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Study to Evaluate the Prevalence, Importance, and Treatment of Women with Congenital Uterine Anomalies

By

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Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

Declaration

Except where acknowledgement is made by reference, the studies undertaken in this thesis were devised and conducted unaided by the author.

No part of this work has been previously accepted for, or is currently being submitted in candidature for, another degree.

Yee Yin Chan

Acknowledgement

I would like to thank the Nottingham University Hospitals NHS Trust for supporting my clinical research fellowship that has given me the opportunity to perform full time clinical research over three years, and thereby to present this thesis.

I am extremely grateful to Mr. Nick Raine-Fenning, Professor Jim Thornton, and Professor Arri Coomarasamy who, as my supervisors, provided support and encouragement throughout my research from its inception to completion of this thesis and helped in developing research skills in me. Without them, none of the work would have been possible.

I am also very grateful to Mr Kannamannadiar Jayaprakasan for helping me in systematic reviews in the first year research work. Special thanks to Mr Lukasz Polanski and Miss Miriam Baumgarten for teaching me the ultrasound techniques and providing invaluable support in the last two years. I will be forever thankful for the help and clinical support Dr Judith Moore has given me over the years, not just as a clinical mentor but also as a friend. I would also like to express my appreciation to Dr Graham Warren and Dr Aurelio Tobias for their expertise in statistics and analyses.

I would also like to thank the Nottingham University Reproduction and Treatment unit in Reproduction (NURTURE) and their valuable staffs for their administrative support and the use of ultrasound scan facilities over the three years period.

I must dedicate special thanks to friends who have supported me over these past few years and made them much more bearable: Lukasz Polanski, Miriam Baumgarten, Kirstyn Sewell, Amarin Narkwichean and Kimberley Swann.

I want to thank mum, dad and my brother Chee Ching for supporting me in whatever I have chosen to do. To my husband Ta Wei, thank you for the unwavering support and continuous encouragement he has provided me with over the last few years. Thank you for being my side through thick and thin. I must also dedicate special thanks to my dear children, Pei Zhen and Jing Cheng, who love me unconditionally and have been very understanding when I could not spend time with them during my research work.

Table of Contents

Declaration ... **ii**
Acknowledgement ... **iii**
Table of Contents ... **v**
Table of Tables ... **viii**
Table of Figures ... **ix**
Table of Abbreviations ... **xi**
Abstract ... **xii**
Peer Reviewed Publications ... **xviii**
Chapter 1 Introduction ... **1**
1.1 Background ... 1
1.2 Embryology and Pathophysiology of the Müllerian System ... 6
1.2.1 Embryology ... 6
1.2.2 Pathophysiology of Müllerian Anomalies ... 10
1.3 Aetiology ... 13
1.4 Classification ... 14
1.5 Clinical Presentations ... 21
1.5.1 Müllerian Duct Agenesis or Hypoplasia and Vaginal Anomalies ... 21
1.5.2 Obstructing Anomalies of the Upper Genital Tract ... 21
1.5.3 Non-obstructing Anomalies of the Upper Genital Tract ... 22
1.6 Investigations and Diagnosis of Müllerian Duct Anomalies ... 23
1.6.1 Pelvic Examination ... 23
1.6.2 Hysterosalpingography (HSG) ... 24
1.6.3 Ultrasonography ... 26
1.6.4 Sonohysterography ... 30
1.6.5 Magnetic Resonance Imaging (MRI) ... 32
1.6.6 Hysteroscopy, Laparoscopy or Laparotomy ... 33
1.7 Management principles ... 35
1.7.1 Conservative Management ... 35
1.7.2 Medical Treatment ... 35
1.7.3 Surgical Treatment ... 36
1.8 Systematic Review and Meta-analysis in Medical Research ... 39
1.8.1 Strengths of Systematic Review and Meta-analysis ... 39
1.8.2 Limitations of Systematic Reviews and Meta-analysis ... 41
1.9 Hypothesis and Objectives ... 44
Chapter 2 Materials and Methods ... **46**
2.1 Introduction ... 46
2.2 Systematic Reviews ... 46
2.2.1 Identification of Literature ... 46
2.2.2 Study Selection ... 47
2.2.3 Quality Assessment and Data Extraction ... 48
2.3 Ultrasound Technique ... 48

2.3.1 Data Acquisitions 49
2.3.2 Ultrasound Data Examination 51
2.4 Statistical Analysis 57
Chapter 3 Results of Systematic Reviews 59
3.1 A Systematic Review – The Prevalence of Congenital Uterine Anomalies in High Risk Populations 59
3.1.1 Introduction 59
3.1.2 Materials and Methods 60
3.1.3 Statistical Analysis 62
3.1.4 Results 63
3.1.5 Discussions 67
3.1.6 Conclusions 80
3.2 The Reproductive Outcomes in Women with Congenital Uterine Anomalies: A Systematic Review 81
3.2.1 Introduction 81
3.2.2 Materials and Methods 83
3.2.3 Statistical Analysis 84
3.2.4 Results 85
3.2.5 Discussions 106
3.2.6 Conclusions 112
3.3 Systematic Review on Treatments of Congenital Uterine Anomalies 113
3.3.1 Introduction 113
3.3.2 Materials and Methods 114
3.3.3 Statistical Analysis 116
3.3.4 Results 117
3.3.5 Discussion 128
3.3.6 Conclusions 132
3.4 Reproducibility of 3D Ultrasound Scan in Diagnosing Congenital Uterine Anomalies: A Systematic Review 133
3.4.1 Introduction 133
3.4.2 Materials and Methods 134
3.4.3 Statistical Analysis 136
3.4.4 Results 136
3.4.5 Discussions 142
3.4.6 Conclusions 146
Chapter 4 Prevalence of Congenital Uterine MAlformations (PUMA) in High Risk Women 147
4.1 Introduction 147
4.2 Materials and Methods 148
4.2.1 Experimental Design 148
4.2.2 Outcome Measures 155
4.2.3 Statistical Analysis 155
4.2.4 Data Collection and Storage 156
4.3 Results 157
4.3.1 Overview 157
4.3.2 Prevalence of Uterine Anomalies 160

4.3.3 Uterine Cavity Length 161
4.3.4 Cervical Length 162
4.3.5 Myometrial Volume of Uterine Body 163
4.3.6 Cervical Volume 164
4.4 Discussions 165
4.4.1 Limitations 169
4.4.2 Future Work or Recommendations 171
4.5 Conclusions 172

Chapter 5 Development of a Randomised Controlled Trial of Hysteroscopic Resection for Uterine Septae 173

5.1 Introduction 173
5.2 Surveys 173
5.2.1 Overview 174
5.2.2 Materials and Methods 175
5.2.3 Results of Surveys 176
5.2.4 Discussions 191
5.2.5 Conclusions 194
5.3 Pilot Trial and Feasibility of Definitive Trial 194
5.3.1 Introduction 194
5.3.2 Study Design 196
5.3.3 Outcomes Measures 200
5.3.4 Statistical Analysis 201
5.3.5 Funding 202
5.3.6 Discussions 202

Chapter 6 General Discussion 204

6.1 A Systematic Review – The Prevalence of Congenital Uterine Anomalies in High Risk Populations 205
6.2 The Reproductive Outcomes in Women with Congenital Uterine Anomalies: A Systematic Review 208
6.3 Systematic Review on Treatments of Congenital Uterine Anomalies 212
6.4 Reproducibility of 3D Ultrasound Scan in Diagnosing Congenital Uterine Anomalies: A Systematic Review 213
6.5 Prevalence of Congenital Uterine Malformations 215
6.6 Development of a randomised controlled trial of hysteroscopic resection for uterine septae 219
6.7 Suggested Directions for Future Work 220
6.7.1 Pathophysiology studies using ultrasound scan 220
6.7.2 Test Accuracy Study of Three-Dimensional Ultrasound Scan 221
6.7.3 Off-line Analysis of UltraSonographic Images Study (OASIS) 222
6.7.4 Multi-centred randomised controlled trial for hysteroscopic resection of uterine septae 223

References 225

Appendix 243

Table of Tables

Table 1: Classification of Müllerian Duct Anomalies by the American Society for Reproductive Medicine Classification (1988) 15
Table 2: Search terms used to generate list of citations 47
Table 3: Criteria for Classification of Congenital Uterine Anomalies (Salim et al., 2003) 54
Table 4: Prevalence of Uterine Anomalies in Different Study Populations 66
Table 5: Characteristic of studies of uterine anomalies versus normal uteri on reproductive outcomes 90
Table 6: Appraisal of methodological quality (Newcastle-Ottawa Scale). 91
Table 7: Reproductive impact of various congenital uterine anomalies 92
Table 8: Characteristics of studies of surgical treatment of uterine anomalies versus no treatment on reproductive outcomes. 120
Table 9: Appraisal of methodological quality (Newcastle-Ottawa Scale). 121
Table 10: Reproductive impact of surgical treatment for congenital uterine anomalies. 122
Table 11: Characteristic of reproducibility studies of 3D ultrasound scan in diagnosis of congenital uterine anomalies 138
Table 12: Evaluation of included studies according to the guidelines GRRAS 139
Table 13: Kappa statistics of included studies 140
Table 14: Baseline characteristics of the study population 158
Table 15: Comparing normal uteri to abnormal uteri in different population 160
Table 16: Prevalence of congenital uterine anomalies in different populations 161
Table 17: Uterine cavity lengths in different population 162
Table 18: Cervical lengths in different populations 163
Table 19: Myometrial volume of uterine body in different populations 164
Table 20: Cervical volume in different populations 165
Table 21: Clinicians' responses based on patients' clinical presentations 181

Table of Figures

Figure 1: Schematic representation of the embryonic development of the female genital system of a 7-week embryo 8
Figure 2: Embryological development of the vagina 9
Figure 3: The American Society for Reproductive Medicine Classification (1988) of Müllerian anomalies 16
Figure 4: Schematic representation of ESHRE/ESGE classification of uterine anomalies 19
Figure 5: Scheme for the classification of female genital tract anomalies according to the new ESHRE/ESGE classification system. 20
Figure 6: Three-dimensional multiplanar display of uterus. 51
Figure 7: Example of post-processing using Volume Contrast Imaging 53
Figure 8: Full uterine volume estimation using VOCAL™ 55
Figure 9: Uterine body volume measurement 55
Figure 10: Cervical volume estimation 56
Figure 11: Study selection process for systematic review on the prevalence of uterine anomalies in unselected population and high risk populations 64
Figure 12: Study selection process for systematic review on the effect of congenital uterine anomalies on reproductive outcome 86
Figure 13: Studies of women with an arcuate uterus versus women with normal uteri for the outcome of clinical pregnancy rate 94
Figure 14: Studies of women with canalization defects versus women with normal uteri for the outcome of clinical pregnancy rate 94
Figure 15: Studies of women with unification defects versus women with normal uteri for the outcome of clinical pregnancy rate 95
Figure 16: Studies of women with an arcuate uterus versus women with normal uteri for the outcome of first trimester miscarriage 96
Figure 17: Studies of women with canalization defects versus women with normal uteri for the outcome of first trimester miscarriage 97
Figure 18: Studies of women with unification defects versus women with normal uteri for the outcome of first trimester miscarriage 97
Figure 19: Studies of women with an arcuate uterus versus women with normal uteri for the outcome of second trimester miscarriage 99
Figure 20: Studies of women with canalization defects versus women with normal uteri for the outcome of second trimester miscarriage 99
Figure 21: Studies of women with unification defects versus women with normal uteri for the outcome of second trimester miscarriage 100
Figure 22: Studies of women with an arcuate uterus versus women with normal uteri for the outcome of preterm birth rate 102
Figure 23: Prospective studies of women with an arcuate uterus versus women with normal uteri for the outcome of preterm birth rate 102
Figure 24: Studies of women with canalization defects versus women with normal uteri for the outcome of preterm birth rate 102
Figure 25: Studies of women with unification defects versus women with normal uteri for the outcome of preterm birth rate 103

Figure 26: Studies of women with an arcuate uterus versus women with normal uteri for the rate of fetal malpresentation at birth.....104
Figure 27: Studies of women with canalization defects versus women with normal uteri for the rate of fetal malpresentation at birth.....105
Figure 28: Studies of women with unification defects versus women with normal uteri for the rate of fetal malpresentation at birth.....105
Figure 29: Study selection process for systematic review on the reproductive impacts of surgical treatments in women with congenital uterine anomalies.....118
Figure 30: Effect of septal divisions on pregnancy rate compared with no surgery.....124
Figure 31: Effect of septal divisions on miscarriage rate compared with no surgery.....125
Figure 32: Effect of septal divisions on term birth rate compared with no surgery.....125
Figure 33: Effect of surgery for unification defects on pregnancy rate compared with no surgery.....126
Figure 34: Effect of surgery for unification defects on miscarriage rate compared with no surgery.....126
Figure 35: Effect of surgery for unification defects on term birth rate compared with no surgery.....127
Figure 36: Study selection process for systematic review on the reproducibility of 3D ultrasound scan in diagnosing congenital uterine anomalies.....137
Figure 37: Pooled kappa statistic for diagnosis of congenital uterine anomalies.....141
Figure 38: Subgroup analysis according to classification system.....141
Figure 39: Participant Recruitment Flow Chart.....158
Figure 40: Number of miscarriage patients/year in other hospitals.....177
Figure 41: Number of patients/year with septate or subseptate.....178
Figure 42: Minimum clinically important difference.....179
Figure 43: Clinicians' opinions on patient recruitment based on patients' clinical presentations.....182
Figure 44: Availability of three-dimensional ultrasound scan.....183
Figure 45: Types of surgical techniques that are acceptable to clinicians.....184
Figure 46: Types of surgical techniques available in the centres clinicians currently working at.....185
Figure 47: Age groups of patients surveyed.....186
Figure 48: Past reproductive history in patients who completed the survey.....187
Figure 49: Patients' responses to willingness to randomisations based on clinical presentations.....190

Table of Abbreviations

2D	Two-dimensional
3D	Three-dimensional
4D	Four-dimensional
ANOVA	Analysis of variance
ASRM	American Society for Reproductive Medicine
Bcl-2	B-cell lymphoma 2
CI	Confidence interval
CONUTA	CONgenital UTerine Anomalies working group
DES	Diethylstilbestrol
EMBASE	Excerpta Medica dataBASE
ESGE	European Society for Gynaecological Endoscopy
ESHRE	European Society of Human Reproduction and Embryology
GRRAS	Guidelines for Reporting Reliability and Agreement Studies
HOX	Homeobox
HTA	Health Technology Assessment Programme
HSG	Hysterosalpingography
ICSI	Intracytoplasmic sperm injection
IRAS	the Integrated Research Application System
IVF	In-vitro fertilisation
Lim1	LIM homeobox 1
LLETZ	Large Loop Excision of the Transformation Zone
MEDLINE	Medical Literature Analysis and Retrieval System Online
MeSH	Medical Subject Headings
MHz	Megahertz
MRI	Magnetic Resonance Imaging
NHS	National Health Service
NIHR	National Institute for Health Research
NURTURE	Nottingham University Research and Treatment Unit in Reproduction
OASIS	Off-line Analysis of UltraSonographic Images Study
Pax2	Paired box gene 2
PhD	Doctor of Philosophy
PREPARE	Patient led Research into Early Pregnancy and Reproduction
PUMA	Prevalence of Congenital Uterine MAlformations
R&D	Research and Development
RR	Relative risks or risk ratios
SD	Standard deviation
SEPTUM	Study to Evaluate the Prevalence, importance and Treatment of women with Uterine Malformation
SPSS	Statistical Package for the Social Sciences
SRI	Speckle reduction imaging
TAS	Transabdominal ultrasound scan
TRUST	The Randomised Uterine Septum Transsection Trial
VCI	Volume Contrast Imaging
VCUAM	Vagina, Cervic, Uterus, Adnexa and Associated Malformations
VOCAL	Virtual Organ Computer-aided Analysis
Wnt-4	Wingless 4 signalling

Abstract

Background

Congenital uterine anomalies result from the abnormal formation, fusion, or resorption of Müllerian duct during fetal life. They are present in 1–10% of the unselected population, 2–8% of infertile women, and 5–30% of women with a history of miscarriage. The discrepancy in the prevalence rates are likely to be related to the use of different diagnostic tests and the use of non-standardized classification systems to diagnose these anomalies. These anomalies have long been associated with increased rates of subfertility, miscarriage, preterm delivery, and other adverse fetal outcomes. However, such associations might be artefactual.

Over the years, surgical treatments have been offered to women diagnosed with congenital uterine anomalies. Abdominal metroplasty is associated with significant morbidities, such as prolonged hospital stay, long post-op recovery, intra-abdominal adhesions, uterine rupture in subsequent pregnancies etc. In the recent years, hysteroscopic metroplasty has taken over abdominal approach as this procedure is deemed safer. However, no randomised controlled trial has been performed to assess its risks and benefits.

Aims

The overall aim of the thesis is to evaluate the prevalence of congenital uterine anomalies; the reproductive impact of these

anomalies; and treatment available for women with uterine malformation (in particularly women with septate or subseptate uteri).

Methods

Systematic reviews

I have performed systematic reviews and meta-analyses of the available literature on four aspects of congenital uterine anomaly research: Prevalence of congenital uterine anomalies; impact of these anomalies on reproductive outcomes; treatment options for congenital uterine anomalies; and finally reproducibility of three-dimensional ultrasound scan in diagnosing these anomalies.

Prevalence of Congenital Uterine MAlformations (PUMA) in High Risk Women

Three groups of women were recruited prospectively into this study over a period of 16 months according to specific inclusion and exclusion criteria. Control group consisted of women who only had previous term deliveries (birth at 37 or more weeks gestation), Preterm group consisted of women with history of preterm +/- miscarriage and finally Miscarriage group consisted of women with history of miscarriage +/- term deliveries only. All these women underwent three-dimensional transvaginal ultrasound scans according to standard operating procedure. Diagnoses of congenital uterine anomalies were recorded. Ultrasound parameters (uterine length, cervical length, uterine volume; and cervical volume) were measured and recorded where possible.

Development of a Randomised Controlled Trial of Hysteroscopic Resection for Uterine Septae

Part of the thesis is to aid the design of a multi-centred randomised controlled trial to assess the effect of hysteroscopic surgery for uterine anomalies on various pregnancy outcomes in women with septate uteri. Two surveys were conducted first, to ask the general public and medical experts with a proven interest, to give their opinion on the need and feasibility of a randomised controlled trial of hysteroscopic resection for uterine septae.

With the survey results from general public and experts available, protocol for a prospective randomised pilot feasibility study was designed as part of the thesis. The primary objective of the pilot study is, to test the hypothesis that hysteroscopic septal resection in women with septate uteri and a history of miscarriage or preterm birth, improves reproductive outcomes. Applications were put in to secure study funding, ethical and R&D approvals.

Results

Systematic reviews

The systematic reviews demonstrated the prevalence of uterine anomalies diagnosed by optimal tests was 5.5% in the unselected population, 6.5% in infertile women, 12.9% in those with miscarriages, 24.5% in those with miscarriages and infertility, and 36% in those undergoing IVF. Arcuate and septate/subseptate uteri were the most common anomalies.

The reviews also showed that women with congenital uterine anomalies appeared to have increased risks of poor reproductive

outcomes (including decreased conception rate; increased miscarriage rate, and preterm birth rate) but this is dependent on the types of anomalies.

It was shown in the systematic review that surgical division of a uterine septum improves reproductive outcome by reducing miscarriage and increasing term birth. However, the exact effects are dependent on the type of surgery performed and the underlying defect. Surgery for unification defects, such as the bicornuate uterus, does not appear to have any impact on reproductive performances.

Our systematic review showed that three-dimensional ultrasound has high inter-observer agreement in diagnosing congenital uterine anomalies. The diagnostic reproducibility was dependent on the classification system used in diagnosing these anomalies.

Unfortunately all these systematic reviews only consisted of observational studies, with varying study qualities and they have significant clinical heterogeneity among studies, including use of different diagnostic tests of unknown accuracy; different classification systems and varying background populations.

Prevalence of Congenital Uterine MAlformations (PUMA) in High Risk Women

In this study, there were a total of 50 women with abnormal shaped uteri and 161 women had normal uteri. There were more abnormal uteri in the miscarriage group than the other groups. 13.2% were abnormal in the control group, 33.7% were abnormal in miscarriage group, and 16.7% were abnormal in the preterm group. The

most common type of anomaly seen was arcuate uterus (90%). High risk women with congenital uterine anomalies were found to have shorter uterine lengths. However, other ultrasound parameters such as cervical length and myometrial volume of uterine body showed no significant difference when compared to control group. The surprising finding was the larger cervical volume in women with history of miscarriage as opposed to those of low risk population.

Development of a Randomised Controlled Trial of Hysteroscopic Resection for Uterine Septae

Survey results from the general public and clinicians demonstrated support and willingness of these people in participating in the trial. We have set up a patient group (Patient led Research into Early Pregnancy and Reproduction: PREPARE) to further help us in study design. A full protocol for a pilot study was designed with input from patients, clinicians, researchers; and statisticians. Application for a Pump Priming grant by the Nottingham University Hospitals Charity and Nottingham University Hospitals Department of Research and Development was approved for this study. Towards the end of my thesis, the pilot trial had obtained ethical and R&D approval and was ready to start.

Conclusions

The systematic reviews carried out as part of the thesis have confirmed the lack of evidence in various aspects of congenital anomalies, due to variable study designs, background population, the lack of standardised diagnostic tools and classification system. Even

though the prevalence of uterine anomalies appeared to be increased in high risk populations, the evidence of their associations with poor reproductive outcomes and the benefit of surgical treatments remain debatable. It was shown by our systematic review that three-dimensional ultrasound scan is a reproducible tool in diagnosing congenital uterine anomalies.

Our prospective study with a control group further confirmed that congenital uterine anomalies are more common in women with history of miscarriage. However, this does not appear to be the case in women with history of preterm births. In this study, women were assessed prospectively using three-dimensional ultrasound scan. Women with history of miscarriage have shorter uterine lengths. Surprisingly, women with history of miscarriage had larger cervical volume than the control group.

By using all the information from systematic reviews and prospective study I performed, the thesis finishes by setting up a pilot study of randomised controlled trial of hysteroscopic resection for uterine septae.

Peer Reviewed Publications

Prevalence of uterine anomalies and their impact on early pregnancy in women conceiving after assisted reproduction treatment

Jayaprakasan K, Chan YY, Sur S, Deb S, Clewes JS and Raine-Fenning NJ
Ultrasound Obstet Gynecol 37:727-732, 2011

The prevalence of congenital uterine anomalies in unselected and high-risk populations: a systematic review

Chan YY, Jayaprakasan K, Zamora J, Thornton JG, Raine-Fenning NJ and Coomarasamy A
Hum Reprod Update 17:761-771, 2011

Reproductive outcomes in women with congenital uterine anomalies: a systematic review

Chan YY, Jayaprakasan K, Tan A, Thornton JG, Coomarasamy A and Raine-Fenning NJ
Ultrasound Obstet Gynecol 38:371-381, 2011

Chapter 1 Introduction

1.1 Background

Müllerian duct anomalies, or more commonly known as congenital anomalies of the female genital tract, result from abnormal formation, fusion or resorption of the Müllerian ducts during fetal life (Moore et al., 2008). They are reported in 1-10% of the normal population (Ashton et al., 1988, Jurkovic et al., 1997, Raga et al., 1997, Simon et al., 1991, Tur-Kaspa et al., 2006, Woelfer et al., 2001), 2-8% of infertile women (Acien, 1993, Godinjak and Idrizbegovic, 2008, Raga et al., 1997, Stillman and Asarkof, 1985) and 5-30% of women with history of miscarriage (Acien, 1993, Ghi et al., 2009, Raga et al., 1997, Weiss et al., 2005). The discrepancy in these prevalence rates presumably relates to the application of different diagnostic methods and the use of different classification systems among investigators to define the abnormalities.

Normal development of the female reproductive tract involves a series of complex processes to form the internal genital tract. When there is an interruption or dysregulation in any of the dynamic processes of differentiation, migration, fusion, and canalization, a wide spectrum of Müllerian duct anomalies can occur. Müllerian anomalies are frequently defined or grouped according to the failed developmental mechanism that gives rise to a given malformation (Acien et al., 2004,

Buttram Jr et al., 1988, Oppelt et al., 2005, Rock, 1986). The most widely used method of categorizing Müllerian duct anomalies is the American Society for Reproductive Medicine Classification (American Fertility Society, 1988).

Müllerian duct anomalies are closely related to an abnormal uterine cavity or vagina, which may have a devastating impact on the potential for sexual activity and reproductive performances. All types of congenital uterine anomalies have long been recognized as a potential cause of poor reproductive outcomes including infertility (Tulandi et al., 1980, Raga et al., 1997), recurrent pregnancy loss (Rackow and Arici, 2007), preterm delivery (Tomazevic et al., 2007) and fetal malpresentation (Stein and March, 1990, Rock and Schlaff, 1985). These are associated with significant physical and psychosocial morbidities, which in turn account for inpatient admissions and time off work. These poor outcomes indirectly contribute to the financial cost of the National Health Service (NHS) and the society. Preterm birth, especially if early delivery before 34 weeks, is the leading cause of perinatal morbidity and mortality in the United Kingdom (Rush et al., 1976). Therefore, the real cost to the society is likely to be substantially higher.

The evidence for the association between Müllerian duct anomalies and abnormal reproductive outcome is, however, unclear as is the effect of the specific type of uterine anomaly. In fact, many women with Müllerian duct anomalies are asymptomatic and have no reproductive problems at all (Ashton et al., 1988, Hunt and Wallach,

1974, Simon et al., 1991). A lot of these anomalies will remain unrecognised and are only discovered incidentally during clinical examination or investigations for unrelated health issues. Nevertheless, women with obstructing anomalies generally present earlier as a result of amenorrhoea or progressive pelvic pain, normally within the first year following menarche (Breech and Laufer, 1999, Croak and Gebhart, 2005), due to the retention of menstrual blood.

The true population prevalence of Müllerian duct anomalies is difficult to assess because of non-standardized classification systems and because the best acceptable diagnostic techniques are invasive and, therefore, rarely applied to the general population. Some diagnostic tests are more sensitive or specific than others. Two-dimensional transvaginal scan or hysterosalpingography (HSG) in isolation have the tendency to underestimate the true prevalence of Müllerian duct anomalies due to their relative poorer sensitivity and specificity as diagnostic tests (Jurkovic et al., 1995, Wu et al., 1997, Andreotti et al., 2006, Guimaraes Filho et al., 2006a, Momtaz et al., 2007, Saravelos et al., 2008). Three-dimensional ultrasound scan and magnetic resonance imaging (MRI) offer a non-invasive method of investigation. Both these imaging modalities were reported to have high accuracy rates in diagnosing Müllerian duct anomalies (Carrington et al., 1990, Fischetti et al., 1995, Ghi et al., 2009, Jurkovic et al., 1995, Olpin and Heilbrun, 2009, Pellerito et al., 1992, Raga et al., 1996, Wu et al., 1997). However, they are relatively new technologies and hence less widely available.

Many authors consider a combination of laparoscopy or laparotomy with hysteroscopy or HSG to be the gold standard in assessing Müllerian duct anomalies (Acien, 1997, Homer et al., 2000, Taylor and Gomel, 2008). However, the final diagnosis is based on the subjective impression of the clinician performing the test and not on strict diagnostic criteria (Woelfer et al., 2001). Besides, the combined approach is also invasive and usually requires general anaesthesia. With the improvements on other diagnostic modalities, the role for these surgical techniques is less certain, especially if adequate information can be obtained using other diagnostic tools.

In the recent years, as three-dimensional transvaginal ultrasound scan is becoming more available, it is becoming the new diagnostic test of choice for a lot of clinicians as it appears to be accurate and reliable, yet cheaper, faster and non-invasive compared to combined hysteroscopy and laparoscopy or magnetic resonance imaging (Deutch and Abuhamad, 2008, Olpin and Heilbrun, 2009). It also allows precise and objective measurements of the uterine dimensions, which is an advantage in classifying different anomalies.

A lot of women with Müllerian duct anomalies do not require any treatment as most of them are asymptomatic and have no sexual or reproductive difficulties. Therefore, treatment is mainly reserved for those who have pain or reproductive or/and sexual difficulties. Unfortunately, most treatments involve surgeries. Over the years, surgical treatment for uterine anomalies has caused a lot of controversies. These interventions are not without risks, such as uterine

perforation, post-operative infertility, uterine rupture in subsequent pregnancies etc. (Ayhan et al., 1992, Lourdel et al., 2007). Whilst many observational studies have reported an improved outcome following surgical intervention (Ayhan et al., 1992, Grimbizis et al., 2001, Homer et al., 2000, Mollo et al., 2009, Valli et al., 2004), there is a need of randomised controlled trials to address the effectiveness and safety of these treatments.

1.2 Embryology and Pathophysiology of the Müllerian System

1.2.1 Embryology

1.2.1.1 Development of the Uterus and Fallopian Tubes

Sexual differentiation of the genital system begins early in the fetal period, at about 6 weeks of development. Earlier in the embryonic development, the male and female genital systems are completely indistinguishable, in which both the Wolffian (mesonephric) and Müllerian (paramesonephric) ducts are present. An absence of testis-determining factor of the Y chromosome in female leads to the lack of testosterone and Müllerian-determining factor. In a normal female (XX) embryo, the Wolffian ducts degenerate due to the lack of testosterone and the Müllerian ducts develop in the absence of Müllerian-determining factor (Gell et al., 1998, Larsen, 1993, Troiano and McCarthy, 2004). It is important to remember that female sexual development does not depend on the presence of ovaries or hormones (Moore et al., 2008). Ovarian development is a separate process from the formation of Müllerian system and hence not usually associated with Müllerian duct anomalies.

Müllerian ducts form as a result of fusion of the coelomic epithelium. They only develop in the presence of Wolffian ducts or

mesonephric ducts. Wolffian ducts act as a precursor and inducer of the female genital tract development (Saravelos et al., 2008). As the Wolffian ducts degenerate, the regressing vestiges of the Wolffian ducts serve as a template for the developing Müllerian ducts. Müllerian ducts grow bilaterally along the lateral aspects of the gonads or developing ovaries. They grow caudomedially, approaching each other and begin to fuse even before they reach the urogenital sinus. The most cranial part of the Müllerian ducts remain unfused and open into the peritoneal cavity to form the fallopian tubes (Puscheck and Cohen, 2008). The caudal part of the ducts fused to form the uterovaginal primordium which gives rise to the uterus, cervix and the superior portion of the vagina (Moore et al., 2008). Figure 1 shows a schematic representation of the embryonic development of the female genital system.

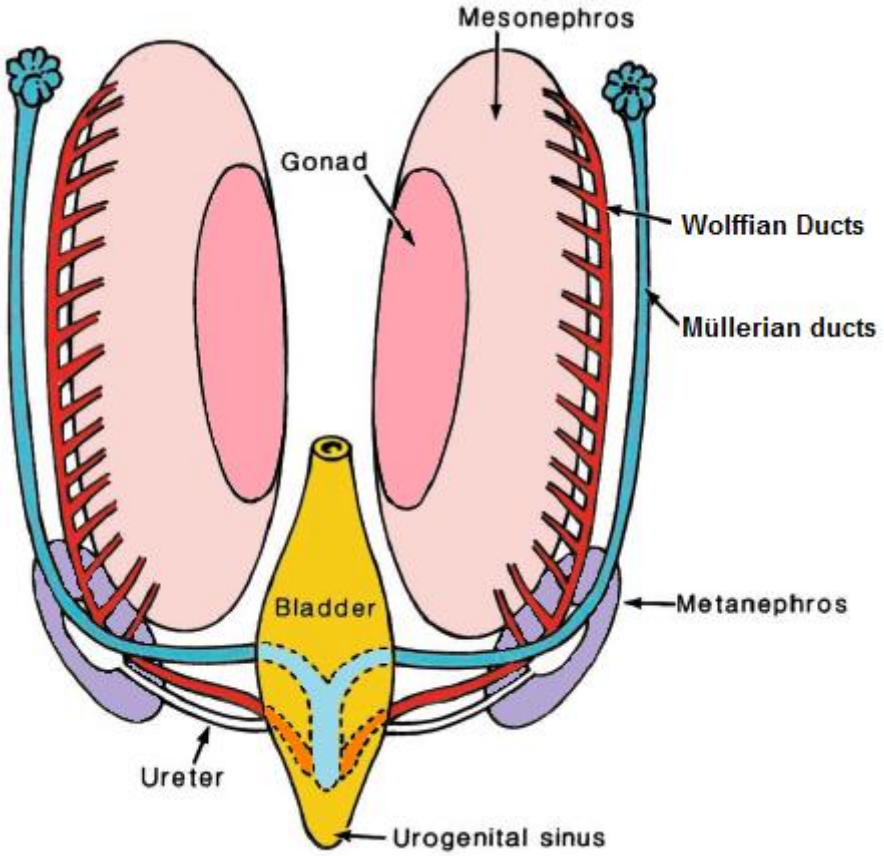


Figure 1: Schematic representation of the embryonic development of the female genital system of a 7-week embryo

Adapted from Moore, K. L., T. V. N. Persaud, et al. (2008). The Urogenital System. Before We Are Born: Essential of Embryology and Birth Defects. Philadelphia, Saunders/Elsevier

After the distal portion of the Müllerian ducts fuse, a central septum is left, separating the uterine cavity. The central septum begins to resorb at about 9 weeks, forming the single lumen of the uterovaginal canal. There are two proposed theories of septal resorption process (Troiano and McCarthy, 2004). The classic theory is the unidirectional resorption hypothesis where the septum regresses from the caudal to cranial end of the uterovaginal canal. However, Muller et al had proposed an alternative hypothesis where the resorption process

proceeds simultaneously in both the cranial and the caudal directions (Muller et al., 1967).

1.2.1.2 Development of the Vagina

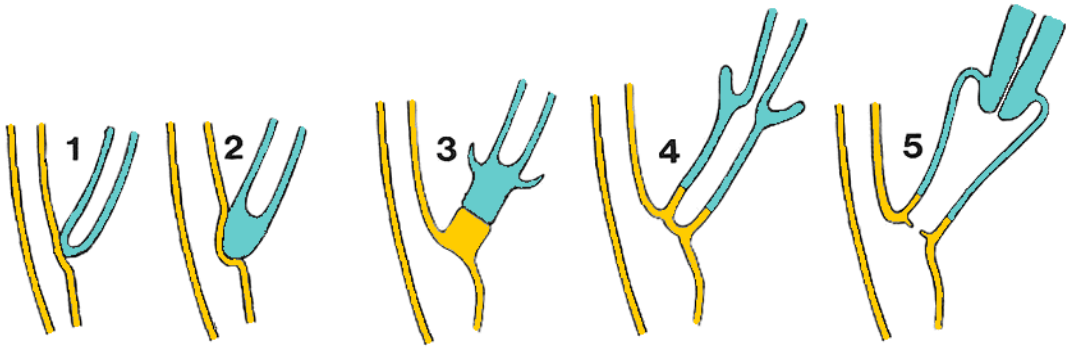


Figure 2: Embryological development of the vagina

Diagrams show that (1), the uterovaginal canal (blue area) reaches the urogenital sinus (yellow area). The vaginal plate starts to form (2), then it proliferates (3), and undergoes canalization (4). The vagina is formed by both the Müllerian ducts and the urogenital sinus (5).

The superior portion of the vagina is derived from the Müllerian ducts (See Figure 2). The vaginal epithelium is derived from the endoderm of the urogenital sinus. The wall of the vagina forms from the surrounding mesenchyma. As the uterovaginal primordium comes in contact with the urogenital sinus, they fuse to form the Müllerian or sinus tubercle. This tubercle subsequently thickens and induces the development of sinovaginal bulbs at its distal end. The sinovaginal bulbs are paired with endodermal evaginations. They grow to form a solid vaginal plate. Vaginal plate first proliferates and elongates, then undergoes canalization, forming the lower vagina (Croak and Gebhart, 2005). The vaginal plate elongates during the 3rd–5th month, and its interface with the urogenital sinus eventually forms the hymen (Moore

et al., 2008), which usually perforates during the perinatal period (Larsen, 1993).

1.2.1.3 Development of the Urinary System

The ureters, renal pelvis, renal calices, and collecting tubules are all formed from mesonephric ducts. Embryologically, the urinary system and the genital systems are closely associated as they both derived from the common ridge of mesoderm, forms on each side of the dorsal aorta (Troiano and McCarthy, 2004). Both these systems depend on the mesonephric ducts or Wolffian ducts for normal development. Hence, abnormal formation or development of the mesonephric ducts will have an effect on the developing urinary system. This explains the frequent associations observed between Müllerian duct anomalies and renal-urinary system malformations (Arnold et al., 2001). Therefore, it is essential to image the renal system in patients with Müllerian duct anomalies and vice versa (Gell et al., 1998).

1.2.2 Pathophysiology of Müllerian Anomalies

Various types of uterine and vaginal anomalies form as a result of developmental anomalies of the uterovaginal primordium. Failure of both Müllerian ducts formation leads to congenital absence of uterus or uterine agenesis. It also leads to vaginal atresia or agenesis due to non-development of the sinovaginal bulbs. Incomplete canalization of the vaginal plate results in vaginal septae (transverse and vertical) or an imperforate hymen.

If one of the Müllerian duct fails to form or develop, this will result in a unicornuate uterus, a uterus formed from only one Müllerian duct. The underdeveloped Müllerian duct may present as a rudimentary horn. Occasionally, the rudimentary horn may not communicate with the cavity of the uterus.

Incomplete fusion of the inferior portion of the Müllerian ducts will give rise to didelphys uterus. It may be associated with a double or single vagina. On the other hand, incomplete fusion of the Müllerian ducts at the level of the fundus will lead to duplication involving the superior part of the uterus, giving rise to bicornuate uterus (Moore et al., 2008).

When there is complete fusion of the Müllerian ducts but absent or incomplete resorption of the uterine septum, a septate or subseptate uterus is formed (Arnold et al., 2001). Hence, septated uteri have normal appearing external fundus.

Arcuate uterus is an anomaly where the uterine fundus displays a mild concave indentation or contour towards the uterine cavity. It is believed to be a near-complete resorption of the uterovaginal septum (Saravelos et al., 2008). However, some authors believe that arcuate uterus is a normal variant rather than a uterine anomaly (Buttram Jr et al., 1988, Heinonen et al., 1982).

Diethylstilbestrol (DES, or stilbestrol) is a recognized human teratogen (Moore et al., 2008). The drug was prescribed to pregnant women in Europe until 1978 (Giusti et al., 1995). It is known to induce

uterine malformation in women exposed to DES in utero. The most common anomaly seen is a T-shaped, hypoplastic uterus (Croak and Gebhart, 2005). The exact pathophysiology mechanism is unclear, though studies have found that DES changes homeobox (HOX) gene expression in the developing Müllerian system (Croak and Gebhart, 2005, Taylor et al., 1997), which causes permanent cellular and molecular changes resulting in altered development of the Müllerian ducts (Newbold, 1995).

1.3 Aetiology

Any disruption during the in utero development of Müllerian duct may lead to formation of Müllerian duct anomalies. However, the exact causes for majority of the Müllerian duct anomalies are unknown, though most are considered to be isolated in nature.

The genetics of Müllerian duct anomalies are complex. Familial factors in Müllerian anomalies formation have been described (Hammoud et al., 2008). However, familial aggregates of Müllerian anomalies are probably best explained on the basis of polygenic or multi-factorial inheritance. Sporadic gene mutations may influence the development of the Wolffian and Müllerian ducts. There are some studies which showed several genes, such as Lim1 (LIM homeobox 1), Pax2 (Paired box gene 2) and Wnt-4 (Wingless 4 signalling) are required for Wolffian and Müllerian duct development (Kobayashi and Behringer, 2003, Kobayashi et al., 2004, Vainio et al., 1999). However, most of these are animal studies using mice. Septal resorption has been proposed to be a result of apoptosis or programmed cell death, regulated by the Bcl2 gene (Lee et al., 1998). Absence of this gene has therefore been associated with the persistence of the uterine septum.

In addition to genetic predisposition, one cannot ignore the role of environmental factors such as drugs with teratogenic effects. For instance, in utero DES exposure can lead to uterine malformation in the affected female offspring. Thalidomide has also been linked to varying degrees of uterine anomalies (Golan et al., 1989, Stelling et al., 1999).

1.4 Classification

Müllerian duct anomalies have been known for years; however we are still uncertain of the prevalence and clinical implications of each anomaly. One of the reasons for this uncertainly is due to the lack of a standard and reliable definition or classification for reporting. If the necessary data are to be collected on Müllerian duct anomalies, it is essential that these anomalies are diagnosed and classified correctly.

Müllerian duct anomalies are frequently grouped according to the failed developmental mechanism that gives rise to a given malformation. Strassman (Strassman, 1907, Strassmann, 1952, Strassmann, 1966) has grouped these anomalies into symmetric double malformations (didelphys, bicornuate and septate) or asymmetric malformations (Unicornuate uterus with or without a rudimentary horn). However, this classification does not provide any meaningful comparisons in the literature as the anomalies are not considered separately.

In 1979, Buttram and Gibbons proposed a classification based on the degree of failure of normal Müllerian development and grouped the anomalies according to their clinical morphology (Buttram and Gibbons, 1979). This was later modified in 1983 and subsequently updated again in 1988 by the American Fertility Society (American Fertility Society, 1988), currently known as the American Society for Reproductive Medicine. This classification is currently the most widely accepted and used. The current American Society for Reproductive

Medicine Classification of Müllerian duct anomalies consists of seven groups, as shown in Table 1 and Figure 3.

Class	Clinical Findings	Description
I	Müllerian agenesis or hypoplasia	Vaginal Cervical Fundal Tubal Any combination of the above
II	Unicornuate Uterus	Communicating rudimentary horn Non-communicating rudimentary horn Rudimentary horn with no cavity No rudimentary horn
III	Didelphys uterus	Failure of lateral fusion involving both the uterus, cervix and often the vagina
IV	Bicornuate uterus	Incomplete fusion of the uterine horns at the level of the fundus Complete Partial
V	Septate uterus	Absent or incomplete resorption of the uterovaginal septum Complete Partial
VI	Arcuate uterus	Mild concave indentation or contour towards the uterine cavity
VII	DES drug related	A T shaped uterus resulting from DES exposure in utero

Table 1: Classification of Müllerian Duct Anomalies by the American Society for Reproductive Medicine Classification (1988)

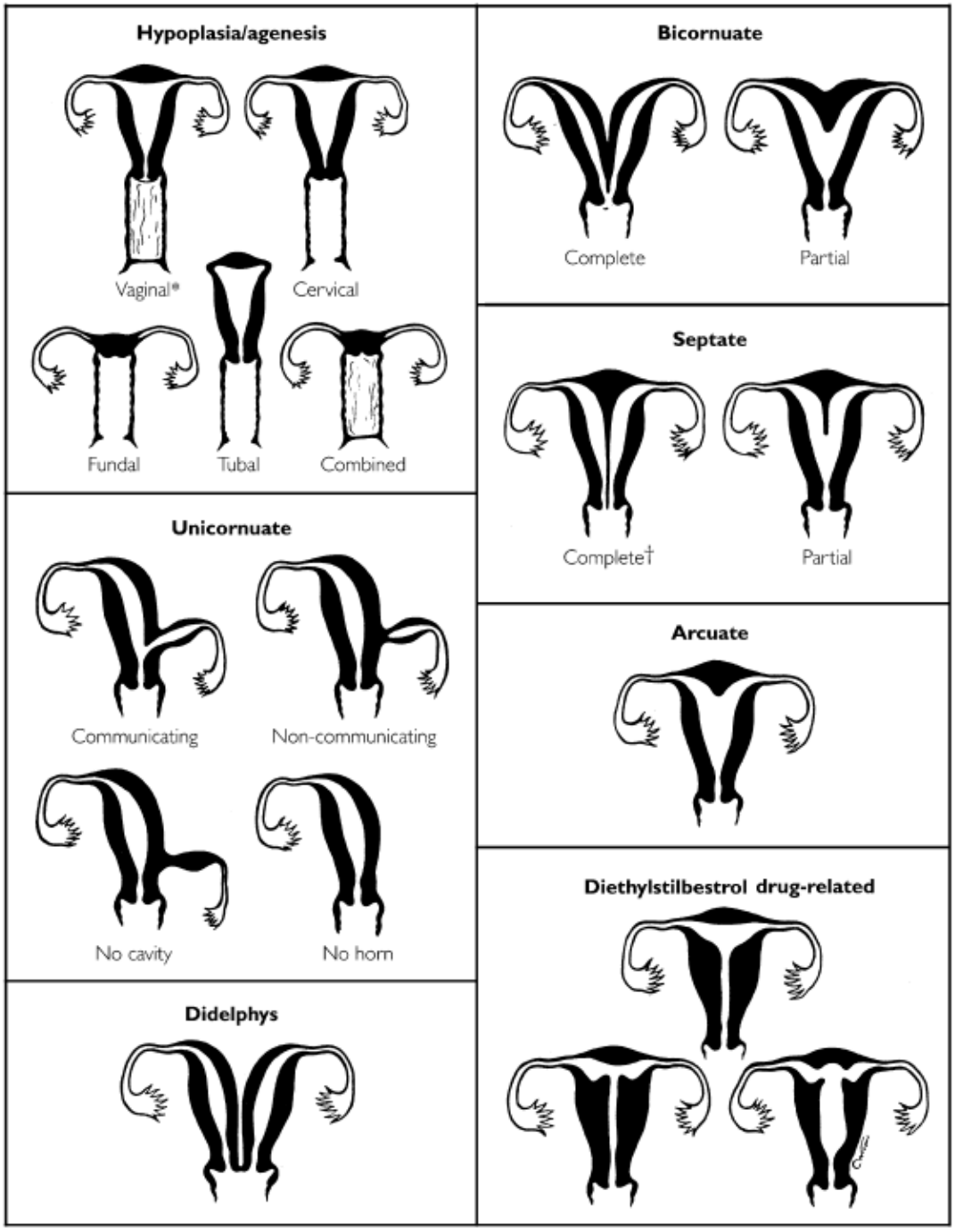


Figure 3: The American Society for Reproductive Medicine Classification (1988) of Müllerian anomalies

From the American Society for Reproductive Medicine Classifications of adnexal adhesions, distal tubal occlusion, tubal occlusion secondary to tubal ligation, tubal pregnancies, Müllerian anomalies and intrauterine adhesions (American Fertility Society, 1988).

American Society for Reproductive Medicine Classification, however, does not specify the diagnostic methods that should be used

for diagnosis. In fact, the final diagnosis is also based on the subjective impression of the clinician or investigator performing the test (Woelfer et al., 2001). In addition, it does not include vaginal, adnexal, or associated renal-urinary malformations. Therefore, this classification is not mutually exclusive. Many Müllerian duct anomalies often coexist and complex. Hence, this classification system should act as a main skeleton for describing Müllerian duct anomalies, instead of a comprehensive list of all anomaly types.

Acien et al presented a classification that is mainly based on the embryological development of the genitourinary tract (Acien et al., 2004). Although this includes anomalies in the uterus, vagina, adnexa and renal-urinary system, this classification is however extremely complex for use in actual clinical practice.

Salim et al has proposed a modified American Society for Reproductive Medicine Classification, in which the diagnostic criteria used were more detailed than previously described and they included cut-offs levels for the fundal shape and distortion (Salim et al., 2003). These cut-offs were necessary to differentiate uterine anomalies with similar morphological features such as subseptate and arcuate uteri. Salim et al has also proposed the use of three-dimensional ultrasound as the diagnostic for this modified classification system.

Some Müllerian duct anomalies can be very complex and difficult to describe. The VCUAM classification (Oppelt et al., 2005) classification makes it possible to describe complex malformations by dividing the external and internal female genital organs in accordance

with the anatomy: vagina (V), cervix (C), uterus (U), adnexa (A) and associated malformations (M). This classification makes it possible to provide more appropriate clinical care for the affected patients. However, again, it does not specify the diagnostic methods that should be used for diagnosis.

In 2013, The European Society of Human Reproduction and Embryology (ESHRE) and European Society for Gynaecological Endoscopy (ESGE) established a common working group named CONUTA (CONgenital Uterine Anomalies), which has developed the new ESHRE/ESGE classification system (Grimbizis et al., 2013) (See Figure 4). Its design is mainly based on the anatomy of the female genital tract and its correlation with clinical presentations. This classification system is supposed to eliminate the subjective diagnoses of the original American Society for Reproductive Medicine Classification, and enable differentiation between septate uterus and other similar anomalies, independent of absolute morphometric criteria. It also included cervical and vaginal anomalies to be classified in independent supplementary sub-classes (Figure 5).

However, ‘Arcuate uterus’ has been removed from the new classification system. The ESHRE/ESGE consensus does not consider arcuate uterus as an anomaly and considers all cases with internal midline indentation <50% of the uterine wall thickness to be a normal uterus. Therefore most of the arcuate uterus by the American Society for Reproductive Medicine would be classified as U0 or U2a. Some studies have suggested that even small deformities of the uterine cavity

could be associated with poor reproductive outcome (Gergolet et al., 2012, Tomazevic et al., 2007). Studies have suggested that the new classification over-diagnose and potentially over-treatment of patients (Ludwin and Ludwin, 2015, Ludwin et al., 2014, Sadek et al., 2016).

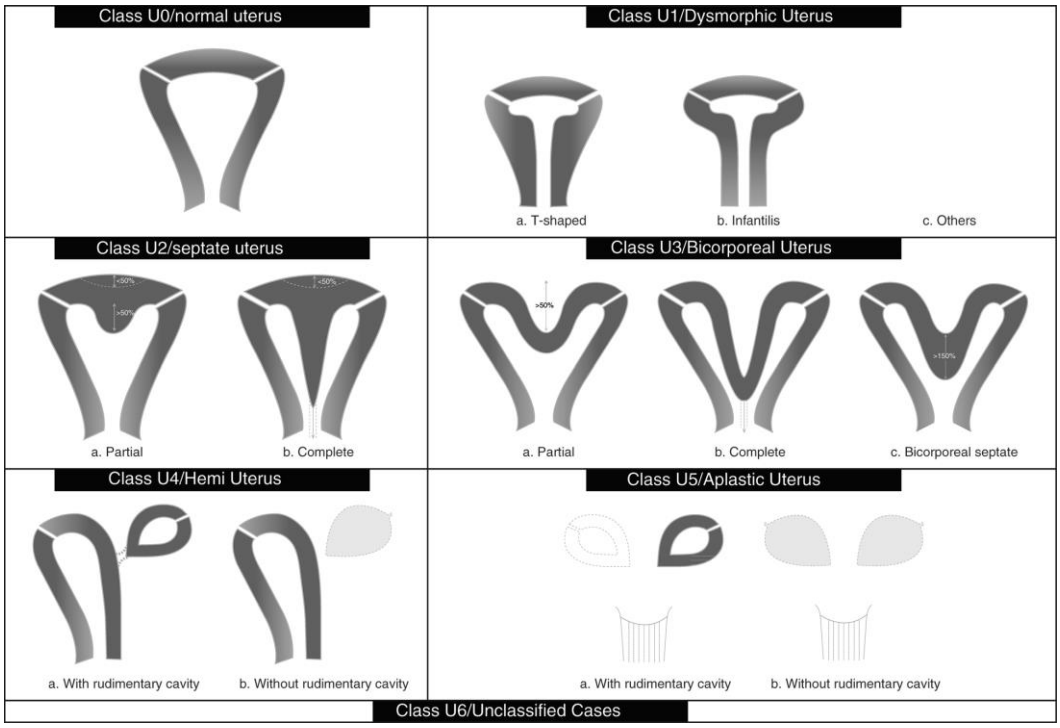
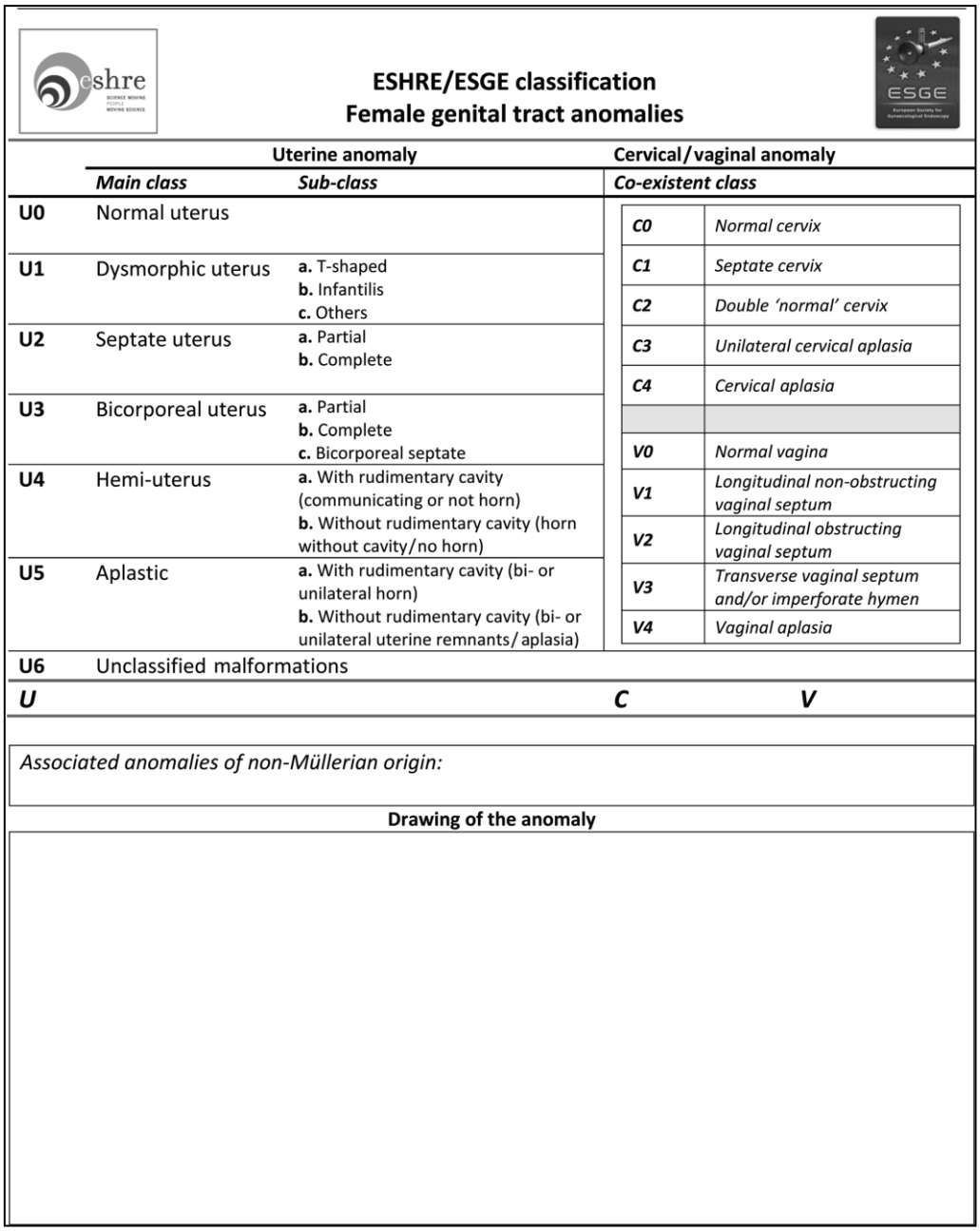


Figure 4: Schematic representation of ESHRE/ESGE classification of uterine anomalies

(Class U2: internal indentation >50% of the uterine wall thickness and external contour straight or with indentation <50%, Class U3: external indentation >50% of the uterine wall thickness, Class U3b: width of the fundal indentation at the midline >50% of the uterine wall thickness).



ESHRE/ESGE classification
Female genital tract anomalies

	Uterine anomaly		Cervical/vaginal anomaly
	Main class	***Sub-class***	***Co-existent class***
U0	Normal uterus		
U1	Dysmorphic uterus	**a.** T-shaped **b.** Infantilis **c.** Others	
U2	Septate uterus	**a.** Partial **b.** Complete	
U3	Bicorporeal uterus	**a.** Partial **b.** Complete **c.** Bicorporeal septate	
U4	Hemi-uterus	**a.** With rudimentary cavity (communicating or not horn) **b.** Without rudimentary cavity (horn without cavity/no horn)	
U5	Aplastic	**a.** With rudimentary cavity (bi- or unilateral horn) **b.** Without rudimentary cavity (bi- or unilateral uterine remnants/ aplasia)	
U6	Unclassified malformations		

C0	*Normal cervix*
C1	*Septate cervix*
C2	*Double 'normal' cervix*
C3	*Unilateral cervical aplasia*
C4	*Cervical aplasia*
V0	*Normal vagina*
V1	*Longitudinal non-obstructing vaginal septum*
V2	*Longitudinal obstructing vaginal septum*
V3	*Transverse vaginal septum and/or imperforate hymen*
V4	*Vaginal aplasia*

U ***C*** ***V***

Associated anomalies of non-Müllerian origin:

Drawing of the anomaly

Figure 5: Scheme for the classification of female genital tract anomalies according to the new ESHRE/ESGE classification system.

1.5 Clinical Presentations

Symptoms at presentation differ in each woman according to the type of Müllerian duct anomaly; however, as mentioned before, many women with Müllerian duct anomalies are asymptomatic and have no reproductive or sexual difficulties (Ashton et al., 1988, Hunt and Wallach, 1974, Simon et al., 1991).

1.5.1 Müllerian Duct Agenesis or Hypoplasia and Vaginal Anomalies

Symptoms at presentation for women with Müllerian duct agenesis or hypoplasia depend on the presence or absence of a functioning endometrium. Women with complete agenesis or without functioning endometrium will manifests with primary amenorrhoea. However, women with a functioning uterus who present with severe cyclical pelvic pain and primary amenorrhoea may reflect isolated vaginal anomalies such as vaginal agenesis, complete transverse vaginal septum or imperforate hymen; leading to haematometra or haematocolpos.

1.5.2 Obstructing Anomalies of the Upper Genital Tract

Women with obstructing uterine anomalies may present with progressive pelvic pain, a pelvic mass (Breech and Laufer, 1999, Croak and Gebhart, 2005), or even urinary retention due to the obstructing

retention of menstrual blood. Therefore, they generally present earlier during puberty. Unicornuate uterus with non-communicating rudimentary horn manifests at menarche with severe and worsening dysmenorrhoea. Very rarely, pregnancy can occur in the non-communicating horn by means of transperitoneal sperm migration (Cash et al., 2006). However, pregnancy within a non-communicating rudimentary horn has a 70-75% risk of rudimentary horn rupture (Nahum, 1998) and may present critically unwell suddenly during pregnancy.

1.5.3 Non-obstructing Anomalies of the Upper Genital Tract

Most non-obstructing Müllerian duct anomalies are asymptomatic. The evidence for the reproductive implications of the different uterine anomaly remains unclear and debatable. However, many studies have shown that congenital uterine anomalies are associated with poor reproductive outcomes (Acien, 1993, Golan et al., 1992, Raga et al., 1997, Rock and Schlaff, 1985, Tomazevic et al., 2007). They may be discovered after investigations for infertility, recurrent pregnancy loss, or preterm delivery. Occasionally, the anomaly is discovered incidentally during imaging evaluation for another unrelated condition or during surgery, such as elective tubal sterilization.

1.6 Investigations and Diagnosis of Müllerian Duct Anomalies

There is huge variation in the prevalence of anomalies in different studies. Apart from the lack of a standardised classification system, the other main reason for this discrepancy is due to the varied sensitivity and specificity of many diagnostic techniques. Over the years, physicians have used many methods to diagnose Müllerian duct anomalies that range from visual and bimanual pelvic examination to non-invasive imaging techniques, to the invasive combined hysteroscopy and laparoscopy/laparotomy.

An accurate diagnosis is essential to determine if surgical intervention is necessary or possible and to counsel the patient appropriately. Here, we discuss briefly the advantages and disadvantages of the most commonly used diagnostic methods for Müllerian anomalies.

1.6.1 Pelvic Examination

The diagnosis of Müllerian anomalies begins with history taking and a complete external physical examination, in particular pelvic examination. Vaginal examination and bimanual palpation is useful mainly to diagnose Müllerian agenesis and vaginal anomalies, such as uterine agenesis, cervical agenesis, vaginal agenesis, vaginal septum, and imperforate hymen. Occasionally, a duplicated cervix is identified (Golan et al., 1989). Bimanual examinations can detect a duplicated

uterus. However, any suspicions of uterine anomalies need to be confirmed using other imaging or surgical diagnostic methods (Raga et al., 2002). Diagnoses made exclusively by pelvic examination are likely to lead to diagnostic errors (Mueller et al., 2007, Raga et al., 2002).

1.6.2 Hysterosalpingography (HSG)

Hysterosalpingography (HSG) was initially described by Rindfleisch in 1910 (Rindfleisch, 1910). Before the advent of ultrasound and MRI, the most widely accepted imaging technique for the assessment of congenital uterine anomalies was HSG.

HSG is an X-ray procedure where iodinated contrast material is injected into the uterine cavity through a catheter or cannula placed in the cervical canal (Olpin and Heilbrun, 2009, Puscheck and Cohen, 2008). Fluoroscopy and a series of X-rays are obtained as the contrast fills the uterine cavity and passes through the fallopian tubes.

This technique assesses the morphology of the internal uterine cavity and endocervical canal (Troiano and McCarthy, 2004). It also provides valuable information regarding tubal patency (Olpin and Heilbrun, 2009) and complex Müllerian communications (Breech and Laufer, 1999). This procedure is simple, versatile and relatively low in cost (Raga et al., 2002).

However, this technique has several limitations. This technique involves inserting the catheter or cannula into the external cervical os; therefore it is technically not possible in patients with imperforate hymen, vaginal atresia or septum (Pui, 2004). Moreover, only the internal

uterine cavity can be seen with HSG and the external contour of the uterus is not evaluated. There can be considerable overlap in findings between anomalies (Olpin and Heilbrun, 2009) and we cannot reliably differentiate between a septate and a bicornuate uterus (Reuter et al., 1989). Whenever HSG shows a unicornuate uterus, it is important to note that blocked or non-communicating rudimentary horns does not appear on HSG (Propst and Hill, 2000). In addition, one must consider the possibility of didelphys uterus, which has two separate uterine cavities and cervixes (Saravelos et al., 2008).

A review by Saravelos et al has shown the weight mean sensitivity and specificity of HSG is approximately 78% and 90% respectively in diagnosing Müllerian anomalies (Saravelos et al., 2008). Others showed similar poor results for HSG accuracy if not worse (Braun et al., 2005, Pellerito et al., 1992, Puscheck and Cohen, 2008, Reuter et al., 1989). Minor anomalies such as arcuate and small subseptate uterus (Homer et al., 2000) can also be missed. Further invasive testing is often required to make the final diagnosis (Puscheck and Cohen, 2008).

HSG is relatively more invasive compared to other imaging techniques (Olpin and Heilbrun, 2009). There is a risk of uterine perforation by means of cannula or catheter insertion and injection of contrast (Pui, 2004, Saravelos et al., 2008). Ayida et al has reported procedure-related pain occurred in up to 72% of the women undergoing HSG (Ayida et al., 1996). In addition, HSG leads to exposure to ionizing radiation for the patient and the physician. Though Karande et al

suggested that the level of radiation is within the safety limits (Karande et al., 1997). Some patients may also have allergic reaction to the iodinated contrast media. Furthermore, HSG can also be complicated by pelvic inflammatory disease, especially if patient has previous tubal disease or confirmed Chlamydia infection (Forsey et al., 1990, Homer et al., 2000).

1.6.3 Ultrasonography

Ultrasonography has been proven to be one of the most essential investigations in obstetrics and gynaecology. It is widely accepted and used in most medical centres or hospitals. There are a few types of ultrasound scan that may be used to assess congenital uterine anomalies: Transabdominal ultrasound, two-dimensional transvaginal ultrasound and three-dimensional transvaginal ultrasound.

Ultrasound scan is preferred in a lot of cases as it is the simplest and least invasive diagnostic method. It allows measurements and quantification of observations to be made. It also allows us to evaluate the uterus as well as other pelvic structures. However, it is limited to assessment of congenital uterine anomalies and rarely used to diagnose vaginal anomalies.

1.6.3.1 Transabdominal Ultrasound Scan (TAS)

When performing ultrasonography to evaluate a suspected Müllerian duct anomaly, transabdominal ultrasound (TAS) is normally attempted first. TAS is easy and quick to perform. In addition, it can be

used to diagnose congenital uterine anomalies in pregnant women. TAS also allows assessment of any associated renal-urinary malformations (Homer et al., 2000). However, TAS is operator dependent (Troiano and McCarthy, 2004) and the image quality may be limited by body habitus, shadowing from bowel peristalsis or bowel gas and uterine position (Olpin and Heilbrun, 2009). TAS can diagnose uterine agenesis (Puscheck and Cohen, 2008) and high-sited rudimentary horn (Saravelos et al., 2008) reliably; however, its ability to classify different forms of double uterus varies (Fedele et al., 1988, Reuter et al., 1989). It has lower sensitivity and specificity than transvaginal ultrasound scan (Raga et al., 2002).

1.6.3.2 Two-Dimensional Transvaginal Ultrasound Scan

Two-dimensional transvaginal scan has the advantage of improved spatial resolution but the field of view will be reduced. It allows better visualisation over TAS as higher frequency probes are used and abdominal subcutaneous fat is avoided (Homer et al., 2000). Typically, it examines the uterus in two planes: transverse and longitudinal planes.

Studies have reported that two-dimensional transvaginal ultrasound scan demonstrated a sensitivity of 100% and a specificity of 80% in the investigation of uterine anomalies (Mendelson et al., 1988, Pellerito et al., 1992). It identifies uterine agenesis, as well as unicornuate uterus with a rudimentary horn (Puscheck and Cohen, 2008) reliably. However, some congenital anomalies are difficult to

distinguish. A unicornuate uterus without a rudimentary horn may be misdiagnosed. As two-dimensional ultrasound scan cannot see the coronal plane of the uterus, it is difficult to differentiate arcuate from septate from bicornuate uterus (Deutch and Abuhamad, 2008). In addition, fibroid, adnexal or other pelvic mass can reduce the image quality and occasionally confused with congenital uterine anomalies (Pui, 2004). However, two-dimensional transvaginal ultrasound scan is a good initial test for congenital uterine anomalies that is widely available (Jurkovic et al., 1995).

1.6.3.3 Three-dimensional Ultrasound Scan

Three-dimensional ultrasound scan is another imaging modality used for diagnosis of congenital anomalies. As in the case of two-dimensional ultrasound scan, three-dimensional ultrasound scan is a non-invasive test.

It is a relatively new technology that creates three-dimensional images from a uterine volume acquisition (Deutch and Abuhamad, 2008, Saravelos et al., 2008). This technique is not operator dependent (Puscheck and Cohen, 2008). Once the volume is obtained, the images can be manipulated to provide three-dimensional images of the uterus from almost any angle (Deutch and Abuhamad, 2008, Olpin and Heilbrun, 2009, Puscheck and Cohen, 2008). As a result, three-dimensional ultrasound scan can view the coronal plane of the uterus and hence able to differentiate arcuate, septate, bicornuate and

didelphys uteri (Ghi et al., 2009, Kupesic, 2005, Kupesic and Kurjak, 2005, Raga et al., 1996).

Reports have shown that three-dimensional ultrasound scan has high sensitivity and specificity, as high as 100% in diagnosing congenital uterine anomalies (Deutch et al., 2006, Saravelos et al., 2008, Wu et al., 1997). Furthermore, it is accurate in differentiating the anomalies (Deutch et al., 2006, Wu et al., 1997). On top of that, the three-dimensional images with the rest of the data once obtained can be stored and re-evaluated later. Salim et al confirmed the reproducibility of three-dimensional ultrasound scan diagnosis of congenital uterine anomalies and for the measurement of uterine cavity dimensions, by using two different observers, who were blinded to each other's findings. There was 99% agreement between the observers (Salim et al., 2003).

Three-dimensional ultrasound scan appears to be an accurate diagnostic test, yet cheaper, faster and safer compared to combined hysteroscopy and laparoscopy or magnetic resonance imaging (Deutch and Abuhamad, 2008, Olpin and Heilbrun, 2009). In most of the cases, women are able to avoid the need for surgery in order to diagnose congenital uterine anomalies.

There are certain limitations to three-dimensional ultrasound scan. Even though it is a very reliable test for diagnosing and classifying the anomalies, there are no universally set criteria for ultrasound diagnosis of congenital uterine anomalies. Many authors have set different classification criteria for ultrasound differentiation of these

anomalies. For example, Troiano and McCarthy diagnose a uterus as septate when the apex is more than 5 mm above the interostial line (Troiano and McCarthy, 2004), whilst Woelfer et al consider a uterus to be septate when the fundal indentation is less than 10 mm (Woelfer et al., 2001).

Fibroids or other large pelvic masses may distort the pelvic or uterine anatomy, causing difficulties in obtaining an adequate examination (Deutch and Abuhamad, 2008, Jurkovic et al., 1997). This will obviously lead to inaccuracy in diagnosis. In addition, it is limited in defining the shape of hypoplastic or T-shape uteri which are more easily identified on HSG (Pui, 2004).

At present, the use of three-dimensional ultrasound scan is restricted as it is still not widely available (Deutch and Abuhamad, 2008, Jurkovic et al., 1997). There are also insufficient trained sonographers or clinicians to perform and analyse three-dimensional ultrasound scans competently (Deutch and Abuhamad, 2008, Olpin and Heilbrun, 2009). With further refinement of three-dimensional ultrasound scanning and sonographers or clinicians become more skilled in performing this technique, it will determine its precise role in the evaluation of Müllerian duct anomalies.

1.6.4 Sonohysterography

Sonohysterography, which is also known as hysterosalpingosonography or hysterosonography, is technically an ultrasound version of an HSG. It works by improving the delineation of

endometrium and internal uterine contour with transcervical instillation of fluid into the uterus (Saravelos et al., 2008). The contrast material injected is saline or sterile water instead of iodinated contrast media. Therefore, there is no risk of radiation that is present in HSG. It is a safe, low-cost, and well tolerated procedure (Alborzi et al., 2003, Brown et al., 2000, Glanc et al., 2008, Guimaraes Filho et al., 2006a, Tur-Kaspa et al., 2006).

Sonohysterography is a useful diagnostic test as it enhances the ultrasound images by distending the uterus and it also provides valuable information on adnexa and tubal patency (Homer et al., 2000). It may be performed with either two-dimensional or three-dimensional ultrasound scans (Sylvestre et al., 2003). A sonohysterography that is performed using two-dimensional ultrasound will share the limitations similar to those of conventional two-dimensional transvaginal ultrasound and unable to view the external contour of the uterus (Devi Wold et al., 2006). Three-dimensional sonohysterography will therefore allow more precise assessment of the uterine anatomy, including the external contour, which is useful for diagnosis of uterine anomalies (Sylvestre et al., 2003). This has been shown by Sylvestre et al (2003), who found that three-dimensional sonohysterography is more precise than two-dimensional sonohysterography (Sylvestre et al., 2003).

Reports (Alborzi et al., 2003, Alborzi et al., 2007, Guimaraes Filho et al., 2006a, Saravelos et al., 2008) have suggested that sonohysterography has high sensitivity and specificity in diagnosing and classifying congenital uterine anomalies. In view of this, it could be a

good alternative in screening for uterine anomalies, especially with three-dimensional sonohysterography.

1.6.5 Magnetic Resonance Imaging (MRI)

Another non-invasive technique used to diagnose Müllerian duct anomalies is magnetic resonance imaging (MRI). MRI has become increasingly popular because of its non-invasive nature. It allows clear assessment of the internal and external uterine contour (Troiano and McCarthy, 2004).

MRI has a lot of advantages over other diagnostic tests. It has no risk of radiation that is posed by HSG (Puscheck and Cohen, 2008). Unlike ultrasound scanning, MRI is not operator dependent and it provides a large field of view. It can overcome the problem of reduced image quality that is caused by air, bone, or depth. It allows simultaneous assessment of the urinary tract associated to Müllerian duct anomalies (Mueller et al., 2007).

MRI has been reported to have 100% accuracy in the evaluation of Müllerian duct anomalies (Carrington et al., 1990, Fedele et al., 1989, Pellerito et al., 1992). T2-weighted images demonstrate the zonal anatomy of the corpus, cervix and vagina (Pui, 2004). T1-weighted images are used to identify blood products, which is a very useful in the presence of obstructed Müllerian anomalies (Olpin and Heilbrun, 2009). However, the high accuracy of MRI has been challenged (Economy et al., 2002, Letterie et al., 1995). This is likely due to the lack of classification system and diagnostic criteria. For example, there are

different reported criteria for distinction between bicornuate and septate uteri (Carrington et al., 1990, Fedele et al., 1989, Letterie et al., 1995, Pellerito et al., 1992). Therefore, more large scale studies are required to confirm the diagnostic accuracy of MRI.

MRI has a few disadvantages. It is more time consuming (Troiano and McCarthy, 2004) and expensive than HSG or ultrasound scanning (Olpin and Heilbrun, 2009). It is not suitable for patients with claustrophobia and patients with pacemakers or other implanted electronic devices (Olpin and Heilbrun, 2009, Puscheck and Cohen, 2008). Assessment of fallopian tubes is difficult on MRI (Carrington et al., 1990), which however, is available with HSG or sonohysterography (Homer et al., 2000).

1.6.6 Hysteroscopy, Laparoscopy or Laparotomy

Hysteroscopy allows direct visualisation of the uterine cavity and ostia. Therefore, it is very reliable in diagnosing the presence of intra-cavity pathologies, such as polyps, submucous fibroids, septum etc. (Homer et al., 2000, Saravelos et al., 2008). It can be performed as an office-based diagnostic test, where patient does not require general anaesthesia (Hinckley and Milki, 2004). Moreover, it can act as a diagnostic tool as well as a therapeutic technique, where removal of intrauterine adhesion, polyps, or septum can be performed (Pui, 2004, Raga et al., 2002).

However, the diagnosis of Müllerian anomaly is still based on the subjective impression of clinician performing the procedure and no

specific strict criteria is available at present (Woelfer et al., 2001). It is not possible to assess tubal patency or evaluate the presence of non-communicating anomalies. Besides, even though it is good in detecting congenital uterine anomalies, hysteroscopy is poor in differentiating the anomalies as it is unable to provide any information on the external uterine contour. Therefore, hysteroscopy is often combined with laparoscopy and very rarely laparotomy to diagnose and differentiate Müllerian anomalies, which many authors consider these combinations to be the gold standard in assessing Müllerian duct anomalies. However, these investigations are invasive and require general anaesthesia (Pui, 2004, Raga et al., 2002, Saravelos et al., 2008). Therefore, a new and ideal gold standard test is needed. Not only the test needs to be accurate and safe, it should allow objective or measurable assessments and classification of the uterine anomalies based on strict diagnostic criteria. It is important to remember that classification of these anomalies is only useful if they can be correlated with clinical and reproductive outcomes.

1.7 Management principles

1.7.1 Conservative Management

A lot of women with Müllerian anomalies are asymptomatic and have no sexual or reproductive difficulties. Therefore, treatments are mainly reserved for those who have pain or reproductive and sexual difficulties. However, it is essential to evaluate possible associated renal anomalies.

However, some clinicians consider women with Müllerian anomalies are high risk for spontaneous late miscarriage or preterm delivery (Airoldi et al., 2005, Berghella et al., 2007, Roberts et al., 1995) and hence often managed under high-risk obstetric care (Ludmir et al., 1990) during pregnancy period.

1.7.2 Medical Treatment

There are very few medical treatments available for Müllerian anomalies as most treatments aim to relieve obstruction or to restore normal anatomy of the female genital tract surgically.

There were some studies that reported the use of oestrogen treatment to improve uterine development in hypoplastic uteri (Cekanski and Peteja, 1980, Chalmers, 1963, Field-Richards, 1955). A few studies have shown a reduction in preterm delivery from progesterone administration in order to reduce myometrial contractility in women with congenital uterine anomalies (Anderson et al., 2009, da Fonseca et al.,

2003, Meis et al., 2003). However the efficacy of these medical treatments is doubtful.

1.7.3 Surgical Treatment

Surgical treatments are generally offered to those with pain or reproductive and sexual difficulties. The treatment varies according to the presenting anomaly.

1.7.3.1 Müllerian Agenesis

The main reason for most patients with Müllerian agenesis undergo surgical treatment is to restore normal sexual function. Surgical creation of neovagina can be offered to those with congenital absence of vagina (American College of Obstetrics and Gynecology, 2002). However, non-surgical creation of the neovagina with the use of successive dilators should be the first-line approach but patients need to be highly motivated.

The reproductive outcome in women with uterine agenesis is obviously poor; however, since these women do have ovaries, they can have their own genetic children through assisted reproductive techniques with embryo transfer to gestational carriers or surrogates (Damario, 2002). However, in recent years, there had been successful cases in uterus transplantation (Brannstrom et al., 2014, Ozkan et al., 2013) which had brought new hope to women with complete uterine agenesis to achieve reproductive ability (Brannstrom et al., 2015, Erman Akar et al., 2013).

1.7.3.2 Vaginal Septums and Impeforate Hymen

Longitudinal vaginal septum can be either partial or complete. Complete septal excision (Gell, 2003) and approximation of the vaginal tissue is performed in these patients. Careful assessment is required to check for two uterine cavities. As for transverse vaginal septum, surgical repair to excise the septum and to relieve obstruction is needed. The surgical technique is dependent upon septal thickness (Breech and Laufer, 1999).

Treatment of imperforate hymen involves a cruciate incision of the hymenal membrane to release the obstructed haematocolpos. The hymen is then excised and the vaginal mucosa re-approximated using delayed absorbable suture (Croak and Gebhart, 2005, Gell, 2003).

1.7.3.3 Other Müllerian Anomalies

Obstructed uterine anomalies normally present with worsening dysmenorrhoea or pelvic mass (Breech and Laufer, 1999). Patients with obstructed uterine horn will often require surgical removal through laparoscopy or laparotomy (Gell et al., 1998). Rudimentary horn has poor distensibility and muscle mass; hence there is an increased risk of rupture (Nahum, 2002) if pregnancy occurs in the rudimentary horn. Therefore, some clinicians may recommend elective removal of rudimentary horn to prevent pregnancy from developing in the rudimentary horn (Gell, 2003) even if they were found incidentally.

Surgical treatment for other non-obstructed uterine anomalies has raised a lot of debates. Septate uterus can be corrected by hysteroscopic or abdominal metroplasty (Homer et al., 2000). However, surgical correction of other non-obstructed uterine anomalies can only be performed by abdominal metroplasty (Breech and Laufer, 1999). These surgeries aim to create a normal uterine cavity. Abdominal metroplasty is associated with a number of complications and risks, including prolonged hospital stay, long recovery period, post-operative adhesions, and risk of uterine rupture during subsequent pregnancy etc. (Ayhan et al., 1992, Homer et al., 2000, Lourdel et al., 2007). In view of these potential problems, abdominal metroplasty is rarely performed nowadays.

There are many observational studies that suggested an improved reproductive outcome following surgical treatment (Ayhan et al., 1992, Grimbizis et al., 2001, Homer et al., 2000, Mollo et al., 2009, Valli et al., 2004), however, there has not been a randomised controlled trial to address the risk and benefit of these surgical treatments.

1.8 Systematic Review and Meta-analysis in Medical Research

Müllerian duct anomalies have been known for years; however there are still a lot of uncertainties in research and clinical practice. It is therefore useful to use systematic reviews and meta-analysis to gather more knowledge of this topic. Systematic review will hopefully provide us with a qualitative review or summary of the current literature on congenital uterine anomalies. Meta-analyses will then combine the quantitative results from studies in systematic review by means of formal statistical methods, to provide a summary result. Systematic reviews may or may not include a meta-analysis depending on the suitability of available data.

Here, we describe the benefits and limitations of systematic reviews and meta-analysis. Only after considering all the advantages and disadvantages, I have decided to use systematic reviews and meta-analyses in this PhD to explore the literature comprehensively.

1.8.1 Strengths of Systematic Review and Meta-analysis

Systematic reviews are a rigorous and transparent form of literature review. They use explicit, fixed, and reproducible methods to systematically search, critically appraise, and synthesize results of multiple primary studies by using strategies that reduce biases and

random errors (Gopalakrishnan and Ganeshkumar, 2013). Clearly this process separates the insignificant, bias and redundant studies in the literature from the critical studies that are worthy of attention. (Mulrow, 1994)

Due to explicit methods, when carried out well, they provide reliable estimates and accurate conclusions addressing a specific clinical question. The presented summary allows the readers to take account of the whole range of relevant findings from research on a particular topic. The results can help to establish whether the findings are consistent and generalizable across populations, treatment effect variations, or variations in particular subgroups (Garg et al., 2008).

Considering the heterogeneous nature and diversity of multiple studies across the topic, systematic review will provide an interpretive context not available in any one study (Mulrow, 1994). The diversity of subjects or treatment variations will hopefully permit a more global solution to a question or hypothesis (Bartolucci and Hillegass, 2010).

Systematic review is an efficient scientific method. Even though some reviews can be time consuming, they are usually quicker and less costly than performing a larger confirmatory study, especially if the evidence from many studies is available, properly handled statistically, and the meta-analysis will provide a sufficiently conclusive result. Meta-analyses are such that most sources of bias, inconsistencies, and heterogeneity can be statistically examined and sensitivity of statistically significant results can be measured (Bartolucci and Hillegass, 2010).

Finally, systematic reviews can be used to identify knowledge gaps and highlight methodological weaknesses or biases in various studies. This is particularly useful for topics such as Müllerian duct anomalies, which are uncommon. Systematic reviews hence will clarify future research priorities and improve methodological qualities in future studies.

1.8.2 Limitations of Systematic Reviews and Meta-analysis

Even though systematic review and meta-analysis are frequently considered the best evidence in answering research question, there are limitations and flaws associated with it. As with other publications, the quality of systematic reviews and meta-analysis varies depending on what was done, what was analysed and clarity of reporting.

Systematic reviews require access to a wide range of literature databases and peer-reviewed journals, which unfortunately can be very challenging and costly for non-academic researchers (Mallett et al., 2012). This made the searching and retrieval of full text literature difficult. Hence, sometimes not all articles necessary for meta-analysis are available and included in the reviews. Unless the data is available and consistent across studies, detection of interaction and trends can be difficult.

A critical problem for systematic review and meta-analysis is the 'file-drawer effect' or publication bias. That is, a study is conducted without a significant or desired result, and it is not published or

publication is likely to be delayed. Published studies, especially those with significant results, are more likely to be published in English, which make them more likely to be identified and included in reviews. A lot of reviews do not assess possible publication bias even though there is significant impact on the results of systematic reviews (Gopalakrishnan and Ganeshkumar, 2013).

While the methodology used in systematic review are supposed to be explicit, it is impossible to know if the steps were followed properly in reality. We always assume the evaluation techniques are consistent and objectively across studies. Strict inclusion and exclusion criteria are used to screen for potential relevant studies objectively. However, there is always subjectivity in this process. Each independent reviewer interprets those criteria slightly differently and inclusion judgments may be influenced by personal knowledge of the particular subject.

The methodological quality of studies included in reviews is important. All studies included in systematic reviews and meta-analyses should ideally be of high methodological quality and free of bias. Biases threaten the validity of studies reviewed. Inclusion of studies with high risk of bias or poor methodological qualities without appropriate weighting of their risk of bias may introduce bias in the systematic review (McDonagh, 2013). When combining studies for analysis, appropriate weighs and scoring must be considered to reduce bias.

Readers and reviewers should certainly be aware of the above issues when reading or appraising these reviews. Systematic reviews

do not always produce accurate and conclusive answers, especially if the reviews are based on small number of studies of doubtful quality.

1.9 Hypothesis and Objectives

The overall aim of the work described is to evaluate the prevalence, importance, and treatment of women with uterine malformation. We have set to examine the following hypotheses:

1. Congenital uterine anomalies are uncommon but not rare and they are more common in high risk groups.
2. Congenital uterine anomalies have negative effect on conception and abnormal reproductive outcomes.
3. Women with congenital uterine anomalies have different anatomical dimensions on three-dimensional ultrasound scans when compared to normal population.
4. Treatment of septate or subseptate uteri improves reproductive outcomes.

In order to test the hypotheses, the following objectives have to be addressed:

1. To comprehensively examine the literature on uterine anomalies to estimate their prevalence in the general population and high risk groups and to assess the degree of association between the anomalies and abnormal reproductive outcomes.
2. To prospectively evaluate the prevalence of congenital uterine anomalies using three-dimensional transvaginal ultrasound in high risk groups: women with history of miscarriage and women with preterm deliveries. The prevalence in these groups will be compared to a control group.

3. To prospectively compare the uterine and cervical dimensions between subjects with uterine anomalies and controls; on both two-dimensional linear and three-dimensional volumetric ultrasound scans.
4. Examine the effect of hysteroscopic septal resection on conception and reproductive outcomes.

Chapter 2 Materials and Methods

2.1 Introduction

The following chapter describes the generic methodologies across the research. A more specific detail of the methodology used or any changes made for each study or investigation are presented in the chapters in which the study or investigation is discussed.

2.2 Systematic Reviews

2.2.1 Identification of Literature

Articles were identified through the following electronic databases: MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, and Web of Science. A combination of Medical Subject Headings (MeSH) and text words were used to generate the list of citations (See Table 2).

Search Terms	Search Terms
Uterine anomal$	Unicornuate
Uterine abnormalit$	Bicornuate
M?llerian anoaml$	Arcuate uter$
M?llerian abnormalit$	Septate$ uter$
Uter$ agenesis	Subseptate$ uter$

Uter$ hypoplasia	Sub-septate$ uter$
Bifid uter$	T shape$ uter$
Didelphys	T-shape$ uter$
Didelphus	

Table 2: Search terms used to generate list of citations

Studies of all types of congenital uterine anomalies were included but limited to 'Humans and Female' to generate a subset of citations relevant to our research question. In addition, the reference lists of all relevant primary studies and review articles were manually searched to identify any additional cited articles not captured by the electronic searches. The searches were conducted independently by two reviewers.

2.2.2 Study Selection

Studies were selected based on a list of predefined inclusion or exclusion criteria in a two-stage process. Firstly, the titles and abstracts from the electronic searches were examined independently by two reviewers and full manuscripts of all citations that met the predefined selection criteria were then obtained. Secondly, examinations of the full manuscripts were done by the two reviewers independently to make final inclusion or exclusion decisions. In cases of duplicates, the most recent or the most complete publication was used. Any disagreements about inclusion were resolved by consensus or arbitration by a third reviewer.

2.2.3 Quality Assessment and Data Extraction

The quality of all selected papers was assessed by the two reviewers, based on different criteria set for each systematic review. From each study, relevant data were extracted by the two reviews. No ethical approval was sought for as these were systematic reviews and meta-regressions of published manuscripts.

2.3 Ultrasound Technique

Many authors consider a combination of laparoscopy or laparotomy with hysteroscopy or HSG to be the gold standard in assessing Müllerian duct anomalies (Acien, 1997, Homer et al., 2000, Taylor and Gomel, 2008). However, due to the invasiveness and subjectivity of these tests, we have instead opted to use three-dimensional ultrasound scan as the diagnostic test in our studies. Three-dimensional transvaginal ultrasound scan has high sensitivity and specificity in diagnosing and classifying congenital uterine anomalies (Grimbizis et al., 2013). It is accurate yet non-invasive and not operator dependent. In addition, the volume datasets can be stored electronically and re-evaluated later.

All ultrasound examinations were performed by the Academic Imaging Team members (Y.Y.C., K.J., J.C., S.S., L.T.P., and M.N.B.) using Voluson E8 Expert machine (GE Healthcare, Kretz, Zipf, Austria) equipped with a volumetric 5-9 MHz four-dimensional transvaginal probe used throughout the research work. All two-dimensional and

three-dimensional scans were performed using the same machine and probe. All three-dimensional volume data were acquired in a similar fashion, the details of which are described below. Ultrasound data examination was conducted using ultrasound machine or 4D View software (version 14.0; GE Kretz, Zipf, Austria).

2.3.1 Data Acquisitions

Each subject was scanned in a supine position with knees flexed and hips abducted. A baseline two-dimensional transvaginal ultrasound scan was first performed in all subjects. A probe programmed with default settings adjusted to provide the best two-dimensional grey scale image was then loaded. While the focal zone and magnification were adjusted on a case-to-case basis, to ensure the best two-dimensional image, all other settings were maintained throughout the study as follows: harmonic frequency low; power 100; gain, 2; speckle reduction imaging (SRI) 2 ; dynamic contrast, 6; and a single focal zone. A routine assessment of the pelvis was first performed to exclude any obvious pathology including adenomyosis, fibroids, endometrial polyps, ovarian cysts, and hydrosalpinges.

A three-dimensional transvaginal ultrasound scan was then subsequently performed. Once a longitudinal view of the uterus, i.e. region of interest, was obtained, the volume mode was entered. An automated slow three-dimensional sweep mode (with a sweep angle of 120º) was used for data acquisition as this provides the largest number of two-dimensional image planes, each of which is used to reconstruct

the three-dimensional dataset, and therefore the highest image quality. This automated sweep mode will automatically present reconstructed images in sagittal, transverse, and coronal planes (hence three-dimensional scan) in an objective way independently of the operator's ability. In two-dimensional ultrasound scan, the final analysis was dependent on the subjectivity criteria of the operator in the selection of the cross section images. In addition, the operator must mentally 'reconstruct' the three-dimensional structure of the uterus, in order to ascertain the shape of the uterus. This mental reconstruction is obviously subjective and dependent on the knowledge and experience of the operator.

The resultant multi-planar display was examined to ensure that the entire uterus had been included in the volume acquisition (See Figure 6). The acquisition process was repeated if there was any doubt about the quality or completeness of the dataset. Every effort was made to avoid movement artefacts by asking the subjects to remain as still as possible and by limiting movements of the transducer by the ultrasonographer. Once an adequate dataset had been acquired, volume datasets were saved to the hard drive of the ultrasound machine using a patient-specific coding system that did not include the patient's name to comply with the Data Protection Act. The stored datasets were transferred onto an external hard drive for subsequent off-line analysis.

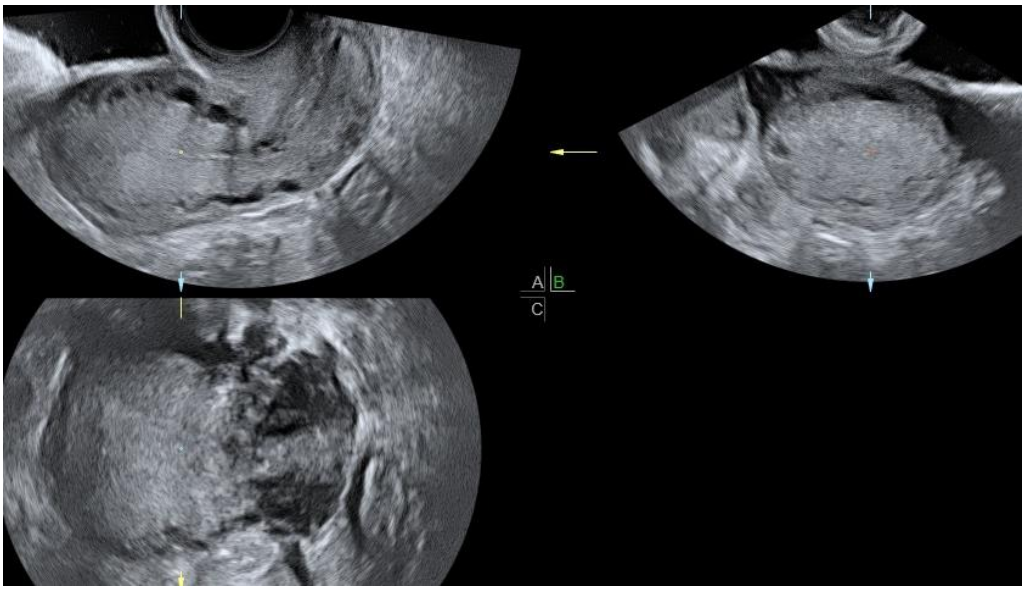


Figure 6: Three-dimensional multiplanar display of uterus.

Longitudinal view or A-plane (upper left); transverse view or B-plane (upper right) and coronal view or C-plane (lower left). Three mutually related orthogonal image planes at 90 degrees to one another. Uterine contour is clearly seen in all three planes. The yellow dot in the A-plane, the red dot in the B-plane, and the blue dot in the C-plane represent the exact same point in each image plane.

2.3.2 Ultrasound Data Examination

All analyses were made on the ultrasound machine or opened and measured using 4D View software (version 14.0; GE Kretz, Zipf, Austria) on a personal computer. This was used to assess uterine morphology or shape of uterine cavity and volume measurements.

The three-dimensional volume dataset was initially displayed in the multiplanar view (Figure 6) which simultaneously displays the longitudinal, transverse, and coronal sections of the acquired data in an orthogonal manner such that each image is at 90-degrees to the other two images (Raine-Fenning et al., 2004). The images can be rotated in any direction and the movement of one plane corresponds to movements of the other two planes in a reciprocal manner. Upon

acquisition of the volume dataset, examination of the dataset is performed in the standardised multi-planar view by adjusting the planes according to steps described by Martins et al (Martins et al., 2011). A standardised multi-planar view reduces inter-observer variation.

2.3.2.1 Assessment of Uterine Morphology or Shape of Uterine Cavity

The dataset was adjusted through the three orthogonal planes separately in all cases, to ensure that the uterine morphology was analysed in a standardized multi-planar view. The uterus was visualized with both the fundal aspect of uterus and upper endometrial cavity demonstrable in the coronal plane, with the interstitial portions of both Fallopian tubes displayed simultaneously. Standardisation of the view is essential to ensure the sensitivity and specificity of the three-dimensional technique to diagnose uterine anomalies (Salim et al., 2003). Image optimization and post-processing functions, such as Volume Contrast Imaging (VCI) could be used to optimise the ultrasound visualisation (see Figure 7). VCI improves depth perception and is particularly useful for improving assessment of the endometrial-myometrial junction (Wong et al., 2015).

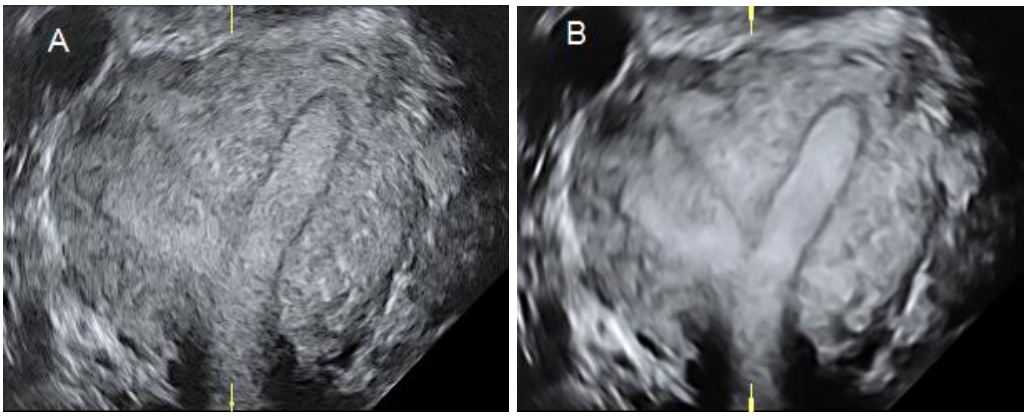


Figure 7: Example of post-processing using Volume Contrast Imaging

VCI enhances the contrast of images and thus improves visualisation of finer details. A: Three-dimensional ultrasound of a septate uterus in coronal plane. B: Three-dimensional ultrasound with volume contrast imaging of the same uterus.

The uterine morphology and shape of the uterine cavity was then assessed in the coronal plane and classified according to modified American Society for Reproductive Medicine Classification proposed by Salim et al (Table 3).

Uterine Morphology	Internal Contour	External Contour
Normal	Straight or convex	Uniformly convex or with indentation < 10mm
Arcuate	Concave fundal indentation with central point of indentation at obtuse angle (> 90 degrees)	Uniformly convex or with indentation <10 mm
Subseptate	Presence of septum, which does not extend to cervix, with central point of septum at an acute angle (<90 degrees)	Uniformly convex or with indentation <10 mm
Septate	Presence of uterine septum that completely divides cavity from fundus to cervix	Uniformly convex or with indentation <10 mm
Bicornuate	Two well-formed uterine	Fundal indentation >10

	cornua	mm dividing the two cornua
Unicornuate with or without rudimentary horn	Single well-formed uterine cavity with a single interstitial portion of Fallopian tube and concave fundal contour	Fundal indentation >10 mm dividing the two cornua if rudimentary horn present

Table 3: Criteria for Classification of Congenital Uterine Anomalies (Salim et al., 2003)

2.3.2.2 Length Measurement

Uterine cavity length is measured in the longitudinal axis from the fundus to the internal cervical os (measured following the curve of endometrium on scan). Cervical length is measured in longitudinal axis from the internal cervical os to the external cervical os.

2.3.2.3 Volume Measurement

Uterine body volume, cervical volume and endometrial volume were estimated with off-line processing using Virtual Organ Computer-aided Analysis (VOCAL^{TM}; GE Medical Systems, Zipf, Austria). VOCAL^{TM} allows the user to rotate the dataset manually through 180° about a fixed axis. Manual contour mode of measurement was selected to allow manual tracing. Once the contour of the object of interest was drawn, the VOCAL^{TM} program automatically generated the three-dimensional reconstructed object and calculated its volume (Figure 8).

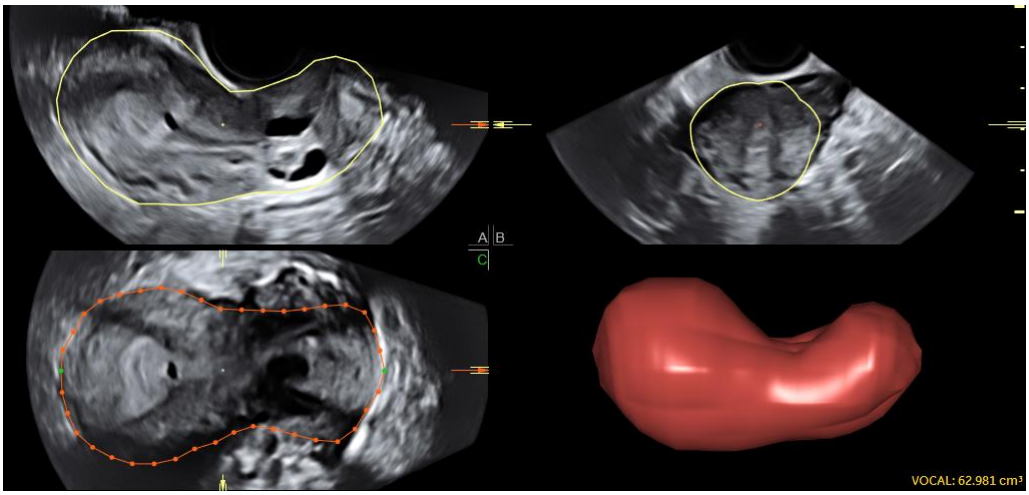


Figure 8: Full uterine volume estimation using VOCAL™

Uterine Body Volume

Uterine body volume measurements were performed with 30° rotation in six steps (Mansour and El-Shalakany, 2012, Yaman et al., 2003) with 1-mm shell thickness starting by the coronal plane. The coronal plane was centralised and the points of start of manual tracing for uterine body volume were positioned at the top of the uterus and at the internal cervical os.

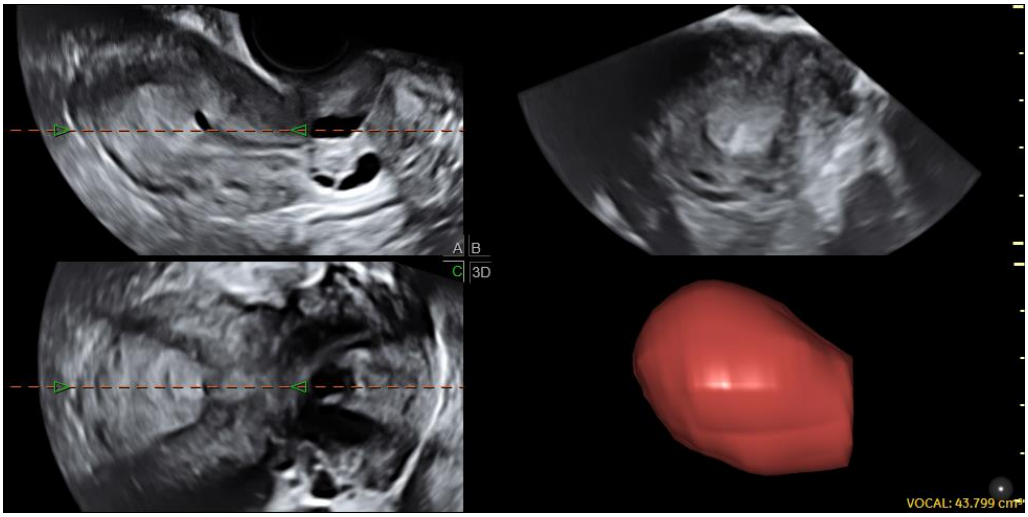


Figure 9: Uterine body volume measurement

Notice the points of start of manual tracing for uterine body volume were positioned at the top of the uterus and at the interval cervical os (In both A-plane and coronal plane)

Myometrial Volume of Uterine Body

In order to assess the volume of the muscular layer of the uterine body, one needs to deduct the endometrial volume from uterine body volume.

Cervical Volume

There were two ways of measuring cervical volume. First option was to deduct uterine body volume from full uterine volume. Second option was to generate cervical volume using techniques similar to uterine body volume measurements (Figure 10), where calculation of cervical volume were performed with 30° rotation with 1-mm shell thickness starting by the coronal plane. The coronal plane was centralised and the points of start of manual tracing for cervical volume were positioned at the internal cervical os and end of the cervix.

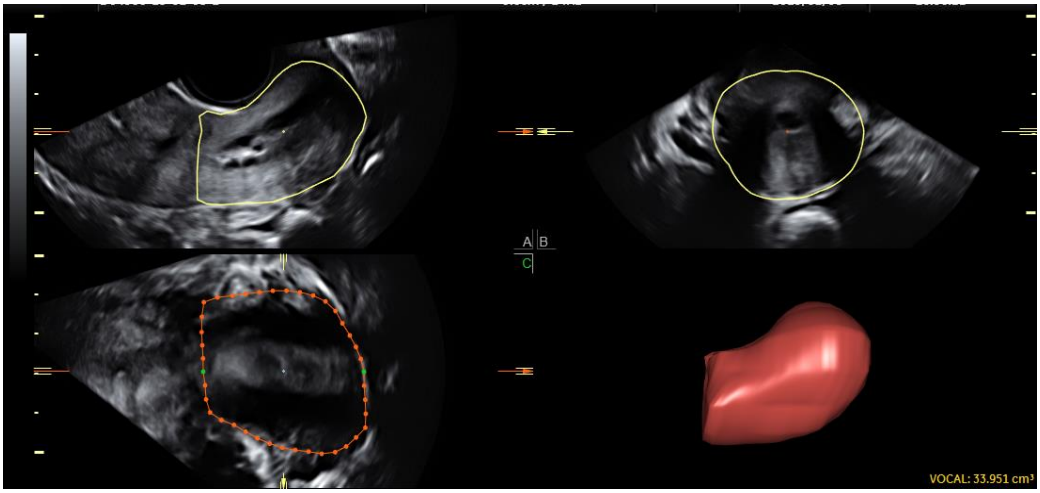


Figure 10: Cervical volume estimation

Endometrial Volume

Endometrial volume measurements were performed through delineation of the endometrium in A-plane instead of C-plane. The

endometrial-myometrial junction was more apparent in A-plane (Martins et al., 2011). A 9° rotation steps were chosen for this work as it provided the best compromised between reliability and time taken for endometrial volume measurement (Raine-Fenning et al., 2002).

2.4 Statistical Analysis

Meta-analyses to establish the prevalence of uterine anomalies in systematic review were performed using Stata 11.0 statistical software (Stata Corp, TX, USA). Log rates were pooled weighting each study by the inverse of its variance, and the summary estimates were exponentiated. Meta-regression was carried to compare the different populations. *P* values <0.05 were considered statistically significant.

Relative risks from individual studies in second and third systematic reviews were meta-analysed using fixed effects model (Mantel and Haenszel, 1959) and random effects model as appropriate (DerSimonian and Laird, 1986). Heterogeneity of the exposure effects was evaluated statistically using I^2 statistic to quantify heterogeneity across studies with I^2 values of <25%, >50% and 75% considered to reflect low, moderate and high levels of heterogeneity respectively (Higgins and Thompson, 2002). These statistical analyses were performed using Review Manager (RevMan) 5.0 (Copenhagen: The Nordic Cochrane Centre) (The Cochrane Collaboration, 2008). *P* values <0.05 were considered statistically significant.

Meta-analysis to establish agreement on diagnosis of congenital uterine anomalies, and their subtypes was performed using Stata 14.0

statistical software (Stata Corp, TX, USA). The pooled Kappa statistic (Sun, 2011) was reported. A random effects model was used for analysis. The heterogeneity across study results was quantified using the Q and I^2 statistic, with I^2 values of <25%, >50% and >75% considered to reflect low, moderate and high levels of heterogeneity respectively (Higgins and Thompson, 2002). P values <0.05 were considered statistically significant.

Other statistical analyses (apart from systematic reviews) were conducted using the Statistical Package for the Social Sciences (Version 21.0; SPSS Inc). The demographic data of the Fisher exact test or Chi-squared analysis was used to test the difference between sets of binomial variables. Continuous data distribution was tested for normality using the Kolmogorov-Smirnov or Shapiro-Wilk statistic tests. Data were presented as mean and standard deviation (SD) or median and range depending on their distribution. Differences between the two sets of data were assessed using students' t-test or Mann-Whitney test for normally distributed and skewed data respectively. Analysis of variance (ANOVA) or Kruskal-Wallis test was used to examine the differences if there were more than two sets of independent variables. A P value of <0.05 was considered significant.

Chapter 3 Results of Systematic Reviews

3.1 A Systematic Review – The Prevalence of Congenital Uterine Anomalies in High Risk Populations

3.1.1 Introduction

As stated before, the reported population prevalence rates have varied between 0.06% and 38% (Acien, 1996, Clifford et al., 1994, Guimaraes Filho et al., 2006b, Homer et al., 2000, Makino et al., 1992, Simon et al., 1991). This wide variation is likely to be due to different patient population studied, non-standardized classification systems and differences in diagnostic techniques used and the test accuracy of the methods employed. Previous reviews have not taken these factors into account when investigating the prevalence of uterine anomalies (Grimbizis et al., 2001, Acien, 1997, Nahum, 1998, Troiano and McCarthy, 2004).

We have therefore conducted a systematic review of studies evaluating the prevalence of congenital uterine anomalies in the general population and in high risk groups including women with infertility,

miscarriage and recurrent miscarriage, and preterm deliveries; and attempted to explore the inconsistencies present in the literature. This new systematic review is not only an update of the work by Saravelos et al (Saravelos et al., 2008), but also represents a different perspective on the classification of optimal and suboptimal tests. We have investigated populations which were not included by Saravelos et al.

3.1.2 Materials and Methods

3.1.2.1 Identification of Literature

On 8th November 2009, articles were identified through the following electronic databases: MEDLINE (1950 to November 2009), EMBASE (1980 to November 2009), Cochrane Central Register of Controlled Trials and Web of Science (1990 to November 2009). Studies were identified as described previously (See 2.2.1 Identification of Literature).

3.1.2.2 Study Selection

Study selection is performed independently by two reviewers (Y.Y.C and K.J.), as described in 2.2.2. Studies were selected if the incidence of any uterine anomalies were investigated. For the purpose of obtaining an overall prevalence of uterine anomalies in the general population, studies that were based upon blatantly biased patient samples (i.e. patients suspected or known to have uterine anomalies) were eliminated in favour of studies that incorporated one of a variety of universal screening techniques such as patients undergoing

laparoscopic or hysteroscopic sterilization or being investigated for non-obstetric or fertility problems. Only cohort studies and large cases series were included. Case reports and series with less than 10 cases were excluded. Studies were also excluded when the population examined or the diagnostic methods used were not accurately defined.

3.1.2.3 Quality assessment and data extraction

The quality of all selected papers was assessed by the two reviewers (Y.Y.C. and K.J.). All selected papers were assessed for the following: study design; adequate sampling (random or consecutive rather than convenient sampling); adequate description of population characteristics; completeness of information in the data sets; and use of a validated diagnostic method.

In assessing the prevalence of congenital uterine anomalies, investigators have used different diagnostic methods, some of which may be more accurate or reliable than others. In view of this, we grouped the studies into two classes according to the diagnostic accuracy of the methods used. Diagnostic methods that we accepted as 'optimal diagnostic tests' included three-dimensional transvaginal ultrasound, laparoscopy or laparotomy in conjunction with hysteroscopy or hysterosalpingography (HSG), magnetic resonance imaging (MRI) and sonohysterography. Suboptimal tests, which are able to identify and differentiate most but not all anomalies, include two-dimensional transvaginal ultrasound scan, hysteroscopy alone, HSG alone, and clinical assessment at caesarean sections.

Arcuate uterus is an anomaly where the uterine fundus displays a mild concave indentation or contour towards the uterine cavity (Salim et al., 2003). It can be difficult to diagnose or differentiate from the subseptate uterus, which in a uterus containing a more acute convex indentation that projects into the cavity but does not reach the cervix (Salim et al., 2003). Some authors believe that the arcuate uterus is a normal variant rather than a true uterine anomaly (Buttram Jr et al., 1988, Heinonen et al., 1982) but this can only be accurately assessed if the true prevalence of the anomaly can be defined and appropriate associations with relevant outcome measures assessed. This has not been achieved, to the best of our knowledge, and the presence of an arcuate uterus should be noted therefore. In view of this, studies that failed to identify or record any arcuate uteri were excluded from the subtype analysis. We were unable to determine if these studies ignored arcuate uteri or if they simply failed to identify them because of a lower sensitivity of the diagnostic tests used.

Data were extracted using a form on patients' characteristics, study quality, inclusion and exclusion criteria, reference period, diagnostic tools used, and anomalies occurrence rates. No ethical approval was sought for this study as it was a systematic review of published manuscripts.

3.1.3 Statistical Analysis

Meta-analyses were performed to establish the prevalence of uterine anomalies, and their subtypes, in each group of women. For

meta-analyses, log rates were pooled weighting each study by the inverse of its variance, and the summary estimates were exponentiated. A random effects model was used for analysis. Comparisons between the unselected population and the high-risk populations were carried out with the aid of meta-regression. Statistical analyses were performed using Stata 11.0 statistical software (Stata Corp, TX, USA).

3.1.4 Results

The search yielded 5952 citations all captured from electronic citations (See Figure 11). Of these, 1309 were excluded as they were duplicates. Another 4129 were excluded as it was clear from the title and abstract that they did not fulfil the selection criteria. Full manuscripts were obtained for the remaining 514 articles, and following scrutiny of these, we identified 87 potentially relevant studies. Three additional studies, identified from manual searches were also included, resulting in a total of 90 studies comprising 84,705 women.

Studies were grouped based on the characteristics of the different patient population, namely: unselected (i.e. the general population), infertility, miscarriage, those with both infertility and recurrent miscarriage, those undergoing in-vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) treatment, and preterm deliveries.

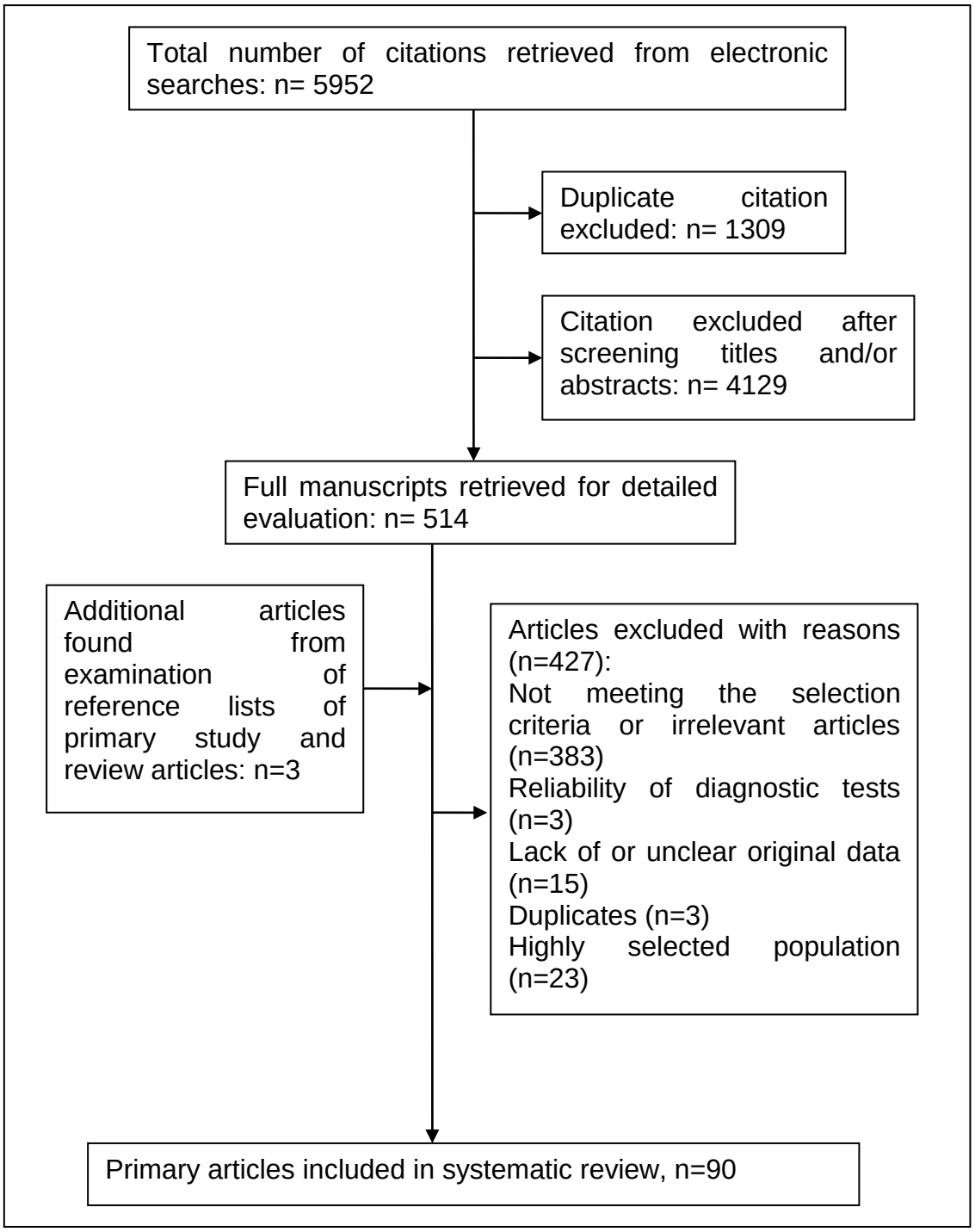


Figure 11: Study selection process for systematic review on the prevalence of uterine anomalies in unselected population and high risk populations

Out of the 90 studies included, 54 were prospective studies while 27 were retrospective studies and 9 did not define the study clearly. 75 studies had consecutive or random patient recruitment. 40 out of 90 studies used optimal diagnostic tests to diagnose congenital uterine

anomalies. Although quality varied among the studies, the majority had important limitations in study design.

Pooled prevalence rates for all uterine anomalies and various subgroups are shown in Table 4. Overall, 5.5% of the unselected population has uterine anomalies diagnosed by optimal tests. The prevalence was not increased in those with infertility (6.5%, *P*=0.498). Women with miscarriages, those with both miscarriages and infertility and those undergoing IVF were shown to have significantly higher rates of uterine anomalies (12.9%, 24.5% and 36% respectively) than the unselected population (*P*=0.045).

We also made comparisons between the unselected population and high risk groups on arcuate, subseptate or septate and bicornuate uteri. Arcuate uteri are common (3.9% in the unselected population), but the prevalence was not increased in those with infertility (1.5%) or miscarriages (2.9%) or those undergoing IVF (0.4%). When the prevalence of subseptate or septate uteri were compared between the unselected populations and high risk groups, the prevalence was approximately the same between the unselected (2.3%) and infertile (2.8%) populations, but significantly higher (*P*<0.05) in the miscarriage population (4.8%) and even higher in those with miscarriages and infertility (15.4%) and those undergoing IVF (35.6%). Bicornuate uteri are uncommon in the unselected population (0.4%), but are increased in those with infertility (1.4%), miscarriages (2.1%) and those with miscarriages and infertility (4.7%).

Population	Diagnostic test	Number of studies	Number of subjects	Prevalence of all anomalies	Arcuate	Subseptate and / or septate	Bicornuate	Unicornuate	Didelphys	Others
Unselected	Optimal	9	5163	5.5 (3.3-8.5)	3.9 (2.1-7.1)	2.3 (1.8-2.9)	0.4 (0.2-0.6)	0.1 (0.1-0.3)	0.3 (0.1-0.6)	0.1 (0-0.2)
	Suboptimal	13	50775	4.9 (2.6-9.2)	2.2 (0.9-5.2)	0.2 (0-1)	0.2 (0-0.6)	0.2 (0.1-0.7)	0.1 (0.1-0.3)	1.4 (0.7-2.9)
Infertility	Optimal	15	7442	6.5 (3.8-11.2)	1.5 (0.2-11.8)	2.8 (1.6-4.9)	1.4 (0.7-2.5)*	0.6 (0.3-1.1)	0.3 (0.2-0.5)	1 (0.6-1.9)
	Suboptimal	18	4801	7.2 (4-12.9)	5.7 (2.7-11.9)	1.9 (0.8-4.4)	1.3 (0.6-2.5)	0.5 (0.2-0.9)	0.5 (0.2-1.3)	0.3 (0.1-1.3)
Miscarriage	Optimal	5	1417	12.9 (6.7-24.8)*	2.9 (0.9-9.6)	4.8 (1.6-14.9)*	2.1 (1.4-3)*	0.5 (0.3-1.1)	0.6 (0.3-1.3)	0.1 (0-0.6)
	Suboptimal	20	4605	16.2 (12.3– 21.3)	10.1 (7.6-13.5)	3.0 (1.4 – 6.4)	2.1 (1.0-4.4)	0.5 (0.3-0.8)	0.5 (0.3-1.1)	0.9 (0.3-3)
Mixed Infertility & Recurrent Miscarriage	Optimal	9	7053	24.5 (18.3-32.8)*	6.6 (2.8-15.7)	15.4 (12.5-19)*	4.7 (2.9-7.6)*	3.1 (2-4.7)	2.1 (1.4-3.2)	0
	Suboptimal	1	66	31.8 (20.7-48.8)	0	0	0	4.5 (1.5-14.1)	0	0
IVF or ICSI Treatment	Optimal	1	267	36 (29.4-43.9)*	0.4 (0.1-2.7)	35.6 (29.1-43.5)*	0	0	0	0
	Suboptimal	11	3701	4.8 (2.7-8.6)	6.6 (4.9-8.9)	2.6 (1.2-5.6)	0.5 (0.2-1.4)	1.2 (0.6-2.5)	0.1 (0-0.4)	0.8 (0.2-3.4)

Table 4: Prevalence of Uterine Anomalies in Different Study Populations

Optimal diagnostic tests: 3-Dimensional transvaginal ultrasound scan, laparoscopy or laparotomy with hysteroscopy or HSG, MRI and sonohysterography.
Suboptimal tests: 2-Dimensional transvaginal ultrasound scan, hysteroscopy, HSG and caesarean sections.
* $P<0.05$, differences are statistically significant when comparing with unselected population

3.1.5 Discussions

This new systematic review has assessed the prevalence of uterine abnormalities in the general population and in various high-risk groups stratified according to the diagnostic accuracy of the tests used to identify and define the anomaly. The review is not only an update of the work by Saravelos et al, but also represents a different perspective on the classification of optimal and suboptimal tests. We have also sub-classified infertility by separating women requiring assisted reproduction from those that have a reduced fecundity and women with miscarriage and infertility as we felt these were separate entities. We have also considered the prevalence of uterine anomalies in women with preterm labour.

3.1.5.1 Diagnostic tests

We agree with the findings by Saravelos et al, but overall we noted a lower prevalence of all anomalies. This reflects the different viewpoints on what constitutes an optimal diagnostic test for the identification and differentiation of uterine anomalies.

In our opinion, optimal tests should be investigations that are capable of accurately identifying and classifying congenital uterine anomalies. Hence, they should be able to assess both the internal and external contours of the uterus. One would expect that suboptimal tests such as two-dimensional transvaginal scan or hysterosalpingography (HSG) in insolation have the tendency to underestimate the true

prevalence of uterine abnormalities due to their relative poorer sensitivity and specificity as diagnostic tests (Jurkovic et al., 1995, Wu et al., 1997, Andreotti et al., 2006, Guimaraes Filho et al., 2006a, Momtaz et al., 2007, Saravelos et al., 2008).

Interestingly, we found that the prevalence of all uterine anomalies detected by optimal test is similar to those detected by suboptimal tests but the distributions of different subtypes vary between the diagnostic test groups. This may reflect that all tests are sensitive in identifying anomalies but suboptimal tests are inadequate in sub-classifying the various subtypes. However, Saravelos et al has found significant differences in the prevalence of all uterine anomalies between those diagnosed by accurate tests and by less accurate tests. Yet, this meta-analysis has considered hysteroscopy alone as an accurate test but MRI is excluded from their analysis. The distension of the uterine cavity during hysteroscopy can potentially eliminate the presence of arcuate uterus. Besides, a lack of awareness of minor uterine anomalies means a lot of clinicians performing hysteroscopy do not even consider looking for minor anomalies such as arcuate or subseptate uteri. The final diagnosis is based on the subjective impression of the clinician performing the test. In addition, hysteroscopy does not allow evaluation of the external contour of the uterus and are unable to reliably differentiate a septate uterus from a bicornuate uterus.

Three-dimensional ultrasound and magnetic resonance imaging (MRI) offer a non-invasive method of investigation. Both imaging modalities were reported to have high accuracy in diagnosing

congenital uterine anomalies (Olpin and Heilbrun, 2009, Raga et al., 1996, Carrington et al., 1990, Pellerito et al., 1992, Jurkovic et al., 1995, Fischetti et al., 1995, Wu et al., 1997, Ghi et al., 2009). Reports have shown that three-dimensional ultrasound scan has high sensitivity and specificity, as high as 100% in diagnosing uterine anomalies (Deutch et al., 2006, Saravelos et al., 2008, Wu et al., 1997). In addition, they offer the ability to simultaneously assess the abdomen which is potentially important due to increased frequency of renal anomalies in patients with uterine anomalies (Li et al., 2000). Three-dimensional ultrasound is preferred by some clinicians who use it as a standard to diagnose congenital uterine anomalies over MRI (Kupesic, 2005, Deutch and Abuhamad, 2008) as MRI is more time consuming and expensive than ultrasound scanning.

Many authors consider the combination of laparoscopy or laparotomy with hysteroscopy or HSG to be the gold standard in assessing congenital uterine anomalies (Hamilton et al., 1998, Acien, 1997, Homer et al., 2000). However, the final diagnosis is based on the subjective impression of the clinician performing the test and in many cases simultaneous views of the external contour and upper cavity are not achieved. The distension of the uterine cavity during hysteroscopy or HSG can potentially eliminate the presence of arcuate uterus and case series limited to these tests do show a much lower prevalence of minor uterine anomalies than those that employ more accurate diagnostic modalities. Besides, the combined approach is also invasive and usually requires general anaesthesia. Whilst we include this

technique as one of the optimal tests, one could argue even this technique is still not ideal as a gold standard due to the weaknesses described. Therefore, a new gold standard test needs to be considered. In our opinion, three-dimensional ultrasound scan, a highly accurate yet non-invasive test, has the potential to emerge as the reference standard for diagnosis of congenital uterine anomalies as this technique becomes more widely available and practitioners become more experienced.

3.1.5.2 Unselected/General Population

In our review, the prevalence of all congenital uterine anomalies diagnosed by optimal tests in the unselected population/general population is 5.5%. This is higher than most literature and reviews, ranging from 0.17%-4.3% (Simon et al., 1991, Raga et al., 1997, Nahum, 1998, Homer et al., 2000, Grimbizis et al., 2001) but lower than a recent systematic review which suggested 6.7% of all women have a uterine anomaly (Saravelos et al., 2008). These differences are likely due to different diagnostic tests used and different clinical background in the study population. The prevalence of all uterine anomalies in studies employing suboptimal tests revealed a prevalence of 4.9% suggesting an under-diagnosis, which was not surprising in view of the presumed lower sensitivity of these tests.

The most common uterine anomaly diagnosed in the unselected population is the arcuate uterus (3.9%), followed by septate uterus (2.3%) and bicornuate uterus (0.4%). This is not in keeping with the

findings from other studies or reviews, which have generally found the septate uterus to be the most common (Nasri et al., 1990, Simon et al., 1991, Raga et al., 1997, Homer et al., 2000, Acien, 1997, Grimbizis et al., 2001). This discrepancy is likely to reflect the lack of a uniform system of classification and possibly the misclassification of some arcuate uteri as subseptate uteri.

Assessing the prevalence of congenital uterine anomalies in the unselected population is difficult. Many anomalies remain asymptomatic and investigations would not be warranted without specific indication. In our review, we have included studies either investigating patients undergoing sterilisation (laparoscopically or hysteroscopically) or being investigated for non-obstetric or fertility problems, such as pelvic pain, abnormal bleeding, ovarian cancer screening, suspected fibroid or ovarian cyst. Hence, the results should reflect the prevalence in the fertile and general population combined. However, the background and various presentations may affect the results. To the best of our knowledge, no studies have really assessed the true unselected population, where subjects undergoing diagnostic tests, are recruited randomly from the general public, and not those who presented to hospital for any medical reason.

3.1.5.3 Infertile Population

The effect of uterine anomalies on fertility is unclear as are the pathophysiological processes underlying any potential detrimental effect. Taylor and Gomel (Taylor and Gomel, 2008) suggested that congenital

anomalies may negatively impact on the complex processes of embryo implantation. Nahum (Nahum, 1998) reported a prevalence of 3.5% in women with infertility, which was 21 times more than the incidence of uterine anomalies in women with normal fertility. However, the author did not consider the reliability of the diagnostic modalities used. In our systematic review, the infertile population included women with primary and secondary infertility. Whilst we found that women with infertility had a slightly higher rate of uterine anomalies (6.5%), this was not statistically significant, regardless of whether the diagnosis was made using optimal or suboptimal tests. This is in agreement with several other studies that have not shown an increased frequency of uterine anomalies in women known to have infertility (Grimbizis et al., 2001, Saravelos et al., 2008, Acien, 1997).

The most common uterine anomaly seen in the infertile population was dependent upon the diagnostic test used. Suboptimal tests revealed a high prevalence of arcuate uteri than in any other subgroup but this association was not seen when optimal tests were used, which identified septate or subseptate uteri the most common uterine anomaly in the infertile population.

When optimal tests were used, the prevalence of septate uteri in the infertile population (2.8%) and the unselected population (2.3%) is similar, agreeing with the findings by Homer et al (Homer et al., 2000). On the other hand, Saravelos et al (Saravelos et al., 2008) demonstrated significant increase in prevalence of septate uteri in infertile women suggesting possible association between septate uteri

and infertility. However, they have considered hysteroscopy as a reliable test to diagnose septate or subseptate uteri. On top of that, the review has different criteria in defining their population. It has included some women with recurrent miscarriage or those undergoing IVF treatment as part of their infertile population. In our systematic review, we have also sub-classified infertility by separating women requiring assisted reproduction from those that have a reduced fecundity and women with miscarriage and infertility.

The prevalence of bicornuate uterus is relatively higher (1.4%) compared to the unselected population (0.4%). This finding is in agreement with findings by Saravelos et al (Saravelos et al., 2008) and Raga et al (Raga et al., 1997). The increased prevalence found in our review would suggest that bicornuate uterus has a potential role in infertility. On the other hand, this does not seem to be the case for arcuate uterus. The pooled result of our data shows that the prevalence of arcuate uterus in the infertile population is similar to that of the unselected population. Large scale observational and prospective cohort studies are required to investigate the relationship between different subtypes of congenital uterine anomalies and infertility.

3.1.5.4 IVF or ICSI Treatment Population

Studies investigating women requiring IVF/ICSI treated population included all patients irrespective of the cause of their infertility. It was not possible to analyse according to the cause and to separately calculate the prevalence in couples with isolated male factor

for example, which would have been interesting and potential control, as no studies provided individual data on this. Furthermore, several studies only included patients with previous unsuccessful IVF/ICSI cycles (Radoncic and Funduk-Kurjak, 2000, Ayida et al., 1997, Rama Raju et al., 2006, Shokeir and Abdelshaheed, 2009) but the duration and degree of infertility, as well as the underlying cause, may also influence the prevalence of uterine anomalies. The results are further compounded by the fact that only one study used an optimal test (Radoncic and Funduk-Kurjak, 2000). The data are limited therefore and we urge caution in their interpretation despite significantly higher prevalence of uterine anomalies (36%) in this group of women.

3.1.5.5 Infertile and Recurrent Miscarriage Population

Studies in this group included those investigating women who suffered from both infertility and recurrent miscarriage. In this review, we included 10 studies which investigated this population. This population of women have a higher prevalence of uterine anomalies overall compared with the general population. However, it is extremely difficult to know if the higher prevalence is due to the varying definitions for recurrent miscarriage or infertility; cumulative number of miscarriages in each study; duration of infertility; cause of infertility; or due to the fact that these women suffered from both infertility and recurrent miscarriage. It was not possible to separate these populations and to separately calculate the prevalence individually as these studies

did not provide individual data on this. Some previous reviews have actually included these studies into infertile population or recurrent miscarriage populations (Grimbizis et al., 2001, Nahum, 1998, Saravelos et al., 2008). In our opinion, however, these women had two clinical problems and should not be included into the infertile population or the miscarriage population.

Same as the other high risk groups, the most commonly seen anomaly is septate uterus (15.4%). The prevalence of septate uterus in this population is significantly higher than the unselected population. Once again, the impact of septate uterus on reproductive outcomes should not be underestimated. High risk patients would most likely benefit from investigations for diagnosing congenital uterine anomalies.

3.1.5.6 Miscarriage Population

The estimated prevalence of uterine anomaly diagnosed by optimal tests in this population is 12.9% and is consistent with the literature (Grimbizis et al., 2001, Saravelos et al., 2008, Raga et al., 1997). The prevalence of anomaly increases to 16.2% when suboptimal tests are used but this is most likely to reflect a lack of specificity and an over-diagnosis.

We appreciate that studies included had different inclusion criteria used by investigators. Most of the studies did not provide clear data if miscarriages occurred during the first of second trimester. They are also different in miscarriage pattern (Consecutive versus non-consecutive) and number of previous miscarriages. It is important to

note that most of the studies included in this review investigated women with two or more miscarriages (Dendrinos et al., 2008, Ghi et al., 2009, Guimaraes Filho et al., 2006b, Raga et al., 1997, Weiss et al., 2005) and the results are not directly applicable to women with previous live birth as this was not assessed or it was not reported as a separate group by any author.

The prevalence of congenital uterine anomalies in women with 2 or more miscarriages is similar to those with 3 or more miscarriages, regardless of diagnostic tests. This is supported by Weiss et al (Weiss et al., 2005) and Saravelos et al (Saravelos et al., 2008) findings. This suggests that women with history of two miscarriages may deserve investigations for diagnosis of a congenital uterine anomaly.

Septate or subseptate uterus is the most commonly seen anomalies in women with miscarriages (4.8%), followed by arcuate uterus (2.9%) then bicornuate uterus (2.1%). However, Saravelos et al suggest that the arcuate uterus is the commonest. This difference is likely to reflect the differences diagnostic tests and possibility the misclassification of some arcuate as subseptate uteri.

The observed prevalence of arcuate uterus in the miscarriage population does not vary greatly from the findings for the unselected population. However, the prevalence of septate uterus and bicornuate uterus in this population are significantly higher than the unselected population. This suggests contributory relations between these types of anomalies and miscarriages. Several studies have shown that these

two types of anomalies are associated with increased risk of miscarriage (Acien, 1993, Shuiqing et al., 2002, Woelfer et al., 2001).

3.1.5.7 Women with Preterm Deliveries

Preterm labour has many aetiologies but congenital anomalies have been suggested as one potential cause. Putative mechanisms include cervical incompetence (Airoldi et al., 2005, Berghella et al., 2007, Roberts et al., 1995), abnormal uterine contractions (Dabirashrafi et al., 1995) and reduced uterine volume (Braun et al., 2005, Pellerito et al., 1992, Puscheck and Cohen, 2008, Reuter et al., 1989). Unfortunately despite these links, no appropriate studies investigating the prevalence of uterine anomalies in women with preterm delivery were identified in the search.

3.1.5.8 Distributions of Congenital Uterine Anomalies

Based on optimal tests only, the most commonly diagnosed anomaly is arcuate uterus in the unselected/general population. However, it is no more prevalent in any high risk group than the general population. Major uterine anomalies (Bicornuate, unicornuate, and didelphys) are generally more prevalent in all high risk groups. Septate uterus is more prevalent in high risk groups with the exception of the infertile population. However, it is important to note that some of the septate uteri may have been diagnosed as arcuate uteri and vice versa. Equally, some septate uteri, particularly large septate extending to the

cervix may have been misdiagnosed as bicornuate uterus in some of these tests.

3.1.5.9 Limitations

Our systematic review's strengths include its extensive electronic and manual search approach. However, it is limited by the retrospective nature of the analysis and heterogeneity of the patient population and diagnostic tests. We were unable to obtain all the clinical information of all the women. We included all studies that meet the selection criteria but did not exclude studies on the basis of inadequate quality. We addressed the problem of clinical heterogeneity by analysing different patient population separately and the results are strengthened by analysing the two groups of diagnostic tests used (suboptimal or optimal testing).

There was clearly a lack of uniformity with the classification of uterine anomalies in the studies included. Most commonly used classification is the American Society for Reproductive Medicine Classification (1988) but it does not specify the diagnostic methods that should be used for diagnosis. In fact, the final diagnosis is based on the subjective impression of the clinician performing the test (Woelfer et al., 2001).

3.1.5.10 Future Work or Recommendations

There are a lot of different diagnostic tests available. In our opinion, three-dimensional ultrasound should be used as a standard in

view of the relatively low cost, non-invasive approach and high accuracy of the test. However, a well-designed test accuracy study is required to determine the best investigation for diagnosis of uterine anomaly.

Salim et al has proposed a modified classification in which the diagnostic criteria used were more detailed than previously described and they included cut-offs levels for the fundal shape and distortion (Salim et al., 2003). These cut-offs were necessary to differentiate uterine anomalies with similar morphological features such as subseptate and arcuate uteri. In the future, this classification should be used as standard to describe uterine anomalies.

Some studies have reported associations between congenital uterine anomalies and poor reproductive outcomes (Acien, 1993, Woelfer et al., 2001, Zupi et al., 1996, Zlopasa et al., 2007). However, further large observational and prospective studies are essential to investigate the reproductive impact of different subtypes of congenital uterine anomalies.

Treatments, such as hysteroscopic resection of uterine septum, have been suggested to improve the reproductive outcomes in these patients (Heinonen, 1997, Maneschi et al., 1993, Valli et al., 2004). However, removal of septum is not without risk and by definition involves damage to the endometrium which must be transected to access the myometrium. Whilst some observational studies have reported an improved outcome following surgical intervention (Homer et al., 2000, Mollo et al., 2009, Taylor and Gomel, 2008), there is a need

for randomised controlled trials to address the effectiveness and safety of such treatment.

3.1.6 Conclusions

In this review, we found that the prevalence of uterine anomalies diagnosed by optimal tests was 5.5% in the unselected population, 6.5% in infertile women, 12.9% in those with miscarriages, 24.5% in those with miscarriages and infertility, and 36% in those undergoing IVF. This concludes that overall congenital uterine anomalies are more prevalent in high risk groups with the exception of the infertile population. The most common anomalies were arcuate uterus in the unselected population and septate uterus in all high risk groups. Major congenital uterine anomalies are more common in the high risk groups than the unselected population which would suggest a causal role in poor reproductive outcomes. The high prevalence of septate or subseptate uteri in high risk population should not be underestimated. It has been suggested that resection of septum improved pregnancy outcome.

3.2 The Reproductive Outcomes in Women with Congenital Uterine Anomalies: A Systematic Review

3.2.1 Introduction

Normal development of the female reproductive tract involves a series of complex processes to form the internal genital tract. When an interruption or dysregulation occurs in any of the dynamic processes of differentiation, migration, fusion, and canalization, a wide spectrum of Müllerian duct anomalies can result. At one end of the spectrum, Müllerian agenesis, characterized by a failure of Müllerian ducts to develop and therefore congenital absence of uterus, has obvious implications for childbearing whilst at the other, the arcuate uterus, characterised by a mild concave indentation or contour towards the uterine cavity, is often thought to represent a normal variant (Heinonen et al., 1982, Buttram Jr et al., 1988) and be of little clinical significance. Other subtypes of uterine anomaly between these two extremes can be categorized by their abnormal embryological development processes; abnormal fusion or unification of Müllerian ducts resulting in unicornuate or bicornuate uterus or didelphys and incomplete canalization or resorption of the midline septum results in a septate or subseptate uterus.

All types of congenital uterine anomalies have long been recognized as a potential cause of poor reproductive outcomes including infertility (Tulandi et al., 1980, Raga et al., 1997), recurrent pregnancy loss (Rackow and Arici, 2007), preterm delivery (Tomazevic et al., 2007) and fetal malpresentation (Stein and March, 1990, Rock and Schlaff, 1985). It is also generally accepted that the various types of Müllerian anomaly are individually associated with these outcomes in different ways and to variable degrees with greater effects being evident in women with more profound defects (Golan et al., 1989, Green and Harris, 1976, Grimbizis et al., 2001, Heinonen et al., 1982, Homer et al., 2000, Lin et al., 2002, Reichman et al., 2009, Stein and March, 1990, Tantini et al., 1996). The evidence for the association between uterine anomalies and abnormal reproductive outcome is, however, unclear as is the effect of the specific type of uterine anomaly, which is important as there are different treatment options available. In view of this, we conducted a systematic review to evaluate the association between the different subtypes of uterine anomaly and various reproductive outcomes. This will be the first systematic review that has assessed the reproductive and obstetric impacts of different subtypes of congenital uterine anomaly by comparing to appropriate control groups. We have also grouped the subtypes of anomalies according to their abnormal embryological development processes, as they may reflect the underlying pathophysiology of poor reproductive outcomes.

3.2.2 Materials and Methods

3.2.2.1 Identification of Literature

Articles were identified through the following electronic databases: MEDLINE (1950 to November 2009), EMBASE (1980 to November 2009), Cochrane Central Register of Controlled Trials and Web of Science (1990 to November 2009). Studies were identified as described previously (See 2.2.1 Identification of Literature).

3.2.2.2 Study Selection

Study selection is performed independently by two reviewers (Y.Y.C and K.J.), as described in 2.2.2 Study Selection. To be eligible for inclusion, studies had to be observational studies or randomised controlled trials comparing the reproductive outcomes between women with known congenital uterine anomalies as the study group and women with no anomalies as the control group. Studies without a control group were not included. Studies involving women with multiple congenital anomalies were excluded as were studies with less than 10 cases. We recorded on the rates of clinical pregnancy, first trimester miscarriage, second trimester miscarriage, preterm birth, and malpresentation at birth.

3.2.2.3 Quality assessment and data extraction

Two reviewers (Y.Y.C. and K.J.) completed the quality assessment. The Newcastle-Ottawa Quality Assessment Scale for

observational studies was implemented (Wells et al., 2000). Items assessed included selection of cases/cohorts and controls, comparability of study design and analysis, outcomes assessment and finally adequacy of follow up. We used allocation of 'stars' for each item included in the Newcastle-Ottawa Quality Assessment Scale to give a quantitative appraisal of overall quality of the individual studies (Wells et al., 2000). A maximum of nine stars can be achieved by any studies. A median score of 6 stars was used to distinguish moderate and high quality studies from poorer quality studies (Allen et al., 2009). From each study, outcome data were extracted in 2 x 2 tables by the two reviewers Y.Y.C. and K.J.

3.2.3 Statistical Analysis

Relative risks from individual studies were meta-analysed using fixed effects model (Mantel and Haenszel, 1959) and random effects model as appropriate (DerSimonian and Laird, 1986). Further analyses relating to different subtypes of unification defects (bicornuate uterus, didelphys, and unicornuate uterus) were also conducted. Heterogeneity of the exposure effects was evaluated graphically using forest plots (Lewis and Clarke, 2001) and statistically using I^2 statistic to quantify heterogeneity across studies with I^2 values of <25%, >50% and 75% considered to reflect low, moderate and high levels of heterogeneity respectively (Higgins and Thompson, 2002). Exploration of the causes of high levels of heterogeneity was planned using variation in features of population, exposure, diagnostic methods, study design, and study

quality. Statistical analyses were performed using Review Manager (RevMan) 5.0 (Copenhagen: The Nordic Cochrane Centre) (The Cochrane Collaboration, 2008). *P* values <0.05 were considered statistically significant.

3.2.4 Results

The search yielded 5952 citations all captured from electronic citations (Figure 12). Of these, 1309 were excluded as they were duplicates. Another 4129 were excluded as it was clear from the title and abstract that they did not fulfil the selection criteria. Of the remaining 514 articles, we obtained full manuscripts, and following scrutiny of these, we identified six potentially relevant studies. 508 articles were excluded because they did not meet the selection criteria, lacked original data (e.g. review articles or letters), were duplicates and/or the same data were used in other included studies. One additional study, identified from the manual search, was also included. A total of seven studies (Acien, 1993, Maneschi et al., 1995, Shuiqing et al., 2002, Sorensen and Trauelsen, 1987, Woelfer et al., 2001, Zlopasa et al., 2007, Zupi et al., 1996), comprising 2989 patients, were finally selected for this review.

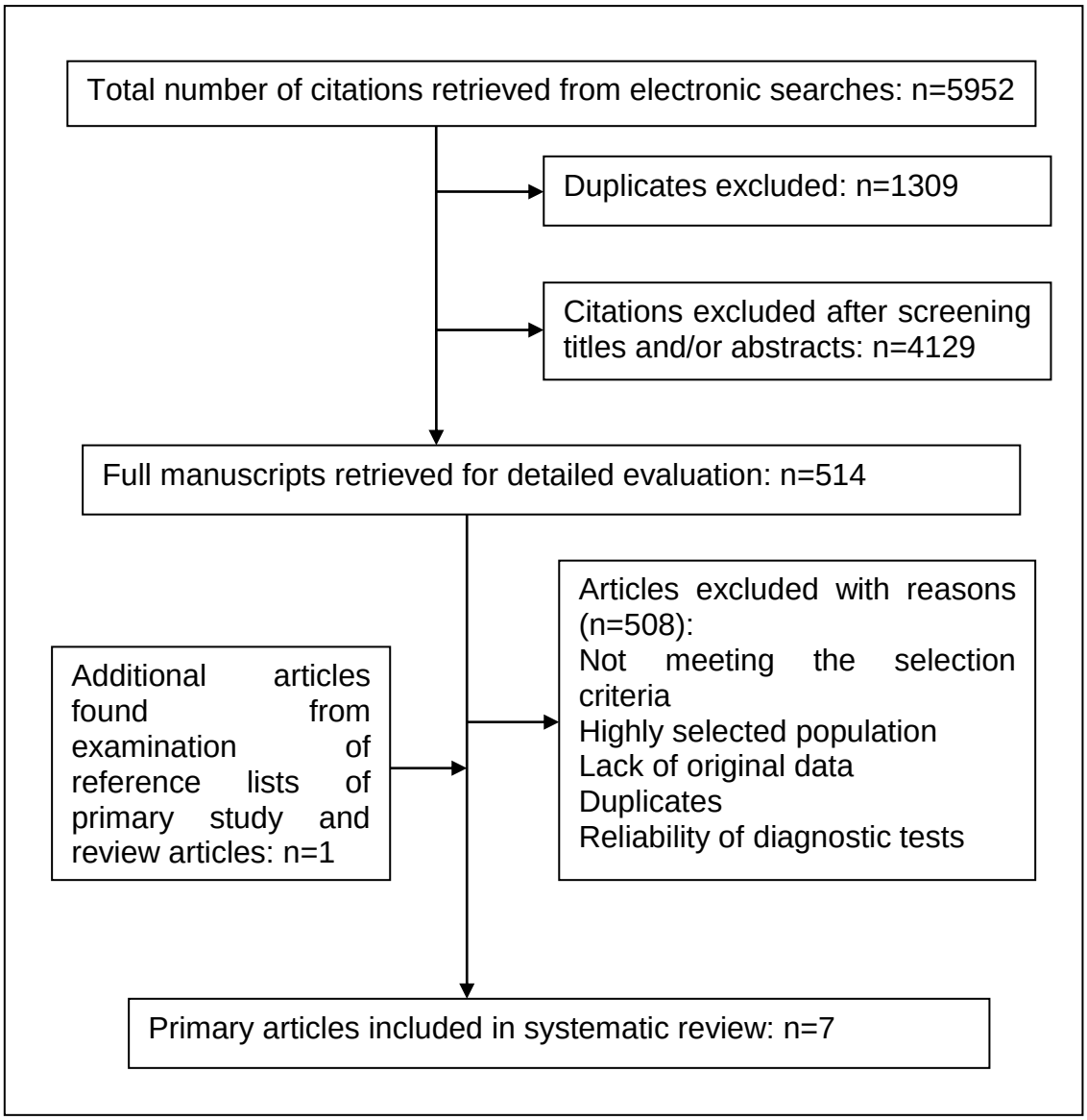


Figure 12: Study selection process for systematic review on the effect of congenital uterine anomalies on reproductive outcome

Study	Types of study	Inclusion criteria	Exclusion criteria	Study groups	Types of anomalies	Outcomes measured
Sorensen & Trauelsen (1987) (n=129)	Retrospective comparative study	Consecutive infertile women with minor uterine anomaly on HSG and consecutive controls without anomaly on HSG	Women with major uterine anomaly on HSG	Study group (n=37) Controls (n=92)	Arcuate uteri (n=37)	Ectopic pregnancy rate Miscarriage rate Preterm birth rate Term birth Caesarean section rate
Acien (1993) (n = 187)	Retrospective cohort study (with some prospective analysis)	Women with uterine anomaly on HSG and controls with urinary anomalies but normal uterus on HSG	Women with Rokitansky syndrome, hypoplastic uterus and incomplete case studies	Study group (n=161) Controls (n=26)	Arcuate uteri (n=38) Unification defects (n=94) Canalization defects (n=29)	Clinical pregnancy rate Ectopic pregnancy rate Miscarriage rate Preterm birth rate Term birth rate Live birth rate Rate of malpresentation at birth Caesarean section rate

Study	Types of study	Inclusion criteria	Exclusion criteria	Study groups	Types of anomalies	Outcomes measured
Maneschi et al. (1995) (n = 309)	Prospective comparative study	Women undergoing hysteroscopy for abnormal uterine bleeding with uterine anomalies and controls with normal uterus	Women not attempted pregnancy, presence of submucous myoma and incomplete medical history	Study group (n=21) Controls (n=288) Matched for age, duration of the menstrual cycle, age at first pregnancy, number of pregnancies	Arcuate uteri (n=21) Unicornuate (n=1) Septate/bicornuate (n=12)	Ectopic pregnancy rate Miscarriage rate Preterm birth rate Term birth rate Rate of malpresentation at birth Caesarean section rate
Zupi et al. (1996) (n = 814)	Retrospective comparative study	Women undergone hysteroscopy for reasons other than infertility with uterine anomalies and consecutive controls with normal uterus	Women with ongoing pregnancy, presence of submucous fibroid or synaechiae, have other reasons for reproductive failure	Study group (n=51) Controls (n=763)	Arcuate uteri (n=49) Unification defects (n=2)	Miscarriage rate Preterm birth rate Term birth rate

Study	Types of study	Inclusion criteria	Exclusion criteria	Study groups	Types of anomalies	Outcomes measured
Woelfer et al. (2001) (n = 1084)	Prospective comparative study	Women undergoing Three-dimensional transvaginal ultrasound with uterine anomalies and controls with normal uterus	Women with ongoing pregnancy, history of infertility or recurrent miscarriage, presence of uterine fibroids that distorted the cavity, previous myomectomy	Study group (n=101) Controls (n=983)	Arcuate uteri (n=72) Canalization defects (n=29)	Clinical pregnancy rate Miscarriage rate Preterm birth rate Term birth rate
ShuiQing et al. (2002) (n = 154)	Retrospective cohort study	Women known to have uterine anomalies and controls with other urogenital malformation but normal uterus		Study group (n=128) Controls (n=26)	Unification defects (n=96) Canalization defects (n=32)	Clinical pregnancy rate Miscarriage rate Preterm birth rate Term birth rate Rate of malpresentation at birth Caesarean section rate

Study	Types of study	Inclusion criteria	Exclusion criteria	Study groups	Types of anomalies	Outcomes measured
Zlopasa et al. (2007) (n = 312)	Retrospective cohort study	Women known to have uterine anomalies and randomly selected matched controls with normal uterus	Other causes for adverse pregnancy outcome and IVF pregnancies	Study group (n=130) Controls (n=182) Matched for age and number of previous pregnancies	Uncertain of the specific numbers for each anomaly but contained arcuate uteri, unification defect and canalization defects	Miscarriage rate Preterm birth rate Term birth rate

Table 5: Characteristic of studies of uterine anomalies versus normal uteri on reproductive outcomes

Study	Case-cohort representative	Selection of non-exposed control	Ascertainment of exposure	Outcome negative at start	Comparability by design	Comparability by analysis	Outcome assessment	Duration of follow up	Adequacy of follow up	Total number of stars
Sorensen & Trauelsen (1987)	*	*	*	*			*	*	*	7
Acien (1993)	*	*	*	*			*	*	*	7
Maneschi et al. (1995)	*	*	*	*			*	*	*	7
Zupi et al. (1996)	*	*	*	*			*	*	*	7
Woelfer et al. (2001)	*	*	*	*		*	*	*	*	8
ShuiQing et al. (2002)	*	*	*	*			*	*	*	7
Zlopasa et al. (2007)	*	*	*	*			*	*	*	7

Table 6: Appraisal of methodological quality (Newcastle-Ottawa Scale).

A star '' is awarded for each item that the study met in the Newcastle-Ottawa Scale.*

All of the seven included studies were observational studies where all of the study and control groups were followed up to the outcomes. The main characteristics of the seven studies and the Newcastle-Ottawa Quality Assessment are presented in Table 5 and Table 6. All studies included women with different types of uterine anomaly but their diagnostic tests were different. The exclusion criteria also varied between the studies (Table 5). Although quality varied among the studies, most had limitations with design or analysis. Only one study (Woelfer et al., 2001) scored 8 on the Newcastle-Ottawa Quality Assessment Scale, the rest scoring seven.

The reproductive impacts of congenital uterine anomalies are summarized in Table 7. Detailed information of how each uterine anomaly affect different reproductive outcomes will be described in turns.

Anomaly	Conception rate	First trimester miscarriage	Second trimester miscarriage	Preterm labour	Malpresentation at birth
Arcuate	No change	No change	Increased	Increased	Increased
Septate or subseptate	Decreased	Increased	No change	Increased	Increased
Bicornuate	No change	Increased	Increased	Increased	Increased
Didelphys	No change	No change	No change	Increased	No change
Unicornuate	No change	Increased	No change	Increased	Increased
Overall Unification defects	Decreased	Increased	Increased	Increased	Increased

Table 7: Reproductive impact of various congenital uterine anomalies

3.2.4.1 Clinical Pregnancy Rate

Three of the seven studies reported clinical or pregnancy or conception rate as an outcome.

Pooled results from two of these studies (Acien, 1993, Woelfer et al., 2001) showed no significant difference in terms of clinical pregnancy rate between women with an arcuate uterus and women with normal uteri (RR=1.03, 95% CI: 0.94-1.12, *P*=0.51, Figure 13). The I^2 value was 63% indicating a moderate level of heterogeneity between studies both of which were of moderate to high quality. The study by Woelfer et al (Woelfer et al., 2001) used three-dimensional ultrasound, generally considered to be a better diagnostic test for uterine anomalies than hysterosalpingography used by Acien (Acien, 1993).

Meta-analysis of the three studies (Acien, 1993, Shuiqing et al., 2002, Woelfer et al., 2001) in which canalization defects were described showed a 14% reduction in clinical pregnancy in women with canalization defects or septate uteri (RR=0.86, 95% CI: 0.77-0.96, *P*=0.009, Figure 14). An I^2 value of 0% indicated statistical homogeneity across these studies.

Pooled analysis of the two studies (Acien, 1993, Shuiqing et al., 2002) in which unification were investigated showed a 13% reduction in clinical pregnancy in women with all unification defects (RR=0.87, 95% CI: 0.76-0.99, *P*=0.03, Figure 15). There was inconsistency between the studies as indicated by an I^2 value of 67% and both studies had small control groups. When further analyses were performed looking at the

different types of unification defects, there was a trend towards a reduction in clinical pregnancy rate in all subtypes though not to the point of statistical significance.

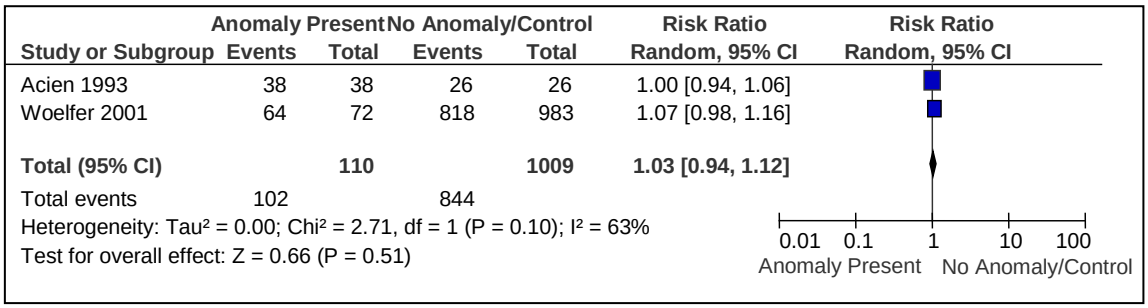


Figure 13: Studies of women with an arcuate uterus versus women with normal uteri for the outcome of clinical pregnancy rate

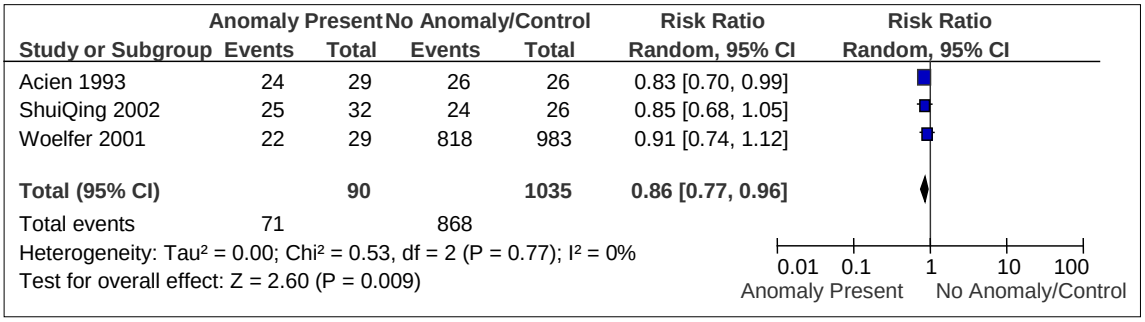


Figure 14: Studies of women with canalization defects versus women with normal uteri for the outcome of clinical pregnancy rate

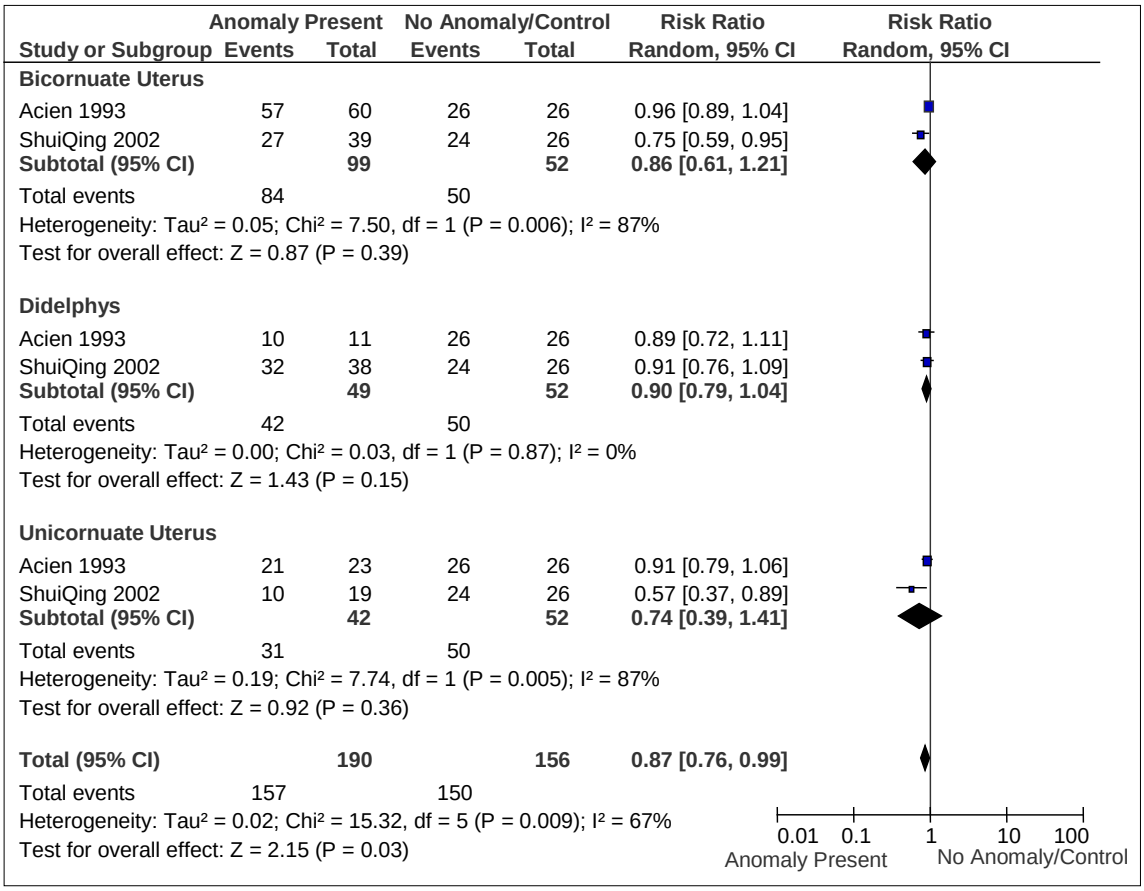


Figure 15: Studies of women with unification defects versus women with normal uteri for the outcome of clinical pregnancy rate

3.2.4.2 First Trimester Miscarriage

Five studies reported first trimester miscarriage as an outcome.

Pooled results from four of these studies (Acien, 1993, Sorensen and Trauelsen, 1987, Woelfer et al., 2001, Zlopasa et al., 2007) showed no significant difference in first trimester loss between women with arcuate uteri and those with a normal uterus (RR=1.45, 95% CI: 0.75-2.78, *P*=0.27, Figure 16). There was a moderate level of heterogeneity across these studies as indicated by an I^2 value of 62%. The inconsistency was primarily due to one study (Acien, 1993) in which much higher rates of miscarriage were seen in the study group.

Pooling of results from four studies (Acien, 1993, Shuiqing et al., 2002, Woelfer et al., 2001, Zlopasa et al., 2007) revealed a significant increase in first trimester miscarriage in women with canalization (RR=2.89, 95% CI: 2.02-4.14, *P*<0.00001, Figure 17). There were inconsistencies across the studies, however, as evident by an I^2 value of 40%

Meta-analysis of three studies (Zlopasa et al., 2007, Shuiqing et al., 2002, Acien, 1993) also showed a statistically significant increase in first trimester in women with unification defects. These women are more than twice as likely to suffer from first trimester pregnancy loss as women with a normal uterus (RR=2.13, 95% CI: 1.24-3.65, *P*=0.006, Figure 18). The I^2 value was 56% indicating moderate level of heterogeneity. Subtype analysis of the unification defects showed women with bicornuate and unicornuate uteri were more likely to suffer from first trimester miscarriage (RR=3.4, 95% CI: 1.18-9.76, *P*=0.02 and RR=2.15, 95% CI: 1.03-4.47, *P*=0.04 respectively) than those with a normal uterus.

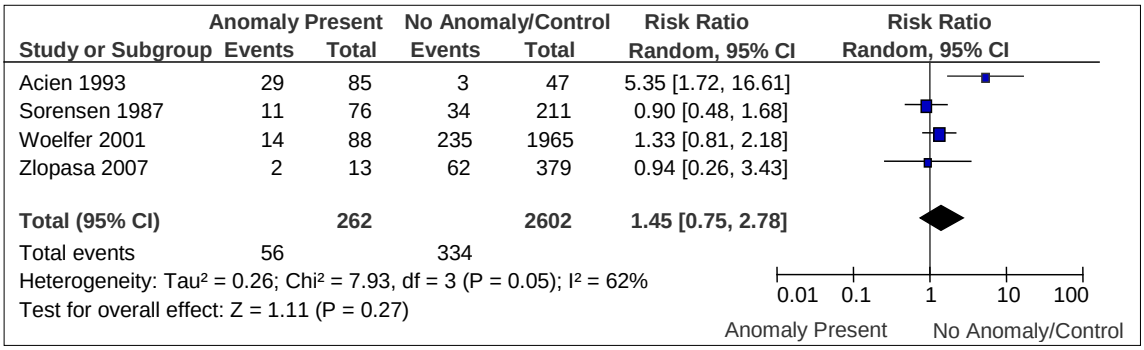


Figure 16: Studies of women with an arcuate uterus versus women with normal uteri for the outcome of first trimester miscarriage

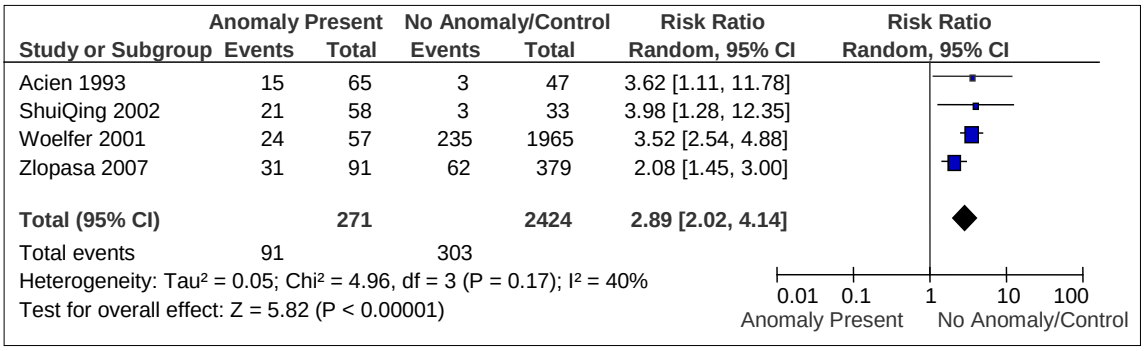


Figure 17: Studies of women with canalization defects versus women with normal uteri for the outcome of first trimester miscarriage

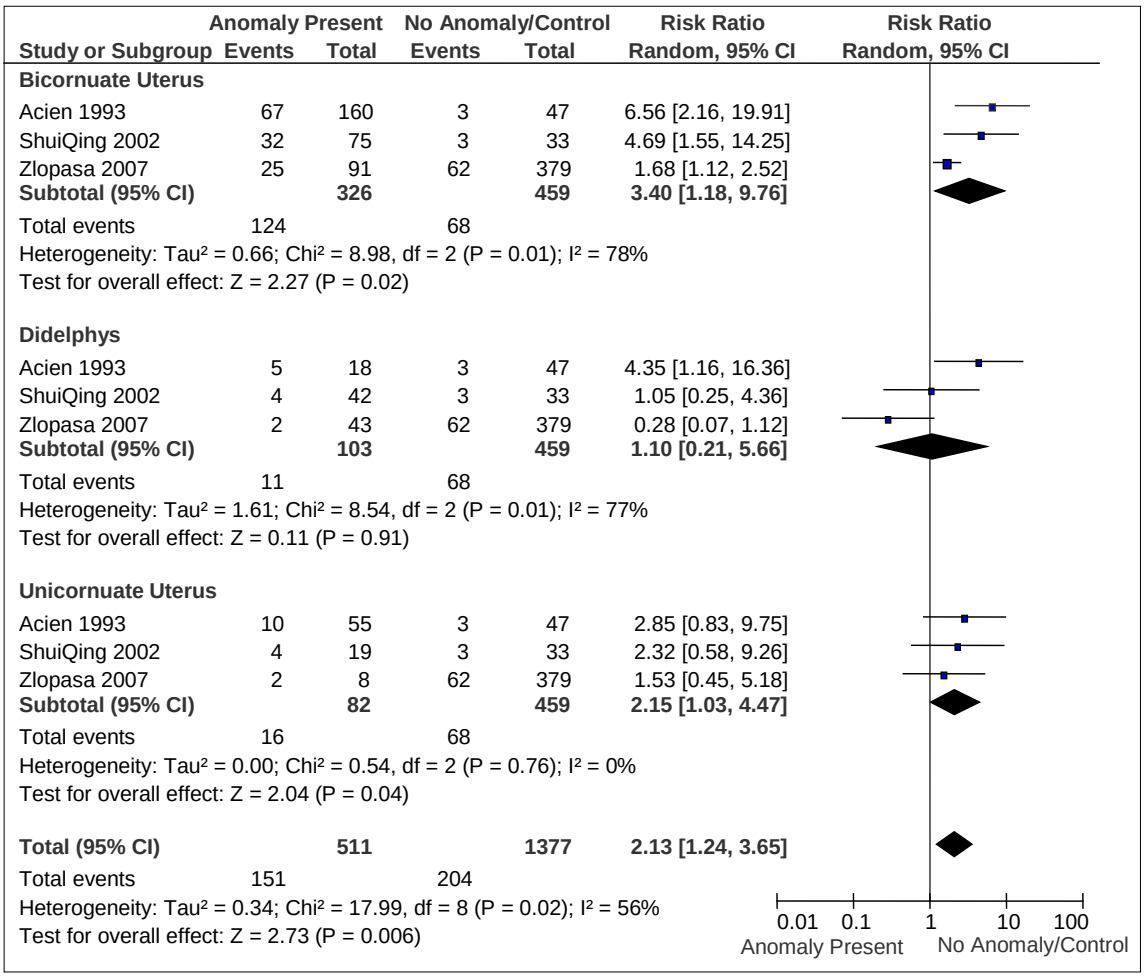


Figure 18: Studies of women with unification defects versus women with normal uteri for the outcome of first trimester miscarriage

3.2.4.3 Second Trimester Miscarriage

Five studies reported second trimester miscarriage as an outcome.

Pooled analysis of four of these studies (Acien, 1993, Sorensen and Trauelsen, 1987, Woelfer et al., 2001, Zlopasa et al., 2007) showed a significant increase in second trimester miscarriage in women with arcuate uteri compared to women with a normal uterus (RR=2.39, 95% CI: 1.33-4.27, *P*=0.003, Figure 19). There was no inconsistency between the studies, as indicated by an I^2 value of 0%.

Meta-analysis of the four studies that considered canalization defects (Acien, 1993, Shuiqing et al., 2002, Zlopasa et al., 2007, Woelfer et al., 2001) showed no effect on second trimester miscarriage rates (RR=2.22, 95% CI: 0.74-6.65, *P*=0.15, Figure 20). There was moderate level of heterogeneity across these studies with I^2 value of 50%.

Pooled result from the three studies that compared women with unification defects and women with a normal uterus (Acien, 1993, Shuiqing et al., 2002, Zlopasa et al., 2007) showed that unification defects as a whole, were associated with a doubling of the risk for second trimester miscarriage (RR=2.01, 95% CI: 1.11-3.64, *P*=0.02, Figure 21). Subgroup analyses according to the different types of unification defect showed the association was restricted to women with bicornuate uteri (RR=2.32, 95% CI: 1.05-5.15, *P*=0.04, I^2=0%). Women with uterus didelphys and unicornuate uteri showed a trend towards an increase in second trimester miscarriage only (RR=1.39, 95% CI: 0.44-4.41, *P*=0.58 and RR=2.22, 95% CI: 0.53-9.19, *P*=0.27 respectively). There was a high level of homogeneity across these studies (I^2=0%) overall and during subgroup analyses.

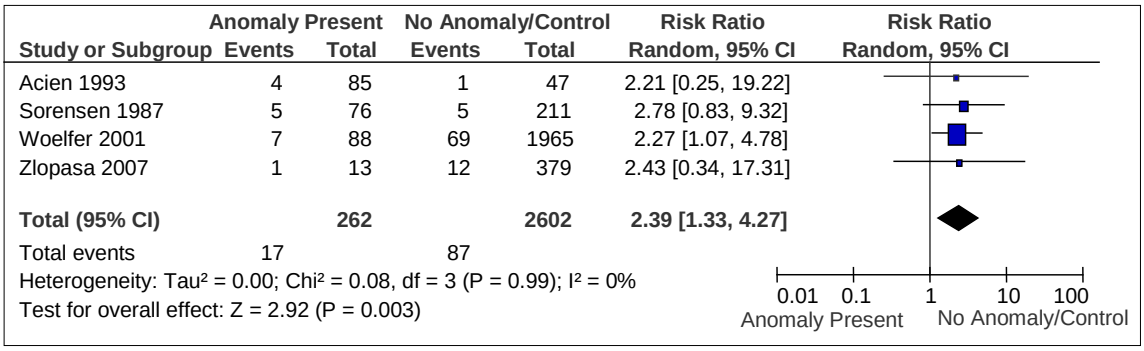


Figure 19: Studies of women with an arcuate uterus versus women with normal uteri for the outcome of second trimester miscarriage

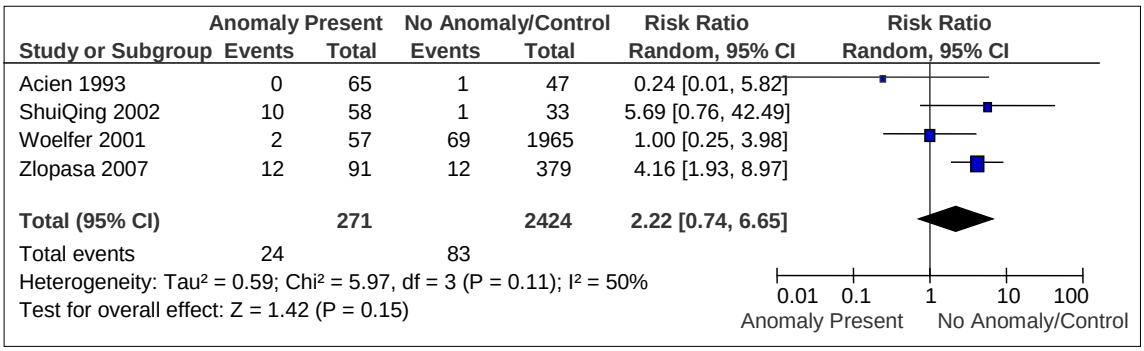


Figure 20: Studies of women with canalization defects versus women with normal uteri for the outcome of second trimester miscarriage

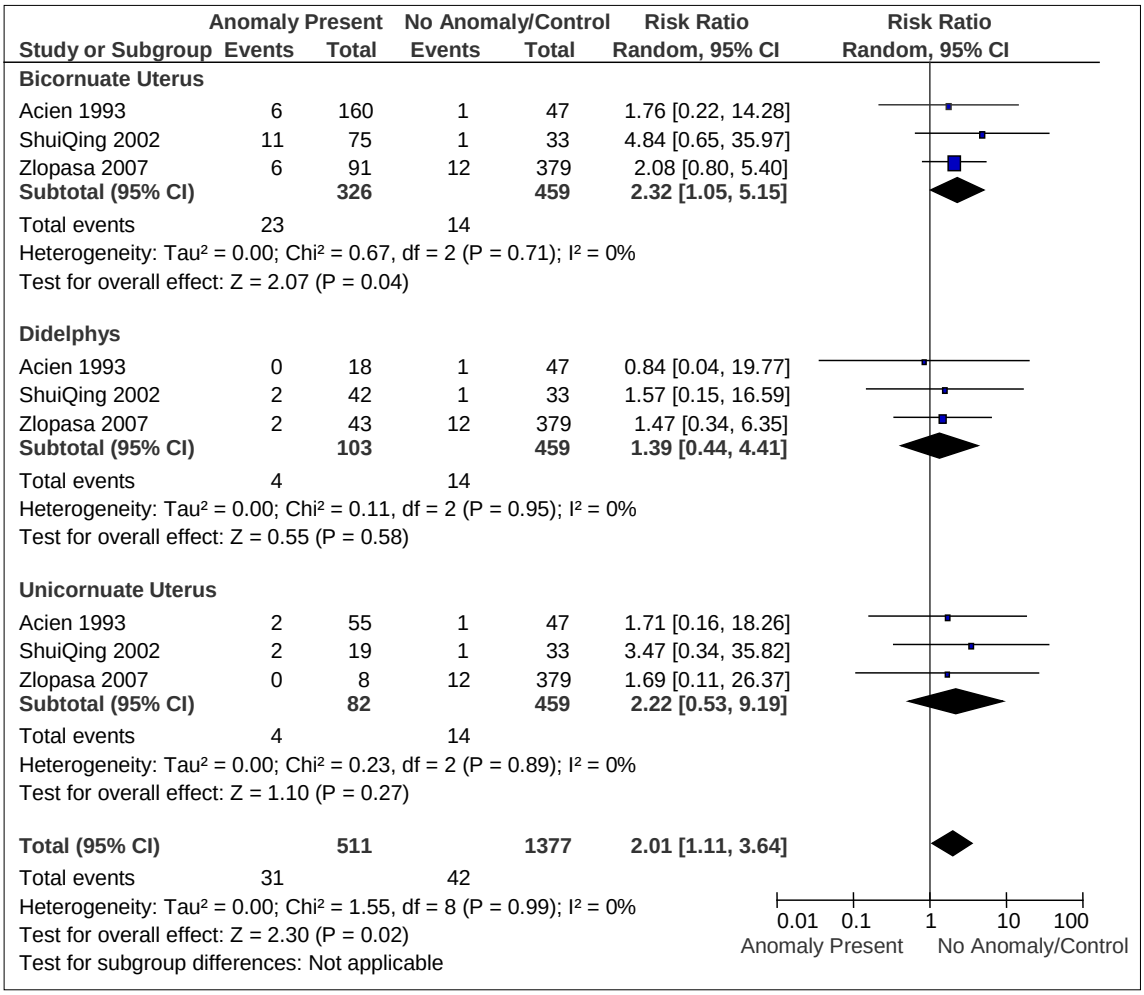


Figure 21: Studies of women with unification defects versus women with normal uteri for the outcome of second trimester miscarriage

3.2.4.4 Preterm Birth Rate

All seven studies reported preterm birth as an outcome.

Pooled analysis of 6 studies (Acien, 1993, Maneschi et al., 1995, Sorensen and Trauelsen, 1987, Woelfer et al., 2001, Zlopasa et al., 2007) showed that preterm birth was not significantly different in women with arcuate uteri compared those with a normal uterus (RR=1.61, 95% CI: 0.67-3.86, *P*=0.29, Figure 22). However, there was a high level of heterogeneity between the studies (I^2=77%). All six studies included in this analysis were of moderate to high quality. To address the high

levels of heterogeneity, further analysis based on study design was performed. Pooled analysis of the two prospective studies (Maneschi et al., 1995, Woelfer et al., 2001) reported a significant increase in preterm birth in women with arcuate uteri (RR=2.02, 95% CI: 1.22-3.34, *P*=0.007, Figure 23). There was no inconsistency between these two studies (I^2 value=0%).

Pooled analysis of the four studies reporting outcome in women with canalization defects (Acien, 1993, Shuiqing et al., 2002, Woelfer et al., 2001, Zlopasa et al., 2007) showed a significant increase in preterm birth (RR=2.27, 95% CI: 1.45-3.45, *P*=0.0003, Figure 24). I^2 value of 0% showed no variability in preterm birth rate between the studies.

Meta-analysis of the four studies in which unification defects were investigated (Acien, 1993, Shuiqing et al., 2002, Zlopasa et al., 2007, Zupi et al., 1996) showed a significant association between these anomalies and preterm birth (RR=3.94, 95% CI: 2.85-5.46, *P*<0.00001, Figure 25). The same relationship was noted during subtypes analysis. The I^2 value was 0% indicating statistically homogeneity across these studies.

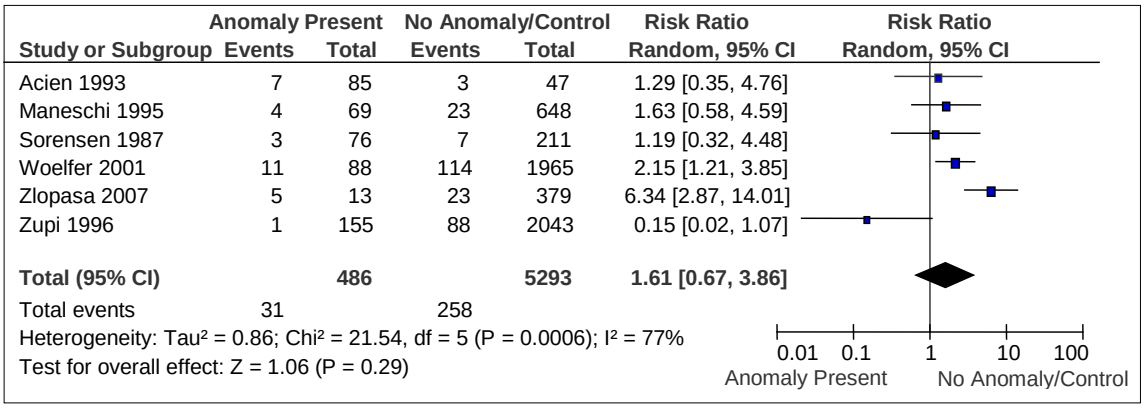


Figure 22: Studies of women with an arcuate uterus versus women with normal uteri for the outcome of preterm birth rate

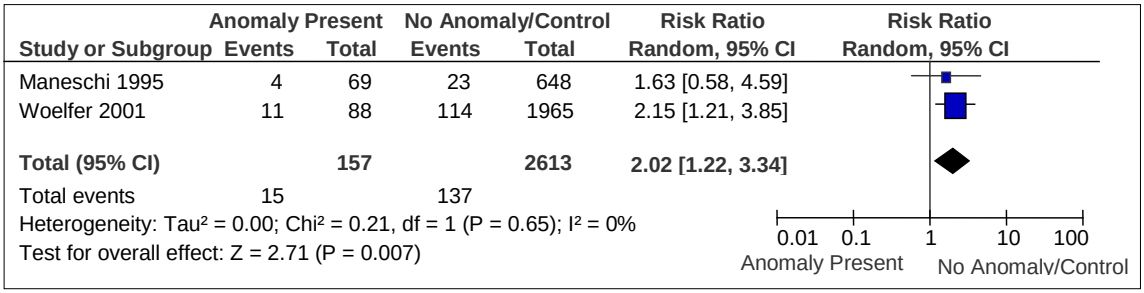


Figure 23: Prospective studies of women with an arcuate uterus versus women with normal uteri for the outcome of preterm birth rate

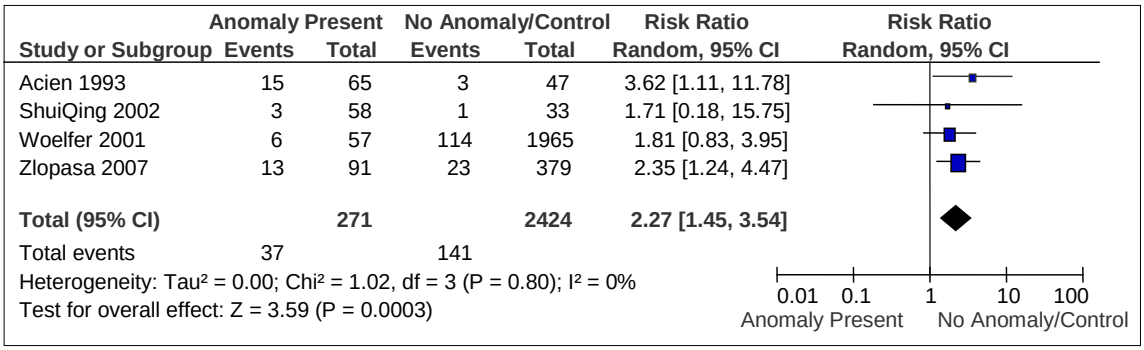


Figure 24: Studies of women with canalization defects versus women with normal uteri for the outcome of preterm birth rate

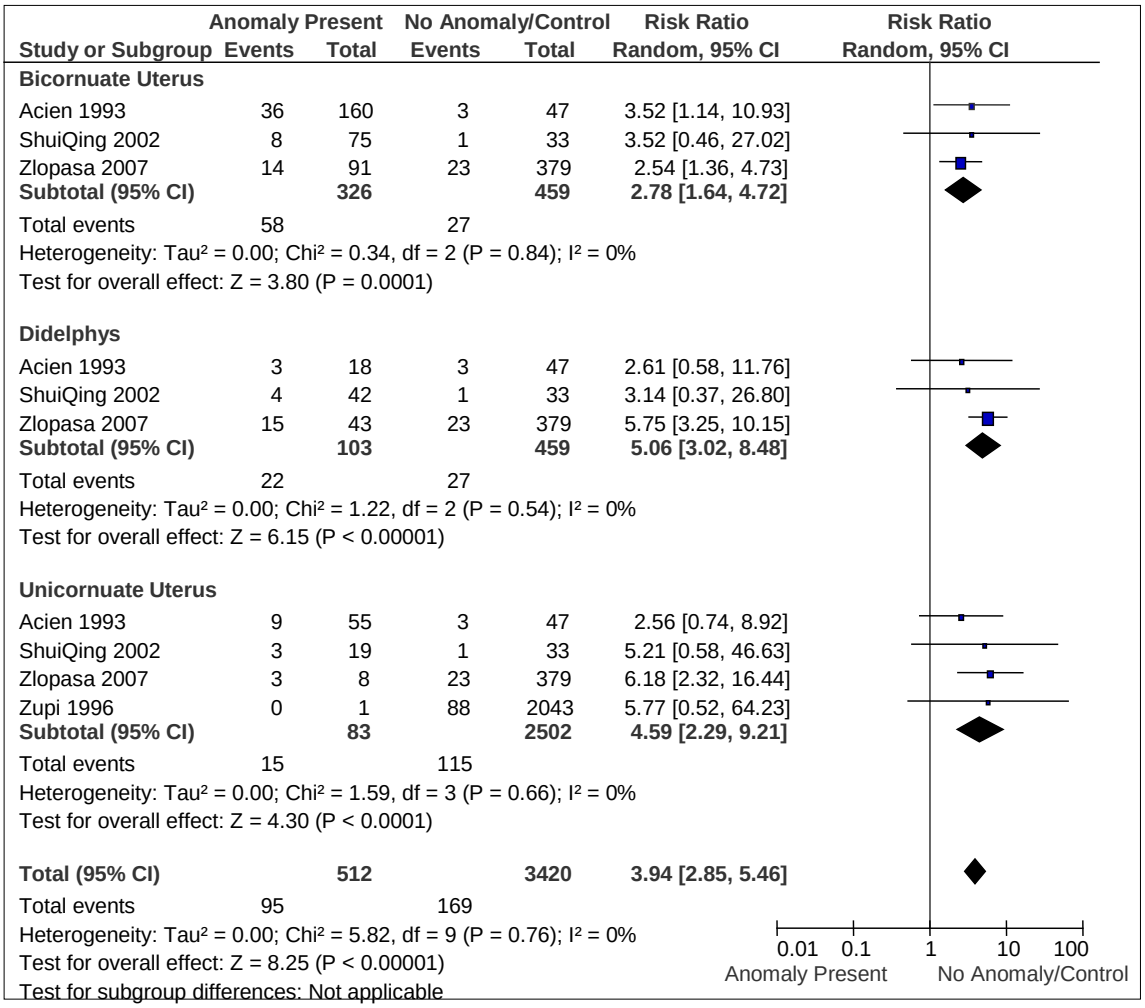


Figure 25: Studies of women with unification defects versus women with normal uteri for the outcome of preterm birth rate

3.2.4.5 Fetal Malpresentation Rate at Birth

Four studies reported the incidence of fetal malpresentation at birth as an outcome.

Pooled results of the three studies (Acien, 1993, Maneschi et al., 1995, Sorensen and Trauelsen, 1987) looking at women with arcuate uteri showed a statistically significant increase in fetal malpresentation in these women compared to those with a normal uterus (RR=2.07, 95% CI: 1.11-3.83, *P*=0.02, Figure 26). There were no inconsistencies across the studies as indicated by I^2 value of 0%.

Meta-analysis of the two studies of women with canalization defects (Acien, 1993, Shuiqing et al., 2002) showed these women have increased rate of fetal malpresentation at birth (RR=4.21, 95% CI: 1.83-9.67, *P*=0.0007, Figure 27). There were no inconsistencies across these two studies (I^2=0%).

Pooled results of the two studies of women with unification defects (Acien, 1993, Shuiqing et al., 2002) showed a significant and consistent increase in fetal malpresentation in women with these anomalies compared to those with a normal uterus (RR=3.3, 95% CI: 1.93-5.66, *P*<0.0001, I^2=0%, Figure 28). Subtype analyses showed that women with bicornuate and unicornuate uteri were more likely to experience fetal malpresentation than women with a normal uterus (RR=3.96, 95% CI: 1.74-8.99, *P*=0.001 and RR=3.29, 95% Ci: 1.93-5.66, *P*=0.009 respectively). There is consistent homogeneity throughout the studies with I^2 value of 0%.

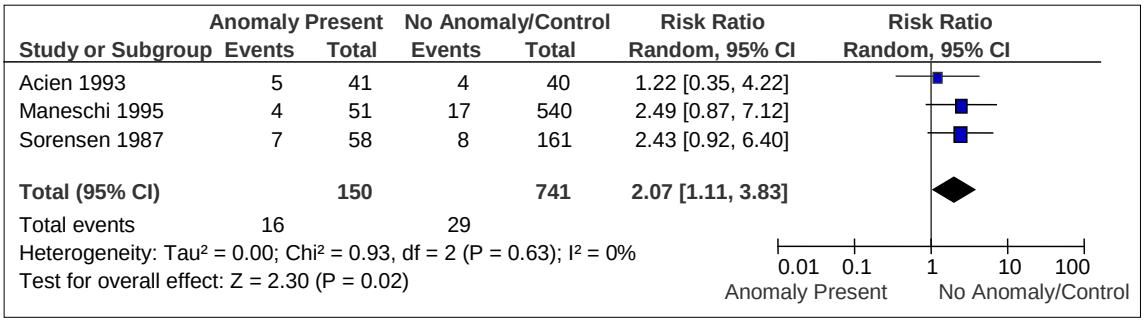


Figure 26: Studies of women with an arcuate uterus versus women with normal uteri for the rate of fetal malpresentation at birth

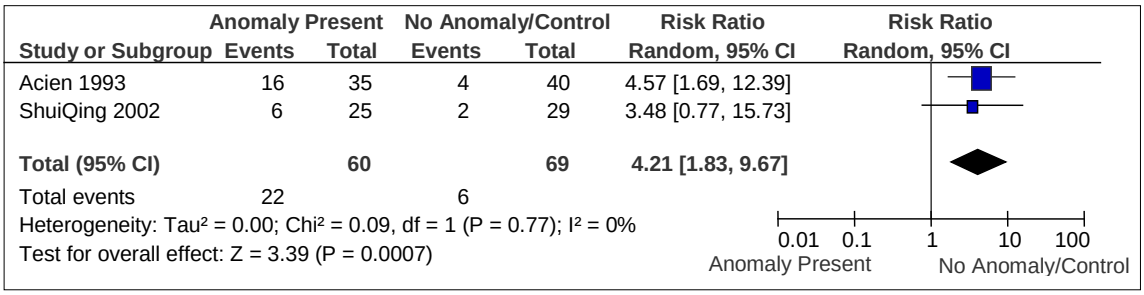


Figure 27: Studies of women with canalization defects versus women with normal uteri for the rate of fetal malpresentation at birth

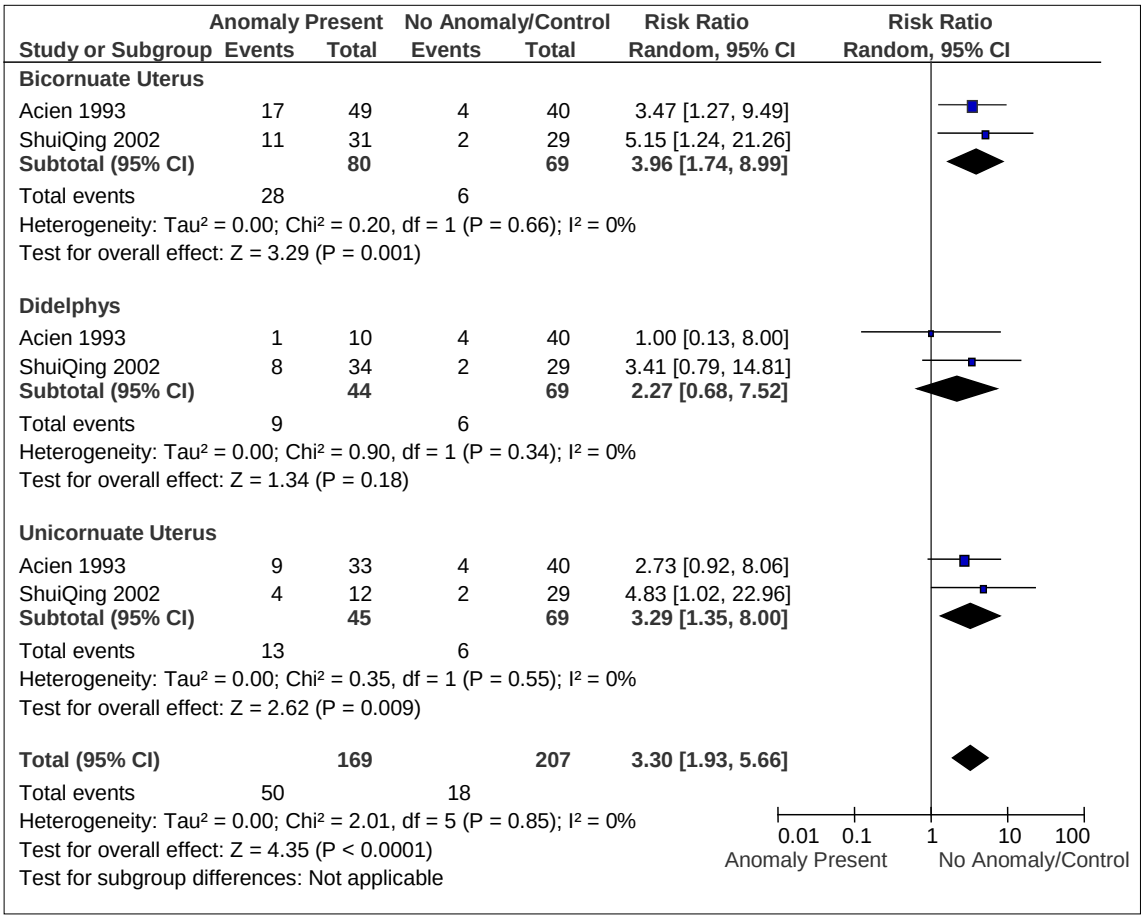


Figure 28: Studies of women with unification defects versus women with normal uteri for the rate of fetal malpresentation at birth

3.2.5 Discussions

To the best of our knowledge, this is the first systematic review evaluating the reproductive impact of congenital uterine anomalies by comparing the reproductive outcomes between women with known congenital uterine anomalies as the study group and women with no anomalies as the control group. Previous reviews by Grimbizis et al (Grimbizis et al., 2001) and Lin (Lin, 2004) have used historical controls. In addition, this review further investigated the effect of each type of uterine anomaly, which is extremely important as there are different treatment options available. This systematic review and meta-analysis has shown that congenital uterine anomalies are associated with poorer reproductive outcomes. All uterine anomalies, including minor ones, do seem to have a negative effect on pregnancy and some also reduce the chance of conception. The exact effects are, however, dependent on the type of anomaly and the outcome in question.

Women with canalization defects, such as septate and subseptate uteri have the poorest reproductive performance and in addition to having a reduced conception rate also appear at increased risk of first trimester miscarriage, preterm birth, and fetal malpresentation at delivery. Whilst the association between canalization defects and suboptimal reproductive performances appears to be generally accepted and supported by the evidence available, the exact aetiology and pathophysiological processes underlying infertility and pregnancy loss remain uncertain. It has been suggested that the

endometrium overlying the septum is abnormal or at least suboptimal and this makes it a poor site for implantation (Candiani et al., 1983, Dabirashrafi et al., 1995, Fedele et al., 1996). Embryos that do implant are more likely to miscarry as a result of this and possibly because the septum itself has a disorderly and decreased blood supply, which is insufficient to support subsequent placentation and embryo growth (Candiani et al., 1983, Homer et al., 2000, Lin, 2004, Rackow and Arici, 2007, Raga et al., 1997, Kupesic, 2001). These hypotheses remain to be proven and there is evidence to contradict these theories (Dabirashrafi et al., 1995, Kupesic, 2001, Pellerito et al., 1992). Other authors have suggested miscarriage and preterm birth may be increased as a result of abnormal uterine contractions (Kupesic, 2001, Dabirashrafi et al., 1995, Rock and Murphy, 1986, Pellerito et al., 1992, Sparac et al., 2001) or reduced space within the uterine cavity (Fedele and Bianchi, 1995). Abnormal uterine contractions and limited cavity space may also explain the increased risk of fetal malpresentation although this may simply reflect the distorted anatomy.

Unification defects, such as the bicornuate and unicornuate uterus and uterus didelphys, do not appear to reduce fertility but are associated with aberrant outcomes throughout the whole course of pregnancy. The exact effects were, again, very dependent on the type of anomaly. Women with bicornuate and unicornuate uteri have an increased risk of miscarriage, preterm birth and fetal malpresentation. These findings are consistent with previous studies (Grimbizis et al., 2001, Heinonen et al., 1982, Lin, 2004, Reichman et al., 2009). By

contrast, uterus didelphys was specifically associated with a modest increased risk of preterm labour alone.

The arcuate uterus, a minor uterine defect, is considered a normal variant rather than an uterine anomaly by many (Heinonen et al., 1982, Buttram Jr et al., 1988) but not all authors (Grimbizis et al., 2001). In this review, the arcuate uterus was associated with an increase in pregnancy loss during the second trimester. Women with an arcuate uterus were also more likely to experience preterm deliveries and fetal malpresentations. Overall, the reproductive performance in women with arcuate uterus is impaired.

3.2.5.1 Limitations and Strengths

Our systematic review has its limitations, mainly due to the clinical heterogeneity seen amongst the studies. Studies included have different inclusion and/or exclusion criteria which meant different populations of women were ultimately investigated, such as those with abnormal uterine bleeding. Whilst some studies excluded women with other known possible causes for reproductive failure, others did not consider this (Acien, 1993, Maneschi et al., 1995, Shuiqing et al., 2002, Sorensen and Trauelsen, 1987). The duration of follow up also varied and whilst most studies followed the majority of women for at least one year a longer follow up period would have provided more information on fertility and on any subsequent pregnancies. Most of the studies did not report adjusted results, so the results from our analyses are primarily based on crude values extracted from the manuscripts and are subject,

therefore, to the unadjusted effects of confounding factors such as age, socioeconomic status, smoking, and body mass index. The most common factor adjusted or matched for in the included studies was age (Maneschi et al., 1995, Woelfer et al., 2001, Zlopasa et al., 2007) but only one ensured the study population was from the same socioeconomic class (Woelfer et al., 2001).

It is highly likely that discrepancies and errors exist in this review as a result of the differences in the sensitivity and specificity of the tests applied. The tests used to diagnose and determine the type of uterine anomaly were inconsistent over the range of studies included. Certain studies used a single diagnostic test (Acien, 1993, Maneschi et al., 1995, Sorensen and Trauelsen, 1987, Woelfer et al., 2001, Zupi et al., 1996), whilst others used a combination of diagnostic tools (Shuiqing et al., 2002, Zlopasa et al., 2007). Studies where two-dimensional transvaginal ultrasound, hysteroscopy, or hysterosalpingography (HSG) have been used in isolation have the tendency to underestimate the prevalence of uterine abnormalities due to an inherent poorer sensitivity and specificity (Jurkovic et al., 1995, Wu et al., 1997, Andreotti et al., 2006, Guimaraes Filho et al., 2006b, Momtaz et al., 2007, Saravelos et al., 2008) They are less accurate in the identification and differentiation of minor uterine anomalies, such as the arcuate and subseptate uterus both of which appeared to have implications for pregnant women in this review. Three-dimensional ultrasound and magnetic resonance imaging offer a more reliable yet, non-invasive method of diagnosing congenital uterine anomalies (Jurkovic et al., 1995, Wu et al., 1997, Ghi et al.,

2009, Olpin and Heilbrun, 2009, Raga et al., 1996), and yet were only used in one of the studies included (Woelfer et al., 2001).

It should be noted that this systematic review consisted of only observational studies. These studies are fraught with potential bias and confounders, leading to spurious results from the meta-analysis and publication bias. We attempted to minimise the effects of publication bias by performing comprehensive searches, yet we were unable to formally test for this due to the small numbers of studies included in the systematic review.

Our results are concordant with the findings of a recent review by Grimbizis et al (Grimbizis et al., 2001) which explored the clinical implications of uterine anomalies but do contrast with those of another review which found the arcuate uterus to have no impact on reproductive outcomes (Lin, 2004). However, the studies in both of these reviews varied considerably in design and analysis, and included studies without control groups or studies that compared their results to historical controls. The major drawbacks in using historical controls are bias in selection of the control population and failure of the historical controls to reflect similar diagnostic criteria as the study groups. Hence, our systematic review should reflect a more genuine association between congenital uterine anomalies and impaired reproductive performances. Our systematic review's strengths also include its extensive electronic and manual search approach and the care taken in study design, data extraction, and analysis. We have used the

Newcastle-Ottawa Quality Assessment Scale to examine the quality of included studies and noted that all studies scored well.

3.2.5.2 Future work

The differences in the sensitivity and specificity of diagnostic tests used are likely to influence the actual prevalence congenital uterine anomalies and the observed reproductive outcomes in these patients. Standardized diagnostic tests and classification system need to be applied to future prospective work to investigate the actual relation between congenital uterine anomalies and various reproductive outcomes. Even though some studies (Ghi et al., 2009, Jurkovic et al., 1995) have investigated the reproducibility of different tests used in routine clinical practice for diagnosis of uterine anomalies, there is more room for a more scientifically robust test accuracy study.

The demonstration of poorer reproductive performances in women with congenital uterine anomalies does not justify advocating routine metroplasty for these women. All major uterine anomalies (bicornuate, unicornuate and uterus didelphys) can only be repaired using abdominal metroplasty. Abdominal metroplasty is associated with a number of complications and risks, including prolonged hospital stay, long recovery period, post-operative adhesions, and risk of uterine rupture during pregnancy etc. (Ayhan et al., 1992, Homer et al., 2000, Lourdel et al., 2007). Uterine septae can, fortunately be treated, and removed fairly easily. However, hysteroscopic metroplasty, a relative quick and simple surgical procedure that can be performed on a day

case basis, is yet to be shown to be a safe or effective procedure for these women. Whilst some observational studies have reported improved reproductive outcomes following surgical intervention (Guimaraes Filho et al., 2006a, Homer et al., 2000), there is a need to conduct a systematic review to investigate if abdominal or hysteroscopic metroplasty improves reproductive outcomes. A randomised controlled trial of metroplasty is much needed to address the actual effectiveness and risk of surgical interventions.

The underlying pathophysiology for poor reproductive outcomes in women with congenital uterine anomalies remains unclear. A lot of different mechanisms have been proposed and further work needs to be carried out to investigate the pathophysiology, such as abnormal uterine contractions and shortening of cervical length and so on.

3.2.6 Conclusions

In conclusion, this new systematic review showed the presence of congenital uterine anomalies is associated with poor reproductive outcome. The exact effect is dependent on the type on anomaly and the outcome being considered but canalisation and unification defects both appear to have a significant effect on clinical pregnancy, miscarriage, and preterm delivery. Arcuate uteri, often considered an incidental benign finding, are specifically associated with second trimester miscarriage. All uterine anomalies appear to increase the chance of fetal malpresentation at birth.

3.3 Systematic Review on Treatments of Congenital Uterine Anomalies

3.3.1 Introduction

For years, various surgical treatments have been offered to women with congenital uterine anomalies to normalise the morphology of the uterine cavity. However, some of these managements are controversial, especially in women with non-obstructive anomalies. Traditionally, abdominal metroplasty was performed to unify or restore the shape of the uterus. However, it is associated with a number of complications and risks, including prolonged hospital stay and recovery period, post-operative intraperitoneal adhesions, and uterine rupture during subsequent pregnancy (Ayhan et al., 1992, Homer et al., 2000, Lourdel et al., 2007). Despite these risks it is still the only surgical treatment available for women with unification defects such as bicornuate or didelphic uteri.

Recently, hysteroscopic metroplasty or resection of the uterine septum has become the established treatment of choice for women with a septate uterus who have experienced infertility, miscarriage, or preterm birth despite an apparent absence of evidence. This approach has replaced abdominal metroplasty for septate uteri as it is associated with lower morbidity, fewer adhesions, and shorter hospital stay. It is not without risk and may be associated with fluid overload, haemorrhage,

intrauterine synaechiae, intra-partum rupture of uterus, and uterine perforation (Ayhan et al., 1992, Homer et al., 2000, Lourdel et al., 2007).

Some observational studies (Ayhan et al., 1992, Grimbizis et al., 2001, Homer et al., 2000, Mollo et al., 2009, Valli et al., 2004) have suggested an improved reproductive outcome following surgical treatment; however, there are no randomised controlled trials to address the risk and benefit of these surgical treatments. Kowalik et al (2011) has recently performed a systematic review comparing hysteroscopic metroplasty and expectant management for women with recurrent miscarriage who have a septate uterus (Kowalik et al., 2011). This review is limited to hysteroscopic septoplasty in this population and does not address the impact of other procedures or other populations.

Based on this, we decided to conduct a systematic review of studies evaluating the impact of all surgical treatment of uterine anomalies on reproductive outcomes and in different populations. This is the first systematic review to assess the reproductive and obstetric impact of surgical treatments for different types of uterine anomaly that has only included studies with a clearly defined study and control group.

3.3.2 Materials and Methods

3.3.2.1 Protocol Registration

The protocol of this review has been registered on the International prospective register of systematic reviews (PROSPERO,

http://www.crd.york.ac.uk/PROSPERO/). The registration number is CRD42014006554.

3.3.2.2 Identification of Literature

Articles were identified through the following electronic databases: MEDLINE (1950 to May 2013), EMBASE (1980 to May 2013), Web of Science (1990 to May 2013) and the Cochrane Central Register of Controlled Trials (The Cochrane Library until January 2013). Studies were identified as described previously (See 2.2.1 Identification of Literature).

3.3.2.3 Study Selection

Study selection is performed independently by two reviewers (Y.Y.C and K.J.), as described in 2.2.2 Study Selection.

Studies of all types of congenital uterine anomalies were included but limited to 'Humans and Female' to generate a subset of citations relevant to our research question. To be eligible for inclusion, studies had to be observational studies or randomised controlled trials comparing the reproductive outcomes in women with known congenital uterine anomalies who had surgical treatment as the study group and women who had no surgical treatment as the control group. Studies that compared their results to historical controls or studies without a control group were not included. Studies that compared reproductive outcome in women before and after surgery, using these women as their own controls were also excluded. Studies were not included when the

surgical treatments used were not accurately defined. Outcome measures of interest include pregnancy rate, miscarriage rate, preterm birth rate, and term birth rate. Only publications in English were considered in our selection.

3.3.2.4 Quality assessment and data extraction

Two reviewers (Y.Y.C and K.J.) completed the quality assessment using the Newcastle-Ottawa Quality Assessment Scale for observational studies (Wells *et al.*, 2000). Items assessed included selection of cases and controls, comparability of study design and analysis, and ascertainment of exposure. We used allocation of 'stars' for each item included in the Newcastle-Ottawa Quality Assessment Scale to give a quantitative appraisal of overall quality of the individual studies (Wells et al., 2000). A maximum of nine stars can be achieved by any studies, and would be considered to have low risk of bias. A median score of six stars was used to distinguish moderate- and high-quality studies from poorer-quality studies (Allen et al., 2009). From each study, outcome data were extracted in 2 x 2 tables, independently by two reviewers (Y.Y.C and K.J.).

3.3.3 Statistical Analysis

Relative risks or risk ratios from individual studies were meta-analysed using fixed effects model (Mantel and Haenszel, 1959) and random effects model as appropriate (DerSimonian and Laird, 1986). Further analyses relating to different types of surgeries (septal division

surgeries or surgeries for unification defects) were also conducted. Heterogeneity of the exposure effects was evaluated graphically using forest plots (Lewis and Clarke, 2001) and statistically using I^2 statistic to quantify heterogeneity across studies with I^2 values of <25%, >50% and >75% considered to reflect low, moderate and high levels of heterogeneity respectively (Higgins and Thompson, 2002). Exploration of the causes of high levels of heterogeneity was planned using variation in features of population, exposure, diagnostic methods, study design, and study quality. Statistical analyses were performed using Review Manager (RevMan) 5.0 (Copenhagen: The Nordic Cochrane Centre) (The Cochrane Collaboration, 2008). *P* values <0.05 were considered statistically significant.

3.3.4 Results

The search yielded 5327 citations all of which were captured from electronic citations (Figure 29). 907 of these were excluded, as they were duplicates. Another 3872 citations were excluded, as it was clear from the title and abstract that they did not fulfil the selection criteria. We obtained full manuscripts of the remaining 548 articles and, following scrutiny of these, we identified four relevant studies (Heinonen, 1997, Maneschi et al., 1993, Pang et al., 2011, Valli et al., 2004). One additional study (Tonguc et al., 2011), identified from manual searches, was also included resulting in five studies comprising 521 patients, were finally selected for this review. 544 articles were excluded because they did not meet the selection criteria, included highly selected populations,

lacked original data (e.g. review articles or letters), were duplicates and/or the same data were used in other included studies.

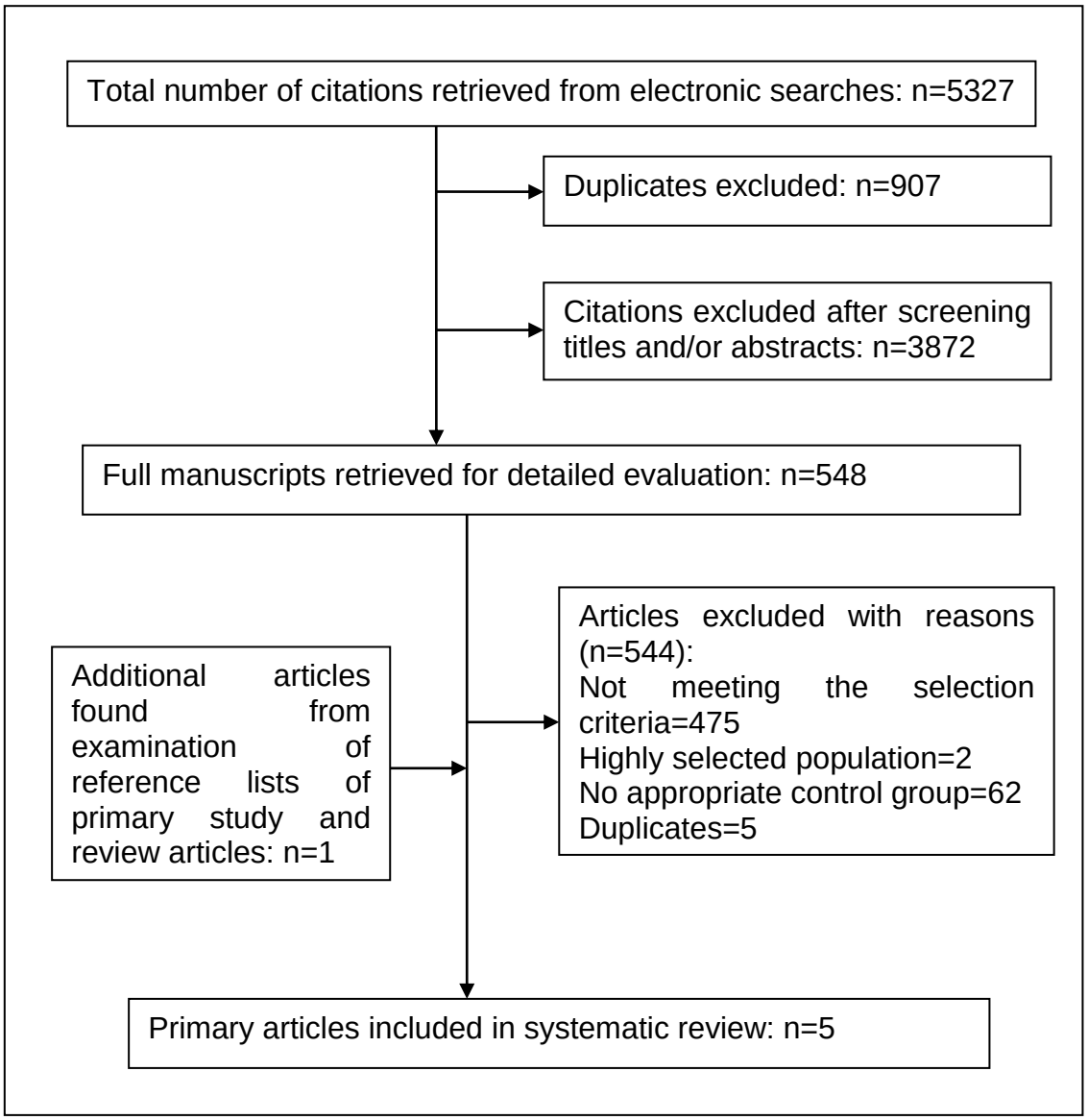


Figure 29: Study selection process for systematic review on the reproductive impacts of surgical treatments in women with congenital uterine anomalies

Study	Design	Number of participants	Intervention	Types of anomalies	Age	Drop outs	Duration of follow up	Outcomes measured
Maneschi et al (1993) (n=21)	Retrospective study. Women with or without previous reproductive problems	Study group (n=8) Control group (n=13)	Bret-Palmer abdominal metroplasty (n=8) and no treatment (n=13)	Bicornuate uteri (confirmed pre-op or intra-op)	27 (range 20-38)	None	4 years (1-17 years)	Pregnancy rate Miscarriage rate Preterm birth rate Term birth rate
Heinonen (1997) (n=192)	Retrospective study. Women with or without previous reproductive problems	Study group (n=52) Control group (n=140)	Hysteroscopic division using semirigid scissors (n=15), hysteroscopic division using resectoscope (n=17), abdominal metroplasty (n=20) and no treatment (n=140)	Septate or subseptate uteri (confirmed pre-op or intra-op)	Hysteroscopic division= 30 (range 18-42) Abdominal metroplasty= 29 (range 20-38)	None	Semirigid scissors= 22-108 months Resectoscope = 3-36 months Abdominal metroplasty= More than 5 years	Pregnancy rate Miscarriage rate Preterm birth rate Term birth rate Complications
Valli et al (2004) (n=43)	Prospective study. Women with two or more first trimester miscarriages	Study group (n=28) Control group (n=15)	Hysteroscopic division using resectoscope (n=28) and no treatment (n=15)	Septate uteri and (confirmed with laparoscopy and hysteroscopy)	Study group = 31.9+/-4.7 Control group = 30.5+/-5.1	2 (4.7%)	36 months	Pregnancy rate Miscarriage rate Preterm birth rate Term birth rate Complications

Study	Design	Number of participants	Intervention	Types of anomalies	Age	Drop outs	Duration of follow up	Outcomes measured
Pang et al (2011)	Prospective study. 1) Women with two or more first trimester miscarriages 2) Women with no history of poor reproductive outcomes	Study group (n=76) Control group (n=62)	Hysteroscopic septum resection (n=76) and no treatment (n=62)	Subseptate uteri (confirmed using 3D transvaginal ultrasound)	No significant differences between groups age	None	15 months	Pregnancy rate Miscarriage rate Preterm birth rate Term birth rate
Tonguc et al (2011)	Retrospective study. Women with unexplained infertility	Study group (n=102) Control group (n=25)	Hysteroscopic division using monopolar knife (n=102) and no treatment (n=25)	Septate/subseptate uteri (confirmed using HSG and laparoscopy), for study group only	Study group= 24.6+/-3.5 Control group = 23.8+/-2.5	None	14 months	Pregnancy rate Miscarriage rate Preterm birth rate Term birth rate Live birth rate

Table 8: Characteristics of studies of surgical treatment of uterine anomalies versus no treatment on reproductive outcomes.

Study	Selection				Comparability		Exposure/Outcome			Total
	Case definition	Representative of the cases	Control selection	Control definition	Most important factor	Additional factors	Ascertainment of exposure	Same method for cases and controls	Non-Response Rate	
Maneschi et al (1993)	*			*			*	*	*	5
Heinonen (1997)		*	*	*			*		*	5
Valli et al (2004)		*	*	*			*	*		5
Pang et al (2011)	*	*	*	*	*		*	*	*	8
Tonguc et al (2011)	*	*	*		*	*	*	*	*	8

Table 9: Appraisal of methodological quality (Newcastle-Ottawa Scale).

A star '' is awarded for each item that the study met in the Newcastle-Ottawa Scale.*

All five studies included in the final analysis were observational studies where all patients within both the study and control group were followed up to the stated outcomes. The main characteristics of the five studies and the Newcastle-Ottawa Quality Assessment are presented in Table 8 and Table 9 respectively. Although quality varied among the studies, all had limitations with design or reporting. Overall, these studies scored moderate to low on the Newcastle-Ottawa Quality Assessment Scale (Table 9). The inclusion and exclusion criteria varied between the studies (Table 8). These studies included women with various presenting problems and their surgical treatments for uterine anomalies were different too.

A summary of the impact of surgical treatments of congenital uterine anomalies on reproductive outcome is given in Table 10.

Surgery		Conception Rate, RR (95% CI)	Miscarriage Rate, RR (95% CI)	Term Birth Rate, RR (95% CI)
Septal division	Hysteroscopic septal division	0.98 (0.77 to 1.25)	* 0.35 (0.22 to 0.56)	* 1.73 (1.08 to 2.77)
	Abdominal approach	0.72 (0.5 to 1.04)	0.49 (0.17 to 1.44)	* 1.43 (1.18 to 1.74
	All	0.92 (0.30 to 2.80)	* 0.37 (0.24 to 0.57)	*1.81 (1.17 to 2.79)
Unification surgery		0.86 (0.63 to 1.18)	0.55 (0.14 to 2.05)	1.5 (0.95 to 2.37)

Table 10: Reproductive impact of surgical treatment for congenital uterine anomalies.

*** *P*<0.05**

Detailed information of how these treatments affect different reproductive outcomes is presented below.

3.3.4.1 Septal division

Four studies reported the reproductive outcome of septal division (Heinonen, 1997, Pang et al., 2011, Tonguc et al., 2011, Valli et al., 2004).

3.3.4.1.1 Pregnancy rate

All four studies showed that septal division does not improve pregnancy rates compared to no treatment (RR 0.92, 95% CI 0.30 to 2.80, *P*=0.89, Figure 30) There is high level of heterogeneity among the studies (I^2=78%) but subgroup analysis showed no difference between the hysteroscopic and abdominal approach (RR 0.98, 95% CI 0.77 to 1.25, *P*=0.89, I^2=66% and RR 0.72, 95% CI 0.5 to 1.04, *P*=0.08 respectively).

3.3.4.1.2 Miscarriage Rate

Meta-analysis of the four studies (Heinonen, 1997, Pang et al., 2011, Tonguc et al., 2011, Valli et al., 2004) showed that women who had septal division were 63% less likely to miscarry compared to those who did not have surgery (RR 0.37, 95% CI 0.24 to 0.57, P<0.00001, I^2=17%, Figure 31). Subgroup analyses showed that this was limited to hysteroscopic septal division (RR 0.35, 95% CI 0.22 to 0.56, *P*=0.0001, I^2=16%) with abdominal metroplasty having no significant impact on miscarriage rate (RR 0.49, 95% CI 0.17 to 1.44, *P*=0.20).

3.3.4.1.3 Term Birth Rate

Meta-analysis showed that septal division improves term birth rate compared to no intervention (RR 1.81, 95% CI 1.17 to 2.79, *P*=0.008, Figure 32). However, there is high level of heterogeneity among the studies (I^2=67%). Further subgroup analyses showed that both hysteroscopic and abdominal metroplasty improves term birth rate (RR 1.73, 95% CI 1.08 to 2.77, *P*=0.01; RR 1.43, 95% CI 1.18 to 1.74, *P*=0.0002 respectively). There is high level of inconsistency amongst the studies investigating hysteroscopy approach (I^2=67%) with only two of the studies received a score of 8 on the Newcastle-Ottawa Quality Assessment Scale. All of the studies included women from different populations and the high level of heterogeneity is also likely due to different study design, patient populations, varied diagnostic tests, and duration of follow up.

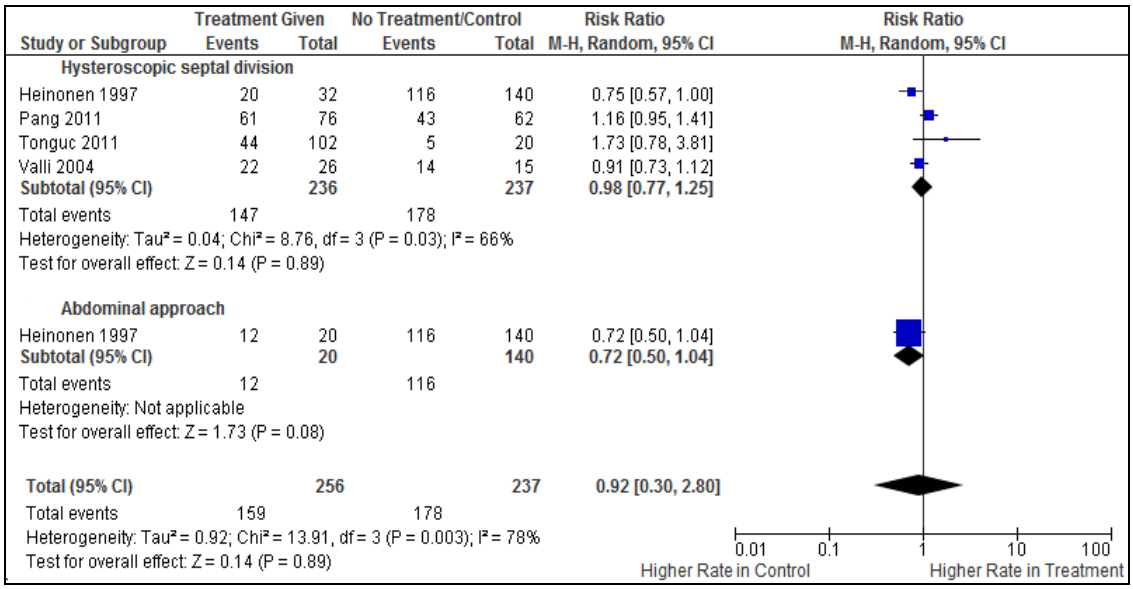


Figure 30: Effect of septal divisions on pregnancy rate compared with no surgery

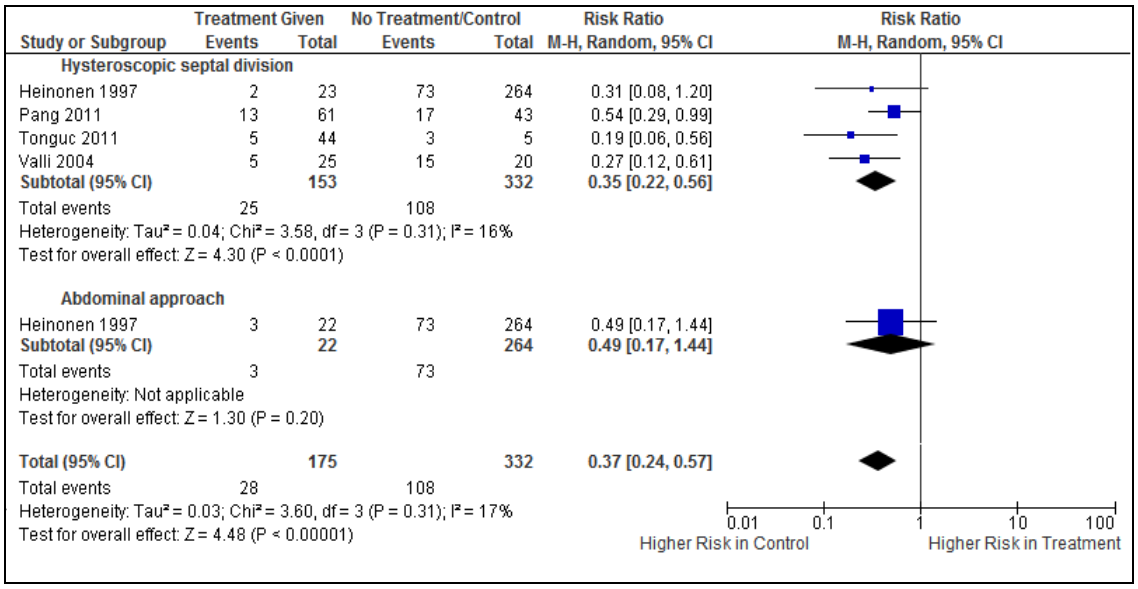


Figure 31: Effect of septal divisions on miscarriage rate compared with no surgery

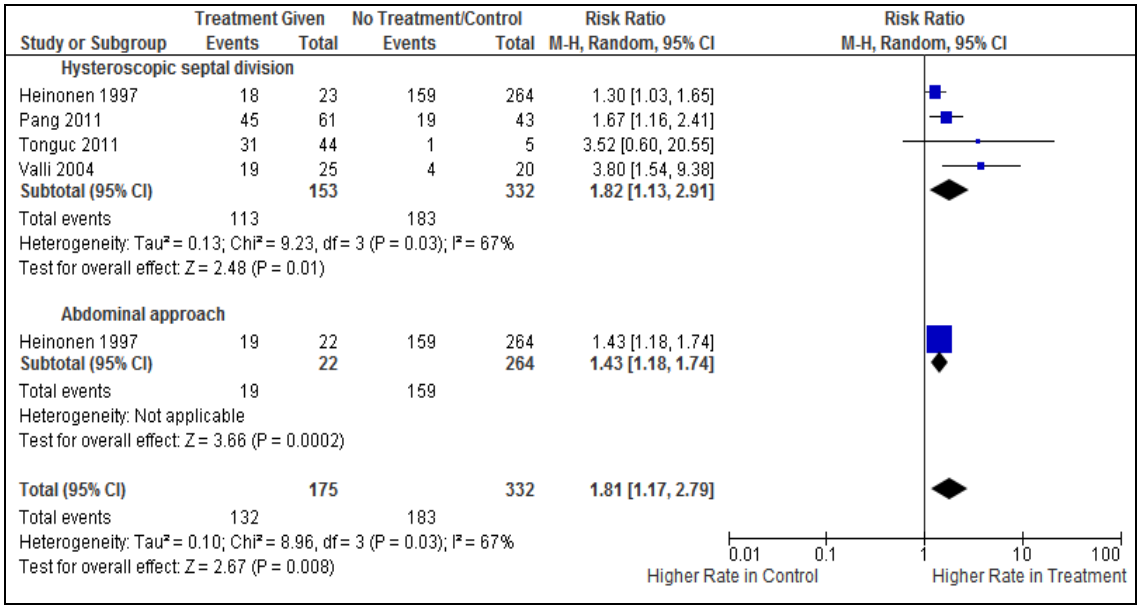


Figure 32: Effect of septal divisions on term birth rate compared with no surgery

3.3.4.2 Surgery for Unification Defects

Only one study (Maneschi et al., 1993) was included in this review precluding meta-analysis. The risk ratio was calculated and showed that abdominal metroplasty for unification defects has no impact on pregnancy, miscarriage or term birth rates (See Figure 33, Figure 34, and Figure 35).

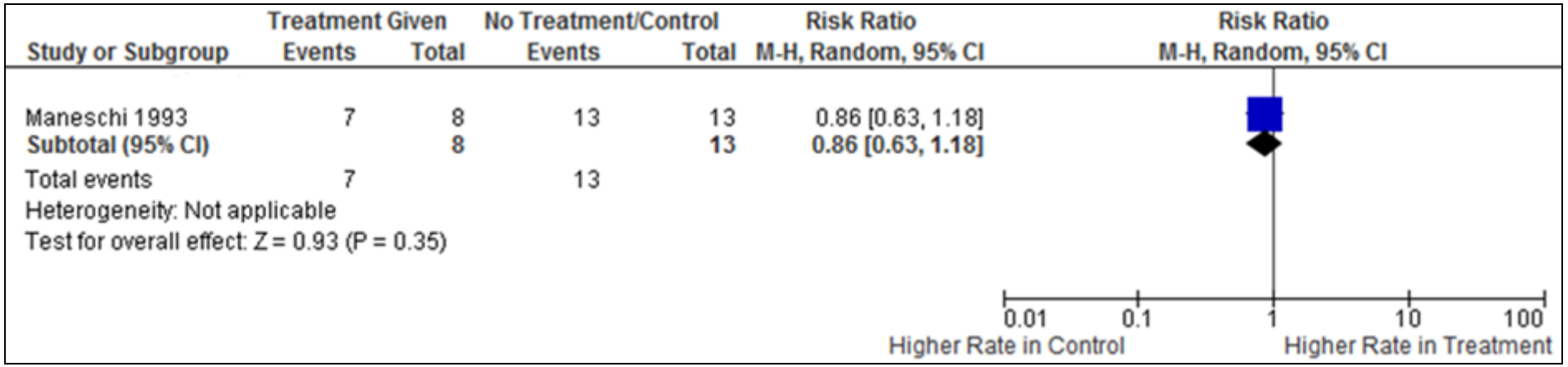


Figure 33: Effect of surgery for unification defects on pregnancy rate compared with no surgery

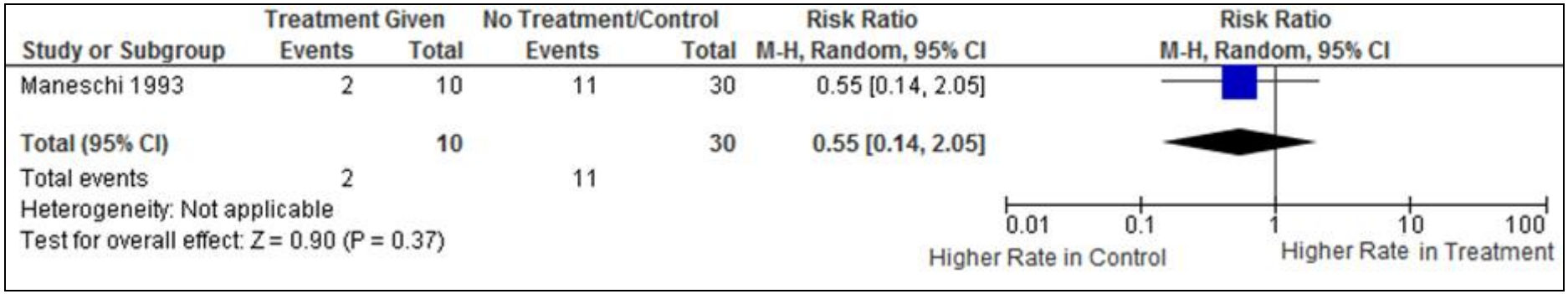


Figure 34: Effect of surgery for unification defects on miscarriage rate compared with no surgery

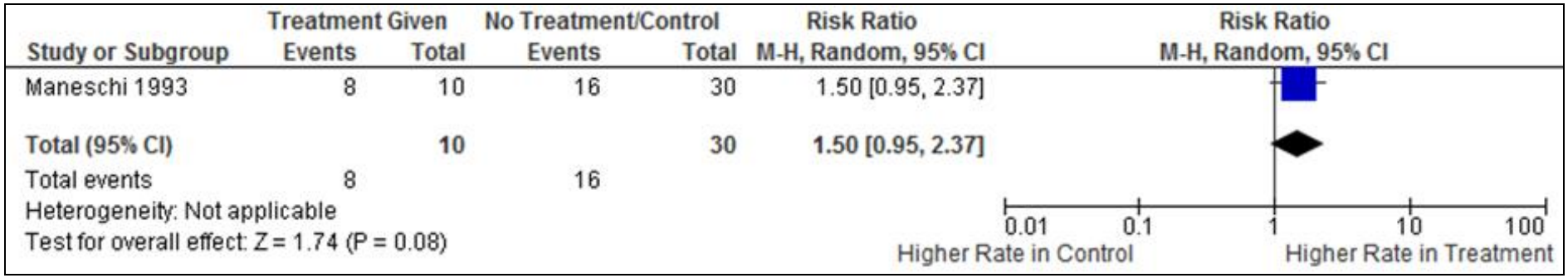


Figure 35: Effect of surgery for unification defects on term birth rate compared with no surgery

3.3.5 Discussion

This systematic review has evaluated the reproductive impact of various surgical treatments of uterine anomalies. It not only updates the work by Nouri et al (2010) and Kowalik et al (2011) on the impact of hysteroscopic septoplasty but provides information on the effect of other forms of corrective surgery for uterine anomalies (Kowalik et al., 2011, Nouri et al., 2010). This systematic review and meta-analysis has shown that septal division is potentially beneficial in terms of reproductive and obstetric outcomes but that the exact effect is dependent on the type of surgery performed.

The systematic review by Nouri et al (2010) focused on the evaluation of reproductive outcome after hysteroscopic septoplasty or hysteroscopic septal division (Nouri et al., 2010). The review did not consider other surgical techniques or surgery for other anomalies. They reported pooled results of studies looking at post-operative outcomes and complication and did not, therefore, use control groups. Kowalik et al (2011) restricted their review to randomised controlled trials of hysteroscopic septal resection in women with recurrent miscarriage and could not find any eligible studies (Kowalik et al., 2011). A new review was required to examine the effect of all forms of corrective surgery and how they performed in different populations with reference to control groups.

Historically septate uteri could only be treated by abdominal metroplasty. This technique is associated with long hospital stays, post-

operative adhesions potentially leading to tubal infertity and uterine rupture during subsequent pregnancy (Ayhan et al., 1992, Homer et al., 2000, Lourdel et al., 2007). Only one study describing the procedure was included in this review and this only considered 20 women (Heinonen, 1997). The data is therefore limited and the various presenting problems may affect the results. Hence, we urge caution in the results interpretation despite significant improved term birth rates in women who had abdominal metroplasty for septate uteri.

Abdominal metroplasty still has a role for the treatment of major unification defects such as bicornuate and didelphic uteri. It is rarely performed however, partly because of the associated intra and post-operative risks but also because there is limited evidence to support its effectiveness. Study by Maneschi et al (1993) does not support the use of abdominal metroplasty for unification defects as it does not improve reproductive or obstetric outcomes (Maneschi et al., 1993).

Abdominal metroplasty for septate uteri has been replaced by hysteroscopic septal division, a relative quick and simple surgical procedure that can be performed on a day case basis. Observational studies have suggested improved reproductive outcomes (Daly et al., 1989, Guimaraes Filho et al., 2006a, Homer et al., 2000, March and Israel, 1987, Mollo et al., 2009, Pabuccu et al., 1995) and our review supports this with an apparent reduction in miscarriage and a concomitant increase in term birth rate. The overall number of women involved is small however and it is not possible to draw any meaningful conclusions on the safety of the procedure.

3.3.5.1 Limitations and Strengths

Our systematic review's strengths include the extensive electronic and manual search performed and in the attention paid to the design of the studies included, data extraction and analysis. The review is limited by the clinical heterogeneity seen amongst the studies which have used a range of different inclusion and/or exclusion criteria resulting in a myriad of populations. Most of the studies did not report adjusted results, so our results are primarily based on crude values extracted from the manuscripts and are subject, therefore, to the unadjusted effects of confounding factors such as age, socioeconomic status, smoking, and body mass index. It is also highly likely that discrepancies and errors exist in this review as a result of the different equipment used to both define the anomaly and repair it. The diagnostic tools and criteria varied considerably as did the techniques and equipment used to perform septal division such as semi-rigid scissors or electro-diathermy. These instruments have different diameters and which may directly cause trauma to the uterine cavity or cervical canal irrespective of the resection. To date, there is no standardised technique or equipment available for metroplasty or septal resection.

It should be noted that this systematic review consisted only of observational studies. These studies are fraught with bias and confounders. We were unable to formally test for publication bias due to the small numbers of studies included in the systematic review. We used the Newcastle-Ottawa Quality Assessment Scale to examine the

quality of included studies and all of studies were considered to be of moderate quality and only two studies were of high quality.

3.3.5.2 Future Work or Recommendations

The suggestion of improved reproductive performances in women after corrective surgeries for congenital uterine anomalies does not justify advocating routine metroplasty for these women. Both abdominal and hysteroscopic metroplasty are associated with surgical risks and increased risk of uterine rupture in subsequent pregnancy. An adequately powered, randomised controlled trial of metroplasty is needed to address the actual effectiveness and risk of surgical interventions. Such a trial needs to carefully consider the population being studied and the diagnostic criteria used to define the anomaly and assess the effectiveness of the surgery, which should also be standardised.

Some authors have suggested that miscarriage and preterm birth may be increased due to abnormal uterine contractions in women with uterine anomalies (Dabirashrafi et al., 1995, Kupesic, 2001, Pellerito et al., 1992, Rock and Schlaff, 1985). Some studies have shown a reduction in preterm delivery from progesterone administration to reduce myometrial contractility (Anderson et al., 2009, da Fonseca et al., 2003, Meis et al., 2003). However, this also requires evaluation in a randomised controlled trial and would be suitable for women not willing to undergone surgery and for those with anomalies considered

unsuitable for surgery such as the arcuate uterus and the more profound unification anomalies.

3.3.6 Conclusions

This systematic review suggests that the surgical division of a uterine septum improves reproductive outcome by reducing miscarriage and increasing term birth. However, the exact effects are dependent on the type of surgery performed and the underlying defect. Surgery for unification defects, such as the bicornuate uterus, does not appear to have any impact on reproductive performances. These results are limited as they have been derived from observational studies involving small numbers of women. There is an urgent need to conduct a multicentre randomised controlled trial of metroplasty in women with uterine anomalies that must be carefully controlled for both the type of anomaly and surgery.

3.4 Reproducibility of 3D Ultrasound Scan in Diagnosing Congenital Uterine Anomalies: A Systematic Review

3.4.1 Introduction

In recent years, three-dimensional ultrasound scan is becoming increasingly popular as the diagnostic test of choice to diagnose congenital uterine anomalies. It is likely due to the high sensitivity and specificity, in diagnosing and classifying congenital uterine anomalies (Deutch et al., 2006, Saravelos et al., 2008, Wu et al., 1997). As discussed in 1.6.3.3, this non-invasive diagnostic test is not operator dependent, cheaper, faster and safer compared to combined hysteroscopy and laparoscopy or magnetic resonance imaging (Deutch and Abuhamad, 2008, Olpin and Heilbrun, 2009). A recent systematic review by Grimbizis et al (Grimbizis et al., 2016) has shown that three-dimensional ultrasound scan has high diagnostic accuracy.

Even though three-dimensional ultrasound scan appears to be an accurate test in diagnosing congenital uterine anomalies, very few reproducibility studies have been performed. The American Society for Reproductive Medicine Classification (1988), which is the most commonly used classification in clinical practice, does not specify the morphological features or cut-offs in classifying these anomalies, making it challenging to examine the reproducibility of ultrasound

diagnosis. In addition, the rarity of congenital uterine anomalies meant it was difficult to recruit sufficient number of patients for studies.

Without an acceptable level of reproducibility, the clinical utility of three-dimensional ultrasound scan becomes substantially compromised. In the last decade, as this diagnostic test becomes more widely available and practitioners become more experienced, researchers and clinicians have published studies evaluating the reproducibility of three-dimensional ultrasound scan diagnoses of congenital uterine anomalies. To date, there is no comprehensive systematic review that has investigated the overall reproducibility of three-dimensional ultrasound scan diagnoses of congenital uterine anomalies. In view of this, we conducted a systematic review to evaluate all three-dimensional ultrasound scan reproducibility studies of congenital uterine anomalies.

3.4.2 Materials and Methods

3.4.2.1 Identification of Literature

Articles were identified through the following electronic databases: MEDLINE (1950 to October 2017), EMBASE (1980 to October 2017), Cochrane Central Register of Controlled Trials and Web of Science (1990 to October 2017). Studies were identified as described previously (See 2.2.1 Identification of Literature).

3.4.2.2 Study Selection

Study selection is performed independently by two reviewers (Y.Y.C and C.C.C.), as described in 2.2.2 Study Selection. To be eligible for inclusion, comparative studies had to be assessing the inter-observer or inter-rater reliability of three-dimensional ultrasound scan in diagnosing congenital uterine anomalies using the kappa statistic (*k*). Studies were excluded when sample size or the diagnostic classification system used were not clearly defined. We recorded the *k* value and the classification system used to diagnose the anomalies.

3.4.2.3 Quality assessment and data extraction

Two reviewers (Y.Y.C and C.C.C) completed the assessment of the quality of the studies based on the Guidelines for Reporting Reliability and Agreement Studies (GRRAS) (Kottner et al., 2011). At least 13 items should be addressed when reliability and agreement are investigated.

From each study, data were extracted independently by two reviewers (Y.Y.C and C.C.C). Extracted data included: First author's last name and year of publication. To establish reproducibility or reliability, the following data were extracted: Reference population, number of scans, sample size, number of assessors, classification system used, and statistical analysis/results (with confidence intervals).

3.4.3 Statistical Analysis

Meta-analysis was performed to establish the pooled Kappa statistic (Sun, 2011), reporting the agreement on diagnosis of congenital uterine anomalies, and their subtypes. For the interpretation of kappa statistic, we used the following cut-off values: <0.20, very poor; 0.21–0.40, poor; 0.41–0.60, moderate; 0.61–0.80, good; and > 0.80, very good (Kottner et al., 2011). A random effects model was used for analysis. The heterogeneity was quantified across study results using the Q and I^2 statistic, with I^2 values of <25%, >50% and >75% considered to reflect low, moderate and high levels of heterogeneity respectively (Higgins and Thompson, 2002). Exploration of the causes of high levels of heterogeneity was planned using subgroup meta-analysis for variations in features of population, classification system, study design, and study quality. Statistical analyses were performed using Stata 14.0 statistical software (Stata Corp, TX, USA). P values <0.05 were considered statistically significant.

3.4.4 Results

The search yielded 9948 citations all of which were captured from electronic citations (Figure 36). 4072 of these were excluded, as they were duplicates. Another 5821 citations were excluded, as it was clear from the title and abstract that they did not fulfil the selection criteria. We obtained full manuscripts of the remaining 55 articles and, following scrutiny of these, we identified five relevant studies (Bermejo et al., 2017, Ludwin et al., 2015, Salim et al., 2003, Tudorache et al.,

2017, Wang et al., 2013), comprising 3367 patients, were finally selected for this review. 50 articles were excluded because they were irrelevant, or did not meet the selection criteria, included lacked sample size data, lacked *k* value or its confidence intervals, were duplicates and/or the same data were used in other included studies.

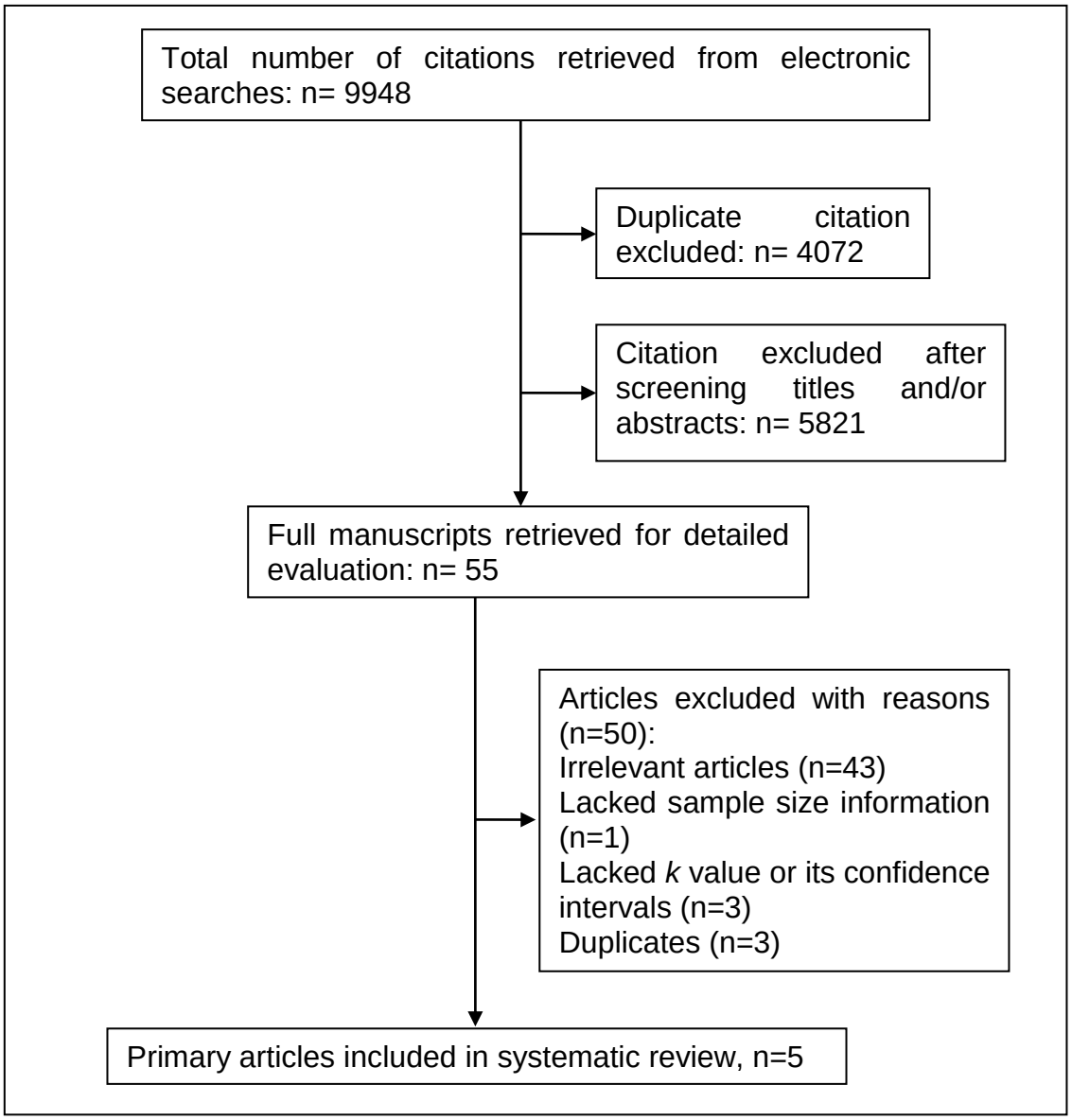


Figure 36: Study selection process for systematic review on the reproducibility of 3D ultrasound scan in diagnosing congenital uterine anomalies

Study	Types of study	Sample size	Sampling method	Classification system	Number of assessors	Kappa (95% CI)
Bermejo et al (2017)	Retrospective	89 mixture of abnormal and normal uteri	Convenience	ESHRE/ESGE	2	0.73 (0.61 – 0.84)
Ludwin et al (2015)	Prospective	50 Abnormal uteri 62 Normal uteri	Consecutive (abnormal) Random (normal)	ESHRE/ESGE ASRM	2	0.8 (0.65 – 0.95) 0.96 (0.85 – 1)
Salim et al (2003)	Retrospective	56 Abnormal uteri 27 Normal uteri	From archive to ensure mixture of cases and normal uteri	ASRM	2	0.97 (0.94 – 1)
Tudorache et al (2017)	Retrospective	294 Abnormal uteri 2861 Normal uteri	Consecutive	ASRM	2	0.89 (0.72 – 0.93)
Wang et al (2013)	Retrospective	49 Abnormal uteri 30 Normal uteri	Consecutive (abnormal) Random (normal)	ASRM	2	0.932 (0.867 – 0.997)

Table 11: Characteristic of reproducibility studies of 3D ultrasound scan in diagnosis of congenital uterine anomalies

ESHRE/ESGE: The European Society of Human Reproduction and Embryology/ the European. Society for Gynaecological Endoscopy Classification

ASRM: American Society for Reproductive Medicine Classification (1988)

Study	Study type in title or abstract	Describe device	Detailed population	Detailed assessors or raters	Sample size	Sampling Method	Rating process	Independence	Statistical analysis	Number of raters and subjects	Describe the raters and subjects	Estimators with uncertainty	Discussed relevance of results
Bermejo et al (2017)	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Ludwin et al (2015)	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Salim et al (2003)	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Tudorache et al (2017) [Abstract only]	Yes	No	No	No	No	Yes	No	Yes	Yes	Yes	No	Yes	No
Wang et al (2013)	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	Yes

Table 12: Evaluation of included studies according to the guidelines GRRAS

All five studies included in the final analysis were observational studies where all patients within both the study and control group were followed up to the stated outcomes. The main characteristics of the five studies and the evaluation of all studies summarized by the GRRAS guidelines are presented in Table 11 and Table 12 respectively. In none of the studies was there a clear description of the population of interest (background history or characteristics). Most studies did not conduct sample size estimation or calculation. One of the studies did not report the confidence intervals of the estimates, but original data was available for us to calculate the confidence intervals.

A summary of the kappa statistics of studies is given in Table 13.

Study	Classification system	Kappa (95% CI)
Bermejo et al (2017)	ESHRE/ESGE	0.73 (0.61 – 0.84)
Ludwin et al (2015)	ESHRE/ESGE ASRM	0.80 (0.65 – 0.95) 0.96 (0.85 – 1)
alim et al (2003)	Modified ASRM	0.97 (0.94 – 1)
Tudorache et al (2017)	ASRM	0.89 (0.72 – 0.93)
Wang et al (2013)	ASRM	0.932 (0.867 – 0.997)

Table 13: Kappa statistics of included studies

Pooled kappa statistic for diagnosis of congenital uterine anomalies is shown in Figure 37 (k=0.90, 95% CI: 0.83-0.96). However, there is high level of heterogeneity among the studies (I^2=76.2%). In view of that, subgroup analysis was performed to investigate the cause of heterogeneity. It appears the inconsistencies among studies were due to the different classification systems used in the studies (Figure 38). Publication bias was not detected in this meta-analysis (P>0.05).

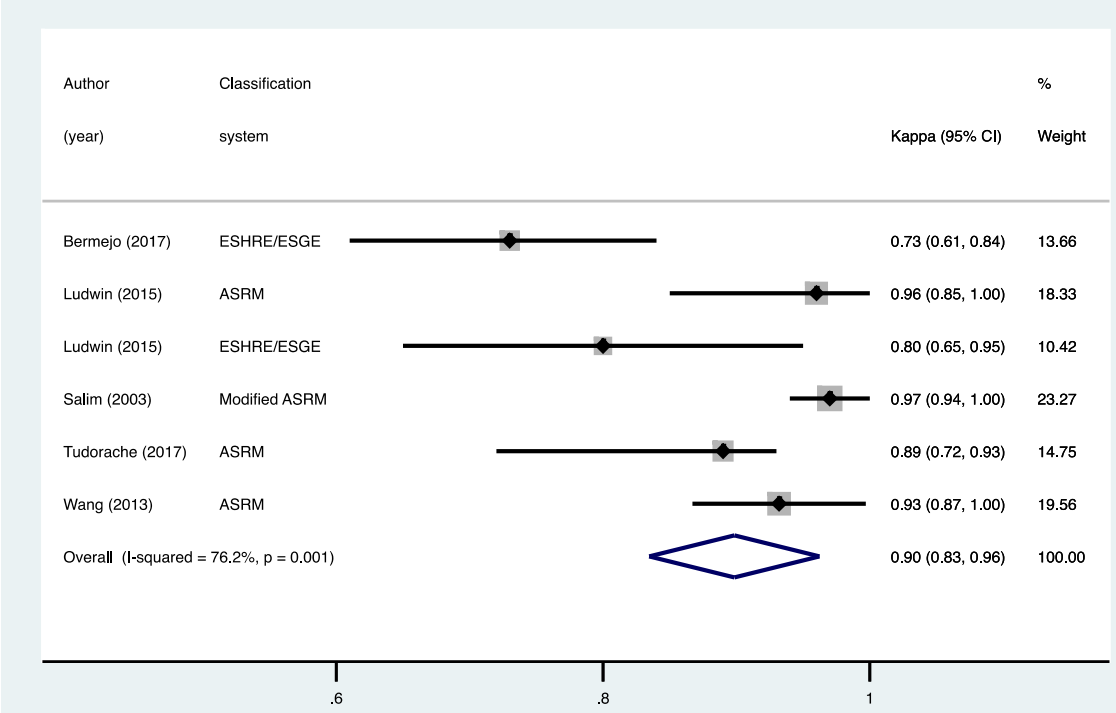


Figure 37: Pooled kappa statistic for diagnosis of congenital uterine anomalies

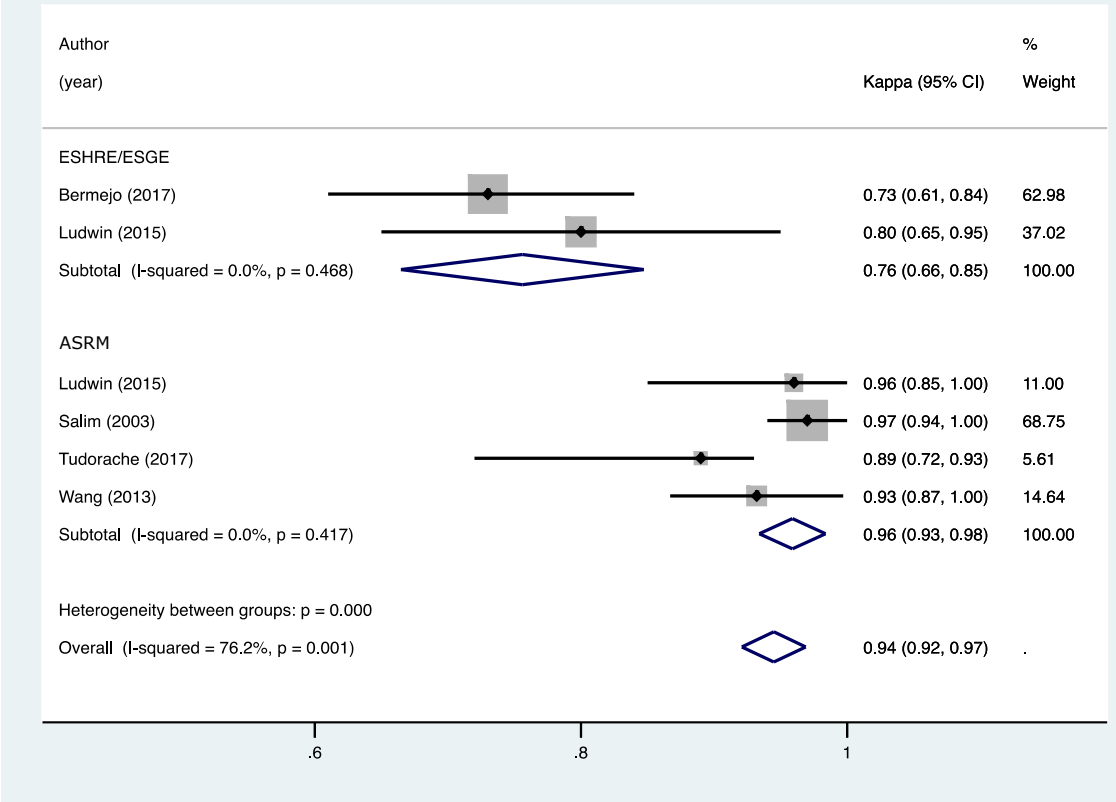


Figure 38: Subgroup analysis according to classification system

3.4.5 Discussions

To the best of our knowledge, this is the first systematic review that investigated the reproducibility of three-dimensional ultrasound scan in diagnosing congenital uterine anomalies. As three-dimensional ultrasound scan becomes more widely available and clinicians more aware of its accuracy in diagnosing congenital uterine anomalies, it is increasingly used in clinical practice. Systematic review by Grimbizis et al (Grimbizis et al., 2016) has demonstrated that three-dimensional ultrasound scan has high diagnostic accuracy for diagnosis of congenital uterine anomalies. However, it is important to ensure that not only this diagnostic test is accurate but also reproducible. Otherwise, the clinical use of this diagnostic tool becomes invalid.

In this systematic review, we have demonstrated that three-dimensional ultrasound scan is highly reproducible in diagnosing congenital uterine anomalies as the pooled kappa was > 0.80. However, it was clear that there were significant inconsistencies among the studies. All but one study were retrospective studies using ultrasound data from archive. Hence, the study design did not explain the inconsistencies. Unfortunately, due to the lack of data on the population being investigated we could not investigate the impact of population features on heterogeneity. We are however, able to perform subgroup analysis on classification system used to diagnosed congenital uterine anomalies. It showed the irregularities among the studies can be explained by the different classification systems used (See Figure 38).

Essentially, there were two main classification systems used in the included studies. The American Society for Reproductive Medicine classification (American Fertility Society, 1988) is the most commonly used classification system for congenital uterine anomalies. However, this classification does not specify the diagnostic methods that should be used for diagnosis and there are no cut-off measurements to classify these anomalies. All diagnoses are based on subjective view of the operator. However, in recent years most clinicians or researchers who used three-dimensional ultrasound scan in diagnoses of congenital uterine anomalies, used the modified American Society for Reproductive Medicine Classification proposed by Salim et al (Salim et al., 2003). This modified classification system included additional morphometric criteria or cut-offs levels for the external uterine contour and cavity distortion. Even though these cut-offs were selected arbitrary, they were necessary to differentiate between uterine anomalies quantitatively. Quantitative measurements certainly made clearer differentiation between uterine anomalies with similar morphological features such as subseptate and arcuate uteri. Even when subjective assessment was used (pattern recognition), instead of using the morphometric criteria, three-dimensional ultrasound scan still has very good inter-observer agreement in assessing congenital uterine anomalies (Tudorache et al., 2017). However, the inter-observer reproducibility is lower than those studies which used the additional morphometric criteria when classifying congenital uterine anomalies.

The CONUTA Working Group, established by the European Society of Human Reproduction and Embryology (ESHRE) and the European Society for Gynaecological Endoscopy (ESGE) developed a new updated classification system in 2013. This classification system supposed to eliminate the subjectivity of the original American Society for Reproductive Medicine Classification. However, this classification system is still relatively new and hence not widely used. A few reproducibility studies were identified using this classification. However, overall, even though the reproducibility of three-dimensional ultrasound scan remained good using this classification, it was lower than the American Society for Reproductive Medicine classification (American Fertility Society, 1988). It may be partly due to lack of experience in using this new classification system. In addition to that, 'Arcuate uterus' has been removed from this classification system. The ESHRE/ESGE consensus does not consider arcuate uterus as an anomaly and considers all cases with internal midline indentation <50% of the uterine wall thickness to be a normal uterus. Therefore most of the arcuate uterus by the American Society for Reproductive Medicine would be classified as U0 or U2a, hence this will cause inconsistencies in classifications.

3.4.5.1 Limitations and strengths

This systematic review is limited by lack of clinical information of the patient population in the studies. We were unable to obtain all the clinical information of all the women. In addition to that, most of the

studies were retrospective observational studies which are obviously prone to selection bias and confounders. We addressed the problem of heterogeneity by analysing different diagnostic classification system. Our systematic review's strengths include its extensive electronic and manual search approach and the care taken in study design, data extraction, and analysis. We have examined the quality of the studies based on existing guidelines for reporting reliability and agreement studies (GRRAS) (Kottner et al., 2011). However, we included all studies that meet the selection criteria but did not exclude studies on the basis of inadequate quality.

3.4.5.2 Future Work and Recommendations

As three-dimensional ultrasound scan becomes more widely available and clinicians more experienced in using them to diagnose congenital uterine anomalies, not only it could be used to quantitatively describe the uterine morphology, it could also describe the severity of the anomalies and its association with adverse pregnancy outcomes. Only with quantitative description of uterine morphology would ensure the standardised diagnostic criteria are used. This would certainly help clinicians to understand the reproductive impact of varying degrees of congenital uterine anomalies and help to refine selection criteria for surgery to correct uterine anomalies. However, even though congenital uterine anomalies are not uncommon, it is still relatively rare and hence any large scale prospective and meaningful studies can only be achieved by multi-centre collaboration.

3.4.6 Conclusions

This systematic review has demonstrated that three-dimensional ultrasound scan for diagnoses of congenital uterine anomalies are highly reproducible, especially when The American Society for Reproductive Medicine Classification (1988) is used with additional morphometric criteria. There is a need to perform prospective studies using three-dimensional ultrasound with quantitatively described classification system, to assess the reproductive impact of congenital uterine anomalies. This will also subsequently lead to further studies to investigate treatment options for congenital uterine anomalies.

Chapter 4 Prevalence of Congenital Uterine MAlformations (PUMA) in High Risk Women

4.1 Introduction

From our systematic review on the prevalence of congenital uterine anomalies, we understand that the prevalence of uterine anomalies varied in different populations. The review concluded that uterine malformations or anomalies are more prevalent in high-risk groups with the exception of subfertile population. Congenital anomalies have been suggested as one potential cause for preterm labour and varies mechanisms including cervical incompetence (Airoldi et al., 2005, Berghella et al., 2007, Roberts et al., 1995), abnormal uterine contractions (Dabirashrafi et al., 1995) and reduced uterine volume (Braun et al., 2005, Pellerito et al., 1992, Puscheck and Cohen, 2008, Reuter et al., 1989) have been suggested. Unfortunately despite these links, no appropriate studies investigating the prevalence of uterine anomalies in women with preterm delivery were identified in our systematic review.

Nottingham Academic Imaging Suite and Nottingham University Research and Treatment Unit (NURTURE) have already established a large three-dimensional pelvic ultrasound database on women requiring assisted reproductive treatment. This ultrasound service has allowed us to gather information on subfertile women (Jayaprakasan et al., 2011) and improve our ultrasound experience and techniques in diagnosing congenital uterine anomalies. Based on our experience and information from systematic reviews, we have decided to carry out a study to investigate the prevalence of uterine anomalies in women with preterm delivery. Not only this study allow us to investigate the prevalence of congenital uterine anomalies in these women, but also allow us to utilise three-dimensional ultrasound scan to assess the uterine, endometrial and cervical dimensions, to examine if these differ between women with uterine anomalies and those with normal uteri. We have included women with history of miscarriage (both early and late) in order to investigate the ultrasound dimensions in these women too.

The main objective of this study was to test the hypothesis that women with miscarriage or preterm birth have higher prevalence of uterine anomalies.

4.2 Materials and Methods

4.2.1 Experimental Design

This study was a prospective dual-centre observational study at the Department of Obstetrics and Gynaecology of the Nottingham

University Hospitals NHS Trust. Participants were recruited from both hospitals of this trust, i.e. Queen's Medical Centre and Nottingham City Hospital.

This study would not change the participants' usual care. However, they would be required to have a single three-dimensional transvaginal ultrasound scan at mutual convenient time. The scan posed no adverse effects. The scan was performed at the University of Nottingham Academic Imaging Suite at Queen's Medical Centre Hospital. The scan was conducted at least eight weeks after the end of last known pregnancy and during the luteal phase (day 14 onwards) of the menstrual cycle. All scans were performed by one of the Academic Imaging Team members (Y.Y.C., L.T.P., and M.N.B.) following standard operating procedures in the department. The ultrasound examination, data acquisition, and measurement techniques are as described in Chapter 2.3.

Due to the study requiring patient participations, ethical and R&D approvals were sought through the Integrated Research Application System (IRAS). The study received full ethical approval from the Nottingham 1 National Research Ethics Service Committee East Midlands (REC Reference: 11/EM/0330). It also obtained R&D approval from the Nottingham University Hospitals NHS Trust Research and Innovation Department (Study Reference: 11GY007). This study is registered with ClinicalTrials.gov (Study Identifier: NCT01487616).

4.2.1.1 Participant Recruitment

Nottingham University Hospitals NHS Trust provides care to over 2.5 million residents of Nottingham its surrounding communities. There is an Early Pregnancy Unit and Recurrent Miscarriage Clinic intended for the care of women with early pregnancy problems. In addition, Queen's Medical Centre and Nottingham City Hospital have approximately 10,000 births per year in total. Therefore, a large number of women with miscarriage or preterm birth presented to this trust.

Women with a history of miscarriage were identified from Early Pregnancy Unit, Recurrent Miscarriage Clinic, and Gynaecology Clinics. Women with a history of preterm birth (< 37 weeks gestation) were identified from labour suite, obstetrics wards, and antenatal clinics. Control group consisted of women with normal term delivery (37 or more weeks of gestation) and they were identified from labour suites, obstetric wards, and antenatal clinics. Women who fit the inclusion criteria were approached verbally or in writing if they would like to participate in this study. The initial approach was usually from a member of the patient's usual care team (which may include one of the study investigators).

Potential participants were fully informed of all aspects pertaining to participation in the study. Written participant information sheets [(see Appendix 1: Participant Information Sheet (Study Group) and Appendix 2: Participant Information Sheet (Control Group)] were given to the women and women would have sufficient time to consider participating

or not (minimum of 24 hours). Informed consent was obtained from each participant before they undergo any investigations related to the study.

4.2.1.2 Inclusion and Exclusion Criteria

Women with a history of miscarriage

<table>
<tr><th>Inclusion criteria</th><th>Exclusion criteria</th></tr>
<tr><td>Age 18 or more</td><td>Miscarriage of multiple pregnancy</td></tr>
<tr><td rowspan="4">Definition of miscarriage: the spontaneous pregnancy loss up until 24 weeks of gestation (Royal College of Obstetricians and Gynaecologists, 2011), where the pregnancy was confirmed histologically or with previous presence of a gestational sac with or without fetal pole and fetal heart activity on ultrasound scanning.</td><td>Miscarriage of pregnancy with known fetal anomalies</td></tr>
<tr><td>Miscarriage after medical intervention, e.g. amniocentesis, chorionic villus sampling, termination of pregnancy, cervical cerclage, induction of labour</td></tr>
<tr><td rowspan="3">Women with known:
a. Antithyroid antibodies
b. Antiphospholipid antibodies, e.g. lupus anticoagulant antibodies, anticardiolipin antibodies, anti-B_2 glycoprotein-I antibodies
c. Thrombophilias
d. Infective cause for miscarriage
e. Pre-existing diabetes before pregnancy</td></tr>
<tr><td>Previous pregnancy history may include term deliveries but not preterm deliveries</td></tr>
<tr><td>Miscarriage may include missed miscarriage which undergone treatment</td></tr>
<tr><td>At least 8 weeks after the end of last pregnancy (to account for involution of the uterus, which usually complete by 6 weeks)</td></tr>
</table>

Women with preterm birth

<table>
<tr><th>Inclusion criteria</th><th>Exclusion criteria</th></tr>
<tr><td>Age 18 or more</td><td>Preterm delivery of multiple pregnancy</td></tr>
<tr><td rowspan="2">Definition of preterm birth: birth at less than 37 weeks of gestation (but 24 weeks or more)</td><td>Preterm delivery of pregnancy with known fetal anomalies</td></tr>
<tr><td>Preterm delivery of stillbirth</td></tr>
<tr><td>Preterm deliveries only included pregnancies with spontaneous labour (regardless of eventual mode of delivery)</td><td>Preterm delivery after medical intervention, e.g. amniocentesis, chorionic villus sampling, termination of pregnancy, cervical cerclage, induction of labour</td></tr>
<tr><td>Previous pregnancy history may include term deliveries and miscarriage</td><td rowspan="2">Women with known:
a. Antithyroid antibodies
b. Antiphospholipid antibodies, e.g. lupus anticoagulant antibodies, anticardiolipin antibodies, anti-B_2 glycoprotein-I antibodies
c. Thrombophilias
d. Infective cause for preterm birth
e. Pre-existing diabetes before pregnancy or diabetes in pregnancy</td></tr>
<tr><td>At least 8 weeks after the end of last pregnancy (to account for involution of the uterus, which usually complete by 6 weeks)</td></tr>
</table>

Controls

Inclusion criteria	Exclusion criteria
Age 18 or more	Had cervical cerclage in pregnancy
Gestation at birth: 37 or more weeks	Pregnancy with known fetal anomalies
All previous pregnancies were delivered at term (37 or more weeks) or ended with medical termination	Delivery of multiple pregnancies
	Delivery of stillbirth
Spontaneous labour or induction of labour	
All vaginal and caesarean deliveries	
At least 8 weeks after the end of last pregnancy (to account for involution of the uterus, which usually complete by 6 weeks)	

4.2.1.3 Other Exclusion Criteria

1. Pregnant on the day of the three-dimensional transvaginal ultrasound scan
2. Recent uterine or endometrial surgery
3. Previous surgery to correct congenital uterine anomalies
4. Unable to tolerate three-dimensional transvaginal ultrasound scan
5. Unable to give informed consent

4.2.2 Outcome Measures

The primary outcome was the number of women diagnosed with congenital uterine anomalies in women with a history of miscarriage or preterm birth (<37 weeks gestation) compared to control group. Secondary outcomes included presence of ultrasound markers on endometrial, uterine, and cervical dimensions in different groups.

4.2.3 Statistical Analysis

4.2.3.1 Sample size calculation

From our recent systematic review in Chapter 3.1 (Chan et al., 2011), the prevalence of uterine malformation is 5.5% in unselected population. A minimum 15% increase in the prevalence of uterine malformations in high-risk population is considered significant.

A 2.5% significance level and 80% power level was used in the power calculations. In view of this, approximately 91 women were needed in each group (Total 273 women).

4.2.3.2 Statistical Methods

All statistical analyses were performed with SPSS version 21.0.

Prevalence data are presented as means or proportions (95% Confidence interval). The Fisher exact test or Chi-square test will be used to determine the differences between the prevalence of uterine anomalies in different populations.

Continuous data distribution was tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk statistic tests. Data were presented as mean and standard deviation (SD) or median and range depending on their distributions. Any differences in three-dimensional transvaginal ultrasound scan measurements between the groups were assessed using Analysis of variance (ANOVA) or Kruskal-Wallis test. A *P* value of <0.05 was considered significant (with Bonferroni correction where appropriate).

4.2.4 Data Collection and Storage

All participants were assigned a study number, which was used in all data storage formats (paper and electronic). There was a separate confidential record of participant's name, date of birth and hospital number; to permit identification of all participants enrolled in the study. This was to ensure that appropriate arrangements could be made to contact participant's General Practitioner or treating clinician as a result of noticing pathology during ultrasound scan, which might require further diagnostic testing and management.

All data were treated as confidential documents and held securely in accordance with regulations. All data were stored on University of Nottingham computers or ultrasound machines but all identifying information were removed. All staff involved endeavoured to adhere to the Data Protection Act, 1998.

4.3 Results

4.3.1 Overview

The participant recruitment flow chart is demonstrated in Figure 39. A total of 212 patients were recruited and scanned over 16-months period (November 2012 until December 2013), of which 38 women were in the control group, 96 women in the miscarriage group and 78 women in the preterm group. Participant recruitment flow chart is demonstrated in Figure 39.

Women in preterm group appeared to be younger compared to the other two groups. The high risk groups had comparable body mass index when compared to the control group. The groups had significantly different total number of pregnancies in each group. The number of women who had cervical surgery such as Large Loop Excision of the Transformation Zone (LLETZ) in different groups was not statistically different. The groups' baseline characteristics were shown in Table 14.

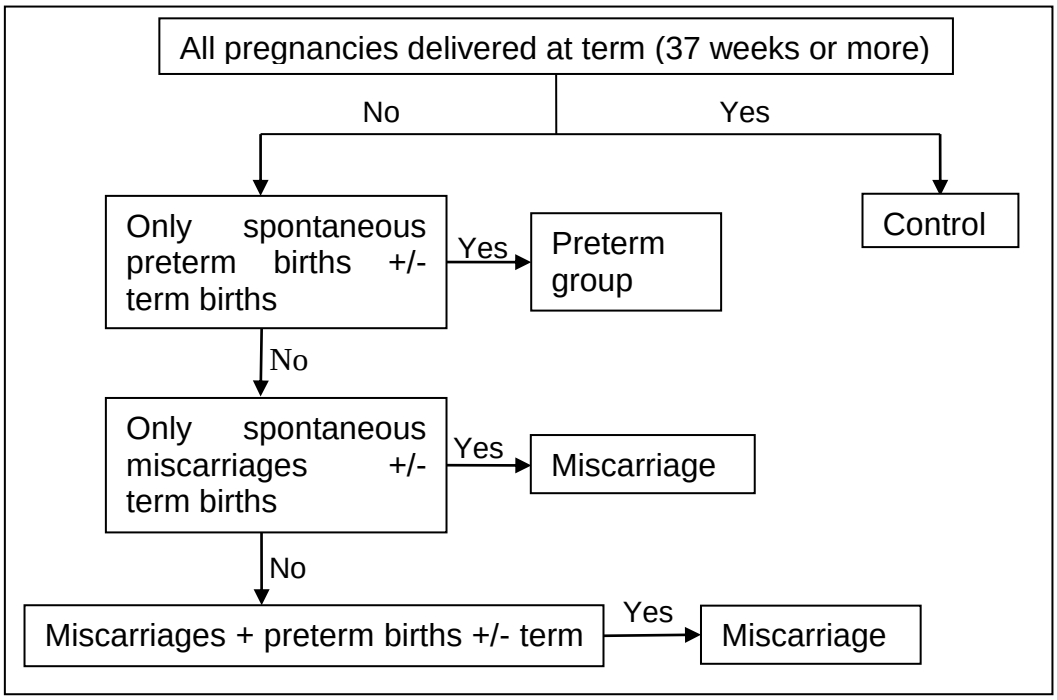


Figure 39: Participant Recruitment Flow Chart

Characteristics	Control (38 women)	Miscarriage (96 women)	Preterm (78 women)
Age (years)	34.42 ± 3.67	34.27 ± 6.07	31.35 ± 5.7*
Body Mass Index (kg/m^2)	24.66 ± 3.91	25.94 ± 5.98	24.55 ± 3.78
Mean number of pregnancies	1.61 ± 0.68*	4.52 ± 2.14*	2.36 ± 1.93*
Number of women with: 1 pregnancy 2 pregnancies ≥ 3 pregnancies	 18 (47.4%) 18 (47.4%) 2 (5.2%)	 15 (15.6%) 19 (19.8%) 62 (64.5%) - 39 of them had ≥ 3 miscarriages	 36 (46.2%) 21 (26.9%) 21 (26.9%)
Number of women with history of cervical surgery	4 (10.5%)	11 (11.5%)	4 (5.1%)

Table 14: Baseline characteristics of the study population

** indicates P<0.05*

Of the 212 women recruited, only 211 ultrasound data was analyse. Overall, 23.7% of the women had congenital uterine anomalies but large majority of them were arcuate uteri (90%). Therefore, the comparisons on ultrasound parameters were between ‘Normal uteri’ and ‘Abnormal uteri’, as number of other types of anomalies were too small for any valid analysis.

A summary of different ultrasound parameters by comparing women with normal uteri to abnormal uteri in different population is given in Table 15.

		Control, mean ± SD	Miscarriage, mean ± SD	Preterm, mean ± SD	Overall, mean difference (p value)
Uterine Length	Normal	3.72 ± 0.45	3.56 ± 0.59	3.62 ± 0.60	0.339 (0.007)
	Abnormal	3.68 ± 0.61	3.27 ± 0.77	2.93 ± 0.69	
Cervical Length	Normal	3.06 ± 0.48	3.26 ± 0.55	3.03 ± 0.51	0.111 (0.281)
	Abnormal	2.87 ± 0.53	3.25 ± 0.47	2.90 ± 0.45	
Myometrial Volume	Normal	47.83 ± 15.53	52.57 ± 19.72	54.68 ± 22.67	- 4.971 (0.225)
	Abnormal	65.41 ± 23.99	54.74 ± 19.96	49.84 ± 15.58	
Cervical Volume	Normal	20.41 ± 9.48	23.98 ± 8.50	18.95 ± 9.37	1.887 (0.326)
	Abnormal	17.68 ± 9.50	25.85 ± 7.64	14.15 ± 7.04	

Table 15: Comparing normal uteri to abnormal uteri in different population

Shaded area indicated statistical difference when comparing normal uteri group with abnormal uteri group

4.3.2 Prevalence of Uterine Anomalies

There were a total of 50 women with abnormal shaped uterus. 161 women had normal uterus. We had to exclude one patient from the analysis as we could not confirm if the endometrial cavity shape was normal due to the presence of multiple fibroids distorting the endometrial cavity.

The chi-squared test statistic is 9.707 with an associated *P*=0.008, which meant there were statistically significant difference in

the proportion of abnormal uterus in each group. Examining the numbers, there are more abnormal uteri in the miscarriage group than the other groups. 13.2% were abnormal in the control group, 33.7% were abnormal in miscarriage group, and 16.7% were abnormal in the preterm group. The most common type of anomaly seen was arcuate uterus (45/50, 90%) (See Table 16).

In view of the small number of other types of anomalies, all anomalies were grouped together (as 'Abnormal uterus' group) for analysis of the remaining of the study.

Groups	Normal Uterus, n (%)	Abnormal Uterus, n (%)	Abnormal Uterus, n (% within the group)		
		All	Arcuate	Septate	Bicornuate
Control	33 (86.8)	5 (13.2)	5 (100)	0 (0)	0 (0)
Miscarriage	63 (66.3)	32 (33.7)*	28 (87.5)	3 (9.4)	1 (3.1)
Preterm	65 (83.3)	13 (16.7)	12 (92.3)	1 (7.7)	0 (0)
Total	161 (76.3)	50 (23.7)	45 (90)	4 (8)	1 (2)

Table 16: Prevalence of congenital uterine anomalies in different populations

4.3.3 Uterine Cavity Length

One of the three-dimensional ultrasound scan parameters was uterine cavity length. All 211 women recruited had their uterine cavity length measured. ANOVA tests showed no significant interaction between the normality of uterine shape and the population groups on uterine cavity length, F (2, 205) = 2.267, *P*=0.106, i.e. the effect of uterine anomaly on uterine cavity length is the same for all population

groups. The overall uterine cavity length in different population groups were not significantly different (F (2, 205) = 2.998, *P*=0.052).

Women with uterine anomalies had significantly shorter uterine cavity length (by 3.9 mm) than those with normal uteri (F (2,205) = 7.495, *P*=0.007). Further pairwise analysis was performed to compare uterine cavity length between those with normal uteri versus abnormal uteri in different populations. Women with abnormal uteri had shorter uterine cavity lengths compared to those with normal uteri within miscarriage group (shorter by 2.9 mm, *P*=0.029) and preterm group (shorter by 6.9 mm, *P*<0.001).

Groups	Normal Uterus	Abnormal Uterus	*P*
Control	3.72 ± 0.45	3.68 ± 0.61	0.895
Miscarriage	3.56 ± 0.59	3.27 ± 0.77	* 0.029
Preterm	3.62 ± 0.60	2.93 ± 0.69	* <0.001

Table 17: Uterine cavity lengths in different population

*The results were shown in mean (cm) ± Standard Deviation. * indicates statistical significance with adjustment for multiple comparisons (Bonferroni)*

4.3.4 Cervical Length

Of the 211 women included in the study, we could not measure the cervical length of two women (from preterm group, with normal endometrial cavity shape) due to the position of the uterus. Women who had cervical surgery were not excluded from analysis as the overall number of those was small.

There was no significant interaction between the normality of uterine shape and the population groups on cervical length, F (2,203) =

0.294, *P*=0.745, i.e. the effect of uterine anomaly on cervical length is the same for all population groups. The main effect of uterine anomalies was not statistically significant (F (1,203) = 0.302, *P*=0.281). Table 18 demonstrated the pairwise comparison of cervical length between women with normal uteri versus abnormal uteri within populations.

There was a significant effect of the population groups on the cervical length (F (2, 203) = 5.667, *P*=0.004). Given that there was main effect of the population group, post hoc tests were performed to establish where the difference lies. Post hoc test demonstrated that women from miscarriage group had statistically longer cervical length (by 2.5 mm, *P*=0.005) than women from the preterm group (not adjusted for uterine anomalies). Further subgroup analysis demonstrated that, the difference was restricted to those with normal uteri (when comparing miscarriage group with preterm group, shorter by 2.3 mm, *P*=0.032). All other mean differences were statistically insignificant.

Groups	Normal Uterus (cm^3) ± SD	Abnormal Uterus (cm^3) ± SD	*P*
Control	3.06 ± 0.48	2.87 ± 0.53	0.440
Miscarriage	3.26 ± 0.55	3.25 ± 0.47	0.866
Preterm	3.03 ± 0.51	2.90 ± 0.45	0.413

Table 18: Cervical lengths in different populations

4.3.5 Myometrial Volume of Uterine Body

Women with fibroids (except pedunculated fibroid that could be seen clearly separated from the rest of the uterus) were all excluded from the measurement myometrial volume analysis as presence of

these fibroids would artificially increase the uterine body volume. As for those with pedunculated fibroids, the volume of the fibroid was not included in the measurement. Due to the presence of fibroids, seven women were excluded from analysis. Therefore, only 204 scans were included in the analysis.

There was no significant interaction between the normality of uterine shape and the population groups on myometrial volume, F (2,198) = 1.928, *P*=0.148, i.e. the effect of uterine anomaly on myometrial volume is the same for all population groups. The main effect of uterine anomalies was not statistically significant (F (1,198) = 1.48, *P*=0.225). Table 19 demonstrated the pairwise comparison of myometrial volume between women with normal uteri versus abnormal uteri within populations. Myometrial volume in different population groups were not significantly different (F (2, 198) = 0.292, *P*=0.747).

Groups	Normal Uterus (cm^3) ± SD	Abnormal Uterus (cm^3) ± SD	*P*
Control	47.83 ± 15.53	65.41 ± 23.99	0.070
Miscarriage	52.57 ± 19.72	54.74 ± 19.96	0.624
Preterm	54.68 ± 22.67	49.84 ± 15.58	0.429

Table 19: Myometrial volume of uterine body in different populations

4.3.6 Cervical Volume

Some datasets for cervical volume were difficult to obtain, as axial or retroverted uterus; or acutely rotated uterus made the acquisition very challenging. 20 women were excluded from analysis as the result of this. Only 191 scans were analysed.

There was no significant interaction between the normality of uterine shape and the population groups on cervical volume, F (2, 185) = 2.090, *P*=0.127, i.e. the effect of uterine anomaly on cervical volume is the same for all population groups. The main effect of uterine anomalies was not statistically significant (F (1, 185) = 0.969, *P*=0.326). There was a significant effect of the population groups on the cervical volume (F (2, 185) = 13.154, *P*<0.001). Table 20 demonstrated the pairwise comparison of cervical volume between women with normal uteri versus abnormal uteri within populations.

Given that there was a main effect of population group, post hoc tests was performed to establish where the difference lies. The tests showed that women from miscarriage group has statistically larger cervical volume than those from control group (by 4.6 cm^3, *P*=0.035) and preterm group (by 6.5 cm^3, *P*<0.001) respectively.

Groups	Normal Uterus (cm^3) ± SD	Abnormal Uterus (cm^3) ± SD	*P*
Control	20.41 ± 9.48	17.68 ± 9.50	0.559
Miscarriage	23.98 ± 8.50	25.85 ± 7.64	0.327
Preterm	18.95 ± 9.37	14.15 ±7.04	0.085

Table 20: Cervical volume in different populations

4.4 Discussions

The study was still ongoing as the target sample size was not achieved by the time the thesis was submitted. The main preliminary finding on this study was the significantly higher prevalence of abnormal uteri in women with miscarriage (33.7%) than the low–risk women (13.2%) or women with history of preterm birth (16.7%). Arcuate uterus

was the most common anomaly across all three groups (up to 90% of all anomalies in the current study). Other anomalies seen were septate and bicornuate uteri. To date, this was the first prospective study using three-dimensional ultrasound scan to evaluate the prevalence of uterine anomalies in women with preterm births.

Comparing to our systematic review (Chan et al., 2011) on prevalence of uterine anomalies (See 3.1), our low-risk or control group had a much higher occurrence of congenital uterine anomalies (5.5% versus 13.2%). Similarly, PUMA study also demonstrated that 33.7% of women with miscarriage had congenital uterine anomalies (as opposed to only 12.9% in systematic review). The higher prevalence was most likely due to the design of PUMA study. It was designed specifically to look for congenital uterine anomalies. Therefore, it was more likely that our current study diagnose all congenital uterine anomalies especially the arcuate uteri, which could otherwise be ignored in a routine scan. A lot of the studies in the systematic reviews were retrospective studies and congenital uterine anomalies, hence some anomalies, especially minor anomalies were easily missed or ignored.

The varied background populations or clinical presentations of women included in the systematic reviews would affect the prevalence of congenital uterine anomalies diagnosed too. For the unselected population, all the studies from the systematic reviews included women who presented to hospitals with different clinical problems, whilst women recruited prospectively in PUMA study were women with only history of full term pregnancies and all within reproductive age, who

were mostly generally fit and healthy, without major medical or gynaecological problems (The reason they were in the hospital was purely to have their babies). For the Miscarriage group, a big proportion of our women had history of recurrent miscarriage compared to studies included in the systematic review, which obviously could be a cause for selection bias as well.

Systematic review suggested that the most commonly seen anomaly in women with miscarriage was subseptate/septate uteri, which was not consistent with our findings in this study. However, the systematic review had included all other acceptable optimal diagnostic tests, which might explain the discrepancy in the results. The final diagnosis of some of these tests was based on the subjective impression of the clinician performing test (Woelfer et al., 2001). As explained before, arcuate uterus (a minor anomaly) was easily missed or ignored in studies in systematic reviews. In addition to that, some arcuate uteri could be misclassified as subseptate/septate uteri.

We decided to perform scans during the luteal phase of the menstrual cycle as oppose to early follicular phase in our observational study in subfertile group (Jayaprakasan et al., 2011). From our experience, it was easier to see the endometrial cavity during the luteal phase and hence making diagnosis easier. In addition to that, luteal phase is the stage where any pregnancy implants and grow, any anomaly seen during this stage may identify any association of uterine anomaly with poor reproductive outcomes.

In the current study, it was shown that women with uterine anomalies had significantly shorter uterine length, especially those from high risk populations. This was consistent with the theory of diminished uterine size by some studies (Reichman and Laufer, 2010).

Overall, women with history of preterm birth appeared to have shorter cervical length than those from miscarriage group. Short cervix in pregnancy is a predictive indicator for preterm labour (Honest et al., 2009). Hence, it made theoretical sense that women in this group had shorter cervix. One wonders if women with preterm birth and congenital uterine anomalies had shortened cervix pre-pregnancy. Yet, this was not the case when comparing between preterm group women with normal uteri and abnormal uteri.

However, as stated before, uterine cavity length or cervical length may not be the best way to measure the uterine or cervical musculature or muscle volume. It is unclear whether diminished muscle mass or volume plays a role in poor reproductive outcomes, especially for second trimester miscarriage and preterm delivery. We have therefore opted to measure the myometrial volumes and cervical volumes in this study. We had modified our techniques in measuring volume as suggested by (Mansour and El-Shalakany, 2012). However, our study did not reveal any difference in myometrial volume, regardless the presence of uterine anomalies or what population.

When comparing cervical volume in different populations during the study, it was very surprising to find that the cervical volume in the miscarriage population to be larger than women from the low risk

groups. Hence, the results from the both the volumetric measurements were surprising as one would expect reduced myometrial volume or cervical volume in women with abnormal uteri or women from high risk populations. However, it was noted that the measurement of both uterine and cervical volume proved to be difficulty. It is likely that the measurements are not reliable. Casikar et al has demonstrated that VOCAL technique for uterine volume systematically underestimated the actual volume measurements but it was no doubt an useful tool (Casikar et al., 2015).

4.4.1 Limitations

There are significant limitations in our prospective study. The study was still recruiting patients to achieve target sample size at the time this thesis was written, and hence the results reported were preliminary findings. As the full sample size was not achieved, the study is under-powered and is likely to subject to bias when the preliminary results were analysed. Full analyses of the whole study would provide a more reliable picture once the sample size is achieved.

There had been difficulty in recruiting patients especially in the Control group, as some patients were reluctant to have any additional research ultrasound scans in addition to their normal clinical care. There were strict exclusion criteria for the high risk groups in order to ensure other causes for miscarriages or preterm deliveries were excluded (See 4.2.1.2), such as diabetes in pregnancy, pre-eclampsia etc. These criteria excluded a proportion of women (especially in

Preterm group) who were initially approached by clinicians regarding the study. However, we did not exclude women who had cervical surgery such as LLETZ. It is thought that women who had cervical surgery such as LLETZ are at increased risk of second trimester miscarriage or preterm delivery (Kyrgiou et al., 2014). None of our patients from Miscarriage group had second trimester miscarriages. Two of the women in the Preterm group who had cervical surgery (total of 4 women) had their surgeries only after they had their preterm deliveries, whilst the other two women in the Preterm group had subsequent full term pregnancies despite LLETZ.

Even though strict inclusion and exclusion criteria were applied in order to recruit women of specific clinical background, there are still different clinical heterogeneity and confounding factors (e.g. smoking status, socioeconomic status) which we had not been able to adjust to.

In addition, some patients presented at Early Pregnancy Unit who had recent diagnosis of miscarriage were uncertain of participation in research study as they were still dealing with their recent pregnancy losses. Therefore, big proportion of the women (40.6%) from Miscarriage group were recruited from Recurrent Miscarriage Clinic. Women from this clinic were more likely to have underlying pathologies due to their clinical background. There is potential selection bias in this group of patients.

Uterine and cervical volume measurements were certainly interesting aspects to be investigated. However, the measurements were obviously challenging and hence likely to be unreliable. Some

authors have suggested that it was difficult to identify the sonographic demarcation between the cervix, the uterine body, and the surrounding tissue (Basgul et al., 2007, Basgul et al., 2006) when measuring these volumes. This would make the measurements less accurate. Reproducibility of volumetric measurements has not been performed in this study as there was insufficient mix of different uterine anomalies. As experience in volumetric measurements grows, intra-observers and inter-observers reliability works would need to be carried out to ensure reproducibility of these measurements. Phantom studies will most certainly be helpful in determining the accuracy of volume measurements.

4.4.2 Future Work or Recommendations

Future studies could concentrate in using three-dimensional ultrasound scan to diagnose congenital uterine anomalies. It is a useful tool to not just diagnose but also perform relevant measurements, which may identify association with poor reproductive outcomes. As stated before, reproducibility studies and phantom studies to ascertain the accuracy of volume measurements and reproducibility studies will be important. Other new features of ultrasound scan such as elastography technique may help to identify if uterine contractility is a potential cause or association of poor reproductive outcomes.

Despite the higher prevalence of uterine anomalies in high risk group, clinicians have to be very careful when counselling high risk women with uterine anomalies in regards to treatment. The most

common type of anomaly is arcuate uterus and there seems to be limited treatment that can be offered to this type of anomaly. In addition, there is no clear evidence demonstrating association of congenital uterine anomalies with poor outcome, and none of the available treatments have been proven useful.

4.5 Conclusions

This study showed that congenital uterine anomalies are not uncommon, particularly in women with history of miscarriage. However, this does not appear to be the case in women with history of preterm births. Uterine length appeared to be shorter in women with uterine anomalies, especially those of high-risk populations, which might indicate the diminished uterine capacity of abnormal uterus. Three-dimensional ultrasound scan is a useful tool for diagnosing congenital uterine anomalies and there is potential in expanding its future use in other ultrasound parameters for congenital uterine anomalies.

Chapter 5 Development of a Randomised Controlled Trial of Hysteroscopic Resection for Uterine Septae

5.1 Introduction

Part of the work is to design a randomised trial to assess the effect of hysteroscopic surgery for uterine anomalies on various pregnancy outcomes in women with septate uteri. This surgery has become the established treatment of choice offered to women with a septate uterus who experience reproductive difficulties (Tomazevic et al., 2007, Valle, 1996, Pellicer, 1997, Saygili-Yilmaz et al., 2003) despite an apparent lack of evidence to support its use. Hysteroscopic metroplasty is not without risk, however and whilst several observational studies (Heinonen, 1997, Valli et al., 2004, Maneschi et al., 1993) have reported an improved outcome following surgical intervention, there is a need to conduct a randomised controlled trial to address the effectiveness and safety of the treatment.

5.2 Surveys

However, before we proceed with this trial, we need to know the awareness and willingness of patients and clinicians to be involved in a randomised controlled of treatment. We have conducted two surveys, to ask the general public and medical experts with a proven interest, both clinical and academic, to give their opinion on the need and feasibility of the study. Their opinion would help us to design the definitive trial.

5.2.1 Overview

Our systematic review has demonstrated a significantly higher prevalence of uterine anomalies in women with recurrent miscarriage compared to the general population. It was also demonstrated in our PUMA study that there was statistically more women with abnormal uteri in the miscarriage group compared to control group (33.7% versus 13.2%). In addition, miscarriage appears to be more common in women with septate and subseptate uteri.

Whilst investigating the association between poor reproductive outcomes and uterine anomalies, we found that patients with septate or subseptate uteri are more likely to suffer from first trimester miscarriage and preterm delivery before 37 completed weeks.

Therefore, we have designed our surveys based on the information we have obtained from our systematic reviews and from PUMA study. The aim of the clinician survey is to obtain information on (1) the attitudes of clinicians towards the proposed trial (2) group of patients to be recruited (3) current surgical techniques that are acceptable and available in different centres or hospitals.

We also conducted a patient survey asking the similar questions. A patients survey is much needed to explore patients' attitudes towards the proposed trial and if they would be happy to be randomised to either treatment or control group. Therefore, a patients questionnaire will be completed prior to the design of the proposed study.

5.2.2 Materials and Methods

Prior to commencing, confirmation was received from the Research and Development Department of Nottingham University Hospitals NHS Trust that ethical approval was not required for the surveys.

The clinician surveys were distributed to members of the United Kingdom Early Pregnancy Clinical Study Group, and also to various clinicians throughout UK. The completed surveys were returned personally, via emails or by post. Clinicians who did not return a completed copy of the questionnaire within two months from the initial contact were contacted again by emails. If one or more questions were not completed, the clinician was contacted again individually to obtain the missing information.

Patient surveys were distributed to patients from fertility unit, gynaecology clinics, and early pregnancy unit at Nottingham University Hospitals NHS Trust, where patients were consulted about various aspects of the trial including the need for the trial and choices of outcomes. The surveys provided current evidence in the literature on congenital uterine anomalies and treatment for septal resection. It also

provided basic details about the proposed clinical trial including the interventions in the study or control group. The clinician survey and patient survey were attached in Appendix 3: Clinician Survey and Appendix 4: Patient Survey.

For each of the questions included in the questionnaire, clinicians or patients were asked to check a box for the appropriate answer. They were allowed to choose more than one answers for each question. Space was provided on the survey to add additional comments or observations which may not have been addressed otherwise.

The results were reviewed by one of the researcher and not the principal investigators. Results from categorical data were presented as percentages in tables and graphs. No formal statistical testing was carried out on the data.

5.2.3 Results of Surveys

5.2.3.1 Clinician Survey

A total of 123 clinicians were approached and 47 completed questionnaires were returned. 40 received via emails and 7 received via paper or postal (from 13 hospitals/centres). This gives an overall response rate from clinicians of 32.5%. However, not all questionnaires were filled in completely as some clinicians were uncertain or unable to provide confident answers. Also, quite a number of the clinicians were

from the same hospitals/centres; in this case, only 10 hospitals/centres in total were included.

Prevalence

In order to find out how feasible it would be to achieve the required sample size, it is crucial to know how many miscarriage patients were seen per annum in other centres. Figure 40 showed that the mode number of patients was 1000-5000 per year. Figure 41 demonstrated number of septate/subseptate patients seen per year in their individual hospitals/centres.

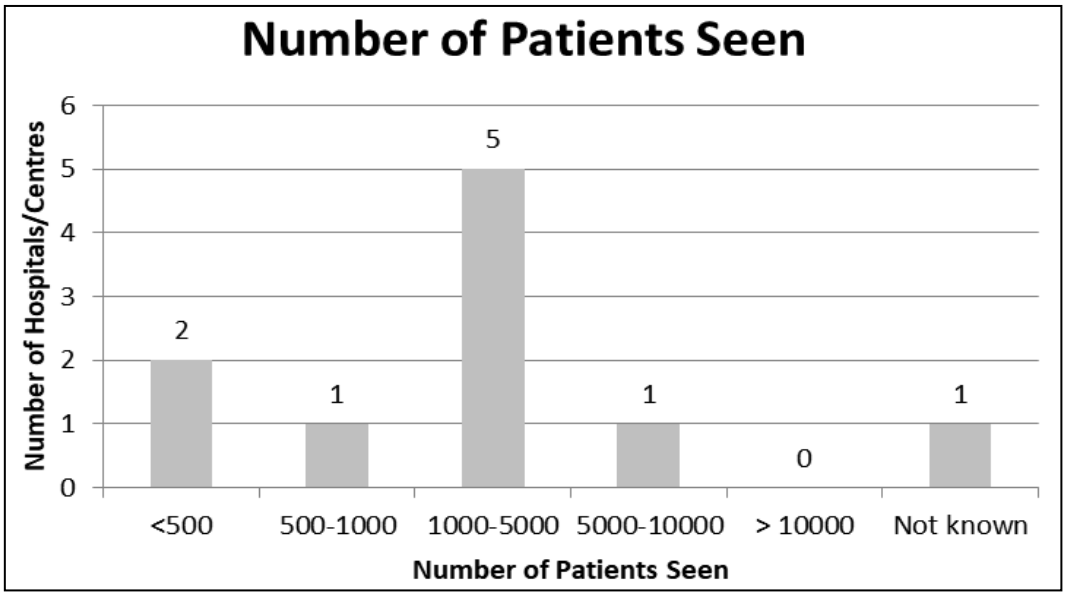


Figure 40: Number of miscarriage patients/year in hospitals

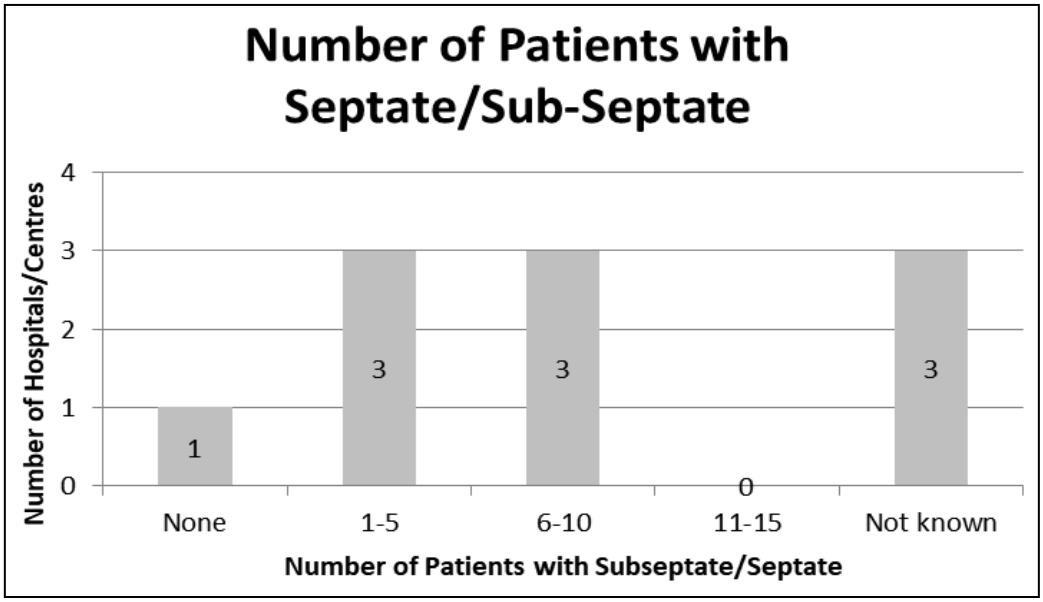


Figure 41: Number of patients/year with septate or subseptate

Minimum Clinically Important Difference

One of the aims of the trial is to test if septal resection improves term birth rate. It is crucial to help us calculate the sample size of the definitive trial. Out of the 47 clinicians, only 20 clinicians answered this question. The remaining clinicians were unable to provide answer due to clinical inexperience or confidence. From Figure 42, most clinicians thought a 20% minimum improvement in term birth rate is required for the trial to be worthwhile, with the average being 15% across all clinicians.

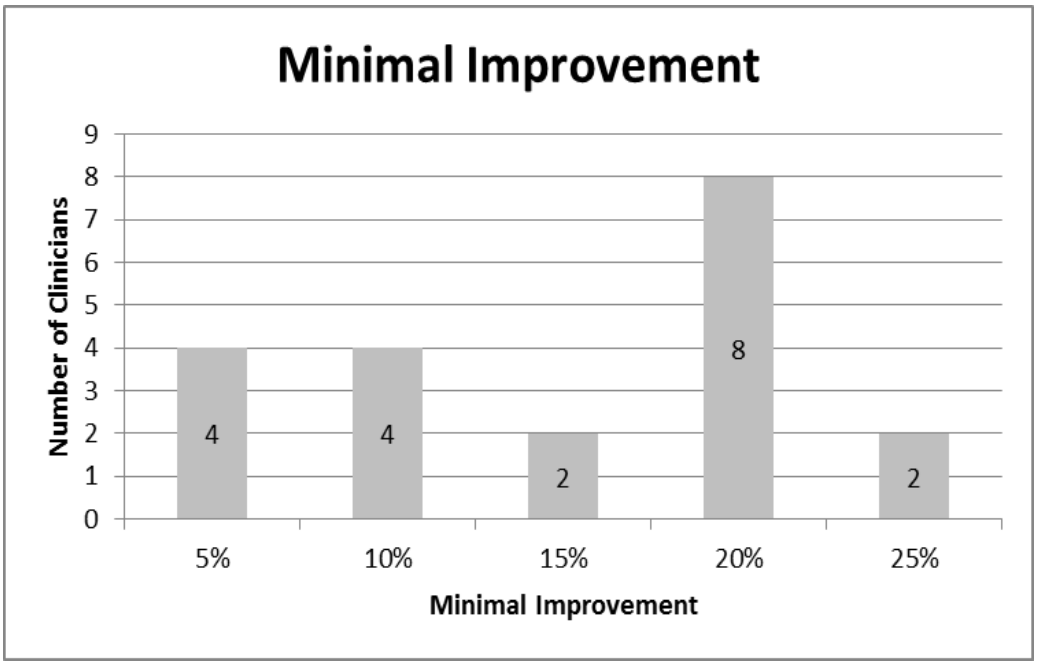


Figure 42: Minimum clinically important difference

Willingness of Clinicians to Randomise

Table 21 (in numbers and percentages) and Figure 43 (in percentages) showed clinicians responses on patient recruitment based on patients' clinical presentation or background.

Clinicians were allowed to choose more than one options which, they consider appropriate (this meant it might be all or none of the options given for each question). Hence, the number of responses for each question would sometimes exceed the total number of clinicians.

Overall, clinicians are willing to recruit patients known to have uterine septae to the proposed trial, in particularly patients with infertility problems and those with recurrent miscarriages or late miscarriage (More than 60% willing to recruit in these cases).

However, some clinicians are reluctant to recruit patients with one or two miscarriages only (27.7% and 19% respectively). 23.4% of

them will not recruit patients with one preterm delivery if the patients have had previous term delivery.

The threshold for treatment drops as patients' clinical presentation becomes recurrent, i.e. recurrent implantation failure and three or more early miscarriages. In these groups, 21.3% and 36.2% of the clinicians respectively, will always offer surgical treatment to patients with uterine septae.

Clinicians' Responses based on Patients Clinical Presentations	Always treat	Agreed to recrutiment	Do not recruit	Do not recruit if previous term delivery	No Comment
Infertility only	6 (12.8%)	35 (74.5%)	3 (6.4%)	9 (19.4%)	2 (4.3%)
Infertility requiring IVF	6 (12.8%)	33 (70.2%)	2 (4.3%)	9 (19.4%)	6 (12.8%)
Recurrent implantation failure	10 (21.3%)	30 (63.8%)	4 (8.5%)	5 (10.6%)	2 (4.3%)
One early miscarriage	5 (10.6%)	27 (57.4%)	13 (27.7%)	7 (14.9%)	0 (0.0%)
Two early miscarriages	10 (21.3%)	22 (46.8%)	9 (19.0%)	7 (14.9%)	5 (10.6%)
Three or more early miscarriages	17 (36.2%)	30 (63.8%)	1 (2.2%)	5 (10.6%)	0 (0.0%)
One late miscarriage	6 (12.8%)	35 (74.5%)	5 (10.6%)	8 (17.0%)	1 (2.1%)
One preterm delivery	9 (19.1%)	29 (61.7%)	8 (17.0%)	11 (23.4%)	0 (0.0%)

Table 21: Clinicians' responses based on patients' clinical presentations

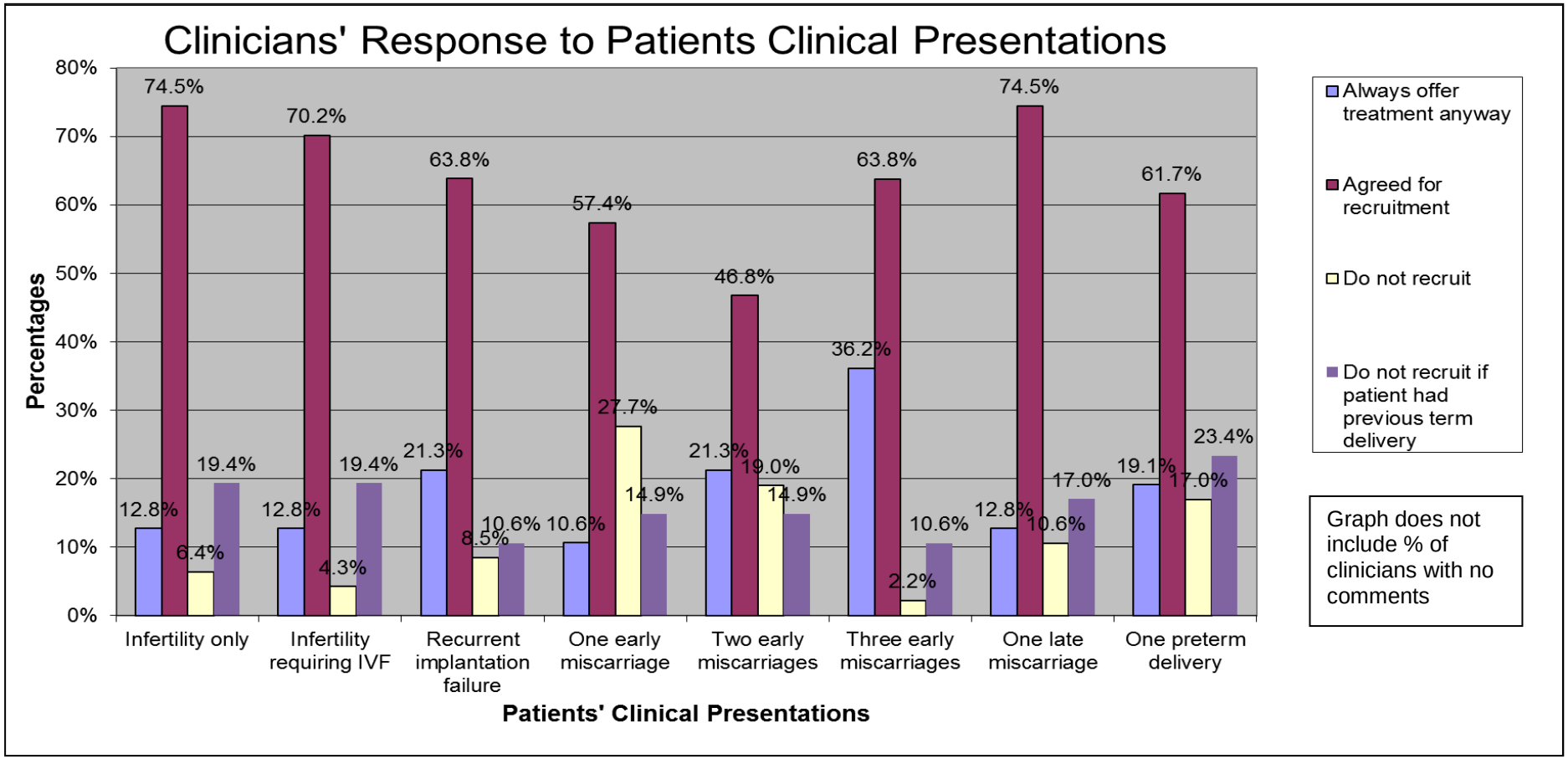


Figure 43: Clinicians' opinions on patient recruitment based on patients' clinical presentations

Technology

Three-dimensional ultrasound scan would be the main diagnostic and follow up imaging tool used in the proposed trial. 60% of the clinicians confirmed the availability of this tool at the centre they're working at (Figure 44).

The survey also enquired about the types of surgical technique that were acceptable for septal resection and what surgical techniques were available in different centres. The results can be seen in Figure 45 and Figure 46. Operating hysteroscope with Versapoint or other bipolar electrode system were the most widely acceptable technique (59.6%) but resectoscope with electrosurgery was the most commonly available surgical equipment (57.4%). Most centres have more than one surgical technique suitable for septal resection.

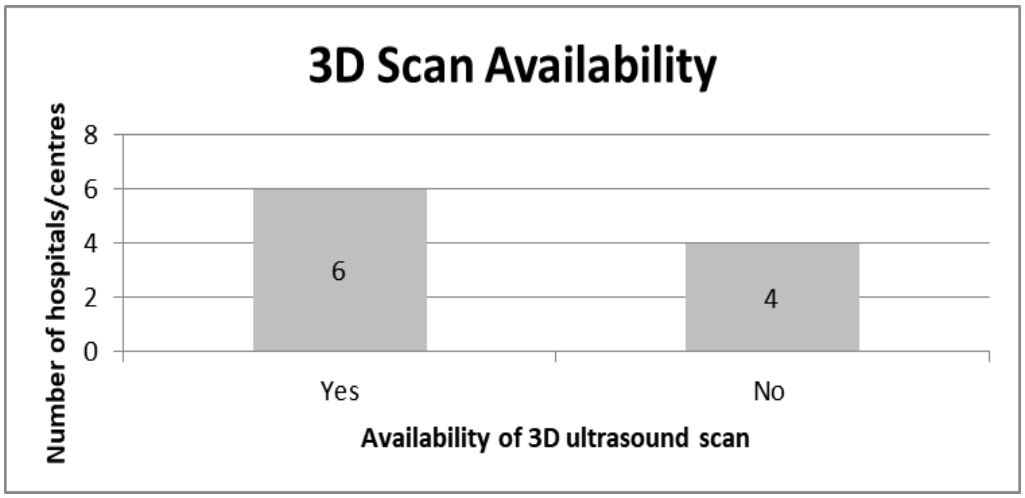


Figure 44: Availability of three-dimensional ultrasound scan

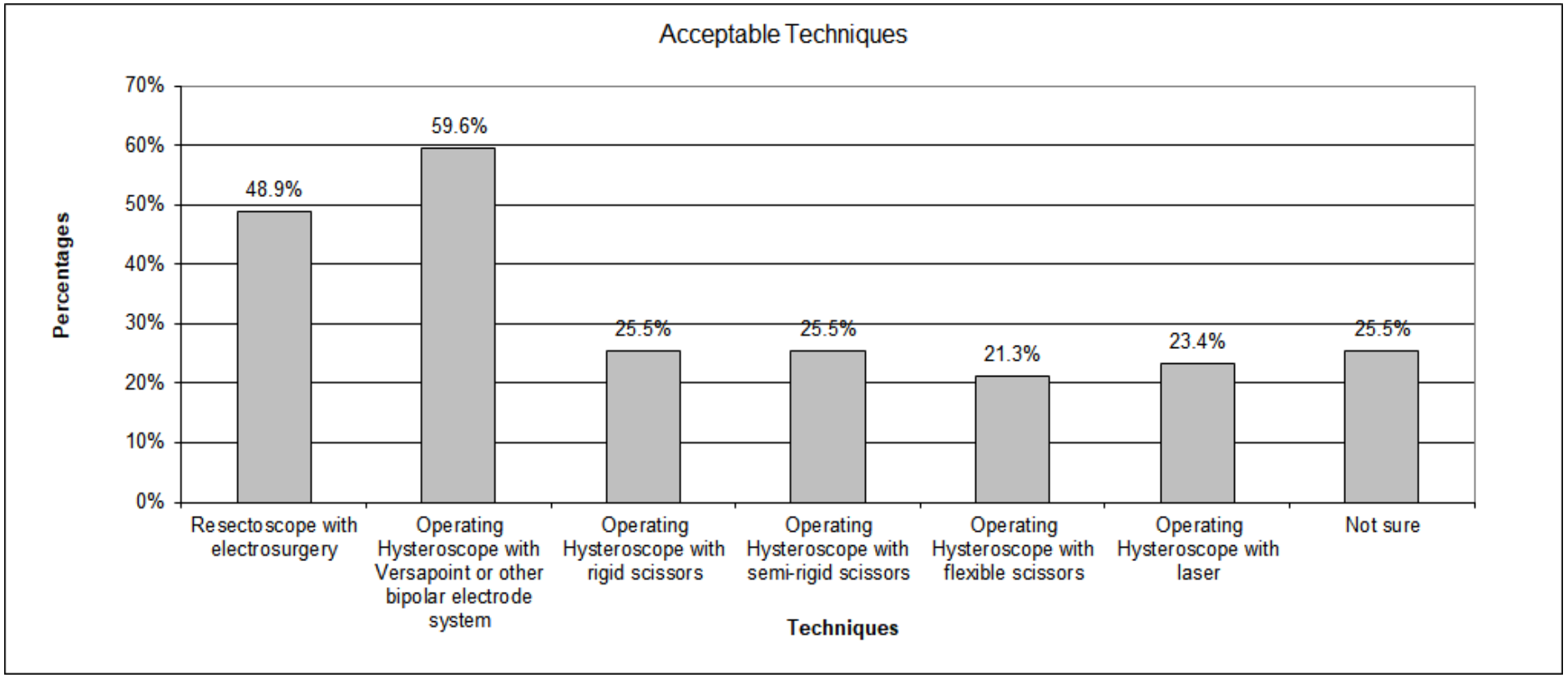


Figure 45: Types of surgical techniques that are acceptable to clinicians

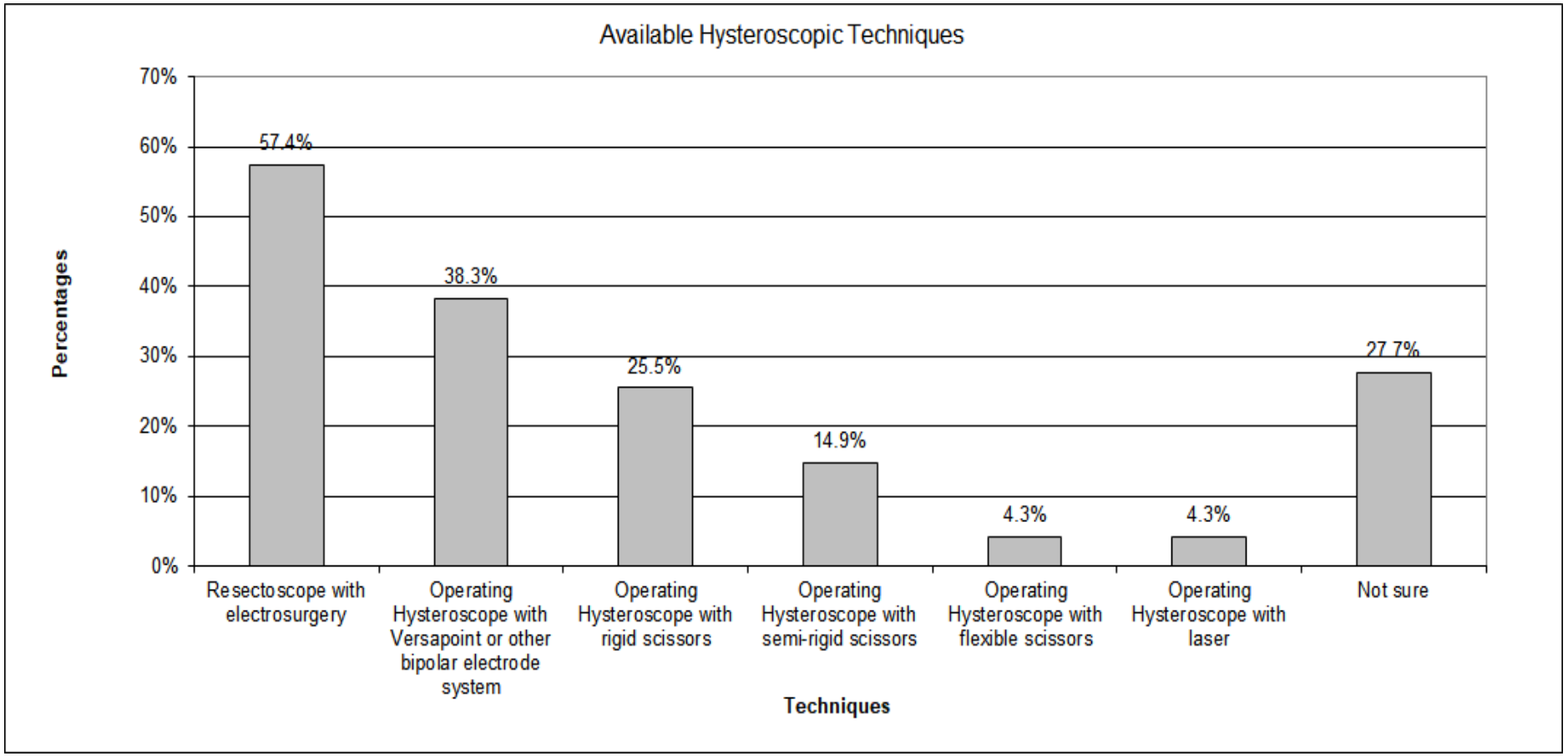


Figure 46: Types of surgical techniques available in the centres clinicians currently working at

5.2.3.2 Patient Survey

A total of 35 patients completed our patient survey. There was unanimous support for the study.

Demographics

Most of the patients surveyed were in the age group 30-40 years old (Figure 47). No patients under the age of 20 surveyed. Most of the patients have some experience in reproductive difficulties. Only 14.3% of the patients had no past difficulties. A high percentage of patients surveyed had subfertility problems (74.3% with subfertility). See Figure 48

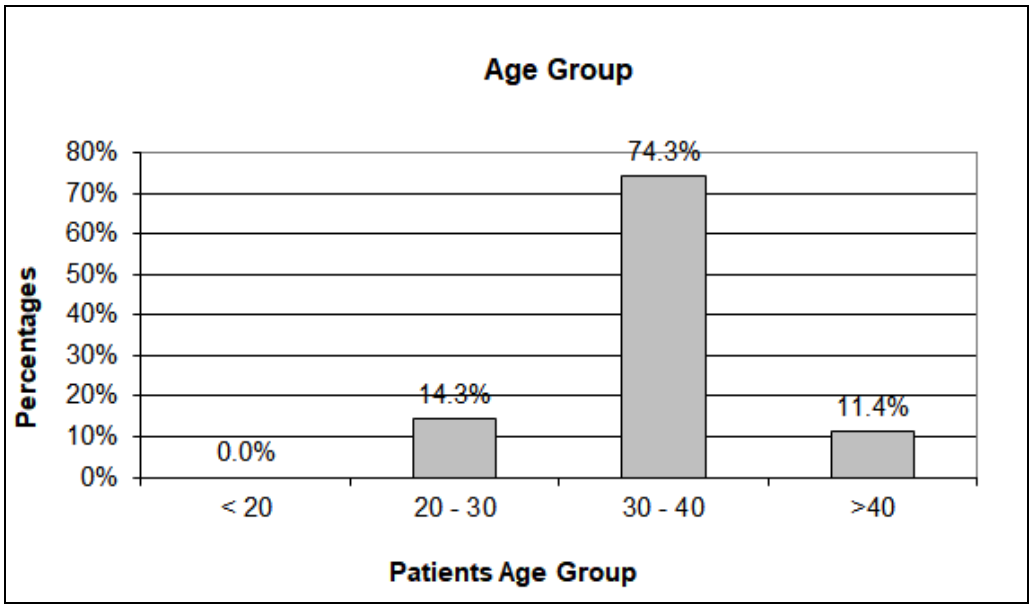


Figure 47: Age groups of patients surveyed

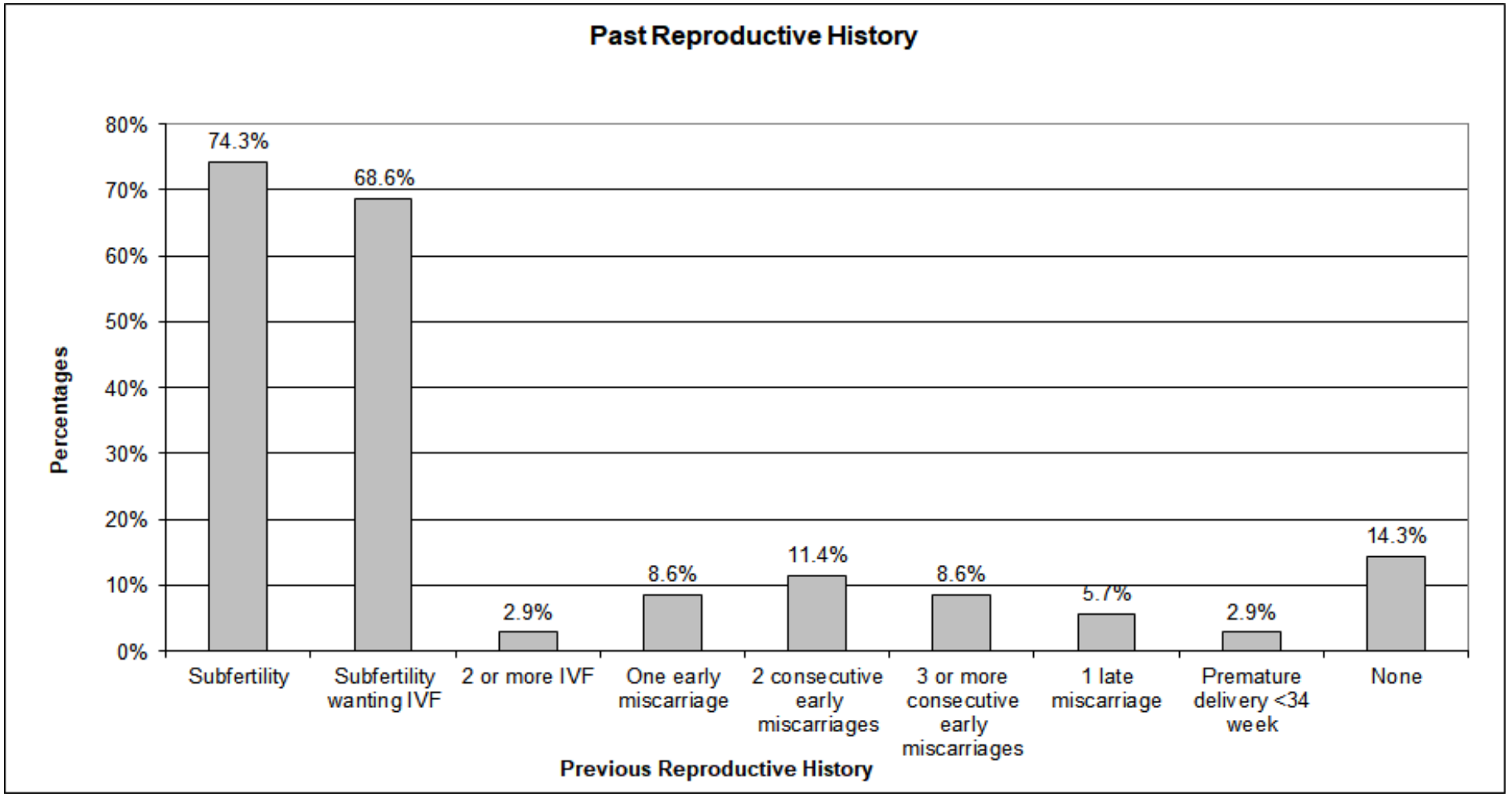


Figure 48: Past reproductive history in patients who completed the survey

Willingness of Patients to be Randomised

Table 22 (in numbers and percentages) and Figure 49 (in percentages) showed patients' responses on patient willingness to enter randomised trial based on patients' clinical presentation or background.

Patients were allowed to choose more than one options which, they consider appropriate (this meant it might be all or none of the options given for each question). Hence, the number of responses for each question would sometimes exceed the total number of patients.

Overall, patients were willing to enter the trial if they were known to have uterine septae with reproductive difficulties. More than 50% of patients would enter the trial in any background history. However, a large proportion would consider also having treatment instead of entering trial when the clinical problems became more recurrent, i.e. those with recurrent implantation failure, and three or more early miscarriages. In these groups, 97.1% and 88.6% respectively, would consider surgical treatment for uterine septae.

Patients' Responses based on Clinical Presentations	Always want treatment	Agreed to enter trial	Refused to enter trial
Infertility only	33 (65.7%)	29 (82.9%)	1 (2.9%)
Infertility requiring IVF	23 (65.7%)	28 (80.0%)	2 (5.7%)
Recurrent implantation failure	34 (97.1%)	26 (74.3%)	1 (2.9%)
One early miscarriage	14 (40.0%)	18 (51.4%)	12 (34.3%)
Two early miscarriages	24 (68.6%)	23 (65.7%)	5 (14.3%)
Three or more early miscarriages	31 (88.6%)	20 (57.1%)	2 (5.7%)
One late miscarriage	9 (54.3%)	22 (62.9%)	9 (25.7%)
One preterm delivery	16 (45.7%)	21 (60.0%)	12 (34.3%)

Table 22: Patients' responses based on clinical presentations

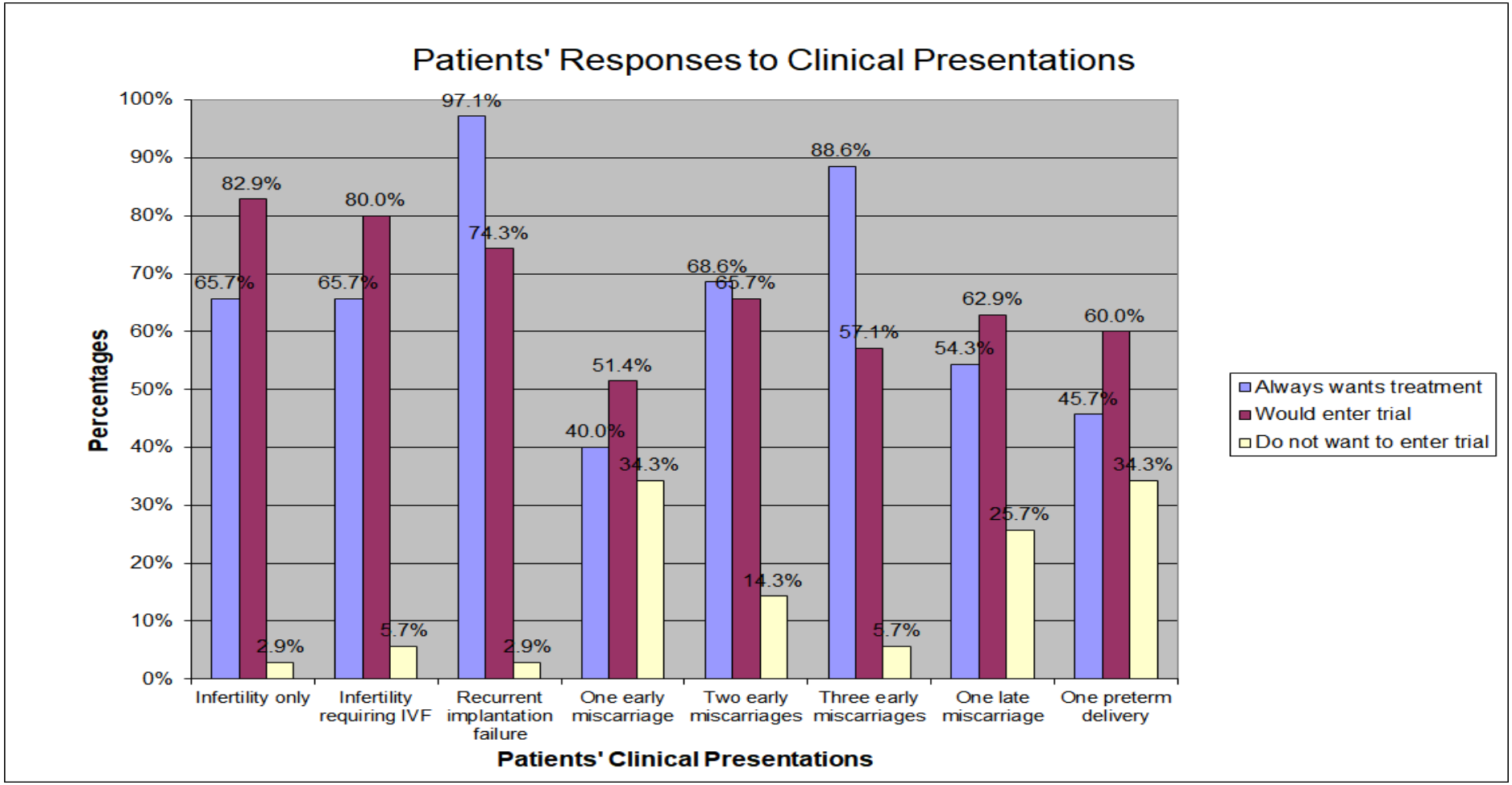


Figure 49: Patients' responses to willingness to randomisations based on clinical presentations

5.2.4 Discussions

This is the first survey, of which we aware, that has formally sought clinicians' views about acceptability of a proposed randomised controlled trial of septal resection.

The key finding in this survey is that, the majority of clinicians, who completed this survey, indicated that they would be prepared to consider entering patients under their care into a randomised controlled trial investigating the effectiveness and risks of hysteroscopic septal resection. Most centres also have the suitable equipment to perform septal resection

This survey has provided important information about reasons why clinicians may not wish to enter some patients under their care into this trial, e.g. in patients with only one miscarriage, as these miscarriages most likely due to chromosomal anomalies of the pregnancy instead of the presence of septum. Hence, offering septal resection would seem over-treating patients at the first instance.

Recruitment has always been a concern in the prospective trial. It was important for us to know how feasible recruitment is in different centres, depending on the number of patients with miscarriage and number of uterine septae case clinicians encountered. Most centres see a large number of miscarriage patients and with improvement in awareness and technology; we expect more uterine septae to be diagnosed. One of the most important aspects of the definitive trial is sample size required. Therefore we had to find out the minimal clinical

improvement in live birth rate required for the trial to be worthwhile. It appears the average was 15% across all clinicians. Based on the prevalence findings in our systematic reviews, we would be able to calculate the sample size required for the definitive trial. For example, in order to improve term live birth by 14%, with a power of 80% and significant threshold of 5%, we need a total of 400 participants in the final definitive trial (with 200 participants in each arm).

There is also concern if medical centres in United Kingdom have the appropriate surgical techniques to perform the surgeries. Therefore, it was important for us to know that most centres in the United Kingdom are willing and capable of participating in the recruitment, with the appropriate techniques (i.e. Versapoint system hysteroscope or resectoscope are the most acceptable and also the most common techniques). Majority of these centres which have the appropriate surgical equipment also have three-dimensional ultrasound scanning facility to organise diagnosis and follow up in trial patients. As three-dimensional ultrasound scan becoming more readily available, this will improve further.

It may be argues that conducting such feasibility surveys could delay the initiation of an important trial. However, in our opinion, the views of clinicians enable an informed decision to be made about the acceptability and feasibility of a proposed trial. The time and effort spent to carry out this survey is minimal when compared to the potential expenditure with a clinical trial that has limited participation from

clinicians leading to poor patient recruitment, especially in trials with patients with rare diseases.

5.2.4.1 Limitations

There are limitations to our questionnaire surveys (both clinicians and patient surveys). First and foremost is the representative of the samples. We have attempted to distribute to clinicians and researchers from different aspects of obstetrics and gynaecology, in order to obtain the opinions of a wide variety of specialists (both academic and non-academics). However, it was expected that only responders with interests in this particular areas were going to respond. Hence, this generated selection bias. Yet, it was still important to have views of these responders as they are most likely those who would participate or give insight into the design of our future proposed trial. They most likely have encounter with women with congenital uterine anomalies in their clinical or academic works.

We have distributed surveys to patients from fertility unit, gynaecology clinics, and early pregnancy unit. We believe these patients were of the target population for our future proposed trial. However, we acknowledged that there is risk of distribution limitations and selection bias. Some patients declined participation due to discomfort with any research interactions. We have reassured all participants that every attempt was made to maintain anonymity of respondents in order to obtain true opinions. Patients were also reassured that their willingness to participate in the survey would not

affect their clinical care. In addition, all survey results and details of the surveys were analysed by one of the researchers and not the principal investigator for the proposed trial.

We understood that survey question answer options could lead to unclear data because certain answer options may be interpreted differently by respondents. Therefore we provided answer options which were as simple as possible yet relevant to our proposed study. We have also provided free text comments option for all respondents to add any additional comments.

5.2.5 Conclusions

This survey confirmed that randomised controlled trial of hysteroscopic resection of septum is a much needed study. Clinicians and patients would be prepared to participate in such a trial. This information is essential to shape the design of the trial and whether it would be feasible to be conducted as a multi-centre trial.

5.3 Pilot Trial and Feasibility of Definitive Trial

5.3.1 Introduction

Our research question is: Does hysteroscopic resection of the uterine septum in women with a septate / subseptate uterus and a history of previous miscarriage or preterm delivery improve outcome in a subsequent pregnancy?

We have conducted a patient survey which showed unanimous support for the study. We conducted a clinician survey in the UK (n=47), and found that at least 10.6% of clinicians perform septal resections on women with uterine septum and history of one early miscarriage; 57.4% reported that they were willing to recruit women with early miscarriage into study, even more (74.5%) for women with late miscarriage.

The absence of prospective randomized studies that include a control group consisting of patients with symptomatic but untreated septate uterus represents a serious limitation in the evaluation of the risks and benefits of hysteroscopic septal resection. Hence a well-designed, multi-centred randomised study is needed.

We intend to submit a formal NIHR/HTA (National Institute for Health Research Health Technology Assessment Programme) application for a randomised controlled trial of hysteroscopic resection of uterine septum. However, we felt this will be a stronger application if we can show that the study is feasible. A pilot study to assess the feasibility of recruitment is therefore essential and will also inform the proposed HTA application. We have therefore designed a pilot study.

The primary objective of the pilot study is, to test the hypothesis that hysteroscopic septal resection in women with septate uteri and a history of miscarriage or preterm birth, improve outcome in a subsequent pregnancy.

A research study group in Netherlands have commenced a similar trial: The Randomised Uterine Septum Transsection Trial

(TRUST), which investigates the efficacy of uterine septum resection in women with recurrent miscarriage (2 or more miscarriages). However, due to the rarity of congenital uterine anomalies and it was only limiting to recurrent miscarriage patients, recruitment proved to be difficult. We have discussed our proposed pilot trial with the research group in Netherlands, whom we agreed that our proposed pilot study would inform us further the feasibility of recruitment of women with different reproductive and clinical background. They have also decided to alter their inclusion criteria in hope to improve recruitment.

5.3.2 Study Design

The study will be a prospective and randomised pilot feasibility two-arm randomised study among women with a septate/subseptate uterus and a history of miscarriage or preterm labour. This will be a single centre study.

The sample population consists of high-risk patients (2 or more early miscarriages before 12 weeks, 1 late miscarriage after 12 weeks, or preterm delivery) who are known to have a subseptate or septate uterus (Diagnosed on Three-dimensional ultrasound, saline hysterosonography, or combined laparoscopy/hysteroscopy which is considered to be gold standard diagnostic tests for major uterine anomalies). All women recruited into the study will have a three-dimensional ultrasound scan to confirm the diagnosis of septate uterus before they are randomised into on the treatment arms. The treatment group participants will undergo a hysteroscopic resection of the uterine

septum while the control participants will have a diagnostic hysteroscopy but the septum will not be removed. Recruitment of the participants will continue until the target sample size is achieved.

Due to the study requiring patient participations, ethical and R&D approvals were sought through the Integrated Research Application System (IRAS). The study received full ethical approval from the Nottingham 1 National Research Ethics Service Committee East Midlands (REC Reference: 13/EM/0382). It also obtained R&D approval from the Nottingham University Hospitals NHS Trust Research and Innovation Department (Study Reference: 13GY007). This study is registered with International Standard Randomised Controlled Number Registry (ISRCTN number: ISRCTN28960271).

5.3.2.1 Participant Recruitment

Potential participants are identified by doctors, research nurses, and nurses in the Gynaecology Clinics, Infertility Clinics, Miscarriage Clinics, and Early Pregnancy Assessment Units. Women who fit the inclusion criteria will be approached verbally or in writing if they would like to participate in this study. The initial approach was usually from a member of the patient's usual care team (which may include one of the study investigators).They will be advised that participation in the study is entirely voluntary with option of withdrawing from the study at any stage, and participation or non-participation will not affect their usual care. They will be provided with the Patient Information Sheet [See Appendix 5: Participant Information Sheet (Pilot randomised, controlled

trial of hysteroscopic septal resection)]and given sufficient time to consider their involvement. Informed consent was obtained from each participant before they undergo any investigations related to the study.

5.3.2.2 Inclusion Criteria

1. Women trying to conceive
2. History of two or more early miscarriages (<12 completed weeks) or one late miscarriage (>12 completed weeks until 23 completed weeks), or preterm delivery, regardless of previous viable or live birth
3. Age: > 18 years at randomisation
4. Confirmed septate uteri (pre-op or intra-op) on three-dimensional ultrasound scan
5. Willing and able to give informed consent
6. No pregnancy or endometrial surgery between diagnosis and treatment or follow up

5.3.2.3 Exclusion Criteria

1. Currently pregnant
2. Current or past treatment for septate uteri
3. Use of any contraception (Females and males)
4. Contraindication to hysteroscopic septal resection
5. Age < 18 years old

6. Exclusion criteria does not include women with:
    - Antithyroid antibodies
    - Antiphospholipid antibodies
    - Lupus anticoagulant antibodies
    - Anticardiolipin antibodies

5.3.2.4 Treatment Assessed

The active treatment group will have hysteroscopic septal resection using an operating hysteroscope with Versapoint or other bipolar electrode system. The control group will have a diagnostic hysteroscopy but the septum will not be removed. Both treatments will be performed as day-case procedures. The choice of distension media and instrument depends on how the surgeon and instruments available and will be performed under general anaesthesia.

5.3.2.5 Randomisation

Participants will be randomised to one of the two treatment arms using sealed envelope randomisation method. Randomisation is not influenced by patients' fertility/pregnancy history, to ensure equal chance of allocation.

5.3.3 Outcomes Measures

5.3.3.1 Primary Outcomes

The primary outcome was the number of live birth surviving until discharge from hospital. This was based on some of the comments expressed by patients in the patient survey, i.e. it is more important for them to be able to go home with babies that have been discharged by the neonatal/paediatric teams.

5.3.3.2 Secondary Outcomes

Secondary outcomes included the following: first trimester miscarriage (12 completed weeks), second trimester miscarriage (>13 weeks until 23 completed weeks), ectopic pregnancy; live birth beyond 28 weeks, 32 weeks, and 37 weeks gestation; mode of delivery; placenta praevia; morbidly adherent placenta; post-partum haemorrhage of > 1000 ml; birth weight; surgical complications: Uterine perforation, fluid overload, endometritis, bleeding, incomplete resection, synechia/adhesions.

5.3.3.3 Safety Outcomes

There are a couple of safety endpoints or outcomes that will be investigated: abnormal three-dimensional ultrasound scans suggesting malignancy; hysterectomy; uterine rupture and death.

5.3.4 Statistical Analysis

5.3.4.1 Sample Size Calculation

As this is a pilot study, we estimated the sample size based on the number of patients we could recruit over a period of one year. From our systematic review, approximately 5.3% of women with miscarriage have septate uteri. Considering a recruitment rate of 20% and there are approximately 1000 women presenting with miscarriage at Nottingham University Hospitals Queen's Medical Centre over one year period, we will be able to recruit 10 participants (Five randomised to the treatment arm and five to control arm) in a year.

At the end, we hope this study will inform us of the recruitment rate and if a multi-centred randomised trial is feasible. We cannot at this stage specify exactly what levels for each parameter would make the main trial feasible. We have intentionally not drawn hard and fast rules for going ahead or not with the main trial.

5.3.4.2 Statistical Methods

Statistical analysis will be performed using the Statistical Package for the Social Sciences (SPSS) software (Version 21.0 for Windows). Results will be summarized and their statistical significance analysed using appropriate statistical techniques. The outcome rates in the intervention group and control group will be compared. Relative risks and 95% confidence intervals will be calculated for the primary outcomes of miscarriage rates. Secondary outcomes will be assessed.

The results will be tabulated. For dichotomous categorical variable, relative risks and 95% confidence intervals will be calculated. For other categorical variables, a Fisher's exact test will be performed. Continuous data distribution was tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk statistic tests. Data were presented as mean and standard deviation (SD) or median and range depending on their distribution. Differences between the two sets of data were assessed using student's t-test or Mann-Whitney test for normally distributed and skewed data respectively. Analysis of variance (ANOVA) or Kruskal-Wallis test was used to examine the differences if there were more than two sets of independent variables. A *P* value of <0.05 was considered significant.

5.3.5 Funding

Application for a Pump Priming grant by the Nottingham University Hospitals Charity and Nottingham University Hospitals Department of Research and Development, has been approved.

5.3.6 Discussions

Based on our systematic review findings, we believe a study to investigate the risk and benefit of hysteroscopic resection for uterine septae is important. Therefore, we intend to submit a formal NIHR/HTA (National Institute for Health Research Health Technology Assessment Programme) application for a randomised controlled trial of hysteroscopic resection of uterine septum.

Using information from clinician surveys and patient surveys, we understood the support and some of the requirements from them in the final design of the multi-centred randomised trial. The surveys helped us to decide the final sample size that will be required in the definitive trial. Further engagement of clinicians and patients would be crucial in designing the trial. We have therefore set up a patient group (Patient led Research into Early Pregnancy and Reproduction: PREPARE) to further help us in this aspect, especially in deciding what important outcomes in a trial would be crucial for patients.

The pilot study was set up and ready to start towards the end of my research study. Therefore, this pilot study will be carried out by the principal investigator with the help of other members of the research team. Despite not being able to participate in the study, the various research methodologies in setting up studies (both observational and randomised study) have improved my knowledge and confidence in future research involvement.

Chapter 6 General Discussion

At the beginning of my PhD, I found very few research studies that have been conducted on congenital uterine anomalies. Published literatures found were mainly case reports or series or reviews. Very few original researches had been performed. The lack of published research studies was most likely due to the rarity of these anomalies and the lack of awareness among clinicians and researchers. In addition to that, the assumed 'gold standard' for diagnosis of congenital uterine anomalies is invasive and hence rarely used in low risk or general population. Non-invasive diagnostic tests such as three-dimensional ultrasound and magnetic resonance imaging scans only become more readily available and reliable in the last decade. It proved to be difficult for researchers and clinicians to know the prevalence of congenital uterine anomalies and to understand the impact of these anomalies. The purpose of my thesis, therefore, was to evaluate the prevalence, importance, and treatment of congenital uterine anomalies.

I started the PhD by performing four systematic reviews in order to understand the current available (or lack of) evidence on four aspects of congenital uterine anomalies: Prevalence of different types of anomalies, their associations with reproductive outcomes, the various treatment options available for women with these anomalies and finally

the reproducibility of three-dimensional ultrasound scan in diagnosing congenital uterine anomalies.

These systematic reviews have confirmed the lack of well-designed prospective studies on congenital anomalies. In view of that, I performed observational studies to investigate the prevalence of uterine anomalies in three different populations for comparison. In our prospective study, women were assessed using three-dimensional ultrasound scan, based on a modified classification system proposed by Salim et al (Salim et al., 2003). This study confirmed higher prevalence of congenital uterine anomalies in certain high risk population (those with miscarriage). By using all the information from systematic reviews and observational study I performed, the PhD finishes by the setting up of a pilot study of randomised controlled trial of hysteroscopic resection for uterine septae.

I shall now outline the major findings in this thesis in respect of the original hypotheses and aims.

6.1 A Systematic Review – The Prevalence of Congenital Uterine Anomalies in High Risk Populations

I began my PhD by performing a systematic review on prevalence of congenital uterine anomalies in various populations (Chapter 3.1). This review has helped me familiarised with steps in

systematic reviews which involved extensive literature search, article reviews, data collection, and meta-analyses.

A total of 90 studies were eventually included in the systematic review and meta-analyses. This systematic review found that the most common anomalies were arcuate uterus in the unselected population and septate uterus in all high risk groups. Also, congenital uterine anomalies diagnosed by optimal tests were more prevalent in high risk populations, in particularly the miscarriage group. The significant increase in prevalence suggested a potential association between congenital uterine anomalies and poor reproductive outcomes. This encouraged us to further explore the reproductive impact of congenital uterine anomalies in our next systematic review.

In order to allow more accurate assessment of congenital uterine anomalies, the included studies were categorised into those with optimal or sub-optimal diagnostic tests. An optimal test should be able to diagnose and classify congenital uterine anomalies accurately, whilst sub-optimal diagnostic tests were able to detect anomalies but not necessarily classifying correctly. Our work agrees with the overall findings by Saravelos et al (Saravelos et al., 2008), but we give a different perspective on the classification of optimal and suboptimal tests. In our opinion, an optimal test therefore should be able to assess both the internal and external contours of the uterus. Therefore, our systematic review should reflect a more accurate picture of the prevalence of various subtypes of congenital uterine anomalies in different populations. It was equally important to classify the subtypes of

congenital uterine anomalies as to diagnose them, since treatment options vary according to the subtypes of anomalies. In addition, only by diagnosing the anomalies accurately, we could assess the risk of adverse reproductive outcomes of different subtypes, and offer treatments (If available).

There is one major downfall for all the studies in this systematic review: the lack of a standardised classification system which describes the morphological features quantitatively. As expected, most of the studies have used the American Society for Reproductive Medicine classification (American Fertility Society, 1988), where final diagnosis is based on the subjective impression of the clinician performing the test (Woelfer et al., 2001) and there is no morphometric criteria or cut-off levels for classifying the subtypes. This undoubtedly poses uncertainties on the diagnosis reliability on some of these studies.

This systematic review was unfortunately limited by the retrospective nature of most of the studies and significant clinical heterogeneity of the patient background history, population, and diagnostic tests used. We included all studies that meet the selection criteria but did not exclude studies on the basis of inadequate quality. We understood the inclusion of studies of inadequate quality may introduce bias in the systematic review. However, the results of this systematic review have essentially confirmed our suspicions of the lack of well-designed prospective prevalence studies using a reliable diagnostic tool.

Despite some studies (Airoldi et al., 2005, Tomazevic et al., 2007) suggesting that congenital uterine anomalies lead to preterm labour, surprisingly, no suitable study was found investigating prevalence of uterine anomalies in women with history of preterm births. This has given us the initial idea of designing a prospective study to investigate prevalence of congenital uterine anomalies in this population.

Finally, the high prevalence of septate or subseptate uteri in high risk population should not be underestimated as these could be treated with hysteroscopic septum resection. This information further supports the need to design a robust randomised trial to assess the risks and benefits of hysteroscopic surgery for uterine anomalies on various pregnancy outcomes in women with septate uteri.

6.2 The Reproductive Outcomes in Women with Congenital Uterine Anomalies: A Systematic Review

As all types of congenital uterine anomalies have long been associated with poor reproductive outcomes (Rackow and Arici, 2007, Raga et al., 1997, Rock and Schlaff, 1985, Stein and March, 1990, Tomazevic et al., 2007, Tulandi et al., 1980), it was prudent to review the evidence for these associations. In addition to that, our prevalence systematic review has found significant increase in prevalence in high risk populations, suggesting a potential association with poor outcomes. We have therefore carried out a systematic review to evaluate the

association between subtypes of congenital uterine anomaly and reproductive outcomes.

Previous reviews by Grimbizis et al (Grimbizis et al., 2001) and Lin (Lin, 2004) have used historical controls or historical data from other studies. We believe the use of historical controls or analyses from other studies poses significant selection bias in systematic review. It would not be possible to ensure these historical controls match the study groups in terms of clinical and demographic background. In our systematic review, we have therefore targeted specifically at randomised trials or case-control studies which compared the reproductive outcomes in women with congenital uterine anomalies (study group) to those with normal uteri (control group). Congenital uterine anomalies are uncommon and therefore only a few comparative observational studies met our inclusion criteria and included in our systematic review.

This systematic review summarised the effect of each type of congenital uterine anomaly. All subtypes of uterine anomalies seem to have a negative effect on pregnancy and conception. The exact effects are dependent on the type of anomaly. Women with canalization defects, such as septate and subseptate uteri, appeared to have the poorest reproductive performance. Not only they had reduced chances of conception, but they also had increased risks of miscarriage, preterm birth, and fetal malpresentation at delivery. Whilst this association was generally supported by the evidence, the exact pathophysiological processes underlying these problems remain unanswered. There were

a variety of hypotheses (such as abnormal endometrial growth; reduced blood supply, disordered uterine contractions or reduced uterine capacity) that had been suggested but there were conflicting studies on these theories.

Unification defects, such as the bicornuate and unicornuate uterus, and uterus didelphys, do not appear to reduce fertility but are associated with aberrant outcomes throughout the whole course of pregnancy. The exact effects were very dependent on the type of anomaly. Morphologically, the structural distortions of unification defects are considered more profound when compared to canalization defects. Hence, it was surprising to find women with canalization defects have the worst reproductive outcomes. Again, the underlying pathophysiological processes for these poor outcomes in various anomalies remain unanswered. This would be an interesting and important area for future studies, in investigating the pathophysiology for poor reproductive outcomes.

Arcuate uterus, which sometimes was considered as a normal variant rather than an actual anomaly, was associated with an increased risk of second trimester miscarriage and preterm births. However, it could be difficult to differentiate arcuate uterus from a mild form of subseptate or bicornuate uterus on some of the diagnostic tests. Therefore, the increased risks in arcuate uterus might be secondary to misclassification.

This systematic review has its limitations. First of all, this systematic review consists of only a small numbers of observational

studies which were likely to be fraught with potential bias and confounders. The included studies had different study populations and unadjusted confounding factors.

One of the biggest limitations was the diagnostic tools used in these studies to diagnose and determine the type of uterine anomaly. The diagnostic tests used were inconsistent across the studies. Some studies in this review used a combination of diagnostic tests, both optimal and sub-optimal tests to confirm diagnosis of congenital uterine anomalies, whilst some used single diagnostic tool. Research studies have shown that two-dimensional transvaginal ultrasound, hysteroscopy, or hysterosalpingography (HSG) when used in isolation, they have the tendency to underestimate the prevalence of uterine abnormalities (Jurkovic et al., 1995, Wu et al., 1997, Andreotti et al., 2006, Guimaraes Filho et al., 2006b, Momtaz et al., 2007, Saravelos et al., 2008). They are less accurate in the identification and differentiation of minor uterine anomalies, such as the arcuate and subseptate uterus. This is likely to influence the actual results of the studies and review.

This systematic review again has raised the issue regarding a robust diagnostic tool to be used in clinical and research setting for congenital uterine anomalies. It is crucial to have a standardised and reliable diagnostic test in all future studies. Only with accurate diagnosis, we could then assess the reproductive outcomes of these anomalies. Bearing that in mind therefore, the demonstration of poorer reproductive outcomes in women with congenital uterine anomalies (especially for arcuate uterus) in this systematic review might be artefactual.

6.3 Systematic Review on Treatments of Congenital Uterine Anomalies

Surgical treatments for congenital uterine anomalies are often performed to relieve any obstructions they caused and they are obviously beneficial in providing symptomatic relief. However, the benefit in performing surgical metroplasty purely to restore the normal anatomy of the female genital tract is less clear. However, these surgical treatments are still being offered to women with congenital anomalies, despite lack of evidence to prove its efficacy or safety. Therefore, we have conducted a systematic review to evaluate the efficacy of all surgical treatment (metroplasty) for uterine anomalies, in all different populations. We anticipated most of the studies would be mainly on hysteroscopic metroplasty instead of abdominal metroplasty due to the significant complications and risks of abdominal metroplasty, including prolonged recovery, post-operative adhesions, and uterine rupture during subsequent pregnancies (Ayhan et al., 1992, Homer et al., 2000, Lourdel et al., 2007). Unfortunately, abdominal metroplasty is the only surgery available for unification defects such as bicornuate or didelphic uteri.

This systematic review only found five studies to be included in the final analysis and all of them were observational studies. No randomised controlled trials were found. This was consistent with systematic reviews performed by Kowalik et al and Rikken et al (Kowalik et al., 2011, Rikken et al., 2017). We analysed the results of

the observational studies. All these studies had varying study quality, and generally had very poor design or reporting systems. There were significant differences in the clinical background and diagnostic tools used in all the included women. Therefore, despite this review showing the apparent reduction in miscarriage and a concomitant increase in term birth rate with hysteroscopic resection of uterine septum, the results of the review had to be interpreted cautiously. Besides, there are different hysteroscopic instruments and techniques that can be used for metroplasty, and these may influence the clinical outcomes of hysteroscopic metroplasty.

This systematic review and previous systematic reviews confirmed there is no evidence to support surgical metroplasty in women with congenital uterine anomalies. These reviews underscore the need for a multi-centred randomised controlled trial of hysteroscopic resection of uterine septae.

6.4 Reproducibility of 3D Ultrasound Scan in Diagnosing Congenital Uterine Anomalies: A Systematic Review

Before any future studies on congenital uterine anomalies can be carried out, it is crucial to have a standardised classification system and a reliable yet reproducible diagnostic tool to diagnose these anomalies. This is even more so if any treatment or intervention is offered for these anomalies.

Studies and reviews have shown that three-dimensional ultrasound scan has high sensitivity and specificity, as high as 100% in diagnosing congenital uterine anomalies (Deutch et al., 2006, Grimbizis et al., 2016, Saravelos et al., 2008, Wu et al., 1997). Furthermore, it is accurate in differentiating the anomalies (Deutch et al., 2006, Wu et al., 1997). However, even though three-dimensional ultrasound scan appears to be an accurate test, very few reproducibility studies have been performed. Without an acceptable level of reproducibility, the clinical utility of three-dimensional ultrasound scan in diagnosing congenital uterine anomalies becomes substantially compromised and uncertain. Therefore, we conducted a systematic review to evaluate the reproducibility of three-dimensional ultrasound scan in the diagnoses of congenital uterine anomalies. We are particularly interested in three-dimensional ultrasound scan because it is a non-invasive, yet highly accurate and becoming more accessible in clinical practice nowadays.

This systematic review demonstrated that three-dimensional ultrasound scan is highly reproducible in diagnosing congenital uterine anomalies. From previous systematic reviews, we recognise the importance of classification systems used in diagnosing congenital uterine anomalies. Hence, further analysis based on classification system was performed to investigate the heterogeneity of this systematic review. It was clear that the American Society for Reproductive Medicine Classification with additional morphometric criteria proposed by Salim et al (Salim et al., 2003) improved the inter-observer agreement in diagnosing congenital uterine anomalies using

three-dimensional ultrasound scan. This showed the importance of having cut-off levels when classifying these anomalies. Some minor congenital uterine anomalies have very similar morphological characteristics; hence quantitative measurements help observers to differentiate these anomalies. It is extremely important for clinicians to be confident with the reproducibility of diagnoses, as treatment availability varies according to the subtypes of the anomaly.

This systematic review has confirmed our confidence in using three-dimensional ultrasound scan as a non-invasive diagnostic tool. However, it is imperative to remember that only a small number of studies were included in this systematic review and most of them are retrospective observational studies which are obviously prone to selection bias and confounders. In addition, the diagnostic accuracy of three-dimensional ultrasound scan is limited with the presence of fibroids or other large pelvic masses. They may distort the pelvic or uterine anatomy, causing difficulties in obtaining an adequate examination (Deutch and Abuhamad, 2008, Jurkovic et al., 1997) and hence uncertainty in diagnosis.

6.5 Prevalence of Congenital Uterine Malformations

Our systematic review showed that no prospective studies have been performed to investigate the prevalence of congenital uterine malformations in women with history of preterm births. In addition, there

have been suggestions that potential mechanisms for congenital uterine anomalies leading to preterm labour were cervical incompetence (Airoldi et al., 2005, Berghella et al., 2007, Roberts et al., 1995) and reduced uterine capacity (Braun et al., 2005, Pellerito et al., 1992, Puscheck and Cohen, 2008, Reuter et al., 1989). In view of these, we have designed a prospective study investigating prevalence of congenital uterine anomalies in women with preterm births. We also extended the study to include women with history of miscarriage, in order to investigate any differences in uterine or cervical parameters when compared to a group of low-risk women who had only full term births (37 or more weeks of gestation).

In order to ensure we can obtain accurate diagnosis and perform measurements of uterine and cervical parameters, we performed three-dimensional ultrasound scans on all women using the standardized settings and classification system for congenital uterine anomalies. We decided to perform scans during the luteal phase of the menstrual cycle as oppose to early follicular phase in our observational study in subfertile group (Jayaprakasan et al., 2011). During the early follicular phase, the endometrial lining is very thin and hence making the diagnosis of uterine cavity shape more challenging. During luteal phase, the endometrium is easily delineated. In addition to that, luteal phase is when embryo implantation occurs, hence any anomaly and difference in measurements at this stage may lead to investigations to potential causes of poor reproductive outcomes.

The preliminary result has shown significantly higher prevalence of uterine anomalies in women with miscarriage than low-risk women or women with preterm birth. Arcuate uterus was the most common anomaly across all these three groups of women. However, women with history of preterm birth do not appear to have increased prevalence of uterine anomalies when compared to low risk women. Our findings showed a significantly higher occurrence of congenital uterine anomalies than our systematic review in women with miscarriage. However, we believe the use of a reliable standardized diagnostic tool and classification system have allowed better diagnostic rate. Besides, this prospective study was designed specifically to investigate prevalence of uterine anomalies and hence it is more likely to diagnose even the mildest form of anomaly. A lot of the studies in the systematic reviews were retrospective studies and congenital uterine anomalies, especially minor anomalies (arcuate or mild subseptate uterus) were easily missed or ignored.

In the current study, it was shown that high risk women with uterine anomalies had significantly shorter uterine length. This was consistent with the theory of diminished uterine size by some studies (Reichman and Laufer, 2010). Overall, women with history of preterm birth appeared to have shorter cervical length. Short cervix in pregnancy is a predictive indicator for preterm labour (Honest et al., 2009), hence this finding was not unexpected. However overall, women with abnormal uteri do not appear to have shorter cervical lengths.

It is unclear whether diminished muscle mass or volume plays a role in poor reproductive outcomes, especially for second trimester miscarriage and preterm delivery. We have therefore opted to measure the myometrial volumes and cervical volumes in this study. However, no significant difference was found for myometrial volume of the uterine body. And surprisingly, women in high risk population actually have larger cervical volume than those of low risk population. However, previous research studies had shown that it was difficult to identify the sonographic demarcation between the cervix, the uterine body, and the surrounding tissue (Basgul et al., 2007, Basgul et al., 2006) when measuring these volumes. Hence we are uncertain how accurate these measurements are when compared to real histology samples from hysterectomies. Reproducibility of volumetric measurements has not been performed as there was insufficient mix of different uterine anomalies.

There are significant limitations in our prospective study. The most significant weakness was that the sample size was not achieved and it is therefore under-powered and is likely to subject to bias when the preliminary results were analysed. Even though strict inclusion and exclusion criteria were applied in order to recruit women of specific clinical background, there is still different clinical heterogeneity and not all confounding factors were adjusted for. These pose risks for biases in the study. There had not been sufficient experience in uterine and cervical volumetric measurements. Hence, the measurements were obviously challenging and unreliable. There is a need to perform

phantom studies to determine the accuracy of volumetric measurements. In addition to that, intra-observers and inter-observers reproducibilityy studies are needed to ensure reproducibility of these measurements, in different types of congenital uterine anomalies.

6.6 Development of a randomised controlled trial of hysteroscopic resection for uterine septae

Part of the PhD work is to design a large randomised trial to assess the effect of hysteroscopic surgery for uterine anomalies on various pregnancy outcomes in women with septate uteri.

Clinician surveys and patient surveys were conducted to assess the need and feasibility of the study. Their opinion is also important in the design of the trial. Clinicians and patients have shown their support and willingness to participate in a randomised controlled trial. Using information from clinician surveys and patient surveys, we obtained vital information and study design requirement in the design of the multi-centred randomised trial. The surveys helped us to decide the final sample size that will be required in the definitive trial. Recruitment has always been a concern in the prospective trial. It was therefore absolutely important to ensure that it was feasible to recruit sufficient number of patients for the definitive trial. Therefore, a pilot trial is helpful to assess the feasibility of a trial.

From the surveys, we realised how important it was to have engagement of clinicians and patients in designing the trial. We have therefore set up a patient group (Patient led Research into Early Pregnancy and Reproduction: PREPARE) to further help us in this aspect, especially in deciding what key outcomes in a trial would be essential for patients and clinicians.

It was quite a journey to set up a pilot study. It all started from systematic reviews, approaching clinicians and patients for opinions and support, followed by study design, eventually applying for ethical and R&D approval. Even though I am not going to be involved in the pilot study or the eventual definitive trial, the various research methodologies in setting up studies (both observational and randomised pilot study) have improved my knowledge and confidence in future research involvement.

6.7 Suggested Directions for Future Work

Various potential research studies or investigations have been mentioned in each chapter; however I would like to highlight a few potential studies here:

6.7.1 Pathophysiology studies using ultrasound scan

Studies and systematic reviews have linked congenital uterine anomalies with various poor reproductive outcomes. However, the underlying pathophysiological processes are not known. There had

been a lot of proposed theories including abnormal endometrial growth; reduced blood supply, disordered uterine contractions or reduced uterine capacity.

As ultrasound scan technology continues to develop, it can be used to investigate some of these theories. Transvaginal colour Doppler or power Doppler angiography can be used to assess the endometrial vascularity of the uterus.

A new method of assessing myometrial contractility is using Elastography technique with ultrasound scan. This technique has been developed to be used clinically in thyroid and breast tissue at present. It would be able to detect any myometrial contractions. This will help us to investigate this as a potential cause for miscarriage and preterm labour in women with congenital uterine anomalies.

As suggested before, as experience in volumetric measurements using three-dimensional ultrasound scans grow, we could further investigate the role of endometrial volume, myometrial volume and cervical volume differences (if any) as causes of poor reproductive outcomes in women with congenital uterine anomalies.

6.7.2 Test Accuracy Study of Three-Dimensional Ultrasound Scan

As three-dimensional ultrasound scan becoming more available and clinicians becoming more aware of its use in the diagnosis of congenital uterine anomalies, it is even more important to confirm its

accuracy and reliability. Even though studies and reviews have been performed to confirm its accuracy with the 'gold standard' diagnostic test, i.e. combined laparoscopy and hysteroscopy, the accuracy of the 'gold standard' has never been tested. Phantom studies or studies comparing ultrasound diagnosis and measurements to hysterectomy specimens will help to confirm its accuracy in diagnosing congenital uterine anomalies. This is especially important if we want to use three-dimensional ultrasound scan to perform volumetric measurements, which to this date, we are uncertain how reliable these measurements are.

6.7.3 Off-line Analysis of UltraSonographic Images Study (OASIS)

Congenital uterine anomalies are uncommon, and hence it is difficult for clinicians to learn and be confident in diagnosing these anomalies when they are not seen regularly in clinical practice. Therefore, a collection of three-dimensional datasets of congenital uterine anomalies accessible anywhere in the world can be useful in helping sonographers and clinicians becoming more familiar with these anomalies.

With the use of such datasets, an Off-line Analysis of UltraSonographic Images Study (OASIS) can be performed. In this study, there will be a series of online three-dimensional volume datasets, including normal and congenital anomalies studies. These datasets are assessed using current techniques and then subsequently

using a set standard operative protocol. The standard operative protocols will describe the technique exactly how to line up a dataset using the Standard Multiplanar Mode and measuring it. The aim of OASIS is to teach and develop a standardised three-dimensional technique for diagnosis of uterine anomalies. The classification of congenital uterine anomalies set will be based on the modified American Society of Reproductive Medicine Classification proposed by Salim et al (Salim et al., 2003). We would be able to improve and also assess the reproducibility of three-dimensional ultrasound scan in diagnosis of congenital uterine anomalies using these online methods.

This can be further developed to perform three-dimensional measurements or standards for other gynaecological pathologies.

6.7.4 Multi-centred randomised controlled trial for hysteroscopic resection of uterine septae

Obviously, one of the most crucial developments I hope to see in the near future is the randomised controlled trial for hysteroscopic resection. This randomised trial hopefully will provide the answers to clinicians regarding the risks and benefits of hysteroscopic septal resection

However, this trial would require a lot of effort and time to set up as it requires a huge financial grant to support the study. Uterine septum is not rare but it is still quite uncommon and it will potentially take a long time to recruit the sufficient number of patients before such study can be completed. It is also more than likely that this trial will be a

multi-centre trial in view of the rarity of septate uterus. Hence, once the pilot study and TRUST trial are completed, we will know how feasible it is for the definitive trial for the future.

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Appendix

Appendix 1: Participant Information Sheet (Study Group)

Participant Information Sheet: Study Group

(Final version 2.0: 11 October 2011)

Title of Study: Prevalence of congenital uterine malformations in high-risk women

Name of Researcher: Mr Nicholas Raine-Fenning, Professor Jim Thornton, Dr Yee Yin Chan

We would like to invite you to take part in our research study. Before you decide, we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

What is the purpose of the study?
Congenital abnormally shaped wombs (uterine malformations) have long been thought to be more common in women with poor pregnancy outcomes, e.g. miscarriage and preterm delivery. However, many women with uterine malformations are asymptomatic and have no fertility or pregnancy problems. There is no conclusive proof or evidence for poor pregnancy outcomes in these women.

We plan to investigate how common uterine malformations are in high-risk women (with history of miscarriage or preterm delivery), by analysing different characteristics in these groups. These will be compared to a group of women who have had term delivery.

This study will also investigate other ultrasound characteristics detected on these women. This study will point towards the possible mechanism of how uterine malformations may affect pregnancy outcomes.

Why have I been invited?
You are being invited to take part because you have a history of preterm delivery or miscarriage. We are inviting 273 participants like you to take part.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep. A member of the research team will contact you to explain the study in details and you will be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights.

What will happen to me if I take part?
If you agree to participate in the study, your usual clinical care will not change, apart from an extra 3-Dimensional transvaginal ultrasound scan appointment at the Academic Imaging Suite, Queen's Medical Centre, Nottingham. This scan will take place at least 8 weeks after your last pregnancy. This ultrasound scan is not associated with any additional risks. Medical history will be taken during the same appointment.

The data that we collect will be retained for a period of 7 years, in line with the University of Nottingham code of research conduct, and will be securely archived, to be accessed strictly by those with authorisation.

Expenses and payments
Participants will not be paid an inconvenience allowance to participate in the study. We will bear the cost of the ultrasound scans that you will receive.

What are the possible disadvantages and risks of taking part?
For 3-Dimensional transvaginal ultrasound scan, a small vaginal probed covered with a sterile condom sheath to avoid infection will be inserted into the vagina. The majority of women find this procedure acceptable with minimal discomfort. A small number of women may find it too uncomfortable, in which case the procedure will be stopped immediately.

Research done at Royal Free Hospital, London in 2003, assessing the acceptability of transvaginal ultrasound scan found that 99% of women who had a transvaginal scan would agree to have it done again in the future, suggesting its wide acceptance among women. All recruited women will most likely have had previous experience of transvaginal ultrasound scan as part of their routine care during their pregnancies. Otherwise, there are no known side effects to the use of ultrasound in women.

What are the possible benefits of taking part?
We cannot promise the study will help you but the information we get from this study will help to provide additional information for women as to the potential cause of poor pregnancy outcomes.

What will happen if any abnormality is found?
Women who were found to have any uterine abnormality on scans will be referred to appropriate gynaecology or obstetric specialists to have a chat or counselling. This is particularly important in women who have had very poor pregnancy or neonatal outcomes, e.g. whose preterm baby has died etc.

What happens when the research study stops?
All the research data will be collected and analysed. The final results will be made available via publications in peer reviewed scientific journals and conference presentation.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. A contact for complaints is Mr Nicholas Raine-Fenning, Consultant Gynaecologist and Reader of Reproductive Medicine and Surgery on 0115 823 0700. If you remain unhappy and wish to complain formally, you can do this by contacting NHS Complaints. Details can be obtained from your hospital.

Will my taking part in the study be kept confidential?
Yes, we will follow ethical and legal practice and all information about you will be handled in confidence. All study staff and investigators will endeavour to protect the rights of the study's participants to privacy and informed consent, and will adhere to the Data Protection Act, 1998.

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the company sponsoring and/or the company organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept **strictly confidential**, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept for a maximum of 12 months after the end of the study so that we are able to contact you about the findings of the study (unless you advise us that you do not wish to be contacted). All other data (research data) will be kept securely for 7 years. After this time, your data will be disposed of securely. During this time, all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

What will happen if I do not want to carry on with the study?
Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis.

Involvement of the General Practitioner/Family doctor (GP)
As part of your consent, your own GP or your usual medical care team will be notified of any suspicious findings on scans in order for you to be referred for future investigations or management; however, the study will not alter your usual clinical care.

Will any genetic tests be done?
No genetic tests will be done

What will happen to the results of the research study?
The research is likely to be published within 12 months of the completion of the study. It will also form part of a PhD thesis. You will not be identified in any report or publication.
If you wish, you may request a summary of the study results.

Who is organising and funding the research?
This research is being organised by the University of Nottingham and is being funded by the Academic Unit of Obstetrics and Gynaecology at the University of Nottingham.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Nottingham Research Ethics Committee.

Further information and contact details

Chief investigator: Mr Nicholas Raine-Fenning
Nottingham University Research and Treatment Unit in Reproduction (NURTURE), B floor, East Block, Queen's Medical Centre, Nottingham NG7 2UH

Appendix 2: Participant Information Sheet (Control Group)

Participant Information Sheet: Control Group

(Final version 1.0: 11 October 2011)

Title of Study: Prevalence of congenital uterine malformations in high-risk women

Name of Researcher: Mr Nicholas Raine-Fenning, Professor Jim Thornton, Dr Yee Yin Chan

We would like to invite you to take part in our research study. Before you decide, we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

What is the purpose of the study?
Congenital abnormally shaped wombs (uterine malformations) have long been thought to be more common in women with poor pregnancy outcomes, e.g. miscarriage and preterm delivery. However, many women with uterine malformations are asymptomatic and have no fertility or pregnancy problems. There is no conclusive proof or evidence for poor pregnancy outcomes in these women.

We plan to investigate how common uterine malformations are in high-risk women (with history of miscarriage or preterm delivery), by analysing different characteristics in these groups. These will be compared to a group of women who have had term delivery (37 weeks or more gestation).

This study will also investigate other ultrasound characteristics detected on these women. This study will point towards the possible mechanism of how uterine malformations may affect pregnancy outcomes.

Why have I been invited?
You are being invited to take part because you have a term delivery (37 weeks or more gestation). We are inviting 273 participants like you to take part.

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep. A member of the research team will contact you to explain the study in details and you will be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights.

What will happen to me if I take part?
If you agree to participate in the study, your usual clinical care will not change, apart from an extra 3-Dimensional transvaginal ultrasound scan appointment at the Academic Imaging Suite, Queen's Medical Centre, Nottingham. This scan will take place at least 8 weeks after your last pregnancy. This ultrasound scan is not associated with any additional risks. Medical history will be taken during the same appointment.

The data that we collect will be retained for a period of 7 years, in line with the University of Nottingham code of research conduct, and will be securely archived, to be accessed strictly by those with authorisation.

Expenses and payments
Participants will not be paid an inconvenience allowance to participate in the study. We will bear the cost of the ultrasound scans that you will receive.

What are the possible disadvantages and risks of taking part?
For 3-Dimensional transvaginal ultrasound scan, a small vaginal probed covered with a sterile condom sheath to avoid infection will be inserted into the vagina. The majority of women find this procedure acceptable with minimal discomfort. A small number of women may find it too uncomfortable, in which case the procedure will be stopped immediately.

Research done at Royal Free Hospital, London in 2003, assessing the acceptability of transvaginal ultrasound scan found that 99% of women who had a transvaginal scan would agree to have it done again in the future, suggesting its wide acceptance among women. All recruited women will most likely have had previous experience of transvaginal ultrasound scan as part of their routine care during their pregnancies. Otherwise, there are no known side effects to the use of ultrasound in women.

What are the possible benefits of taking part?
We cannot promise the study will help you but this provides an opportunity to detect any pelvic abnormality early. You will be referred to appropriate specialists for future investigations or management if any abnormality is found on scan.

What will happen if any abnormality is found?
Women who were found to have any uterine abnormality on scans will be referred to appropriate gynaecology specialists to have a chat or counselling.

What happens when the research study stops?
All the research data will be collected and analysed. The final results will be made available via publications in peer reviewed scientific journals and conference presentation.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. A contact for complaints is Mr Nicholas Raine-Fenning, Consultant Gynaecologist and Reader of Reproductive Medicine and Surgery on 0115 823 0700. If you remain unhappy and wish to complain formally, you can do this by contacting NHS Complaints. Details can be obtained from your hospital.

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If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the company sponsoring and/or the company organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept **strictly confidential**, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept for a maximum of 12 months after the end of the study so that we are able to contact you about the findings of the study (unless you advise us that you do not wish to be contacted). All other data (research data) will be kept securely for 7 years. After this time, your data will be disposed of securely. During this time, all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

What will happen if I do not want to carry on with the study?
Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis.

Involvement of the General Practitioner/Family doctor (GP)
As part of your consent, your own GP or your usual medical care team will be notified of any suspicious findings on scans in order for you to be referred for future investigations or management; however, the study will not alter your usual clinical care.

Will any genetic tests be done?
No genetic tests will be done

What will happen to the results of the research study?
The research is likely to be published within 12 months of the completion of the study. It will also form part of a PhD thesis. You will not be identified in any report or publication.
If you wish, you may request a summary of the study results.

Who is organising and funding the research?
This research is being organised by the University of Nottingham and is being funded by the Academic Unit of Obstetrics and Gynaecology at the University of Nottingham.

Who has reviewed the study?
All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Nottingham Research Ethics Committee.

Further information and contact details
Chief investigator: Mr Nicholas Raine-Fenning
Nottingham University Research and Treatment Unit in Reproduction (NURTURE), B floor, East Block, Queen's Medical Centre, Nottingham NG7 2UH

Appendix 3: Clinician Survey

Study to **E**valuate the **P**revalence, importance and **T**reatment of women with **U**terine **M**alformation (**SEPTUM**)

Could we please ask you to complete a short survey to help us decide on the need for, and the feasibility of, a randomised study?

The study we are considering conducting is a UK randomised trial designed to assess the effect of hysteroscopic surgery for uterine abnormalities on various pregnancy outcomes in women with septate uteri. Your response will be instrumental in deciding whether we should proceed with this trial.

Here is some background information that may help you answer the questions:

Based on our own systematic reviews of the literature, the rate of all uterine abnormalities in unselected populations of women of reproductive age is approximately 5.5%. An estimated 2.3% of all women have a septate or subseptate uterus.

There are studies (Nahum, 1998; Taylor and Gomel, 2008) that suggest congenital uterine anomalies impede embryo implantation. Our systematic review shows a slight but non-significant increase in the prevalence of uterine anomalies in women with infertility. Congenital uterine anomalies, and septate and subseptate uteri in particular, do however appear to be significantly more common in women undergoing assisted reproduction treatment including IVF.

There is a strong association between uterine abnormalities and miscarriage. Our systematic review has demonstrated a significantly higher prevalence of uterine anomalies in women with recurrent miscarriage (12.9%) compared to the general population (5.5%). Miscarriage appears to be more common in women with septate and subseptate uteri.

Whilst investigating the association between miscarriage and uterine anomalies we found that patients with septate or subseptate uteri are more likely to suffer from first trimester miscarriage (Risk Ratio 2.89: 95% CI = 2.02 – 4.14) but not second trimester miscarriage.

To the best of our knowledge, there are no appropriately designed observational studies to quantify the prevalence of uterine anomalies in women affected by preterm delivery. Women with septate or subseptate uteri do, however, seem to have an increased risk of delivering before 37 completed weeks (Risk Ratio 2.18: 95% CI = 1.13 – 4.22).

We do not know how prior pregnancies affect these risks. For example, is the risk of miscarriage and preterm delivery less in women who have previously had term pregnancies?

The surgical management of uterine anomalies is controversial but many clinicians offer hysteroscopic resection of uterine septae (Valle, 1996; Pellicer, 1997; Saygili-Yimaz, 2003; Tomazevic, 2007). Hysteroscopic metroplasty is not without risk, however, and whilst several observational studies (Maneschi, 1993; Heinonen, 1997; Valli, 2004) have reported an improved outcome following surgical intervention there is paucity of randomised controlled trials to address the effectiveness and safety of such treatment.

Recent studies (Raju, 2006; El-Toukhy, 2008) and a systematic review (Bosteels, 2010) have suggested that outpatient hysteroscopy alone may improve pregnancy rates in women who have recurrent IVF implantation failure. The mechanism for this is not known.

Proposed Study Design

The study we are planning involves randomly allocating high-risk patients, who are known to have a septate uterus, to one of two groups:

Group 1: The active treatment group.
Women allocated to this group will have hysteroscopic septal resection

Group 2: The control group.
Women allocated to this group will have a diagnostic hysteroscopy but the septum will not be removed

The study will be blinded and as both groups will have a hysteroscopy patients will not know if they have had their septum removed or not.

The diagnosis of a septate uterus can be made by 3D ultrasound, MRI, combined hysteroscopy / laparoscopy and 2D saline hysterosonography.

Please note: Please fill or put an ‘X’ in the box as appropriate

Name:

1. Please select your specialty:

- ☐ Obstetrician/Gynaecologist
- ☐ Gynaecologist
- ☐ Obstetrician
- ☐ Paediatrician/Neonatologist
- ☐ Other

2. … and your grade:

- ☐ NHS Consultant (Substantive)
- ☐ NHS Consultant (Honorary)
- ☐ Senior Trainee (ST 6-7)
- ☐ Junior Trainee (ST 1-5)
- ☐ Other

If you selected "other" to Question 1, please tell us your specialty below:

If you selected "other" to Question 2, please tell us your grade below:

3. Having considered the background information given above please let us know which of the options you consider appropriate (you may identify all or none of the options) for each of the following. Please put an 'X' in the box for each selected option:

Subfertility

☐ TREAT (all or sub- group of) women with uterine septae AND subfertility to improve the chance of conception

☐ RANDOMISE women with uterine septae AND subfertility, who are planning to conceive, to a trial to compare the effects (both beneficial and harmful) of hysteroscopic septal resection to no treatment

☐ WOULD NOT TREAT or RANDOMISE to such a trial

☐ WOULD NOT TREAT or RANDOMISE to such a trial if had previous term delivery

Subfertility requiring IVF

☐ TREAT (all or sub- group of) women with uterine septae AND subfertility who are planning to have IVF treatment to improve the chance of conception

☐ RANDOMISE women with uterine septae AND subfertility, who are planning to have IVF, to a trial to compare the effects (both beneficial and harmful) of hysteroscopic septal resection to no treatment

☐ WOULD NOT TREAT or RANDOMISE to such a trial

☐ WOULD NOT TREAT or RANDOMISE to such a trial if had previous term delivery

Recurrent Implantation Failure (≥ 2 failed IVF cycles, including both fresh and frozen embryos)

☐ TREAT (all or sub- group of) women with uterine septae AND 'recurrent implantation failure', who are planning to have further IVF, to improve the chance of conception

☐ RANDOMISE women with uterine septae AND 'recurrent implantation failure', who are planning to have further IVF, to a trial to compare the effects (both beneficial and harmful) of hysteroscopic septal resection to no treatment

☐ WOULD NOT TREAT or RANDOMISE to such a trial

☐ WOULD NOT TREAT or RANDOMISE to such a trial if had previous term delivery

One Early Miscarriage (≤ 12 weeks)

- ☐ TREAT (all or sub- group of) women with uterine septae AND a single miscarriage, who are planning to conceive, to reduce the risk of further miscarriage and preterm birth
- ☐ RANDOMISE women with uterine septae AND a single miscarriage, who are planning to conceive, to a trial to compare the effects (both beneficial and harmful) of hysteroscopic septal resection to no treatment
- ☐ WOULD NOT TREAT or RANDOMISE to such a trial
- ☐ WOULD NOT TREAT or RANDOMISE to such a trial if had previous term delivery

Two Consecutive Early Miscarriages (≤ 12 weeks)

- ☐ TREAT (all or sub- group of) women with uterine septae AND two consecutive early miscarriages, who are planning to conceive, to reduce the risk of miscarriage and preterm birth
- ☐ RANDOMISE women with uterine septae AND two consecutive early miscarriages, who are planning to conceive, to a trial to compare the effects (both beneficial and harmful) of hysteroscopic septal resection to no treatment
- ☐ WOULD NOT TREAT or RANDOMISE to such a trial
- ☐ WOULD NOT TREAT or RANDOMISE to such a trial if had previous term delivery

Three or more Consecutive Early Miscarriages (≤ 12 weeks)

- ☐ TREAT (all or sub- group of) women with uterine septae AND three or more consecutive early miscarriages, who are planning to conceive, to reduce the risk of miscarriage and preterm birth
- ☐ RANDOMISE women with uterine septae AND three or more consecutive early miscarriages, who are planning to conceive, to a trial to compare the effects (both beneficial and harmful) of hysteroscopic septal resection to no treatment
- ☐ WOULD NOT TREAT or RANDOMISE to such a trial
- ☐ WOULD NOT TREAT or RANDOMISE to such a trial if had previous term delivery

Late miscarriage (>12 weeks)

☐ TREAT (all or sub- group of) women with uterine septae AND a history of late miscarriage, who are planning to conceive, to reduce the risk of miscarriage and preterm birth

☐ RANDOMISE women with uterine septae AND a history of late miscarriage, who are planning to conceive, to a trial to compare the effects (both beneficial and harmful) of hysteroscopic septal resection to no treatment

☐ WOULD NOT TREAT or RANDOMISE to such a trial

☐ WOULD NOT TREAT or RANDOMISE to such a trial if had previous term delivery

Preterm Birth (< 34 weeks)

☐ TREAT (all or sub- group of) women with uterine septae AND a history of preterm birth, who are planning to conceive, to increase the chance of delivery after 34 weeks gestation

☐ RANDOMISE women with uterine septae AND a history of preterm birth, who are planning to conceive, to a trial to compare the effects (both beneficial and harmful) of hysteroscopic septal resection to no treatment

☐ WOULD NOT TREAT or RANDOMISE to such a trial

☐ WOULD NOT TREAT or RANDOMISE to such a trial if had previous term delivery

4. Please let us know which surgical techniques you consider appropriate for hysteroscopic septal resection (you may identify all or none of the options)

☐ Resectoscope with electrosurgery

☐ Operating Hysteroscope with Versapoint or other bipolar electrode system

☐ Operating Hysteroscope with rigid scissors

☐ Operating Hysteroscope with semi-rigid scissors

☐ Operating Hysteroscope with flexible scissors

☐ Operating Hysteroscope with laser

5. Please let us know which surgical techniques (you may identify all or none of the options) are available at your hospital

☐ Resectoscope with electrosurgery

☐ Operating Hysteroscope with Versapoint or other bipolar electrode system

- ☐ Operating Hysteroscope with rigid scissors
- ☐ Operating Hysteroscope with semi-rigid scissors
- ☐ Operating Hysteroscope with flexible scissors
- ☐ Operating Hysteroscope with laser

6. Please share any comments you may have with us:

7. Please let us know the contact details (telephone or e-mail) for any other colleagues who may be willing to answer this questionnaire:

End of questionnaire

Thank you for taking the time to answer these questions. Your feedback and personal opinion mean a great deal to us and will ultimately decide if such a study is feasible.

If you would like further information, please contact us by email at the following addresses:

Nick Raine-Fenning nick.fenning@nottingham.ac.uk

Arri Coomarasamy a.coomarasamy@bham.ac.uk

Yours sincerely,

Nick Raine-Fenning & Arri Coomarasamy

NRF is a Clinical Senior Lecturer at the University of Nottingham

AC is a Clinical Senior Lecturer at the University of Birmingham

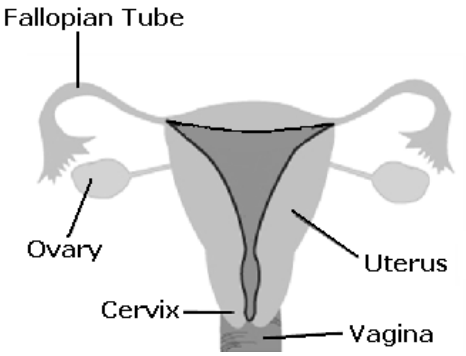
Appendix 4: Patient Survey

Study to **E**valuate the **P**revalence, importance and **T**reatment of women with **U**terine **M**alformation (**SEPTUM**)

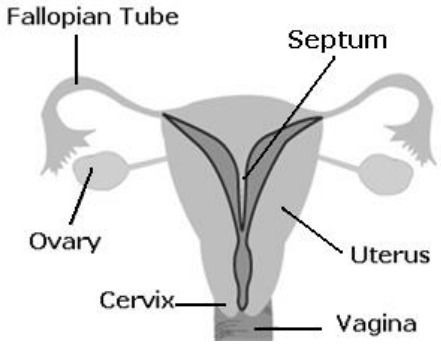
We are considering conducting a study to assess the role of a keyhole surgery of the womb for women with a particular type of abnormally shaped womb called a septate uterus. These women appear to be at an increased risk of miscarriage and possibly infertility and premature delivery. The septum or piece of muscle, that divides the womb cavity into two separate compartments can be removed or divided in an attempt to make the uterus appear normal again. This operation, which is known as “hysteroscopic septal resection”, is performed regularly but we do not know for sure if it is beneficial.

We are interested in conducting a large national study to address these questions and critically assess if this operation should be offered to all women with a septate uterus or only to high risk patients such as those who have had several miscarriages. Before we made a decision on whether we proceed or not we would like to know what prospective patients think about the proposal. You have been asked to complete this questionnaire as you have been shown to have an abnormally shaped womb. Your response will be instrumental in deciding whether we should proceed with this study.

The normal uterus (womb) has a single regularly shaped cavity whereas a septate uterus is essentially a womb divided into two separate compartments by a central piece of muscle (see diagrams).



Normal Uterus/Womb



Septate Uterus

Here is some background information that may help you answer the questions below. This information has been derived by a six month study in which we have reviewed the entire medical literature on uterine abnormalities. During this review we found:

- 5.5% of women in general have an abnormally shaped uterus (all types) and an estimated 2.3% have a septate uterus

- An uncertain link between an abnormally shaped womb and infertility. Certain studies (Nahum, 1998; Taylor and Gomel, 2008) have suggested there is a link whilst others contradict this finding (Grimbizis, 2001; Saravelos, 2008). Uterine abnormalities do appear more common, however, in women undergoing IVF treatment (35.6%).

- There is a strong association between an abnormally shaped womb and miscarriage. Almost 13% of women who suffer from repeated miscarriage are subsequently shown to have an abnormally shaped womb and most often a septate uterus. Women with septate uterus are approximately 3 times more likely to suffer from an early miscarriage (before 12 weeks).

- A woman with a septate uterus also appears to be at an increased risk of delivering their babies prematurely, before the 37th week of pregnancy. Premature babies are more vulnerable to a number of problems, such as breathing difficulties, infections, and brain haemorrhage and are more likely to die or to have learning difficulties if they survive .

- The muscular septum that divides the uterus can be easily removed with a fairly simple keyhole surgical technique that involves stretching the cervix (the neck of the womb) and the use of scissors or an electrical cutting instrument. Whilst there are some obvious risks associated with the procedure it has been performed for many years without any major complications.

- We do not know if surgery is beneficial in terms of improving fertility or reducing the risk of miscarriage and/or premature delivery.

- Recent studies (Raju, 2006; El-Toukhy, 2008) have suggested that a simple diagnostic hysteroscopy, where a telescope is inserted into the womb, may improve the chance of conception in women who have had two unsuccessful attempts at IVF. The mechanism for this is not known. The surgery is straightforward and is performed as a day case.

The study we are planning involves randomly allocating certain patients, such as those who have suffered from subfertility, miscarriage or preterm birth, who are known to have a septate uterus to one of two groups:

Group 1: the active treatment group. Women allocated to this group will have keyhole surgery to remove the septum.

Group 2: the control group. These women will also have an operation called a “diagnostic hysteroscopy” where a telescope is inserted through the neck of the womb allowing the doctor to visualise the lining of the womb and cavity. It is identical to the operation above but the septum will not be removed.

NB both groups will have keyhole surgery involving the insertion of a telescope into the womb but neither will know if they have had their septum removed. This is important as it prevents doctors and patients bias, and may influence the outcome of the study.

Please note: Please put an 'X' in the box as appropriate

1. Please select your age group:

- ☐ Under 20
- ☐ 20 to 30
- ☐ 30 to 40
- ☐ >40

2. Have you suffered from or experienced:

- ☐ Subfertility or infertility (Trying for a baby for more than 12 months)
- ☐ Subfertility or infertility and are considering IVF
- ☐ 2 or more unsuccessful IVF cycles and are planning to go for further IVF treatment
- ☐ One early miscarriage (<12 weeks pregnant)
- ☐ Two consecutive (one after another) early miscarriages (<12 weeks pregnant)
- ☐ Three or more consecutive (one after another) early miscarriages (<12 weeks pregnant)
- ☐ Late miscarriage (> 12 weeks pregnant)
- ☐ Premature delivery (<34 weeks pregnant)
- ☐ None of the above

3. Having considered the background information given above, please answer all the following sections even if they do not apply to you, i.e. answer all of them hypothetically. Please put an 'X' in the box for each selected option:

Infertility (Trying for a baby for more than 12 months)

☐ WOULD prefer to have definitive surgery (surgical resection) to improve the chance of pregnancy if you have a septate uterus AND suffered from subfertility but are trying for a baby

☐ WOULD be willing to enter the trial outlined above to compare the effects (both beneficial and harmful) of surgery and to be randomised to treatment or no treatment

☐ WOULD NOT AGREE to treatment or ENTER to such a trial

Infertility requiring IVF

☐ WOULD prefer to have definitive surgery (surgical resection) to improve the chance of pregnancy if you have a septate uterus AND suffered from infertility and considering IVF treatment

☐ WOULD be willing to enter the trial outlined above to compare the effects (both beneficial and harmful) of surgery and to be randomised to treatment or no treatment

☐ WOULD NOT AGREE to treatment or ENTER to such a trial

Recurrent Unsuccessful IVF Treatment (≥ 2 unsuccessful IVF cycles)

☐ WOULD prefer to have definitive surgery (surgical resection) to improve the chance of pregnancy if you have a septate uterus AND have had recurrent unsuccessful IVF cycles and considering further IVF treatment

☐ WOULD be willing to enter the trial outlined above to compare the effects (both beneficial and harmful) of surgery and to be randomised to treatment or no treatment

☐ WOULD NOT AGREE to treatment or ENTER to such a trial

One Early Miscarriage (≤ 12 weeks)

☐ WOULD prefer to have definitive surgery (surgical resection) if you have a septate uterus AND one previous early miscarriage, to reduce the risk of further miscarriage and premature delivery in a subsequent pregnancy

☐ WOULD be willing to enter the trial outlined above to compare the effects (both beneficial and harmful) of surgery and to be randomised to treatment or no treatment

☐ WOULD NOT AGREE to treatment or RANDOMISE to such a trial

Two Consecutive Early Miscarriages (≤ 12 weeks)

☐ WOULD prefer to have definitive surgery (surgical resection) if you have a septate uterus AND two previous consecutive (one after another) early miscarriages, to reduce the risk of further miscarriage and premature delivery in a subsequent pregnancy

☐ WOULD be willing to enter the trial outlined above to compare the effects (both beneficial and harmful) of surgery and to be randomised to treatment or no treatment

☐ WOULD NOT AGREE to treatment or ENTER to such a trial

Three or more Consecutive Early Miscarriages (≤ 12 weeks)

☐ WOULD prefer to have definitive surgery (surgical resection) if you have a septate uterus AND three or more previous consecutive (one after another) early miscarriages, to reduce the risk of further miscarriage and premature delivery in a subsequent pregnancy

☐ WOULD be willing to enter the trial outlined above to compare the effects (both beneficial and harmful) of surgery and to be randomised to treatment or no treatment

☐ WOULD NOT AGREE to treatment or ENTER to such a trial

Late miscarriage (>12 weeks)

☐ WOULD prefer to have definitive surgery (surgical resection) if you have a septate uterus AND one previous late miscarriage, to reduce the risk of further miscarriage and premature delivery in a subsequent pregnancy

☐ WOULD be willing to enter the trial outlined above to compare the effects (both beneficial and harmful) of surgery and to be randomised to treatment or no treatment

☐ WOULD NOT AGREE to treatment or ENTER to such a trial

Premature Delivery (< 34 weeks)

☐ WOULD prefer to have definitive surgery (surgical resection) if you a have septate uterus AND a history of preterm delivery, to increase the chance of delivery after 34 weeks in a subsequent pregnancy

☐ WOULD be willing to enter the trial outlined above to compare the effects (both beneficial and harmful) of surgery and to be randomised to treatment or no treatment

☐ WOULD NOT AGREE to treatment or ENTER to such a trial

4. Please share any comments you may have with us:

Thank you for taking the time to answer these questions. Your feedback and personal opinion mean a great deal to us and will ultimately decide if such a study is feasible.

If you would like further information, please contact us by email at the following addresses:

Nick Raine-Fenning nick.fenning@nottingham.ac.uk
Arri Coomarasamy a.coomarasamy@bham.ac.uk
Yee Yin Chan mgxyyc@nottingham.ac.uk

Yours sincerely,

Nick Raine-Fenning, Arri Coomarasamy & Yee Yin Chan

Nick Raine-Fenning is an Associate Professor at the University of Nottingham

Arri Coomarasamy is an Associate Professor at the University of Birmingham

Yee Yin Chan is a Clinical Research Fellow at the University of Nottingham

Appendix 5: Participant Information Sheet (Pilot randomised, controlled trial of hysteroscopic septal resection)

Participant Information Sheet

(Version 1.2: 23 October 2013)

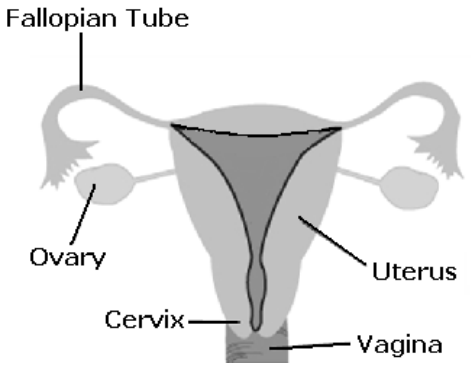
Pilot randomised, controlled trial of hysteroscopic septal resection

Researchers: Mr Nicholas Raine-Fenning, Professor Jim Thornton, Dr Yee Yin Chan

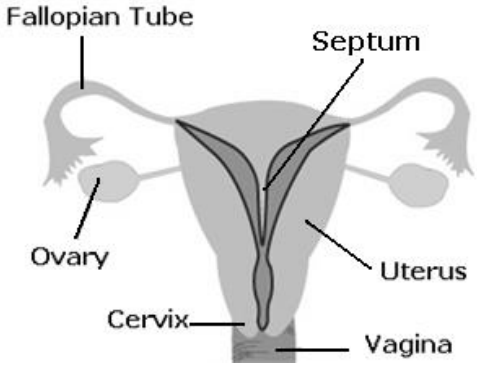
We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. We also recommend that you talk to others about the study if you wish. Ask us if there is anything that is not clear.

What is the purpose of the study?
It is thought that miscarriages and preterm deliveries are more common in women with a particular type of abnormally shaped womb called a septate uterus. The septum is a piece of muscle, which divides the womb cavity into two separate compartments. The septum can be removed or divided in an attempt to make the womb appears normal again. This surgery, which is known as "hysteroscopic septal resection" uses a telescope (hysteroscopy) to remove septums. It is performed regularly but we do not know if it is beneficial.

The normal uterus (womb) has a single regularly shaped cavity whereas a septate uterus/womb is essentially a womb divided into two separate compartments by a central piece of muscle.



Normal Uterus/Womb



Septate Uterus/Womb

This study aims to see if hysteroscopic septal resection improves the pregnancy outcomes in women with a septate uterus, who have a history of miscarriage or preterm delivery. We also want to find out if this surgery is safe.

Why have I been invited?
You are being invited to take part because you have a history of preterm delivery or miscarriage. We are inviting 10 participants like you to take part.

Do I have to take part?
It is up to you to decide to join the study. We will describe the study and go through this information sheet. If you do decide to take part you will be given this information to keep and be asked to sign a consent form. You are still free to withdraw at any time, without giving a reason. This would not affect your legal rights or the standard of care you receive.

What will happen to me if I take part?

VISIT 1: Interview and 3-Dimensional Scan
If you agree to participate in the study, you will be asked to attend an interview with a member of the research team to sign a consent form. Once you sign the consent form, you will need to have a 3-Dimensional transvaginal ultrasound scan to confirm the diagnosis. The interview and scan will normally be combined and performed on the same day you consent to the study, so that you don't need to make any additional visits to the hospital at this stage of the study. The ultrasound scan is not associated with any addition risks.

VISIT 2: Treatment
We put people into groups and give each group a different treatment so that we could compare the results to see if one is better. To try to make sure the groups are the same to start with, each patient is put into a group by chance (randomly). We will randomly allocate you to one of the two groups (50-50 chance):

Group 1: the Metroplasty group. Women allocated to this group will be scheduled to have "hysteroscopic septal resection" where a telescope is inserted through the neck of the womb allowing the doctor to visualise the lining of the womb at cavity. The uterine septum will then be removed under direct vision using the telescope. This will be performed as day-case surgery.

Group 2: the Diagnostic group. These women will have an operation called a "diagnostic hysteroscopy" which is identical to the operation in Group 1 but the septum will not be removed.

VISIT 3: Post-operative review and 3-dimensional ultrasound scan
We will see you 6-8 weeks after your operation with another 3-dimensional ultrasound scan. This appointment allows us to ensure that you have recovered well from your operation. After this appointment, you will be encouraged to start trying to become pregnant. You will then be followed up for approximately 24 to 34 months (duration depending if you become pregnant after treatment).

Expenses and payments
Participants will not be paid to participate in the study. We will bear the cost of the ultrasound scans and operations that you will receive. The researchers are not receiving any payment for being involved in this study

What are the possible disadvantages and risks of taking part?
Before participating you should consider if this will affect any health insurance you have and seek advice if necessary

3-Dimensional transvaginal ultrasound scan
For 3-Dimensional transvaginal ultrasound scan, a small vaginal probed covered with a sterile condom sheath to avoid infection will be inserted into the vagina. The majority of women find this procedure acceptable with minimal discomfort. A small number of women may find it too uncomfortable, in which case the procedure will be stopped immediately.

Research done at Royal Free Hospital, London in 2003, assessing the acceptability of transvaginal ultrasound scan found that 99% of women who had a transvaginal scan would agree to have it done again in the future, suggesting its wide acceptance among women.

Diagnostic hysteroscopy (with or without septal resection)
All women will be having a day-case hysteroscopy under general aneesthesia. The procedures will be performed by competent and well-trained clinicians only to ensure procedure-related complications are minimised.

Hysteroscopy is a common procedure in gynaecology. Procedure-related complications included are bleeding (1 in 1000), infection (1 in 250), accidental damage to the womb or neck of womb (1 in 135) and damage or perforation to the womb (1 in 500 women). Patients may develop fluid overload if excessive amount of irrigation fluid is used during hysteroscopic resection.

Complication rates have been described following a multicentre study involving 82 hospitals and 13600 procedures in the Netherlands. Diagnostic hysteroscopy had a lower complication rate than operative hysteroscopy (0.13 versus 0.95 %).

What are the possible benefits of taking part?
The current evidence suggested improved reproductive outcomes (reduced miscarriage and preterm delivery rates) in patients who had hysteroscopic septal resections (Homer, 2000; Grimbizis, 2001). If you agree to do the study, you have a 50-50 chance of being randomised to the group who will undergo septal resection. We are doing this study to see if the operation improves the pregnancy outcomes, and until we have the results of this study, we cannot promise this study will help you.

It has been shown that a simple diagnostic hysteroscopy improves the chance of conception in women undergoing IVF. Therefore, if you are randomised to the control group (septums not removed), a diagnostic hysteroscopy may improve the chance of you getting pregnant.

What happens when the research study stops?
The results obtained during the study will be analysed by the researchers and will be published in medical journals, and national and international scientific conferences.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers contact details are given at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this by contacting NHS Complaints. Details can be obtained from the Queens Medical Centre Patient Advice and Liaison Service (PALS) by phone on 0800 183 0204 or email on pals@nuh.nhs.uk. In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in the study be kept confidential?
Yes, we will follow ethical and legal practice and all information about you will be handled in confidence.

If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the University of Nottingham and Nottingham University Hospitals NHS Trust. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

All information which is collected about you during the course of the research will be kept **strictly confidential**, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept for a maximum of 10 years after the end of the study so that we are able to contact you about the findings of the study or *possible follow-up studies* (unless you advise us that you do not wish to be contacted). All other data (research data) will be kept securely for 10 years by the research team. After this time, your data will be disposed of securely. During this time, all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

What will happen if I do not want to carry on with the study?
Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis to avoid biasing trial results.

Involvement of the General Practitioner/Family doctor (GP)
We will let your family doctor or GP know that you are taking part in the study.

Will any genetic tests be done?
No genetic tests will be done

What will happen to the results of the research study?
The study results will be presented and published in national/international meetings or journals, but you will not be referred to by name or identified in any report or publication. The results of this pilot study will be used to design a larger study. All results will be published following closure of the study; however some intermediate results might be published earlier.

Who is organising and funding the research?
This research is being organised by the University of Nottingham and is being funded by the Nottingham University Hospitals Charity and the Department of Research and Development.

Who has reviewed the study?
All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Nottingham 1 Research Ethics Committee.

Further information and contact details
Thank you for taking time to read and consider this information sheet.
Before, during or after the study if you have any concerns or complaints, please contact Dr Nicholas Raine-Fenning or Dr Yee Yin Chan at Nottingham University Research and Treatment Unit in Reproduction (NURTURE), B floor, East Block, Queen's Medical Centre, Nottingham NG7 2UH. Telephone: 0115 8230700