeclampsia in the aspirin arm.[5] Our study differs on several counts; (i) routine aspirin arm – use of a third arm assessing provision of routine aspirin assessed the acceptability and feasibility of this policy; (ii) aspirin dosage (150mg vs. 75mg) – in light of limited evidence on dosage and effect, the safest lowest effective dose was selected. A recent meta-analysis, published since completion of this study suggests that there is an aspirin dose-response effect, with higher doses of aspirin commenced prior to 16-weeks gestation, associated with a greater reduction in preeclampsia and fetal growth restriction compared to standard lower doses.[18] When supported by robust safety data when using higher dosing, this is something to consider in future studies and clinical practice; (iii) adverse events – rates of PPH and vaginal bleeding were reported. This information would be useful from the FMF

study in light of the higher aspirin dosing regime and; (iv) our study was not powered to detect a difference in clinical outcome, with the primary focus feasibility and acceptability.

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

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

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

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

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. This 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.

COMPETING INTERESTS STATEMENT: Authors report no conflict of interest

FUNDING STATEMENT: This work was supported by Perinatal Ireland, HRB and HRB Mother and Baby Clinical Trials Network.

CONTRIBUTION OF AUTHORSHIP: (i) Conceived and designed the experiments: FM, CM, PMcP, FB, PD, DM, MC, AS, FC, JM, SD, JH, AC, ET, PD, ZA, FDM, FMcA; (ii) Performed the experiments: FM, CM, FC ; (iii) Analyzed the data: FM, PD, ZA, FMcA; (iv) Contributed reagents/materials/analysis/tools: PMcP, FB, PD, DM, MC, AS, JM, SD, JH, AC, AH, ET, PD, ZA, FDM, FMcA; (v) Wrote the paper: FM, CM, PMcP, FB, PD, DM, MC, AS, FC, JM, SD, JH, AC, AH, ET, PD, ZA, FDM, FMcA

ACKNOWLEDGEMENTS: The women that took part in the study

DATA SHARING STATEMENT: Dataset available from corresponding author on request

REFERENCES

1. Henderson JT, Whitlock EP, O'Connor E, Senger CA, Thompson GH, Rowland MG. Low-dose aspirin for prevention of morbidity and mortality from preeclampsia: A systematic evidence review from the U.S. Preventative Services Task Force. *Ann Intern Med.* 2014; 160: 695-703

2. Sibai BM, Caritis SN, Thom E, Klebanoff M, McNellis D, Rocco L, *et al*. Prevention of preeclampsia with low-dose aspirin in healthy, nulliparous pregnant women. The National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. *N Eng J Med* 1993; 329(17): 1213-8

3. Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. *Fetal Diagn Ther.* 2013;33(1): 8-15

4. Mone F, Mulcahy C, McParland, McAuliffe FM. Should we recommend universal aspirin for all pregnant women? *Am J Obstet Gynecol.* 2017; 216(2): 141.e1-141.e5

5. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallaa C, *et al*. Aspirin versus placebo in pregnancies at high risk for preterm preeclampsia. *N Eng J Med* 2017; 377: 613-22

6. O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, de Alvarado M, *et al*. Multicenter screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks' gestation: comparison to NICE guidelines and ACOG recommendations. *Ultrasound Obstet Gynecol* 2017; 49(6):756-760

7. Meads CA, Cnossen JS, Meher S, Juarez-Garcia A, ter Riet G, Duley L, *et al.* Methods of prediction and prevention of pre-eclampsia: systematic reviews of accuracy and effectiveness literature with economic modelling. *Health Technol Assess.* 2008;12(6).

8. Mone F, Mulcahy C, McParland P, *et al.* An open-labeled controlled trial of low dose aspirin with an early screening test for pre-eclampsia and growth restriction (TEST): Trial Protocol. *Contemp Clin Trials* 2016; 49: 143-8

9. NICE. Guideline CG107 : Hypertension in Pregnancy : The management of hypertensive disorders during pregnancy. National Institute for Health and Clinical Excellence 2010. (Accessed Sept 5, 2016, at https://www.nice.org.uk/guidance/cg107)

10. Perneby C, Vahter M, Akesson A, Bremme K, Hjemdahl P. Thromboxane metabolite excretion during pregnancy – influence of preeclampsia and aspirin treatment. *Thromb Res* 2011;127(6):605-6

11. Poon LCY, Zymery NA, Zamprakou A, Syngelaki A, Nicolaides KH. Protocol for measurement of mean arterial pressure at 11-13 weeks' gestation. *Fetal Diagn Ther.* 2012;31(1):42-48

12. Bhide A, Acharya G, Bilardo CM, Brezinka C, Cafici D, Hernandez-Andrade E, *et al.* ISUOG Practice guidelines: use of Doppler ultrasonography in obstetrics. *Ultrasound Obstet Gynecol.* 2013; 41(2), 233-239

13. International society for the study of hypertension in pregnancy. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health* 2014;4:97-104

14. Billingham SAM, Whitehead AL, Julious SA. An audit of sample sizes for pilot and feasibility trials being undertaken in the United Kingdom registered in the United Kingdom Clinical Research Network database. *BMC Med Res Methodol* 2013;13:104
15. Chiswick C, Reynolds RM, Denison F, Drake AJ, Forbes S, Newby DE, *et al*. Effect of metformin on maternal and fetal outcomes in obese pregnant women (EMPOWaR): a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol 2015;3(10):778-786
16. Navaratnam K, Alfirevic A, Alfirevic Z. Low dose aspirin and pregnancy: how important is aspirin resistance? *BJOG* 2016;123(9):1481-7
17. McCambridge J, Witton J, Elbourne DR. Systematic review of the Hawthorne effect: New concepts are needed to study research participation effects. *J Clin Epidemiol* 2014; 67(3): 267-77
18. Roberge S, Nicolaides K, Demers S, Hyett J, Chaillet N, Bujold E. The role of aspirin dose on the prevention of preeclampsia and fetal growth restriction: systematic review and meta-analysis. *Am J Obstet Gynecol* 2017;216(2):110-20
19. Thapa K, Sanghvi H, Rawlins B, Karki YB, Regmi K, Arval S, *et al.* Coverage, compliance, acceptability and feasibility of a program to prevent pre-eclampsia and eclampsia through calcium supplementation for pregnant women: an operations research study in one district of Nepal. *BMC Pregnancy Childbirth*. 2015; 16: 241
20. Kulkarni B, Christian P, LeClerq SC, Khatry SK. Determinants of compliance to antenatal micronutrient supplementation and women's perceptions of supplement use in rural Nepal. *Public Health Nutr.* 2010; 13(1): 82-90
21. Seck BC and Jackson RT. Determinants of compliance with iron supplementation among pregnant women in Sengal. *Public Health Nutr.* 2008; 11(6): 596-605
22. De Jonge L, de Walle HEK, de Jong-van den Berg LTW, van Langen IM, Bakker

MK. Actual Use of Medications Prescribed During Pregnancy: A Cross-Sectional Study Using Data from a Population-Based Congenital Anomaly Registry. *Drug Saf.* 2015; 38: 737-47

23. Caron N, Rivard GE, Michon N, Morin F, Pilon D, Moutquin JM, Rey E. Low dose ASA response using the PFA-100 in women with high risk pregnancy. *J Obstet Gynaecol Can*. 2009; 31: 1022-7
24. Schisterman EF, Silver RM, Lesher LL, Faraggi D, Wactawski-Wende J, Townsend JM, *et al.* Preconception low-dose aspirin and pregnancy outcomes: results from the EAGeR randomised trial. *Lancet.* 2014; 384: 29-36
25. Golding J. A randomised trial of low dose aspirin for primiparae in pregnancy: The Jamaica Low Dose Aspirin Study Group. *BJOG*. 1998; 105(3): 293-9

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]

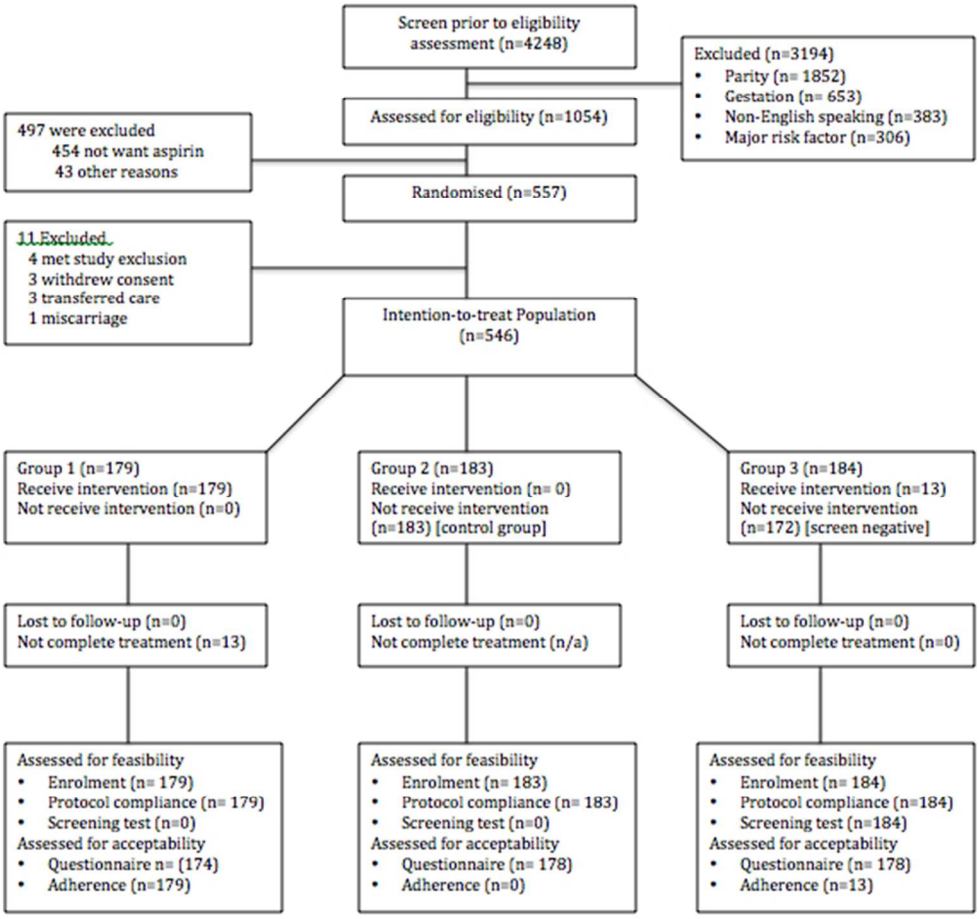
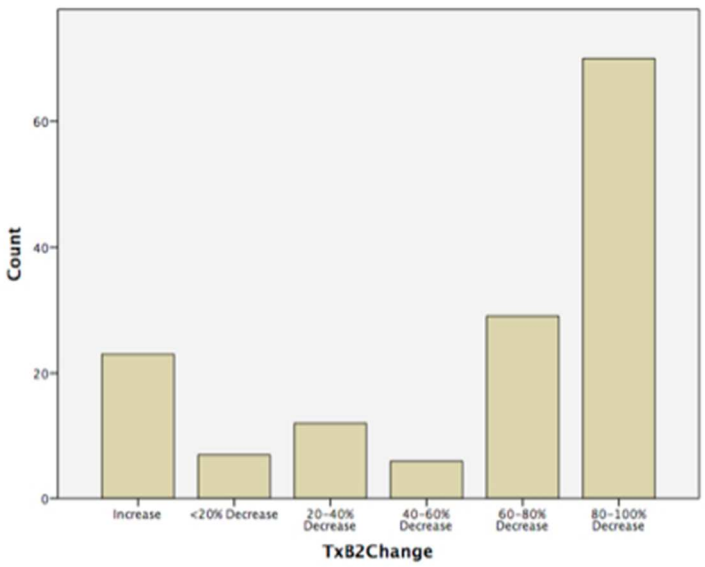


Figure 1 – Consort diagram

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 <10th 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. (%) Pre-eclampsia <34-weeks Pre-eclampsia <37-weeks	2 (15.4) 0 (0) 2 (15.4)	5 (2.9) 2 (1.2) 2 (1.2)	0.13 0.70 0.02
Birthweight <10th 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 Stillbirth Neonatal death	 13 (100) 0 (0) 0 (0)	 169 (98.8) 2 (1.2) 0 (0)	 0.70 0.70 --

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* >1000mls	 25 (13.0) 7 (3.6%)	 9 (4.9) 1 (0.5)	 12 (6.5) 4 (2.2)	 0.004 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

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.

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-022056.R2
Article Type:	Research
Date Submitted by the Author:	24-May-2018
Complete List of Authors:	Mone, Fionnuala; UCD Perinatal Research Centre, Obstetrics and Gynaecology, School of Medicine, University College Dublin, Mulcahy, Cecilia; UCD Perinatal Research Centre, Obstetrics and Gynaecology, School of Medicine, University College Dublin McParland, Peter; UCD Perinatal Research Centre, Obstetrics and Gynaecology, School of Medicine, University College Dublin Brethnach, FIONNUALA; Royal College of Surgeons in Ireland Downey, Paul; Department of Pathology and Laboratory Medicine, National Maternity Hospital McCormack, Dorothy; Department of Pharmacy, National Maternity Hospital Culliton, Marie; Department of Pathology and Laboratory Medicine, National Maternity Hospital Staunton, Alice; Royal College of Surgeons in Ireland Cody, Fiona; Royal College of Surgeons in Ireland Morrison, John; National University of Ireland Galway, Obstetrics and Gynaecology Daly, Sean; Coombe Women and Infants University Hospital, Obstetrics and Gynaecology Higgins, John; Department of Obstetrics and Gynaecology, University College Cork Cotter, Amanda; Department of Obstetrics and Gynaecology, Graduate Entry Medical School, University of Limerick Hunter, Alyson; Royal Maternity Hospital, Grosvenor Road, BT12 6BB Tully, Elizabeth; Royal College of Surgeons in Ireland, Perinatal Ireland Dicker, Patrick; Perinatal Ireland Alfirevic, Zarko; University of Liverpool, Department of Women's and Children's Health Malone, Fergal; Royal College of Surgeons in Ireland, Obstetrics and Gynaecology McAuliffe, Fionnuala; University College Dublin, School of Medicine; National Maternity Hospital, Obstetrics and Gynaecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Health policy, Public health
Keywords:	preeclampsia, screening, aspirin, feasibility, low risk

TITLE: **T**rial of feasibility and acceptability of routine low dose aspirin versus **E**arly **S**creening **T**est indicated aspirin for preeclampsia prevention [**TEST** Study] – A multicenter randomised controlled trial

AUTHORS: Fionnuala MONE[1], Ms. Cecilia MULCAHY[1], Peter McPARLAND[1], Fionnuala BREATHNACH[2], Paul DOWNEY[3], Ms. Dorothy MCCORMACK[4], Ms. Marie CULLITON[3], Alice STANTON[5], Ms. Fiona CODY[2], John MORRISON[6], Sean DALY[7], John HIGGINS[8], Amanda COTTER[9], Alyson HUNTER[10], Elizabeth TULLY[2], Patrick DICKER[2], Zarko ALFIREVIC[11], Fergal D MALONE [2], Fionnuala M McAULIFFE[1]*

AFFILIATIONS:

1.UCD Perinatal Research Centre, School of Medicine, University College Dublin, National Maternity Hospital, Dublin, Ireland. 2. Department of Obstetrics and Gynaecology, Royal College of Surgeons in Ireland, Rotunda Hospital, Parnell Square, Dublin 1, Ireland. 3. Department of Pathology and Laboratory Medicine, National Maternity Hospital, Holles St, Dublin 2, Ireland. 4. Department of Pharmacy, National Maternity Hospital, Holles St, Dublin 2, Ireland. 5. Molecular and Cellular Therapeutics, Royal College of Surgeons in Ireland, Dublin 2, Ireland. 6. Department of Obstetrics and Gynaecology, National University of Ireland, Galway, Ireland. 7. Department of Obstetrics and Gynaecology, Coombe Women's and Infant's University Hospital, Dublin 8, Ireland. 8. Department of Obstetrics and Gynaecology, University College Cork, Cork, Ireland. 9. Department of Obstetrics and Gynaecology, Graduate Entry Medical School, University of Limerick, Limerick, Ireland. 10. Royal Jubilee Maternity Hospital, Belfast, UK. 11. Department of women's and Children's Health, Institute of Translational Medicine, University of Liverpool, Liverpool, UK

*Corresponding author: Prof FM McAuliffe. UCD Perinatal Research Centre, School of Medicine, University College Dublin, National Maternity Hospital, Dublin, Ireland. T:+353 1 6373216 E: Fionnuala.mcauliffe@ucd.ie

WORD Count: 3, 927 words

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).

Conclusion: Low-risk nulliparous women are open to taking aspirin in pregnancy and had high levels of adherence. Aspirin use was associated with greater rates of vaginal bleeding. An appropriately powered randomized controlled trial is now required to address the efficacy and safety of universal low dose aspirin in low-risk pregnancy compared to a screening approach.

Trial Registration: www.isrctn.com/ISRCTN15191778

Funding: Perinatal Ireland, HRB and the Mother and Baby Clinical Trials Network, HRB

ARTICLE SUMMARY

Strengths and limitations of this study

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

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.

106

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 published[8] 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 erythematosis, 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

the research clinician at the first trimester antenatal booking visit.

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-dehydroxo-thromboxane-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

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

Study assessments at the time of the recruitment visit included the FMF screening test, the results of which were assessed for those in Group 3 (screen positive and received aspirin (3A) and screen negative no aspirin (3B)). The FMF screening test was not routine practice within Ireland. Components of the screening test included; maternal history (including ovulation induced conception, race, body mass index, age, mother with preeclampsia); mean arterial blood pressure (MAP); uterine artery Doppler pulsatility index; and pregnancy associated plasma protein-A (PAPP-A) and placental like growth factor (PLGF) multiples of the median. To determine risk of preeclampsia, the FMF algorithm was used and based upon a screen positive rate of 5%, a cut off for preeclampsia prior to 42-weeks at greater than 1:8 was used.[3] This cut-off was selected with the aim of capturing the majority of pre-eclamptics; both pre and post-term and at the time of study commencement this was the optimal screening algorithm for detection of any preeclampsia. Two un-blinded trained clinical research sonographers performed the first trimester uterine artery Doppler waveforms and MAP and interpreted findings. MAP was assessed using an automated blood pressure monitoring device as outlined by the technique stipulated by the FMF.[11] Uterine artery Doppler velocimetry was obtained using Viewpoint® Version 5.6.16 GE Healthcare, 2012 and Voluson Expert 730®, GE 2012 using the technique outlined from by the International Society of Ultrasound in Obstetrics and Gynecology. The pulsatility index was measured from both uterine arteries and an average value was calculated.[12]

A maternal blood sample was analyzed for PAPP-A and PLGF under standard conditions using a 6000 DELFIA® Xpress, PerkinElmer, 2014 clinical random access screening platform in the hospital clinical biochemistry laboratory. A quantitative immunoturbimetric

TxBCardio® immunoassay was used to determine TxB2 levels in urine samples obtained both study visits. These were then standardized as a ratio with creatinine levels and expressed as pg/mg creatinine.

Outcomes

The primary objective of this study 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 symphysio-fundal-height and or fetal biometrical ultrasound assessment as appropriate. - was defined based upon the definition from the ISSHP with new onset hypertension (>140mmHg systolic or >90mmHg diastolic) after 20-weeks gestation associated with; (i) proteinuria of at least 1g/L [2+] on urine dipstick testing, and or; (ii) maternal organ dysfunction ; an or fetal growth restriction.[13] Suspicion of a diagnosis of pre-eclampsia at an antenatal visit prompted further investigation in the fetal assessment unit with clinical examination, blood testing (urea and creatinine, liver function tests and full blood picture), 24-hour urine collection for proteinuria and departmental fetal ultrasound assessment with final diagnosis made by an obstetrician.

Safety data were reported as adverse and serious adverse events and participants discontinued from the study were recorded in addition to the reason for discontinuation and outcome. As an assessment of post-partum haemorrhage, blood loss was weighed at time of delivery.

Statistical Analysis

As outlined in the published study protocol, the projected sample size for this study was 500 women across two sites with 18,000 deliveries per annum.[8] To determine preeclampsia as a primary outcome; the anticipated number of patients required is over 15,000 women. As this study aimed to determine the feasibility of such a study, 500 participants were more than adequate as 3% of the number required for a substantive study is required (n=450).[14] Accounting for a drop-out rate of 10% (n=45), 500 participants were adequate to obtain the

primary outcome. Analysis was performed by a statistician using SAS v.20 on the intention-to-treat (ITT) population, which included all participants randomised, which completed the full second trimester assessment. Measures of variance included standard deviation. Follow-up of serious adverse events continued until 28-days following delivery. Adverse events were reported as odds ratios (OR) and uncertainty was expressed using 95% confidence intervals. No hypothesis tests were performed.

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. Baseline characteristics were similar and the summaries are presented in 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 Black Asian Other	 181 (97.9) 1 (0.5) 3 (1.6) 0	 179 (95.7) 2 (1.1) 6 (3.2) 0 (0)	 180 (97.3) 0 (0) 5 (2.7) 0 (0)
Completed secondary school – No.(%)	136 (73.5)	143 (76.4)	152 (82.2)
BMI (kg/m2)	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 ICSI Ovulation induction Spontaneous	 5 (2.7) 3 (1.6) 5 (2.7) 172 (93.0)	 9 (4.8) 4 (2.1) 6 (3.2) 168 (89.9)	 8 (4.3) 3 (1.6) 6 (3.2) 170 (91.9)
Previous miscarriage – No.	20 (10.8)	31 (16.6)	31 (16.8)

(%)			

Table 1: Baseline characteristics of the study population. Where number (No.) percentage is not expressed average and range are demonstrated.

Primary outcomes

(i) The proportion of eligible women agreeing to participate in a trial where aspirin is prescribed routinely (feasibility); 1054 women were approached that were eligible to partake. 497 were subsequently not enrolled as they did not want to take aspirin n=454 or for an alternative reason n=43 e.g. appointment did not suit. Hence 546/1054 (51.8%) women were willing to partake in a study where they may have to take aspirin routinely.

(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);

(a) Of those women included in analysis that were taking aspirin (n=192), the average adherence based upon patient reported diary cards was 96.0% and based upon tablet counts 95.0%. Seven women were non-adherent and 19 (10.0%) poorly compliant (<80%). Average adherence was 95.0% in both the test indicated aspirin group (3a) and routine aspirin group (1) [Table 2]. The median first trimester pre-aspirin urine TxB2 level was 8662.2 pg/mg (IQR 2014.5-9931.5) and second trimester (post-aspirin) 2285.1 pg/mg (IQR 591.0-2300.1). The percentage change in TxB2 was then assessed for all paired samples (n=147) and found that 124/147 (84.4%) of subjects had a fall in TxB2 levels between the first and second trimesters versus 23/147 (15.6%) who had an increase. The greater the reduction in urinary TxB2 pre- and post- aspirin dose the greater the degree of aspirin adherence, as

demonstrated in Figure 2. Patient groups were similar (routine aspirin and screen positive aspirin) and percentage change in urine TxB2.

(b) Of those that underwent randomization (n=557), eleven were excluded prior to fulfillment of study participation requirements (attendance at second study visit). Of the eleven, three withdrew consent for participation as they decided that they did not wish to take aspirin following randomization.

(c) Of all 546 subjects collection of outcome measures and variables were obtained for all apart from the questionnaire on patient acceptability, which was completed in 97.1% (530/546).

d) Six protocol violations were recorded (0.01 per 100 participants) including women transferring care to another hospital (n=3), incorrect randomization of women that did not meet inclusion criteria (n=2) and a subject in the non-aspirin group commencing aspirin by their clinician (n=1).

(iii) The proportion of women in whom it was possible to obtain first trimester trans-abdominal uterine artery Doppler velocimetry (feasibility); The FMF screening test was completed in 98.4% (181/184) following successful uterine artery Doppler velocimetry acquisition, of which one was obtained vaginally due to challenges with abdominal acquisition, with an overall sonographer reported ease of acquisition 3.1 (SD +/- 0.91) (score 1 (easy) to 5 (unobtainable)) [Table 2].

(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

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 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%). 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

The adverse event profile differed between groups but not the serious adverse event profile [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 an observable 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. Similarly, the rate of PPH >1000mls was higher in the aspirin group. However, the numbers were small. Rates of blood transfusion or significant hemoglobin drop to <8g/dL were similar.

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 >1000mls Blood transfusion Hb drop <8g/dL		 26(13.5) 7 (3.6) 3 4	 20 (5.6) 5 (1.4) 4 7	 2.6 (1.4-4.8) 2.8 (0.9-9.0) 0.5 (0.1-2.7) 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)

362

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

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 over-sight 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

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

Interpretation

A recently published large RCT from the FMF found that, following application of FMF screening and subsequent randomization of women deemed to be at risk of preterm preeclampsia to aspirin 150mg versus placebo, there was a reduction in the incidence of preterm preeclampsia in the aspirin arm.[5] Our study differs on several counts; (i) routine aspirin arm – use of a third arm assessing provision of routine aspirin assessed the acceptability and feasibility of this policy; (ii) aspirin dosage (150mg vs. 75mg) – in light of limited evidence on dosage and effect, the safest lowest effective dose was selected. A recent meta-analysis, published since completion of this study suggests that there is an aspirin dose-response effect, with higher doses of aspirin commenced prior to 16-weeks gestation, associated with a greater reduction in preeclampsia and fetal growth restriction compared to standard lower doses.[18] When supported by robust safety data when using higher dosing, this is something to consider in future studies and clinical practice; (iii) adverse events – rates of PPH and vaginal bleeding were reported. This information would be useful from the FMF

study in light of the higher aspirin dosing regime and; (iv) our study was not powered to detect a difference in clinical outcome, with the primary focus feasibility and acceptability.

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

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

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

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

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. This study was not powered to 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.

COMPETING INTERESTS STATEMENT: Authors report no conflict of interest

FUNDING STATEMENT: This work was supported by Perinatal Ireland, HRB and HRB Mother and Baby Clinical Trials Network.

CONTRIBUTION OF AUTHORSHIP: (i) Conceived and designed the experiments: FM, CM, PMcP, FB, PD, DM, MC, AS, FC, JM, SD, JH, AC, ET, PD, ZA, FDM, FMcA; (ii) Performed the experiments: FM, CM, FC ; (iii) Analyzed the data: FM, PD, ZA, FMcA; (iv) Contributed reagents/materials/analysis/tools: PMcP, FB, PD, DM, MC, AS, JM, SD, JH, AC, AH, ET, PD, ZA, FDM, FMcA; (v) Wrote the paper: FM, CM, PMcP, FB, PD, DM, MC, AS, FC, JM, SD, JH, AC, AH, ET, PD, ZA, FDM, FMcA

ACKNOWLEDGEMENTS: The women that took part in the study

DATA SHARING STATEMENT: Dataset available from corresponding author on request

REFERENCES

1. Henderson JT, Whitlock EP, O'Connor E, Senger CA, Thompson GH, Rowland MG. Low-dose aspirin for prevention of morbidity and mortality from preeclampsia: A systematic evidence review from the U.S. Preventative Services Task Force. *Ann Intern Med.* 2014; 160: 695-703

2. Sibai BM, Caritis SN, Thom E, Klebanoff M, McNellis D, Rocco L, *et al*. Prevention of preeclampsia with low-dose aspirin in healthy, nulliparous pregnant women. The National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. *N Eng J Med* 1993; 329(17): 1213-8

3. Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for preeclampsia by biophysical and biochemical markers. *Fetal Diagn Ther.* 2013;33(1): 8-15

4. Mone F, Mulcahy C, McParland, McAuliffe FM. Should we recommend universal aspirin for all pregnant women? *Am J Obstet Gynecol.* 2017; 216(2): 141.e1-141.e5

5. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallaa C, *et al*. Aspirin versus placebo in pregnancies at high risk for preterm preeclampsia. *N Eng J Med* 2017; 377: 613-22

6. O'Gorman N, Wright D, Poon LC, Rolnik DL, Syngelaki A, de Alvarado M, *et al*. Multicenter screening for preeclampsia by maternal factors and biomarkers at 11-13 weeks' gestation: comparison to NICE guidelines and ACOG recommendations. *Ultrasound Obstet Gynecol* 2017; 49(6):756-760

7. Meads CA, Cnossen JS, Meher S, Juarez-Garcia A, ter Riet G, Duley L, *et al.* Methods of prediction and prevention of pre-eclampsia: systematic reviews of accuracy and effectiveness literature with economic modelling. *Health Technol Assess.* 2008;12(6).

8. Mone F, Mulcahy C, McParland P, *et al.* An open-labeled controlled trial of low dose aspirin with an early screening test for pre-eclampsia and growth restriction (TEST): Trial Protocol. *Contemp Clin Trials* 2016; 49: 143-8

9. NICE. Guideline CG107 : Hypertension in Pregnancy : The management of hypertensive disorders during pregnancy. National Institute for Health and Clinical Excellence 2010. (Accessed Sept 5, 2016, at https://www.nice.org.uk/guidance/cg107)

10. Perneby C, Vahter M, Akesson A, Bremme K, Hjemdahl P. Thromboxane metabolite excretion during pregnancy – influence of preeclampsia and aspirin treatment. *Thromb Res* 2011;127(6):605-6

11. Poon LCY, Zymery NA, Zamprakou A, Syngelaki A, Nicolaides KH. Protocol for measurement of mean arterial pressure at 11-13 weeks' gestation. *Fetal Diagn Ther.* 2012;31(1):42-48

12. Bhide A, Acharya G, Bilardo CM, Brezinka C, Cafici D, Hernandez-Andrade E, *et al.* ISUOG Practice guidelines: use of Doppler ultrasonography in obstetrics. *Ultrasound Obstet Gynecol.* 2013; 41(2), 233-239

13. International society for the study of hypertension in pregnancy. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health* 2014;4:97-104

14. Billingham SAM, Whitehead AL, Julious SA. An audit of sample sizes for pilot and feasibility trials being undertaken in the United Kingdom registered in the United Kingdom Clinical Research Network database. *BMC Med Res Methodol* 2013;13:104

15. Chiswick C, Reynolds RM, Denison F, Drake AJ, Forbes S, Newby DE, *et al*. Effect of metformin on maternal and fetal outcomes in obese pregnant women (EMPOWaR): a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol 2015;3(10):778-786

16. Navaratnam K, Alfirevic A, Alfirevic Z. Low dose aspirin and pregnancy: how important is aspirin resistance? *BJOG* 2016;123(9):1481-7

17. McCambridge J, Witton J, Elbourne DR. Systematic review of the Hawthorne effect: New concepts are needed to study research participation effects. *J Clin Epidemiol* 2014; 67(3): 267-77

18. Roberge S, Nicolaides K, Demers S, Hyett J, Chaillet N, Bujold E. The role of aspirin dose on the prevention of preeclampsia and fetal growth restriction: systematic review and meta-analysis. *Am J Obstet Gynecol* 2017;216(2):110-20

19. Thapa K, Sanghvi H, Rawlins B, Karki YB, Regmi K, Arval S, *et al.* Coverage, compliance, acceptability and feasibility of a program to prevent pre-eclampsia and eclampsia through calcium supplementation for pregnant women: an operations research study in one district of Nepal. *BMC Pregnancy Childbirth*. 2015; 16: 241

20. Kulkarni B, Christian P, LeClerq SC, Khatry SK. Determinants of compliance to antenatal micronutrient supplementation and women's perceptions of supplement use in rural Nepal. *Public Health Nutr.* 2010; 13(1): 82-90

21. Seck BC and Jackson RT. Determinants of compliance with iron supplementation among pregnant women in Sengal. *Public Health Nutr.* 2008; 11(6): 596-605

22. De Jonge L, de Walle HEK, de Jong-van den Berg LTW, van Langen IM, Bakker

MK. Actual Use of Medications Prescribed During Pregnancy: A Cross-Sectional Study Using Data from a Population-Based Congenital Anomaly Registry. *Drug Saf.* 2015; 38: 737-47

23. Caron N, Rivard GE, Michon N, Morin F, Pilon D, Moutquin JM, Rey E. Low dose ASA response using the PFA-100 in women with high risk pregnancy. *J Obstet Gynaecol Can*. 2009; 31: 1022-7
24. Schisterman EF, Silver RM, Lesher LL, Faraggi D, Wactawski-Wende J, Townsend JM, *et al.* Preconception low-dose aspirin and pregnancy outcomes: results from the EAGeR randomised trial. *Lancet.* 2014; 384: 29-36
25. Golding J. A randomised trial of low dose aspirin for primiparae in pregnancy: The Jamaica Low Dose Aspirin Study Group. *BJOG*. 1998; 105(3): 293-9

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]

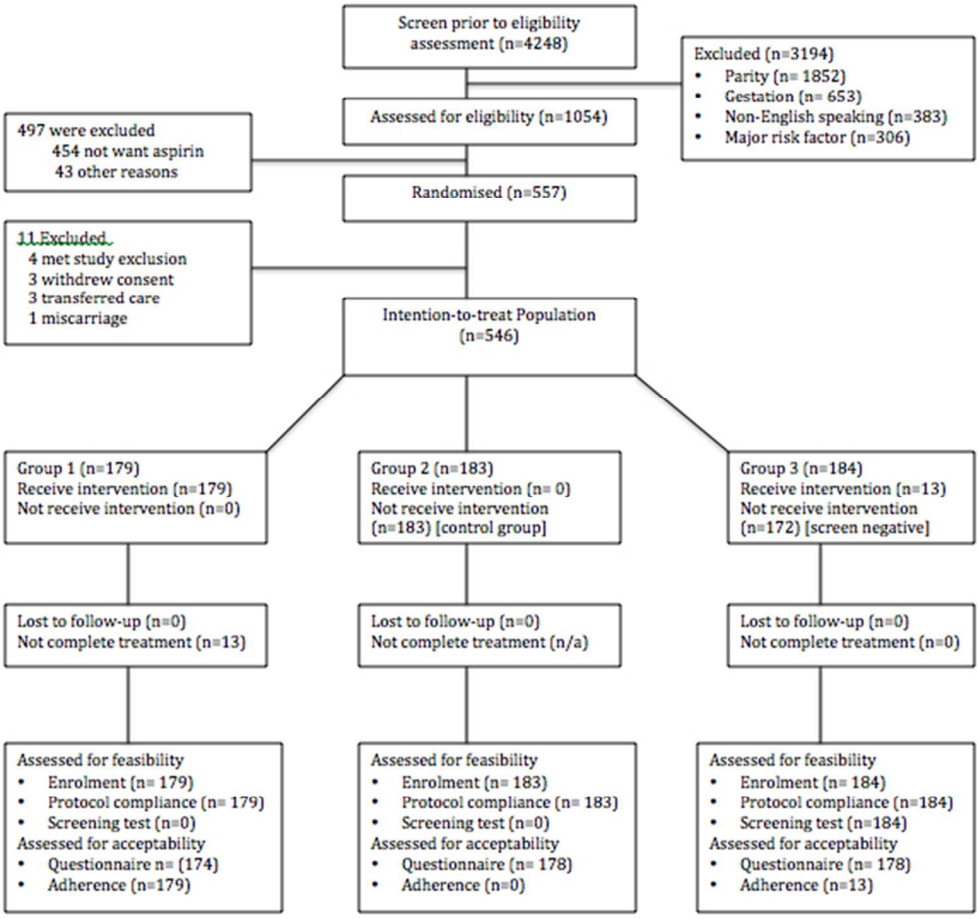
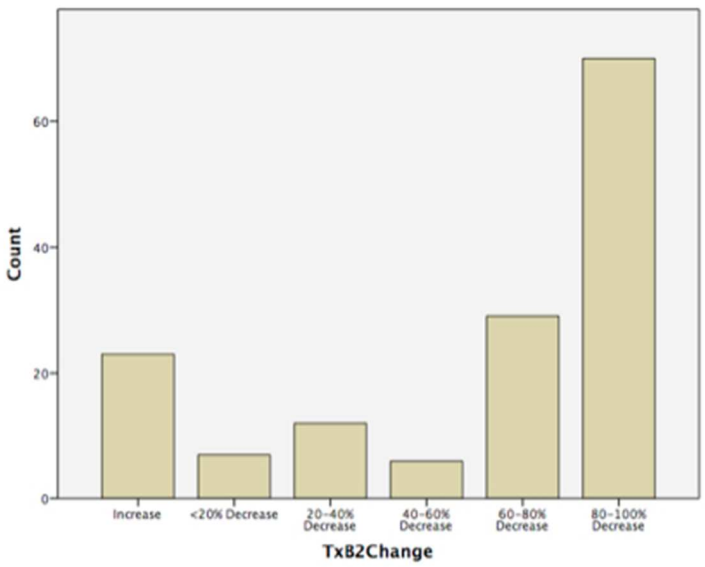


Figure 1 – Consort diagram

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
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 <10th centile No. (%)	14 (8%)	18(10%)	25 (14%)
Mode of delivery No. (%) Spontaneous Instrumental Caesarean	 85 (47.5) 56 (31.3) 38 (21.2)	 95 (52.0) 47 (25.7) 41 (22.3)	 88 (47.8) 51 (27.7) 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. (%) Preeclampsia <34-weeks Preeclampsia <37-weeks	8 (4.5) 0 (0) 2 (1.1)	7 (3.8) 2 (1.1) 2 (1.1)	7 (3.8) 1 (0.5) 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 Stillbirth Neonatal death	 177 (98.9) 2 (1.1) 0 (0)	 181 (99.0) 1 (0.5) 1 (0.5)	 182 (98.9) 0 (0) 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. (%) Pre-eclampsia <34-weeks Pre-eclampsia <37-weeks	2 (15.4) 0 (0) 2 (15.4)	6 (3.5) 2 (1.2) 2 (1.2)	5.0 (0.9 – 27.7) - 15.4 (2.0 – 120)
Birthweight <10th 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 Stillbirth Neonatal death	 13 (100) 0 (0) 0 (0)	 169 (98.8) 2 (1.2) 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 >1000mls	 25 (13.0) 7 (3.6%)	 9 (4.9) 1 (0.5)	 12 (6.5) 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

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.