Human Reproduction, Vol.28, No.7 pp. 1743–1752, 2013
Advanced Access publication on April 12, 2013 doi:10.1093/humrep/det117

human reproduction

ORIGINAL ARTICLE *Early pregnancy*

A feasibility trial of screening women with idiopathic recurrent miscarriage for high uterine natural killer cell density and randomizing to prednisolone or placebo when pregnant

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Submitted on September 13, 2012; resubmitted on March 13, 2013; accepted on March 22, 2013

STUDY QUESTION: Is it feasible to screen women with idiopathic recurrent miscarriage (RM) for high uterine natural killer (uNK) cell density and then randomize them to prednisolone or placebo when pregnant?

SUMMARY ANSWER: It was feasible to recruit women with idiopathic RM into a 'screen and treat' trial despite their desire for active medication.

WHAT IS KNOWN ALREADY: Clinical trials of immunotherapy in women with idiopathic RM have failed to substantiate efficacy in preventing miscarriage. Preimplantation uNK cell density is higher in women with RM and can be reduced with prednisolone.

STUDY DESIGN, SIZE, DURATION: In a pilot RCT, 160 eligible women were screened with an endometrial biopsy and those with high uNK cell density were invited to return when pregnant for randomization to prednisolone (20 mg for 6 weeks, 10 mg for 1 week, 5 mg for 1 week) or identical placebo tablets. Randomization was by random number generation and patients, clinicians and outcome assessors were blinded to allocation. The study size and duration was determined by funding, which was for a feasibility trial, for 2 years, sufficient to screen 150 women and randomize 40 women. The outcome measures were recruitment rate, women's perspectives, compliance, live birth and miscarriage rates and pregnancy complications.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The trial was advertised nationally in the UK. Women who attended research clinics run by one consultant (SQ) with three or more consecutive idiopathic miscarriages were included. Women's perspectives of the trial were sought through a questionnaire. The endometrium was sampled 5–9 days after the LH surge, stained using immunohistochemistry for CD56 and the sub-epithelial region analysed with image analysis. Women with a high uNK cell density (>5%) were invited to contact the clinic at 4–6 weeks gestation for randomization. Compliance with medication was assessed using a daily log, and side effects recorded by the women in a diary and on a structured proforma completed in the clinic at the end of the first trimester. All women had ultrasound scans every 2 weeks until 14 weeks' gestation and growth scans at 28 and 34 weeks' gestation in addition to routine antenatal care and a follow-up in person or by telephone 6 weeks after delivery.

MAIN RESULTS AND THE ROLE OF CHANCE: Despite the fact that 85% of women said they would prefer the active treatment, the trial recruitment occurred at the planned rate. Eligible women ($n = 160$) attended the research clinics and had the uNK test, 72 were screen positive and 40 returned when pregnant for randomization. Compliance with medication was reported to be 100%. The active treatment was associated with side effects of insomnia and flushing. The live birth rate was 12/20 (60%) with prednisolone and 8/20 (40%) with placebo (Risk Ratio 1.5, 95% confidence interval (CI) 0.79–2.86, absolute difference 20% CI-10%, +50%), and hence, this was not significant. There were no pregnancy complications or serious adverse fetal outcomes.

LIMITATIONS, REASONS FOR CAUTION: This was a feasibility trial so of insufficient size to assess efficacy or safety. There was inconsistency in the start date of the trial medication and this may have affected the outcome in the active treatment group.

WIDER IMPLICATIONS OF THE FINDINGS: It was feasible to recruit women with idiopathic RM into a 'screen and treat' trial despite their desire for active medication. Our data also suggest that in future trials the primary outcome measure is live birth rate after 24 weeks gestation.

STUDY FUNDING/COMPETING INTEREST(S): Moulton charitable trust funded this study. There are no competing interests.

TRIAL REGISTRATION NUMBER: Current Controlled Trials No: ISRCTN28090716.

Key words: recurrent miscarriage / uterine natural killer cells / prednisolone

Introduction

Recurrent miscarriage (RM), defined as the loss of three or more consecutive pregnancies, is a distressing condition for patients and clinicians for which there are few treatments of proven efficacy. All Cochrane reviews of RM suggest that further trials are needed (Empson *et al*., 2005; Porter *et al*., 2006; Hass and Ramsey, 2008; Kaandorp *et al*., 2009; Kowalik *et al*., 2011). An endometrial contribution to RM has been frequently suggested, however, there are no treatments of proven efficacy based on endometrial pathology. The involvement of an immune mechanism in RM has also been proposed. However, trials of immunotherapy in women with idiopathic RM have not demonstrated benefit in improving subsequent live birth rates in these women (Porter *et al*., 2006; Stephenson *et al*., 2010). One potential solution to this situation would be a test that identifies the subgroup of women with RM that could benefit from treatment (Porter *et al*., 2006).

Uterine natural killer (uNK) cells have been postulated to play a role in reproductive failure, including RM, owing to the temporal and spatial distribution of these cells. They form the largest population of leucocytes in the endometrium, varying in density throughout the menstrual cycle (Bulmer *et al*., 1991). UNK cells increase in density towards the mid-luteal phase and, if implantation occurs, they peak in density in the first trimester of pregnancy. UNK cells are detected near endometrial glands and blood vessels, and are adjacent to trophoblast cells in early pregnancy. Direct antigen-receptor interactions between uNK cells and extravillous trophoblast occur (Moffett-King, 2002) and polymorphisms in the uNK cell receptor and trophoblast antigen have been associated with RM (Hiby *et al*., 2008). A function of uNK cells is to regulate endometrial angiogenesis, an important factor in implantation (Hanna *et al*., 2006; Quenby *et al*., 2009).

Many studies have associated a high density of uNK cells with RM (Lachapelle *et al*., 1996; Clifford *et al*., 1999; Quenby *et al*., 1999; 2005, 2009; Tuckerman *et al*., 2007). However, it remains debatable whether high uNK cell density is associated with subsequent adverse pregnancy outcomes. Our systematic review of this association found only two studies that reported pregnancy outcomes, with small numbers of women in each study (Tang *et al*., 2011). The evidence that high levels of uNK cells are predictive of subsequent miscarriage in idiopathic RM remains inconclusive.

UNK cells express glucocorticoid receptors (Henderson *et al*., 2003). A prospective study carried out to investigate the effect of steroids on uNK cells showed a significant decrease in uNK cell density in women with RM after 3 weeks of prednisolone treatment (Quenby *et al*., 2005). Prednisolone was chosen as the placenta metabolizes it via 11 beta-hydroxysteroid dehydrogenase 2. Thus, very little of the drug administered orally reaches the fetus (Addison *et al*., 1993). Furthermore, prednisolone has been given in the first trimester of pregnancy to many women, with asthma (Nelson-Piercy, 2001), rheumatoid arthritis (Ostensen, 2001) and hyperemesis gravidarum (Yost *et al*., 2003) with no ill effects, even in recent trials with high-quality post-natal follow-up (Nelson-Piercy *et al*., 2001; Miller *et al*., 2004).

Given the limited evidence about the efficacy of prednisolone in RM further trials are needed. A large trial with adequate sample size is needed to test whether prednisolone, when given to women with a high density of uNK cells, has an impact on important clinical outcomes. Such a large study would face a range of obstacles and this feasibility study was designed to address some of these before designing a large definitive trial. Our aim was to assess whether women with RM would attend for a screening visit consisting of an endometrial biopsy to assess uNK cell density and, if screen positive, return when pregnant for randomization to placebo or prednisolone. We also aimed to assess women's perceptions of the trial and whether prednisolone, which is known to have side effects, would be tolerated in pregnant women with RM.

Methods

Study population

The trial was advertised nationally in the UK using conference lectures, newspaper, radio and television media, trial website and the Miscarriage Association website and locally. The women then discussed the trial with their local consultant or general practitioner who were persuaded by the women to refer them to one of two research clinics run by a single investigator (SQ). Recruitment into the study occurred between August 2008 and August 2010. Women who were <40 years old and had three or more consecutive miscarriages were eligible for this study. The exclusion criteria for recruitment were pregnancy or a known factor that could be contributory to RM. These factors were antiphospholipid syndrome (two positive tests for immunoglobulin (Ig)G or IgM anticardiolipin antibodies or lupus anticoagulant), abnormal parental karyotype, known congenital uterine abnormality (septate or bicornuate uterus), abnormal thyroid function tests or a positive thrombophilia screen (factor V Leiden mutation, activated protein C resistance, prothrombin G20210A mutation, protein C or S deficiency or antithrombin deficiency) (RCOG, 2011). Women were also not eligible if there was a

contraindication to prednisolone treatment, such as hypertension, diabetes mellitus, obesity with a BMI > 35 kg/m^2 or severe mental health problems.

Women's perspective

The women's perspective was assessed with a simple questionnaire asking whether they thought they had an endometrial cause to the miscarriages and their preference of treatment allocation if they had an option to choose one rather than be randomized. This questionnaire was given to 78 consecutive women at the screening visit in the middle phase of the trial (January to December 2009).

Study design and treatment regimes

Women were advised to use barrier methods of contraception for one cycle. They then telephoned the clinic at the time of the urinary LH surge and were given an appointment at one of two research clinics both run by a single investigator, 6–9 days later. At this appointment written informed consent was obtained for screening and women had a

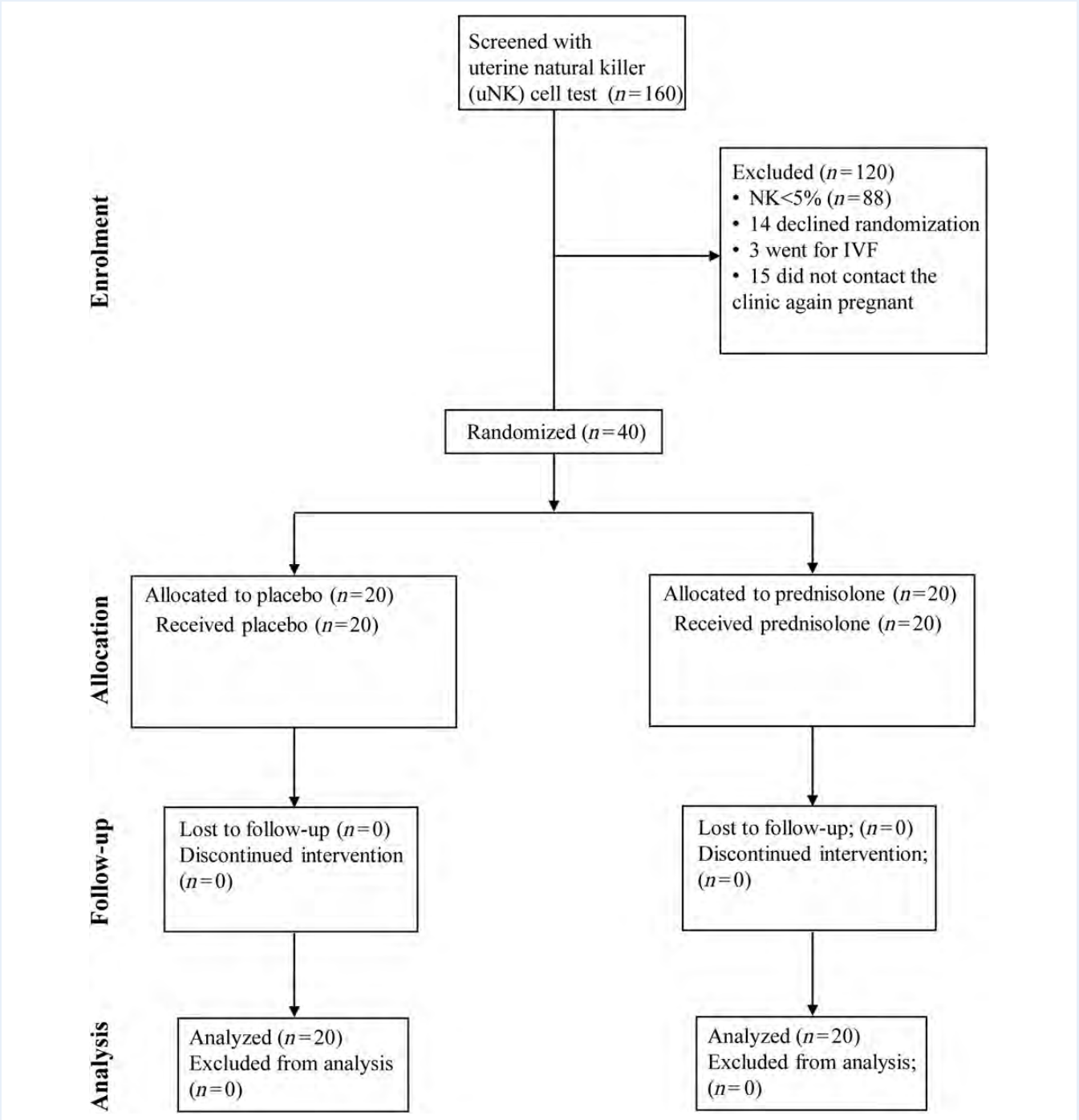

Figure 1 CONSORT statement flow diagram.

transvaginal ultrasound scan of their pelvis and an endometrial biopsy (Wallach endocell sampler) was taken and put in formalin. The biopsy was embedded in paraffin, cut into sections and immunohistochemistry was used to identify uNK cells using an antibody to CD56 (Quenby *et al*., 1999, 2005, 2009). CD56 was used as previous work had found that CD56+ cells discriminated between women with RM and those with normal obstetric histories better than other leucocyte markers, including CD16 (Quenby *et al*., 1999). The uNK cell density was determined as the percentage of uNK cells relative to stromal cells. A cut-off of normality of 5% uNK cell density was used, as determined by previous studies of women with RM compared with those with normal obstetric histories where 5% represented the upper end of the interquartile range of control samples (Quenby *et al*., 1999, 2005, 2009). However, a lower inter- and intra-observer variation is needed for clinical trial entry than for cross-sectional comparative studies published in this area (Clifford *et al*., 1999; Quenby *et al*., 1999, 2005, 2009; Tuckerman *et al*., 2007; Mariee *et al*., 2012). Hence, an improved methodology of uNK cell density assessment was developed specifically for this trial and a standard operating procedure was adhered to. This involved: (i) using the same control samples in each batch to ensure the staining was consistent; (ii) excluding glands and vessels from the count; (iii) only counting CD56+ cells near the epithelial edge as uNK cell density varies with endometrial depth; (iv) counting a minimum of eight randomly selected high powered fields and 5000 stromal cells per patient; (v) defining a CD56-positive cell as one that had a nucleus (CD56 stains the cell surface); (vi) using image analysis and (vii) external quality control advice from Dr Bulmer (Drury *et al*., 2011).

Women were informed of their results by letter and had a further telephone consultation. Women with a normal uNK cell density of <5% were discharged back to standard care, while those with a density of ≥5% uNK cells were advised to contact the clinic again as soon as they were pregnant (4–6 weeks after their last menstrual period) for randomization into the trial.

Pregnancy was confirmed by a urinary test for β-hCG and a transvaginal ultrasound scan was performed. If the pregnancy location was uncertain, women were still invited to be randomized and follow up ultrasound scans were arranged. Written informed consent was obtained for a second time prior to randomization, to either prednisolone or placebo. Randomization was performed using a computerized random number generator in blocks of 20. The allocation list was kept in pharmacy where a dedicated pharmacist dispensed the tablets. Both the trial investigators and women were blinded to the treatment allocation. Both prednisolone (manufactured by Wockhardt UK Ltd) and placebo (manufactured by Quay Pharmaceuticals) tablets were similar in size and colour and dispensed in identical packaging. Women took four tablets a day for 6 weeks, two tablets a day for 1 week and then one tablet a day for the final week. Women were advised to take the tablets early in the morning. The active tablets consisted of 5 mg of prednisolone. Once randomized, patients were reviewed fortnightly with ultrasound scans until 14 weeks gestation. Growth scans were carried out at 28 and 34 weeks gestation, in addition to routine antenatal care. All women were followed up 6 weeks after delivery, either in the clinic or through a telephone consultation. Women who miscarried while taking trial medication were advised to tail off medication slowly, two tablets a day for 3 days then one tablet a day for 3 days. Women were counselled on the options of conservative, medical or surgical management for the miscarriage.

Outcome measures

The main outcomes measures of interest were: recruitment rate for screening and randomization, women's perspective about the trial procedures, intervention and willingness for and compliance with randomization.

Table I Baseline characteristics of women ($n = 160$) with idiopathic RM who were screened for high uNK cell density.

Characteristics	<5% ($n = 88$)	≥5% ($n = 72$)
Age (years)	34 (23–39)	34 (18–29)
% UNK	2.8 (0.2–4.7)	7.7 (5–23)*
Previous live birth	29 (33%)	15 (21%)
Number of previous miscarriages	5 (3–14)	5 (3–15)
Number of previous fetal losses	1.6 (0–7)	1.5 (0–7)
Number of previous empty sac and biochemical losses	3.4 (0–13)	3.3 (0–12)
Previous 2nd trimester miscarriage	2 (2.3%)	5 (6.9%)
Previous ectopic pregnancy	7 (8%)	6 (8.3%)

Data are mean (range) or *n* (%). UNK: uterine natural killer cell.
*Statistically significant $P < 0.01$.

Table II Baseline characteristics of women ($n = 40$) in the pilot RCT who were randomized when pregnant to prednisolone or placebo.

	Prednisolone ($n = 20$)	Placebo ($n = 20$)
Age(years)	34 (25–39)	33 (27–39)
% UNK	8.3 (5–22.8)	7.2 (5–18.3)
Previous live birth	4 (20%)	3 (15%)
Number of previous miscarriages	4 (3–8)	5 (3–15)
Number of previous fetal loses	1.05 (0–4)	1.35 (0–5)
Number of previous empty sac and biochemical losses	3.3 (1–6)	3.8 (1–10)
Previous 2nd trimester miscarriage	0	2 (10%)
Previous ectopic pregnancy	1 (5%)	2 (10%)
BMI (kg/m^2)	26 (20–34)	26 (21–32)
Trial pregnancy (*n*)		
Folic Acid	20	19
−400 mcg	15	17
−5 mg	5	2
Low-dose aspirin	4	5
Sac present at randomization	15	17
Fetal heartbeat present at randomization	3	1

Data are mean (range) or *n* (%). All comparisons between groups NS.

All randomized women had compliance to medication assessed through a daily medication log and side effects were assessed using a proforma completed at the end of first trimester during a clinic review. A diary was also completed by the woman whilst taking the trial medication. The women were asked to record any unusual symptoms, hospital admissions or side effects.

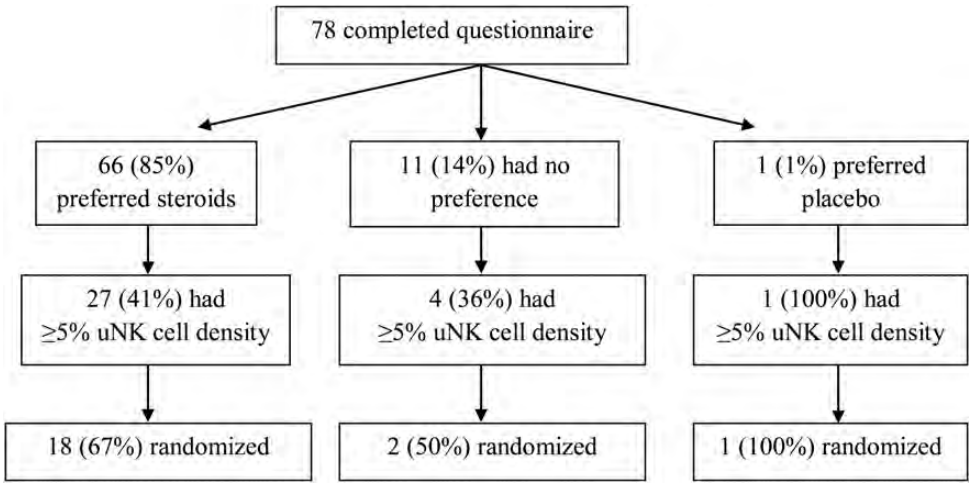

Figure 2 Women's perspective of the trial.

Clinical outcomes were live birth, mode of delivery, gestational age at delivery, congenital or neonatal abnormality, and pregnancy complications including intrauterine growth restriction (IUGR) (<5th centile of customized birthweight chart), macrosomia (>90th centile of customized birthweight chart), gestational hypertension, pre-eclampsia and gestational diabetes mellitus. The outcomes for miscarriages included: gestation week at which miscarriage occurred; type of miscarriage (biochemical loss; sac loss or fetal loss as defined by the European Society of Human Reproduction and Embryology (Farquharson *et al*., 2005)); karyotype of miscarried pregnancies and intrauterine fetal death (fetal death after 24 weeks gestation).

Case report forms (CRF) were completed at 14 weeks' gestation, after delivery and 6 weeks after delivery. Completed CRFs were given to an independent research administrator who entered the information on a database and kept them as confidential trial documentation. Reports were generated to the data monitoring committee (DMC) as necessary.

Study oversight

The approval to conduct the trial was granted by the Liverpool Local Research Committee, and Medicines and Healthcare products Regulatory Agency. The trial was included on the National Institute for Health Research (NIHR) Clinical Research Portfolio (NIHR CRN ID: 6567) and was registered with the European and international clinical trials database (EUDRACT No: 2005-003307-36, ISRCTN28090716). The trial was co-sponsored by Liverpool Women's NHS Foundation Trust and the University of Liverpool. The trial protocol was published (Tang *et al*., 2009). A trial steering committee and trial management group supervised and managed the trial and was responsible for approving the core protocol and any subsequent amendments. They met every 6 months. A DMC which was independent of the trial investigators provided independent review of unblinded data after 1 year of recruitment to ensure that no harm was caused by the treatment. The accuracy of CRFs to the case notes was intermittently checked by the sponsors of the trial at random. There was also double entry data management to minimize human error.

Sample size

As this study involved a controversial area with novel methodology, the feasibility of this trial RCT was uncertain. Hence a significant number of women needed to be recruited to prove feasibility. We obtained funding for 2 years that was sufficient to screen 150 women, and randomize 40 women. The trial was not designed to test efficacy or safety of the intervention but was designed to demonstrate feasibility.

Statistical analysis

Data were cleaned prior to any analysis. The proportion of women who screened positive for a high uNK cell density and returned for randomization into the trial was recorded. Their willingness for randomization was also assessed using the screening visit questionnaire. Demographic information for all women screened was tabulated. Similarly, summary statistics of demographic information relating to the allocation groups of women who were randomized were tabulated and examined to ensure the treatment and placebo groups were similar. Compliance with medication and side effects of steroids were evaluated. All adverse events were also noted according to allocation group. Clinical outcomes of live birth rate in each group were expressed as a risk ratio (RR) with 95% confidence intervals (CIs). Other outcomes, such as the rates of miscarriage, type of miscarriage (biochemical, sac or fetal loss), karyotype of miscarried pregnancies, gestational age at delivery, mode of delivery and pregnancy complications, were tabulated.

Results

Feasibility of recruitment

In the 2-year period 160 eligible women consented to be screened for uNK cell density (Fig. 1) and 40 women were randomized. Hence the recruitment rate met the planned targets. Of these 160 women, 72 (45%) were found to have ≥5% uNK cells, and were suitable to be randomized to either prednisolone or placebo when pregnant. Eighty-eight women were informed of the normal results and discharged back for standard care. Of the 32 women with ≥5% uNK cells and not randomized, 14 conceived but declined randomization, 3 decided to seek assisted reproduction and 15 women did not contact the clinic again because they did not conceive before the trial ended.

The demographic characteristics of women in both screen positive (≥5% uNK) and screen negative groups were similar (Table I). The

demographic characteristics of those who agreed to be randomized were similar to those who were not, as seen by comparing Tables I and II. The demographic characteristics of the patients randomized to placebo were similar to those randomized to prednisolone (Table II).

Patient perspective

Almost half the women who attended for screening (78/160, 49%) were given and completed a questionnaire. Fifty-three (68%) women thought they had abnormal uNK cells, and thus attended screening. There was little relationship between the patient's perception of their result and their actual result: 23 of 53 (43%) who thought they had high uNK cell density did so and 7 of 17 (41%) who thought they had normal uNK cell density did so. Sixty-six (85%) women wanted prednisolone treatment if they were given an option (Fig. 2). Many commented that the desire for a child far outweighed the experience of side effects and potential risk of any empirical treatment. Of those wanting active medication, 27 (41%) were found to have high uNK cells and 18 of the 27 (67%) were randomized. Eleven (14%) women had no preference for either group and four (36%) were found to have high uNK cells. Of these four women, two (50%) were randomized. Only one woman preferred to have placebo because of a fear of side effects from prednisolone. She was found to have a high uNK cell density, and returned for randomization when pregnant.

Table III Side effects profile of women who were randomized in the trial.

Side effects (*n* (%))	Prednisolone (*n* = 20)	Placebo (*n* = 20)	Relative risk (95% CI)
Acne	5 (25%)	2 (10%)	2.5 (0.5–11.4)
Bruising	0	0	–
Flushing	4 (20%)	3 (15%)	1.3 (0.3–5.2)
Gastrointestinal problems (Reflux)	5 (25%)	3 (15%)	1.6 (0.4–6.0)
Infections	0	0	–
Insomnia	7 (35%)	1 (5%)	7.0 (0.9–51.0)
Joint pain	0	2 (10%)	0.2 (0.01–3.9)
Mood changes	3 (15%)	2 (10%)	1.3 (0.3–8.0)
Others	8 (40%)	4 (20%)	2.0 (0.7–5.9)

Acceptability of trial medication

Side effects were more commonly reported in the 20 women in the prednisolone group compared with the placebo group (Table III). However, none were severe enough for women to stop taking medication and compliance with treatment was reported to be 100%. Women were advised to discontinue treatment when a miscarriage was confirmed. Despite the fact that women were advised to take the tablets early in the morning, the most common side effect was insomnia (Table III). There were no reports of bruising or infection. In the patient diaries, eight women in the prednisolone group reported additional side effects. Two women reported increased appetite, one headache and hallucinations, one reported palpitations, one reported hirsutism, one worsening of her irritable bowel syndrome and two complained of nausea. In the placebo group, two complained of side effects (one headache and one bloating). There were no suspected unexpected serious adverse reactions (SUSAR).

Clinical outcomes

The live birth rate was 12/20 (60%) in the prednisolone group and 8/20 (40%) in the placebo group, and this was not significant (RR 1.5, 95% CI 0.8–2.9) (Table IV). There was only one pre-term delivery of <37 weeks in the prednisolone group, and all others delivered at term. The Caesarean section rate was high in both groups but the reason for this was not clear. The mean birthweight was similar in the

Table IV Clinical outcomes of women randomized in the trial.

	Prednisolone (*n* = 20)	Placebo (*n* = 20)	Relative risk (95% CI)
Live birth *n* (%)	12 (60)	8 (40)	1.5 (0.8–2.9)
Delivery at <37 weeks *n* (%)	1 (8.3)	0	3.00 (0.1–69.5)
Vaginal Delivery (%)	3 (25)	4 (50)	0.75 (0.2–2.9)
Caesarean Section Delivery (%)	9 (75)	4 (50)	2.25 (0.8–6.1)
Elective	2	1	
Emergency	7	3	
Birthweight (g) mean (range)	3522 (2350–4110)	3547 (3176–3970)	
Admission to Neonatal Unit *n* (%)	1 (8.3)	1 (12.5)	1.00 (0.07–14.9)
Miscarriages *n* (%)	8 (40)	12 (60)	0.67 (0.4–1.3)
Biochemical Loss	2	1	
Sac Loss	2	3	
Fetal Losses	4	6	
Normal karyotype	2	2	
Trisomy 22	1	1	
Ectopic (treated with methotrexate)	0	2	

two groups, with no pregnancy complications of IUGR or macrosomia. There were also no reports of gestational hypertension, pre-eclampsia or gestational diabetes in either group. There were no adverse fetal outcomes. In one baby in the prednisolone group mild hearing problems were detected post-natally and in one baby in the placebo group renal tract abnormalities were detected at an antenatal check. Both the admissions to neonatal unit were for observation only and both babies were discharged home with the mother. The baby from the prednisolone group had mild jaundice and hypoglycaemia and the baby in the placebo group was treated with antibiotics for the renal tract abnormality.

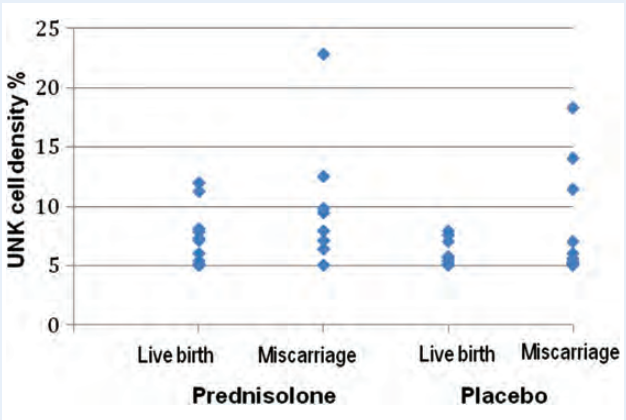

Figure 3 UNK cell density in the sub-epithelial layer of endometrium and pregnancy outcome in women with RM.

Table V Forrest plot of live birth rates in the control group in other similar recent trials compared with the present pilot RCT.

Author	Date	Patients	Control	Live birth rate (95% CI)
Current pilot RCT		Raised uNK cell density	Placebo	40%
Visser *et al.*	2011	Idiopathic	Aspirin	64%
Stephenson *et al.*	2010	Secondary recurrent miscarriage	Placebo	63%
Kaandorp *et al.*	2010	Idiopathic and thrombophilia	Placebo	67%
Clarke *et al.*	2010	Idiopathic and thrombophilia	Ultrasound scans	80%
Laskin *et al.*	2009	Thrombophilia	Aspirin	69%
El-Zibdeh	2005	Idiopathic	Placebo	71%
Total other trials				72%

There were 8/20 (40%) miscarriages in the prednisolone group and 12/20 (60%) miscarriages in the placebo group (Table IV). There were similar numbers of biochemical, sac and fetal losses in both groups. Karyotypes of six miscarriages were obtained, three in each group, and 67% were normal in each group. Products of conception were not readily available for the other miscarriages as most women chose to have conservative or medical management of the miscarriage. Women with a very high uNK density (>10%) had poor outcomes, with 3/3 on placebo miscarrying and 2/4 on prednisolone miscarrying (Fig. 3). In this feasibility trial, the live birth rate in the placebo group was low compared with that in the control group of other RCTs in similar populations of women but this finding did not reach statistical significance (Table V).

Discussion

The lack of proven efficacy in current RCTs of miscarriage prevention suggests that a new approach to RM is needed. This trial has found that a stratification and treatment trial based upon endometrial uNK cell density is feasible and acceptable to the patients thus allowing new approaches to the management of RM to be developed. Enthusiasm of women for the trial was demonstrated by the fact that some women travelled from all over the UK to participate in this study, funding their own transport both for screening and randomization visits. Despite the fact that the majority of screen positive women had a preference for the treatment arm, over half returned to be randomized to that or placebo. It was the women's commitment to this trial that enabled us to complete the planned recruitment with the limited budget provided in the pre-agreed time frame. Although side effects were reported by the women, compliance with treatment was reported to be 100% with no discontinuation because of these side effects. Reassuringly, there were also no SUSARs reported from treatment with prednisolone.

There has been a series of publications providing convincing evidence for a defect in endometrial stromal cell differentiation in RM (Salker *et al.*, 2010, 2011). This defective decidualization correlates with a uNK cell density >5% and a lack of 11-β hydroxyl steroid dehydrogenase type 1 in stromal cells (Kuroda *et al.*, 2012). 11-β hydroxyl steroid dehydrogenase type 1 actives cortisol, hence administration of the glucocorticoid prednisolone may overcome this deficit. Furthermore, prednisolone reduced endometrial uNK cell density, the production of angiogenic cytokines known to be secreted by uNK cells and endometrial angiogenesis (Quenby *et al.*, 2009; Lash *et al.*, 2011). Hence we argue that a new approach is needed for effective treatment for RM, one based on stratification of risk using an endometrial test and then treatment. Furthermore, we have demonstrated that it is feasible to fully test this new approach with high-quality RCTs.

In contrast, recently published high-quality RCTs have been pragmatic and have failed to demonstrate effective prevention of subsequent miscarriage using heparin and/or aspirin (Laskin *et al.*, 2009; Clark *et al.*, 2010; Kaandorp *et al.*, 2010; Visser *et al*, 2011) and intravenous immunoglobulin G (Stephenson *et al.*, 2010; Ata *et al.*, 2011). This lack of proven efficacy may be related to the tradition of stratifying women according to the clinical history. This means that the participants can be at high or low risk of subsequent miscarriage when randomized in

the trials. Thus any possible differential treatment effect related to risk status is diluted by the pragmatic nature of the trials.

This trial has a number of limitations. Importantly, as a feasibility study the trial was not powered to test the efficacy or safety of prednisolone. There were some problems with the trial design that should be addressed in future trials of this nature. The aim of the trial was to treat women who had an endometrial component associated with their pregnancy losses. Hence it was logical to include women with a history of very early or biochemical losses. However, the location of the pregnancy is uncertain in biochemical losses, as they could have been tubal pregnancies. Thus, including women with biochemical losses and those with previous ectopic pregnancies may explain the ectopic pregnancies in women in this trial (3/40). The trial protocol stated that we would initiate the medication at '4–6 weeks gestation'. This led to inconsistency in start date of the trial medication, four women (two in each group) started immediately after a positive pregnancy test at 4 weeks gestation, others started after an ultrasound scan revealed an intrauterine sac at 4–5 weeks' gestation (18 in each group). This meant that some women were included (ectopic pregnancy, biochemical pregnancy) in whom the active treatment could not have prevented their loss. Furthermore some women were randomized before (15 prednisolone, 17 placebo) and others after (3 prednisolone, 1 placebo) a fetal heart was visualized. In all cases who were randomized before the fetal heart was visualized, the gestation sac size was consistent with the last menstrual period date. In future trials we recommend that the time of entry to the trial is more consistent. There was a low rate of karyotype results from the miscarriages (30%) for the following reasons: some women had biochemical miscarriages, with no tissue for karyotyping, some had medical and conservative management for their miscarriage. We could not determine what type of pregnancy loss prednisolone could potentially prevent, as the number of losses in each category was too small. The multiplicity of outcomes meant that none would reach a significance threshold of 0.01 needed for all outcomes even in a larger trial. Hence in future trials we recommend that the primary outcome measure is live birth rate after 24 weeks' gestation. The limited financial resources meant that it was not possible to follow up obstetric outcomes in all the women screened and this meant that important information was lost.

Currently, uNK cell density assessment varies greatly from laboratory to laboratory and there is also some variation between menstrual cycles (Mariee *et al.*, 2012). It is important to realize that many laboratories undertaking uNK cell density assessment use different methodologies, so the cut-off of normality cannot be compared between publications. Indeed there are insufficient prospective data published to validate uNK cell density as a clinical test (Tang *et al.*, 2011). Mariee *et al.* (2012), Tuckerman *et al.* (2007) and Clifford *et al.* (1999) counted CD56+ cells in the deeper layers of the endometrium where CD56+ cell density is considerably higher and the variation greater than in the sub-epithelial region used in the current trial. This trial has allowed a re-assessment of the uNK cell density test. For the test to select women who need treatment the live birth rate in the screen positive and placebo group needs to be lower than that expected in trials involving similar women. In this feasibility trial, the live birth rate in the placebo group was 40% (CI 12%, 68%), hence lower than the live birth rate in the control group of other recently published RCTs in women with idiopathic RM (Table V). However, the current trial was not of sufficient size for this finding to reach significance (Table V). Hence larger trials are needed to confirm or refute the clinical utility of the uNK cell density assessment. Women with very high density of uNK cells (>10%) appeared to have worse outcomes than those with density of 5–10% (Fig. 3) again suggesting that the test has value in stratification of pregnancy outcome. Hence for clinical research on uNK cells to be assessed and for uNK cell density to be used clinically an international standardization is urgently needed and this issue is currently being addressed. Importantly, in this trial the uNK cell density was assessed in a single laboratory to ensure consistency.

The live birth rate was 12/20 (60%) with prednisolone and 8/20 (40%) with placebo (RR 1.5, 95% CI 0.8–2.9, absolute difference 20% CI −10%, +50%). This is a preliminary observation that needs to be interpreted with caution as it does not demonstrate efficacy. Larger trials are needed to demonstrate efficacy and safety.

In conclusion, this pilot RCT has demonstrated that it is feasible and acceptable to the patients to perform an endometrial-based, screen and treat, double-blind, randomized, placebo-controlled trial in women with RM. In the context of idiopathic RM with no other treatments of proven efficacy, we suggest that stratification of pregnancy outcome using an endometrial-based test then randomization to treatment is a potentially important strategy. We have demonstrated that patient willingness for randomization is not a barrier to further trials.

Acknowledgements

The authors thank all the women who took part. They also thank Liverpool Women's Hospital staff, particularly, Diane Manifold for her invaluable administrative support to the patients for this study, Lisa Heathcote for technical assistance with sample processing and immunohistochemistry, Pharmacy department and research and development department, Dr J Bulmer for Newcastle University for providing quality control advice regarding uNK cell testing, The Miscarriage Association and Ruth Bender-Atik for providing advise, independent patient support and advertising the study and also Prof. A. Sheenan, chair of the DMC.

Authors' roles

A.-W.T: principle investigator, Liverpool; Z.A.: member of trial management group (TMG) and trial steering committee (TSC); M.A.T.: member of TSC; J.A.D.: member of TMG and co-ordinator of laboratory analysis; R.S.: principle investigator, Birmingham; S.Q.: chief investigator.

Funding

The Moulton Charitable Foundation provided funding for the study.

Conflict of interest

None declared.

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