

A Thesis Submitted for the Degree of PhD at the University of Warwick

Permanent WRAP URL:

<http://wrap.warwick.ac.uk/127558>

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



WARWICK

**Does The DPP4 Inhibitor Sitagliptin Increase the
Endometrial Mesenchymal Stem Cell Count in Those
with Recurrent Pregnancy Loss?**

Shreeya Tewary

MBChB, MRCOG

Submitted to the University of Warwick

For the Degree of Doctor of Medicine

December 2017

Student ID: 1592109

TABLE OF CONTENTS

Declaration	8
Acknowledgements	8
Dedication	10
Abstract	11
List of Abbreviations	13
List of figures and tables	15
Chapter 1: INTRODUCTION	17
1.1 Recurrent Miscarriage.....	18
1.1.1 Overview	18
1.2 Recurrent Miscarriage: Definition	19
1.3 Known Causes of Miscarriage.....	19
1.3.1 Maternal Age.....	20
1.3.2 Previous Reproductive History	21
1.3.3 Smoking and Alcohol	21
1.3.4 Obesity	22
1.3.5 Infection	22
1.3.6 Genetics.....	23
1.3.7 Family history	24
1.3.8 Acquired Thrombophilia.....	24
1.3.9 Inherited Thrombophilia	26
1.3.10 Immunological Causes.....	27
1.3.11 Endocrine Factors.....	27
1.3.12 Uterine Structural Anomalies	29
1.3.13 Male Factor	30
1.3.14 Stress	31
1.3.15 Unexplained Recurrent Pregnancy Loss	31
1.3.16 Recurrent Miscarriage: Psychological Morbidity.....	34
1.3.17 The Recurrent Pregnancy Loss Clinic.....	34
1.3.18 What We Know So Far.....	35
1.4 The Human Endometrium	36
1.4.1 Implantation and Decidualisation	36
1.4.2 Embryo Selection and Implantation	38
1.5 Stem cells and The Endometrium	41
1.6 Mechanisms of Stem Cell Recruitment	44
1.6.1 CXCL12 and CXCR4 Pathway.....	44
1.6.2 Regeneration From the Basalis Layer	45
1.6.3 Other Evidence	46
1.6.4 Increasing Stem cell recruitment.....	47
1.7 DPP4 Inhibitors	48
1.7.1 Safety of DPP4 inhibitors.....	49
1.8 Endometrial Biopsy/scratch	50
1.9 conclusion.....	51
Chapter 2: TRIAL DESIGN AND PROTOCOL DEVELOPMENT.....	53

2.1 Aims and Objectives.....	54
2.2 Trial Planning and Design	55
2.2.1 Basic Trial Idea:.....	55
2.2.2 Basic Flow Diagram for Trial Pathway	57
2.3 Sample Size Calculations	58
2.4 Ethical Considerations.....	59
2.4.1 Endometrial Biopsy	59
2.4.2 Trial Medication Safety and Side Effects	59
2.4.3 Safety of Sitagliptin in Healthy Non-Diabetic Individuals	60
2.4.4 Safety of Sitagliptin in Pregnancy.....	61
2.4.5 Investigation Medicinal Product and Placebo	63
2.5 Eligibility Criteria.....	63
2.6 Patient Specific Documents.....	66
2.7 Patient and Public Involvement	67
2.8 Peer review.....	69
2.9 Randomisation Procedures	70
2.10 Unblinding	71
2.11 Application TO REC, MHRA and Site Activation	72
CHAPTER 3: TRIAL PROTOCOL.....	74
3.1 Study Summary.....	75
3.2 Study Aims and Objectives	80
3.3 Outcome Measures.....	80
3.3.1 Primary Outcome Measure	80
3.3.2 Secondary Outcome Measures	80
3.4 Study Design	80
3.5 Ethical considerations	81
3.6 Sample Size and Power	81
3.7 Participant Entry	81
3.8 Inclusion and Exclusion Criteria.....	82
3.8.1 Inclusion criteria	82
3.8.2 Exclusion criteria	82
3.9 Trial Pathway	83
3.9.1 Enrolment Procedure	83
3.9.2 Endometrial Biopsy	84
3.9.3 Randomisation procedure	85
3.9.4 Follow up	85
3.9.5 Final Visit	86
3.10 Monitoring For Pregnancy.....	86
3.11 Trial Medication.....	87
3.11.1 Prescribing and Dispensing.....	87
3.11.2 Treatment.....	87
3.11.3 Dose and Route of Administration	87
3.11.4 Dose Modifications for Toxicity.....	88
3.11.5 Supply of Sitagliptin and Placebo	88
3.11.6 Labelling	88
3.11.7 Compliance Monitoring, Accountability, Reconciliation and Destruction.....	89
3.11.8 Interaction with Other Drugs	89
3.12 Data Collection And Management.....	89
3.13 Confidentiality	90

3.14 Research Ethics Committee (REC) And Medicines Health Regulatory Authority Review	91
3.15 Peer Review	91
3.16 Public and Patient Involvement.....	92
3.17 Regulatory Compliance	92
3.18 Protocol Compliance	92
3.19 Notification of Serious Breaches to GCP and/or the protocol	93
3.20 Audit And Inspection.....	93
3.21 Pharmacovigilance and Safety Monitoring	93
3.21.1 Adverse Events	94
3.21.2 Adverse Reactions	94
3.21.3 Serious Adverse Events	95
3.21.4 Reporting Procedures.....	95
3.21.5 Reporting AEs/ARs.....	96
3.21.6 Reporting Serious Adverse Events.....	96
3.22 In Event of Pregnancy	96
3.23 Unblinding	97
3.24 Early Termination Visit.....	98
3.25 Database.....	98
3.26 Statistics and Data Analysis.....	99
3.26.1 Primary Endpoint.....	99
3.26.2 Statistical Analysis	99
3.27 Trial Closure	100
3.28 Definition of the End of the Trial	100
3.29 Trial Registration.....	100
CHAPTER 4: CLONOGENIC ASSAY.....	101
4.1 Aim	102
4.2 Important points.....	102
4.3 Materials and reagents	102
4.4 Procedure	105
4.4.1 Digestion Media Preparation	106
4.4.2 Sample Chopping.....	106
4.4.3 Adding Digestion Media And Washing	106
4.4.4 Cryopreservation	107
4.4.5 Colony Forming Unit (CFU) Assay	108
chapter 5: TRIAL MANAGeMENT	111
5.1 Roles and Responsibilites.....	112
5.1.1 Trial Sponsor.....	112
5.1.2 Chief Investigator And Principle Investigator	112
5.1.3 Trial Co-ordinator and Trial Manager	113
5.1.4 Trial Pharmacist.....	114
5.2 Risk Assessment.....	115
5.3 Research Team And Site Initiation Visit.....	116
5.4 Data Collection and Management	116
5.5 Trial Management Group Meetings.....	117
5.6 Trial Steering Committee (TSC) and Data Monitoring COMMITTEE(DMC)	119
5.7 Research Governance	120
5.8 Trial Suspension.....	121
5.9 TRIAL MONITORING.....	121

5.9.1 Internal Monitoring	121
5.9.2 External Monitoring	122
5.10 Code Break Test	122
5.11 Amendments	122
5.11.1 Non Substantial Amendments.....	122
5.11.2 Substantial amendments.....	123
Chapter 6: TRIAL SUSPENSION	124
6.1 Events Surrounding Suspension	125
6.2 Substantial Amendment 1.....	127
6.3 Substantial Amendment 2 +3	128
6.4 Trial Restart	131
6.5 Lessons Learned.....	132
chapter 7: TRIAL RECRUITMENT.....	133
7.1 Trial Pathway	134
7.1.1 Participant Entry.....	134
7.1.2 Participants Not Eligible After Screening.....	134
7.1.3 Withdrawals Prior To Randomisation.....	135
7.1.4 Withdrawals After Randomisation	135
7.2 Enrolment procedure	136
7.3 Intervention Visit	137
7.4 Biopsy Transportation And Cryopreservation.....	138
7.5 Randomisation.....	139
7.6 Follow up Visit 1	140
7.7 Follow up Visit 2	140
7.8 Intervention Visit 2	141
Chapter 8: STATISTICAL ANALYSIS PLAN	142
8.1 Statistical Analysis Plan	143
8.1.1 Consort Diagram.....	143
8.1.2 Clonogenic Assay	143
8.1.3 Patient Demographics	145
8.1.4 eMSC counts.....	145
8.2 Primary Outcome Measure	146
8.3 Secondary Outcome Measures.....	146
8.4 Other Data Analysis	146
CHAPTER 9: RESULTS	148
9.1 Consort Diagram	149
9.2 Recruitment.....	150
9.3 Patient Demographics.....	151
9.4 Distribution Of Miscarriages In Each Group	154
9.5 Reationship Between Patient Demographics and Initial eMSC Count	155
9.5.1 Age and eMSC count	155
9.5.2 BMI and eMSC count	155
9.5.3 Number of T1 miscarriages and eMSC count	156
9.6 The eMSC count Profiles	157
9.7 Primary Analysis (Unadjusted Analysis).....	158
9.8 Secondary Analysis (Adjusted Analysis).....	159
9.8.1 Primary Outcome Measure	160

9.8.2 Other Results.....	160
9.9 Secondary Outcome Measures.....	162
9.9.1 Adverse Events and Side Effects.....	162
9.10 Questionnaire	163
9.10.1 Study Acceptability.....	163
9.10.2 Study Medication Acceptability.....	165
9.10.3 Acceptability of Participant Information Leaflet	165
9.10.4 Care Received By Research Team.....	165
9.10.5 Improving Care	166
Chapter 10: DISCUSSION.....	168
10.1 Recurrent Miscarriage.....	169
10.2 The Endometrium and Recurrent Miscarriage	170
10.3 The SIMPLANT Study – Set Up	171
10.4 The SIMPLANT Study - Recruitment.....	172
10.5 The SIMPLANT Study: Trial Management	173
10.6 SIMPLANT: Results.....	173
10.7 Change in eMSC Count in the Initial and Final Biopsy	174
10.8 Other Results	174
10.9 Adverse Events and Study Acceptability	175
10.10 Limitations	176
10.11 Future work	178
10.12 Conclusions	179
Key Dates	180
References	182
Appendices	195
Appendix 1	195
Appendix 2 – Consent Form	199
Appendix 3 – Letter to General Practitioner	200
Appendix 4 – Symptom Diary.....	202
Appendix 5 - Questionnaire	207
Appendix 6 – Emergency Contact Card.....	210
Appendix 7 – Peer Review from Dr Nigel Simpson	211
Appendix 8 – Peer Review from Dr O’Hare.....	212
Appendix 9 – Funding Letter	213
Appendix 10 – Initial Recommendation Response from the REC	214
Appendix 11 – Response to Initial REC Recommendations	221
Appendix 12 – Approval Letter from HRA	225
Appendix 13 – Trial Medication Label	227
Appendix 14 – Screening log	228
Appendix 15 – Recruitment Log	229
Appendix 16 – Pharmacy IMP Accountability Log.....	230
Appendix 17 – Sample of CRF pack – Baseline Visit	231
Appendix 18 – Adverse Event Reporting Form	240
Appendix 19 – Serious Adverse Event Reporting Form	241
Appendix – 20 Final TMG Meeting Summary	245
Appendix 21 – Notification of Trial Suspension	253
Appendix 22 – Letter to REC and MHRA with Proposed Changes.....	255

Appendix 23 – Approval Letter from REC to Restart	264
Appendix 24 – Notification of External Audit	265

DECLARATION

I, Shreeya Tewary, declare that

1. My research has been conducted ethically under the supervision of Professor Siobhan Quenby.
2. All participants were recruited by myself and Professor Siobhan Quenby. All work presented in this thesis has been performed by myself unless otherwise stated.
3. Endometrial biopsies were taken by me with the support of nursing colleagues acting as chaperones.
4. The endometrial biopsies were analysed by Dr Emma Lucas who is a post-doctoral research fellow at the University of Warwick with many years of experience in advanced laboratory skills which were required for the clonogenic assays.
5. The data collected and results presented are original and obtained during my research.
6. I have referenced appropriately through-out my thesis ensuring that acknowledgement and recognition of concepts and ideas have been made.
7. The thesis submitted is within the required word limit specified by the University of Warwick.

ACKNOWLEDGEMENTS

I am forever indebted and most grateful to my supervisors; Professor Siobhan Quenby and Professor Jan Brosens for their propitious and most inestimable help in providing me with a window of opportunity to be a part of their research team. In addition, I am mindful that this work would not have come to fruition without their understanding and generosity in terms of time invested and patient explanations offered at numerous junctions along the way.

Hailing from a clinical background, I was new to the methodology of laboratory work, and needed a step-by-step instruction in order to proceed with my project. I was fortunate to encounter a group of highly motivated and very dedicated individuals who had the patience to instruct me and support my tottering steps in an entirely unfamiliar environment.

I owe a special debt of thanks to Dr Emma Lucas for her invaluable help in analysing the samples in the laboratory. I am mindful of the labour-intensive process involved as it takes two weeks to analyse each representative sample.

I must credit Peter Kimani with special thanks for guiding me through the maze of statistics and helping me analyse and present my data. I am thankful to Mr Mojid Khan for supporting and guiding me through the strict regulations for trials of medicinal products.

This acknowledgement would be wholly incomplete without a special mention to all my colleagues in the Biomedical Research Unit. They have been extremely patient and flexible, and have encouraged me towards completion of this work. Their positive reinforcements helped me in difficult times, and they eased our patients through the sample collection and biopsies. My gratitude extends to my colleague and good friend Dr. Lacey and also Dr. Ewington for their help with the final patients.

This study would not have been possible without the sponsorship and guidance from the University Hospital of Coventry and Warwickshire, and the funding from Tommy's Charity.

DEDICATION

This thesis is dedicated to my parents Ashok Tewary and Manju Tewary and my beloved brother Shravan Tewary.

This work would not have materialised without their gentle but consistent persuasion, their self-belief installed in me over the years and their good wishes and prayers.

ABSTRACT

Introduction: Miscarriage is the most common complication of pregnancy. It is estimated that approximately 1% of women suffer three or more consecutive miscarriages.

Chromosomal abnormalities are the most common cause of first trimester miscarriage. Maternal medical conditions such as uncontrolled diabetes, thyroid disease, thrombophilia's, infection and immunological disorders have all been implicated as a cause for recurrent pregnancy loss (RPL). Other important risk factors include advanced maternal age and lifestyle factors such as stress, smoking, alcohol intake and body mass index.

Research conducted at Warwick Medical School recently discovered that RPL is associated with stem cell deficiency as well as enhanced cellular senescence and disordered inflammation of the endometrium.

Stem cell recruitment normally occurs in response to menstruation, childbirth, miscarriage and curettage of the uterus. The process of stem cell recruitment may be deficient in those with RPL but has been shown in animal studies to be enhanced with DPP4 inhibitors.

Hypothesis: We hypothesised that DPP4 inhibition with Sitagliptin given prior to conception will increase the endometrial mesenchymal stem cell (eMSC) count during menstruation.

Methods/Design: The SIMPLANT study (**S**itagliptin for **I**MPLANTation) was a double blind randomised feasibility study conducted at University Hospital Coventry and Warwickshire.

The study was aimed at women aged 18 – 42 with 3 or more miscarriages. Participants were randomised to one of two groups; an endometrial scratch and 100mg Sitagliptin taken once daily for 3 months or an endometrial scratch and placebo taken once daily for 3 months. Group allocation was unknown at the time of analysis and so have been labelled as Group 0 and Group 1.

Our primary outcome measure was the eMSC count after 3 months of Sitagliptin versus 3 months of placebo, measured using a clonogenic assay.

Results: The results of this feasibility study showed no significant difference in the final eMSC count between the two treatment groups. The study was acceptable to participants and very few side effects to study medication experienced.

The study has shown a significant increase in the eMSC count in one treatment group (Group1)

when the baseline eMSC count was compared to the eMSC count after 3 months.

Conclusion: Our results have shown that the eMSC count after 3 months of treatment of Sitagliptin was not significantly different to the eMSC count after 3 months of placebo. Significant confounding and limiting factors within this feasibility study design have been discussed.

Results have shown that we may be able to increase the eMSC count in certain patients which should in turn improve decidualisation of the endometrium and the environment for implantation; however much further work is required before investigating this.

LIST OF ABBREVIATIONS

ACOG	American College of Obstetrics and Gynaecology
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Transferase
AR	Adverse Reaction
BFG	Basic Fibroblast Growth Factor
BMI	Body Mass Index
bmMSC's	Bone Marrow Mesenchymal Stem Cells
BR	Bilirubin
BRU	Biomedical Research Unit
CRF	Case Report Form
cTIMP	Clinical Trial of Investigational Medicinal Product
DMSO	Dimethyl Sulfoxide
eGFR	Estimated Glomerular Filtration Rate
eMSC's	Endometrial Mesenchymal Stem Cells
eSFs	Endometrial stromal fibroblasts
ESHRE	The European Society of Human Reproduction and Embryology
FDA	Food and Drug Administration
FDA	Food and Drug Administration
FISH	Fluorescent in situ hybridisation
G.P	General Practitioner
GCP	Good Clinical Practice
GCSF	Growth Colony Stimulating Factor
GMP	Good Manufacturing Practice
HCG	Human Chorionic Gonadotrophin
HESC's	Human Endometrial Stromal Cells
HRA	Health regulations Authority
IL-1 β	Interleukin 1 beta
IMP	Investigational Medicinal Product
IMS	Industrial Methylated Spirit
IRAS	Integrated Research Applications System
IVF	In-Vitro Fertilisation
IVIG	Intravenous Immunoglobulin
LBR	Live Birth Rate
LH	Luteinising Hormone
LMWH	Low Molecular Weight Heparin
MHRA	Medicines Health Regulations Authority

MIA (IMP)	Manufacturers Importers License for an Investigational Medicinal Product
MSC's	Mesenchymal Stem Cells
MSD	Merck Sharp and Dohme
NBF	Neutral Buffered Saline
NHS	National Health Service
NIHR	National Institute of Health Research
PBS	Phosphate Buffered Saline
PCOS	Polycystic Ovarian Syndrome
PGD	Pre-implantation Genetic Diagnosis
PGS	Pre-implantation Genetic Screening
PIL	Patient Information Leaflet
pNK cells	Peripheral Natural Killer Cells
PPI	Patient and Public Involvement
PTSD	Post-Traumatic Stress Disorder
QP	Qualified Person
RCOG	The Royal College of Obstetricians and Gynaecologists
RCT	Randomised Controlled Trial
RD&I	Research Development and Innovation
REC	Research Ethics Committee
RM	Recurrent Miscarriage
RPL	Recurrent Pregnancy Loss
SAE	Serious Adverse Event
SPC	Summary of Product Characteristics
TMF	Trial Master File
TMG	Trial Management Group
TNF - α	Tumour Necrosis Factor alpha
U.K	United Kingdom
UFH	Unfractionated Heparin
UHCW	University Hospital Coventry and Warwickshire
uNK cells	uterine Natural Killer Cells

LIST OF FIGURES AND TABLES

Figure 1.0.1: Number Of Conceptions To Reach Each Stage Of Pregnancy. Diagram Adapted From: (Rai & Regan, 2006).	Error! Bookmark not defined.
Figure 1.0.2: Embryo Encapsulation By Decidualising Stroma.....	40
Figure 1.0.3: Embryo Selection At Implantation.....	41
Figure 1.0.4: Stem Cell-Ness Of The Endometrium In Those With RPL.....	42
Figure 1.0.5: Number Of Miscarriages And Relationship To eMSC Count.....	43
Figure 1.0.6: Migration Of bmMSC's To The Endometrium.....	47
Figure 1.0.7: DPP4 Expression At The Endometrium In Those With Recurrent Miscarriage	49
Figure 2.0.1: Basic Flow Diagram For Trial Pathway.....	57
Figure 6.0.1: Statement From Dr O'hare	Error! Bookmark not defined.
Figure 9.0.1: Trial Consort Diagram	149
Figure 9.0.2: SIMPLANT Monthly Recruitment Chart	150
Figure 9.0.3: SIMPLANT Cumulative Recruitment Chart.....	150
Table 9.0.1: Table Of Patient Demographics.....	151
Figure 9.0.4: Distribution Of T1 Miscarriages In Each Treatment GroupError! Bookmark not defined.	
Figure 9.0.5: Distribution Of T1 Miscarriage In Each Treatment GroupError! Bookmark not defined.	
Figure 9.0.6: Scatter Graph Of The Relationship Between Age And Initial eMSC Count	Error! Bookmark not defined.
Figure 9.0.7: Scatter Graph Showing The Relationship Between BMI And Initial eMSC Count.....	Error! Bookmark not defined.
Figure 9.0.8: Scatter Graph Showing The Relationship Between T1 Miscarriages And eMSC Count.....	Error! Bookmark not defined.
Figure 9.0.9: eMSC Count Profile In Each Treatment Group	Error! Bookmark not defined.
Table 9.0.2: Estimated Mean eMSC Count At Final Biopsy Between The Two Treatment Groups	Error! Bookmark not defined.
Table 9.0.3: Adjusted Analysis Of eMSC Counts Between Baseline And Final Biopsy And Between Both Treatment Groups.....	Error! Bookmark not defined.
Figure 9.0.10: Graphical Representation Of Adjusted Secondary AnalysisError! Bookmark not defined.	
Table 9.0.4: Adverse Effects And Side Effects Of Each Treatment Group.....	162

Table 9.0.5: Questionnaire Results.....	164
Table 9.0.6: Study Medication Acceptability	165
Table 9.0.7: Study Medication Acceptability and Compliance.....	165

CHAPTER 1: INTRODUCTION

1.1 RECURRENT MISCARRIAGE

1.1.1 OVERVIEW

Miscarriage, defined as the loss of pregnancy before the fetus reaches viability at 24 weeks gestation, is the most common complication of pregnancy (Rai & Regan, 2006). As many as 15-25% of clinically diagnosed pregnancies end in miscarriage (Lucas *et al.*, 2016b; Rai & Regan, 2006). When combined with pre-clinical losses, the true incidence is closer to 50% (Macklon *et al.*, 2002). Figure 1.0.1 shows how a significant proportion of pregnancies end before actually being clinically recognised.

FIGURE 1.0.1: Number of Conceptions to Reach Each Stage of Pregnancy.

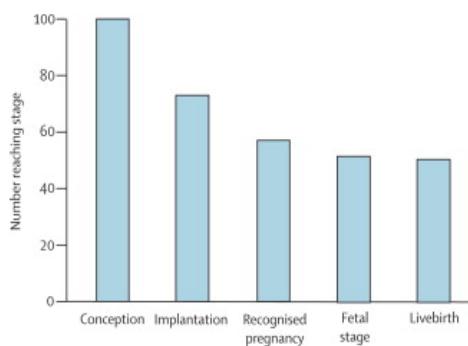


Diagram Adapted From: (Rai & Regan, 2006).

The number of miscarriages in the UK is estimated to be approximately 200,000 per year (Royal College of Obstetricians & Gynaecologists, 2011). Most miscarriages are sporadic and occur before 12 weeks' gestation (Rai & Regan, 2006).

Recurrent pregnancy loss (RPL) or recurrent miscarriage (RM) is a condition unique and distinct to sporadic miscarriages. It is estimated that 5% of women experience two consecutive miscarriages and approximately 1 - 2% suffer three or more consecutive miscarriages (Ford & Schust, 2009; Stirrat, 1990).

The observed frequency of RPL is higher than would be expected by chance alone (1% vs 0.4%) suggesting that RPL is a specific type of reproductive failure (Regan, 1991).

1.2 RECURRENT MISCARRIAGE: DEFINITION

The definition of RPL and when investigations should be instigated has been debated.

The American College of Obstetrics and Gynaecology (ACOG) 2001 and the Dutch Society of Obstetrics and Gynaecology (NVOG) 2007 define RPL as 2 consecutive pregnancy losses whereas The European Society of Human Reproduction and Embryology (ESHRE) 2006 and The Royal College of Obstetricians and Gynaecologists (RCOG) 2003 define RPL as 3 or more consecutive miscarriages (ACOG, 2001; NVOG, 2007) (ESHRE, 2017). The new EHSRE guideline published in November 2017 has recently changed the definition for diagnosis of RPL to 2 or more pregnancy losses (ESHRE, 2017). It is thought that this will help with facilitating research and providing support to couples earlier.

In those without a previous live birth, the risk of a further miscarriage after 2 miscarriages is 30%. The risk of a miscarriage after 3 losses is 33% (Ford & Schust, 2009) suggesting that there is a strong reason to look for a cause after 2 miscarriages.

1.3 KNOWN CAUSES OF MISCARRIAGE

Risk factors such as maternal age and reproductive history have been shown to be associated with RPL. Lifestyle factors such as smoking, alcohol, exercise, medical factors, genetics, immunological and anatomical causes have all also been linked.

General practitioners refer their patients to specialist clinics for investigations and possible treatment options.

Here I will discuss what is known already about conditions associated with RPL. I will also assess the evidence for them being causative. A **causative** factor in miscarriage would need to be more prevalent in those with RPL, its presence would need to predict miscarriage, and treating the condition would have to prevent miscarriage.

1.3.1 MATERNAL AGE

Increasing maternal age and associated ageing oocytes is known to be the most common cause of a single miscarriage (Royal College of Obstetricians & Gynaecologists, 2011).

A large prospective study has shown that maternal age is a strong risk factor for miscarriage due to an increase in chromosomally abnormal oocytes (Nybo Andersen *et al.*, 2000). This population based register linkage study has shown that at the age of 42 the risk of miscarriage is more than 50% and reaches 75% in those over the age of 45. Regardless of previous miscarriages or parity, high maternal age was found to be a significant risk factor for miscarriage (Nybo Andersen *et al.*, 2000).

However, the prevalence of chromosomal abnormalities decreases with an increasing number of miscarriages and the incidence of euploidic foetal loss increases with each additional miscarriage (Ogasawara *et al.*, 2000) implying that there are other fundamental factors in addition to age implicated in RPL.

A cohort study looking at the live birth rate in those with RPL found that the rate of live birth within five years of attending the RPL clinic was 67% but also the chance of live birth decreased with increasing maternal age (Lund *et al.*, 2012) emphasising the role of advanced maternal age in the role of conception, miscarriage and live birth.

Patients need to be carefully counselled about the effect of age on the risk of miscarriage so that careful decisions can be made between the couple about further attempts to pregnancy.

1.3.2 PREVIOUS REPRODUCTIVE HISTORY

The risk of miscarriage in a first pregnancy is 5% and this risk decreases to 4% in those who have had a previous successful pregnancy. The risk of miscarriage in those who have had a previous miscarriage is 19% (Regan *et al.*, 1989).

45% of those with RPL have had previous live births showing those with previous live births are not precluded from RPL (Rai & Regan, 2006). The risk of further miscarriage has been repeatedly shown to increase after each successive pregnancy loss, reaching 45% after three consecutive losses (Nybo Andersen *et al.*, 2000; Regan *et al.*, 1989).

However, it has also been demonstrated that even after five consecutive miscarriages, the likelihood of a live birth is more than 50% (Brigham *et al.*, 1999; Rai & Regan, 2006).

Many studies have found that the number of miscarriages is an important prognostic factor for live birth (Quenby & Farquharson, 1993). It is recommended practice to obtain a thorough reproductive history from couples who attend a RPL clinic.

1.3.3 SMOKING AND ALCOHOL

There are strong links between cigarette smoking and intrauterine growth restriction, placental abruption and stillbirth. Smoking may also be associated with RPL.

A retrospective study has shown that exposure to tobacco smoke significantly increases the risk of RPL and the risk is greater the longer the exposure (Zhang *et al.*, 2010).

Conversely, a case control study in 2003, has shown that those women who consume even over 20 cigarettes per day do not have a significantly increased risk of miscarriage (Rasch, 2003).

A cohort study including over 24 000 singleton pregnancies has shown that alcohol consumption even at 3-5 units per week increases the risk of first trimester miscarriage (Kesmodel *et al.*, 2002). This has also been proven in a case control study performed in 2003 (Rasch, 2003).

A case control study has shown a dose-dependent relationship between miscarriage and alcohol. There is an increased risk of miscarriage in those who

drink at least once per week compared to those who do not drink at all (Maconochie *et al.*, 2007). These studies however have been performed in those with sporadic miscarriage rather than RPL.

Although there is a lack of good quality evidence showing that smoking increases the risk of RPL, smoking cessation is recommended to all patients due to the health benefits for themselves and also because of the clear increased risk of obstetric complications once they become pregnant such as intrauterine growth restriction, placental abruption and stillbirth. The same applies for alcohol consumption which is associated with foetal alcohol syndrome. We also know that alcohol and smoking consumption can affect semen quality and so this lifestyle advice should be given to both the patient and the partner.

1.3.4 OBESITY

A systematic review has shown a higher prevalence of RPL in women with a BMI >30 compared with those with a BMI<30 (0.4% vs. 0.1%; OR 2.51; 95% CI 1.03–12.01) (Boots & Stephenson, 2011; Coomarasamy *et al.*, 2015).

The frequency of euploidic miscarriage among obese women is higher than those with a normal BMI (58% vs 37% respectively) (Zhang *et al.*, 2010) which suggests that obesity is an independent risk factor for miscarriage.

On the converse, being underweight with a BMI <18.5 has been found to be significantly associated with first trimester miscarriage (Maconochie *et al.*, 2007). However, this has not been consistently proven. There are other studies which have not found an increased risk of RPL in those who are underweight (Lo *et al.*, 2012).

We know that weight loss improves fertility rates and can reduce the risk of obstetric complications such as gestational diabetes and therefore patients in RPL clinics should be advised on a healthy lifestyle to help achieve a healthy BMI.

1.3.5 INFECTION

The overall prevalence of chronic endometritis from endometrial biopsies performed on women with RPL has been shown to be 9% (McQueen *et al.*, 2014). The prevalence of chronic endometritis in RPL patients detected at hysteroscopy has

been reported to be 58% (Cicinelli *et al.*, 2014). The effect of treatment and subsequent live birth rate is yet to be tested in a randomised controlled trial (Boots *et al.*, 2014).

1.3.6 GENETICS

Chromosomal anomalies are known to be the most common reason for a sporadic miscarriage. The prevalence of chromosomal abnormalities in a single miscarriage has been shown to be slightly higher but still comparable to the prevalence in a subsequent miscarriage after RPL (45% vs 39%) (van den Berg *et al.*, 2012).

It is possible to analyse foetal tissue for genetic abnormalities using fluorescence in situ hybridisation (FISH) or array-based comparative genomic hybridisation (array CGH) which is more accurate than FISH. This can provide a reason to the couple for their pregnancy loss.

In a study of nearly 800 couples with RPL, 3.5% were found to have a chromosomal abnormality (Flynn *et al.*, 2014). More than 50% of these abnormalities were balanced reciprocal translocations. In the same study however, they did also reassuringly find a 64% cumulative live birth rate in patients with RPL.

It has also been shown that the risk of future miscarriage is dependent on the nature of the parental karyotype abnormality. There appears to be increased miscarriage in those with reciprocal translocations rather than Robertsonian translocations or other types of abnormalities (Sugiura-Ogasawara *et al.*, 2004).

Parental karyotyping can provide couples with a possible cause for fetal chromosomal abnormalities and may help them decide if they want to continue trying to conceive or chose prenatal testing or preimplantation genetic diagnosis with in vitro fertilisation (IVF).

It must be noted that even if a parental karyotype abnormality is found, the cumulative live birth rate has still been shown to be good despite a higher risk of another miscarriage (Flynn *et al.*, 2014).

Couples with chromosomal anomalies on fetal testing or parental karyotyping should be offered genetic counselling.

Pre-implantation genetic screening (PGS) or pre-implantation genetic diagnosis (PGD) can be considered where chromosomal anomalies are thought to be the cause of RPL. This involves IVF with screening for or diagnosing chromosomal anomalies prior to embryo transfer.

PGD may be of particular interest to those couples at high risk of transmitting genetic disorders especially structural chromosomal abnormalities which are found in RPL patients. A systematic review on the use of PGD on RPL patients with a structural chromosomal abnormality actually showed no improvement in live birth rate. However, the studies in this review were not of high quality (Franssen *et al.*, 2011).

1.3.7 FAMILY HISTORY

Studies have proven that sporadic miscarriage and RPL can run in families. RPL is more common in first degree relatives when compared to controls (Kolte *et al.*, 2011). This study and others which have confirmed the same however are open to much reporting bias. Couples may only talk of their experience of miscarriage if others in the family have experienced the same.

1.3.8 ACQUIRED THROMBOPHILIA

Antiphospholipid syndrome (APLS) is widely recognised as a risk factor for pregnancy complications such as miscarriage, intrauterine growth restriction, pre-eclampsia and intrauterine death (Ziakas *et al.*, 2010). APLS is an acquired thrombophilia diagnosed when there is a combination of antiphospholipid antibodies with pregnancy complications (two or more pregnancy losses) and/or venous thromboembolism (van den Boogaard *et al.*, 2013). Antiphospholipid antibodies include lupus anticoagulant, anticardiolipin antibodies and B₂ glycoprotein antibodies.

There is a strong association of lupus anticoagulant with late RPL and of anticardiolipin antibodies with early RPL (Opatrny *et al.*, 2006). The association with B2 glycoprotein is not as strong.

There have been many randomised controlled trials (RCT's) over the years looking at treatment of APLS to try and reduce the rate of miscarriage in these patients.

There is very little data on the use of aspirin alone in patients with APLS. Empson et al have concluded that the use of aspirin alone does not help to prevent RPL in those with APLS when compared to no treatment (Empson *et al.*, 2005).

A meta-analysis performed in 2002 showed that aspirin and low molecular weight heparin (LMWH) may reduce miscarriage risk in those with APLS by 54% when compared to aspirin alone (Empson *et al.*, 2002). However, it was also concluded that this meta-analysis was based on only two small trials and one lacked adequate allocation concealment.

The Cochrane review of this meta-analysis (Empson *et al.*, 2005) is the strongest analysis of treatment for APLS we have and so, patients with APLS are currently advised to have LMWH and aspirin from the date of a positive pregnancy test and this is favoured over no treatment.

There are some conflicting reports. An RCT including 98 women with RPL showed that the live birth rate (LBR) in those randomised to heparin and aspirin was not significantly higher than those randomised to aspirin alone (72% vs 78%) (Farquharson *et al.*, 2002).

The meta-analysis by Empson et al in 2005 also examined outcomes of many other treatment options such as steroids and intravenous immunoglobulin (IVIG) in patients with APLS and RPL. It was found that steroid therapy did not increase LBR when used with aspirin or aspirin plus heparin. The safety of any intervention is important to assess. Prednisolone was found to significantly increase the risk of prematurity and gestational diabetes. The safety of heparin has also been assessed with regards to risks of prematurity/fetal growth restriction/risk of haemorrhage/bone mineral density and fractures. Empson et al 2005 also concluded from a systematic review of three RCT's that IVIG does not reduce the chance of miscarriage.

The lack of knowledge on the optimal dose of heparin to maximise benefit and minimise harm has also been commented on (Empson *et al.*, 2002; Ziakas *et al.*, 2010). Anticoagulation may need to be adjusted according to weight and renal clearance changes in pregnancy.

Ziakas et al have questioned whether LMWH is the right choice of anticoagulant to result a real benefit in those with APLS (Ziakas *et al.*, 2010). In this meta-analysis,

treatment with unfractionated heparin (UFH) plus aspirin proved to be more efficacious than aspirin alone in reducing first trimester miscarriages and LMWH plus aspirin compared with aspirin alone showed no significant benefit in preventing pregnancy loss. These results however could have been due to the small study samples.

APLS in those with RPL is not common (15% of cases). In the presence of APLS clinicians often tell the patient that a cause has been found and that treatment is available. However, it must be noted that clear evidence on APLS is hindered by studies with small sample sizes, lack of laboratory standardisation and differences in the clinical picture of those with APLS. Matched case control studies to help overcomes the huge differences found between patients with APLS which accounts for age, number of miscarriage and autoimmune status are necessary (Ziakas *et al.*, 2010).

1.3.9 INHERITED THROMBOPHILIA

Inherited thrombophilias have been implicated as a cause for RPL. Inherited thrombophilias include Factor V Leiden mutation, Prothrombin mutation, Protein C, Protein S and antithrombin III deficiency. So far, studies looking at the association and treatment of inherited thrombophilia and RPL have been inconsistent.

Protein C, protein S deficiency and antithrombin III deficiency are less common than factor V leiden and prothrombin mutations but more strongly associated with venous thromboembolism. Even so, only weak associations have been found. A meta-analysis found no difference in live birth rate when using LMWH vs no LMWH in those with RPL and inherited thrombophilia (Skeith *et al.*, 2016). This meta-analysis however had a limited sample size ($n = 66$) and so definite conclusions could not be made.

The ALIFE-2 trial is currently being conducted to evaluate the effect of LMWH versus standard pregnancy surveillance in those with an inherited thrombophilia.

1.3.10 IMMUNOLOGICAL CAUSES

There are many immunological biomarkers that have been associated with RPL. However, to date their ability to predict pregnancy outcome in women with RPL has rarely been adequately assessed (ESHRE, 2017).

Measuring anti-HY antibodies and HLA determination in women with RPL is currently not recommended. The association between subsequent pregnancy outcome and HLA polymorphisms in couples with RPL has not been sufficiently studied. Although there is an association between cytokines such as tumour necrosis factor alpha (TNF- α) and RPL (Piosik *et al.*, 2013), no treatment options are available and prognosis for subsequent pregnancies is unknown.

Some studies have shown a significant association between peripheral natural killer cells (pNK) and RPL (Karami *et al.*, 2012), but there have also been studies which did not find this association (Liang *et al.*, 2012).

Equally, there is conflicting evidence about uterine natural killer cell (uNK) levels in patients with RPL. In 1996 Lachapelle *et al* found a significantly higher number of CD56^{dim}CD16+ NK cells in endometrial biopsies in women with RPL using flow cytometry (Lachapelle *et al.*, 1996). Using immunohistochemistry several other authors found increased uNK cells (CD56⁺) in the endometrium of women with RPL (Quenby *et al.*, 2005). Furthermore, a study has demonstrated that prednisolone treatment significantly reduced the number of uterine NK cells (Quenby *et al.*, 2005). A pilot randomised controlled trial of offering prednisolone to women with RPL and a high uNK cell density suggested an improvement in pregnancy outcome, however, this finding has not been confirmed in a large-scale trial (Tang *et al.*, 2013).

There is inadequate evidence to suggest that screening and treating immunological abnormalities should be introduced into routine clinical practise but further work in this area is needed.

1.3.11 ENDOCRINE FACTORS

Hypothyroidism is associated with miscarriage, intrauterine growth restriction and. The neurocognitive development of the fetus can also be affected if hypothyroidism is not treated appropriately. Treatment of hypothyroidism and optimisation of thyroid

status prior to conception is recommended especially to reduce these potential complications of pregnancy.

Subclinical hypothyroidism has been detected in over 20% of patients with RPL (Lata *et al.*, 2013). There is conflicting evidence as to whether subclinical hypothyroidism should be treated to manage RPL. Bernardi *et al* concluded that there was no significant difference in the LBR of those with RPL when comparing treatment of those with subclinical hypothyroidism and those without (Bernardi *et al.*, 2013). The European Thyroid Association Guidelines for the Management of Subclinical Hypothyroidism in Pregnancy and Children advises treatment of subclinical hypothyroidism detected pre-conception and in pregnancy. So far there are no large RCT's to come to a firm conclusion.

There is however a clear association between thyroid autoantibodies (anti-thyroglobulin (TG-Ab), TPO-Ab or anti-TSH receptor autoantibodies) and RPL (Ticconi *et al.*, 2011). We await the results of the TABLET trial to advise whether treatment of those with thyroid autoimmunity will reduce miscarriage or increase live birth.

Also, looking at subclinical hypothyroidism and thyroid autoantibodies in those with RPL, the presence of subclinical hypothyroidism has been found to be significantly higher in those with TPO-Ab's (Lata *et al.*, 2013). For this reason, thyroid screening and TPO-antibody testing is recommended in patients with RPL (ESHRE, 2017).

Progesterone is vital for maintenance of pregnancy and so, progesterone support has been studied in the women with RPL. The largest and most recent randomised controlled trial showed that progesterone did not lead to a significant increase in LBR when compared to placebo (RR1.04, CI 0.94 – 1.15, P 0.45) for those with unexplained RPL (Coomarasamy *et al.*, 2015).

Polycystic ovarian syndrome (PCOS) is known to be associated with subfertility and pregnancy complications such as gestational diabetes. A clear association between PCOS and RPL is difficult to define due to the multi-factorial nature of the syndrome including obesity, hyperinsulinaemia and hyperandrogenism but there have been studies showing PCOS being more prevalent in those with RPL when compared to parous women (Sagle *et al.*, 1988; Watson *et al.*, 1993).

Although exposure to metformin in pregnancy is regarded as generally safe, there is not enough evidence to recommend its use in those with RPL.

Vitamin D deficiency is related to antenatal complications such as intrauterine growth restriction, pre-eclampsia and gestational diabetes (Aghajafari *et al.*, 2013). It is therefore advisable to consider supplementation in those found to be deficient. However, there is little published data to assess the effectiveness of vitamin D in improving the chance of live birth.

Prolactin testing, androgen testing, ovarian reserve testing, luteal phase insufficiency testing, luteinising hormone (LH) testing and homocysteine plasma levels are not recommended for patients with unexplained RPL due to the paucity of evidence associating these and RPL and the absence of any prospective or randomised data (ESHRE, 2017).

1.3.12 UTERINE STRUCTURAL ANOMALIES

An association between congenital uterine anomalies and RPL has been reported (Saravelos *et al.*, 2008). Arcuate uteri are associated with second trimester losses whereas septate uteri are associated with first trimester miscarriages when compared to women with a normal uterus (ESHRE, 2017). Other congenital uterine anomalies include unicornuate, didelphic and bicornuate uteri.

Acquired uterine anomalies such as myomas or endometrial polyps have also been found to be prevalent in those with RPL but the real clinical significance of this is unclear.

Three-dimensional USS (3-D USS) is now the gold standard and should be offered to RPL patients as it allows visualisation of the inside and outside of the uterus and gives high definition pictures. 3-D USS should be used wherever possible due to higher specificity and sensitivity to two-dimensional (2-D USS). If pelvic ultrasound reveals an anomaly then combined hysteroscopy and laparoscopy are useful for diagnosis (Saravelos *et al.*, 2008).

Sonohysterography is also accurate in diagnosing congenital uterine anomalies and will provide more information than a hysterosalpingogram or an ultrasound scan as saline is used to improve the ultrasound images. This can be uncomfortable for the patients but may help to avoid the need for more invasive procedures with more risks such as a laparoscopy (ESHRE, 2017).

Surgery for congenital uterine anomalies can be considered however surgery may be at the cost of an effect on fertility and there have been no adequately powered randomised controlled trials to confirm efficacy as recruitment to these trials has proven to be not feasible.

There are no well powered studies on the effect of surgery for fibroids in those with RPL. There has been work done to show that subserosal and intramural fibroids are unlikely to contribute to RPL but in selected patients treatment of submucous fibroids may reduce the risk of miscarriage (Jaslow, 2014).

Overall there is no conclusive evidence that treatment of endometrial polyps or fibroids leads to improved outcomes for those with RPL.

Intrauterine adhesions from previous uterine surgery are often seen in those with RPL and small observational studies have recommended removal of adhesions (Jaslow, 2014), but further work is required before confidently recommending treatment which may actually predispose to further adhesions.

1.3.13 MALE FACTOR

Many studies have shown a link between lifestyle factors such as obesity, smoking and alcohol consumption and sperm quality (Anifandis *et al.*, 2014), (Li *et al.*, 2011). Medication such as selective serotonin reuptake inhibitors, corticosteroids and antibiotics are known to have effects on sperm function (Sharma *et al.*, 2013). The effects of these medications are reversible and so it is important to take a detailed history from the male.

We also know that semen from couples with RPL have been shown to have reduced viability, morphology and progressive motility (Ruixue *et al.*, 2013). Therefore, it is reasonable to address male lifestyle factors when assessing a couple with RPL. Advise on a healthy diet, exercise, the intake of antioxidants such as Vitamin C and E and minerals such as iron and zinc will help reduce levels of reactive oxygen species (ESHRE, 2017).

Recent studies have looked at male genetic defects such as DNA fragmentation and Y chromosome deletions. Robinson et al have performed a meta-analysis of studies looking at sperm DNA damage. This showed a significant increase in miscarriage rates in patients with high sperm DNA damage when compared to low

sperm DNA damage (RR 2.16; 95%CI 1.54 – 3.03) (Robinson *et al.*, 2012). However, the best test to detect sperm DNA damage has yet to be determined. There have been not been any randomised controlled trials of antioxidants that have demonstrated an improved live birth rate. DNA damage is aggravated by smoking and obesity and so again, it is important to assess male lifestyle factors in RPL clinics.

1.3.14 STRESS

There are no high quality studies assessing the association between maternal stress and risk of miscarriage. It is also difficult to ascertain cause or effect in this situation.

1.3.15 UNEXPLAINED RECURRENT PREGNANCY LOSS

Unfortunately, a cause for RPL is not found for the majority of patients attending the RPL clinic. To get an overview of treatment options available for such patients I performed a PubMed search of RCT's and systematic reviews conducted over the last ten years evaluating the treatment for preventing miscarriage or improving LBR in those with unexplained RPL. This PubMed search was by no means an in-depth or comprehensive review of all available evidence but gave me some insight into the research conducted within this area.

The Pubmed search was as follows:

```
Search (((fetal loss[Title/Abstract]) OR (((((recurrent miscarriages[MeSH Terms])  
OR ((recurrent[Title/Abstract]) AND miscarr*[Title/Abstract]))) OR  
((RECURRENT[Title/Abstract]) AND ((spontaneous abortion[MeSH Terms]) OR  
early pregnancy loss[Title])))) OR (((habitual abortion[MeSH Terms]) OR habitual  
abortion[Title/Abstract]) AND "last 10 years"[PData] AND Humans[Mesh])) AND "last  
10 years"[PData] AND Humans[Mesh])) AND TREAT*[Title]
```

Comment | Office|: I can confirm that my search was fetal loss with a space in it – had saved search criteria.

The search revealed two systematic reviews looking into IVIG for unexplained primary RPL which concluded that they are of no benefit in this patient group (Ata *et al.*, 2011; Hutton *et al.*, 2007). One of these systematic reviews concluded that there is a significant increase in LBR in those with secondary RPL (Hutton *et al.*, 2007) but this finding was not replicated in 2 RCT's done more recently (Christiansen *et al.*, 2015; Stephenson *et al.*, 2010).

There has also been report of a higher LBR if IVIG is started pre-conceptually in the follicular phase before pregnancy (Hutton *et al.*, 2007) but this conclusion did not apply when data from women with 3 or more miscarriages was exclusively analysed (Coulam *et al.*, 1995; Stephenson *et al.*, 1998).

Progesterone is essential to maintain pregnancy (Saccone *et al.*, 2017) and it has been suggested that deficiency of the hormone in the luteal phase may be linked to miscarriage. However, the PROMISE trial has shown that progesterone does not increase LBR when compared to placebo in those with unexplained RPL (Coomarasamy *et al.*, 2015). Fortunately, there was no increase in frequency of adverse effects with progesterone when compared to placebo. Further work may be needed on the type, route and dose of progesterone used.

Another systematic review looking into progesterone for unexplained RPL by Saccone *et al* (Saccone *et al.*, 2017) included 10 RCT's and 1586 women which actually concluded that progestogens do reduce the risk of RPL (RR 0.72, CI 0.53 – 0.97) and result in a higher LBR (RR 1.07, CI 1.02 – 1.15). However, this systematic review included seven studies which were done before 1990, only eight were double blind, more than half of the patients came from one trial and different preparations, doses and routes of progesterone were used. Therefore, it was concluded that the route or dose that would have a significant effect cannot be commented on. Further trials would be needed to address this question.

The role of anticoagulants have also been tested in the quest to find a combination that would successfully treat patients with unexplained RPL. The PubMed search revealed RCT's looking at LMWH + folic acid versus folic acid alone (Shaaban *et al.*, 2017), LMWH versus placebo (Pasquier & Martin, 2015) and LMWH versus multivitamins (Schleussner & Petroff, 2015) which all showed slightly inconsistent results. This was likely to be confounded by different outcome measures either being 'take home baby' rate, live birth rate and ongoing pregnancy at 24/40 respectively.

A systematic review looking at aspirin and/or heparin to treat those with or without inherited thrombophilia (de Jong *et al.*, 2014) concluded that anticoagulants for unexplained RPL cannot be supported. There was also a similar conclusion in a systematic review where there were similar birth rates in the aspirin and placebo group (Kaandorp *et al.*, 2009).

Lymphocyte immunotherapy has been evaluated as a treatment option for unexplained RPL based on immunological mechanisms thought to reject the 'allograft embryo' (Cavalcante *et al.*, 2017). A systematic review and meta-analysis performed in 2017 supported the efficacy and safety of lymphocyte immunotherapy for unexplained RPL (Cavalcante *et al.*, 2017), however, there was not a vigorous assessment of the quality research in this meta-analysis

In 2016 Liu *et al* conducted a meta-analysis including 18 RCT's which showed that lymphocyte immunisation significantly improved LBR (OR 5.25, CI 4.16 ~6.64). LBR was improved whether paternal lymphocytes or unrelated donor lymphocytes were used (Liu *et al.*, 2016). Again there was inadequate assessment of trial quality in this meta-analysis. When the more vigorous Cochrane meta-analysis was undertaken there was no benefit over placebo from leucocyte immunisation in terms of improving LBR (Wong *et al.*, 2014)

Human Chorionic Gonadotrophin (HCG), granulocyte colony stimulating factor (GCSF) and luteinising hormone (LH) suppression have all also been evaluated as treatment options for unexplained RPL (Cavalcante *et al.*, 2017; Morley *et al.*, 2013; Scarpellini & Sbracia, 2009) . None of the randomised, placebo controlled trials from this PubMed search demonstrated that these interventions led to an improvement in pregnancy outcome.

Many other smaller studies investigating other treatment options were found in this search such as treatment of chronic endometritis, treatment of fibroids and treatment of varicoceles which although gave new information to build from, did not give any definite treatment strategies to implement.

A Systematic review looking at Chinese herbal medicine (Yang *et al.*, 2013)also concluded that it is not possible to recommend such treatment for unexplained RPL. There was however much heterogeneity and bias in the studies included in this systematic review.

Performing this PubMed search provided me with useful insight into the almost infinite quest to find a treatment for unexplained RPL. Overall, Lymphocyte immunisation, IVIG, prednisolone, heparin, aspirin, progesterone, intralipid and G-CSF have all been tested in those with unexplained RPL but there is not enough good quality evidence to recommend any of these options in those with unexplained RPL.

1.3.16 RECURRENT MISCARRIAGE: PSYCHOLOGICAL MORBIDITY

Recurrent pregnancy loss is a debilitating disorder, associated with considerable psychological morbidity. Miscarriage is associated with feelings of loss and grief and these feelings are intensified with each loss.

A national survey of public perceptions of miscarriage including over 1000 participants showed that over 40% felt they had done something wrong, nearly 50% felt guilty and 28% felt ashamed (Bardos *et al.*, 2015). These emotions then lead to low mood and anxiety.

A third of women attending recurrent pregnancy loss clinics are clinically depressed with 20% having high levels of anxiety (Rai & Regan, 2006). A prospective survey study has shown that 39% of women suffering RPL meet the criteria for moderate to severe post-traumatic stress disorder (PTSD) and 20% meet the criteria for moderate – severe anxiety compared to 10% of the general population (Farren *et al.*, 2016). PTSD was higher at 3 months after the event compared to 1 month after the event. It is important to appreciate that going through miscarriage has an impact on the quality of life, relationships and physical health.

It has been shown that compassionate care, acknowledgement of reproductive history and recognition that RPL is a significant life event, will affect an individual's experience in the RPL clinic.(Musters *et al.*, 2013).

1.3.17 THE RECURRENT PREGNANCY LOSS CLINIC

This clinic offers specialist investigations, research opportunities and possible treatment to those with RPL. Along with specialist medical staff there may also be psychologists accessible for the couples.

At the RPL clinic at University Hospital Coventry and Warwickshire patients are first seen by Professor Quenby. The patients' reproductive history is established, a medical and family history is noted and a plan for investigations are made.

Patients are aware that despite extensive investigations, often a direct cause is not actually found. This is understandably distressing for patients even though unexplained RPL has a good prognosis for a future successful pregnancy outcome.

Patients are offered entrance into research trials which will potentially give them the opportunity to try new treatment or at least, support the feeling of helplessness.

Regardless of treatment plans or entrance into research studies, plans for support are made for the subsequent pregnancy. All patients have the contact details for the Biomedical Research Unit where research midwives are available for support.

Patients are able to call once they have a positive pregnancy test and an early ultrasound scan is booked for 6 weeks gestation with regular 2 weekly follow up for ultrasound scans and supportive care until 12 weeks' gestation.

1.3.18 WHAT WE KNOW SO FAR

Despite many randomised controlled trials having been undertaken in RPL, the only treatment with demonstrated significant efficacy for the prevention of RPL is heparin and aspirin for those women with APLS (Empson *et al.*, 2005). APLS occurs in only 15% women with RPL thus there is no effective treatment for 85% of RPL patients.

This brings us onto the research being conducted at The University of Warwick into reasons behind RPL. This paradigm is based on the fundamental relationship and interaction between the conceptus and the endometrium to help achieve a successful pregnancy.

1.4 THE HUMAN ENDOMETRIUM

The human endometrium is one of the most dynamic human tissues (Du & Taylor, 2009) undergoing approximately 400 cycles of regeneration in a woman's lifetime (Du *et al.*, 2012; Mutlu *et al.*, 2015). Not only does the endometrium undergo monthly shedding, regeneration and differentiation with menstrual cycles but also regenerates following childbirth, endometrial resection and in post-menopausal women taking hormone replacement therapy (Gargett, 2007).

The endometrium is made of an upper superficial layer called the functionalis and a lower deeper layer called the basalis. The basal layer contains glands and small arteries which are embedded in stroma. The stroma is made up of fibroblasts and collagen which make up the extracellular matrix providing a structural framework for the tissue and is vital for wound healing.

Implantation in humans has always been largely described as a process where the blastocyst firstly adheres to the endometrium and then breaches and invades through the luminal epithelium. With this view, the maternal tissues need to tolerate the blastocyst. Inadequate invasion can lead to complications such as miscarriage and on the converse, if invasion through maternal tissues is excessive then this too can lead to unfortunate circumstances such as placenta accreta (Quenby & Brosens, 2013).

Emerging insights into the cyclic changes to the endometrium and the mechanisms that govern embryo implantation have provided new ontological dimensions to early pregnancy loss. We now know that implantation of the human embryo is much more dynamically controlled by the endometrium than previously appreciated (Quenby & Brosens, 2013).

1.4.1 IMPLANTATION AND DECIDUALISATION

Successful implantation is believed to be the result of appropriate preparation of the endometrium for pregnancy (decidualisation) and also as a result of appropriate signalling between the conceptus and the endometrium in that particular cycle (Lucas *et al.*, 2016b).

Decidualisation is a process whereby stromal fibroblasts transform into secretory

cells (Macklon & Brosens, 2014). It is a transformative process which involves the endometrium passing through a pro-inflammatory phase through to an anti-inflammatory phase, all prior to conception, in the mid luteal phase of the menstrual cycle (Lucas *et al.*, 2016a).

Pro-inflammatory signals trigger the expression of genes for endometrial receptivity. This is followed by an anti-inflammatory response driven by the secretion of progesterone which is essential for the support of an embryo. (Salker *et al.*, 2012). Usually these pro-inflammatory and anti-inflammatory waves within a menstrual cycle mark a narrow window of implantation. Within this narrow window of implantation, the endometrial environment is optimised for the successful implantation of the blastocyst (Salker *et al.*, 2012). A narrow window of implantation with an appropriately receptive and selective endometrium is vital for meaningful implantation.

Menstrual cycles, monthly endometrial regeneration and an optimised implantation window all come together as a method of quality control for any new fertilisation. The endometrium is normally able to mount an implantation response tailored to each distinct pregnancy. (Brosens *et al.*, 2014). The cumulative birth rate for patients with RPL is high which suggests that the embryo-endometrial interface actually adapts and changes with each cycle allowing a pregnancy to be finally successful.

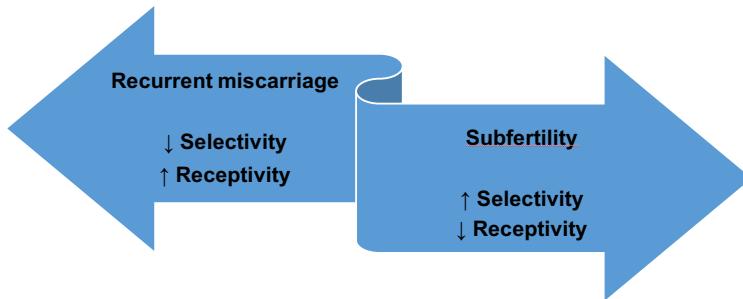
Decidualisation is vital for the successful preparation of pregnancy and recent studies show that the hallmark for RPL is aberrant decidualisation of the endometrium (Salker *et al.*, 2010; Salker *et al.*, 2012).

In patients with RPL the window of implantation is disordered and prolonged, leading to 'super fertility' with a high rate of early pregnancy loss due to disabled embryo selection (Lucas *et al.*, 2016a). In this situation, endometrial stromal cells do not respond to deciduogenetic cues in the post-ovulatory phase of the menstrual cycle resulting in a defect in cellular maturation and this leads to abnormal decidualisation (Lucas *et al.*, 2016b). Abnormal decidualisation is characterised by a prolonged inflammatory response, causing the loss of a selectivity checkpoint, rendering the endometrium to be excessively receptive but unable to sustain the pregnancy (Gellersen & Brosens, 2014).

On the opposite side of the spectrum is when the decidualisation process is far too excessive. This restrains receptivity and leads to conception delay as seen in

subfertility patients. (Macklon & Brosens, 2014).

The concept of super-fertility is supported by the common theme we see in RPL clinics where patients state they are able to get pregnant very easily however, as they have a prolonged and disordered window of implantation they then miscarry very early too due to abnormal embryo selection. 40% of patients with RPL can achieve a pregnancy within three cycles (Orlando & Coulam, 2014). This is in contrast to the subfertility patients who struggle to get pregnant because the endometrium is far too selective and not receptive.



This is a diagrammatic representation of the spectrum of reproductive difficulties. For a successful pregnancy, there needs to be a balance of receptivity and selectivity.

1.4.2 EMBRYO SELECTION AND IMPLANTATION

Human embryos have been shown to be exceptionally diverse. Blastocyst analysis has shown that the transfer of mosaic embryos can still lead to a successful pregnancy with a healthy euploid baby at birth (Greco *et al.*, 2015). This finding has emphasised the presence of an intrinsic and distinct pre-implantation selection process whereby those blastocysts even with mosaicism implant, adapt and result in successful outcomes.

Normally, human endometrial stromal cells (HESC's) become sensitive to embryonic protease signals upon decidualisation and respond to low quality embryos by inhibiting secretion of implantation factors such as cytokines and growth

factors such as interleukin-1 beta (IL1- β) and heparin-binding EGF-like growth factor (Teklenburg *et al.*, 2010).

HESC's therefore, serve as biosensors of embryos that have breached the luminal epithelium (Brosens *et al.*, 2014; Teklenburg *et al.*, 2010).

This observation leads to the concept of 'natural selection'. Appropriately decidualised endometrium responds to embryonic signals and either supports development of the embryo (positive selection) or leads to pregnancy failure through menstruation-like shedding (negative selection) (Brosens *et al.*, 2014; Lucas *et al.*, 2016b). This process protects maternal resources to invest in a future successful pregnancy.

So, appropriately decidualising HESC's migrate towards the trophoblast rather than the trophoblast invading the endometrium as previously considered (Gellersen *et al.*, 2013). The embryo will then implant at receptive sites where HESC's have migrated. HESC's then encapsulate the conceptus and create a specific environment for optimal implantation and development (Quenby & Brosens, 2013).

This concept of endometrial cell migration and the mutual attraction between the embryo and endometrium to select and invest in the best quality embryo has also been demonstrated by Weimer *et al* (Weimar *et al.*, 2012). In vitro studies of fertile women have shown decidualising cells normally selectively migrate to high quality embryos but not low quality ones but decidualising cells from patients with miscarriage were not able to discriminate between embryo quality (Weimar *et al.*, 2012).

These concepts demonstrate that in RPL, rather than maternal rejection of the embryo there is actually a lack of correct selection so abnormal embryos that should be lost with menstruation are allowed to implant. The patient then suffers pain, bleeding and pregnancy loss. In addition, normal embryos are more supported and are also at risk of miscarriage hence suboptimal selection at implantation increases the rate of miscarriage.

Figure 1.0.2: Embryo Encapsulation by Decidualising Stroma

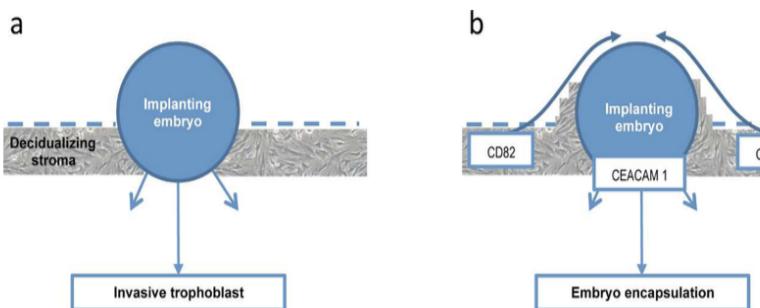
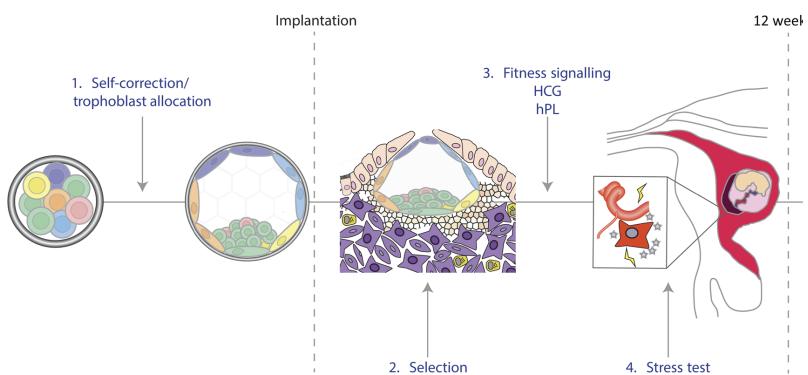


Figure 1.0.2 taken from (Quenby & Brosens, 2013) shows embryo encapsulation rather than invasion of the trophoblast into the decidualising stroma.

It makes sense that there are several check points and stress tests for an embryo to overcome for maternal investment into pregnancy. Intrinsic biomarkers and signalling between the embryo and the endometrium lead to self-correction of mosaicism and implantation (Lucas *et al.*, 2016a). The syncytiotrophoblast cells must secrete enough β HCG to help maintain progesterone secretion from the corpus luteum. The next stress test is when the placenta must take over to support the pregnancy. If there is a failure of the pregnancy to overcome the burden of any of these checkpoints it results in a clinical miscarriage. This does not however explain the reason for pregnancy loss prior to implantation (Macklon & Brosens, 2014).

Figure 1.0.3: Embryo Selection at Implantation



The diagram in figure 1.0.3, taken from (Lucas *et al.*, 2016a) shows the check points the embryo must pass to prevent a prolonged commitment into a pregnancy that will fail. Initially blastocysts are often mosaic. A combination of embryo self-correction and decidualised endometrial cells appropriately screening for embryo quality are the first hurdles to overcome followed by sufficient β HCG secretion and then the inception of placental perfusion at the placental-endometrial interface.

1.5 STEM CELLS AND THE ENDOMETRIUM

Stem cells are undifferentiated cells, present in most organs, capable of self-replication and differentiation into multiple cell types (Watt & Hogan, 2000). Their unique property of self-renewal and differentiation has generated a lot of interest all over the world in their potential use in injured and damaged tissue.

The first study demonstrating the presence of mesenchymal stem cells (eMSC's) in the endometrium was reported only 12 years ago (Chan *et al.*, 2004). Stem cell populations in the endometrium are highly dynamic because of their continuous recruitment and activation in response to menstruation, miscarriage, or parturition (Gellersen & Brosens, 2014; Macklon & Brosens, 2014). Normally, monthly menstrual cycles lead to the activation of endometrial stem/progenitor cells, tissue regeneration and maturation of endometrial stromal cells (Lucas *et al.*, 2016b).

There are three different types of endometrial stem cells; epithelial progenitor cells, endometrial mesenchymal stem cells and endothelial progenitor cells (Gargett & Masuda, 2010). Endometrial mesenchymal stem cells are shed during menstruation (Gargett & Masuda, 2010).

Research conducted recently at the University of Warwick has shown that RPL is strongly associated with endometrial mesenchymal stem cell deficiency. The endometrium in those with RPL lacks plasticity due to stem cell deficiency, increased cellular senescence and limited differentiation potential (Lucas *et al.*, 2016b). Low levels of eMSC's, cellular senescence, disordered inflammation all contribute to abnormal endometrial decidualisation (Lucas *et al.*, 2016b).

These findings are based on results from clonogenic assays performed on endometrial biopsies of patients attending the implantation clinic. These patients either have a history of RPL or of recurrent implantation failure. Figure 1.0.4 shows that those patients with less than 3 miscarriages (control) have a higher number of eMSC's (assessed by a clonogenic assay) compared to patients with three or more miscarriages (RPL).

FIGURE 1.0.4: Stem Cell-Ness of the Endometrium in Those with RPL.

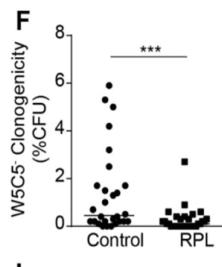
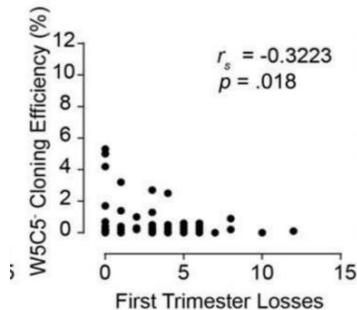


Figure 1.0.5 taken from (Lucas *et al.*, 2016b) shows that the stem cell-ness of the endometrium decreases with increasing numbers of miscarriages.

FIGURE 1.0.5: Number of First Trimester Miscarriages and the Relationship to the eMSC Count



(Lucas *et al.*, 2016b)

Endometrial stroma consists of MSC's, fibroblasts and senescent cells. This stem cell deficiency at the endometrium has an impact on the overall composition of the endometrial stroma. Accumulation of senescent cells in a stroma deficient in clonogenic stem cells results in the release of inflammatory cytokines and chemokines (Acosta *et al.*, 2013).

This environment negatively impacts the plasticity of the endometrium for appropriate signalling to occur between the endometrium and the conceptus.

Endometrial stem cell deficiency is the foundation for my research project. We wanted to develop a treatment for endometrial stem cell deficiency to improve the environment for implantation.

1.6 MECHANISMS OF STEM CELL RECRUITMENT

Intrinsic inflammatory actions lead to stem cell recruitment. Bone marrow mesenchymal stem cells (bmMSCs), and (eMSCs) have been shown to be highly proliferative and migratory (Khatun *et al.*, 2017).

eMSC's have been shown to reside in the perivascular space of the endometrium contributing to monthly regeneration with menstrual cycles (Gargett *et al.*, 2009; Gargett & Ye, 2012). These stem cells are unique, highly proliferative, have the ability for self-renewal and can differentiate into other cell lineages such as osteocytes and chondrocytes. (Gargett *et al.*, 2009). It is thought that endometrial stromal fibroblasts (eSFs) which are the most common cell type in the endometrial stroma are descendants of eMSC's.

Several other studies have shown that eMSCs actually originate from the bone marrow and migration of these cells towards the endometrium occurs in response to tissue injury (menstruation). Here they differentiate into eMSC's contributing to endometrial regeneration (Taylor, 2004).

1.6.1 CXCL12 AND CXCR4 PATHWAY

Cytokines and chemokines released in response to hypoxia that occurs in the secretory phase of the menstrual cycle are thought to enhance recruitment of bmMSC's to the endometrium (Hu *et al.*, 2013). bmMSC's are undifferentiated and have been shown to travel to distant organs to contribute to tissue repair (Du & Taylor, 2007).

The first study to report bone marrow derived endometrial stem cells at the endometrium looked at the endometrial biopsies of those who received HLA-mismatched bone marrow transplants (Taylor, 2004). Donor endometrial cells (epithelial and stromal cells) were detected in the biopsy from all bone marrow recipients suggesting that bone marrow derived stem cells contribute to endometrial regeneration (Taylor, 2004).

In 2012, Du *et al* were able to show that when male to female bone marrow transplantation was performed in mice, a uterine injury resulted in a 2-fold increase in bone marrow derived stem cell recruitment to the endometrium (Du *et al.*, 2012).

This effect was independent of whether oestrogen was present or not suggesting that these bone marrow stem cells are recruited at times of injury but not with monthly cyclic regeneration of the endometrium in mice.

Bone marrow derived stem cells have also been shown to travel to help the repair and regeneration of diabetic skin wounds in mice (Castilla *et al.*, 2012).

One of the postulated methods of stem cell recruitment from the bone marrow is via the CXCL12 and CXCR4 pathway. The CXCL12 (SDF-1) and CXCR4 pathway has been shown to be vital for chemo-attraction of bone marrow derived mesenchymal stem cells to peripheral tissues (Döring *et al.*, 2014; Wang *et al.*, 2015). It has been repeatedly shown that when CXCL12 production is increased at sites of injury in the body, for example in bone injury or myocardial injury, stem cell recruitment is enhanced allowing wound healing and functional recovery (Liu *et al.*, 2013; Penn *et al.*, 2012). This highlights the activation of this pathway in many areas of the body in response to injury.

CXCL12 is also produced by endometrial stromal cells and is the primary chemokine that recruits bone marrow derived cells to the uterus. CXCR4 is the receptor for CXCL12 expressed on the surface of bmMSC's to modulate migration of stem cells to the endometrium (Sahin Ersoy *et al.*, 2017; Wang *et al.*, 2015). The interaction between CXCR4 and CXCL12 is vital for stem cell mobilisation (Sahin Ersoy *et al.*, 2017).

An antagonist to CXCR4 has been shown to block migration of stem cells indicating that the CXCR4 receptor is vital for chemoattraction of bone marrow cells to human endometrial cells (Wang *et al.*, 2015).

Also, oestradiol is known to induce the expression of CXCL12 and CXCR4 indicating that stem cell recruitment would actually increase in response to hormonal changes with the menstrual cycle.

1.6.2 REGENERATION FROM THE BASALIS LAYER

It has also been postulated that eMSC's reside in the endometrial basalis and help to maintain the regenerative capacity of the endometrium each month. Hormonal changes with the ovarian cycle and increase in oestradiol is thought to result in the migration of endogenous stem cells to the functional layer of the endometrium to

help with regeneration of the endometrium every month (Chan *et al.*, 2004).

Gargett et al have shown that the human endometrium contains small populations of epithelial and stromal stem cells which are clonogenic and are involved in monthly regeneration of the glands and stroma. Gargett et al used a label retaining cell method to identify stem cells and their locality. This experiment demonstrated epithelial and stromal stem cells in mouse endometrium.

This theory is also supported by a study which showed that although MSC's from the bone marrow help with repair after uterine injury, these specific MSC's are not involved in this cyclic regeneration of the endometrium with menstruation. This was demonstrated in mice studies where endometrial repair with bmMSCs occurred with or without oestrogen (Du *et al.*, 2012).

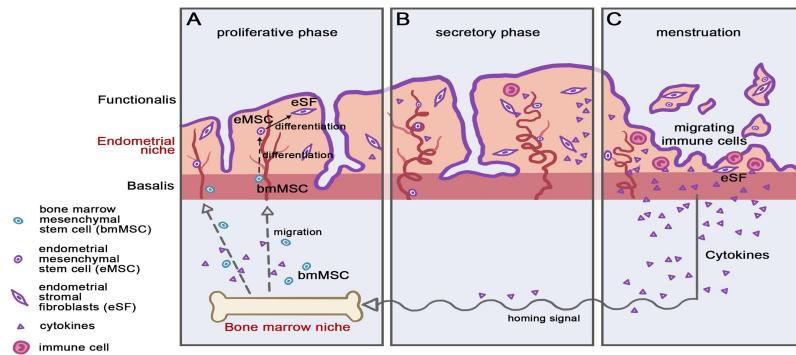
It appears that local signals may be more important in enhancing mobilisation of bmMSCs to sites of injury rather than hormonal changes with monthly menstrual cycles.

1.6.3 OTHER EVIDENCE

A recent study by Khatun et al reported an experiment where eMSC's from endometrial biopsies and bmMSC's from bone marrow aspirates were studied. Khatun et al looked at surface marker characteristics, migration potential and cytokine profiles between bmMSC's, eMSCs and eSFs. This study demonstrated that eMSC's are more likely to originate from the bone marrow rather than descending from the haematopoietic stem cell lineage because all three cell types were negative for haematopoietic cell surface antigens. It has been demonstrated that both bmMSCs and eMSCs have similar surface marker profiles with high levels of migration towards areas of inflammation.

Figure 1.0.6 taken from (Khatun *et al.*, 2017) shows the migration of bmMSCs from the bone marrow to the endometrium in the proliferative phase of the menstrual cycle after signals are received by the bone marrow at a time of menstrual injury to the endometrium. Differentiation to eMSC's and eSF's then occurs.

Figure 1.0.6: Migration of bmMSC's to the Endometrium



It has also been shown that bmMSCs are recruited by IL-1 β . This cytokine is highly expressed during hypoxia and menstruation and may be a recruiting signal to bmMSCs (Rossi *et al.*, 2005). It appears that IL-1 β is able to trigger migration of bmMSCs **and** eMSCs to areas of inflammation (Khatun *et al.*, 2017). Interestingly, IL-1 β also promotes CXCL12 expression (Peng *et al.*, 2006) and as mentioned above, CXCL12 produced by endometrial stromal cells is the primary chemokine that recruits bone marrow derived cells to the uterus.

1.6.4 INCREASING STEM CELL RECRUITMENT

Migration of bone marrow derived stem cells to the endometrium is known to be enhanced after ischaemic injury (Wolff *et al.*, 2011). The potential of increasing mesenchymal stem cells at the endometrium as treatment has been demonstrated in both animals and humans.

Infusion of stem cells into the uterus of women with Asherman's syndrome has shown to increase the endometrial thickness in women undergoing IVF (Nagori *et al.*, 2011; Singh *et al.*, 2014). Studies on mice with simulated Asherman's syndrome have shown that after a male bone marrow transplant, there is evidence of Y+ cells in the endometrium and also improved conception rates (Alawadhi *et al.*, 2014).

The discovery of endometrial mesenchymal stem cell deficiency in those with recurrent miscarriage has provided further insight into the cause of abnormal decidualisation patterns of the endometrium. Stem cell recruitment can be enhanced and this could potentially allow improved decidualisation and an improved environment for implantation which in turn could improve pregnancy outcomes.

1.7 DPP4 INHIBITORS

DPP4 inhibitors are new class of medication for glycaemic control in diabetic patients. They are well accepted and tolerated by diabetic patients because they are taken orally (rather than by an injection) and are safe. They also have advantages of not causing weight gain or hypoglycaemia (Ahrén, 2007).

DPP4 inhibitors are particularly relevant here because inhibition of DPP4 retards the degradation of CXCL12 and therefore should increase the recruitment of stem cells to sites of injury through enhancement of the CXCL12-CXCR4 axis (Brenner *et al.*, 2014; Jungraithmayr *et al.*, 2012).

A mouse model found that DPP4 inhibitors increased stem cell recruitment to the lung after an ischemic-reperfusion injury by enhancing the CXCL12-CXCR4 axis (Hopman & DiPersio, 2014).

Furthermore, DPP4 expression has been found to be higher in the endometrium from women with RPL compared to control subjects (Lucas *et al.*, 2016b). This is demonstrated in figure 1.0.7. This may explain the lack of stem cell recruitment due to curtailment of the CXCL12/CXCR4 pathway. This also suggests that DPP4 inhibition would be beneficial in women with repeated miscarriage.

figure 1.0.7: DPP4 Expression at the Endometrium in those with Recurrent Miscarriage.

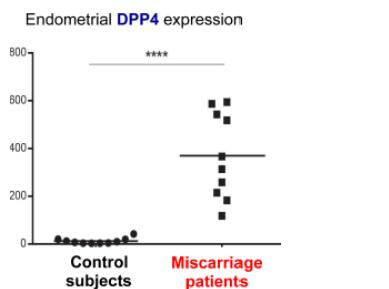


Diagram adapted from (Lucas *et al.*, 2016b)

1.7.1 SAFETY OF DPP4 INHIBITORS

So far, there is good data to suggest that DPP4 inhibitors are not only safe but also beneficial. A meta-analysis has shown that DPP4 inhibitors may decrease the risk of adverse cardiovascular events which diabetic patients are (Patil *et al.*, 2012).

The TECOS trial, published in the New England Journal of Medicine in July 2015 also looked at the most commonly used DPP4 inhibitor; Sitagliptin and cardiovascular outcomes (Green *et al.*, 2015). This large randomised double blind placebo controlled trial revealed very reassuring data about the safety of Sitagliptin. Over 14,000 patients were assigned to either Sitagliptin or placebo. This RCT showed that the use of Sitagliptin in diabetic patients, who are already at high cardiovascular risk, did not increase the risk of cardiovascular complications. Sitagliptin was also not associated with a significant increase in the rate of severe hypoglycaemia when compared to placebo.

There have been case reports of pancreatitis associated with the use of Sitagliptin. The incidence is not known but presumed to be very low (Merck & Co, 2015). The TECOS trial also showed no significant difference in rates of acute pancreatitis in the Sitagliptin and placebo groups.

Additional effects of DPP4 inhibitors are to improve endothelial function, reduce pro-oxidative and pro-inflammatory states which are all potentially favourable factors for

pregnancy (Avogaro *et al.*, 2014).

The Summary of Product Characteristics for Sitagliptin states that it does not cause hypoglycaemia when used as monotherapy. There is an increase in the risk of hypoglycaemia if used with other diabetic medication. There have been reports of severe arthralgia and some reports of hypersensitivity including anaphylaxis and exfoliative skin conditions. Adverse reactions occurring in over 5% of patients include an upper respiratory tract infection, nasopharyngitis and a headache. The safety of Sitagliptin in those under the age of 18 has not yet been established. Assessment of renal function is recommended before starting a patient on Sitagliptin as dose adjustments are needed for moderate or severe renal disease. With regards to drug interactions there is a small increase in the peak drug concentration of digoxin. No dose adjustment is necessary but if both digoxin and Sitagliptin are being taken, patients should be monitored carefully (Merck & Co, 2015).

With regards to safety in pregnancy, animal studies have shown that a dose 12 times higher than the normal recommended dose did not harm the developing fetus. Sitagliptin given to rats and rabbits in the period of organogenesis did not result in any teratogenic effects at 20-30 times the recommended maximum dose. At doses 100 times the recommended dose there was an increase in rib malformations in rat studies (Co, 2017).

Sitagliptin has been deemed as a category B drug for pregnancy by The Food and Drug Administration (FDA) Agency of the United States Department of Health and Human Services. This means that it should only be used if clearly needed. It is not known if Sitagliptin is secreted in breast milk so caution is advised.

Sitagliptin is the most commonly used DPP4 inhibitor. The recommended dose of Sitagliptin is 100mg once daily.

1.8 ENDOMETRIAL BIOPSY/SCRATCH

An endometrial scratch is a procedure commonly offered to patients undergoing IVF with an aim to improve pregnancy outcomes.

It is thought that an endometrial scratch may help with decidualisation and release cytokines and growth factors which are all important for successful implantation(Laird *et al.*, 2006; Tiboni *et al.*, 2011).

A meta-analysis has shown that clinical pregnancy rate in those couples having IVF is significantly improved when an endometrial scratch has been performed (El-Toukhy *et al.*, 2012) . A meta-analysis performed by Potdar et al in 2012 has also shown that endometrial injury improves the LBR for those women going through IVF(Potdar *et al.*, 2012). This finding needs to be confirmed with a well conducted randomised controlled study. Also, the effect of an endometrial scratch on pregnancy outcome has not yet been assessed in an RCT for patients with RPL.

It is important to note that the endometrial scratch in itself may act as an injury promoting stem cell recruitment as part of a wound healing response.

1.9 CONCLUSION

A national survey carried out by Bardos et al revealed that 78% of patients want to know a cause for their miscarriage even if there is no intervention to prevent it as this may affect their psychological response (Bardos *et al.*, 2015). The James Lind Alliance; a priority setting partnership between patients and health care professionals identified new treatments to prevent miscarriage as the number one research priority in this area (Prior *et al.*, 2017). It is vital therefore that we continue to undertake discovery science into new causes and potential new treatments for miscarriage prevention in patients with RPL.

In this literature review I have demonstrated the need to improve the uterine, endometrial environment for implantation. I have demonstrated that a lack of stem cell-ness of the endometrium may contribute to the failure of implantation. I have found some evidence to support the hypothesis that DPP4 inhibitors could improve endometrial stem cell-ness by increasing eMSC recruitment by enhancing the CXCL12/CXCR4 pathway.

I have therefore designed, set up, managed and performed an early phase randomised feasibility study to investigate whether Sitagliptin and an endometrial scratch improves the stem cell-ness of the endometrium in those with RPL.

CHAPTER 2: TRIAL DESIGN AND PROTOCOL DEVELOPMENT

2.1 AIMS AND OBJECTIVES

In my literature review in chapter one I have demonstrated the existence of a complex interaction between the endometrium and the blastocyst.

There is a lack of eMSC's and an increased expression of DPP4 in the endometrium of women with recurrent miscarriage.

An increase in stem cell recruitment to the endometrium should improve the process of decidualisation, optimise the balance of endometrial receptivity and selectivity and in turn improve the quality of the endometrium and its ability to support implantation and early pregnancy.

Currently, most treatment options for patients with RPL start once they have had a positive pregnancy test. The aim of this study is to develop a pre-conception treatment aimed at enhancing the environment for implantation for those with recurrent miscarriage.

We **hypothesised** that DPP4 inhibition with Sitagliptin given prior to conception will increase eMSC recruitment above that of endometrial scratch, via an enhancement of the CXCL12-CXCR4 axis during menstruation. The DPP4 inhibitor Sitagliptin is cheap, safe and well tolerated by diabetic patients and widely used in the UK.

We set up a randomised, double blind feasibility study to test the **null hypothesis** that the mean number of colonies of stem cells per 1500 endometrial stromal cells for the control group (placebo group) is equal to that of the intervention group (Sitagliptin group).

In this study we have also assessed whether the DPP4 inhibitor Sitagliptin is well tolerated by non-diabetic individuals by performing a qualitative analysis of adverse events reported in each treatment group (Sitagliptin vs. placebo). We have also performed a qualitative analysis of the acceptability of the study design.

2.2 TRIAL PLANNING AND DESIGN

The Clinical Trials Toolkit published by the National Institute of Health Research (NIHR) was used to understand the legal and good practise requirements of setting up a Clinical Trial of an Investigational Medicinal Product (CTIMP) (NIHR, 2017).

This toolkit describes the importance of having a robust trial design to ensure a clinically useful trial outcome. It also states the need to have a trial design which is conceptually simple and tailored to the patient group.

2.2.1 BASIC TRIAL IDEA:

The foundation of this feasibility study was to assess whether the eMSC increased after 3 months of treatment with Sitagliptin when compared with placebo. Our primary outcome measure would therefore be a laboratory based one. We planned to perform the same clonogenic assay performed in the original research this study was based upon.

We considered other outcome measures such as live birth rate. This could not be justified however for many reasons. Primarily, we needed to test the hypothesis that DPP4 inhibition with Sitagliptin given prior to conception increases the eMSC count above that of an endometrial scratch. Without the laboratory based evidence we would not be able to scientifically explain any clinical results found.

Live birth rate could therefore not be used due to time constraints. The set-up of this study was going to take one year and we anticipated that recruitment of all participants would take just under one year. The study pathway lasted 3 months for each participant and so to then look at pregnancy outcomes once participants had finished their journey through the study would take at least another year.

We therefore focused the primary outcome measure on our hypothesis and decided to look at the acceptability of the study design and side effects experienced as part of our secondary outcome measures to help assess the feasibility of the study design for a larger pragmatic trial if the results were positive.

Various arms to this randomised study were considered. Essentially, we could look at randomisation to an endometrial biopsy and Sitagliptin for 3 months and randomisation to an endometrial biopsy and placebo for 3 months. We also considered randomisation to an endometrial biopsy with neither Sitagliptin or placebo (i.e. no treatment) or having either Sitagliptin or placebo for 3 months with only one endometrial biopsy at the end of the 3 months and no initial endometrial biopsy which could in itself positively or negatively affect the results.

We felt that having a third arm to the study of not receiving any medication (Sitagliptin or placebo) could result in difficulties with recruitment. Patients were more likely to accept the study if they had a 50% chance of receiving a treatment which may help reduce the risk of miscarriage.

We also decided that performing an endometrial biopsy on all participants to ascertain their baseline eMSC count would also allow us to ascertain how the eMSC count changed over the 3-month period in each participant. We do not know what defines a high or low eMSC cell count and so the initial endometrial biopsy would be particularly important in establishing baseline information and looking into whether previous data could be reproduced.

We felt a 3-month treatment period was a good compromise between allowing sufficient time for the eMSC count to increase and a suitable time period for participants to be committed to taking part in a clinical trial.

The flow diagram below represents the basic trial idea. It is important to note that there are 3 menstrual cycles within the treatment period where the endometrium would be regenerated.

I presented this basic trial design to the Research, Development and Innovation department at UHCW. They were keen to be involved in the development of the study design and help gain sponsorship from UHCW.

2.2.2 BASIC FLOW DIAGRAM FOR TRIAL PATHWAY

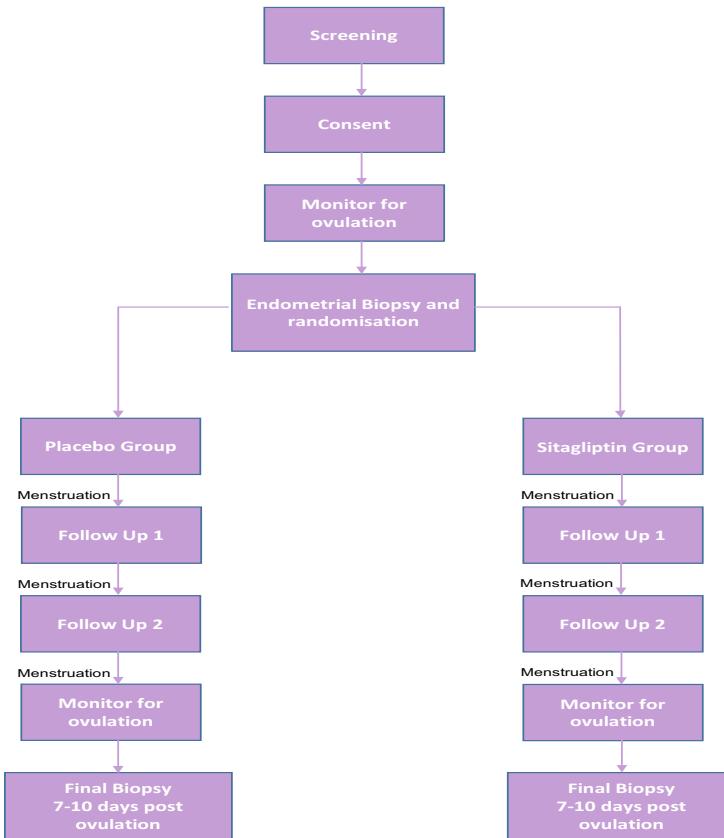


FIGURE 2.0.1: Basic Flow Diagram for the Trial Pathway

2.3 SAMPLE SIZE CALCULATIONS

To start this project, I looked at the original data that demonstrated the deficiency of stem cells at the endometrium of those with RPL.

I reviewed the results of clonogenic assays done at the Warwick Clinical Sciences Research Laboratory in 2014. There were results for 34 patients who had attended the Implantation Clinic, all with different reproductive histories in terms of number of miscarriages. The mean number of colonies of stem cells per thousand endometrial stromal cells in those without RPL (those with less than 3 miscarriages) was 14 colonies and the mean number of stem cells per thousand endometrial stromal cells in those with RPL (3 or more miscarriages) was 3.4 colonies.

The patients defined as having less than 3 miscarriages were patients who had attended the clinic with a history of less than 3 miscarriages or had a history of recurrent implantation failure related to primary or secondary subfertility. Some of these patients may have had a previous live birth and some may never have achieved a clinical pregnancy. The implications of this are discussed in chapter 10. My first task in setting up this trial was to establish an appropriate sample size to ensure it had sufficient power to test the null hypothesis. The null hypothesis was that the mean number of colonies of stem cells per thousand endometrial stromal cells for the placebo group is equal to that of the Sitagliptin group.

The trial statistician and I used the existing data to formulate accurate power and sample size calculations to form the basis of this clinical trial.

When 4 outlying observations for women without recurrent miscarriages were excluded because they may have been due to experimental error, the mean for women with no recurrent miscarriages was 7.0 colonies per thousand cells.

For the power calculation, we assumed that the mean number of colonies per 1000 cells would remain 3.4 for the control group and the number of colonies would increase to 6 for the intervention group (slightly less than the mean for the normal women). Using simulation, the power to detect this difference at 10% significance level was 95%.

We calculated that we would need to have a sample size of 30 participants for the study to have 95% power at a 10% significance level.

2.4 ETHICAL CONSIDERATIONS

Participants in this study would need to consent to having 2 endometrial biopsies and take oral trial medication every day for 3 months.

2.4.1 ENDOMETRIAL BIOPSY

Endometrial biopsies are taken with the Wallach endocell endometrial cell sampler, which is a simple and safe manual suction device routinely used to take endometrial biopsies in gynaecology clinics.

Endometrial biopsies take approximately three minutes to perform in an outpatient setting however, they are intrusive and cause discomfort. Hence, all women would be given verbal and written information regarding the biopsy procedure to ensure they were adequately informed of this prior to seeking their consent for this study and the biopsy.

In mitigations of discomfort of the procedure it is possible that the biopsy itself is beneficial to future pregnancy outcome. Along with the injury of menstruation it has been demonstrated in chapter one that performing an endometrial biopsy initiates a wound healing response which may enhance stem cell recruitment to the endometrium.

2.4.2 TRIAL MEDICATION SAFETY AND SIDE EFFECTS

Participants in this study would also need to consent to taking study medication every day. There was a 50% chance of this being Sitagliptin and a 50% chance of this being placebo. There are always ethical considerations to consider with the use of placebo but we also had to explore the safety of Sitagliptin for this non-diabetic patient cohort.

Sitagliptin is routinely used by patients with type 2 diabetes who often have multiple co-morbidities with a very low incidence of reported side effects. It is used as monotherapy or dual therapy in combination with insulin, metformin or sulphonylureas.

I studied the Summary of Product Characteristics (SPC) for Sitagliptin from the electronic Medicines Compendium (EMC) which contains up to date information about medicines licensed for use in the U.K. I was able to establish common and uncommon side effects, contraindications, interactions with other medication and pre-prescribing checks.

Sitagliptin was developed by Merck & Co (MSD). The lead pharmacist at UHCW for clinical trials requested information from MSD on the safety of Sitagliptin in healthy humans and also on the safety of Sitagliptin in pregnancy. These documents were reviewed thoroughly by myself, the pharmacy team and the Research and Development governance team at UHCW.

Sitagliptin has a high safety profile. The SPC states that the only common side effect (frequency $\geq 10\%$) is a headache. All other known side effects occur less frequently. Side effects occurring in approximately 5% include upper respiratory tract infections and nasopharyngitis. Up to 5% have been known to suffer with osteoarthritis or pain in extremities.

Side effects for which the frequency is unknown includes hypersensitivity, interstitial lung disease, vomiting, acute pancreatitis, angioedema, rash, urticaria, exfoliative skin conditions, arthralgia, myalgia, and impaired renal function. These have mainly been reported as individual case reports.

Some adverse reactions are observed mainly in those using a combination of Sitagliptin and other diabetic medication together rather than with Sitagliptin monotherapy. These include hypoglycaemia, influenza, nausea and vomiting, constipation, peripheral oedema and dry mouth.

2.4.3 SAFETY OF SITAGLIPTIN IN HEALTHY NON-DIABETIC INDIVIDUALS

The various RCT's looking into the safety of Sitagliptin in healthy patients showed that Sitagliptin monotherapy did not lead to hypoglycaemia (Devin *et al.*, 2014; Herman *et al.*, 2005; Mistry *et al.*, 2008), ECG changes (Mistry *et al.*, 2008) or altered immune function (Price *et al.*, 2013).

There were reports of transient light headedness and nausea which both resolved after stopping the medication. Overall there were no adverse drug events and

Sitagliptin was well tolerated at 25/50/100/200/400 mg doses (Bergman *et al.*, 2007).

The protocol for the study was peer reviewed by Dr O'Hare who is a consultant diabetologist. Dr O'Hare with clinical experience of using Sitagliptin confirmed that we would not need to monitor blood glucose levels of participants and confirmed safety of the medication in the healthy population.

2.4.4 SAFETY OF SITAGLIPTIN IN PREGNANCY

It was expected that participants entering this study would need to avoid pregnancy for the duration of the study to allow them to have two endometrial biopsies over 3 months and also allow time for this pre-conception treatment to take effect.

I did however have to consider the safety of Sitagliptin use in pregnancy in the event of pregnancy occurring in any of the participants while on study medication.

There is lack of human data on Sitagliptin to currently recommend its safe use in pregnancy however there are post marketing reports of exposure during pregnancy on the Merck & Co pregnancy register.

Merck pregnancy registries have prospective and retrospective data collection systems aimed at detecting adverse effects of certain drug use in pregnancy. This register receives voluntary reports from women or healthcare providers for women who have taken Sitagliptin or Sitagliptin and metformin during pregnancy. The importance of reporting all outcomes of exposure to Sitagliptin in pregnancy as early as possible is emphasised. This facilitates the collection of prospective unbiased information. It is important to note that retrospective reports to the company pregnancy register will contain bias towards reporting mainly abnormal outcomes.

The fifth annual cumulative review produced by Merck & Co for Sitagliptin/Sitagliptin +metformin contained post marketing pregnancy reports received from reports from August 2006 to August 2011. There was a total of 16 complete prospective reports which included 14 live births (one set of twins) and three spontaneous miscarriages. No congenital anomalies were reported in any of the exposed pregnancies (Merck & Co, 2015).

A pre and postnatal development study performed in rats showed no adverse effects with the use of Sitagliptin. Reproduction studies on rats and rabbits given

doses 12 times the maximum human dose did not impair fertility or harm the fetus (Co, 2017).

Most case reports on outcomes of fetuses exposed to Sitagliptin are from mothers with uncontrolled diabetes despite the use of metformin or insulin. It is difficult to ascertain if miscarriage is a result of embryonic abnormality, diabetic embryopathy or polytherapy.

The SPC for Sitagliptin states that 'there is no adequate data from the use of Sitagliptin in pregnant women. Studies in animals have shown reproductive toxicity at high doses. Due to lack of human data, Sitagliptin should not be used during pregnancy'.

Overall, there is not enough data in the pregnancy register to allow an analysis of pregnancy outcomes by type or by timing of exposure. The SPC does not recommend the use of Sitagliptin in pregnancy and this needed to be taken into account for the purpose of the study.

Sitagliptin has been assigned FDA pregnancy category B (Co, 2017). This American pregnancy risk category classification (A, B, C, D or X) indicates the potential risk of a drug to be teratogenic or harmful if used in pregnancy. FDA category B indicates that animal studies have failed to show a risk to the fetus but there are no adequately well controlled studies in humans.

Although there are no adequate and well controlled studies in pregnant women there were no teratogenic effects when female rats were administered 20 – 30 times the maximum recommended human dose (25mg/kg in rats and 125mg/kg in rabbits). Doses approximately 100 times the maximum human dose increased the risk of rib malformations in offspring. Because of the high safety margins, these findings do not suggest a relative risk for human reproduction.

When Sitagliptin was administered to rats at a dose 100 times that of maximum human exposure from 6 weeks' gestation to 21 days post-natal there was a reduction in body weight in offspring. No functional behavioural toxicity was observed in the offspring of the rats.

In order to reduce the risk to any fetus, a series of measures were put into place to minimise the risk of women conceiving on the study medication. We also included measures to ensure that if pregnancy did occur, it was detected early and the study

medication was discontinued as soon as possible. Details of the measures put in place by the trial management group are in the trial protocol.

2.4.5 INVESTIGATION MEDICINAL PRODUCT AND PLACEBO

Once it was deemed safe to give Sitagliptin to healthy non diabetic participants I started contacting companies who made placebo's for the supply of Sitagliptin and placebo. We had quotes from three separate companies.

After close liaison with the trial pharmacist we felt that Sharp Clinical Services were best placed and best value for money to manufacture and supply the study requirements.

Sharp clinical services were responsible for the packaging, labelling and transport of the study medication to the research study site.

Sitagliptin is usually issued in a tablet form. To ensure the study was blinded, the Sitagliptin tablets were converted to capsule form to make them identical to the placebo.

Sitagliptin was encapsulated because it was substantially cheaper to produce placebo in capsule form rather than in tablet form. The encapsulated Sitagliptin then had to undergo disintegration testing to ensure it had the same bioavailability and shelf life as the original tablet form.

2.5 ELIGIBILITY CRITERIA

The endometrial biopsy and use of Sitagliptin in healthy volunteers had been considered as safe and ethical for the purpose of this study.

I needed to explore other variables to ensure the utmost safety for participants and homogeneity of the study. These considerations formed the eligibility criteria for the study.

Factors which would determine the eligibility criteria involved

1. Menstrual cycle length and duration of treatment

We decided that participants would need to have a regular 28-30-day cycle for inclusion to the study. With a regular 30-day cycle there would be three menstruations and therefore three opportunities for stem cell recruitment to occur within the treatment period for each participant. Participants with longer cycles would not have the same number of opportunities for stem cell recruitment in the treatment period. Participants with a cycle length that is consistent would be more homogenous and therefore would minimise confounding factors.

2. Safety of Sitagliptin in healthy non diabetic individuals

The SPC for Sitagliptin was initially reviewed by myself, the pharmacy advisor and the hospital (UHCW) research governance manager. A plan was made with the trial sponsor (UHCW) for a formal review of the SPC every three months for the duration of the study. This would ensure the most up to date reference safety information was used and consistent with the protocol and participant specific documents. I also set up an email address specifically for the trial called simplantstudyoffice@uhcw.nhs.uk and received any MHRA drug alerts to trigger extra reviews and updates.

We carefully studied the SPC for Sitagliptin to ensure our inclusion and exclusion criteria would only include participants in whom using Sitagliptin would be safe.

3. Concomitant Medications

Plasma monitoring of Digoxin would be required if taking it along with Sitagliptin. Sitagliptin appears to alter the hypotensive effects of enalapril. For this reason, those patients on digoxin or enalapril were not eligible for the study.

4. Renal Function

The SPC for Sitagliptin states that an assessment of renal function is recommended prior to its initiation because dose adjustment is required if there is mild, moderate or severe renal impairment. In order to accurately assess the true effect of Sitagliptin on the endometrium we needed all participants to be on the same dose. Therefore, we decided that all participants needed normal renal function in order to be entered to the study.

5. Hepatic function

The SPC states that care should be exercised in the use of Sitagliptin in those with severe hepatic impairment. Only patients with normal hepatic function were therefore entered to the study.

6. Diabetes

Sitagliptin should not be used in patients with type one diabetes and dual therapy of Sitagliptin and other anti-diabetic medication is known to be associated with an increased risk of side effects such as hypoglycaemia which would require increased monitoring. Therefore, patients with type 1one or type two diabetes were not included in the trial.

7. Age

The safety of Sitagliptin has not been studied in patients under the age of 18. This was therefore the lower age limit for inclusion to the study. As the risk of miscarriage naturally sharply increases after the age of 42 this was the upper limit of inclusion to the study.

8. Body mass index (BMI)

There is no dose adjustment of Sitagliptin required for BMI. BMI has no clinically meaningful effect on the pharmacokinetics of Sitagliptin. Therefore, we did not need to limit inclusion to the study according to BMI.

9. Breast feeding

Caution is advised when prescribing Sitagliptin to breastfeeding mothers as it is not known if it is secreted in breast milk. Breast feeding patients would not be eligible for the study.

10. Safety of Sitagliptin in pregnancy

We needed to assess the safety of Sitagliptin in pregnancy in case pregnancy did occur during the treatment period. We contacted MSD and obtained reference safety information. This was studied carefully and has been considered in section 2.4 (Ethical Considerations).

There is too little data in the MSD company register to make a conclusion on the potential risk for humans and so Sitagliptin should not be used during pregnancy.

11. Avoiding pregnancy

Not only is Sitagliptin not recommended in pregnancy but the start and end of this study was marked with an endometrial biopsy. This obviously could not be performed if the participant was pregnant.

We decided that we will inform participants at the time of consent, that they must not try for a pregnancy in the cycle leading up to the first biopsy or for the duration of the study. The consent form required participants to be willing not try for a pregnancy and also to use barrier methods of contraception in the cycle leading up to the biopsy and for the whole duration of the study. Barrier contraception (condoms) were provided to participants at the time of consent.

If the couple had unprotected intercourse in the cycle leading up to the endometrial biopsy the procedure was cancelled.

After a trial management group (TMG) meeting with the sponsor (UHCW) it was decided that there needs to be clear pathways of checking for pregnancy through the study. This is detailed in the trial protocol.

2.6 PATIENT SPECIFIC DOCUMENTS

As all these ideas came together, the trial protocol was becoming layered with deeper and finer details of the study design.

As well as developing the protocol I developed a participant information leaflet, a consent form, a GP letter, a symptom diary, a participant questionnaire and an emergency contact card (appendix 1 – 6 inclusive).

Local standard operating procedures were used to formulate these documents. There was a section specific on the consent form for this study which required participants to agree not to try to get pregnant and be willing to use barrier contraception for the duration of the study.

2.7 PATIENT AND PUBLIC INVOLVEMENT

We gained permission from a patient who had previously been involved in a research trial for recurrent miscarriage to appraise the basic trial idea and the associated documents.

Her feedback:

Hi Dr Tewary,

I have had a read through the documents today and found that the patient information document was very clear and informative, it explains the trial well and I didn't have any other questions after reading this that hadn't already been answered.

The consent form is clear and I personally wouldn't have an issue signing any part of it. I think the GP letter was informative and had enough information to keep them informed of the trial, the only thing I would add to this is that the patient is not to get pregnant on the trial, only because this leaps out at me as an important part of the trial and may be worth advising the GP on.

Hope this helps, anything else I can do to help just let me know

Kind regards
XXX

As a result of this feedback, the G.P letter was amended to include information about avoiding pregnancy for the duration of the study.

Before the protocol and participant specific documents were submitted to the Research Ethics Committee (REC) we discussed the proposed trial idea at our local 'Patient Public Involvement Group' (PPI Group).

This is an initiative set up with the help of a public engagement fund from the University of Warwick Medical School. A meeting is held once every two months where patients with a history of RPL or the experience of a clinical trial in pregnant are invited to discuss potential upcoming research ideas and their sustainability in this target group.

The meeting for the SIMPLANT study was held in the Clinical Sciences building at UHCW and attended by five patients and four members of staff.

A basic description of research terminology was given to the participants to assist them with the understanding of a clinical study. I did a formal presentation on the basic study design.

The attendees explored the practicalities of the study and gave me a lot of insight into questions and concerns that patients approached for the study may have.

There were no objections from any members to the idea of two endometrial biopsies as part of the study or the potential side effects of Sitagliptin. Patients also expressed the willingness to accept a 50% chance of receiving a placebo tablet on the basis that there would be a 50% chance they would receive a treatment which may help to prevent further miscarriages which is not otherwise available.

There was also constructive feedback on how to improve patient documents to improve the quality of information and maximise the impartiality of any information given back on the symptom diaries.

Some of the suggestions included:

- The group felt that if the symptom diary contained a suggestive list of side effects such as a dry cough and headache for participants to tick from, they may record symptoms which are not real. They recommended to replace it with a free text box to help participants record only those side effects actually experienced.
- The group wanted a simplified explanation of the trial pathway. The participant information leaflet was amended to include a step-by-step guide on what would be involved as part of the study pathway.
- Members of the group suggested more information about the timing of the biopsy. This was included.
- Patients suggested providing an emergency contact number in case of any untoward side effects, concerns or hospital admissions while taking the trial medication. We created an emergency contact card which was the size of a bank card so that participants could keep it in their wallet. We made formal arrangements with the hospital switchboard for participants to be able to contact Professor Quenby or Dr Tewary 24 hours a day 7 days a week.

I wanted to ensure the participant information leaflet was easy to read. Members in the PPI group were given the participant information leaflet in 2 two different formats. One in A4 and one in an A5 folded booklet. The members felt that the A5 booklet form was too small and the small font made it difficult to read.

I also provided two different designs. One was black and white with the characteristics of the local trust policy guidelines whereas the other was

divided into coloured sections. The following feedback was given regarding the two designs:

White standard layout	Coloured box layout
Difficult for dyslexic to read as text jumps	Short snippets of information make it easier to understand and digest
Can key information be bullet pointed? Too much text to digest	Text needs to be centred in boxes
Boring	Better font
Too wordy	Better layout
Bland	People want to read the information
Not clear on what trial is about	More interested in the trial with this layout
Summary box needs to be same alignment as rest of text	
Side effects would be beneficial in box as makes it easier to find	
Ensure spacing, underlining and text is uniformed and formatted the same	

All this feedback was taken on board before the final patient specific documents were finalised to be submitted to the NHS national REC. We developed a participant information leaflet in line with hospital guidelines but also with colour and summary boxes making information easier to digest.

The PPI groups' unreserved critique of the documents enabled me to make the required changes to address patient concerns and encompass the needs of the trial. As a result, when the trial opened for recruitment I was confident that the documents were comprehensive to the needs of the participants.

2.8 PEER REVIEW

The NIHR describes the importance of a peer review to assess the design, feasibility, acceptability and importance of the topic in question (NIHR, 2017).

The scientific, methodological and statistical aspects of the trial protocol was reviewed by two reviewers: Dr Nigel Simpson (External) and Dr Paul O'Hare (Internal) (Appendix 7 and 8).

The review from Dr O'Hare confirmed the safety of using Sitagliptin proven in very large clinical trials and also complimented the study design. Dr O'Hare also confirmed that we will not need to carry out blood sugar monitoring as the absence of hypoglycaemia is a major reason for the use of DPP4 inhibitors like Sitagliptin in patients with diabetes. His overall conclusion was that this is extremely important research which should constitute minimal risks to participants.

The peer review from Dr Nigel Simpson has also confirmed safety of the trial especially with the plans for careful counselling and constant surveillance to prevent pregnancy.

2.9 RANDOMISATION PROCEDURES

We had to ensure randomisation procedures were carefully thought out and controlled.

Production of a randomisation list, medication allocation, blinding and unblinding were discussed repeatedly at the TMG meetings until agreements were made by all.

A coding convention was developed:

- Participants would be assigned a screening number once consented to the trial – e.g. S001, S002.
- Participants randomised would be assigned a participant trial ID e.g. R01, R02.
- Pack numbers of the trial drug would be allocated from 1001 to 1120.

Random Allocation Sequence

It was agreed that Sharp Clinical Services who were manufacturing and supplying the trial medication would provide a list of pack numbers and contents to the trial statistician.

e.g.

Pack No	Pack contents
2351	Sitagliptin (B)
1498	Sitagliptin
5641	Sitagliptin
6315	Placebo (A)

7531	Placebo
5975	Placebo

The trial statistician would be unblinded to the treatment allocation and be responsible for creating the master randomisation list.

Each treatment pack would contain one months' supply of medication. Each participant would require three months' supply of IMP/placebo, therefore three unique pack numbers will be assigned to each participant (three placebo packs or three treatment packs depending on group allocation).

We also decided to have a second check of the study office master randomisation list to confirm that the three packs allocated to each participant all contained either Sitagliptin or placebo.

A computer-generated blocked randomisation sequence would be used, to create the master randomisation list.

Study ID	Pack No
1001	6315, 7531, 5975

2.10 UNBLINDING

We had to consider the need for unblinding a participant. We decided that the trial statistician would create the master randomisation list and he would also create two sets of opaque code break envelopes containing pack numbers and associated contents in that pack. We decided to have a separate envelope for each pack number so that unblinding would be specific to the pack number assigned to the participant at that time.

If unblinding became necessary, then the envelope labelled with the current pack number that had been assigned to the participant would be opened.

Both sets of code break envelopes would be delivered to the pharmacy at UHCW to confirm that the envelopes were sealed and intact. One set of envelopes would be kept in a locked cabinet with the medical team in the Biomedical Research Unit

(BRU) for use in-case of emergency unblinding, and the second set was kept in a locked cabinet with the Sponsor in the Research and Innovation Office as a backup.

We created a system for unblinding to be possible 24 hours a day and 7 days a week. During office hours (8 am to 4pm) participants or healthcare providers could call the research office on 02476 967890 or out of ours by contacting the medical team via the main hospital switchboard on 02476964000 (ask for Dr Shreeya Tewary or Professor Quenby). The contact details were on the emergency contact card to be given to participants once randomised. This process of contacting the team out of hours through switchboard was formally approved with the manager at the hospital switchboard and tested. The unblinding procedure was tested and found to be effective prior to initiation of the trial.

2.11 APPLICATION TO REC, MHRA AND SITE ACTIVATION

After detailed discussions at TMG meetings, UHCW confirmed sponsorship and issued confirmation of capacity and capability approval.

I was then able to submit the application on the Integrated Research Application System (IRAS). This is an electronic system for permission for research in the U.K which meets all regulatory and governance requirements.

This is an extensive form capturing all the information needed for the REC, HRA and MHRA approval and takes away the need to do replicate applications. We registered the trial on the European Clinical Trials Database and obtained a EudraCT number (2016-001120-54).

Health Regulation Authority (HRA) and Research Ethics Committee (REC) Approval

The role of the HRA is to assess the governance and legal compliance of a proposed research project. The role of the REC is to ensure the proposed research project maintains safety, dignity and well-being of participants taking part in research.

After submitting the application via IRAS we were invited for a formal REC meeting. This was attended by myself and Professor Quenby. This meeting involved a panel of over ten members where the design of the study was discussed in detail.

We received feedback and recommended changes before a favourable opinion could be granted (Appendix 10). The requested changes were mainly to do with the participant information leaflet. Changes were made and a written response was submitted (Appendix 11).

Submission to the MHRA was postponed due to delays in receiving the technical agreement from Sharp Clinical Services. I made an appointment with a representative from the company and expedited the finalisation of the technical agreement.

Once we had this approval from the REC and the technical agreement was confirmed between Sharp Clinical Services and the sponsor (UHCW) we were able to make an application to the MHRA. We received approval from the MHRA on the 25th July 2016.

Once we had permission from the REC, HRA and MHRA, site specific training took place with the clinical team, randomisation team and pharmacy team.

A code break test for unblinding and an 'out of hours' emergency call' test were performed. Case report form packs were all printed, prepared and stored in the research unit.

We received the green light for site activation from the sponsor on the 14th September 2016. I recruited the first patient on the 15th September 2016.

CHAPTER 3: TRIAL PROTOCOL

This Chapter includes the study protocol which was submitted to the REC, MHRA and HRA.

3.1 STUDY SUMMARY

FULL TITLE	Does the DPP4 Inhibitor (Sitagliptin) Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage?	
ACRONYM	SIMPLANT (Sitagliptin for IMPLANTation)	
CLINICAL PHASE	Phase II study	
HYPOTHESIS	DPP4 Inhibitors increase endometrial stem cells, assessed by a clonogenic assay in women with RPL	
TRIAL DESIGN	Single-centre, double-blind, randomised, parallel-group, placebo-controlled trial	
TRIAL POPULATION	Women, aged 18-42 years, with a history of recurrent miscarriage (≥ 3) for whom a cause has not been identified	
PLANNED SAMPLE SIZE	30 patients to have completed study	
TREATMENT DURATION	13 weeks	
OUTCOME MEASURES	<u>Objectives</u>	<u>Outcome measures</u>
<u>Primary</u>	To determine the effect of Sitagliptin on endometrial mesenchymal stem cell count	The number of colonies per thousand endometrial stromal cells after 3 months of Sitagliptin (100mg) vs. 3 months of placebo, determined by a clonogenic assay
<u>Secondary</u>	To determine how well the IMP dosing schedule is tolerated by participants	Qualitative analysis of adverse events/serious adverse events reported in each treatment group (Sitagliptin vs. placebo)

	To determine the acceptability of the protocol to study participants	Qualitative analysis of process evaluation questionnaire in participants receiving IMP vs placebo.
<u>INVESTIGATION MEDICINAL PRODUCT</u>	1:1 randomisation to: o Sitagliptin o Placebo	
<u>FORMULATION, DOSE, ROUTE OF ADMINISTRATION</u>	Formulation: Encapsulated tablet containing either 100mg of active Sitagliptin or placebo Route of administration: Oral Dose: <i>Intervention group (100mg Sitagliptin):</i> once daily administration of one tablet containing 100mg of active Sitagliptin for 3 months from day 1 post-randomisation <i>Control group (placebo):</i> once daily administration of one tablet containing placebo, identical in size, colour and weight to the active Sitagliptin tablet, for 3 months from day 1 post-randomisation	
<u>ISRCTN number</u>	67932311	
<u>Duration of trial</u>	15 months from 15/09/2017	
<u>Study Sponsor</u>	Research, Development and Innovation University Hospital Coventry and Warwickshire	
<u>Study Funder</u>	Tommy's Charity	

Keywords: Sitagliptin, recurrent miscarriage, implantation, endometrium, stem cells, ischaemic injury

VISIT	Miscarriage Clinic visit	Baseline visit	Ovulation testing at home daily from day 10 of regular cycle	Endometrial biopsy clinic visit	Home pregnancy test	4 week clinic visit	Home pregnancy test	8 week clinic visit	Home pregnancy test	Ovulation testing at home daily from day 10 of regular cycle	12 week endometrial biopsy clinic visit	Early termination visit**
TIME POINT	Standard Care Patient information leaflet given			7-10d post-ovulation If not positive to ovulation test in first cycle then biopsy arranged 1 week prior to next period due	2 weeks ±3d post-biopsy	4 weeks ±4 days post-biopsy	6 weeks ±3d post-biopsy	8 weeks ± 4 days post-biopsy	10 weeks ±3d post-biopsy		7-10d post-ovulation	
Patient demographics	X											
Standard care blood tests	X											
Eligibility assessment		X										
Informed consent		X										
Medical history		X										
Current medications		X										
Ovulation testing			X							X		
Pregnancy test in clinic				X		X		X			X	

Transvaginal ultrasound scan				X						X	
Endometrial biopsy				X						X	
Randomisation				X							
IMP dispensed				X		X		X			
Study drug intake				X						x	
Pregnancy test at home					X		X		X		
Side effect diary review						X		X			X
Study drug compliance review						X		X			X
AEs/SAEs				X						x	X
Process evaluation questionnaire										X*	X
Pregnancy outcome recorded (via appointment or medical records)				X	X	X	X	X		X	X
End of study										X	X

3.2 STUDY AIMS AND OBJECTIVES

The primary objective of the SIMPLANT study is to test the hypothesis that Sitagliptin increases the endometrial mesenchymal stem cell count in those with RPL.

The secondary objectives are to determine how well the IMP dosing schedule is tolerated by participants and to determine the acceptability of the study protocol to participants.

3.3 OUTCOME MEASURES

3.3.1 PRIMARY OUTCOME MEASURE

- The number of colonies per thousand endometrial stromal cells after three months of the IMP determined by a clonogenic assay.

3.3.2 SECONDARY OUTCOME MEASURES

- Review of adverse events and serious adverse events reported by the participant or healthcare providers in the duration of the trial.
- Participant acceptability of the trial determined by a questionnaire filled in once each participant has completed the trial.

3.4 STUDY DESIGN

This is a pilot randomised double blind, parallel-group, placebo controlled feasibility study carried out at a single Centre; University Hospital Coventry and Warwickshire.

3.5 ETHICAL CONSIDERATIONS

Participants approached and recruited to the SIMPLANT study would have been fully investigated for all known causes of miscarriage. Some will have tried various empirical treatment options such as heparin injections to try and prevent miscarriage. This cohort of patients would not have any other treatment options to help prevent another miscarriage. We were therefore not withholding any treatment when they entered the study.

We know that performing endometrial biopsies is intrusive and can cause some discomfort but many patients approached for this trial would be familiar with the concept of an endometrial biopsy. Performing endometrial biopsies are safe and generally well tolerated.

We have established that Sitagliptin is safe for non-diabetic individuals.

When presented at our PPI group there were no objections to the idea of two endometrial biopsies in the space of three months.

3.6 SAMPLE SIZE AND POWER

We calculate that we would need to have a sample size of 30 participants for the study to have 95% power at a 10% significance level.

As participants will be in the study for a total of three months we anticipate a drop-out rate of more than 10%. Therefore, we aim to recruit 34 participants.

3.7 PARTICIPANT ENTRY

Patients invited to participate in this trial will be identified from the RPL clinic or the implantation clinic. There is a RPL clinic every week at UHCW lead by Professor Quenby. There are two implantation clinics every week at UHCW lead by Professor

Quenby and Professor Brosens who will first approach any of the patients who will be recruited to this study.

Patients will be advised that participation is completely voluntary and that their choice to participate or not participate will not affect their usual care.

3.8 INCLUSION AND EXCLUSION CRITERIA

3.8.1 INCLUSION CRITERIA

- Provision of informed written consent
- History of RPL
- Regular menstrual cycle, up to 30 days in length
- Age 18-42 years at consent
- Any BMI
- Willing and able to give consent for the study and endometrial biopsy
- Ability to fully understand the requirements of the protocol
- Negative pregnancy test on the day of randomisation

3.8.2 EXCLUSION CRITERIA

- Under 18 years of age
- Type I Diabetes
- Type II Diabetes – based on medical history
- Pregnancy
 - Tested at multiple points in trial – see schedule of events
- Breast feeding
- Known hypersensitivity to Sitagliptin
- Not taking any medications with potential to react with interventional product
 - Digoxin
 - Enalapril
- Previous diagnosis of pancreatitis
- Renal impairment (eGFR<50ml/min or AKI ≥1 as this would require dose adjustment of IMP)

- Hepatic impairment defined as Alanine Transferase (ALT) > 38 U/L, ALP> 105U/L Bilirubin (BR) >20 umol
- Inclusion in another intervention trial
- Unwilling to use effective contraception for the duration of the trial (from consent)
- Allergy/sensitivity to excipients of the IMP/placebo

*Note: eGFR will be calculated using the Cockcroft Gault Equation:

$$\text{eGFR} = \frac{(140 - \text{Age}) \times \text{Weight (Kg)} \times 1.04}{\text{Serum Creatine (micromol/L)}}$$

These inclusion and exclusion criteria are revised criteria which were approved by the REC and MHRA after a substantial amendment was made due to the suspension of the trial from 21st October 2016 to 15th December 2016. The trial was suspended just over one month after opening for recruitment on the basis of concerns with screening and eligibility. Details of the trial suspension and the changes which were put in place are detailed in chapter six.

3.9 TRIAL PATHWAY

3.9.1 ENROLMENT PROCEDURE

Participants will be given the Patient Information Leaflet (PIL) for the study at their initial consultation at the recurrent miscarriage clinic.

After all known causes for miscarriage are excluded and eligibility criteria is confirmed they will be able to consent to the study. We will make sure the patient has read and understood the PIL, been given the opportunity to ask questions and encouraged to discuss their involvement with their GP family and friends. Consent will be taken by the chief investigator or myself using the trial specific consent form (Appendix 2).

If patients refuse to participate with or without a reason, this will be respected. Information on any reasons for refusal of participation will be recorded on the trial

database. Patients will also be made aware that they are free to withdraw at any stage of the study without giving reasons.

Three copies of consent will be obtained – one for the site file, one for the hospital notes and one for the patient to keep.

Consented participants will be asked to monitor for ovulation with a simple urinary ovulation kit (provided by Clear Blue). They will be given a phone number and a trial specific email address to contact once ovulation had occurred.

A biopsy will then be arranged for 7-10 days' post ovulation.

Participants must not be pregnant at the time of the biopsy. We will provide barrier contraception for participants while they are in the study. Hormonal contraception cannot be used whilst in this study as it would alter menstrual cycles and the endometrial biopsies. If the couple have had unprotected intercourse since the last menstrual cycle the procedure will be cancelled.

Patients will be asked about any episodes of unprotected intercourse

- When they call to book the biopsy
- On the day of the biopsy
- At each face to face consultation through the trial.

Patients will be asked to do a pregnancy test every two weeks once randomisation has taken place. Pregnancy test kits will be provided. They will have been given instructions on what to do if the pregnancy test is positive. They will be advised to immediately stop taking the trial medication and we will arrange an appointment at the trial centre.

3.9.2 ENDOMETRIAL BIOPSY

The endometrial biopsy will be taken by one of the co-investigators in the presence of a chaperone. Participants will have a transvaginal scan prior to the biopsy.

The participants will be warned beforehand that the sampler can cause some pelvic pain and cramps due to uterine contractions. They will be advised that they can take 400mg of Ibuprofen and 1g of Paracetamol prior to their visit. Entonox is

available to use when the biopsy is being taken. They will also be told to bring a sanitary pad as they may experience some spotting after the procedure.

If the uterus is acutely anteverted or retroverted a plastic disposable vulcellum may be used to help hold the cervix while the biopsy is being taken.

3.9.3 RANDOMISATION PROCEDURE

Block randomisation, in a 1:1 ratio of active:placebo will be used to ensure balanced recruitment to each arm over the course of the study.

Randomisation will be done by the Research, Development and Innovation (RD&I) randomisation team via a phone call. The clinical research team can request randomisation during office hours (Monday – Friday 0930 – 1700).

The randomisation team and study team will remain blinded to treatment allocation. Eligibility and consent will be confirmed and 3 pack numbers will be assigned to the participant.

Sponsor confirmation of randomisation will then be sent to the trial office specific email address (simplantstudyoffice@uhcw.nhs.uk) and to pharmacy.

G.P's will have been informed in writing (with the patient's consent) about participation in the trial and about the possibility of being on Sitagliptin.

Participants will also be given an emergency contact card with contact details of the trial team in case of any emergency admissions and or unblinding is required.

3.9.4 FOLLOW UP

Participants will be reviewed every 4 weeks +/- 4 days after starting treatment. This will be by a face to face consultation with a doctor. This visit will include a pregnancy test and review of the symptom diary. Continued willingness to participate will be confirmed and a prescription for the next months' supply of medication will be completed.

About a week after the first endometrial biopsy participants will have their expected menstrual period. They will attend for the first follow up 4 weeks +/- 4 days after

starting the treatment and then the second follow up 4 weeks⁺⁻ 4 days after this. The final visit is to have a biopsy in the third menstrual cycle.

During the trial period participants should have three menstrual cycles and therefore three opportunities for the eMSC to increase.

3.9.5 FINAL VISIT

After the third menstrual cycle participants will start to monitor for ovulation again. Once the test is positive they will contact the trial team by a phone call or by sending an email to the trial specific email address to arrange their final biopsy 7-10 days after ovulation.

The day this biopsy is taken marks the end of the study. Participants will be given a questionnaire to fill in after this visit is complete. We will request for the questionnaire to be sent back within four weeks of the second biopsy. The aim of this questionnaire is to assess the acceptability of the trial.

Any pregnancy which occurs after the trial period has finished will occur after the endometrium has been renewed following menstruation. Participants will then be offered standard RPL clinic care which includes fortnightly scans in the first trimester and high risk antenatal care as is standard practice.

3.10 MONITORING FOR PREGNANCY

Participants will be carefully monitored for any unplanned pregnancies occurring during the trial period. Monitoring points for pregnancy will be every two weeks. A urinary pregnancy test will be done on the day of randomisation. We will provide participants with urinary pregnancy tests so that they can do another test at home two weeks after randomisation. They would then have a pregnancy test at the hospital follow up appointment two weeks later. This pattern will follow until the end of the study. If the test is positive at any time they will be asked to stop the medication and ring the trial-coordinating centre to arrange follow up.

3.11 TRIAL MEDICATION

3.11.1 PRESCRIBING AND DISPENSING

A trial specific prescription has been produced and will be completed by one of the doctors in the research unit.

Dispensing will be done by the site hospital pharmacy. Participants will be dispensed the study drug on a monthly basis according to assigned pack numbers.

3.11.2 TREATMENT

Trial Interventions:

Arm A – Active treatment: Sitagliptin in capsule form, 100mg orally, once daily for up to 13 weeks

Arm B – Control: Matching placebo, oral capsule, once daily for up to 13 weeks

The placebo is a dummy capsule identical in colour, shape and weight to Sitagliptin. Sitagliptin has been converted to capsule form for the purpose of the study. No pre-medication is required.

Treatment will be initiated on the day of the initial endometrial biopsy and continued up until the day of the second endometrial biopsy due 7-10 days after ovulation of the third menstrual cycle within the trial.

Capsules are to be taken orally with water at approximately the same time each day. If a dose is missed a double dose should not to be taken on the same day.

3.11.3 DOSE AND ROUTE OF ADMINISTRATION

Evidence on the optimal dose of Sitagliptin shows that 100mg once daily dosing gives the best efficacy in terms of glucose control and DPP4 inhibition. The capsule will be taken orally with water at the same time each day.

3.11.4 DOSE MODIFICATIONS FOR TOXICITY

There are no dose modifications for toxicity. If there are any significant side effects treatment will be stopped and participants will be withdrawn from the study.

3.11.5 SUPPLY OF SITAGLIPTIN AND PLACEBO

The trial drug will be supplied by Sharp Clinical Services. Commercially available Sitagliptin has been over-encapsulated and matching placebo capsules manufactured.

The manufacture will be done under an MIA (IMP) license following Good Manufacturing Practice (GMP) & Medicines for Human Use (Clinical Trials) Regulations 2004 as amended. Sharp Clinical Services will assign a qualified person (QP) to be responsible for batch release and provide support for product complaints/queries for the duration of the study. Details of specific arrangements have been documented in a GMP Technical Agreement with the Sponsor.

The trial medication must be stored at ambient temperature 15-25 degrees Celsius and will be supplied to the site at the start of the study.

The trial drug will be supplied as blinded packs of Sitagliptin/placebo 100mg capsules, each containing 32 tablets to last one cycle. Each pack had a unique 4-digit pack number.

3.11.6 LABELLING

A label for the trial drug has been designed to ensure traceability and identification of the trial, identification of the product and facilitate proper use of the trial medication in accordance with Volume 4 of Good Manufacturing Practices, Annex 13 (Manufacture of IMPs). The label for the Sitagliptin and placebo is identical.

Sample labels designed by us have been created by Sharp Clinical Services and been provided to the MHRA for approval prior to manufacture (Appendix 13).

We have added an additional dispensing label which includes patient specific

details to the trial drug as part of standard hospital dispensing practice.

3.11.7 COMPLIANCE MONITORING, ACCOUNTABILITY, RECONCILIATION AND DESTRUCTION

The dispensing of the trial medication will be recorded in pharmacy on the Pharmacy IMP Accountability Log (Appendix 16).

Within the participant symptom diary there is a compliance-monitoring chart. Participants will be asked to fill this in daily and bring in left over capsules at the end of the study period. We will ensure that the compliance recorded matches up with the number of left over capsules.

At the end of the patient's participation in the study any un-used study drug will be returned to pharmacy to be counted and destroyed.

At the end of the study any unused IMP will be quarantined pending Sponsor approval for destruction.

3.11.8 INTERACTION WITH OTHER DRUGS

There is a low risk of drug-drug interactions as Sitagliptin is primarily eliminated by the kidney with hepatic metabolism playing only a small role in clearance.

If patients are started on any anti-diabetic drugs, digoxin or enalapril, they should be withdrawn from the study.

3.12 DATA COLLECTION AND MANAGEMENT

Every patient given a PIL will be entered onto the screening log (Appendix 14). They will have a unique screening number (e.g. S001). Once patients have been randomised they will have a unique Randomisation number (e.g. R01).

Case report forms will be filled in from the day of consent (Appendix 17). These have been formulated to collect all required trial data. The information will be stored on a secure trial database.

The paper copies will be locked in a secure cabinet in the Biomedical Research Unit (BRU) while the trial is running and then saved for 25 years in accordance with the UHCW NHS Trust archiving procedures.

3.13 CONFIDENTIALITY

The confidentiality of all participants will be preserved under the Data Protection Act 1998.

Participants will all be assigned a 'randomisation number' which will be used for the case report forms (CRF) and this number will also be used to enter information onto the secure password encrypted electronic database provided by MedScieNet, for randomisation. There is also specific coding for the endometrial samples. Details of this coding can be found in chapter 4.4.

Consent from participants will have been obtained to use the endometrial sample for future research projects. If the patient does not consent to this process the sample will be discarded as per UHCW Trust policy on disposal of tissue.

During the project, the patient can withdraw their consent at any time and the sample will be discarded according to Trust policy.

All data collected as part of the trial will be stored securely in password protected files (electronic data) or in locked filing cabinets in secure entry-card protected areas (hard copy data).

All the staff involved in the SIMPLANT study will share the same duty of care to prevent any unauthorised disclosure of personal information. No data that could be used to identify an individual will be published.

3.14 RESEARCH ETHICS COMMITTEE (REC) AND MEDCINES HEALTH REGULATORY AUTHORITY REVIEW

Approval for the trial protocol and supporting documents will be sought from a Research Ethics Committee (REC), the local NHS Trust R&D department and the Medicines Health Regulatory Authority (MHRA).

Substantial amendments that require review by a REC will not be implemented until the REC and the local R&D department grants a favourable opinion and all correspondence with the REC and R&D department will be retained in the Trial Master File (TMF).

An annual progress report will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended.

If the study is ended prematurely, the Chief Investigator will notify the REC, including the reasons for the premature termination. Within one year after the end of the study, the Chief investigator will submit a final report with the results, including any publications/abstracts, to the REC.

3.15 PEER REVIEW

The scientific, methodological and statistical aspects of the trial protocol have been reviewed by two reviewers: Dr Nigel Simpson (External) and Dr Paul O'Hare (Internal).

3.16 PUBLIC AND PATIENT INVOLVEMENT

A participant in a previous miscarriage research study has been consulted on the design and conduct of this research study. Patient specific documents have been reviewed by our local patient and public involvement group.

3.17 REGULATORY COMPLIANCE

The protocol and trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any amendments and the trial will not commence until a Clinical Trial Authorisation (CTA) has been obtained from the MHRA. All correspondence with regulatory bodies will be kept in the Trial Master File.

3.18 PROTOCOL COMPLIANCE

Prospective, planned deviations from the protocol are not permitted under the UK regulations on clinical trials and will not be used. Should accidental deviations from the protocol occur the deviation will be documented and reported to the Chief Investigator and Sponsor immediately. Protocol deviations which frequently recur will require immediate action and may be classified as a serious breach following investigation by the Sponsor.

3.19 NOTIFICATION OF SERIOUS BREACHES TO GCP AND/OR THE PROTOCOL

A 'serious breach' will be defined as a deviation from the protocol that is likely to affect the safety or physical or mental integrity of the subjects of the trial or the scientific value of the trial.

The sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase and the sponsor will notify the licensing authority in writing of any serious breach of the conditions or principles of Good Clinical Practice (GCP) in connection with the trial or the trial protocol.

3.20 AUDIT AND INSPECTION

The study will be subject to inspection and audit by the sponsor to ensure adherence to GCP. A trial monitoring plan will be developed and agreed by a joint Trial Steering Committee (TSC) and a Data Monitoring Committee (DMC) based on the risk assessment undertaken by the sponsor prior to commencement of the trial.

Direct access to the study documents and data will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

3.21 PHARMACOVIGILANCE AND SAFETY MONITORING

The summary of product characteristics (SPC) for Sitagliptin will be reviewed on a formal basis every three months for the duration of the study to ensure the most up to date reference safety information was used and consistent with the protocol and participant documents. simplantstudyoffice@uhcw.nhs.uk will receive any MHRA drug alerts to trigger extra reviews and updates.

3.21.1 ADVERSE EVENTS

An adverse event (AE) is an unintentional and unfavourable sign or symptom that occurs while being in the trial. It may or may not have a causal relationship with the medication being taken. When taking the endometrial biopsy pain, bleeding and feeling faint straight after the biopsy are expected outcomes and so will not be recorded as adverse events or reported.

3.21.2 ADVERSE REACTIONS

An adverse reaction (AR) is an unintended sign or symptom judged to have 'reasonable causal relationship' to the IMP. As such, the distinguishing feature between an AR and AE is whether there is evidence to suggest there is a causal relationship between the event and the IMP.

The following adverse reactions, which are side effects, thought to be 'common' i.e. occurring 1/10 – 1/100 people are of special interest in this trial:

- Nasopharyngitis
- Upper respiratory tract infections,
- Osteoarthritis
- Headache

As they are listed as common side effects in the SPC for Sitagliptin they do not have to be reported to the regulatory authorities. If the outcome to the side effect is serious then a Serious Adverse Event form should be completed (Appendix 18).

Frequency not known/Case reports:

- Hypersensitivity including anaphylactic responses
- Interstitial lung disease
- Acute pancreatitis
- Angioedema
- Cutaneous vasculitis
- Exfoliative skin conditions including Stevens-Johnson syndrome
- Renal impairment/renal failure

The causality behind any AE or AR will be made by the investigator responsible for the patients' care. The assignment of causality will be defined as unrelated/unlikely/possible/probable/definitely or not assessable.

3.21.3 SERIOUS ADVERSE EVENTS

Any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening *
- Requires hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity; or
- Consists of a congenital abnormality or birth defect
- Requires medical intervention to prevent one of the above, or is otherwise considered medically significant by the investigator.

* Life-threatening refers to an event where the participant was at risk of death at the time of the event; not to an event that hypothetically might have caused death if it was more severe. A medical review must be undertaken in deciding whether an SAE / SAR is serious in other situations. Those events that are not immediately life-threatening or do not result in death or hospitalisation, but may jeopardise the subject or may require intervention to prevent one or more of the other outcomes listed, should be considered serious.

A Serious Adverse Reaction (SAR) meets the criteria of being an AR and is serious. The SmPC contains the known side effects of Sitagliptin, however there needs to be a clinical judgement for expecting an unexpected SAR.

3.21.4 REPORTING PROCEDURES

Patients will have direct contact with the trial team by telephone and email to report any events themselves. Adverse events will be reviewed and unscheduled clinic visits will be arranged if further clinical care is required.

Should any participant be admitted as an emergency to another department of the hospital e.g. Accident and Emergency they will have been informed to give their 'SIMPLANT Emergency Contact Card' to the clinician looking after them. The card

provides contact details to get in touch with either Professor Quenby (chief investigator) or Dr Tewary (trial co-ordinator). The chief investigator will then review the event to determine causality and report if necessary.

3.21.5 REPORTING AES/ARS

AEs can be observed directly when they attend for their face to face consultations or volunteered by the patient. All non-serious AEs/ARs should be reported to the sponsor as soon as possible but no later than one month and then recorded onto the trial database. If the outcome to the AE is serious then an SAE form should be completed (Appendix 19).

3.21.6 REPORTING SERIOUS ADVERSE EVENTS

Serious Adverse Events (SAE) should be reported to the sponsor within 24 hours of research staff becoming aware of an event. An initial report may be made orally but must be followed up promptly by a detailed written report. An SAE form will be completed together with relevant supporting documents, including an assessment of severity, causality and expectedness, as reviewed by the chief investigator. SAE form should be submitted to the sponsor's office at RD&Isponsorship@uhcw.nhs.uk.

Any change of condition or other follow-up information should be emailed to the Sponsor as soon as it is available or at least within 24 hours of the information becoming available. Events will be followed up until the event has resolved or a final outcome has been reached. The Sponsor will inform the regulatory authorities within the required expedited reporting timescales.

3.22 IN EVENT OF PREGNANCY

As shown in the timeline of events there are many checks in place to prevent pregnancy.

- Patients will sign a consent form to agree to not try to become pregnant while in the study and will be supplied with barrier contraception.
- The biopsy will not be performed if unprotected intercourse has taken place in the cycle leading up to visit.
- Patients will do a pregnancy test every 2 weeks for the duration of the trial.
- A pregnancy test will be done before each endometrial biopsy

In the event of a positive pregnancy test patients will be asked to stop the medication immediately and a trial centre visit will be organised. The Chief Investigator will be informed, it will be recorded on the trial database and the patient will be withdrawn from the trial.

3.23 UNBLINDING

Participants, investigators, research midwives and nurses will remain blinded to the trial drug allocation throughout the duration of the trial.

Unblinding in the study is not allowed unless there are medical or safety reasons to do so. Unblinding will only occur when all participants have had their endometrial biopsy 3 months after treatment.

If there is a serious adverse event, then the management of the patient should be started as if the patient is on Sitagliptin. If the event or reaction is thought to be related to Sitagliptin then unblinding will be considered.

Unblinding requests may be made by the chief investigator, the chief investigators designee or, in the event of an emergency, the participants health care provider.

Unblinding can occur 24 hours a day and 7 days a week. During office hours (8 am to 4pm) participants or healthcare providers can call the research office on 02476 967890 or out of ours by contacting the medical team via the main hospital switchboard on 02476964000 (ask for Dr Shreyya Tewary or Professor Quenby). The contact details are on the emergency contact card given to participants once randomised.

3.24 EARLY TERMINATION VISIT

A participant will be required to discontinue the drug under the following circumstances

- Participant becomes pregnant or is no longer willing to use reliable contraception
- Participant wishes to withdraw for any reason
- Presence of any medical condition that may jeopardise the participant's safety e.g. development of type two diabetes
- Non compliance
- Determination by the investigator that the discontinuation is in the best interest of the participant.
- Adverse event related
- Sponsor's decision
- Lost to follow up

If there is early discontinuation of the medication the patient will be asked to attend for an early termination visit. An end of study form in the CRF pack will be completed.

3.25 DATABASE

A database compliant with GCP requirements which will be password protected, encrypted and stored on a secure drive to NHS standards will be developed for the purpose of this trial.

3.26 STATISTICS AND DATA ANALYSIS

3.26.1 PRIMARY ENDPOINT

The primary end point is the stem cell assay after 3 months of treatment. This will be assessed by doing a clonogenic assay. The number of colonies formed reflects the stem cell count.

For each biopsy, 500 cells will be seeded in 3 separate wells of a colony forming unit assay plate. We will look at the number of colonies formed per 500 cells seeded for each participant in the study.

3.26.2 STATISTICAL ANALYSIS

Data exploration will compare baseline characteristics for the women in the intervention group (women randomised to receive Sitagliptin) and the control group (women randomised to receive placebo).

For example, for continuous characteristics such as age, we will report the mean and the standard deviation and for count data such as number of previous miscarriages, we will report the median and the interquartile range (See template table below).

Characteristic	Intervention	Control
Age (years)		
Mean (SD)		
Number of previous miscarriages		
Median (IQR range)		

The primary analysis will compare the number of colonies per 500 cells between the Sitagliptin group and the placebo group. We will test the null hypothesis that the

mean number of colonies for the control (placebo) group is equal to that of the intervention group (Sitagliptin) after three months of treatment.

We will fit a Poisson regression model, which is appropriate for count data. If the baseline characteristics are imbalanced between the control and the intervention groups, we will perform secondary analysis in which the Poisson regression model will adjust for the baseline characteristics.

Because this is a pilot study, we will test hypothesis tests at 10% significance level.

3.27 TRIAL CLOSURE

The trial will be stopped early if

- Requested by Ethics Committee
- Mandated by the data monitoring committee on safety grounds
- Decided by the Sponsor
- Funding for the trial ceases

The REC and MHRA will be informed if the trial is terminated earlier than planned

3.28 DEFINITION OF THE END OF THE TRIAL

This will be when the last patient has had their second endometrial biopsy.

Following the discharge of the patient from the trial, patients will go back to standard care. This involves being given a contact number to phone to book an appointment to the RPL clinic as soon as they are next pregnant.

3.29 TRIAL REGISTRATION

The trial has been registered on the International Standard Randomised Controlled Trial Number registry prior to the start of recruitment (ISRCTN 67932311).

CHAPTER 4: CLONOGENIC ASSAY

This chapter outlines the standard operating procedure developed from which to perform the clonogenic assay. These assays were performed in the Warwick Medical School Clinical Sciences Research Laboratory based at UHCW.

Each assay took two weeks to complete and required advanced laboratory experience. They were performed by Dr Emma Lucas, Dr Katherine Fishwick and Dr Paul Brighton. I followed through the first 14 participants with Dr Lucas to get a good understanding of how the assays are performed.

4.1 AIM

1. Isolate the endometrial stromal cells from the endometrial biopsy
2. Plate the endometrial stromal cells for a colony forming unit (CFU) assay.
 - Each colony formed is a marker of stem-ness of the sample. A property of the stem cells is to self-replicate and form colonies – other types of cells don't do this.

4.2 IMPORTANT POINTS

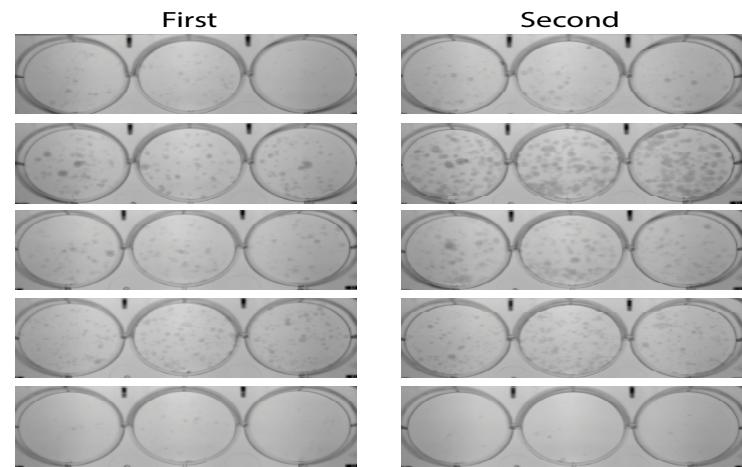
- a) Clean the biosafety unit from the back to the front with 70% industrial methylated spirit (IMS)
- b) Use a class II cell culture microbiological safety cabernet and aseptic techniques to avoid microbiological contamination
- c) Ensure to wear a lab coat and gloves. Gloves need to cover the cuff of the lab coat

4.3 MATERIALS AND REAGENTS

1. 70% industrial methylated spirit (IMS)
2. 60mm sterile petri dish (untreated)
3. Falcon tubes – 50ml/15ml/14ml
4. 20 ml syringe
5. 0.4 um cell strainer
6. Filter

7. Glass pipettes
8. Strippette
9. t25 flask
10. Scalpel for chopping
11. Digestion media
14ml falcon tube, 10ml DMEM/F12(additive free, phenol non red),
100ul DNAase (10mg/ml), 100ul collagenase
12. Colony Forming unit (CFU) Assay plates (6 well plate)
13. 2% DCC (serum containing) = dextra charcoal coated
DMEM/F12 **without phenol red** containing 2 % DCC, 2 mM L-glutamine
and 1X antibiotic-antimycotic mix
14. 10% DCC (DMEM/F12 containing 10 % dextran-coated charcoal filtered
FBS (DCC), 2 mM L-glutamine, 1X antibiotic-antimycotic mix, 1 nM estradiol
(E2) and 2 µg/ ml insulin)
15. 10% DMEM/T12
500mls DMEM + 50 mls 10% serum, 5mls L-Glutamine, oestradiol
(mitogen), insulin
16. Trypsin-EDTA (0.25 %)
17. sterile filtered phosphate buffered saline (pbs)
18. plasma fibronectin (1mg/ml)
19. Basic fibroblast growth factor (BFGF) (1 microlitre/ml)
20. microcentrifuge tube
21. trypan blue
22. 10% neutral buffered formalin (NBF)
23. 10% dimethyl sulfoxide (DMSO)
24. harris haematoxylin
25. neubauer improved haemocytometer
26. counting slide
27. luna cell counter.

A sample of CFU images for 5 patients. The three wells on the left represent colonies of stem cells in the baseline biopsy and the 3 wells on the right demonstrate colonies of stem cells in the final biopsy.



4.4 PROCEDURE

The endometrial biopsy was obtained using a Wallach Endometrial Cell Sampler.

Each participant had two endometrial biopsies; one at the start of the trial and one at the end of the trial. These were labelled as 'biopsy A' and 'biopsy B'. Patient 1 = R01. The biopsy portion used for stem cell analysis was labelled R01A and R01B. These were analysed together to ensure that the paired analysis had minimal errors due to minor alterations in the laboratory environment.

The biopsy portion sent to tissue bank could not be labelled with letters. These were therefore replaced with numbers

R	9
Biopsy A	1
Biopsy B	2

Therefore, R01a and R01b was labelled as 9001 1 and 9001 2
R02a and R02b was labelled as 9002 1 and 9002 2

The biopsy was divided into 4 portions.

- a. A small piece (0.5 – 1cm long) was snap frozen in liquid nitrogen for protein analyses.
- b. A small piece (0.5 – 1cm long) was preserved in RNA later for transcriptomic analyses.
- c. Another piece (1-2cm) was fixed in 10% neutral buffered formalin (NBF) for histology and immunohistochemistry.
- d. The remainder was placed into 10% DMEM/F12 and processed for cell culture.

4.4.1 DIGESTION MEDIA PREPARATION

1. A 14ml Falcon tube was used to prepare the digestion medium.
2. 10mls of DMEM/F12 (without phenol red free/additive free), 100ul DNase I and 100ul collagenase Ia were mixed together into the 14ml Falcon tube.
 - a. The collagenase breaks down the extracellular matrix between the cells.
 - b. The DNase removes the DNA which would be released from the cells after chopping the sample. Removal of the DNA is necessary to prevent the DNA causing apoptosis of other cells.

4.4.2 SAMPLE CHOPPING

The biopsy was then transferred from the 10% DMEM/F12 vial into a sterile petri dish. The tissue was separated from the liquid portion (DMEM/F12) by pouring the liquid portion into the petri dish and pouring the endometrial biopsy into the upturned lid of the petri dish.

1. A pipette was used to remove any remaining fluid or any mucus from the biopsy material.
2. The biopsy was then chopped with a scalpel for 5 minutes determined by a countdown timer into a 'milkshake' texture. This increases the surface area for digestion.

4.4.3 ADDING DIGESTION MEDIA AND WASHING

1. A filter was attached to the tip end of a syringe.
2. The pre-prepared digestion media was put into the barrel of the syringe and pushed through the filter directly onto the endometrial sample in the sterile petri dish.
 - a. The enzymes within the digestion medium break down the interaction between the cells in the biopsy to produce a single cell suspension.
3. The contents of the petri dish were washed by collecting the mixed digestion medium and endometrial sample into a stripette and adding it to a Falcon tube.
4. The flask was shaken vigorously for 15 seconds and placed in an incubator for 1 hour at 37°C, 5% CO₂.

5. During the hour the sample was shaken vigorously every 20 minutes for approximately 15 seconds.
6. At 1 hour the digestion media was cloudy and no visible pieces of tissue remained.

4.4.4 CRYOPRESERVATION

1. The digested sample was poured through a 40 μ M cell strainer into a 50ml falcon tube. The strainer prevented glandular clumps going into the falcon tube.
2. The 40 μ M cell strainer was flipped over and placed onto another falcon tube and backwashed into a separate tube with additive free media to collect the glandular clumps.
3. 10 ml of 10% DCC (DMEM/F12 containing 10 % dextran-coated charcoal filtered FBS (DCC), 2 mM L-glutamine, 1X antibiotic-antimycotic mix, 1 nM estradiol (E2) and 2 μ g/ ml insulin) was pipetted through the strainer to wash.
4. The 40 μ M cell strainer was flipped over and placed onto another falcon tube and backwashed into a separate tube with additive-free media (DMEM/F12 **without phenol red**) to collect the glandular clumps.
5. The two falcon tubes (one with the glandular layer and one with the stromal layer) were centrifuged at 270 x g for 5 minutes to pellet cells.
6. The media above the pellet of epithelial cells was aspirated.
7. The glandular pellet clump was then digested for a further 10 minutes in Trypsin-EDTA (0.25 %) at 37 °C and then washed in 10 ml 10 % DCC. The media above the pellet was aspirated again.
 - i. The 10%DCC inactivates the trypsin
8. Epithelial cells were cryopreserved in 10 % DMSO in DCC (2 ml per vial) with the stromal compartment split into two or three vials depending on the original biopsy size.
9. After controlled cooling in a Mr Frosty container, samples were transferred to liquid nitrogen for longer term storage.

4.4.5 COLONY FORMING UNIT (CFU) ASSAY

1. Prepare CFU plates

- a. Make a solution of PBS and 1microlit/ml fibronectin (amount of solution prepared depended on number of biopsies being processed)
 - i. E.g. 3 participants = Total of 6 biopsies as each participant has 2 biopsies. Therefore, 6 CFU plates would be required
 - ii. Each CFU plate has 6 wells. Each well needs 1ml of solution therefore 36ml solution needed.
- b. Pipette 1 ml of solution to each well
- c. Put wells in incubator for 20 mins (37 degrees)

2. Thaw the sample

- a. Cryopreserved stromal cells were thawed for 3 minutes at 37 °C in the water bath
- b. The 2ml sample was then transferred immediately into 8 ml pre-warmed 10 % DCC.
- c. The sample was centrifuged at 270 X g for 5 mins to produce a pellet
 - The pellet at the bottom of the falcon tube contains 3 layers - a buffer, a layer of red blood cells and the stromal cells
- d. The supernatant was aspirated to leave only the pellet at the bottom of the falcon tube
- e. The cells were re-suspended in 10ml 10% DCC and cryoprotectant
- f. This solution was also then aspirated media from around sample

3. Count cells

- a. 15microlitres of the stromal cell sample was mixed with 15microlitres of trypan blue (put both into a micro-centrifuge tube)
- b. Viable stromal cells were counted on a Neubauer Improved haemocytometer
 - i. Dead cells stain blue as the dye enters the cells
 - ii. Live cells don't stain and so the dye doesn't enter the cell membrane
- c. 10ul of the cell suspension was added to each groove of a cell counting slide (2 grooves) using a p10 pipette

- d. The Luna Cell Counter was used to count the number of stromal cells per ml

An example of a stromal cell count

Average cell count from 4 x 16 squares

$$\begin{aligned} &= 13.5 \\ &= 13.5 \times 10^4 \times 2 \text{ (to account for trypan blue dilution)} = 27 \times 10^4/\text{ml} \\ &= 270000 \text{ cells/ml} \\ &= 270 \text{ cells/ul} \end{aligned}$$

If we want a baseline of 500 cells seeded for the CFU analysis, we could now calculate how much of the solution is needed to be put on the CFU plate

500 cells = 1.85ul of the solution (=500/270)

We wanted to put 500 cells in 3 wells and so 1500 stromal cells were suspended in media with FGF before plating.

4. Suspend sample in media with FGF

- a. 9mls of 10%DCC with FGF were put into a falcon tube
- b. 1500 stromal cells were put into the media (as per calculation above)

5. Plate cells

- a. The solution already lining the CFU plate wells (PBS and FN) was aspirated
- b. 3mls of media containing 500 stromal cells was put into each well
- c. Plates were cultured in 5 % CO₂ at 37 °C, left undisturbed for 3 days after which growth was monitored to ensure colonies arose from single cells.

6. Media Change

- a. Media was half-changed at 7 days of culture.

7. Counting Colonies

- a. On day 10 cultures were washed in phosphate buffered saline (PBS), fixed in 10 % NBF for 10 minutes at room temperature,

washed extensively in sterile water and then stained with Harris Hematoxylin for 4 minutes.

- b. For counting colonies, plates were imaged using a G:Box dark room imager and GeneSys software.
- c. Images were analysed in ImageJ by a single operator using the cellcounter plugin to count colonies of 50 cells or larger.

CHAPTER 5: TRIAL MANAGEMENT

This chapter outlines the team work involved in the management of this study, the risk assessments performed and important information on research governance.

5.1 ROLES AND RESPONSIBILITES

5.1.1 TRIAL SPONSOR

The NIHR toolkit on clinical trials states that The Research Governance Framework requires that all health research should have a formal sponsor (NIHR, 2017).

The sponsor of this trial was University Hospitals Coventry and Warwickshire NHS Trust (UHCW). The sponsor was responsible for ensuring the study met all regulatory standards and arrangements were in place for initiation, management, reporting, monitoring and financing of the trial.

Within this trial the sponsors were involved in the trial set up and thoroughly reviewed the protocol and all patient specific documents before they were sent to the REC, HRA and MHRA for approval.

They ensured that principles of GCP were adhered to and when they felt that there was a breach of protocol, the trial was suspended until appropriate measures were put into place.

5.1.2 CHIEF INVESTIGATOR AND PRINCIPLE INVESTIGATOR

The chief investigator and principle investigator for this trial was Professor Siobhan Quenby who is a world renowned professor and has been conducting research in RPL for over 20 years. Her guidance was instrumental to the set up and running of the trial.

The protocol was thoroughly reviewed by the chief investigator to ensure the trial could run in concordance with how it was set out in the protocol.

The chief investigator delegated trial related responsibilities to suitably trained staff in the research unit. These responsibilities included helping booking appointments, being an empathetic and understanding chaperone for the endometrial biopsies, collecting medication from pharmacy and helping with follow up visits. All respected roles were captured and signed on the delegation log.

The chief investigator reviewed the trial master file and ensured all CV's and evidence of appropriate training of all site staff was present in the file.

All adverse events were reviewed and signed by the chief investigator before they were sent to the sponsor. The chief investigator ensured that the trial was run in accordance with GCP principles.

The chief investigator also ensured all source data was complete as an 'end of study sign off' was completed each time a participant completed their journey in the trial.

5.1.3 TRIAL CO-ORDINATOR AND TRIAL MANAGER

I was the principle trial co-ordinator and responsible for the daily running of the trial. I have been heavily involved in the care of all the participants in the SIMPLANT trial. I approached and counselled all patients in the RPL clinic with Professor Quenby.

I followed patients up with phone calls and emails to answer any further questions and concerns they may have.

I have been present to consent all participants and have performed all the endometrial biopsies for participants recruited during my time as a research fellow. There were just 2 participants randomised by research fellows continuing to work in the research unit after I finished my post.

I have been present for the whole trial journey for most of the participants and rarely been away when they have attended for follow up visits. I have therefore understood their journey through the trial very well. Also, performing 94% of the biopsies myself has also allowed standardisation in the methods used to collect the biopsies.

I have been present for all the trial management group meetings and trial steering committee meetings. Working closely with the chief investigator and the trial manager on a daily basis, as well as planning and attending these meetings has given me a good understanding of how to conduct a clinical trial.

The day-to-day running of the trial involved co-operation between the research team, the laboratory team, the pharmacy team and the trial manager. Putting in

measures to ensure there were no missing lines in communication and good working relations between all those involved helped to improved my team working and team leadership skills.

I was supported by a trial manager; Mrs Katie Bruce who helped with the set-up of the study. Her role was taken over by Dr Indrani Manoharan just before the trial opened for recruitment. Having a new trial manager just before the trial opened for recruitment brought about new challenges but at this point I appreciated the importance of having an organised trial master file and an organised system for all trial specific documents and correspondence to help make the change of trial managers efficient.

All correspondence to the sponsor was done through the trial manager. All adverse events and a monthly tracker on the status of the trial were reported to the trial manager.

The trial master file was set up and maintained by myself and the trial manager. This was stored in the RD & I department on the 4th floor at UHCW. We had a site file in the BRU which contained documents needed for the daily running of the trial.

All the staff in the BRU were involved in the running of the trial. This included myself, a lead research midwife for the trial, the clinical trials assistants and the secretarial staff. A site initiation visit was done by myself and separately by the trial manager for all staff.

5.1.4 TRIAL PHARMACIST

We had expert advice during the set up and running of the trial from the trial pharmacist; Mr Mojid Khan.

He was responsible for finalising agreements and quotes from Sharp Clinical Services. A visit to the main site of manufacture was made to ensure systems were in place to conform with regulatory requirements. He was heavily involved in the finalising of the technical agreement between Sharp Clinical Services and the sponsor. This technical agreement was necessary to set out roles and responsibilities between the sponsor (UHCW) and the manufacturer of IMP (Sharp Clinical Services). This agreement contained finer details such as recall responsibilities, temperature and storage monitoring.

The trial pharmacist was responsible for overseeing the disintegration testing which had to be performed when Sitagliptin was converted to capsule form to ensure it had the same bioavailability and shelf life as the tablet form.

I had meetings with the trial pharmacist to confirm capsule size, packaging and labelling. The trial manager was responsible for the code break test and also making plans for accountability of trial medication and destruction when the trial ended.

The trial pharmacist overlooked the pharmacy team responsible for checking prescriptions and issuing the trial medication.

5.2 RISK ASSESSMENT

A risk assessment is designed to identify potential vulnerabilities in trial design and to prepare trial management and monitoring plans to minimise the risks. The risk assessment addressed the investigational medicinal product (IMP) risk category and devised a clear risk monitoring plan. The risk assessment was submitted to the MHRA.

This trial was categorised as a type A study (i.e. no higher risk than that of standard medical care) because

1. There are no substantial dose modifications made in comparison to the licensed indication.
2. There are no combinations of medication for which interactions are suspected.
3. There is no reason to suspect a different safety profile in the trial population. This was supported by the evidence from MSD Ltd provided on the use of Sitagliptin in healthy volunteers.

The extensive risk management and risk management strategies were signed by the chief investigator, the sponsor, the research governance manager and the research pharmacist.

5.3 RESEARCH TEAM AND SITE INITIATION VISIT

Before the study was opened for recruitment I carried out a site initiation training session for all staff involved in the trial. This was attended by the research unit staff, the randomisation team, the pharmacy team, the trial manager, one member from the sponsor team and the chief investigator.

This involved a formal presentation on the scientific basis of the trial and an explanation of the patient pathway within the trial. I discussed everybody's roles and responsibilities and ensured there were no concerns.

I performed the training on two separate occasions so that everyone had an opportunity to attend. All those involved in the trial, including the laboratory team had GCP certification and had good experience of clinical trials already.

5.4 DATA COLLECTION AND MANAGEMENT

Data management processes are essential to ensure data included in trial reports and publications are accurate. A data management plan with clear data entry instructions were created by the trial manager.

I designed the case report forms (CRF) for the trial. Each participant was assigned a case report form pack which contained all the paper work for all the hospital visits for that participant within the trial (screening, consent, intervention 1 & randomisation, follow up visit 1, follow up visit 2 and intervention visit 2) (Appendix 17 - Sample of screening and consent CRF).

The CRF packs were reviewed at trial management meetings before submission to the sponsor for local approval. The paper case report forms were filled in by myself at the participants' clinic visit. Original CRF's have been stored in locked filing cabinets in the Biomedical Research Unit. A paper copy of each case report pack

will be securely saved for 25 years in accordance with the UHCW NHS Trust archiving procedures.

The CRF's allowed us to capture important information from each hospital visit but also included a pregnancy test log as participants had to complete one every two weeks. This was entered into an electronic pregnancy test log weekly so that I was able to constantly keep up to date with pregnancy test tracking. If there were any pregnancy tests that were overdue, I would alert the lead research midwife who would ring or email the participants.

An agreement with MedSciNet was made for development of the electronic trial database. The database is compliant with GCP requirements and is password protected. Validation testing was conducted by myself and the trial manager. Any anomalies were resolved in liaison with the database manufacturer before the start of data entry.

Once a participant had completed their journey in the trial the trial manager was emailed. The CRF pack for that patient was photocopied without any identifiable data. Data from the CRF was entered onto the electronic database by the trial manager. The photocopied version was then scanned and stored onto the electronic research drive and the photocopied paper copy stored in the CRF folder in the R&D offices at UHCW.

5.5 TRIAL MANAGEMENT GROUP MEETINGS

There were regular trial management meetings (TMG) from the initial set up and design of the trial and then quarterly during the running of the trial.

The TMG team consisted of

- Chief investigator: Siobhan Quenby
- Myself: Shreeya Tewary
- RD+I manager: Becky Chadwick
- The trial manager: Katie Bruce then Dr Indrani Manoharan
- A member of the UHCW governance team: Isabella Petrie
- The trial pharmacist Mojid Khan

- The trial statistician Peter Kimani

At the first meeting we discussed the study design and objectives. We also discussed timelines, recruitment targets, funding and processes to secure trust sponsorship.

Between the first and second TMG meeting I had to finalise the trial protocol, organise peer reviews and the risk assessment from pharmacy and RD&I Governance.

The next meeting was held one month later where sponsorship by UHCW was confirmed following a low risk assessment. Funding by Tommy's charity was also confirmed at this meeting.

Quotes that I had received from various companies to supply the placebo had been evaluated by the trial pharmacist and discussed at the trial management meetings. After discussion with everybody at the meeting it was decided that Sharp Clinical Services would be best to deliver the study requirements.

We also discussed and confirmed methods of randomisation with the trial statistician.

There were four further TMG meetings before the trial commenced to ensure there was no room for error when the trial opened. The agenda for each meeting would follow the same pattern which included review of minutes and action points from previous meetings, updates on progress & recruitment, pharmacy updates, review of any substantial or non-substantial amendments, monitoring plans and safety reporting.

There was an extensive checklist to be completed at the TMG meeting on the 23rd August 2016 to ensure there was tight control on all aspects of trial management before the trial was officially opened (Appendix 20).

The trial then opened for recruitment on the 15th September 2016. We have had quarterly TMG meetings since to review the progress of the trial.

5.6 TRIAL STEERING COMMITTEE (TSC) AND DATA MONITORING COMMITTEE(DMC)

As this was a small single centre study with a laboratory rather than a clinical outcome, the sponsors agreed that a Joint TSC/DMC would be appointed.

The members were:

- Independent chair: Professor Arri Coomarasamy (University of Birmingham),
- Independent members: A consultant obstetrician, Dr Gupta (Heart of England NHS Trust)
- An independent statistician: Dr Tobias (Spanish Council for Scientific Research)
- The non-independent members were the chief investigator, the trial manager, the trial statistician and myself.

The TSC/DMC charter was developed by the trial manager to be signed by all members of the TSC/DMC to commit to join the SIMPLANT TSC/DMC.

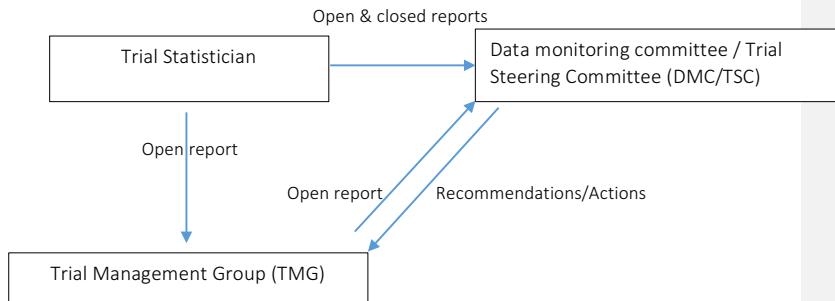
The joint Trial Steering Committee and Data Monitoring Committee (TSC/DMC) provided independent overall supervision of the trial, in particular with respect to the progress of the trial, adherence to the protocol, participant safety and review of any new information, particularly with medication safety.

At the first TSC/DMC meeting I presented an overview on the scientific basis behind the trial. We then discussed the safety data and information on optimal dosing of Sitagliptin. We also discussed the validity of the sample size calculations with the trial statistician.

The Trial Steering Committee had reviewed and agreed the final version of the Protocol before submission to the REC and MHRA.

Once the trial had opened for recruitment, telephone conference meetings were held at six monthly intervals. The trial manager and myself were responsible for producing an update report and circulating this to the TSC/DMC one week ahead of the meeting. This would involve up-to-date information on recruitment targets with a consort diagram and adverse event reporting.

The following diagram shows the flow of information between the TSC/DMC and the Trial Management Group (TMG). I would receive an open report from the TSC/DMC and a closed report would be shared between the trial statistician and the TSC/DMC.



5.7 RESEARCH GOVERNANCE

Appropriate and effective research governance ensures the proposed research protects the safety of patients and so monitoring and audit measures are put in place for any research trial.

A formal monitoring plan which was put in place by Isabella Petrie (Research Governance Manager). This was approved by the TMG Committee at a meeting before the trial opened for recruitment.

This monitoring plan included

- Three monthly TMG meetings
- Six monthly TSC meetings by teleconference
- A site visit to view set up and team understanding of trial
- Pharmacovigilance plans to regularly monitor any SAE's and a list of trigger factors which would lead to a monitoring visit (e.g. persistent late reporting of AE/SAE's, concerns with data collection, protocol deviation).

This monitoring plan also details governance procedures which will take place at the end of the trial which include:

- Communication of closure of trial to participants registered but not randomised
- Site visit and closure notification
- IMP accountability
- Provisions for archiving.

We had an internal and external audit of this trial which forms a large part of research governance procedures.

5.8 TRIAL SUSPENSION

Despite thorough review of all documents and many TMG meetings to agree and finalise how the trial would be managed and run, the trial was suspended on the 21st October 2016 due to a serious breach of the trial protocol. Details of this trial suspension and actions taken to overcome it are detailed in chapter six.

5.9 TRIAL MONITORING

5.9.1 INTERNAL MONITORING

In November 2016 when the trial was actually suspended and applications were being made to the REC and MHRA to restart the trial, we had an internal monitoring visit from the sponsor.

This involved a review of all relevant documentation, the trial protocol, CRF's and patient notes.

In the final report there were some areas of concern, mainly to do with data entry which were easily rectified but overall the report commented on good practice.

5.9.2 EXTERNAL MONITORING

External monitoring forms an integral part of effective Research Governance and ensures levels of control are being implemented.

On the 27th January 2017, just six weeks after the trial had been re-opened we were informed of an external audit and monitoring exercise which was going to take place on the 8th February 2017 by Trueman Hall Associates Ltd (Appendix 24).

We were informed that access to all research files and patient hospital notes would be necessary and we would need to be available to answer any queries. All participant hospital notes and case report forms were made available for review.

We received feedback on the audit and produced a response to some concerns raised. Overall, the concerns raised were actually due to certain information not being available on the day of the audit. This was rectified very quickly. There were some concerns about the protocol, sample consent form, and participant information card in the investigator site file not being signed by all three parties. Again, all these issues were addressed. Overall, the sponsor was pleased with the outcome of the external audit.

5.10 CODE BREAK TEST

Along with the code break test which occurred before the trial opened for recruitment there was another code break test while the trial was running on the 19th January 2017. This confirmed the safety and integrity of the systems in place to contact the clinical team and also unblind participants if necessary.

5.11 AMENDMENTS

5.11.1 NON SUBSTANTIAL AMENDMENTS

A non-substantial amendment was made soon after the trial opened to provide clarification on the contact details on the emergency contact card. Originally the contact card had a phone number on it and stated to ask for the SIMPLANT research team in case of an emergency. This was thought to be a bit vague with

possibilities of confusion between the participant and switchboard. We changed the card to state that participants should specifically ask for Dr Tewary or Professor Quenby when calling switchboard out of hours in an emergency situation. It was felt that this change would facilitate appropriate re-direction of the call by switchboard staff.

Before this non substantial amendment was submitted to the REC it was tested with a 'out of hours' test call'. We had written confirmation from switchboard that their staff had all been made aware of possible calls relating to the trial and where to transfer the call.

5.11.2 SUBSTANTIAL AMENDMENTS

Two substantial amendments were made when the trial was suspended. These are detailed in the chapter six titled 'Trial Suspension'.

The third substantial amendment was made in relation to our required sample size. It was calculated that 30 participants would be needed for the trial have a power of 95% at a 10% significance level. To allow for a 10% drop-out rate we had planned to recruit 34 participants.

At the point of 20 participants being randomised we had 4 participants who had already been withdrawn. This meant that if there were any further withdrawals we would not have reached our planned sample size of 30 participants by recruiting 34 participants.

We therefore made an application for a substantial amendment to our recruitment target. Our amendment stated that we would like to randomise up to 40 participants as the supply of the study medication would allow for this but would stop recruitment once 30 participants have completed the study.

Also, to allow time to recruit the additional participants we amended the protocol to extend the recruitment period from 12 months (ending June 2017) to 18 months (ending December 2017). The study was funded for 24 months and therefore the existing funding would cover the extended recruitment period. This extended recruitment period would not affect the shelf life of the study drug. This amendment was approved swiftly by the REC, MHRA and HRA.

CHAPTER 6: TRIAL SUSPENSION

6.1 EVENTS SURROUNDING SUSPENSION

The SIMPLANT study opened for recruitment on the 15th September 2016. We had randomised 4 participants within the first month. On the 21st October 2016 the study was suspended by the sponsor due to a serious breach of the trial protocol. This breach was related to screening and eligibility criteria.

As described in the protocol, normal renal function was necessary for participants to be eligible for the study. This is because the SPC for Sitagliptin states that before initiating Sitagliptin at the standard dose of 100mg, normal renal function should be confirmed and for patients with an estimated glomerular filtration rate (eGFR) below 50mL/min the dose of Sitagliptin should be lowered. We needed all participants to be on the same dose to look at the true effect of Sitagliptin on the eMSC count.

Therefore, normal renal function was part of the eligibility criteria so that a standard dose of 100mg of Sitagliptin could be safely administered.

The eligibility criteria on the original proposed protocol stated 'normal renal and hepatic function'. When the initial application with this protocol was made to the REC, one of the recommendations was the need to define 'normal renal and hepatic function'.

The protocol was amended and I had defined normal renal function with the reference ranges from the laboratory at UHCW (Urea 2.5 – 7.8mmol/L, Creatinine 50 -90umol/L, potassium 3.5 – 5.3mmol/L, Sodium 133 -146mmol/L). I had no reason to suspect that the young and healthy group of patients who would be approached for this study would not have normal renal function and hepatic function.

Some of the above components of a renal or liver function test can however be altered by hydration status. For example, a slightly low creatinine or urea level is of no clinical significance and actually only expresses normality for this population of young fit and healthy women. If a creatinine level is lower than the normal range or a bilirubin level is lower than the normal range, this result is actually viewed as better than normal. Clinically, these slight deviations from the specific laboratory reference ranges would not be viewed as abnormal.

Soon after the trial opened for recruitment we realised that the inclusion and exclusion criteria were too tight. This issue became apparent when a patient was deemed eligible to enter the study as her renal function was better than normal as she had a marginally lower urea level than the normal range. This was however unacceptable due to the reference ranges in the protocol. At this point I realised that clinical judgement had no bearing on defining eligibility of the patient. This only came to light when I called the randomisation team on the day of her first intervention visit.

After her endometrial biopsy was performed the randomisation team revealed that they would not be happy to perform randomisation as the participant did not actually fulfil eligibility criteria. Long discussions between the chief investigator and the trial manager left us in a position only to be told to break the news to the participant that she had to be withdrawn from the study.

When explaining the situation to the participant she explained that she had 3 litres of water to drink just before her blood test was performed and questioned whether this was the reason her blood test was abnormal.

With this new information we concluded that her blood tests were actually clinically invalid and needed to be repeated. We repeated her renal function tests which were now within normal range. The randomisation team were called with the new set of results and she was successfully randomised. She started medication that day.

Repeating the blood tests and effectively re-screening the participant to confirm eligibility was identified by the sponsor as a serious breach of the protocol. This lead to a local formal investigation straight away.

An investigation of all patients who had been screened for the study took place by the sponsor. There were other patients who had been deemed eligible as their renal and hepatic function were clinically normal. However, there were certain values within the tests which were marginally out of the reference range, in fact their results were better than average, however, were not within the range specified in the eligibility criteria and so the sponsor deemed this as persistent non-compliance with the protocol and was therefore reported as a serious breach of protocol.

The trial was suspended on the 21st October 2016. The notification of this serious breach of the trial protocol was made to the REC and MHRA by the sponsor on the 24th October 2016.

The sponsors directed me to

1. Recall and remove the patient who had been screened twice prior to randomisation
2. Remove patients who had been consented based on their blood test results which did not actually meet eligibility criteria.

These were difficult phone calls to make. Fortunately, the patients were very understanding and appreciative of the care they had received so far. They were all asked if they would like to participate in any other RPL trials in the future which they were all happy for.

Between the 21st October 2016 and 15th December 2016 I worked hard with the chief investigator and sponsors to get the trial open again. We submitted 2 substantial amendments through the REC and MHRA.

6.2 SUBSTANTIAL AMENDMENT 1

Notification of the suspension which was sent via IRAS to the REC on the 25th October 2016. On the 26th October 2016 the REC acknowledged receipt of the amendment.

On the 9th November 2016 the REC gave favourable ethical opinion of the suspension amendment and action which had been undertaken as a result of the suspension. On the 14th November 2016 the MHRA accepted the amendment and the actions that had been put in place.

It had taken nearly one month to receive acknowledgment from the MHRA of the trial suspension. Although they responded within a standard time frame, I realised that opening the trial for recruitment again would take a very long time considering two separate amendments would have to be sent; firstly proposing changes that would be made to the current trial pathway and then secondly to request to restart

the trial. I had a meeting with the trial sponsor and after discussion with the MHRA, an agreement was made to send the second and third amendment together.

6.3 SUBSTANTIAL AMENDMENT 2 +3

I formulated a statement of all the changes that would be put in place to ensure safe running of the trial (Appendix 22).

Issues we had to address were

- Defining normal renal function
- Defining normal hepatic function

I had to change the eligibility criteria on the protocol and the information about blood tests on the participant information leaflet. I then also had to amend the consent form to ensure it referred to the correct new version of the participant information leaflet.

Changes instigated to renal and hepatic function:

Renal Function

Eligibility Criteria on Original Protocol

- Inclusion Criteria: Adequate renal function defined as Urea 2.5 – 7.8mmol/L, Creatinine 50 – 90 umol/L, potassium 3.5 – 5.3mmol/L, sodium 133 – 146mmol/L.
- Exclusion Criteria: Renal impairment with eGFR<50mL/min.

The SPC for Sitagliptin actually uses eGFR (estimated glomerular filtration rate) to define renal function which is a calculated result relative to a participants age, weight and creatinine level. eGFR would give us a more accurate assessment of renal function. The SPC states that if the eGFR is <50mL/min then dose adjustment is required and this is why it was originally part of the exclusion criteria.

In the original protocol, there were participants who had normal renal function and this would have been reflected with a normal eGFR however, were rendered

ineligible according to the original eligibility criteria because one of the parameters of the renal function tests fell out of reference range e.g. urea 2.4mmol/L with reference range 2.5 – 7.8mmol/L.

After discussion at a TMG meeting we decided to use eGFR as a more important and accurate way of assessing renal function. This would also take away the possibility of the result being altered by the patients' hydration status. eGFR is calculated using the Cockcroft Gault Equation.

I contacted Dr Paul O'Hare who is a consultant physician and the endocrinology advisor for the trial and also Mr Mojid Khan, the clinical trials pharmacist at UHCW who agreed that eGFR will be a more accurate way of measuring renal function and at the same time will ensure that fit and healthy patients are not rendered ineligible. A written report from Dr O'Hare was provided for the application.

Revised Eligibility Criteria on New Protocol

- Inclusion Criteria: No longer includes renal function – only in exclusion criteria
- Exclusion Criteria: Renal impairment (eGFR <50mL/min or AKI >1)
 - *Note: eGFR will be calculated using the Cockcroft Gault Equation:

$$\text{eGFR} = \frac{(140 - \text{Age}) \times \text{Weight (Kg)} \times 1.04}{\text{Serum Creatine (micromol/L)}}$$

Figure 6.0.1: Statement from Dr O'Hare

Re: SIMPLANT TRIAL AMENDMENT

From O'Hare, Paul 
To shreeya.tewary@doctors.org.uk , Quenby, Siobhan 
Copy Jones Ceri (RKB) Head of R,D&I 
Date 2016-10-21 11:38

Message 1 of 1 <

Dear Siobhan and Shreya

I was sorry to hear you have had difficulty over the lower end of the normal range and that their inclusion now requires you to do an amendment which I hope will not delay you on your recruitment to this trial. values below the normal range in my clinical experience are of no relevance and often just reflect a baseline characteristic of the population. A low creatinine relevant in this group of young women does not reflect disease but the small muscle mass of some young women.

I would totally agree you should use the upper limits but I would recommend not using Cr Clearance but EGFR that the labs calculate based on weight, age and creatinine and is now standard for defining renal function. You can only find a Cr clearance by undertaking a 24 hour or fixed time urine collection and I am sure you do not want or need to do this! with LFTS use the upper limit but best to include alk phosphatase as occasionally you see 1 biliary cirrhosis and would want to exclude and further investigate.

Make sure you use same definitions and wordings in inclusion and exclusion.

In inclusion I do not like "adequate" and would affirm "normal". On the rescreening issue often in trials a time period is stated eg after 3 weeks if there is a remediable factor eg alcohol or poor control and it protects against patients being rescreened too often to no avail.

Paul

*LFTS – Liver function tests

Hepatic Function

Eligibility Criteria on Original Protocol version 1.2, 11th May 2016

Inclusion criteria: Adequate hepatic function defined as total protein 60 – 80g/L, Albumin 35 – 50g/L, Bilirubin 4 – 20umol/L, Alkaline Phosphatase (ALP) 35 – 105U/L, Alanine Transferase (ALT) 5 – 38u/l.

Exclusion criteria: Liver impairment, defined as any value out of normal range (total protein 60 – 80g/L, Albumin 35 – 50g/L, Bilirubin 4 – 20umol/L, Alkaline Phosphatase (ALP) 35 – 105U/L, Alanine Transferase (ALT) 5 – 38u/l).

With this original eligibility criteria, patients with a bilirubin <2 umol/L (range 4 – 20umol/L) or an ALP of 34 U/L (range 35 – 105 U/L) with otherwise normal blood

tests were not eligible for the study. Again, these results are out of the reference range but of no clinical significance. They actually tell us that the blood test results are better than would be expected and so patients were being unfairly excluded.

The SPC for Sitagliptin states that Sitagliptin has not been studied in patients with severe hepatic impairment and care should be exercised.

After discussion with Dr Paul O'Hare it was decided that ALT, ALP and bilirubin are sufficient markers of hepatic function but values below the lower limit of the reference range are clinically insignificant in this situation.

Revised Eligibility Criteria on New Protocol

Inclusion criteria: No longer includes hepatic function.

Exclusion criteria: Liver impairment defined as any value above the normal reference range (alt >38U/L, ALP >105U/L, Bilirubin >20umol).

6.4 TRIAL RE-START

Approval for substantial amendment 2.0 & 3.0 was received from the REC on the 6th December 2016 and from the MHRA on the 7th December 2016 (Appendix 23).

The sponsor had set out further action points to be completed before the suspension could be lifted.

The chief investigator and I had to read and sign all the local standard operating procedures for the running of a clinical trial.

We had to re-train all staff involved in the study. This included training for the randomisation team and pharmacy team as well as a new site initiation visit for the clinical research team.

The principle investigator and co-investigators also had to attend a GCP refresher course.

The trial opened again on the 15th December 2016.

6.5 LESSONS LEARNED

The suspension of this trial which lasted two months taught me that a trial protocol must be written extremely carefully taking into consideration the safety of participants but also ensuring that patients are not unfairly excluded.

I learnt skills of team working at this difficult time. I had to liaise with the trial management team, the sponsor, the REC and MHRA to make changes and prove that I could run this trial safely.

I learnt skills of time management as I ensured all new documents, cover letters, statements and peer reviews were ready in good time to send as soon as possible.

I facilitated discussions with the MHRA to help us to submit amendment 2+3 together which was a fundamental part of accelerating the process. This is because receipt of each amendment takes 32 days to be acknowledged and then decision making with a formal response can take a long time.

I also learnt that a trial protocol is not like a clinical guideline which is essentially used as a guide and can be overridden if clinically justified. When I write another protocol I would use a very different thought process.

CHAPTER 7: TRIAL RECRUITMENT

7.1 TRIAL PATHWAY

7.1.1 PARTICIPANT ENTRY

All the patients invited to participate in this trial were identified from the recurrent miscarriage clinic or the implantation clinic at UHCW.

70 patients were screened and given information on the study at the recurrent miscarriage clinic.

During my time as a clinical research fellow I was present at each of these clinics to further counsel patients about the trial once they had seen Professor Quenby or Professor Brosens.

I discussed the scientific basis of the trial with every participant approached for the study and explained the requirements of the trial. This visit would typically last an hour. Patients would understandably have questions and concerns as it was a big commitment for any patient to enter this trial.

Patients were given the PIL for the trial at this visit and given contact details for the research unit. All patients given a PIL were entered onto a screening log (Appendix 14).

I had specifically set up an email address for the participants taking part in the trial called simplant@uhcw.nhs.uk. This was given to all patients approached for the study so that they could send any questions they had about the trial once they had left the clinic.

Patients were advised that participation was completely voluntary and that their choice to participate or not participate would not affect their usual care.

7.1.2 PARTICIPANTS NOT ELIGIBLE AFTER SCREENING

Five of the seven patients did not meet the eligibility criteria for the trial due to abnormal liver function tests or renal function tests.

There was one patient who was not eligible as she turned 43 years old before she re-attended to the clinic to take part and consent to the trial.

There was one patient who was on metformin and therefore not eligible as there is a risk of hypoglycaemia when Sitagliptin is used with other diabetic medication.

7.1.3 WITHDRAWALS PRIOR TO RANDOMISATION

There were four participants who consented to take part in the study but were withdrawn prior to randomisation. Two of these patients self-withdrew as they changed their mind about postponing attempts to pregnancy for three months which was a requirement of the trial.

One of the participants had to be withdrawn because an endometrial biopsy could not be obtained despite attempts by myself and the chief investigator. We referred her on to have a hysteroscopy by one of the consultants in reproductive medicine.

The fourth participant was withdrawn at the time of the trial suspension. She had been considered eligible to enter the trial by the co-ordination team as her renal and liver function tests were clinically normal however one of the parameters in the liver function tests was out of range (result: 34U/L, range: 35 – 105 U/L) and therefore according to the eligibility criteria of the original protocol she was not actually eligible although her results were actually better than the normal range. Details of this withdrawal, the trial suspension and the substantial amendment submitted to the REC and MHRA and are detailed in chapter 6.

7.1.4 WITHDRAWALS AFTER RANDOMISATION

There were five participants who were withdrawn after randomisation. The first participant was withdrawn in concordance with the trial suspension as she was considered not actually eligible for the trial on the basis of her renal function test (result: urea 2.3 mmol/L, range 2.5 – 7.8mmol/L). Again, her results were better than the normal range. Details of the trial suspension and her withdrawal can be found in chapter six.

There were two participants who became pregnant after randomisation while taking trial medication and were therefore withdrawn. Both of these participants conceived in the cycle after the initial biopsy in the first month of taking medication. They both stopped taking the medication immediately and arranged a follow up visit at the research unit as soon as possible.

Both of these patients have been followed up every three months as per the sponsors risk assessment protocol. I am pleased to report that one of them has given birth to a healthy baby.

There was one participant who was withdrawn by the trial co-ordination team. She did not attend for her scheduled follow up appointment one month after the biopsy. After repeated attempts to email and telephone the participant she was withdrawn due to non-compliance with the trial pathway. These follow up appointments were a fundamental part of the trial pathway to assess for any side effects to the medication, exclude pregnancy and also to issue the next months' supply of medication. We did find out over one month after her follow up was due that she had not attended and changed her home and mobile phone number due to personal reasons.

There was one participant who withdrew the day the endometrial biopsy was taken. When she received the treatment pack and realised that the medication was in capsule form she questioned the presence of gelatine in the capsule. We confirmed the ingredients did actually include gelatine and so for religious reasons the participant decided to withdraw herself from the study before any medication was taken.

7.2 ENROLMENT PROCEDURE

Once patients who had been invited to participate had sufficient time to read the information and make a decision about enrolment an appointment was made with me at the research unit to confirm eligibility and sign consent forms.

As this was a Clinical Trial of an Investigational Medicinal Product (CTIMP), consent had to be taken by a doctor. I consented all participants for the trial myself.

There were 39 participants who consented to take part in the study. Once consent forms were signed, participants were given an ovulation kit, barrier contraception and contact details to get in touch once ovulation had occurred. It was emphasised that they must not have had unprotected intercourse in the cycle leading up to the biopsy.

7.3 INTERVENTION VISIT

Consented participants would then email me once they had a positive ovulation test. The intervention visit would then be planned for 7-10 days after ovulation, during the implantation window, when the endometrium is receptive to the implanting embryo.

The first participant attended for the endometrial biopsy and randomisation on the 16th September 2016. In total there were 35 participants who attended for the initial intervention visit and were randomised.

This intervention visit had to be arranged at a time to suit the patient, the laboratory team, pharmacy team and the randomisation team. Participants were warned beforehand that the endometrial biopsy may cause some pelvic pain and cramps due to uterine contractions. I advised them to take 400mg of ibuprofen and 1g of paracetamol prior to their visit.

At the intervention visit consent would be confirmed. Then, a pregnancy test would be undertaken and it would be confirmed that no unprotected intercourse had occurred in the month leading up to the visit.

The participant would be asked to empty their bladder before a pelvic ultrasound scan was performed. Patients with RPL are all familiar with transvaginal scans from previous pregnancies. During the pelvic ultrasound I was able to assess the endometrial thickness and also the axis of the uterus. The scan provided me with the location of the endometrial cavity relative to the cervix so that the biopsy could be obtained as quickly and easily as possible to reduce patient discomfort.

I would then discuss what to expect from the procedure. I always explained that the first part of the procedure was similar to a smear test in that they would be in the same position and a speculum would be inserted. The aim of this explanation was to help normalise the procedure to help reduce the anxiety associated with having this intrusive test performed.

I would explain that a small catheter would be inserted through the neck of the womb into the uterus to take the biopsy and induce a wound healing response and that once the catheter was inside the uterus I would count back from 10 to 1 and although the procedure may cause some period-like cramps it is quite quick. Participants were always informed that the procedure could be stopped at any point they felt necessary. Entonox was available to use when the biopsy was being taken.

Taking the endometrial biopsy involved inserting a Cusco's speculum into the vagina to visualise the cervix. The cervix was then cleaned with a cotton tip dipped in normal saline. A Wallach Endocell sampler was used to take the biopsy. This is a simple manual suction device commonly used in gynaecology clinics used to screen for carcinoma, pre-cancerous conditions and menstrual disorders. It is mouldable for easy insertion into an acutely anteverted or acutely retroverted uterus. No cervical dilatation is required. It provides excellent suction by elastomeric seal piston plunger. The sampler is inserted through the cervix into the uterus to take the endometrial biopsy.

A research fellow from the laboratory was always present at the time of taking a sample so that there was minimal but consistent time taken in the transport of the sample from the participant to its initial processing.

7.4 BIOPSY TRANSPORTATION AND CRYOPRESERVATION

A member of the laboratory team present at the time of the biopsy would take the biopsy to the clinical sciences research laboratory for immediate preparation and cryopreservation.

The time the biopsy was taken and the time it was stored for cryopreservation was recorded in the laboratory data tracker.

The average time from collection of the biopsy to cryopreservation was 114.3 minutes (range 105 – 120 minutes).

7.5 RANDOMISATION

Once the biopsy was taken and transported to the laboratory for initial processing, randomisation was performed.

This involved a phone call to the randomisation team in RD&I where consent and eligibility criteria were checked again before the participant was allocated three pack numbers, each containing one months' supply of medication. These three pack numbers were documented on the case report form.

A prescription for the first pack was completed, signed and sent to pharmacy. In pharmacy, the prescription and medication was checked by two pharmacists before it was made ready for collection.

The participant was then given the treatment pack which came as a bottle, along with:

1. A pregnancy test to complete in two weeks at home
2. A symptom diary
3. An emergency contact card.
4. Barrier contraception
5. A follow up appointment in 4 weeks +/- 4 days

In total, 35 patients were randomised. Randomised participants would then be added onto the recruitment log (Appendix 15).

I would then send the letter approved by the REC and HRA to the G.P informing them of participation in the trial and the possibility of the participant being on Sitagliptin.

7.6 FOLLOW UP VISIT 1

At 4 weeks +/- 4 days the participant would attend for a follow up visit. The symptom diary would be collected and reviewed. Any adverse events would be reported to the sponsor and a pregnancy test would be completed.

I would confirm that no new medication had been started, there was no change in medical history, there were no concerning adverse events and the participant was happy to continue in the trial.

Safety of continuing in the trial would be confirmed before another prescription for the next pack number assigned to the participant would be completed. Medication for the following month would be issued.

Participants would also be given another symptom diary for the next month and another pregnancy test to complete in two weeks at home. Further barrier contraception would be given to couples.

The five participant withdrawals after randomisation all occurred within the first month of treatment after the baseline endometrial biopsy. Therefore, there were 30 participants in total who attended the first follow up visit.

7.7 FOLLOW UP VISIT 2

At 4 weeks +/- 4 days, participants would attend for a second follow up visit. Once again, the symptom diary would be collected and reviewed, any adverse events would be reported to the sponsor, a pregnancy test would be completed and safety of continuing in the trial would be confirmed.

At this 2nd follow up visit, the 3rd month of medication would be issued. A pregnancy test would be issued to be completed two weeks later at home.

This time, participants would also be issued an ovulation kit to plan for the second endometrial biopsy to mark the end of the trial.

Participants would email me directly at simplant@uhcw.nhs.uk once their ovulation test was positive and a second endometrial biopsy was arranged for 7-10 days later, again after liaising with the laboratory team for availability.

7.8 INTERVENTION VISIT 2

In total, there were 30 participants who attended for the final visit of the trial pathway. This visit marked the end of the trial period.

Symptom diaries were reviewed and any left-over medication was collected and sent to pharmacy for accountability and destruction.

The procedure of this visit was exactly the same as the first intervention visit where patients would have a pregnancy test before proceeding to a pelvic ultrasound scan and endometrial biopsy.

Participants were given a questionnaire to fill in about their experience of being in a clinical trial.

Follow up after the second biopsy was part of standard clinical care. Patients who become pregnant after having been seen in the recurrent miscarriage clinic or implantation clinic routinely get regular early pregnancy scans from six weeks' gestation.

CHAPTER 8: STATISTICAL ANALYSIS PLAN

8.1 STATISTICAL ANALYSIS PLAN

8.1.1 CONSORT DIAGRAM

We summarised the patient pathway using a consort diagram adapted from the consort-statement.org. The consort diagram can be found at the start of the results chapter.

The whole study team and myself were blinded to treatment allocation when results were analysed.

All participants in one treatment arm were labelled as Group 0 and all participants in the other treatment arm were labelled as Group 1. This is also reflected in the consort diagram at the start of the results chapter.

8.1.2 CLONOGENIC ASSAY

When performing the clonogenic assays we performed the stem cell count for each participant in 3 wells to ensure there is uniformity across the sample and results were replicable.

The table below shows an example of results for two participants.

- RX has a count of 8, 6, 6 colonies in each well per 500 endometrial stromal cells in the baseline biopsy and a count of 8, 5, 15 colonies per 500 endometrial stromal cells in the final biopsy

RXx has a count of 8, 5, 3 colonies in each well per 500 endometrial stromal cells in the baseline biopsy and a count of 8, 5, 15 colonies per 500 endometrial stromal cells in the final biopsy.

RID	Well 1a 500 cells	Well 2a 500 cells	Well 3a 500cells	sumA/ 1500cells	well 1b 500 cells	well 2b 500 cells	well 3b 500 cells	SumB/ 1500 cells
RX	8	6	6	20	8	5	15	28
RXx	8	5	3	16	3	6	4	13

We decided to take the sum of the colonies in all 3 wells to get a colony count per 1500 endometrial stromal cells rather than an average of all three wells so that we were able to include all results obtained. It is biologically plausible that one well from the same sample could contain a different number of clonogenic cells to another and so, at a meeting with the investigators and the statistician it was decided not to exclude outliers.

Exclusion of outlying assay results when the assay is done in triplicate is standard laboratory practise when there is a large sample size as the variability can be explained by laboratory error.

Therefore, through the results chapter, the endometrial mesenchymal stem cell count (eMSC count) is a calculated as a count of the colonies of stem cells grown per 1500 endometrial stromal cells.

8.1.3 PATIENT DEMOGRAPHICS

Continuous and ordinal characteristics were summarised using the mean, median, standard deviation (sd), interquartile range (IQR) and range. Categorical characteristics were summarised using the count and equivalent percentage.

Demographic data compared between treatment groups included

- Age
- Baseline eMSC count
- Number of previous miscarriages
- Body Mass Index
- Surgical Management of Miscarriage in the past (ERPC)

All characteristics between the two treatment groups were compared using the Mann Whitney U test.

Any statistically significant difference between the two treatment groups was accounted for in the secondary data analysis when fitting the adjusted Poisson Regression Model.

As discussed in chapter one, Asherman's syndrome and/or intrauterine adhesions from repeated evacuation of retained products of conception (ERPC) has been linked to RPL. I wanted to ensure there was no difference in the number of ERPC's performed between the two treatment groups.

8.1.4 eMSC COUNTS

Our primary outcome measure as outlined in the trial protocol was the eMSC count after three months of Sitagliptin versus three months of placebo determined by a clonogenic assay. We fitted a Poisson model to compare the eMSC counts in the two treatment groups at three months. The only predictor in the model was the intervention group allocation.

Although we have 30 participants who successfully completed the study we have 29 complete clonogenic assays from the set of initial (baseline) endometrial biopsies. This is because we have one sample which could not be analysed due to

heavy yeast contamination which prevent the stem cell growth and colony formation.

Also, we have 29 results in the set of 30 final endometrial biopsies. Again, there is one sample with heavy yeast contamination and so stem cell colonies could not be counted.

8.2 PRIMARY OUTCOME MEASURE

Our primary end point as outlined in the trial protocol was the eMSC count after 3 months of Sitagliptin versus 3 months of placebo assessed by a clonogenic assay.

8.3 SECONDARY OUTCOME MEASURES

We have used a Fisher's Exact Test to assess the significance of difference in reports of adverse events between the two groups.

The questionnaire results have been expressed as ratios and comments received by participants have been reviewed to evaluate any concerns with tolerability of the study medication and also the acceptability of the study.

8.4 OTHER DATA ANALYSIS

We were also interested in evaluating the increase in the eMSC count from the baseline biopsy to the final biopsy between the two groups and so we needed a model which allowed for this.

Although there was not a significant difference in the baseline eMSC count between group 0 and group 1, the mean baseline count for group 0 was higher than group 1 and so we wanted to fit a model that adjusts for this as well.

Also, there were some participants whose eMSC count changed from one colony in the first (baseline) biopsy to three colonies in the final biopsy. This is a higher relative rise than for those whose count changed from 20 in the first (baseline)

biopsy to 28 in the final biopsy.

The Poisson regression model analysed the baseline eMSC and the final eMSC as the dependent variable, and age, intervention group, time (baseline/final biopsy) and an interaction term for each group and time.

The interaction term was included to allow for the different gradients between the baseline and final biopsy in each group and also to allow for the possibility of different magnitudes of difference in the eMSC count between each group firstly at the baseline biopsy and then at the final biopsy.

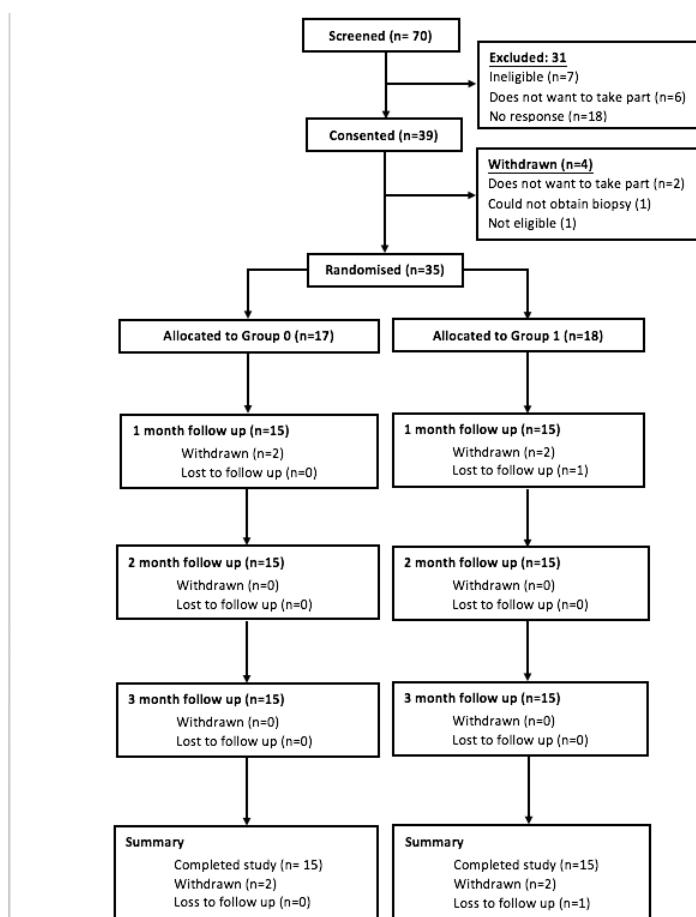
To adjust for the fact that a woman's baseline and final biopsy are likely to be corrected, we included a random effects term; a random effects Poisson regression model.

CHAPTER 9: RESULTS

9.1 CONSORT DIAGRAM

In the 30 participants who completed the trial, despite five withdrawals, there was equal randomisation among the two groups with 15 participants randomly allocated to Group 0 and 15 participants randomly allocated to Group 1.

Figure 9.0.1: Trial Consort Diagram



9.2 RECRUITMENT

Figure 9.0.2: Monthly Recruitment Chart

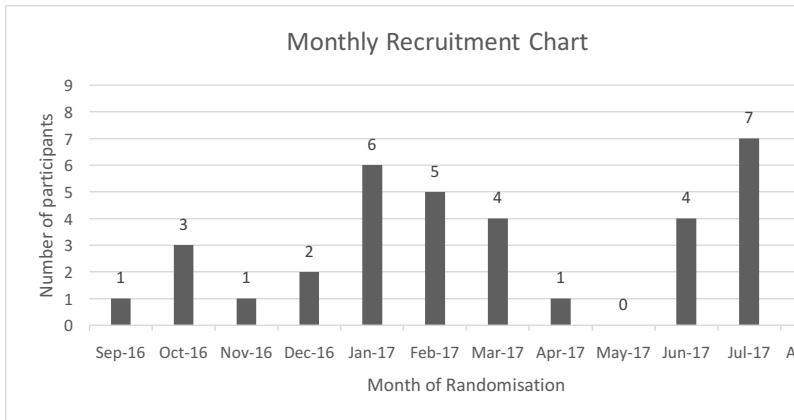
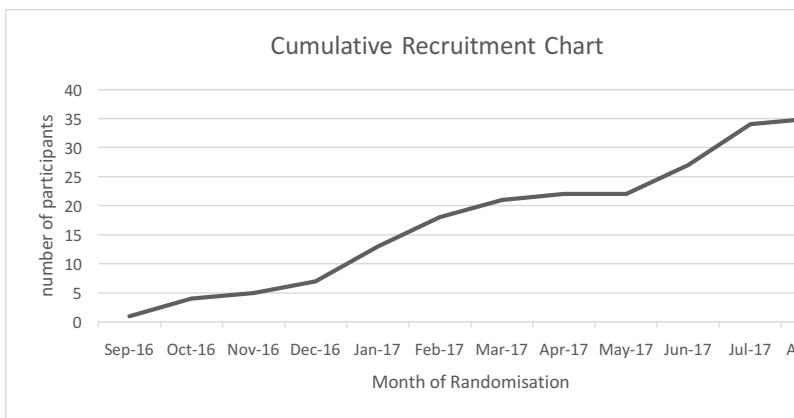


FIGURE 9.0.3: Cumulative Recruitment Chart



The cumulative chart in figure 9.0.3 shows a steady rate of recruitment over 12 months. The monthly recruitment chart in figure 9.0.2 highlights the decrease in recruitment during November and December due to the trial suspension. There were no participants randomised in May but screening and consent occurred which is then reflected in improved recruitment from June to August 2017.

9.3 PATIENT DEMOGRAPHICS

Table 9.0.1 summarises the age, baseline eMSC count, number of first trimester miscarriages, BMI and number of ERPC's between group 0 and group 1.

TABLE 9.0.1: Table of Patient Demographics

Characteristic	All women (n=30), n(%)	Group 0 (n=15), n(%)	Group 1 (n=15), n(%)	P-value[†]
Age, Years				
Mean (Sd)	32.90 (4.47)	31.30 (4.0)	34.5 (4.4)	0.037
Median (IQR)	33.00 (29.75 – 37.00)	32 (28.0- 34.0)	36 (31.0 – 38.0)	
Range	24 - 40	24 - 37	26 - 40	
Baseline eMSC count [¤]				
Mean (Sd)	20.34	26.64 (26.56)	14.47 (19.19)	
Median (IQR)	9.00 (6.0-28.0)	15.50 (6.0 – 52.0)	8.00 (4.0 –11.0)	0.252
Range	1 - 77	2 - 78	1 - 74	

Number of T1 miscarriages				
2	1 (3)	0 (0)	1 (6.7)	
3	3 (10)	2 (13.3)	1 (6.7)	
4	5 (17)	3 (20)	2 (13.3)	
5	6 (20)	3 (20)	3 (20.0)	
6	2 (7)	2 (13.3)	0 (0)	0.595
7	5 (17)	2 (13.3)	3 (20)	
8	1 (3)	0 (0)	1 (6.7)	
9	2 (7)	1 (6.7)	1 (6.7)	
10	1 (3)	0 (0)	1 (6.7)	
11	0 (0)	0 (0)	0 (0)	
12	2 (7)	1 (0)	1 (6.7)	
13	1 (3)	0 (0)	1 (6.7)	
14	1 (3)	1 (6.7)	0 (0)	
Total	30	15	15	
BMI kg/m²				
Mean (Sd)	26.94	26.55 (4.76)	27.33	
Median (IQR)	25.20 (23.72 – 28.88)	25.6 (23.20 – 28.20)	27.40 (23.90 -30.50)	0.412
Range	19.5 – 38.4	21.1 – 38.4	19.5 – 35.7	
Number of ERPC's				
0	14 (47)	7 (46.7)	7 (46.7)	
1	2 (7)	2 (13.3)	0 (0)	
2	7 (23)	4 (26.7)	3 (20.0)	
3	3 (10)	2 (13.3)	1(6.7)	
4	2 (7)	0 (0)	2 (13.3)	
5	2 (7)	0 (0)	2 (13.3)	0.436

[†] The p-value compares groups 0 and 1 using the Mann Whitney U test.

[∞] eMSC count in Group 0 (n = 14), eMSC count in Group 1(n = 15)

We can see that overall participants in Group 1 seemed generally older than group 0. The mean age in group 0 was 31.3 and the mean age in group 1 was 34.5. The difference in age between the two groups was statistically significant (p = 0.037). Our calculations showed a statistically significant difference in the age of participants between Group 0 and Group 1 and therefore this needed to be adjusted for in our analyses.

The difference in the baseline eMSC count was not statistically significant ($p=0.252$) between the two groups and therefore these results show that both groups were balanced in terms of the baseline eMSC count.

The mean number of first trimester miscarriages among the whole patient cohort was 6.5 miscarriages ($sd=3.16$, range 2 – 12). There were five participants who had suffered over 10 first trimester miscarriages.

In group 0 there was one participant who suffered 14 first trimester miscarriages. In group 1 there was one participant who suffered 13 first trimester miscarriages.

In group 0, 40% of participants suffered six or more miscarriages. In group 1, 54% of participants suffered 6 or more miscarriages.

The difference in the number of miscarriages between the two groups was not significant ($p=0.595$), which means that both the control (placebo) and intervention (Sitagliptin) groups were balanced for the number of first trimester miscarriages.

The overall mean BMI in the whole patient cohort was $26.9\text{kg}/\text{m}^2$ which is considered overweight by international standards of BMI calculation.

The mean BMI for group 0 was 26.55 (range 21.1 – 38.4) and the mean BMI for group 1 was 27.33 (range 19.5 – 35.7). The difference in the BMI values between the two groups is not statistically significant ($p=0.412$). This tells us that although the patient cohort was generally in the overweight category, the mean BMI was overall balanced between the two groups.

There was a non-significant difference ($p=0.436$) in the number of ERPC's performed in both group 0 and group 1.

9.4 DISTRIBUTION OF MISCARRIAGES IN EACH GROUP

Figure 9.0.4 below complements table 9.0.1 to show the number of women and the number of miscarriages they suffered between each treatment group.

FIGURE 9.0.4: Distribution of T1 Miscarriages in Each Treatment Group

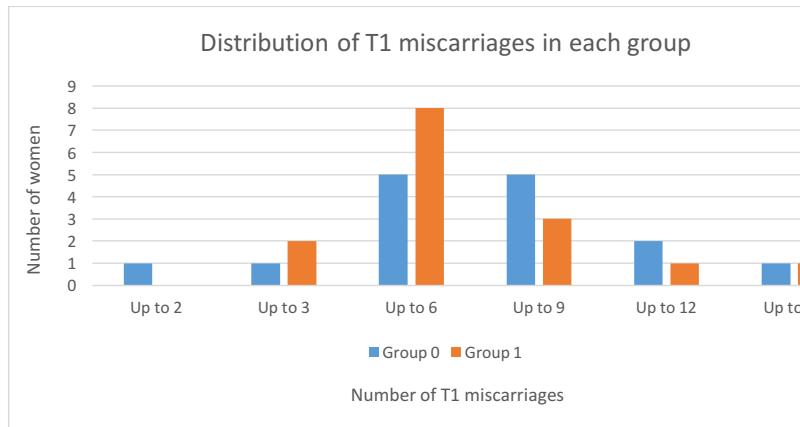
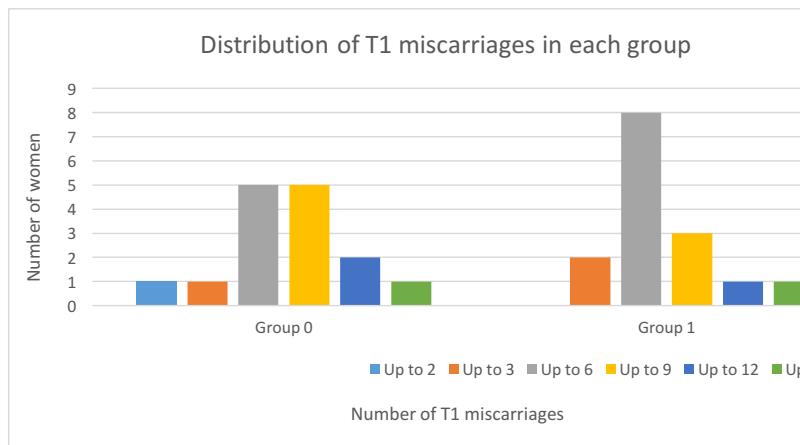


FIGURE 9.0.5: Distribution of T1 Miscarriage in Each Treatment Group

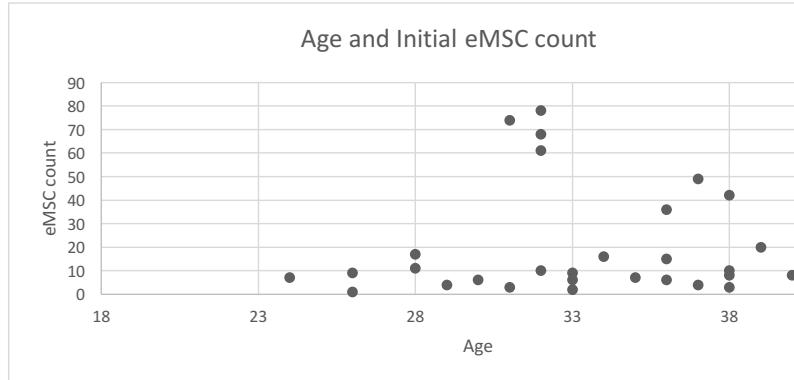


9.5 RELATIONSHIP BETWEEN PATIENT DEMOGRAPHICS AND INITIAL EMSC COUNT

9.5.1 AGE AND eMSC COUNT

The scatter graph in figure 9.0.6 shows that there was no relationship between age and the initial eMSC count over the whole participant cohort.

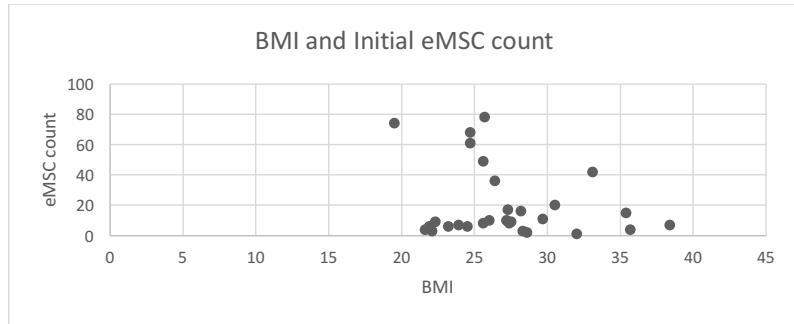
FIGURE 9.0.6: Scatter Graph of the Relationship between Age and Initial eMSC Count



9.5.2 BMI AND eMSC COUNT

The scatter graph in figure 9.0.7 shows that there was no relationship between BMI and the initial eMSC count over the whole participant cohort.

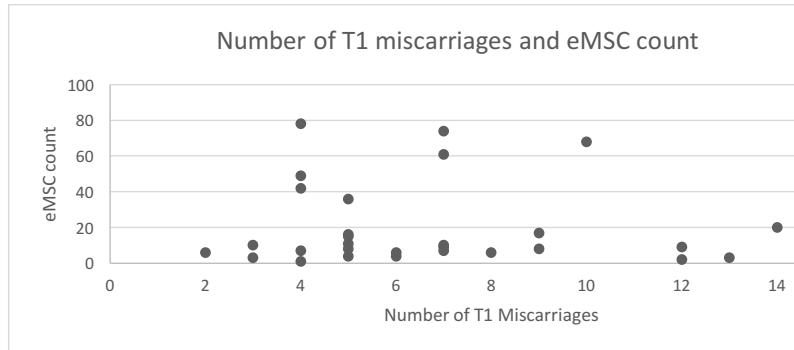
FIGURE 9.0.7: Scatter Graph Showing the Relationship Between BMI and Initial eMSC Count



9.5.3 NUMBER OF T1 MISCARRIAGES AND eMSC COUNT

The scatter graph in figure 9.0.8 shows that there is no relationship between the baseline eMSC count and the number of T1 miscarriages.

Figure 9.0.8: Scatter Graph Showing the Relationship Between T1 Miscarriages and eMSC Count



9.6 THE EMSC COUNT PROFILES

Figure 9.0.9: eMSC Count Profile in Each Treatment Group

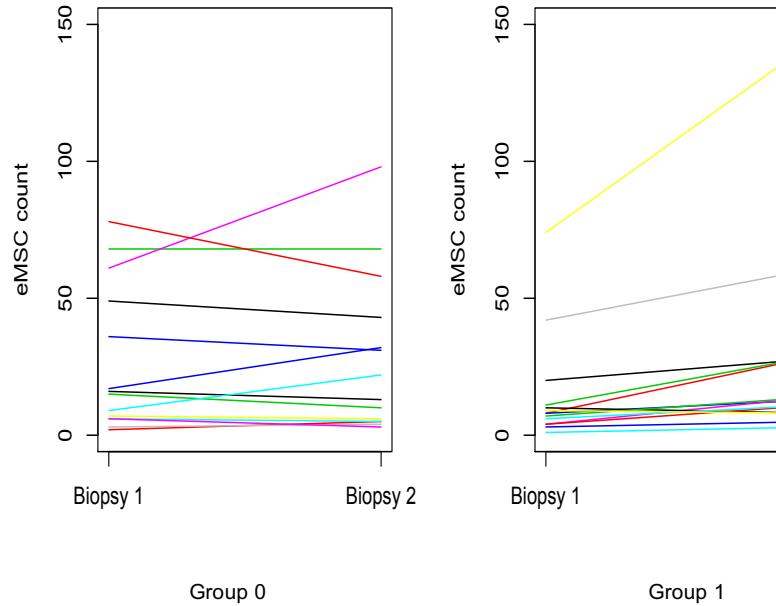


Figure 9.0.9 show the profiles of eMSC counts between the initial baseline biopsy (Biopsy 1) and the final biopsy (Biopsy 2) for each participant in the two groups.

The beginning of each coloured line represents the baseline initial eMSC count and the end point of the line represents the final eMSC count for each participant separately.

9.7 PRIMARY ANALYSIS (UNADJUSTED ANALYSIS)

Table 9.0.2: Estimated Mean eMSC Count At Final Biopsy Between The Two Treatment Groups

Group	Estimated mean eMSC count at final biopsy (month 3)	Difference	P=Value
0	3.30	0.0026	0.971
1	3.30		

Table 9.0.2 shows the mean count at the final biopsy for each group using a Poisson regression model which takes into account that the data is not normally distributed.

This analysis does not account for variables such as the mean age difference in the two groups and does not tell us the change in eMSC count compared to the baseline biopsy, which is relative.

Using a Poisson model that compares the final eMSC count between groups 0 and 1, the mean eMSC count for Group 0 is 3.30 and the mean for Group 1 is 3.30 (rounded to 2 decimal places).

The difference in the mean count at the final biopsy between the two groups (mean of group 1 – mean group 0) is 0.0026 (95% CI -0.1376 to 0.1427). This difference is not statistically significant ($p=0.971$).

9.8 SECONDARY ANALYSIS (ADJUSTED ANALYSIS)

Table 9.0.3 gives an estimate of the difference in mean eMSC at baseline between the two groups (Row 1) and an estimate of the difference in the mean eMSC at the final biopsy between the two groups (Row 2). It also gives an estimate of the difference in the mean eMSC between the final biopsy and the baseline biopsy in each treatment group separately (Row 3 and Row 4).

TABLE 9.0.3: Adjusted Analysis of eMSC Counts Between Baseline and Final Biopsy and Between Both Treatment Groups

Row	Effect	Difference Estimate (95% CI)	P-value
1	Month 1 (Baseline biopsy) Group 1- Group 0	-0.72 (-1.52, 0.0870)	0.071
2	Month 3 (Final biopsy) Group 1 – Group 0	-0.17 (-0.9709, 0.6239)	0.660
3	Group 0 Month 3 – Month 1	0.06(-0.0775, 0.2051)	0.3750
4	Group 1 Month 3 – Month 1	0.61 (0.4375, 0.7761)	<0.001

Row 1 gives an estimate of the difference in the mean eMSC count between groups 0 and 1 at baseline. The difference is -0.72 (Group 1 – Group 0) (95% CI -1.52, 0.09), which is almost one extra colony of stem cells in group 0 at baseline than group 1. Participants in Group 0 were also on average older than Group 1. Although the difference is not statistically significant the p-value is small ($p=0.071$) and the confidence interval varies from -1.52 to 0.08 suggesting a trend towards a difference. These results reflect the profiles in figure 9.0.9 where the groups did not seem well balanced at the baseline biopsy.

9.8.1 PRIMARY OUTCOME MEASURE

Row 2 gives an estimate of the difference in mean eMSC count between groups 0 and 1 at the final biopsy (month three). This is effectively our primary outcome measure; the stem cell count after three months.

The difference between the two groups is - 0.17 (Group 1 – Group 0) which is small and not statistically significant ($p=0.660$).

The results reflect the profiles in Figure 9.0.9, where the eMSC count at 3 months for the two groups overall seemed similar.

The unadjusted analysis revealed the mean difference in the eMSC count between the two groups at the final biopsy to be 0.0026 ($p=0.971$). The adjusted and unadjusted analyses were different as we adjusted for the difference in age and the baseline eMSC count between the two groups.

9.8.2 OTHER RESULTS

Although there was no significant difference in the eMSC count after three months between Group 0 and Group 1 there are some other interesting results.

We looked at the difference in the eMSC count between the initial and final biopsy between the two treatment groups.

Row 3 of Table 9.0.3 gives an estimate of the difference in the mean eMSC count between the baseline and final biopsy in group 0. The difference is 0.06 (month 3 – month 1) which is very small and not statistically significant ($p=0.370$).

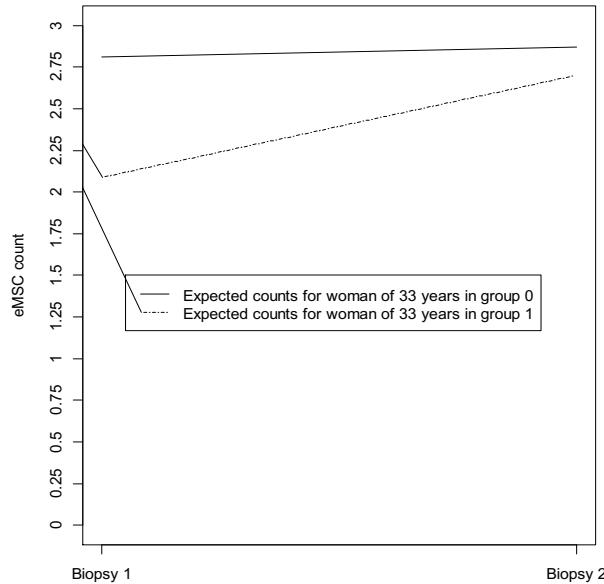
These results again are reflected in the profiles in 9.0.10 where it can be seen that there were only very few participants in this group who appeared to have a large change in the eMSC count between the baseline and final biopsy.

Row 4 gives an estimate of the difference in the mean eMSC count between the final and baseline biopsy in group 1. The difference is 0.61 (month 3 – month 1) which is 10 times higher than the change in group 0. The difference in group 1 is highly statistically significant ($p = <0.001$) and the confidence interval is narrow.

This significant result for participants in group 1 may indicate a therapeutic target. This difference of nearly one colony per 1500 cells is biological significant. This is

because each colony contains in the region of 100-1000 cells that have originated from one cell in just the 10 days of the assay. If this is viewed in the context of the whole endometrium with millions of cells, one extra stem cell colony with the ability to reproduce exponentially and rapidly per 1500 cells would translate to thousands of additional colonies each able to make thousands more cells. Hence one extra stem cell-forming colony per 1500 cells could make a large biological difference to endometrial regeneration.

Figure 9.0.10: Graphical Representation Of Adjusted Secondary Analysis
(Expected profiles for women of 33 years (median age in the whole cohort) in groups 0 and 1)



The graph in figure 9.0.10 represents the information in the table of results from the adjusted secondary analysis.

The final eMSC count in group 0 and group 1 was not significantly different. The null hypothesis is therefore correct.

Other observations were that there is a higher baseline mean count in group 0 than group 1, Group 0 did not have a statistically significant increase in the eMSC count between the baseline and the final biopsy and Group 1 had a statistically significant increase in the eMSC count between the baseline and final biopsy.

9.9 SECONDARY OUTCOME MEASURES

9.9.1 ADVERSE EVENTS AND SIDE EFFECTS

Table 9.0.4: Adverse Effects and Side Effects in Each Treatment Group

Adverse event/Side Effect	Group 0 n= 15 Events (participants, %)	Group 1 n = 15 Events (participants,%)
Headache	26 events (7 participants, 47%)	7 (3 participants, 20%)
Dizziness	0	1
Nausea	0	0
Thirst	0	1
Myalgia	1	1
Diarrhoea	2 (2 participants)	0
Dry mouth	1	1
Rash	2 (1 participant)	0
Chills	1	1
Night sweats	0	1
Mouth ulcers	2 (1 participant)	0
Nose bleed	1	0
UTI	0	1

Reported adverse reactions related to Sitagliptin which occur in >5% of patients include symptoms of an upper respiratory tract infection, nasopharyngitis and headache. In our study there were 7 participants (47%) who suffered with at least one headache in the three-month period in Group 0 and 3 participants (20%) in Group 1 who suffered with at least one headache in the three-month period.

Using the Fisher's Exact Test, the difference in participants experiencing headaches between the two groups is not significant ($p = 0.245$).

All other adverse events occurred in a maximum of one participant in one group. There was one participant who suffered with diarrhoea once and mouth ulcers on twice.

9.10 QUESTIONNAIRE

9.10.1 STUDY ACCEPTABILITY

We received 29 questionnaires of the 30 participants who completed the study (97% return rate).

Participants did not always answer all parts of the questionnaire and so percentages in table 9.0.5 are displayed in a ratio of how many responses we received for each question.

We assessed acceptability of the study with the following questions:

1. I found taking part in this study a worthwhile experience
2. Given the choice, I would like to continue taking part in this study
3. I would recommend taking part in the study to others
4. I found taking part in the study supportive to my care
5. I would be open to taking part in other research studies

The aim of these questions was to assess if participants had a positive experience and would recommend this to family and friends. If participants had a positive experience they are more likely to continue, recommend it to others and be open to taking part in other research studies.

Participants were asked to use a scale to answer the questions

- 5 = Strongly agree
- 4 = Agree
- 3 = Neither agree/disagree
- 2 = Disagree
- 1 = Strongly disagree

The overall results of the questionnaire are displayed in Table 9.0.5

Table 9.0.5: Questionnaire Results

	Strongly Agree n (%)	Agree n (%)	Neither Agree or Disagree n (%)	Disagree n (%)	Strongly Disagree n (%)
I found taking part in this study a worthwhile experience	26 (93)	2 (7)	0 (0)	0 (0)	0 (0)
Given the choice, I would like to continue taking part in this study	22 (79)	3 (11)	1 (4)	2 (7)	0
I would recommend taking part in the study to others	27 (93)	2 (7)	0 (0)	0 (0)	0 (0)
I found taking part in the study supportive to my care	27 (93)	2 (7)	0 (0)	0 (0)	0 (0)
I would be open to taking part in other research studies	21 (72)	5 (17)	2 (7)	1 (3)	0 (0)

26 of 28 participants found taking part in the study worthwhile and 27 of 29 participants would recommend the study to others and felt well supported.

9.10.2 STUDY MEDICATION ACCEPTABILITY

There were three questions on the questionnaire related to study medication. The results of these are displayed in table 9.0.6

Table 9.0.6: Study Medication Acceptability

	Strongly Agree n(%)	Agree n(%)	Neither Agree or Disagree n(%)	Disagree n(%)	Strongly Disagree n(%)
Medication was easy to take	25 (86)	3 (10)	0 (0)	0 (0)	1 (3)
Side effects of the medication were easy to manage	22 (81)	2 (7)	2 (7)	1 (4)	0 (0)

Table 9.0.7: Study Medication Acceptability and Compliance

	Often	Sometimes	Rarely n(%)	Never n(%)
I forgot to take the study medication	0	0	5 (18)	23 (82)

96% of participants strongly agreed or agreed that the medication was easy to take and 88% either strongly agreed or agreed that the side effects were easy to manage.

Even though we do not have responses from two participants we can see that at least 23 of the 30 participants never forgot to take the study medication.

9.10.3 ACCEPTABILITY OF PARTICIPANT INFORMATION LEAFLET

All the 29 participants who returned the questionnaire reported that the participant information leaflet described the study clearly. There were no extra comments in the free space regarding the information leaflet.

9.10.4 CARE RECEIVED BY RESEARCH TEAM

We asked participants if they felt the hospital visits within the study supportive to their care and if they were able to contact the research team when needed. 28 of the 29 participants found the hospital follow up visits supportive to their care and were able to contact the research team easily.

28 of the 29 participants reported no problems with booking their appointments for the biopsy visits or the follow up visits. One participant had trouble booking her second biopsy appointment due to work commitments, living very far away and also having a narrow window of when the appointment could be made (7-10 days post ovulation).

9.10.5 IMPROVING CARE

As part of the questionnaire we asked participants if they felt any part of their journey could have been improved.

- There were six participants who answered 'no' to this question with no other comments. All other comments are listed here:
- No the team were amazing in every way
- No, been really helpful and friendly
- None whatsoever!
- No it was explained well to me and I was supported throughout
- More local clinics
- Was very well organised
- Not having to attend for the monthly visits or doing it over the phone, but I understand the necessity.
- It would have been good to see the same midwife/nurse each time.
- None - although it would be good to receive updates on the research in an email post completion of my involvement in the study.
- All the staff were lovely and very supportive. I quite enjoyed the experience.
- Everyone was really fantastic!
- Fantastic service
- No! Husband just said dedicated parking
- No - felt very looked after

We also gave free space for participants to leave any other comments. The comments we received were:

- Could not thank the team enough for all the care and support. It's been so exciting to be part of this trial. Made even better by Dr. Tewary and Angela Polanco, they have been amazing.
- The first time I have felt cared for within the NHS is with your staff - A*

- Dr Tewary was very accommodating. Very lovely team & always friendly.
- I felt supported every step of the way. Contacting the doctor was easy with both phone numbers and email addresses. All my questions and worries were answered and reassured.
- It was very helpful to be able to email staff rather than phone because of my work.
- Thank you for the opportunity and looking after us!
- I found everyone very helpful and supportive, thank you
- The team are amazing and very supportive
- The whole team were very supportive & friendly - making the whole experience so much easier.
- Everyone we have dealt with have been incredible! Thank you
- Everyone involved were so supportive and empathetic
- Fantastic supportive care
- Staff were incredibly supportive and took time to talk things through with me
- The team and Shreeya were flexible for our visits from West Wales
- The team and staff have been very supportive and professional. Thank you.
- Parking is a problem at the hospital, meaning participation took longer than anticipated and I had to leave plenty of time in advance to get to the hospital to park the car in order not to be late to the appointment.
- Everyone made me feel very relaxed every time I came to my appointments.
- Thank you for your support throughout.

CHAPTER 10: DISCUSSION

10.1 RECURRENT MISCARRIAGE

Miscarriage is the most common complication of pregnancy. RPL is considered a distinct and unique disorder affecting 1-5% of couples (Ford & Schust, 2009).

Chapter one gives some insight into the difficulties we face giving couples with RPL a real cause and a prevention strategy. And ultimately, after dealing with the emotional, psychological and physical morbidity of a miscarriage, an explanation is most important to couples to help them move forward.

Risk factors such as maternal age, obesity, smoking and reproductive history have been shown to be associated with RPL (ESHRE, 2017). We spend time in clinic counselling couples about weight loss, reducing smoking and alcohol consumption which is which very important to reduce risks to health in general but also to reduce the risk of obstetric complications. There have not been any well conducted trials demonstrating that these health care interventions prevent miscarriage as these trials would incur ethical and feasibility problems.

Medical co-morbidities such as endocrine, haematological, cardiovascular and immunological disorders have all been associated with RPL. In line with pre-pregnancy counselling, thyroid disorders, diabetic and hypertensive management are optimised to improve pregnancy outcomes, one of which is miscarriage.

Despite many randomised controlled trials looking into causes and treatment of RPL, the only treatment with demonstrated significant efficacy for the prevention of RPL is heparin and aspirin for those women with APLS (Empson *et al.*, 2005). Others are categorised as 'unexplained RPL'. Understandably, this is generally not acceptable to them and so most couples are willing to consider research projects in the hope that experimental treatment may help them.

There is emerging evidence that the human endometrium plays an important role in determining the success of implantation. There have already been huge advances in the research into this paradigm.

10.2 THE ENDOMETRIUM AND RECURRENT MISCARRIAGE

The human endometrium is one of the most unique and dynamic tissues in the body (Du & Taylor, 2009). It is able to prepare for potential pregnancy, is shed and then regenerates every month in response to hormonal signalling and does so approximately 400 times in a woman's lifetime (Mutlu *et al.*, 2015).

Decidualisation of the endometrium is vital for successful pregnancy (Salker *et al.*, 2010). When the endometrium has been appropriately decidualised it responds to embryonic signals and leads to positive or negative selection of the embryo. There is parallel but mutual signalling between endometrium and the embryo where selectivity and receptivity of the endometrium is defined. Decidualisation is known to be dysfunctional in those with RPL. Low levels of endometrial mesenchymal stem cells, cellular senescence and disordered inflammation all contribute to abnormal decidualisation (Lucas *et al.*, 2016b). Abnormal decidualisation results in the loss of a selectivity checkpoint, resulting in the endometrium being excessively permissive to implantation but unable to sustain the pregnancy (Gellersen & Brosens, 2014; Macklon & Brosens, 2014). For patients, this means the exposure to repeated miscarriage which comes with significant psychological, emotional and physical morbidity. Endometrial mesenchymal stem cell deficiency contributing to abnormal decidualisation was the focus point of this research project.

There have already been huge advances in research into endometrial mesenchymal stem cells, their origin and their therapeutic use in the last 10 years. Du *et al* have demonstrated that ischaemia/reperfusion injuries in the uterus promote bone marrow derived stem cells to migrate to that area of injury (Du *et al.*, 2012). There have been alternate hypotheses about endometrial stem cells residing in the basal layer of the endometrium which are activated in response to certain signals and released at the time of menstruation or when injury occurs leading to a regenerated functionalis layer with a new niche of eMSC's(Gargett *et al.*, 2009).

Endometrial stem cell therapy has already proven to be an exciting venture. For example, Bone marrow derived eMSC's have been used to treat Asherman's syndrome in mice studies. Asherman's syndrome was mimicked in a mouse model and a Y+ bone marrow transplant was performed. Not only were Y+ cells detected at the endometrium but conception rates also improved (Alawadhi *et al.*, 2014). This

study suggested that after endometrial injury, bone marrow derived stem cells not only migrate to the endometrium but also improve fertility. In humans, bone-marrow derived stem cells have been directly injected into the uterine artery leading to successful pregnancies (Santamaria *et al.*, 2016). However, isolating mesenchymal stem cells of sufficient quality to give back to humans is costly and time consuming and injecting the uterine artery is invasive and could lead to serious complications. Hence, we attempted to increase the natural trafficking of stem cells. We investigated a DPP4 inhibitor, that reduces the degradation of CXCL12. Tissue injury derived CXCL12 enhances trafficking of stem cells expressing CXCR4 the CXCL2 receptor to the site of injury.

10.3 THE SIMPLANT STUDY – SET UP

This feasibility study was the first ever randomised study performed to attempt to improve the natural trafficking of bone marrow derived mesenchymal stem cells to human endometrium using medication.

As this was a completely novel concept, the set-up of the study underwent extensive scrutiny by the hospital research governance team and sponsor before applications could be made to the national REC and MHRA. This was understandably necessary to ensure the safety and wellbeing of patients who would take part in the trial.

Also, this was the first time UHCW sponsored an early phase clinical trial of an investigational medicinal product (CTIMP). This meant that the set-up of this study was a steep learning curve for the whole team. The study pathway was carefully reviewed and we did extensive research into the safety of Sitagliptin in healthy, non-diabetic individuals who were at risk of pregnancy while taking medication. We had many TMG meeting to finalise plans for robust blinding and randomisation methods. These methods were successful because at no point in the study were these matters compromised.

I had to learn about the production and supply of investigational medicinal products (IMP) before approaching manufacturers. I learnt that for the manufacture, packaging and labelling for any IMP, a Manufacturer/Importer Authorisation (MIA)

license is needed. I contacted many companies about meeting the requirements of the study in a specific time frame and at a reasonable cost. The trial pharmacist was invaluable in helping to review quotes and agreements to ensure there were no hidden costs or concerning features which would not be so obvious to me.

Sharp Clinical Services provided a good service with timely supply of the IMP and a continued point of contact within the company.

Patient and public involvement was vital for the set-up of the trial. I needed to be confident that the trial pathway would be acceptable to the cohort of patients that would be approached for the study.

The trial pathway, the format and content of patient specific documents were reviewed by patients and their unreserved critique enabled me to make changes to address patient concerns but at the same time encompass GCP requirements.

As a result, when the trial opened for recruitment I was confident the trial would be acceptable to patients with RPL and the documents were comprehensive to patient needs.

10.4 THE SIMPLANT STUDY - RECRUITMENT

Patients who see Professor Quenby in the recurrent miscarriage clinic are often at a state of despair having been tested for known causes of miscarriage, had cytogenetic testing on products of conception and feel disillusioned about where to go next.

Original research had shown that the higher the number of first trimester miscarriages the lower the eMSC count. The SIMPLANT study was therefore offered to patients with high order first trimester miscarriages as this severe phenotype would be likely to have low eMSC's and therefore potentially benefit more if the treatment worked. This is reflected in the average number of miscarriages over the whole patient group being 6.5 ($sd = 3.16$, range 2-12).

Entering this trial was a big commitment for any patient. They had to effectively consent to having two endometrial biopsies, take a capsule every day, attend the hospital for regular follow up and avoid pregnancy for three months.

Recruitment however, was generally not a problem and the trial was popular with couples with severe phenotype despite the multiple clinic visits needed. We managed to recruit all participants within one year, despite the trial being suspended for two months.

Some patients understandably found the prospect of losing three opportunities to become pregnant at the cost of taking medication which could be placebo too much to accept and therefore declined taking part.

10.5 THE SIMPLANT STUDY: TRIAL MANAGEMENT

I was supported very well by the chief investigator who was involved with the day to day running of the trial. The trial manager was always available to help, to liaise with any issues which needed to be addressed with the sponsor and to help with daily governance issues.

10.6 SIMPLANT: RESULTS

Among the 30 participants who completed the study we had equal randomisation to each treatment group.

The five withdrawals all occurred within the first month and no patient was withdrawn due to difficulties with tolerating the medication or difficulties accepting the study pathway. We did not do an intention to treat analysis as the outcome measure was dependant on the participants having a second biopsy and it was not appropriate for any of the five withdrawn patients to return for the second biopsy. This issue was discussed at the joint DMC/TSC and agreed.

94% of the endometrial biopsies were taken by myself and 100% were analysed by Dr Emma Lucas allowing standardisation in methods of collection and analysis.

The participants in each group were matched for age, baseline eMSC count, number of miscarriages and BMI.

The results of this feasibility study have shown no significant difference in the eMSC count between the intervention group and the placebo group after 3 months

of treatment ($p=0.660$). There are various confounding factors which are discussed later in this chapter.

10.7 CHANGE IN eMSC COUNT IN THE INITIAL AND FINAL BIOPSY

In Group 0 there is a non-significant change in the eMSC count between the baseline and the final biopsy ($p = 0.37$). In Group 1 there was a significant increase in the eMSC count between the baseline and the final biopsy result.

The small change in eMSC count seen in Group 0 between the baseline and final biopsy may be because the mean eMSC count at baseline in this group was actually higher.

The adjusted Poisson regression model showed that the difference in the eMSC count between the two groups at baseline was 0.72 colonies. This was not statistically significant but the p value was small (0.071) and there is a difference of nearly one colony between the two groups at baseline in a small sample size.

This imbalance in the overall mean at baseline between the two groups may have been because of experimental error or, more likely, because of the small sample size and the wide eligibility criteria for inclusion to the study in terms of patient demographics. A larger sample size with tighter inclusion and exclusion criteria would be needed to confirm or refute this. The difference in the mean eMSC count between the baseline and final biopsy in group 1 is 0.61 colonies which is ten times higher than the change in the eMSC count in group 0 (0.06 colonies).

The difference in group 1 is highly statistically significant ($p = <0.001$) and the confidence interval is narrow. This has demonstrated that in some patients we can increase the eMSC count at the endometrium. This finding and the significance of this needs further investigation.

10.8 OTHER RESULTS

The research conducted at Warwick Medical School that this work was based on had demonstrated that not only do RPL patients have a deficiency in endometrial stem cells but also that the stem cell-ness of the endometrium decreases with an increase in the numbers of miscarriages (Lucas *et al.*, 2016b). This original research was however conducted via a retrospective analysis of endometrial biopsies and the reproductive history was known when performing the analysis. Retrospective analyses are subject to type 1 error with the risk of reporting outcomes which may be due to chance.

Analysis of 29 endometrial biopsies in the SIMPLANT study has shown no relationship between the eMSC count with the number of previous first trimester miscarriages. As this was a prospective randomised study we can be more confident that there is actually no relationship in the eMSC count with the number of previous miscarriages. A larger sample size would be needed to confirm this.

10.9 ADVERSE EVENTS AND STUDY ACCEPTABILITY

The only reported adverse event which occurred more than once in more than one participant was a headache. Headaches are a common side effect of Sitagliptin (>5% of patients) but there was not a statistically significant difference in the headaches reported between the two groups ($p = 0.245$). Given these results we can be confident that the headaches were unlikely to be related to the study medication.

Questionnaires were completed by participants to assess the acceptability of the trial. 97% of participants returned the questionnaire. 93% found taking part in the study worthwhile and would recommend taking part to others. This gives me confidence that the trial was acceptable to participants and if we performed a larger pragmatic trial recruitment and compliance would not be an issue.

We did not have any concerns with patient compliance to trial medication and there were no participants who withdrew because of difficulties taking the medication or side effects. All participants who continued in the study attended for their final endometrial biopsy. This tells me that none of them found the biopsy too difficult to

tolerate at the baseline visit discouraging them or stopping them attending for the second biopsy.

10.10 LIMITATIONS

There are some promising results and findings from this study which could potentially be translated into a clinical outcome. Limitations of this study must be addressed before further work can be conducted.

The sample size for this study was calculated using the results of clonogenic assays done at the Warwick Clinical Sciences Research Laboratory in 2014 which was the original work demonstrating that those with RPL had eMSC deficiency.

This analysis was derived from dividing results obtained into those patients without RPL (those with less than 3 miscarriages) and those patients with RPL (3 or more miscarriages). Those patients with less than 3 miscarriages were those who had booked into the clinic for investigations after having up to 3 miscarriages, or, actually had primary or secondary unexplained subfertility with recurrent implantation failure

These original stem cell assay results were therefore from patients of two different spectra of reproductive failure. There may have been a smaller difference in the average eMSC count between those with successful pregnancies when compared to those with RPL rather than comparing patients with implantation failure with patients with RPL.

We do not have any stem cell assay results for patients without a history of miscarriage or implantation failure and had successful pregnancies. Although this would give us more of an idea of the stem cell count associated with successful pregnancy, these biopsies and assays are not possible to obtain.

The significant difference in the eMSC count found in those with or without RPL in the patient cohort used may have actually resulted in an over-estimation in the expected effect size.

Also, there was large interpatient variation in the baseline eMSC count between all participants. The baseline eMSC count among all participants varied from 1 to 145

colonies. The eMSC count did not seem to be related to age, the number of previous miscarriages or BMI.

We may have seen this large variation in the stem cell count as our small sample size was small. This variation may have been more narrow if we had a larger sample size. This variation may even be due to cyclical variation of eMSC's within the same patient.

The variation in the baseline eMSC count is more likely because of the large variation in patient demographics. When setting up this study we needed to consider how to optimise trial recruitment but also needed to ensure the eligibility criteria ensured patient safety. Patients with an age of 18-42 and patients with any BMI could be included. This was because the safety of Sitagliptin had been proven in those above 18 and also because the pharmacokinetics of Sitagliptin are not altered by BMI.

These criteria were perhaps too wide leading to a high variation in baseline eMSC counts amongst the participant cohort. We had young patients with a high BMI and older patients with a low BMI all with different reproductive histories which may have led to a wide variation in the results obtained.

Our required sample size was 30 participants. Despite five withdrawals after randomisation we had 30 participants complete the study. The overall number of clonogenic assay results however, were affected by yeast contamination. This made it impossible to accurately assess the eMSC at the final biopsy in one of the participants. This feasibility study demonstrated that should a similar outcome measure be used for a further trial; the sample size needs to account for this possible hindrance.

94% of the endometrial biopsies were taken by myself. The same method to scrape four walls of the uterus using the Endocell Wallach Catheter was used for every participant to maintain standardisation in the methods used to collect the endometrial biopsies. This catheter creates a suction effect to collect an endometrial biopsy. The superior wall of the uterus would always be scraped first which meant that the majority of the biopsy obtained was from the superior wall of the uterus in every participant. It must be appreciated however that there may have been a variation in the stem cell count in different parts of the endometrium for each participant. This possibility is impossible to overcome as there is no way of

predicting where there would be a higher or lower concentration of stem cells in different parts of the endometrium in different participants.

The CFU assay takes 10 days to complete. It is expensive, time consuming and requires advanced laboratory skills. This study was possible to conduct with this small sample size but this assay would not be practical in a large sample setting.

10.11 FUTURE WORK

Although our primary outcome measure showed no difference in the final eMSC between the two treatment groups, once we have been unblinded to study treatment we will be able to establish in which group there was an increase in the eMSC count from baseline.

The fact that there was no difference in the final eMSC count between the two treatment groups may reflect a ceiling of maximal stem cell-ness of the endometrium rather than a non-significant result. This needs to be explored.

The significant increase in eMSC in one group may mean that we can increase trafficking of eMSC to the endometrium in certain patients. This is an area for further work as it may provide a therapeutic target.

We firstly need to develop a new method of analysing the stem cell-ness of the endometrium using markers which are more practical, cheaper and less time consuming to conduct than the clonogenic assay which takes two weeks for each sample, so that we can conduct research with a bigger sample size.

Once we have developed a new method of assessing the stem cell-ness of the endometrium we can look at the eMSC count in a larger group of patients. We can establish what patient factors are associated with a lower eMSC count, establish more accurately if there is a certain reference range of stem cell-ness within the endometrium and if there is an overall maximum eMSC count.

It would also be useful to look at cycle-cycle variation within the same patient which would be possible to do as patients now attend the Implantation Clinic in two cycles and have two endometrial biopsies performed.

Future work would also involve looking at pregnancy outcomes and see if they relate to eMSC counts or the change in eMSC counts over 3 months. Ultimately, would want to design a project to look into whether the eMSC count can predict pregnancy outcomes.

This study had a scientific primary outcome. We eventually need to conduct this an study with a clinical outcome such as live birth rate.

10.12 CONCLUSIONS

Patients with RPL have a deficiency of eMSC's which contributes to abnormal patterns of decidualisation. Improving this deficiency is potential therapeutic target. If we are able to improve decidualisation of the endometrium there will be improved signalling between the embryo and the endometrium leading to improved endometrial selection of the appropriate embryo rather than repeatedly investing in pregnancies which lead to miscarriage.

There was no significant difference in the eMSC after 3 months in the two treatment groups but participants in Group 1 had statistically significant increase in the eMSC count after 3 months when compared to baseline. The study was also acceptable to participants with few side effects. This feasibility study has provided us with a foundation from which to perform further work.

KEY DATES

Protocol and supporting documents*	November 2015 – March
2016	
Tommy's Grant Approval Letter:	08/02/2016
Ethics Application	08/04/2016
Research Ethics Committee (REC) Meeting	27/04/2016
Response from REC	10/05/2016
Amendments sent	18/05/2016
Favourable opinion from REC	14/06/2016
MHRA approval	
Site initiation visit and training	23/08/2016
Sponsor greenlight	14/09/2016
Recruitment commencement date	14/09/2016
Trial Suspension	21/10/2016
Internal Monitoring	14/11/2016
Major Amendment 1.0 approval	9/11/2016 from REC 18/11/2016 from MHRA
Major Amendment 2.0 approval	06/12/2016 from REC 07/12/2016 from MHRA
Lift of suspension & trial restart	15/12/2016
External Audit:	08/02/2017

Major Amendment 3.0 approval

2/05/2017

Trial Steering Committee Meetings

10/06/2016, 08/02/2017, 31/05/2017

Trial management Group Meetings

02/02/2016, 22/02/2016, 31/03/2016, 19/05/2016, 23/07/2016,
23/08/2016, 30/01/2017, 08/05/2017

*This included development of trial design, sample size calculations, contact with pharmaceutical companies, agreement of costs, manufacture and supply of IMP and placebo, method of randomisation, methods for un-blinding procedures, safety measures, peer review, statistician review, R&D review, sponsorship and funding agreements

REFERENCES

- ACOG (2001) Management of Recurrent Early Pregnancy Loss.
- Acosta, J. C., Banito, A., Wuestefeld, T., Georgilis, A., Janich, P., Morton, J. P., Athineos, D., Kang, T. W., Lasitschka, F., Andrusis, M., Pascual, G., Morris, K. J., Khan, S., Jin, H., Dharmalingam, G., Snijders, A. P., Carroll, T., Capper, D., Pritchard, C., Inman, G. J., Longerich, T., Sansom, O. J., Benitah, S. A., Zender, L. & Gil, J. (2013) A complex secretory program orchestrated by the inflamasome controls paracrine senescence. *Nat Cell Biol*, 15 (8): 978-990.
- Aghajafari, F., Nagulesapillai, T., Ronksley, P. E., Tough, S. C., O'Beirne, M. & Rabi, D. M. (2013) Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. *BMJ*, 346 f1169.
- Ahrén, B. (2007) DPP-4 inhibitors. *Best Pract Res Clin Endocrinol Metab*, 21 (4): 517-533.
- Alawadhi, F., Du, H., Cakmak, H. & Taylor, H. S. (2014) Bone Marrow-Derived Stem Cell (BMDSC) transplantation improves fertility in a murine model of Asherman's syndrome. *PLoS One*, 9 (5): e96662.
- Anifandis, G., Bounartzi, T., Messini, C. I., Dafopoulos, K., Sotiriou, S. & Messinis, I. E. (2014) The impact of cigarette smoking and alcohol consumption on sperm parameters and sperm DNA fragmentation (SDF) measured by Halosperm®. *Arch Gynecol Obstet*, 290 (4): 777-782.
- Ata, B., Tan, S. L., Shehata, F., Holzer, H. & Buckett, W. (2011) A systematic review of intravenous immunoglobulin for treatment of unexplained recurrent miscarriage. *Fertil Steril*, 95 (3): 1080-1085.e1081-1082.
- Avogaro, A., de Kreutzenberg, S. & Fadini, G. (2014) Dipeptidyl-peptidase 4 inhibition: linking metabolic control to cardiovascular protection. *Curr Pharm Des*, 20 (14): 2387-2394.
- Bardos, J., Hercz, D., Friedenthal, J., Missmer, S. A. & Williams, Z. (2015) A national survey on public perceptions of miscarriage. *Obstet Gynecol*, 125 (6): 1313-1320.
- Bergman, A., Mistry, G. C., Luo, W. L., Liu, Q., Stone, J., Wang, A., Zeng, W., Chen, L., Dilzer, S., Lasseter, K., Herman, G. A., Wagner, J. A. & Krishna, R. (2007) Dose-proportionality of a final market image sitagliptin formulation, an oral dipeptidyl peptidase-4 inhibitor, in healthy volunteers. *Biopharm Drug Dispos*, 28 (6): 307-313.

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.

Boots, C. & Stephenson, M. D. (2011) Does obesity increase the risk of miscarriage in spontaneous conception: a systematic review. *Semin Reprod Med*, 29 (6): 507-513.

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.

Brenner, C., Kränkel, N., Kühlenthal, S., Israel, L., Remm, F., Fischer, C., Herbach, N., Speer, T., Grabmaier, U., Laskowski, A., Gross, L., Theiss, H., Wanke, R., Landmesser, U. & Franz, W. M. (2014) Short-term inhibition of DPP-4 enhances endothelial regeneration after acute arterial injury via enhanced recruitment of circulating progenitor cells. *Int J Cardiol*, 177 (1): 266-275.

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.

Brosens, J. J., Salker, M. S., Teklenburg, G., Nautiyal, J., Salter, S., Lucas, E. S., Steel, J. H., Christian, M., Chan, Y. W., Boomsma, C. M., Moore, J. D., Hartshorne, G. M., Sućurović, S., Mulac-Jericevic, B., Heijnen, C. J., Quenby, S., Koerkamp, M. J., Holstege, F. C., Shmygol, A. & Macklon, N. S. (2014) Uterine selection of human embryos at implantation. *Sci Rep*, 4: 3894.

Castilla, D. M., Liu, Z. J., Tian, R., Li, Y., Livingstone, A. S. & Velazquez, O. C. (2012) A novel autologous cell-based therapy to promote diabetic wound healing. *Ann Surg*, 256 (4): 560-572.

Cavalcante, M. B., Sarno, M., Araujo Júnior, E., Da Silva Costa, F. & Barini, R. (2017) Lymphocyte immunotherapy in the treatment of recurrent miscarriage: systematic review and meta-analysis. *Arch Gynecol Obstet*, 295 (2): 511-518.

Chan, R. W., Schwab, K. E. & Gargett, C. E. (2004) Clonogenicity of human endometrial epithelial and stromal cells. *Biol Reprod*, 70 (6): 1738-1750.

Christiansen, O. B., Larsen, E. C., Egerup, P., Lunoe, L., Egestad, L. & Nielsen, H. S. (2015) Intravenous immunoglobulin treatment for secondary recurrent miscarriage: a randomised, double-blind, placebo-controlled trial. *BJOG*, 122 (4): 500-508.

Cicinelli, E., Matteo, M., Tinelli, R., Pinto, V., Marinaccio, M., Indraccolo, U., De Ziegler, D. & Resta, L. (2014) Chronic endometritis due to common bacteria is prevalent in women with

recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. *Reprod Sci*, 21 (5): 640-647.

Co, M. (2017) *Merk Pregnancy Registries*. [online] Available from: (Accessed <http://www.merckpregnancyregistries.com/januvia.html>, 30/11/2017).

Coomarasamy, A., Williams, H., Truchanowicz, E., Seed, P. T., Small, R., Quenby, S., Gupta, P., Dawood, F., Koot, Y. E., Bender Atik, R., Bloemenkamp, K. W., Brady, R., Briley, A. L., Cavallaro, R., Cheong, Y. C., Chu, J. J., Eapen, A., Ewies, A., Hoek, A., Kaaijk, E. M., Koks, C. A., Li, T. C., MacLean, M., Mol, B. W., Moore, J., Ross, J. A., Sharpe, L., Stewart, J., Vaithilingam, N., Farquharson, R. G., Kilby, M. D., Khalaf, Y., Goddijn, M., Regan, L. & Rai, R. (2015) A Randomized Trial of Progesterone in Women with Recurrent Miscarriages. *N Engl J Med*, 373 (22): 2141-2148.

Coulam, C. B., Krysa, L., Stern, J. J. & Bustillo, M. (1995) Intravenous immunoglobulin for treatment of recurrent pregnancy loss. *Am J Reprod Immunol*, 34 (6): 333-337.

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.

Devin, J. K., Pretorius, M., Nian, H., Yu, C., Billings, F. T. & Brown, N. J. (2014) Dipeptidyl-peptidase 4 inhibition and the vascular effects of glucagon-like peptide-1 and brain natriuretic peptide in the human forearm. *J Am Heart Assoc*, 3 (4):

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.

Du, H. & Taylor, H. S. (2009) Stem cells and female reproduction. *Reprod Sci*, 16 (2): 126-139.

Döring, Y., Pawig, L., Weber, C. & Noels, H. (2014) The CXCL12/CXCR4 chemokine ligand/receptor axis in cardiovascular disease. *Front Physiol*, 5 212.

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.

Empson, M., Lassere, M., Craig, J. C. & Scott, J. R. (2002) Recurrent pregnancy loss with antiphospholipid antibody: a systematic review of therapeutic trials. *Obstet Gynecol*, 99 (1): 135-144.

ESHRE (2017) Recurrent Pregnancy Loss: A Guideline of the European Society of Human Reproduction and Embryology.

Farquharson, R. G., Quenby, S. & Greaves, M. (2002) Antiphospholipid syndrome in pregnancy: a randomized, controlled trial of treatment. *Obstet Gynecol*, 100 (3): 408-413.

Farren, J., Jalmbrant, 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.

Flynn, H., Yan, J., Saravelos, S. H. & Li, T. C. (2014) Comparison of reproductive outcome, including the pattern of loss, between couples with chromosomal abnormalities and those with unexplained repeated miscarriages. *J Obstet Gynaecol Res*, 40 (1): 109-116.

Ford, H. B. & Schust, D. J. (2009) Recurrent pregnancy loss: etiology, diagnosis, and therapy. *Rev Obstet Gynecol*, 2 (2): 76-83.

Franssen, M. T., Musters, A. M., van der Veen, F., Repping, S., Leschot, N. J., Bossuyt, P. M., Goddijn, M. & Korevaar, J. C. (2011) Reproductive outcome after PGD in couples with recurrent miscarriage carrying a structural chromosome abnormality: a systematic review. *Hum Reprod Update*, 17 (4): 467-475.

Gargett, C. E. (2007) Uterine stem cells: what is the evidence? *Hum Reprod Update*, 13 (1): 87-101.

Gargett, C. E. & Masuda, H. (2010) Adult stem cells in the endometrium. *Mol Hum Reprod*, 16 (11): 818-834.

Gargett, C. E., Schwab, K. E., Zillwood, R. M., Nguyen, H. P. & Wu, D. (2009) Isolation and culture of epithelial progenitors and mesenchymal stem cells from human endometrium. *Biol Reprod*, 80 (6): 1136-1145.

Gargett, C. E. & Ye, L. (2012) Endometrial reconstruction from stem cells. *Fertil Steril*, 98 (1): 11-20.

Gellersen, B. & Brosens, J. J. (2014) Cyclic decidualization of the human endometrium in reproductive health and failure. *Endocr Rev*, 35 (6): 851-905.

Gellersen, B., Wolf, A., Kruse, M., Schwenke, M. & Bamberger, A. M. (2013) Human endometrial stromal cell-trophoblast interactions: mutual stimulation of chemotactic migration and promigratory roles of cell surface molecules CD82 and CEACAM1. *Biol Reprod*, 88 (3): 80.

Greco, E., Minasi, M. G. & Fiorentino, F. (2015) Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts. *N Engl J Med*, 373 (21): 2089-2090.

Green, J. B., Bethel, M. A., Armstrong, P. W., Buse, J. B., Engel, S. S., Garg, J., Josse, R., Kaufman, K. D., Koglin, J., Korn, S., Lachin, J. M., McGuire, D. K., Pencina, M. J., Standl, E., Stein, P. P., Suryawanshi, S., Van de Werf, F., Peterson, E. D., Holman, R. R. & Group, T. S. (2015) Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med*, 373 (3): 232-242.

Herman, G. A., Stevens, C., Van Dyck, K., Bergman, A., Yi, B., De Smet, M., Snyder, K., Hilliard, D., Tanen, M., Tanaka, W., Wang, A. Q., Zeng, W., Musson, D., Winchell, G., Davies, M. J., Ramael, S., Gottesdiener, K. M. & Wagner, J. A. (2005) Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. *Clin Pharmacol Ther*, 78 (6): 675-688.

Hopman, R. K. & DiPersio, J. F. (2014) Advances in stem cell mobilization. *Blood Rev*, 28 (1): 31-40.

Hu, C., Yong, X., Li, C., Lü, M., Liu, D., Chen, L., Hu, J., Teng, M., Zhang, D., Fan, Y. & Liang, G. (2013) CXCL12/CXCR4 axis promotes mesenchymal stem cell mobilization to burn wounds and contributes to wound repair. *J Surg Res*, 183 (1): 427-434.

Hutton, B., Sharma, R., Fergusson, D., Tinmouth, A., Hebert, P., Jamieson, J. & Walker, M. (2007) Use of intravenous immunoglobulin for treatment of recurrent miscarriage: a systematic review. *BJOG*, 114 (2): 134-142.

Jaslow, C. R. (2014) Uterine factors. *Obstet Gynecol Clin North Am*, 41 (1): 57-86.

Junggraithmayr, W., De Meester, I., Mattheeuw, V., Baerts, L., Arni, S. & Weder, W. (2012) CD26/DPP-4 inhibition recruits regenerative stem cells via stromal cell-derived factor-1 and beneficially influences ischaemia-reperfusion injury in mouse lung transplantation. *Eur J Cardiothorac Surg*, 41 (5): 1166-1173.

Kaandorp, S., Di Nisio, M., Goddijn, M. & Middeldorp, S. (2009) Aspirin or anticoagulants for treating recurrent miscarriage in women without antiphospholipid syndrome. *Cochrane Database Syst Rev*, (1): CD004734.

Karami, N., Boroujerdnia, M. G., Nikbakht, R. & Khodadadi, A. (2012) Enhancement of peripheral blood CD56(dim) cell and NK cell cytotoxicity in women with recurrent spontaneous abortion or in vitro fertilization failure. *J Reprod Immunol*, 95 (1-2): 87-92.

Kesmodel, U., Wisborg, K., Olsen, S. F., Henriksen, T. B. & Secher, N. J. (2002) Moderate alcohol intake in pregnancy and the risk of spontaneous abortion. *Alcohol Alcohol*, 37 (1): 87-92.

Khatun, M., Sorjamaa, A., Kangasniemi, M., Sutinen, M., Salo, T., Liakka, A., Lehenkari, P., Tapanainen, J. S., Vuolteenaho, O., Chen, J. C., Lehtonen, S. & Piltonen, T. T. (2017) Niche matters: The comparison between bone marrow stem cells and endometrial stem cells and stromal fibroblasts reveal distinct migration and cytokine profiles in response to inflammatory stimulus. *PLoS One*, 12 (4): e0175986.

Kolte, A. M., Nielsen, H. S., Moltke, I., Degn, B., Pedersen, B., Sunde, L., Nielsen, F. C. & Christiansen, O. B. (2011) A genome-wide scan in affected sibling pairs with idiopathic recurrent miscarriage suggests genetic linkage. *Mol Hum Reprod*, 17 (6): 379-385.

Lachapelle, M. H., Miron, P., Hemmings, R. & Roy, D. C. (1996) Endometrial T, B, and NK cells in patients with recurrent spontaneous abortion. Altered profile and pregnancy outcome. *J Immunol*, 156 (10): 4027-4034.

Laird, S. M., Tuckerman, E. M. & Li, T. C. (2006) Cytokine expression in the endometrium of women with implantation failure and recurrent miscarriage. *Reprod Biomed Online*, 13 (1): 13-23.

Lata, K., Dutta, P., Sridhar, S., Rohilla, M., Srinivasan, A., Prashad, G. R., Shah, V. N. & Bhansali, A. (2013) Thyroid autoimmunity and obstetric outcomes in women with recurrent miscarriage: a case-control study. *Endocr Connect*, 2 (2): 118-124.

Li, Y., Lin, H. & Cao, J. (2011) Association between socio-psycho-behavioral factors and male semen quality: systematic review and meta-analyses. *Fertil Steril*, 95 (1): 116-123.

Liang, P., Mo, M., Li, G. G., Yin, B., Cai, J., Wu, T., He, X., Zhang, X. & Zeng, Y. (2012) Comprehensive analysis of peripheral blood lymphocytes in 76 women with recurrent miscarriage before and after lymphocyte immunotherapy. *Am J Reprod Immunol*, 68 (2): 164-174.

Liu, X., Zhou, C., Li, Y., Ji, Y., Xu, G., Wang, X. & Yan, J. (2013) SDF-1 promotes endochondral bone repair during fracture healing at the traumatic brain injury condition. *PLoS One*, 8 (1): e54077.

Liu, Z., Xu, H., Kang, X., Wang, T., He, L. & Zhao, A. (2016) Allogenic Lymphocyte Immunotherapy for Unexplained Recurrent Spontaneous Abortion: A Meta-Analysis. *Am J Reprod Immunol*, 76 (6): 443-453.

Lo, W., Rai, R., Hameed, A., Brailsford, S. R., Al-Ghamdi, A. A. & Regan, L. (2012) The effect of body mass index on the outcome of pregnancy in women with recurrent miscarriage. *J Family Community Med*, 19 (3): 167-171.

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.

Macklon, N. S. & Brosens, J. J. (2014) The human endometrium as a sensor of embryo quality. *Biol Reprod*, 91 (4): 98.

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.

Macdonochie, N., Doyle, P., Prior, S. & Simmons, R. (2007) Risk factors for first trimester miscarriage--results from a UK-population-based case-control study. *BJOG*, 114 (2): 170-186.

McQueen, D. B., Bernardi, L. A. & Stephenson, M. D. (2014) Chronic endometritis in women with recurrent early pregnancy loss and/or fetal demise. *Fertil Steril*, 101 (4): 1026-1030.

Merck & Co (2015) *Annual Cumulative Review for Januvia and Janumet*. Co, M.

Mistry, G. C., Bergman, A. J., Zheng, W., Hreniuk, D., Zinny, M. A., Gottesdiener, K. M., Wagner, J. A., Herman, G. A. & Ruddy, M. (2008) Sitagliptin, an dipeptidyl peptidase-4 inhibitor, does not alter the pharmacokinetics of the sulphonylurea, glyburide, in healthy subjects. *Br J Clin Pharmacol*, 66 (1): 36-42.

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

Musters, A. M., Koot, Y. E., van den Boogaard, N. M., Kaaijk, E., Macklon, N. S., van der Veen, F., Nieuwkerk, P. T. & Goddijn, M. (2013) Supportive care for women with recurrent miscarriage: a survey to quantify women's preferences. *Hum Reprod*, 28 (2): 398-405.

Mutlu, L., Hufnagel, D. & Taylor, H. S. (2015) The endometrium as a source of mesenchymal stem cells for regenerative medicine. *Biol Reprod*, 92 (6): 138.

Nagori, C. B., Panchal, S. Y. & Patel, H. (2011) Endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman's syndrome. *J Hum Reprod Sci*, 4 (1): 43-48.

NIHR (2017) *Clinical Trials Toolkit*. [online] Available from: (Accessed <http://www.ct-toolkit.ac.uk/>, 21/11/2017).

NVOG (2007) Utrecht Guideline: Recurrent Miscarriage

Nybo Andersen, A. M., Wohlfahrt, J., Christens, P., Olsen, J. & Melbye, M. (2000) Maternal age and fetal loss: population based register linkage study. *BMJ*, 320 (7251): 1708-1712.

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

Opatrny, L., David, M., Kahn, S. R., Shrier, I. & Rey, E. (2006) Association between antiphospholipid antibodies and recurrent fetal loss in women without autoimmune disease: a metaanalysis. *J Rheumatol*, 33 (11): 2214-2221.

Orlando, J. & Coulam, C. (2014) Is superfertility associated with recurrent pregnancy loss? *Am J Reprod Immunol*, 72 (6): 549-554.

Pasquier, E. & Martin, L. e. S. (2015) Low-Molecular-Weight Heparin for Women With Unexplained Recurrent Pregnancy Loss. *Ann Intern Med*, 163 (6): 484-485.

Patil, H. R., Al Badarin, F. J., Al Shami, H. A., Bhatti, S. K., Lavie, C. J., Bell, D. S. & O'Keefe, J. H. (2012) Meta-analysis of effect of dipeptidyl peptidase-4 inhibitors on cardiovascular risk in type 2 diabetes mellitus. *Am J Cardiol*, 110 (6): 826-833.

Peng, H., Erdmann, N., Whitney, N., Dou, H., Gorantla, S., Gendelman, H. E., Ghorpade, A. & Zheng, J. (2006) HIV-1-infected and/or immune activated macrophages regulate astrocyte SDF-1 production through IL-1beta. *Glia*, 54 (6): 619-629.

Penn, M. S., Pastore, J., Miller, T. & Aras, R. (2012) SDF-1 in myocardial repair. *Gene Ther*, 19 (6): 583-587.

Piosik, Z. M., Goegebeur, Y., Klitkou, L., Steffensen, R. & Christiansen, O. B. (2013) Plasma TNF- α levels are higher in early pregnancy in patients with secondary compared with primary recurrent miscarriage. *Am J Reprod Immunol*, 70 (5): 347-358.

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.

Price, J. D., Linder, G., Li, W. P., Zimmermann, B., Rother, K. I., Malek, R., Alattar, M. & Tarbell, K. V. (2013) Effects of short-term sitagliptin treatment on immune parameters in healthy individuals, a randomized placebo-controlled study. *Clin Exp Immunol*, 174 (1): 120-128.

Prior, M., Bagness, C., Brewin, J., Coomarasamy, A., Easthope, L., Hepworth-Jones, B., Hinshaw, K., O'Toole, E., Orford, J., Regan, L., Raine-Fenning, N., Shakespeare, J., Small, R., Thornton, J. & Metcalf, L. (2017) Priorities for research in miscarriage: a priority setting partnership between people affected by miscarriage and professionals following the James Lind Alliance methodology. *BMJ Open*, 7 (8): e016571.

Quenby, S. & Brosens, J. J. (2013) Human implantation: a tale of mutual maternal and fetal attraction. *Biol Reprod*, 88 (3): 81.

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

Quenby, S. M. & Farquharson, R. G. (1993) Predicting recurring miscarriage: what is important? *Obstet Gynecol*, 82 (1): 132-138.

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

Rasch, V. (2003) Cigarette, alcohol, and caffeine consumption: risk factors for spontaneous abortion. *Acta Obstet Gynecol Scand*, 82 (2): 182-188.

Regan, L. (1991) Recurrent miscarriage. *BMJ*, 302 (6776): 543-544.

Regan, L., Braude, P. R. & Trembath, P. L. (1989) Influence of past reproductive performance on risk of spontaneous abortion. *BMJ*, 299 (6698): 541-545.

Robinson, L., Gallos, I. D., Conner, S. J., Rajkhowa, M., Miller, D., Lewis, S., Kirkman-Brown, J. & Coomarasamy, A. (2012) The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. *Hum Reprod*, 27 (10): 2908-2917.

Rossi, M., Sharkey, A. M., Viganò, P., Fiore, G., Furlong, R., Florio, P., Ambrosini, G., Smith, S. K. & Petraglia, F. (2005) Identification of genes regulated by interleukin-1beta in human endometrial stromal cells. *Reproduction*, 130 (5): 721-729.

Royal College of Obstetricians & Gynaecologists (2011) *Recurrent Miscarriage, Investigation and Treatment of Couples (Green-top Guideline No. 17)*. London: Royal College of Obstetricians & Gynaecologists.

Ruixue, W., Hongli, Z., Zhihong, Z., Rulin, D., Dongfeng, G. & Ruizhi, L. (2013) The impact of semen quality, occupational exposure to environmental factors and lifestyle on recurrent pregnancy loss. *J Assist Reprod Genet*, 30 (11): 1513-1518.

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.

Sagle, M., Bishop, K., Ridley, N., Alexander, F. M., Michel, M., Bonney, R. C., Beard, R. W. & Franks, S. (1988) Recurrent early miscarriage and polycystic ovaries. *BMJ*, 297 (6655): 1027-1028.

Sahin Ersoy, G., Zolbin, M. M., Cosar, E., Moridi, I., Mamillapalli, R. & Taylor, H. S. (2017) CXCL12 Promotes Stem Cell Recruitment and Uterine Repair after Injury in Asherman's Syndrome. *Mol Ther Methods Clin Dev*, 4 169-177.

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., 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.

Santamaría, 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.

Saravelos, S. H., Cocksedge, K. A. & Li, T. C. (2008) Prevalence and diagnosis of congenital uterine anomalies in women with reproductive failure: a critical appraisal. *Hum Reprod Update*, 14 (5): 415-429.

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.

Schleussner, E. & Petroff, D. (2015) Low-Molecular-Weight Heparin for Women With Unexplained Recurrent Pregnancy Loss. *Ann Intern Med*, 163 (6): 485.

Shaaban, O. M., Abbas, A. M., Zahran, K. M., Fathalla, M. M., Anan, M. A. & Salman, S. A. (2017) Low-Molecular-Weight Heparin for the Treatment of Unexplained Recurrent Miscarriage With Negative Antiphospholipid Antibodies: A Randomized Controlled Trial. *Clin Appl Thromb Hemost*, 23 (6): 567-572.

Sharma, R., Biedenharn, K. R., Fedor, J. M. & Agarwal, A. (2013) Lifestyle factors and reproductive health: taking control of your fertility. *Reprod Biol Endocrinol*, 11 66.

Singh, N., Mohanty, S., Seth, T., Shankar, M., Bhaskaran, S. & Dharmendra, S. (2014) Autologous stem cell transplantation in refractory Asherman's syndrome: A novel cell based therapy. *J Hum Reprod Sci*, 7 (2): 93-98.

Skeith, L., Carrier, M., Kaaja, R., Martinelli, I., Petroff, D., Schleußner, E., Laskin, C. A. & Rodger, M. A. (2016) A meta-analysis of low-molecular-weight heparin to prevent pregnancy loss in women with inherited thrombophilia. *Blood*, 127 (13): 1650-1655.

Stephenson, M. D., Dreher, K., Houlihan, E. & Wu, V. (1998) Prevention of unexplained recurrent spontaneous abortion using intravenous immunoglobulin: a prospective, randomized, double-blinded, placebo-controlled trial. *Am J Reprod Immunol*, 39 (2): 82-88.

Stephenson, M. D., Kutteh, W. H., Purkiss, S., Librach, C., Schultz, P., Houlihan, E. & Liao, C. (2010) Intravenous immunoglobulin and idiopathic secondary recurrent miscarriage: a multicentered randomized placebo-controlled trial. *Hum Reprod*, 25 (9): 2203-2209.

Stirrat, G. M. (1990) Recurrent miscarriage. *Lancet*, 336 (8716): 673-675.

Sugiura-Ogasawara, M., Ozaki, Y., Sato, T., Suzumori, N. & Suzumori, K. (2004) Poor prognosis of recurrent aborters with either maternal or paternal reciprocal translocations. *Fertil Steril*, 81 (2): 367-373.

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.

Taylor, H. S. (2004) Endometrial cells derived from donor stem cells in bone marrow transplant recipients. *JAMA*, 292 (1): 81-85.

Teklenburg, G., Salker, M., 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., Brosens, J. J. & Macklon, N. S. (2010) Natural selection of human embryos: decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation. *PLoS One*, 5 (4): e10258.

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.

Ticconi, C., Giuliani, E., Veglia, M., Pietropolli, A., Piccione, E. & Di Simone, N. (2011) Thyroid autoimmunity and recurrent miscarriage. *Am J Reprod Immunol*, 66 (6): 452-459.

van den Berg, M. M., van Maarle, M. C., van Wely, M. & Goddijn, M. (2012) Genetics of early miscarriage. *Biochim Biophys Acta*, 1822 (12): 1951-1959.

van den Boogaard, E., Cohn, D. M., Korevaar, J. C., Dawood, F., Vissenberg, R., Middeldorp, S., Goddijn, M. & Farquharson, R. G. (2013) Number and sequence of preceding miscarriages and maternal age for the prediction of antiphospholipid syndrome in women with recurrent miscarriage. *Fertil Steril*, 99 (1): 188-192.

Wang, X., Mamillapalli, R., Mutlu, L., Du, H. & Taylor, H. S. (2015) Chemoattraction of bone marrow-derived stem cells towards human endometrial stromal cells is mediated by estradiol regulated CXCL12 and CXCR4 expression. *Stem Cell Res*, 15 (1): 14-22.

Watson, H., Kiddy, D. S., Hamilton-Fairley, D., Scanlon, M. J., Barnard, C., Collins, W. P., Bonney, R. C. & Franks, S. (1993) Hypersecretion of luteinizing hormone and ovarian steroids in women with recurrent early miscarriage. *Hum Reprod*, 8 (6): 829-833.

Watt, F. M. & Hogan, B. L. (2000) Out of Eden: stem cells and their niches. *Science*, 287 (5457): 1427-1430.

Weimar, C. H., Kavelaars, A., Brosens, J. J., Gellersen, B., de Vreeden-Elbertse, J. M., Heijnen, C. J. & Macklon, N. S. (2012) Endometrial stromal cells of women with recurrent miscarriage fail to discriminate between high- and low-quality human embryos. *PLoS One*, 7 (7): e41424.

Wolff, E. F., Gao, X. B., Yao, K. V., Andrews, Z. B., Du, H., Elsworth, J. D. & Taylor, H. S. (2011) Endometrial stem cell transplantation restores dopamine production in a Parkinson's disease model. *J Cell Mol Med*, 15 (4): 747-755.

Wong, L. F., Porter, T. F. & Scott, J. R. (2014) Immunotherapy for recurrent miscarriage. *Cochrane Database Syst Rev*, (10): CD000112.

Yang, G. Y., Luo, H., Liao, X. & Liu, J. P. (2013) Chinese herbal medicine for the treatment of recurrent miscarriage: a systematic review of randomized clinical trials. *BMC Complement Altern Med*, 13 320.

Zhang, B. Y., Wei, Y. S., Niu, J. M., Li, Y., Miao, Z. L. & Wang, Z. N. (2010) Risk factors for unexplained recurrent spontaneous abortion in a population from southern China. *Int J Gynaecol Obstet*, 108 (2): 135-138.

Ziakas, P. D., Pavlou, M. & Voulgarelis, M. (2010) Heparin treatment in antiphospholipid syndrome with recurrent pregnancy loss: a systematic review and meta-analysis. *Obstet Gynecol*, 115 (6): 1256-1262.

APPENDICES

APPENDIX 1

PARTICIPANT INFORMATION LEAFLET

Does Sitagliptin Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage?

Chief Investigator: Professor Siobhan Quenby

Version 2.0 17/11/2016

Invitation to join the SIMPLANT study

You are being invited to take part in a pilot research study called SIMPLANT. This study is for the women who are suffering with recurrent miscarriages.

It is important for you to read this leaflet to understand why the research is being done and what it involves. This leaflet explains why we are doing the study and outlines the benefits and risks of taking part. The results of this study will form part of a doctorate which will be supervised by Professor Siobhan Quenby. Please take some time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. If you have any questions, please ask the Research Team who will be happy to help.

What is the purpose of this study?

Research has shown that women with recurrent miscarriage have low numbers of stem cells in the lining of the womb (endometrium). Sitagliptin, a medication, which is routinely used in treating patients with diabetes, has been shown to be successful in increasing the number of stem cells in other areas of the body where wound healing is taking place. This study will test whether giving Sitagliptin to women with recurrent miscarriage, increases the number of stem cells in the lining of the womb after an endometrial scratch (biopsy). If Sitagliptin is successful in increasing the number of stem cells, this in turn may help improve the chance of a successful pregnancy by improving the environment for implantation.

Where is this study being carried out?

The study is being carried out at the Biomedical Research Unit at University Hospitals Coventry and Warwickshire NHS Trust.

Why have you been chosen to take part?

You have been invited to be part of this study because you have experienced 3 or more miscarriages and your health care team has not been able to find a cause.

What does taking part in this study involve?

If you are happy to take part, we will check all the routine blood tests that you would have had to look for causes of your miscarriages to ensure you are eligible to take part in the study. We would also need to ensure you have regular periods with a 28 – 30 day cycle.

If the blood tests are normal you will be asked to agree to take part in the study. This will involve being assigned at random to receive either Sitagliptin or placebo (dummy) tablets, which you will take for the duration of the study (3 months). You will have two endometrial biopsies: one just before treatment starts and one after 3 months of treatment.

You will have an equal chance of receiving the Sitagliptin or the placebo treatment. Neither you nor the study team can influence which treatment you receive; a computer will make this decision. During the study, neither you nor the study team will know which treatment you are on, however it will be possible to access this information should it become necessary for your clinical care.

You will be given a patient diary to keep a record of taking the tablets and any side effects you are experiencing.

We will ask you to come to the Biomedical Research Unit every 4 weeks +/- 4 days to review this diary and make sure you are well.

Summary of what is involved

1. We will check you are eligible to take part in the study.
2. We will ask you to sign a consent form signed for the study
3. Your GP will be informed by writing of your inclusion into trial with your consent
4. You will be asked to attend for an endometrial biopsy 7-10 days after ovulation. We will ask you to sign a consent form for an endometrial biopsy - this is then performed the same day
5. You will be assigned at random to either Sitagliptin or Placebo (dummy) tablet. Each pack will contain 32 tablets.
6. One tablet needs to be taken every day for a total of 3 months up until the day of the second endometrial biopsy.
7. We will ask you to visit the research unit for general health check every 4 weeks +/- 4 days. We will give you a simple symptom diary to fill in so that we can monitor any problems closely.
8. At each monthly visit we will give you the following months' supply of tablets as long as you are happy to carry on.
9. An endometrial biopsy will be performed again at the end of the 3-month period

During the study we will ask you to do a pregnancy test at home every 2 weeks. Pregnancy test kits will be provided. It is important you do not get pregnant while participating in this trial as we do not clearly know of the safety of Sitagliptin in pregnancy.

Once you have had your second endometrial biopsy we will ask you to fill in a simple questionnaire related to your experience of being in a research trial. Your replies will be anonymous and will help to improve services in the future.

Once results have been analysed you will be able to discuss your results in the context of the other participant's results with the research team.

What is an Endometrial Biopsy?

An Endometrial Biopsy is a sample of the lining of the womb. A transvaginal (internal) ultrasound scan be done to assess the thickness of the lining of the womb and to determine which way the womb is tilted that we know how to take the biopsy. To take the biopsy a thin plastic straw will be passed through the cervix into the uterus. Gas and air (Entonox) is available if needed. The procedure usually takes a couple of minutes and will be done in clinic. Following the procedure the small sample of your womb lining will be frozen and stored in UHCW's Tissue Bank for analysis of stem cells and genetic analysis. With permission, any tissue left over after the analysis is complete will be stored anonymously in the Tissue Bank for use in future research studies. You do not have to give your consent for long term storage of your tissue samples if you do not wish.

What are the possible risks of being in the study?

Sitagliptin has been given to patients routinely for many years as part of their treatment for diabetes and is very safe. However, as with all medication, there are some side effects (affecting less than 1 in 10 people) associated with taking Sitagliptin that may or may not affect you. These include a sore throat, runny nose, headache. Other less common side effects include change of bowel habit. There have also been some reports of skin conditions, muscle pain and allergic reactions in patients taking Sitagliptin. In extremely rare cases Sitagliptin may cause inflammation of the pancreas causing severe abdominal pain. We will monitor you closely throughout the study and you will be given a point of contact should you develop any worrying symptoms while in the study.

The Endometrial Biopsy may cause some cramping abdominal pain at the time of the biopsy. Taking Paracetamol and Ibuprofen an hour before the biopsy can help with this. We also have gas and air available to use while having the biopsy. Some patients have also reported some spotting after the biopsy is taken but this will resolve quickly on its own.

What are the possible benefits of being in the study?

We do not know if there is any benefit of taking Sitagliptin to the endometrium. It is possible that both Sitagliptin and the placebo have no effect on the endometrium. It is however important to have some patients on a placebo tablet so that we can accurately look at whether Sitagliptin increases the stem cell count.

We know that taking an endometrial biopsy causes a wound healing response in the lining of the womb which has been shown to improve pregnancy rates in women having IVF, so there may be some benefit from having an endometrial biopsy in recurrent miscarriage as well.

What if I become pregnant during the study?

It is very important that you do not try to become pregnant while you are taking part in the study. If you decide to take part in the study you will be asked to take a pregnancy test before you are enrolled or study and then every 2 weeks for the duration of the study. Pregnancy test kits will be provided. You will also be provided with and required to use a reliable, non-hormonal contraceptive throughout your enrolment in the study. Once you have finished taking part in the study it is safe for you to try to become pregnant again straightforwardly if you wish.

If you do become pregnant during the study, you should stop taking the study medication immediately and call the study team to arrange an appointment.

Do I have to take part?

Participation in the study is entirely voluntary. If you do not wish to take part you will not have to give reason and your decision will not affect the care you receive. If you do decide to participate we would be most grateful if we could use any extra tissue from the endometrial biopsy for research purposes by our research team. The biopsy would not be used by any other team and the tissue sample would be frozen and stored in the Tissue Bank at UHCW anonymously. The sample would not be shared externally.

If you would like to take part in the study but would not like your tissue to be used for other research purposes other than those for this study this will be respected and reflected on the consent form you sign.

What if I wish to withdraw from the study?

If you do decide to take part in the study you are free to withdraw yourself and your data from the study and stop taking the study medication at any time. You do not have to give a reason. If you decide to withdraw from the study we would ask you to let us know when you stopped taking the medication.

Will the information you collect be kept private?

All the information collected during the study will remain confidential like your medical records. With your consent we will inform your GP that you are taking part in the study. All information collected during the study will be recorded on a secure database, which will only be accessible to authorised people. You will have a personal 'trial number' which will be used to identify all of your information and test results. If the results of the study are available they will be circulated to relevant medical staff and they will be published in medical journals, but there will be no details included that would allow identification of patients involved. The personal information collected about you as part of this study e.g. your name and contact details, will be stored securely at UHCW for up to 3 years. Only authorized members of the research team will be allowed access to your personal information and it will not be shared with any third party without your express permission.

Who can I contact about any questions or problems?

If you have any questions or concerns about any aspect of this study or would like to take part, please contact the study office on **024 769 67528** and a member of the research team will be able to help.

Prof. Siobhan Quenby is in charge of the study at this hospital, please see contact details below:

PA to Prof. Quenby, Kerri Geraghty on 024 769 67528.

Contact information for any complaints:

If you experience any problems as a result of taking part in this study, or if you wish to make a formal complaint you can do so by writing to:

Biomedical Research Unit, UHCW, Clifford Bridge Road, Coventry CV2 2DX or at simplant@uhcw.nhs.uk.

For independent advice on research, you can contact PALS (Patient Advice and Liaison Service) on freephone 0800 028 4203 or email: Feedback@uhcw.nhs.uk

Who has reviewed the study?

To protect your interests, all research in the NHS is looked at by a Research Ethics Committee. This study has been reviewed and given favourable opinion by the HRA & South Central – Hampshire B Research Ethics Committee.

APPENDIX 2 – CONSENT FORM

CONSENT FORMScreening Number: S -

Study full title: Does the DPP4 inhibitor Sitagliptin increase endometrial mesenchymal cells in women with recurrent miscarriage?

Short title: SIMPLANT study

Principal Investigator: Professor Siobhan Quenby
Version 2.0, 17/11/2016

Please Initial

1. I confirm that I have read and understood the information leaflet version 2.0, 17/11/2016 for the above trial and consent to the procedures within the trial. I have had the opportunity to consider the information and ask questions. I have received satisfactory answers to all my questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my future medical care or legal rights being affected.

3. I understand that relevant sections of my medical records and/or data collected as part of the study may be looked at by responsible individuals from regulatory authorities or from the NHS Trust where it is relevant to my taking part in this study. I give my permission for these individuals to have access to these records.

4. I understand that my information, and tissue samples collected as part of the study will be frozen and stored in the Tissue Bank at UHCW and analysed for stem cells and genetic analysis as part of this study.

5. I agree for my tissue samples to be frozen and stored in the Tissue Bank at UHCW anonymously for use in future research by the research team. The sample would not be shared externally. Please initial in ONE of the two boxes.

YES	NO
<input type="checkbox"/>	<input type="checkbox"/>

6. I agree for my G.P to be informed about my inclusion to this study.

7. I understand that I must not try to get pregnant while I am taking part in this study and I agree to use reliable contraception for the duration of my involvement in the study.

8. I agree to take part in the study

.....
Name of participant
(BLOCK CAPITALS) Date Signature

.....
Name of person taking consent
(if different from researcher) Date Signature

.....
Name of researcher Date Signature

SIMPLANT study consent form_v2.0 17/11/2016 IRAS No:

APPENDIX 3 – LETTER TO GENERAL PRACTITIONER

<GP NAME>
<PRACTICE NAME>
<PRACTICE ADDRESS>
<DATE>

Dear <GP NAME>

RE: SIMPLANT study
Does the DPP4 Inhibitor (Sitagliptin) Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage?

Patient name	
DOB	
Address	

The Biomedical Research Unit at University Hospitals Coventry and Warwickshire NHS Trust are currently running the SIMPLANT study. This is a single centre pilot randomised double blind controlled trial looking at the effect of Sitagliptin in increasing the mesenchymal stem cell count at the endometrium.

Your patient, <patient name>, agreed to take part in the trial when she attended hospital on <date of appointment> and was randomised to receive either:

1. Intervention group: 100mg of Sitagliptin daily for 3 months
2. Control group: oral placebo daily for 3 months

As part of their involvement in the study your patient will also undergo two endometrial biopsies; one at the start and one at the end of the treatment period. It is therefore important they do not get pregnant during the trial.

As this is a double blind study, neither the participant nor our study investigators know the treatment allocation of your patient. Your patient has the contact details of our research team in case of any difficulties. Patients in this study also carry a participant information card in case they are admitted as an emergency. This card can be given to the clinician looking after the patient and the research team can be contacted.

Please find a copy of the information leaflet that was given to your patient enclosed for your information. If you would like any further details about the study, please feel free to contact our Trial Coordination team via simplantstudyoffice@uhcw.nhs.uk or 024 7696 7528.

Yours Sincerely,

Prof Siobhan Quenby

SIMPLANT study GP letter_v1.0_08.04.2016

APPENDIX 4 – SYMPTOM DIARY

Patient Initials:

--	--	--

Screening ID:

	-			
--	---	--	--	--

Participant Trial ID:

R		
---	--	--

SIMPLANT

Participant Symptom Diary

Does the DPP4 Inhibitor (Sitagliptin) Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage?

Follow Up Appointment

Patient Initials:

Screening ID: -

Participant Trial ID:

Patient Initials:

Medication Compliance

Day	Time
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	
26	
27	

Screening ID: -

Participant Trial ID: R

If you have filled all the above boxes please contact the Research Unit to discuss your symptoms further

Patient Initials:

--	--	--

Screening ID:

	-			
--	---	--	--	--

Participant Trial ID:

R		
---	--	--

28	
29	
30	
31	

Menstrual Diary		
Length of period	_____ days	
Heavy Days?	Yes	No
	If Yes Number of days _____	

Other Comments?	

Patient Initials:

Screening ID: -

Participant Trial ID: R

Research Team contact details

**If you have any concerns or your pregnancy test
is positive, Please contact us on**

simplant@uhcw.nhs.uk

02476967528 (Kerri Geraghty – Secretary to Professor Quenby)



The image shows the front cover of the SIMPLANT Participant Questionnaire. The cover has a pink-to-purple gradient background with a wavy white line. At the top center, the word "SIMPLANT" is written in large, bold, white capital letters. Below it, in smaller white capital letters, is "PARTICIPANT QUESTIONNAIRE". In the center of the cover, there is a white rectangular area containing the study title: "Does the DPP4 Inhibitor (Sitagliptin) Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage?". At the bottom left of this white area, the text "Thank you for your participation in the SIMPLANT study." is printed in bold black font. Below this, a message in regular black font reads: "We would really value your opinion about your experience and would be grateful if you could complete this questionnaire. This will help us to ensure future research projects take your opinion on board." Further down, another message says: "Please complete this after your cycle starts and post it back in the self-addressed envelope". At the very bottom of the cover, there is a small white box containing the text "Thank you for your time. Your information is valuable to us. If you wish us to contact you to discuss feedback this can be arranged. Please ring Kerri Geraghty on 02476967528".

SIMPLANT_Participant questionnaire V1.2_02.06.2016

IRAS No196058

**University Hospitals
Coventry and Warwickshire
NHS Trust**
SIMPLANT
PARTICIPANT QUESTIONNAIRE

Scale: Please use the following scale to answer the following questions

Strongly agree	5
Agree	4
Neither agree/disagree	3
Disagree	2
Strongly disagree	1

I found taking part in the study a worthwhile experience	5	4	3	2	1
Given the choice, I would like to continue taking part in this study	5	4	3	2	1
I would recommend taking part in the study to others	5	4	3	2	1
I found taking part in the study supportive of my care	5	4	3	2	1
I would be open to taking part in other research studies	5	4	3	2	1

I found taking the study medication easy	5	4	3	2	1
I found the side effects from the medication easy to manage	5	4	3	2	1

I forgot to take the study medication	Often	Sometimes	Rarely	Never
---------------------------------------	-------	-----------	--------	-------

University Hospitals
Coventry and Warwickshire
SIMPLANT
NHS Trust
PARTICIPANT QUESTIONNAIRE

1. Did the patient information leaflet describe the study clearly?

Yes / No

If No what do you think could be improved?.....

.....

2. Did you any difficulties booking your appointment for your biopsy?

a. Biopsy 1 – Yes / No

b. Biopsy 2 – Yes / No

If yes, please give details

.....

.....

3. Did you find it difficult to book the monthly hospital visits?

Yes / No

If yes, please give details

.....

.....

4. Did you find the hospital visits supportive to your care?

Yes / No

If No what do you think could be improved?.....

.....

.....

5. Were you able to contact the research team for support when needed?

Yes / No

If No, please give details

.....

.....

6. Do you have any suggestions as to how the whole experience could be improved?

.....

.....

7. Any other comments?

.....

APPENDIX 6 – EMERGENCY CONTACT CARD



APPENDIX 7 – PEER REVIEW FROM DR NIGEL SIMPSON

University Hospitals 
Coventry and Warwickshire
NHS Trust

UHCW NHS Trust R,D & I Independent Peer Review Form

1. Full Project Title: (The project protocol should be attached to this form) Does the DPP4 Inhibitor (Sitagliptin) Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage?		
2. Short Title: SIMPLANT - Sitagliptin for IMPLANTation		
3. Investigators		
(a) Chief Investigator (For UHCW NHS Trust sponsored studies)	Name Professor Siobhan Quenby	Department
(b) Principal Investigator (For External Sponsors)		
4. Application Details		
Funding Body	Tommys (NB needs substituting for 'i4i' on p34 para 19.1)	
Sponsor (if External)		
5. INDEPENDENT PEER REVIEW: Please comment on the following areas:		
Area Reviewed	Comments	
a) The originality of the research	This is innovative and translational work, following earlier scientific studies and designed to optimise effective implantation in women with recurrent pregnancy loss	
b) The study design	A prospective double-blinded RCT into the ability of a (in this context) novel therapy of a DPP4 inhibitor to effectively assist endometrial MSC numbers, sufficiently-powered, with secondary outcomes that include successful implantation	
c) The research methods - appropriateness and achievability of the chosen methods and outcome measures in meeting the objectives of the study	Satisfactory	
d) Sampling – the appropriateness of the sampling methods and the inclusion/exclusion criteria	Satisfactory	

Version 1.0 February 2016

APPENDIX 8 – PEER REVIEW FROM DR O'HARE



REVIEW OF SIMPLANT PILOT RCT – S TEWARY AND SIOBHAN QUENBY

27/02/2016

RESEARCH IDEA/CONCEPT

This is highly innovative and has potential of producing a game changing approach to the treatment of some infertile woman using a drug that has over 10 years has established a strong safety record in the treatment of type 2 diabetes and its safety has been extensively scrutinised in very large scale clinical trials

SUGGESTIONS

This appears to be a well designed pilot study where clearly this proof of concept is the first step necessary before data would be available to define subsequent larger studies. 30 seems a practical number to look at.

Investigators seem to have addressed the ethical scrutiny whether there is enough animal data or models that could be done before moving on to patients. The answer lies in interspecies differences that would make animal models of little value.

Investigators need to mention on safety the recent 2015 Tecnos study (Nejm) that represents the long term use of sitagliptin and the relatively clean results putting to rest concerns about possible pancreatitis, heart disease or cancer over the many years of study in thousands of subjects.

Indemnity issues will no doubt be raised and the sponsors Univ/UHCW have insurance cover for this unless there has been a recent change. Clearly if these sort of innovations by our academics are to be fostered and insurers reassured that the intervention with sitagliptin will not carry serious risk to these volunteers and the potential benefits for infertile women could be substantial.

This class of drugs the gliptins do not require patients with diabetes to carry out blood sugar monitoring and the absence of hypoglycaemia is a major reason for their current use in diabetes.

CONCLUSION

Extremely important research that should constitute minimal risk to participants and if successful could lead on to groundbreaking treatments for infertility and much patient benefit from a relatively cheap drug on a worldwide scale. There appears little risk to the sponsor and a potential for considerable gain and considerable impact from the innovation.

Peer review – Internal – SIMPLANT – Paul O'Hare – 27/02/2016

APPENDIX 9 – FUNDING LETTER



University Hospitals **NHS**
Coventry and Warwickshire
NHS Trust

Research, Development & Innovation Department
University Hospitals Coventry & Warwickshire NHS Trust
4th Floor Rotunda, Rm: ADA40017
University Hospital
Clifford Bridge Road
Coventry
CV2 2DX

Commercial enquiries: 02476 966196
Governance/Non-commercial enquiries: 02476 966196
Innovation & Communication enquiries: 02476 964746
Research Funding & Grant enquiries: 02476 964956
Email: RD&I@uhcw.nhs.uk

18th May 2016

Professor Siobhan Quenby
Biomedical Research Unit
University Hospitals Coventry & Warwickshire
Coventry
CV2 2DX

Dear Professor Quenby

Study Title: SIMPLANT: Does the DPP4 inhibitor (Sitagliptin) increase endometrial mesenchymal stem cells in recurrent miscarriage?
RD&I No: SQ167015

Further to your successful application for Trust sponsorship I am writing to confirm the funding arrangements for the above research study. As you are aware the funding for the SIMPLANT study (previously referred to as DPP4) has been awarded £123,728 by Tommy's as part of a research collaboration under the umbrella 'The Tommy's National Centre for Miscarriage Research'.

In accordance with the funding award letter dated 8th February and the budget for Work Package 3 outlined in Schedule 1 attached, the Trust will enter into a contractual agreement with the University of Warwick for recovery of trial related costs.

The contract between the Trust and the University of Warwick has yet to be fully executed and therefore in anticipation of this and in the unlikely event that this funding is not recovered I can confirm that the Research, Development and Innovation Department will underwrite the cost of this study in full.

I will confirm the financial arrangements on execution of the agreement.

We wish you every success with your project.

You're sincerely

Mrs Ceri Jones
Head of Research, Development & Innovation



We Care. We Achieve. We Innovate.

APPENDIX 10 – INITIAL RECOMMENDATION RESPONSE FROM THE REC



South Central - Hampshire B Research Ethics Committee

Level 3 Block
Whitefriars
Lewins Mead
Bristol
BS1 2

Telephone: 0207 104 8

10 May 2016

Professor Siobhan Quenby
University Hospital Coventry and Warwickshire
Clifford Bridge Road
Coventry
CV22DX

Dear Professor Quenby

Study Title: Does the DPP4 Inhibitor (Sitagliptin) Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage?
REC reference: 16/SC/0229
Protocol number: SQ167015
EudraCT number: 2016-001120-54
IRAS project ID: 196058

The Research Ethics Committee reviewed the above application at the meeting held on 27 April 2016. Thank you for attending to discuss the application.

Provisional opinion

The Committee was unable to give a favourable opinion based on the information and documentation received so far.

The Committee requested the following information before confirming its final opinion:

Participant Information Sheet

1. Please amend the Participant Information Sheet to state that women would have to have regular periods for inclusion in the study and to advise whether they would be given a 28 or 35 day cycle of Sitagliptin. It should also be made clear in the PIS that they need to continue using the treatment up until the time of the second biopsy.
2. Please amend the Participant Information Sheet to include information about the storage and subsequent use of participants' samples, and that they would only be used by this research team.

A Research Ethics Committee established by the Health Research Authority

3. Please indicate in the Participant Information Sheet that pregnancy test kits would be provided.
4. Please amend the Participant Information Sheet and Consent Form so as to inform participants that, with their consent, their samples would be subject to genetic analysis.
5. Please amend the Participant Information Sheet to make it clear that this is a pilot study and would form part of a doctorate.
6. Please amend the Participant Information Sheet to explain how post-treatment follow-up of any subsequent pregnancy would work. This should include whether the researcher would remain blinded at this stage and how treating clinicians might be made aware of any results relevant to clinical care.
7. Please indicate in the Participant Information Sheet that participants will be asked to complete a questionnaire about their experience during the study.
8. Please add to the "Summary of what is involved" items referring to keeping a symptom diary and completing an end of study questionnaire. These documents should be titled 'Participant Questionnaire' and Participant Symptom Diary'.
9. Please consider whether you would be able to offer participants reimbursement of travel expenses and if so then please update the Participant Information Sheet accordingly.

The Committee delegated authority to confirm its final opinion on the application to the Chair and Sue Edwards.

The Committee nominated Georgina Castledine to be the point of contact should further clarification be sought from the applicant upon receipt of the decision letter.

The Committee nominated the Chair to be the point of contact for the REC Manager if further information was required.

When submitting a response to the Committee, the requested information should be electronically submitted from IRAS. A step-by-step guide on submitting your response to the REC provisional opinion is available on the HRA website using the following link:
<http://www.hra.nhs.uk/nhs-research-ethics-committee-rec-submitting-response-provisional-opinion/>

Please submit revised documentation where appropriate underlining or otherwise highlighting the changes which have been made and giving revised version numbers and dates. You do not have to make any changes to the REC application form unless you have been specifically requested to do so by the REC.

The Committee will confirm the final ethical opinion within a maximum of 60 days from the date of initial receipt of the application, excluding the time taken by you to respond fully to the above

A Research Ethics Committee established by the Health Research Authority

points. A response should be submitted by no later than 09 June 2016.

Summary of the discussion at the meeting

Other ethical issues were raised and resolved in preliminary discussion before your attendance at the meeting.

Ethical issues raised by the Committee in private discussion, together with responses given by the researcher when invited into the meeting

The Committee welcomed the researcher to the meeting.

Recruitment arrangements and access to health information, and fair participant selection

The Committee noted that there was a placebo group so as to eliminate any confounding effect of the biopsy, but asked whether this was really necessary.

You said that the problem was that women who suffered from repeated miscarriages may react to the biopsy differently to other women, which is why the repeated miscarriage placebo group would be needed for comparison purposes.

The Committee accepted this response.

Favourable risk benefit ratio: anticipated benefit/risks for research participants (present and future)

The Committee sought clarification as to whether you had asked the manufacturers of Sitagliptin for safety data in respect of use in pregnancy arising from its use in other studies.

You said that there was not enough data to confirm that the drug was safe in the case of pregnancy but that it had been administered up to 12 times in animals and had not caused any problems with fertility or pregnancy. You said that if a participant became pregnant, you would stop the treatment straight away and hoped that the treatment would become a pre-conception treatment to revolutionise treatment for those who suffer miscarriage and to give them a good womb to enable them to get pregnant.

The Committee accepted this response.

The Committee asked whether there would be any way that the treatment would adversely impact on someone getting pregnant after the trial and asked whether it was possible that it could lower endometrial stem cell numbers.

The Committee stated that it should be made clear in the Participant Information Sheet that participants should not try to get pregnant whilst taking part in the trial.

You said that there was no risk that the treatment would make a woman's chance of getting pregnant any worse but admitted that the drug might not help. You agreed to update the Participant Information Sheet.

The Committee asked whether there had been any epidemiological study on Sitagliptin.

A Research Ethics Committee established by the Health Research Authority

You said that no there had not been an epidemiological study as Sitagliptin was a new drug offered to those with Type 2 diabetes and those beyond reproductive life.

The Committee accepted this response.

Care and protection of research participants; respect for potential and enrolled participants' welfare and dignity

Prior to the researcher entering the meeting the Committee raised the following point;

The Committee asked whether consideration could be taken to reimburse participants travel expenses and stated that this should be made clear in the Participant Information Sheet.

The Committee asked how long stem cell numbers stayed in the women's endometrium.

You said that in previous studies stem cells had been placed directly in the women's endometrium, but some had suffered problems from this surgical procedure, but the stem cells numbers were usually maintained for up to three months. Professor Quenby said that they were looking for a less invasive procedure for women who had previously had miscarriages.

The Committee asked how women would be followed-up and whether they would come into the clinic for this.

You said that the participants involved in the study would be local patients who were well known and that they would be followed-up in the clinic where they knew the team well and would feel comfortable.

The Committee accepted this response.

The Committee asked whether women would have to have regular periods for inclusion in the study and asked whether they would be given a 28 or 35 day cycle of Sitagliptin and stressed that participants should not run out of pills. The Committee stated that it was important to ensure that women were still using the treatment when they received the second biopsy. The Committee stated that the Participant Information Sheet should include this information.

You said that the longest cycle would be 35 days and confirmed that participants would not run out of Sitagliptin and that they would still be receiving the treatment when they had their second biopsy. You agreed to revise the Participant Information Sheet to make this clear to participants.

The Committee noted that there was mention of storage and future use of participant samples and asked whether they would be held exclusively for this research team's use or whether they would be shared externally.

You said that samples would only be used for this research team's use and agreed to make this clear in the Participant Information Sheet.

The Committee asked whether the pregnancy test kits would be provided at no expense to the participants and stated that if so the Participant Information Sheet should be updated.

A Research Ethics Committee established by the Health Research Authority

You confirmed that pregnancy test kits would be provided and agreed to update the Participant Information Sheet.

Informed consent process and the adequacy and completeness of participant information

Prior to the researcher entering the meeting the Committee raised the following point;

The Committee stated that the Participant Information Sheet and consent form needed updating to inform participants that their samples would be subject to genetic analysis.

The Committee stated that the Participant Information Sheet should make it clear that this would be a pilot study and would form part of a doctorate.

The Committee noted that the Participant Information Sheet did not mention the use of an end of study questionnaire, and that neither this questionnaire nor the symptom diary was mentioned in the Summary of what is involved. This should be rectified and the titles of both documents should be amended by replacing the word 'Patient' by 'Participant'.

The Committee stated that the Participant Information Sheet should explain how post-treatment follow-up of any subsequent pregnancy would work. This should include whether the researcher would remain blinded at this stage and how treating clinicians might be made aware of any results relevant to clinical care.

The researcher left the meeting and the Committee discussed the application further.

Documents reviewed

The documents reviewed at the meeting were:

Document	Version	Date
Covering letter on headed paper [Covering Letter]	v1.0	08 April 2016
GP/consultant information sheets or letters [GP letter]	v1.0	08 April 2016
Investigator's brochure / IMP Dossier [Investigator's brochure]		12 April 2016
Letter from sponsor [Letter from sponsor]		22 February 2016
Letter from statistician [Letter from statistician]		18 March 2016
Non-validated questionnaire [Patient questionnaire]	v1.0	08 April 2016
Other [Participant emergency contact card]	v1.0	08 April 2016
Other [PPI summary report]		01 April 2016
Other [PPI - Letter from lay TSC member]		08 April 2016
Other [Student CV]		12 April 2016
Other [Letter from funder]		08 February 2016
Participant consent form [Informed consent form]	v1.0	08 April 2016
Participant information sheet (PIS) [PIS]	v1.0	08 April 2016
REC Application Form [REC_Form_08042016]		08 April 2016
Referee's report or other scientific critique report [Peer Review - Dr. Nigel Simpson]		01 February 2016

A Research Ethics Committee established by the Health Research Authority

You confirmed that pregnancy test kits would be provided and agreed to update the Participant Information Sheet.

Informed consent process and the adequacy and completeness of participant information

Prior to the researcher entering the meeting the Committee raised the following point;

The Committee stated that the Participant Information Sheet and consent form needed updating to inform participants that their samples would be subject to genetic analysis.

The Committee stated that the Participant Information Sheet should make it clear that this would be a pilot study and would form part of a doctorate.

The Committee noted that the Participant Information Sheet did not mention the use of an end of study questionnaire, and that neither this questionnaire nor the symptom diary was mentioned in the Summary of what is involved. This should be rectified and the titles of both documents should be amended by replacing the word 'Patient' by 'Participant'.

The Committee stated that the Participant Information Sheet should explain how post-treatment follow-up of any subsequent pregnancy would work. This should include whether the researcher would remain blinded at this stage and how treating clinicians might be made aware of any results relevant to clinical care.

The researcher left the meeting and the Committee discussed the application further.

Documents reviewed

The documents reviewed at the meeting were:

Document	Version	Date
Covering letter on headed paper [Covering Letter]	v1.0	08 April 2016
GP/consultant information sheets or letters [GP letter]	v1.0	08 April 2016
Investigator's brochure / IMP Dossier [Investigator's brochure]		12 April 2016
Letter from sponsor [Letter from sponsor]		22 February 2016
Letter from statistician [Letter from statistician]		18 March 2016
Non-validated questionnaire [Patient questionnaire]	v1.0	08 April 2016
Other [Participant emergency contact card]	v1.0	08 April 2016
Other [PPI summary report]		01 April 2016
Other [PPI - Letter from lay TSC member]		08 April 2016
Other [Student CV]		12 April 2016
Other [Letter from funder]		08 February 2016
Participant consent form [Informed consent form]	v1.0	08 April 2016
Participant information sheet (PIS) [PIS]	v1.0	08 April 2016
REC Application Form [REC_Form_08042016]		08 April 2016
Referee's report or other scientific critique report [Peer Review - Dr. Nigel Simpson]		01 February 2016

A Research Ethics Committee established by the Health Research Authority

South Central - Hampshire B Research Ethics Committee

Attendance at Committee meeting on 27 April 2016

Committee Members:

Name	Profession	Present	Notes
Dr Diane Ackerley	Retired Doctor	Yes	
Mrs Lisa Armstrong	Senior Lecturer Social Work	No	
Mrs Ravina Barrett	Pharmacist	Yes	
Mr Brian Birch (AVC)	Consultant Urological Surgeon	Yes	
Mrs Janet Brember	Pharmacist	Yes	
Ms Julie Brinton	Speech and Language Therapist	Yes	
Mr Mark Cassidy	Senior Lecturer in Radiography	Yes	
Dr Alessandro di Nicola	Lecturer in Philosophy	Yes	
Ms Susan Edwards	Lead Contract Manager, NHS South West and Central Contract Support Unit	Yes	
Mrs Angela Iveson	Acute Oncology Clinical Nurse Specialist	Yes	
Professor Ron King (Chair)	Mathematician (Retired)	Yes	
Mr Geoff Lowndes	Chartered Engineer (Retired)	Yes	
Miss Becci Petch	Clinical Trial Coordinator	Yes	
Dr Andrew Scott (Vice Chair)	Course Leader, M.Sc. Clinical Exercise Science	No	

Also in attendance:

Name	Position (or reason for attending)
Miss Georgina Castledine	REC Manager
Mrs Samantha Trace	Research Assistant

A Research Ethics Committee established by the Health Research Authority

APPENDIX 11 – RESPONSE TO INITIAL REC RECOMMENDATIONS



University Hospitals Coventry and Warwickshire NHS Trust
Clifford Bridge Road
Walsgrave
Coventry
CV2 2DX

Dear Ms. Edwards,

RE: SIMPLANT study (REC ref: 16/SC/0229)

Thank you for your provisional response with the changes to be considered for the SIMPLANT study. We have closely looked at the information requested and amended the documents accordingly.

Please find below responses to all queries. All updates have been highlighted in yellow on the Participant Information Leaflet, Participant Questionnaire and Symptom Diary.

- 1. Please amend the Participant Information Sheet to state that women would have to Have regular periods for inclusion in the study and to advise whether they would be given a 28 or 35 day cycle of Sitagliptin. It should also be made clear in the PIS that they need to continue using the treatment up until the time of the second biopsy.**

The Participant Information Leaflet has now been amended to state that women should have a 28-30 day cycle for inclusion to the study. This is because, patients with a cycle length that is consistent will be more homogenous and thus we wish to minimise confounding factors.

The Participant Information Leaflet also now states that they will be supplied with a pack of 32 tablets at a time. After giving participants the first pack of medication we will see them for a review in the research unit at 4 weeks +/- 4 days (28+/- 4 days) when they will get the medication for the next hospital visit.

It also states that 'one tablet needs to be taken every day for a total of 3 months up until the day of the second endometrial biopsy.'

- 2. Please amend the Participant Information Sheet to include information about the storage and subsequent use of participants' samples, and that they would only be used by this research team.**

The Participant Information Leaflet now states

'Following the procedure the small sample of your womb lining will be frozen and stored in UHCW's Tissue Bank for analysis of stem cells and genetic analysis. With your permission, any tissue left over after the analysis is complete will be stored anonymously in the Tissue Bank for use in future research studies. You do not have to give your consent for long term storage of your left over tissue samples if you do not wish.'

In another section of the Participant Information Leaflet it also states:

'If you do decide to participate we would be most grateful if we could use any extra tissue from the endometrial biopsy for research purposes by our research team. The biopsy would not be used by any other team and the tissue sample would be frozen and stored in the Tissue Bank at UHCW anonymously. The sample would not be shared externally.'

3. Please indicate in the Participant Information Sheet that pregnancy test kits would be provided

The Participant Information Leaflet now states:

'During the study we will ask you to do a pregnancy test at home every 2 weeks. Pregnancy test kits will be provided. It is important you do not get pregnant while participating in this trial as we do not clearly know of the safety of Sitagliptin in pregnancy.'

4. Please amend the Participant Information Sheet and Consent Form so as to inform participants that, with their consent, their samples would be subject to genetic analysis.

The Participant Information Leaflet now states that

'Following the procedure the small sample of your womb lining will be frozen and stored in UHCW's Tissue Bank for analysis of stem cells and genetic analysis. With your permission, any tissue left over after the analysis is complete will be stored anonymously in the Tissue Bank for use in future research studies. You do not have to give your consent for long term storage of your left over tissue samples if you do not wish.'

The consent form has been amended to clarify that the participant's left over tissue will be stored anonymously in UHCW's Tissue Bank for future research by the research team and will not be shared externally. This element of the study is optional.

5. Please amend the Participant Information Sheet to make it clear that this is a pilot study and would form part of a doctorate

It is now stated in the Participant Information Leaflet that this is a pilot study and would form part of a doctorate supervised by Professor Siobhan Quenby. (please see highlighted in yellow)

6. Please amend the Participant Information Sheet to explain how post-treatment follow-up of any subsequent pregnancy would work. This should include whether the researcher would remain blinded at this stage and how treating clinicians might be made aware of any results relevant to clinical care.

It was originally stated in the Protocol V1.0 section 20.2 that the definition of the end of the trial will be when the last patient has had their second endometrial biopsy. The secondary outcome measures and objectives have now been amended not to include pregnancy outcomes. Follow up after the second biopsy is part of standard clinical care and not part of the study.

This trial is designed to examine the effect of Sitagliptin on the endometrium and not to assess the effect of Sitagliptin on pregnancy outcome as this would require a larger study. The results of the project are novel and will not be given to treating clinicians as their relevance is not known and they are thus uninterruptable clinically and therefore not relevant. For example, if a patient has a low number of endometrial stem cells we do not know at present what effect this would have on her pregnancy, nor do we have any preventative action to take. Thus in this instance such information is not helpful.

Furthermore, any pregnancy will occur after the trial has finished and occur after the endometrium has renewed following menstruation. We have no idea at present whether Sitagliptin will affect the endometrium so the treatment allocation is of no relevance to the clinician looking after the patient in pregnancy.

However, women who participated in the research project will be offered standard recurrent miscarriage clinic care which includes fortnightly scans in the first trimester and high risk antenatal care as is standard practise.

7. Please indicate in the Participant Information Sheet that participants will be asked to complete a questionnaire about their experience during the study.

This has now been included in the Participant Information Leaflet as:

'Once you have had your second endometrial biopsy we will ask you to fill in a simple questionnaire related to your experience of being in a research trial. Your replies will be anonymous and will help to improve services in the future.'

8. Please add to the "Summary of what is involved" items referring to keeping a symptom diary and completing an end of study questionnaire. These documents should be titled 'Participant Questionnaire' and Participant Symptom Diary'.

The Participant Information Leaflet now states

'We will ask you to visit the research unit for general health check every 4 weeks +/- 4 days. We will give you a simple symptom diary to fill in so that we can monitor any problems closely'

We have changed the titles of the two documents and resubmitted the new versions.

9. Please consider whether you would be able to offer participants reimbursement of travel expenses and if so then please update the Participant Information Sheet accordingly.

We would not be able to offer participants reimbursement of travel expenses. Participants who have been involved in the PPI group are aware of this but do not have concerns.

10. The Committee stated that it should be made clear in the Participant Information Sheet that participants should not try to get pregnant whilst taking part in the trial.

The Participant Information Leaflet now states

'During the study we will ask you to do a pregnancy test at home every 2 weeks. Pregnancy test kits will be provided. It is important you do not get pregnant while participating in this trial as we do not clearly know of the safety of Sitagliptin in pregnancy.'

It also states

'It is very important that you do not try to become pregnant while you are taking part in the study. If you decide to take part in the study you will be asked to take a pregnancy test before you are enrolled on the study and then every 2 weeks for the duration of the study. Pregnancy test kits will be provided.'

- 11. You said that there was no risk that the treatment would make a woman's chance of getting pregnant any worse but admitted that the drug might not help. You agreed to update the Participant Information Sheet.**

This has been included in the original Patient Information Leaflet as:

'We do not know if there is any benefit to taking Sitagliptin to the endometrium. It is possible that both the Sitagliptin and the placebo have no effect on the endometrium'.

Other issues raised:

As per requests from the HRA we have added in more details about the side effect profile on the Participant Information Leaflet of Sitagliptin to allow full disclosure to participants.

Since the meeting on the 27th April we have had a meeting with Professor Arvanitis who is the Head of Research at the Institute of Digital Healthcare who has been approached about database creation for the trial. He has now been included in the list of study contacts.

In light of responses above other changes made to the protocol which include:

1. In section 4 this has been corrected to say that the study will be funded for 24 months and open to recruitment for 12 months. This was an oversight in the original protocol and has been amended to be in line with the original study summary on page 6 of the protocol.
2. After our meeting with Professor Arvanitis the protocol has been corrected to make it clear that the data in the database will be link anonymised and not anonymous as has been stated in the IRAS form.

I hope these amendments help us to reach a favourable opinion.

Thank you again for your time.

Yours Sincerely,

Professor Siobhan Quenby

APPENDIX 12 – APPROVAL LETTER FROM HRA



Professor Siobhan Quenby
University Hospital Coventry and Warwickshire
Clifford Bridge Road
Coventry
CV22DX

Email: hra.approval@nhs.n

01 August 2016

Dear Professor Quenby,

Letter of HRA Approval

Study title: Does the DPP4 Inhibitor (Sitagliptin) Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage?
IRAS project ID: 196058
EudraCT number: 2016-001120-54
Protocol number: SQ167015
REC reference: 16/SC/0229
Sponsor University Hospitals Coventry and Warwickshire NHS Trust

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. Please read **Appendix B** carefully, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

Page 1 of

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact detail and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the *After Ethical Review* document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the [HRA website](#), and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the [HRA website](#).

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at <http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/>.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

Page 2 of

APPENDIX 13 – TRIAL MEDICATION LABEL

1

Sharp Clinical Services (UK) Ltd		Label Design Form		
Client: University Hospitals Coventry & Warwickshire		Study Reference: SIMPLANT	Label Code: SIM 001	
Rev No: 01	Supersedes Rev No: N/A	Requested by date: <i>26/07/16</i>	Designed by date: <i>26/07/16</i>	Pending: <i>26/07/16</i>
SAMPLE				
<p style="text-align: center;">FOR CLINICAL TRIAL USE ONLY SIMPLANT Clinical Trial (EudraCT No: 2016-001120-54)</p> <p style="text-align: center;">SITAGLIPTIN/PLACEBO 10MG CAPSULES Take ONE capsule DAILY daily. Take orally swallow whole with water, do not open capsules.</p> <p style="text-align: center;">STORE AT ROOM TEMPERATURE AT 15-25°C KEEP OUT OF REACH AND SIGHT OF CHILDREN</p> <p style="text-align: right; font-size: small;">Print Name: <i>Natalia V. Blatnikova</i> Email: <i>vblatnikova@cskuk.org</i> Date: <i>26/07/16</i></p> <hr/> <p style="text-align: center;">Patient Trial ID: <i>None</i> Date of Dispensing: <i>None</i></p> <p style="text-align: center;">Investigator Name: Professor Slobhan Quenby Investigator Site: University Hospitals Coventry & Warwickshire Clifford Bridge Rd, Coventry, CV2 2DX</p> <hr/> <p style="text-align: center;">Sponsor: University Hospitals Coventry & Warwickshire, Research Development & Innovation, Clifford Bridge Road, Coventry CV2 2DX, UK. Tel: +44(0)2476969937 / 6195</p>				
Clients amendments required: <i>None.</i>				
Client Approval (Label accepted by, please print name, sign & date): <i>NOJIA KHAN</i> <i>26/7/16</i>		SCS QA/QC Approved by/ date:		

Form: LDF0001

Revision Number: 11

Issue Date: 13 Feb 13

APPENDIX 14 – SCREENING LOG

SCREENING

SIMPLANT study

Chief Investigator: Professor Siobhan Quenby

Site name: University Hospitals Coventry and Warwickshire

REC no.: 16/S/0229

Sponsor No.: SQ167015

Screening No.	Subject initials	Date of birth (dd/mm/yyyy)	Date of screening (dd/mm/yyyy)	Eligible? (Y/N)	Enrolled? (Y/N)	Date of consent (dd/mm/yyyy)	If YES, Trial ID*	If No, reason for refusal/ineligibility
S052								
S053								
S054								
S055								
S056								
S057								
S058								
S059								
S060								

*Enter the participant's unique trial ID

SIMPLANT_screening log_v3.0_06.04.201

APPENDIX 15 – RECRUITMENT LOG

RECRUITMENT

SIMPLANT study

Chief Investigator: Professor Siobhan Quenby
REC no: 16/SCI/0229

Site name: University Hospitals Coventry and Warwickshire

Trial ID	Participant's Name	Participant's Hospital Number	Date of birth (dd/mm/yyyy)	Date of Consent (dd/mm/yyyy)	Screening no.	Date of Randomisation (dd/mm/yyyy)	Did participant complete study? (Y/N)	If participant did not study, give reason
R24								
R25								
R26								
R27								
R28								
R29								
R30								
R31								

1

SIMPLANT Recruitment Log_V3.0_02.05.2017

APPENDIX 16 – PHARMACY IMP ACCOUNTABILITY LOG

SIMPLANT Does the DPP4 Inhibitor (Sitagliptin) Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage?		University Hospitals Coventry and Warwickshire NHS Trust Sponsor: University Hospitals Coventry & Warwickshire NHS EudraCT: 2016-001420-54								
Pharmacy IMP Accountability Log										
For use by Sponsor/Sponsor Representative only Initial delivery of IMP to site										
All IMP is delivered at the start of the study and entered into quarantine. IMP checked and received by _____		Print name: _____ Sign: _____ Date: _____								
IMP is undamaged, labelled correctly and fit for use. IMP released from quarantine and available for use at site.		Print name: _____ Sign: _____ Date: _____								
Principal Investigator: _____		Hospital Site: University Hospital Coventry	Manufacturer: Sharp Clinical Services							
Pack Number	Participant Trial ID	Patient Initials	Batch Number	Expiry	Completed by		Quantity Returned & destroyed	Completed by		Monitor Initials
					Initials (Disp/Chk)	Date		Initials (Disp/Chk)	Date	
					I			I		
					I			I		
					I			I		
					I			I		
					I			I		

Pharmacy IMP Accountability Log

Document Version 1 Date: 03/05/2016

Page: _____

CASE REPORT FORM BOOKLET SIMPLANT

Chief Investigator	Professor Siobhan Quenby
Sponsor Number:	SQ167015
EudraCT Number	2016 – 001120 - 54
Name of site	University Hospital Coventry and Warwickshire NHS Trust
CRF Version Number	V 2.0, 16.12.2016

Patient Initials

<input type="text"/>	<input type="text"/>	<input type="text"/>
----------------------	----------------------	----------------------

Screening ID:

S - To be used until randomisation

Participant Trial ID:

R Used from randomisation

CRF Completion Guidelines

General

Complete the CRF using a **black ballpoint pen** and ensure that all entries are complete and legible. Avoid the use of abbreviations and acronyms. The CRF should be completed as soon as possible after the scheduled visit. Do not use participant identifiers anywhere on the CRF, such as name, hospital number etc., in order to maintain the confidentiality of the participant. Ensure that the header information (i.e. participant's initials and ID number) is completed consistently throughout the CRF. Missing initials should be recorded with a dash (i.e. D-L).

Each CRF page should be signed and dated by the person completing the form. The 'completed by' Name in the footer of each page must be legible and **CRFs should only be completed by individuals delegated to complete CRFs on the Site Delegation log (and signed by the PI)**.

Ensure that all fields are completed on each page:

- If a test or investigation was Not Done record **ND** in the relevant box(es)
- Where information is Not Known write **NK** in relevant box(es)
- Where information is not applicable write **NA** in the relevant box(es)

Corrections to entries

If an error is made, draw a single line through the item, then write the correct entry on an appropriate blank space near the original data point on the CRF and initial and date the change.

Do NOT

- Obscure the original entry by scribbling it out
- Try to correct/ modify the original entry
- Use Tippex or correction fluid

Medications taken by the participant during the trial should be recorded on the "Concomitant Medications Log" using the generic name whenever possible, except combination products which will be recorded using the established trade name.

Verbatim Adverse Event terms (initial medical term) should be recorded as the final diagnosis whenever possible.

Complete all **dates** as day, month, year i.e. 13/NOV/2016. Partial dates should be recorded as NK/NOV/2016

Source documents such as lab reports, biopsy results etc. should be filed separately from the CRF (if not in the medical notes) for each participant and be signed and dated by a delegated Investigator as proof of review of the assessment during the trial. Questionnaires should be considered as the CRF appendices (excluding standard approved questionnaire e.g. EQ-5D)

If a participant prematurely withdraws from the trial a single line must be drawn across each uncomplete page to correspond with the last visit of the participant as mentioned on the "End of Study" page.

The protocol deviation log should be used to record comments relating to each CRF visit that cannot be captured on the page itself. This includes reason for delayed or missed protocol visits or trial assessments, unscheduled visits etc.

If additional pages are required of the Concomitant Medications or Adverse Event Forms please print the continuation page and mark the page as 'a', 'b' etc as required.

The Chief Investigator (for lead site)/Principal Investigator is responsible for the accuracy of the data reported on the CRF. The CI/PI must sign and date the Principal Investigator's Sign Off page to certify accuracy, completeness and legibility of the data reported in the CRF.

Serious Adverse Events (SAEs)

SAEs should be scanned and emailed **within 24 hours** of the site being aware of the event using the trial specific SAE report form to RD&ISponsorship@uhcw.nhs.uk.

Storage

CRF documents should be stored in a locked, secure area when not in use where confidentiality can be maintained. Ensure that they are stored separately to any other documents that might reveal the identity of the participant.

SIMPLANT	Pt Screening No: <input type="text" value="S"/> <input type="text"/> <input type="text"/> Initials: <input type="text"/> <input type="text"/> <input type="text"/>
-----------------	--------------------------------------------------------------------------------------------------------------------------------------------------------------------

BASELINE VISIT

Date of Baseline Visit:

ELIGIBILITY REVIEW		
PARTICIPANT ELIGIBILITY CRITERIA: The following criteria must be 'YES' to be recruited to the trial		
	YES	NO
Provision of written informed consent	<input type="checkbox"/>	<input type="checkbox"/>
History of recurrent miscarriage - 3 or more miscarriages	<input type="checkbox"/>	<input type="checkbox"/>
Regular menstrual cycle, up to 30 days in length	<input type="checkbox"/>	<input type="checkbox"/>
Age 18 – 42 years at consent	<input type="checkbox"/>	<input type="checkbox"/>
Willing to consent to give consent for the study and endometrial biopsy	<input type="checkbox"/>	<input type="checkbox"/>
Ability to fully understand requirements of protocol	<input type="checkbox"/>	<input type="checkbox"/>
PARTICIPANT EXCLUSION CRITERIA: The following criteria must be 'NO' to be recruited to the trial:		
	YES	NO
Type I Diabetes	<input type="checkbox"/>	<input type="checkbox"/>
Type II Diabetes	<input type="checkbox"/>	<input type="checkbox"/>
Pregnancy	<input type="checkbox"/>	<input type="checkbox"/>
Breastfeeding	<input type="checkbox"/>	<input type="checkbox"/>
Known sensitivity to Sitagliptin	<input type="checkbox"/>	<input type="checkbox"/>
Taking any medications with potential to react with IMP (Digoxin or Enalapril)	<input type="checkbox"/>	<input type="checkbox"/>
Previous diagnosis of pancreatitis	<input type="checkbox"/>	<input type="checkbox"/>
Renal impairment with *eGFR <50 ml/min or AKI ≥1	<input type="checkbox"/>	<input type="checkbox"/>
Hepatic impairment, defined as Alanine Transferase (ALT) >38 U/L, ALP>105 U/L Bilirubin (BR) >20 µmol	<input type="checkbox"/>	<input type="checkbox"/>
Inclusion in another interventional trial	<input type="checkbox"/>	<input type="checkbox"/>
Unwilling to use effective contraception for duration of trial (from consent)	<input type="checkbox"/>	<input type="checkbox"/>
Allergy/sensitivity to excipients of the IMP/placebo	<input type="checkbox"/>	<input type="checkbox"/>

Completed by: _____ Date: _____ / _____ / _____
 Name _____ Sign _____ (DD / MMM / YYYY)

SIMPLANT CRF v2.0, 16.12.2016

SIMPLANT	Pt Screening No: <input type="text" value="S"/> <input type="text"/> <input type="text"/> Initials: <input type="text"/> <input type="text"/> <input type="text"/>
-----------------	--------------------------------------------------------------------------------------------------------------------------------------------------------------------

*eGFR will be calculated using the Cockcroft Gault Equation:

$$\text{eGFR (ml/min)} = \frac{(140 - \text{Age}) \times \text{Weight (Kg)}}{1.04} \times 1.04$$

Serum Creatinine (umol/L)

Variables	Values																		
Age	yrs																		
Weight	kg																		
Serum Creatinine	umol/L																		
eGFR	ml/min																		
AKI	(or) <input type="checkbox"/> tick here if not reported																		
Participant Eligibility Investigator Sign-Off																			
Is the participant eligible to participate in the trial?	<input type="checkbox"/> Yes																		
Investigator Name: _____	<input type="checkbox"/> No, complete below																		
Investigator Sign: _____																			
Date: <table style="margin-left: auto; margin-right: auto;"><tr><td>D</td><td>D</td><td>M</td><td>M</td><td>M</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td></tr><tr><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td></tr></table>	D	D	M	M	M	Y	Y	Y	Y	<input type="text"/>									
D	D	M	M	M	Y	Y	Y	Y											
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>											
Reason(s) for failure:	1. 2. 3.																		

Informed Consent																			
Date of signed written Informed consent:	<table style="margin-left: auto; margin-right: auto;"><tr><td>D</td><td>D</td><td>M</td><td>M</td><td>M</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td></tr><tr><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td></tr></table>	D	D	M	M	M	Y	Y	Y	Y	<input type="text"/>								
D	D	M	M	M	Y	Y	Y	Y											
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>											
Version no and date of consent form:	Version No: Date: <table style="margin-left: auto; margin-right: auto;"><tr><td>D</td><td>D</td><td>M</td><td>M</td><td>M</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td></tr><tr><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td><td><input type="text"/></td></tr></table>	D	D	M	M	M	Y	Y	Y	Y	<input type="text"/>								
D	D	M	M	M	Y	Y	Y	Y											
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>											
Name of individual taking informed consent:																			

Completed by: _____ Date: _____ / _____ / _____
 Name _____ Sign _____ (DD / MMM / YYYY)

SIMPLANT CRF v2.0, 16.12.2016

SIMPLANT	Pt Screening No: <input type="text" value="S"/> <input type="text"/> <input type="text"/> <input type="text"/>	Initials: <input type="text"/> <input type="text"/> <input type="text"/>
-----------------	----------------------------------------------------------------------------------------------------------------	--------------------------------------------------------------------------

Demographic Data (collected retrospectively from 1st miscarriage clinic)

Date of 1st miscarriage clinic appt:	<input type="text"/> D <input type="text"/> D <input type="text"/> M <input type="text"/> M <input type="text"/> M Y <input type="text"/> Y <input type="text"/> Y <input type="text"/> Y				
Date of Birth	<input type="text"/> D <input type="text"/> D <input type="text"/> M <input type="text"/> M <input type="text"/> M Y <input type="text"/> Y <input type="text"/> Y <input type="text"/> Y				
Ethnicity	Complete below				
White	White British <input type="checkbox"/>	White Irish <input type="checkbox"/>	White Other <input type="checkbox"/>		
Mixed race	White & Black Caribbean <input type="checkbox"/>	White & Black African <input type="checkbox"/>	White & Asian <input type="checkbox"/>	Other mixed background <input type="checkbox"/>	[]
Asian or Asian British	Indian <input type="checkbox"/>	Bangladeshi <input type="checkbox"/>	Pakistani <input type="checkbox"/>	Other Asian background <input type="checkbox"/>	[]
Black or Black British	Caribbean <input type="checkbox"/>	African <input type="checkbox"/>	Black Other <input type="checkbox"/>		
Chinese or other ethnicity	Chinese <input type="checkbox"/>	Other <input type="checkbox"/>			

Clinical Assessments

Height	<input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> cm
Weight	<input type="text"/> <input type="text"/> <input type="text"/> kg
BMI	<input type="text"/> <input type="text"/>

Medical History

Has the Patient had any relevant medical history?	<input type="checkbox"/> No <input type="checkbox"/> Yes, complete below		
Condition / illness / procedure	Start date (DD/MMM/YYYY)	Stop Date (DD/MMM/YYYY)	Or tick ongoing at visit
	<input type="text"/> / <input type="text"/> /	<input type="text"/> / <input type="text"/> /	<input type="checkbox"/>
	<input type="text"/> / <input type="text"/> /	<input type="text"/> / <input type="text"/> /	<input type="checkbox"/>
	<input type="text"/> / <input type="text"/> /	<input type="text"/> / <input type="text"/> /	<input type="checkbox"/>
	<input type="text"/> / <input type="text"/> /	<input type="text"/> / <input type="text"/> /	<input type="checkbox"/>
	<input type="text"/> / <input type="text"/> /	<input type="text"/> / <input type="text"/> /	<input type="checkbox"/>

Completed by: _____
Name _____ Sign _____ Date: _____ / _____ / _____
(DD / MMM / YYYY)

SIMPLANT CRF v2.0, 16.12.2016

SIMPLANT	Pt Screening No: <input type="text" value="S"/> <input type="text"/> <input type="text"/> <input type="text"/>	Initials: <input type="text"/> <input type="text"/> <input type="text"/>
-----------------	----------------------------------------------------------------------------------------------------------------	--------------------------------------------------------------------------

	____ / ____ / ____	____ / ____ / ____	<input type="checkbox"/>
	____ / ____ / ____	____ / ____ / ____	<input type="checkbox"/>
	____ / ____ / ____	____ / ____ / ____	<input type="checkbox"/>

Lifestyle	
Alcohol	<input type="checkbox"/> Drinker <input type="checkbox"/> Non-drinker Average no of units / week <input type="text"/> <input type="text"/>
Smoking	Has participant ever smoked? <input type="checkbox"/> No <input type="checkbox"/> Yes, complete below <input type="checkbox"/> Current smoker <input type="checkbox"/> Ex smoker, ceased smoking ____ / ____ / ____ Smoked for ____ years / months (delete as appropriate) Avg daily no of cigarettes/cigars/pipes _____

Completed by: _____
 Name _____ Sign _____ Date: ____ / ____ / ____
 (DD / MMM / YYYY)

SIMPLANT CRF v2.0, 16.12.2016

SIMPLANT Pt Screening No: Initials:

Pregnancy history - Detail all previous pregnancies, use codes below for fields marked by *

Outcome	1 - Live birth	2 - Still birth	3 - Early misc <14/40	4 - Late misc 14- <24
Type of management	5 - Ectopic pregnancy	6 - Molar pregnancy	7 - Resolved PUL	8 - Termination
Mode of Delivery	1 - Expectant	2 - Medical	3 - Surgical	
	1 - Normal vaginal delivery	2 - Instrumental delivery	3 - Elective C/S	4 - Emergency C/S
	5 - Vaginal breech	6 - N/A		

Completed by: _____ Date: ____ / ____ / ____
Name _____ Sign _____ (DD / MMM / YYYY)

SIMPLANT CRF v2.0, 16.12.2016

SIMPLANT	Pt Screening No:	<input type="text"/> S <input type="text"/> <input type="text"/> <input type="text"/> Initials: <input type="text"/> <input type="text"/>									
Biochemistry											
Clinical Biochemistry Laboratory tests performed?		<input type="checkbox"/> No (Comment Below) <input type="checkbox"/> Yes, Complete below Comment*: _____									
Date of Sample (collected at 1st miscarriage clinic) :		<table style="width: 100%; text-align: center;"> <tr> <td><input type="text"/> D</td> <td><input type="text"/> D</td> <td><input type="text"/> M</td> <td><input type="text"/> M</td> <td><input type="text"/> M</td> <td><input type="text"/> Y</td> <td><input type="text"/> Y</td> <td><input type="text"/> Y</td> <td><input type="text"/> Y</td> </tr> </table>	<input type="text"/> D	<input type="text"/> D	<input type="text"/> M	<input type="text"/> M	<input type="text"/> M	<input type="text"/> Y	<input type="text"/> Y	<input type="text"/> Y	<input type="text"/> Y
<input type="text"/> D	<input type="text"/> D	<input type="text"/> M	<input type="text"/> M	<input type="text"/> M	<input type="text"/> Y	<input type="text"/> Y	<input type="text"/> Y	<input type="text"/> Y			
BIOCHEMISTRY Laboratory Parameter	Value	Unit	If parameter indicated as out of normal range on report, please check if clinically significant IF CS consider it is an AE and add to log (if appropriate)::								
SODIUM		mmol/L	<input type="checkbox"/> No	<input type="checkbox"/> Yes							
POTASSIUM		mmol/L	<input type="checkbox"/> No	<input type="checkbox"/> Yes							
UREA		mmol/L	<input type="checkbox"/> No	<input type="checkbox"/> Yes							
CREATININE		umol/L	<input type="checkbox"/> No	<input type="checkbox"/> Yes							
TOTAL PROTEIN		g/L	<input type="checkbox"/> No	<input type="checkbox"/> Yes							
TOTAL BILIRUBIN		mmol/L	<input type="checkbox"/> No	<input type="checkbox"/> Yes							
ALBUMIN		g/L	<input type="checkbox"/> No	<input type="checkbox"/> Yes							
ALK PHOS (ALP)		U/L	<input type="checkbox"/> No	<input type="checkbox"/> Yes							
ALT		U/L	<input type="checkbox"/> No	<input type="checkbox"/> Yes							
AKI (eGFR)		If Yes – 1, 2, 3 (Please circle)	<input type="checkbox"/> No	<input type="checkbox"/> Yes							

Completed by: _____ Date: ____ / ____ / ____
Name _____ Sign _____ (DD / MMM / YYYY)

SIMPLANT CRF v2.0, 16.12.2016

SIMPLANT	Pt Screening No: <input type="text" value="S"/> <input type="text"/> <input type="text"/> <input type="text"/> Initials: <input type="text"/> <input type="text"/> <input type="text"/>
-----------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Menstrual Cycles										
Length of cycle (bleed days / cycle length)	<input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/>									
Heavy	<input type="checkbox"/> Yes <input type="checkbox"/> No									
Previous history (ensure documented on medical history)	<table style="margin-left: auto; margin-right: auto;"> <tr> <td>Fibroids</td> <td><input type="checkbox"/> Yes</td> <td><input type="checkbox"/> No</td> </tr> <tr> <td>Endometriosis</td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> <tr> <td>Uterine anomaly</td> <td><input type="checkbox"/></td> <td><input type="checkbox"/></td> </tr> </table>	Fibroids	<input type="checkbox"/> Yes	<input type="checkbox"/> No	Endometriosis	<input type="checkbox"/>	<input type="checkbox"/>	Uterine anomaly	<input type="checkbox"/>	<input type="checkbox"/>
Fibroids	<input type="checkbox"/> Yes	<input type="checkbox"/> No								
Endometriosis	<input type="checkbox"/>	<input type="checkbox"/>								
Uterine anomaly	<input type="checkbox"/>	<input type="checkbox"/>								

End of Baseline Visit –Checklist 1:		
	Yes	No
1. Does the participant satisfy the inclusion and exclusion criteria to date?	<input type="checkbox"/>	<input type="checkbox"/>
3. Have the Medical History and Concomitant Medication pages been completed?	<input type="checkbox"/>	<input type="checkbox"/>
4. Is the participant still willing to proceed in the trial?	<input type="checkbox"/>	<input type="checkbox"/>
5. Has the participant signed a consent form for the study	<input type="checkbox"/>	<input type="checkbox"/>
6 Ovulation Kits supplied	<input type="checkbox"/>	<input type="checkbox"/>

Completed by: _____ Date: _____ / _____ / _____
 Name _____ Sign _____ (DD / MMM / YYYY)

SIMPLANT CRF v2.0, 16.12.2016

APPENDIX 18 – ADVERSE EVENT REPORTING FORM

Page 26 of 30

SIMPLANT	Patient Trial ID: <input type="text" value="R"/> <input type="text"/> Initials: <input type="text"/> <input type="text"/>
----------	---------------------------------------------------------------------------------------------------------------------------

ADVERSE EVENTS PAGE

AE No	Event Name (Please give Diagnosis if known)	Start date (DD/MMM/YYYY)	Stop date (DD/MMM/YYYY)	Serious? If serious, please complete SIMPLANT SAE form	Con- comitant Medication given	Severity 0 - Mild 1 - Moderate 2 - Severe	Study Drug Action 0 - None 1 - Temporarily discontinued with sequelae 2 - permanently withdrawn	Outcome 0 - Resolved 1 - Temporarily discontinued with sequelae 2 - Not resolved	Relationship to Study Dr 0 - Definit 1 - Probabl 2 - Possibl 3 - Unlikel 4 - Not rela 5 - Not assas
1		/_/_	/_/_	<input type="checkbox"/> No <input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Yes				
2		/_/_	/_/_	<input type="checkbox"/> No <input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Yes				
3		/_/_	/_/_	<input type="checkbox"/> No <input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Yes				
4		/_/_	/_/_	<input type="checkbox"/> No <input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Yes				
5		/_/_	/_/_	<input type="checkbox"/> No <input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Yes				
6		/_/_	/_/_	<input type="checkbox"/> No <input type="checkbox"/> Yes	<input type="checkbox"/> No <input type="checkbox"/> Yes				

I have reviewed the AEs on this page and have assessed them for seriousness, causality, severity and outcome and confirm that, to the best of my knowledge, it accurately reflects the study information obtained for this participant

PI signature _____ Date: _____ Please check box if this is the last page use

Completed by: Name _____ Sign _____ Date: ____/____/_____
(DD / MMM / YYYY)

SIMPLANT CRF v2.0, 16.12.2016

APPENDIX 19 – SERIOUS ADVERSE EVENT REPORTING FORM

University Hospitals
Coventry and Warwickshire
NHS Trust

SERIOUS ADVERSE EVENT REPORT FORM FOR UHCW SPONSORED CLINICAL TRIALS OF INVESTIGATIONAL MEDICINAL PRODUCTS

Sponsor Reference Number	
Study Title:	
Patient Study Number and Initials	
Centre:	

This form is to be completed within 24 hours of becoming aware of the Serious Adverse Event

1. Type of Report Initial Follow Up Final Initial & Final
(Tick relevant box)

Date of Report

--	--	--

--	--	--

Serious Adverse Event: _____

Date of Onset

--	--	--

--	--	--

Date of Study
Team Aware

--	--	--

--	--	--

NB If the event is a pregnancy it should be reported on a UHCW Pregnancy Notification Form

2. Serious Criteria:

- Resulted in death
- Life threatening
- In-patient hospitalisation or prolongation of existing hospitalisation
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Other

3. Narrative -Briefly describe the event (attach supporting documentation if applicable)

Admission Date

--	--	--

--	--	--

 Discharge Date

--	--	--

--	--	--

--

SIMPLANT SAE Report Form, Version 2.0; 03.11.2016

Both the Causality & Expectedness MUST be completed by the Chief/Principal Investigator or other medically qualified Investigator as agreed by the Sponsor for all IMP studies

4a. Causality and Expectedness

Evaluation of causal relationship with study drug 1

Related- If the causal relationship between the IMP and the SAE is at least a reasonable possibility

Un Related- If there is no causal relationship between the IMP and the SAE

(Name of drug)

Related

Unrelated

4b.

The assessment of expectedness must be based on the information contained in the Investigator Brochure and/or the Summary of Product Characteristics

Expected

Unexpected

If the event is related and unexpected it is a Suspected Unexpected Serious Adverse Reaction (SUSAR) and requires expedited reporting. **Inform the Sponsor immediately**

Telephone number: 02476 966907/966195 Email: RD&Isponsorship@uhcw.nhs.uk.

Evaluation of causal relationship with study drug 2

(Name of drug)

Related

Unrelated

If causal relationship is 'related' was the event 'expected'

The assessment of expectedness must be based on the reference safety information contained in the Investigator Brochure and/or the Summary of Product Characteristics

Expected

Unexpected

If the event is related and unexpected it is a Suspected Unexpected Serious Adverse Reaction (SUSAR) and requires expedited reporting. **Inform the Sponsor immediately**

Telephone number: 02476 966907/966195 Email: RD&Isponsorship@uhcw.nhs.uk.

5. Is the Study**Investigational Medicinal Product Blinded or Unblinded?**Blinded Unblinded **6. Was the event related to a protocol violation?**Yes No **7. Study Medication Information:**

Participant has been Administered Study Drug?	<input type="checkbox"/> Yes (Provide details in box below) <input type="checkbox"/> No (Give reason i.e. screening)				
Name of Medication	Indication(s) for Use	Dose (units)	Route of Administration	Date of First Administration	Date of Last Administration

8. Action taken with investigational product due to event:

- Dose not changed
- Temporarily discontinued Date: _____
- Permanently discontinued Date: _____
- Dose reduced - provide details _____
- Other – provide details _____
- Not applicable

9. Was the patient withdrawn from the study as a result of this event? Yes No **10. Outcome of the Event**

- Resolved Date of Resolution:
 - Resolved with Sequelae
 - Ongoing
 - Unknown at present
 - Fatal Date of Death: _____
 - Cause of Death: _____
 - Cause of death obtained from (tick one)
 - Working Diagnosis
 - Coroners Inquest
 - Death Certificate
- Supporting documentation to be supplied with SAE*

SIMPLANT SAE Report Form, Version 2.0; 03.11.2016

Reporting Person:	Principal Investigator/Delegated medically qualified individual as agreed by the sponsor:
Name:	Name :
Role:	Role:
Signature:	Signature:
Date:	Date:
Contact No:	Contact No:

Please return the completed form and copies of any additional documents to the Research Governance Office, by email to isabella.petrie@uhcw.nhs.uk

Reporting of SUSARs to the Research Ethics Committee and Regulatory Authority for UHCW sponsored studies will be undertaken in accordance with SOP 17 – Safety Reporting (CTIMPs)

APPENDIX – 20 FINAL TMG MEETING SUMMARY

Final CTIMP TMG Meeting Summary

Short title:	SIMPLANT
RD&I Number:	SO167015
EudraCT Number:	2016-001120-54
Chief Investigator:	Prof Siobhan Quenby
Trial Manager:	Becky Haley (Acting)
Date of Meeting	23/08/2016
Location of Meeting:	Cancer Seminar Room, 3 rd Floor opposite Ward 34
Attendees:	Prof Siobhan Quenby, Chief Investigator (SQ) Dr Shreeya Tewary, Trial Manager (ST) Becky Haley, (Acting) Trial Manager (BH) Mojid Khan, Sponsor Pharmacy Lead (MK) Emma Lucas (EL)
Apologies:	Isabella Petrie, Research Governance Manager (IP) Peter Kimani, Trial Statistician (PK)

Item 1.0 TRIAL TEAM	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
1.1 Coordinating Team		✓			Indrani Manoharan, the new Trial Manager starts on September 5 th and will lead on SIMPLANT. BH has acted as interim Trial Manager following Katie Bruce's maternity leave. Shivam Joshi will provide study support e.g. data management and recruitment uploads. IP sends her apologies due to impending HTA inspection. She will review minutes and TMF/ final document set prior to Sponsor approval.
1.2 Supporting Departments	✓				Emma Lucas attended the meeting to discuss arrangements for analysis of the endometrial biopsies.

Item 2.0 STUDY DETAILS	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
2.1 Protocol		✓			Protocol v1.2, 18.05.16 approved by CI, Sponsor and Statistician, signed copy retained in TMF.
2.2 Study procedures discussed, specify:		✓			There are four study specific procedures, listed in appendix 1. Emma Lucas attended the meeting to discuss the analysis of the biopsies. The procedure was outlined as per the study specific procedure: colony forming assay.

2.3	Study specific SOPs, please list:	✓		<p>Randomisation and unblinding - The TMG were satisfied with outlined process and PK has reviewed this outside of the meeting. MK to review prior to finalising and sending to PK to complete randomisation. PK has received the treatment pack allocation list from Sharp so is ready to proceed with randomisation once document finalised.</p> <p>Key documents will be reviewed and signed off by appropriate personnel.</p>
2.4	Randomisation procedures in place	✓		<p>The participant hospital number should be included in the 'confirmation of randomisation' email to support correct patient identification prior to discharge. BC to action.</p> <p>A copy of the randomisation confirmation email should be kept with the code break envelopes in the event of the patient not knowing the pack IDs. BC to add to study specific procedure.</p> <p>Admin support within the core team will be requested to help create the envelopes. BC to request.</p> <p>RD&I governance and grants team (independent of the trial team) will perform by the randomisation handlers. Staff to receive training to undertake role. BC to action.</p>
2.5	Code break procedures	✓		<p>Details reviewed as per study specific procedure: Randomisation and Unblinding. Patients issued with emergency contact cards with BRU and Switchboard detailed but this also needs to state to ask for Dr Tewary or Prof Quenby.</p> <p>Amendment to be submitted to Sponsor inbox for review. BC to action.</p>
2.6	Study emergency Out of Hours contact and unblinding in place and tested? (Documented evidence in TMF)	✓		<p>24-hour code break - Switchboard have agreed to facilitate and forward any calls to ST or SQ whose details have been added to their system. ST and BC to formalise arrangements with Switchboard Manager. ST and SQ coordinate leave to ensure that at least one investigator is always available.</p> <p>MK has Pharmacy SOP for code break which could be used to test the 24-hour code break procedure. MK to forward to BC for review.</p> <p>Code break testing to be undertaken once unblinding envelopes have been delivered to site. BC / MK to action.</p> <p>MK advised that Annex 13 requirements provide the option for the label to include emergency contact details. MK noted that the label already states that patients will carry an emergency contact card providing such details.</p>

2.7	Confirmation of recruitment target and timelines	✓		Planned start date is early September 2016. Recruitment of 34 patients is expected to take 9 months and therefore expected closure to recruitment by end May 2017. LPLV will therefore be end August 2017. See Appendix 2. The expiry date of the IMP/placebo is February 2018 so this allows for a little slippage in recruitment (last patient must be recruited by November 2016).
-----	--------------------------------------------------	---	--	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Item	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
3.0 DATA COLLECTION					
3.1	Case Report Form reviewed and finalised	✓			The primary outcome data in the CRF currently includes the percentage but is to include the number of endometrial colonies. ST/EL to review the primary outcome data and provide data points ahead of finalising CRF – PK to review prior to finalising. Statistician (PK) to analyse primary outcome and AE/SAE and process evaluation secondary outcomes only. Other secondary outcome data will not be included within the CRF as data set too large – RNA sequencing, methylation status and immunohistochemistry. EL to confirm locations of each source document. <i>Specific changes –</i> <ul style="list-style-type: none">• eGFR should be listed under biochemistry but is referred to as AKI (Acute Kidney Injury) – ST to provide updated CRF datapoint, eligibility criteria to be updated accordingly to include both terms – BC to action• ST to find out how eGFR is calculated as this can vary. BH to ask Indrani Manaharan to create a source document locations list once in post.
3.2	Case Report Form sign off - by CI, Sponsor and Statistician?	✓			SQ approved current content and changes outlined in meeting. CRF Review/Approval form to be completed once finalised. ST to action
3.3	Database and validation	✓			Once the CRF is finalised the database programming can begin. MedSciNet is to be used for data capture with data transposed once implemented. Design and build takes c. 6-8 weeks. Indrani will lead on this once in post.
3.4	Data entry and query resolution	✓			Data queries will be sent from Coordinating team back to site for resolution. Data query form to be created. BC/Indrani Manoharan to action once in post.

2.7	Confirmation of recruitment target and timelines	✓		Planned start date is early September 2016. Recruitment of 34 patients is expected to take 9 months and therefore expected closure to recruitment by end May 2017. LPLV will therefore be end August 2017. See Appendix 2. The expiry date of the IMP/placebo is February 2018 so this allows for a little slippage in recruitment (last patient must be recruited by November 2016).
-----	--------------------------------------------------	---	--	----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Item	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
3.0 DATA COLLECTION					
3.1	Case Report Form reviewed and finalised	✓			<p>The primary outcome data in the CRF currently includes the percentage but is to include the number of endometrial colonies. ST/EL to review the primary outcome data and provide data points ahead of finalising CRF – PK to review prior to finalising.</p> <p>Statistician (PK) to analyse primary outcome and AE/SAE and process evaluation secondary outcomes only. Other secondary outcome data will not be included within the CRF as data set too large – RNA sequencing, methylation status and immunohistochemistry. EL to confirm locations of each source document.</p> <p><i>Specific changes –</i></p> <ul style="list-style-type: none"> • eGFR should be listed under biochemistry but is referred to as AKI (Acute Kidney Injury) – ST to provide updated CRF datapoint, eligibility criteria to be updated accordingly to include both terms – BC to action • ST to find out how eGFR is calculated as this can vary. <p>BH to ask Indrani Manaharan to create a source document locations list once in post.</p>
3.2	Case Report Form sign off - by CI, Sponsor and Statistician?	✓			SQ approved current content and changes outlined in meeting. CRF Review/Approval form to be completed once finalised. ST to action
3.3	Database and validation	✓			Once the CRF is finalised the database programming can begin. MedSciNet is to be used for data capture with data transposed once implemented. Design and build takes c. 6-8 weeks. Indrani will lead on this once in post.
3.4	Data entry and query resolution	✓			Data queries will be sent from Coordinating team back to site for resolution. Data query form to be created. BC/Indrani Manoharan to action once in post.

Item	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
5.0 REGULATORY AND ETHICAL APPROVALS					
5.1 Ethics Committee	✓				Received 14 June 2016. HRA Approval received 1 August 2016
5.2 MHRA	✓				Received 25 July 2017.
5.3 Process for Sponsor Green Light	✓				Documents to be reviewed by IP / Governance team prior to Sponsor Green Light. Actions from this meeting needed ahead of this will need to be completed.
5.4 Site activation checklist and SIV	✓				To be completed as part of Sponsor Green Light process. BC/IP to complete checklist.

Item	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
6.0 TRIAL DOCUMENTATION AND TMF					
6.1 TMF/ISF complete	✓				As this is a single-centre, single sponsor study the TMF and ISF will be combined. The TMF will be held in RD&I with file notes specifying documents to be held in a folder within the BRU.
6.2 Amendments	✓				To be submitted to RD&Isponsorship@uhcw.nhs.uk for sponsor review ahead of ethical and regulatory approvals (as required).
6.3 Non-compliance	✓				A protocol deviation log will need to be completed for any protocol non-compliances. BC/Indrani to create and include within TMF.

Item	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
7.0 MONITORING & COMMITTEES					
7.1 Monitoring plan finalised and signed by CI and Sponsor	✓				Monitoring arrangements reviewed at and approved at DMEC/TSC. Monitoring Plan to be finalised with IP following meeting ahead of trial initiation.
7.2 Monitoring and audit arrangements	✓				Monitoring to be conducted as per monitoring plan. Study will be audited by Independent Auditor at next annual review – anticipated prior to end of 2016.
7.3 Trial Committee scheduled as per monitoring plan	✓				First meeting has taken place. Next meeting to be scheduled in December 2016 (six monthly).
7.4 Members all signed DMEC/TSC Charter	✓				DMEC/TSC Charters received from all but Aurelio Tobias and Nicola Haddon. ST/BC to chase.
7.5 Calendar updated with reminders of committee dates	✓				New Trial Manager to add all key trial dates to diary once in post.

Item	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
8.0 DELEGATION					
8.1	Sponsor to CI delegation reviewed	✓			CI responsibilities form completed and in TMF.
8.2	Requirement to comply with UHCW SOPs - read and sign	✓			BC to outline SOPs that will need to be read by the trial team prior to trial initiation.
8.3	Staff training and SIV	✓			Dr Tewary will lead SIV visits. SIV to be conducted twice to ensure all staff are able to attend. Pharmacy invited to attend. Attendance register to be completed, including SIV slides version number. BC to add version number and send attendance log to ST. CV and GCP certificates to be collected from all staff and stored in TMF. ST to action. Emma Lucas to complete GCP training. Details for eLearning course to be sent. BC to send.
8.4	Signed copy of delegation log received and sent to Pharmacy	✓			Coordinating centre delegation log started, to be completed. BC to action. Site delegation log to be completed at SIV and forwarded to Pharmacy once complete.

Item	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
9.0 FUNDING & CONTRACTS					
9.1	Contracts checklist completed and signed?	✓			Warwick University contract signed, awaiting subcontract with UHCW to be signed.
9.2	Funding arrangements	✓			Study funding part of programme funding from Tommy's Charity. As contracts had not been signed a letter from the Sponsor confirmed that the study costs would be underwritten in full.

Item	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
10.0 PHARMACOVIGILANCE					
10.1	Pharmacovigilance arrangements discussed and agreed	✓			Reporting, management and monitoring to be performed as per Study specific procedure: Safety reporting v1.0, 28.06.16.
10.2	AE recording	✓			Recorded in CRF and reviewed as part of ongoing monitoring. Forms reviewed and approved by TMG.
10.3	SAE and SUSAR reporting	✓			SAE form to be completed by site team and sent to Sponsor within 24-hours.

10.4	24-hour unblinding	✓			Refer to 1.6
------	--------------------	---	--	--	--------------

Item	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
11.0 LABORATORY AND EQUIPMENT					
11.1 Details of laboratories and analysis		✓			Blood samples to be analysed as per Trust standard practice (collected as part of standard care). Emma Lucas to lead on analysis of biopsy as per Colony Forming Assay study specific procedure.
11.2 Normal laboratory ranges		✓			Lab ranges documented in the eligibility criteria in the protocol are stated according to Trust normal ranges. ST to provide confirmation of normal ranges e.g. Pathology handbook to include within TMF.
11.2 Destruction of samples		✓			The cells cultured from the endometrial biopsy do not fall under the HTA. Samples to be destroyed according to Trust policy (incineration).
11.3 Patient supplies		✓			Patient to be supplied with pregnancy and ovulation tests from NHS stock. Patient diaries to be printed ready to be issued to patients. BC to request RD&I admin support to prepare.
11.4 Calibration of equipment		✓			Conducted and documentation by Vinci. Stored centrally. Z:\Equipment - Clinical and Non Clinical\Medical Equipment Certificates\2nd Floor\PRI Suite Opp Ward 24

Item	(Any item marked "No" requires a comment)	Yes	No	N/A	Comment
12.0 ANNUAL REPORTS					
12.1 Calendar updated with reminders?		✓			Date of First APR: 14 June 2017 Date of First DSUR: 25 July 2017 Dates to be added to calendar of Trial Manager once in post.
12.2 Anticipated closure date		✓			Expected last patient recruitment by end May 2017, LPLV therefore end August 2017.

Summary of actions:			
No	Section	Action	Delegated individual
1	2.3	To review Randomisation and Unblinding study specific procedure prior to finalising.	MK
2	2.4	Hospital number to be added to 'Confirmation of randomisation' email	BC
3	2.4	Clarify randomisation and unblinding study specific procedure to include requirement to keep copy of randomisation confirmation email with code break envelopes	BC

4	2.4	Complete training for undertaking randomisation call handlers	BC
5	2.4	Request RD&I admin support to create code break envelopes (and CRF packs). Packs to be created.	BC
6	2.5	Amendment to emergency contact cards to include specific names to contact, submit to Sponsor for review	BC
7	2.6	Meet with Switchboard Manager to formalise code break arrangements	ST/BC
8	2.6	Provide copy of pharmacy code break procedure for testing and documenting 24-hour code-break	MK
9	2.6	Test and document outcome of 24-hour code break once envelopes delivered	MK / BC
10	3.1	Review the primary outcome data and provide data points ahead of finalising CRF	ST/EL
11	3.1	Provide details of source document locations for laboratory analysis	EL/ST
12	3.1	eGRF to be updated in CRF to AKI (as appears on lab reports), eligibility criteria to be updated for clarity	ST / BC
13	3.1	Determine how eGFR/AKI calculated as this can differ	ST
14	3.1	Request Indrani to create source document location list once in post	BC
15	3.1	CRF review/approval form to be completed once finalised	ST/SQ
16	3.4	Data query form to be created	BC/IM
17	4.2	Complete Shipment Request Form and return to Sharpe	MK
18	4.6	Send all RSI documents to SQ and ST	MK
19	4.6	Add file note to TMF to outline RSI documents	BC
20	4.7	SPC log to be updated to review every 3 months	BC
21	5.4	Complete sponsor green light checklist	BC/IP
22	6.3	Protocol deviation log to be completed	BC/IP
23	7.1	Monitoring plan to be finalised	IP
24	7.4	Outstanding TSC/DMEC charters to be chased	BC/ST
25	7.5	Add all key trial dates to calendar	BC/I
26	8.2	Provide list of SOPs to read and review to site team	BC
27	8.3	SIV to be completed including attendance register and collection of CV/GCP certificates for all staff	ST
28	8.3	EL to complete GCP training	EL

Actions highlighted in blue must be completed prior to Sponsor Green Light.

APPENDIX 21 – NOTIFICATION OF TRIAL SUSPENSION



University Hospitals **NHS**
Coventry and Warwickshire
NHS Trust

Research, Development & Innovation Department
University Hospitals Coventry & Warwickshire NHS Trust
4th Floor Rotunda, ADA4001
University Hospital
Clifford Bridge Road
Coventry
CV2 2E

21st October 2016

Professor Siobhan Quenby
Biomedical Research Unit
UHCW NHS Trust

Commercial enquiries: 02476 96619
Governance/Non-commercial enquiries: 02476 96619
Innovation & Communication enquiries: 02476 96474
Research Funding & Grant enquiries: 02476 96495
Email: RD&I@uhcw.nhs.uk

Dear Siobhan

URGENT ACTION REQUIRED: STUDY TO BE SUSPENDED WITH IMMEDIATE EFFECT

Study Title: SIMPLANT - Sitagliptin for IMPLANTation
Protocol Number: version 1.2 18th May 2016
RD&I No: SQ167015

Following discussions this week about a potential non-compliance with the study protocol a
additional investigation into this matter, the view of the Sponsor is as follows:

Section 9 of the protocol is explicit in that the research will not involve taking any extra blood
Section 10 of the protocol requires that, only once blood tests are reviewed, written consent for t
study will be taken.

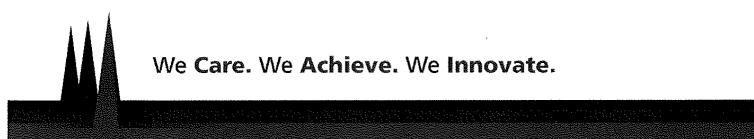
It has been found that:

1. Routine clinical bloods for woman Screening Number S-003 / Participant Trial ID R03 showed a urea value outside of the range stipulated within the protocol.
2. Another, second, blood test was carried out to check that the woman had no underlying disorders.
3. The results of the second blood test were used to confirm eligibility for the research.
4. The woman was then randomized and is receiving study treatment.
5. There is a suggestion that the patient was consented before the blood results were available.
6. Potentially, other patients were not screened or consented in accordance with the protocol.

Given the above, I am sufficiently concerned that the issues raised to date suggest non-compliance with GCP and so I am suspending the study with immediate effect.

Actions you must take:

- Do not approach or consent any further patients into the study
- Do not randomise any further patients
- Recall randomized patient R03 and remove her from the study, following the procedure outlined in the protocol



We will be carrying out a monitoring visit on Monday 24th October which will help to inform us as the actions required to re-start the study.

Yours sincerely



Mrs Ceri Jones
Head of Research, Development & Innovation

Cc:

Dr Indrani Manoharan, Trial Manager
Dr Shreeya Tewary, Clinical Research Fellow
Angela Polanco, Research Nurse
Mojid Khan, Pharmacy UHCW NHS Trust
Isabella Petrie, Research Governance Manager
Prof Chris Imray, RD&I Clinical Director

APPENDIX 22 – LETTER TO REC AND MHRA WITH PROPOSED CHANGES



Professor Siobhan Quenby
Professor / Honorary Consultant
University of Warwick & University Hospitals Coventry & Warwickshire NHS Trust
Division of Reproductive Health
Clinical Sciences Research Institute
Warwick Medical School
Clifford Bridge Road
Coventry CV2 2
Tel: 0121 42 437
Fax: 0121 42 431
Email: s.quenby@warwick.ac.uk

Dear Sir/Madam,

Ref: Major Amendment 2.0 dated 15/11/2016

Study title:	Does the DPP4 Inhibitor (Sitagliptin) Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage?
IRAS project ID:	196058
EudraCT number:	2016-001120-54
Sponsor reference:	SQ167015
Protocol version	V1.2 dated 18 th May 16
REC reference:	16/SC/0229
MHRA serious breach reference number	13268/0003/001-0002
Sponsor	University Hospitals Coventry and Warwickshire NHS Trust





Part 1: Amendment to request the restart of the trial

We write this letter based on the RD&I investigational report and CAPA (dated 28/10/2016) agreed by the Sponsor along with the Governance and Trial Team, we request restart of the trial.

Please find the final report attached with this submission for further details. Please note that we have received favourable ethical opinion from South Central - Hampshire B Research Ethics Committee 09/11/2016 for Notice of Substantial Amendment 1.0 (suspension of trial by Sponsor).

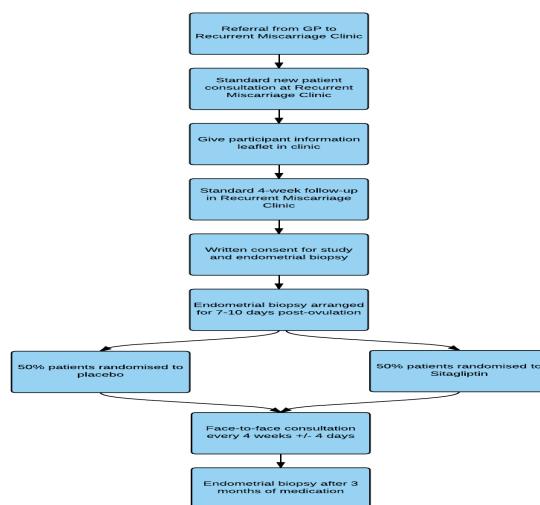


Part 2: Amendment to trial protocol

Section 1: Trial Flowchart and Schedule of Events

Current protocol (version 1.2 dated 11th May 16):

Trial flowchart



Limitations with current protocol:

After patients have had their initial and follow up consultations in the recurrent miscarriage clinic they attend for a new appointment after each miscarriage. In the case of >5 miscarriages this can mean >5 'new appointments' for the same patient along with the follow up visits that come with it.

The trial flow chart has been revised to take away the GP referral as this does not occur for every new appointment for the same patient in standard practice. Also, the new trial flow chart takes away



the '4 week follow up appointment' as sometimes patients are brought back for follow up sooner than this.

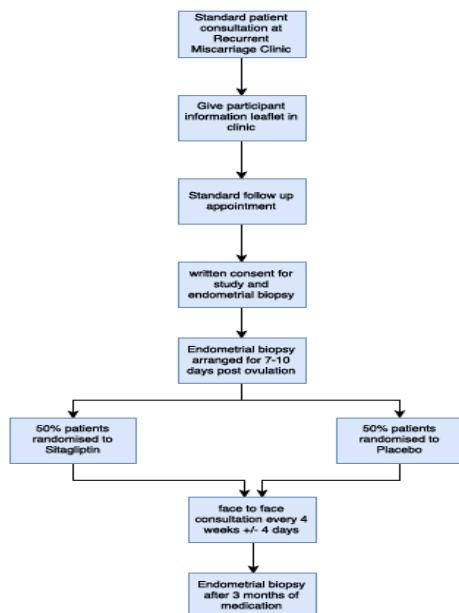
The schedule of events has also been changed to reflect the new trial flow chart.

Other changes made to the schedule of events only clarifies details within the original protocol. TI includes

1. Inclusion of statement to say that 'If no positive ovulation test in first cycle then biopsy arranged 1 week prior to next period due'.
2. Process Evaluation questionnaire will be returned by the participant within 4 weeks of second endometrial biopsy visit
3. The baseline visit for the trial remains the same

Amended protocol (1.4, dated: 15/11/2016):





Section 2: Participant Entry

Current protocol (version 1.2 dated 11th May 16):

Patients invited to participate in this trial will be identified from the recurrent miscarriage clinic. This is a recurrent miscarriage clinic every week at UHCW lead by Professor Quenby, a world renowned Professor in the area of recurrent miscarriage. Professor Quenby or Professor Brosens will approach any of the patients who will be recruited to this study.



Limitations with current protocol:

The current protocol did not mention approaching patients in The Implantation Clinic at UHCW where patients with recurrent miscarriage also attend.

Amended protocol (v1.4, dated: 15/11/2016):

Patients invited to participate in this trial will be identified from the recurrent miscarriage clinic or the implantation clinic. There is a recurrent miscarriage clinic every week at UHCW lead by Professor Quenby, a world renowned Professor in the area of recurrent miscarriage. There are two implantation clinics every week at UHCW lead by Professor Quenby and Professor Brosens who will first approach any of the patients who will be recruited to this study.

Section 3: Inclusion/exclusion criteria regarding renal function

Current protocol (version 1.2 dated 11th May 16):

- Inclusion criteria: Adequate renal function, defined as Urea 2.5 – 7.8mmol/L, Creatinine 51 – 99umol/L, potassium 3.5 – 5.3mmol/L, Sodium 133 -146mmol/L
- Exclusion criteria: Renal impairment with eGFR/AKI<50 mL/min

Limitations with current protocol:

We are confirming normal renal function prior to potentially starting patients on Sitagliptin. This is because the SmPC (last updated: 14-Mar-16) states:

'When considering the use of sitagliptin, its conditions for use in patients with renal impairment should be checked.
For patients with mild renal impairment [creatinine clearance [CrCl] ≥ 50mL/min], no dose adjustment is required.
For patients with moderate renal impairment [CrCl; ≥30 to < 50mL/min], the dose of Januvia is 50 mg once daily.'

There have been patients who have normal renal function and this is reflected with a normal CrCl/eGFR (creatinine clearance/estimated glomerular filtration rate). However, with the current inclusion criteria they are rendered ineligible for the trial but because one of the parameters of renal function tests falls out of reference range. For example, patients with a urea <2.4 mmol (range 2.5 – 7.8mmol/L) or Creatinine <50umol/L with otherwise normal blood tests are currently not eligible for the trial. These results are of no clinical significance and actually only express normal for this population of young fit and healthy women.

We would like to use eGFR exclusion criteria as this is a more important and accurate way of assessing renal functions. This also takes away the possibility of results being affected by patients hydration status. eGFR will be calculated using the Cockcroft Gault Equation as per standard practice of measuring eGFR.



Dr Paul O'Hare who is a consultant physician and our endocrinology advisor and Mr Mojid Khan, t clinical trials pharmacist at UHCW have been informed of the problem and have agreed that eGf will be a more accurate way of measuring renal function but at the same time will ensure that fit a healthy patients are not rendered ineligible. Please find statement from Dr. O'Hare attached with tl submission.

Amended protocol (v1.4, dated: 15/11/2016):

- Inclusion criteria: No longer includes renal function – in exclusion criteria
- Exclusion criteria: Renal impairment (eGFR 50mL/min/AKI >1*)

Note: eGFR will be calculated using The Cockcroft Gault Equation ($1.09 \times (190 - \text{Age}) / \text{weight} / \text{Creatinine}$)

Section 4: Inclusion/exclusion criteria regarding hepatic function

Current protocol (version 1.2 dated 11th May 16):

- **Inclusion criteria:** Adequate hepatic function, defined as total protein 60 – 80g/L, Albumin 35-50g/L, Bilirubin 4-20umol/L, Alkaline Phosphatase (ALP) 35-105U/L, Alanine Transferase (ALT) 5-38 U/L

Exclusion criteria: Liver impairment, defined as any value out of normal range (total protein – 80g/L, Albumin 35-50g/L, Bilirubin 4-20umol/L, Alkaline Phosphatase (ALP) 35-105U, Alanine Transferase (ALT) 5-38 U/L)

Limitations with current protocol:

Patients with a bilirubin <2 umol/L (range 4-20umol/L) or ALP 34 U/L (range 35-105U/L) w otherwise normal blood tests are currently not eligible for the trial. These results are of no clinic significance and actually only express a normality for this population of young fit and healthy women.

These results are out of the reference ranges but clinically insignificant and so currently fit a healthy individuals cannot be included with these current reference ranges stated in the exclusi criteria.

Also, the SmPC for Sitagliptin and hepatic impairment states:

'No dose adjustment is necessary for patients with mild to moderate hepatic impairment. Sitagliptin has not been studied in patients with severe hepatic impairment and care should be exercised'

ALT, ALP and bilirubin are sufficient markers of hepatic function to exclude any hepatic impairment

Amended protocol (v1.4, dated: 15/11/2016):

- Inclusion Criteria: No longer includes hepatic function
- Exclusion Criteria: Liver impairment, (defined as any value out of normal range Alanine Transferase (ALT) >38 U/L, ALP >105U/L, Bilirubin (BR) >20umol*)



Section 5: Pre-randomisation, Evaluation and Screening

Current protocol (version 1.2 dated 11th May 16):

'In the recurrent miscarriage clinic patients routinely have their initial consultation and a range of blood tests looking for causes of recurrent miscarriage performed on the day of the consultation. They are then followed up four weeks later in clinic to discuss results of these investigations.

To recruit participants to SIMPLANT we will give information leaflets to those eligible for the study their initial appointment at the recurrent miscarriage clinic. The blood tests looking for causes of recurrent miscarriage will already include the pre-randomisation blood tests that are needed prior to initiating Sitagliptin. This will not involve taking any extra blood.

Limitations with current protocol:

The protocol has been amended to clarify the sequence of events for recruiting patients to the trial.

1. We have clarified when the participant information leaflet will be given to patients to coincide with the schedule of events
2. We have changed 'four weeks' to a 'few weeks' as patients can be called back to clinic sooner than this.
3. We have clarified what we would do about recruiting patients who have been investigated in the last 3 months or been seen in other hospitals
4. The protocol now also clarifies that we may email or post the participant information leaflet to patients.

Amended protocol (v1.4, dated: 15/11/2016):

In the recurrent miscarriage clinic patients routinely have their consultation and a range of blood tests looking for causes of recurrent miscarriage performed on the day of the consultation. They will be given the information leaflet for the trial at this visit. They are then followed up a few weeks later in clinic to discuss results of these investigations.

The blood tests looking for causes of recurrent miscarriage will already include the pre-randomisation blood tests that are needed prior to initiating Sitagliptin. This will not involve taking any extra blood.

Patients who have already been investigated in the last 3 months or seen in other hospitals may show interest in the trial in which case we may also telephone patients and provide the participant information leaflet by email or post before the patient is brought back to clinic.

Patients identified from the implantation clinic will be referred to the recurrent miscarriage clinic to have routine blood tests to ensure there is no cause for their miscarriages before being recruited to SIMPLANT.

We Care. We Achieve. We Innovate.

Section 6: Enrolment procedure

We would like to obtain informed consent at a time convenient for the patient. Also patients are sometimes brought back for follow up of investigations sooner than 4 weeks.

Current protocol (version 1.2 dated 11th May 16):

'The consent will be taken at the follow up visit in the recurrent miscarriage clinic. By this time patients will have had 4 weeks to think about participation and will have had an opportunity to answer questions at two clinic visits. We will make sure the patient has read and understood the patient information leaflet, been given the opportunity to ask questions and encouraged to discuss their involvement with their GP family and friends.'

Amended protocol (v1.4, dated: 15/11/2016):

Consent will be taken at a time that suits the patient after they have had sufficient time to read the patient information leaflet and eligibility has been confirmed.

Section 7: Pharmacovigilance and Safety monitoring

This section has been edited to reflect correct reporting procedures to the Sponsor and regulatory authorities. Please find changes in the tracked version of the protocol.

Section 8: Unblinding

Originally, Sharpe Clinical Services were going to make the master unblinding list and provide the code break envelopes however this was done by an independent statistician.

The location of the code break envelopes and the unblinding procedures remain the same.

Yours Sincerely,

Prof. Siobhan Quenby
15/11/2016

We **Care**. We **Achieve**. We **Innovate**.

APPENDIX 23 – APPROVAL LETTER FROM REC TO RESTART



South Central - Hampshire B Research Ethics Committee

Level 3 Block
Whitefriar
Lewins Mead
Bristol
BS1 2N

Tel: 0207104801

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

07 December 2016

Mrs Ceri Jones
University Hospital Coventry and Warwickshire
Clifford Bridge Road
Coventry
CV2 2DX

Dear Mrs Jones

Study title: Does the DPP4 Inhibitor (Sitagliptin) Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage?
REC reference: 16/SC/0229
Protocol number: SQ167015
EudraCT number: 2016-001120-54
Amendment number: 2
Amendment date: 18 November 2016
IRAS project ID: 196058

The above amendment was reviewed 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:

Document	Version	Date
Covering letter on headed paper		17 November 2016
Notice of Substantial Amendment (CTIMP)	2	18 November 2016
Other [internal investigation report]		04 November 2016
Other [Supporting Statement from Dr O'Hare.]		21 October 2016

A Research Ethics Committee established by the Health Research Authority

APPENDIX 24 – NOTIFICATION OF EXTERNAL AUDIT



Research, Development & Innovation Department
University Hospitals Coventry & Warwickshire NHS Trust
4th Floor Rotunda, Rm: ADA40001;
University Hospital
Clifford Bridge Road
Coventry
CV2 2DX

27 January 2017

Professor Siobhan Quenby
Professor of Obstetrics
BRU,UHCW NHS Trust,
Clifford Bridge Road,
Coventry
CV2 2DX

Commercial enquiries: 02476 966196
Governance/Non-commercial enquiries: 02476 966196
Innovation & Communication enquiries: 02476 964741
Research Funding & Grant enquiries: 02476 964956
Email: RD&I@uhcw.nhs.uk

Dear Professor Quenby,

Study Title: Does the DPP4 Inhibitor (Sitagliptin) Increase Endometrial Mesenchymal Stem Cells in Women with Recurrent Miscarriage (**SIMPLANT**)

In line with the Research Governance Framework for Health and Social Care, the Trust Board requires assurance that an appropriate and effective system of Research Governance is in place and that the levels of controls and monitoring are being implemented.

On 08 February 2017, a Contract Research Organisation, Trueman Hall Associates Ltd, will be working with the R,D&I Department to undertake a monitoring exercise on a selection of active research projects. David Ollier, Trueman Hall Associates Ltd, will be undertaking the monitoring visit, when he is entitled full access to all project and patient records that may be deemed necessary.

The above project was selected from the Trust database to be included in our monitoring. David Ollier will require access to all research files associated with this project and will make checks, e.g. on adherence to protocol, consent, ethics and Trust R&D approval. You will need to ensure that he has access to the files at all times during these days and be available to answer any queries. **Patient notes must be requested beforehand and kept readily available for review.**

David Ollier will liaise with you and where possible, arrange any briefings to suit your timetable so as to be less disruptive. Your project is one of four that have been chosen at random and its inclusion in this monitoring exercise may be subject to change depending on the time available.

External monitoring of this sort will continue at the Trust to ensure that the Trusts' ethics and governance systems are robust. We ask that you co-operate fully with Trueman Hall Associates Ltd during this exercise.

If you have any queries relating to this, please do not hesitate to contact Isabella Petrie, 02476 966069

Yours sincerely,

Ceri Jones
Head of Research & Development

