Recombinant Human Granulocyte – Colony Stimulating Factor in women with unexplained Recurrent Pregnancy Losses: a randomised clinical trial

A Eapen, M Joing, P Kwon, J Tong, E Maneta, C De Santo, F Mussai, D Lissauer★ , D Carter for the RESPONSE study group

★Corresponding Author: Dr David Lissauer, Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research, University of Birmingham, Mindelsohn way, Birmingham, West Midlands, UK. B152TG. Email: d.m.lissauer@bham.ac.uk

Running title: A randomised clinical trial of rhG-CSF in recurrent miscarriage.

Extended Title:
Recombinant Human Granulocyte – Colony Stimulating Factor in women with unexplained Recurrent Pregnancy Losses (RESPONSE Study): double-blind, placebo controlled, randomised trial

Tables: 2
Figures: 2
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Abstract

Study question:

Does administration of recombinant human granulocyte colony stimulating factor in the first trimester improve pregnancy outcomes, among women with a history of unexplained recurrent pregnancy loss?

Summary answer:

Recombinant human granulocyte colony stimulating factor administered in the first trimester of pregnancy did not improve outcomes among women with a history of unexplained recurrent pregnancy loss.

What is known already:

The only previous randomised controlled study of granulocyte colony stimulating factor in recurrent miscarriage in sixty-eight women with unexplained primary recurrent miscarriage found a statistically significant reduction in miscarriage and improvement in live birth rates. A further four observational studies where G-CSF was used in a RM population was identified in lierature, two of which confirmed statistically significant increase in clinical pregnancy and live birth rates.

Study design, size, duration:

A randomised, double-blind, placebo controlled clinical trial involving 150 women with a history of unexplained recurrent pregnancy loss was conducted at 21 sites with established recurrent

54 miscarriage clinics in the United Kingdom between 23 June 2014 and 05 June 2016. The study
55 was coordinated by University of Birmingham, UK.
56
57 **Participants/materials, setting, methods:**
58 150 women with a history of unexplained recurrent pregnancy loss: 76 were randomised to
59 recombinant human granulocyte – colony stimulating factor and 74 to placebo. Daily
60 subcutaneous injections of recombinant human granulocyte – colony stimulating factor 130 mcg
61 or identical appearing placebo from as early as three to five weeks of gestation for a maximum of
62 9 weeks. The trial used central randomisation with allocation concealment. The primary outcome
63 was clinical pregnancy at 20 weeks of gestation, as demonstrated by an ultrasound scan.
64 Secondary outcomes included miscarriages, livebirth, adverse events, stillbirth, neonatal birth
65 weight, changes in clinical laboratory variables following study drug exposure, major congenital
66 anomalies, preterm births and incidence of anti-drug antibody formation. Analysis was by
67 intention to treat.
68
69 **Main results and the role of chance:**
70 A total of 340 participants were screened for eligibility of which 150 women were randomised.
71 76 women (median age, 32[IQR, 29-34] years; mean BMI, 26.3[SD, 4.2] and 74 women (median
72 age, 31[IQR, 26-33] years; mean BMI, 25.8[SD, 4.2] were randomised to placebo. All women
73 were followed-up to primary outcome, and beyond to live birth. The clinical pregnancy rate at
74 20 weeks, as well as the live birth rate, was 59.2% (45/76) in the rhG-CSF group, and 64.9%
75 (48/74) in the placebo group, giving a relative risk of 0.9 (95% CI: 0.7 to 1.2; p=0.48). There was
76 no evidence of a significant difference between the groups for any of the secondary outcomes.

Adverse events (AEs) occurred in 52 (68.4%) participants in rhG-CSF group and 43 (58.1%) participants in the placebo group. Neonatal congenital anomalies were observed in 1/46 (2.1%) of babies in the rhG-CSF group versus 1/49 (2.0%) in the placebo group (RR of 0.9; 95% CI: 0.1 to 13.4; p=0.93).

Limitations, reasons for caution:

This trial was conducted in women diagnosed with unexplained recurrent pregnancy loss and therefore no screening tests (commercially available) were performed for immune dysfunction related pregnancy failure/s.

Wider implications of the findings:

To our knowledge, this is the first multicentre study and largest randomised clinical trial to investigate the efficacy and safety of granulocyte human colony stimulating factor in women with recurrent miscarriages. Unlike the only available single centre RCT, our trial showed no significant increase in clinical pregnancy or live births with the use of rhG-CSF in the first trimester of pregnancy.

Study funding/competing interest(s):

Mark Joing, Paul Kwon, Jeff Tong and Darryl Carter were or are employees of Nora Therapeutics, Inc. Arri Coomarasamy received consulting fees from Nora therapeutics through his employer, University of Birmingham. Ruth Bender Atik received consulting fees from Nora therapeutics which was paid to The Miscarriage Association, UK. No other potential conflict of

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Trial registration number:

EUDRACT No: 2014-000084-40; ClinicalTrials.gov Identifier: NCT02156063

Trial registration date:

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Date of first patient's enrolment:

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Key words:

recombinant human granulocyte colony stimulating factor

recurrent pregnancy loss

semi-allogenic fetus

immune mediated miscarriages

unexplained recurrent miscarriages

Introduction

Recurrent pregnancy losses (RPL) defined as the loss of three or more pregnancies, affects approximately 1-3% of couples attempting to have a child (Ford et al., 2009). Investigations do not provide a cause for recurrent pregnancy losses in approximately half of those investigated, and such couples are said to have unexplained recurrent pregnancy losses (Yadava et al., 2014). Whilst a range of treatments are currently offered to couples with unexplained recurrent pregnancy losses (uRPL), no effective treatment has yet been identified (ESHRE Recurrent Pregnancy Loss Guidelines., 2017).

Immune mediated mechanisms are thought to contribute to recurrent pregnancy losses. In particular, a failure of the maternal immune system to adapt to accommodate the semi-allogenic fetus may be important. Despite a lack of good evidence of their efficacy, a range of immunomodulatory treatments including, paternal lymphocyte infusion therapy (Cavalcante et al., 2014, Mowbray et al., 1985), corticosteroids, intravenous immunoglobulin therapy, (Nyborg et al., 2014), intravenous intralipid infusion (Meng et al., 2016) and anti–TNFα monoclonal antibody therapy (Clark et al., 2009) to modulate the maternal immune response are offered to women with recurrent pregnancy losses.

Granulocyte colony stimulating factor (G - CSF) is a cytokine conventionally viewed as important in stimulating the proliferation and differentiation of neutrophils (Thomas et al., 2002). It is widely used in treatments associated with severe chronic neutropenia and chemotherapy induced neutropenia for mobilization of neutrophils (Carton et al., 2013). In addition, G-CSF is postulated to have immunomodulatory properties by inducing peripheral regulatory T-cells and myeloid derived suppressor cells, which have an important function curbing the immune response to infection, inflammation, and autoimmunity (Williams et al., 2012). Furthermore,

studies in both humans and animals have showed that administration of G-CSF improves endometrial thickness (Gleicher et al 2011), ovarian follicular function and oocyte quality (Salmassi et al., 2004), which may enhance embryo implantation (Ledee et al., 2008).

A phase 1 randomised double blind, placebo-controlled dose escalation (65 mcg,130 mcg and 260 mcg) study of rhG-CSF in 48 healthy female volunteers suggested changes in peripheral blood subsets including a temporary induction of toleragenic cell subsets and decreased percentages of pro-inflammatory and cytotoxic cell subsets, without evidence of global immune changes or suppression. These specific changes were observed only in the multidose groups and not in single dose or placebo groups (unpublished data; available on request through the corresponding author).

Furthermore, a single centre, randomised controlled trial of 68 women diagnosed with recurrent pregnancy losses, suggested that recombinant G-CSF may be an effective treatment (Scarpellini et al., 2009). Although the clinical evidence is limited (ESHRE Recurrent Pregnancy Loss Guidelines., 2017), the use of G-CSF to treat recurrent miscarriage and implantation failure appears to be increasing. Therefore, there was an urgent need to determine the efficacy and safety of this treatment in a multicentre trial.

We conducted a multicentre, randomised, double-blind, placebo-controlled trial to evaluate the efficacy and safety of recombinant human granulocyte colony stimulating factor (rhG-CSF) in women with a history of unexplained recurrent pregnancy loss to provide a definitive answer on whether rhG-CSF administration improved pregnancy outcomes.

Methods

Study design

RESPONSE study was a multicentre, double blind; placebo controlled randomised clinical trial conducted to determine the effect of recombinant human granulocyte – colony stimulating factor in women with a history of unexplained recurrent pregnancy loss. All the eligible participants gave their written informed consent. The participants in the RESPONSE trial were recruited from 21 hospitals with established recurrent pregnancy loss clinics located across the United Kingdom. Study enrolment occurred between June 2014 and June 2016. The study was approved by NRES Committee North West – Greater Manchester Central (REC Ref.No: 14/NW/0130) and the individual research and development departments at respective hospital sites.

Participants

Women were eligible for enrolment in the study: (1) if they were aged 18 to 37 years with a BMI of 19-35 (at the time of consent), and (2) with regular ovulatory menstrual cycles and those who were actively trying to conceive naturally after being diagnosed with a history of unexplained recurrent pregnancy loss (three or more consecutive or non-consecutive first trimester losses of which at least 2 were confirmed by ultrasound or by histology). Age criterion was applied because the likelihood of miscarriages due to chromosomal aberrations is higher in older women, with such miscarriages unlikely to be prevented by immune-modulation.

Participants were excluded if ***any*** of the following criteria were applicable (a) greater than 5 completed weeks of gestation (i.e. greater than 3 completed weeks since ovulation as indicated by ovulation monitoring) when presenting for randomisation, (b) known karyotype abnormalities in either the participant or her current male partner, (c) congenital malformations and uncorrected major and minor uncorrected intrauterine abnormalities (as assessed by ultrasound,

hysterosonography, hysterosalpingography, or hysteroscopy within 3 years prior to screening), (d) vaginal bleeding of unknown cause, (e) diagnosis of infertility, (f) current or past diagnosis of the following: systemic autoimmune disease (e.g. systemic lupus erythematosus, Hashimoto's thyroiditis, Graves' disease, rheumatoid arthritis), antiphospholipid syndrome, or other thrombophilic disorder, (g) presence of anti-thyroid antibodies, lupus anticoagulant, anti-cardiolipin antibodies, or anti-β2 GP1 antibodies, (h) hyperprolactinemia, (i) any uncontrolled clinically significant medical condition (e.g. asthma, type II diabetes, infection), (j) the following laboratory abnormalities at initial screening or within 3 months prior to randomisation: thrombocytopenia or thrombocytosis (platelet count < 75,000/μL or > 500,000/μL), neutropenia or neutrophilia (absolute neutrophil count < 1500/μL or > 10,000/μL), leucopenia or leucocytosis (white blood cell count < 3000/μL or > 15,000/μL), and creatinine, hepatic transaminases, lactate dehydrogenase (LDH), alkaline phosphatase, or uric acid ≥ 1.5x upper limit of normal (ULN), (k) use of lithium within 1 month prior to screening, (l) known hypersensitivity to any rhG-CSF drug product, any of its components, or any E. coli-derived proteins, (m) history of any of the following conditions: human immunodeficiency virus (HIV) infection, (n) malignancy within the past 5 years other than treated basal cell carcinoma or squamous cell carcinoma of the skin, (o) splenomegaly or splenic rupture, (p) adult respiratory distress syndrome (ARDS), acute lung injury (ALI), or pulmonary edema, (q) sickle cell anemia, (r) acute myocardial infarction, stroke, or revascularization (coronary or cerebral), (s) previous rhG-CSF therapy for any indication, or (t) in the investigator's opinion, any contraindication to use of the investigational drug.

Randomisation, masking and procedures

Participants in RESPONSE trial were randomised to receive rhG-CSF 130 mcg or placebo in a 1:1 ratio. Stratified permuted block randomization was used with number of prior miscarriages

(3, >3), and age (<35, 35-37 years) as the stratification factors. An interactive central web response system (IWRS) was used for randomization.

Eligible participants were identified from recurrent pregnancy loss clinics and underwent comprehensive screening tests for eligibility evaluation. Once eligibility was established, the participant began ovulation monitoring and attempts at spontaneous conception. The participant performed daily urine pregnancy tests from 6 days after ovulation. After reporting a positive home urine pregnancy test, the participant scheduled a visit to the study site for a repeat urine pregnancy test, randomization into the study and initiation of study drug treatment. The study site visit took place within 4 days of the positive home urine pregnancy test. Once randomised, the participant self-administered rhG-CSF or placebo as a subcutaneous injection for a maximum of 9 weeks (up to 12^{th} week of gestation) or until pregnancy failure.

Recombinant human granulocyte colony stimulating factor (rhG-CSF) and placebo were supplied to the investigative site in single-use 1 mL prefilled syringes. Each prefilled syringe contained 0.5 mL of rhG-CSF 260 mcg/mL for the 130 mcg dose, or identical appearing placebo. Participants, doctors and trial nurses remained unaware of study assignments. The first dose of study drug was administered at the investigative site. All subsequent doses were administered by the participant once daily at approximately the same time each day (within 20 to 28 hours after the previous dose).

All study data except central laboratory and immunogenicity data were recorded in an electronic case report form (eCRF). Research personal allocated for the trial at individual sites were responsible for entering these data.

Immunogenicity and safety analysis

All participants receiving study drug had serum samples collected prior to study drug administration, and at 6, 12 and 16 weeks of gestation or 4 week post drug follow up (for participants diagnosed with pregnancy loss) for the presence of anti-drug antibodies (ADAs). Safety was monitored through the assessment of adverse events, vital signs, physical examinations, and clinical laboratory variables throughout the treatment period and 4-week post drug follow-up period by a designated medical monitor. In order to minimize unnecessary exposure to study drug, any randomised participant who was no longer pregnant discontinued study drug prior to the completion of the treatment period and was followed for a minimum of 4 weeks after last dose of study drug.

An external and independent Data Monitoring Committee (DMC) facilitated close monitoring of safety data, including any deaths, serious adverse events, anti-drug antibody formation, and adverse events of special interest including splenic rupture, anaphylaxis, acute respiratory distress syndrome (ARDS) or acute lung injury (ALI), and major cardiovascular event.

Study blinding

This study was randomised, double-blinded, and placebo-controlled to minimize potential bias in treatment assignment, subject monitoring, and endpoint evaluations. All participants, subjects, investigative centre study staff, and investigative centre monitors were blinded to treatment assignment. In addition, the laboratory results for white blood cells (WBC) and WBC subset counts, alkaline phosphatase, uric acid, lactate dehydrogenase (LDH) and anti-drug antibody (ADA) was blinded, as it had the potential for unblinding the intervention.

Outcomes

The primary outcome of ongoing clinical pregnancy was assessed via ultrasound examination at 20 weeks of gestation. All participants were monitored for adverse events. All participants who received at least one dose of study drug were followed for safety for a minimum of 4 weeks following the last dose of study drug.

The secondary outcome measures were: (a) live birth, (b) ongoing clinical pregnancy at weeks 6 and 12 of gestation, (c) spontaneous pregnancy loss, (d) elective abortion, (e) stillbirth, (f) neonatal birth weight, (g) maternal adverse events and serious adverse events during the treatment period and within 4 weeks of the last dose of study drug, (h) changes in clinical laboratory variables following study drug exposure, (i) major congenital anomalies, (j) preterm births and (k) incidence of anti-drug antibody (ADA) formation.

For participants who maintained pregnancy through 20 weeks of gestation, phone visits were conducted every 8 weeks during pregnancy to assess pregnancy status/outcomes and prescription medication use. Within one month of delivery, additional information was obtained, including pregnancy outcome, gestational age at delivery, mode of delivery, birth weight, and Apgar scores.

Sample size calculations

The target sample size was a total of 150 participants. Participants were randomised in a 1:1 ratio to the two treatment arms. This sample size had been selected to achieve >90% power to detect a difference in ongoing clinical pregnancy rates of 60% for the placebo group and 80% in the active treatment group. Efficacy analyses was based on an intent-to-treat principle. The difference in the primary efficacy outcome measure (ongoing clinical pregnancy rate at week 20

of gestation) between rhG-CSF and placebo was tested using a Cochran Mantel Haenszel (CMH) test.

Statistical analysis

The statistician who conducted the analysis was blinded to group allocation. Efficacy analysis was based on an intention to treat principle. The relative risks (RRs) with 95% CI s were calculated for the primary and secondary outcomes. Subgroup analysis was performed using the RR for the stratification factors. Missing data were imputed only for those with a clinical pregnancy and livebirth. This implied that summary measures were limited to participants who remained pregnant at that time point. The statistical analysis plan (SAP) is available in supplement file.

Results

Between 23 June 2014 and 05 June 2016, a total of 340 women were screened for inclusion criteria at 21 different recruiting centres in the United Kingdom. One hundred and ninety women did not meet the inclusion criteria. One hundred and fifty women who conceived naturally and remained willing to participate in the trial were randomised to receive either rhG-CSF (76 women) or placebo (74 women) (Fig.1). Overall, 140/150 women (93.3%) completed the study and 10/150 women (6.7%) discontinued the study prematurely for reasons including loss to follow up, withdrawal of consent, and other reasons (for e.g. use of any excluded therapy).

All participants were followed to primary outcome, and beyond to live birth. The baseline characteristics of the study population were similar across the study groups (Table 1). The median age at the time of recruitment was 30.6 years (IQR 29 to 34 years) and 37 participants (24.7%) had experienced more than three previous miscarriages. The mean BMI at the time of randomisation was 26.06 kg/m^2. Ethnicity data was available for all 150 randomised women, and

of these, 134 (89%) were white, 9 (6%) were Asian, 2 (1%) were black and 5 (3%) were from other ethnic groups. Most of the women were non-smokers (123/150, 82%). Study records of concurrent medications showed that 3 (2%) participants were taking metformin at the time of participation, and 23 (15.3%) were taking low dose aspirin.

Treatment compliance

Subject compliance with study drug dosing was accessed via a site review of the returned syringes and the compliance record maintained by the participant. From these results, summaries of treatment compliance and exposure were produced. In the overall population, the mean (SD) compliance rate was 98.67% (3.987).

Outcomes of the participants

The clinical pregnancy rates at 20 weeks of gestation was 59.2% (45/76) in the rhG-CSF group, compared with 64.9% (48/74) in the placebo group, giving a RR of 0.9 (95% CI: 0.7 to 1.2; p=0.48), (Table 2). There were no pregnancy losses in the time period from primary outcome to live birth; therefore the live birth rate was 59.2% (45/76) in the rhG-CSF group, and 64.9% (48/74) in the placebo group, giving a relative risk of 0.9 (95% CI: 0.7 to 1.2; p=0.48). During the study, clinical pregnancies were confirmed by ultrasound scan at six weeks of gestation in 136 (90.7%) of the 150 randomised participants [67/76, 88.2% in the rhG-CSF group vs 69/74, 93.2% in the placebo group, RR of 0.9 (95% CI: 0.9 to 1.0; p=0.28)]. Ongoing pregnancies were confirmed at approximately 12 weeks in 96 (64.0%) of the women [45/76, 59.2% in the rhG-CSF group vs 51/74, 68.9% in the placebo group, RR of 0.9 (95% CI: 0.7 to 1.1; p=0.224)].

Miscarriage rates were not significantly different between the study groups (rhG-CSF 28/76, 36.8% vs placebo 25/74, 33.8%, RR of 1.1 (95% CI: 0.7 to 1.7, p=0.70). Amongst the 28 pregnancies that ended in miscarriage for participants receiving rhG-CSF, the median gestation

was 6 weeks (IQR 6 to 7 weeks). Amongst the 25 pregnancies that ended in miscarriage for participants receiving placebo, the median gestation was 6.5 weeks (IQR 6 to 9 weeks). The distributions of gestational age at live birth delivery for the rhG-CSF and placebo groups are given in Figure 2. All infants were discharged home alive from the hospital.

Adverse events (AEs) occurred in 52 (68.4%) participants in rhG-CSF group and 43 (58.1%) participants in the placebo group (supplementary table 1). Neonatal congenital anomalies were observed in 1/46 (2.1%) of babies in the rhG-CSF group versus 1/49 (2.0%) in the placebo group (RR of 0.9; 95% CI: 0.1 to 13.4; p=0.93).

Findings of subgroup analyses are given in supplementary table 2; no significant subgroup effects were identified. Exploratory analyses for a number of key obstetric outcomes did not find any significant differences between the rhG-CSF and placebo arms.

Discussion

This randomised clinical trial investigating the effect of recombinant human granulocyte colony stimulating factor in the first trimester of pregnancy in women, diagnosed with unexplained recurrent pregnancy loss, found no improvement in clinical pregnancies at 20 weeks, or live births, compared to placebo.

Comparison with previous studies

The findings from this large multicentre, randomised, placebo-controlled trial do not support the findings of the only previous randomised control study evaluating recombinant G-CSF in recurrent pregnancy loss (Scarpellini et al., 2009). In this previous, smaller, single centre study by Scarpellini and Sbracia, sixty eight women with a previous history of unexplained recurrent miscarriage were randomised to G-CSF or placebo. Women in the intervention group were

started on a dose of 1mcg/kg/day starting on sixth day after ovulation. The live birth rates in the rhG-CSF group was 82.8% versus 48.5% in the placebo group (p=0.0061, OR=5.1; 95% confidence interval 1.5 -18.4), suggesting a statistically significant improvement in outcomes.

There are also 2 retrospective cohort studies in women with recurrent miscarriage which suggested improved outcomes with administration of G-CSF(Santjohanser et al., 2013, Wurfel et al., 2013). Observational data from 2 separate population registry was also identified. Boxer et al, identified 224 pregnancy events in women diagnosed with chronic neutropenia and identified a decrease in abortion rates with no adverse side effects(Boxer et al., 2010). However, Zeidler et al, used data from severe chronic neutropenia international registry (SCNIR) and observed no improvement in pregnancy outcomes after administration of G-CSF(Zeidler et al., 2014). All the above studies used a varying dose and duration of G-CSF and were of poor quality (ESHRE Recurrent Pregnancy Loss Guidelines., 2017).

Recombinant human granulocyte colony stimulating factor is also widely used in assisted conception treatment. A previous review of G-CSF in reproductive medicine studies also suggested therapeutic benefit based on body weight dependent target dose or use of G-CSF as an intrauterine infusion (Cavalcante et al., 2015). This review included 1 RCT, 5 cohort studies and 1 case report in a varied range of patients. The included studies were of poor quality and the researchers called for larger well-designed studies. A more recent randomised open label clinical trial of G-CSF (using a single dose of 300 µg as an intrauterine infusion on day of oocyte recovery) in 100 infertile women undergoing in-vitro fertilization treatment did not show a benefit of G-CSF in improving pregnancy outcomes (Eftekhar et al., 2016).

Strength and limitations of this study

The strengths of our study include the multicentre study design involving 21 hospitals spread across the United Kingdom. After standard screening tests, as practiced in the United Kingdom, 150 women with unexplained recurrent pregnancy losses from different ethnic background were randomised. Thus, our study represents the largest placebo-controlled randomised control study of rhG-CSF in women with unexplained recurrent pregnancy losses. Participant compliance rate was high and all participants were followed up until completion of study endpoints, as appropriate. Unlike other studies where a varying dose of G-CSF was utilized, we initiated optimal dosage of rhG-CSF treatment as soon as the pregnancy was confirmed, which started as early as 7 days after ovulation. A Phase 1, randomized, double-blind, placebo-controlled, dose-escalation study was conducted prior to this study for dosage determination. This study consisted of 6 single- and multiple-dose cohorts with 8 participants in each dose cohort, randomized in a 3:1 ratio to receive either rhG-CSF or placebo. Transient neutrophilia and increases in white blood cell (WBC) counts were observed following both single and multiple doses. Changes in peripheral blood cell subsets were observed consistent with supporting a state of maternal-fetal immune tolerance. These changes include the temporary induction of toleragenic cell subsets including an increase in toleragenic myeloid derived suppressor cells (MDSC) and a decrease in cytotoxic natural killer (NK) cells, without evidence of global immune changes or suppression. These changes were observed only in the multidose rhG-CSF treatment groups, and not in any placebo group. The weakness of our study was that women were not screened prior to inclusion to demonstrate immune dysfunction as the reason for their pregnancy losses. This is because there is no accepted test(s) for immune dysfunction in reproductive immunology.

There was no increased risk of congenital anomalies among offspring of women treated with rhG-CSF, although the study was not powered for such rare outcomes.

Conclusion

Among women with a history of unexplained recurrent pregnancy loss, administration of rhG-CSF in the first trimester of pregnancy, compared with placebo, did not improve the clinical pregnancy rates at 20 weeks or live birth rates.

Appendix:

RESPONSE study group consist of : A Eapen[1,20], M Joing[2], P Kwon[2], J Tong[2], D Carter[2], E Maneta[1], C De Santo[1], F Mussai[1], A Ahmed[3], C Bass[4], R Bender-Atik[5], R Bhattacharya[6], Y Cheong[7], F Dawood[8], I Granne[9], P Gupta[10], A Horne[11], P Manda[12], L Mohiyiddeen[13], J Moore[14], S Quenby[15], R Rai[16], J Shillito[17], J Stewart[18], E Truchanowicz[19], L Dwyer[13], R Small[10], L Sharpe[4], A Smith[8], D Lissauer[1], A Coomarasamy[1]

(1) Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research, University of Birmingham, UK. B15 2TG, (2) Nora Therapeutics, California, USA. 94301, (3) Sunderland Royal Hospital, Sunderland, UK. SR4 7TP, (4) St. Peters Hospital, Ashford, UK. KT16 OPZ, (5) Miscarriage Association, UK. WF1 3QW, (6) Derriford Hospital, Plymouth, UK. PL6 8DH, (7) University of Southampton, UK. SO17 1BJ, (8) Liverpool Women's Hospital, UK. L8 7SS, (9) University of Oxford, UK. OX1 2JD, (10) Birmingham Heartlands Hospital, UK. B9 5SS, (11) MRC Centre for Reproductive Health, Edinburgh, UK. EH16 4TJ, (12) James Cook University Hospital, Middlesborough, UK. TS4 3BW, (13) St Mary's Hospital, Manchester, UK. M13 9WL, (14) Queens Medical Centre, Nottingham, UK. NG7 2UH, (15) University Hospital, Coventry, UK. CV2 2DX, (16) St Mary's Hospital, London, UK. W2 1NY, (17) St James' Hospital, Leeds, UK. LS9 7TF, (18) Royal Victoria Infirmary, Newcastle upon Tyne, UK. NE1 4LP, (19) Alta Innovations Ltd, UK. B15 2SQ, (20) University of Iowa Hospitals and Clinics, Carver college of Medicine, Iowa, USA. 52242

Abey Eapen (Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research, University of Birmingham,UK & Carver college of Medicine, University of Iowa), Ebtehaj Maneta, Carmela De Santo, Francis Mussai, David Lissauer and Arri Coomarasamy (Tommy's National Centre for Miscarriage Research, Institute of Metabolism and Systems Research, University of Birmingham, UK); Mark Joing, Paul Kwon, Jeffrey Tong, Darryl Carter (Nora Therapeutics, California, USA); Amna Ahmed (Sunderland Royal Hospital, UK); Claire Bass and Lisa Sharpe (St. Peters Hospital, Ashford, UK); Ruth Bender-Atik (Miscarriage Association, UK); Rukma Bhattacharya (Derriford Hospital, Plymouth, UK); Ying Cheong (University of Southampton, UK); Feroza Dawood and Amy Smith (Liverpool Women's Hospital, UK); Ingrid Granne (University of Oxford, UK); Pratima Gupta and Rachel Small (Birmingham Heartlands Hospital, UK); Andrew Horne (MRC Centre for Reproductive Health, Edinburgh, UK); Padma Manda (James Cook University Hospital, Middlesborough, UK); Lamiya Mohiyiddeen and Lucy Dwyer (St Mary's Hospital, Manchester, UK); Judith Moore (Queens Medical Centre, Nottingham, UK); Siobhan Quenby (University Hospital, Coventry, UK); Raj Rai (St Mary's Hospital, London, UK); Jane Shillito (St James' Hospital, Leeds,UK); Jane Stewart (Royal Victoria Infirmary, Newcastle upon Tyne, UK) and Ewa Truchanowicz (Alta Innovations Ltd, UK)

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Author Roles: The first, second, and last author vouch for the accuracy of the data and analyses and for the fidelity of the study to the protocol.
Conception of the study: Darryl Carter, Mark Joing, Paul Kwon, Jeffrey Tong and Arri Coomarasamy.
Chief Investigator and design of the study: Prof.Arri Coomarasamy.
Data interpretation and writing of the report: Abey Eapen, Mark Joing, David Lissauer, Darryl Carter and Arri Coomarasamy.
Members of trial steering committee: Abey Eapen, Mark Joing, Paul Kwon, Ruth Bender-Atik, Ewa Truchanowicz and Arri Coomarasamy.
Data analysis committee: Mark Joing, Paul Kwon, Jeffrey Tong and Arri Coomarasamy.
Principal investigators at local study sites: Amna Ahmed, Claire Bass, Rukma Bhattacharya, Ying Cheong, Feroza Dawood, Ingrid Granne, Pratima Gupta, Andrew Horne, Padma Manda, Lamiya Mohiyiddeen, Judith Morre, Siobhan Quenby , Raj Rai, Jayne Shillito and Jane Stewart.
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Competing interests
Mark Joing, Paul Kwon, Jeff Tong and Darryl Carter were or are employees of Nora Therapeutics, Inc. Arri Coomarasamy received consulting fees from Nora therapeutics through his employer, University of Birmingham. Ruth Bender Atik received consulting fees from Nora therapeutics which was paid to The Miscarriage Association, UK. No other potential conflict of interest relevant to this article was reported. Disclosure forms provided by the authors are available with full text of this article.

Ethical approval: The study was approved by NRES Committee North West – Greater Manchester Central (REC Ref.No: 14/NW/0130) and the individual research and development departments at respective hospital sites.

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Figure 1. Participant flow diagram

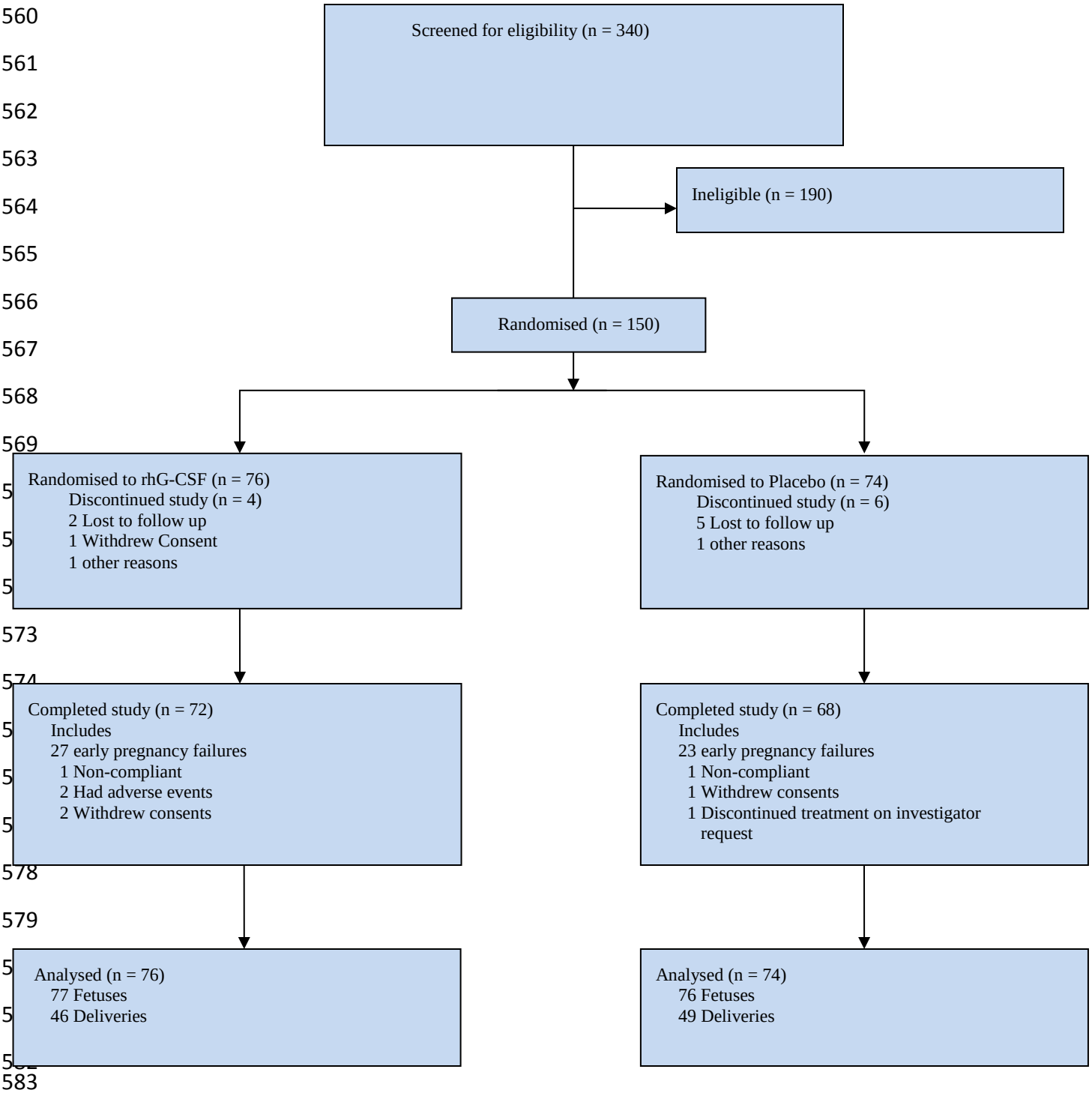


Table 1. Baseline Characteristics of the Participants (Intention to Treat Analysis)*

Characteristics	rhG-CSF (N= 76)	Placebo (N=74)
Maternal age – yr†		
Median	32	31
Interquartile range	29-34	26-33
Maternal BMI	26.3±4.2	25.8±4.2
Maternal BMI > 30 – no. (%)	17(22.4)	13(17.6)
Maternal race – no. (%)‡		
White	71(93.4)	63(85.1)
Black	0	2(2.7%)
Asian	4(5.3)	5(6.8)
Other, including mixed race	1(1.3)	1(5.4)
Maternal smoking –no (%)		
Nonsmoker	62(81.6)	61(82.4)
<10 cigarettes/day	10(13.2)	7(9.5)
10 to 19 cigarettes/day	3(3.9)	6(8.1)
>=20 cigarettes/day	1(1.3	0
Alcohol use – no. (%)§		
None	36(47.4)	35(47.3)
≤3 units/day	27(35.5)	26(35.1)
>3 to ≥20 units/day	13(17.1)	13(17.6)
>20 units/day	0	0
Parity		
Previous live birth – no. (%)	38(50.0)	37(50.0)
≥4 previous miscarriages – no. (%)	40(52.6)	40(54.1)
Previous pregnancy losses – no.		
Median	4.0	4.0
Interquartile range	3-5	3-5
Clinical risk factors – no. (%)		
Polycystic ovaries	2(2.6)	6(8.1)
Fibroids	5(6.6)	3(4.1)
Large-loop excision of the cervical transformation zone	2(2.6)	8(10.8)
Concurrent medications – no. (%)		
Metformin	1(1.3)	2(2.7)
Aspirin	13(17.1)	10(13.5)

* Plus – minus values are means ± SD. The baseline data (age, body mass index [BMI; the weight in kilograms divided by the square of the height in metres], maternal race, smoking status, and parity) of the participants were similar in the two study groups.

† Listed is the maternal age at the time of randomisation.

‡ Race was self-reported

§ One unit is 10 g of pure alcohol.

Table 2. Primary Outcome and Secondary Outcomes of participants in this trial

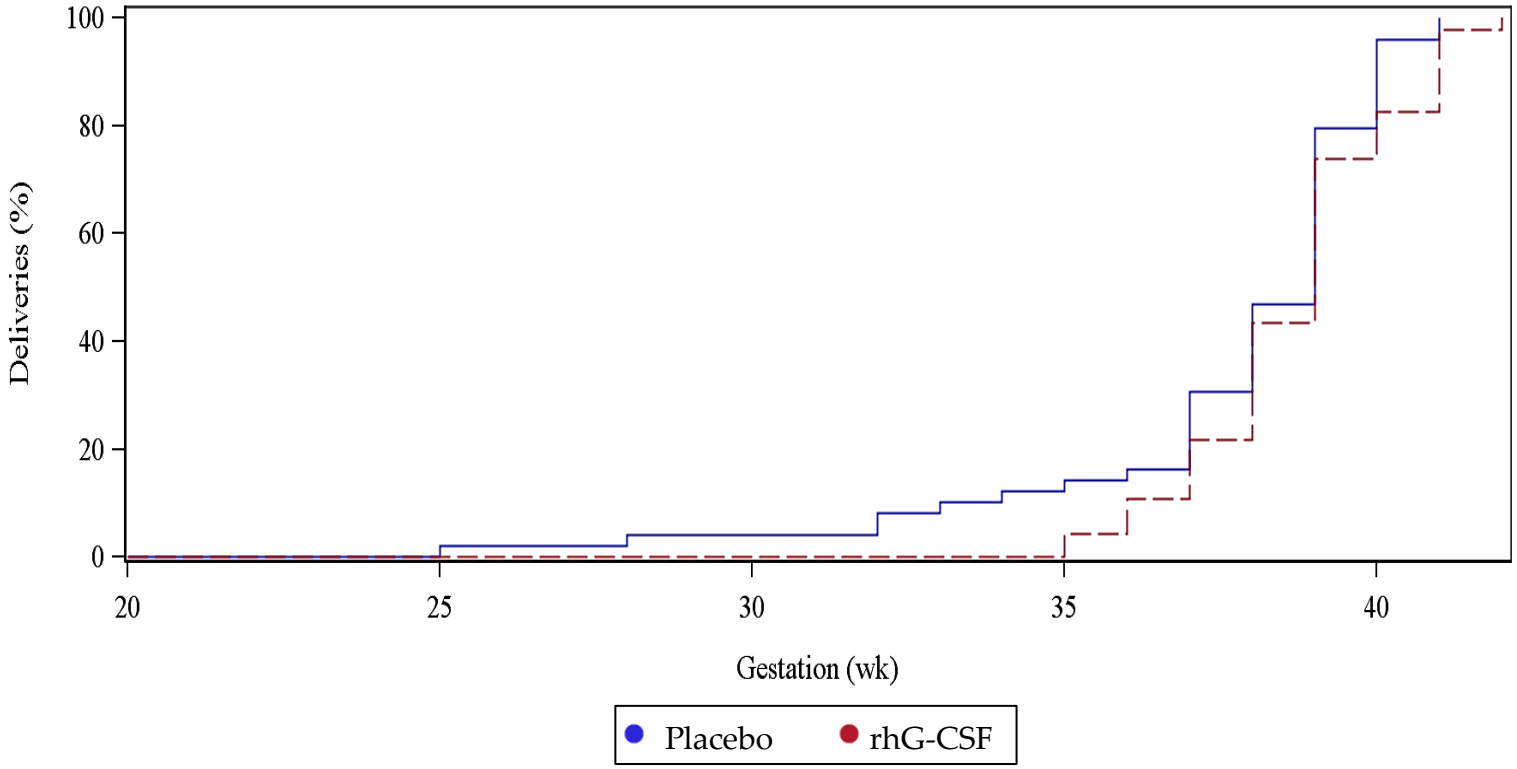
	rhG-CSF no./total no. (%)	Placebo no./total no. (%)	Relative Risk (95%CI)	P Value
Primary Outcome				
Live birth after 20 weeks of gestation	45/76(59.2)	48/74(64.9)	0.9 (0.7, 1.2)	0.48
Secondary Outcomes				
Pregnancy outcomes				
Clinical pregnancy at 6 weeks	67/76(88.2)	69/74(93.2)	0.9 (0.9, 1.0)	0.28
Ongoing pregnancy at 8 weeks	51/76(67.1)	59/74(79.7)	0.8 (0.7, 1.0)	0.09
Ongoing pregnancy at 12 weeks	45/76(59.2)	51/74(68.9)	0.9 (0.7, 1.1)	0.22
Live birth after 24 weeks of gestation	45/76(59.2)	48/74(64.9)	0.9 (0.7, 1.2)	0.48
Live birth after 34 weeks of gestation	45/76(59.2)	42/74 (56.8)	1.0 (0.8, 1.4)	0.76
Ectopic pregnancy	1/76(1.3)	0/74(0.0)	NA	NA
Miscarriage*	28/76(36.8)	25/74(33.8)	1.1 (0.7, 1.7)	0.70
Stillbirth	0/76(0.0)	0/76(0.0)	NA	NA
Preterm birth (before 37 weeks 0 days of gestation)	5/45(11.1)	8/48(16.7)	0.7 (0.3, 2.0)	0.54
Infant birth weight (g)				
Median	3420.0	3300.0	NA	NA
Range	3005-3920	2690-3610	NA	NA
Neonatal outcomes†				
Infants discharged alive from hospital	46/46(100.0)	49/49(100.0)	NA	NA
Any congenital anomaly	1/46(2.2)	1/49(2.0)	0.9 (0.1, 13.4)	0.93
Adverse events§	n/N (%)	n/N(%)		
Maternal adverse events	52/76(68.4)	43/74(58.1)	1.2 (0.9, 1.5)	0.20
Serious adverse events	4/76(5.2)	2/74(2.7)	1.9 (0.3, 10.3)	0.43
Incidence of anti-drug antibody formation	0/76(0.0)	NA	NA	NA

*Miscarriage was defined as spontaneous loss of a pregnancy less than 24 weeks of gestation; the median gestational age at miscarriage was 6.0 weeks (interquartile range, 6 to 7) in the rhG-CSF and 6.5 weeks (interquartile range, 6 to 9) in the placebo group. There were 3 pregnancies of unknown location in the rhG-CSF group.

†The end point is listed per neonate.

§Please see supplementary table 1 for details.

Figure 2. **Distribution of gestational age according to study group assignment.**
Only pregnancies which continued beyond 24 weeks are shown.



Supplementary Table S1 - Adverse Events				
Adverse events	rhG-CSF n(n/N)	Placebo n(n/N)	Relative Risk (95% CI)	P value
Total number of participants	76	74		
Participants with AE	52	43		
Blood and lymphatic system disorders	4 (5.3)	1(1.4)	3.9 (0.4, 34.2)	0.22
Cardiac disorders	1(1.3)	NA[Y]	NA	
Gastrointestinal disorders	33(43.4)	24(32.4)	1.3 (0.9, 2.0)	0.17
General disorders and administration site conditions	10(13.2)	13(17.6)	0.7 (0.4, 1.6)	0.44
Hepatobiliary disorders	1(1.3)	NA	NA	
Immune system disorders	2(2.6)	NA	NA	
Infections and infestations	16(21.1)	10 (13.5)	1.6 (0.8, 3.2)	0.23
Injury, poisoning and procedural complications	2(2.6)	3(4.1)	0.6 (0.1, 3.7)	0.63
Significantly deranged serum parameters	8(10.5)	5(6.8)	1.6 (0.5, 4.6)	0.42
Musculoskeletal and connective tissue disorders	20(26.3)	6(8.1)	3.2 (1.4, 7.5)	0.01
Nervous system disorders	21(27.6)	14(18.9)	1.5 (0.8, 2.6)	0.22
Pregnancy, puerperium and perinatal conditions	8(10.5)	4(5.4)	2.0 (0.6, 6.1)	0.25
Psychiatric disorders	1(1.3)	NA	NA	
Renal and urinary disorders	1(1.3)	1(1.4)	1.0 (0.1, 15.5)	0.99
Reproductive system and breast disorders	19(25.0)	1(14.9)	1.7 (0.9, 3.3)	0.13
Respiratory, thoracic and mediastinal disorders	4(5.3)	2(2.7)	1.9 (0.4, 10.3)	0.44
Skin and subcutaneous tissue disorders	8(10.5)	2(2.7)	3.9 (0.9, 17.8)	0.08
Vascular disorders	1(1.3)	1(1.4)	1.0 (0.1, 15.2)	0.98

[X] Among these, serious adverse events in the rhG-CSF group comprised of 2 occurrences of gastrointestinal disorders, a diagnosis of lower respiratory tract infection and 1 occurrence of severe headache, whereas serious adverse events in the placebo group comprised of a diagnosis of pneumonia and a diagnosis of endometritis.

[Y] NA indicates there were no events in the group.

[Z] Please note that this table is a summary of all adverse events. Some participants had multiple system involvement and therefore numbers in each system class will not add up to the total number of participants with AE.

Supplementary Table S2 - Subgroup Analyses of Primary Endpoint

Subgroup	rhG-CSF n/N(%)	Placebo n/N(%)	Relative risk (95% confidence interval)	P value
Age*				
< 35 years	36/62(58.1)	40/60(66.7)	0.9 (0.7, 1.2)	0.33
≥ 35 years	9/14(64.3)	8/14(57.1)	1.1 (0.6, 2.1)	0.71
Previous miscarriages				
3	23/36(63.9)	23/34(67.6)	0.9 (0.7, 1.3)	0.67
4	12/16(75.0)	13/20(65.0)	1.3 (0.8, 2.0)	0.26
5	4/7(57.1)	4/10(40.0)	1.3 (0.5, 3.6)	0.58
>5	6/17(35.3)	8/10(80.0)	0.5 (0.2, 1.0)	0.06
Gestation at treatment start				
≤4 weeks	42/72(58.3)	44/68(64.70	0.9 (0.7, 1.2)	0.43
>4 weeks	3/4 (75.0)	4/6(66.7)	1.1 (0.6, 2.1)	0.73

*Maternal age at time of randomization

680 **Supplementary Table S3: Trial recruitment number by centres**

Hospital	PI	Screened	Randomised
Liverpool Womens Hospital	Dawood	30	19
Birmingham Heartlands Hospital	Gupta	49	17
University Hospital Coventry	Quenby	31	17
St Mary's Hospital Manchester	Mullan	28	9
St Mary's Hospital London	Rai	21	9
James Cook University Hospital Middlesborough	Manda	20	9
Queens Medical Centre Nottingham	Moore	15	9
St James' Hospital Leeds	Shillito	17	7
Southampton General Hospital	Cheong	15	7
Newcastle Royal Victoria Infirmary	Stewart	12	7
Birmingham Women's Hospital	Coomarasamy	23	6
Sunderland Royal Hospital	Ahmed	10	6
Oxford John Radcliffe Hospital	Granne	10	6
Royal Infirmary of Edinburgh	Horne	16	5
Plymouth Derriford Hospital	Bhattacharya	8	5
Ashford St Peter's Hospital	Bass	9	4
Frimley Park Hospital	Chandra	8	4
Guy's Hospital London	Khalaf	9	3
South Tyneside Hospital	Al-Inizi	3	1
Wansbeck Hospital	Raheja	4	0
Royal Stoke Hospital	Misra	2	0
	TOTAL	**340**	**150**

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