

**A Thesis Submitted for the Degree of MD at the University of Warwick**

**Permanent WRAP URL:**

<http://wrap.warwick.ac.uk/153049>

**Copyright and reuse:**

This thesis is made available online and is protected by original copyright.

Please scroll down to view the document itself.

Please refer to the repository record for this item for information to help you to cite it.

Our policy information is available from the repository home page.

For more information, please contact the WRAP Team at: [wrap@warwick.ac.uk](mailto:wrap@warwick.ac.uk)



The effect of endometrial scratch on  
pregnancy outcomes in women with  
recurrent pregnancy loss

**Valarmathy Kandavel**

Submitted to the University of Warwick

Division of biomedical sciences

Warwick Medical School

**October 2020**

## Table of Contents

Table of Contents .....	2
List of Tables and Figures .....	12
List of Figures .....	12
List of Tables .....	13
Acknowledgments .....	14
Dedication .....	16
Declaration .....	17
Abstract .....	18
Abbreviations .....	19
Chapter 1: .....	24
Chapter 1: Introduction .....	25
1.1 Definition: .....	25
1.1.1 Epidemiology: .....	25
1.1.2 Burden of Miscarriage: .....	26
1.1.3 Terminologies Used: .....	26
1.1.4 Types of Miscarriage: .....	27
1.2 Recurrent Pregnancy Loss (RPL) .....	28
1.2.1 Definition: .....	28
1.2.2 Incidence: .....	29
1.3 Non-modifiable Risk Factors: .....	29
1.3.1 Previous Miscarriages: .....	29
1.3.2 Genetic Factors: .....	30
1.3.3 Maternal Age: .....	32
1.4 Lifestyle Factors and Modification: .....	32
1.4.1 Maternal BMI: .....	32
1.4.2 Smoking: .....	33
1.4.3 Caffeine Intake: .....	33
1.4.4 Alcohol: .....	34
1.4.5 Stress: .....	34
1.4.6 Exercise: .....	34
1.4.7 Use of E-cigarettes: .....	34
1.4.8 Pre-conception Options: .....	35
1.5 Conditions Associated with RPL: .....	36
1.5.1 Acquired Thrombophilia .....	36
1.5.2 Hereditary Thrombophilias: .....	38
1.5.3 Endocrine Factors: .....	38

1.6 Unexplained Recurrent Pregnancy Loss:.....	40
1.7 Possible Treatment Considerations for Unexplained RPL: .....	40
1.7.1 Progesterone:.....	41
1.7.2 Heparin:.....	43
1.7.3 Aspirin: .....	44
1.7.4 Steroids: .....	44
1.7.5 Immunotherapy:.....	45
1.7.6 Use of Granulocyte-Colony Stimulating Factor (G-CSF): .....	48
1.7.7 Use of Human Chorionic Gonadotropin (hCG): .....	49
1.7.8 Bromocriptine: .....	50
1.8 Psychological Impact of RPL: .....	50
1.9 Dedicated Recurrent Pregnancy Loss Clinics: .....	51
1.9.1 Ultrasound in Early Pregnancy for Reassurance:.....	52
1.9.2 Research Participation in Dedicated Clinics: .....	53
1.9.3 Supportive Care:.....	53
<b>Chapter 2</b> .....	<b>56</b>
Chapter 2: Endometrium .....	57
2.1 Introduction: .....	57
2.2 Implantation: .....	57
2.3 RPL and Endometrium:.....	59
2.4 Uterine Natural Killer (uNK) Cells and RPL:.....	61
2.5 Endometrial Scratch (ES):.....	64
2.6 Mechanism of a Possible Beneficial Effect of Scratch:.....	64
2.6.1 Stem Cell Recruitment:.....	64
2.6.2 Improved Decidualisation: .....	65
2.6.3 Synchronicity Hypotheses: .....	65
2.6.4 Inflammation Hypothesis: .....	65
2.6.5 Immune System Cell Recruitment:.....	66
2.6.6 Hypothesis Concerning Events Accompanying Wound Healing:.....	67
2.6.7 Gene Expression Theory:.....	67
2.6.8 The Neoangiogenesis Theory: .....	68
2.6.9 Clinical Correlation: .....	68
2.7 Role of Endometrial Scratch in In-Vitro Fertilisation (IVF): .....	68
2.7.1 Method of Injury:.....	74
2.7.3 Impact on miscarriages: .....	75
2.7.4 Complications:.....	75
2.8 Interpretation of IVF Data:.....	76

2.9 Endometrial Scratch and RPL:.....	76
Conclusion:.....	77
<b>Chapter 3</b> .....	<b>78</b>
Chapter 3: Trial Planning and Set Up.....	79
3.1 Hypothesis:.....	79
3.1.1 Aims and Objectives:.....	79
3.2 Research Question:.....	79
3.2.1 Research Design and Methods:.....	80
3.2.2 Feasibility Study:.....	80
3.3 Trial Methods- Randomised Controlled Trial.....	80
3.3.1 Patients:.....	80
3.3.2 Set-up:.....	81
3.3.3 My Role in the Trial:.....	81
3.3.4 Recruitment:.....	81
3.3.5 Transvaginal Ultrasound Scan:.....	82
3.3.6 Speculum Examination:.....	82
3.3.7 Phase of the Cycle:.....	82
3.3.8 Additional Considerations for Sampling in Luteal Phase-Pregnancy:.....	83
3.3.9 Intervention Arm- Endometrial Scratch:.....	83
3.4 Ethical Considerations in the Control Arm:.....	84
3.4.1 Need for a Control Arm:.....	84
3.4.2 What Would Mimic Endometrial Scratch in the Control Arm?.....	84
3.5 Patient and Public Involvement:.....	84
3.5.1 Question and Method of Testing:.....	85
3.5.2 Setting for Questionnaire Testing:.....	85
3.5.3 Outcome from the Questionnaire for Sham Procedure:.....	86
3.6 Blinding in the Trial:.....	87
• Methods for Ensuring Blinding:.....	87
• Methods for Unblinding the Trial:.....	87
3.7 Methods and Assessments:.....	87
3.7.1 Schedule of Delivery of Intervention and Data Collection:.....	87
3.7.2 Outcome Measure Assessment:.....	88
3.8 Power and Sample Size:.....	89
3.8.1 Statistical Analysis of Efficacy and Harms:.....	89
3.9 Development of Supporting Documents for the Trial:.....	90
3.9.1 Protocol:.....	90
3.9.2 Patient information sheet (PIS):.....	90

3.9.3 Poster: .....	90
3.9.4 GP letter: .....	90
3.9.5 Letter of invitation: .....	90
3.9.6 Consent form: .....	91
3.9.7 Telephone interview questionnaire: .....	91
3.9.8 Questionnaire for assessing acceptability: .....	91
3.9.9 Case Report Form (CRF): .....	91
Adverse event form (AE): .....	92
Conclusion: .....	92
<b>Chapter 4</b> .....	<b>93</b>
Chapter 4: Trial Methods .....	94
4.1 Study Summary: .....	94
4.1.1 Outcome measures: .....	95
4.1.2 Study details: .....	95
4.2 Trial Organisation and Oversight: .....	95
4.2.1 Sponsor and Governance Arrangements: .....	95
4.2.2 Regulatory Authorities/Ethical Approval: .....	95
4.2.3 Research Ethics Committee (REC) Review: .....	96
4.4 Recruitment and Randomisation: .....	98
4.4.1 Eligibility Criteria: .....	98
4.4.2 Recruitment: .....	99
4.4.3 Consent Visit: .....	99
4.5 Randomisation: .....	100
4.5.1 Randomisation Visit: .....	100
4.5.2 Data Management: .....	101
4.5.3 Post-Randomisation Withdrawals and Exclusions: .....	101
4.6 Trial Details .....	101
4.6.1 Outcome Measures: .....	101
4.6.2 Pathways for Outcomes in the Trial: .....	102
4.6.3 Laboratory Assessments: .....	105
4.6.4 Adverse Event Management: .....	105
4.7 Data Management: .....	107
4.7.1 Data Collection and Management: .....	107
4.7.2 Database: .....	108
4.7.3 Data Storage: .....	108
4.7.4 Archiving: .....	108
4.7.5 Administration: .....	108

<b>Chapter 5</b> .....	109
Chapter 5: Trial Management.....	110
5.1 Roles and Responsibilities:.....	110
5.1.1 Trial Sponsor:.....	110
5.1.2 Chief Investigator:.....	110
5.1.3 Trial Co-ordinator:.....	111
5.2 Trial Submission: .....	112
5.2.1 Feedback from Ethics Committee:.....	112
5.2.2 Trial Registration: .....	113
5.2.3 Site Initiation Visit: .....	113
5.2.4 Laboratory procedures: .....	114
5.3 Trial Monitoring: .....	114
5.3.1 Trial Management Committee: .....	114
5.3.2 Internal Audit: .....	115
5.3.3 Trial Management Group (TMG): .....	115
5.3.4 Audit and Inspection:.....	116
5.4 Trial Issues and Challenges:.....	116
5.4.1 Change of the Lead Midwife:.....	116
5.4.2 Recruitment issues:.....	117
5.4.3 Process Issue -Ovulation test kits:.....	117
5.4.4 Patient who Conceived in the Same Cycle of Randomisation: .....	118
5.4.5 Adverse Events and Serious Adverse Events in the Trial:.....	119
5.4.6 Issues with Randomisation Envelope:.....	120
5.4.7 Amendments: .....	121
5.5 Lessons Learnt during the Conduct of the Trial: .....	122
<b>Chapter 6</b> .....	123
Chapter 6: Results.....	124
6.1 Trial results: .....	124
6.1.1 Trial Recruitment: .....	124
6.1.2 Trial Design and Conduct: .....	124
6.1.3 CONSORT Diagram SiM.....	125
6.1.4 Trial Enrolment: .....	126
6.1.5 Trial Randomisation:.....	127
6.2 Results:.....	128
6.2.1 Study Population Demographics: .....	128
6.3 Results of Primary Outcome: .....	131
6.3.1 Time to Conception between Pregnant and Non-Pregnant Women:.....	132

6.4 Results of Secondary Outcomes:.....	132
6.4.1 Miscarriages: .....	132
6.4.2 Ectopic pregnancy: .....	133
6.4.3 Pregnancy Complications:.....	133
6.4.4 Trial Acceptability: .....	133
6.5 Results of the Questionnaire Analysis:.....	134
6.5.1 Response: .....	134
6.5.2 Trial Pathway Evaluation Questions:.....	134
• How did you find out about the study? .....	135
• Was the patient information sheet (PIS) helpful? .....	136
• Difficulties encountered during appointment booking? .....	136
• Experience of participating in the study.....	136
6.6 Questions to Evaluate the Procedure Experience:.....	138
6.6.1 Infection:.....	138
6.6.2 Bleeding: .....	138
6.6.3 Pain: .....	139
6.7 Endometrial Results:.....	140
6.8 Limitations of the Questionnaire Analysis: .....	141
6.9 Conclusions from the Questionnaire Analysis:.....	142
<b>Chapter 7</b> .....	<b>144</b>
Chapter 7: Discussion .....	145
7.1 Introduction: .....	145
7.2 Strengths of the Study:.....	145
7.2.1 Patients: .....	145
7.2.2 Intervention:.....	145
7.2.3 Sham Procedure:.....	145
7.2.4 Randomisation:.....	146
7.2.5 Endometrial Factors: .....	146
7.2.6 Emotional Support:.....	146
7.2.7 Primary outcome measure definition: .....	146
7.3 Weaknesses of the Study: .....	147
7.3.1 Superfertility: .....	147
7.3.2 Analysis: .....	148
7.4 The Lessons learnt which can be used for Future Study Design: .....	148
7.4.1 Population Likely to Benefit: .....	148
7.4.2 Outcome Measures: .....	150
7.4.3 Size of the Study: .....	151

7.5 Other learning points from the trial:.....	151
7.5.1 Use of social media platforms: .....	151
<b>Chapter 8</b> .....	152
Chapter 8: Conclusions .....	152
<b>Bibliography</b> .....	154
<b>Appendix 1</b> .....	177
<b>Appendix 2</b> .....	178
Trial Protocol .....	178
TABLE OF CONTENTS for contents of appendix.....	182
List of abbreviations/GLOSSARY.....	185
1. Background .....	187
Epidemiology and burden of the condition.....	187
Existing knowledge .....	187
<b>1.1</b> Hypothesis .....	194
<b>1.2</b> Need for a trial .....	194
<b>1.3</b> Ethical considerations .....	195
<b>1.4</b> CONSORT .....	196
2. Trial Design .....	196
<b>2.1</b> Trial summary and flow diagram .....	196
3. ....	199
<b>3.1</b> Aims and objectives .....	200
3.1.1 Primary objective.....	200
3.1.2 Secondary objective.....	200
<b>3.2</b> Outcome measures .....	200
3.2.1 Efficacy .....	200
3.2.2 Safety .....	200
<b>3.3</b> Eligibility criteria .....	200
3.3.1 Inclusion criteria.....	200
3.3.2 Exclusion criteria .....	201
2.5 Informed consent .....	201
<b>3.6</b> Recruitment and randomisation.....	201
3.6.1 Recruitment .....	201
3.6.2 Randomisation .....	202
2.6.2.1 Post-randomisation withdrawals and exclusions.....	202
<b>3.7</b> Trial treatments / intervention.....	202
3.7.1 Trial treatment(s) / intervention .....	202

3.7.2	Control intervention .....	203
3.7.3	Drug storage and dispensing.....	203
3.7.4	Drug accountability.....	203
3.7.5	Compliance/contamination .....	203
<b>3.8</b>	Blinding .....	204
3.8.1	Methods for ensuring blinding .....	204
3.8.2	Methods for unblinding the trial .....	204
<b>3.9</b>	Concomitant illness and medication .....	204
3.9.1	Concomitant illness.....	204
3.9.2	Concomitant medication .....	204
<b>3.10</b>	End of trial .....	204
<b>4.</b>	METHODs and assessments .....	205
<b>4.1</b>	Schedule of delivery of intervention and data collection.....	205
<b>4.2</b>	Laboratory assessments .....	205
<b>5.</b>	adverse event management.....	206
<b>5.1</b>	Definitions .....	206
5.1.1	Adverse Events (AE) .....	206
5.1.2	Adverse Reaction (AR).....	206
5.1.3	Serious Adverse Events (SAEs) and Suspected Unexpected Serious Adverse Events (SUSARS) .....	206
<b>5.2</b>	Reporting SAEs and SUSARs .....	206
<b>5.3</b>	Procedures in case of overdose .....	207
<b>5.4</b>	Procedures in case of pregnancy .....	207
<b>6.</b>	Data management .....	208
<b>6.1</b>	Data collection and management .....	208
<b>6.2</b>	Database.....	208
<b>6.3</b>	Data storage.....	209
<b>6.4</b>	Data access and quality assurance .....	209
<b>6.5</b>	Archiving.....	209
<b>7.</b>	Statistical analysis .....	209
<b>7.1</b>	Power and sample size .....	209
<b>7.2</b>	Statistical analysis of efficacy and harms .....	210
<b>7.3</b>	Health Economic Evaluation.....	210
<b>8.</b>	Trial organisation and oversight .....	211
<b>8.1</b>	Sponsor and governance arrangements .....	211
<b>8.2</b>	Regulatory authorities/ethical approval .....	211

8.3	Trial Registration .....	211
8.4	Indemnity .....	211
8.5	Trial timetable and milestones .....	211
8.6	Administration .....	211
8.7	Trial Management Group (TMG) .....	211
8.8	Trial Steering Committee (TSC).....	212
8.9	Data Monitoring Committee (DMC).....	212
8.10	Essential Documentation .....	212
9.	Monitoring and Quality assurance of trial procedures .....	212
10.	Patient and Public Involvement (PPI).....	213
11.	Dissemination and publication.....	213
12.	References.....	213
	<b>Appendix 3:</b>	
	Patient Questionnaire: .....	217
	<b>Appendix 4</b> .....	219
	Adverse events form for SiM study- Scratch in Miscarriage study .....	219
	<b>Appendix 5</b> .....	221
	Patient questionnaire for assessment of ‘sham’ procedure .....	221
	<b>Appendix 6</b> .....	222
	Joint poster.....	222
	<b>Appendix 7</b> .....	223
	Letter of invitation for SiM Study .....	223
	<b>Appendix 8</b> .....	225
	Patient information sheet.....	225
	<b>Appendix 9</b> .....	229
	GP LETTER.....	229
	<b>Appendix 10</b> .....	231
	SiM (Scratch in Miscarriage) Study Screening Log .....	231
	<b>Appendix 11</b> .....	233
	SiM Study- Site Signature and Delegation Log.....	233
	<b>Appendix 12</b> .....	235
	Letter to Regional Ethics Committee.....	235
	<b>Appendix 13</b> .....	236
	Amendment Letter to REC .....	236
	<b>Appendix 14</b> .....	238
	Telephone interview questionnaire after live birth for SiM study .....	238

<b>Appendix 15</b> .....	239
Case Report Form (CRF) for the SiM Study .....	239
<b>Appendix 16</b> .....	243
Consent Form .....	243
<b>Appendix 17</b> .....	245
Letter from BJOG.....	245
<b>Appendix 18</b> .....	248
Approval letter from Ethics Committee.....	248
<b>Appendix 19</b> .....	251
Substantial amendment approval letter.....	251

# List of Tables and Figures

## List of Figures

Figure 1. 1 The 'black box' of early pregnancy loss.....	25
Figure 1. 2 Miscarriage and karyotype. Reproduced with permission from (Ogasawara et al., 2000) .....	30
Figure 2. 1 Cyclic Decidualisation of Human endometrium .....	60
Figure 2. 2 Forest plot of comparison: Endometrial injury vs no injury .....	71
Figure 2. 3 Forest plot of comparison: Livebirth per randomly assigned woman .....	72
Figure 2. 4 Forest plot for clinical pregnancy rate (Sar-Shalom Nahshon et al., 2019). The Forest plot showed that the CPR is improved in women who underwent endometrial injury. ....	73
Figure 2. 5 Forest plot for livebirth rate (Sar-Shalom Nahshon et al., 2019). ....	73
Figure 2. 6 Forest plot for miscarriage rate (Sar-Shalom Nahshon et al., 2019). ....	73
Figure 3. 1 Image of a Wallach catheter.....	83
Figure 4. 1 Trial Flow chart .....	97
Figure 4. 2 Pregnancy outcomes flowchart .....	103
Figure 6. 1 Consort diagram of the SiM study .....	125
Figure 6. 2 Enrolment Graph .....	126
Figure 6. 3 Randomisation Graph.....	127
Figure 6. 4 Mean time to conception between women in the two groups .....	130
Figure 6. 5 Distribution of miscarriages between the groups.....	130
Figure 6. 6 Mean time to conception between the pregnant (n=66) and non-pregnant women (n=43). ....	132
Figure 6. 7 Sources of recruitment into the study .....	135
Figure 6. 8 Comparison of reported bleeding between groups.....	139
Figure 6. 9 uNK cell density distribution.....	140
Figure 6. 10 uNK cell density comparison between groups .....	141

## List of Tables

Table 1. 1 Types of non visualised pregnancy loss as defined by ESHRE early pregnancy special interest group (Kolte et al., 2015a).....	27
Table 1. 2 Types of miscarriage as defined by ESHRE early pregnancy special interest group (Kolte et al., 2015a). .....	28
Table 1. 3 Summary of trials and interventions in Immunotherapy.....	47
Table 3. 1 Schedule of delivery of interventions in the trial.....	88
Table 4. 1 Summary of the SiM study .....	94
Table 4. 2 Outcome measures in the study .....	95
Table 4. 3 Eligibility criteria for inclusion and exclusion in the study .....	98
Table 4. 4 Schedule of delivery of care during pregnancy .....	104
Table 6. 1 Table of demographic characteristics .....	129
Table 6. 2 Results of the study .....	131
Table 6. 3 Summary of cytogenetics reported on the miscarriages .....	133

## **Acknowledgments**

I would like to express my sincere thanks and gratitude to my research supervisors Professor Quenby and Professor Brosens.

Professor Quenby has been a guiding beacon and support in helping me understand the research process. She has facilitated my navigation through the process of writing this MD and I will always be grateful for the opportunity to work with her.

Professor Brosens never ceases to amaze me with his sense of humour, scientific acumen and passion for research.

I am indebted to my clinical supervisor Mr. Keay for his flexibility and his mentorship. I hope, one day, to emulate your quick thinking and hard work. I would like to thank Dr. Agrawal who taught me all the practical procedures and is an example of effective communication with the patients. Mr. Ghobara for his surgical training and methodical approach to patient care. The staff in CRM are some of the most talented and brilliant team members that I have had the pleasure to work with.

I would like to acknowledge my friend and colleague Dr. Lokman for her recruitment into the trial during my clinical block and invaluable help. Dr. Tewary who has helped me immensely during the randomisation process. I would like to thank Dr. Lacey for her support in the first year of my post and continued input even after I finished my job.

This trial would not have been possible without the brilliant support team in the BRU. They are an amazing, friendly team with whom I have forged lifelong friendships during the process. My thanks particularly to Debbie Bullen, Lyndsey Prue and Jane Hillen, who filled in when Debbie moved onto a new job. There was so much learning, fun, tears, cakes and teas during this journey. My special thanks to Natalie Morris and Hannah for their file tracking and maintenance.

My special thanks extend to the UHCW research team and Warwick CTU for their support with the trial. I would like to thank all my other friends for their encouragement and steering me in the right path.

My biggest thank you and gratitude extends to all the women who considered our trial and went on to participate. I have had the honour of working with some of the most resilient, strong and brave women who face a difficult situation with such dignity and fortitude. The most humbling experience was to be part of their journey which consisted of hope, joys, tears and courage.

## **Dedication**

I dedicate this thesis to my family.

The love of my life, my children Nethraa and Mukundh. All the words in the world are insufficient to express how much joy, and love they shower upon me with the sacrifices they have endured from mum being absent even during weekends whilst working on the thesis.

My husband Vinod for his support, understanding and childcare.

My dad for believing in me and instilling a strong sense of work ethic and resilience: always leading by example.

My mum, brother and sister-in-law for their love, constant encouragement and a listening ear.

## **Declaration**

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Medicine. It has been composed by myself and has not been submitted in any previous application for any degree.

I, Valarmathy Kandavel, declare that:

1. My research has been conducted ethically and all of the work presented in this thesis, except where specifically stated, was original research performed by myself under the supervision of Professor Siobhan Quenby.
2. Participants were recruited by myself and Professor Quenby. The sample and data collection during the study were performed by myself with the support and help of my colleagues at the University of Warwick and University Hospitals Coventry and Warwickshire NHS Trust.
3. The endometrial biopsies were obtained by myself, Dr. Shreeya Tewary and Dr. Mariam Lokman during the trial with BRU research team members acting as chaperones. The figures used in Chapter 6 are the work of my fellow researcher Dr. Mariam Lokman who studied the biopsy samples obtained from women in the trial. I have used these for the discussion of the results.
4. The data and the results presented are genuine and obtained during my research.
5. I have appropriately acknowledged and referenced within my thesis, where I have drawn on the work, ideas and help of others.
6. The thesis submitted is within the required word limit as specified by the University of Warwick.

## **Abstract**

**Introduction:** Recurrent pregnancy loss (RPL) is defined as two or more consecutive pregnancy losses before 24 weeks of gestation, which affects between 3-5 % of couples. There are psychological, emotional, physical and financial costs associated with miscarriages.

The majority of the losses happen in the first trimester before 13 weeks and are due to chromosomal abnormalities in the embryo. Standard care involves investigations after which around 50% of the couples are labelled as “unexplained RPL” and current management options are limited to supportive care in the next pregnancy. Endometrial factors are being explored to explain and treat this enigmatic condition.

Women with RPL may share a common contributory endometrial factor as women with recurrent implantation failure. Prior to starting this thesis, evidence suggested that women with RIF had improved pregnancy outcomes, if they underwent an endometrial scratch in the cycle preceding embryo transfer. The feasibility study was to explore this option in women with RPL.

**Hypothesis:** Preconception endometrial scratching prevents miscarriage in women with recurrent miscarriage.

**Method:** The **SiM** study (**S**cratch in **M**iscarriage), was a single centre randomised controlled trial of the effects of endometrial scratch on pregnancy outcomes in women with RPL, in women aged 18-42 with two or more previous miscarriages. The women were randomised to either having a luteal phase endometrial scratch or a ‘sham’ procedure.

**Primary outcome:** Livebirth after 24 weeks of gestation in women who conceived within the three cycles following randomisation.

**Results:** The study showed a trend toward improvement of livebirth rate in the endometrial scratch group. However, this did not reach statistical significance.

**Conclusions:** The role of the scratch in improving implantation in some women needs to be explored in a bigger RCT.

## **Abbreviations**

AACE	American Association of Clinical Endocrinologists
ACA	Anti-Cardiolipin Antibody
AE	Adverse Event
ALIFE2	<b>Anticoagulants for living FoEtus</b> in women with recurrent miscarriage and inherited thrombophilia
APC	Antigen Presenting Cells
APS	Anti Phospholipid Syndrome
Array CGH	Array Comparative Genomic Hybridisation
ART	Assisted Reproductive Technology
ASRM	American Society of Reproductive Medicine
ATA	American Thyroid Association
BJOG	British Journal of Obstetrics and Gynaecology
BMI	Body Mass Index
BRU	Biomedical Research Unit
BRU-RH	Biomedical Research Unit – Reproductive Health
CAMs	Cellular Adhesive Molecules
CARE	Care and Reproductive Health study
CD	Cluster of Differentiation
CG	Clinical Guideline
CI	Confidence Interval
CI	Chief Investigator
CONSORT	Consolidated Standards of Reporting Trials
CPR	Clinical Pregnancy Rate
CRF	Case Report Form
CRM	Centre of Reproductive Medicine
CSRL	Clinical Skills Research Laboratory
CTU	Clinical Trials Unit
CUME	Congenital Uterine Malformation by Experts
DCs	Dendritic Cells
DNA	Deoxyribosenucleic Acid
DNA	Did Not Attend

DOH	Department of Health
EAGeR	Effects of Aspirin in Gestation and Reproduction study
EBCOG	European Board and College of Obstetricians and Gynaecology
eMSC	Endometrial Mesenchymal Stem Cell
EPAU	Early Pregnancy Assessment Unit
ES	Endometrial Scratch
ESGE	European Society of Gynaecological Endoscopy
ESHRE	European Society of Human Reproduction and Embryology
ESI	Endometrial Scratch Injury
FET	Frozen Embryo Transfer
FISH	Fluorescence In situ Hybridisation
FU	Follow up
G-CSF	Granulocyte-Colony Stimulating Factor
GCP	Good Clinical Practice
GP	General Practitioner
Groa	Growth-related oncogene a
HB-EGF	Heparin-Binding Endothelial-like growth factor
hCG	Human Chorionic Gonadotrophin
HESCs	Human Endometrial Stromal Cells
HRA	Health Research Authority
HSD	Hydroxysteroid dehydrogenase
HTA	Human Tissue Authority
ICSI	Intracytoplasmic Sperm Injection
IFN	Interferon
IGFBP-1	Insulin-like Growth Factor Binding Protein – 1
IL	Interleukin
IRAS	Integrated Research Application System
IUI	Intrauterine Insemination
IVF	In-Vitro Fertilisation
IVIG	Intravenous Immunoglobulin
LB	Live birth
LBR	Live Birth Rate

LDA	Low Dose Aspirin
LH	Luteinising Hormone
LMWH	Low Molecular Weight Heparin
MDI	Major Depressive Inventory
MHTFR	Methylenetetrahydrofolate Reductase
MIB-1B	Macrophage Inflammatory Protein 1B
MRI	Magnetic Resonance Imaging
MUC-1	Mucin-1
NGS	Next Generation Sequencing
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute of Health Research
Non-CTIMP	Non-clinical Trial of an Investigational Medicinal Product
NRES	National Research Ethics Service
NTDs	Neural Tube Defects
NVP	non-visualised Pregnancy
OI	Ovulation Induction
OPN	Osteopontin
OR	Odds Ratio
PCRI	Positive Reinforcement and Coping Intervention
PET	Pre-eclamptic Toxaemia
PGD	Pre-Implantation Genetic Diagnosis
PGS	Pre-Implantation Genetic Screening
PGT	Pre-Implantation Genetic Testing
PI	Principal Investigator
PID	Pelvic Inflammatory Disease
PIS	Patient Information Sheet
PRISM	PRogesterone in Spontaneous Miscarriage Trial
PROMISE	A randomized trial of progesterone in women with recurrent miscarriages
PSP	Priority Setting Partnerships
PSS	Perceived Stress Scale
PTSD	Post-Traumatic Stress Disorder

PUL	Pregnancy of unknown location
RCOG	Royal College of Obstetricians and Gynaecologists
RCT	Randomised Controlled Trial
R & D	Research and Development
REC	Research Ethics Committee
RESPONSE	A multi-centre, placebo-controlled study to evaluate NT100 in pregnant women with a history of unexplained recurrent pregnancy loss
RIF	Recurrent Implantation Failure
RM	Recurrent miscarriage
RMC	Recurrent Miscarriage Clinic
RPL	Recurrent Pregnancy Loss
RR	Relative Risk
SA	Substantial Amendment
SAE	Serious Adverse Event
SCH	Subclinical Hypothyroidism
SCRaTCH	Endometrial scratching in women with implantation failure after a first IVF/ICSI cycle; does it lead to a higher live birth rate? A RCT
SiM	<b>S</b> cratch in <b>M</b> iscarriage trial
SGA	Small Gestational Age
SOP	Standard Operating Procedure
SP (cells)	Side Population of bone marrow cells
SPIRIT	Standard Protocol Items: Recommendations for Interventional Trials
TABLET	Randomised controlled trial of the efficacy and mechanism of levothyroxine treatment on pregnancy and neonatal outcomes in women with thyroid antibodies
Th	T-helper cells
TMC	Trial Management Committee
TMG	Trial Management Group
TNF $\alpha$	Tumour Necrosis Factor $\alpha$
TPO	Thyroid Peroxidase
TROPHY	Hysteroscopy in recurrent in-vitro fertilisation failure trial
TRUST	The randomised uterine septum transection trial

TSH	Thyroid-stimulating Hormone
TTP	Time to Pregnancy
TVS	Transvaginal Ultrasound
UHCW	University Hospital Coventry and Warwickshire NHS Trust
uNK	uterine Natural Killer cell
UK	United Kingdom
USA	United States of America
USS	Ultrasound Scan
3D-USS	3-Dimensional ultrasound scan
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organisation

# Chapter 1:

# Chapter 1: Introduction

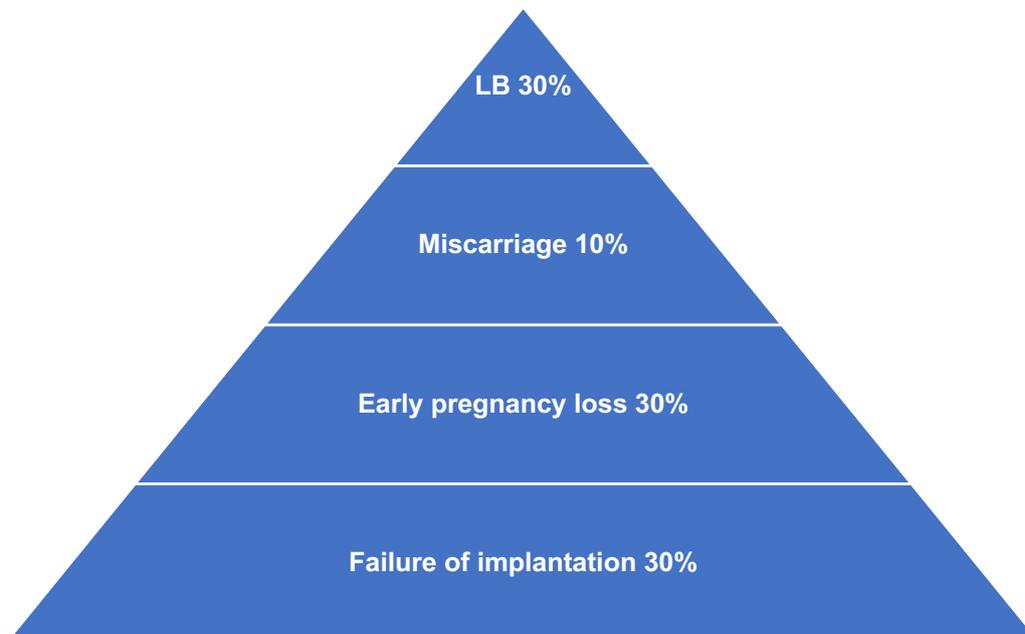
In this chapter I will review the current literature regarding the causes, associated risk factors and management of recurrent pregnancy loss (RPL).

## 1.1 Definition:

Miscarriage is the most common complication of pregnancy (NICE, 2012). Miscarriage is defined as spontaneous pregnancy loss of an intrauterine pregnancy before the period of viability. There is considerable variation in the upper defined gestation cut off for miscarriage, varying between 20 weeks (WHO 2018) to 23+6 weeks (Miscarriage Association; RCOG, 2011). For the purpose of this thesis I will use the RCOG definition, loss of pregnancy up to 23+6 weeks gestation.

### 1.1.1 Epidemiology:

Around 30% of all conceptions progress to a livebirth (LB), with the majority of human pregnancies lost due to implantation failure or before the pregnancy is visualised on an ultrasound scan, termed early pregnancy loss. This is illustrated in Figure 1.1.



*Figure 1. 1 The 'black box' of early pregnancy loss*

Figure 1.1 illustrates the fact that livebirth represents the tip of the iceberg of all conceptions, the majority of pregnancy losses are pre-clinical (not visualised on ultrasound

scan) and about 10% are clinical miscarriages (visualised on ultrasound scan). Adapted from (Macklon et al., 2002).

The background risk of miscarriage in pregnancies that are recognised by patients using home pregnancy tests including some pre-clinical and all clinical losses is around 20% or one in five pregnancies (NICE, 2012). The incidence of miscarriage in clinically recognised pregnancy on ultrasound scan is between 10-15% (Saravolos & Li, 2012). The majority of clinically recognised miscarriages, around 98%, occur in the first trimester of pregnancy, defined as less than 13 completed weeks of pregnancy (NICE, 2012; RCOG, 2011). A prospective observational study published by Zinaman and co-workers estimates that the efficiency of the human reproduction in terms of positive urine pregnancy test is at best 30% per cycle, and was maximal in the first two cycles and then gradually tapered. In healthy couples, 82% conceived over a period of 12 months (Zinaman *et al.*, 1996).

#### 1.1.2 Burden of Miscarriage:

The major burden of miscarriage is psychological with significant anxiety and depression reported in the first month after miscarriage and evidence of post-traumatic stress disorder (Farren *et al.*, 2016). In addition, there is the physical burden of pain, bleeding and potential complications with surgical management of miscarriage. There are economic implications to the NHS with a third of inpatient admissions in women being secondary to miscarriage. In England and Wales, in one year between 2012- 2013 there were 39,800 miscarriages that led to a hospital stay (Statistics, 2012-2013).

#### 1.1.3 Terminologies Used:

There are variable terminologies used to describe the type of miscarriages in the literature. The use of a consistent terminology worldwide would enable better communication between researchers and may improve the sensitivity with which women are spoken to and counselled after miscarriage. The European Society of Human Reproduction and Endocrinology (ESHRE) has published a consensus statement on the terminology of pregnancy loss and it is these that will be used in my thesis (Kolte *et al.*, 2015a).

#### 1.1.4 Types of Miscarriage:

Early pregnancy loss: Spontaneous demise of pregnancy less than ten weeks gestational age.

Non-visualised pregnancy loss: Spontaneous pregnancy demise based on decreasing serum or urinary  $\beta$ -hCG (human chorionic gonadotropin) levels and non-localisation on ultrasound, if performed. The types of non-visualised pregnancy loss are shown in Table 1.1.

<b>Types of non-visualised pregnancy loss</b>	<b>Definition</b>
Biochemical pregnancy loss	Spontaneous pregnancy demise based on decreasing serum or urinary $\beta$ -hCG levels, without an ultrasound evaluation
Resolved pregnancy loss of unknown location (resolved PUL)	Pregnancy demise not visualised on transvaginal ultrasound with resolution of serum $\beta$ -hCG after expectant management or after uterine evacuation without chorionic villi on histology
Treated pregnancy loss of unknown location (treated PUL)	Pregnancy demise not visualised on transvaginal ultrasound with resolution of serum $\beta$ -hCG after medical management

*Table 1. 1 Types of non visualised pregnancy loss as defined by ESHRE early pregnancy special interest group (Kolte et al., 2015a).*

Miscarriage: Intrauterine pregnancy demise confirmed by ultrasound or histology.

The various types of miscarriage terminologies are shown in Table 1.2. I will be using these terminologies throughout my thesis.

Type of miscarriage	Definition
Early miscarriage	Intrauterine pregnancy loss less than 10 weeks' size on ultrasound
Anembryonic (empty sac) miscarriage	Intrauterine pregnancy loss with a gestational sac but without a yolk sac or an embryo on ultrasound
Yolk sac miscarriage	Intrauterine pregnancy loss with a gestational sac and yolk sac, without an embryo on ultrasound
Embryonic miscarriage	Intrauterine pregnancy loss with an embryo without cardiac activity on ultrasound
Fetal miscarriage	Pregnancy loss $\geq 10$ weeks' size with a fetus ( $\geq 33$ mm) on ultrasound

*Table 1. 2 Types of miscarriage as defined by ESHRE early pregnancy special interest group (Kolte et al., 2015a).*

## 1.2 Recurrent Pregnancy Loss (RPL)

### 1.2.1 Definition:

There are variations in the definitions of recurrent pregnancy losses as discussed below, which can be interchangeably used with recurrent miscarriages.

The UK (RCOG, 2011) definition is three or more consecutive pregnancy losses before viability.

The ESHRE guideline on recurrent pregnancy loss 2017, defines RPL as two or more pregnancy losses including non visualised pregnancy losses but excludes ectopic pregnancy and molar pregnancy (Ruth Bender Atik et al., 2018).

The American Society of Reproductive Medicine (ASRM) definition of recurrent miscarriage is loss of two or more clinical pregnancy losses visualised either by scan or confirmed by histology (ASRM, 2012; Pfeifer *et al.*, 2013).

For the purposes of this thesis I will use the ESHRE guideline definition of RPL.

### 1.2.2 Incidence:

The quoted incidence of recurrent pregnancy loss after three or more losses is 1% (RCOG, 2011). The incidence increases to 2-3 % in other studies (Christiansen, 1996; Larsen & Christiansen, 2012; Quenby *et al.*, 2002; Stephenson *et al.*, 2002) with the inclusion of non-visualised pregnancy loss and up to 5 % if the definition of two or more pregnancy losses are used (Li *et al.*, 2002).

There are several associations which are considered in this chapter along with a discussion regarding the postulated treatment options. I will be focusing predominantly on the recurrent first trimester losses throughout this chapter. The aim of management of couples with RPL is to prevent the loss of normal pregnancies.

### 1.3 Non-modifiable Risk Factors:

The aim is to prevent the loss of normal pregnancies in RPL. The current evidence suggests that the prognosis in women with RPL depends on the age of the woman and number of previous miscarriages (Ruth Bender Atik *et al.*, 2018).

#### 1.3.1 Previous Miscarriages:

The prognosis for a successful pregnancy outcome in women with RPL is influenced by the number of previous miscarriages. This was noted in two studies a decade apart. Brigham *et al.*, undertook a longitudinal follow up study of pregnancy outcomes in patients with a diagnosis of idiopathic miscarriages and reported that the livebirth rate was affected by the preceding number of miscarriages (Brigham *et al.*, 1999).

This result was again replicated in the descriptive cohort study by Lund *et al.*, in Denmark. Increasing number of miscarriages was associated with decreased chance of at least one subsequent livebirth. The women who achieved a livebirth in the five years after consultation fell from around 75 % after three miscarriages to 50% after six or more miscarriages (Lund *et al.*, 2012).

In addition Ogasawara et al 2000 showed that the frequency of normal karyotype losses significantly increased with the number of previous miscarriages (Ogasawara *et al.*, 2000).

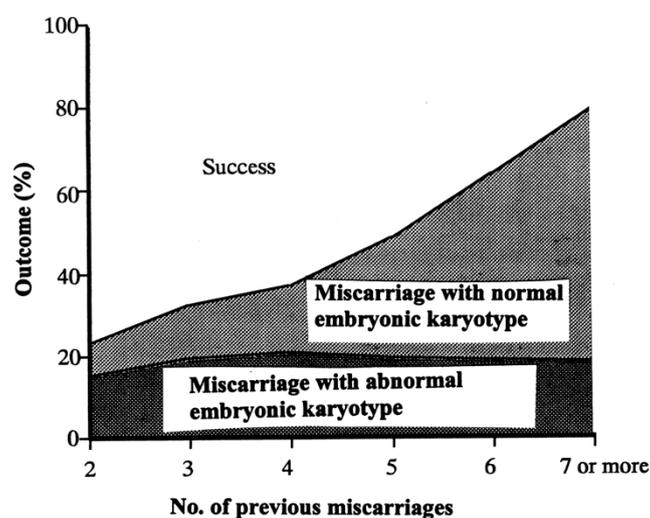


Figure 1. 2 Miscarriage and karyotype. Reproduced with permission from (Ogasawara *et al.*, 2000)

Figure 1.2 illustrates that with increasing number of previous miscarriages the proportion of miscarriages with abnormal karyotype remains fairly static but the proportion of normal karyotype (potentially preventable) losses increase.

### 1.3.2 Genetic Factors:

#### 1.3.2.1 Parental Karyotyping:

There is an increased incidence of carrier status of chromosomal rearrangements in couples with recurrent miscarriages. This incidence is quoted at around 5.2% after three miscarriages (compared to 0.7% in the general population to 2.2% after one miscarriage and 4.8% after two miscarriages) (van den Boogaard *et al.*, 2011). The commonly detected

chromosomal rearrangements are Robertsonian and balanced translocations (Ruth Bender Atik *et al.*, 2018).

RCOG guideline No.17 recommends consideration of parental peripheral blood karyotyping if the result of fetal karyotyping is an unbalanced structural chromosomal abnormality (RCOG, 2011). Selective karyotyping models based on maternal age at second miscarriage, personal history and family history of recurrent miscarriages have suggested parental karyotyping if the risk of carrier status exceeded 2.2% (Franssen *et al.*, 2005). Although this strategy was adopted by the ESHRE guideline, it is yet to be adopted in the UK. Cumulative livebirth rates are high even in couples carrying a balanced translocation, as evidenced by similar livebirth rates of 64% (Flunn *et al.*, 2014) and 63% (Sugiura-Ogasawara *et al.*, 2008).

Couples with a carrier status should be followed up with genetic counselling to aid in their decision making regarding their future options including consideration of preimplantation genetic testing and/or prenatal diagnosis (RCOG, 2011; Ruth Bender Atik *et al.*, 2018).

#### 1.3.2.2 Fetal Karyotyping:

Analysis of miscarried tissue has found some type of cytogenetic abnormality in 50-70% of cases, with autosomal trisomy's being the commonest at 60% followed by monosomy and polyploidy and chromosome mosaicism (8%) (Goddijn & Leschot, 2000; Hyde & Schust, 2015; Silver & Branch, 2007). Unusual aneuploidies dominate the early gestation losses and the trisomy's at later gestation (Wapner & Lewis, 2002). When considering clinically recognised pregnancies, aneuploidy pregnancy accounts for 5-10% of all pregnancies, the majority of which result in early pregnancy loss (Suzumori & Sugiura-Ogasawara, 2010). Most karyotype abnormalities are thought to be due to random errors in female germ cell development. Hence the future pregnancy prognosis is better after an aneuploidy miscarriage in comparison to euploid loss (Carp *et al.*, 2001; Ogasawara *et al.*, 2000; Warburton *et al.*, 1987). This is because euploid loss is more likely to be due to an underlying maternal cause for the loss.

Fetal karyotyping technique such as culture and fluorescence in situ hybridisation (FISH), have reported a high prevalence of chromosomal abnormalities but the technique was limited by

maternal blood or decidua contamination making the interpretation of the results difficult. ESHRE RPL guideline acknowledges that since the introduction of array CGH, false negative results are lower and is the preferred method (Ruth Bender Atik *et al.*, 2018). However, array CGH is limited by its ability to detect balanced rearrangements and low-level mosaicisms (Sahoo *et al.*, 2017). Array CGH, in addition, has low sensitivity to detect copy number variants (Freeman *et al.*, 2006). The emerging technique is next generation sequencing (NGS), which may have an application in the near future (Shamseldin *et al.*, 2013).

As a result of uncertainties, the current ESHRE recommendation is to consider karyotyping of miscarried tissue for explanatory purposes and not routinely recommended (Ruth Bender Atik *et al.*, 2018). However, the current RCOG guideline No.17 recommends cytogenetic analysis of the pregnancy tissue from the third and subsequent consecutive miscarriages and consideration of parental peripheral blood karyotyping only if the result is an unbalanced structural chromosomal abnormality (RCOG, 2011).

### 1.3.3 Maternal Age:

Maternal age is a non-modifiable risk factor and has an impact on subsequent livebirth rate after adjusting for the number of previous miscarriages (Brigham *et al.*, 1999; Lund *et al.*, 2012). This is because the frequencies and types of the chromosomal abnormalities vary with maternal age with increasing incidence of autosomal trisomy with increasing maternal age (Eiben *et al.*, 1990; Hassold & Chiu, 1985; Hassold T, 1984).

## 1.4 Lifestyle Factors and Modification:

The following factors discussed below should be optimised to improve outcomes.

### 1.4.1 Maternal BMI:

Miscarriage has been associated with underweight (BMI<18) and obese women (BMI>30) in pregnancy, in the retrospective cohort studies and matched case control studies (Lashen *et al.*, 2004; Metwally *et al.*, 2010).

A meta-analysis by Metwally *et al.*, showed a significantly higher odds of further miscarriage in women with BMI>25, OR-1.67; (95% CI, 1.76 -14.83), irrespective of the method of conception (Metwally *et al.*, 2008). A higher reported frequency of euploid miscarriages was found in obese women compared to non-obese women (Boots *et al.*, 2014). In addition a shortened time to conception and miscarriage was found in obese women with RPL suggestive of impaired decidualisation and selectivity, secondary to the metabolic effects of obesity on the endometrium (Bhandari *et al.*, 2016). However, there have been no randomised controlled trial (RCT) demonstrating that weight reduction in obese women with RPL prevents miscarriage. A Cochrane review found no suitable trial for consideration for the review (Opray *et al.*, 2015).

ESHRE RPL guidelines suggests that it would be sensible to offer pre-pregnancy advice on diet and exercise to women with high BMI presenting to a recurrent miscarriage (RM) clinic (Ruth Bender Atik *et al.*, 2018). This is because, care of couples in dedicated RM clinics offers the unique opportunity for pre-pregnancy counselling and advice to modify lifestyle factors including optimisation of BMI can be undertaken.

#### 1.4.2 Smoking:

The review by Pineles *et al.*, showed an increased risk of miscarriage with active smoking (summary RR ratio =1.23) and found a dose dependent association between smoking and miscarriages (Pineles *et al.*, 2014).

#### 1.4.3 Caffeine Intake:

The current evidence appears to suggest increased risk of pregnancy loss with higher caffeine intake (Chen *et al.*, 2016; Li *et al.*, 2015) and an increased risk of fetal growth restriction (CARE, 2006). WHO 2018 recommends that pregnant women with high daily caffeine intake (more than 300 mg per day), should consider lowering their daily intake during pregnancy, to reduce the risk of pregnancy loss and low-birth-weight neonates (WHO, 2018).

#### 1.4.4 Alcohol:

Some studies have reported an association between early miscarriages and consumption of alcohol (Avalos *et al.*, 2014). As there is no established safe limit in pregnancy, a precautionary approach is taken and women are advised to not drink any alcohol both preconceptionally and during pregnancy, similar to the UK Chief Medical Officers' Low Risk Drinking Guidelines 2016 and European Board and College of Obstetrics and Gynaecology (EBCOG) position paper on alcohol and pregnancy.

#### 1.4.5 Stress:

There is some emerging evidence on the association between stress and miscarriage (Li *et al.*, 2012). The systematic review and meta-analysis by Qu F *et al.*, suggested that the risk of miscarriage was significantly higher in women with a history of exposure to psychological stress (OR 1.42, 95% CI 1.19–1.70) (Qu *et al.*, 2017). However, Kolte *et al.*, 2015 found no association between a depression score and chance of on-going pregnancy and livebirth rate (Kolte *et al.*, 2015b). Hence the ESHRE RPL guideline acknowledged the association between RPL and stress but found that there was insufficient evidence for this to be causative (Ruth Bender Atik *et al.*, 2018).

#### 1.4.6 Exercise:

There is published evidence of improvement in maternal fitness with exercise (Kramer & McDonald, 2006; MS & SW, 2006). The EAGeR study found improved fecundability by physical activity of up to four hours per week in women with two or more previous miscarriages (Russo *et al.*, 2018).

#### 1.4.7 Use of E-cigarettes:

Although e-cigarettes help with quitting smoking the long-term safety is uncertain (Cochrane review 2016). There is no published evidence of benefit of use in pregnant women. Until more evidence is available, the advice would be not to recommend e-cigarettes (Britton, 2016).

#### 1.4.8 Pre-conception Options:

##### 1.4.8.1 Multi -Vitamin Supplementation:

The Cochrane review on vitamin supplementation by Balogun *et al.*, considered the effects of vitamin C, vitamin A, folic acid, multivitamins in combination with iron and folic acid or iron+ folic acid on its own, for prevention of miscarriage. In this review, none of the vitamins including anti-oxidant vitamin supplementation reduced the risk of miscarriage (Balogun *et al.*, 2016).

##### 1.4.8.2 Folic Acid:

Folic acid is recommended as a pre-conception vitamin supplementation from three months pre-conceptually to the end of the first trimester in order to prevent neural tube defects. Folic acid deficiency can also contribute to anaemia in the mother. The Cochrane review by De-Regil *et al.*, found there was insufficient data to evaluate the effects of folic acid supplementation in prevention of miscarriage (RR 1.10, 95% CI 0.94- 1.28) (De-Regil *et al.*, 2015).

##### 1.4.8.3 Vitamin D

A recent study has established that up to 50% of the population in the UK has vitamin D deficiency with a higher prevalence in obese women. NICE Clinical Guidance (CG 62), recommends routine calcium and vitamin D supplementation for pregnant women due to the prevalence and the increased demand of calcium metabolism during pregnancy (NICE, 2018).

There is some biological plausibility for the role of vitamin D deficiency as a cause for miscarriage:

- Deficiency of vitamin D is associated with pre-eclampsia, gestational diabetes, preterm delivery and growth restriction all conditions that may have a similar aetiology to RPL
- Vitamin D is essential for calcium homeostasis and calcium metabolism

- Vitamin D has an immune modulatory role

There is some evidence of beneficial effect of vitamin D supplementation in pregnancy. A Cochrane review by De-Regil *et al.*, 2016 included 15 randomised controlled trials involving 2833 women. Nine trials compared the effects of vitamin D alone with no supplementation or a placebo and six trials compared the effects of vitamin D and calcium with no supplementation (De-Regil *et al.*, 2016). With Vitamin D supplementation, the 25-hydroxyvitamin D concentrations at term improves. This reduces the risk of a low birth weight baby (less than 2500g) and of both preterm delivery less than 37 weeks and developing high blood pressure. However, it appears that when vitamin D and calcium are combined, the risk of preterm birth is increased. Data on adverse effects for the mother were not well reported. The authors concluded that further randomised trials are required to confirm the effects of vitamin D supplementation and effects on birth weight and blood pressure (De-Regil *et al.*, 2016). Hence, an association between vitamin D deficiency and miscarriage is plausible but no causality has been established.

## 1.5 Conditions Associated with RPL:

### 1.5.1 Acquired Thrombophilia

- Antiphospholipid syndrome (APS)

APS is a well-recognised cause of RPL and reported in 7-42% of women (Greaves *et al.*, 2000). Treatment of women with APS improves pregnancy outcomes (RCOG, 2011). The diagnosis of APS is based on the fulfilment of at least one of the clinical criteria as outlined below, along with the persistent elevation of either Lupus anticoagulant (LA) or anti cardiolipin antibodies (ACA) with  $\beta$ -2 glycoprotein.

The clinical criteria as defined by Wilson et al (Wilson *et al.*, 1999) include;

1. the loss of three or more embryos before the 10<sup>th</sup> week of gestation,
2. and/or one or more otherwise unexplained fetal deaths beyond the 10<sup>th</sup> week of pregnancy,

3. and/or the premature birth of a morphologically normal neonate before the 34<sup>th</sup> week of gestation because of eclampsia, severe pre-eclampsia or placental insufficiency

The mechanisms of pregnancy morbidity are due to inhibition of trophoblastic function and differentiation, activation of complement pathways at maternal-fetal interface and thrombosis of the uteroplacental vasculature in later pregnancy (Peaceman & Rehnberg, 1993).

Rai *et al.*, reported that untreated women with APS and recurrent miscarriage had a livebirth rate as low as 10% (Rai *et al.*, 1995). Two single centre randomised controlled trials (RCTs) reported that treatment with heparin and aspirin improves pregnancy outcome in women with APS (Kutteh, 1996; Rai *et al.*, 1997). The Cochrane review by Empson *et al.*, in 2005 concluded that in APS and recurrent miscarriage, unfractionated heparin is effective at preventing miscarriage (relative risk (RR) 0.46, 95% CI : 0.29 to 0.71) when compared with aspirin alone (Empson *et al.*, 2005). Combined unfractionated heparin and aspirin may reduce pregnancy loss by 54%. However, the trial which reported this was not blinded and the method of randomisation was poor.

There is mechanistic data to support the use of heparin. Heparin has been shown to reverse the negative effects of APS on trophoblast function (Bose *et al.*, 2004; Quenby *et al.*, 2005b) and complement activation in in-vitro studies (Girardi *et al.*, 2004). Heparin does not cross the placenta and has no effect on the fetus. Heparin does however have side effects and is associated with bruising, bleeding, osteopenia with long term use and rarely heparin induces thrombocytopenia.

Low molecular weight Heparin (LMWH), is a longer acting agent with a more predictable therapeutic effect needing only once a day administration compared to the several times a day and coagulation monitoring with unfractionated heparin. The one RCT of LMWH in women with APS and RPL showed no benefit in preventing miscarriage (Empson *et al.*, 2005). The improvement in pregnancy outcome reported by Empson *et al.*, 2005 was of such a large magnitude that it is now routine practice to give LMWH to women with APS and recurrent

miscarriage (Greaves *et al.*, 2000), commenced with the identification of an intra-uterine pregnancy on ultrasound scan (USS).

### 1.5.2 Hereditary Thrombophilias:

There are many recognised thrombophilia defects that have been associated with RPL. Factor V Leiden mutation is the most common affecting 3-4 % of the UK population (Li *et al.*, 2002). Some studies reported a positive association with a factor V Leiden mutation and increased risk of miscarriage (Grandone *et al.*, 1997) while other studies failed to find this association (Rai *et al.*, 2001). Rey E *et al.*, conducted a meta-analysis of retrospective studies in 2003 and reported an association between Factor V Leiden, protein S deficiency and a prothrombin gene mutation with miscarriage and recurrent miscarriage (Rey *et al.*, 2003). However, Walker *et al.*, concluded that routine testing is not recommended due to the uncertainty of the effect. The variation in the effects could be explained due to case selections and small sample size in the studies (Walker, 2000).

There is conflicting evidence of benefit of anti-coagulants in women with inherited thrombophilia and RPL. Gris *et al.*, conducted a RCT in women with late (>10 weeks' gestation) first trimester miscarriages and inherited thrombophilia's. They reported livebirth rate of 86% in women treated with enoxaparin in comparison to 29% in women who took aspirin alone (OR 15.5, 95% CI: 7-34)(Gris *et al.*, 2004). However, there have been no RCTs of heparin in women with recurrent first trimester miscarriage and inherited thrombophilia. Both the Cochrane review and the ESHRE guidelines have highlighted the need for such a trial (de Jong *et al.*, 2014; Ruth Bender Atik *et al.*, 2018). The current ongoing multi-centre RCT (ALIFE2) on the treatment of women with inherited thrombophilia with RPL may answer this question in the near future (de Jong *et al.*, 2015). So, the treatment of hereditary thrombophilia with LMWH for RPL is not indicated outside clinical trials (Li *et al.*, 2002).

### 1.5.3 Endocrine Factors:

#### 1.5.3.1 Subclinical Hypothyroidism and Thyroid auto antibodies:

There has been a recent debate regarding the role of thyroxine in women with thyroid autoimmunity and its effect on the pregnancy outcomes in women who suffer from RPL. The recommendation by the American Association of Clinical Endocrinologists (AACE) in association with American Thyroid Association (ATA) is to maintain the thyroid stimulating hormone (TSH) level below 2.5mIU/L in women who are pregnant and planning to get pregnant (Garber *et al.*, 2012).

An increased risk of miscarriage in women with subclinical hypothyroidism (SCH) and miscarriage has been reported (Kim *et al.*, 2011) and this risk was higher in the group associated with thyroid autoantibody (RR=2.47, 95% CI 1.77-3.45,  $p<0.01$ ) (Yibing Zhang *et al.*, 2017). The prospective cohort study by Uchida *et al.*, in women with unexplained RPL and SCH, defined by elevated TSH>4.5mIU/L in the presence of normal circulating free T4 included 317 women who were followed up for two years. Although there was a difference in the pregnancy loss < 22 weeks between the groups (29% in borderline SCH vs 17.9% in the euthyroid group), it did not reach statistical significance (Uchida *et al.*, 2017). The pregnancy outcomes were not different in women with SCH who were treated with thyroxine in comparison to no treatment (Bernardi *et al.*, 2013; Plowden *et al.*, 2016). However, a Cochrane review found that there was a treatment effect with thyroxine and a fall in miscarriage in the two small scale RCTs with a RR of 0.48, 95%, CI 0.25-0.92,  $p=0.03$  (Thangaratinam *et al.*, 2011).

A meta-analysis of cohort studies in women with thyroid antibodies but normal thyroid function test showed a tripling of the odds of miscarriage compared to control patients in both cohort studies (OR 3.90,95%, CI 2.48 to 6.12,  $p<0.001$ ) and case control studies (Thangaratinam *et al.*, 2011). The Cochrane review of trials in treatment of euthyroid women with thyroid peroxidase (TPO) antibodies by Reid SM *et al.*, showed a trend towards reduction of miscarriage in the women treated with levothyroxine compared with no treatment (RR 0.25, 95%, CI 0.06 to 1.15,  $p=0.07$ ). There was no difference in the women treated with selenium (Reid SM *et al.*, 2010).

The TABLET trial was a large double blind placebo controlled RCT that investigated whether levothyroxine treatment would increase the livebirth rates in women who were euthyroid

with TPO and a history of miscarriage or infertility. The patients who fell pregnant in the study (56% vs 58% in the two groups), were treated with either levothyroxine 50mcg or placebo from conception to end of pregnancy. The livebirth rate after 34 weeks was similar in both groups. The study concluded that the use of levothyroxine in euthyroid women with TPO antibodies did not result in higher live births in comparison to placebo. There was no difference in the rate of miscarriage between the two groups (Dhillon-Smith *et al.*, 2019). The study was well powered and had a good follow up rate of 98.7%. The drawbacks of the study included the widening of the inclusion criterion to include both subfertile women and those with miscarriage, the use of different assays to measure TPO and the use of a standard dose of 50mcg which did not vary with the BMI, level of TPO antibodies or thyrotropin concentration.

Hence, currently there is no role for use of thyroxine in women with RPL with normal thyroid function tests.

### 1.6 Unexplained Recurrent Pregnancy Loss:

There are multiple conditions that have been postulated as being associated with RPL. Despite screening for these conditions, approximately 50% of the women being evaluated for RPL have a negative investigation screen (Li *et al.*, 2002). These couples are categorised as unexplained recurrent pregnancy loss.

This is an enigmatic condition with many associations but with lack of robust data to support the various management options, other than in the context of a research setting. I have now considered the various investigations and management options in women with unexplained RPL.

### 1.7 Possible Treatment Considerations for Unexplained RPL:

The current evidence does not support any pharmacological management options for treatment of unexplained RPL. However, the treatment option with the most conflicting and

sometimes confusing evidence concerns the use of progesterone for prevention of miscarriage in women with unexplained RPL.

### 1.7.1 Progesterone:

Progesterone's physiological role is to prepare the uterus for the implantation of the embryo, enhance uterine quiescence and suppress uterine contractions, hence, it may play a role in preventing rejection of the embryo. Inadequate secretion of progesterone in early pregnancy has been linked to the aetiology of miscarriage and progesterone supplementation has been used as a treatment for threatened miscarriage to prevent spontaneous pregnancy loss. The published evidence for the use of progesterone in prevention of recurrent miscarriages is a series of conflicting results.

In support of Progesterone being effective at miscarriage prevention;

- The review in 2011 of four trials found a benefit to miscarriage prevention (Coomarasamy *et al.*, 2011)
- The Cochrane review suggests that luteal phase progesterone improves outcome in pregnancies secondary to assisted reproduction. However, once pregnancy is achieved it is less clear when to discontinue the treatment (van der Linden *et al.*, 2015)
- A systematic review of 14 randomized controlled trials (2158 women) found that women who have suffered three or more miscarriages may benefit from progestogen during pregnancy (Haas & Ramsey, 2013)
- A recent systematic review by Saccone *et al.*, included 10 trials of 1586 women and reported a lower risk of miscarriage (RR 0.72, 95% CI 0.53–0.97) and higher livebirth rate (RR 1.07, 95% CI 1.02–1.15) in women with unexplained recurrent miscarriages who were randomised in the first trimester before 16 weeks of pregnancy. This review included women who received synthetic progestogens as opposed to the natural progesterone (Saccone *et al.*, 2017)
- The updated Cochrane review, by Haas *et al.*, published in 2018, assessed the efficacy and safety of progestogens for preventing idiopathic recurrent miscarriage. The review included all the trials with allocation to progesterone versus placebo or no treatment but cross over trials were not included. Women achieving pregnancy

through in-vitro fertilisation (IVF) were also excluded. The primary outcome was miscarriage defined as pregnancy loss less than 20 weeks' gestation. 13 trials were included in the review and they differed in the formulations of progesterone used, routes of administration, duration of treatment, inclusion criterion and outcome measures. The trials were a mix of multicentre and single-centre trials, conducted in Egypt, India, Jordan, UK and USA. The majority of trials were at low risk of bias for most domains. The meta-analysis of 11 trials of moderate quality involving 2359 women, suggests that there is probably a reduction in the number of miscarriages for women given progestogen supplementation compared to placebo/controls (average risk ratio (RR) 0.69, 95% CI 0.51 to 0.92). This benefit was more apparent in women with three or more prior miscarriages. The meta-analysis of seven trials of 2086 women noted a probable slight benefit for women receiving progestogen for livebirth rate (RR 1.11, 95%, CI 1.00 to 1.24) and a reduction in stillbirth and preterm birth (Haas *et al.*, 2018). There have been no reported differences in the adverse events in either the mother or baby

- In the case of threatened miscarriage, a systematic review of seven trials involving 696 women compared the use of progestogens in the treatment of threatened miscarriage with either placebo or no treatment. These suggest that progestogens are effective in the treatment of threatened miscarriage. This is found in both oral and transvaginal route of administration (Wahabi *et al.*, 2018)

Against the use of progesterone in miscarriage prevention:

- A systematic review of 14 randomized controlled trials (2158 women) found no evidence that routine use of progestogens can prevent miscarriages (Haas *et al.*, 2013). Four trials showed a decrease in miscarriage compared with placebo or no treatment in these women; but the trials were of poorer methodological quality (Haas & Ramsey, 2013)
- This was followed by the publication of the robust large multicentre, double blinded randomised controlled trial (PROMISE trial) which did not show any reduction in the miscarriage rates or improvement in livebirth rates in women who suffered from recurrent miscarriages. In this trial women were randomised to progesterone support or placebo in early pregnancy (Coomarasamy *et al.*, 2016)

- The updated Cochrane review by Haas *et al.*, 2018 noted that there was high heterogeneity in the subgroup of women with three or more prior miscarriages. The data must be interpreted with caution as the major contributor to the result was from a single trial (Haas *et al.*, 2018). As most of the included trials had different entry criteria and duration of therapy, determining a uniform protocol for treatment may be difficult
- The PRISM trial was a double-blind placebo controlled RCT conducted to evaluate progesterone in women with bleeding in early pregnancy. This large study randomised 4153 women to either receiving 400mg progesterone twice daily or placebo from the first presentation with vaginal bleeding until 16 weeks of gestation. The primary outcome was livebirth achieved after 34 weeks of gestation. This was similar in both groups with 75% in the treatment group and 72% in the placebo group with  $p=0.08$ . The study concluded that treatment with progesterone in early pregnancy bleeding did not result in higher livebirths. However, a subgroup analysis showed a beneficial effect in women with three or more miscarriages with a statistically significant improvement in livebirth rates of 72% in the treatment group vs 57% in the control group with  $p=0.007$ . The drawback of the study included the route and preparation of progesterone used and the start point of the medication. This trial examined the use of micronized progesterone only and therefore the conclusions cannot be applied to other progesterone preparations and routes of administration (Coomarasamy *et al.*, 2019).

The variation in the trial results reflect that progesterone only benefits a subgroup of women, who are currently poorly defined, for example women with a severe phenotype of RPL or threatened miscarriage. The subgroup that benefit could be defined as those with the loss of a normal pregnancy.

#### 1.7.2 Heparin:

LMWH has been demonstrated to have no effect at preventing miscarriage during pregnancy in idiopathic recurrent miscarriage in several trials (Clark *et al.*, 2010; Kaandorp *et al.*, 2010b). The systematic review by de Jong *et al.*, who reported no benefit from either unfractionated

heparin or LMWH in preventing miscarriage in unexplained recurrent miscarriage without thrombophilia (de Jong *et al.*, 2014; de Jong *et al.*, 2015).

### 1.7.3 Aspirin:

Low-dose aspirin is indicated for women who are considered high risk for developing pre-eclampsia. In the United Kingdom low-dose Aspirin (75mg) is recommended to be commenced from 12 weeks of pregnancy until labour in women at high risk of hypertensive disease in pregnancy (Hypertension guideline NICE 2011).

A systematic review by de Jong *et al.*, showed no benefit from low dose aspirin in preventing miscarriage in unexplained RPL (de Jong *et al.*, 2014). There was a lower livebirth rate in women on aspirin and unexplained RPL (Clark *et al.*, 2010; Kaandorp *et al.*, 2010a). There is no role for aspirin in prevention of miscarriages in unexplained RPL.

### 1.7.4 Steroids:

RPL is thought to be potentially due to an immunological mechanism of imbalance between the anti-inflammatory and pro-inflammatory mechanisms that underpin early pregnancy (RCOG, 2011). It is postulated that there could be an imbalance in Th-1/Th-2 systems, with a prevalence during pregnancy of the cytotoxic T-helper immunity with cytokines such as interleukin 2 (IL-2) and Tumour Necrosis Factor  $\alpha$  (TNF $\alpha$ ) and hence the use of immunomodulatory approach. One intervention that has been considered is prednisolone. This is lipophilic and, in the placenta, it is inactivated by the 11 $\beta$  hydroxyl steroid dehydrogenase (HSD)2. Fetal uptake is also reduced by active retrograde transport by P-glycoprotein, a mechanism that reduced the fetal concentration by 8-10-fold in comparison to the mother.

Quenby *et al.*, observed that women with RPL had a higher endometrial NK cell density in comparison to fertile control women. Uterine NK cell density was significantly reduced with the use of prednisolone ( $p=0.004$ ) (Quenby & Farquharson, 2006). A pilot double-blind placebo controlled RCT of first trimester prednisolone for the prevention of miscarriage in

women with high uNK cell levels and recurrent miscarriage showed a trend of improvement in the treatment arm, this was not statistically significant. The livebirth rate was 80% in the treatment arm and 60% in the placebo group (Tang *et al.*, 2013).

The use of steroids in pregnancy is not without its risks. Silver *et al.*, observed that use of prednisolone in early pregnancy has been associated with gestational diabetes and preterm birth (Silver *et al.*, 1997).

In conclusion there is paucity of data regarding the dose and timing of steroids and large RCTs are required to establish the efficacy of prednisolone. Until then the use of steroids should be conducted in a research setting.

#### 1.7.5 Immunotherapy:

The immune mechanisms of recurrent implantation failure (RIF) and RPL postulate the rejection of the embryo by the mother by mounting an immune system response (Cochrane review 2014). The hallmark of the endometrium in recurrent miscarriage is a disordered and prolonged pro-inflammatory decidual response. This excessive inflammatory response is thought to prolong the 'window of receptivity' thereby promoting out-of-phase implantation and disabling embryo selection (Lucas *et al.*, 2016a; Salker *et al.*, 2010; Salker *et al.*, 2011; Salker *et al.*, 2012). The ratio of TNF- $\alpha$  producing T cells seems to be significantly higher in patients with RIF and RPL in comparison to the fertile controls (Santjohanser *et al.*, 2013). Lucas *et al.*, conducted transcriptome studies of mid-luteal biopsies and observed that in women with RPL, there was excessive inflammation with heightened influx of immune cells. This is postulated to be associated with cellular stress and activation of wound healing pathways (Lucas *et al.* 2016a).

The various modalities grouped under immunotherapy consists of:

- Injection of paternal leucocytes or third-party donors in early pregnancy was done initially, to overcome the postulated rejection phenomenon
- Intravenous immunoglobulin, which has been subject to a series of RCTs and has potential side effects including anaphylaxis

- Trophoblast membrane infusion to tolerise the pregnant women to allogeneically dissimilar fetus
- TNF- $\alpha$  inhibitors which have been associated with severe reactions such as immunosuppression and granulomatous disease. This is to suppress the production of pro- inflammatory Th-1 cytokines.

Cochrane review of immunotherapy for recurrent miscarriages by Wong *et al.*, in 2014, reviewed the effects of any immunotherapy on the livebirth rates (after 20 weeks' gestation) in women with recurrent unexplained miscarriages and with no more than one previous livebirth (Wong *et al.*, 2014).

20 RCTs involving 1137 women between 1985 and 2004 were included in the review. 19 studies were excluded from the review. The overall risk of bias was considered to be low. The summary of the trials, intervention and outcomes are summarised in the table 1.3.

<b>Intervention</b>	<b>Number of trials, participants</b>	<b>Timing of treatment (n= number of trials)</b>	<b>Outcomes</b>
Partner leucocytes	12 trials, 641 women	Prior to pregnancy n=10, double n=1 During pregnancy n=1	No increase in LB OR- 1.23 CI 0.89 to 1.70
Third party donor leucocytes	3 trials, 156 women	All trials prior to pregnancy	No increase in LB OR 1.39 CI 0.68 to 2.82
Trophoblast membranes	1 trial, 37 women	Prior to pregnancy	No increase in LB OR 0.40 CI 0.11 to 1.45
IVIG	8 trials, 303 women	During pregnancy, n=6 Prior to pregnancy, n=2	No increase in LB OR 0.98 CI 0.72 to 1.93

*Table 1. 3 Summary of trials and interventions in Immunotherapy*

Table 1.3 summarises the various trials and the interventions which were considered in the Cochrane review of Immunotherapy in recurrent miscarriage. The most widely studied interventions were treatment with IVIG and use of partner leucocytes as summarised in the table 1.3. Some other interventions included the use of third party donor leucocytes and trophoblast membranes.

The limitations of the study outcome measures are heterogeneity in dose, duration and timing of immunotherapy. The quality of the studies included in the review was moderate to high. However, current meta-analysis of evidence has shown no benefits from the immunotherapy approaches in preventing miscarriage. The research suggestion is that the future work should focus on identifying the group of women who will benefit from the use of immunomodulatory interventions (Wong et al., 2014).

There is no role for immunomodulation therapy for women with RPL due to the lack of benefit and potential side effects.

#### 1.7.6 Use of Granulocyte-Colony Stimulating Factor (G-CSF):

G-CSF is a cytokine that stimulates neutrophil granulocyte proliferation and differentiation, has receptors expressed in placental tissue and is expressed and produced by decidua cells. G-CSF is secreted by immune and endometrial cells (Waseda *et al.*, 2008). The postulated effects of G-CSF are induction of Th-2, inhibition of NK cells and blood mononuclear cells. Furthermore, in vitro G-CSF application seems to be favourable on the decidualisation process of endometrial stromal cells. But the specific effects on the endometrium as well as the fetomaternal interphase are not fully understood yet (Santjohanser *et al.*, 2013). Animal studies have evaluated its anti-abortion activity (Litwin *et al.*, 2005; S *et al.*, 1995). No embryotoxic effect was reported from previous use in treatment of neutropenia in cancer chemotherapy.

Interest in G-CSF was evoked after the publication of the RCT of treatment of women with G-CSF (Scarpellini & Sbracia, 2009). 68 women with unexplained RPL and with four or more previous miscarriages were randomised to treatment at a dose of 1 µg/kg/day or placebo from day six of ovulation until onset of menstruation or the end of ninth week of pregnancy. The livebirths in the treatment group in comparison to the placebo group was significantly higher at 82.8% vs 48.5% respectively with  $p=0.0061$  (OR= 5.1, CI- 1.5-18.4) (Scarpellini & Sbracia, 2009). A further retrospective cohort study by Santjohanser *et al.*, also compared the use of G-CSF in women with RPL and infertility along with two other arms of either treatment with other medications and no treatment. Both the pregnancy rates and livebirth rates were higher in the G-CSF group in comparison to the other two groups. This result reached statistical significance and the reported pregnancy rate was 47% and livebirth rate of 32%. The study drawbacks included the retrospective nature and the need for several cycles of IVF/ICSI treatment (Santjohanser *et al.*, 2013).

However, a subsequent study by Barad *et al.*, 2014 showed no difference in the pregnancy outcomes with intra-uterine instillation with G-CSF (Barad *et al.*, 2014). In addition, the

RESPONSE study was a randomised double-blind placebo-controlled trial of the use of NT 100, a recombinant form of G-CSF, in women with unexplained RPL (n=150). The study found no significant difference in the clinical pregnancy rates or livebirth rate (LBR) between the groups. This RCT suggest that the use of G-CSF is not an effective treatment for patients with RPL.

In conclusion there is no evidence to continue the use of G-CSF outside the research setting.

### 1.7.7 Use of Human Chorionic Gonadotropin (hCG):

The role of hCG is postulated to:

- Sustain the corpus luteum and progesterone action
- Possibly prolong the implantation window by a combination of action on Insulin-like Growth Factor Binding Protein-1 (IGFBP-1) and the impact on the protease enzyme that has a role in endometrial breakdown
- Increase in vascular angiogenesis with significant increase in endometrial vascular endothelial growth factor (VEGF) (Licht *et al.*, 2007)

Quenby *et al.*, reported improved miscarriage rates in women with oligomenorrhoea and suspected luteal phase deficiency after hCG treatment (Quenby & Farquharson, 1994). The Cochrane review by Devaseelan *et al.*, 2010, did not find any difference in the miscarriage rates in women with or without treatment with hCG (Devaseelan *et al.*, 2010). The Cochrane review by Morley *et al.*, 2013 included five RCTs (involving 596 women) that used hCG to prevent miscarriages rather than treat them. The review suggested a statistically significant reduction in miscarriage rate in women using hCG. Nevertheless, this was not replicated when the two poor quality studies were removed. Hence the evidence was considered as equivocal (Morley *et al.*, 2013).

Thus, the current evidence is inadequate to support the use of hCG in prevention of recurrent miscarriages or treatment of threatened miscarriages.

### 1.7.8 Bromocriptine:

Hirahara *et al.*, conducted a clinical trial of 48 women, with either overt or occult hyperprolactinemia and compared the pregnancy outcomes between those who had treatment with bromocriptine and those without treatment. The women in the treatment group had bromocriptine prescribed pre-conception and up to nine weeks of pregnancy, with a test in between to evaluate the prolactin levels in a dose of 2-5mg on an individual response-based dosing. The pregnancy rate was very high during the one year period of observation. The livebirth rate in women who were treated was 85.7% and in those without treatment was 52.4% ( $p < 0.05$ ). Although the definition had a strict cut off value, the study itself was small, underpowered and non-blinded (Hirahara *et al.*, 1998).

ESHRE 2017 guideline group deemed that there was insufficient evidence to recommend screening RPL women for hyperprolactinemia and of treatment with bromocriptine (Ruth Bender Atik *et al.*, 2018).

### 1.8 Psychological Impact of RPL:

Women who suffer from RPL are prone to heightened anger, depression, anxiety and feelings of guilt and grief (Miscarriage Association). This is evidenced by several studies. Rai & Regan reported that one in five women who experience miscarriage have anxiety levels similar to people attending the psychiatric outpatient services. One third of the women attending specialist clinics as a result of miscarriage are clinically depressed (Rai & Regan, 2006).

A small prospective study evaluating the psychological impact of pregnancy loss was performed in 45 women who had two previous unexplained first trimester miscarriages. Self-reported questionnaires and interviews before their next pregnancy showed that 10 pregnancies (22.2%) resulted in a miscarriage. The degree of baseline depressive symptoms predicted the rate of miscarriage (Sugiura-Ogasawara *et al.*, 2002).

Farren *et al*, reported a pilot trial in which 39% of the women who participated in the study, met the criterion for probable moderate-to severe post-traumatic stress disorder (PTSD) 3 months after suffering an early pregnancy loss. The anxiety score was also higher with 20% who meet the criteria for moderate-to-severe anxiety compared with 10% in a control population. Levels of anxiety and depression dropped between one and three months, but the symptoms associated with PTSD persisted (Farren *et al*, 2016).

A study from Japan showed statistically significant higher scores of mental distress as assessed by the Kessler score in women with unexplained RPL. The study acknowledged that the significance of mental state and the cardiovascular risk factors in women with unexplained RPL needs to be clarified (Kataoka *et al.*, 2015).

Kolte *et al.*, 2015 also encountered indices suggestive of high levels of stress on the perceived stress scale (PSS) among women who attended their specialist clinic. Feelings of guilt and self-blame typical of depressive disorders, was highly prevalent in women with RPL. Though the score was high on the Major Depressive Inventory (MDI), there was no association between MDI score and lower chance of on-going pregnancy and livebirth rate. However, a successful outcome lowered the scores in a follow up assessment. The outcomes of this study can be used to reassure women that the emotional effects of negative reproductive outcomes do not adversely affect their future pregnancy outcomes (A.M.Kolte *et al.*, 2015).

In summary couples who undergo RPL are faced with the unaddressed burden of psychological distress with particular feelings of isolation especially in the male partner. They should be referral to specialist dedicated RPL clinics which has the experience to handle such couples with compassion and evidence-based care with access to counselling services to deal with the previous traumatic experiences, in a select group of patients.

### 1.9 Dedicated Recurrent Pregnancy Loss Clinics:

Referral to a specialist is variable in the UK and may happen after two/three or more consecutive miscarriages (RCOG, 2011). Dedicated specialist clinics would reduce the variation of practise among the clinicians and reduce the widespread use of empirical

treatment. The recommendation is that the management of couples who experience recurrent miscarriage should occur in a specialised multi-disciplinary team setting (Little, 1988). The outcomes were better following referral to a specialised clinic (Clifford *et al.*, 1997). One paper recommends having a specialist clinic for a population of two million to adequately counsel, manage and support the couples using the service (T.C.Li, 1998). The dedicated clinics follow evidence based practise with individualised care for the couple with support available during some of the challenging times during their subsequent pregnancy. A well-established team, including access to counselling, would ensure familiarity for patients and continuity of care. This helps the couple to build rapport with the clinician and have an agreed discussion based on the expectations for the individual couple. This ensures compliance to advice and management plan.

The RPL clinic run by Professor Quenby aims to deliver high quality evidence based and individualised care to couples with RPL. They are also presented with the opportunity to actively participate in research projects which would help answer some of the ambiguity in the understanding and management of this enigmatic condition.

Tommy's is a charity involved in promoting research in pregnancy to prevent outcomes such as miscarriages, stillbirth and preterm deliveries. The Tommy's National Early Miscarriage Centre was set up in 2016 as a partnership of three universities: The University of Birmingham, The University of Warwick, and Imperial College London. The three sites run specialist clinics enabling 24,000 women per year to access treatment and support and participate in Tommy's research studies.

### 1.9.1 Ultrasound in Early Pregnancy for Reassurance:

The expectations of patients with previous pregnancy loss, would be for earlier access to ultrasound assessment of pregnancy and repeated reassurance scans. This was further confirmed by the qualitative research by Musters *et al.*, examining the supportive care options for women who suffered from RPL (Musters *et al.*, 2011). This was an explorative semi-structured in-depth interview of 20 different options that were presented to the 17 participating women. The data from 15 participants were published.

The most preferred options to emerge from the study were:

- *β- hCG monitoring in early pregnancy*
- *Early and frequent monitoring of the pregnancy by ultrasound*
- *Advice regarding life style and diet*
- *Counselling and emotional support*
- *Clear formulated plan including medications for the 12 weeks of pregnancy*

Although women acknowledged the heightened anxiety in the lead up to the scans, they still preferred the reassurance elements from a scan and the certainty that the pregnancy was progressing well. The identification of a viable pregnancy can further allay anxiety as the risk of miscarriage declines significantly with increasing gestation.

Most specialist clinics would offer fortnightly scans from six weeks until the dating scan with the flexibility of additional scans based on the clinical outcome and the patient's expectations.

### 1.9.2 Research Participation in Dedicated Clinics:

The additional advantage of care of women in a dedicated clinic includes the opportunity for participation in research and clinical trials to guide evidence-based management, reducing the ambiguity and variation in the management of early pregnancy issues. Musters *et al.*, also reported the willingness of women to participate in scientific research. The reasons behind the participation were two fold, contribution to the greater good and personal gains for themselves (Musters *et al.*, 2011). Not all women with the above care package will have a successful outcome. However, the support that they received in the pregnancy will equip them to face and prepare for the pregnancy with confidence.

### 1.9.3 Supportive Care:

Definition: A tailored approach to offer women support, advice and reassurance ultrasound in a dedicated setting such as early pregnancy unit or RPL clinic with a multi-disciplinary team of doctors, midwives, nurses and/or psychologists.

Recurrent pregnancy losses place huge stress and strain in the relationship of couples as the coping mechanisms adopted by men and women may vary. This can sometimes lead to the woman feeling isolated with lack of acknowledgement. Hence any support or therapy should ideally be directed to the couple rather than just the woman. Men usually feel that they are not spoken to or in the periphery of the decision-making process. The acknowledgement and support from friends and family contributes positively to the emotional wellbeing of the couple.

A clinical nurse with training in counselling skills or a professional psychologist can offer counselling. The women in the study by Kolte *et al.*, rejected the option of counselling by their GP. Acknowledgement of the anxiety and support by the caring clinician helps women cope with the pregnancy and the outcomes even when it's negative (Kolte *et al.*, 2015b).

The earliest evidence for the efficacy of supportive care is from Pedersen and Pedersen, who assigned women with unexplained RPL to tailored supportive antenatal care or no specific care in a subsequent pregnancy. 86% of the pregnancies in the supportive group reached term gestation in comparison to 67% in the other group ( $p < 0.001$ ). This study was non-randomised and the supportive care was only offered to patients who lived close to the hospital, hence subject to bias. Nevertheless it highlighted the novel concept of supportive care in pregnancy (Stray-Pedersen & Stray-Pedersen, 1984). A subsequent study by Clifford *et al.*, from UK also found that the rates of miscarriage was significantly lower in women who attended the early pregnancy clinic at 26% vs. the 51% repeat miscarriage in women who did not attend the specialist clinic (Clifford *et al.*, 1997). Liddell *et al.*, reported a livebirth rate of 86% in women with recurrent ( $\geq 3$ ) miscarriages who were enrolled into a program of emotional support, compared to 33% in similar women who had no formal supportive care (Liddell *et al.*, 1991). However, all these studies were limited by small numbers and higher loss to follow up rates (Liddell *et al.*, 1991; Stray-Pedersen & Stray-Pedersen, 1984).

Bailey *et al.*, undertook a feasibility study to assess the efficacy of Positive Reinforcement and Coping Intervention (PRCI) for women who are in the waiting period of their next pregnancy. Though 92% women preferred follow-up, it was only offered to 30% of the women. In the

NHS setting, access to support and therapy is limited. PCRI is a novel self-administered supportive technique, that shows significant promise of improving patient coping mechanism in early pregnancy (Bailey *et al.*, 2015).

The exact mechanism by which supportive care improves outcomes is not understood. There is dearth of good quality RCT's conducted to address the question the role of psychological and support interventions in pregnancy outcome. The evidence discussed so far points to the limitations of robust explanations of causations and therefore treatment for unexplained RPL.

The current management of RPL leaves a large population of women with no explanation for their pregnancy losses. Therefore, in the next chapter I will consider the evidence that supports the role of the endometrium in the causation and potentially modify the outcomes in couples with RPL.

# Chapter 2

## Chapter 2: Endometrium

### 2.1 Introduction:

The shift to the endometrium as a causative and modifiable aspect of the management of women with RPL underpins the research work at the Warwick Medical School Laboratory. The Warwick paradigm is that the endometrial and embryo interaction is paramount to the success of a pregnancy. With increasing number of miscarriages, the frequency of normal karyotype losses significantly increased (Lund *et al.*, 2012; Ogasawara *et al.*, 2000). Li *et al.*, suggested a correlation between increased miscarriages and the significance of endometrial factors (Li *et al.*, 2002). Thus, there is a proportion of preventable miscarriages that could be attributed to endometrial factors (Ogasawara *et al.*, 2000).

This discussion starts with the understanding of the process of implantation and the role of uterine natural killer cells (uNK) followed by the evidence for endometrial scratching.

### 2.2 Implantation:

The physiology of very early pregnancy is a complex mechanism that happens over a narrow window of opportunity against a background of synchronised hormonal and immune factors that lead up to the adequate preparation of the endometrium for the implanting embryo. During a woman's reproductive cycle, the opportunities for a pregnancy with intercourse are on the day of ovulation and the preceding two days (Cunningham, 2014). There are marked changes that occur within the endometrium which contribute to the "window of implantation" on day 20- 24 of a patient with a regular 28-day cycle. Increasing circulating oestrogen levels lead to positive feedback upon the anterior pituitary gland causing the release and surge of luteinising hormone (LH). The LH surge triggers ovulation and can be detected 10-12 hours prior to ovulation. The endometrium shows changes associated with the pre-decidual transformation of the upper two thirds of the functionalis layer. The glands exhibit extensive coiling and luminal secretions become visible. Epithelial cells show decreased microvilli and cilia along with appearances of luminal protrusions of the apical cell

surface. These pinopodes are important in preparation for the blastocyst to implant. They also coincide with changes on the surface of the glycocalyx that allows the acceptance of a blastocyst (Aplin, 2006; Aplin & Kimber, 2004). This is further facilitated by the modulations in the expressions of various cytokines, growth factors and adhesion molecules (Achache & Revel, 2006).

The endometrium transforms into a highly modified endometrium referred as decidua. Decidualisation involves the transformation of endometrial stromal cells into specialised secretory decidual cells, imperative for successful implantation and placentation (Gellersen & Brosens, 2014). This process is dependent upon oestrogen, progesterone and factors secreted by the implanting blastocyst. The decidua is classified into three parts based on anatomical location. The decidua directly beneath the blastocyst implantation site is modified by trophoblast invasion and becomes the decidua basalis. The decidua capsularis overlies the enlarging blastocyst and initially separates the conceptus from the rest of the uterine cavity. The remainder of the uterus is lined by the decidua parietalis. The decidual reaction is completed with blastocyst implantation. However, predecidual changes commence during the mid-luteal phase in endometrial stromal cells adjacent to the arterioles and spiral arteries (Cunningham, 2014).

The embryo implants between six to seven days after fertilisation, in the uterine wall. This involves three processes

1. Apposition - initial contact of the blastocyst to the uterine wall
2. Adhesion - increased physical contact between the blastocyst and the endometrial epithelium
3. Invasion - penetration and invasion of extra villous cytotrophoblasts into the endometrium, inner third of myometrium and uterine vasculature (Fatemi & Popovic-Todorovic, 2013).

Adherence is mediated by endothelial cell surface receptors at the implantation site that interact with blastocyst receptors (Carson *et al.*, 2002; Lessey, 2002; Lindhard *et al.*, 2002; Paria *et al.*, 2002). If the blastocyst approaches the endometrium after day 24, the potential

for adhesion is diminished because antiadhesive glycoprotein synthesis prevents receptor interactions (Navot & Bergh, 1991).

Successful endometrial blastocyst adhesion involves modification in the expression of cellular adhesion molecules (CAMs). The integrin's are one of the four families of CAMs. They are cell surface receptors that mediate cell adhesion to extracellular matrix proteins (Lessey, 2002). Endometrial integrin's are hormonally regulated, and a specific set of integrin's is expressed during implantation. Specifically,  $\alpha V\beta 3$  and  $\alpha 4\beta 1$  integrins expressed on endometrial epithelium are considered as receptivity markers for blastocyst attachment (Lessey, 1997). Recognition site blockade on integrin's for binding to extracellular matrix molecules such as fibronectin will prevent blastocyst attachment (Kaneko *et al.*, 2013).

Normal pregnancy may be the result of a predominantly Th-2 cytokine response with anti-inflammatory markers such as IL-4, IL-6 and IL-10. RPL patients have a bias towards mounting a Th-1 cytokine response with pro-inflammatory markers such as TNF- $\alpha$ , Interferon (IFN) and IL-2 (RCOG, 2011).

Another component of decidualisation is the infiltration of large populations of decidual leucocytes to the implantation site that has been observed in both the human and the mouse. Of these cells 65-70% are uterine specific natural killer cells (uNKs). 10-20% is antigen presenting cells (APC) such as macrophages and dendritic cells (DC's) (Granot *et al.*, 2012). Uterine NK cells are postulated to play a role in angiogenesis and trophoblast invasion. CD56 + uNK cells show a dramatic rise in absolute numbers and as a percentage of stromal cells from day 22- 28 of a 28 day menstrual cycle.

### 2.3 RPL and Endometrium:

The current hypothesis that is being assessed in Warwick is that suboptimal selection at implantation is associated with increased risk of clinical miscarriages.

The process of preparation for an embryo starts with a pro-inflammatory response followed by an anti-inflammatory response (Gellersen & Brosens, 2014; Salker *et al.*, 2011). However, Salker *et al.*, demonstrated that RPL is associated with a prolonged pro-inflammatory decidual

response which leads to out of phase implantation (of poor quality embryos) or in a hostile environment (of high quality embryos) thus predisposing to miscarriages (Salker *et al.*, 2011; Salker *et al.*, 2012). Salker *et al.*, reported on a retrospective study of time to pregnancy (TTP) in 560 women with RPL. 40% had a TTP of three months or less which could be considered as ‘superfertile’. The rest will either not achieve a pregnancy or not have a short TTP. This would be dependent on the presence or absence of concurrent reproductive disorders. A function of decidualisation is the selection of normal from abnormal embryos. A widely held paradigm for RPL is that there is reduced selectivity at implantation. Women with RPL have impaired decidualisation leading to implantation of non-optimum embryos and subsequent miscarriage, the term coined as ‘superfertility’ (Salker *et al.*, 2010).

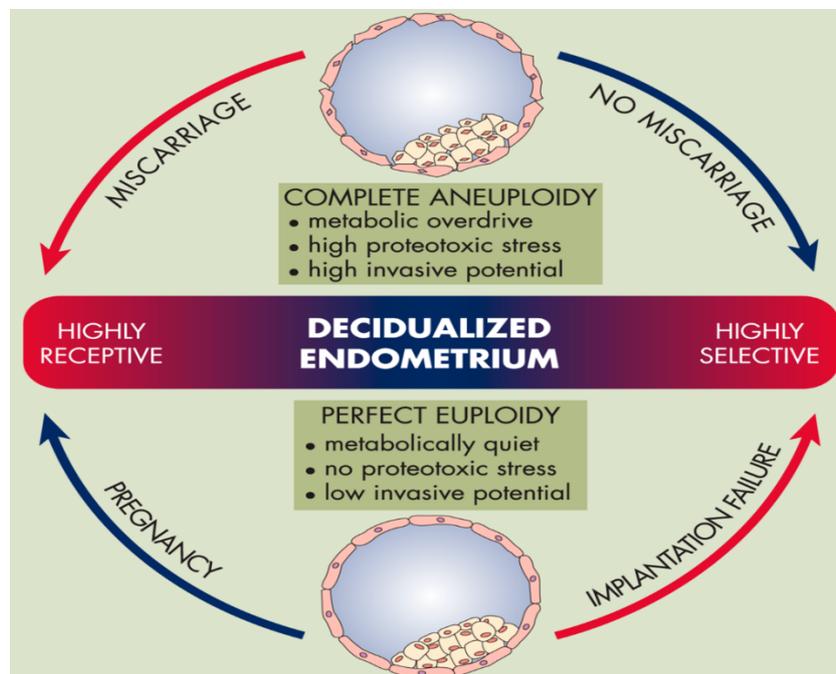


Figure 2. 1 Cyclic Decidualisation of Human endometrium

Figure 2.1 represents the role of the decidualised endometrium including embryo selectivity. If the endometrium is highly receptive, it allows for implantation of abnormal embryos thus manifesting as RPL. In contrast a highly selective endometrium manifests itself as RIF. Thus, a fine balance between the opposing elements are essential. Used with permission from Endocr Rev 2014 (Salker *et al.*, 2010).

Several studies have shown evidence of an association between impaired decidualisation and adverse reproductive outcomes such as RPL and RIF (Brighton *et al.*, 2017; Gellersen *et al.*, 2007; Lucas *et al.*, 2016b; Weimar *et al.*, 2013).

There is emerging evidence regarding the role of endometrial stem cells in disordered decidualisation in women with RPL (Lucas *et al.*, 2016a). The combination of lower stem cell count and the decreased stem cell-ness of the endometrium with increasing order of miscarriages, is postulated to contribute to abnormal decidualisation and miscarriages (Lucas *et al.*, 2016a). The observation was that endometrial mesenchymal stem cell (eMSC) deficiency leads to the accumulation of senescent cells in the endometrial stromal cells. Cellular senescence is associated with distinct pro-inflammatory cytokines, chemokines, growth factors and matrix metalloproteinases, similar to the secretory response of cultures of endometrium from women with RPL. Lucas *et al.*, showed from their work that the endometrium of women with RPL exhibits loss of plasticity defined by stem cell deficiency, heightened senescence and limited differentiation potential (Lucas *et al.*, 2016b). The speculation is that the persistence of this defect over several cycles contributes to the consecutive nature of miscarriages in women with RPL. However, the predictive value of endometrial assessments on future pregnancy outcomes will need validation from further longitudinal studies.

#### 2.4 Uterine Natural Killer (uNK) Cells and RPL:

Uterine NK cells have been associated with reproductive failure including RPL. Uterine NK cells vary in density during a menstrual cycle and are the dominant leucocyte population in the endometrium, during the window of implantation in the mid luteal phase of the menstrual cycle (Bulmer *et al.*, 1991). The uNK cell density increases towards the mid luteal phase and achieve their peak density in the first trimester of pregnancy if pregnancy occurs (King & Loke, 1991). Uterine NK cells are localised around the spiral arteries, near endometrial glands and in the extra villous trophoblast. They fluctuate throughout the menstrual cycle (Tang & Quenby, 2010). Uterine NK cells are thought to play an important role in implantation and maintenance of early pregnancy (Liu *et al.*, 2014). Direct antigen-receptor interactions between uNK cells and extra villous trophoblast occur (Moffett-King, 2002). Polymorphisms in the uNK cell receptor and trophoblast antigen have been associated with RPL (Hiby *et al.*, 2008). One of the postulated functions of the uNK cells is to regulate endometrial angiogenesis, which is an important factor in implantation (Hanna *et al.*, 2006; Quenby *et al.*,

2009). Active uNK cells are thought to secrete IL-15 and increase production of cytokines, growth factors (Hanna *et al.*, 2006) all essential for decidualisation and embryo implantation (Manaster *et al.*, 2008).

There are studies that have shown a raised uNK cell density in the luteal phase endometrial biopsies in women who suffer from RPL (Clifford *et al.*, 1999; Kuon *et al.*, 2017; Li *et al.*, 2002; Michimata *et al.*, 2002; Quenby *et al.*, 2005a; Tuckerman *et al.*, 2007). Some studies noted the correlation between the raised uNK cell numbers/density with adverse reproductive outcomes. Tuckerman *et al.*, showed that uNK cell count did not predict the pregnancy outcome in women with RPL (Tuckerman *et al.*, 2007). This was again confirmed in a subsequent study by Liu *et al.*, in 2014. They did not find a significant correlation of uNK cells on their own with the pregnancy outcome, but a combination with histological dating (significantly correlating with pregnancy outcome) improved the prognostic value of uNK cell count. The upper limit of normality for uNK in this study was 13.9% (Liu *et al.*, 2014).

Although the causal mechanism between raised uNK and RPL is poorly explained, there are several postulated theories for the association and their use as a biomarker for those women who would benefit from prednisolone for miscarriage prevention

- Uterine NK cells express the glucocorticoid receptor (RCOG, 2016a). Raised uNK cells could be the result of a relative steroid deficiency in the local milieu due to a lack of 11- $\beta$  HSDB type 1, which activates cortisol, in stromal cells (Kuroda *et al.*, 2013a). 11- $\beta$  HSD type 1 is induced from stromal cells in decidualisation but this is impaired in RPL (Kuroda *et al.*, 2013b). Quenby *et al.*, observed that women with RPL had a higher uNK count in comparison to their controls and this was significantly reduced with the use of prednisolone ( $p=0.004$ ) (Quenby *et al.*, 2005a). Hence supplementation with prednisolone may overcome the effect of defective decidualisation of stromal cells in RPL.
- Prednisolone reduces uNK cell density and angiogenic cytokine production and thus could reduce over-oxygenation of the decidua associated with raised uNK cells to maintain trophoblast invasion (Lash *et al.*, 2011; Quenby *et al.*, 2009)

- Trophoblast invasion is facilitated by a hypoxic environment. Uterine NK cells produce angiogenic cytokines which in turn promote endometrial angiogenesis (Lash *et al.*, 2011; Quenby *et al.*, 2009). Hence a high uNK cell density could lead to excessive angiogenesis thereby over-oxygenating the decidua which in turn would limit trophoblast invasion. Quenby *et al.*, demonstrated that raised uNK density of >5% was associated with a lower density of stromal cells with increasing spaces between the stromal cells, due to stromal oedema. The study also found positive correlation between uNK density and endometrial angiogenesis and decreased uterine artery resistance (Quenby *et al.*, 2009). This led to the postulated mechanism that increased peri-implantation angiogenesis leads to early maternal circulation and miscarriage by the final common pathway of excessive oxidative stress
- Brighton *et al.*, showed that the uNK cells are responsible for clearing the senescent cells. Excessive clearance of the stromal cells leads to decidual breakdown and miscarriage. This may be ameliorated by the use of prednisolone (Brighton *et al.*, 2017).

There are several uncertainties for the use of uNK cells as a biomarker to determine which women would benefit from prednisolone for miscarriage prevention. These are the inter-cycle variations, difficulty in standardisation of counting methods and therefore the cut-off thresholds for treatment (Lash *et al.*, 2016). Whilst the current evidence points towards an association between elevated uNK cells and their role in RPL, the role of very low uNK cells is poorly understood.

There is a debate surrounding whether the variation in the uNK density reflects a true increase in their numbers or is due to a relative reduction of stromal cell numbers or a variation in the normal pattern of oedema, all which are essential for normal implantation. Although there are basic science mechanistic explanations for the association between the raised uNK cells and RPL, due to the various uncertainties as discussed above, its role in causation of RPL and consequent treatment of elevated levels, are still contentious.

## 2.5 Endometrial Scratch (ES):

Endometrial scratch or injury is defined as an intentional minor damage to the endometrium performed with the objective of improving the reproductive outcomes of women desiring pregnancy (Nastri *et al.*, 2012).

## 2.6 Mechanism of a Possible Beneficial Effect of Scratch:

Endometrial injury triggers a series of biological responses. Siristatidis *et al.*, 2014, concluded in their review that it is likely that the endometrial injury/trauma elicits a cluster of events, some explained and the other's as yet unknown, that resets the endometrium and benefits embryo implantation (Siristatidis *et al.*, 2014).

The postulated mechanisms by which the endometrial injury could improve implantation and clinical pregnancy rates are:

### 2.6.1 Stem Cell Recruitment:

Adult stem cells play a crucial role in tissue homeostasis by replacing cells as a physiologic response to apoptosis or tissue damage due to injury. This happens following menstruation, trauma or parturition. Du and Taylor previously demonstrated that tissue repair and regeneration following trauma was carried out by circulating bone marrow derived mesenchymal stem cells or stem cells harbored in bone marrow. This recruitment of stem cells from bone marrow, to peripheral blood, then to the endometrium was demonstrated in the murine models, presumed to be following the signals emitted from the injury site. The injury is thus responsible for and aid wound healing. The tissue wound healing or reconstruction is in turn postulated to restore the capacity of the endometrium to accommodate embryonic attachment and development thereby promoting a favorable environment for successful implantation and embryonic migration (Du & Taylor, 2007; HS, 2004). Hyodo *et al.*, used mouse model to provide evidence that endometrial injury induces a rapid increase in stem cells in endometrium. They showed that the side population (SP) cells reside in the basal layer of the endometrium and could provoke endometrial regeneration and proliferation, critical for implantation (Hyodo *et al.*, 2011). Other studies

also postulated that there is a possible increase in stem cell recruitment following the endometrial injury (Du *et al.*, 2012; Lucas *et al.*, 2016b; Siristatidis *et al.*, 2014).

#### 2.6.2 Improved Decidualisation:

In 1907, Loeb L demonstrated that endometrial injury of the progestational uterus in guinea pigs leads to enhanced receptivity. This was linked to decidualisation via a rapid multiplication of endometrial cells, comparable to multiplication of decidual cells in pregnancy (Loeb, 1907). Other studies also reported similar outcomes by the use of oil or suturing the uterine horns (Finn & Martin, 1972; Humphrey, 1969). The converse was true when antihistamines and chemical histamine releasers prevented this phenomenon (Basak *et al.*, 2002; Finn & Martin, 1972). Local injury to the endometrium might induce decidualisation of endometrium and increase the implantation rate (Tiboni *et al.*, 2011).

#### 2.6.3 Synchronicity Hypotheses:

The paper by Li & Hao., 2009 proposed that the endometrial injury delays endometrial maturation and results in enhanced synchronicity between the endometrium and transferred embryos (Li & Hao, 2009). The backward development hypothesis by Zhou *et al.*, 2008 postulates that endometrial injury induces synchronicity between endometrium and embryo stage. It is postulated that in stimulated cycles the endometrium is ahead by two to four days in its development, in comparison to natural cycles. Endometrial injury may induce a partial arrest (lag) due to the wound which delays endometrial development so that the endometrium and embryo are in the same phase of development (Almog *et al.*, 2010; Zhou *et al.*, 2008).

#### 2.6.4 Inflammation Hypothesis:

Local endometrial inflammation is necessary for implantation, linked to high levels of chemokines, cytokines and leucocytes, promoting a crucial dialogue between endometrium and embryo promoting implantation. Injury induces an inflammatory reaction causing an inflow of dendritic cells and macrophages and an upsurge of pro-inflammatory cytokines. Analysis of endometrial samples retrieved from biopsy-treated patients revealed an increased

expression of the chemokine growth-related oncogene a (Groa), osteopontin (OPN), IL-15, macrophage inflammatory protein 1B (MIP-1B), and TNF- $\alpha$  (Dong *et al.*, 2009; Gnainsky *et al.*, 2010).

The proposed mode of action in the improvement of implantation could be explained by one of two mechanisms. The most likely mechanism is by a direct effect on trophoblast migration. The alternative postulate is by attraction of macrophages and dendritic cells (DC's) to the site of implantation leading to "snowball phenomenon". DC's and macrophages increase cytokine and chemokine secretion which in turn leads to further recruitment of these cells to the implantation site. TNF- $\alpha$  and OPN is thought to mediate MIP-1B in the window of implantation. Zheng *et al.*, 2009 postulated that the interaction of molecules with blastocyst facilitates its apposition to uterus and prevents rejection (Zheng *et al.*, 2009).

Dekel *et al.*, studied the relation between DC's/cytokines/chemokines production and local degradation of Mucin-1 (Muc-1). Muc-1 is the glycoprotein that modulates blastocyst attachment to a specific area in the endometrium. This group suggested that the trauma regulated the action of stored and newly produced adhesion molecules and their reorganisation at the site of implantation, thereby increasing receptivity (Dekel *et al.*, 2010).

#### 2.6.5 Immune System Cell Recruitment:

The endometrial injury attracts immune cells to the site, some of which may be long lived and able to differentiate into macrophages or dendritic cells (Dekel *et al.*, 2010; Gnainsky *et al.*, 2010; Luster *et al.*, 2005) playing a direct role in decidual development and embryo implantation (Granot *et al.*, 2012). These cells in conjunction with uNK cells trigger the production of chemokines and cytokines and adhesion molecules. Uterine NK cells modulate stromal differentiation and trophoblast invasion through production of IL-8 and IFN-inducible protein 10. DC's in turn regulate uNK differentiation via the IL-5 and IL-12 secreted by them (Blois *et al.*, 2011). Thus, injury may lead to an upsurge of pro inflammatory cytokines and growth factors and enzymes which all are beneficial for embryo implantation (Laird *et al.*, 2006).

Junovich *et al.*, 2011 reported that the uNK cells are reduced with controlled ovarian stimulation but are increased in the late proliferative phase following endometrial injury. Thus injury activates uNK cells by endocrine regulated stromal signals leading to the synthesis of the immunomodulatory cytokines particularly IFN  $\gamma$  which significantly upregulates pro-implantation chemokines, enzymes and transcription factors (Junovich *et al.*, 2011).

#### 2.6.6 Hypothesis Concerning Events Accompanying Wound Healing:

Wound healing is a dynamic process distinguished by three characteristic phases of inflammation, tissue formation and remodeling (Salamonsen, 2003). The role of stem cells in this process has been discussed in section 2.6.1.

Grant *et al.*, 2012 suggested that the local response to the injury promoting wound healing like process are characterised by pro-inflammatory Th-1 response. This process is mediated and amplified via the secretion of other cytokines, growth factors and enzymes such as Leukemia inhibitory factor (LIF) 11, Heparin binding endothelial growth like factor (HB-EGF), IL-4, IL-6, IL-10, IL-13, IL-15, TNF  $\alpha$ . This inflammatory response upregulates vascular, connective tissue and epithelial cell remodeling. This response is then thought to produce an environment for successful blastocyst attachment to the endometrium (Granot *et al.*, 2012).

#### 2.6.7 Gene Expression Theory:

There is differential gene expression at the site of implantation in women with subfertility, RIF and RPL with endometrial dysregulation reflects the impaired ability of the endometrium to co-act with the embryo (Siristatidis *et al.*, 2014). Injury altered expression of genes that regulate the implantation process. Local injury modulates and/or upregulates the expression of a wide variety of genes, such as phospholipase A2, MUC-1, glycodeilin A, crystallin alpha B, endometrial stem cells expressed genes, laminin alpha 4, MMP1, bladder transmembranal uroplakin IB, phospholipase A2, and apolipoprotein D gene (APOD) (Almog *et al.*, 2010; Dekel *et al.*, 2010; Gnainsky *et al.*, 2010; Granot *et al.*, 2000; Kalma *et al.*, 2009; Zhou *et al.*, 2008). Kalma *et al.*, reported a 2 to 10-fold increase in the range of expression of 183 genes, including 13 which were downregulated, in response to endometrial injury. Gene modulation was due

to the alteration in the chemokine/cytokine profile and was essential for preparation of endometrium and receptivity (Kalma *et al.*, 2009).

#### 2.6.8 The Neoangiogenesis Theory:

Ruszczak and Schwartz theorised that endometrial trauma stimulated angiogenesis which in turn improved receptivity (Ruszczak & Schwartz, 2000). Injury can contribute to angiogenesis through the IL-12 and IL-18 pathway that regulate the uNK cell activation and the positive effect on local vascular transformation (Ruszczak & Schwartz, 2000). Trauma causes differentiation of uNK cells leading to a positive effect on vascularization, providing adequate blood flow and preventing embryo rejection (Blois *et al.*, 2011).

#### 2.6.9 Clinical Correlation:

There is evidence in support of endometrial injury improving pregnancy outcomes. The earliest evidence was by Karrow *et al.*, 1971, who reported only two miscarriages in 28 women who underwent endometrial biopsy in the luteal phase and conceived in the same cycle (Karrow *et al.*, 1971). The publication by Barash *et al.*, in 2003, reported a doubling of take home pregnancy rate in women with preceding endometrial injury prior to IVF has since led to several studies which are discussed below (Barash *et al.*, 2003).

I identified a series of systematic reviews that I am now going to discuss and the subsequently published evidence.

#### 2.7 Role of Endometrial Scratch in In-Vitro Fertilisation (IVF):

Several RCTs, cohort studies showed an improvement in outcomes with endometrial scratch (Güven *et al.*, 2014; Helmy *et al.*, 2017; Kara *et al.*, 2012; Karimzadeh *et al.*, 2009; Li & Hao, 2009; Mahran *et al.*, 2016; Matsumoto *et al.*, 2017; Narvekar *et al.*, 2010; Nastri *et al.*, 2013; Shohayeb & El-Khayat, 2012; Singh *et al.*, 2015; Zhou *et al.*, 2008).

An equal number of studies published contradicted the positive findings and found no beneficial effect (Aflatoonian *et al.*, 2016; Aleyamma *et al.*, 2017; Baum *et al.*, 2012; Dain *et*

*al.*, 2014; Gibreel *et al.*, 2013; Gibreel *et al.*, 2015; Levin *et al.*, 2017; Liu *et al.*, 2017; Mak *et al.*, 2017; Safdarian *et al.*, 2011; Shahrokh-Tehraninejad *et al.*, 2016; Yeung *et al.*, 2014).

The selection of the population varied between highly selected cohort of women, for example with RIF to unselected group of women who underwent the scratch before the first/second cycle of IVF, frozen embryo transfer (FET), intra-uterine insemination (IUI), ovulation induction (OI).

Several published systematic reviews considered a combination of studies and assessed the effect of endometrial scratch on pregnancy outcomes (El-Toukhy *et al.*, 2012; Lensen *et al.*, 2016; Nastri *et al.*, 2015; Potdar *et al.*, 2012; Sar-Shalom Nahshon *et al.*, 2019). The consistent finding is that the studies had heterogeneity, risk of bias and possibility of some amount of injury in both the groups, especially in the earlier studies. This has led to conflicting evidence and sometimes widespread use of the procedure even without strong evidence to back the practice.

El-Toukhy *et al.*, conducted a systematic review and meta-analysis on local endometrial injury and IVF outcomes. They assessed the IVF outcomes in the subsequent cycle to the endometrial injury. The meta-analysis demonstrated that the clinical pregnancy rate was significantly improved after the injury in both the randomised (RR= 2.63) and non-randomised studies (RR=1.95). However, the livebirth rates did not reach statistical significance in the one RCT that reported it (RR=2.29, CI 0.86-6.11). The review reported on the outcomes of 901 participants, the meta-analysis included outcomes from two RCTs and six non-randomised studies. There was no statistical difference in the livebirth outcomes. The limitation of this review was the inclusion of both randomised and non randomised studies (El-Toukhy *et al.*, 2012).

A second systematic review and meta-analysis on endometrial injury in RIF was published by Potdar *et al.*, in 2012. This review studied the outcome of 2062 women with RIF and included four RCTs and three non RCTs. The pooled relative risk for clinical pregnancy rate showed that local injury is 70% more likely to result in a clinical pregnancy when compared to no injury. The effect was higher in women who underwent endometrial scratch (RR=2.32, CI 1.72-3.13)

than in women with RIF where hysteroscopy has been used as a method of injury (RR=1.51 CI 1.30-1.75) (Potdar *et al.*, 2012).

These reviews seemed to suggest a difference in clinical pregnancy rates in women who underwent the endometrial scratch. However, the reviews considered different population of women and considered different studies in their reviews with hysteroscopy being an additional mode of injury in the Potdar review. Although there was an increase in clinical pregnancy rate in women being offered the scratch, the reviews did not specify the population that would benefit from scratch.

The subsequent Cochrane meta-analysis in March 2015 of endometrial injury in assisted reproduction considered 14 RCTs. Of these, women in 13 trials underwent the endometrial biopsy between day seven of the previous cycle to seven days of the embryo transfer cycle. The livebirth rate and ongoing pregnancy rate was significantly increased in the injury group with a RR of 1.42 (Nastri *et al.*, 2015). Similarly, the clinical pregnancy rate was higher in the endometrial injury group with a RR of 1.34 ( $p=0.002$ ) (Nastri *et al.*, 2015). The meta-analysis did not reveal any difference in miscarriage rate, but the evidence was rated as low quality, because of insufficient number of participants in the study. A large proportion of the studies included had important limitations in the methods they used, including selection bias, different outcome measures and varying degree and timing of injury in the intervention and differing degrees of uterine manipulations in the control groups. The procedure was associated with mild pain, which was transient (Nastri *et al.*, 2015). Figures 2.2 and 2.3 illustrates the forest plot of outcomes compared to injury vs no injury and the population respectively.

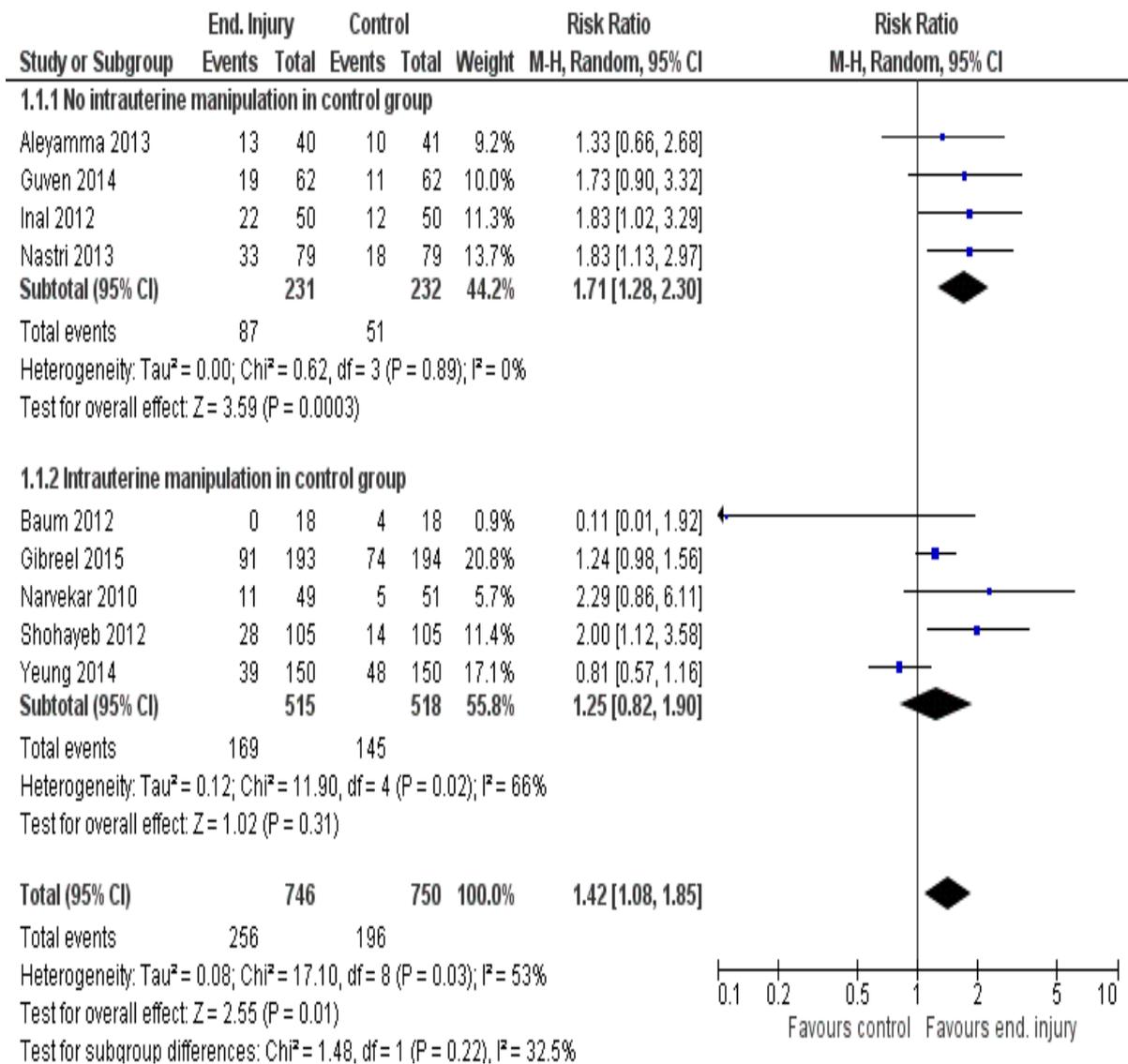


Figure 2. 2 Forest plot of comparison: Endometrial injury vs no injury

Figure 2.2 compares the outcomes of livebirth between groups who had no injury vs some injury in the control group. Used with permission (Nastri et al., 2015).

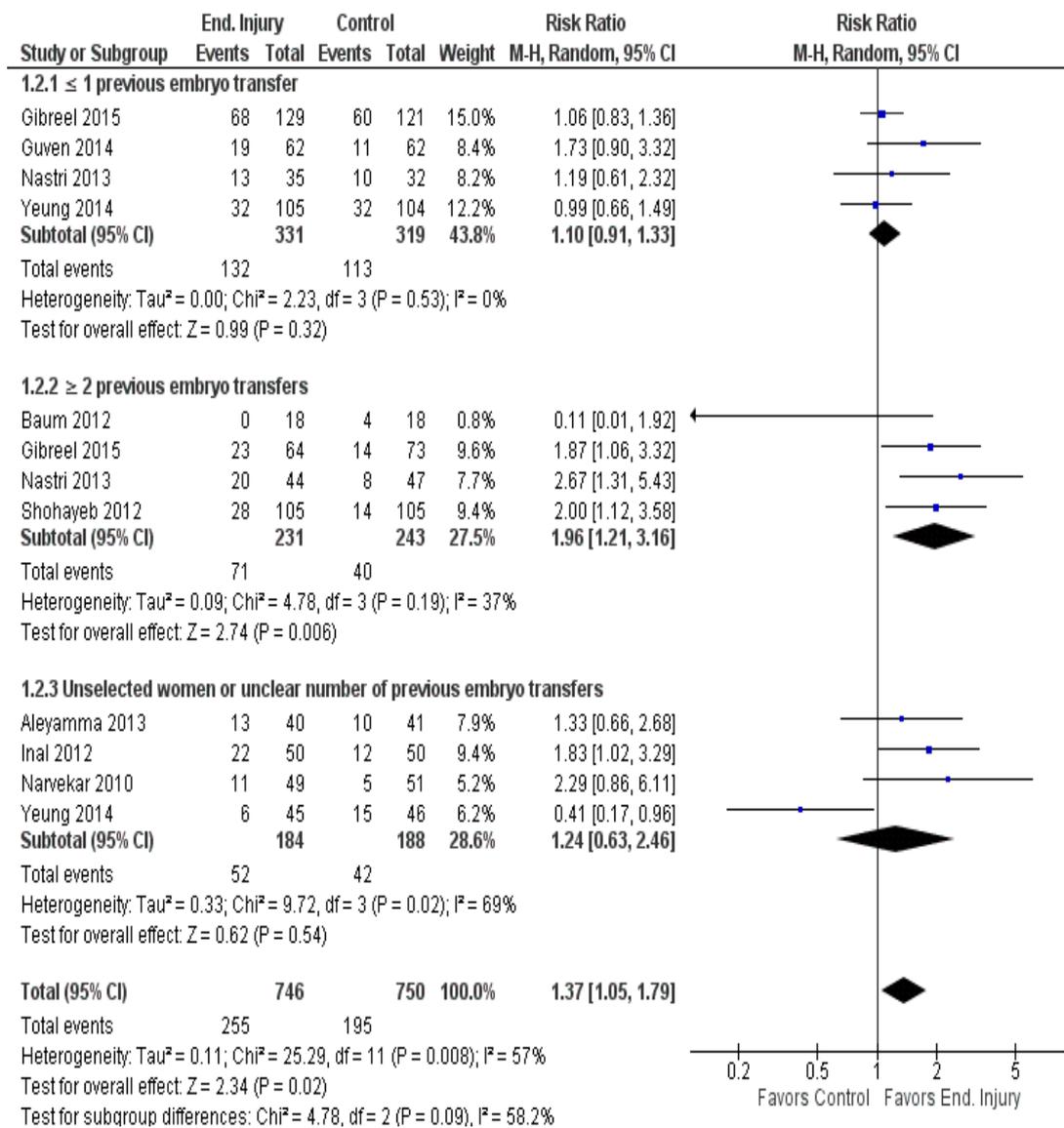


Figure 2. 3 Forest plot of comparison: Livebirth per randomly assigned woman

Figure 2.3 demonstrates the difference in outcomes between the different groups of women who underwent endometrial scratch. The endometrial injury improves the outcomes in women with two or more previous failed transfers rather than an unselected population. Reproduced with permission (Nastri *et al.*, 2015).

There was no difference in effect size between clinical pregnancy and livebirth and therefore miscarriage rates. When comparing the timing or the multiplicity of the endometrial scratch, there was no difference in the outcomes.

The TROPHY trial (Lancet 2015) results also found that hysteroscopy, prior to IVF, did not lead to an improvement in pregnancy outcomes (El-Toukhy *et al.*, 2016).

The latest published meta-analysis by Sar-Shalom *et al.*, assessed the impact of intentional endometrial injury on reproductive outcomes. They found that the clinical pregnancy rate (CPR) was improved after one previous failed cycle but was not improved by injury after two or more failed transfers, or in older women (Sar-Shalom Nahshon *et al.*, 2019).

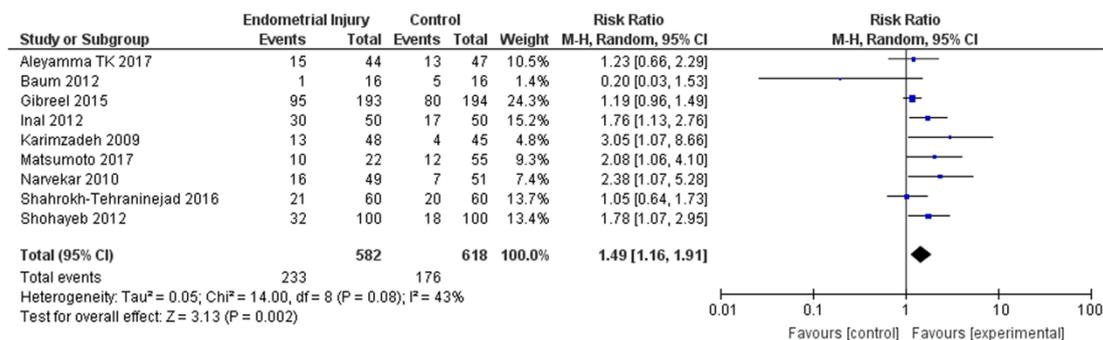


Figure 2. 4 Forest plot for clinical pregnancy rate (Sar-Shalom Nahshon *et al.*, 2019). The Forest plot showed that the CPR is improved in women who underwent endometrial injury.

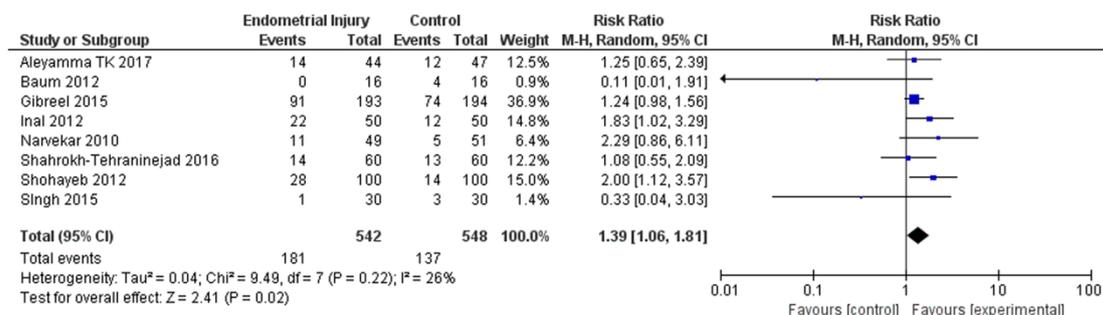


Figure 2. 5 Forest plot for livebirth rate (Sar-Shalom Nahshon *et al.*, 2019).

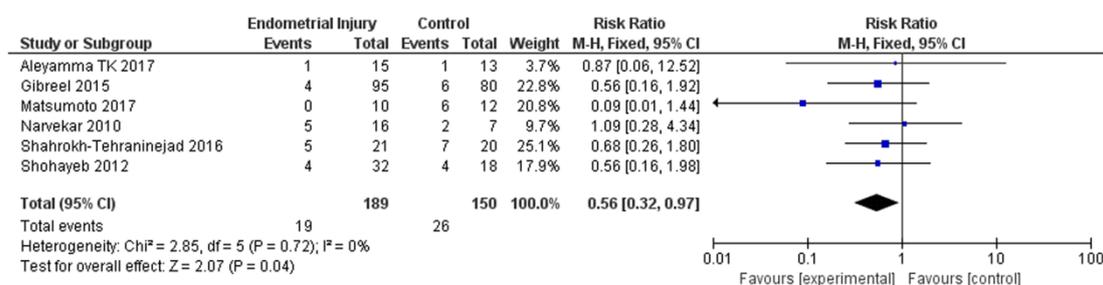


Figure 2. 6 Forest plot for miscarriage rate (Sar-Shalom Nahshon *et al.*, 2019).

The Forest plots in Figures 2.4 to 2.6 are used with permission from Hum Reprod Update (Sar-Shalom Nahshon *et al.*, 2019).

The review concluded that whilst hysteroscopy may improve outcomes in women with underlying uterine abnormalities its role in women with normal uterine cavity is not well established. The conclusion from the authors, Sar-shalom *et al.*, 2018, of this recent systematic review and meta- analysis is that endometrial injury improves the CPR and LBR in women with previous failed IVF cycles, with the basic science explanation supporting the finding. The influencing factors on success are the number of previous failed treatment cycles and maternal age. Hence the authors recommend a restrictive use of endometrial scratch procedure (Sar-Shalom Nahshon *et al.*, 2019).

Since my trial has finished, the latest published RCT from New Zealand by Lensen *et al.*, studied if endometrial scratch would increase the probability of pregnancy in women undergoing IVF by facilitating embryo implantation. The study randomised 1364 women and 26.1% of women achieved a pregnancy in both the ES and control group. There was no significant difference in the secondary outcomes considered including miscarriage rates. Thus, the authors found that endometrial scratch prior to their cycle of IVF did not improve livebirth (Lensen *et al.*, 2019). The median pain score was reported as 3.5 in the study group. In their subgroup analysis the authors noted a negative trend towards the outcome in women who had two or more previous failed embryo transfers thus questioning the role of endometrial scratch injury (ESI) that was widely used in women with RIF to improve implantation.

#### 2.7.1 Method of Injury:

The studies have considered the method of injury using either pipelle (Nastri *et al.*, 2012), Novak curette (Karimzade *et al.*, 2010) or hysteroscopy (Demireol & Gurgan, 2004; Makrakis *et al.*, 2009; Rama Raju *et al.*, 2006). The most common method used is the pipelle. The Novak catheter achieves a higher degree of injury.

### 2.7.2 Timing of Injury:

Karimzade *et al.*, found that endometrial injury on the day of oocyte retrieval had a negative impact on pregnancy rate (Karimzade *et al.*, 2010). The postulated explanation is that this could be due to the acute rejection of the embryo due to the acute inflammatory reaction (Siristatidis *et al.*, 2014). The beneficial effect of inducing injury was demonstrated when it precedes the cycle of ovarian stimulation. The effect has been shown to last in the subsequent cycle, the authors argued that this was possibly because the monocytes recruited to the injured sites are long lived and reside in tissues for a long time (Yeung *et al.*, 2014). Other authors argued that injury in the luteal phase is likely to induce more decidualisation due to the progesterone influence (Kumbak *et al.*, 2014).

Studies have compared if a single injury produces the same beneficial effect as multiple injuries. Shohayeb & El-Khayat concluded that a single luteal phase endometrial injury was found to be as effective as multiple injuries without the risks of potential risk of infection from repeated instrumentation, the possible negative effects due to endometritis and the additional costs of the procedure (Shohayeb & El-Khayat, 2012).

### 2.7.3 Impact on miscarriages:

Yeung TW *et al.*, found that there was a difference in the miscarriage rates between those with injury and those without, but it did not reach statistical significance (30.3% vs 18.6%) (Yeung *et al.*, 2014). However, the systematic reviews found no impact on miscarriage rate, but with the acknowledgment that the studies were of very low quality with high risk of bias (El-Toukhy *et al.*, 2012; Lensen *et al.*, 2016; Nastri *et al.*, 2015; Potdar *et al.*, 2012). The review by (Sar-Shalom Nahshon *et al.*, 2019) suggests that there is an improvement in miscarriages in the injury group.

### 2.7.4 Complications:

The consistent finding is the fact that very few studies (Polanski *et al.*, 2014; Yeung *et al.*, 2014) reported on the pain scores and therefore there is less evidence if women find the procedure acceptable. The average pain scores were between 5-6 (Nastri *et al.*, 2015). Only one study reported on the pain score and had an average score of 6 (Lensen *et al.*, 2016).

## 2.8 Interpretation of IVF Data:

Endometrial injury was shown to improve the IVF outcomes in younger women with good ovarian reserve and good response to ovarian stimulation but not in women with poor ovarian reserve (Baum *et al.*, 2012). The intervention is not expected to improve IVF outcomes in the presence of reduced ovarian reserve or poor embryo quality. However, when women with poor ovarian reserve received egg donation and hence good quality embryos there was still no effect of injury suggesting ovarian hormones are involved in the positive benefits of endometrial injury (Dain *et al.*, 2014). Kitaya *et al.*, suggested that local endometrial injury is most effective for improving the pregnancy rates and outcomes in patients with uncompromised ovarian reserve (Kitaya *et al.*, 2016). Sar-Shalom *et al.*, in their review recommend that the ideal population of women who should be tested for the effect of endometrial scratch are the recipients of ovum donation cycles (Sar-Shalom Nahshon *et al.*, 2019).

The current ongoing RCTs will further shed light to the question if endometrial scratching is applicable for a subset of population-RIF as the current evidence suggests or in a wider group of patients. This includes the ongoing SCRaTCH trial in Denmark in couples who have one failed cycle of treatment (van Hoogenhuijze *et al.*, 2017). The UK wide Endometrial scratch trial is aimed at couples who undergo endometrial scratch in their first cycle of IVF/ICSI (Pye *et al.*, 2018). This would help to define the population that are likely to benefit from endometrial scratch.

## 2.9 Endometrial Scratch and RPL:

There are no trials of endometrial scratch in women with RPL. The role of endometrial scratch in women with RIF has been widely studied and necessitated the need for conduct of a similar trial in women with RPL, the first of its kind in the world. The literature review suggests that endometrial injury is beneficial in women with a likely endometrial factor such as repeated implantation failure and good ovarian reserve. Women with RPL also fall into this category of a likely associated endometrial factor. The most convincing data is that RPL has been

associated with endometrial stem cell deficiency and endometrial scratch injury is thought to increase endometrial stem cell recruitment.

## Conclusion:

My review of the scientific evidence suggests that endometrial scratch may benefit women with RPL. Series of credible scientific mechanisms, as discussed, by which scratch could improve endometrium and reduce pregnancy loss contributed by endometrial dysfunction.

The clinical evidence also suggests that scratch would benefit a subgroup of women with reproductive failure, contributed to by endometrial dysfunction. There has been no RCT of endometrial scratch in women with RPL. RCOG scientific impact paper No 54, 2016 acknowledges that the effect of endometrial trauma on pregnancy outcomes in women with RPL has not been evaluated (RCOG, 2016b).

Therefore, at the end of the literature search, we aimed to answer the following questions

1. Is trial of endometrial scratch in RPL women feasible?
2. Does uNK cell density identify those in whom scratch is followed by livebirth compared to repeated miscarriage?
3. Does endometrial scratch improve endometrial selectivity, prevent implantation of abnormal embryos in superfertile women and thereby increase livebirths?

The set up and conduct of the trial will be discussed in Chapters 3-5.

# Chapter 3

## Chapter 3: Trial Planning and Set Up

This chapter discuss the issues faced in the trial planning and design.

### 3.1 Hypothesis:

The trial was therefore designed to see if we could assess the hypothesis that endometrial scratch in women with unexplained RPL improves the livebirth rate.

#### 3.1.1 Aims and Objectives:

##### *3.1.1.1 Primary*

To assess whether a trial of endometrial scratch is feasible in women with recurrent pregnancy loss.

##### *3.1.1.2 Secondary*

- To accumulate a tissue bank of samples from women with recurrent miscarriages and pregnancy outcomes and compare the endometrium of different groups
- To compare the pre-pregnancy endometrium of those with miscarriage and livebirth
- To assess whether scratch benefits those with clinical evidence of failure of a functional role of decidualisation, selectivity i.e. those presenting with superfertility.

The aim was to generate data needed to support the design of a definitive trial. The trial design involved discussions between myself, the Chief Investigator-Professor Siobhan Quenby, Professor Jan Brosens, Peter Kimani the University statistician, my co-investigators and the biomedical research unit (BRU) team members. Their input was valuable in re-evaluating the different steps of the process. Questions and ambiguity during the trial set up was answered in this team, guided by potential patients themselves.

### 3.2 Research Question:

Is it feasible to do a trial investigating whether endometrial scratch improves pregnancy outcomes in women with RPL?

### 3.2.1 Research Design and Methods:

The first question was should we do a feasibility or a pilot trial. Clinical trials are expensive, and the likelihood of successful recruitment/completion can be assessed if the question, “Can this study be done?” is answered. Thus, feasibility studies are used to estimate important parameters that are needed to fulfil the design of a larger trial. The key to the design of a large RCT is through an initial pilot study. Pilot studies, as defined by National Institute of Health Research (NIHR) are “a version of the main study that is run in miniature to test whether the components of the main study can all work together. It is focused on the processes of the main study, for example to ensure recruitment, randomisation, treatment, and follow-up assessments all run smoothly. It will therefore resemble the main study in many respects, including an assessment of the primary outcome”. Feasibility studies are the initial step in the setting up of a novel intervention. This is not a hypothesis testing process but it is a, guide to early phase developmental function for a future successful RCT (Leon *et al.*, 2011).

We decided that a feasibility trial was needed as there were a series of questions that needed to be addressed in order to design a large scale trial.

### 3.2.2 Feasibility Study:

The aims of the study were to assess patient suitability, acceptability, recruitment rate, pregnancy outcomes, endometrium and trial procedures. The study results are needed to determine the optimal inclusion criteria, recruitment process, primary outcome measures and guide a power calculation for a larger multicentre randomised controlled trial.

## 3.3 Trial Methods- Randomised Controlled Trial

### 3.3.1 Patients:

We decided to be pragmatic and ask all women aged 18-42 with two or more idiopathic miscarriages, that we could approach, if they would agree to be in the trial.

### 3.3.2 Set-up:

This was a single centred study that was conducted in University Hospitals of Coventry and Warwickshire (UHCW) NHS Trust, led by Professor Siobhan Quenby as the Chief investigator (CI).

### 3.3.3 My Role in the Trial:

My role was to set up the trial including the essential documentation, completion of the Integrated Research Application System (IRAS) application, attendance at the ethics committee meeting and to secure other approvals including the trust research and development (R&D) department. I co-ordinated the running of the trial including randomisation, support of the research midwives and resolved any issues arising from running the trial. I organised regular trial management meetings to discuss trial progress and plan ongoing recruitment. I maintained the database for the outcome of the patients and carried out clinics where patients presented for reassurance scans.

### 3.3.4 Recruitment:

The study aimed to try and identify and recruit patients from the recurrent miscarriage clinic (RMC), the early pregnancy assessment unit and patient self-referral after advertising via the Tommy's charity website. The RMC is run weekly by Professor Quenby at the centre for reproductive medicine (CRM) at UHCW and accepts patient referrals from all over the country after two previous miscarriages. The patients are investigated for conditions commonly associated with RPL and then given the option to participate in various relevant trials within the unit. The clinic is supported by a team of research midwives, trial assistants and medical team comprising of research fellows and clinical lecturers. The women are followed up through the study protocol of the corresponding studies. In addition, they are also offered reassurance scans in a subsequent pregnancy on a fortnightly basis from 6 weeks of pregnancy until their dating scan.

On a pragmatic basis the study aimed to recruit patients for a year from NRES approval and the follow up continued until the six weeks postnatal telephone consultation of the last woman who had achieved a live birth within the trial.

### 3.3.5 Transvaginal Ultrasound Scan:

We decided to incorporate a transvaginal scan prior to the procedure to complete a baseline assessment of the uterine cavity and ovaries. This provided additional reassurance for the women and had the potential to identify any pathology which was otherwise not anticipated including uterine anomalies and ovarian pathology. The ultrasound scan had an additional advantage for women in the biopsy group. It would guide the investigator regarding the uterine position and flexion in relation to the cervix and thereby alter the technique as the anatomy demands. This would include anticipation of potential difficulties in the procedure in case of acutely anteverted or retroverted uterus.

### 3.3.6 Speculum Examination:

This is a common procedure carried out in different settings such as obtaining cervical smear, in early pregnancy bleeding, insertion of coils and obtaining swabs. Most women tolerate the speculum examination and the procedure is quick.

### 3.3.7 Phase of the Cycle:

The selection of the luteal phase of the cycle was because decidualisation and implantation occurs in this phase. Previous evidence for endometrial injury in women with RIF was based on endometrial scratch performed in this phase. In addition, the scratch was timed, to 7-10 days post LH surge so that any tissue obtained could be used for other ethically approved studies on endometrial factors contributing to miscarriage. We decided to ask the patients to organise their randomisation visit between LH+7 to LH+10, based on home ovulation testing. Free ovulation test kits were issued to the patient (courtesy of Clearblue) to both the groups of patients. We randomised women on the day of the procedure so that we did not lose women between randomisation and intervention.

### 3.3.8 Additional Considerations for Sampling in Luteal Phase-Pregnancy:

In order to minimise the risk of pregnancy at the time of sampling, the patient information sheet (PIS) had clear instructions about avoiding trying for pregnancy in the month of randomisation, either by abstinence or using barrier contraception. The patients were counselled regarding the potential of cancellation should there be any risk of very early pregnancy.

### 3.3.9 Intervention Arm- Endometrial Scratch:

A Wallach endometrial sampler was introduced through the cervix, into the uterine cavity until the fundus was reached. Then the piston was withdrawn and the sampler was moved up and down the cavity at multiple angles for 10 seconds.



*Figure 3. 1 Image of a Wallach catheter*

We aimed to obtain adequate scratching as assessed by the quantity of endometrium in the sampler, with good patient tolerability with 10 seconds of sampling. The sample obtained from the process was stored in the tissue bank so that it could be analysed for uNK cell density to assess the correlation with the pregnancy outcome.

The intervention was planned to be carried out by a gynaecologist on the delegation log, all of whom had been trained to perform the procedure and who undertake the procedure in this setting on a regular basis.

### 3.4 Ethical Considerations in the Control Arm:

Many scratch trials in the context of IVF had no “sham procedure’. We wanted to explore this option and therefore decided that women in the control arm should also undergo a transvaginal ultrasound for reassurance regarding uterine and ovarian abnormalities.

#### 3.4.1 Need for a Control Arm:

We explored the use of a ‘Sham’ procedure that would be similar to the intervention arm so that women would not know which group they were allocated to. In addition, we wanted the outcome assessors to be blind to the allocations. Hence, we explored the option of performing a speculum examination in both groups.

#### 3.4.2 What Would Mimic Endometrial Scratch in the Control Arm?

The procedure on the cervix, which would mimic the intervention arm, had to consider the possibility of unintended endometrial injury during the sham procedure. We decided that the sham procedure should not proceed beyond the internal cervical os. The options for the sham procedure included touching and cleaning the cervix for 10 seconds to mimic the timing similar to the intervention group. Alternative option of touching the cervix with the Wallach sampler was considered as it then improves the procedure similarity and the patient and partner perception. The sampling device would be securely received back into an opaque paper container and taken out of the room for further processing.

We decided to consider speculum examination and touching of the cervix with the Wallach catheter in the control arm.

### 3.5 Patient and Public Involvement:

Patient involvement in assessing the acceptability of a sham procedure in the control arm of the trial during the consultation phase was explored.

### 3.5.1 Question and Method of Testing:

Is it acceptable to subject the patient to an invasive procedure such as speculum examination and the sham procedure? We decided that the best way to answer this question was to ask potential patients themselves. We did this by using a semi-structured questionnaire. The questionnaire was designed to provide insight of patient's views and acceptability of the sham procedure, prior to presenting this to the ethics committee. If the majority of the patients had felt that it was not acceptable, then there was an opportunity to reconsider the sham procedure in the control arm.

### 3.5.2 Setting for Questionnaire Testing:

The setting for the questionnaire was considered. Options considered were, the early pregnancy assessment unit (EPAU), the implantation clinic and the recurrent miscarriage clinic. The Implantation clinic is a biweekly research clinic, for patients with RPL and RIF, led by Professor Brosens and Professor Quenby. The EPAU clinic was a diverse setting with women presenting with various problems which would include miscarriages. It was concluded that this setting may not be the most appropriate for patient feedback due to introduction of bias in selecting the appropriate patients and it was thought to be unfeasible in a busy emergency situation.

The alternative option was the implantation clinic. This is a self-funded research clinic where women with both recurrent implantation failure and recurrent miscarriages attend. The sample population was better representative of the cohort of the patients being tested and there were less time pressures in the clinic. However, all women attending this clinic were expecting an endometrial biopsy, because of written information given before attending, so this was also thought to be a biased population.

The decision was made to collect the questionnaires from 10 women and their partners attending the recurrent miscarriage clinic. This was initiated by the other co-investigator in the trial and discussed in the pre-trial meeting before it was presented to the patients.

The responses from the women were collected over a period of one month. The patients were approached by the clinical trials assistant who requested that they completed a simple quick questionnaire prior to their appointment in the waiting area. The patients were aware that participation was entirely voluntary and would not impact on the treatment provided if they did not wish to participate. If the women showed interest they were provided with the questionnaire and the results were briefly discussed by the co- investigator.

Patient feedback was particularly focussed on the timing of the biopsy and the invasiveness of the speculum. The patient questionnaire is in appendix 5.

### 3.5.3 Outcome from the Questionnaire for Sham Procedure:

Ten couples were approached for their opinions and all agreed to share their thoughts. Nine couples out of the ten approached responded that they would accept a sham procedure. The remaining patient did not object to the speculum examination but merely wished that all patients in the trial should have the endometrial scratch.

Three women left comments as follows:

1. *'As long as full communication and consent is given.'*
2. *'Because most of women they do not know the cause of miscarriage, so research might help to find out.'*
3. *'With plenty of information given and support for both male and female.'*

One patient felt it was unacceptable to offer only a speculum examination in a single-blinded trial as the 'placebo group'. This patient's comment was as follows:

*'Because it is about trying every treatment for pregnancy to happen.'*

The responses validated our thinking that patients would accept a sham procedure if it was essential to blinding, randomisation and trial outcomes. The responses from the ten patients

were presented to the ethics committee. The information provided was instrumental in obtaining permission to undertake a sham procedure in the control arm.

Feedback was obtained from the research team members regarding the PIS (Appendix 8) and the language and layout was changed to reflect their suggestions.

### 3.6 Blinding in the Trial:

There was a consideration of whether the trial should be single or double blinded? After the outcomes from the patient questionnaire it was felt that it is feasible to blind the patients. The issue of blinding the outcome assessors was discussed. This included the CI and research midwives. Though the lack of blinding would be unlikely to affect the pregnancy outcomes of miscarriage or livebirth for the patient, we felt blinding the staff assessing outcomes would minimise any reporting bias and inadvertent disclosure of randomisation to the patient during the follow up period.

- Methods for Ensuring Blinding:

The patient and the outcome assessor (BRU research midwives) would be blinded. However, the investigators would be aware of the allocated group. The investigators would know whether or not they took the biopsy.

- Methods for Unblinding the Trial:

It was thought to be unlikely that unblinding would be necessary, however in the event of complications in the week after the biopsy, unblinding was available. The group allocation was available in the files in the BRU. This BRU has a security pass access and locked office and cupboard. However, the investigator team were able to access the BRU at any time in a 24-hour period.

### 3.7 Methods and Assessments:

#### 3.7.1 Schedule of Delivery of Intervention and Data Collection:

<b>Visit</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
<b>Visit Window timeline</b>	<b>Recruitment</b>	<b>Consent</b>	<b>4-12 wks after V 2 randomisation</b>	<b>4wks after V3</b>
Informed consent		✓		
Issue of ovulation test kits		✓		
Medical History	✓			
Screening Urinalysis BP measurement	✓			
Inclusion/exclusion criteria	✓	✓		
Intervention			✓	
Questionnaire				✓

*Table 3. 1 Schedule of delivery of interventions in the trial*

### 3.7.2 Outcome Measure Assessment:

As infusing stem cells into the uterine artery has been demonstrated to last 3 months (Santamaria *et al.*, 2016), we assumed the effect of endometrial scratch on increasing endometrial stem cells was at best likely for three months. Therefore, we decided to only investigate pregnancies that occur within three cycles of the scratch. We thought that it was biologically implausible for scratch to affect endometrium longer than three menstrual cycles. In addition, if scratch improved decidualisation it should improve selectivity. This means that we aimed at preventing the losses in the superfertile group of women who lose abnormal pregnancies and conceive within three months.

### 3.8 Power and Sample Size:

Peter Kimani the trial statistician advised that although this was a feasibility trial, a sample size, power calculation would be useful. The aim was to test the null hypothesis of equal birth rates at two-sided 20% significance level (Schoenfeld, 1980). We assumed that the birth rate in the control is 60% (Coomarasamy *et al.*, 2016) and that the scratch will improve birth rate by 20% to 80%. The power to detect this difference for a study with a sample size of 100 (50 in each group) at 20% significance was 82%. We aimed to allocate 50 patients to each group (100 in total).

We thought that a 20% difference was a very optimistic assessment of the efficacy of scratch. He recommended that we performed a power calculation so that we were aware of the effects size that was detectable. We anticipated that nine women seen in RMC per month would meet the inclusion criterion. This would equate to 108 women in 12 months. We expected 100 women to consent and proceed with randomisation. We expected 92.6% of the women that consent would attend the randomisation visit. We aimed to use the 50 women enrolled in the intervention arm to assess the acceptability rate for the scratch intervention. We expected that the scratch would be acceptable to 95% of the women. If the acceptability rate was 95%, with 50 women, the 95% confidence interval was 89% to 100%, which was sufficiently precise for a feasibility study.

#### 3.8.1 Statistical Analysis of Efficacy and Harms:

The statistical analysis was planned to assess the primary outcome measure of livebirth. We planned to compare the birth rates for each group and compare them using a chi-squared test. We decided it would be considered worthwhile, to conduct a larger study if the p-value was less than 0.2. A larger cut-off for p-value was chosen because it was a trial to consider whether it was worthwhile to conduct a larger study in which type I error rate will be controlled at a lower rate (Schoenfeld, 1980).

We planned to assess recruitment by assessing the number of eligible women who consented and the number of consented women who were randomised. We planned to report the

proportion (and the 95% confidence interval) for the women who consider the scratch procedure (intervention) acceptable compared to the sham procedure.

### 3.9 Development of Supporting Documents for the Trial:

The following documents were developed in support of the protocol and the delivery of the trial for the ethics committee.

#### 3.9.1 Protocol:

The protocol was developed using the university of Warwick template non CTIMP trial protocol. The protocol captured the aims and objectives, including the eligibility criterion and outcome measures (Appendix 2).

#### 3.9.2 Patient information sheet (PIS):

The patient information sheet was developed in accordance with the University of Warwick stipulations along with Trust guidelines. The PIS was intended to be short with pictorial representation along with bullet points with simple language, avoiding the use of medical jargon (Appendix 8).

#### 3.9.3 Poster:

The poster was one of the advertising materials intended to be a short communication regarding the study to patients attending the EPAU clinic or miscarriage support group (Appendix 6).

#### 3.9.4 GP letter:

This is a mandatory letter requesting the patient's consent of disclosure of their participation in the trial to their GP (Appendix 9).

#### 3.9.5 Letter of invitation:

This was developed for use in conjunction with the poster and contained the contact information for the interested patient (Appendix 7).

### 3.9.6 Consent form:

This was produced in accordance with the University of Warwick guidelines (Appendix 16).

### 3.9.7 Telephone interview questionnaire:

There were two questionnaires that were designed for use in the trial. The first of which was the follow up telephone questionnaire that was incorporated in the CRF. This was a non-validated questionnaire aiming to collect the outcome measures in women who had a livebirth (Appendix 14).

### 3.9.8 Questionnaire for assessing acceptability:

One of the aims of the trial was to ascertain the acceptability of the endometrial scratch and also the response rate. This was another non-validated questionnaire given to patients on the day of randomisation to both the group of patients. This was designed to try and answer the question regarding the efficacy of blinding from the patient's perspective. The results of the questionnaire analysis will be discussed in Chapter 5. The questionnaire contained an objective score of pain, bleeding. Pain was scored between none, mild, moderate and severe. The bleeding was scored in comparison to their periods. There was a space for free comments from patients including some questions to evaluate the service provision with a view to change or improve in future larger studies (Appendix 3).

### 3.9.9 Case Report Form (CRF):

The case report form was formulated based on the University of Warwick template. The first page captured the demographic details along with the previous pregnancy outcomes and relevant medical and surgical history. This was completed on the day of obtaining consent along with the details of the person obtaining the consent. The clinical page was filled in on the day of randomisation including the scan findings. The clinical pregnancy outcomes were recorded on the final page along with the details of the pregnancy scans. The last page was for the completion of the data collection with a telephone interview at 6 weeks in patients who had achieved a livebirth (Appendix 15).

### Adverse event form (AE):

This form was developed using the University of Warwick template. The causality would be ascertained by the CI (Appendix 4).

### Conclusion:

Thus, a feasibility randomised controlled trial was set up to evaluate the effect of endometrial scratch on pregnancy outcomes in women with unexplained RPL.

# Chapter 4

## Chapter 4: Trial Methods

This chapter describes the trial protocol. The trial protocol was developed in accordance with the SPIRIT guidelines. The University of Warwick template was used as a guide for the development of the protocol.

### 4.1 Study Summary:

**Full title-** A pilot randomised controlled trial of the effects of endometrial scratch in women with recurrent miscarriage, on their pregnancy outcomes

**Acronym:** SiM (Scratch in Miscarriage) study

**Hypothesis:** Does endometrial scratch improve pregnancy outcomes in women with unexplained recurrent miscarriages?

<b>Population</b>	Women aged 18-42 with two or more idiopathic miscarriages
<b>Intervention</b>	Endometrial scratch in the luteal phase at LH+7 to 10
<b>Comparator</b>	Sham procedure- speculum examination and touching/cleaning of cervix
<b>Outcomes</b>	Pregnancy outcome in the three cycles after the randomisation visit

*Table 4. 1 Summary of the SiM study*

#### 4.1.1 Outcome measures:

Primary	Secondary
Livebirth after 24 weeks	Miscarriage- types, gestation, karyotype of the loss, ectopic pregnancy Pregnancy complications- placenta praevia, foetal growth restriction, preterm labour Acceptability of the intervention by questionnaire analysis

*Table 4. 2 Outcome measures in the study*

#### 4.1.2 Study details:

**Trial design:** Single centre, double blind, randomised trial. The patient and outcome assessor were blinded to the procedure.

**Method of randomisation** - Sealed sequential envelope method with random treatment allocation

**Sponsor-** University of Warwick

**Funding-** Biomedical Research Unit and Tommy's Charity Coventry

**Recruitment:** RMC, EPAU, self-referral

#### 4.2 Trial Organisation and Oversight:

##### 4.2.1 Sponsor and Governance Arrangements:

The primary sponsor for the study was University of Warwick and the Warwick CTU Standard Operating Procedure (SOP) standards was followed.

##### 4.2.2 Regulatory Authorities/Ethical Approval:

The IRAS application form was submitted to the North Birmingham research ethics committee and a favourable opinion was obtained on 16<sup>th</sup> October 2015. The trial commenced after the trust R&D approval on 30<sup>th</sup> November 2015. A substantial amendment was submitted in

February 2016 and was approved. The first patient was consented on 30<sup>th</sup> November 2015 and the last patient was recruited in July 2017. The trial was registered on the [clinicaltrials.gov.uk](http://clinicaltrials.gov.uk) website. The Trial Registration number is NCT02681627.

#### 4.2.3 Research Ethics Committee (REC) Review:

The trial was submitted for REC approval and recruitment was commenced once favourable opinion had been obtained and the Trust R&D approval was in place. The documentation was filed in the Trial Master File. All substantial amendments were implemented after REC and R&D approval had been obtained. The CI notified the REC when the trial was formally concluded with a follow up final report on the study results.

### 4.3 Trial Summary and Flow Chart

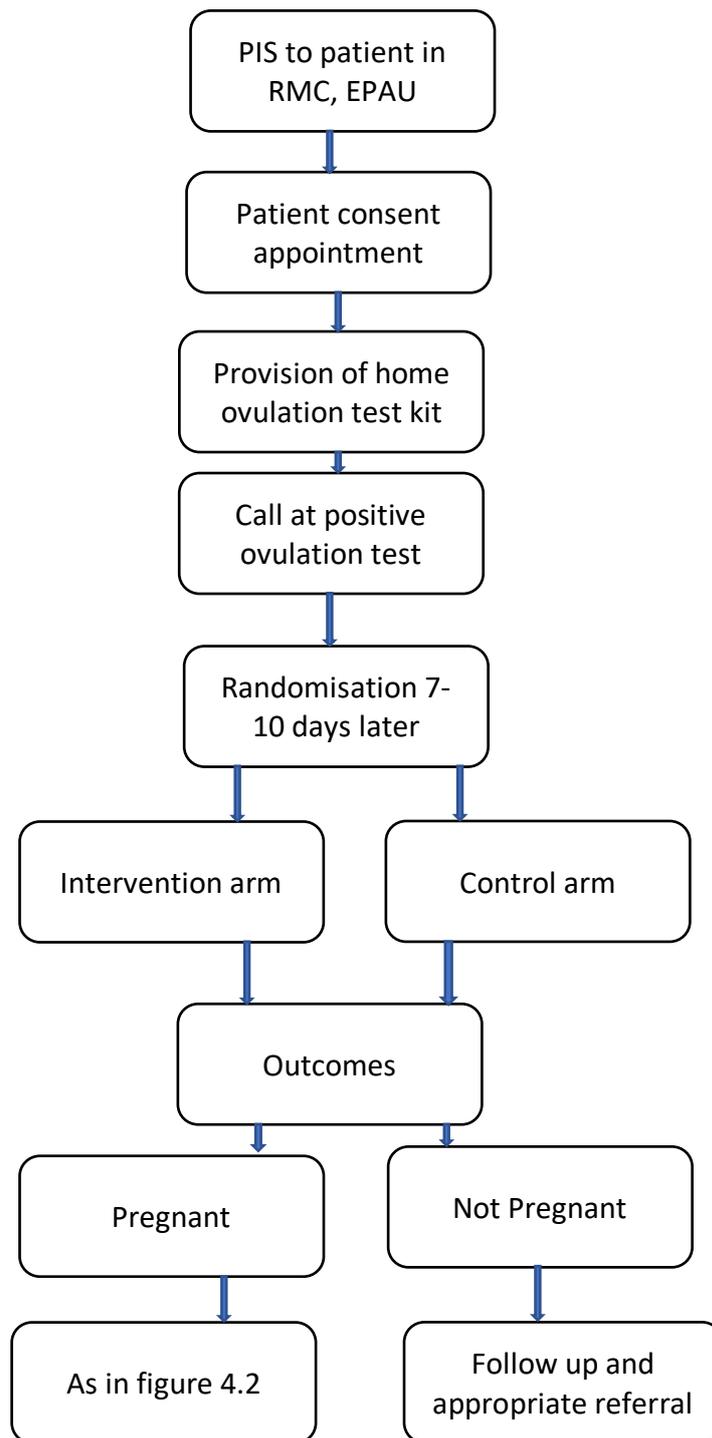


Figure 4. 1 Trial Flow chart

#### 4.4 Recruitment and Randomisation:

##### 4.4.1 Eligibility Criteria:

Patients were eligible to be included in the trial if they meet the following criteria:

Inclusion Criterion	Exclusion criterion
<ul style="list-style-type: none"><li>• Actively trying for a pregnancy</li><li>• Unexplained RPL</li><li>• Age 18-42</li><li>• Able to consent to the procedure</li></ul>	<ul style="list-style-type: none"><li>• No active treatment during the pregnancy</li><li>• Inherited or acquired thrombophilia</li><li>• Medical conditions- diabetes, hypertension, thyroid disorders</li><li>• Inability to tolerate internal examinations</li><li>• Uterine anomalies</li><li>• Previous entry or randomisation in the present trial.</li></ul>

*Table 4. 3 Eligibility criteria for inclusion and exclusion in the study*

The trial aimed to report on the livebirth after 24 weeks of gestation of the pregnancies achieved within the three cycles of randomisation. Hence, we aimed to recruit those couples who were actively trying for a further pregnancy and in the age group with a high possibility of achieving a spontaneous conception.

The aim was to actively assess the effect of endometrial scratch on pregnancy outcomes. Any co-existing conditions that necessitated treatment either pre-pregnancy or during pregnancy were excluded to prevent confounding. As the women in both groups would undergo internal examinations, anyone with an inability to tolerate internal examinations were also excluded.

#### 4.4.2 Recruitment:

The study aimed to recruit women from the EPAU clinic, self-referral and from the RMC. New patients in the RMC were given information about the trial at the time of their investigation screen. If their investigation screen was negative, they were offered attendance at the biomedical research unit-reproductive health (BRU-RH) for their consent visit. Women who have had a diagnosis of miscarriage in the EPAU clinic were offered the invitation letter (Appendix 7) regarding the study. Interested women were sent a PIS, when they contacted the BRU. PIS was issued and consent for participation in the study was obtained after at least 24 hours of the receipt of the PIS. Women attending the recurrent miscarriage clinic were offered the opportunity to have information and the explanation of the trial by members of the research team with provision of PIS. The Tommy's Miscarriage Association website also contained some information about the trial. Information about the trial was made available on the local BRU-RH website. Social media such as Twitter and UHCW Facebook account was used to advertise the trial. This was to enable women to self-refer for the trial. All advertising material had ethics committee approval prior to use.

Eligibility for participation in the trial was assessed at the first visit and the criteria were checked. Consent for participation was confirmed on the day of the visit. We aimed to recruit a total of 108 patients over a period of 9-12 months. The recruitment was planned to continue for the duration even if the required numbers were achieved.

#### 4.4.3 Consent Visit:

During the consent visit, the eligibility criterion was once again confirmed by team members followed by the signing of the consent form as per Warwick Clinical Trials Unit (CTU) specifications. The consent was obtained by the investigators with the initials of the woman. Three copies were obtained, one each for the CRF, hospital notes and the patient. The consent included disclosure of information to GP, access to hospital records and contact by telephone for interview, six to eight weeks after delivery. The GP letter was posted at recruitment (Appendix 9).

The demographic details were filled in the CRF at this visit. Women were then issued with home ovulation test kit for a month with the instructions of use. The woman should have had at least one normal cycle after the miscarriage, before the endometrial scratch. They were given advice to use barrier contraception in the cycle when they would have the randomisation visit in order to prevent any unplanned pregnancy.

If the patients were from out of the local area, the consent and randomisation visit happened on the same visit day. The contact details were also shared for booking appointments and for seeking advice. Participation in the study was voluntary. We aimed to consent women after they have had at least 24 hours to read the PIS (Appendix 8) and consider the information.

## 4.5 Randomisation:

### 4.5.1 Randomisation Visit:

Once the ovulation test was positive the patient was required to telephone the BRU-RH and make an appointment for a randomisation visit between 7-10 days after the positive test. The patients were randomised from Monday to Friday from 8am to 6pm. Provisions for randomisation and undertaking procedures for Saturday were made in individual circumstances. The BRU- RH was open on Saturday with availability of staff for randomisation and chaperone.

At the randomisation appointment, consent was confirmed and a paper Trust consent was obtained and filed in the CRF and hospital notes. This included the risks and benefits of the procedure and the use of endometrial tissue obtained for further research projects.

The randomisation was through a sealed sequential envelope method with random allocation stratified for every 10 numbers. The envelope was picked by a member of staff at BRU-RH and opened in the clinic. The patient was randomised to either sham or endometrial biopsy.

Trust consent form was completed for the procedure of endometrial biopsy/sham procedure. This second consent was obtained by the investigator for the day in the clinic. The procedure was undertaken in the presence of a chaperone. Inhalational analgesia in the form of entonox through a disposable mouth valve was available for all women during the intervention. The

women then had the transvaginal ultrasound scan (TVS) and the findings were explained to her and partner. The trolley with the necessary equipment for the procedures was set up at the bottom end of the table. The investigator then proceeded with the speculum examination followed by the endometrial biopsy/sham procedure. The withdrawn Wallach catheter was returned into an opaque envelope and taken to the adjacent room. The procedure was carried out by three investigators only-myself, Dr. Lokman and Dr. Tewary, who were the research fellows for the BRU-RH. The findings from the TVS and the procedure details were captured on the CRF. The patient's participation in the trial was documented in the hospital notes along with a copy of the consent form and PIS. The research midwives were blinded to allocation.

#### 4.5.2 Data Management:

The patient details were then entered in a password protected Microsoft excel sheet. At the end of the appointment the woman was given a questionnaire along with a self-addressed envelope. She was requested to complete and post the form within 4weeks of the intervention and preferably not to complete it on the day of the procedure. This was to evaluate the patient's acceptability of the trial and feedback from the appointment. The data was analysed at the end of the trial.

#### 4.5.3 Post-Randomisation Withdrawals and Exclusions:

The patients were made aware that participation in the study was voluntary and they had the option of withdrawal from the study at any time. The reason for the withdrawal would be collected and documented. If the patient withdrew, from the study she would be offered the same follow up as she would have had, if she continued in the trial. This would be dependent on the patient's willingness to engage in further follow up. As there were no medicinal products involved in the trial, withdrawal by the investigator was deemed unlikely and did not occur.

### 4.6 Trial Details

#### 4.6.1 Outcome Measures:

The primary outcome measure was:

- Livebirth rate after 24 weeks of gestation.

The primary aim of the trial was to evaluate if endometrial scratch prevents further miscarriages. The most meaningful outcome for the patient is a livebirth and hence this was decided to be used as the primary outcome measure. This was assessed by patient self-reporting, access of hospital records and telephone follow up at six weeks after their expected date of delivery.

The secondary outcome measures were:

- 3 Miscarriage before 23+6 weeks of pregnancy, gestation at miscarriage and the type of miscarriage (biochemical pregnancy, delayed miscarriage, second trimester loss), ectopic pregnancy along with karyotype results
- 4 Pregnancy complications such as SGA, PET, abruption, placenta praevia, placenta accreta, preterm delivery
- 5 Acceptability of the intervention by questionnaire analysis and also by the above methods

#### 4.6.2 Pathways for Outcomes in the Trial:

##### *4.6.2.1 In case of pregnancy:*

The primary endpoints in this study were pregnancy achieved within three cycles after randomisation and their outcomes. As soon as conception occurred, patients contacted the clinic and fortnightly scans from six weeks at fortnightly intervals until the dating scan and the results were recorded in the CRF. These scans were to allay anxiety and provide reassurance. Additional scans were organised if the patient experienced symptoms such as pain, bleeding or in the event of an inconclusive scan.

We recorded the expected date of delivery and contacted the women by telephone and also encouraged women to contact us for the birth outcomes. The pregnancy outcomes were collected and presented with the data. This included livebirths after 24 weeks, miscarriage, termination of pregnancy and stillbirth.

The scans were undertaken as part of the care package available for women who attend the recurrent miscarriage clinic. Hence women who fell pregnant after three menstrual cycles were also offered follow up scans as part of their pregnancy management from the RMC. All reports of congenital abnormalities and/or birth defects would have been reported and followed up as a SAE. There were none in the study.

### Pregnancy Outcomes Flowchart

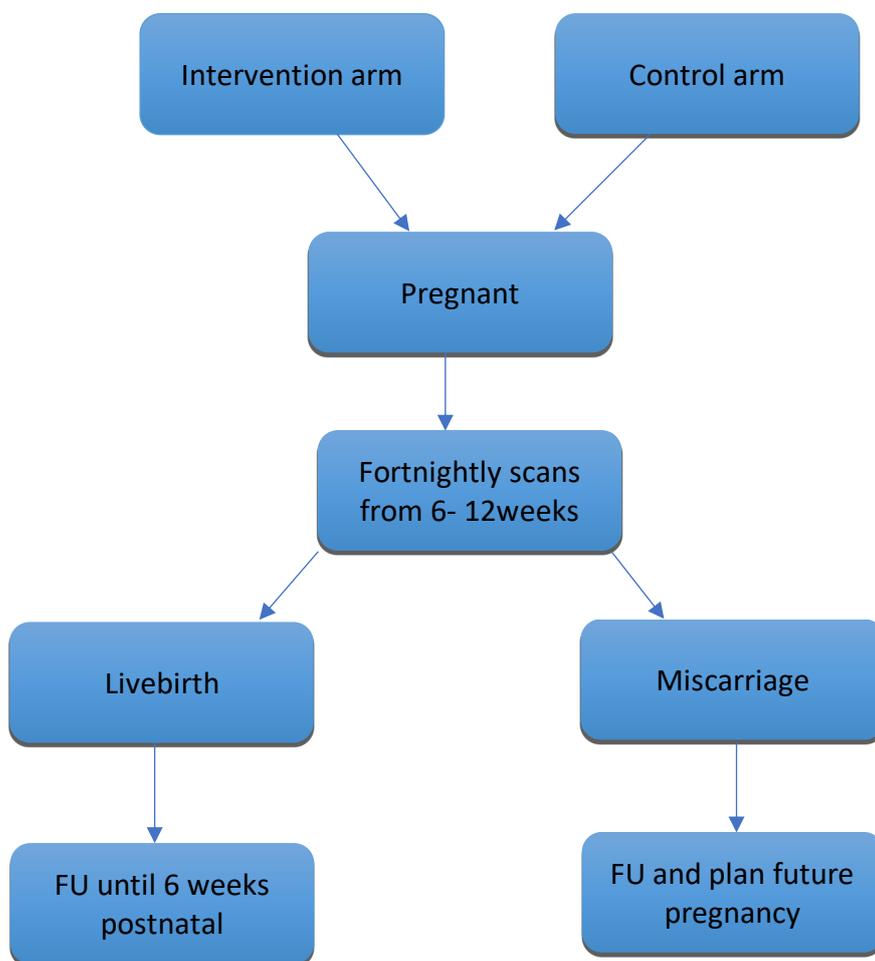


Figure 4. 2 Pregnancy outcomes flowchart

### When Patient is pregnant

Visit	1	2	3	4	5
Visit Window No. Weeks ( $\pm$ No. Days)	6Wk (+/- 7d)	8wk ( $\pm$ 7d) After V 1	10 Wk. ( $\pm$ 17d) After V2	8-9mon ( $\pm$ 14d) After V 3	6- 8wks ( $\pm$ 7d) After V 4
Ultrasound	✓	✓	✓		
Outcomes				✓	✓
Questionnaire					✓

Table 4. 4 Schedule of delivery of care during pregnancy

#### 4.6.2.2 In the event of another miscarriage:

If the patient miscarried, then we aimed for the pregnancy tissue to be sent for cytogenetics testing. She was offered a follow up in clinic with Professor Quenby to discuss the results of tests on the pregnancy tissue, if obtained, and to formulate future management plans. The patients in the control arm were offered the option of endometrial scratch outside the research setting. This was arranged through the recurrent miscarriage clinic. The participants in the trial would be informed of the results of the trial on conclusion of the study.

#### 4.6.2.3 In the event of not achieving a pregnancy after three months:

As the trial progressed it was apparent that there was a proportion of patients who failed to achieve a pregnancy. They were offered follow up with Professor Quenby and myself due to my experience working as a clinical fellow in reproductive medicine. This meant that the patient had some baseline investigations conducted for secondary subfertility, if they fulfilled the criterion, and a referral to the fertility clinic was initiated.

#### *4.6.2.4 Safety:*

Adverse Events (AE's) and Serious adverse events (SAE's) were reported to the sponsor and the UHCW NHS Trust R&D department.

#### *4.6.2.5 End of trial:*

The trial end was after the pregnancy outcomes of all the trial participants were known and recorded. This included the 6 weeks telephone follow up for the patients who have had a successful livebirth.

The other reasons for a trial to be stopped early would have included

- Mandated by the REC
- Mandated by the trial management committee following the review of AE's and SAE's.
- Funding for the trial ceases

The REC would have been notified in writing if the trial had been concluded or terminated early.

#### *4.6.3 Laboratory Assessments:*

In order to achieve the secondary objective of the trial, patient consent was obtained regarding the use of the endometrial sample for future research projects. If the patient didn't consent to the process, it was discarded as per the UHCW NHS Trust policy on disposal of biological waste. If the patient consented to the use of the material, it would be stored at the UHCW Arden tissue bank for 10 years. The samples were anonymised with no identifiable patient information. The patient could withdraw consent at any time and the sample was disposed as per policy.

#### *4.6.4 Adverse Event Management:*

##### *4.6.4.1 Adverse Events (AE):*

An Adverse Event (AE) was defined as any untoward medical occurrence in a participant and which does not necessarily have a causal relationship with this treatment/intervention.

Expected outcomes during the procedure:

Pain

Bleeding

Feeling faint

The above were not reported as adverse events.

The expected outcomes in pregnancy are:

Nausea, vomiting

Pelvic discomfort

Early pregnancy spotting

Dizziness

Swelling of both legs in the third trimester, without pain

Stretch marks in the abdomen

If the patient had been admitted for hyperemesis or evaluation of the above symptoms they were not reported as adverse events. Any deviation from the normal expected course of pregnancy or admission for any other reasons were reported as adverse events or serious adverse events. The assessment of the causality of any AE was made by the CI and defined as unrelated/unlikely/possible/probable/definitely/not assessable.

*4.6.4.2 Serious Adverse Events (SAEs):*

A Serious Adverse Event is an AE that fulfils one or more of the following criteria:

Results in death

Is immediately life-threatening

Requires hospitalisation or prolongation of existing hospitalisation

Results in persistent or significant disability or incapacity, or

Is a congenital abnormality or birth defect?

Considered to be an important medical condition by the investigator

#### *4.6.4.3 Reporting of SAEs:*

All SAEs were reported using the SAE form in the participant's CRF. The causality of SAEs (i.e. relationship to trial treatment) was assessed by the CI on the SAE form. The SAE form was completed at the resolution of the condition and documented in the hospital records and in the patients' case file in the research unit. The completed SAE form was reported to the sponsor.

### **4.7 Data Management:**

#### **4.7.1 Data Collection and Management:**

Personal data collected during the trial was handled and stored in accordance with the 1998 Data Protection Act. The data from the CRF was entered on a secure database for the analysis of the study data. The patients were assigned a unique study number and there was no patient identifiable information on the CRFs. The data was stored on the University of Warwick secure drive.

The data was entered on a Microsoft Excel spreadsheet and was password protected. The data entry was undertaken by myself with cross check of my data entry by my co-investigator at regular intervals in order to maintain standards. The data collection and storage adhered to the University of Warwick Research Data and Management Policy and Information Security Framework. The data collection and storage were also in line with the UHCW NHS Trust policy.

The CRF (Appendix 15) was developed to collect all required trial data. CRF was completed for each patient by all involved in the patient recruitment and consent and will be securely saved for 10 years at the BRU-RH suite in a locked storage. The women were given a questionnaire along with a self-addressed envelope, at the randomisation visit, for completion and return within 4 weeks. Their consent included the access of the hospital notes and telephone interviews, for the outcomes of the pregnancy. All patients would be contacted for a telephone consultation 6 week after delivery of the baby. This way all the patients who have not delivered at University hospitals of Coventry and Warwickshire (UHCW) NHS Trust were followed up and data collected for reporting. The data from the CRF's were entered on a secure database for the analysis of the study data. The data collection and

storage adhered to the University of Warwick Research Data and Management Policy and Information Security Framework.

#### 4.7.2 Database:

I was responsible for maintaining the database in my role as the trial co-ordinator. Data entry checks was made by Dr Lokman. The BRU-RH research midwives collected pregnancy outcome data so were blinded to the treatment allocation group.

The database was an excel spread sheet with the data entered for the following measures:

- demographic details
- group allocation
- outcomes of the pregnancy- miscarriage, livebirth
- pregnancy complications
- gestation at delivery, weight of baby
- 6-8 weeks telephone follow up for neonatal health
- reasons for discontinuation in trial or lost for follow up

This was kept on the University secure drive only.

#### 4.7.3 Data Storage:

All essential documentation and trial records will be stored by BRU-RH at UHCW NHS Trust, in conformance with the applicable regulatory requirements with access to stored information restricted to authorised personnel.

#### 4.7.4 Archiving:

Trial documentation and data will be archived for at least ten years after completion of the trial as per Warwick Research Data Management policy.

#### 4.7.5 Administration:

The trial co-ordination was at the BRU-RH, Coventry.

# Chapter 5

## Chapter 5: Trial Management

This chapter describes the trial management.

### 5.1 Roles and Responsibilities:

#### 5.1.1 Trial Sponsor:

The role of a sponsor in a clinical trial is set out in the Research Governance Framework and is, defined by the Health Research Authority policy (HRA) on planning and improving research publication, responsible for the initiation, management, planning of the trial including financing of the trial (HRA, 2018). Though a sponsor is not legally required for a non-clinical trial of an investigational medicinal product (non-CTIMP) trial, the University of Warwick was the sponsor of this trial. The documents necessary for the trial were all produced according to the University of Warwick SOP's. The trial was also monitored by the UHCW NHS Trust R&D department. They were available for guidance, recruitment drive by posting in their social media, feedback with AE and conducted an audit of documentation maintenance of 10 case notes and CRF.

#### 5.1.2 Chief Investigator:

CI is defined, by the HRA, as an individual who is responsible for the conduct of the whole project and must be able to supervise the research effectively and be readily available to communicate with the REC and other review bodies during the application process and where necessary during the conduct of the research (HRA, 2018). At this was a single centre trial, the CI and PI roles were managed by one person.

Professor Siobhan Quenby was the CI and PI for the trial. She is an acclaimed researcher and a world-renowned expert on recurrent miscarriage with extensive experience in the conduct and completion of several trials under her supervision. Her passion, supervision and guidance ensured that the trial was set up and completed successfully.

The CI was involved in all aspects of the trial planning, set up and running with delegation of the role to myself and other members of the research team, in accordance to their training

and roles. A delegation log assigned the various different roles and responsibilities to all the team members other than the clerical staff who co-ordinated the booking of the appointments.

The CI oversaw the trial protocol, feedback and review of the supporting documents, the trial documentation files such as the master file. The CI completed the AE forms and assigned causality. She led the trial management meetings and ensured support for recruitment and resolution of all issues arising within the trial operations.

### 5.1.3 Trial Co-ordinator:

I was delegated the task of running the trial and co-ordinating the different aspects of the trial. This was undertaken during my job as a Clinical Research Fellow in Reproductive Medicine. I undertook the Good Clinical Practice (GCP) training prior to starting my research job in order to understand the various terminologies and the process involved in clinical research as I was a novice in this area. I was then guided by the CI to attend the chief investigators course, organised by University of Warwick, in January 2015 in order to familiarise myself with the trial process. This helped me understand the various framework and regulations that underpin research. I also attended the training on human tissue handling, including completion of an e-module and obtained a certificate for the Human Tissue Authority (HTA) regulations compliance.

My job as a clinical research fellow meant that I had assigned research time in 4 monthly blocks alternating with clinical work, working in reproductive medicine. This was a truly team effort with tight co-ordination of the schedules and constant communication with the team members, especially the research midwives. There were initially two assigned research midwives for the study which then changed midway through the study.

My fellow research colleagues filled in for myself during my clinical and on call commitments and kindly undertook the procedures on my behalf. As previously discussed we have all been trained to the same standards in running of the various trials within the BRU-RH as we were

involved in the recruitment into the other portfolio trials and understand that research is team work.

I had a weekly meeting with the research midwives during my clinical block so that I could ensure that I was up-to date with the recruitment and support them in any issues arising in the interim. I have been flexible to come out of hours or stay back later during my work to undertake follow up visits or the scans suited to the patients' needs and clinical presentation.

The running of the trial involved close working with the secretarial staff, research midwives, clinical trials assistants and the laboratory staff. I organised the trial management meetings on a regular basis and communicated the minutes to the other members of the team and maintained the log of the minutes. I ensured that the database was complete and up-to date on a weekly basis. This was helpful as the trial progressed with the increase in patient numbers and at varying points in their journey.

The Trial Master File with the index pages with the contents of the key study documents to be filed, in accordance with the University of Warwick SOPs, and all source documentation was maintained in the BRU-RH locked cupboard by the research midwives along with myself.

## 5.2 Trial Submission:

### 5.2.1 Feedback from Ethics Committee:

The initial IRAS application was submitted in August 2015. I attended the West Midlands South Birmingham Research Ethics Committee (REC) meeting along with my CI and my colleague in September 2015. There was an active discussion involved with the ethics committee members. The feedback concerned the PIS. It was felt that the information was not laid out well and suggested the inclusion of flow diagram in order to make it easy for patients to navigate the steps required post ovulation. The comments also included the fact that the contact numbers in the event of complaints regarding the trial was not very clear on the PIS. Further suggestions included the alteration of some of the wordings in the PIS to

maintain equipoise and to alter the poster to include more information and changes to the GP letter.

The feedback from the ethics committee was taken on board and the appropriate changes instituted encompassing the changes suggested in both the PIS and the protocol. The trial obtained approval from the REC on 21<sup>st</sup> October 2015 (Appendix 17). The trial then was given the UHCW NHS Trust R&D approval on 23<sup>rd</sup> November 2015 and the first patient was enrolled on 30<sup>th</sup> November 2015.

### 5.2.2 Trial Registration:

The trial was registered on the [clinicaltrials.gov.uk](http://clinicaltrials.gov.uk) website as mandated by the guidelines for publication of trials, Trial Number NCT0268162. This was completed in December 2015.

A favourable mention of the trial was published in the BJOG and appeared online in May 2016, under the section of exciting ongoing trials (Appendix 1). This led to an increase in self recruitment and contact from patients directly quoting this reference.

### 5.2.3 Site Initiation Visit:

This was a single centre trial and I set up a meeting of all the research team members including the clinical trials assistants, research midwives and research fellows and undertook a formal power point presentation of the trial set up and the pathway.

As another source of patients who may be eligible for the trial was the EPAU, I also set up a meeting and explanation of the trial with the EPAU lead consultant and the nurse practitioners. Copies of the PIS and advertisement materials were kept in the scan room for easy access to patients.

In order to improve the awareness of the clinicians in my department I presented the trial in the departmental teaching session and enlisted the help of the registrars and consultants to sign post their patients following a miscarriage.

#### 5.2.4 Laboratory procedures:

An additional training session was undertaken with the laboratory staff and the unique identifiers for the biopsy samples for patients in the trial was agreed upon. The meeting was attended by the manager of the pathology laboratory, post-doctoral researcher who will be working with the endometrial samples from the trial, CI, Professor Brosens, myself and my colleague researcher Dr. Lokman. The discussion in the meeting surrounded the method of identification of the samples from the trial, reporting of the results, transport to the pathology lab and storage for future use. It was agreed that in order to positively identify the endometrial samples, separate to the implantation clinic samples, we would use a bright blue coloured label over the pot for distinction. The numbering of the samples would also be different and would be coded as X followed by the year of the sample and the number in sequential order (four-digit format with the last three digits corresponding to the randomisation number), e.g. sample 1 will be labelled as X15-1001. Sample 24 in the year 2016 would be labelled as X16-1024 with the no patient identifiable labels on the sample pot. The co-relation to the patient was through the recorded entry in a separate book, which was securely stored in a locked cupboard in BRU-RH with access only to myself. The patient was correlated to the sample by the randomisation order number and the study ID number. The sample will then be taken to the pathology laboratory and will be processed for uNK testing. The results will then be available on the Warwick secure drive with restricted access only to those with a Warwick id. This was applicable to all the 50-55 patients who were to be randomised to the intervention arm. The remainder of the samples from the patients in the intervention arm will be stored in the Arden Tissue Bank for future use. The plan for the future would be apply for separate ethics approval for the processing and work on the endometrial samples in the direction of future research.

#### 5.3 Trial Monitoring:

##### 5.3.1 Trial Management Committee:

I set up a trial management committee (TMC) for the monitoring of the trial progress. This comprised of the following members

- CI- Professor Siobhan Quenby

- Myself- Valarmathy Kandavel
- Dr. Mariam Lokman my co-investigator
- Lyndsey Prue- co-ordinating research midwife
- Debbie Bullen until September 2016- Co-ordinating research midwife
- Jane Hillen- from September 2016 onwards
- Sean James – Manager of the CSRL laboratory, until august 2016

The trial statistician attended the first meeting to discuss the issues surrounding the randomisation envelopes. The committee met at regular monthly intervals commencing from January 2016 to July 2016. Thereafter it was conducted every other month due to the changes in the various team members roles.

Mr. Feras Izzat (EPAU lead for UHCW NHS Trust) was the assigned person of contact if there were any outstanding issues that were unresolved from the TMC meetings. A final meeting was conducted at the end of the trial recruitment in July 2017. This was when the last patient was enrolled in the trial. However, the trial follow up was still ongoing as the outcome is livebirth after 24 weeks and the trial completion happened at the end of the 6-week postnatal telephone questionnaire completion.

### 5.3.2 Internal Audit:

This was undertaken by the UHCW NHS Trust R&D manager Nick Aldridge after the first 10 patients were randomised. This was a review of the case notes, documentation, site file, master file maintenance, database entry. Feedback was provided and the suggestions were implemented.

### 5.3.3 Trial Management Group (TMG):

The Trial Management Group consisting of the project staff and co-investigators was set up and met up regularly every four to six weeks to discuss the trial progress and resolve any issues arising. A Trial Master File was set up according to Warwick CTU SOP and held securely at the coordinating centre. The trial protocol was sent to Mr. Mostafa Metwally for peer review and feedback. The peer review was also undertaken by my fellow colleague and research fellow Dr. Lauren Lacey.

#### 5.3.4 Audit and Inspection:

The trial was open for regular audits and inspections by authorised representatives of the sponsor. In addition, inspection and audit was undertaken by the trust R&D department.

#### 5.4 Trial Issues and Challenges:

The trial was well planned and enrolled patients from 30<sup>th</sup> November 2015 until July 2017. There were some challenges and issues that were dealt with as discussed in sections 5.4.1 to 5.4.6. During this period, we enrolled 133 women of whom 109 women proceeded to randomisation. This was possible by the extensive team work, commitment and working for a common cause by all the members of the research unit, under the able guidance of the CI.

The trial process emphasised the importance of team work, communication, emphasis on patient care and able leadership. The lessons learnt from the challenging situations were valuable personal experiences for myself in both my role as a clinician and researcher.

Despite the fulfilment of the various pathways leading to the successful trial set up, there were issues faced along the way that were not anticipated. This chapter discusses some of the situations that arose during the trial process such as change of assigned team, randomisation issues and clinical scenarios of two women in the trial.

##### 5.4.1 Change of the Lead Midwife:

At the start of the trial, two of the senior research midwives agreed to be the assigned midwives for the running of the trial. The research unit has ongoing portfolio trials and it was felt that two midwives would be required along with myself and the clinical trials assistant in the effective running of the trial and for continuity of patient care. My clinical block commenced in December 2015 coinciding with the start of recruitment. I was able to continue recruitment by a combination of communication, teamwork and forward planning including utilising my time off from the rota for research work.

One of the assigned full-time midwife in the trial team then moved onto a new position, eight months after the start of the trial and her position became vacant. The timing of her leaving coincided when the recruitment had reached the halfway point and the initial patients were going through pregnancy or having follow up scans and needing additional support in the event of a miscarriage. There was still ongoing recruitment and we had to request another part-time research midwife to support us in the trial. She kindly agreed and had to have a handover of the current patients and continue with the rest of her portfolio studies. This was a testing time for the team and the other team members showed enormous support and the trial continued to be conducted smoothly. This was also a time when I learnt about forward planning, good communication and team support.

#### 5.4.2 Recruitment issues:

We found that some women did not achieve positive ovulation test despite regular cycles. We did not exclude them from the study but we amended the protocol to accommodate them in the study. If the patient has tried ovulation test kits for two months without success, we aimed to perform a transvaginal scan in the luteal phase of her cycle, 7-10 days prior to her expected period. If the endometrial thickness was greater than 5mm she was randomised on the day and proceeded with the study. Five women underwent randomisation through this approach.

#### 5.4.3 Process Issue -Ovulation test kits:

We had a process issue due to differing perception of the nurses and gynaecologists who thought that ovulation kits were part of usual gynaecological history taking and the Warwick CTU auditor who thought they were trial related procedure. This meant that the practise of sending, ovulation kits out prior to consent for patient convenience was criticised by the auditor as a trial deviation. The action from this was to make this issue clear in future trial protocols and to ensure audits were done early in trial so that similar misperception could be acted on in a timely fashion.

#### 5.4.4 Patient who Conceived in the Same Cycle of Randomisation:

The initial protocol and PIS of the trial advised patients against trying for a pregnancy in the same cycle as randomisation. This was because a previous study in patients who underwent embryo transfer in the same cycle as the endometrial scratch, reported that this was associated with poorer pregnancy outcomes (Karimzade *et al.*, 2010). The PIS simply advised patient to use barrier contraception and avoid pregnancy in the same cycle.

Patient number 006 was randomised to the endometrial biopsy group. The pre-randomisation screening questions check was undertaken and the patient was randomised. The procedure went well and the ultrasound scan was unremarkable. The patient informed us of her delayed period and undertook the pregnancy test on our advice. The pregnancy test was positive 11 days after randomisation. This was an unexpected outcome as patients are advised against getting pregnant in the same cycle of randomisation. She was offered regular early pregnancy scans from six weeks onwards until her dating scan. She was booked at UHCW NHS Trust and continued to have surveillance during her pregnancy. She had a livebirth at term with no complications.

An AE form and an incident form were completed and submitted to the sponsor and the trust R&D department. It was difficult to ascertain the exact period of conception. It was likely that the conception happened around the time of the biopsy. This event proved that despite advice it is still possible to have unplanned pregnancy. The ultrasound was not going to pick up the gestation so early.

A face to face meeting was organised with the trust R&D manager to discuss the incident and future steps that would be needed to avoid a recurrence. We amended the protocol to include a pregnancy test on the day of randomisation to rule out inadvertent unplanned pregnancy. After that single episode, no further patients became pregnant in the same cycle as the randomisation.

#### 5.4.5 Adverse Events and Serious Adverse Events in the Trial:

There were two patients with notified AE's during the trial. One of which involved participant no 006, as discussed in section 5.4.4.

The second AE was for participant number 016. She had a series of SAE's completed during the trial. She was gravida 7 para 4 with three previous miscarriages. She was randomised to the endometrial biopsy group. The procedure was uneventful and she fell pregnant in the second cycle post randomisation. She had an uneventful first trimester and was diagnosed with placenta praevia in her mid-trimester anomaly scan. She had an admission at 22+5 weeks of her pregnancy with PV bleeding and was discharged home and this generated the first SAE. The causation was classified as unlikely to be related to the primary event by the CI.

She was then admitted at 26 weeks of gestation with sudden onset severe abdominal pain. A clinical diagnosis of placental abruption was made and she underwent delivery by emergency caesarean section. She delivered a live male infant who was admitted to the neonatal unit and discharged home at 6 months of age. This admission event generated another SAE. Again, the event was unrelated to the endometrial biopsy as patients with placenta praevia are at high risk of placental abruption needing delivery.

The patient had a difficult post-operative recovery period with non-resolution of her pain. She underwent a laparotomy and surgery for caecal torsion on the second post-operative day due to her worsening abdominal pain and clinical symptoms. She recovered from surgery and was subsequently discharged home on seventh post-operative day after the laparotomy.

Due to the prolonged admission in the hospital a third SAE was generated and submitted by the CI. The patient had regular phone calls from myself and the team to offer further support if she required. The baby was doing well at the six weeks telephone interview.

#### 5.4.6 Issues with Randomisation Envelope:

The randomisation method planned with the statistician was to use a computer generated randomisation code to put group allocation in sequential envelopes. This was deemed adequate for a pilot trial and the statistician agreed on doing the randomisation process and formulated 110 envelopes with the allocated groups for use during the trial. The envelopes were brown opaque sealed envelopes. The agreed code for the randomisation was A and B. The statistician allocated the code B as E as he felt that this was tamper proof and would appear similar even when exposed to bright light. This was to reduce any chance of introducing bias during randomisation. The code was stratified so that there was equal number allocated to each group in 10 patient blocks.

When the first patient was randomised the team picked the first envelope and were faced the letter E. During the previous set up meeting the co-investigators were made aware of the code and the allocation to groups. As the letter code was changed after that, the team discarded the first envelope as they were not sure what it meant. They randomly picked up the next envelope and proceeded with biopsy as denoted by B. A further random envelope was picked for another patient by my co-investigator.

I immediately consulted this issue with my CI. I then set up an urgent meeting with the trial statistician and explained the issues with the envelopes and discussed the way forward. We decided to discard the unblinded envelope. Therefore, three replacement envelopes had to be done again. The rest of the envelopes were opened in sequential number order. We decided to skip envelope numbers 12, 13 and proceed with the numbers after these. Hence there was no envelope 1 allocated to any patient, similarly for envelope 12 and 13. There were three duplicate envelope numbers- 37,28,36. The duplicate numbers were identified by the numbers 99 in front of them. This was discussed with the CI who approved the decision from the meeting. The entire envelope sequence was repeated by the trial statistician in order to maintain equal allocation to both the groups. We then proceeded with using the second set of envelopes in sequence until the end of the trial.

Another drawback of the randomisation was that it did not match to case numbers that were allocated in order of consent but were allocated in order of arrival for randomisation visit. As they were stratified in batches of 10 numbers, there was an ability to predict which group they may be allocated to within the group of doctors. This was because they could remember the allocation of the previous 7,8 or 9 envelopes and able to predict what the likely allocation would be near the end of the batch. The envelope method of randomisation was chosen due to funding restraints and was not ideal.

#### 5.4.7 Amendments:

One submission for substantial amendment (SA) was made in March 2016, five months after the trial commenced. This was approved on 06/04/2016 (appendix 18). The SA concerned three points

1. Increasing the upper age entry into the trial to 42 years. The initial age of entry into the trial was inclusive from 18 to 40 years of age. This increase was to bring the age criterion in line with the other trials in recurrent miscarriages prevention offered in the research unit. The discrepancy in age inclusion criteria between trials was noted by one of the prospective patients and caused confusion amongst the team members. This issue improved after the amendment.
2. The guidance for randomisation appointment booking in patients who did not achieve a positive ovulation test result at home. We amended the protocol to include these women if their endometrial thickness was greater than 5mm by TVS on the day of randomisation. Five women underwent randomisation even in the absence of positive ovulation.
3. A joint poster for ALIFE 2 trial and the SiM trial was submitted for approval (Appendix 5). This was also submitted to the REC committee for the ALIFE 2 trial separately. During the time that the trial commenced, Coventry was chosen as one of the sites for Tommy's National Miscarriage research. The joint poster was to appear in the Tommy's website to encourage information and possible self-referral from women themselves, without being referred to the RMC.

## 5.5 Lessons Learnt during the Conduct of the Trial:

The issues with the changeover of the team member during the active phase of recruitment coincided with my clinical block of my fellowship. The trial recruitment continued due to the support and teamwork of all the members of the research team. My resilience and time management were developed further during the trial recruitment phase. I learnt that the timely and effective resolution of any issues, especially with the envelope issue, would be vital to continue the seamless running of the trial. Perhaps this could have been avoided by maintaining lines of communication and confirmation with the statistician on receipt of the envelopes for randomisation. The co-ordination of the trial was a valuable experience in not only enhancing my knowledge of research methods but also the appreciation of the volume of work and research governance that guides the safe and effective conduct of research studies. It helped me to enhance my negotiating skills, leadership, ownership and responsibilities with patient centred care. It was a truly humbling experience to have had the opportunity to have met some of the most courageous and selfless patients who despite enduring the pain of recurrent miscarriages, were willing to participate in research not only to enhance the understanding and the science behind miscarriages but also with an altruistic approach to help women in the future.

The set-up and running of the trial have helped me gain confidence in my future role as a researcher within my area of work and to continue my role in active participation and recruitment into research studies, to enhance our knowledge and move the evidence forward.

# Chapter 6

## Chapter 6: Results

### 6.1 Trial results:

#### 6.1.1 Trial Recruitment:

The recruitment for the trial was predominantly from the UHCW NHS Trust recurrent RMC. The women were reviewed by the CI in the clinic and a discussion happened regarding the management options including the participation in the various trials. The women were then met by myself or the research team midwives and the trial process was explained to the patient. A PIS was provided if the patient expressed an interest in the trial. If a PIS was given out, minimal patient identifiers (initials) were entered into a screening log. If the patient returned the reason for the participation or not in the trial was recorded. The patients were made aware that the participation in the trial was entirely voluntary and their withdrawal or declining to participate would not affect their ongoing management in their future pregnancy. During the trial we screened 274 women for eligibility for participation in the trial. Of these, 50 women were lost to follow up because they did not contact the BRU-RH to consent to the trial or request a randomisation appointment probably because they decided against it having read the PIS at home. Forty patients, had a positive investigation result that found a possible modifiable factor associated with their recurrent miscarriage and were therefore ineligible. Twenty-five women were already pregnant when they returned for their consent appointment and eighteen declined to participate in the trial. The other reasons included not meeting the age criterion, opting to have IVF treatment or not actively trying for a pregnancy at that time.

#### 6.1.2 Trial Design and Conduct:

Only 18/274 (6.5%) declined the trial to the BRU team but 50/275 (18.2%) were lost to follow up probably because they decided not to participate. Hence the majority (75.3%) of eligible women did return to the research unit and consented to the study. A total of 133 patients consented to participate and 109 women proceeded with randomisation. Of the 24 women who were not randomised, nine women became pregnant prior to attending for the randomisation visit. The further 15 women were lost for a variety of reasons as shown in the

CONSORT diagram (Figure 6.1). There was no loss to follow up after the process of randomisation.

### 6.1.3 CONSORT Diagram SiM

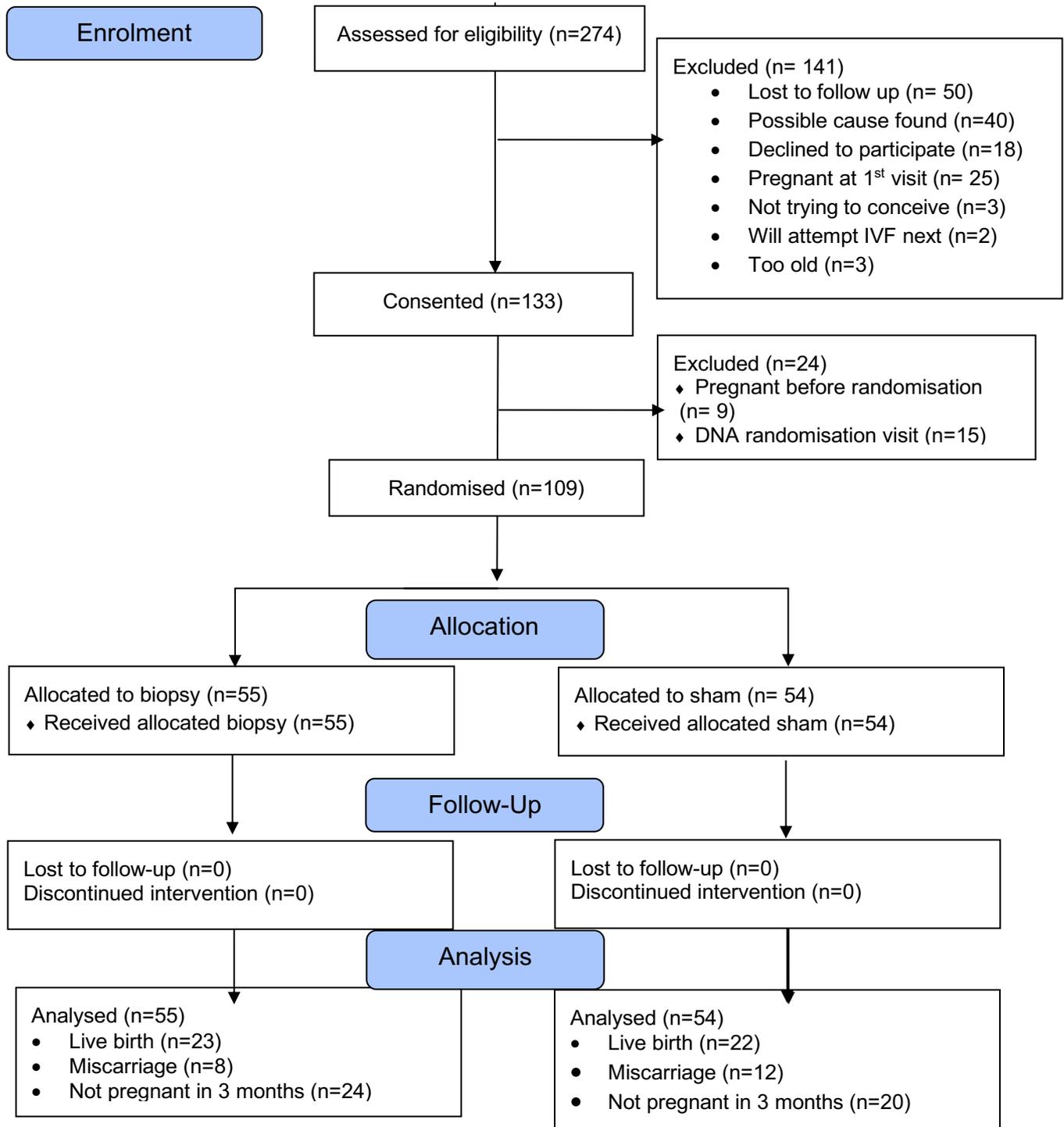


Figure 6. 1 Consort diagram of the SiM study

### 6.1.4 Trial Enrolment:

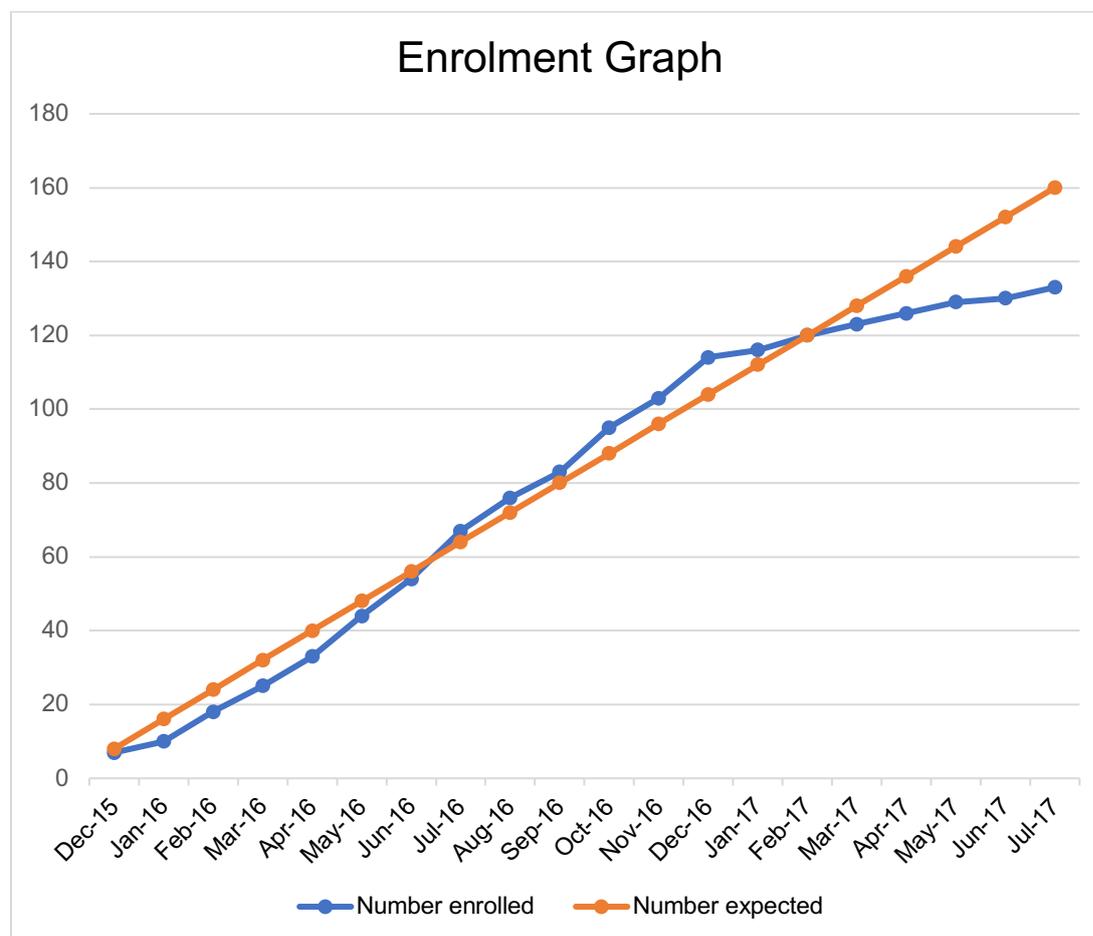


Figure 6. 2 Enrolment Graph

Figure 6.2 illustrates the trend of the enrolment into the study. There was near expected recruitment to the study until the last 4 months of the study. The recruitment into the study commenced on 30<sup>th</sup> November 2015. The projected recruitment was around nine patients per month and therefore the anticipated completion of recruitment of 110 patients was estimated at 12-13 months. However due to the drop out of 24 participants between consent and randomisation and the slowing down of recruitment in the final months there was a trial extension leading to the trial being open for the consenting and recruitment for 20 months in total. The most common reason for the drop out was due to the participants achieving a spontaneous pregnancy prior to the appointment for randomisation. The last patient underwent randomisation in July 2017.

### 6.1.5 Trial Randomisation:

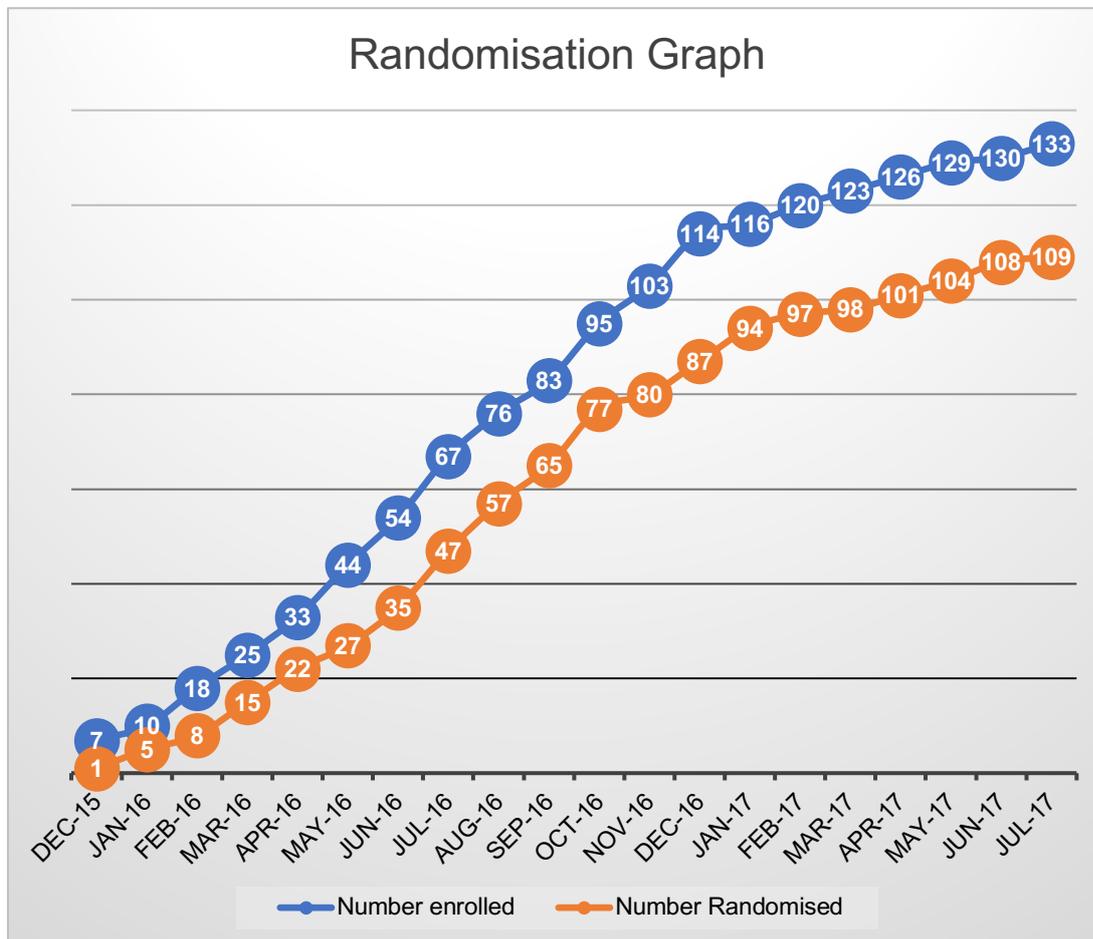


Figure 6. 3 Randomisation Graph

Figure 6.3 demonstrates the difference between the actual number of patients consented into the study and those that were randomised in the study. The graph explains the need to continue with the recruitment due to the drop out from the study after enrolment. The first patient was randomised in December 2015 and the last patient in July 2017.

## 6.2 Results:

### 6.2.1 Study Population Demographics:

**Table 1: Baseline characteristics of the randomised patients**

Characteristic	Biopsy group (n=55)	Non-biopsy group (n=54)	p-value
Age (years)			
Median (IQR, Range)	33.0 (29.0-37.0, 22-42)	33.0 (31.0-36.3, 24-41)	0.62 <sup>†</sup>
Mean (SD)	32.9 (5.08)	33.3 (4.11)	
BMI			
Median (IQR, Range)	25.8 (21.8- 29.0, 18.5-	25.7 (23.2-30.3, 19.3-	0.22 <sup>†</sup>
Mean (SD)	37.3)	39.0)	
	25.7 (4.44)	27.0 (4.96)	
Smoking status, n (%)		*	
Non-smoker	39 (72.2)	31 (57.4)	0.26 <sup>§</sup>
Ex-smoker	10 (18.5)	14 (25.9)	
Smoker	5 (9.3)	9 (16.7)	
Ethnicity, n (%)			
Caucasian	48 (87.3)	44(81.5)	0.27 <sup>†</sup>
European	1(1.8)	4 (7.4)	
Asian	3 (5.5)	4 (7.4)	
Middle east	1(1.8)	1(1.9)	
Black/Caribbean	2(3.6)	1(1.9)	
Previous livebirth, n (%)			
0	31 (56.4)	30 (55.6)	0.52 <sup>†</sup>
1	20(36.4)	21 (38.9)	
2	1(1.8)	3(5.6)	
3	1 (1.8)	0(0)	
4	2(3.6)	0(0)	

Previous miscarriages, n (%)			
2	14 (25.5)	17 (31.5)	0.72 <sup>†</sup>
3	24 (43.6)	19 (35.2)	
4	6 (10.9)	8 (14.8)	
5	3 (5.5)	5 (9.3)	
6	1 (1.8)	1(1.9)	
7	2 (3.6)	1 (1.9)	
8	1 (1.8)	0 (0)	
9	2 (3.6)	0 (0)	
10	1 (1.8)	2 (3.7)	
16	1 (1.8)	0 (0)	
17	0 (0)	1 (1.9)	

<sup>†</sup> P-value obtained using the Mann-Whitney U test; <sup>§</sup> P-value obtained using the chi-squared test;

<sup>‡</sup> P-value obtained using the Fisher's exact test; \*Observation for one woman missing.

Table 6. 1 Table of demographic characteristics

The women were equally distributed and matched for their demographic characteristics between the two groups as seen in the Table 6.1. This again validates that randomisation process worked and the women were evenly distributed between the groups. The average age of the women in the two groups was 33, with a spread across age groups of 22-42. The median BMI between the two groups was 25.1 with no participant above the BMI of 40. Though there appeared to be more women in the control group who were current smokers, the difference was not statistically significant. Although the majority of the participants were of Caucasian ethnicity, 17 women who participated in the study represented other ethnic groups. This group accounted for 16% of the participants across both the groups. Just over half of the participants in the trial were nulliparous women. In the multiparous group, most women had 1 previous livebirth.

The time to conception before trial entry for women were put into categories;

- Less than 3months, n= 25
- Greater than 3 months but less than 6 months, n= 34

- Greater than 6 months up to 12 months, n= 26
- Greater than 12 months, n= 8
- Unable to calculate, n=16

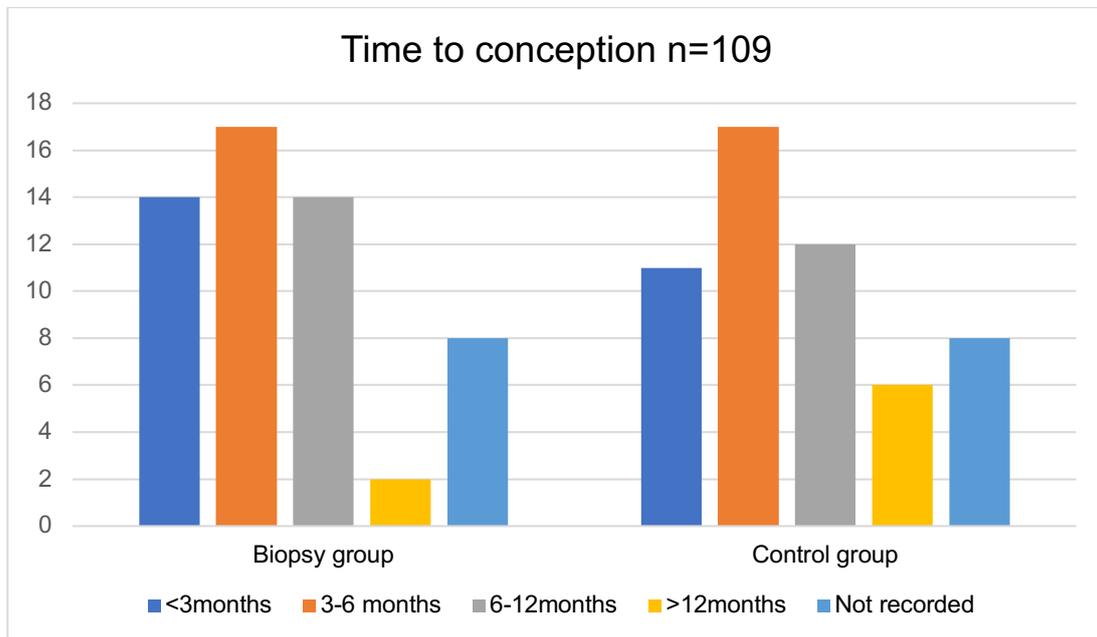


Figure 6.4 Mean time to conception between women in the two groups

Figure 6.4 is the graph that shows the mean time to conception between the two groups allocated to the biopsy and control arm was similar. The x axis shows the frequency of distribution between the groups and the y axis the subgroup to time points to conception.

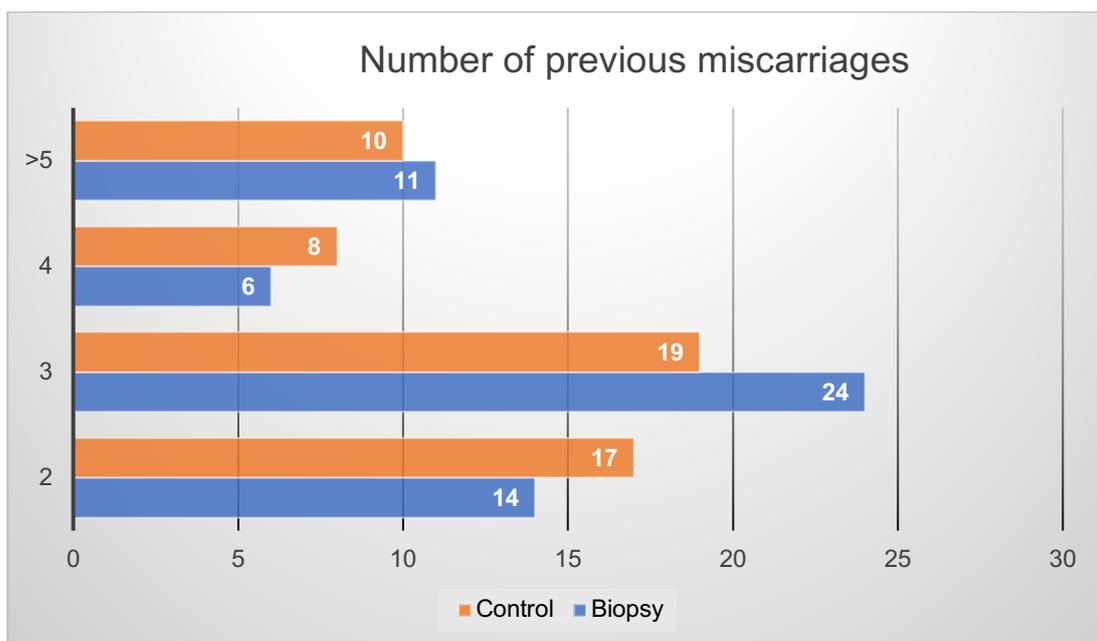


Figure 6.5 Distribution of miscarriages between the groups

Figure 6.5 is the graph that shows the distribution of the miscarriages between the two groups. The x axis shows the number of previous miscarriages and the y axis the frequency of distribution between the groups. The spread of the miscarriages was similar in both the groups. The number of participants who suffered from higher order miscarriages were very small.

### 6.3 Results of Primary Outcome:

The primary outcome measure of the trial was livebirth after 24 weeks of gestation. This was reported in women who achieved their pregnancy within three menstrual cycles after randomisation. This was because the effect of the biopsy was postulated to last for up to three months.

	<b>Livebirth, n (%)</b> <b>Biopsy group</b>	<b>Livebirth, n (%)</b> <b>Control group</b>	<b>OR (95% CI)</b>	<b>p-value</b>
<b>All women (n=109)</b>	23 (41.8)	22 (40.7)	1.05(0.49-2.42)	0.91
<b>Pregnant women (n=65)</b>	23 (74.2)	22 (64.7)	1.57 (0.54-4.57)	0.41

*Table 6. 2 Results of the study*

The chi squared test was used to compare the outcomes between the groups as described in the protocol. All the women in each group and the percentages of livebirths was calculated. This was 41.8% in the biopsy group and 40.7% in the control group. The difference in livebirths between the two groups was not statistically significant (p=0.91).

The percentages of livebirths in the women who did achieve a pregnancy within three months of the biopsy was calculated. In this analysis the livebirths percentage in the biopsy group was 74.2% and 64.7% in the control group. This result reflected the expected pregnancy rates in women with recurrent miscarriage from other trials. The results revealed a difference of 9.5% in livebirths between the groups. This trend towards more livebirths in the biopsy group did

not reach statistical significance by the chi-squared test ( $p= 0.41$ ). This did not meet the pre-specified  $p$  value ( $p<0.2$ ) for the feasibility trial. This is likely to be due to the small number of patients in the trial.

### 6.3.1 Time to Conception between Pregnant and Non-Pregnant Women:

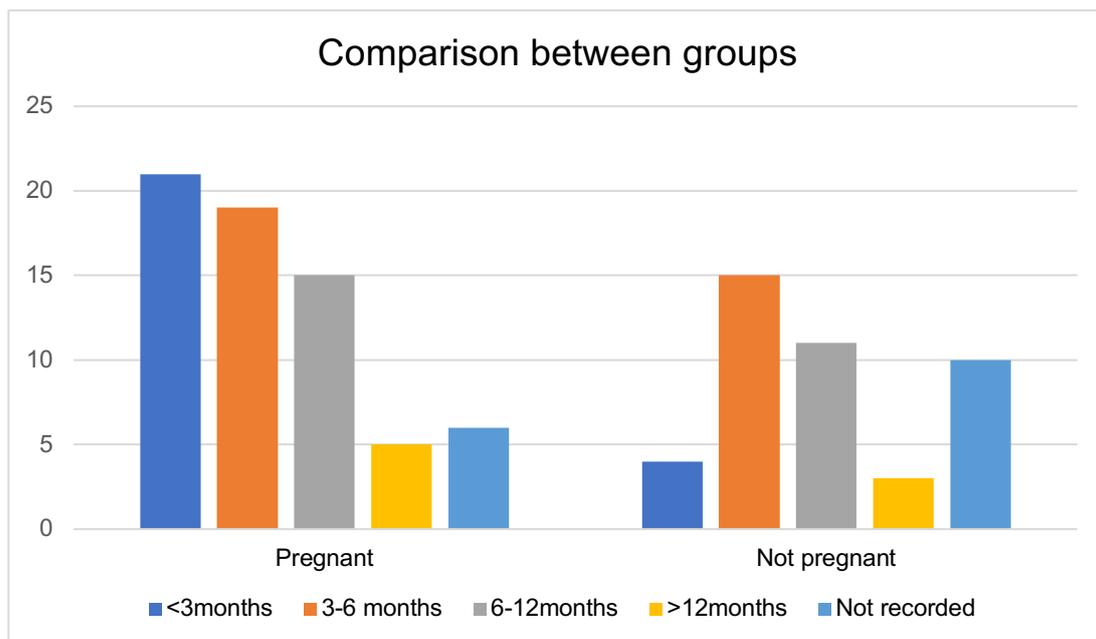


Figure 6. 6 Comparison of the mean time to conception of their previous pregnancies between pregnant ( $n=66$ ) and non-pregnant women ( $n=43$ ) in the study.

The x axis shows the frequency of distribution between the groups and the y- axis the subgroup to time points to conception. As illustrated in figure 6.6, a greater number of women in those who conceived had a previous time to conception of less than three months between pregnancies than in those who failed to achieve a pregnancy ( $p=0.04$  by chi-squared test).

## 6.4 Results of Secondary Outcomes:

### 6.4.1 Miscarriages:

This included gestational losses before 23+ 6 weeks. 20 women experienced a repeat miscarriage during the study, of which eight were in the biopsy group and 12 in the control

group. Of these, 15 women experienced embryonic pregnancy loss and five had non-visualised pregnancy loss (n=5).

Cytogenetic analysis of the products of conception using next generation sequencing (NGS) was sent for ten patients and results were obtained for nine patients. Of these, five results were abnormal. This is summarised in the table 6.3.

<b>Cytogenetics results (n=9)</b>	<b>Biopsy Group</b>	<b>Control Group</b>
Trisomy 22	1	2
Trisomy 16	1	
Trisomy 13		1
No copy number variants	1	1
Normal karyotype	2	

*Table 6. 3 Summary of cytogenetics reported on the miscarriages*

#### 6.4.2 Ectopic pregnancy:

One participant had an ectopic pregnancy in the trial, which was treated surgically and confirmed with histology.

#### 6.4.3 Pregnancy Complications:

This included SGA, PET, abruption, placenta praevia, placenta accrete and preterm delivery. Only one participant randomised to the biopsy arm had a pregnancy complication. She had a preterm delivery at 26 weeks for suspected placental abruption with a low-lying placenta. The outcomes have been discussed in Chapter 5 under trial issues (section 5.4.4).

#### 6.4.4 Trial Acceptability:

This was assessed by the analysis of the semi-structured patient questionnaire (Appendix 2) to evaluate the following questions

1. Is it possible to blind patients?
2. Acceptability of the procedure
3. Feedback of the trial pathway and patient's perceptions

The questionnaire was provided to all of the 109 patients who underwent randomisation, in a self-addressed envelope. They were requested to complete the questionnaire and return it within four weeks of the randomisation. The women were instructed not to complete the questionnaire on the day of the procedure. This was to avoid a biased opinion which may be influenced by the experience of the day.

The questionnaire consisted of two parts-trial pathway evaluation and procedure experience. Objective scoring of the common expected outcomes such as bleeding, pain, infection and the use of analgesia prior to the procedure was recorded. There was a column for the trial pathway evaluation with comments on the process of booking appointments and feedback on the PIS. We were also interested to ascertain the source of referral and the ease of booking for the randomisation appointment. The questionnaire also included the time for response between the procedure and completion of the questionnaire.

## 6.5 Results of the Questionnaire Analysis:

### 6.5.1 Response:

The response rate for the questionnaire was 63.3% (69/109) with near equal responses from both the groups (33 patients in the biopsy group and 35 patients from the control group). One was discarded as there was no patient identifiable information and was therefore difficult to identify the randomisation group. The majority of women returned their response within 2 weeks from randomisation-76.5% (52/68).

### 6.5.2 Trial Pathway Evaluation Questions:

We aimed to evaluate the trial pathway by including the questions as discussed below along with the discussion of the results from the responses. The trial evaluation pathway questions and the results are discussed as follows:

- How did you find out about the study?

This was to identify the source of referral for the study. 50% (34/68) of the patients who returned the questionnaire first heard about the study in the recurrent miscarriage clinic run within the UHCW NHS Trust. The remainder of the recruited patients were from EPAU (n=7), Tommy's website (n=13), online (n=8) and directly via the research unit (n=3). This is illustrated in Figure 6.7.

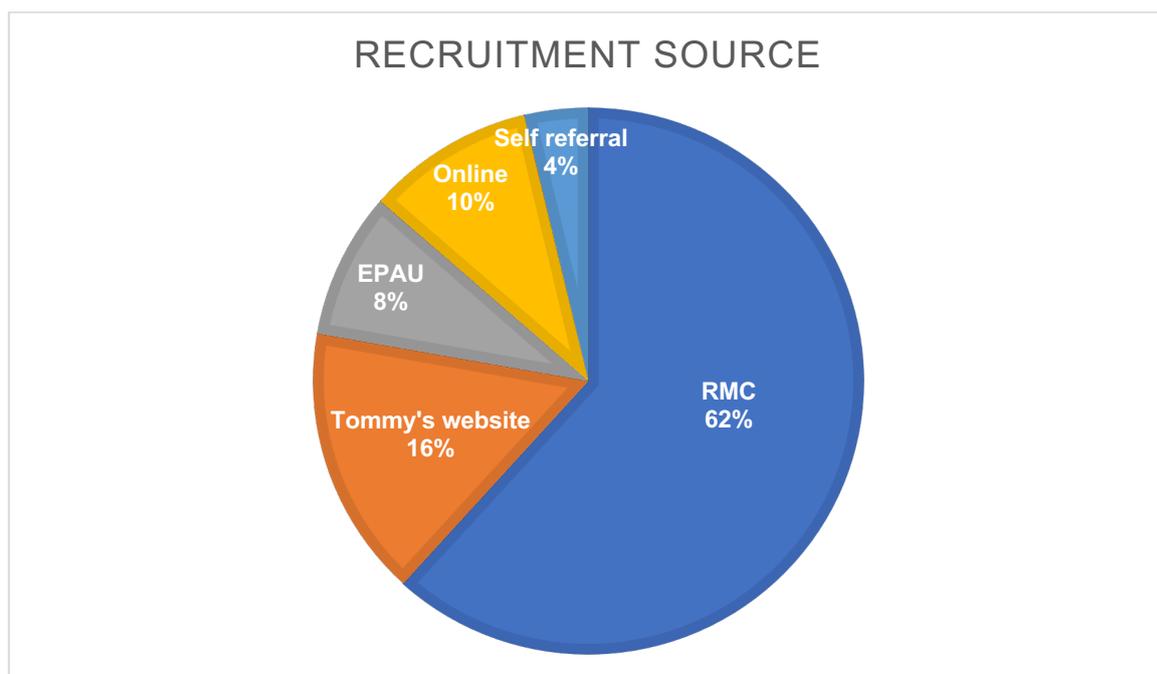


Figure 6. 7 Sources of recruitment into the study

During the study period, I received a number of direct emails from women who did an online search and found information about trials in miscarriage. The design of a future study should focus on spreading information through the internet and social media via use of a dedicated Facebook page, twitter feeds and on website most frequently accessed by women for example, Tommy's, Miscarriage Association.

- Was the patient information sheet (PIS) helpful?

All the 68 returned questionnaires answered that the PIS was useful. There were fifteen comments in the free text box. Five participants commented that the PIS was helpful and informative while the rest found the instructions to be clear.

The two comments that suggested improvement included “no explanation regarding how/why the endometrial scratch (ES) will be helpful” and “needed more information particularly regarding the pregnancy test”.

The comments reinforced that the PIS did provide adequate information but could be improved with more information regarding the potential benefits of scratch.

- Difficulties encountered during appointment booking?

The vast majority of the respondents did not encounter any problems with the booking system for the randomisation appointment.

Three patients left the following comments

“Monday, Friday appointment was difficult, but changed to Tuesday on request”

“Emailed and phoned several times before response”

“Several attempts to book appointment”

There were no comments in the free text box for contact information for further feedback to discuss the problems encountered during booking. Therefore, the exact nature of the problems encountered by the above patients could not be quantified.

Overall it appeared that the assigned Monday and Friday clinic booking worked as it was accepted by 96% of the patients. This interval between clinics also captured the window of opportunity for randomisation post positive ovulation.

- Experience of participating in the study.

All the women had positive comments about their participation in the study. They felt hopeful for the future and commented on the positive attitude from the staff.

The common words used were:

*Hopeful for the future*

*Feel supported*

*Reassuring*

*Positive*

*Clear and straight forward*

*Happy to participate in research trial*

- What do you think went well for you?

The responses were all positive and listed below were the most commonly observed comments for this questionnaire

*Supportive and caring staff*

*Hope due to participation in trial*

*Quick and easy to access appointments*

*Reassurance from the ultrasound*

*Professionalism of the staff*

*Good communication*

- What do you think we could improve on?

There were comments from twenty respondents. There were no consistent remarks to suggest a pattern. The suggestions included:

*Longer time slots*

*No randomisation*

*Option to collect more ovulation test kits if needed*

*Unsure what would happen if further miscarriage or not getting pregnant*

*Waiting for appointment*

*Car parking was a hassle*

- Did you find the staff helpful during the study?

All the 68 women who responded to the questionnaire had positive feedback regarding the staff they encountered during the study. The comments remarked how kind, caring, supportive and knowledgeable they were.

- Are there any other comments that you wish to make?

The responses were all positive with expression of *thank you* from patients and hoping that the trial will be a success and the outcomes for them were better.

One response verbatim was *“Better emotional recovery due to support. Trials need to understand the emotional and medical component of miscarriages”*.

This summarised the value of supportive care for women with RPL and the importance of incorporating this essential element in future trial designs.

## 6.6 Questions to Evaluate the Procedure Experience:

### 6.6.1 Infection:

The question on infection had a direct yes/no response. There was no self-reported infection reported in the women who returned the completed questionnaires.

### 6.6.2 Bleeding:

Bleeding was quantified in relation to the patient’s usual period if answered. The options were spotting, less/like/more than a period. Bleeding was reported in 52.9% of the participants (36/68). Among the respondents, more women in the biopsy group (28/33) experienced bleeding than in the control group (8/35). The reported bleeding however was less than or like a period in most of the women (n=34/36). The remaining women did not report any bleeding (32/68) which included five women in the biopsy group. This is summarised in Figure 6.8.

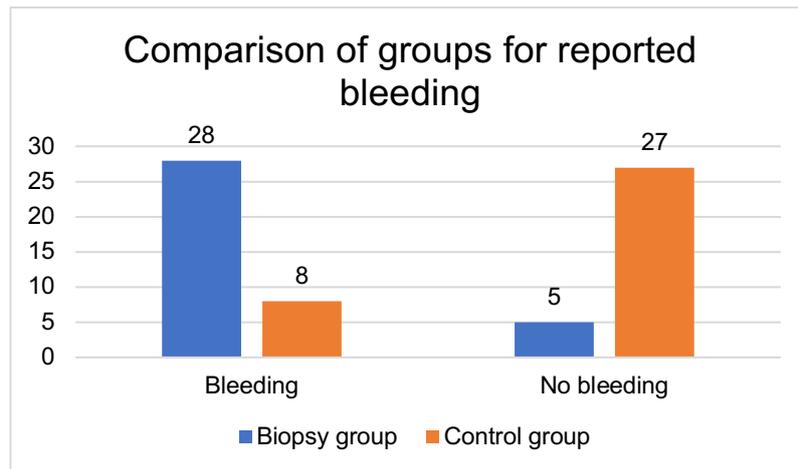


Figure 6. 8 Comparison of reported bleeding between groups

The drawback was that some women's periods are variable.

### 6.6.3 Pain:

Pain was quantified as mild, moderate and severe along with the duration of <10mins, <1hour and >1 hour. The use of previous pain relief was also recorded along with a space for free text box comments for any other symptoms that the patient experienced.

Women in both the groups experienced pain. This was in almost all with the exception of three patients in the biopsy group (n=30/33) in comparison to the control group (n=20/35). The pain score was reported as mild by the majority of women in both the groups. Of the 20 women who reported pain in the control group, nine described it as moderate intensity pain. Women who experienced severe pain (n=4) were all in the biopsy group. Less than half the patients followed the analgesia advice (30/68). Despite the varied pain, the duration was short lasting (< 10 minutes) in most women. Four patients each in the two groups experienced pain for one hour.

The possible explanation for the experience of pain in the control group could be due to:

- Perception of being in the endometrial scratch group and the anticipation of pain
- The speculum examination in itself can be painful for some women

Although the pain symptoms were scored in the questionnaire, none of the women commented that they could not tolerate the procedure or that it would influence their future

participation adversely. No patients experienced the expected outcome of feeling faint during the procedure.

### 6.7 Endometrial Results:

The results as shown in Table 6.2 showed a positive trend towards livebirth in the biopsy group of patients. This is encouraging results with a possible explanation provided by the analysis of the endometrium, performed by my fellow researcher Dr. Lokman as discussed in Figure 6.9 and 6.9.1.

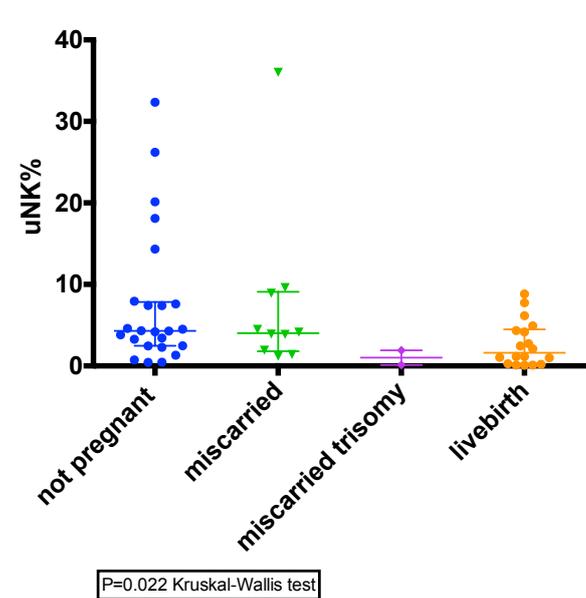


Figure 6. 9 uNK cell density distribution

The uNK cell density in women with RPL who were allocated to Group A/B/C/D. A- not conceived within three cycles, B- miscarried, C-miscarried trisomy, D- livebirth. Used with permission from Dr. Lokman.

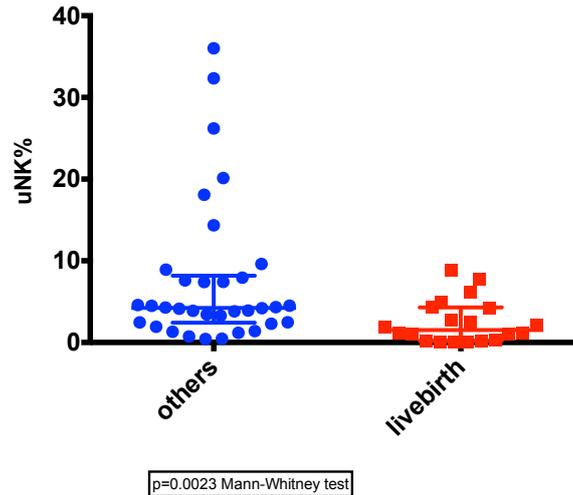


Figure 6. 10 uNK cell density comparison between groups

Figure 6.10 shows that the uNK cell density was lower in those who conceived within three months of the biopsy and had a livebirth compared to those who did not. Used with permission from Dr. Lokman.

### 6.8 Limitations of the Questionnaire Analysis:

This was a feasibility trial and we acknowledge that the numbers are small. The response rate to the questionnaire was just over 63%. The possibility of bias is likely as the most motivated group of women may have chosen to respond to the questionnaire. There may be an argument that the other 50 women who did not respond post randomisation either had a positive experience, no symptoms or otherwise. As there was no loss to follow up after randomisation, they were all ongoing participants in the trial process and had some point of contact with the research team. It would have been highly likely that the research team would have been made aware of any ongoing issues. Therefore, it can be suggested that in even in the non-responders to the questionnaire the trial pathway and the randomisation process worked. The time limit of four weeks was set in order to account for the variation in the cycle length and the receipt of the postal envelopes. We were confident that by the time the women responded they wouldn't have known the outcome of their subsequent cycle thereby introducing bias into the results of the study. Nevertheless, it is still likely that there was a possibility of recall bias introduced especially for those whose response were delayed.

This may have been influenced by the expectations of the women and the experience on the day of the randomisation.

## 6.9 Conclusions from the Questionnaire Analysis:

The questionnaire response analysis provided valuable feedback on the trial set up, randomisation process and answered some of the questions that would be required if a future larger trial was to be set up. It demonstrated that women with RPL are willing to participate and accept randomisation into a trial with a 'sham' procedure arm.

Moving forward the response rates could have been improved perhaps if there was an option to complete it online rather than post the questionnaires back, which in hindsight was a more time-consuming process.

We are confident that both the randomisation and the blinding process worked well. This is due to the fact that women experienced pain in both groups during the procedure, it is possible that they were truly blinded at the point of the procedure. None of the responses indicated that women would not accept the procedure despite experiencing some pain and bleeding. The bleeding episodes were more frequent in the women who underwent biopsy but this was not statistically significant ( $p=0.41$  by chi-squared test).



# Chapter 7

## Chapter 7: Discussion

### 7.1 Introduction:

In this thesis I have assessed the feasibility of endometrial scratch in miscarriage prevention in women with recurrent pregnancy loss. My overall conclusion is that a large scale RCT of endometrial scratch for the prevention of recurrent miscarriage is feasible. Although the difference in livebirth between the groups did not achieve statistical significance, there was a 9.5% improvement in livebirth rate in the women who underwent endometrial scratch. The feasibility study was not powered to detect this difference but this difference is clinically significant.

### 7.2 Strengths of the Study:

#### 7.2.1 Patients:

75% of the patients who were approached, agreed to participate in the trial. The RMC was the best source of patient recruitment and women with RPL did participate in the trial. The recruitment rate was good and as predicted. Once the women had been randomised we did not lose any one to follow up.

#### 7.2.2 Intervention:

The intervention was acceptable to the women despite the reports of pain and bleeding associated with the intervention. Women were unanimously positive towards the trial. This was because participating in the trial meant that they felt they had good emotional support from the team.

#### 7.2.3 Sham Procedure:

The trial demonstrated that successful recruitment is possible into a RCT with a 'sham procedure' arm and involving an invasive examination as women are keen to seek an understanding of their condition and contribute to the improvement of the science and evidence behind the management of this enigmatic condition. We demonstrated that sham

procedure was acceptable and blinding was maintained. This sham procedure reduced the risk of bias in our study.

#### 7.2.4 Randomisation:

The randomisation process was effective as evidenced by the equal distribution of women between the groups.

#### 7.2.5 Endometrial Factors:

The study provided prospective data to assess the uNK cell test. High uNK cell density was a predictor of poor reproductive outcomes as those women who conceived within three months of the scratch and had a livebirth, had lower uNK cell density than those who did not.

#### 7.2.6 Emotional Support:

The positive contribution of emotional support and care during the participation in the trial process could have influenced the pregnancy outcomes. It is not always possible to discern the beneficial placebo effect of being cared for in a specialist clinic and/or participation in the trial. The reason for this is that if women are well supported during pregnancy loss they will try again, increasing their chances of a livebirth.

#### 7.2.7 Primary outcome measure definition:

Our primary outcome measure was a livebirth conceived within three months of the intervention. In this trial two women who had biochemical loss in cycle one following the intervention then conceived immediately in cycle three and achieved a livebirth. A biochemical loss is one that is not visualised on an ultrasound scan so the location is not known. Hence, we followed the pre-trial analysis plan that meant that these two women were categorised as having a livebirth. The definition of primary outcome measure is an important aspect to consider in future trials of miscarriage prevention. Other trials have reported the outcome in the first pregnancy following an intervention, had we followed this definition then these two women with biochemical losses immediately followed by conception and livebirth would have been reported as a miscarriage. The trial experience has suggested that a patient

orientated outcome measure of cumulative livebirth rate following the intervention is preferable and an important trial design consideration for future trials.

### 7.3 Weaknesses of the Study:

#### 7.3.1 Superfertility:

In our trial 40.4% (44 women out of 109) did not achieve a pregnancy in the three menstrual cycles after the biopsy. The number of women who did not achieve a pregnancy within the study period was not different between the groups. Hence, we have no evidence that scratch affects conception rates but the trial was not powered for this outcome measure.

One of the trial aims was to investigate whether there was a sub-group with the RPL population who would be more likely to benefit from the intervention. In the trial planning, we considered the possibility that the group of women with RPL and superfertility were those who had a problem with decidualisation that leads to lack of endometrial selectivity. Hence it was the superfertile women who were to benefit from the trial. Recruitment was of all eligible women that we could approach. However, we failed to recruit the superfertile RPL couples. This was because many of the women were pregnant before we randomised them, 25 were pregnant at the first clinic visit and a further nine women fell pregnant between consenting to participate in the trial and the randomisation/intervention visit. Hence, 34 of the most fertile women with RPL were not recruited. This meant that our recruited population was a mixture of women with RPL and subfertility and others with RPL and less fertile. However, those superfertile patients were lost whilst waiting for their first appointment and achieved a pregnancy between receipt of information and consent and similarly between consent and randomisation

Salker *et al.*, 2010, observed that 40% of patients attending a RPL clinic were super fertile with a TTP of 3 months or less, with the remainder either not having a short TTP or being sub fertile. In a further retrospective study of 158 fertile patients with RPL 98 patients achieved a spontaneous conception (62%). 88% of this cohort conceived within 6 months with a median time of 2 months (range 1-10 months) (Perfetto *et al.*, 2015). Hence our trial with 60% of the

patients achieving a pregnancy during the study period of three months is comparable to previous studies.

### 7.3.2 Analysis:

We did not meet our pre-specified feasibility criteria of a p value of  $<0.2$  between the randomised groups, it was  $p=0.41$ .

## 7.4 The Lessons learnt which can be used for Future Study Design:

This study has informed the design of future trials

### 7.4.1 Population Likely to Benefit:

The first issue is identifying who would benefit from an endometrial scratch? This would be influenced by the number and type of miscarriages and the time to conception. Each of these factors are considered in the discussion below.

- Number of previous miscarriages

This procedure has a low cost and is well tolerated by participants of this study. It is therefore, a reasonable intervention in women with less severe phenotype encompassing those with a history of two to four miscarriages. The reason we chose women with two or more miscarriages was in line with ESHRE and ASRM definitions of RPL and published work advocating investigating younger women after two clinical pregnancy losses (Saravolos & Li, 2012). The problem with investigating and treating women with a lower number of miscarriages is that they already have a high livebirth rate without intervention and therefore any intervention that only makes such a small difference in the population with an existing high livebirth rate that it is very difficult to detect and may not be clinically meaningful. We found that including those with mild phenotype led to a livebirth rate of 69% in those who conceived, meaning 31% miscarried. This 31% can be assessed in the context of the 15% miscarriage rate in the general population. Hence, recruiting a population with mild phenotype did select a population of patients whose miscarriage rate was potentially reducible.

- Type of previous pregnancy loss

As the scratch is thought to improve implantation it seems reasonable to include women with biochemical losses as these may well represent very early implantation problems. However, the definition of miscarriage is loss of an intra-uterine pregnancy. Biochemical losses are not visualised (NVP) so include both early implantation failures and early tubal losses. This issue was illustrated by the fact that we did have an outcome of ectopic pregnancy. We found that many women attending clinic give a history of miscarriage without the pregnancy being visualised on ultrasound scan. Hence on a practical level we need to include NVP losses but be aware of the limitations of this approach.

- Time to conception for outcome measure

There is biological plausibility for the effect of scratch to last three months, however it is difficult to argue that any positive effect on the endometrium lasts any longer than this, in a tissue that is regenerated monthly. Hence on reflection this three month limit on time to conception for the outcome measure was sensible. However, only 60% of the women conceived within three months. We found that the time to conception before the trial was shorter in those that conceived compared to those that did not.

- Inclusion criteria

We found that the time to conception before the trial was shorter in those that conceived compared to those that did not. Hence, in any future trial the inclusion criteria could specify time to conception. Using an inclusion criteria of mean time to conception of less than or equal to three months would mean that more randomised women conceived within three months of the biopsy. However, this short time to conception inclusion criteria is problematic because; it is difficult to ascertain from the clinical history due to patient uncertainty, would mean excluding many women so that recruitment is slow and would have excluded women who did in fact conceive within three months.

Hence from the experience of this trial we recommend that any future trial would need to devise strategies to approach women earlier, so that they were not pregnant at their first clinic visit and the randomisation and intervention occurred promptly. This would mean contacting women before their first visit to the recurrent miscarriage clinic or in the early pregnancy unit soon after their miscarriage.

The recently published systematic review concluded that there may be a role for endometrial scratch in younger women (Sar-Shalom Nahshon *et al.*, 2019). This was illustrated in our trial where the majority of the women who failed to achieve a pregnancy were above 37 years old. Therefore, we recommend serious consideration of the inclusion criterion regarding maternal age. Restricting the trial to women less than 37 years of age would enrich the population in terms of those who do not conceive within three months but again exclude many women, making recruitment difficult and exclude some who do conceive within three months.

#### 7.4.2 Outcome Measures:

We analysed livebirth after 24 weeks as the primary outcome measure. This had the advantage of being a robust measure that can be ascertained by telephone call. Hence, we had 100% ascertainment of the measure. However, accuracy of the miscarriage outcome data was more difficult to determine. One of the setbacks of RPL trials is establishing which miscarriages could have potentially been avoided. Ideally this would mean identifying losses due to abnormal karyotype, which would enable a more accurate assessment of the effectiveness of an intervention.

The trial included the offer of cytogenetics testing of fetal tissue through NGS. Despite strenuous efforts by the trial team we only got a result in nine out of the 15 women who experienced pregnancy loss. This highlights the inherent difficulties in obtaining karyotype information. The problem was that many women miscarried at home or were too early to bring their miscarried tissue to the unit to be analysed. Five of these women had an abnormal karyotype and went in some way to provide explanation for the loss. This information is important for counselling regarding future pregnancy as the resultant pregnancy loss was anticipated. There was no evidence that scratch reduced the number of abnormal pregnancy

or improved endometrial selectivity although we did not have enough pregnancy losses karyotyped to support or refute this possibility. The fact that the women miscarried aneuploidy pregnancies in the biopsy group, shows that scratch did not always lead to ideal selectivity whereby all abnormal pregnancies do not implant and are lost in menstruation.

#### 7.4.3 Size of the Study:

The experience from larger RCTs has opened up discussions on the effect of an intervention and setting the appropriate clinically significant difference in the outcome. Even in large multi-centre studies which are adequately powered (PROMISE trial), an effect has been difficult to ascertain. The 10% difference of livebirth between groups would have had an influence on the sample size. Using an online sample size calculator, to detect and increase the livebirth rate from 64% to 74%, with alpha 5% and 90% power we would need 894 women to be randomised and conceive. Using the same recruitment procedures, this would mean screening of 3768 women ( $274/65 \times 894 = 3,768$ ). Thus, a large number of women are needed for the study in order to establish the possible efficacy of endometrial scratch in miscarriage prevention.

### 7.5 Other learning points from the trial:

#### 7.5.1 Use of social media platforms:

The use of multiple platforms such as Tommy's website, Trust R&D Facebook, Twitter pages could be used effectively to spread the message regarding the trial and aid recruitment.

# Chapter 8

Chapter 8: Conclusions

I have reflected on the MD thesis using the Driscoll's model of reflection.

1. I have learnt about trial design and importance of planning and consequences of the decisions made at planning to the results and acceptability of the trial.
2. PPI involvement in design of the trial was crucial and allowed us to have sham biopsy in the control arm accepted by the ethics committee and turned out to be acceptable in the trial.
3. I have developed confidence to be involved as a PI in future studies and my role in them. I have gained confidence in understanding the considerations needed in the design of future studies based on clinical needs.
4. I have learnt about the critical appraisal of evidence and interpreting studies and results.
5. I have understood that the role of endometrium needs further study and clarification

Endometrial scratch may improve implantation and reduce miscarriage in some women with recurrent pregnancy loss. Based on the findings and what has been learnt from this study, it is feasible to design a large RCT with the aim of confirming or refuting this hypothesis.

## Bibliography

A.M.Kolte, L.R.Olsen, Mikkelsen, E. M., Christiansen, O. B. & Nielsen, H. S. (2015) Depression and emotional stress is highly prevalent among women with recurrent pregnancy loss. *Human Reproduction*, no.4 777-782.

Achache, H. & Revel, A. (2006) Endometrial receptivity markers, the journey to successful embryo implantation. *Human Reproduction Update*, 12 (6): 731–746,.

Aflatoonian, A., Baradaran Bagheri, R. & Hosseiniadat, R. (2016) The effect of endometrial injury on pregnancy rate in frozen-thawed embryo transfer: A randomized control trial. *Int J Reprod Biomed (Yazd)*, 14 (7): 453-158.

Aleyamma, T., Singhal, H., Premkumar, P. S., Acharya, M., Kamatha, M. S. & George, K. (2017) Local endometrial injury in women with failed IVF undergoing a repeat cycle: A randomized controlled trial. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 214 109-114.

Almog, B., Shalom-Paz, E., Dufort, D. & Tulandi, T. (2010) Promoting implantation by local injury to the endometrium. *Fertil Steril*, 94 (6): 2026-2029.

Aplin, J. D. (2006) Embryo implantation: the molecular mechanism remains elusive. *Reprod Biomed Online*, 13 (6): 833-839.

Aplin, J. D. & Kimber, S. J. (2004) Trophoblast-uterine interactions at implantation. *Reprod Biol Endocrinol*, 2 48.

ASRM (2012) Evaluation and treatment of recurrent pregnancy loss: a committee opinion. *Fertility and Sterility*, Volume 98 (5): 1103-1111.

Avalos, L. A., Roberts, S. C., Kaskutas, L. A., Block, G. & Li, D.-K. (2014) Volume and Type of Alcohol During Early Pregnancy and the Risk of Miscarriage. *Substance use and misuse*, 49 (11): 1437-1445.

Bailey, S., Bailey, C., Boivin, J., Cheong, Y., Reading, I. & Macklon, N. (2015) A feasibility study for a randomised controlled trial of the positive reappraisal coping intervention, a novel supportive technique for recurrent miscarriage. *BMJ Open*, 5:e007322

Balogun, O. O., da Silva Lopes, K., Ota, E., Takemoto, Y., Rumbold, A., Takegata, M. & Mori, R. (2016) Vitamin supplementation for preventing miscarriage. *Cochrane Database Syst Rev*, (5): CD004073.

Barad, D. H., Yu, Y., Kushnir, V. A., Shohat-Tal, A., Lazzaroni, E., Lee, H. J. & Gleicher, N. (2014) A randomized clinical trial of endometrial perfusion with granulocyte colony-stimulating factor in in vitro fertilization cycles: impact on endometrial thickness and clinical pregnancy rates. *Fertil Steril*, 101 (3): 710-715.

Barash, A., Dekel, N., Fieldust, S., Segal, I., Schechtman, E. & Granot, I. (2003) Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing in vitro fertilization. *Fertil Steril*, 79 (6): 1317-1322.

Basak, S., Dubanchet, S., Zourbas, S., Chaouat, G. & Das, C. (2002) Expression of pro-inflammatory cytokines in mouse blastocysts during implantation: modulation by steroid hormones. *Am J Reprod Immunol*, 47 (1): 2-11.

Baum, M., Yerushalmi, G. M., Maman, E., Kedem, A., Machtinger, R., Hourvitz, A. & Jehoshua, D. (2012) Does local injury to the endometrium before IVF cycle really affect treatment outcome? Results of a randomized placebo controlled trial. *Gynecological Endocrinology*, 28 933-936.

Bernardi, L. A., Cohen, R. N. & Stephenson, M. D. (2013) Impact of subclinical hypothyroidism in women with recurrent early pregnancy loss. *Fertil Steril*, 100 (5): 1326-1331.

Bhandari, H. M., Tan, B. K. & Quenby, S. (2016) Superfertility is more prevalent in obese women with recurrent early pregnancy miscarriage. *BJOG*, 123 (2): 217-222.

Blois, S. M., Klapp, B. F. & Barrientos, G. (2011) Decidualization and angiogenesis in early pregnancy: unravelling the functions of DC and NK cells. *J Reprod Immunol*, 88 (2): 86-92.

Boots, C. E., Bernardi, L. A. & Stephenson, M. D. (2014) Frequency of euploid miscarriage is increased in obese women with recurrent early pregnancy loss. *Fertil Steril*, 102 (2): 455-459.

Bose, P., Black, S., Kadyrov, M., Bartz, C., Shlebak, A., Regan, L. & Huppertz, B. (2004) Adverse effects of lupus anticoagulant positive blood sera on placental viability can be prevented by heparin in vitro. *Am J Obstet Gynecol*, 191 2125-2131.

Brigham, S. A., Conlon, C. & Farquharson, R. G. (1999) A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. *Hum Reprod*, 14 (11): 2868-2871.

Brighton, P., Maruyam, Y., Fishwick, K., Vrljicak, P., Tewary, S., Fujihara, R., Muter, J., Lucas, E., Yamada, T., Woods, L., Lucciola, R., Hou Lee, Y., Takeda, S., Ott, S.,

Hemberger, M., Quenby, S. & Brosens, J. (2017) Clearance of senescent decidual cells by uterine natural killer cells in cycling human endometrium. *ELife*,

Britton, J. (2016) Electronic cigarettes and smoking cessation in England

BMJ 2016; 354 doi: <https://doi.org/10.1136/bmj.i4819> (Published 13 September 2016) Cite this as: BMJ 2016;354:i4819. *BMJ*,

Bulmer, J. N., Morrison, L., Longfellow, M., Ritson, A. & Pace, D. (1991) Granulated lymphocytes in human endometrium: histochemical and immunohistochemical studies. *Hum Reprod*, 6 (6): 791-798.

CARE, s. g. (2006) Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. *BMJ*,

Carp, H., Toder, V., Aviram, A., Daniely, M., Mashiach, S. & Barkai, G. (2001) Karyotype of the abortus in recurrent miscarriage. *Fertility and Sterility*, 75 (4): 678-682.

Carson, D. D., Lagow, E., Thathiah, A., Al-Shami, R., Farach-Carson, M. C., Vernon, M., Yuan, L., Fritz, M. A. & Lessey, B. (2002) Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. *Mol Hum Reprod*, 8 (9): 871-879.

Chen, L., Wu, Y., Neelakantan, N., Chong, M., Pan, A. & van Dam, R. (2016) Maternal caffeine intake during pregnancy and risk of pregnancy loss: a categorical and dose-response meta-analysis of prospective studies. *Public Health Nutr.*, 19 (7): 1233-1244.

Christiansen, O. B. (1996) A fresh look at the causes and treatments of recurrent miscarriage, especially its immunological aspects. *Hum Reprod Update*, 2 (4): 271-293.

Clark, P., Walker, I. D., Langhorne, P., Crichton, L., Thomson, A., Greaves, M., Whyte, S., Greer, I. A. & collaborators, S. P. I. S. S. (2010) SPIN (Scottish Pregnancy Intervention) study: a multicenter, randomized controlled trial of low-molecular-weight heparin and low-dose aspirin in women with recurrent miscarriage. *Blood*, 115 (21): 4162-4167.

Clifford, K., Flanagan, A. M. & Regan, L. (1999) Endometrial CD56+ natural killer cells in women with recurrent miscarriage: a histomorphometric study. *Hum Reprod*, 14 (11): 2727-2730.

Clifford, K., Rai, R. & Regan, L. (1997) Future pregnancy outcome in unexplained recurrent first trimester miscarriage. *Hum Reprod*, 12 (2): 387-389.

Coomarasamy, A., Devall, A. J., Cheed, V., Harb, H., Middleton, L. J., Gallos, I. D., Williams, H., Eapen, A. K., Roberts, T., Ogwulu, C. C., Goranitis, I., Daniels, J. P., Ahmed, A., Bender-Atik, R., Bhatia, K., Bottomley, C., Brewin, J., Choudhary, M., Crosfill, F., Deb, S., Duncan, W. C., Ewer, A., Hinshaw, K., Holland, T., Izzat, F., Johns, J., Kriedt, K., Lumsden, M. A., Manda, P., Norman, J. E., Nunes, N., Overton, C. E., Quenby, S., Rao, S., Ross, J., Shahid, A., Underwood, M., Vaithilingam, N., Watkins, L., Wykes, C., Horne, A. & Jurkovic, D. (2019) A Randomized Trial of Progesterone in Women with Bleeding in Early Pregnancy. *N Engl J Med*, 380 (19): 1815-1824.

Coomarasamy, A., Truchanowicz, E. G. & Rai, R. (2011) Does first trimester progesterone prophylaxis increase the live birth rate in women with unexplained recurrent miscarriages? *BMJ*, 342 d1914.

Coomarasamy, A., Williams, H., Truchanowicz, E., Seed, P. T., Small, R., Quenby, S., Gupta, P., Dawood, F., Koot, Y. E., Atik, R. B., Bloemenkamp, K. W., Brady, R., Briley, A., Cavallaro, R., Cheong, Y. C., Chu, J., Eapen, A., Essex, H., Ewies, A., Hoek, A., Kaaijk, E. M., Koks, C. A., Li, T. C., MacLean, M., Mol, B. W., Moore, J., Parrott, S., Ross, J. A., Sharpe, L., Stewart, J., Trépel, D., Vaithilingam, N., Farquharson, R. G., Kilby, M. D., Khalaf, Y., Goddijn, M., Regan, L. & Rai, R. (2016) PROMISE: first-trimester progesterone therapy in women with a history of unexplained recurrent miscarriages - a randomised, double-blind, placebo-controlled, international multicentre trial and economic evaluation. *Health Technol Assess*, 20 (41): 1-92.

Cunningham, F. G., editor (2014) *Williams obstetrics* 24 edn. McGraw-Hill Education/Medical.

Dain, L., Ojha, K., Bider, D., Levron, J., Zinchenko, V., Walster, S. & Dirnfeld, M. (2014) Effect of local endometrial injury on pregnancy outcomes in ovum donation cycles. *Fertil Steril*, 102 (4): 1048-1054.

de Jong, P. G., Kaandorp, S., Di Nisio, M., Goddijn, M. & Middeldorp, S. (2014) Aspirin and/or heparin for women with unexplained recurrent miscarriage with or without inherited thrombophilia. *Cochrane Database Syst Rev*, (7): CD004734.

de Jong, P. G., Quenby, S., Bloemenkamp, K. W., Braams-Lisman, B. A., de Bruin, J. P., Coomarasamy, A., David, M., DeSancho, M. T., van der Heijden, O. W., Hoek, A., Hutten, B. A., Jochmans, K., Koks, C. A., Kuchenbecker, W. K., Mol, B. W., Torrance, H. L., Scheepers, H. C., Stephenson, M. D., Verhoeve, H. R., Visser, J., de Vries, J. I., Goddijn, M. & Middeldorp, S. (2015) ALIFE2 study: low-molecular-weight heparin for women with recurrent miscarriage and inherited thrombophilia--study protocol for a randomized controlled trial. *Trials*, 16 208.

De-Regil, L., Palacios, C., Lombardo, L. & Peña-Rosas, J. (2016) Vitamin D supplementation for women during pregnancy.

Dekel, N., Gnainsky, Y., Granot, I. & Mor, G. (2010) Inflammation and implantation. *Am J Reprod Immunol*, 63 (1): 17-21.

Demiroglu, A. & Gurgan, T. (2004) Effect of treatment of intrauterine pathologies with office hysteroscopy in patients with recurrent IVF failure. *Reprod Biomed Online*, 8 (5): 590-594.

Devaseelan, P., Fogarty, P. P. & Regan, L. (2010) Human chorionic gonadotrophin for threatened miscarriage. *Cochrane Database Syst Rev*, (5): CD007422.

De-Regil, L., Peña-Rosas, J., Fernández-Gaxiola, A. & Rayco-Solon, P. (2015) Effects and safety of periconceptional oral folate supplementation for preventing birth defects. *Cochrane Database of Systematic Reviews*, (12):

Dhillon-Smith, R. K., Middleton, L. J., Sunner, K. K., Cheed, V., Baker, K., Farrell-Carver, S., Bender-Atik, R., Agrawal, R., Bhatia, K., Edi-Osagie, E., Ghobara, T., Gupta, P., Jurkovic, D., Khalaf, Y., MacLean, M., McCabe, C., Mulbagal, K., Nunes, N., Overton, C., Quenby, S., Rai, R., Raine-Fenning, N., Robinson, L., Ross, J., Sizer, A., Small, R., Tan, A., Underwood, M., Kilby, M. D., Boelaert, K., Daniels, J., Thangaratinam, S., Chan, S. Y. & Coomarasamy, A. (2019) Levothyroxine to increase live births in euthyroid women with thyroid antibodies trying to conceive: the TABLET RCT. In:

Dong, D. Z., Aplin, A. & Nicosia, R. (2009) Regulation of angiogenesis by macrophages, dendritic cells, and circulating myelomonocytic cells. *Current Pharmaceutical Design*, 15 365-379.

Du, H., Naqvi, H. & Taylor, H. S. (2012) Ischemia/reperfusion injury promotes and granulocyte-colony stimulating factor inhibits migration of bone marrow-derived stem cells to endometrium. *Stem Cells Dev*, 21 (18): 3324-3331.

Du, H. & Taylor, H. S. (2007) Contribution of bone marrow-derived stem cells to endometrium and endometriosis. *Stem Cells*, 25 (8): 2082-2086.

Eiben, B., Bartels, I., Bähr-Porsch, S., Borgmann, S., Gatz, G., Gellert, G., Goebel, R., Hammans, W., Hentemann, M. & Osmers, R. (1990) Cytogenetic analysis of 750 spontaneous abortions with the direct-preparation method of chorionic villi and its implications for studying genetic causes of pregnancy wastage. *American Journal of Human Genetics*, 47 (4): 656-663.

El-Toukhy, T., Campo, R., Khalaf, Y., Tabanelli, C., Gianaroli, L., Gordts, S. S., Gordts, S., Mestdagh, G., Mardesic, T., Voboril, J., Marchino, G. L., Benedetto, C., Al-Shawaf, T., Sabatini, L., Seed, P. T., Gergolet, M., Grimbizis, G., Harb, H. & Coomarasamy, A. (2016)

- Hysteroscopy in recurrent in-vitro fertilisation failure (TROPHY): a multicentre, randomised controlled trial. *Lancet*, 387 (10038): 2614-2621.
- El-Toukhy, T., Sunkara, S. & Khalaf, Y. (2012) Local endometrial injury and IVF outcome: a systematic review and meta-analysis. *Reprod Biomed Online*, 25 (4): 345-354.
- Empson, M., Lassere, M., Craig, J. & Scott, J. (2005) Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. *Cochrane Database Syst Rev*, (2): CD002859.
- Farren, J., Jalbrant, M., Ameye, L., Joash, K., Mitchell-Jones, N., Tapp, S., Timmerman, D. & Bourne, T. (2016) Post-traumatic stress, anxiety and depression following miscarriage or ectopic pregnancy: a prospective cohort study. *BMJ Open*, 6 (11): e011864.
- Fatemi, H. M. & Popovic-Todorovic, B. (2013) Implantation in assisted reproduction: a look at endometrial receptivity. *Reprod Biomed Online*, 27 (5): 530-538.
- Finn, C. A. & Martin, L. (1972) Endocrine control of the timing of endometrial sensitivity to a decidual stimulus. *Biol Reprod*, 7 (1): 82-86.
- Freeman, J., Perry, G., Feuk, L., Redon, R., McCarroll, S., Altshuler, D., Aburatani, H., Jones, K., Tyler-Smith, C., Hurles, M., Carter, N., Scherer, S. & Lee, C. (2006) Copy number variation: new insights in genome diversity. *Genome Res*, 16 (8): 949-961.
- Garber, J. R., Cobin, R. H., Gharib, H., Hennessey, J. V., Klein, I., Mechanick, J. I., Pessah-Pollack, R., Singer, P. A. & Woeber, K. A. (2012) CLINICAL PRACTICE GUIDELINES FOR HYPOTHYROIDISM IN ADULTS: COSPONSORED BY THE AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS AND THE AMERICAN THYROID ASSOCIATION.
- Gellersen, B., Brosens, I. A. & Brosens, J. J. (2007) Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. *Semin Reprod Med*, 25 (6): 445-453.
- Gellersen, B. & Brosens, J. J. (2014) Cyclic decidualization of the human endometrium in reproductive health and failure. *Endocr Rev*, 35 (6): 851-905.
- Gibreel, A., Badawy, A., El-Refai, W. & El-Adawi, N. (2013) Endometrial scratching to improve pregnancy rate in couples with unexplained subfertility: a randomized controlled trial. *J Obstet Gynaecol Res*, 39 (3): 680-684.
- Gibreel, A., El-Adawi, N., Elgindy, E., Al-Inany, H., Allakany, N. & Tournaye, H. (2015) Endometrial scratching for women with previous IVF failure undergoing IVF treatment. *Gynecological Endocrinology* 31 (4): 313-316.

Girardi, G., Redecha, P. & Salmon, J. E. (2004) Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. *Nature Medicine*, 10 1222–1226.

Gnainsky, Y., Granot, I., Aldo, P. B., Barash, A., Or, Y., Schechtman, E., Mor, G. & Dekel, N. (2010) Local injury of the endometrium induces an inflammatory response that promotes successful implantation. *Fertil Steril*, 94 (6): 2030-2036.

Goddijn, M. & Leschot, N. J. (2000) Genetic aspects of miscarriage. *Baillieres Best Pract Res Clin Obstet Gynaecol*, 14 (5): 855-865.

Grandone, E., Margaglione, M., Colaizzo, D., D'Addeda, M., Cappucci, G., Vecchione, G., Sciannamé, N., Pavone, G. & Di Minno, G. (1997) Factor V Leiden is associated with repeated and recurrent unexplained fetal losses. *Thrombosis and Haemostasis*, 77 (5): 822-824.

Granot, I., Dekel, N., Bechor, E., Segal, I., Fieldust, S. & Barash, A. (2000) Temporal analysis of connexin43 protein and gene expression throughout the menstrual cycle in human endometrium. *Fertil Steril*, 73 (2): 381-386.

Granot, I., Gnainsky, Y. & Dekel, N. (2012) Endometrial inflammation and effect on implantation improvement and pregnancy outcome. *Reproduction*, 144 (6): 661-668.

Greaves, M., Cohen, H., Machin, S. J. & Mackie, I. (2000) Guidelines On The Investigation and Management Of The Antiphospholipid Syndrome. *British Journal of Haematology*, 109 (4): 704-715.

Gris, J., Mercier, E., Quéré, I., Lavigne-Lissalde, G., Cochery-Nouvellon, E., Hoffet, M., Ripart-Neveu, S., Tailland, M., Dauzat, M. & Marès, P. (2004) Low-molecular-weight heparin versus low-dose aspirin in women with one fetal loss and a constitutional thrombophilic disorder. *Blood*, 103 (10): 3695-3699.

Güven, S., Kart, C., Unsal, M. A., Yildirim, O., Odaci, E. & Yulug, E. (2014) Endometrial injury may increase the clinical pregnancy rate in normoresponders undergoing long agonist protocol ICSI cycles with single embryo transfer. *Eur J Obstet Gynecol Reprod Biol*, 173 58-62.

Haas, D. M., Hathaway, T. J. & Ramsey, P. S. (2018) Progestogen for preventing miscarriage in women with recurrent miscarriage of unclear etiology. *Cochrane Database Syst Rev*, 10 CD003511.

Haas, D. M. & Ramsey, P. S. (2013) Progestogen for preventing miscarriage. *Cochrane Database Syst Rev*, (10): CD003511.

Hanna, J., Goldman-Wohl, D., Hamani, Y., Avraham, I., Greenfield, C., Natanson-Yaron, S., Prus, D., Cohen-Daniel, L., Arnon, T. I., Manaster, I., Gazit, R., Yutkin, V., Benharroch, D., Porgador, A., Keshet, E., Yagel, S. & Mandelboim, O. (2006) Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med*, 12 (9): 1065-1074.

Hassold, T. & Chiu, D. (1985) Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. *Human Genetics*, 70 (1): 11-17.

Hassold T, W. D., Kline J, Stein Z. (1984) The relationship of maternal age and trisomy among trisomic spontaneous abortions. *Am J Hum Genet*, 36 (6): 1349-1356.

Helmy, M. E. E., Maher, M. A., Elkhoully, N. I. & Ramzy, M. (2017) A randomized trial of local endometrial injury during ovulation induction cycles. *Int J Gynaecol Obstet*, 138 (1): 47-52.

Hiby, S. E., Regan, L., Lo, W., Farrell, L., Carrington, M. & Moffett, A. (2008) Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage. *Hum Reprod*, 23 (4): 972-976.

Hirahara, F., Andoh, N., Sawai, K., Hirabuki, T., Uemura, T. & Minaguchi, H. (1998) Hyperprolactinemic recurrent miscarriage and results of randomized bromocriptine treatment trials. *Fertil Steril*, 70 (2): 246-252.

HRA (2018) Research Planning.

HS, T. (2004) Endometrial cells derived from donor stem cells in bone marrow transplant recipients. *JAMA*, 292 81-85.

Humphrey, K. W. (1969) Interactions between oestradiol 3, 17 beta and progesterone on the induction and growth of deciduomata in ovariectomized mice. *Aust J Biol Sci*, 22 (3): 689-699.

Hyde, K. J. & Schust, D. J. (2015) Genetic Considerations in Recurrent Pregnancy Loss. *Cold Spring Harb Perspect Med*, 5 (3):

Hyodo, S., Matsubara, K., Kameda, K. & Matsubara, Y. (2011) Endometrial injury increases side population cells in the uterine endometrium: a decisive role of estrogen. *Tohoku J Exp Med*, 224 (1): 47-55.

Junovich, G., Mayer, Y., Azpiroz, A., Daher, S., Iglesias, A., Zylverstein, C., Gentile, T., Pasqualini, S., Markert, U. R. & Gutiérrez, G. (2011) Ovarian stimulation affects the

levels of regulatory endometrial NK cells and angiogenic cytokine VEGF. *Am J Reprod Immunol*, 65 (2): 146-153.

Kaandorp, S., M, G., JAM, v. d. P., Hutten, B., HR, V. & Hamulyak (2010a) Aspirin plus Heparin or Aspirin Alone in Women with Recurrent Miscarriage. *New England Journal of Medicine*, 362 (17): 1586-1596.

Kaandorp, S. P., Goddijn, M., van der Post, J. A., Hutten, B. A., Verhoeve, H. R., Hamulyák, K., Mol, B. W., Folkeringa, N., Nahuis, M., Papatsonis, D. N., Büller, H. R., van der Veen, F. & Middeldorp, S. (2010b) Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. *N Engl J Med*, 362 (17): 1586-1596.

Kalma, Y., Granot, I., Gnainsky, Y., Or, Y., Czernobilsky, B., Dekel, N. & Barash, A. (2009) Endometrial biopsy-induced gene modulation: first evidence for the expression of bladder-transmembranal uroplakin Ib in human endometrium. *Fertil Steril*, 91 (4): 1042-1049, 1049.e1041-1049.

Kaneko, Y., Murphy, C. R. & Day, M. L. (2013) Extracellular matrix proteins secreted from both the endometrium and the embryo are required for attachment: a study using a co-culture model of rat blastocysts and Ishikawa cells. *J Morphol*, 274 (1): 63-72.

Kara, M., Aydin, T., Turktekin, N. & S, K. (2012) Efficacy of the local endometrial injury in patients who had previous failed IVF-ICSI outcome. *Iran J Reprod Med*, 10 (6): 567-570.

Karimzade, M. A., Oskouian, H., Ahmadi, S. & Oskouian, L. (2010) Local injury to the endometrium on the day of oocyte retrieval has a negative impact on implantation in assisted reproductive cycles: a randomized controlled trial. *Arch Gynecol Obstet*, 281 (3): 499-503.

Karimzadeh, M. A., Ayazi Rozbahani, M. & Tabibnejad, N. (2009) Endometrial local injury improves the pregnancy rate among recurrent implantation failure patients undergoing in vitro fertilisation/intra cytoplasmic sperm injection: a randomised clinical trial. *Aust N Z J Obstet Gynaecol*, 49 (6): 677-680.

Karow, W. G., Gentry, W. C., Skeels, R. F. & Payne, S. A. (1971) Endometrial biopsy in the luteal phase of the cycle of conception. *Fertil Steril*, 22 (8): 482-495.

Kataoka, K., Tomiya, Y., Sakamoto, A., Kamada, Y., Hiramatsu, Y. & Nakatsuka, M. (2015) Altered autonomic nervous system activity in women with unexplained recurrent pregnancy loss. *The Journal Of Obstetrics And Gynaecology Research*, 41 (6): 912-918.

Kim, C., Ahn, J., Kang, S., Kim, S., Chae, H. & Kang, B. (2011) Effect of levothyroxine treatment on in vitro fertilization and pregnancy outcome in infertile women with subclinical hypothyroidism undergoing in vitro fertilization/intracytoplasmic sperm injection. *Fertility and Sterility*, 1650-1654.

King, A. & Loke, Y. W. (1991) On the nature and function of human uterine granular lymphocytes. *Immunol Today*, 12 (12): 432-435.

Kitaya, K., Matsubayashi, H., Takaya, Y., Nishiyama, R., Yamaguchi, K. & Ishikawa, T. (2016) Clinical background affecting pregnancy outcome following local endometrial injury in infertile patients with repeated implantation failure. *Gynecol Endocrinol*, 32 (7): 587-590.

Kolte, A. M., Bernardi, L. A., Christiansen, O. B., Quenby, S., Farquharson, R. G., Goddijn, M. & Stephenson, M. D. (2015a) Terminology for pregnancy loss prior to viability: a consensus statement from the ESHRE early pregnancy special interest group. *Human Reproduction*, Vol.30 495 – 498.

Kolte, A. M., Olsen, L. R., Mikkelsen, E. M., Christiansen, O. B. & Nielsen, H. S. (2015b) Depression and emotional stress is highly prevalent among women with recurrent pregnancy loss. *Hum Reprod*, 30 (4): 777-782.

Kramer, M. & McDonald, S. (2006) Aerobic exercise for women during pregnancy.

Kumbak, B., Sahin, L., Ozkan, S. & Atilgan, R. (2014) Impact of luteal phase hysteroscopy and concurrent endometrial biopsy on subsequent IVF cycle outcome. *Arch Gynecol Obstet*, 290 (2): 369-374.

Kuon, R. J., Weber, M., Heger, J., Santillán, I., Vomstein, K., Bär, C., Strowitzki, T., Markert, U. R. & Toth, B. (2017) Uterine natural killer cells in patients with idiopathic recurrent miscarriage. *Am J Reprod Immunol*, 78 (4):

Kuroda, K., Venkatakrisnan, R., James, S., Šucurovic, S., Mulac-Jericevic, B., Lucas, E. S., Takeda, S., Shmygol, A., Brosens, J. J. & Quenby, S. (2013a) Elevated periimplantation uterine natural killer cell density in human endometrium is associated with impaired corticosteroid signaling in decidualizing stromal cells. *J Clin Endocrinol Metab*, 98 (11): 4429-4437.

Kuroda, K., Venkatakrisnan, R., Salker, M. S., Lucas, E. S., Shaheen, F., Kuroda, M., Blanks, A., Christian, M., Quenby, S. & Brosens, J. J. (2013b) Induction of 11 $\beta$ -HSD 1 and activation of distinct mineralocorticoid receptor- and glucocorticoid receptor-dependent gene networks in decidualizing human endometrial stromal cells. *Mol Endocrinol*, 27 (2): 192-202.

- Kutteh, W. H. (1996) Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. *Am J Obstet Gynecol*, 174 (5): 1584-1589.
- Laird, S., Tuckerman, E. & Li, T. (2006) Symposium: embryo implantation failure and recurrent miscarriage: cytokine expression in the endometrium of women with implantation failure and recurrent miscarriage. *Reproductive BioMedicine Online*, 13 13–23.
- Larsen, E. C. & Christiansen, O. B. (2012) [Recurrent miscarriage--causes, diagnostics and treatment]. *Ugeskr Laeger*, 174 (41): 2448-2452.
- Lash, G. E., Bulmer, J. N., Li, T. C., Innes, B. A., Mariee, N., Patel, G., Sanderson, J., Quenby, S. & Laird, S. M. (2016) Standardisation of uterine natural killer (uNK) cell measurements in the endometrium of women with recurrent reproductive failure. *J Reprod Immunol*, 116 50-59.
- Lash, G. E., Naruse, K., Robson, A., Innes, B. A., Searle, R. F., Robson, S. C. & Bulmer, J. N. (2011) Interaction between uterine natural killer cells and extravillous trophoblast cells: effect on cytokine and angiogenic growth factor production. *Hum Reprod*, 26 (9): 2289-2295.
- Lashen, H., Fear, K. & Sturdee, D. W. (2004) Obesity is associated with increased risk of first trimester and recurrent miscarriage: matched case-control study. *Hum Reprod*, 19 (7): 1644-1646.
- Lensen, S., Osavlyuk, D., Armstrong, S., Stadelmann, C., Hennes, A., Napier, E., Wilkinson, J., Sadler, L., Gupta, D., Strandell, A., Bergh, C., Vigneswaran, K., Teh, W. T., Hamoda, H., Webber, L., Wakeman, S. A., Searle, L., Bhide, P., McDowell, S., Peeraer, K., Khalaf, Y. & Farquhar, C. (2019) A Randomized Trial of Endometrial Scratching before In Vitro Fertilization. *N Engl J Med*, 380 (4): 325-334.
- Lensen, S. F., Manders, M., Nastri, C. O., Gibreel, A., Martins, W. P., Templer, G. E. & Farquhar, C. (2016) Endometrial injury for pregnancy following sexual intercourse or intrauterine insemination. *Cochrane Database Syst Rev*, (6): CD011424.
- Leon, A., Davis, L. & Kraemer, H. (2011) The role and interpretation of pilot studies in clinical research. *J Psychiatr Res*, 45 (5): 626-629.
- Lessey, B. A. (1997) Integrins and the endometrium: new markers of uterine receptivity. *Ann N Y Acad Sci*, 828 111-122.
- Lessey, B. A. (2002) Adhesion molecules and implantation. *J Reprod Immunol*, 55 (1-2): 101-112.

Levin, D., Hasson, J., Cohen, A., Or, Y., Ata, B., Barzilay, L. & Almog, B. (2017) The effect of endometrial injury on implantation and clinical pregnancy rates. *Gynecol Endocrinol*, 33 (10): 779-782.

Li, J., Zhao, H., Song, J. M., Zhang, J., Tang, Y. L. & Xin, C. M. (2015) A meta-analysis of risk of pregnancy loss and caffeine and coffee consumption during pregnancy. *Int J Gynaecol Obstet*, 130 (2): 116-122.

Li, R. & Hao, G. (2009) Local injury to the endometrium: its effect on implantation. *Curr Opin Obstet Gynecol*, 21 (3): 236-239.

Li, T., Makris, M., Tomsu, M., Tuckerman, E. & Laird, S. (2002) Recurrent miscarriage: aetiology, management and prognosis. *Hum Reprod Update*, 8 (5): 463-481.

Li, W., Newell-Price, J., Jones, G., Ledger, W. & Li, T. (2012) Relationship between psychological stress and recurrent miscarriage *Reprod Biomed Online*, 25 (2): 180-189.

Licht, P., Fluhr, H., Neuwinger, J., Wallwiener, D. & Wildt, L. (2007) Is human chorionic gonadotropin directly involved in the regulation of human implantation? *Mol Cell Endocrinol*, 269 (1-2): 85-92.

Liddell, H. S., Pattison, N. S. & Zanderigo, A. (1991) Recurrent miscarriage--outcome after supportive care in early pregnancy. *Aust N Z J Obstet Gynaecol*, 31 (4): 320-322.

Lindhard, A., Bentin-Ley, U., Ravn, V., Islin, H., Hviid, T., Rex, S., Bangsbøll, S. & Sørensen, S. (2002) Biochemical evaluation of endometrial function at the time of implantation. *Fertil Steril*, 78 (2): 221-233.

Little, A. B. (1988) There's many a slip 'twixt implantation and the crib. *N Engl J Med*, 241-242.

Litwin, S., Lagadari, M., Barrientos, G., Roux, M. E., Margni, R. & Miranda, S. (2005) Comparative immunohistochemical study of M-CSF and G-CSF in feto-maternal interface in a multiparity mouse model. *Am J Reprod Immunol*, 54 (5): 311-320.

Liu, B., Mariee, N., Laird, S., Smith, J., Li, J. & Li, T. C. (2014) The prognostic value of uNK cell count and histological dating in the mid-luteal phase of women with reproductive failure. *Eur J Obstet Gynecol Reprod Biol*, 181 171-175.

Liu, W., Tal, R., Chao, H., Liu, M. & Liu, Y. (2017) Effect of local endometrial injury in proliferative vs. luteal phase on IVF outcomes in unselected subfertile women undergoing in vitro fertilization. *Reprod Biol Endocrinol*, 15 (1): 75.

Loeb, L. (1907) The experimental proof changes in the decider of guinea pig after mating. *Zentralbl Allg Pathol* 18 563-565.

Lucas, E. S., Dyer, N. P., Fishwick, K., Ott, S. & Brosens, J. J. (2016a) Success after failure: the role of endometrial stem cells in recurrent miscarriage. *Reproduction*, 152 (5): R159-166.

Lucas, E. S., Dyer, N. P., Murakami, K., Lee, Y. H., Chan, Y. W., Grimaldi, G., Muter, J., Brighton, P. J., Moore, J. D., Patel, G., Chan, J. K., Takeda, S., Lam, E. W., Quenby, S., Ott, S. & Brosens, J. J. (2016b) Loss of Endometrial Plasticity in Recurrent Pregnancy Loss. *Stem Cells*, 34 (2): 346-356.

Lund, M., Kamper-Jørgensen, M., Nielsen, H. S., Lidegaard, Ø., Andersen, A. M. & Christiansen, O. B. (2012) Prognosis for live birth in women with recurrent miscarriage: what is the best measure of success? *Obstet Gynecol*, 119 (1): 37-43.

Luster, A. D., Alon, R. & von Andrian, U. H. (2005) Immune cell migration in inflammation: present and future therapeutic targets. *Nature Immunology*, 6

Macklon, N. S., Geraedts, J. P. & Fauser, B. C. (2002) Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. *Hum Reprod Update*, 8 (4): 333-343.

Mahrn, A., Ibrahim, M. & Bahaa, H. (2016) The effect of endometrial injury on first cycle IVF/ICSI outcome: A randomized controlled trial. *Int J Reprod Biomed (Yazd)*, 14 (3): 193-198.

Mak, J. S. M., Chung, C. H. S., Chung, J. P. W., Kong, G. W. S., Saravelos, S. H., Cheung, L. P. & Li, T. C. (2017) The effect of endometrial scratch on natural-cycle cryopreserved embryo transfer outcomes: a randomized controlled study. *Reprod Biomed Online*, 35 (1): 28-36.

Makrakis, E., Hassiakos, D., Stathis, D., Vaxevanoglou, T., Orfanoudaki, E. & Pantos, K. (2009) Hysteroscopy in women with implantation failures after in vitro fertilization: findings and effect on subsequent pregnancy rates. *J Minim Invasive Gynecol*, 16 (2): 181-187.

Manaster, I., Mizrahi, S., Goldman-Wohl, D., Sela, H. Y., Stern-Ginossar, N., Lankry, D., Gruda, R., Hurwitz, A., Bdolah, Y., Haimov-Kochman, R., Yagel, S. & Mandelboim, O. (2008) Endometrial NK cells are special immature cells that await pregnancy. *J Immunol*, 181 (3): 1869-1876.

Matsumoto, Y., Koikeguchi, S. & Shiotani, M. (2017) Effects of endometrial injury on frozen-thawed blastocyst transfer in hormone replacement cycles. *Reprod Med Biol*, 16 (2): 196-199.

Metwally, M., Ong, K. J., Ledger, W. L. & Li, T. C. (2008) Does high body mass index increase the risk of miscarriage after spontaneous and assisted conception? A meta-analysis of the evidence. *Fertil Steril*, 90 (3): 714-726.

Metwally, M., Saravelos, S. H., Ledger, W. L. & Li, T. C. (2010) Body mass index and risk of miscarriage in women with recurrent miscarriage. *Fertil Steril*, 94 (1): 290-295.

Michimata, T., Ogasawara, M. S., Tsuda, H., Suzumori, K., Aoki, K., Sakai, M., Fujimura, M., Nagata, K., Nakamura, M. & Saito, S. (2002) Distributions of endometrial NK cells, B cells, T cells, and Th2/Tc2 cells fail to predict pregnancy outcome following recurrent abortion. *Am J Reprod Immunol*, 47 (4): 196-202.

Miscarriage Association *Talking about research*. [online] Available from: (Accessed

Moffett-King, A. (2002) Natural killer cells and pregnancy. *Nature Reviews Immunology*, 2

Morley, L. C., Simpson, N. & Tang, T. (2013) Human chorionic gonadotrophin (hCG) for preventing miscarriage. *Cochrane Database Syst Rev*, (1): CD008611.

MS, K. & SW, M. (2006) Aerobic exercise for women during pregnancy.

Musters, A. M., Taminiau-Bloem, E. F., van den Boogard, E., van der Veen, F. & Goddijn, M. (2011) Supportive care for women with unexplained recurrent miscarriage: patient's perspectives. *Human Reproduction*, 26 (No.4): 873-877.

Narvekar, S. A., Gupta, N., Shetty, N., Kottur, A., Srinivas, M. & Rao, K. A. (2010) Does local endometrial injury in the nontransfer cycle improve the IVF-ET outcome in the subsequent cycle in patients with previous unsuccessful IVF? A randomized controlled pilot study. *J Hum Reprod Sci*, 3 (1): 15-19.

Nastri, C. O., Gibreel, A., Raine-Fenning, N., Maheshwari, A., Ferriani, R. A., Bhattacharya, S. & Martins, W. P. (2012) Endometrial injury in women undergoing assisted reproductive techniques. *Cochrane Database Syst Rev*, (7): CD009517.

Nastri, C. O., Lensen, S. F., Gibreel, A., Raine-Fenning, N., Ferriani, R. A., Bhattacharya, S. & Martins, W. P. (2015) Endometrial injury in women undergoing assisted reproductive techniques. *Cochrane Database Syst Rev*, (3): CD009517.

Nastri, C. O., Teixeira, D. M. & Martins, W. P. (2013) Endometrial injury in the menstrual cycle prior to assisted reproduction techniques to improve reproductive outcomes. *Gynecol Endocrinol*, 29 (5): 401-402.

Navot, D. & Bergh, P. (1991) Preparation of the human endometrium for implantation. *Ann N Y Acad Sci*, 622 212-219.

NICE (2012) *Ectopic pregnancy and miscarriage: diagnosis and initial management* [online] Available from: (Accessed

NICE (2018) Antenatal care for uncomplicated pregnancies.

Ogasawara, M., Aoki, K., Okada, S. & Suzumori, K. (2000) Embryonic karyotype of abortuses in relation to the number of previous miscarriages. *73* (2): 300-304.

Opray, N., Grivell, R. M., Deussen, A. R. & Dodd, J. M. (2015) Directed preconception health programs and interventions for improving pregnancy outcomes for women who are overweight or obese. *Cochrane Database Syst Rev*, (7): CD010932.

Paria, B. C., Reese, J., Das, S. K. & Dey, S. K. (2002) Deciphering the cross-talk of implantation: advances and challenges. *Science*, 296 (5576): 2185-2188.

Peaceman, A. & Rehnberg, K. (1993) The effect of immunoglobulin G fractions from patients with lupus anticoagulant on placental prostacyclin and thromboxane production. *Am J Obstet Gynecol*, 169 1403-1406.

Perfetto, C. O., Murugappan, G. & Lathi, R. B. (2015) Time to next pregnancy in spontaneous pregnancies versus treatment cycles in fertile patients with recurrent pregnancy loss. *Fertil Res Pract*, 1 5.

Pfeifer, S., Goldberg, J., Lobo, R., Thomas, M., Widra, E., Licht, M., Collins, J., Cedars M, Vernon, M., Davis, O., Gracia, C., Catherino, W., Thornton, K., Rebar, R. & La Barbera, A. (2013) Definitions of infertility and recurrent pregnancy loss: a committee opinion. Practice Committee of American Society for Reproductive Medicine. *Fertil Steril* 99 (1):

Pineles, B. L., Park, E. & Samet, J. M. (2014) Systematic review and meta-analysis of miscarriage and maternal exposure to tobacco smoke during pregnancy. *Am J Epidemiol*, 179 (7): 807-823.

Plowden, T. C., Schisterman, E. F., Sjaarda, L. A., Zarek, S. M., Perkins, N. J., Silver, R., Galai, N., DeCherney, A. H. & Mumford, S. L. (2016) Subclinical Hypothyroidism and Thyroid Autoimmunity Are Not Associated With Fecundity, Pregnancy Loss, or Live Birth. *J Clin Endocrinol Metab*, 101 (6): 2358-2365.

Polanski, L. T., Barbosa, M. A., Martins, W. P., Baumgarten, M. N., Campbell, B., Brosens, J., Quenby, S. & Raine-Fenning, N. (2014) Interventions to improve reproductive outcomes in women with elevated natural killer cells undergoing assisted reproduction techniques: a systematic review of literature. *Hum Reprod*, 29 (1): 65-75.

Potdar, N., Gelbaya, T. & Nardo, L. G. (2012) Endometrial injury to overcome recurrent embryo implantation failure: a systematic review and meta-analysis. *Reprod Biomed Online*, 25 (6): 561-571.

Pye, C., Chatters, R., Cohen, J., Brian, K., Cheong, Y. C., Laird, S., Mohiyiddeen, L., Skull, J., Walters, S., Young, T. & Metwally, M. (2018) Induced endometrial trauma (endometrial scratch) in the mid-luteal menstrual cycle phase preceding first cycle IVF/ICSI versus usual IVF/ICSI therapy: study protocol for a randomised controlled trial. *BMJ Open*, 8 (5): e020755.

Qu, F., Wu, Y., Zhu, Y., Barry, J., Ding, T., Baio, G., Muscat, R., Todd, B., Wang, F. & Hardiman, P. (2017) The association between psychological stress and miscarriage: A systematic review and meta-analysis. *Sci Rep*. 2017 May 11;7(1):1731. doi: 10.1038/s41598-017-01792-3., 7 (1):

Quenby, S. & Farquharson, R. (2006) Uterine natural killer cells, implantation failure and recurrent miscarriage. *Reprod Biomed Online*, 13 (1): 24-28.

Quenby, S. & Farquharson, R. G. (1994) Human chorionic gonadotropin supplementation in recurring pregnancy loss: a controlled trial. *Fertil Steril*, 62 (4): 708-710.

Quenby, S., Kalumbi, C., Bates, M., Farquharson, R. & Vince, G. (2005a) Prednisolone reduces preconceptual endometrial natural killer cells in women with recurrent miscarriage. *Fertil Steril*, 84 (4): 980-984.

Quenby, S., Mountfield, S., Cartwright, J. E., Whitley, G. S., Chamley, L. & Vince, G. (2005b) Antiphospholipid antibodies prevent extravillous trophoblast differentiation. *Fertil Steril*, 83 (3): 691-698.

Quenby, S., Nik, H., Innes, B., Lash, G., Turner, M., Drury, J. & Bulmer, J. (2009) Uterine natural killer cells and angiogenesis in recurrent reproductive failure. *Hum Reprod*, 24 (1): 45-54.

Quenby, S., Vince, G., Farquharson, R. & Aplin, J. (2002) Recurrent miscarriage: a defect in nature's quality control? *Hum Reprod*, 17 (8): 1959-1963.

Rai, R., Clifford, K., Cohen, H. & Regan, L. (1995) High prospective fetal loss rate in untreated pregnancies of women with recurrent miscarriage and antiphospholipid antibodies. *Hum Reprod*, 10 (12): 3301-3304.

Rai, R., Cohen, H., Dave, M. & Regan, L. (1997) Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). *BMJ*, 314 (7076): 253-257.

Rai, R. & Regan, L. (2006) Recurrent miscarriage. *Lancet*, 368 (9535): 601-611.

Rai, R., Shlebak, A., Cohen, H., Backos, M., Holmes, Z., Marriott, K. & Regan, L. (2001) Factor V Leiden and acquired activated protein C resistance among 1000 women with recurrent miscarriage. *Hum Reprod*, 16 (5): 961-965.

Rama Raju, G., Shashi Kumari, G., Krishna, K., Prakash, G. & Madan, K. (2006) Assessment of uterine cavity by hysteroscopy in assisted reproduction programme and its influence on pregnancy outcome. *Arch Gynaecol Obstet*, 274 160-164.

RCOG (2011) *The Investigation and Treatment of Couples with Recurrent First-trimester and Second-trimester Miscarriage*. [online] Available from: (Accessed

RCOG (2016a) *The Role of Natural Killer Cells in Human Fertility*. [online] Available from: (Accessed

RCOG (2016b) *Local Endometrial Trauma (Endometrial Scratch): A Treatment Strategy to Improve Implantation Rates*. [online] Available from: (Accessed

Reid SM, Middleton P, Crossich MC & CA, C. (2010) Interventions for clinical and subclinical hypothyroidism in pregnancy.

Rey, E., Kahn, S., David, M. & Shrier, I. (2003) Thrombophilic disorders and fetal loss: a meta-analysis. *Lancet*, 361 901-908. .

Russo, L. M., Whitcomb, B. W., Mumford, S. L., Hawkins, M., Radin, R. G., Schliep, K. C., Silver, R. M., Perkins, N. J., Kim, K., Omosigho, U. R., Kuhr, D. L., Holland, T. L., Sjaarda, L. A. & Schisterman, E. F. (2018) A prospective study of physical activity and fecundability in women with a history of pregnancy loss. *Hum Reprod*, 33 (7): 1291-1298.

Ruszczak, Z. & Schwartz, R. A. (2000) Modern aspects of wound healing: An update. *Dermatol Surg*, 26 (3): 219-229.

Ruth Bender Atik, Christiansen, O. B., Elson, J., Astrid Marie Kolte, Lewis, S., Saskia Middeldorp, Willianne Nelen, Braulio Peramo, Siobhan Quenby, Nathalie Vermeulen & Mariëtte Goddijn (2018) ESHRE Guideline: Recurrent Pregnancy Loss. *Human Reproduction Open*, 2018 (2):

- S, S., R, F., M, I. & S., N. (1995) Expression of granulocyte colony-stimulating factor and its receptor at the fetomaternal interface in murine and human pregnancy. *Growth Factors*, 10 (2): 135-143.
- Saccone, G., Schoen, C., Franasiak, J. M., Scott, R. T. & Berghella, V. (2017) Supplementation with progestogens in the first trimester of pregnancy to prevent miscarriage in women with unexplained recurrent miscarriage: a systematic review and meta-analysis of randomized, controlled trials. *Fertil Steril*, 107 (2): 430-438.e433.
- Safdarian, L., Movahedi, S., Aleyasine, A., Aghahosaini, M., Fallah, P. & Rezaian, Z. (2011) Local injury to the endometrium does not improve the implantation rate in good responder patients undergoing in-vitro fertilization. *Iran J Reprod Med*, 9 (4): 285-288.
- Sahoo, T., Dzidic, N., Strecker, M. N., Commander, S., Travis, M. K., Doherty, C., Tyson, R. W., Mendoza, A. E., Stephenson, M., Dise, C. A., Benito, C. W., Ziadie, M. S. & Hovanes, K. (2017) Comprehensive genetic analysis of pregnancy loss by chromosomal microarrays: outcomes, benefits, and challenges. *Genet Med*, 19 (1): 83-89.
- Salamonsen, L. (2003) Tissue injury and repair in the female human reproductive tract. *Reproduction*, 125 (3): 301-311.
- Salker, M., Teklenburg, G., Molokhia, M., Lavery, S., Trew, G., Aojanepong, T., Mardon, H. J., Lokugamage, A. U., Rai, R., Landles, C., Roelen, B. A., Quenby, S., Kuijk, E. W., Kavelaars, A., Heijnen, C. J., Regan, L., Macklon, N. S. & Brosens, J. J. (2010) Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. *PLoS One*, 5 (4): e10287.
- Salker, M. S., Christian, M., Steel, J. H., Nautiyal, J., Lavery, S., Trew, G., Webster, Z., Al-Sabbagh, M., Puchchakayala, G., Föller, M., Landles, C., Sharkey, A. M., Quenby, S., Aplin, J. D., Regan, L., Lang, F. & Brosens, J. J. (2011) Deregulation of the serum- and glucocorticoid-inducible kinase SGK1 in the endometrium causes reproductive failure. *Nat Med*, 17 (11): 1509-1513.
- Salker, M. S., Nautiyal, J., Steel, J. H., Webster, Z., Sućurović, S., Nicou, M., Singh, Y., Lucas, E. S., Murakami, K., Chan, Y. W., James, S., Abdallah, Y., Christian, M., Croy, B. A., Mulac-Jericevic, B., Quenby, S. & Brosens, J. J. (2012) Disordered IL-33/ST2 activation in decidualizing stromal cells prolongs uterine receptivity in women with recurrent pregnancy loss. *PLoS One*, 7 (12): e52252.
- Santamaria, X., Cabanillas, S., Cervelló, I., Arbona, C., Raga, F., Ferro, J., Palmero, J., Remohí, J., Pellicer, A. & Simón, C. (2016) Autologous cell therapy with CD133+ bone

marrow-derived stem cells for refractory Asherman's syndrome and endometrial atrophy: a pilot cohort study. *Hum Reprod*, 31 (5): 1087-1096.

Santjohanser, C., Knieper, C., Franz, C., Hirv, K., Meri, O., Schleyer, M., Würfel, W. & Toth, B. (2013) Granulocyte-colony stimulating factor as treatment option in patients with recurrent miscarriage. *Arch Immunol Ther Exp (Warsz)*, 61 (2): 159-164.

Sar-Shalom Nahshon, C., Sagi-Dain, L., Wiener-Megnazi, Z. & Dirnfeld, M. (2019) The impact of intentional endometrial injury on reproductive outcomes: a systematic review and meta-analysis. *Hum Reprod Update*, 25 (1): 95-113.

Saravelos, S. H. & Li, T. C. (2012) Unexplained recurrent miscarriage: how can we explain it? *Hum Reprod*, 27 (7): 1882-1886.

Scarpellini, F. & Sbracia, M. (2009) Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: a randomised controlled trial. *Hum Reprod*, 24 (11): 2703-2708.

Schoenfeld, D. (1980) Statistical considerations for pilot studies. *Int J Radiat Oncol Biol Phys*, 6 (3): 371-374.

Shahrokh-Tehraninejad, E., Dashti, M., Hossein-Rashidi, B., Azimi-Nekoo, E., Haghollahi, F. & Kalantari, V. (2016) A Randomized Trial to Evaluate the Effect of Local Endometrial Injury on the Clinical Pregnancy Rate of Frozen Embryo Transfer Cycles in Patients With Repeated Implantation Failure. *J Family Reprod Health*, 10 (3): 108-114.

Shamseldin, H. E., Swaid, A. & Alkuraya, F. S. (2013) Lifting the lid on unborn lethal Mendelian phenotypes through exome sequencing. *Genet Med*, 15 (4): 307-309.

Shohayeb, A. & El-Khayat, W. (2012) Does a single endometrial biopsy regimen (S-EBR) improve ICSI outcome in patients with repeated implantation failure? A randomised controlled trial. *Eur J Obstet Gynecol Reprod Biol*, 164 (2): 176-179.

Silver, R. M., Branch, D. W. & Scott, J. R. (1997) Prednisone and aspirin in women with recurrent fetal loss. *N Engl J Med*, 337 (22): 1629-1630.

Silver, R. M. & Branch, W. D. (2007) Sporadic and recurrent pregnancy loss. In: Hobbins, E. A. R. J. C., ed. *Handbook of Clinical Obstetrics: The Fetus and Mother*. Second edn. 41-46.

Singh, N., Toshyan, V., Kumar, S., Vanamail, P. & Madhu, M. (2015) Does endometrial injury enhances implantation in recurrent in-vitro fertilization failures? A prospective randomized control study from tertiary care center. *J Hum Reprod Sci*, 8 (4): 218-223.

Siristatidis, C., Vrachnis, N., Vogiatzi, P., Chrelias, C., Retamar, A. Q., Bettocchi, S. & Glujovsky, D. (2014) Potential Pathophysiological Mechanisms of the Beneficial Role of Endometrial Injury in In Vitro Fertilization Outcome. *Reprod Sci*, 21 (8): 955-965.

Statistics, H. E. (2012-2013) (Hospital Episode Statistics: Maternity Statistics, 2012-2013).

Stephenson, M. D., Awartani, K. A. & Robinson, W. P. (2002) Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. *Hum Reprod*, 17 (2): 446-451.

Stray-Pedersen, B. & Stray-Pedersen, S. (1984) Etiologic factors and subsequent reproductive performance in 195 couples with a prior history of habitual abortion. *AM. J. OSTET. GYNECOL*, 148

Sugiura-Ogasawara, M., Aoki, K., Fujii, T., Fujita, T., Kawaguchi, R., Maruyama, T., Ozawa, N., Sugi, T., Takeshita, T. & Saito, S. (2008) Subsequent pregnancy outcomes in recurrent miscarriage patients with a paternal or maternal carrier of a structural chromosome rearrangement. *J Hum Genet*, 53 (7): 622-628.

Sugiura-Ogasawara, M., Furukawa, T., Nakano, Y., Hori, S., Aoki, K. & Kitamura, T. (2002) Depression as a potential causal factor in subsequent miscarriage in recurrent spontaneous aborters. *Hum Reproduction.*, 17 2580-2584.

Suzumori, N. & Sugiura-Ogasawara, M. (2010) Genetic factors as a cause of miscarriage. *Curr Med Chem*, 17 (29): 3431-3437.

T.C.Li (1998) Recurrent Miscarriage: principles of management. *Human Reproduction*, 13 (no.2): 478-482.

Tang, A. & Quenby, S. (2010) Recent thoughts on management and prevention of recurrent early pregnancy loss. *Curr Opin Obstet Gynecol*, 22 (6): 446-451.

Tang, A. W., Alfirevic, Z., Turner, M. A., Drury, J. A., Small, R. & Quenby, S. (2013) 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. *Hum Reprod*, 28 (7): 1743-1752.

Thangaratinam, S., Tan, A., Knox, E., Kilby, M. D., Franklyn, J. & Coomarasamy, A. (2011) Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. *BMJ*, 342 d2616.

- Tiboni, G. M., Giampietro, F., Gabriele, E., Di Donato, V. & Impicciatore, G. G. (2011) Impact of a single endometrial injury on assisted reproductive technology outcome: a preliminary observational study. *J Reprod Med*, 56 (11-12): 504-506.
- Tuckerman, E., Laird, S. M., Prakash, A. & Li, T. C. (2007) Prognostic value of the measurement of uterine natural killer cells in the endometrium of women with recurrent miscarriage. *Hum Reprod*, 22 (8): 2208-2213.
- Uchida, S., Maruyama, T., Kagami, M., Miki, F., Hihara, H., Katakura, S., Yoshimasa, Y., Masuda, H., Uchida, H. & Tanaka, M. (2017) Impact of borderline-subclinical hypothyroidism on subsequent pregnancy outcome in women with unexplained recurrent pregnancy loss. *J Obstet Gynaecol Res*, 43 (6): 1014-1020.
- van den Boogaard, E., Hermens, R., Verhoeve, H., Kremer, J., van der Veen, F., Knegt, A. & Goddijn, M. (2011) Selective karyotyping in recurrent miscarriage: are recommended guidelines adopted in daily clinical practice? *Hum Reprod.*, 26 (8): 1965-1970.
- van der Linden, M., Buckingham, K., Farquhar, C., Kremer, J. & Metwally, M. (2015) Luteal phase support for assisted reproduction cycles. *Cochrane Database of Systematic Reviews*, (7): 1465-1858.
- van Hoogenhuijze, N. E., Torrance, H. L., Mol, F., Laven, J. S. E., Scheenjes, E., Traas, M. A. F., Janssen, C., Cohlen, B., Teklenburg, G., de Bruin, J. P., van Oppenraaij, R., Maas, J. W. M., Moll, E., Fleischer, K., van Hooff, M. H., de Koning, C., Cantineau, A., Lambalk, C. B., Verberg, M., Nijs, M., Manger, A. P., van Rumste, M., van der Voet, L. F., Preys-Bosman, A., Visser, J., Brinkhuis, E., den Hartog, J. E., Sluijmer, A., Jansen, F. W., Hermes, W., Bandell, M. L., Pelinck, M. J., van Disseldorp, J., van Wely, M., Smeenk, J., Pieterse, Q. D., Boxmeer, J. C., Groenewoud, E. R., Eijkemans, M. J. C., Kasius, J. C. & Broekmans, F. J. M. (2017) Endometrial scratching in women with implantation failure after a first IVF/ICSI cycle; does it lead to a higher live birth rate? The SCRaTCH study: a randomized controlled trial (NTR 5342). *BMC Womens Health*, 17 (1): 47.
- Wahabi, H. A., Fayed, A. A., Esmail, S. A. & Bahkali, K. H. (2018) Progestogen for treating threatened miscarriage. *Cochrane Database Syst Rev*, 8 CD005943.
- Walker, I. D. (2000) Thrombophilia in pregnancy. *Journal of Clinical Pathology*, 53 573-580.
- Wapner, R. J. & Lewis, D. (2002) Genetics and metabolic causes of stillbirth. *Seminars in Perinatal Pathology*, 26 (1): 70-74.
- Warburton, D., Kline, J., Stein, Z., Hutzler, M., Chin, A. & Hassold, T. (1987) Does the karyotype of a spontaneous abortion predict the karyotype of a subsequent abortion?

Evidence from 273 women with two karyotyped spontaneous abortions. *Am J Hum Genet*, 41 (3): 465-483.

Waseda, T., Tomizawa, H., Fujii, R., Makinoda, S. & Hirosaki, N. (2008) Granulocyte colony-stimulating factor (G-CSF) in the mechanism of human ovulation and its clinical usefulness. *Current Medicinal Chemistry*, 15 (6): 604-613.

Weimar, C. H., Macklon, N. S., Post Uiterweer, E. D., Brosens, J. J. & Gellersen, B. (2013) The motile and invasive capacity of human endometrial stromal cells: implications for normal and impaired reproductive function. *Hum Reprod Update*, 19 (5): 542-557.

WHO. (2018) *WHO recommendation on caffeine intake during pregnancy*.

Wilson, W., Gharavi, A., Koike, T., Lockshin, M., Branch, D., Piette, J., Brey, R., Derksen, R., Harris, E., Hughes, G., Triplett, D. & Khamashta, M. (1999) International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum.*, 42 (7): 1309-1311.

Wong, L. F., Porter, T. F. & de Jesus, G. R. (2014) Recurrent early pregnancy loss and antiphospholipid antibodies: where do we stand? *LUPUS*, 23 (12): 1226-1228.

Yeung, T. W., Chai, J., Li, R. H., Lee, V. C., Ho, P. C. & Ng, E. H. (2014) The effect of endometrial injury on ongoing pregnancy rate in unselected subfertile women undergoing in vitro fertilization: a randomized controlled trial. *Hum Reprod*, 29 (11): 2474-2481.

Yibing Zhang, Haoyu Wang, Xifeng Pan, Weiping Teng & Shan, Z. (2017) Patients with subclinical hypothyroidism before 20 weeks of pregnancy have a higher risk of miscarriage: A systematic review and meta-analysis. (April): Available from: (Accessed

Zheng, W., Li, R., Pan, H., He, D., Xu, R., Guo, T. B., Guo, Y. & Zhang, J. Z. (2009) Role of osteopontin in induction of monocyte chemoattractant protein 1 and macrophage inflammatory protein 1beta through the NF-kappaB and MAPK pathways in rheumatoid arthritis. *Arthritis Rheum*, 60 (7): 1957-1965.

Zhou, L., Li, R., Wang, R., Huang, H. X. & Zhong, K. (2008) Local injury to the endometrium in controlled ovarian hyperstimulation cycles improves implantation rates. *Fertil Steril*, 89 (5): 1166-1176.

Zinaman, M. J., Clegg, E. D., Brown, C. C., O'Connor, J. & Selevan, S. G. (1996) Estimates of human fertility and pregnancy loss. *Fertility and Sterility*, Volume 65 (3): 503- 509.



## Clinical trials

Clinicians keen to keep up-to-date regarding clinical studies that are currently recruiting may find the following informative.

### SiM (Scratch in Miscarriage) study (SiM)

<https://clinicaltrials.gov/ct2/show/NCT02681627>

**Registration Description** This phase 1, pilot, randomised controlled trial aims to determine whether endometrial scratch with a Wallace catheter during the luteal phase improves the live birth rate in women with recurrent miscarriage.

**Outcome measures** **Primary:** Live birth rate after 24 weeks of gestation. **Secondary:** Miscarriage until 23<sup>rd</sup> weeks of gestation; pregnancy complications; acceptability of the intervention.

**Study site** Coventry and Warwick, UK. **Anticipated study end date** September 2017.

## Appendix 1

DOI:10.1111/1471-0528.14032. [www.bjog.org](http://www.bjog.org) April 2016

## Appendix 2

Trial Protocol



## PROTOCOL

Pilot randomised controlled trial of the effect of endometrial scratch in recurrent miscarriage on pregnancy outcomes.  
SiM study (Scratch in Miscarriage)

Sponsor: University of Warwick  
Funding Body: Biomedical research Unit- Reproductive Health  
Ethics Approval NRES South Birmingham Reference: 15/WM/0295  
Date: 26/10/2015

Version: 3  
Date: 17/02/2016  
Stage:  
CI Signature:

### Protocol Amendments:

Amendment No.	Date of Amendment	Date of Approval
1	28/09/2015	21/10/2015
2	02/02/2016	

**CONTACT NAMES AND NUMBERS**

<b>Role</b>	<b>Name, address, telephone</b>
<b>Sponsor:</b>	Mrs. Jane Prewett University of Warwick, Research & Impact Services University House, Kirby Corner road Coventry CV4 8 UW, UK Tel: [REDACTED] Email: [REDACTED]
<b>Chief Investigator:</b>	Professor. Siobhan Quenby B.Sc.,MBBS,MD,FRCOG Professor in Obstetrics, Division of Reproductive Health Clinical Sciences Research Laboratory Warwick Medical School, University of Warwick University Hospitals Coventry and Warwickshire NHS Trust Clifford Bridge Road, Coventry CV2 2DX, UK Tel: [REDACTED] Email: [REDACTED]
<b>Trial Co-ordinator:</b>	Dr.Valarmathy Kandavel MBBS, DGO, MRCOG Clinical Research Fellow , Centre for Reproductive Medicine University Hospitals Coventry and Warwickshire NHS Trust Clifford Bridge Road, Coventry CV2 2DX, UK Tel: [REDACTED] Email: [REDACTED]

<b>Role</b>	<b>Name, address, telephone</b>
<b>Co-investigators:</b>	<p>Dr.Valarmathy Kandavel MBBS, DGO, MRCOG  Clinical Research Fellow , Centre for Reproductive Medicine  University Hospitals Coventry and Warwickshire NHS Trust  Clifford Bridge Road, Coventry CV2 2DX, UK  Tel: [REDACTED]  Email: [REDACTED]</p> <p>Dr. Mariam Lokman MRCOG  Clinical Research Fellow , Centre for Reproductive Medicine  University Hospitals Coventry and Warwickshire NHS Trust  Clifford Bridge Road, Coventry CV2 2DX, UK  Tel: [REDACTED]  Email: [REDACTED]</p>
<b>Statistician:</b>	<p>Dr.Peter Kimani  Assistant Professor, Statistics and Epidemiology Health  sciences  Warwick Medical School  The University of Warwick  Coventry  CV4 7AL  Tel: [REDACTED]  Fax:  Email: [REDACTED]</p>
<b>Health Economist:</b>	NA
<b>Trial Steering Committee:</b>	<p>Chair: Mr.Feras Izzat MBChb, MRCOG  Consultant and EPAU lead  University Hospitals Coventry and Warwickshire NHS Trust  Clifford Bridge Road, Coventry CV2 2DX, UK  Tel: [REDACTED]  [REDACTED]</p> <p>Two others will be appointed</p>
<b>Data Monitoring Committee:</b>	N/A as pilot/feasibility trial only

**Role****Name, address, telephone**

For general queries and supply of trial materials please contact the coordinating centre:

BRU University Hospitals Coventry and Warwickshire NHS Trust

Tel: 02476967528

Fax: 02476967375

## TABLE OF CONTENTS FOR CONTENTS OF APPENDIX

	TABLE OF CONTENTS	182
	List of abbreviations/GLOSSARY	185
1.	Background	187
1.1	Epidemiology and burden of the condition	187
1.2	Existing knowledge	187
1.3	Hypothesis	153
1.4	Need for a trial	194
1.5	Ethical considerations	80
1.6	CONSORT	89
2.	Trial Design	196
2.1	Trial summary and flow diagram	96
2.2	Aims and objectives	200
2.2.1	Primary objective	<b>Error! Bookmark not defined.</b>
2.2.2	Secondary objective	<b>Error! Bookmark not defined.</b>
2.3	Outcome measures	104
2.3.1	Efficacy	200
2.3.2	Safety	105
2.3.3	Others	105
2.4	Eligibility criteria	98
2.4.1	Inclusion criteria	<b>Error! Bookmark not defined.</b>
2.4.2	Exclusion criteria	<b>Error! Bookmark not defined.</b>
2.5	Informed consent	<b>Error! Bookmark not defined.</b>
2.6	Recruitment and randomisation	98
2.6.1	Recruitment	98
2.6.2	Randomisation	100
2.6.2.1	Post-randomisation withdrawals and exclusions	216
2.7	Trial treatments / intervention	202
2.7.1	Trial treatment(s) / intervention	202
2.7.2	Placebo treatment(s) / control intervention	203
2.7.3	Drug storage and dispensing	203
2.7.4	Drug accountability	203

2.7.5	Compliance/contamination	203
2.8	Blinding	<b>Error! Bookmark not defined.</b>
2.8.1	Methods for ensuring blinding	87
2.8.2	Methods for unblinding the trial	87
2.9	Concomitant illness and medication	204
2.9.1	Concomitant illness	204
2.9.2	Concomitant medication	204
2.10	End of trial	105
3.	METHods and assessments	87
3.1	Schedule of delivery of intervention and data collection	87
3.2	Laboratory assessments	105
4.	adverse event management	105
4.1	Definitions	206
4.1.1	Adverse Events (AE)	105
4.1.2	Adverse Reaction (AR)	206
4.1.3	Serious Adverse Events (SAEs) and Suspected Unexpected Serious Adverse Events (SUSARS)	<b>Error! Bookmark not defined.</b>
4.2	Reporting SAEs and SUSARs	107
4.3	Procedures in case of overdose	207
4.4	Procedures in case of pregnancy	102
5.	Data management	107
5.1	Data collection and management	107
5.2	Database	108
5.3	Data storage	108
5.4	Data access and quality assurance	<b>Error! Bookmark not defined.</b>
5.5	Archiving	108
6.	Statistical analysis	<b>Error! Bookmark not defined.</b>
6.1	Power and sample size	<b>Error! Bookmark not defined.</b>
6.2	Statistical analysis of efficacy and harms	<b>Error! Bookmark not defined.</b>
6.3	Health Economic Evaluation	210
7.	Trial organisation and oversight	95
7.1	Sponsor and governance arrangements	95
7.2	Regulatory authorities/ethical approval	95
7.3	Trial Registration	95
7.4	Indemnity	211

7.5	Trial timetable and milestones	211
7.6	Administration	108
7.7	Trial Management Group (TMG)	115
7.8	Trial Steering Committee (TSC)	212
7.9	Data Monitoring Committee (DMC)	212
7.10	Essential Documentation	212
8.	Monitoring and Quality assurance of trial procedures	212
9.	Patient and Public Involvement (PPI)	213
10.	Dissemination and publication	213
11.	References	213
12.	Appendices	<b>Error! Bookmark not defined.</b>

## **LIST OF TABLES**

**PAGE**

Table 1	Trial assessments	<b>Error! Bookmark not defined.</b>
---------	-------------------	-------------------------------------

## **LIST OF FIGURES**

**PAGE**

Figure 1	Trial flow diagram	197
----------	--------------------	-----

## LIST OF ABBREVIATIONS/GLOSSARY

Abbreviation	Explanation
AE	Adverse Event
AR	Adverse Reaction
BRU-RH	Biomedical Research Unit- Reproductive Health
CI	Chief Investigator
CONSORT	<i>Consolidated Standards of Reporting Trials</i>
CRF	Case Report Form
CTA	Clinical Trials Authorisation
CTIMP	Clinical Trial of an Investigational Medicinal Product
CTU	Clinical Trials Unit
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IRAS	Integrated Research Application System
ISRCTN	International Standard Randomised Controlled Trial Number
MHRA	Medicines and Healthcare products Regulatory Agency
MRC	Medical Research Council
PI	Principal Investigator
PPI	Patient & Public Involvement
RM	Recurrent Miscarriage
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
R&D	Research and Development
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction

TSC  
WCTU

Trial Steering Committee  
Warwick Clinical Trials Unit

---

# 1. Background

## Epidemiology and burden of the condition

Miscarriage is the loss of pregnancy at less than 24 weeks. The definition of recurrent miscarriages in the UK is three or more consecutive pregnancy losses. The background risk of miscarriages is quoted around 20% or 1: 5 pregnancies. The majority of losses occur in the first trimester, defined as less than 13 completed weeks of pregnancy. (NICE Ectopic Pregnancy and Miscarriage Guideline( CG 154), 2012).

The pregnancy loss may be either biochemical, prior to the scan diagnosis or a missed miscarriage where there is evidence of an intra- uterine pregnancy on scan. In England and Wales Between 2012- 2013 there were 39,800 miscarriages that led to a hospital stay (Hospital Episode Statistics: Maternity Statistics, 2012- 2013).

Miscarriage is distressing for the patient and evidence suggests that 1: 5 women who experience miscarriage have anxiety levels similar to people attending the psychiatric outpatient services. One third of the women attending specialist clinics as a result of miscarriage are clinically depressed (Rai & Regan, 2006).

## Existing knowledge

During a women's reproductive cycle, pregnancy occurs with intercourse within 2 days of ovulation. There are marked changes that occur within the endometrium which contribute to the "window of implantation" on day 20- 24. Increasing circulating oestrogen levels lead to positive feedback upon the anterior pituitary gland causing the release and surge of Luteinising hormone. The surge triggers ovulation and can be detected 10- 12 hours prior to ovulation. The endometrium shows changes associated with the pre-decidual transformation of the upper 2/3<sup>rd</sup> of the functionalis layer. The glands exhibit extensive coiling and luminal secretions become visible. Epithelial cells show decreased microvilli and cilia along with appearances of luminal protrusions of the apical cell surface. These pinopodes are important in preparation for the blastocyst to implant. They also coincide with changes on the surface of the glycocalyx that allows the acceptance of a blastocyst.

Decidua is a specialised highly modified endometrium of pregnancy.

Decidualisation is the transformation of secretory endometrium to decidua and is dependent upon oestrogen, progesterone and factors secreted by the implanting blastocyst. The decidua is classified into three parts based on anatomical location. The decidua directly beneath the blastocyst implantation site is modified by trophoblast invasion and becomes the decidua basalis. The decidua capsularis overlies the enlarging blastocyst and initially separates the conceptus from the rest of the uterine cavity. The remainder of the uterus is lined by the decidua parietalis. The decidual reaction is completed with blastocyst implantation. However, predecidual changes commence during the mid-luteal

phase in endometrial stromal cells adjacent to the arterioles and spiral arteries. (Cunningham, et al., 2014)

The embryo implants 6- 7 days after fertilisation, in the uterine wall. This involves three processes

1. Apposition- initial contact of the blastocyst to the uterine wall
2. Adhesion- increased physical contact between the blastocyst and the endometrial epithelium
3. Invasion- penetration and invasion of syncytiotrophoblast and cytotrophoblasts into the endometrium, inner third of myometrium and uterine vasculature. (Fatemi & Popovic-Todorovic, 2013)

Adherence is mediated by endothelial cell- surface receptors at the implantation site that interact with blastocyst receptors (Carson, 2002: Lessy 2002: Lindhard 2002: Paria, 2002). If the blastocyst approaches the endometrium after day 24, the potential for adhesion is diminished because antiadhesive glycoprotein synthesis prevents receptor interactions (Navot, 1991).

Successful endometrial blastocyst adhesion involves modification in the expression of cellular adhesion molecules (CAMs). The integrin's are one of the four families of CAMs. They are cell surface receptors that mediate cell adhesion to extracellular matrix proteins (Lessey, 2002).

Endometrial integrin's are hormonally regulated, and a specific set of integrin's is expressed during implantation (Lessey, 1996). Specifically,  $\alpha V\beta 3$  and  $\alpha 4\beta 1$  integrins expressed on endometrial epithelium are considered as receptivity marker for blastocyst attachment. Recognition site blockade on integrins for binding to extracellular matrix molecules such as fibronectin will prevent blastocyst attachment (Kaneko, 2013).

Infiltration of large populations of decidual leucocytes to the implantation site has been observed in both the human and the mouse. Of these cells 65-70% is uterine specific natural killer cells (uNKs). 10-20% is antigen presenting cells (APC) such as macrophages and dendritic cells (DCs) (Granot, Gnainsky, & Dekel, Endometrial Inflammation and effect on implantation improvement and pregnancy outcome, 2012).

Uterine natural killer cells are postulated to play a role in angiogenesis and trophoblast invasion. CD56 + (uNK) cells show a dramatic rise in absolute numbers and as a percentage of stromal cells from day 22- 28 of a 28 day menstrual cycle.

Normal pregnancy may be the result of a predominantly Th-2 cytokine response with anti- inflammatory markers such as IL-4, IL-6 and IL-10.

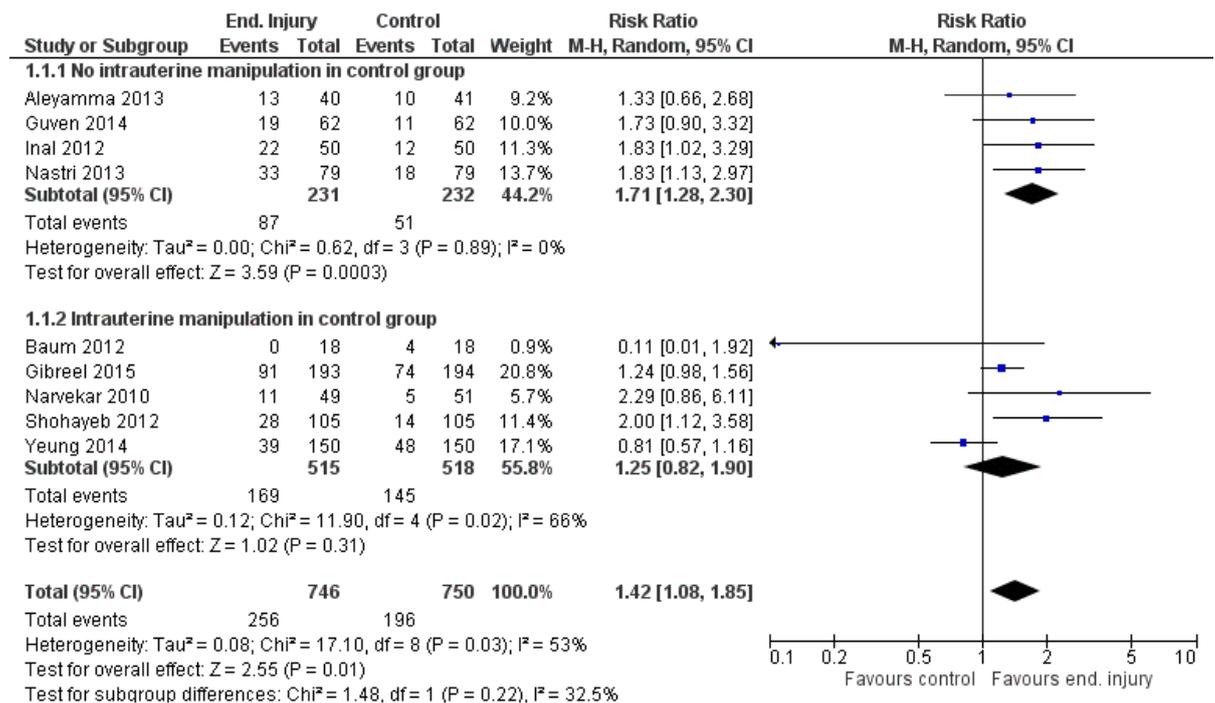
Recurrent miscarriages have a bias towards mounting a Th-1 cytokine response with pro- inflammatory markers such as TNF-  $\alpha$  (tumour necrosis factor –alpha), IFN (Interferon) and IL-2 (interleukin-2) (Recurrent Miscarriage, Investigation and Treatment of Couples( Green-top Guideline No.17), 2011).

**Endometrial scratch or injury** is defined as an intentional minor damage to the endometrium performed with the objective of improving the reproductive outcomes of women desiring pregnancy. The most common method of injury is by using a Pipelle catheter. (Nastri , et al., July 2012). Novak curette achieves a higher degree of injury. The evidence in favour of endometrial injury has been obtained from studies in women undergoing IVF and recurrent implantation failures.

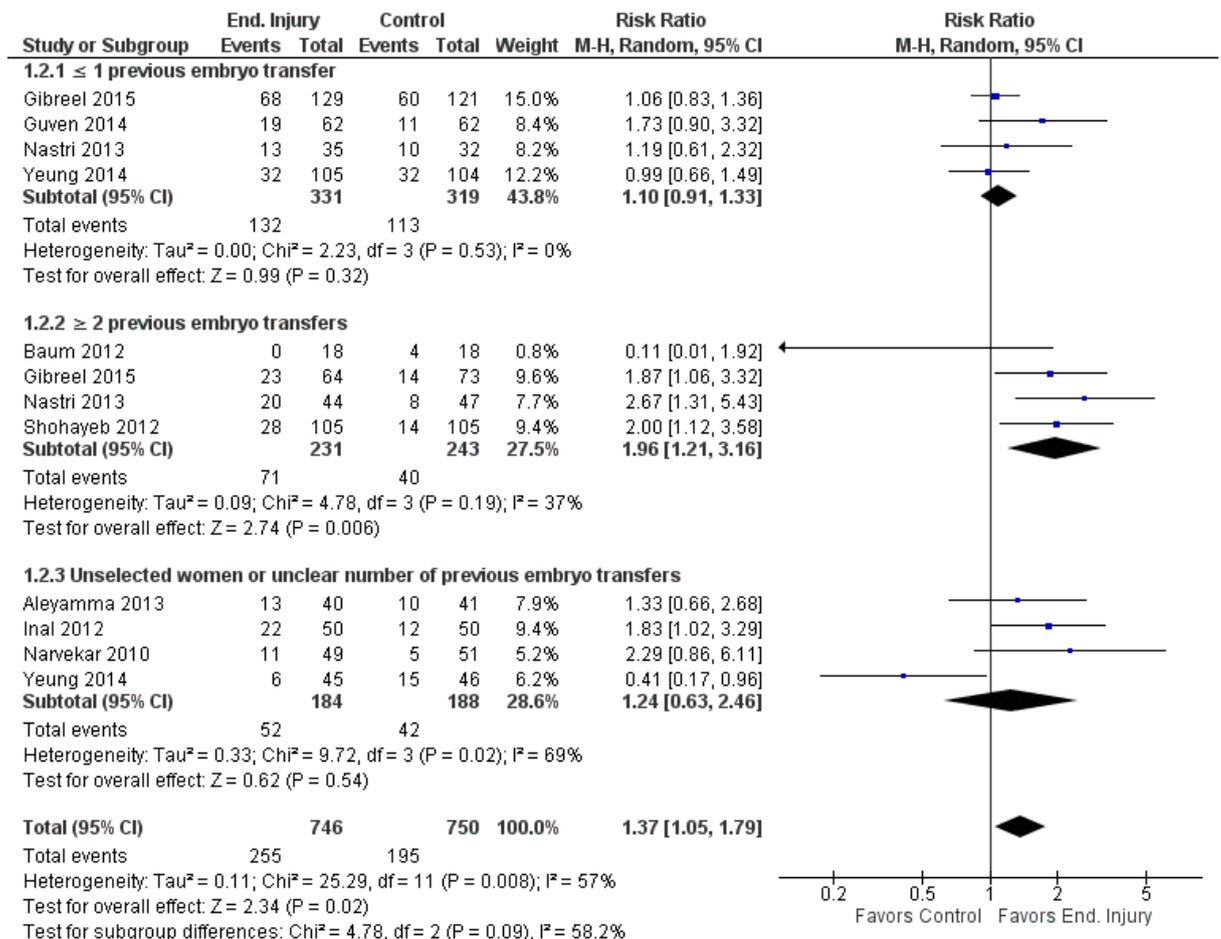
Earliest evidence is from Karrow *et al* who in 1971 reported there were only 2 miscarriages in 28 women who underwent endometrial biopsy in the luteal phase and conceived in the same cycle.

Cochrane meta- analysis (March 2015) of endometrial injury in assisted reproduction considered 14 randomised controlled trials. Of these, women in 13 trials underwent the endometrial biopsy between 7 days of previous cycle to 7 days of the embryo transfer cycle. The livebirth rate and ongoing pregnancy rate was significantly increased in the injury group with a RR (risk ratio) of 1.42.

Similarly, the clinical pregnancy rate was higher in the endometrial injury group with a risk ratio of 1.34 (P value=0.002). The Meta –analysis did not reveal any difference in miscarriage rate, but the evidence was very low quality. The procedure was associated with mild pain, which was transient.



Forest plot of comparison: 1 Endometrial injury vs no injury, outcome: 1.2 Live birth/Ongoing pregnancy per randomly assigned woman (Cochrane 2015)



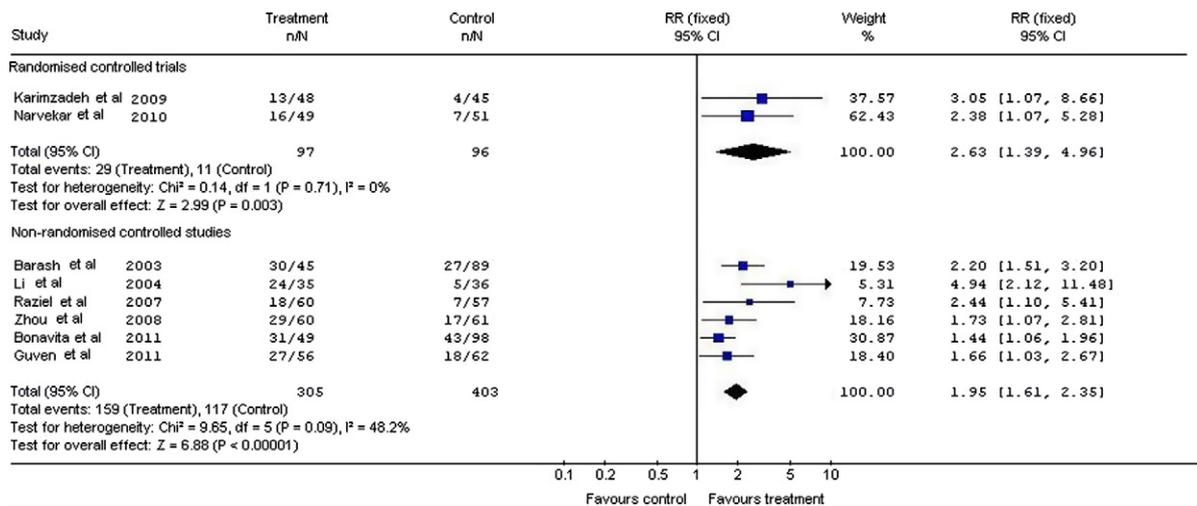
Forest plot of comparison: 1 Effectiveness, outcome: 1.1 Live birth per randomly assigned woman

Cochrane Database of Systematic Reviews 22 MAR 2015 DOI: 10.1002/14651858.CD009517.pub3

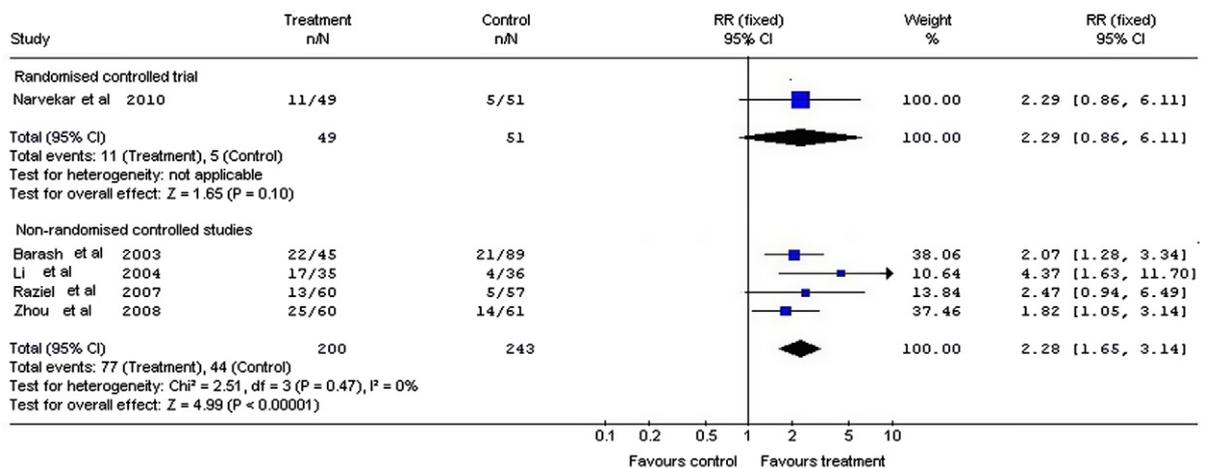
A single sampling in the proliferative phase was also found to be adequate.

However local injury on the day of oocyte retrieval had a negative impact on pregnancy rate. (Karimzade, Oskouian, Ahmadi, & Oskouian, 2010)

El-Toukhy et al (El-Toukhy, Sunkara, & Khalaf, 2012) conducted a systematic review and meta-analysis on Local endometrial injury and IVF outcome. They measured the IVF outcomes in the subsequent cycle to the endometrial injury. The meta-analysis showed that the clinical pregnancy rate was significantly improved after the injury in both the randomized and non randomised studies.



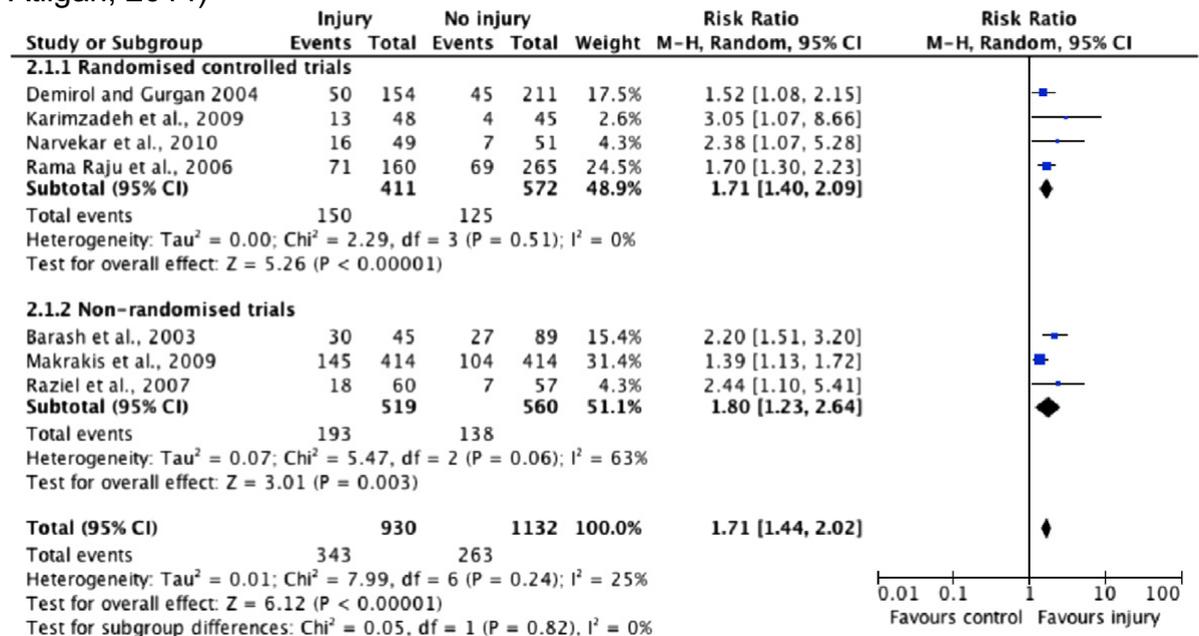
Summary of clinical pregnancy rate for the eight studies included in the systematic review. Local endometrial injury and IVF outcome (El-Toukhy 2012)



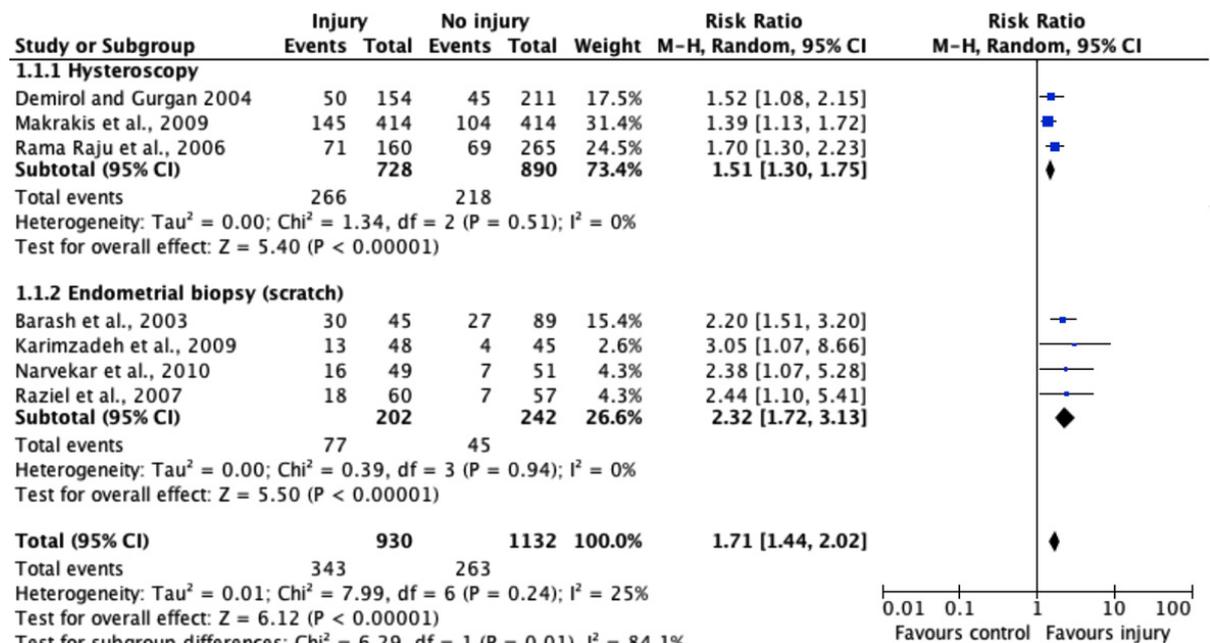
Summary of the livebirth/ongoing pregnancy rate for the five studies included in the systematic review. Local endometrial injury and IVF outcome (El-Toukhy 2012)

The systematic review and meta-analysis on endometrial injury in recurrent implantation failure by N Potdar et al in 2012 (Potdar, Gelbaya, & Nardo, 2012), also favours the endometrial injury group. The pooled relative risk for clinical pregnancy rate shows that local injury is 70% more likely to result in a clinical pregnancy when compared to no injury. The beneficial effect of inducing injury is when it precedes the cycle of ovarian stimulation. The effect has been shown to last in the subsequent cycle, possibly because the monocytes recruited to the injured sites are long lived and reside in tissues for a long time (Yeung, Chai, Li, Lee, Ho, & Ng, 2014). Injury in the luteal phase is likely to induce more

decidualization due to the progesterone influence. (Kumbhak, Sahin, Ozkan, & Atilgan, 2014)



Forest plot for clinical pregnancy rate in the randomized and non-randomized studies for endometrial injury and control groups. (N.Potdar et al 2012) in recurrent implantation failure



hysteroscopy and endometrial biopsy) and control group

Forest plot for clinical pregnancy rate in the endometrial injury (hysteroscopy and endometrial biopsy) and control groups. Endometrial injury to overcome recurrent embryo implantation failure, N. Potdar et al 2012)

A single luteal phase endometrial injury was found to be as effective as multiple injuries. This avoids increasing the risk of infection from repeated instrumentation and the possible negative effects due to endometritis, and the additional costs of the procedure. (Shohayeb & El-Khayat, 2012)

There was no significant difference in the miscarriage rates in patients who underwent endometrial injury with IVF or IUI. (Nastri, Ferriani, Raine-Fenning, & Martins, 2013)

The mechanisms by which the endometrial injury favours a receptive endometrium for implantation and improve clinical pregnancy rates are:

1. Local injury to the endometrium might induce decidualisation of endometrium and increase the implantation rate. (Tiboni, Giampietro, Gabriele, Di Donato, & Impicciatore, 2011)
2. It may provoke wound healing involving increased secretion of different cytokines and growth factors including Leukaemia inhibitory factor, IL-11, Heparin binding endothelial growth like factor (HB-EGF), which are beneficial for embryo implantation.
3. The backward development hypothesis. Delayed endometrial development inducing synchronicity between endometrium and embryo stage. It is postulated that in stimulated cycles the endometrium is ahead by 2-4 days in its development, in comparison to natural cycles.
4. Hydo et al (2011) used mouse model to provide evidence that endometrial injury induces a rapid increase in SP (side population) progenitor cells in endometrium. SP cells reside in the basal layer of the endometrium and could provoke endometrial regeneration and proliferation, which are critical for implantation.
5. Modulation of gene expression of factors required for implantation including glycodelin A, Laminin  $\alpha$ 4, integrin  $\alpha$ 6 and MMP1 (Matrix Metalloproteinase 1)

Endometrial injury triggers a series of biological responses, but the findings suggest that no single pathway is solely adequate to explain the association between trauma and improved pregnancy rates. The probable explanation is rather a cluster of events in response to trauma, which benefits embryo implantation in ways both known and unknown to the scientific community. (Siristatidis, et al., 2014)

Changes induced are:

1. Decidualisation

2. Development of secretory glandules, pinopodes and microvilli on luminal epithelium
3. Modulation in expression of different cytokines, growth factors and adhesion molecules.

### 1.1 Hypothesis

The Warwick BRU-RH paradigm is that failed pregnancies are the result of failed decidualisation of the endometrium. The hypothesis is that endometrial scratch improves pregnancy outcomes in recurrent miscarriage patients. The endometrial scratch is of proven benefit to patients with recurrent implantation failure in IVF, defined as the transfer of at least 10 good grade embryos. It has also proven benefit in patients who undergo IVF without implantation failure. The endometrial injury was proven to improve the IVF outcomes in younger women with intact ovarian reserve and good response to ovarian stimulation. (Baum, et al., 2012) This was in all the 8 studies included in the meta- analysis conducted by El-Touhky et al (2012). The intervention is not expected to improve IVF outcome in the presence of reduced ovarian reserve or poor embryo quality. There was no difference in the pregnancy rates in women who underwent endometrial injury and ovum donation cycles. (Dain, et al., 2014) The success with IVF is modest and therefore the results of the endometrial scratch are important, as it is a relatively quick, inexpensive and safe procedure with no significant complications. The outcomes from the meta- analysis support the application of this intervention in recurrent miscarriage patients to improve pregnancy outcomes.

### 1.2 Need for a trial

ASRM (American Society of Reproductive Medicine) definition of recurrent miscarriage is two or more failed clinical pregnancies. Clinical evaluation may proceed following two first trimester pregnancy losses. This accounts for fewer than 5% of women. A small prospective study of 45 women evaluating the psychological component of pregnancy loss was done after two first trimester miscarriages, with other causes eliminated. Self report questionnaires and interviews before their next pregnancy showed that 10 pregnancies (22.2%) resulted in a miscarriage. The degree of baseline depressive symptoms predicted the rate of miscarriage. (American society for Reproductive Medicine, 2012)

Recurrent pregnancy loss patients are prone to heightened anger, depression, anxiety and feelings of guilt and grief. (Miscarriage Association). The Miscarriage Association Patient Information Leaflet acknowledges that taking part in a clinical trial can be helpful, even if the treatment does not turn out to be effective. That “patients taking part in the trial tend to get additional care and

monitoring and there is some evidence that enhanced care can have a positive impact in reducing miscarriage rates”.

The hypothesis is that idiopathic recurrent miscarriage is due to a primary endometrial problem. The effects of endometrial scratch are proven in recurrent implantation failure and IVF patients. The application of this hypothesis necessitates the need to conduct the trial and evaluate the effects in recurrent miscarriage women. The intervention has not been done in this subset of women previously.

This study is aimed as a feasibility study. Pilot studies are the initial step in the setting up of a novel intervention. This is not a hypothesis testing process; however, it will guide as the early phase developmental function for a future successful RCT. The outcomes from this pilot study are to assess feasibility of the study, recruitment, enrolment and acceptability of the intervention. (Leon, Davis, & Kraemer, 2011)

This study is a pilot randomised study of endometrial scratch in the luteal phase and effects on pregnancy outcomes after two miscarriages. The pilot feasibility study will aim to recruit patients from the Early Pregnancy Unit at UHCW NHS trust, RM clinic and self- referral. The aims of the pilot study are to assess patient suitability, acceptability, and recruitment rate and pregnancy outcomes such as Live birth rate. The study outcome will be used as a guide to perform power calculation for a larger multicentre randomised controlled trial. The study will be open for patient recruitment for a year from Ethical approval and the data will be published when the outcomes from the last patient recruited to the study are known and completed.

### 1.3 Ethical considerations

Recruitment will commence only after a favourable ethical approval has been obtained.

There are no previous trials exploring the hypothesis whether endometrial scratch improves pregnancy outcomes in women with recurrent miscarriages. Current RCOG recommendations are for intervention only after three consecutive miscarriages.

The trial involves an intervention and control arm, both of which involve a transvaginal ultrasound, speculum examination. This is a common procedure carried out in different settings such as obtaining cervical smear, in early pregnancy bleeding, insertion of coils and obtaining swabs. Most women tolerate the speculum examination and the procedure is quick.

Women in the intervention arm will undergo the endometrial scratch.

Experience from the vast number of patients who underwent the scratch from the implantation clinic at UHCW NHS Trust, suggests that it is generally, a well tolerated procedure. The issue of pain is addressed by the advice to take analgesics 1-2 hours prior to the procedure and the availability of inhalational analgesia such as Entonox.

The patients in the control group will be subject to a sham procedure for the purpose of blinding, after the randomisation. This involves a transvaginal scan,

speculum examination and cleaning of the cervix with a cotton wool tip, dipped in saline. As the current practise is no intervention after two miscarriages, recruitment to the trial which involves an intervention and control arm is acceptable.

The trial will be conducted in full conformance with the principles of the Declaration of Helsinki and to ICH/MRC Good Clinical Practice (GCP) guidelines. It will also comply with Human Tissue Authority legislation and Warwick Standard Operating Procedures (SOPs). All data will be stored securely and held in accordance with Data Protection Act 1998.

## 1.4 CONSORT

The trial will be reported in line with the CONSORT (*Consolidated Standards of Reporting Trials*) statement (Lancet 2001, **357**: 1191-1194).

*Up-to-date information on CONSORT revisions, downloadable check lists and flow chart are available on the CONSORT web site: <http://www.consort-statement.org/>*

## 2. Trial Design

### 2.1 Trial summary and flow diagram

The trial is intended to be a pilot feasibility single centre study. The study will aim to recruit women from the EPAU clinic, self-referral and from recurrent miscarriage clinic. The patient will be given an invitation letter after the miscarriage. Patient will be sent out the information leaflet when she contacts the BRU. She will be seen for counselling after the miscarriage when it is suitable for her. Consent for participation in the study will be obtained after at least 24 hours of the receipt of the patient information sheet. The woman should have at least one normal cycle after the miscarriage, before the endometrial scratch. The woman will be counselled regarding home ovulation test kits and the process of using them. They will be provided with them at counselling with instructions for use. Increasing numbers of women do use the home ovulation kits and are familiar with the instructions.

Once the ovulation test is positive she will be required to attend for the endometrial scratch 7-10 days after the positive test. If the patient has tried ovulation test kits for 1 month without success, we aim to perform a transvaginal scan in the luteal phase of her cycle, 7-10 days prior to her expected period. If the endometrial thickness is greater than 5mm she will be randomised on the day and proceed with the study. The couple will be advised to use barrier contraception in the cycle of endometrial scratch and to try for a pregnancy from the next cycle. The evidence from IVF suggests that the outcomes are worse off when the scratch was performed on the day of oocyte retrieval. The procedure will be organised between 8am to 6pm from Monday to Friday. Visits can also be arranged on a Saturday if it is convenient for the patient.

At the appointment, written consent for the procedure and the use of the endometrial tissue for research projects will be obtained. The patient will

undergo a transvaginal ultrasound scan to assess the uterine cavity and endometrial thickness. The patient will need to have an empty bladder before the procedure. The procedure involves insertion of a medium size Cusco's speculum and visualisation of the cervix. The cervix will be cleaned with a cotton tip dipped in saline. The endometrial scratch will be performed with a Wallach sampler. The sampler will be inserted through the cervix until the resistance is felt at the level of the fundus of the uterus. The sampler will be rotated and moved up and down the uterine cavity for 10- 20 seconds, to obtain the endometrial biopsy. The endometrial sample is obtained by manual suction, provided by elastometric seal piston plunger. The patient will experience cramps due to uterine contractions. The patient will be advised to have 1gm paracetamol and/or 400mg ibuprofen 1-2 hours prior to the procedure. The patient may experience bleeding which may be spotting or bleeding like a period after the procedure. In some women additional interventions such as use of plastic cervical dilators or plastic vosellum to hold the cervix may be necessary. This is particularly possible in patients who have a retroverted uterus, acutely flexed uterus or previous treatment on the cervix. Entonox will be available to breathe through a disposable mouthpiece, for analgesia. The procedure will be performed by the co-investigators and in the presence of a chaperone.

The control group of women will undergo the transvaginal scan, speculum examination with cleaning of the cervix but no endometrial scratch. The patients in both the control and intervention group will be advised regarding oral analgesics and the availability of Entonox.

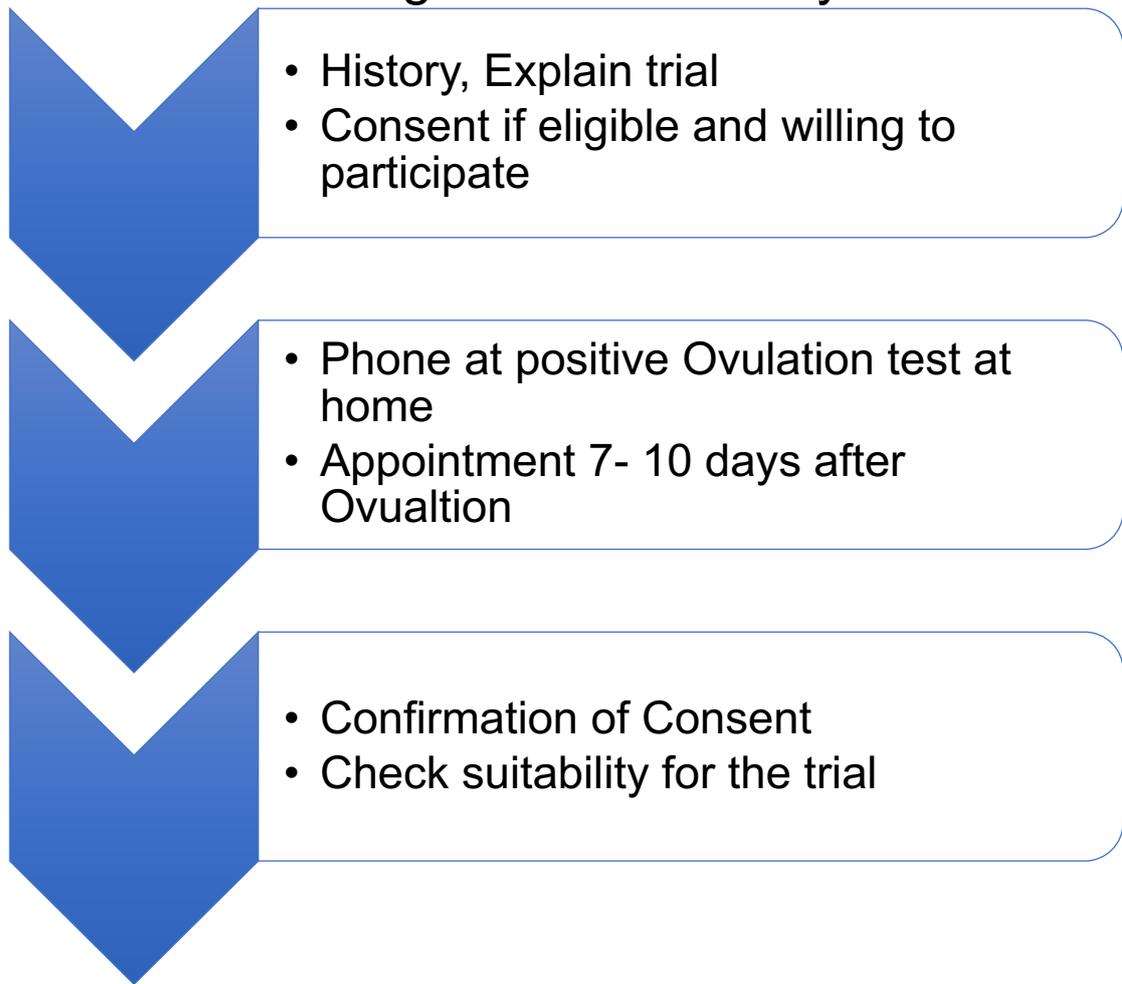
At the end of the appointment the woman will be given a questionnaire along with a self-addressed envelope. She will be requested to complete the form within 4weeks of the intervention, preferably not on the day of the procedure. This is to evaluate the patient's acceptability of the trial and feedback from the appointment. The data will be analysed at the end of the trial.

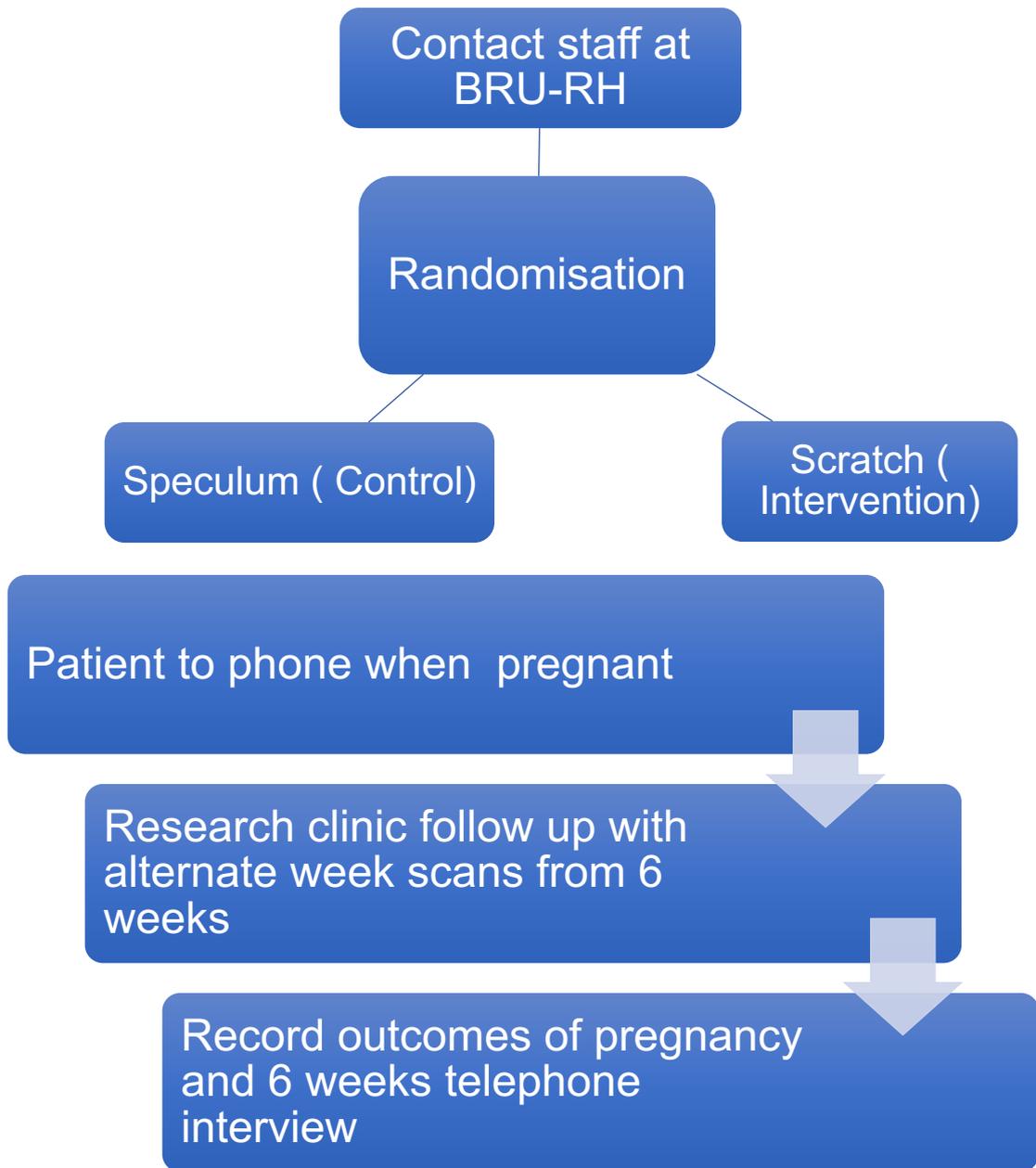
Consent will be obtained to access the pregnancy outcomes of the patients when they deliver.

They will also be consented for a telephone interview 6 weeks after her delivery.

.  
Figure

## Flow diagram of SiM Study





3.

### 3.1 Aims and objectives

#### 3.1.1 Primary objective

To assess whether endometrial scratch prevents miscarriage in women with recurrent miscarriages.

#### 3.1.2 Secondary objective

To accumulate a tissue bank of samples from women with recurrent miscarriages and pregnancy outcomes.

### 3.2 Outcome measures

#### The primary outcome measure is:

- Live birth rate after 24 weeks of gestation.

#### The secondary outcome measures are:

- Miscarriage until 23+6 weeks of pregnancy, gestation at miscarriage and the type of miscarriage (Biochemical pregnancy, delayed miscarriage, second trimester loss)
- Ectopic pregnancy
- Pregnancy complications such as SGA, PET, abruption, placenta praevia, placenta accreta, preterm delivery
- Acceptability of the intervention

.

#### 3.2.1 Efficacy

The study primary outcome (live birth rate) will be used as a guide to perform power calculation for a larger multicentre randomised controlled trial.

The study will be open for patient recruitment for a year from Ethical approval and the data will be published when the outcomes from the last patient recruited to the study are known and completed.

Secondary outcomes will be obtained through hospital notes and telephone interview.

#### 3.2.2 Safety

Adverse events and SUSAR's will be reported to the sponsor and the UHCW NHS Trust Research Development and Innovation department.

### 3.3 Eligibility criteria

Patients are eligible to be included in the trial if they meet the following criteria:

#### 3.3.1 Inclusion criteria

1. Provision of written informed consent

2. Women aged 18-42 years after two idiopathic miscarriages
3. Actively trying for a further pregnancy

### 3.3.2 Exclusion criteria

1. No active treatment during the pregnancy
2. Inherited or acquired thrombophilia
3. Medical conditions- diabetes, hypertension, thyroid disorders
4. Inability to tolerate internal examinations
5. Uterine anomalies
6. Previous entry or randomisation in the present trial.

## 2.5 Informed consent

Participation in the study will be entirely voluntary. We aim to consent women after they have had at least 24 hours to read the patient information leaflet and consider the information.

Consent will be obtained at the initial visit if they are eligible, in the consent form as per Warwick CTU specifications. The consent will be obtained by the investigators with the initials of the woman. Three copies will be obtained, one each for the CRF, Hospital notes and the patient. The consent will include disclosure of information to GP, access to hospital records and contact by telephone for interview, 6- 8 weeks after delivery. The GP letter will be posted at recruitment.

A paper UHCW NHS Trust consent will be obtained and filed in the CRF and hospital notes on the day of intervention. This will include the risks and benefits of the procedure and the use of endometrial tissue obtained for further research projects.

## 3.6 Recruitment and randomisation

### 3.6.1 Recruitment

We aim to recruit women from the Early pregnancy assessment Unit at UHCW NHS Trust. Women who have had a diagnosis of miscarriage will be offered the invitation letter regarding the study. Interested women will be sent a patient information sheet, when they contact the BRU- RH.

Women attending the recurrent miscarriage clinic will be offered the trial and information leaflet will be given.

Information about the trial will be made available on the local BRU-RH website. Social media such as Twitter and UHCW Facebook account will be used to advertise the trial. We aim to advertise for the trial in a joint poster with ALIFE 2 trial, on the Tommy's website. This will enable women to self-refer for the trial. All advertising material will be sent to the ethics committee for approval prior to use.

Eligibility for participation in the trial will be assessed at the first visit and the criteria will be checked. Consent for participation will be confirmed on the day of the visit.

We aim to recruit 100 patients over a period of 9- 12 months. The recruitment will continue for the duration even if the required numbers are achieved.

### 3.6.2 Randomisation

The patients will be randomised to the intervention and control arm.

The randomisation will be through a sealed envelope with random numbers picked up by a member of staff at BRU-RH. The patient details will then be entered in a password protected Microsoft excel sheet. The patients will be randomised from Monday to Friday from 8am to 6pm. Provisions for randomisation and undertaking procedures will be made for Saturday, if patient wishes to. The BRU- RH is open on Saturday with availability of staff for randomisation and chaperone.

The patient's participation in the trial will be documented in the hospital notes along with a copy of the consent form and Patient information leaflet.

#### 2.6.2.1 Post-randomisation withdrawals and exclusions

Participants may be discontinued from the trial treatment and/or the trial at any time without prejudice. Unless a participant explicitly withdraws their consent, they should be followed-up wherever possible and data collected as per the protocol until the end of the trial.

Participation in the study is voluntary and the patient has the option of withdrawal from the study at any time. The reason for the withdrawal will be collected and documented. As there are no medicinal products involved in the trial, withdrawal by the investigator is unlikely.

When the patient withdraws from the study she will be offered scan at 6 weeks of her next pregnancy, if the patient wishes.

Participants may be withdrawn from the trial at the discretion of the investigator and/or Trial Steering Committee due to safety concerns.

## 3.7 Trial treatments / intervention

### 3.7.1 Trial treatment(s) / intervention

At the appointment, written consent for the procedure and the use of the endometrial tissue for research projects will be obtained.

The patient will undergo a transvaginal ultrasound scan to assess the uterine cavity and endometrial thickness. The patient will need to have an empty bladder before the procedure. The procedure involves insertion of a medium size Cusco's speculum and visualisation of the cervix. The cervix will be cleaned with a saline dipped cotton tip. The endometrial scratch will be performed with a Wallach sampler. The sampler will be inserted through the cervix until the resistance is felt at the level of the fundus of the uterus. The sampler will be rotated and moved up and down the uterine cavity for 10- 20 seconds, to obtain the endometrial biopsy. The endometrial sample is obtained by manual suction, provided by elastometric seal piston plunger.

The patient will experience cramps due to uterine contractions. The patient will be advised to have 1gm paracetamol and/or 400mg ibuprofen 1-2 hours prior to the procedure. The patient may experience bleeding which may be spotting or bleeding like a period after the procedure.

In some women additional interventions such as use of plastic cervical dilators or plastic vosellum to hold the cervix may be necessary. This is particularly possible in patients who have a retroverted uterus, acutely flexed uterus or previous treatment on the cervix. Entonox will be available to breathe through a disposable mouthpiece, for analgesia. The procedure will be performed by the co-investigators and in the presence of a chaperone.

The control group of women will undergo the transvaginal scan, speculum examination with cleaning of the cervix but no endometrial scratch.

If we are unable to enter the uterine cavity and hence not perform the endometrial scratch, we will record this and will still monitor the outcome of her subsequent pregnancy.

### 3.7.2 Control intervention

The control group of women will undergo the transvaginal scan, speculum examination with cleaning of the cervix and no endometrial scratch.

### 3.7.3 Drug storage and dispensing

Not applicable

### 3.7.4 Drug accountability

Not applicable

### 3.7.5 Compliance/contamination

The women will be offered instructions on the use of the home ovulation test kits and how to read positive results. They should ring the BRU for an appointment on the day of positive ovulation test. If it happens over the weekend they can contact us by email, to the co-investigators or leave a message on an answering machine of the BRU secretary Kerri Gerraghty. The appointment will be scheduled between 7- 10 days after the positive ovulation test or the expected date of her next period. The couple will be instructed to use barrier contraception in the cycle of the scratch and not to try for a pregnancy.

If the couple have had unprotected intercourse, the procedure will be cancelled. Compliance monitoring is not applicable.

### **3.8 Blinding**

#### **3.8.1 Methods for ensuring blinding**

The patient will be blind to the allocated group. The outcome assessor (BRU research midwives) will also be blinded. However, the investigators will be aware of the allocated group.

#### **3.8.2 Methods for unblinding the trial**

It is unlikely that unblinding would be necessary, however in the event of complications in the week after the biopsy, unblinding would be available. The group allocation will be available in the files in the BRU as a security pass access and locked office and cupboard. However, the investigator team are able to access the BRU at any time in a 24 hour period.

### **3.9 Concomitant illness and medication**

#### **3.9.1 Concomitant illness**

Not applicable as these patients would be excluded.

#### **3.9.2 Concomitant medication**

Not applicable as these patients would be excluded.

### **3.10 End of trial**

The trial will end after the pregnancy outcomes of all the trial participants are known and recorded. This includes the 6 weeks telephone follow up for the patients who have had a successful pregnancy outcome.

The trial will be stopped prematurely if:

- Mandated by the Ethics Committee
- Mandated by the trial steering committee following the review of AE's and SUSAR's.
- Funding for the trial ceases

The Research Ethics Committee will be notified in writing if the trial has been concluded or terminated early.

If the patient has another miscarriage during the trial, she will be offered referral to the recurrent miscarriage clinic with Professor Quenby. The patients in the control arm will be offered the option of endometrial scratch outside the research setting. This will be arranged through the recurrent miscarriage clinic. The participants in the trial will be informed of the results of the trial on conclusion of the study.

## 4. METHods and assessments

### 4.1 Schedule of delivery of intervention and data collection

Visit	1	2	3	4
<b>Visit Window</b> (No. Weeks $\pm$ No. Days)			3m ( $\pm$ 1 m) after V 2	1m ( $\pm$ 2 wks) after V3
Informed consent		✓		
Issue of ovulation test kits		✓		
Medical History	✓			
Screening Urinalysis BP measurement	✓			
Inclusion/exclusion criteria	✓	✓		
Intervention			✓	
Questionnaire				✓

#### **When Patient is pregnant**

Visit	1	2	3	4	5
<b>Visit Window</b> (No. Weeks $\pm$ No. Days)	6W(+/- 1W)	8 w ( $\pm$ 1w) After V 1	10 W ( $\pm$ 1 w) After V2	8-9m ( $\pm$ 2w) After V 3	6- 8wks ( $\pm$ 1w) After V 4
Ultrasound	✓	✓	✓		
Outcomes				✓	✓
Questionnaire					✓

### 4.2 Laboratory assessments

Consent will be obtained regarding the use of the endometrial sample for future research projects. If the patient does not consent to the process, it will be discarded as per the UHCW NHS Trust policy on disposal of biological waste. If the patient consents to the use of the material, it will be stored at the UHCW Arden tissue bank for 10 years. The samples will be anonymised with no identifiable patient information. The patient can withdraw consent at any time and the sample will be disposed as per policy.

## 5. adverse event management

### 5.1 Definitions

#### 5.1.1 Adverse Events (AE)

An Adverse Event (AE) is defined as any untoward medical occurrence in a participant and which does not necessarily have a causal relationship with this treatment/intervention.

During the scratch- pain, bleeding, and feeling faint are expected outcomes and will not be reported as adverse events.

The expected outcomes with pregnancy are:

Nausea, vomiting

Pelvic discomfort

Early pregnancy spotting

Dizziness

Swelling of both legs in the third trimester, without pain

Stretch marks in the abdomen

If the patient had been admitted for hyperemesis or evaluation of the above symptoms they should not be reported as adverse events.

Any deviation from the normal expected course of pregnancy or admission for any other reasons will be reported as adverse events.

#### 5.1.2 Adverse Reaction (AR)

Not Applicable

#### 5.1.3 Serious Adverse Events (SAEs) and Suspected Unexpected Serious Adverse Events (SUSARS)

A Serious Adverse Event is an AE that fulfils one or more of the following criteria:

Results in death

Is immediately life-threatening

Requires hospitalisation or prolongation of existing hospitalisation

Results in persistent or significant disability or incapacity

Is a congenital abnormality or birth defect

Is an important medical condition.

Suspected Unexpected Serious Adverse Reactions (SUSARs) are SAEs that are considered to be related to the administration of the trial drug and are also unexpected i.e. their nature or severity is not consistent with the IB/Summary of Product Characteristics.

### 5.2 Reporting SAEs and SUSARs

*Describe the system for assessing seriousness, causality & expectedness and who will be responsible for review and assessment.*

*State safety reporting period* - All SAEs or SUSARs that occur between X e.g. date of *consent* and Y e.g. *end point (often 30 days after the end of the trial drug/intervention)* will be reported. SAEs and SUSARs will be reported using the SAE form in the participant's CRF. The Principal Investigator in each centre must report any SAEs and <<SUSARs/related and unexpected SAEs>> to the trial coordinating centre within 24 hours of them becoming aware of the event. The SAE form should be completed and faxed to the dedicated fax at Warwick CTU: 02476 150549. The trial coordinator will liaise with the investigator to compile all the necessary information. The trial coordinating centre is responsible for reporting serious adverse events to the sponsor, REC and MHRA within required timelines. The causality of SAEs (i.e. relationship to trial treatment) will be assessed by the investigator(s) on the SAE form.

<b>Relationship to trial medication</b>	<b>Description</b>
Unrelated	There is no evidence of any causal relationship
Unlikely to be related	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication or device). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).
Possible relationship	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication or device). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
Probable relationship	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely related	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

*State which of the criteria in the table above constitutes a relationship e.g. events which are possibly, probably or definitely related will be reported immediately.*

*For CTIMPs, state which document(s) (IB/SMPC) is/are to be used to assess expectedness of events.*

*State type & duration of follow-up of participants after adverse events.*

### **5.3 Procedures in case of overdose**

Not applicable

### **5.4 Procedures in case of pregnancy**

Pregnancy and the outcomes are the primary endpoints in this study. When the pregnancy test is positive, she will be offered an early scan at 6 weeks of gestation. This is calculated from her last menstrual period or 2 weeks after the first positive pregnancy test. Further follow up scans will be arranged at 2-3 weekly intervals for reassurance until the dating scan at 11- 13 weeks of pregnancy. Additional scans will be accommodated if the patient experiences symptoms such as pain, bleeding or inconclusive scan. The pregnancy

outcomes will be collected and presented with the data. This will include live births after 24 weeks, miscarriage, termination of pregnancy and stillbirth. The scans will be undertaken as part of the care package available for women who attend the recurrent miscarriage clinic.

All reports of congenital abnormalities/birth defects must be reported and followed up as a SAE.

## 6. Data management

Personal data collected during the trial will be handled and stored in accordance with the 1998 Data Protection Act. The data from the case report forms and will also be entered on a secure database for the analysis of the study data. The patient will be assigned a unique study number and there will be no patient identifiable information on the CRFs. The data will be stored on the University of Warwick secure Drive.

The data collection and storage will adhere to the University of Warwick Research Data and Management Policy and Information Security Framework. The data collection and storage will also be in line with the UHCW NHS Trust policy.

### 6.1 Data collection and management

The Case Report Forms (CRFs) will be developed to collect all required trial data.

Case report forms will be completed for each patient by the chief investigators. Case report form will be filled for each patient and securely saved for 10 years at the BRU suite in a locked storage. The patient questionnaire will be given after the procedure, for completion and return within 4 weeks. The consent will include the access of the hospital notes and telephone interviews, for the outcomes of the pregnancy. All patients will be contacted for a telephone consultation 6 weeks after delivery of the baby.

This way all the patients who have not delivered in the UHCW NHS Trust will be followed up and data collected for reporting.

The data from the case report forms will also be entered on a secure database for the analysis of the study data. The patient will be assigned a unique study number and there will be no patient identifiable information on the CRFs.

The data collection and storage will adhere to the University of Warwick Research Data and Management Policy and Information Security Framework.

### 6.2 Database

The database will be maintained by the trial co-ordinator Dr Kandavel. Data entry checks will be made by Dr Lokman. The BRU research midwives will collect pregnancy outcome data so will be blind to the treatment allocation group.

The database will be an excel spread sheet with the data entered for the following measures:

- demographic details
- group allocation
- outcomes of the pregnancy- miscarriage, live birth
- Pregnancy complications
- gestation at delivery, weight of baby
- 6-8 weeks telephone follow up for neonatal health
- reasons for discontinuation in trial or lost for follow up

This will be kept on the University secure drive only.

### 6.3 Data storage

All essential documentation and trial records will be stored by BRU- RH at UHCW

in conformance with the applicable regulatory requirements and access to stored information will be restricted to authorised personnel.

### 6.4 Data access and quality assurance

The patient will be assigned a unique study number and will be entered on the database. The consent will involve the initials and a copy will be kept in her CRF. A further copy will be filed in the main hospital notes, in case of emergency admissions.

The database will be password protected and will only be accessed by the Chief investigator and the co- investigators on a University of Warwick secure drive only. Consent will be obtained for sharing of the information with the GP and acted upon accordingly.

### 6.5 Archiving

Trial documentation and data will be archived for at least ten years after completion of the trial as per Warwick Research Data Management policy.

## 7. Statistical analysis

### 7.1 Power and sample size

The sample size is determined by adequacy to provide sufficient power in comparing efficacy between the control and intervention groups and adequacy to provide precise estimates for consent rate and acceptability rate. We will

allocate 50 patients to each group (100 in total). Because this is a pilot/feasibility study, we will test the null hypothesis of equal birth rates at two-sided 20% significance level [Schoenfeld, 1980]. Assuming that the birth rate in the control is 60% and that the scratch improves birth rate by 20% to 80%, the power to detect this difference for a study with a sample size of 100 (50 in each group) at 20% significance is 82%.

We anticipate that we will identify 9 eligible women every month and we will recruit for 12 months so that in total 108 women are invited to join the study. Of the 108 women, we expect 100 women to consent to enrol in the study, which corresponds to a consent rate of 92.6%. With 108 women, if the consent rate is 92.6%, the 95% confidence interval for the consent rate is 87.6% to 97.5%, which is sufficiently precise for a feasibility study.

We will use the 50 women enrolled in intervention arm to assess the acceptability rate for the scratch intervention. We believe that the scratch will be acceptable to 95% of the women. If the acceptability rate is 95%, with 50 women, the 95% confidence interval is 89% to 100%, which is sufficiently precise for a feasibility study.

## 7.2 Statistical analysis of efficacy and harms

The statistical analysis will assess efficacy, consent rate and acceptability rate for the scratch (intervention).

To assess efficacy, we will compare the birth rates for the scratch (intervention) and speculum (control) arms. We will report the birth rates for each group and compare them using a chi-squared test. It will be considered worthwhile, to conduct a larger study if the p-value is less than 0.2. A larger cut-off for p-value is chosen because it is a trial to consider whether it is worthwhile to conduct a larger study in which type I error rate will be controlled at a lower rate [Schoenfeld, 1980].

We will assess the consent rate by reporting the proportion (and the 95% confidence interval) of eligible women who consent to be enrolled in the study. To assess acceptability rate, we will only use the women recruited into the intervention arm. We will report the proportion (and the 95% confidence interval) for the women who consider the scratch procedure (intervention) acceptable.

## 7.3 Health Economic Evaluation

Not Applicable

## 8. Trial organisation and oversight

### 8.1 Sponsor and governance arrangements

The primary sponsor for the study will be the University of Warwick and the Warwick CTU SOP standards will be followed.

### 8.2 Regulatory authorities/ethical approval

THE IRAS application form with REC will be submitted and the trial will commence recruitment after a favourable ethical approval is obtained. The UHCW NHS Trust Research, Development and Innovation department approval will be obtained prior to enrolment of participants to the trial.

### 8.3 Trial Registration

The trial will be registered on the Clinical Trials.gov. This is a registry and results database of publicly and privately supported clinical studies of human participants conducted around the world.

### 8.4 Indemnity

NHS indemnity covers NHS staff, medical academic staff with honorary contracts, and those conducting the trial. NHS bodies carry this risk themselves or spread it through the Clinical Negligence Scheme for Trusts, which provides unlimited cover for this risk. The University of Warwick provides indemnity for any harm caused to participants by the design of the research protocol.

### 8.5 Trial timetable and milestones

	Month	Recruitment
Set-up	April- Aug 15	n/a
Pilot study	Sep 15- Aug 16	100
Follow up	Oct 15- June 17	n/a
Analysis	June 17- Dec 17	n/a

### 8.6 Administration

The trial co-ordination will be based at WCTU, University of Warwick.

### 8.7 Trial Management Group (TMG)

The Trial Management Group, consisting of the project staff and co-investigators involved in the day-to-day running of the trial, will meet regularly throughout the project. Significant issues arising from management meetings will be referred to the Trial Steering Committee or Investigators, as appropriate.

## 8.8 Trial Steering Committee (TSC)

The trial will be guided by a group of respected and experienced personnel and trialists as well as at least one 'lay' representative. The TSC will have an independent Chairperson. Face to face meetings will be held at regular intervals determined by need but not less than once a year. Routine business is conducted by email, post or teleconferencing.

The Steering Committee, in the development of this protocol and throughout the trial will take responsibility for:

- Major decisions such as a need to change the protocol for any reason
- Monitoring and supervising the progress of the trial
- Reviewing relevant information from other sources
- Considering recommendations from the DMC
- Informing and advising on all aspects of the trial

The membership of the TSC will be determined in the future.

## 8.9 Data Monitoring Committee (DMC)

Not applicable as this is a pilot trial.

## 8.10 Essential Documentation

A Trial Master File will be set up according to WCTU SOP and held securely at the coordinating centre.

# 9. Monitoring and Quality assurance of trial procedures

The University of Warwick and UHCW will audit the quality of data collection and adherence to GCP.

*Detail what types of monitoring will take place. Provide assurance on GCP, quality of data collection. (Refer to monitors and their role – if appropriate).*

*Detail frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor.*

*Consider potential 'triggers' for on-site monitoring visits (e.g. sites persistently late in reporting SAEs, receipt of multiple late/poorly completed CRFs from a site).*

*Sponsor to ensure investigator(s)/institutions will permit trial-related monitoring, audits, REC review and regulatory inspections, providing direct access to source data/documents.*

*For complex interventions include methods used to ensure consistency and delivery across sites and over time and how they will be monitored.*

## 10. Patient and Public Involvement (PPI)

### **Analysis of patient opinion on ‘Endometrial Scratch test’**

10 women with recurrent miscarriage agreed to take part in this questionnaire on patient opinion. These opinions were collated over a period of a month in a clinic setting.

The main focus of this opinion poll was to determine whether women find it acceptable for patient’s to undergo a speculum examination without the scratch test as part of a single blinded trial.

9 out of 10 women feel that this is acceptable. 3 women left comments as below:

1. *‘As long as full communication and consent is given.’*
2. *‘Because most of women they do not know the cause of miscarriage, so research might help to find out.’*
3. *‘With plenty of information given and support for both male and female.’*

1 patient felt it was unacceptable to offer only a speculum examination in a single-blinded trial as the ‘placebo group’. This patient’s comment was as follows:

*‘Because it is about trying every treatment for pregnancy to happen.’*

## 11. Dissemination and publication

The results of the trial will be reported first to trial collaborators. The main report will be drafted by the trial co-ordinating team, and the final version will be agreed by the Trial Steering Committee before submission for publication, on behalf of the collaboration.

The success of the trial depends on the collaboration of doctors, nurses and researchers from across the UK. Equal credit will be given to those who have wholeheartedly collaborated in the trial.

The trial will be reported in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines ([www.consort-statement.org](http://www.consort-statement.org)).

*Describe the plans for investigators and sponsor to communicate trial results to participants, health care professionals, the public and other relevant groups (e.g. via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions. State authorship eligibility guidelines and any intended use of professional writers.*

*Detail plans, if any, for granting public access to full protocol, participant level data set, and/or statistical code. (Baum, et al., 2012)*

## 12. References

1. American society for Reproductive Medicine. (2012). Evaluation and treatment of recurrent pregnancy loss: A committee opinion. *Fertility and Sterility* , 1103-11.
2. Baum, M., Yerusalmi, G., Maman, E., Kedem, A., Machtinger, R., Hourvitz, A., et al. (2012, dec). Does local injury to the endometrium before ivf cycle really affect treatment outcome? results of a randomized placebo controlled trial. *Gynecol Endocrinol* , 933-6.
3. Williams Obstetrics. In G. Cunningham, K. J. Leveno, S. L. Bloom, C. Y. Spong, J. S. Dashe, B. S. Hoffman, et al., *Williams Obstetrics* (pp. 86-95). Mcgrwa-Hill Education,2014.
4. D, S. (1980). Statistical consideration for pilot studies. *International Journal of Radiation Oncology, Biology and Physics* , 371-374.
5. Dain, L., Ojha, K., Bider, D., Levron, J., Zinchenko, V., Walster, S., et al. (2014). Effect of local endometrial injury on pregnancy outcomes in ovum donation cycles. *Fert Steril* , 1048-54.
6. El-Toukhy, T., Sunkara, S., & Khalaf, Y. (2012). Local Endometrial injury and IVF outcome: a systematic review and meta-analysis. *reprod biomed Online* , 25 (4), 345-54.
7. Fatemi, H., & Popovic-Todorovic, B. (2013). Implantation in assisted reproduction:a look at endometrial receptivity. *Reprod Biomed Online* , 27 (5), 530-8.
8. Gibreel, A., Badaway, A., El-Refai, W., & El-Adawi, N. (2013). Endometrial scratching to improve pregnancy rate in couples with unexplained subfertility:a randomized placebo controlled trial. *J Obstet Gynaecol Res* , 933-6.
9. Gnainsky, Y., Granot, I., Aldo, P., Barash, A., Or, Y., Schechtman, E., et al. (2010). Local injury of the endometrium induces an inflammatory response that promotes successful implantation. *Fertil Steril* , 2030-6.
10. Granot, I., Gnainsky, Y., & Dekel, N. (2012). Endometrial Inflammation and effect on implantation improvement and pregnancy outcome. *Reproduction* , 661-8.
11. Granot, I., Gnainsky, Y., & Dekel, N. (2013). Implantation in assisted reproduction:a look at endometrial receptivity. *reprod Biomed Online* , 530-8.
12. Junovich, G., Mayer, Y., Azpiroz, A., Daher, S., Iglesias, A., Zylverstein, C., et al. (2011). Ovarian stimulation affects the levels of regulatory endometrial NK cells and angiogenic cytokine VEGF. *AM J Reprod Immunol* , 146-53.
13. Karimzade, M., Oskouian, H., Ahmadi, S., & Oskouian, L. (2010). Local injury to the endometrium on the day of oocyte retrieval has a negative impact on implantation in assisted reproductive cycles: a randomized controlled trial. *Archives of gynecology and Obstetrics* , 499-503.

14. Kumbhak, B., Sahin, L., Ozkan, S., & Atilgan, R. (2014). Impact of luteal phase hysteroscopy and concurrent endometrial biopsy on subsequent IVF cycle outcome. *Arch Gynecol Obstet* , 369-74.
15. Leon, A. C., Davis, L. L., & Kraemer, H. C. (2011). The Role and Intepretation of Pilot Studies in Clinical research . *J Psychiatr Res* , 629-629.
16. Nastri , C., Gibreel, A., Raine-Fenning, N., Maheshwari, A., Ferriani, R., Bhattacharya, S., et al. (July 2012). *Endometrial injury in women undergoing assisted reproductive techniques*. Cochrane Database Syst Rev.
17. Nastri, C., Ferriani, R., Raine-Fenning, N., & Martins, W. (2013). Endoemtrial scratching performed in the non-transfer cycle and outcome of assisted reproduction;a randomized controlled trial. *Ultrasound Obstet Gynecol* , 375-82.
18. Nastri, C., Larsen, S., Gibreel, A., Raine- Fenning, N., Ferriani, R., Bhattacharya, S., et al. (2015, march). *Endometrial Injury in women undergoing assisted reproductive techniques*. From Cochrane Database of Systematic reviews.
19. *NICE Ectopic Pregnancy and Miscarriage Guideline( CG 154)*. (2012, December). From NICE.
20. Potdar, N., Gelbaya, T., & Nardo, L. (2012, Dec). Endometrial Injury to overcome recurrent embryo implantation failure: a systematic review and meta-analysis. *Reprod Biomed Online* , 561-71.
21. Rai, L., & Regan, L. (2006). recurrent Miscarriage. *Lancet* , 601-11.
22. *Recurrent Miscarriage, Investigation and Treatment of Couples( Green-top Guideline No.17)*. (2011, may 19). From RCOG:  
<https://www.rcog.org.uk/en/guidelines-research-services/guidelines/gtg17/>
23. Shohayeb, A., & El-Khayat, W. (2012). Does a single endometrial biopsy regimen (S-EBR) improve ICSI outcome in patients with repeated implantation failure? A randomised controlled trial. *European Journal of Obstetrics & Gynecology and Reproductive Biology* , 176-9.
24. Siristatidis, C., Vrachnis, N., Vogiatzi, P., Chrelias, C., Retamar, A., Bettocchi, S., et al. (2014). Potential Pathophysiological Mechanisms of the Beneficial Role of Endometrial Injury in In Vitro Fertilization Outcome . *Reprod Sci* , 966-965.
25. Tiboni, G., Giampietro, F., Gabriele, E., Di Donato, V., & Impicciatore, G. (2011). imapct of a single endometrial injury on assisted reproductive techonology outcome:a preliminary observation study. *J Reprod Med* , 504-6.
26. Yeung, T., Chai, J., Li, R., Lee, V., Ho, P., & Ng, E. (2014). The effect of endometrial injury on ongoing pregnancy rate in unselected subfertile women undergoing in vitro fertilization:a randomized controlled trial. *Hum Reprod* , 2474-81.

27. Hospital Episode Statistics: Maternity Statistics. (2012- 2013).
28. Retrieved from Miscarriage Association.
29. Retrieved from Tommys Charity.
30. Retrieved from NHS Choices.

### **Appendix 3:**

Patient Questionnaire:

#### **SiM Study (Scratch in Miscarriage)**

#### **Pilot randomised controlled trial of the effect of endometrial scratch in recurrent miscarriage on pregnancy outcomes**

Name of Researchers: Prof. S Quenby, Dr. V Kandavel

Patient Name:

Date of completion of questionnaire: DD/MM/YYYY

Date of procedure: DD/MM/YYYY

We would like to thank you for your participation in the study.

We value your opinion and would be grateful if you could complete the questionnaire.

This will provide us with feedback and help us to improve our services.

Please fill in the questionnaire when your cycle starts after the procedure and post it to us in the self-addressed envelope within 4 weeks.

1. How did you find out about the study?
2. Was the patient information sheet helpful?
3. Did you encounter any difficulties in booking the appointment?
4. Please describe in your words, the experience of participating in the study.
5. What do you think went well for you?
6. What do you think we could improve on?
7. Did you find the staff helpful during the study?
8. Are there any other comments that you wish to make?
9. Please complete the following symptom questionnaire

- a. Bleeding: Yes/No, if so describe as below (tick/circle the applicable box)
  - Spotting:  Less/ like/more, than a period
- b. Infection: Yes/No, if so did you need treatment
- c. Pain: Yes/No, if yes, describe as below (tick the applicable box)
  - Pain score: 1- mild  2- moderate  severe
  - Duration of pain: 1- 10 minutes  11 mins to 1hour  > 1 hour
- d. Did you take any painkillers before the procedure: Yes/No?
- e. Any other symptoms that you experienced?

10. If you wish us to contact you to discuss regarding the feedback, please provide the time, date and number to contact you.

## Appendix 4

Adverse events form for SiM study- Scratch in Miscarriage study

Trial ID:                      Participant Initials:                      DOB:

### Event date:

1. **Event:** (select from the following and circle the choices)

- Heavy pv bleeding requiring admission
- Admission with pain in early pregnancy
- Threatened preterm labour
- Pre-eclampsia
- Abruption
- Preterm delivery
- Fetal growth restriction
- IUD
- Stillbirth
- Unexpected admission to neonatal unit
- Major congenital abnormalities

2. **Severity assessment:**

- Mild - Does not interfere with the patients usual functioning
- Moderate – Interferes to some extent with patients usual functioning
- Severe- Interferes significantly with patients usual functioning
- Fatal/life threatening - Causes death or risk of death, organ damage or disability

3. **Causality:** In the opinion of the reporting clinician

- Was the event related to the trial intervention?

definitely <input type="checkbox"/>	probably <input type="checkbox"/>	po <input type="checkbox"/> ibly	<input type="checkbox"/> hlikely	<input type="checkbox"/> unrelated
-------------------------------------	-----------------------------------	----------------------------------	----------------------------------	------------------------------------

Clinician initials: \_\_\_\_\_

4. **Outcome of event:** (please select one only)

1. Resolved

Date of Resolution:

- 2. Ongoing
- 3. Death

5 Form completed by (print name):

Signature:

Date signed:

## Appendix 5

### Patient questionnaire for assessment of 'sham' procedure

1. Do you consent to participate in the filling of the questionnaire?

2. Have you had a speculum examination before?

if so, the what was the reason?

is it painful?

if so, score it from 1-10

is it uncomfortable?

is it acceptable?

3. Would you accept a speculum examination in a research study in a 'placebo group'?

4. Please add your suggestions to improve your experience of a speculum examination.

## Appendix 6

Joint poster

Tommy's Centre for Early Pregnancy Research @ University Hospital, Coventry

[Can you help us find answers to prevent recurrent miscarriage?](#)

The team at the Tommy's centre for early pregnancy research are asking women who have suffered miscarriages to join a research trial. We are trying to understand the reasons for and improve treatment for women with recurrent miscarriage.

[Can you answer yes to the following questions?](#)

1. Have you had two or more miscarriages?
2. Are you aged 18 to 42 years?
3. Are you able to travel to University Hospital, Coventry?
4. Are you trying for a baby but are not pregnant?

If you can answer yes to all of the above you may be able to take part in one of the following trials:

### **SiM:**

Assessing whether scratching the lining of the womb prevents miscarriage.



Assessing whether anti-coagulant (blood thinning) treatment reduces the risk of miscarriage in women with inherited thrombophilia. Thrombophilia is when your blood has a tendency to clot more than normal.

If you were to take part in one of these trials you will be randomly assigned to receive either standard care or standard care plus the trial treatment/intervention.

[If you would like more information:](#)

Please ring **02476 964 983** to speak to the Clinic Secretary about your interest in this research. Your eligibility for the study will be assessed and if you are eligible you will be given further information about how to take part.

## Appendix 7

### Letter of invitation for SiM Study

Title of Study: Scratch in Miscarriage Study (SiM Study)

Chief Investigator: Prof. Siobhan Quenby **B.Sc.**, MBBS, MD, FRCOG

I, Dr. Valarmathy Kandavel, Clinical Research Fellow of Prof. Quenby at the Biomedical research unit from the Department of Obstetrics and Gynaecology at UHCW NHS Trust, invite you to participate in a research project titled SiM Study.

The aim of this study is to evaluate if the scratch of the lining of the womb improves the pregnancy outcomes in women who have suffered from recurrent miscarriages. This is a randomized control trial, which means that you may be either in the intervention group or the control group. Should you choose to participate, you will be asked to contact the secretary at the biomedical research unit on ( [REDACTED] ) for a Patient Information leaflet.

If you are interested, you will need to attend for a screening visit during which history, examination and consent for participation in the trial will be done. If you are eligible and you consent to participate in the study, you will be expected to do home based ovulation testing. You should ring when the ovulation test is positive and you will be offered appointment 7-10 days after the positive test. If randomized to the intervention group, you will have a scan, speculum and endometrial scratch. The scratch involves obtaining a biopsy of the lining of the womb, using a fine plastic catheter. The control group would have an internal scan and speculum only. You will be given a questionnaire with a self-addressed envelope to be completed and returned within 4 weeks of your appointment.

If you become pregnant, contact us after your positive pregnancy test, to book for early pregnancy scans. You will be offered scans every 2-3 weeks from 6 weeks until your dating scan at 12 weeks.

Your participation will be during your early pregnancy for the scans and the telephone interview (6-8 weeks after delivery), if you have a successful outcome. We will obtain your consent to access your medical records for further information.

If you consent, the sample obtained will be used for further research projects.

The study will be conducted at UHCW NHS Trust and the sponsor is University of Warwick. The study is a single site project conducted by the named researchers above.

This study has been reviewed and received favourable opinion through West Midlands REC 15/WM/0295.

If you have any questions about your rights as a research participant, please contact the Research, Development & Innovation Department on 02476 96 7478 or email RD&I@uhcw.nhs.uk

If you have any questions, please feel free to contact me (see below for contact information).

Thank you

Dr. Valarmathy Kandavel  
Clinical research fellow in reproductive medicine  
UHCW NHS Trust  
Coventry



## Appendix 8

Patient information sheet

**Trial: Pilot randomised controlled trial of the effect of endometrial scratch in recurrent miscarriage on pregnancy outcomes**

**Study Title: SiM Study (Scratch in Miscarriage)**

**Chief Investigator: Professor. Siobhan Quenby B.Sc., MBBS, MD, FRCOG**

### **Patient Information Sheet**

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the information carefully and ask us if there is anything that is not clear or if you would like more information.

### ***This study involves***

This study is offered to women who have had two or more previous miscarriages and who meet the inclusion criteria. Currently there is no intervention available on the NHS after two miscarriages.

We think the underlying cause for recurrent miscarriages is in the lining of the womb (endometrium). Endometrial scratch may improve the lining of the womb. We propose to recruit women into a pilot randomised control trial, to find out whether endometrial scratch prevents miscarriages.

The endometrial scratch involves taking a biopsy from the lining of the womb, between 7- 10 days after ovulation. The evidence from large number of women, who underwent the endometrial scratch prior to IVF, shows that it improves pregnancy outcomes. We know from other studies that there was no harm to the lining of the womb from the

endometrial scratch. However, this is the first trial to test if the endometrial scratch improves pregnancy outcomes in women with repeated miscarriages.

***What happens?***

If you are eligible for the trial, you will be requested to consent to the study.

We advise you to take home-based ovulation tests. You will be offered the endometrial scratch between 7-10 days after your ovulation test is positive. On the day of the appointment your consent will be confirmed.

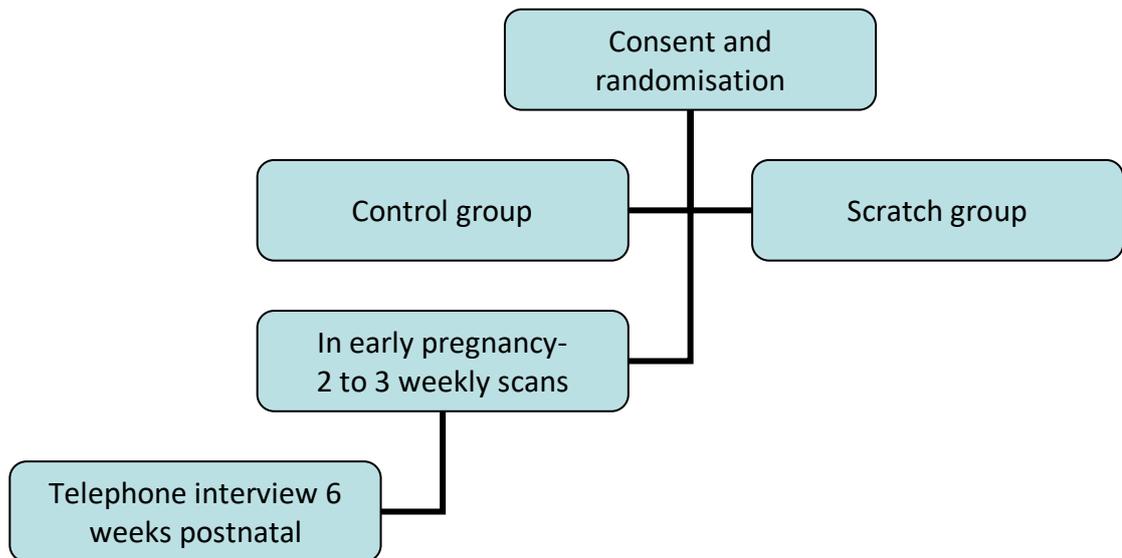
You will be randomly allocated to the treatment or the control group of the trial. You will not be told which group you are in until after the study is completed.

Both groups will have an internal scan followed by insertion of a speculum, similar to having a smear test. If randomised to scratch group, additionally a fine plastic tube is introduced through the cervix into your womb and the lining of the womb is sampled for 10 seconds. You may experience crampy pains at the time of the procedure and for 1-2 minutes thereafter. You may also experience bleeding, which may vary from being light or similar to a period. It is important that you do not try for a pregnancy in the cycle that the test is offered. You may wish to take two 500mg paracetamol tablets or two 200mg ibuprofen tablets up to an hour prior to the procedure. We will offer you gas and air during the procedure if you are uncomfortable. The procedure will be carried out in the presence of a chaperone.

***What happens if I agree to take part in the study?***

- Perform home based ovulation test and ring us ( [REDACTED] ) when it is positive. You will attend for scratch procedure/speculum examination
- Use barrier contraception during the menstrual cycle of the procedure
- Complete and post the patient questionnaire

- Actively try for a pregnancy following the next menstrual period after the procedure
- If you become pregnant, ring us for early pregnancy scan appointments
- If you have a successful outcome, be available for telephone interview 6 weeks after birth of baby



***What happens if I do not wish to take part in the study?***

Participation in the study is entirely voluntary. You will continue to book with your GP/midwife during your pregnancy and have the routine care.

***What data will be collected?***

We will collect information regarding your age, height, weight, ethnicity, medical history and previous pregnancy outcomes. We will be obtaining your consent for using the tissue sample obtained, for other research projects looking into the causes of miscarriages.

***Confidentiality***

There will be no patient identifiable information and you will be assigned a unique trial number. The data collected from you will be entered through a secure system and will only be accessible to the research team.

***What happens to the results of the study***

You are welcome to ring us for a copy of the study result when it becomes available. Please be aware that it will take up to 2 years before the results are published.

***Advice***

For independent advice on research, you can contact PALS (Patient Advice and Liaison Service) on Freephone 0800 028 4203. Local contact details can be found on <http://www.pals.nhs.uk/>

***Complaints***

After participating in the study if you experience any problems specific to the study or if you wish to make a formal complaint you can do so by writing to:

Ceri Jones  
Head of Research & Development  
Research & Development Department  
University Hospitals Coventry & Warwickshire  
University Hospitals  
Coventry CV2 2DX

Direct Tel: [REDACTED]

**Appendix 9**  
GP LETTER

Scratch in Miscarriage (SiM study)

CHIEF INVESTIGATOR: Prof. Siobhan Quenby B.Sc., MBBS, MD, FRCOG

DATE:

RE: Patient Name:

DOB:

Hospital Number:

Dear Doctor (Name of GP) \_\_\_\_\_

This is to inform you that your patient above, who has been found to have recurrent miscarriages, has consented to participate in the study for a period of up to 12 months.

**This study:**

The study is a 'Pilot randomised controlled trial of the effect of endometrial scratch on pregnancy outcomes. The endometrial scratch will be performed in the luteal phase of the cycle. The participants may be either in the control group with no scratch or in the intervention group. The procedure involves transvaginal scan, speculum and scratch in the intervention group. The scratch is endometrial biopsy obtained by passing a fine plastic catheter into the uterus. The control group will have transvaginal scan and insertion of speculum only. The participants will be followed up during their pregnancy until the outcomes are known, including outcomes for baby and a telephone consultation at 6 weeks post-delivery. The trial will be conducted at the UHCW NHS Trust, with sponsorship from University of Warwick.

**Aim of this study:**

To test the hypothesis that recurrent miscarriage is due to a primary endometrial problem. The evidence from studies in women who underwent IVF is, endometrial scratch improves live births. The effect is particularly so in women with two or more implantation failures. The basis of the trial is to test the hypothesis whether endometrial scratch improves the pregnancy outcomes and prevents further miscarriages, in women who have suffered from two or more miscarriages. This is a pilot study for a year with the aim to recruit 100 patients. If the trial results are positive, this may lead to a further randomised control trial.

A copy of the patient information sheet is enclosed which describes the study in more detail. If you require further information, please do not hesitate to contact Valarmathy Kandavel on [REDACTED]

Many thanks for your help.

Yours sincerely

Name

Title

## Appendix 10

### SiM (Scratch in Miscarriage) Study Screening Log

<b>Site Name</b>	UHCW NHS Trust	<b>Principal Investigator</b>	Prof. Siobhan Quenby
------------------	----------------	-------------------------------	----------------------

Date screening initiated	Patient's initials	DOB	Screen fail patients		Registered patients		Site staff initials
			Main reason	Please specify if other reasons	number	date	

## **SIM (Scratch in Miscarriage) study Screening Log**

### **Reasons for non- registration:**

1. Age is above 40 years
2. Screen positive for tablet trial
3. Screen positive for A-life 2 trial
4. Medical disorders such as diabetes, hypertension
5. Need for progesterone supplementation due to past obstetric history
6. Uterine anomalies
7. Unable to tolerate speculum examination
8. Previous participation in the same trial
9. Patient refusal (give reason)

## Appendix 11

### SiM Study- Site Signature and Delegation Log

#### SiM study- Scratch in Miscarriage

Site Name: UHCW NHS Trust

Print Name	Official job title	Trial responsibilities	Signature	Initials	Date start in trial	Date stop in trial	CI initials	Date (dd/mm/yyyy)

## SiM Study- Site Signature and Delegation Log

Site Name: UHCW NHS Trust

Please use the 'Trial Responsibilities Key' below to indicate which responsibilities have been agreed locally. Only members of the research team who are authorised by the Principal Investigator are permitted to undertake the trial responsibilities.

Should any member of the research team who has been delegated responsibilities below either join or leave post, an updated Site Signature and Delegation Log must be updated, to indicate who the responsibility has now been transferred to.

Trial Responsibility Key:

1	Overall responsibility for the trial at the site	9	Investigator site file set up and maintenance
2	Explain trial to participants	10	Recording/Reporting deviations/Violations
3	Obtain medical history	11	Other
4	Perform inclusion and exclusion assessments		
5	Obtain informed consent		
6	Data entry into CRF's		
7	Medical care of participants		
8	SAE reporting to sponsor		

I confirm that I take overall responsibility for the conduct of this study at UHCW NHS Trust, and the trial personnel listed below are authorised to perform trial responsibilities on my behalf as indicated, within the dates indicated. I confirm that they agree to take on these responsibilities and are qualified and appropriately informed about the trial.

Name of Principal Investigator:

Signature:

Initials:

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Date:

## Appendix 12

Letter to Regional Ethics Committee

23<sup>rd</sup> October 2015

Dr John Cochrane

Vice

Chair,

NRES

Committee

West Midlands- South Birmingham Research Ethics Committee  
Birmingham

Dear Dr. Cochrane,

<b>Study Title:</b>	<b>Pilot randomised controlled trial of the effect of endometrial scratch in recurrent miscarriage on pregnancy outcomes.</b>
<b>REC reference:</b>	<b>15/WM/0295</b>
<b>IRAS project ID:</b>	<b>182998</b>

We thank you for the favourable opinion with conditions for the above trial from the 21<sup>st</sup> October 2015.

We have incorporated the following changes to the Participant Information Sheet as requested by the Chair and Lead Reviewer:

Section: '*What happens if I agree to take part in the study?*' for clarity and ease of understanding:

- A second point should be added after '*...when it is positive*', reading '*Attend for scratch procedure/speculum examination*'. This has been done and highlighted in version 3 of the PIS
- Point three should read '*Use barrier contraception during the menstrual/monthly cycle of the procedure*'. This has been changed to the menstrual cycle and highlighted in the PIS
- Point five should read '*Actively try for a pregnancy following the next menstrual period*' after the procedure. This has been changed as per the advice.

Thanking you

Yours sincerely,

Professor. Siobhan Quenby

UHCW NHS Trust

Coventry

## **Appendix 13**

Amendment Letter to REC

22<sup>nd</sup> March 2016

Dr John Cochrane

Vicechair,

NRES

Committee

West Midlands- South Birmingham Research Ethics Committee

Birmingham

Dear Dr. Cochrane,

<b>Study Title:</b>	<b>Pilot randomised controlled trial of the effect of endometrial scratch in recurrent miscarriage on pregnancy outcomes.</b>
<b>REC reference:</b>	<b>15/WM/0295</b>
<b>IRAS project ID:</b>	<b>182998</b>

We thank you for the favourable opinion for the above trial from the 26<sup>th</sup> October 2015. We would like to submit the following amendments:

1. The protocol amendments include:
  - Age inclusion criteria increased from age 40 to 42, in line with the other trials being currently conducted in our research unit.
  - Guidance for randomisation of women for the trial in the luteal phase after a transvaginal scan proven endometrial thickness of 5mm. This will be applicable for those patients who have negative home ovulation tests after a month of testing.
  - Advertisement on the Tommy's website by a joint poster with the ALIFE 2 trial. This is to improve recruitment to the trial and opportunity for women to self-refer.

The updated protocol Version is 3 dated 17<sup>th</sup> February 2016.

2. The Patient Information Sheet (PIS) amendments include guidance for women who are unsuccessful after a month of home ovulation testing. They can arrange an appointment in the luteal phase of the menstrual cycle. The new PIS Version is 4 dated 17<sup>th</sup> February 2016.

3. The participant invitation letter has been amended with information regarding the option of arranging for appointment in the luteal phase of the menstrual cycle. The updated participant invitation letter version is 3 dated 17<sup>th</sup> February 2016.
4. The amended CRF with the additional input for date of the last menstrual period and the day of the cycle when the patient is randomised. The amended CRF version is 2 dated 17<sup>th</sup> February 2016.
5. The Joint poster with the ALIFE 2 trial is attached, for advertisement on the Tommy's website. The ALIFE2 SiM UHCW patient poster is V1.0 dated 07/03/2016.

The ALife2 study received favourable opinion from the Coventry and Warwickshire Ethics Committee on 21<sup>st</sup> December 2015. Ref: 15/WM/0261. EudraCT number: 2015-002357-35. Sponsor UK: UHCW, Funding Body: NIHR.

Thanking you  
Yours sincerely,  
Professor. Siobhan Quenby  
UHCW NHS Trust  
Coventry  
Cv2 2DX

## Appendix 14

Telephone interview questionnaire after live birth for SiM study

Trial ID:

Live birth:  gestation at delivery:

Pre-term:  gestation at delivery:

Mode of delivery: NVD  CS  Instrumental Delivery

Any maternal complications: PET  Abruptio  placenta praevia

Placenta accreta  other:

Weight of baby:                      centile:

SGA: Yes  No

Apgar scores:

Any neonatal complications: Yes  No

If yes, specify:

Anomalies: Yes  No

Thriving: Yes  No

Any ongoing issues: Yes  No

If yes, specify:

## Appendix 15

### Case Report Form (CRF) for the SiM Study

(Pilot randomised controlled trial of the effect of endometrial scratch in recurrent miscarriage on pregnancy outcomes)

Screening visit:

Hospital Number:

Date of recruitment:

Age of the patient:

BMI:

BP:

Urine analysis:

Smoker: Yes  No  Ex-Smoker  No per day if current smoker:

Ethnicity: Caucasian  Asian  Black  Mixed

Number of previous miscarriages:

No	Gestation	Outcome	Management	Complications

Live birth if any:

Any medical or surgical problems:

Significant Gynae history:

Regular cycles: Yes  No

Allergies: Yes  No

Latex allergy: Yes  No

Eligible for TABLET: Yes  No

Patient information leaflet given: Yes  No

Consent visit

Patient ID for the study:

Eligibility criteria met: Yes  No

If not met, the reason:

Patient agrees to the study: Yes  No

Consent obtained: Yes  No

Consent obtained by:

Ovulation kits issued: Yes  No

Instructions given: Yes  No

Intervention visit:

Patient ID for the study:

Randomisation on:                      Method:

Scratch  No Scratch

Date of positive ovulation test:

Chaperone present: Yes  No                       Name:

Scan findings:

Uterus: anteverted  retroverted  axial

Endometrial thickness:

Ovaries: normal  cystic  corpus luteum

If cyst or corpus luteum, state the side and nature:

Cervix at speculum: normal  ectropion

Scratch performed on day \_\_\_\_ after ovulation

Scratch successful: Yes  No

If no, state the reason:

Analgesia taken by the patient: Yes  No

Entonox offered: Yes  No                       Used: Yes  No

Patient questionnaire given : Yes  No

Any complications :

Pregnancy visit

Scans results:

Scan date	Gestation	GS	Yolk sac	Fetal pole, CRL	Fetal heart	Diagnosis

Outcomes of Pregnancy:

First Trimester miscarriage:  gestation at miscarriage

Type of miscarriage: Biochemical  Delayed  GS/YS/fetal pole/absent FH

Late miscarriage (13- 23+6 weeks):  gestation:

Live birth:  gestation at delivery:

Pre-term:  gestation at delivery:

Mode of delivery: NVD  CS  Instrumental Delivery

Any maternal complications: PET  Abruption  placenta praevia   
Placenta accreta  other:

Weight of baby:                      centile:                      SGA: Yes  No

Apgar scores:

Any neonatal complications:

Telephone interview at 6 weeks postnatal: Yes  No

Anomalies: Yes  No

Thriving: Yes  No

## Appendix 16

Consent Form

Consent Form

Study Number:

Participant Identification Number for this trial:

### CONSENT FORM

Title of Project: Pilot randomised controlled trial of the effect of endometrial scratch in recurrent miscarriage on pregnancy outcomes

(Scratch in **M**iscarriage - **SiM** study)

Name of Researcher: Professor. Siobhan Quenby

Please initial box

1. I confirm that I have read the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
  
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.
  
3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.
  
4. I consent to the participation in the study which involves 
  - Performing home ovulation tests, pregnancy tests and ringing for appointment
  - Randomisation to either the control or intervention arm of the study

- Completion of questionnaire and attending for scans from 6 to 12 weeks of pregnancy
- Telephone interview between 6-8 weeks after the pregnancy

4. I understand that the tissue sample collected from me will be used to support other research in the future, and may be shared anonymously with other researchers.

5. I agree to my General Practitioner being informed of my participation in the study.

6. I agree to take part in the above study.

\_\_\_\_\_  
Name of Participant                      Date                      Signature

\_\_\_\_\_  
Name of Researcher                      Date                      Signature

## Appendix 17

Letter from BJOG

----- Original message -----

From: BJOG <[REDACTED]>  
Date: 01/06/2016 14:40 (GMT+00:00)  
To: "[REDACTED]" <[REDACTED]>, [REDACTED] <[REDACTED]>  
Cc: 'Aris Papageorghiou' <[REDACTED]>  
Subject: Your trial: NCT02681627 highlighted in BJOG

Dear Dr Kandavel and Dr Lokman

I am very interested to see that you are currently involved in the study "Sim (Scratch in Miscarriage) Study".

Your work has come to my attention as Executive Scientific Editor of *BJOG: An International Journal of Obstetrics and Gynaecology* (BJOG). As part of my role, I edit our monthly column 'Insights from Outside BJOG' which highlights top, ongoing clinical trials in the field of women's health. In the May issue of BJOG, a brief synopsis of your study has been included. I attach a copy of the column for your reference.

Your work is relevant to our worldwide readership. I hope that when your study is complete, you and your collaborators will consider submitting your paper to BJOG.

For your information I have included below some details on the benefits of publishing in BJOG:

**Global research** – publish with authors from all over the world

- 78% submissions to BJOG are from authors based outside the UK
- Submissions from over 70 countries in 2014

**Global reach** – disseminate your work worldwide

- 90% of institutional subscriptions for BJOG are from outside the UK

- Over 1.3 million full text downloads per year, of which 37% are from US readers
- BJOG is available to all RCOG fellows and members worldwide
- Strong social media presence
- Regular press releases and coverage

**We work for you to select, validate and improve your paper**

- Fast turnaround times
- Rigorous peer-review with unique editor consultations
- Constructive and comprehensive feedback to authors
- Impact factor: 3.448
- Value-added content for selected articles: commentaries, journal clubs, BJOG perspectives

If you do decide to submit your work to BJOG, please do mention this invitation in your cover letter. Our instructions for authors can be found at [www.BJOG.org](http://www.BJOG.org). All submissions to BJOG are subject to our standard peer review process and editorial policies, details of which can also be found on our website.

All the best for your study

Best wishes

Aris Papageorghiou

Executive Scientific Editor, BJOG

**Louisa Waite**  
Content Development Editor, BJOG  
Royal College of Obstetricians and Gynaecologists

T: [REDACTED]

F: [REDACTED]

E: [REDACTED]

W: [www.rcog.org.uk](http://www.rcog.org.uk)

27 Sussex Place, Regent's Park, London, NW1 4RG, UK

Switchboard: [REDACTED]

Registered Charity No: 213280

BJOG: An International Journal of Obstetrics and Gynaecology

Reporting the best research in women's health worldwide since 1902

[www.bjog.org](http://www.bjog.org) | [www.twitter.com/BJOGTweets](https://www.twitter.com/BJOGTweets) | [www.linkedin.com](http://www.linkedin.com)



This email and any attachments to it may be confidential and are intended solely for the use of the individual to whom it is addressed. Unless otherwise stated, any views or opinions expressed are solely those of the author and do not necessarily represent those of the Royal College of Obstetricians and Gynaecologists (RCOG).

If you are not the intended recipient of this email, you must neither take any action based upon its contents, nor copy or show it to anyone. Please contact the sender if you believe you have received this email in error.

The RCOG may monitor, analyse and archive email traffic, data and the content of email for the purposes of security, legal compliance and staff training.

RCOG is a registered charity in England & Wales (No. 213280)

Web: [www.rcog.org.uk](http://www.rcog.org.uk)

## Appendix 18

Approval letter from Ethics Committee



West Midlands - South Birmingham Research Ethics Committee

Royal Standard Place  
NottinghamNG1S

Tel: [REDACTED]

26 October 2015

Professor  
Siobhan  
Quenby  
UHCW NHS  
Trust CV22DX

Dear Professor Quenby

<b>Study title:</b>	<b>Pilot randomised controlled trial of the effect of endometrial scratch in recurrent miscarriage on pregnancy outcomes.</b>
<b>REC reference:</b>	<b>15/WM/0295</b>
<b>Protocol number:</b>	<b>1</b>
<b>IRAS project ID:</b>	<b>182998</b>

Thank you for your letter of 23 October 2015. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 21 October 2015

Documents received

The documents received were as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Covering letter on headed paper [covering letter]		23 October 2015
IRAS Checklist XML [Checklist_23102015]		23 October 2015
Participant information sheet (PIS) [PIS CLEAN VERSION]	VERSION 3	22 October 2015
Participant information sheet (PIS) [PIS TRACKED VERSION3]	VERSION 3	22 October 2015

Approved documents

The final list of approved documentation for the study is therefore as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Copies of advertisement materials for research participants [patient poster]	version 2	01 October 2015
Covering letter on headed paper [covering letter]		13 October 2015
Covering letter on headed paper [covering letter]		23 October 2015

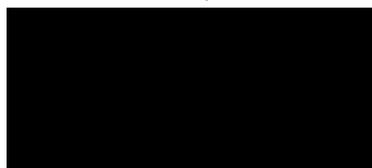
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [certificate of insurance]		31 July 2014
GP/consultant information sheets or letters [Specimen GP LETTER v2]	version 2	01 October 2015
IRAS Checklist XML [Checklist_23102015]		23 October 2015
Letter from funder [funder letter]		21 July 2015
Letters of invitation to participant [LETTER OF INVITATION]	VERSION 2	01 October 2015
Non-validated questionnaire [questionnaire]	Version 1	31 July 2015
Other [GCP Certificate CI]		
Other [GCP Certificate student]		
Other [MARIAM LOCKMAN CV]		
Other [gcp certificate lokman]		
Other [TELEPHONE INTERVIEW QUESTIONNAIRE]	Version 1	06 August 2015
Other [confirmation of insurance]		
Other [professional indemnity]		
Other [pubic liability]		
Other [confirmation of insurance liability]		
Participant consent form [consent form]	Version 1	31 July 2015
Participant information sheet (PIS) [PIS TRACKED VERSION3]	VERSION 3	22 October 2015
Participant information sheet (PIS) [PIS CLEAN VERSION]	VERSION 3	22 October 2015
REC Application Form [REC_Form_07082015]		07 August 2015
Research protocol or project proposal [protocol]	VERSION 2	01 October 2015
Summary CV for Chief Investigator (CI) [cv]		
Summary CV for student [cv student]		
Summary CV for supervisor (student research) [cv]		
Summary, synopsis or diagram (flowchart) of protocol in non-technical language [flow diagram of SiM study]		

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

**15/WM/0295**

**Please quote this number on all**

Yours sincerely



Nicola Kohut REC Assistant

E-mail: [nrescommittee.westmidlands-southbirmingham@nhs.net](mailto:nrescommittee.westmidlands-southbirmingham@nhs.net)

Copy to: Mrs Jane Prewett Ms Ceri Jones, UHCW

## Appendix 19

Substantial amendment approval letter



**West Midlands - South Birmingham Research Ethics Committee**

Royal Standard Place  
Nottingham NG1 6FS

13 April 2016

Prof Siobhan Quincey  
UHCW NHS trust  
CV22DX

Dear Prof Quincey

Study title: Pilot randomised controlled trial of the effect of endometrial  
**scratch in recurrent miscarriage on pregnancy outcomes.**

**REC reference: 15/WM/0295**

**Protocol number: 1**

**Amendment number: SA#01**

**Amendment date: 06 April 2016**

**IRAS project ID: 182998**

The above amendment was reviewed on 08 April 2016 by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Copies of advertisement materials for research participants	1	07 March 2016
Covering letter on headed paper [from Prof Quincey]		22 March 2016

Letters of invitation to participant	3	17 February 2016
Notice of Substantial Amendment (non-CTIMP) [182998/953318/13/419/52191]	SA#01	06 April 2016
Participant information sheet (PIS)	4	17 February 2016
Research protocol or project proposal	3	17 February 2016

#### Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

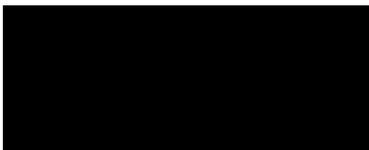
Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at <http://www.hra.nhs.uk/hra-training/>

**15/WM/0295:                    Please quote this number on all correspondence**

Yours sincerely



Professor Simon Bowman Chair

E-mail: [nrescommittee.westmidlands-southbirmingham@nhs.net](mailto:nrescommittee.westmidlands-southbirmingham@nhs.net)

*Enclosures:                    List of names and professions of members who took part in the review*

*Copy to:                        Ms Ceri Jones, UHCW  
Mrs Jane Prewett*

West Midlands - South Birmingham Research Ethics Committee Attendance at Sub-Committee of the REC meeting on 08 April 2016

**Committee Members:**

<i>Name</i>	<i>Profession</i>	<i>Present</i>	<i>Notes</i>
Professor Simon Bowman	Consultant Rheumatologist	Yes	Chair
Dr John David Cochrane	Retired GP	Yes	

**Also, in attendance:**

<i>Name</i>	<i>Position (or reason for attending)</i>
Mr Tad Jones	REC Assistant

