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An investigation of basic science and clinical research methodologies to benefit clinical practice

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Contents of Thesis

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* There has been repetition in the listing of these tables and figures in the Thesis text. This has been adopted in order to help the reader when reading the particular section, rather than the reader having to cross-reference to other chapters of the Thesis in order to locate the relevant item.

PRELIMINARIES

Abstract

The aim of this PhD thesis was to produce research that could inform and benefit clinical practice by exploring the application of basic science and clinical research methodologies to disorders in obstetrics and gynaecology. **Chapter 1's** investigation of endometriosis is the first to 1) report detailed genetic mapping of endometriosis-associated ovarian cancer, 2) report the existence of micro-LOH (loss of heterozygosity) in ovarian endometriosis through a SNP 100K DNA array. **Chapter 2** explores the efficacy of interventions to treat menstrual abnormalities using clinical cohort studies. Furthermore, **Chapter 2** highlights how negligence in female sterilization failure may be mathematically (Bayesian) modelled. **Chapter 3** explores the value of systematic reviews for preventing preterm delivery and use of LNG-IUS (Mirena coil). The clinical guidelines published in **Chapter 4** include: vaginal birth after previous caesarean, ectopic pregnancy, safe laparoscopic entry and minimising risk of sterilisation failure. The thesis concludes (**Chapter 5)** by suggesting strategies to augment the research methodological approaches evaluated in this thesis in order fulfill the aim of benefitting clinical practice. Work included in this PhD thesis has been orally presented at international conferences, published in peer-reviewed journals, and published as a national clinical guideline by the Royal College of Obstetricians and Gynaecologists, UK (RCOG).

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Introduction to Thesis

Clinical research, although a commonly used term, is actually difficult to achieve a consensus definition for. A definition stated by Department of Health (United Kingdom) is that research is "*the attempt to derive generalisable new knowledge by addressing clearly defined questions with systematic and rigorous methods*". This definition includes studies that aim to generate hypotheses as well as studies that aim to test them [1] . The Medical Research Council (United Kingdom) aims to support research that is aimed at " *maintaining and improving human health"* [2]; a commitment endorsed by all other research funding bodies, professional medical colleges and National Health Service (UK).

There are numerous basic science and clinical research methodologies employed in clinical research. I suggest that these may be depicted as components of a 'research pyramid' (**Figure A**). For research to ultimately translate to clinical benefit, there needs to be *ascension* of the pyramid to its peak through appropriate selection of the 'next level" research methodology. The graphical depiction is useful as it highlights methodologies existing within the context of a particular level that corresponds to the level of evidence that is considered during guideline development (**Table A**). Furthermore, the pyramid shape mirrors the typical frequency of publications on disease, with several existing at the base, and fewer identified as the pyramid is ascended.

Multiple components are necessary to ensure that research is relevant, effective, efficient, ethical, and will ultimately translate to health gain. The aim of this PhD thesis was to produce research that could inform and benefit clinical practice. The chapters have been ordered to follow a stepwise ascension of the research methodology pyramid (**Figure A**).

Each chapter illustrates the use of a specific research methodology by considering selected disorders in obstetrics and gynaecology. In **Chapter 1**, the thesis explores the molecular aetiology of endometriosis and tests whether it behaves as a neoplastic precursor to ovarian cancer. Maintaining a gynaecological theme, **Chapter 2** explores the efficacy and effectiveness of interventions to treat menstrual abnormalities using clinical cohort studies; this work also led to the production of a RCOG educational module for specialist trainees in abnormal uterine bleeding [3]. Furthermore, **Chapter 2** highlights how rare outcome measures, such as failed sterilisation, may be adequately explored using cohort study design and Bayesian mathematical modelling. **Chapter 3** explores the clinical value and potential drawbacks of systematic review by assessing screening-preventative interventions to reduce the risk of preterm delivery. In addition, the chapter includes a systematic review of the non-contraceptive uses of Levonorgestrel-releasing hormone system. The publications of the clinical guidelines in **Chapter 4** are likely to have immediate and maximal benefit on clinical practice. The production of clinical guidelines adopted a structured approach and considered all levels of research evidence, not just systematic reviews or RCTs, to generate recommendations for best medical practice. The guidelines included: vaginal birth after previous caesarean (RCOG national guideline), ectopic pregnancy (BMJ Clinical evidence), safe gynaecological laparoscopic entry, and minimising the risk of sterilisation failure. The thesis concludes (**Chapter 5**) by summarising the benefits to clinical practice for each research methodology. The chapter also suggests future research themes that may augment the research methodological approaches evaluated in this thesis in order to benefit clinical practice.

Table A Classification of evidence used by RCOG guideline development

Classification of Evidence Levels

Ia Evidence obtained from meta-analysis of randomised controlled trials.

Ib Evidence obtained from at least one randomised controlled trial.

IIa Evidence obtained from at least one well-designed controlled study without randomisation.

IIb Evidence obtained from at least one other type of well-designed quasi-experimental study.

III Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case studies.

IV Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities.

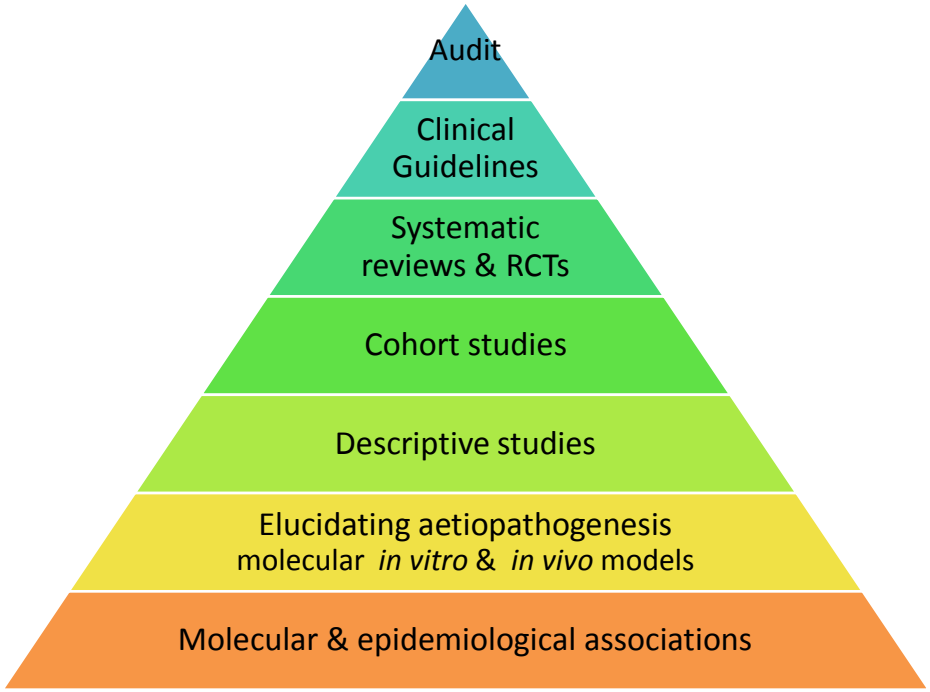
Grades of Recommendations

A	Requires at least one randomised controlled trial as part of a body of literature of overall good quality and consistency addressing the specific recommendation. (Evidence levels Ia, Ib)
B	Requires the availability of well controlled clinical studies but no randomised clinical trials on the topic of recommendations. (Evidence levels IIa, IIb, III)
C	Requires evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities. Indicates an absence of directly applicable clinical studies of good quality. (Evidence level IV)

Good Practice Point

✓	Recommended best practice based on the clinical experience of the guideline development group

Figure A. Ascension of research pyramid



Footnotes

RCT randomised controlled clinical trials

Audit refers to clinical audit to assess impact of clinical guidelines

Publications and presentations arising from Thesis

CHAPTER ONE	
Prowse AH, Manek S, Varma R, Liu J, Godwin AK, Maher ER et al. Molecular genetic evidence that endometriosis is a precursor of ovarian cancer. Int J Cancer 2006; 119(3):556-562.	4
Varma R, Rollason T, Gupta JK, Maher ER. Endometriosis and the neoplastic process. Reproduction 2004; 127(3):293-304.	5
Genome wide SNP 100K analysis of endometriosis: demonstration of genomic imbalance, Oral Presentation, British Congress of Obstetrics and Gynaecology RCOG, London, July 2007.	6
Genetic evidence for malignant transformation of endometriosis (1st July 2007, Oral Presentation, European Society for Human Reproduction & Embryology (ESHRE), Lyon, Paris.	7
CHAPTER TWO	
Varma R, Gupta JK. Predicting negligence in female sterilization failure using time interval to sterilization failure: analysis of 131 cases. Hum Reprod 2007; 22(9):2437-2443.	8
Varma R, Soneja H, Bhatia K, Ganesan R, Rollason T, Clark TJ et al. The effectiveness of a levonorgestrel-releasing intrauterine system (LNG-IUS) in the treatment of endometrial hyperplasia--a long-term follow-up study. Eur J Obstet Gynecol Reprod Biol 2008; 139(2):169-175.	9
Varma R, Soneja H, Samuel N, Sangha E, Clark TJ, Gupta JK. Hospital recovery following Thermachoice ablation is not dependent on setting (outpatient or daycase) or rescue analgesia: unexpected result. Eur J Obstet Gynecol Reprod Biol 2008; 140(1):76-81.	10
Varma R, Soneja H, Clark TJ, Gupta JK. Outpatient Thermachoice endometrial balloon ablation: long term, prognostic and quality of life measures. European Journal of Obstetrics & Gynaecology and Reproductive Biology. In submission, 2008.	11
Varma R, Soneja H, Clark TJ, Gupta JK. Hysteroscopic myomectomy for menorrhagia using Versascope trade mark bipolar system: Efficacy and prognostic factors at a minimum of one year follow up. Eur J Obstet Gynecol Reprod Biol 2008. In Press	12
Varma R, Gupta JK. Royal College of Obstetricians and Gynaecologists. Abnornal Uterine Bleeding. Module 13. StratOG.net. Stuctured Training Resource to Assist Trainees in Obstetrics and Gynaecology. June 2007.	3

CHAPTER THREE	
Varma R, Gupta JK, James DK, Kilby MD. Do screening-preventative interventions in asymptomatic pregnancies reduce the risk of preterm delivery--a critical appraisal of the literature. Eur J Obstet Gynecol Reprod Biol 2006; 127(2):145-159.	[13]
Varma R, Gupta JK. Antibiotic treatment of bacterial vaginosis in pregnancy: multiple meta-analyses and dilemmas in interpretation. Eur J Obstet Gynecol Reprod Biol 2006; 124(1):10-14.	[14]
Varma R, Sinha D, Gupta JK. Non-contraceptive uses of levonorgestrel-releasing hormone system (LNG-IUS)--a systematic enquiry and overview. Eur J Obstet Gynecol Reprod Biol 2006; 125(1):9-28.	[15]
CHAPTER FOUR	
Varma, R, Gupta, J.K., Smith, G.C. Birth after previous caesarean section. Royal College of Obstetricians and Gynaecologists Clinical Green top guideline No.45. February 2007. http://www.rcog.org.uk/index.asp?PageID=1913	[16]
Varma R, Smith GC. Management of women with previous caesarean section. In Press. In: Warren R, Arulkumaran S, editors. Best Practice in Labour and Delivery. Cambridge University Press, Cambridge, UK.; 2008.	[17]
Varma R, Gupta JK. Ectopic Pregnancy. http://clinicalevidence.bmj.com/ceweb/conditions/pac/1406/1406_background.jsp . BMJ Clinical Evidence . 2006.	[18]
Varma R, Gupta JK. Laparoscopic entry techniques: clinical guideline, national survey, and medicolegal ramifications. Surg Endosc 2008; 22(12):2686-2697.	[19]
Varma R, Gupta JK. Failed sterilisation: evidence-based review and medico-legal ramifications. BJOG 2004; 111(12):1322-1332.	[20]
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CHAPTER 1: INVESTIGATING THE GENETIC AND MOLECULAR AETIOLOGY OF ENDOMETRIOSIS

Introduction

Endometriosis is a gynaecological disorder affecting 10-15% of women of reproductive age. The condition often presents with infertility and pelvic pain, causing significant impairment of quality of life. The precise aetiology of endometriosis is unclear, but is considered to involve multiple genetic, environmental, immunological, angiogenic and endocrine processes. Although endometriosis is a benign disorder, recent studies suggest endometriosis could be viewed as a neoplastic process. This chapter presents a basic science investigation of the genetic and molecular aetiology of endometriosis. The similarities between endometriosis and neoplasia have been used to investigate endometriosis using techniques normally applied in cancer biology. Initially, the chapter presents the epidemiological, genetic and molecular evidence that justifies the rationale for using a cancer biology model to study endometriosis. Thereafter, the chapter discusses various genetic and immunohistochemistry techniques used in the investigation. Traditional approaches (such as microsatellite genetic marker genetic mapping) are contrasted with newer technologies (laser capture microdissection and Affymetrix SNP 100K DNA microarray). The aim was to identify the key genes involved in the initiation, proliferation and malignant transformation of endometriosis to enable development of improved screening-preventative therapies for both endometriosis and ovarian cancer.

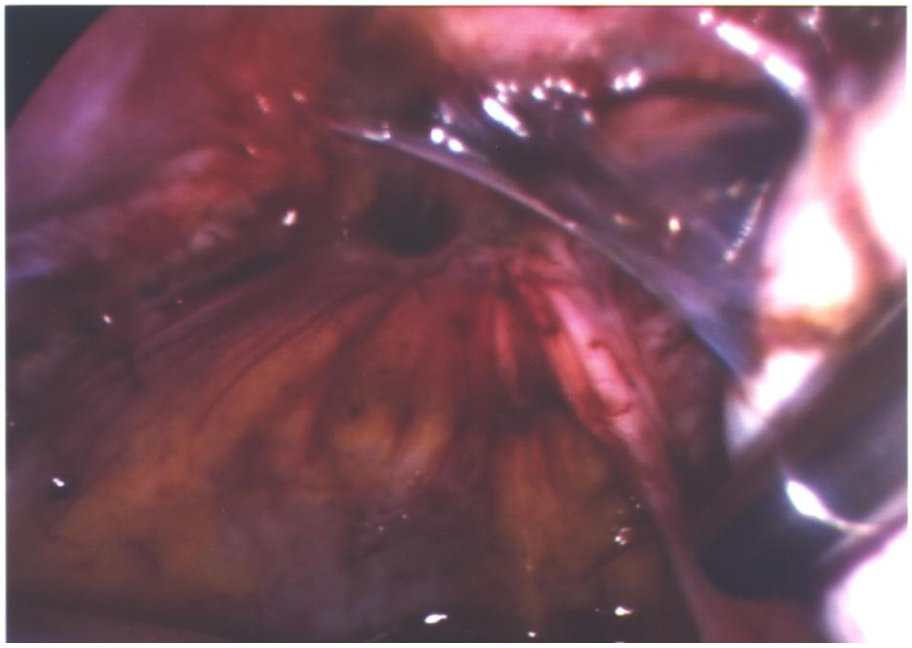
1.1: Exploring the hypothesis that endometriosis is a neoplastic precursor to ovarian cancer

Introduction

Endometriosis is defined as the implantation of endometrium-like glandular and stromal cells outside their normal location in the uterus. Endometriotic lesions are usually identified at laparoscopy localised to ovaries and the Pouch of Douglas (**Figure 1.1**). Endometriosis is diagnosed in 30% of cases referred for infertility investigation [1] and in 10%-70% of women with pelvic pain [1] . Overall, studies estimate that endometriosis may affect around 7-15% of women of reproductive age, thus making this a common condition.

Endometriosis has been considered a 'disease' because it is often identified when investigating women with infertility, pelvic pain, dyspareunia (pain on intercourse) and dysmenorrhoea (painful periods). Traditionally the classification of endometriosis has been made by anatomical (surgical staging by revised American Fertility Society score) and histopathological (atypical and non-atypical endometriosis) criteria [2]. However, this combined approach of classification does not correlate closely with pelvic pain or reproductive outcome, and is prone to inter-observer error. Furthermore, the emphasis of targeting the endometriotic lesion, by surgical removal or hypo-oestrogenic inactivation, does not necessarily correct the aberrant underlying molecular mechanism(s). This explains why current endometriosis treatment does not alleviate clinical symptoms in all cases, and recurrence is common [3].

Figure 1.1. Image of an endometriotic lesion surrounded by adhesions



These disparities suggest that endometriosis may not be a true 'disease' but a heterogeneous entity with differing subtypes. One subtype may be capable of causing symptomatic disease directly consequent to endometriotic pathology (e.g. ovarian endometriomas, pelvic adhesions), whereas, another subtype, may be associated with symptoms without obvious endometriotic-lesion basis. Another subtype may be clinically asymptomatic and its presence be considered a normal 'non-pathogenic' phenomena. Consequently the current focus on treating the endometriotic lesion should be reconsidered, and efforts to understand the pathogenesis of endometriosis, and its temporo-spatial relationship to symptomology, should be increased.

Traditionally, endometriosis research has focused on the lesion itself and comparing molecular processes between ectopic and eutopic endometrium[4]. This has identified multiple anomalies in genetic, environment, angiogenic, endocrine, metabolic, and immunological mechanisms. Some of these correlate with the severity of endometriosis and/or associated clinical sequelae implying a causative rather than simply associative role. However, the major obstacle has been the difficulty in discriminating between processes fundamental to endometriosis aetiopathogenesis and epiphenomena. Importantly, these physiological differences are multi-compartment (endometrium, peritoneal fluid, follicular fluid and blood) and not just localised to the site of the endometriotic lesion, implying a fundamental widespread alteration in reproductive tract function. This multifactorial multi-compartment pathogenesis, coupled with the clinical heterogeneity, has created a confusion of data, with little consensus on a unifying mechanism. Nevertheless, since Sampson first reported in 1925[5] that endometriosis may give rise to malignant change, and proposed criteria for diagnosis of malignancy arising in endometriosis, extensive evidence for an association between endometriosis and cancer (especially ovarian) has now accumulated.

Aim To evaluate the hypothesis that endometriosis is a neoplastic precursor to the development of ovarian cancer based on systematic literature search and critical appraisal of clinical and basic science data

Methods All observational and experimental studies examining the relationship between endometriosis and ovarian cancer were retrieved from MEDLINE (1966-2004) and EMBASE (1980-2004) medical databases using combination of specific keywords and MeSH terms. The following search terms and word variants were used: 'endometriosis', 'endometriotic', or 'endometrio$' combined with "AND" to 'ovarian neoplasms', 'neoplasms', 'carcinoma' 'genital neoplasms, female', 'carcinogens', 'carcinogen$', 'carcinogens, enivronmental', 'tumo$', 'malignan$', 'cancer$', or 'neoplas$'. In addition, bibliographies of retrieved articles were examined to identify further relevant studies. The search was completed in April 2004. At the time of submission of this PhD thesis, a further search of the medical databases was performed and specific key articles have been included where they substantially alter the evidence-base. The hypothesis was examined by examining by considering the following methodological approaches:

A. **Clinico-pathological epidemiological** data

B. **Genetic and molecular data of endometriosis** and cancer. In particular, considering how endometriosis demonstrates a molecular cancer phenotype according to **Hanahan's 'Hallmarks of Cancer**[6]' criteria for a cancer cell [defined as seven critical features of the cancer phenotype **(Tables 1.1 and 1.2**)]

C. Testing association vs. causality of endometriosis and cancer using **Bradford-Hill (1965) epidemiological causality criteria**[7] (**Table 1.3**).

D. **Summary** of the published evidence that supports or refutes the hypothesis that endometriosis is a neoplastic precursor to the development of ovarian cancer.

Table 1.1 Hanahan's criteria of properties exhibited by a cancer cell: 'the hallmarks of cancer' [6]

1	Self-sufficiency in growth signals	Mitotic growth signals are needed for cells to move from a quiescent state into active proliferative state. These signals are transmitted into the cell by transmembrane cell-surface receptors that bind to: diffusible growth factors, ECM components, cell-to-cell adhesion interaction molecules
2	Insensitivity to antiproliferative signals	Growth inhibitory signals (soluble or immobilized in ECM and on surfaces of nearby cells) are received by transmembrane cell-surface receptors coupled to intracellular signaling circuits
3	Resistance to Apoptosis	Evasion mechanisms of programmed cell death
4	Limitless replicative potential	Disruption of intrinsic cell-autonomous program that limits their multiplication. This program operates independently of the cell-to-cell signaling pathways described above
5	Sustained angiogenesis	Virtually all cells in a tissue are obligated to reside within 100um of a capillary blood vessel to allow adequate permeation of oxygen and nutrients crucial for cell survival The cells within aberrant proliferative lesions initially lack angiogenic ability, but in order to progress, incipient neoplasias must develop angiogenic ability
6	Tissue invasion and metastasis	This enables cancer cells to escape the primary tumour mass and colonise new sites where, at least initially, nutrients and space are not limited
7	Genomic instability	Mutations or inactivation/activation of tumour suppressor genes, oncogenes, DNA monitoring and repair enzymes, checkpoint systems at mitosis. These are carried out by intragene (e.g. mutation, deletion) and epigenetic (e.g. promoter hypermethylation) mechanisms

Footnotes

The listed capabilities are mostly acquired directly, or indirectly, through changes in the genomes of cancer cells.

Table 1.2 How endometriosis displays the 'Hallmarks of cancer'[6]

Hallmarks of cancer		How endometriosis demonstrates the signified hallmark
1	Self-sufficiency in growth signals	Increased local production of estrogen and responsiveness to estrogen[8] Inherited genetic polymorphisms in drug-metabolizing enzymes (CYP1A1, CYP19, and GSTM1) which predispose to endometriosis[9] and ovarian endometrioid and clear cell cancer[10]
2	Insensitivity to antiproliferative signals	Expression of the inhibitory progesterone receptor isoform PR-A instead of the stimulatory isoform PR-B [11] Altered expression of p27Kip1 protein (cdk inhibitor) in active and inactive endometriotic lesion, and increased p21 expression in endometriomas compared with benign and malignant ovarian tumours [12;13]
3	Resistance to Apoptosis	Elevated soluble Fas ligand and IL-8 in endometriotic peritoneal fluid.[14] Germline and somatically acquired inactivating mutations of p53 gene [15;16] Up-regulation of survivin, matrix metalloproteinases, and bcl-2, and decreased BAX [17-19]
4	Limitless replicative potential	No studies examining telomerase function in endometriosis, but it is noted estrogen and progesterone stimulate, whilst tamoxifen and wild-type (normal variant) p53 inhibit, telomerase activity in estrogen dependent neoplasms (breast and endometrial cancer cells) [20;21] which endometriosis represents.
5	Sustained angiogenesis	Pathological angiogenesis, immune cell suppression and immune cell activation co-exist in endometriosis[22] and cancer processes[23]. Mediators of angiogenesis exhibit genetic polymorphisms that either predispose to endometriosis (e.g. ICAM-1, IL-6 and IL-10 gene promoters)[24-26] or ovarian cancer (e.g. IL-6, MMP-1, integrin beta3, TGFBR1 ,IL-1R antagonist)[27-31]
6	Tissue invasion and metastasis	Endometriosis exhibits invasiveness that is mediated through de-regulation of similar cell adherence signaling (such as integrins, beta-catenin, cadherins and matrix metalloproteinases[17-19;32-34] to cancer. Beta catenin mutations occur in endometrial and ovarian endometrioid cancers [35;36] but have not been investigated for in endometriosis.
7	Genomic instability	Like cancer, endometriosis is monoclonal[37] and shows allelic imbalance[38]. Mutations of tumour suppressor genes occur in endometriosis[39;40], which are in some cases similar to those ovarian cancers arising directly from the endometriosis[41].

Table 1.3 Criteria and fulfillment of Bradford Hill criteria[7] of causality for endometriosis and ovarian cancer

Listed factor	Causality criteria	Comments	Strength of supporting evidence identified by this review
1	Temporal sequence	Did exposure precede outcome?	Weak
2	Strength of association	How strong is the effect, measured as relative risk or odds ratio?	Moderate
3	Consistency of association	Has the effect been seen by others?	Strong
4	Biological gradient	Does increased exposure result in more of the outcome (dose-dependency)?	None
5	Specificity of association	Does exposure lead only to outcome?	Weak
6	Biological plausibility	Does the association make pathophysiological sense?	Moderate
7	Coherence with existing knowledge	Is the association consistent with available evidence?	Weak
8	Experimental evidence	Has a randomized controlled trial been done?	Human-Weak Animal-Strong
9	Analogy	Is the association similar to others?	Weak

A) Clinico-pathological AND epidemiological data

1.Histopathology. Like malignancy, endometriosis displays features of atypia, adherence, invasion and metastases**.** Atypical endometriosis is characterized histologically by endometrial glands with cytological or architectural atypia [42], and has been observed in 12%-35% of ovarian endometriosis [43-45]. Around 60%-80% of cases of endometriosis-associated ovarian cancer (EAOC) occur in the presence of atypical ovarian endometriosis[46-48]. Of these cases, 25% showed direct continuity of the atypical ovarian endometriosis with ovarian cancer[49] , underlying a potential 'premalignant' transition spectrum of non-atypical to atypical and malignant variants.

2. Nuclear morphometry This involves a structured histological approach to grading mitotic activity using nuclear size and pleomorphism. Morphometric analysis of cancer has been shown to correlate to clinical prognosis [50;51]. There is published data on nuclear morphometric analysis of endometriosis (and related adenomyosis), albeit most limited to mainly non-prognostic correlations[52-61]. Morphometric analysis of non-atypical endometriosis showed no difference between active (red lesions) and inactive (black or white lesions) lesions; it is yet to be studied in atypical endometriosis[62] . Nonetheless, mild cytological atypia in the glandular epithelium of endometriotic cysts has been associated with normal DNA diploid patterns, whereas severe atypia may be associated with aneuploidy[63] . Furthermore, the existence of morphometric differences between peritoneal, ovarian and rectovaginal endometriosis supports the earlier stated hypothesis that endometriosis at different anatomical locations are likely to be molecularly diverse entities[53;55;56].

3.Ovarian malignancy may arise directly from ovarian endometriosis Around 60% of EAOC occurs with the cancer adjacent to endometriosis or arising directly from ovarian endometriosis, with the remaining 40% occurring with distant endometriotic disease [64;65]. Clear cell and endometrioid carcinomas are the commonest EAOCs with ovarian endometriosis, while clear cell adenocarcinoma and adenosarcoma the commonest EAOCs in extraovarian endometriosis[66-70]**.** The risk of direct malignant transformation of ovarian endometriosis has been estimated as 0.7% to 1.6% over an average of eight years [43;44]. Interestingly, there is a common unexplained left-sided predominance for endometriotic cysts, and ovarian endometrioid and clear-cell cancers[71,72]

4. Increased risk of ovarian cancer in women with endometriosis, irrespective if endometriosis is distant or adjacent to ovarian tumour. The age standardised incidence of ovarian cancer in women in the UK is 21.9 per 100,000 (0.02%), with around 75% of cases diagnosed in postmenopausal women [73]. If there were no association between cancer and endometriosis then the incidence of endometriosis in women with ovarian cancer would be similar to that in the general population. However, the incidence of endometriosis in women with ovarian cancer is 8%-30% [46;74;75]. This compares to a background incidence of endometriosis of 7-15% in women of reproductive age, and less than 2% in postmenopausal women [1]**.** This data correlates with the finding from a Swedish population study, where the risk of ovarian cancer was increased 4.2-fold (95% confidence interval 2.0 to 7.7) in the presence of endometriosis [76]. Furthermore, the histology of EAOC (40-55% clear cell , 20-40% endometrioid and <10% serous and mucinous subtypes)[77-79] differs considerably from that seen in all ovarian cancers (FIGO 1998 annual report 55% serous, 13% mucinous, 14% endometrioid, 6% clear cell) [80].

5.Increased risk of synchronous endometrial and ovarian cancers, especially endometrioid type, in presence of endometriosis. Simultaneously detected endometrial and ovarian carcinomas are most often associated with endometrioid subtypes, and ovarian endometriosis was identified in around 30% of these cases[68;70;81].

6.Clinical behavior and prognosis of endometriosis associated ovarian cancer (EAOC) differs from matched ovarian cancer subtypes not associated with endometriosis. EAOC compared to ovarian cancer without endometriosis presents at a less advanced stage, lower grade, predominantly endometrioid and clear cell type, and has a better overall survival [82;83].

7.Increased risk of extra-ovarian cancers. Around 80% of intraperitoneal cancers associated with endometriosis relate to ovarian cancer, with the remainder extra-ovarian[84]. A separate study showed an increased risk of extra-pelvic cancers (breast and non-Hodgkin's lymphoma) in women with endometriosis [85].

B) Genetic and molecular data

These have been considered according the SEVEN listed criteria that a cancer cell possess according to Hanahan and Weinberg (2000)[6] (Tables 1.1 and 1.2).

1.Self-sufficiency in growth signals: Like uterine and breast cancer, endometriosis behaves as an oestrogen dependent neoplasm. Endometriosis has specifically adapted to oestrogen-induced signaling by:

- *Increased local production of oestrogen* through increased expression of aromatase cytochrome P450 expression but deficient 17B-hydroxysteroid dehydrogenase (17B-HSD) type 2 expression (which impairs inactivation of potent oestradiol E2 to less potent oestrone E1) [86].

- *Increased responsiveness to oestrogen*. There is increased oestrogen receptor (ER-alpha) expression in active (red lesions) than inactive (black lesions) endometriosis [87].

- *Inherited genetic polymorphisms in oestrogen and progesterone receptors, which predispose to endometriosis*[88;89]

- *Inherited genetic polymorphisms in drug-metabolizing enzymes (CYP1A1, CYP19, and GSTM1) which predispose to endometriosis*[9;90-92] *and ovarian endometrioid and clear cell cancers* [93]. These alterations may induce endometriosis or cancer by altering a dioxin-induced oestrogen growth signal. Dioxins have been shown to induce endometriosis-like and oestrogen-dependent tumours in animal models [94]. Of importance, there is a doubled risk of developing endometriosis amongst women with high serum dioxin levels [95] . Activation of oestrogen receptors in endometriosis may occur indirectly through upregulated CYP1A1 activity, which causes increased aromatase P450 and oestrogen production [96], or directly by dioxin activated aryl hydrocarbon receptor [97].

Other growth factors, such as transforming growth factor alpha (TGF-alpha) and insulin-like growth factor-1 (IGF-1) have also been implicated in endometriosis and cancer development[98]. IGF-1 signaling is required for cell cycle progression and appears to be a pre-requisite for malignant transformation and implantation. A higher risk for cervical, ovarian and endometrial cancer is related to high IGF-1 levels in post- and premenopausal women. Plasma IGF-1 levels are higher in cases of severe endometriosis; however, in endometriosis IGF-1 levels locally in the endometrium are reduced [99].

2.Insensitivity to antiproliferative signals

Cell division relies on the activation of Cyclins (e.g. Cyclin D1), which bind to cyclin-dependent kinases (cdk) to induce cell-cycle progression towards S phase and later to initiate mitosis. Since uncontrolled cdk activity is often the cause of human cancer, their function is tightly regulated by cdk inhibitors (e.g. p21 and p27 Cip/Kip proteins). For example, increased expression of Cyclin D1 and cdk occurs in breast cancer and is associated with poor outcome. At the cellular level, differences in expression of p27Kip1 protein (cdk inhibitor) in active and inactive endometriotic lesions[13], coupled with increased p21 expression in endometriomas compared with benign and malignant ovarian tumours [12], suggests a role for increased cyclin-dependent kinase activity through reduced cell-cycle inhibitor activity; which is an imbalance frequently seen in cancer. At the tissue level, endometriosis may resist the anti-proliferative effect of progesterone by the predominant expression of the inhibitory progesterone receptor isoform PR-A instead of the stimulatory isoform PR-B [11].

3.Resistance to Apoptosis

Malignancy commonly displays overexpression of anti-apoptotic (Bcl-2), under-expression of pro-apoptotic (BAX) factors, and inactivation of p53 gene (p53 is a tumour suppressor gene whose protein is pro-apoptotic) through mutation. Similarly, endometriotic lesions have also evolved strategies to evade apoptosis by:

- Increased bcl-2, and decreased BAX[100].
- Up-regulation of survivin and matrix metalloproteinases (MMPs)[17-19].
- Elevated soluble Fas ligand and IL-8 in endometriotic peritoneal fluid. The increased FasL expression by IL-8 may induce apoptosis of T lymphocytes and thus enable endometriosis to evade immune mediated cell death[14].
- Germline [16] and somatically acquired [101] inactivating mutations of p53 gene.

4.Limitless replicative potential

With each replicative cycle, telomeres (repetitive DNA sequences capping each chromosome) become progressively shorter, eventually resulting in cell senescence and cell death. Tumours commonly express the enzyme telomerase, which protects the telomeres from shortening and thus preventing 'cell ageing'. Oestrogen and progesterone stimulate, whilst tamoxifen and wild-type (normal variant) p53 inhibit, telomerase activity in breast and endometrial cancer cells[20;21]. Although there are no published studies examining telomerase function in endometriosis, it is notable that oestrogen dependent neoplasms are potentially susceptible to telomerase control.

5.Sustained angiogenesis

Pathological angiogenesis, immune cell suppression and immune cell activation co-exist in endometriosis and cancer processes [22;23]. Genetically transmitted or environmentally induced (e.g. exposure to dioxins) alterations in the angiogenic and/or immune response may predispose women to the ectopic implantation of endometrial cells, transported into the peritoneal cavity by retrograde menstruation which thereby lead to endometriosis. Significantly, both cancer and endometriosis share some of the mediators implicated in this 'inflammatory angiogenesis' model. Furthermore, the genes of these mediators exhibit genetic polymorphisms that either predispose to endometriosis (e.g. ICAM-1, IL-6 and IL-10 gene promoters) [102-104] or cancer (e.g. IL6, IL8, TNF-alpha, NFKB1, and PPAR-gamma genes) [105-109].

Anti-angiogenic therapy involves the inhibition of pro-angiogenic factors (e.g. anti-vascular endothelial growth factor VEGF monoclonal antibodies) or activation of endogenous inhibitors of angiogenesis (e.g. endostatin and angiostatin). Pre-clinical studies have shown that endostatin effectively inhibits tumor growth and shrinks existing tumor blood vessels. Phase 1 clinical cancer trials of endostatin and angiostatin are ongoing, and preliminary results show minimal toxicities. Similarly, anti-angiogenic strategies for treating endometriosis exist, but are still at the experimental phase[110]. Soluble truncated receptor (flt-1) and an affinity-purified antibody to human VEGF-A, significantly inhibited the growth of endometrial explants in a mouse *in vivo* model of endometriosis by disrupting the vascular supply. Gene transfection (using a replication-deficient adenovirus vector Ad-Angiostatin) of the endogenous angiogenesis inhibitor angiostatin to the peritoneum of a mouse was successful in treating a mouse *in vivo* model of endometriosis[111].

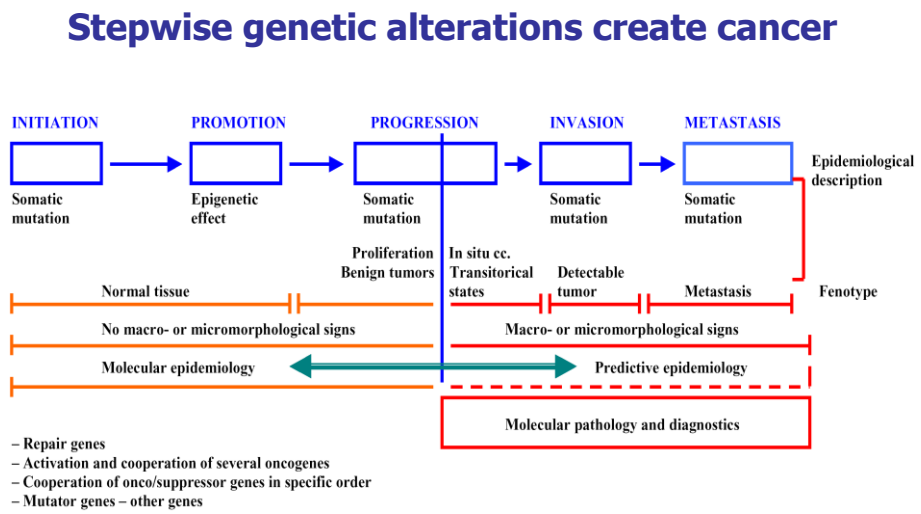
6.Tissue invasion and metastasis

The ability to invade through the basement membranes characterizes the transition from non-invasive to invasive cancer. Tumours secret proteases (e.g. matrix metalloproteinases MMPs) to degrade the basement membrane and surrounding stroma. Expression of MMP-2 and MMP-9 is correlated to grade and stage of many cancers. Likewise, MMP activity is upregulated in endometriotic lesions [112]. De-regulation of cell adherence signaling involving integrins, beta-catenin, E-Cadherin and P-Cadherin has been demonstrated in the genesis of a number of malignancies[113], and has also been implicated in endometriosis aetiopathogenesis [32;33;114]. Beta-catenin mutations have been identified in endometrial and ovarian endometrioid cancers[35;36]but have not been invesitgated in endometriosis. Cytokeratin-positive and E-Cadherin-negative endometriotic cells have an invasive phenotype in an *in vitro* collagen invasion assay similar to metastatic carcinoma cells [115].

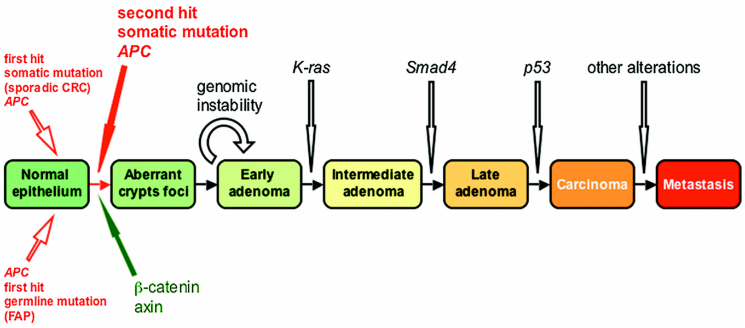
7.Genomic Instability

The classical model of malignant transformation of the cell involves the stepwise acquisition of multiple genetic alterations, which confers a clonal selective advantage at each step predisposing to the next step (Fearon and Vogelstein [116;117], Figure 1.2). This is often accompanied by activation of proto-oncogenes to oncogenes (transformation of normal cellular growth, proliferation and differentiation genes) and inactivation of tumour suppressor genes (TSG) (genes that encode for proteins which inhibit excess cellular proliferation and malignant transformation). The genetic alterations can occur at different levels and include single nucleotides, small stretches of DNA [microsatellites], whole genes, chromosomal components or whole chromosomes. The genetic alterations can be intragene or epigenetic (e.g. gene silencing by promoter hypermethylation).

Figure 1.2, Acquired stepwise genetic somatic mutations that predispose to development of cancer (Fearon and Vogelstein 1990[116;117])



Genetic model of colorectal carcinogenesis
[Fearon and Vogelstein (1990)]



Six principle genetic mechanisms have been identified to contribute to genomic instability in cancer, but only the first three have been examined for in endometriosis:

- **Gain in oncogenic activity.**
- **Inactivation of TSG** (loss of both gene copies of allele confers functional loss), or inactivation of haploinsufficient TSG (loss of only a single gene copy of allele confers functional loss)
- **Anomalies in DNA mismatch repair** enzymes, identified by microsatellite instability
- **Inactivation of genes that monitor genomic instability** at cell cycling (e.g. mitotic spindle assembly checkpoint genes)
- **Telomere dysfunction** (provokes chromosomal aberrations initiating carcinogenesis) and telomerase-mediated telomere maintenance (enables cells to achieve a fully malignant endpoint and metastasis).
- **Hypermethylation**. These mechanisms often act in synergy to promote genomic instability and tumour cell proliferation. For example, deficiency of the TSG p53 alters the cellular response to DNA damage, in that it leaves cells with attenuated DNA damage checkpoint controls and a reduced propensity for apoptotic cell death. Thus, although the DNA repair capacity of these cells is reduced, survival is increased. This promotes genomic instability and contributes to the resistance of p53-deficient cells to cytotoxic agents.

Importantly, pre-malignant lesions display similar genetic aberrations to established cancer. Loss of mismatch repair enzyme activity, and loss of PTEN (phosphatase and tensin homolog gene) and p53 TSGs frequently occurs in premalignant and malignant stages of breast, endometrial and ovarian carcinomas [118;119]. Furthermore, epithelial-stromal interactions are

important in tumour microenvironment and tumour development. In a similar manner, endometriosis demonstrates somatically acquired genetic alterations analogous to those found in cancer, resulting in the clonal expansion of genetically abnormal cells. The genetic evidence supporting the 'pre-neoplastic' state of endometriosis involves:

- **Monoclonality.** Most neoplasms are monoclonal in origin and evidence for monoclonality of endometriosis has been demonstrated in several studies [120-122], although these findings have been challenged recently [123].

- **Comparative genomic hybridization (CGH**) has shown over-representation (increased copy-number) of chromosomes 1, 2, 3, 5, 6p, 7, 16, 17q, 20, 21q and 22q in an endometriosis cell culture line FbEM-1, while chromosomes 5p, 6q, 9q, 11p, 12, 13q, 18 and X were under-represented. CGH repeated in endometriotic tissue revealed loss of DNA copy number on 1p, 22q and chromosome X, while gain on 6p and 17q. FISH analysis confirmed that the gain at 17q includes amplification of the proto-oncogene HER-2/neu[124;125]**.**

- **Fluorescent in situ hybridization (FISH)** analysis of late stage endometriotic lesions showed monosomy of chromosome 17, and loss of TP53 (17p13.1) locus. Because not all endometriotic cells displayed this genetic alteration it was suggested that this was a somatically acquired mutation, perhaps occurring in mainly advanced endometriosis states[126;127].

- **Loss of heterozygosity (LOH)** commonly indicates regions of TSG inactivation, and has been identified in endometriosis and endometriosis derived cell lines at 5q, 6q, 9p, 10q, 11q, 22q, p16 (Ink4), GALT, p53, APOA2 [128-133]. Importantly, cases with ovarian cancer adjacent to endometriosis or arising from endometriosis showed common genetic LOH

alterations in endometriosis and cancer indicating a possible malignant genetic transition spectrum between endometriosis and cancer [134;135].

- **Microsatellite Instability (MSI)** Hypermethylation of hMLH1 (gene product is a component of the DNA mismatch repair pathway), with concurrent absence of hMLH1 protein expression, is noted in 8.6% of endometriotic lesions [136].

- **Somatic mutations in TSGs**. Mutations of PTEN, a TSG, were identified in 20% of ovarian endometrioid carcinomas (EAOC and sporadic) and 20% of solitary endometrial cysts, suggesting that inactivation of the PTEN is an early event in the malignant transformation of endometriotic implants[137]. A separate study identified reduced PTEN protein expression in 15% of endometriosis cases [136].

- **Germline mutations in Tumour suppressor genes (TSGs).** As stated earlier, germline and somatically acquired [138] inactivating mutations of p53 gene.

- **Activation of oncogene.** Both human [139;140] and mouse model[141] studies of endometrosis have shown that activation of the K-ras oncogene promotes the development of ovarian cancer, even though the mutation appears not be present in the adjacent endometriosis.

- **Evidence from endometriosis associated ovarian cancer (EAOC) arising from endometriosis.** Endometrioid EAOC arising from endometriosis shows higher expression of p53 and c-erB-2 oncoproteins than similar ovarian endometrioid cancers without endometriosis [142]. The different pattern of expression in the two groups suggests different molecular pathways and could explain variations in cancer subtype and prognosis between the two groups [66;143].

C) Testing causality of endometriosis and ovarian cancer using Bradford Hill criteria

Causality for aetiological factors is normally assessed using the following study designs: randomized controlled clinical trials (RCTs), cohort, case-control, cross-sectional analyses and biological models (*in vivo, ex vivo, in vitro*). Studies would normally be subjected to critical analysis according to established causal inference methods, the most widely used being the criteria suggested by Austin Bradford Hill (1965) [7] and listed in **Table 1.3**. The strength of the causal relationship between endometriosis and ovarian cancer is assessed using such epidemiological causality criteria.

1. **Temporal sequence** The natural history of the development and progression of endometriosis and ovarian carcinoma is not known. No studies have examined women with initially normal pelvices, who then develop endometriosis, and prospectively followed them with a control cohort to establish the relative risk of developing ovarian cancer; or the need for endometriosis as a pre-requisite that precedes the onset of ovarian cancer. However, indirect evidence exists that supports this concept.

Cross sectional studies indicate that the peak age range for endometriosis diagnosis is 25-30 years [144] and for sporadic ovarian cancer, the age range is 50-55 years[80], thus fulfilling the criteria for temporal sequence. However, studies mainly reported estimates of incidence of symptomatic endometriosis and ovarian cancer diagnosis rather than their actual incidence of onset. There is little evidence to support that endometriosis onset necessarily coincides with symptom onset. Furthermore, delays in diagnosis may also exist. The time elapsed from onset of symptoms to diagnosis of endometriosis varies from 3-13 years for women mainly complaining of pelvic pain, and 2-6 years for infertility [145;146] .The time from symptom onset

to diagnosis in ovarian cancer is four to six weeks[147], although the cancer is often at a significantly advanced stage at diagnosis. Thus, even taking into account the symptom free intervals before the diagnosis of endometriosis and ovarian cancer, the criteria for temporal sequence remains valid. One study retrospectively assessed ovarian cancer cases (n=573) to investigate whether ovarian pathology had been identified 12 months previously[148]. This study showed that within this limited period approximately half of ovarian carcinomas developed secondarily from preexisting benign-appearing cysts or endometriotic cysts, and the remainder appeared to develop from an ovary of normal appearance. A case report has described the continuous transition from benign endometrioid epithelium through epithelial atypia to invasive ovarian carcinoma within a three year period [149], again suggesting causality.

2. **<u>Strength of Association</u>** Strong associations imply causality, whereas weak associations are more likely to have arisen or been influenced by unsuspected bias. It has been suggested that relative risks more than 3 in cohort studies, or odds ratios greater than 4 in case-control studies, provide strong support for causation[150]. Strong evidence to support this component of causality testing was identified by demonstrating:-

- **Increased prevalence of ovarian cancer in women with endometriosis**

Several studies have found an increased ovarian cancer incidence in women with endometriosis: the odds ratios range from 0.8 to 4.2 (studies are listed in **Table 1.4)**.

- **Increased prevalence of endometriosis in women with ovarian cancer**

The age standardised incidence of ovarian cancer in women in the UK is 21.9 per 100,000 (0.02%), with around 75% of cases being diagnosed in postmenopausal women [73]. If there

were no association between cancer and endometriosis then the prevalence of endometriosis in women with ovarian cancer would be similar to that in the age-matched general population, and would be similar across all ovarian cancer subtypes. However, the prevalence of endometriosis is increased in women with ovarian cancer (7.7%-29%)[46;47;67;74;151-158], in comparison to a background prevalence of endometriosis of 5%-15% in women of reproductive age and 3%-5% in postmenopausal women[144] . Furthermore, endometrioid and clear cell ovarian cancer subtypes are more likely in the presence of endometriosis than those ovarian cancers occurring in the absence of endometriosis: odds ratios range from 1.87 to 5.36 for endometrioid, and range from 1.05 to 7.30 for clear cell subtypes, and these are shown in **Table 1.5**. This table also shows increased odds ratios for 'mixed epithelial' and 'other types' of ovarian cancer, but these tumours are generally uncommon and contain mixed varieties of endometrioid, clear cell and adenosquamous cells; the significance of this association is unclear. Nonetheless, summarizing comparative and non-comparative studies [46;152;153;155;156;158-161], the prevalence of endometriosis for each ovarian cancer subtype is: 0-8% of serous, 0-6% of mucinous, 8%-74% of clear cell, and 9%-43% of endometrioid subtypes.

3. **Consistency of association** Since Sampson's first report in 1925 [5], numerous reports have described cases of ovarian cancer arising from pre-existing endometriosis or associated with ovarian cancer. This observation is consistently repeated in different populations. Furthermore, all the studies depicted in **Tables 1.4 and 1.5**, apart from one, found consistent and similar increases in risk of ovarian cancer and distribution of histological subtypes, and thus further emphasizing the validity of this association.

Table 1.4. Risk of ovarian cancer and other types of cancer in women with endometriosis

Type of study	Risk of ovarian cancer in women with endometriosis	Other cancers in women with endometriosis	Reference and source of data
Case control study Examining 28,163 women with endometriosis	OR 1.34 (95% CL 1.03-1.75)	Not reported	[162] Swedish Hospital Discharge Register
Pooled analysis of eight case-control studies	OR 1.73 (95% CL 1.10-2.71)	Not reported	[163] Studies from US, Canada, Australia, Denmark
Cohort study of women with Self-reported endometriosis Up to 13 year follow up	RR 0.8 (95% CL 0.2-2.4)	Non Hodgkin's lymphoma RR 1.8 (95% CL 1.0-3.0)	[164] Iowa Women's Health Study
Case control study examining 20,686 women with endometriosis	OR 1.9 (95% CL 1.3-2.8) OR 4.2 (95% CL 2.0-7.7) for long-standing endometriosis	Non-Hodgkin's lymphoma OR 1.8 (95% CL 1.2-2.6) Breast OR 1.3 (95% CL 1.1-1.4)	[165] Swedish Inpatient Register and National Swedish Cancer Registry

Table 1.5. Prevalence of ovarian cancer in women with and without endometriosis

Subtype of epithelial ovarian cancer	Prevalence of ovarian cancers in association with endometriosis	Prevalence of ovarian cancers in absence of endometriosis	Odds Ratio With (95% confidence interval)	Reference	Pooled epidemiological studies of ovarian cancer[80] (prevalence of endometriosis not stated)
Serous	32% (8/25) 15% (8/52) 11% (4/37) 13% (6/48) 7% (4/58) 21% (3/14)	57% (84/147) 51% (212/414) 62% (56/90) 44% (57/131) 52% (121/232) 38% (56/146)	0.35 (0.28-0.42) 0.17 (0.14-0.21) 0.07 (0.03-0.12) 0.19 (0.13-0.24) 0.07 (0.04-0.10) 0.44 (0.36-0.52)	[166] [156] [46] [74] [66]* [47]	55%
Mucinous	4% (1/25) 11% (6/52) 0% 4% (2/48) 2% (1/58) 14% (2/14)	23% (34/147) 21% (88/414) 19% (17/90) 25% (33/131) 11% (25/232) 14% (21/146)	0.14 (0.09-0.19) 0.48 (0.44-0.53) - 0.13 (0.08-0.18) 0.15 (0.10-0.19) 0.99 (0.98-1.01)	[166] [156] [46] [74] [66]* [47]	13%
Mixed epithelial	0% 22% (13/58) 28% (4/14)	0% 5% (11/232) 23% (33/146)	- 5.80 (5.2-6.41) 1.37 (1.26-1.48)	[166] [66]* [47]	3%
Endometrioid	12% (3/25) 58% (30/52) 41% (9/22) 8% (3/37) 27% (13/48) 57% (33/58) 28% (4/14)	7% (10/147) 20% (84/414) 24% (14/57) 4% (4/90) 13% (18/131) 27% (62/232) 10% (14/146)	1.87 (1.68-2.06) 5.36 (4.92-5.80) 2.13 (1.79-2.47) 1.90 (1.67-2.12) 2.33 (2.07-2.59) 3.62 (3.26-3.97) 3.77 (3.27-4.27)	[166] [156] [154] [46] [74] [66]* [47]	14%
Clear Cell	52% (13/25) 15% (8/52)	13% (19/147) 7% (30/414)	7.30 (6.29-8.31) 2.33 (2.17-2.49)	[166] [156]	6%

	32% (7/22) 81% (30/37) 56% (27/48) 10% (6/58) 7% (1/14)	18% (10/57) 14% (13/90) 18% (23/131) 5% (11/232) 7% (10/146)	2.19 (1.84-2.55) 25.38 (21-29) 6.04 (5.23-6.85) 2.32 (2.12-2.52) 1.05 (1.01-1.08)	[154] [46] [74] [66]* [47]	
Other types	0% 2% (1/58) 0%	0% 1% (2/232) 8% (12/146)	- - 2.02 (1.85-2.18)	[166] [66]* [47]	9%

Footnotes *** Age matched nested case control study**

4. **Biological gradient (dose-response relationship)** No studies were identified that correlated volume and extent of endometriosis with acquisition of ovarian cancer. Interestingly, there is a common unexplained left-sided predominance for endometriotic cysts, and ovarian endometrioid and clear-cell cancers [167], which may suggest a 'spatial' biological gradient.

5. **Specificity** This criterion relates to a specific cause producing a specific effect. Importantly, the occurrence of endometriosis need not cause ovarian cancer, pelvic pain or infertility. Similarly ovarian cancer, as well as pelvic pain and infertility, may occur without endometriosis.

6. **Biological plausibility** There is extensive histopathological, molecular and genetic evidence showing that endometriosis may be considered a neoplastic process with potential for malignant transformation (discussed earlier [168]).

7. **Coherence with existing knowledge** There is strong evidence to support this causality component, as several of the risk factors known to increase or decrease susceptibility to endometriosis are also common to those of epithelial ovarian cancer. These

factors may indicate a common aetiological mechanism for endometriosis and ovarian cancer. Alternatively, these factors could act as confounders in the association between endometriosis and ovarian cancer. However, data of their presence in endometriosis associated ovarian cancers compared to matched (by age, histological subtype, grade and FIGO stage) ovarian cancers is unobtainable in most studies, which precludes any analysis of their confounding influence. The risk factors currently identified are:

- **Infertility and nulliparity**- both of multifactorial aetiology and positively associated with endometriosis [144] and ovarian cancer[169].

- **Unopposed estrogen replacement therapy (ERT)-** this is associated with malignant transformation of endometriosis [170;171] and increased the risk of **endometrioid or clear cell epithelial ovarian tumours (OR 2.56; 95% CL 1.32-4.94)[172;173].** Importantly, a confounding effect is unlikely with ERT as most studies reporting prevalence of endometriosis associated ovarian cancer were based on women not taking ERT.

- **Multiple lifetime ovulations**- this increases the risk of epithelial ovarian cancer[174]. The combined oral contraceptive pill, which is known to reduce ovulations, has been shown to reduce the risk of ovarian cancer[175;176] and endometriosis.

- **Tubal ligation**- this reduces the risk of ovarian cancer[177], particularly endometrioid and clear cell types[178;179]. No prospective trials exist showing tubal ligation to reduce endometriosis occurrence, progression or recurrence, However, assuming retrograde menstruation to be a main mechanism for endometriosis, it is plausible that tubal ligation suppresses retrograde menstruation and endometriosis which consequently suppresses endometriosis associated ovarian cancer development.

8. **Experimental evidence** Animal models (mice, rat or baboon) of endometriosis may be created by surgically implanting host (or human) endometrial cells in to the peritoneal cavity and promoting proliferation of the cells by administration of supraphysiological estrogens[141;180-186]. Of these animal models, a sentinel paper by Dinulescu (2005) [141] induced ovarian lesions with an endometrioid glandular pre-neoplastic morphology by activating an oncogenic K-ras allele and deletion of the PTEN tumour suppressor gene; hence, fulfilling Fearon and Vogelstein's classic stepwise model of cancer development (**Figure 1.2**)[116;117] . Human studies demonstrating induction of endometriosis or its malignant transformation are highly unlikely as such research would be deemed unethical.

9. **Analogy** Malignant transformation of endometriosis is not restricted to the ovary. Several studies have reported analogous malignant transformation at extra-ovarian locations, such as the rectovaginal septum, vulva, and colon[187]. Principle malignancies include endometrial stromal sarcoma, endometrioid adenocarcinoma, clear cell carcinoma, with histological confirmation of tumour and adjacent endometriosis in all cases. Furthermore, malignant transformation of adenomyosis, considered the 'uterine' variant of endometriosis, has been observed and results in similar histological subtypes to that found for endometriosis related malignancies[188].

D. Summarising the published evidence whether endometriosis is a neoplastic precursor to ovarian cancer

Based on the methodological approaches discussed earlier (A: clinicopathological, B: Genetic and molecular hallmarks of cancer, C: Bradford-Hill causality criteria) **there is inadequate evidence** to support the hypothesis that 'ENDOMETRIOSIS IS A NEOPLASTIC PRECURSOR TO OVARIAN CANCER' **(Table 1.6).**

Table 1.6. Summarising the published evidence that supports or refutes the hypothesis that endometriosis is a neoplastic precursor to the development of ovarian cancer

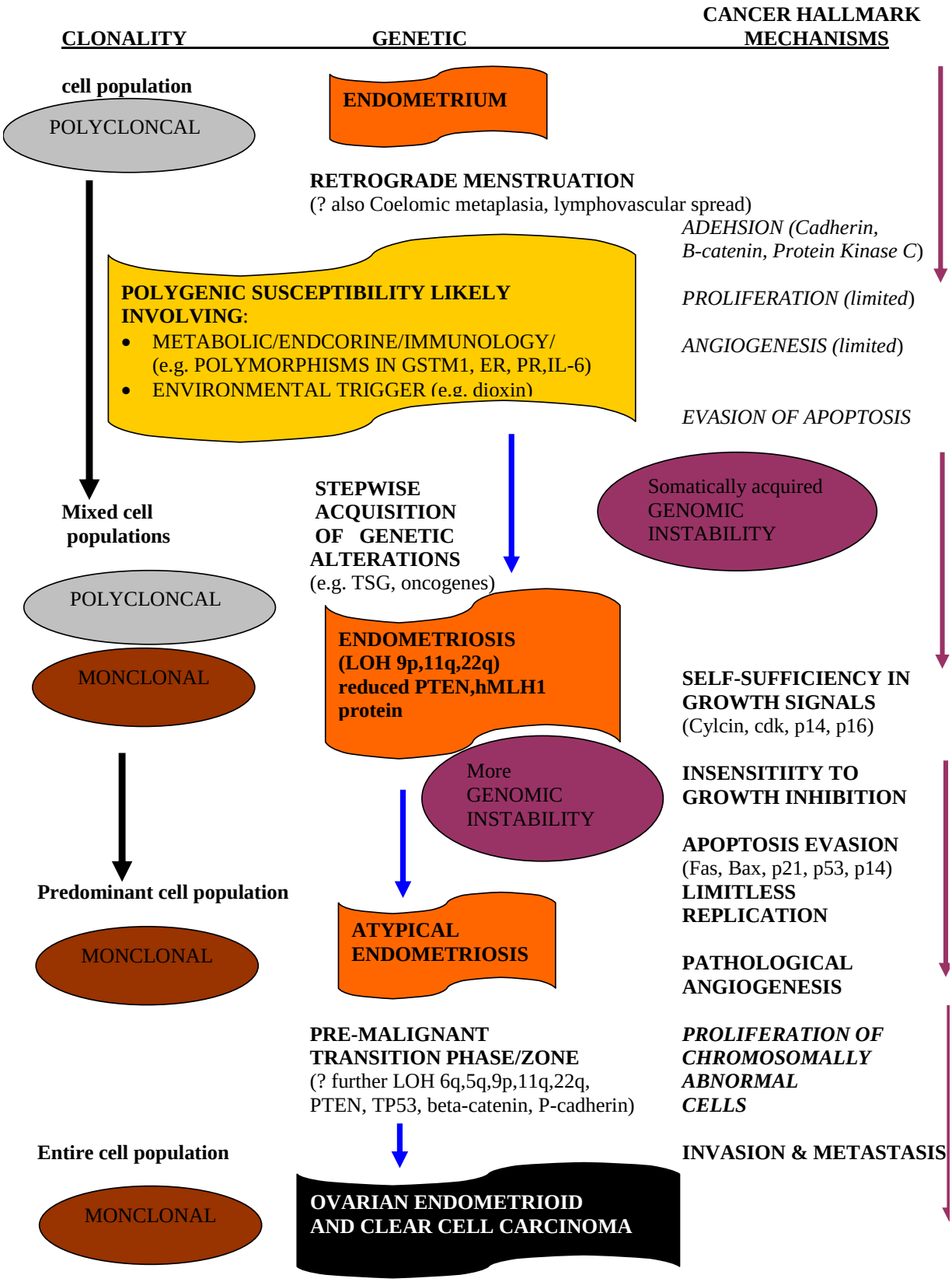
	Supporting evidence for endometriosis	**Refuting evidence for endometriosis**	**Overall strength that hypothesis is true**
A. Clinico-pathological epidemiological data	Cancer arises directly from endometriosis Increased risk of certain ovarian cancer subtypes	Inconsistency of histological observations Weak association	Weak
B. Genetic and molecular 'Hallmarks of Cancer[6]' criteria	Self-sufficiency Insensitivity to anti-proliferative signals Resistance to apoptosis Angiogenesis Genomic instability	Limitless replication potential Tissue invasion and metastasis	Moderate
C. Association vs. causality using Bradford-Hill (1965) epidemiological criteria[7]	Experimental evidence (animal model) Biological plausibility Experimental analogy	Strength of association Specificity of association Inconsistency of association Temporal sequence Biological gradient (dose response relationship) Experimental evidence (humans)	Weak

Nevertheless, the identification of an association between endometriosis and ovarian cancer may suggest alternative hypotheses:

- Only specific endometriotic implants may directly undergo malignant transformation, perhaps through environmental exposure via an atypical endometriosis transition phase, analogous to the genetic cancer model of colon cancer where colonic epithelium acquires stepwise somatic genetic mutations to transform to colonic polyp, adenoma and finally to colonic carcinoma **(Figure 1.2)**[116;117]. Therefore, like most types of sporadic cancer [189], endometriosis may be exposed to complex interactions between inherited germline polygenic low-penetrance alleles (polymorphisms) [190;191], somatically acquired genetic alterations [192] and environmental factors [94] . A visual summary of the main pathways of this hypothesis is shown in **Figure 1.3**.

- Both endometriosis and cancer share common antecedent mechanisms and/or predisposing factors (e.g. genetic susceptibility, immune/angiogenic dysregulation, environmental toxin exposure), with obvious divergence in molecular pathways downstream.

Figure 1.3 Proposed genetic and molecular aeitopathogenesis of endometriosis



Discussion of methodology used in testing hypothesis

A strength of this work has been the utilization of a systematic literature search and combining this with established research methodological approaches. However, it is accepted there may still be grounds to challenge our conclusion.

To some extent, my conclusions may be less certain, as most included studies were of small sample size, retrospective design, and suffered from selection bias (incomplete case ascertainment and unmatched populations), information bias (varying histological criteria for cancer arising from endometriosis and atypical endometriosis) and confounding to varying degrees. Such problems of interpreting epidemiological studies involving endometriosis have also been observed by others[193;194].

Significantly, my research aim of using Bradford-Hill criteria to test causality was adopted by another group (Vigano 2007[195]) investigating the causal link of endometriosis and cancer. Their work[195], which partly included and referenced my work[168],concluded that there was only a weak causal link. However, Vigano's group did not perform a systematic literature search (and omitted key references that we have included above) and utilised modified causality criteria. I therefore believe my conclusion is more likely to be accurate, and in fact, has been further validated through the experimental work discussed below and orally presented at international conferences[196;197].

1.2. Experimental investigation of endometriosis and EAOC

Introduction

Experimental studies on primary endometriotic tissue and endometriotic-cell lines has shown endometriosis and cancer to share similar molecular (limitless replicative potential, self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, sustained angiogenesis) and genetic(monoclonality, genetic instability) characteristics[168;198;199]. Allelic loss in endometriosis and sporadic ovarian cancer has been demonstrated in similar chromosomal regions 1p, 1q21, 5p, 5q, 6q, 7p, 9p , 9q, 11q, 17p13.1, 17q and 22q [200;201]. Furthermore, a recent *in vivo* mouse model study demonstrated induction of endometriosis-like and ovarian cancer tissue through introduction of oncogenic K-ras and conditional deletion of PTEN [202]. Endometriosis-associated ovarian cancer (EAOC) (25-35% of all ovarian cancers) appears to be a separate entity from sporadic ovarian cancer without endometriosis (SOC). Epidemiological studies have shown EAOC tends to present in younger aged women, has better survival, and more likely to be a low-grade endometrioid or clear cell cancer subtype [82;203-205]. However, there is only limited data on the genetic alterations in EAOC, which to date is mainly confined to the roles of PTEN and K-ras [139;140] and a limited genome-wide (n=14 cases)[206] LOH screen and CGH analysis (n=4 cases)[207;208].

Hypothesis

Although the supporting evidence is weak (**Table 1.6**), we could assume that, in some cases, it is possible that endometriosis behaves as a neoplastic precursor to the development of ovarian cancer (**Figure 1.3**). If this is valid, then we could better understand the genetic aetiopathology of both endometriosis by deliberately selecting endometriosis-associated ovarian cancer (EAOC) as a model and testing the ovarian cancer and adjacent endometriosis

for common or dissimilar genetic abnormalities. Therefore, in this chapter, I will explore whether normal ovarian surface epithelium and adjacent endometriosis and adjacent ovarian cancer display a stepwise accumulation of LOH events analogous to the stepwise accumulation of LOH (due to inactivation of tumour suppressor genes TSG) observed in Fearon and Vogelstein's model for colon cancer (**Figure 1.2**). The following patterns of results may be generated by adopting this approach:

- LOH is only demonstrated in the ovarian cancer, and not in endometriosis. *This would suggest acquired somatic genetic events occur, perhaps due to the presence of endometriosis, that cause malignant transformation to ovarian cancer.*

- LOH is demonstrated in endometriosis when compared against matched normal ovarian surface epithelium. *This would suggest that inactivation of particular TSGs were responsible for either the initiation or progression of endometriosis.*

- Similar chromosomal regions of LOH occur in endometriosis and ovarian cancer. *This would suggest that a particular set of TSGs are in involved in both the initiation, progression and malignant transformation of endometriosis and ovarian cancer, and that endometriosis and ovarian cancer share common antecedent genetic events.*

- Additional LOH events are identified in ovarian cancer compared to LOH events identified in adjacent endometriosis. *This would confirm a stepwise accumulation of specific inactivating TSG(s) (equating to the additional LOH events) are responsible for the malignant transformation of endometriosis.*

Fine mapping the LOH regions would therefore allow us to select candidate TSGs that were either responsible for the initiation and progression of endometriosis (pattern B), malignant

transformation of endometriosis to ovarian cancer (pattern D), or common to development of both endometriosis and ovarian cancer (pattern C).

Plan of investigation

- Investigate epidemiological and prognostic factors associated with endometriosis associated ovarian cancer (EAOC) and sporadic ovarian cancer (SOC), matched for endometrioid and clear cell ovarian cancer subtypes. **(Tables 1.7, 1.8, 1.9; Figures 1.7, 1.8, 1.9)**

- Loss of heterozygosity (LOH) mapping of EAOC and SOC-fine mapping of chromosomes 9 and 11 using multiple microsatellite genetic markers to identify candidate tumour suppressor genetic loci**. (Tables 1.10, 1.11;** Figure 1.4-LOH mapping output**; Figures 1.10 and 1.11).**

- Analyse survival prognostic significance of LOH at chromosomes 9 and 11. Combine information narrowed fine-mapped genetic region of LOH, frequency of LOH and prognostic significance of loci, to select candidate tumour suppressor genes for further investigation (**Figure 1.12**).

- Laser Capture Microdissection of endometriosis adjacent to ovarian cancer and perform LOH using candidate genetic microsatellite markers. Compare findings to similar study by collaborators (we have donated our samples to their unit). (Figure 1.5-importance of LCM; **Table 1.12**).

- Immunohistochemical investigation of candidate disease-modifying genes in endometriosis adjacent to and distant from EAOC. Selected gene products are **Glycodelin (9q34.3)** and **Progesterone receptor (11q22).** (**Table 1.13; Figures 1.13, 1.14, 1.15**). Correlation of immunohistochemical expression to disease development .

- Preliminary nuclear morphometry analysis of transition zone between endometriosis, atypical endometriosis and ovarian cancer that exist in direct continuum. **(Figure 1.16).**
- Single nucleotide polymorphism genome wide analysis of endometriosis using Affymetrix 100K SNP microarray **(**Figure 1.6-SNP microarray advantages**; Figures 1.17, 1.18, 1.19, 1.20).**

Experimental Methods

Ethics: South Birmingham Local Research Ethics Committee has given full approval to all work included in this thesis chapter (LREC reference No: 2002/057, August 2002).

Clinical material Cases of EAOC and SOC of endometrioid and clear cell subtype were identified by interrogation of a computerized histopathological database at Birmingham Women's Hospital. All cases were gynaecological cancers operated on from 1995-2001 at Birmingham Women's Hospital. Five micron thick paraffin embedded slides were used for DNA extraction and three micron thick slides were cut from selected cases for immunohistochemical analysis. Realising that molecular genetic alterations of ovarian cancer vary according to histological subtype [209], we ensured our comparative analysis of allelic loss between EAOC and SOC were matched for endometrioid and clear cell subtypes of ovarian cancer. Chromosomal regions showing greatest frequency of LOH in EAOC and SOC and that appeared to reside within a consistent minimal region of LOH loss were prioritized for further study.

DNA Extraction

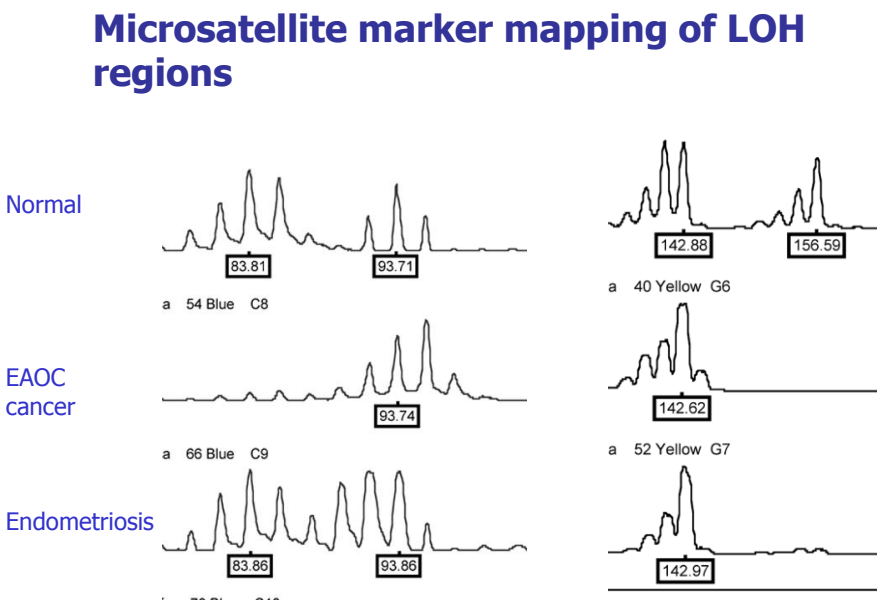
Cancer and matched normal DNA were extracted from five micron glass slides using one of two methods depending on slide content and composition. Needle microdissection was used to collect histologically labeled endometriosis and cancer. DNA was extracted from micro-dissected material held in an eppendorf using a microwave-based method as previously described [210] . Briefly, retrieved tissue material was placed in an eppendorf containing 400µl TrisT-EDTA buffer and heated in a 600W microwave for one minute in 15 second bursts. Following centrifugation the upper paraffin layer was discarded and the supernatant incubated for 48 hours with 4µl of proteinase K 20mg/ml (Sigma-Genosys) with continuous gentle agitation. Proteinase K was inactivated by heating to 95ºC for ten minutes and the supernatant aliquoted for DNA studies.

LOH Analysis

Highly polymorphic microsatellite markers spanning the full length of chromosomes 9 and 11 at approximately 20cM intervals (Wellcome Trust) were kindly provided by Oxford Group, Dr. Stephen Kennedy. Detailed genetic fine mapping was performed using customised microsatellite markers (Sigma) spaced approximately 10cM apart and in between the previous Wellcome markers. The forward primers were 5' end-labeled with FAM. A 25-µl PCR reaction volume containing 1xAB Gene Buffer (ABGene), Magnesium Chloride (ABGene), 100 µM each of dATP, dCTP, dGTP, and dTTP; 0.5 unit of DNA Taq polymerase (AB Gene); sterile DNAse and RNAse free water (Sigma), and 2 pmol of reverse primer, 2ul (approximately 100 ng) of genomic DNA. PCR cycling conditions were as follows: (*a*) 5 min at 94°C; (*b*) 30 cycles of 30 s at 94°C, 30 s at the appropriate annealing temperature (usually 55°C), and 30 s at 72°C; and a final step of 72°C for 10 min. The

reaction products were then diluted 1:15 with sterile water. One microlitre of the diluted PCR product was added to 10ul of 95% formamide, and 0.02ul of LIZ dye and subsequently denatured for 5 minutes at 95°C and snap frozen with ice. PCR products were run on ABI 377 gel electrophoresis analyser and fragment sizes were recorded using GeneScan software analysis. LOH was scored based on the absence of alleles in tumour-derived DNA compared to normal DNA or a loss of at least 70% in the relative size of alleles in the tumour-derived DNA compared to normal tissue; examples of GeneScan images are shown in **Figure 1.4**

Figure 1.4. Genetic allelic products images observed following microsatellite amplification of target DNA and analysis on ABI Prism analyser



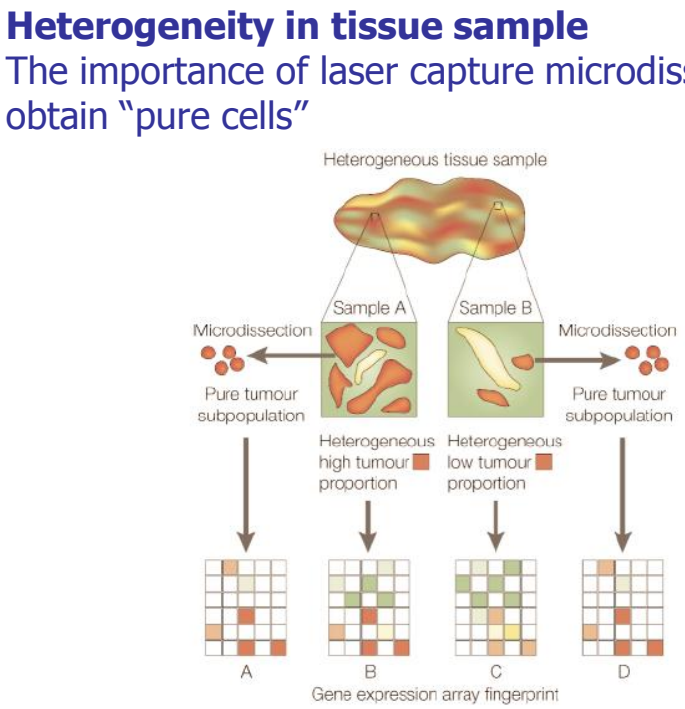
Immunohistochemistry and nuclear morphometry

Monoclonal antibodies were obtained for p53, CD10 (Santa Cruz), Progesterone receptor (isoforms A and B) (AbCAM) and Glycodelin (AbCAM). Immunohistochemistry was performed according to standardised protocols using the Dako Chem Mate antigen detection kit. Briefly, 3 micron slides were deparaffinised through stepwise Xylene, ethanol, water and methanol washes; endogenous peroxidase was subsequently blocked by 20 minutes incubation with 0.5% hydrogen peroxide/methanol mixture. Antigen exposure was achieved by pressure cooker boiling for 5 to 7 minutes with pH 6 citric acid buffer. Primary antibodies were diluted to concentrations of 1 in 200 to 1 in 1000 in TBS Tris buffered saline (pH 7.6) and 200 microlitres were applied to each slide. The Dako Chem Mate protocol (yellow and red antibody washes) followed by DAB chromagen/substrate then copper sulphate solution staining was performed. Brief dips in Haemotoxylin, acid-alcohol dip and Scott's Media followed by tap water wash allowed final ascending alcohol/xylene and coverslip slide creation.

Laser Capture microdissection

A PALM microlaser was used. EAOC paraffin 3micron thick cut slides were de-waxed and suspended in aqueous buffer. Endometriotic epithelium was separated 'purely' using laser blot and line cutting according to the manufacturer's guidance (**Figure 1.5).** Particles were catapaulted on to the inside lid surface of a single PALM 1cm^3 opaque lid eppendorf. QUIAGEN mini-DNA prep kit buffer was placed in the conical base of the eppendorf and the lid closed after particle deposition and eppendorf was then inverted. DNA was extracted and cleaned according to the QUIAGEN mini-columns and centrifuge protocol.

Figure 1.5. Importance of Laser capture microdissection of target disease (such as endometriosis epithelium glandular lining) from surrounding tissue (such as endometriosis stroma)



Affymetrix SNP 100K Microarray

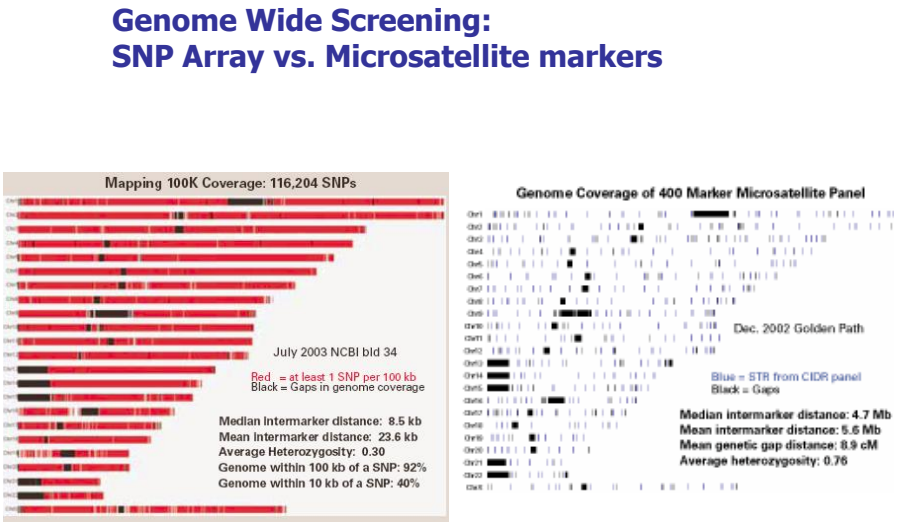
Ovarian endometriosis and matched normal ovarian surface epithelium were needle micro-dissected immediately at the time of surgical extraction from the patients with their documented informed consent, and then promptly snap frozen in liquid nitrogen and held at -77°C. DNA was extracted by crushing the tissue in PureGene extraction buffer and following the PureGene centrifugation and incubation protocol. The quality and concentration of extracted DNA was determined by spectrophotometry at A260/280. The Affymetrix GeneChip® Mapping Assay, in conjunction with the GeneChip Human Mapping 100K Set, is designed to detect > 100,000 Single Nucleotide Polymorphisms (SNPs) in samples of genomic DNA. The Mapping 100K Set is comprised of two arrays (*Mapping 50K Array Xba*

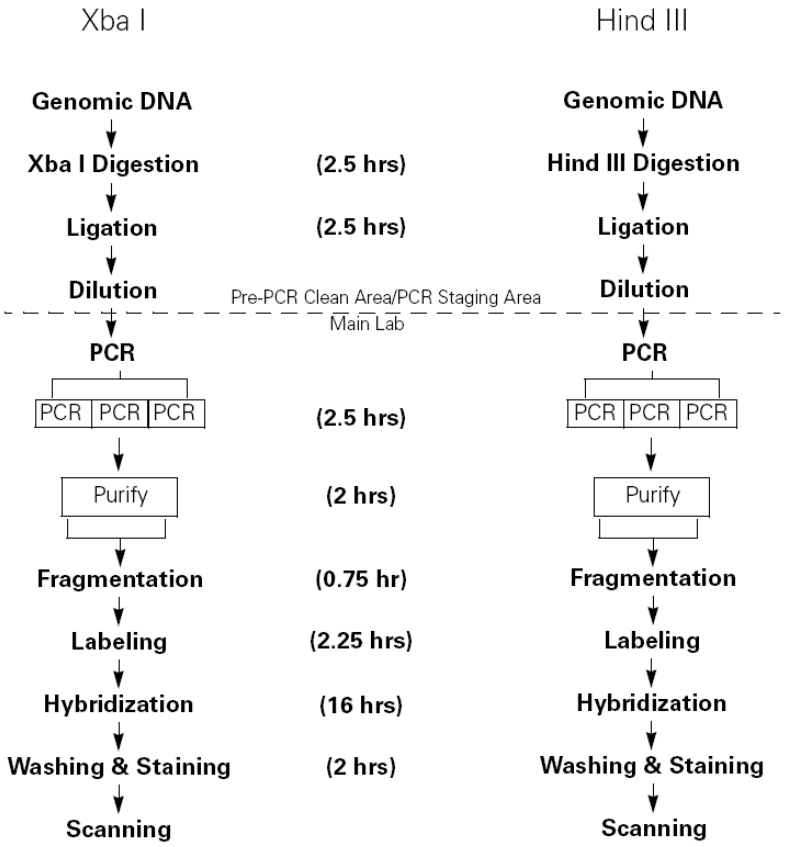
240 and Mapping 50K Array Hind 240) and two assay kits (containing either Hind III, XbA 1 restriction enzymes). Each array and its corresponding assay kit are processed independently from the second enzyme. The protocol starts with 250 ng of genomic DNA per array and will generate SNP genotype calls for more than 50,000 SNPs for each array of a two array set. The assay first digests the genomic DNA with the Xba I or Hind III restriction; an overview of the remainder of the assay protocol is shown in **Figure 1.6**. The final PCR products (amplicons) are fragmented, end-labelled, and hybridized to either the Xba 1 or Hind III GeneChip array. Scanned images obtained from the GeneChip Mapping 50K Array Xba 240 and the GeneChip Mapping 50K Array Hind 240 are digitally combined and displayed by GeneChip Operating Software (GCOS).

Statistical analysis

Statistical data were analysed with the use of SPSS version 13 (SPSS Inc, USA). Continuous variables were analysed by T-test, Mann-Whitney U and ANOVA tests . Categorical variables were analysed by Chi-square. Survival regression was analysed using either the Kaplan-Meier or Cox proportional Hazards model, depending on the parameters employed. A p-value less than 0.05 was considered statistically significant, although a Bonferonni correction was considered (p value<0.001) when multiple testing (>10) may have led to increased risk of type 1 error.

Figure 1.6. Increased genetic resolution of Affymetrix Single Nucleotide Polymoprhism DNA microarray compared to 'traditional' multiple microsatellite marker genome wide mapping





1.3 Investigation of epidemiological factors associated with EAOC and SOC

At total of 62 cases were identified from the histopathological database; their epidemiological characteristics are shown in **Table 1.7.** Of these, paraffin tissue blocks were retrieved for 50 cases, and these were subjected to genetic investigation; the epidemiological characteristics are shown in **Table 1.8**.

Ovarian cancer survival was statistically significantly associated with clear cell or endometrioid subtype, cancer stage and the presence of synchronous endometrial and ovarian cancer [regression model Chi-sq 14.1, p=0.003). Clear cell compared to endometrioid subtype of cancer increases the odds of dying earlier by 2.2 (i.e. the probability of dying earlier is 69%). An advanced cancer stage increases the odds of dying earlier by 1.6 (i.e. the probability of dying earlier is 62%). Synchronous cancers compared to solitary ovarian cancers decreases the odds of dying earlier by 0.13 (i.e. the probability of dying earlier is 12%). The presence or absence of endometriosis did not influence survival, as did other factors as listed in **Table 1.9.** These observations are graphically depicted by the survival curves (**Figures 1.7, 1.8, 1.9**).

Odds=Prob/1-Prob

Prob= Odds/1+Odds

Table 1.7. Characteristics of endometriosis associated ovarian cancer (EAOC) and sporadic ovarian cancer (SOC), matched for endometrioid and clear cell histologies, used in epidemiological analysis (N=62)

	EAOC N=34	**SOC** N=28	**P value**
Clear cell	18	9	
Endometrioid	16	19	
Age mean [range]	57.7 [32-79]	60.4 [32-84]	n.s.
Cancer stage: 1	21	13	0.150
2	7	5	
3	5	9	
4	1	1	
Mean	1.59	1.93	
Sidedness: Left	14	8	**0.002**
Right	18	8	
Bilateral	2	12	
Endometriosis proximity to tumour:		Not relevant	
Distant	11 (32%)		
Adjacent	12 (35%)		
Tumour arising from endometriosis	11 (32%)		
Synchronous uterine & ovary cancer	5	0	**0.034**
Uterine hyperplasia	11	8	n.s
Leiomyoma	25	17	n.s
Adenomyosis	14	11	n.s
Tumour in lymph nodes	2	3	n.s
Tumour in omentum	4	6	n.s.
Ascites	12	10	n.s.

Table 1.8. Characteristics of endometriosis associated ovarian cancer (EAOC) and sporadic ovarian cancer (SOC), matched for endometrioid and clear cell histologies, used in genetic analysis(N=50)

	EAOC Clear cell	**EAOC Endometrioid**	**SOC Clear cell**	**SOC Endometrioid**	**Statistical Testing P value**
Number of cases	15	12	7	16	
Mean Age	57.2	59.9	61.6	59.2	0.865**
Age:lower to upper quartile	51-65	56-66	45-72	51-67	
Staging of ovarian cancer Stage 1 Stage 2 Stage 3 Stage 4	 9 3 3 0	 8 3 0 1	 3 0 3 1	 9 3 3 1	0.486
Ascites	5	4	4	5	0.660
Synchronous endometrial and ovarian cancer	0	5 (cases 17,19,36,37,38)	0	1	0.222
Presence of endometriosis directly adjacent to ovarian cancer	7/15 (cases 2, 3, 4,5,10,11,13)	5/12 (cases 17,18,19,24, 26)	N/A	N/A	
Surviving >48 months >36 months >24 months >12 months <12 months	 3 3 6 10 5	 4 7 9 10 2	 0 0 3 5 2	 5 8 12 13 3	 0.337 **0.023** 0.112 0.707 0.707

Table 1.9. Multivariate survival regression analysis (N=62 combined cases of EAOC and SOC)

Variables included in multivariable Cox regression analysis	P-value of variable	Odds of dying earlier Expressed as Hazard Ratio
Clear cell vs. Endometrioid subtype	0.019	2.16 (95% CI 1.14 - 4.10)
Advancing cancer stage	0.016	1.56 (95% CI 1.09 - 2.24)
Synchronous cancer vs. ovarian cancer	0.012	0.13 (95% CI 0.03 - 0.64)
Endometriosis presence	0.80	not significant
Age	0.72	not significant
CA125	0.43	not significant
Tumour in Lymph nodes	0.65	not significant
Tumour in omentum	0.92	not significant
Ascites	0.70	not significant
Sidedness of tumour	0.56	not significant
Proximity of endometriosis to tumour	**	

Footnotes

Probability corresponds to HR/1+HR

Interpreting Hazard Ratio results: when all variables are combined in a survival regression analysis, only *histological subtype, cancer stage and presence of synchronous uterine and ovarian cancer* statistically significantly impact on cancer survival [Chi-sq 14.1, p=0.003) :

Clear cell compared to endometrioid subtype of cancer increases the odds of dying earlier by 2.2:1 (i.e. probability of dying earlier is 69%)

An advanced cancer stage increases the odds of dying earlier by 1.6:1 (i.e. probability of dying earlier is 62%).

Synchronous cancers compared to solitary ovarian cancers increases the odds of dying earlier by 0.13:1 (i.e. probability of dying earlier is 12%)

Figure 1.7 Survival differences between subtypes of ovarian cancer

There is no statistically significant association between the four individual cancer subtypes and survival. However, there is a statistically significant association for clear cell vs. endometroid types of ovarian cancer (Hazard Ratio 2.16 (95% CI 1.14-4.10)), as depicted in the figure i.e. Clear cell compared to endometrioid subtype of cancer increases the odds of dying earlier by 2.2:1 (i.e. probability of dying earlier is 69%).(Hazard analysis results are depicted in Table 1.9).

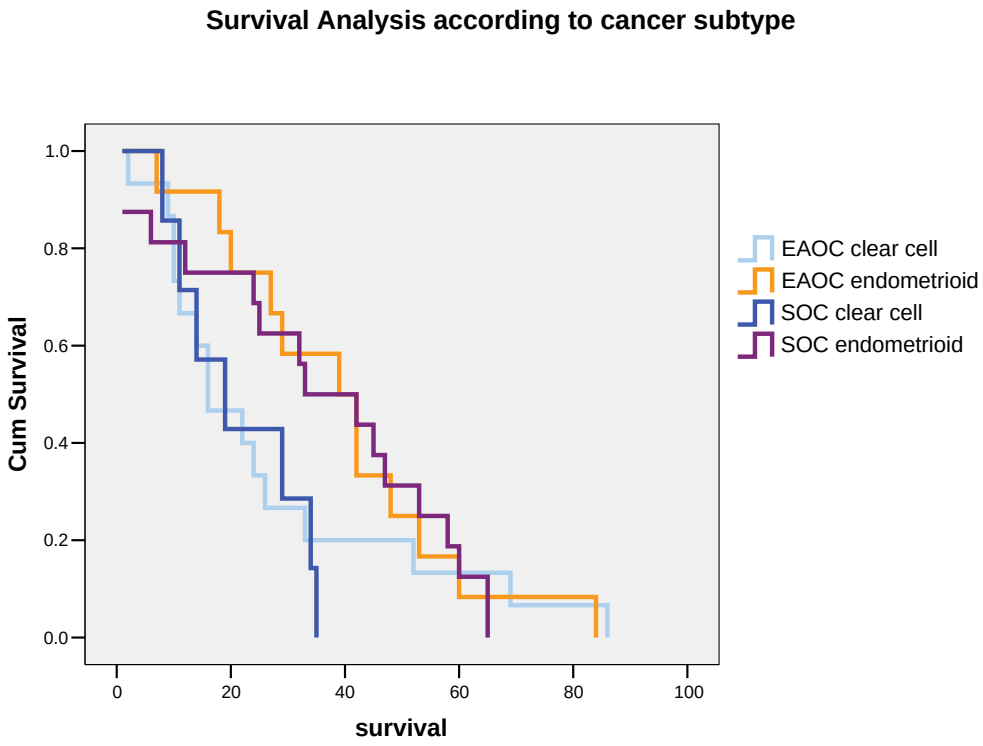


Figure 1.8. Survival analysis according to stage of ovarian cancer.

There is a statistical significant association between ovarian cancer staging and cancer survival (Hazard Ratio 1.56 (95% CI 1.09-2.24)) i.e. an advanced cancer stage increases the odds of dying earlier by 1.6:1 (i.e. probability of dying earlier is 62%).(Hazard analysis results are depicted in Table 1.9).

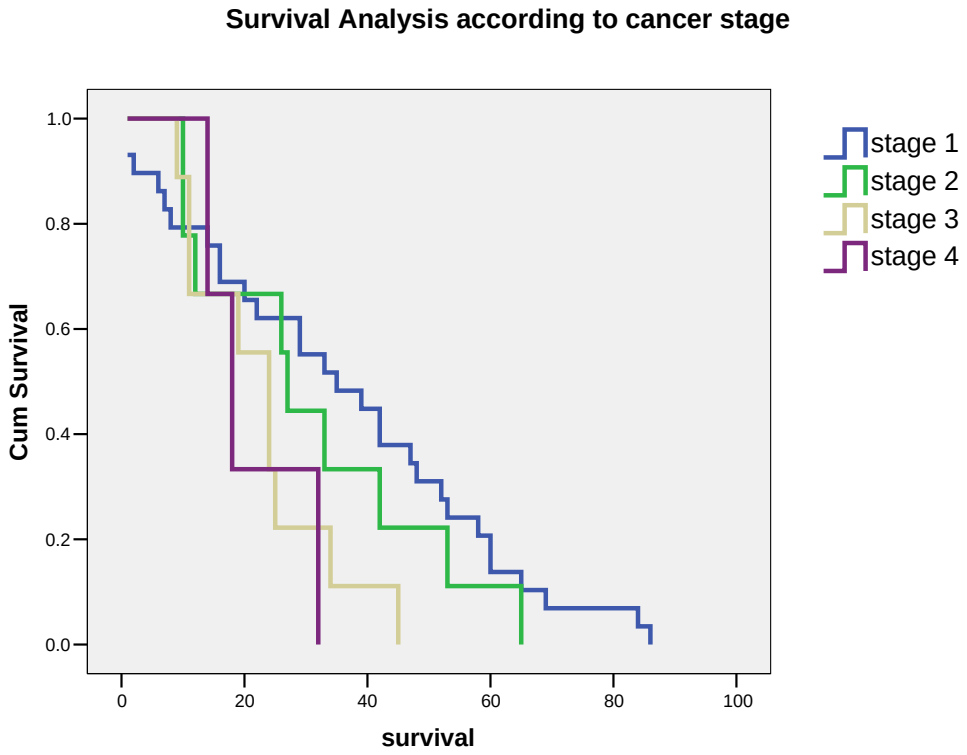
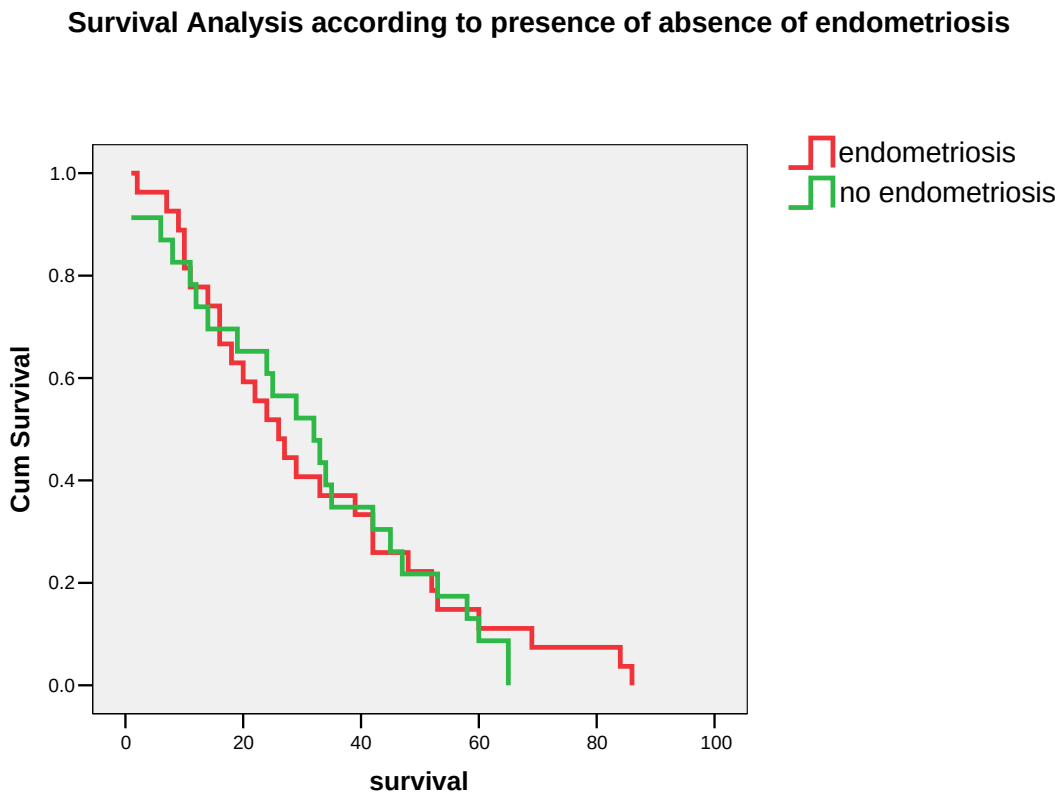


Figure 1.9. Survival analysis according to presence of endometriosis

There is no statistical association between the presence of endometriosis and survival for all cancers (Log Rank Mantel Cox p=0.80). (Hazard analysis results are depicted in Table 1.9).



1.4. Loss of heterozygosity (LOH) fine mapping of EAOC and SOC at chromosomes 9 and 11 using multiple microsatellite genetic markers and their prognostic significance.

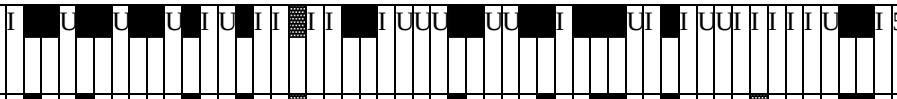
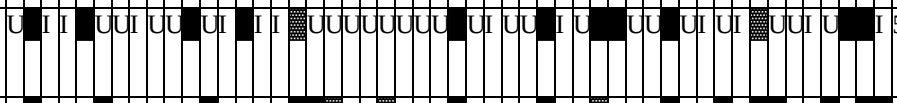
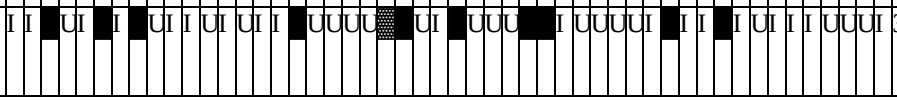
Based on previous published research, chromosomes 9 and 11 were selected for LOH mapping as chromosomes most likely to harbor tumour suppressor genes (TSG) for either endometriosis or ovarian cancer[201;211].

Preliminary microsatellite markers demonstrated LOH at chromosomes 9 and 11 for both EAOC and SOC. Microsatellite markers that mapped to genetic loci no greater than 10cM apart, were selected and used to create a fine map of LOH at chromosomes 9 (**Table 1.10**) and chromosome 11 (**Table 1.11**). The background frequency of genome-wide LOH observed was 30-40% for chromosome 9 (**Figure 1.10**) and 20-40% for chromosome 11(**Figure 1.11**). High frequency LOH was observed at 9q32 (65%), 9q34.3 (78%), 11q22.1 (57%), 11q24.1 (60%), and 11q25 (64%). There were no significant differences in the patterns of LOH between EAOC and SOC (**Figures 1.10 and 1.11**).

Survival analysis showed LOH at 9q34.3 correlated to poorer survival , suggesting that this region of high frequency LOH may harbor a candidate TSG (Figure 1.12). Conversely, survival analysis showed LOH at 11q 23.3 correlated to improved survival, suggesting that this region of high frequency LOH may harbor a candidate oncogene (**Figure 1.12**).

Table 1.10. Allelic Loss at chromosome 9

Genetic Marker	DeCODE CM	Cytogenetic	EAOC CLEAR CELL															EAOC ENDOMETRIOID												SOC CLEAR CELL							SOC ENDOMETRIOID																OVERALL LOH FREQUENCY
			01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	
D9S171	45.57	9p21.3	U	U	U	I	U	I	U	U	I	U	U	I	I	U	I	I	I	I	I	U	I	I	■	U	I	■	■	I	U	■	U	U	■	I	U	■	I	U	I	U	■	U	■	U	U	I	U	■	U	I	32%
D9S273	66.75	9q21.11	U	I	I	U	U	I	I	■	U	U	I	I	■	U	I	I	I	I	I	I	I	■	U	U	U	U	■	I	I	■	U	I	■	■	■	U	■	U	I	I	■	U	I	■	I	I	U	I	U	I	32%
D9S933	78.26	9q21.31	U	I	I	U	I	I	I	I	U	■	■	I	I	I	U	I	■	U	I	■	U	I	U	U	U	U	■	I	I	■	I	I	■	■	U	U	I	■	■	I	■	I	I	I	■	U	U	U	I	■	37%
D9S283	94.85	9q22.2	U	U	U	■	U	U	U	U	U	U	■	■	U	I	■	U	I	I	U	U	U	I	U	U	U	U	■	U	■	U	U	U	U	▒	U	I	U	■	U	I	■	U	I	I	■	I	I	■	I	U	50%
D9S1816	101.8	9q22.32	U	▒	U	U	U	▒	I	■	U	U	U	U	I	U	U	U	U	U	I	I	■	U	U	▒	U	U	U	I	U	I	■	I	I	▒	▒	U	U	I	I	U	■	■	U	U	U	U	U	U	U	I	48%
D9S287	98.7	9q22.32	I	I	I	U	I	U	U	U	U	U	■	■	I	■	U	I	U	I	I	■	U	▒	U	U	U	U	U	■	I	■	■	I	■	U	U	U	U	■	I	I	U	▒	I	U	U	U	U	U	■	U	48%
D9S1690	104.08	9q31.1	I	■	U	U	U	U	U	U	U	U	■	■	I	■	■	I	U	I	U	■	U	■	U	U	U	U	U	U	I	■	■	I	U	■	U	U	U	■	I	I	U	■	I	U	U	■	U	■	■	■	64%
D9S1677	112.85	9q31.3	I	■	I	U	U	U	■	■	▒	U	■	■	U	■	I	U	U	I	I	I	U	▒	U	U	U	U	I	■	U	■	U	U	U	■	■	U	■	■	I	U	U	U	U	U	■	■	U	■	U	U	68%
D9S930	116.75	9q32	I	■	U	U	I	I	U	U	U	U	■	■	I	U	I	I	I	I	I	■	I	U	U	U	U	U	■	I	U	■	■	■	U	■	■	U	■	U	I	U	■	I	I	I	U	I	U	U	■	I	43%
D9S1776	121.62	9q32	I	U	I	U	U	I	U	U	U	■	■	■	I	■	■	U	▒	■	U	U	U	U	■	■	I	■	■	■	U	■	■	I	■	■	■	U	I	■	U	I	■	I	I	■	■	U	■	■	■	■	71%
D9S934	126	9q33.1	■	I	I	I	I	U	I	I	I	U	I	■	I	I	I	I	I	■	I	U	I	U	U	U	I	U	■	I	U	■	■	U	■	U	■	U	I	■	I	U	■	I	I	■	U	I	U	U	■	U	35%
D9S1685	132	9q33.2	■	U	I	U	U	I	U	U	U	I	■	U	U	U	U	U	U	U	U	U	■	U	U	▒	I	■	U	■	U	U	U	U	■	■	■	I	I	■	I	U	U	I	I	U	I	U	U	U	U	I	48%
D9S1682	128.77	9q33.2	U	I	I	U	U	U	U	U	U	U	■	U	■	I	I	I	▒	I	U	■	U	U	■	■	I	■	■	■	■	■	■	■	■	■	■	I	I	■	U	■	■	■	U	■	U	I	I	■	■	I	66%
D9S290	136.4	9q34.11	U	U	■	U	U	U	I	U	U	U	I	U	I	I	U	I	U	I	U	I	U	U	U	U	U	U	U	U	U	■	■	I	■	■	■	U	I	■	I	■	■	I	I	U	■	U	U	I	■	U	46%
D9S260	141	9q34.11	■	I	I	U	I	■	■	U	U	U	■	U	U	I	I	U	I	I	■	U	U	U	U	U	U	U	■	U	U	U	■	U	U	■	U	U	U	U	U	U	■	I	U	U	U	I	U	■	U	U	53%
D9S1830	145.65	9q34.13	I	I	I	U	I	■	U	U	■	■	■	■	U	I	I	I	▒	I	■	■	I	▒	■	▒	I	■	U	U	■	U	■	I	■	U	■	U	U	■	I	I	U	I	I	U	I	I	U	■	■	I	51%

D9S2157	146.54	9q34.2		57%
D9S1826	157.73	9q34.3		52%
D9S158	161.71	9q34.3		74%
D9S1838	164	9q34.3		37%

Footnotes

■ indicates informative loci that showed LOH

▒ indicates MSI

I indicates informative loci, but no LOH

U indicates uninformative loci, therefore unable to determine absence or presence of LOH

Last column refers to overall frequency of LOH and MSI combined at informative loci.

Table 1.11 Allelic loss at chromosome 11

Genetic Marker	DeCODE cM	Cytogenetic	EAOC CLEAR CELL															EAOC ENDOMETRIOID												SOC CLEAR CELL							SOC ENDOMETRIOID																OVERALL LOH FREQUENCY
			01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	
D11S1338	9.77	11p15.4	I	■	I	I	I	I	U	U	I	U	■	I	I	U	I	U	▒	I	I	■	U	■	■	U	■	■	■	I	■	U	■	■	U	U	U	U	U	■	I	U	I	U	I	U	I	I	■	■	U	U	47%
D11S902	25.69	11p15.1	I	U	U	U	■	I	U	U	I	U	I	U	U	I	I	U	U	I	I	U	U	U	▒	U	U	U	■	I	I	U	U	■	■	U	■	U	U	U	I	I	U	I	U	U	■	I	U	U	U	I	32%
D11S4204	43.12	11p14.2	I	■	I	■	■	I	U	I	■	U	U	U	I	I	U	■	I	■	U	■	U	I	U	U	U	U	I	U	U	■	■	■	■	■	U	U	I	I	I	I	■	I	I	I	■	I	I	I	I	I	40%
D11S935	52.94	11p13	I	U	I	■	■	U	■	I	■	U	■	I	■	I	I	I	I	U	I	U	■	▒	U	U	■	U	■	U	I	■	U	■	■	■	U	I	U	I	U	U	U	U	I	U	U	U	U	U	U	U	52%
D11S1993	59.21	11p11.2	I	■	U	U	I	U	■	I	I	■	I	▒	U	I	U	I	▒	I	■	■	▒	U	I	I	■	▒	U	I	▒	U	■	U	■	U	■	I	I	I	I	I	I	I	I	■	I	I	■	U	■	I	44%
D11S4191	64.96	11q12.1	I	■	I	U	U	I	■	■	U	I	U	I	I	U	U	I	▒	U	I	■	■	I	▒	U	▒	■	■	U	I	U	■	I	U	I	▒	I	■	I	■	I	■	I	I	I	I	I	U	U	I	I	41%
D11S987	72.17	11q13.2	I	U	U	I	I	U	U	U	I	U	U	I	I	I	I	I	▒	I	I	U	I	I	U	U	■	■	U	I	I	■	I	U	■	■	U	I	U	I	I	I	I	U	U	■	U	U	U	U	U	I	24%
D11S971	76.76	11q13.4	U	■	U	U	U	I	U	U	U	U	U	I	U	U	I	U	▒	I	U	■	I	U	U	U	U	■	U	I	I	■	U	U	■	U	U	■	U	I	U	I	U	U	U	■	U	U	U	U	I	I	42%
D11S937	83.73	11q14.1	I	■	I	I	I	I	I	I	U	I	I	I	U	I	I	■	▒	I	I	■	U	▒	U	U	■	■	■	I	I	■	I	U	■	U	U	U	I	U	I	I	I	I	I	■	■	I	U	■	U	I	34%
D11S2002	87.25	11q14.1	I	■	I	I	U	I	I	I	■	U	U	U	I	I	■	I	▒	I	I	■	I	I	U	U	I	■	■	U	I	■	I	■	■	I	U	U	I	■	I	I	I	U	I	■	I	U	I	▒	I	U	34%
D11S919	98.31	11q21	U	U	I	U	I	I	U	U	▒	U	U	U	U	I	U	U	U	U	U	U	U	U	U	U	U	■	■	I	U	■	U	U	■	U	■	■	U	■	I	I	I	I	I	■	U	■	■	U	I	I	48%
D11S898	103.59	11q22.1	I	■	U	I	I	I	U	I	■	U	■	U	I	■	I	U	■	I	I	■	■	■	■	▒	■	■	■	U	U	■	I	U	■	U	■	U	■	■	I	I	I	I	■	▒	■	U	■	I	■	I	59%
D11S2000	106	11q22.3	I	■	I	I	U	■	■	I	▒	U	U	U	I	I	■	I	■	I	I	U	U	■	U	U	I	U	■	■	I	■	■	■	■	■	■	U	I	■	I	■	I	U	I	■	■	U	U	U	I	U	54%

D11S1986	110	11q23.1	I	█	I	I	█	█	▒	I	█	I	█	█	I	I	I	I	█	█	I	█	I	█	I	▒	█	█	▒	I	I	I	█	U	█	█	█	█	I	█	I	I	I	I	I	█	█	█	█	I	I	I	51%
D11S3179	112.7	11q23.1	I	█	I	I	U	U	U	U	U	█	U	U	U	I	U	U	▒	I	U	█	U	█	█	█	I	U	█	I	U	U	U	U	█	I	U	U	U	U	I	I	U	U	U	U	U	U	U	U	U	U	47%
D11S908	116.46	11q23.3	I	U	I	U	U	U	█	U	▒	U	U	I	I	I	█	U	█	U	U	█	▒	█	▒	U	▒	█	U	U	█	█	U	█	█	█	█	I	U	U	I	█	U	U	U	█	U	█	U	U	█	I	71%
D11S1998	119.99	11q23.3	I	U	I	U	I	I	U	U	I	U	I	U	U	I	I	U	█	▒	U	█	█	█	U	U	U	█	U	I	U	U	█	U	█	I	█	U	U	█	U	U	I	I	I	█	U	█	█	U	█	I	50%
D11S4089	124.37	11q23.3	I	█	I	U	U	I	█	U	U	U	I	I	U	I	I	I	▒	█	█	█	█	U	I	U	█	█	█	I	I	█	I	U	█	█	█	█	U	U	U	I	I	I	U	U	█	U	█	█	█	I	54%
D11S4464	130.43	11q24.1	U	█	U	I	U	█	I	I	U	U	U	█	█	I	I	U	█	I	I	█	█	█	U	U	U	▒	█	I	I	U	█	U	U	I	█	I	I	█	I	U	U	I	I	█	U	█	█	U	█	I	52%
D11S933	131.38	11q24.2	I	U	I	U	I	U	U	U	I	U	U	U	U	I	I	I	█	U	U	I	U	U	U	U	U	U	█	U	▒	U	I	U	U	I	█	U	I	█	I	█	I	U	U	█	█	█	U	U	U	I	39%
D11S4150	132.95	11q24.3	I	█	U	█	U	I	I	I	U	U	U	U	I	I	U	U	U	U	I	I	U	I	U	U	U	U	U	U	U	█	I	█	█	U	U	U	I	U	I	U	I	U	U	█	I	I	█	█	█	I	36%
D11S2018	142	11q24.3	U	U	U	█	█	I	I	I	I	I	U	I	I	U	I	I	U	I	█	I	█	I	█	I	I	I	I	I	U	█	U	I	I	█	I	I	I	U	█	█	I	I	U	█	I	I	█	U	U	I	29%
D11S1320	146.94	11q25	I	█	I	U	U	I	U	U	I	U	U	I	I	I	I	U	▒	U	█	U	U	█	U	U	U	█	█	I	I	█	U	U	U	█	I	I	U	U	I	U	I	U	I	█	U	U	U	█	█	I	41%
D11S1304	148.52	11q25	U	█	I	U	U	I	I	U	U	U	I	U	U	I	I	█	▒	I	I	U	█	█	U	U	█	█	U	I	█	U	I	U	U	U	I	U	U	I	U	U	I	U	█	█	U	U	U	U	█	█	48%
D11S969	151.02	11q25	I	█	U	U	I	U	U	U	U	█	I	U	U	█	I	I	U	I	I	█	█	█	█	U	U	█	U	I	▒	█	█	█	█	U	I	█	U	I	I	I	U	█	U	█	█	█	█	█	U	U	63%
D11S4125	152.45	11q25	I	█	I	U	I	U	█	U	U	U	U	I	█	█	I	I	U	I	I	█	U	▒	U	U	U	█	█	U	█	█	█	U	U	█	█	U	U	U	U	I	I	I	I	█	█	I	█	█	█	U	58%
D11S968	152.45	11q25	█	█	I	█	I	I	I	I	U	U	U	I	I	U	I	█	▒	█	I	█	█	▒	U	U	U	█	█	I	I	█	█	█	█	I	█	I	U	U	U	I	U	U	I	U	I	U	U	U	I	U	48%

Footnotes

█ indicates informative loci that showed LOH

▒ indicates MSI

I indicates informative loci, but no LOH

U indicates uninformative loci, therefore unable to determine absence or presence of LOH

Last column refers to overall frequency of LOH and MSI combined at informative loci.

Figure 1.10. Contribution to allelic loss at chromosome 9 by each cancer subtype

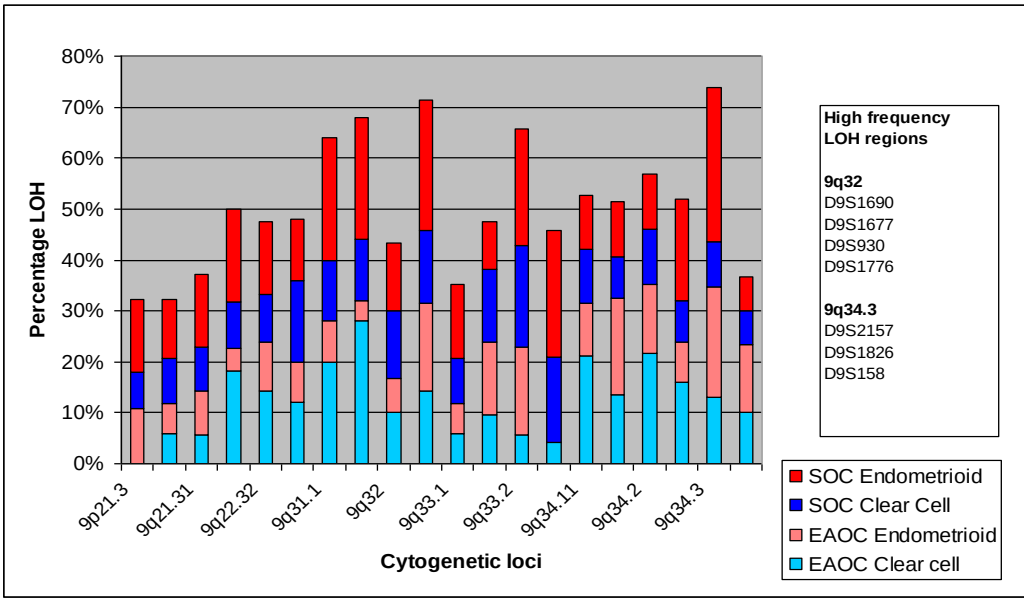


Figure 1.11. Contribution to allelic loss at chromosome 11 by each cancer subtype

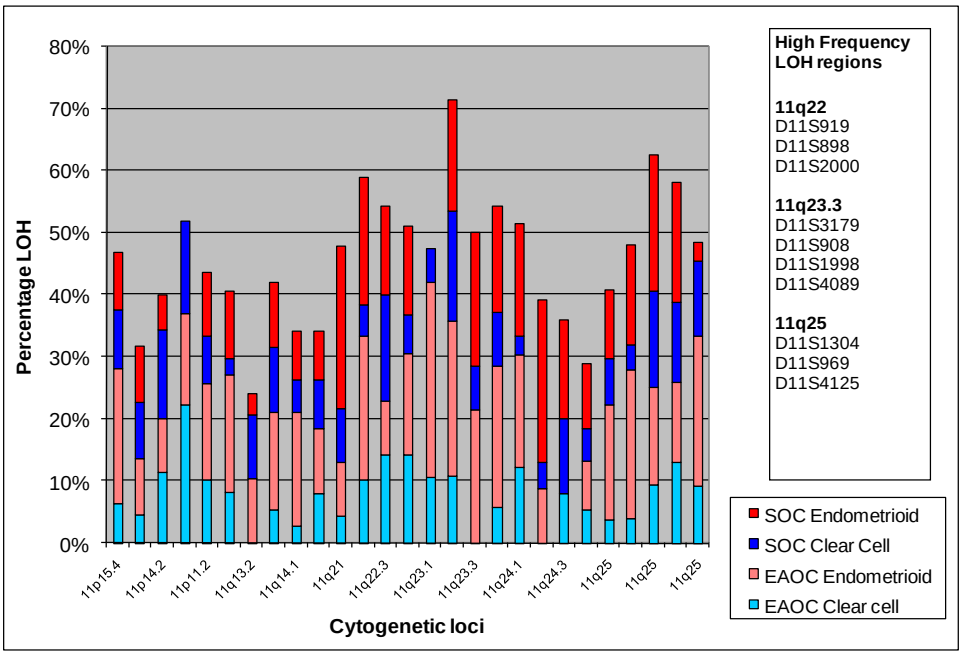
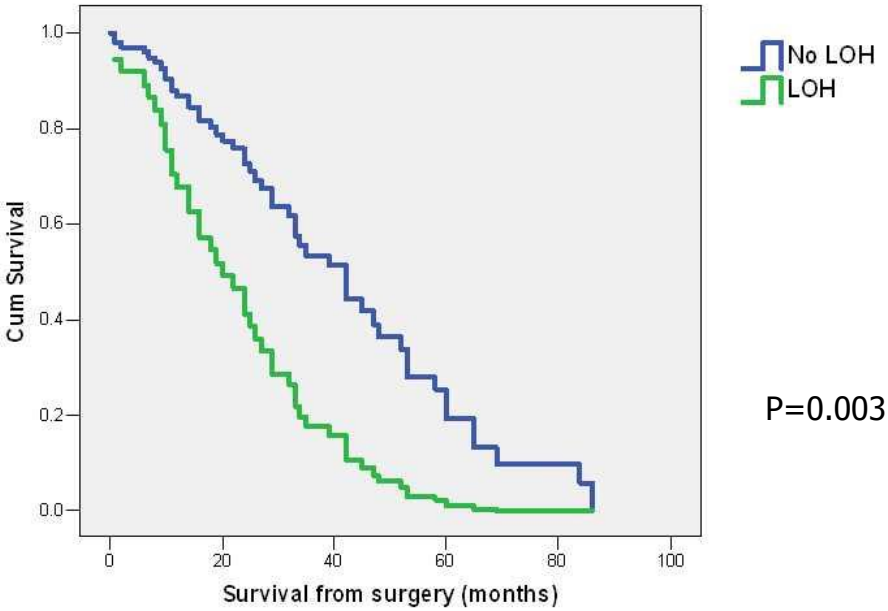


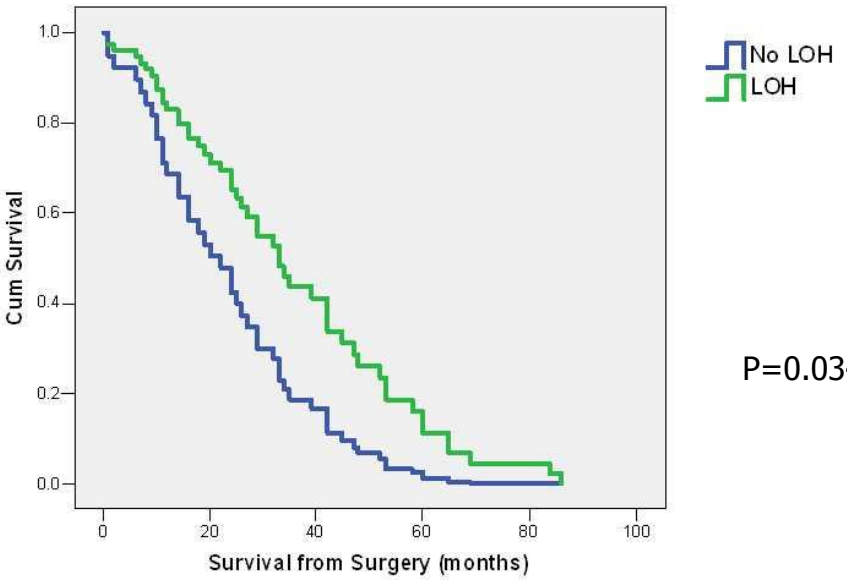
Figure 1.12. Significant association of LOH at 9q34.3 and 11q 23.3 on survival of all ovarian cancers according to Cox Proportional Hazards survival analysis

Cox survival analysis for LOH at 9q34.3



P=0.003

Cox survival analysis for LOH at 11q23.3



P=0.034

Rationale for selecting Glycodelin and Progesterone Receptor as candidate disease-modifying genes

A bioinformatic search was performed to examine the published data on genetic expression and functional taxonomy of genes at these two genetic loci to select candidate disease-modifying genes. Previous work had identified glycodelin (9q34) expression to be significantly altered in endometriosis and it had also been implicated in tumourigenesis[212-215]. Progesterone had been implicated in both endometriosis proliferation and anti-proliferation and ovarian cancer[11;211;216-218]; mutations of the Progesterone receptor (PROGINS)(11q22) had been associated with development of endometriosis[219].

1.5. Laser Capture Microdissection (LCM) of endometriosis and selected LOH mapping

Endometriosis adjacent to EAOC was extracted by LCM and its DNA subjected to LOH mapping using the 4 microsatellite markers at chromosome 9 and 11. LOH was identified in 1/7 cases at 9q34 and 1/7 cases for LOH 11q23.3. Our results did not show strong evidence that LOH events occurred in endometriosis. However, our research collaborators, who utilised our Birmingham Women's EAOC/SOC samples we had donated, showed LOH to occur more frequently when they microsatellite mapped their LCM endometriosis, particularly when the endometriosis LOH corresponded to an adjacent ovarian cancer LOH event **(see Table 1.12)**.

Table. 1.12. Genome wide microsatellite analysis of endometriosis adjacent to ovarian cancer (Prowse, Varma 2006) [220]

10 EAOC cases; LCM of endometriosis from EAOC Genome wide 82 markers A total of 63 LOH events were detected in EAOC 22/63 of these were also detected in the corresponding endometriosis sample, **involving the same allele in each case** No marker was specific to only LOH in endometriosis

1.6 Immunohistochemical investigation of EAOC using Glycodelin (9q34.3) and Progesterone receptor (11q22)

A summary collation of the immunohistochemical staining for 6 EAOC cases (3 endometrioid, 3 clear cell) is depicted in **table 1.13 and images are depicted (Figure 1.13, Figure 1.14, Figure 1.15). Glyocdelin** staining was absent in the ovarian cancer and present in the endometriosis distant to the ovarian cancer, but not so strongly expressed in endometriosis adjacent to ovarian cancer; this is weak evidence that endometriosis adjacent may be a differing molecular entity to distant endometriosis, and that glycodelin is possibly involved in causing this difference. No significant differences were observed for PR-A or PR-B staining.

Table 1.13. Summary of immunohistochemistry findings

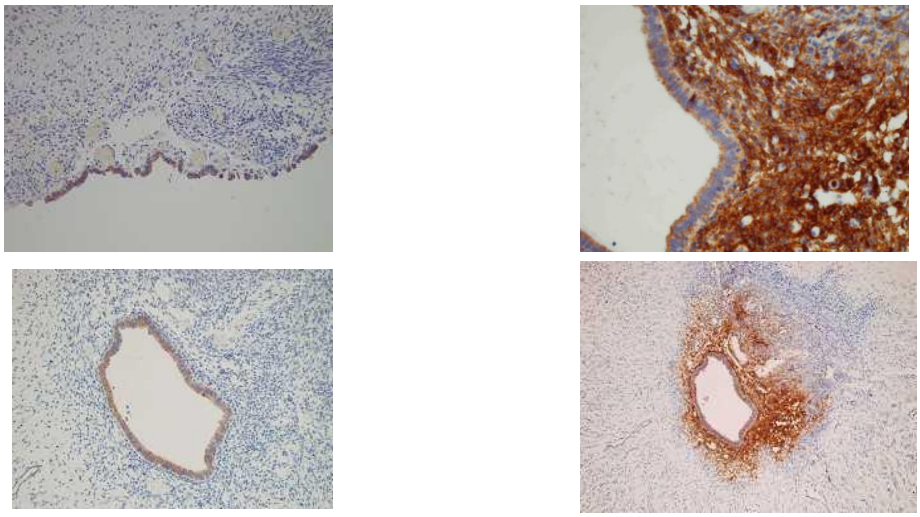
	Endometrioid EAOC patient	Clear Cell EAOC patient
Endometriosis distant from ovarian cancer	Moderate Glycodelin Strong PR-A,PR-B	Moderate glycodelin Strong PR-A,PR-B
Endometriosis adjacent to ovarian cancer	Weak Glycodelin Strong PR-A, PR-B	Weak Glycodelin Strong PR-A,PR-B
Ovarian cancer	Absent Glycodelin Absent PR-A, Patchy positive PR-B	Absent Glycodelin Absent PR-A PR-B

Footnotes

CD10 used as a positive control for identification of endometriosis[221]-see Figure 1.13

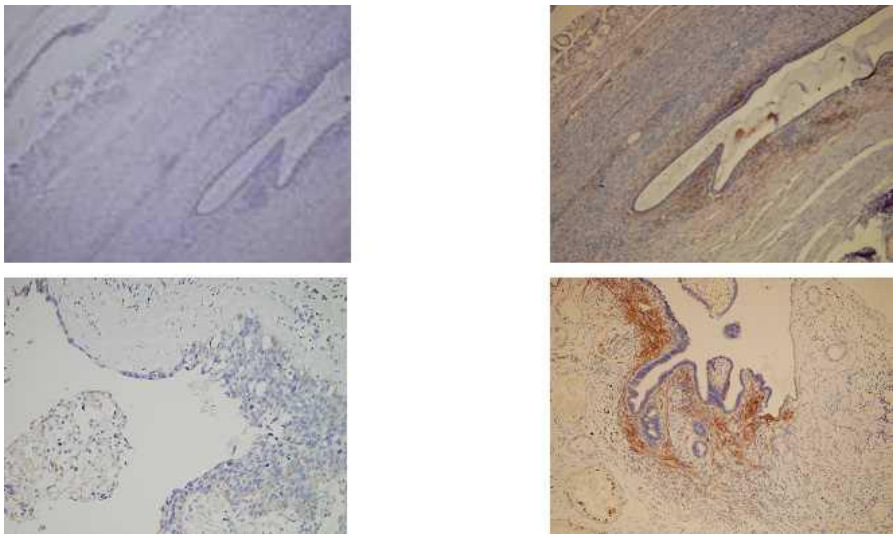
Figure 1.13. Immunohistochemistry images of endometriosis and ovarian cancer using Glycodelin and CD10

Glycodelin (9q34.3) and endometriosis distant from cancer



Glycodelin CD10

Glycodelin (9q34.3) and endometriosis adjacent to cancer



Glycodelin CD10

Figure 1.13 continued. Immunohistochemistry images of endometriosis and ovarian cancer using Glycodelin and CD10

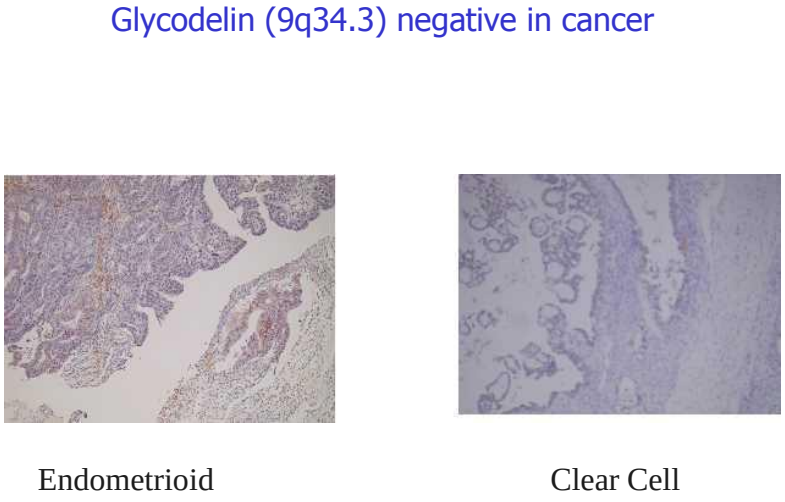


Figure 1.14. Immunohistochemistry images of endometriosis and ovarian cancer using Progesterone receptor subtypes A and B (individually labeled)

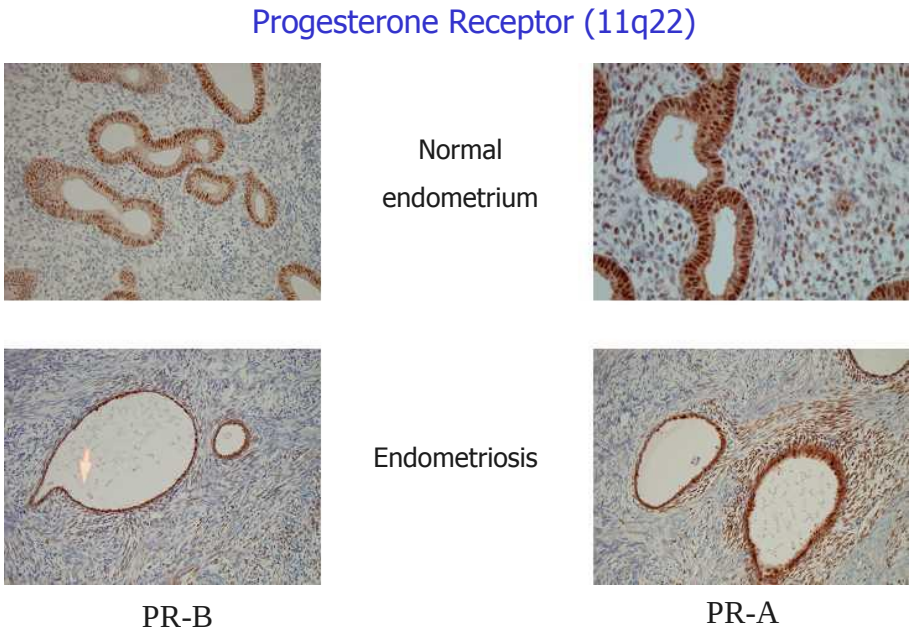
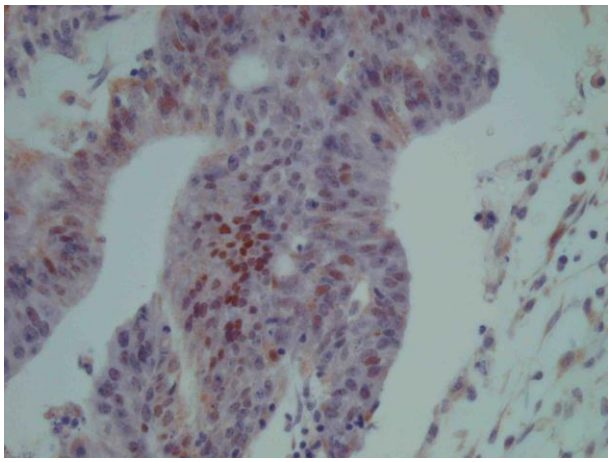
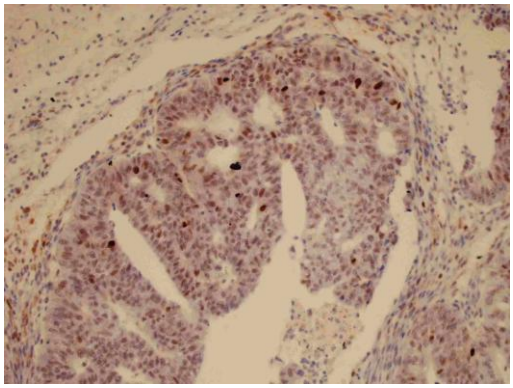


Figure 1.15. Immunohistochemistry: patchy positive staining of PR-B in endometrioid cancer



PR-B patchy positive in endometrioid cancer

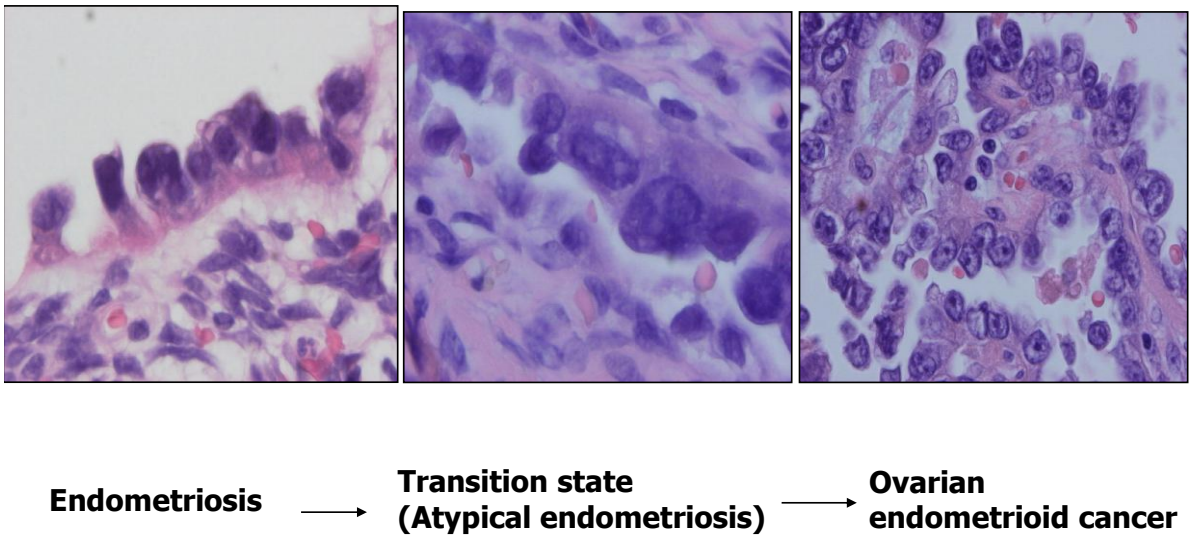
Figure 0.1 Immunohistochemistry of PR-B in endometrioid cancer



1.7. Preliminary nuclear morphometric analysis of endometriosis adjacent to ovarian cancer

In the single case identified, there was increasing nuclear diameter and pleomorphism in the direct continuum transition between endometriosis, atypical endometriosis and EAOC **(Figure 1.16).**

Figure 1.16. Nuclear morphometric analysis of endometriosis, atypical endometriosis and ovarian cancer that appear as one continuum on the histology slide



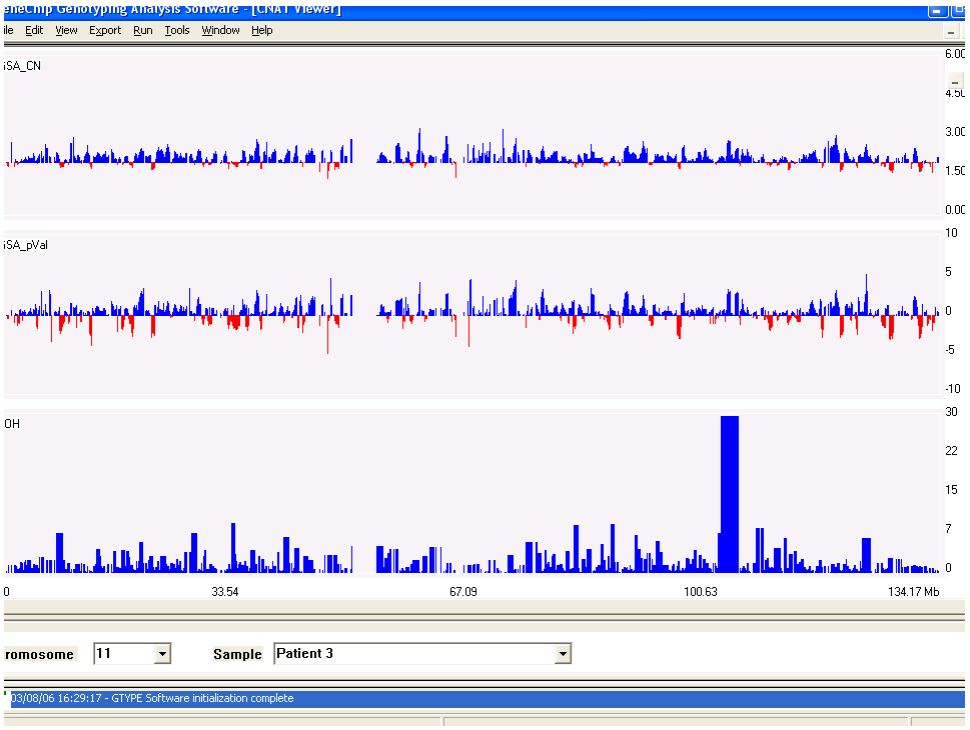
1.8. Affymetrix SNP DNA microarray genotyping of ovarian endometriosis

DNA from 10 patients, 5 matched ovarian endometriosis and ovarian surface epithelium and 5 only ovarian endometriosis DNA, were subjected to SNP microarray analysis. Multiple, extremely small genetic distance areas of LOH were observed in ovarian endometriosis when compared to its matched ovarian surface epithelium control, without alteration of the DNA copy number at that genetic locus. There was no genome-wide consistency of the chromosome or chromosomal region affected by this 'micro-LOH' (summarized in **Table 1.14**). However, regions on chromosome 11 **(Figure 1.17)**, 15 **(Figure 1.18)**, 21 (**Figure 1.19),** 6 and X **(Figure 1.20)** showed considerable LOH prominence. These regions of LOH need to be validated by confirmatory microsatellite marker analysis.

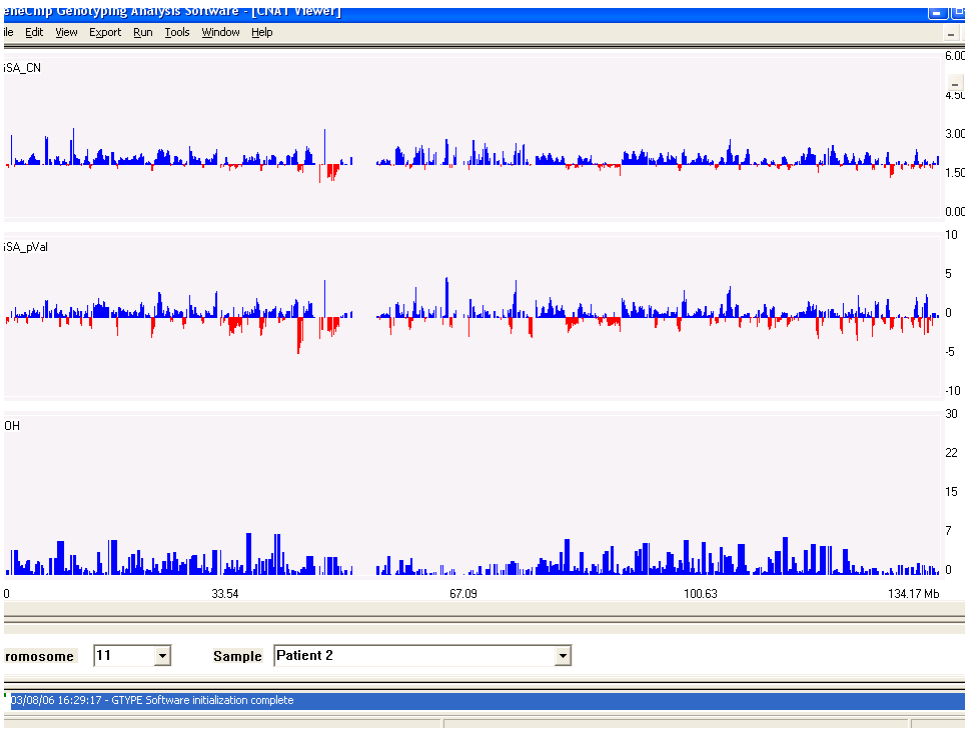
Table 1.14. Summarising genome-wide LOH regions identified in ovarian endometriosis through SNP Affymetrix microarray analysis

Chromosomal region where LOH identified	Number of ovarian endometriosis cases (N= 5)	Proximity to regions of LOH identified in ovarian cancer
1q	One case	
2q	One case	
3q	One case	
6p	One case	
9q	No cases	9q32 9q34.3
11q	Two cases	11q23.3 YES 11q222 YES 11q25 NO
15p	One case	
21p	Two cases	
Xp	Two cases	
Xq	One case	

Figure 1.17. Selected images of chromosomal abnormality (chrom 11) in ovarian endometriosis (patient 3) compared to their matched normal ovarian surface epithelium (patient 2)

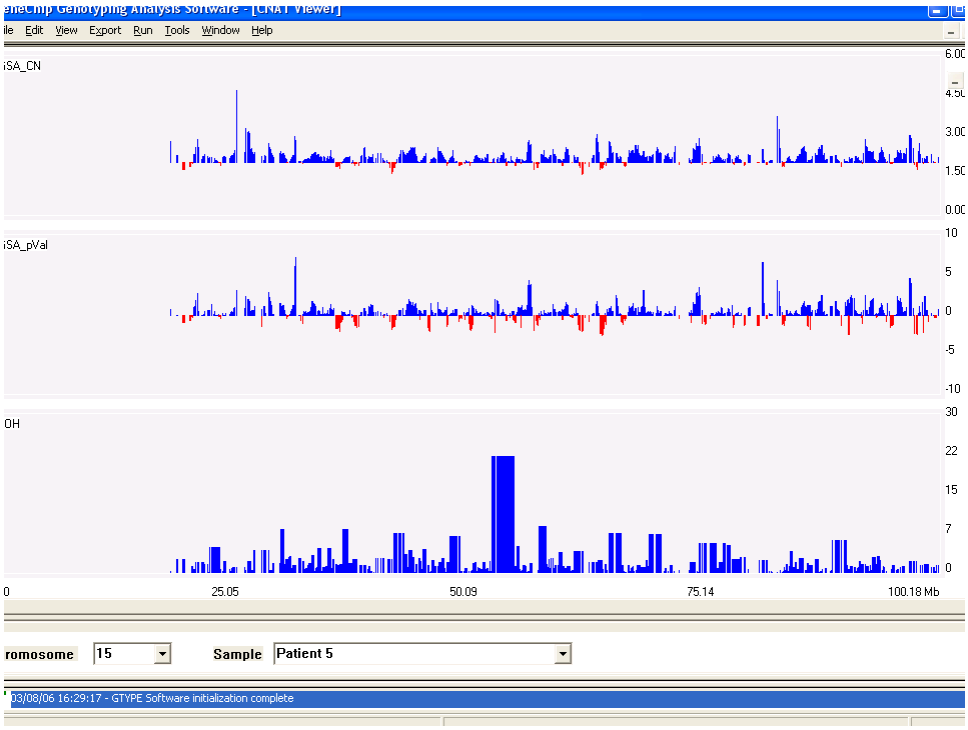


Ovarian endometriosis LOH at 11q

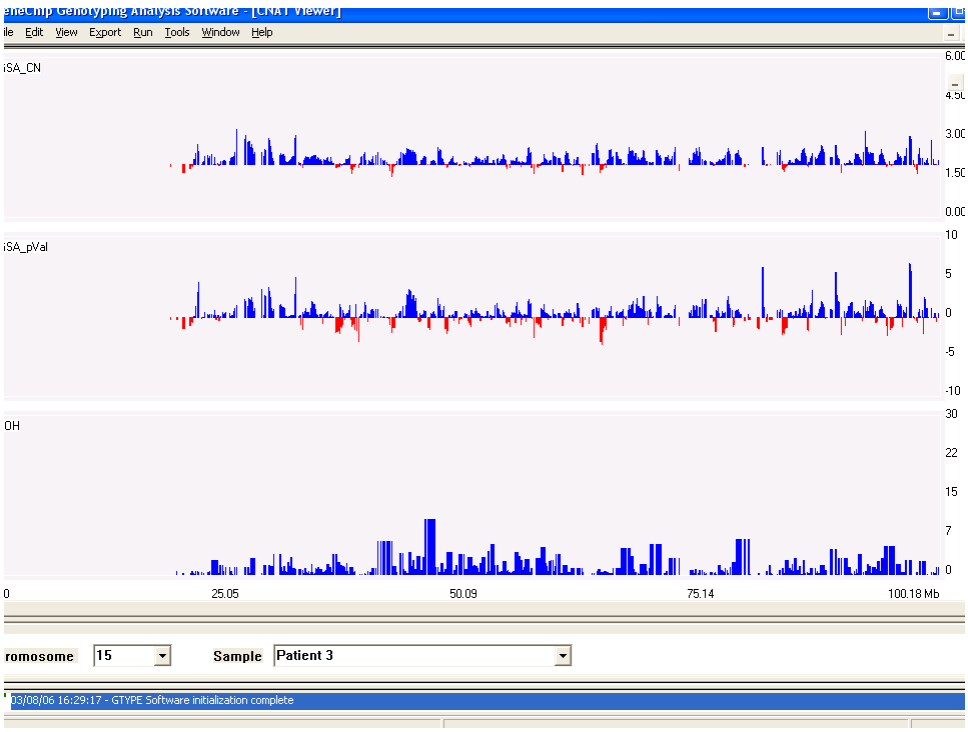


Matched normal ovary

Figure 1.18. Selected images of chromosomal abnormality (chrom 15) in ovarian endometriosis (patient 5) compared to their matched normal ovarian surface epithelium (patient 3)

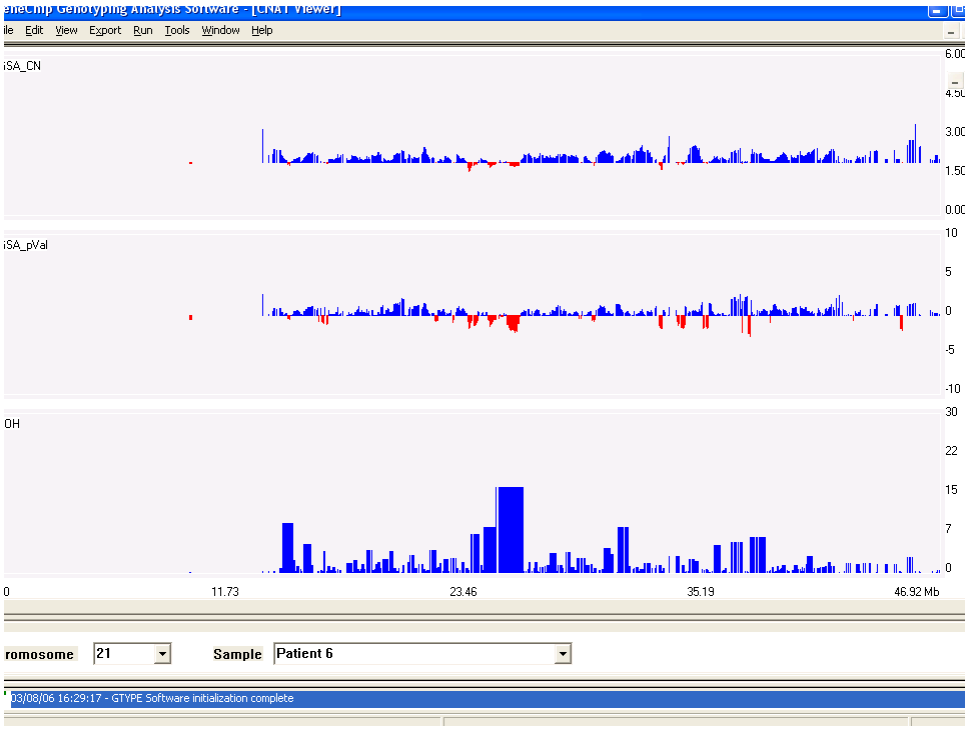


Ovarian endometriosis LOH at 15p

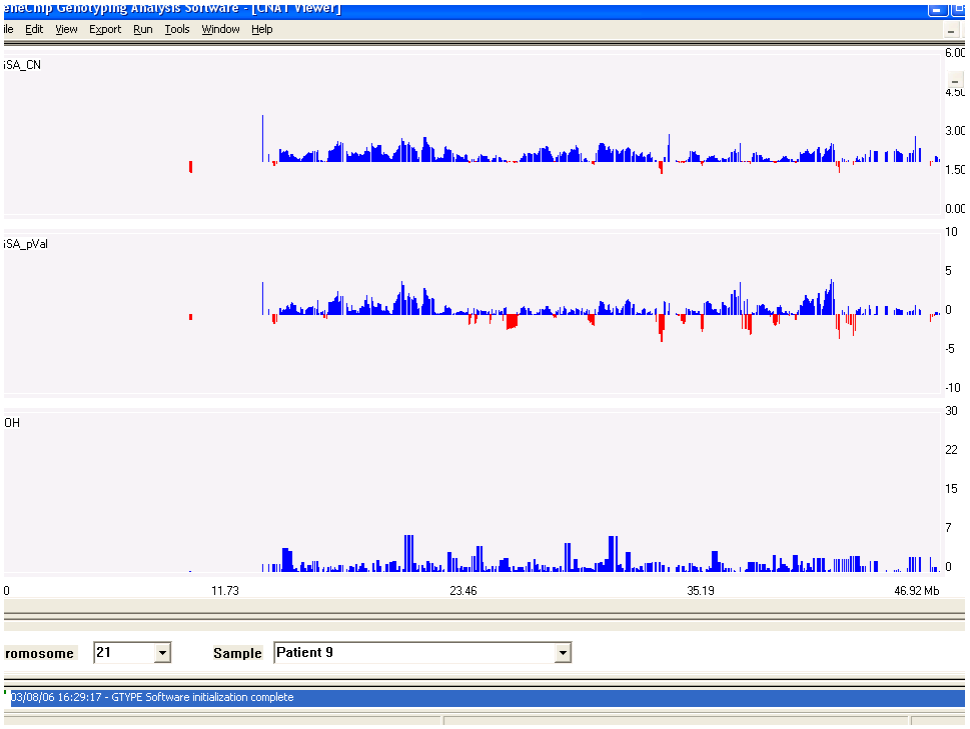


Matched normal ovary

Figure 1.19. Selected images of chromosomal abnormality (chrom 21) in ovarian endometriosis (patient 6) compared to their matched normal ovarian surface epithelium (patient 9)

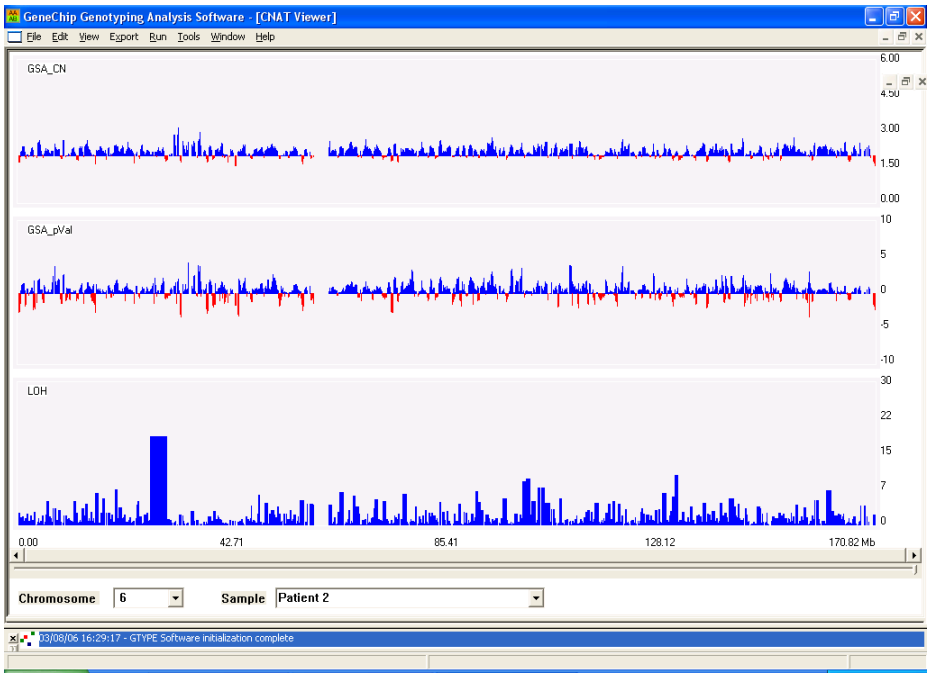


Ovarian endometriosis LOH at 21p

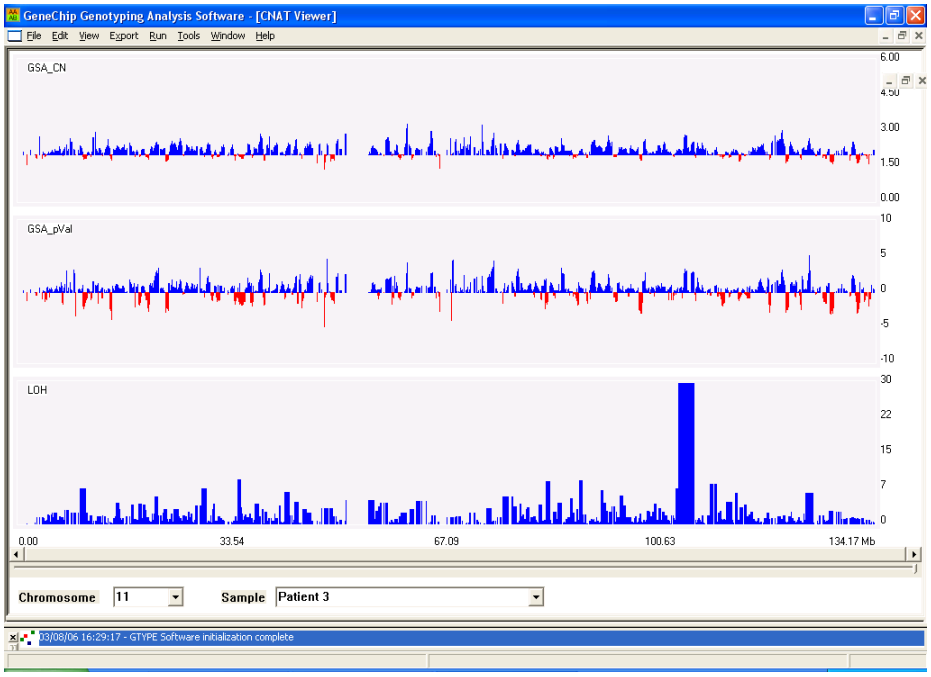


Matched normal ovary

Figure 1.20. Selected images of chromosomal abnormality (chrom 6 and chrom 11 and chrom X) in ovarian endometriosis for patient 2 and patent 3 and patient 6, respectively.

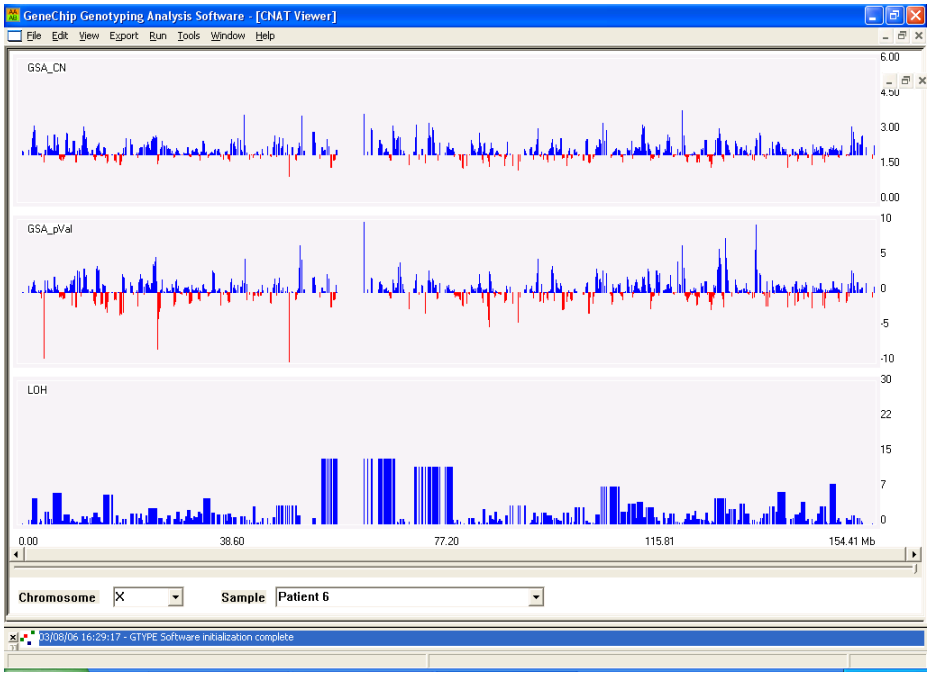


Ovarian endometriosis LOH at 6p



Ovarian endometriosis LOH at 11q

Figure 1.20 continued. Selected images of chromosomal abnormality (chrom 6 and chrom 11 and chrom X) in ovarian endometriosis for patient 2 and patent 3 and patient 6, respectively.



Ovarian endometriosis LOH at Xp, Xq

Chapter 2. ANALYTICAL OBSERVATIONAL STUDIES

Introduction The use of cohort and case-control studies to benefit clinical practice.

Appraising the clinical value of cohort and case-control studies.

Results Examination of methodology through five topics in gynaecology

Chapter	Title
2.1	Predicting negligence in female sterilization failure using time interval to sterilization failure: analysis of 131 cases
2.2	The effectiveness of a levonorgestrel-releasing intrauterine system (LNG-IUS) in the treatment of endometrial hyperplasia – a long-term follow-up study.
2.3	Hospital recovery following Thermachoice ablation is not dependent on setting (outpatient or daycase) or rescue analgesia: unexpected result
2.4	Outpatient Thermachoice endometrial balloon ablation: long term, prognostic and quality of life measures
2.5	Long term outcomes following hysteroscopic myomectomy for abnormal uterine bleeding

Introduction

Cohort and case-control study methodologies are the main types of analytical observational study. Randomised controlled clinical trials are considered a superior methodology in the hierarchy of evidence, because they limit the potential for selection bias and minimise the influence of confounding due to differences between the two comparison groups (**Table 2A**). However, it is either impractical or unethical to perform RCTs to answer all clinical scenarios. Furthermore, cohort studies may provide important preliminary evidence to suggest whether a RCT is actually warranted or not. Both Cohort and RCT studies are able to determine relative risk as both measure incidence. The differences between cohort and RCT design are depicted in the table below. There are many famous longstanding cohort studies in medicine (e.g. Framingham in Heart Study) and obstetrics (e.g. UK Confidential enquiry into maternal and perinatal mortalities coordinated by CEMACH).

The aim of this chapter was to assess the effectiveness of menstrual treatments (Outpatient Thermachoice endometrial balloon ablation and Hysteroscopic myomectomy) over a long time period in a pragmatic clinical setting (rather than highly selected population). It was felt that the best study design would be a prospective cohort analysis. The chapter discusses the findings in applying the cohort study design to this situation, how reliable data interpretation can be given the study design, and the practical beneficial clinical impact the study has achieved. Furthermore, the cohort study design is applied to a rare outcome measure that tends to occur after considerable time (failed female sterilisation). The cohort study design is chosen to test a mathematical (Bayesian) hypothesis that time interval to sterilisation failure is predictive of negligence rather than non-negligence. The publication of this work has clarified the medico-legal probability of negligence in those cases where the failure mechanism is unknown, and has therefore had profound medico-legal impact.

Furthermore, in our end of thesis conclusion (**chapter 5**), we suggest that the cohort design may be under-utilised, and ways to address this. For example, provided the cohort design adopts strict case ascertainment and selection criteria (i.e. minimises selection bias), is sufficiently powered to identify and correct for known confounders in comparison groups, and utilises sophisticated statistical techniques in the analysis, then the results of the cohort analysis may be at least (if not more) as reliable as those obtained by a suitably powered RCT. To achieve this, robust large scale all inclusive prospective cohort databases are needed-akin to the electronic Patient Medical Records database envisaged for both USA and UK.

Table 2A.Advantages and Disadvantages as displayed by Centre for Evidence-Based Medicine (Oxford, UK; www.cebm.net)

Cohort Study	**Randomised Controlled Clinical Trial**
Advantages: ethically safe subjects can be matched can establish timing and directionality of events eligibility criteria and outcome assessments can be standardised administratively easier and cheaper than RCT	**Advantages** unbiased distribution of confounders blinding more likely randomisation facilitates statistical analysis.
Disadvantages: controls may be difficult to identify exposure may be linked to a hidden confounder blinding is difficult randomisation not present for rare disease, large sample sizes or long follow-up necessary	**Disadvantages:** expensive: time and money volunteer bias ethically problematic at times

2.1. Predicting negligence in female sterilization failure using time interval to sterilization failure: analysis of 131 cases

BACKGROUND: Sterilization failure due to 'tubal non-occlusion' or 'wrong structure sterilization' is considered negligent, whereas 'spontaneous tubal recanalization' or 'fistula formation' is considered non-negligent. We examined whether interval to pregnancy failure was predictive of a negligent rather non-negligent failure mechanism. We aim to test this hypothesis in a selected population series of known mechanisms of sterilization failure and their time interval to failure.

METHODS: Analyses of 131 failed sterilizations pooled from UK (NHS Litigation Authority, Medical Protection Society and our hospital), Australia and a qualitative systematic review.

RESULTS: We identified 88 negligent and 43 non-negligent sterilization failures. Filshie and ring methods failed earlier than diathermy and Pomeroy methods. Sterilization failure occurred significantly earlier in negligent than non-negligent failure mechanisms [median failure intervals 7.0 versus 12.0 months; Hazard ratio (2.35 95% CI 1.31–4.21)]. Knowing that sterilization failure occurred early, increased the probability that the failure mechanism was likely to be negligent rather than non-negligent.

CONCLUSIONS: A short interval to failure is suggestive of a negligent failure mechanism. There is less certainty in the predictive value of longer time intervals on the mechanism of failure due to a paucity of cases. A national register of failed sterilizations that have been systematically investigated is needed to improve our understanding of negligent and non-negligent failure mechanisms.

INTRODUCTION

Female sterilization is one of the commonest procedures performed worldwide. In 1999 around 50,000 female sterilisations were performed in England in the NHS and charitable sectors [1] . The procedure is performed on mainly healthy women at their request. Where resources permit, the preference is to use a laparoscopic technique that occludes tubal patency through tubal application of a mechanical device (e.g. Filshie, Hulka clip or Fallope ring) or electrocautery. Tubal excision and separation and related techniques (e.g. Pomeroy procedure) are preferred if sterilisation is performed at caesarean delivery. Conception that occurs after sterilisation is termed failed sterilisation and can occur several years after the procedure. Two large population-wide studies have reported the ten-year cumulative probability of pregnancy of 18.5 per 1000 procedures (US CREST study) [2] and 8 per 1000 procedures (Canada) [3] (**Table 2.1)**. Differences in sterilisation failure rates arise due to variation in: the characteristics of the women undergoing sterilisation; operator experience; operating centre; sterilisation method chosen, and the time interval to resuming sexual activity post sterilisation and its frequency. However, neither of these studies reported on the precise mechanism of sterilisation failure. In the UK, the RCOG [1] recommends laparoscopic sterilisation by either Filshie clip or ring. The 10-year sterilisation failure rate for Filshie clip has been reported by studies as 2-3 per 1000 procedures (**Table 2.1**).

Table 2.1. Filshie Clip sterilisation failure rates

Study	Period data are collected from	Sterilisations Performed	Sterilisation method	Outcome	Type of study
Peterson[2] **US Collaborative review of Sterilisation (CREST)**	1978-1986	10,685	Various methods. Hulka spring clip (1595) Silicone Rubber band (3329)	Overall 18.5 per 1000 over 10 years Hulka 36.5 per 1000 Silicone rubber band 17.7 per 1000	Prospective cohort multicentre
Trussell [3]	1980-1999	311,960	Mainly Laparoscopic Filshie clip	8 per 1000 [2496 failures]	Retrospective multicentre
Kovacs [6]	1994-1998	30,000 (estimate)	All Filshie	2.4 per 1000 [73 failures][a]	Retrospective multicentre
Filshie [7]	1982-1992	First 202 responders from a series of 434	All Filshie	2.3 per 1000 [1 failure at 6 months]	Case series
Birdsall [8]	1988-1989	1094	Mainly Laparoscopic Filshie clip	12 per 1000 at 12 months [b]	Case series
Sokal [9]	1984-1990	2746	Filshie clips vs. Rings [2 in each group became pregnant]	1.7 per 1000 for both Ring and Filshie clip groups at 12 months	RCT
Dominik [10]	1984-1990	2126	Filshie clips vs. Hulka clips [11 pregnancies occurred: 9 Hulka, 2 Filshie]	At 12 months 1.1 per 1000 for Filshie Clip 6.9 per 1000 for Hulka Clip group. At 24 months, 9.7 per 1000 for Filshie and 28.1 per 1000 for Hulka	RCT

Footnotes to Table 2.1

[a] Kovacs: Of the 73 failures, 14 cases were due to operator error, 29 were properly applied clips and 30 cases had unknown reason for failure.
[b] Birdsall: Registrars had a 1.3% failure rate, consultants 1.9% and when both a consultant and registrar performed the procedure a failure rate was 0.7%. Eighty-six percent (6/7) of failed sterilisations were due to operator error (wrong structure, initial non-occlusion).

The psychological and physical morbidity following failed sterilisation often leads to litigation [4]. Women who have undergone sterilisation performed negligently are entitled to recover damages according to wrongful conception, negligence, and wrongful birth. Also, women are entitled to recover general damages for pain and suffering during pregnancy and delivery, and loss of earnings during pregnancy. A recent judgment in the Australian High Court [5] led the Australian government to amend the Civil Liberty Act to restrict the amount of damages that could be awarded in such situations.

Despite intense medico-legal activity, research into the prevention and causation of sterilisation failure is lacking. The mechanism of failure should be identified through a systematic assessment of fallopian tube histology, X-ray hysterosalpingography and direct pelvic visual inspection. If the mechanism of failure is due to 'tubal non-occlusion' or 'wrong structure sterilisation ', these are considered negligent mechanisms, whereas 'spontaneous tubal recanalisation' or 'fistula formation' mechanisms of failure are considered non-negligent. However, in the majority of failed sterilisation cases, even those in the advanced stages of litigation, the mechanism of failure remains unknown as there is no uniform requirement for such cases to undergo systematic enquiry or to be reported to any supervisory national registry. The RCOG should consider this requirement at the time of the sterilisation guideline review in 2006. [1]

Thus, a common scenario in the legal setting is to cast judgment on the likelihood of negligence or non-negligence in cases with unknown mechanisms of sterilisation failure. Our qualitative systematic review [4] pooled 81 cases of sterilisation failure that had documented both interval to pregnancy and mechanism of failure. We showed that a greater proportion of early (within 12 months from operation) than late (after 12 months from operation) sterilisation failures occurred by a negligent mechanism. We therefore propose that interval to sterilisation failure may represent a surrogate marker of negligence and non-negligence. Our aim was to:-

1. Determine if sterilisation failure occurred earlier in negligent than non-negligent groups.

2. Determine if time interval to sterilisation failure was predictive of negligence.

We aimed to test this hypothesis in a selected population series of known mechanisms of sterilisation failure and their time interval to failure.

METHODS

A written application was made to NHS Litigation Authority (NHSLA), Medical Defence Union (MDU) and Medical Protection Society (MPS) requesting anonymised information on failed sterilisation cases. The NHSLA provided 16 cases and the MPS provided 8 cases. Similar anonymised failed sterilisation cases that had been subject to litigation proceedings were retrieved from our hospital legal services department (n=12) and a series from an Australian population (n=14) [11]. These cases were pooled with those identified in our previously published qualitative systematic review [4] (n=81). A total of 131 failed sterilisation cases were identified that reported mechanism of sterilisation failure, interval to pregnancy and method used for each case. We have only included cases where the cause of

sterilisation failure has been established either by direct pelvic visualization or histology of the fallopian tubes or a combination of both. Most of our data series examines Filshie clip sterilisation failures as our data set emanates from countries where Filshie clip predominates as the preferred sterilisation method (i.e. UK and Australia). The derivation of this set is shown in **Table 2.2**.

STATISTICAL ANALYSES

Statistical analysis was undertaken using SPSS version 13. Geometric means were derived by exponentiating the means from the logarithm transformed interval to pregnancy data. Categorical correlations were assessed by Chi-squared analysis. Time-to-event methods (Kaplan-Meier and Cox regression) were used to investigate covariates impacting on time interval to pregnancy. Graphs of log cumulative hazard for failure against time interval for negligent and non-negligent cases were found to be parallel indicating that the proportional hazards assumption was true validating the use of the Cox proportional Hazard regression model. The probability that a randomly selected case was negligent given sterilisation failure before a specified time interval was calculated using Bayes' Theorem.

Table 2.2. Databases used to acquire failed sterilisation records

Source of cases	NHSLA	MPS	BWH	Australian Series	Qualitative Systematic review	Used in Study
Dates of sterilisation procedure	1995-2004	1990-2004	1987-1996	1990-2000	1966-2005	
Filshie	70 [b]	6	13	31 [b]	17	62+[2]
Diathermy	0	4	0	0	20	24
Ring	1	0	0	0	24	24
Hulka [b]	0	1	0	0	1	[2]
Pomeroy	0	0	0	0	19	19
Total included in study [a]	16	8	12	14	81	**131**

Footnotes:-

NHSLA National Health Service Litigation Authority

MPS Medical Protection Society, UK

BWH Birmingham Women's Hospital

Australian series This was published in our qualitative systematic review[4].

[a] Only cases that included all three components (mechanism of failure, interval to pregnancy and sterilisation method used) were included in the study's analysis.

[b] Individual separate analysis of 2 Hulka clip cases would be extremely limited, therefore these were included with the Filshie clip category as both methods utilise similar mechanical tubal occlusive devices.

RESULTS

1. Overall interval to pregnancy The mean age for the group was 33.2 years (SD 4.4; 95% CI 31.9-34.4; age range 24-42 years). The arithmetic mean interval to pregnancy was 13.0 months (SD 14.2; 95% CI 10.6-15.5; range 1 to 102 months). The greatest proportion of sterilisation failures occurred by 12 months (72.5%) in a markedly positively skewed frequency distribution. The distribution was normalised by natural log transformation of the interval to pregnancy times to give a geometric mean interval to pregnancy of 9.3 months (SD 2.2 months; 95% CI 8.1-10.6). Unlike the arithmetic mean, the geometric mean is not overly influenced by the large values in a skewed distribution, and so gives a better representation of the average for the purposes of this study.

2. Negligent and non-negligent failure group compositions and intervals to pregnancy Filshie and Ring sterilisation methods failed significantly earlier than diathermy and Pomeroy methods (Log Rank p=0.037); the mean and range intervals to pregnancy are shown in **Table 2.3.** Non-occlusion and wrong structure mechanisms of failure occurred significantly earlier than fistula and recanalisation methods (Log Rank p=0.001); the mean intervals for negligent and non-negligent failure were 7.5 and 14.2 months respectively [**Table 2.4].** There is a significant association between sterilisation method used and negligent and non-negligent mechanism of sterilisation failure (Chi-square, p= 0.001). The Filshie clip, most often failing due to non-occlusion or wrong structure, is the predominant method in negligent failures (71% of cases) **[Tables 2.3, 2.4].** Whereas, Pomeroy, only failing by recanalisation and fistula, is the predominant method in non-negligent failures (44% of cases) **[Tables 2.3,2.4]**.

Table 2.3. Sterilisation method and time interval to pregnancy

Method of sterilisation		Filshie	Diathermy	Ring	Pomeroy or related surgical method	Overall all Groups	P value
Number in group		64	24	24	19	131	
Interval to pregnancy (months)							
Geometric Mean		7.6	11.9	8.2	14.2	9.3	$ *0.037*
95% confidence interval		6.1-9.5	8.5-16.6	7.6-9.9	11.4-17.9	8.1-10.6	
Range of time intervals to pregnancy (months) for each method							
Negligent	**Non-occlusion**	2-38	3-10	4-5	No cases		
	Wrong structure	1-102	9*	7-20	No cases		
Non-negligent	**Fistula**	14*	3-44	6-10	10-48		
	Recanalisation	10*	60*	6-13	4-18		

Footnotes

$ Kaplan-Meier Log Rank (Mantel-Cox) test for interval to pregnancy difference

* Single case only, therefore no range

Table 2.4. Negligent and Non-negligent failure group compositions and intervals to pregnancy

MECHANISM OF FAILURE	NEGLIGENT		NON-NEGLIGENT		P value
Number in group	88		43		
mean interval to pregnancy and 95% CI	7.5 [6.4-8.8]		14.2 [11.8-17.2]		$ *0.001*
median interval to pregnancy and 95% CI	7.0 [6.1-8.0]		12.0 [10.6-13.5]		
Composition by method of sterilisation **Number of cases / [%]**	Filshie Diathermy Ring Pomeroy	62 [71%] 13 [15%] 13[15%] 0 [0%]	Filshie Diathermy Ring Pomeroy	2 [5%] 11 [26%] 11 [26%] 19 [44%]	*<0.001
Composition by mechanism of failure					
Mechanism	Non- occlusion 45 [51%]		Fistula 19[44%]		
mean interval to pregnancy and 95% CI	6.4 [5.2-7.9]		17.1 [12.1-24.1]		$ *0.001*
Mechanism	Wrong structure 43 [49%]		Recanalisation 24[56%]		
mean interval to pregnancy and 95% CI	8.9 [6.9-11.3]		12.4 [10.2-14.9]		

Footnotes

* Pearson Chi-Square for category composition difference

$ Kaplan-Meier Log Rank (Mantel-Cox) test for interval to pregnancy difference

3. Regression analysis of interval to failure

Given that the interval to sterilisation failure was associated with sterilisation method and mechanism of failure, and that both of these latter variables may interact with each other, a Cox regression analysis was performed. The regression showed that negligence compared to non-negligence significantly increased the hazard potential for sterilisation failure, and that negligence (p=0.004) was the only statistically significant covariate when adjusting for sterilisation method (p=0.237). The unadjusted Hazard Ratio for negligence was 1.91 (95% CI 1.31-2.77), and adjusted Hazard Ratio was 2.35 (95% CI 1.31-4.21). Therefore, interval to pregnancy was predictive of a negligent compared to a non-negligent failure mechanism, irrespective of the sterilisation method used. Specifically, the earlier the time interval to failure the greater the likelihood of negligence than non-negligence. This is graphically illustrated in **Figure 2.1.**

4. Probability of negligence for any case given the interval to pregnancy

We have assumed that sterilisation failure occurring before time t represents a test of negligence. We have calculated the various test positive (failed before or at time t) and test negative (failed after time t) likelihood ratios (LR) for negligence at various time intervals using Bayes' Theorem (**Table 2.5).** This table shows statistically significantly increasing Likelihood Ratios for negligence at successive earlier time interval increments. This is consistent with a mathematical trend that negligence is more likely the earlier the sterilisation failure occurs.

Table 2.5. Empirical probabilities and likelihood ratios at incremental time intervals.

Time interval that sterilisation failure has occurred	Negligent (n=88)	Non-Negligent (n=43)	Probability that randomly selected case is negligent from the study series given failure with time interval	Likelihood Ratio of negligence given failure within time interval (LR test positive)
Not stated	88	43	0.67*	-n/a-
0 ≤ 6	40	4	0.91	4.89 (1.87-12.77)
0-≤ 9	61	7	0.90	2.48 (1.51-4.10)
0 ≤ 12	73	22	0.77	1.62 (1.19-2.20)
0 ≤ 18	81	33	0.71	1.20 (1.01-1.43)
0 ≤ 24	83	34	0.71	1.19 (1.01-1.40)
0 ≤ 48	86	42	0.67	1.15 (1.01-1.32)

Footnotes to Table 2.5

* The pretest probability of negligence from our case series is 0.67. This corresponds to the probability of a randomly selected case of sterilisation failure being negligent when selected from our case series. However, knowledge of the time interval to sterilisation failure either increases or decreases the probability of the case being negligent as shown in the table.

Likelihood ratios (LR) are derived by dividing the cumulative probabilities of sterilisation failure occurring at or before a certain time interval (t) according to Bayes' Theorem. For example, if we consider a test as failure at or before t=10 months then the LR for test positive is

$$\frac{P(\text{Fail} \mid \text{Neg})}{P(\text{Fail} \mid \text{NonNeg})} = \frac{0.7386}{0.2791} = 2.65$$

and, the LR for test negative is

$$\frac{P(\text{NoFail<10m} \mid \text{Neg})}{P(\text{NoFail<10m} \mid \text{NonNeg})} = \frac{1-0.7386}{1-0.2791} = 0.36$$

Thus, the probability that a randomly selected case is negligent may be calculated by knowing the time interval to failure, the Likelihood Ratios at that time interval (as displayed in **Table 2.5)** and the Bayesian equation:

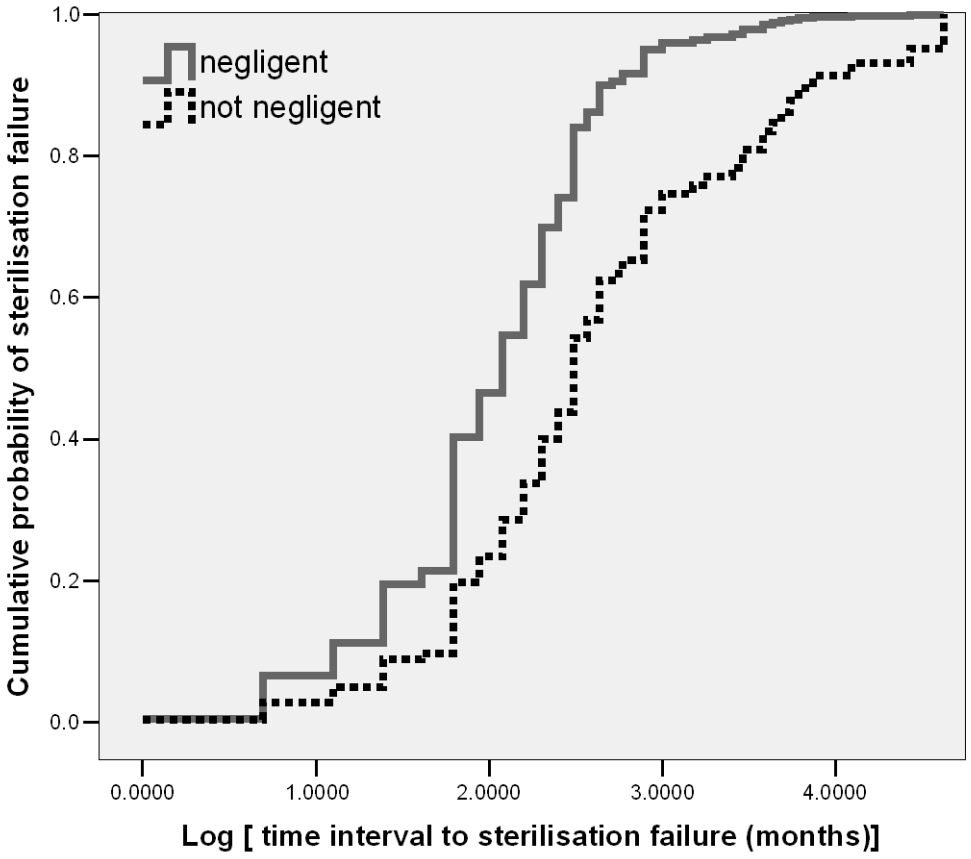
PRE TEST ODDS	X	LIKELIHOOD RATIO FOR THAT TIME INTERVAL	=	POST TEST ODDS

$$\text{Odds} = \frac{\text{Prob.}}{1-\text{Prob.}} \qquad \text{Prob.} = \frac{\text{Odds}}{1+\text{Odds}}$$

From our case series (88 negligent, 43 non-negligent), the pre-test probability of negligence was 0.67 (88/88+43). However, our case series is highly selected. Therefore we suggest using a pre-test probability of negligence of 0.5 (Odds=0.5/1-0.5= 1). This pre-test probability would correspond to that used in legal proceedings in cases with unknown mechanism of failure and therefore derivation of the post-test probability of negligence (using the Bayesian equation or Fagan's nomogram) would be useful within this medicolegal context..

Let us suppose that a sterilisation failure occurred at 8 months and the pre-test probability of negligence is 0.5. The post-test probability of negligence for a case that fails before or at 8 months is 0.73 (pre-test odds of 1 x LR 3.70=3.70 post test odds; probability is 3.70/1+3.70= 0.79). In contrast, the post-test probability of negligence if failure had occurred after 8 months is 0.32 (pre-test odds of 1 x LR 0.48=0.48 post test odds; probability is 0.48/1+0.48). This suggests that failure at 8 months is likely to be negligent because the probability distribution is greater in the negligent (0.73) than non-negligent (0.32) direction from a pre-test probability of 0.5 **(see Figure 2.1**).

Figure 2.1. The probability of sterilisation failure for negligent and non-negligent cases against time interval to failure (Cox Regression model)



Footnotes

The graph depicts the 1-minus survival function plot of the adjusted Cox regression model function i.e. incorporates both sterilisation method and failure mechanism covariates. All cases have ultimately failed, therefore for both negligent and non-negligent cases the cumulatively probability is 1 at the maximum recorded time interval for each group.The hazard ratio corresponds to the odds that a case in the negligent group fails before a case in the non-negligent group. Thus, there is a 70% probability (converting Hazard odds of 2.35 to probability by 2.35/ (1+2.35)) that sterilisation failure will occur earlier in a negligent case than a non-negligent case, irrespective of the sterilisation method used. Furthermore, comparing median times (**Table 2.3)**, negligence reduces the time interval to failure by approximately 5 months (or 42%) compared to non-negligence.

Let us suppose that a sterilisation failure occurred at 18 months and the pre-test probability of negligence is 0.5. The post-test probability of negligence for any case that fails before or at 18 months is 0.55 (pre-test odds 1 x LR 1.20=1.20 post test odds; probability is 1.20/1+1.20= 0.79). In contrast, the post-test probability of negligence if failure had occurred after 18 months is 0.25 (pre-test odds 1 x LR 0.34=0.34 post test odds; probability is 0.34/1+0.34). This suggests that failure at 18 months is likely to be non-negligent because the probability distribution is greater in the non-negligent (0.25) than negligent (0.55) direction from a pre-test probability of 0.5 (**see Figure 2.1**).

DISCUSSION Analysis of our selected series of failed sterilisations has shown that a short interval to failure, and a long interval to failure are suggestive of a negligent and non-negligent failure mechanism, whilst intervals between the two extremes are less reliable indicators of the mechanism of failure. Negligence compared to non-negligence reduces the interval to failure by 5 months. A test of negligence may be applied to any case of sterilisation failure having been provided the time interval to pregnancy and the pre-test probability, as we have obtained likelihood ratios for the test at various time intervals. Such a test may have important medico-legal ramifications in cases with unknown mechanism of failure.

Our case series represents the world's largest number of failed female sterilisations with concurrent knowledge of their mechanism of sterilisation failure and interval to pregnancy. Until this study, issues involving mechanism of failure, had not been addressed by the two largest studies of sterilisation failure [2;3] or the Cochrane review [12]. We had predicted this hypothesis in our earlier qualitative systematic review [4]. Previous studies had showed differences in time interval to failure for different sterilisation methods [2]and patient age [3].

We agree there may be caveats when interpreting our results, particularly as our data series is selective. Firstly, our data series is composed of cases from 1975 onwards. Advances in training in laparoscopic procedures and laparoscopic video imaging may be under-represented in our data series leading us to overestimate the proportion of negligence (operator-fault) that may occur with earlier (1970-1990s) sterilisation failures. Secondly, our study sample is not derived from a repository of systematically investigated and recorded sterilisation failures. Thirdly, although NHSLA has systematically collected data on litigated cases in England since 1995, there are many exclusion criteria allowing hospitals to locally manage some failed sterilisation cases thereby limiting case ascertainment. We were unable to examine the individual records from the NHSLA and MPS databases to verify the accuracy of the failure mechanism reported. Consequently, we are uncertain whether there are inconsistencies in the classification of failure mechanism used. Fourthly, we anticipate a general under-reporting of non-negligent sterilisation failures in the published literature and in the legal databases that we used for the study. Therefore, it is likely that our overall estimate of the prevalence of negligence (i.e. pre-test probability of 0.67, 88/88+43) from our case series is likely to exceed the upper limit of prevalence that would be obtained from the true population of systematically acquired sterilisation failures.

Negligence litigation in the UK is based on the claimant producing the burden of proof (prove negligent action has occurred) and the standard of proof is the civil standard (balance of probabilities). The claimant has to show that the harm suffered (i.e. failed sterilisation) on the balance of probabilities, is more likely than not to be caused by a negligent action than non-negligent action. In this legal situation, an unknown mechanism of sterilisation failure could be presumed to have a pre-test probability of negligence of 0.5 (legal equivalence). If a case had failed at say 8 months, then applying our test of failure before or at 8 months (post

test probability of 0.73) and failure after 8 months (post test probability of 0.32) indicates that failure at 8 months is more likely to be negligent than non-negligent. Furthermore, for any given interval to pregnancy, the post-test probabilities of negligence for failure before or after a specified time interval could be derived using the Bayesian methodology discussed in this manuscript. Although our test provides an overall probability of negligence >0.5 or <0.5 and therefore satisfies the legal test of negligence or non-negligence, we would always endorse that the actual negligent or non-negligent cause of sterilisation failure can only be established after a systematic clinical, histopathological and X-ray examination process.

A national register of systematically collected and investigated failed sterilisations, as recommended by the RCOG [1], would quantify the exact prevalence (pre-test probability) of negligent and non-negligent failure mechanisms, and show how this proportion is distributed amongst the various sterilisation methods, enabling its use in the legal situation described above. Little is known on non-negligent failure mechanisms due to poor case ascertainment, but such a registry may show that the probability of a non-negligent sterilisation failure equated to the probability of a negligent sterilisation failure for a particular sterilisation method, which would then make any legal claim for negligent sterilisation unlikely to succeed. Furthermore, such a registry could identify areas of substandard care that could be used as an impetus to improve medical training and design effective clinical risk prevention strategies.

2.2. The effectiveness of a levonorgestrel-releasing intrauterine system (LNG-IUS) in the treatment of endometrial hyperplasia – a long-term follow-up study.

OBJECTIVES: Medical treatment of non-atypical endometrial hyperplasia with oral progestogens has limited efficacy and poor compliance. A levonorgestrel-releasing intrauterine system (LNG-IUS) has been shown to successfully treat hyperplasia in small-sized studies. Our aim was to examine the effectiveness of LNG-IUS in a larger study with long term follow up.

METHODS: Prospective observational study of 105 women diagnosed with endometrial hyperplasia and treated with LNG-IUS between 1999-2004 at a University Teaching hospital. Baseline characteristics and outpatient endometrial Pipelle sampling was undertaken at 3 and 6 months post LNG-IUS insertion and 6-monthly intervals thereafter in all cases. Outcome included histological data derived from both Pipelle and uterine histologies at one and two years LNG-IUS therapy.

RESULTS: LNG-IUS achieved endometrial regression in 90% (94/105) of cases by two years, with a significant proportion (96%, 90/94) achieving this within one year. Regression occurred in 88/96 (92%) of non-atypical and 6/9 (67%) of atypical hyperplasias, and in all 22 cases of endometrial hyperplasia associated with HRT. Regression rates did not differ between histological types of hyperplasia. Twenty-three (22%) underwent hysterectomy of which 13 were indicated and 10 were performed at patient request despite regressed endometrium. Two cases of cancer (one uterine and one ovarian) were identified.

CONCLUSION: LNG-IUS is highly effective at treating endometrial hyperplasia. Beneficial effects are observed by the majority within one year. Treatment can be reliably monitored through regular 6-montly outpatient endometrial Pipelle surveillance. LNG-IUS treatment of non-atypical hyperplasias is likely to reduce the number of hysterectomies performed in this subgroup.

INTRODUCTION Endometrial hyperplasia may be divided into three principal histological categories listed in the order of ascending architectural and cytological abnormality: simple, complex and atypical hyperplasia [13] . Cytological atypia is the most important prognostic factor for progression to carcinoma [14]. Around 1-3% of non-atypical hyperplasias progress to endometrial carcinoma, over a mean duration of 10 years. In contrast, 8-30% of atypical hyperplasias progress to carcinoma over a mean duration of 4 years [15]. Pooling three observational studies [16-18] the rates of spontaneous regression after expectant treatment for non-atypical (n=129) and atypical hyperplasia (n=28) are around 72% and 54% respectively.

The objectives of treating women with endometrial hyperplasia are to reduce abnormal bleeding symptoms and to prevent progression to endometrial cancer[18-20]. In view of an increased oncogenic potential with atypical endometrial hyperplasia, hysterectomy is generally recommended unless fertility issues or significant risk factors for surgery preclude this. However, for non-atypical endometrial hyperplasia, there is debate as to whether hysterectomy is 'over-treatment' given the low risk of malignant transformation, high probability of possible spontaneous resolution, low risk of coexistent uterine cancer and high therapeutic responsiveness to oral progestogen therapy. Nonetheless, oral progestogens are associated with poor compliance and systemic side effects that may limit overall efficacy [18;19;21]. Levonorgestrel-releasing intrauterine system (LNG-IUS) may be used to successfully treat endometrial hyperplasia without incurring the disadvantages of oral progestogens. This finding has been demonstrated in two recently published observational studies [22;23], together with a systematic review [24] that included four limited sized studies[25-28]. Our objective was to examine the effectiveness of LNG-IUS to treat endometrial hyperplasia in a larger prospective observational study with a long-term follow-up period.

METHODS All women participating in this study had presented to our hospital (Birmingham Women's Hospital, England) for the investigation of abnormal uterine bleeding. Their reasons for referral included: women aged 40 years and over with heavy menstrual bleeding or intermenstrual bleeding aged unresponsive to medical therapies (such as tranexamic acid, combined oral contraceptive or oral progestins), post-menopausal bleeding and unscheduled bleeding whilst on hormone replacement therapy or tamoxifen. Natural menopause was recognised to have occurred if there had been at least 12 consecutive months of amenorrhoea, for which there was no other obvious pathological or physiological cause. Clinical investigation involved transvaginal pelvic sonography, outpatient endometrial Pipelle sampling (Laboratoire C.C.D, Paris, France) and outpatient hysteroscopy in all cases. Intrauterine polyps that were identified at hysteroscopy were removed using outpatient local anaesthetic Versapoint® (Gynecare, Ethicon Inc. USA) polyp resection or blind polypectomy techniques.

Endometrial hyperplasia was subdivided into three categories: simple, complex and atypical. For the purposes of this study, we grouped simple atypical and complex atypical hyperplasias as one atypical hyperplasia group. The criteria for diagnosing endometrial hyperplasia and endometrial regression of hyperplasia following LNG-IUS use was as we[29] and others [13;30-32] have previously described. Typically, LNG-IUS resulted in atrophy of glands separated by plump, polygonal, pseudodecidualised stromal cells. These were accompanied by varying degrees of secretory glandular changes and Metaplasia of the lining epithelium. These changes have been collectively and loosely termed as "regression" of hyperplasia in this article. This is not a defined histological entity except in the context of follow up of endometrial hyperplasia. Similar morphology can be seen with both oral progestogens and intrauterine progestogen (LNG-IUS) when used for other clinical indications.

Our study included cases where hyperplasia was only present in the endometrial polyp but not the background endometrium, a phenomenon also described by a previous study [33].All histopathological diagnoses were undertaken by two experienced consultant histopathologists (TR, RG) working independently; referral to the other pathologist for a second opinion was made in cases where there was diagnostic doubt, and a mutual consensus was then achieved.

Throughout the study period (January 1999-January 2004) there were 114 women diagnosed with non-atypical hyperplasia. All were offered oral progestogens, LNG-IUS insertion (Mirena®, Schering Health Care, Burgess Hill, UK) or hysterectomy as part of our routine practice; those opting for LNG-IUS (n=105) were included in our study cohort. Women diagnosed with atypical endometrial hyperplasia were recommended to undergo hysterectomy. Women who declined surgery or who were medically unfit to undergo surgery were offered oral progestogens or LNG-IUS insertion; the latter LNG-IUS treated group (n=9) were included our study cohort. Women diagnosed with non-atypical endometrial hyperplasia whilst using hormone replacement therapy (HRT) were offered either withdrawal of HRT and LNG-IUS, withdrawal of HRT and oral progestagens, or HRT (either estrogen replacement therapy or continuous combined preparations) and LNG-IUS; those opting for combinations involving LNG-IUS (n=22) were included in our study cohort.

Baseline data and study design

Insertion of LNG-IUS took place between January 1999 and January 2004. For all women in the study (n=105) anonymised baseline data was recorded on: histological subtype, sociodemographic characteristics [with emphasis on risk factors for endometrial hyperplasia such as parity, body mass index, diabetes, hypertension], use of exogenous hormones (e.g. hormone replacement therapy, tamoxifen), and presenting with abnormal bleeding symptoms. Study participants underwent regular outpatient clinic review and endometrial histological surveillance by outpatient Pipelle sampling. Histological surveillance was performed at 3-months and 6-months following LNG-IUS insertion, and continued thereafter at 6-monthly intervals in all cases (n=105). We present the outcome for participants at 1 and 2 years post LNG-IUS insertion, however, in clinical practice, we are continuing to prospectively record outcome beyond this time, even in cases that show endometrial regression. LNG-IUS treatment was abandoned and hysterectomy recommended if:-

1. There was no histological evidence of partial or complete regression of the hyperplasia by 12 months of LNG-IUS use.

2. There was histological evidence of endometrial cancer or progression of endometrial hyperplasia to atypia.

3. There was reversion to the original endometrial histology showing hyperplasia following a period of endometrial regression.

4. The primary outcome was the proportion of women with complete regression of the endometrial hyperplasia according to both outpatient endometrial Pipelle and uterine histologies at hysterectomy. Secondary outcomes included time to disease regression, the proportion of women undergoing hysterectomy (histologically indicated or non-histologically indicated) and the accuracy of outpatient Pipelle compared to uterine histology at hysterectomy.

Statistical analysis

SPSS version 13 for Windows (Release 13.0, 1 Sep 2004, SPSS Inc.) was used. The significance of different histological subtypes and other covariates on time interval to regression was determined by Kaplan-Meier and Cox-regression survival analysis. A P value less than 0.05 was considered statistically significant. Sensitivity, Specificity and Likelihood Ratios were derived by constructing a 2 by 2 table and using standard techniques[34].

RESULTS

Baseline characteristics

There were 105 women with endometrial hyperplasia (simple 16, complex 80, atypical 9) included in the 5-year study period. A summary of the baseline characteristics and presenting symptoms are shown in **Table 2.6**. The mean age was 54.5 ± SD 10.1 years (range 37-88). The study comprised of 37 premenopausal and 68 postmenopausal women. Most women presented with postmenopausal bleeding (n=68). Endometrial polyps were visualised in 36/105 (34%) cases at hysteroscopy. Hyperplasia in the endometrial polyp, but not in the background endometrium, occurred in 16% (17/105) of cases; all remaining cases had endometrial hyperplasia identified within the endometrium.

Endometrial regression at 2 years post LNG-IUS insertion

Figure 2.2 summarises the outcome of the 105 hyperplasias that received LNG-IUS according to pre-treatment and 2-year *outpatient endometrial Pipelle histologies*. In contrast, **Table 2.7** summarises the outcome of the study according to histological data derived from *both outpatient endometrial Pipelle and hysterectomy histologies* at 1 and 2 years post LNG-IUS insertion. The derivation for the data are explained in the footnotes to **Figure 2.2** and **Table 2.7**.

Table 2.6. Baseline characteristics (n=105) of LNG-IUS treatment of endometrial hyperplasia

Characteristic	Size of parameter
Age (years)	Mean 54.5 (St Dev 10.1, Range 37-88)
Weight (kg)	Mean 86.0 (St Dev 28.0, Range 50-168
BMI kg/m^2	Mean 32.0 (St Dev 8.8. Range 18-67)
Characteristic	**Percentage of cases in study group (equals number of cases)**
Parity [a]	21% (22) Parity 0 43% (45) Parity 1 or 2 23% (24) Parity 3 or higher Mean 1.87; St Dev 1.34, Range 0-5
Menopausal status	35% (37) Premenopausal; 65% (68) Postmenopausal
Diabetes	18% (19)
Hypertension	30% (31)
Exogenous HRT	21% (22)
Exogenous tamoxifen	1% (1)
Abnormal bleeding symptoms on presentation	27% (28) Premenopausal, abnormal uterine bleeding 9% (9) Premenopausal, unscheduled bleeding with HRT 51% (54) Postmenopausal bleeding 13% (14) Postmenopausal, unscheduled bleeding with HRT or tamoxifen

Footnotes

[a] Missing parity data in 14 cases

Table 2.7. Outcome of the study according to histological data derived from outpatient endometrial Pipelle and hysterectomy histologies

Endometrial Hyperplasia (number of cases at study commencement)	Total number of cases regressing with LNG-IUS	[a] Mean time for regression (months) and 95% Confidence limits	Proportion achieving regression [b] by 12 months of LNG-IUS	Proportion achieving regression [b] by 24 months of LNG-IUS
Simple (n=16)	15 (94%)	6.2 (4.4-8.0)	15/16	15/16
Complex (n=80)	73 (92%)	9.4 (7.0-11.7)	69/80	73/80
Atypical (n=9)	6 (67%)	8.2 (5.2-11.3)	6/9	6/9
Overall group (n=105)	94 (90%)	9.0 (7.0-11.1)	[c] 90/105	[c] 94/105

2. 1 Outcome of study according to histology from Pipelle or hysterectomy

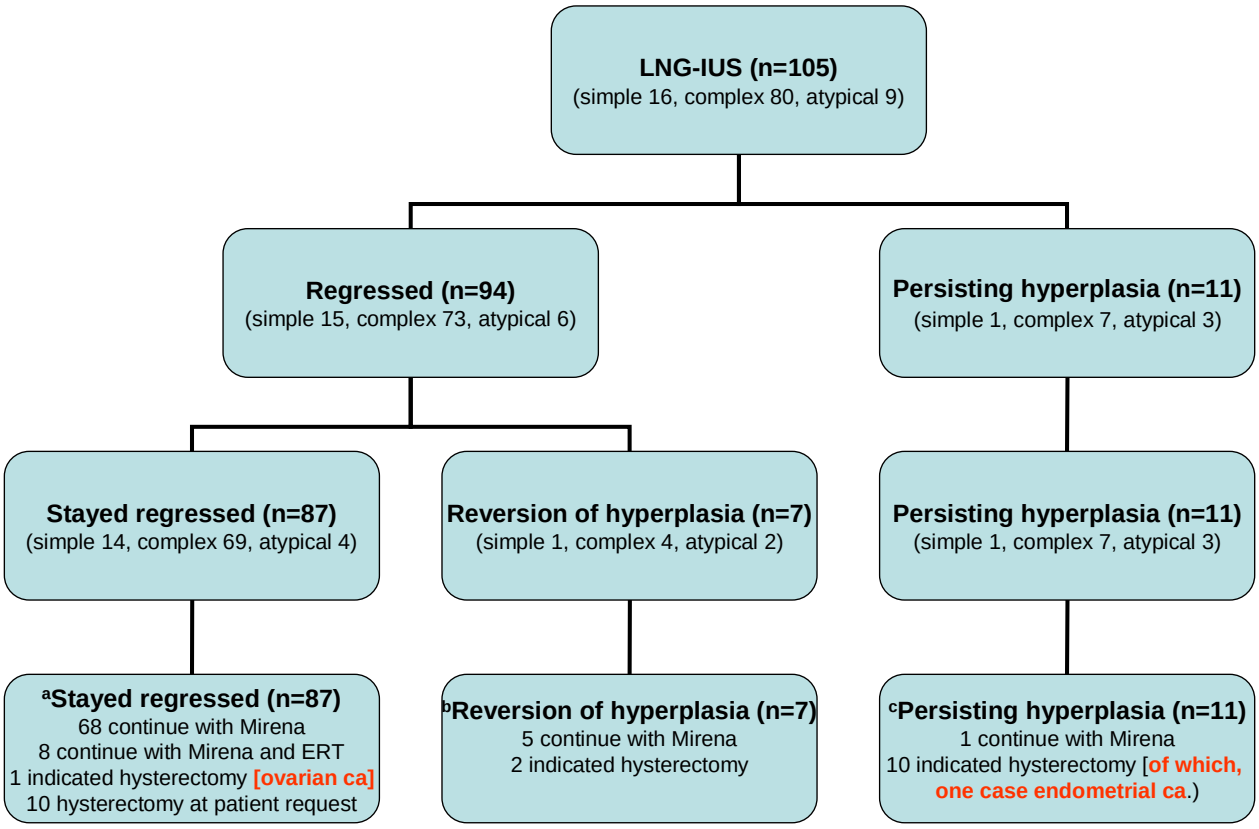
Footnotes

[a] There are no statistically significant differences in probabilities of regression over time between simple, complex and atypical hyperplasias [Kaplan-Meier Log Rank Mantel-Cox (p=0.20)).

[b] Data on histological regression is derived from combined use of outpatient endometrial Pipelle and hysterectomy histologies.

[c] Two of the 94 cases that shown regression on Pipelle, were subsequently identified to have atypical hyperplasia (one case, formerly simple hyperplasia) and ovarian cancer (one case, formerly complex hyperplasia). The former case underwent hysterectomy at patient request due to troublesome abnormal bleeding side effects with LNG-IUS despite Pipelle regression. The latter case underwent hysterectomy as this was indicated through ongoing sonographic surveillance for a postmenopausal cyst concurrent with the regressed Pipelle. Both cases were identified within one year of LNG-IUS treatment.

Figure 2.2. Outcome of study according to outpatient endometrial Pipelle histology at pre-treatment and 2-years following LNG-IUS insertion



Footnotes

[a] Of the 10 hysterectomies at patient request from the stayed regressed group, histologies from the uteri showed nine regressed uteri and one atypical endometrial hyperplasia. This is further explained in **Table 2.7, footnote c**.

[b] Of the 7 reverted hyperplasias, all were non-atypical hyperplasias on Pipelle, all were offered hysterectomy, five declined hysterectomy in favour of continuing with LNG-IUS. Of the 2 indicated hysterectomies performed, histological analysis showed one had regressed and one had complex hyperplasia.

[c] Of the 11 persisting hyperplasias, all were offered hysterectomy, one declined hysterectomy in favour of continuing with LNG-IUS. Of the 10 indicated hysterectomies performed, histological analysis showed two had regressed, one simple, four complex, two atypical hyperplasias persisted and one case of Stage 1A endometrial cancer.

Outpatient endometrial Pipelle regression was observed in 94/105 cases, and of these, 87/94 continued to maintain endometrial regression at 2 years follow up **(Figure 2.2).** Failed treatment, indicated by persisting Pipelle hyperplasia or hyperplasia that regressed then reverted to hyperplasia, occurred in 18/105 cases **(Figure 2.2).**

Overall, 90% (94/105) of the study participants achieved endometrial regression according to combined outpatient Pipelle and hysterectomy histologies (**Table 2.7**). A significant proportion (96%, 90/94) had achieved this by one year of LNG-IUS use.

Survival analysis methods (Kaplan-Meier, Cox proportional hazard) showed there was no statistically significant difference between the types of hyperplasia in terms of the time interval to regression **(Table 2.7).** The overall mean interval to regression was 9 months (95% CI 7.0-11.1) for the overall group **(Table 2.7).** Furthermore, survival analysis showed no statistically significant association of baseline covariates (age, parity, menopausal status, BMI, diabetes, hypertension, exogenous estrogen or tamoxifen use) on the rate of regression.

Endometrial hyperplasia associated with Hormone Replacement Therapy (HRT)

Of the 22 cases of HRT associated endometrial hyperplasia and treated subsequently with LNG-IUS, 2 stopped HRT, 17 continued with cyclical combined HRT and 3 opted for estrogen only HRT. All were non-atypical hyperplasias (19 complex and 3 simple), and all, apart from one case, showed endometrial regression with LNG-IUS therapy. The non-regressed complex hyperplasia underwent hysterectomy and uterine histology subsequent confirmed endometrial regression had in fact occurred. There was a single case of tamoxifen associated complex hyperplasia which initially regressed with LNG-IUS then reverted back to complex hyperplasia; uterine histology at hysterectomy confirmed complex hyperplasia.

Two cases of cancer

Two cases of cancer were identified. One case was Stage 1B ovarian cancer, which had been identified in a complex hyperplasia that had regressed at 3 months with LNG-IUS but had been under ultrasonographic surveillance for a persistent postmenopausal ovarian cyst. The other case was Stage 1A endometrial cancer, which had been identified in a case of complex hyperplasia that had shown non-regression at 12 months with LNG-IUS and therefore underwent indicated hysterectomy (**Figure 2.2**).

Hysterectomy and correlation with endometrial Pipelle

Hysterectomy occurred in 23/105 women, and a summary of the origin and indication for hysterectomy is shown in **Figure 2.2**. Most hysterectomies (12/23) were performed for persisting hyperplasia and reversion to hyperplasia following initial regression to normal histology. However, 10/23 hysterectomies were performed in women with endometrial regression on Pipelle histology. The reasons cited included: worsening or persistence of abnormal bleeding symptoms (3), patient request (4), patient fear of progression to cancer (1), uterine prolapse (1) and concurrent cervical intraepithelial neoplasia (1). In all these cases the endometrium was extensively sampled, including the cornual aspects, and showed changes secondary to the local progestogen therapy without any evidence of hyperplasia. Using histology of the uterus at hysterectomy as the "gold standard" and the preceding endometrial Pipelle biopsy as a diagnostic test, then Pipelle had a sensitivity of 83% and specificity of 73% for identifying endometrial regression **(Table 2.8**).

Table 2.8 Correlation between endometrial Pipelle histology and hysterectomy histology (n=23 hysterectomies)

		Uterine Histology at Hysterectomy	
		Regressed endometrium	**Not regressed endometrium**
Endometrial Pipelle biopsy	Test positive: showing regression	10	3
	Test negative: showing non-regression	2	8

Sensitivity 83%
Specificity 73%
Likelihood ratio (95% confidence interval)
LR (positive test) 3.06 (1.23-8.74)
LR (negative test) 0.23 (0.06-0.70)

DISCUSSION

LNG-IUS is highly effective at treating endometrial hyperplasia, irrespective of whether non-atypical or atypical hyperplasia is being treated. Beneficial effects are observed by the majority within one year of treatment. Treatment success can be reliably monitored through regular 6-monthly outpatient endometrial Pipelle surveillance. Future widespread use of LNG-IUS to treat non-atypical hyperplasias is likely to reduce the number of hysterectomies performed for this condition, and thereby avoid exposing women to unwarranted surgical risks.

This is the largest published series of the use of LNG-IUS to treat endometrial hyperplasia [24;26;28;35-38]. Furthermore, we believe this is the first study to examine the use of LNG-IUS to treat endometrial hyperplasia occurring in HRT users. The prospective design and strict data collection proforma used in this study ensured uniform inclusion/exclusion criteria and

reliable collection of all outcome measures. The study was designed as a pragmatic measure of the effectiveness of LNG-IUS at one and two-years, therefore our results are applicable to current clinical practice.

Our study could be criticised for not incorporating a control (expectant management) or cohort (e.g. oral progestogens) comparison group. Furthermore, our study is under- powered to detect genuine differences in subtypes of endometrial hyperplasia, as well as investigate their significance along with other covariates (e.g. diabetes, hypertension, HRT) on the likelihood of regression with LNG-IUS treatment.

It has been established that outpatient endometrial biopsy is accurate in diagnosing endometrial hyperplasia [39]. However, we accept there may be uncertainty in our estimations of sensitivity and specificity of endometrial Pipelle in correlating to uterine histology. This is because we only performed hysterectomy and obtained 'gold standard' uterine histology in around a quarter of study participants, and there may be differences in histological criteria used by others and our own group. Nonetheless, by finding similar degrees of test accuracy as previous authors [18;40-43]we believe our results are at least consistent with the published literature. Furthermore, we minimised the histopathological bias by utilising strict predefined histological criteria and limiting the histological interpretation to two experienced Histopathologists.

Overall, our study's 90% (94/105) endometrial regression rate incorporates regression rates of 92% (88/96) and 67 % (6/9) for non-atypical and atypical hyperplasias, respectively. A higher regression rate of 95% (19/20) with regression rates of 100% (12/12) and 88%(7/8) for non-atypical and atypical hyperplasias had been observed in a recently published long-term study [44]. This difference could be explained by the longer duration of follow up in the published study [45]. Nevertheless, our study's non-atypical regression rate is similar to the oral progestogen treatment regression rate (93%, n=134) [46]and exceeds the expectantly managed regression rate of 72% (93/129) identified by pooling studies [16-18]. This study's atypical regression rate does not significantly differ from the expectant regression rate of 54% (15/28) identified from the same pooled studies. Importantly, this study suggests a trend for intrauterine progestogen therapy to regress non-atypical rather than atypical hyperplasia, which is a finding that has also been suggested by other groups [46-51].

We would have expected LNG-IUS use in our study to have led to a greater reduction in hysterectomy treatment for hyperplasia. However, for a variety of unexpected reasons (e.g. personal choice, fear of progression) in addition to those due to failed medical treatment or unwanted side-effects with LNG-IUS, women opted for hysterectomy. We were unable to further explore how such patient preferences could impact on patient satisfaction, compliance and cost-effectiveness of LNG-IUS compared to hysterectomy treatment alternatives. Furthermore, as we were dealing with a pre-malignant condition, in an age group not requiring to conserve the uterus for fertility, this would lead to an increased risk of favouring a hysterectomy decision, irrespective of whether endometrial regression had been successful or unsuccessful.

Both cases of cancer identified in the study were Stage I tumours, and were readily identified within one year of insertion of LNG-IUS. It could be argued that earlier hysterectomy, instead of LNG-IUS medical treatment, would have prevented cancer development or improved prognosis if cancer was identified earlier. In this context, our study suggests around 50 hysterectomies would be needed to prevent (NNT) one case of gynaecological cancer in women with endometrial hyperplasia.

Oral progestagens and hysterectomy are widely accepted treatment options for endometrial hyperplasia [18;19;52]. Newer therapies under evaluation include endometrial ablation [53] and aromatase inhibitors[54]. Nonetheless, we believe that the success of this study, utilising LNG-IUS therapy, should provide an impetus for future robust randomised controlled trials to evaluate the effectiveness of medical and surgical treatments in treating endometrial hyperplasia. Successful validation of the treatment potential of LNG-IUS for endometrial hyperplasia will undoubtedly reduce the number of women undergoing hysterectomies for this condition and avoid exposing them to unwarranted surgical risks.

2. 3. Hospital recovery following Thermachoice ablation is not dependent on setting (outpatient or daycase) or rescue analgesia: unexpected result

Background: Thermal balloon endometrial ablation (TBEA) is increasingly being performed in the outpatient setting under local anaesthesia (LA) rather than in a daycase setting under general anaesthesia (GA). Our aim was to compare the post operative *rescue* analgesia requirements and duration of hospital say in women undergoing outpatient (LA) and daycase (GA) TBEA.

Methods: Prospective observational study of consecutively recruited women who underwent outpatient (LA) TBEA (n=51) and daycase (GA) TBEA (n=50) over the same time period. Analgesia that was provided additional to the standard administered analgesic regimen was considered *rescue analgesia*. The main outcome measures were requirement for *rescue analgesia* and duration of hospital stay in both cohorts.

Result(s): LA compared to GA cohorts had shorter hospital stays (11 hours [95% CI 9-13] vs. 17 hours [95% CI 14-20]) and lower analgesia requirements. However, multivariate regression, correcting for all known confounders, showed that duration of stay was independent of setting for ablation or amount of rescue analgesia.

Conclusion(s): Duration of hospital stay is not entirely dependent on whether outpatient or daycase endometrial ablation is considered. This unexpected preliminary finding deserves to be validated in future confirmatory trials that compare outpatient and daycase treatments. We also discuss the confounding factors that should be considered when designing such trials.

Abbreviation(s): TBEA: Thermal Balloon Endometrial Ablation; LA: Local anaesthesia; GA: General Anaesthesia; CI: Confidence Interval.

INTRODUCTION Menorrhagia has a considerable impact on many women's lives [55]. Endometrial ablation is being increasingly used as a treatment option [56] and is endorsed by National Institute for Health and Clinical Excellence, NICE, UK [55]. There is wide variation in the preferred endometrial ablation device and whether treatment should be performed in the outpatient local anaesthetic (LA) or daycase general anaesthesia (GA) setting [57-61].

Outpatient therapy has obvious advantages in terms of safety, convenience and short discharge time for the woman, and may be preferred over GA for women with high risk medical conditions[62;63]. We [64], along with other groups [65-68], have had considerable experience and success in performing outpatient thermal balloon endometrial ablation (TBEA). We perform local anaesthetic (LA) thermal balloon endometrial ablation in the conscious patient without sedation at any time in the menstrual cycle and without prior endometrial preparation.

There is considerable heterogeneity in postoperative pain and duration of hospital stay reported for LA and GA endometrial ablations. This may be partly explained by differences in peri-operative analgesic regimens adopted by such studies. Even if such confounding influences are minimized, it remains unclear whether women experience higher levels and/or prolonged duration of pain during and after LA TBEA compared to GA TBEA. This information would be particularly important when counseling women on their choices between LA and GA TBEA. Given the paucity of robust data to answer this concern[64;69;70], we conducted a prospective study to compare rescue analgesia requirement and duration of hospital stay in LA and GA TBEA.

MATERIALS AND METHODS:

Patient Population Pre-menopausal women with subjectively defined heavy menstrual bleeding were referred by primary care (GP) and / or by secondary care physicians for assessment in our menstrual disorders clinic. Our routine practice was to offer a first line trial of medical treatments for at least 6 months if there was no clinical suspicion of underlying pathology. The medical treatments included Levonorgestrel-releasing intrauterine hormone system (LNG-IUS; Mirena®, Schering Health Care), combined oral contraceptive, progestogens (oral and long-acting), tranexamic acid and /or mefenamic acid.

All women underwent transvaginal pelvic sonography, hysteroscopy and outpatient endometrial Pipelle biopsy (Laboratoire C.C.D, Paris, France) investigations. Any significantly sized intrauterine polyps (greater than 2cm in size) were excised by either blind polyp forcep avulsion or Versapoint [Gynecare, Ethicon Inc. USA) resection. Women were excluded from the study if there were significantly sized uterine fibroids (fibroids greater than 3cm size in any uterine location), enlarged uterine size (uterine cavity length greater than 10cm), abnormally shaped uterine cavity (e.g. bicornuate uterus), endometrial hyperplasia or cancer, or active pelvic infection.

Women with normal sized uteri (less than 10cm cavity size), no underlying structural uterine pathology and unresponsive to medical therapy commenced by their GP or secondary care, were offered thermal balloon endometrial ablation TBEA (either under LA or GA) and hysterectomy as second-line treatments. Those women who opted for TBEA were given the choice of undertaking the procedure under LA or GA.

Study design Recruitment for the study occurred in a prospective continuous manner between June 2003 and June 2005. During this time period, two prospective consecutively recruited cohorts were established: LA TBEA and GA TBEA i.e. both cohorts were constructed and evaluated over the same time period in parallel.

Intervention Endometrial ablation was performed using a Thermachoice III (Gynecare®, Menlo Park, California, USA) device according to the manufacturer's guidance.

Local Anaesthetic TBEA This was performed in our ambulatory gynaecological clinic according to our previously described protocol [64], which included:-

Pre-procedure analgesic regimen (one to two hours prior to TBEA):

All women received diclofenac 100mg rectally, oral co-dydramol 10/500 (two tablets) and oral cyclizine 50 mg. Tramadol hydrochloride 100mg was used if non steroidal analgesia was contra-indicated.

Local anaesthetic: The cervix was directly injected in a circumferential manner with three 2mL cartridges containing 3% prilocaine hydrochloride (30 mg/mL) and felypressin 0.03 unit/mL (citanest with octapressin®, Dentslply, UK) using a 27G dental syringe.

Dedicated patient nurse: A particular nurse was allocated to provide continuous supportive care to the patient during the procedure. The nurse engaged the patient in conversation ('distraction' analgesia effect termed "vocal local") and often held the patient's hand throughout the procedure.

Post ablation day case bed stay: All women recovered in a day case bed and were allowed home after a minimum stay of 2 hours. A strict protocol of post-procedure pain relief was adhered to. A patient information leaflet was provided detailing expected symptoms and analgesic advice post LA TBEA.

General Anaesthetic TBEA Women, fasted for at least 6 hours, were admitted to hospital on the day of the procedure. In a minority of cases, women with high risk medical disorders (e.g. diabetes) were admitted the day before the planned procedure. TBEA was carried out in gynaecology theatres after induction of general anaesthesia. All women received diclofenac 100mg and 1g paracetamol rectally (or paracetamol alone if diclofenac was contraindicated) just prior to performing TBEA. Infiltration of the cervix with a local anaesthetic was not done in these women. The TBEA surgical procedure, post-procedure analgesia regimen and day case bed stay for GA TBEA were identical to the LA TBEA procedure described above.

Outcome measures Initial baseline data recorded were: age, body mass index, menorrhagia alone or combined with dysmenorrhoea, menstrual cycle phase, ultrasound and hysteroscopy findings and uterine axis. In relationship to TBEA procedure, the following data were recorded: mean intrauterine ablation pressure, successful completion of TBEA procedure, procedure related complications (e.g. vasovagal episodes for LA TBEA) and duration of hospital stay following the TBEA procedure. All women were asked to record the pain they experienced immediately following LA TBEA on a graduated Visual Analogue Scale (VAS), ranging from 0 (no pain) to 10 (worst imaginable pain), which had been validated in our previous study [64].

Rescue analgesia This refers to analgesia that was administered post TBEA that was additional to the routinely supplied peri-operative analgesia regimen. Rescue analgesia was administered at the request of the woman following nurse-led enquiry. The amount of rescue analgesia was determined according to the woman's VAS score at the time of enquiry and hierarchy of analgesia that was available on a standardised 'as required' drug prescription

chart. To quantify the amount of rescue analgesia we utilised a numerical (morphine equivalent dose) and an ordinal (mild, moderate, severe) scale was created according to the following:

a) *Morphine Equivalent Dose*. This is the estimated bioequivalent dose of morphine sulphate (in milligrams) that corresponds to the oral analgesic preparations (such as codeine phosphate, dihydrocodeine) given according to an accepted validated conversion scale [71].

b) *An ordinal ranking scale* of none, mild, moderate, strong, very strong rescue analgesia. This scale was created by the study authors, and recorded as mild (paracetamol <2g or diclofenac <100mg only), moderate (paracetamol <2g and diclofenac <100mg or low morphine equivalent dose <15mg) or strong (paracetamol>2g or diclofenac>100mg or high morphine [>15mg] dose) or very strong (paracetamol> 2g and diclofenac>100mg and morphine>15mg or high morphine [>30mg] dose) grading for strength of analgesia usage.

Post TBEA procedure (common to both LA and GA TBEA cohorts)

All women recovered in a daycase bed. Women were discharged home according to a Nurse-led care plan that required patients to have tolerated oral diet, voided urine, and have adequate pain control. All women were discharged with a patient information leaflet that described expected postoperative symptoms and were given instructions to take regular analgesics for the first 24 hours (diclofenac 50 mg three times daily and/or co-dydramol 10/500 two tablets four times daily). In addition, all women were contacted by telephone at home the following day to check on their progress.

Statistical analysis:

Dichotomous data were presented as simple proportions. SPSS version 13 was used to undertake univariate linear regression and multivariate regression analysis and to conduct Chi-square and Mann-Whitney U test for comparing the difference between the two groups. $P < 0.05$ was considered statistically significant.

Sample size & Power calculation

There was no pilot data of the expected mean and standard deviation values for the amount of analgesia used or hospital stay. Hence, a sample size calculation was not performed a priori to study commencement. However, if we assume that a clinically significant difference of the mean between two groups is 0.5 Standard Deviations, then the sample size required for an alpha of 0.05 and a power of 80% is 64 in each group. Hence, as our study recruited 101 subjects, it approaches the power required to detect this accepted clinically significant difference.

RESULTS: There were 51 and 50 women in LA and GA TBEA cohorts respectively. Baseline characteristics are depicted in **Table 2.9**. The procedure was completed successfully in all women in both cohorts. There was no serious morbidity in either cohort. Individual requirements for different analgesics are shown in **Table 2.10**. The strength of rescue analgesia was found to be statistically significantly lower in the LA compared to GA cohort: 8/51 compared to 47/50 women required moderate to strong analgesia, respectively (**Table 2.10**).

On univariate analysis, duration of hospital stay correlated to strength of rescue analgesia and type of TBEA; significantly lower in LA (11 hours; 95% CI 9 - 13 hours) compared to GA (17 hours; 95% CI 14 - 20 hours) cohorts (**Tables 2.10, 2.11 and Figure 2.3**). However, multivariate regression, correcting for identifiable confounding influences (listed in **footnotes of Table 2.11**), showed that duration of hospital stay was independent of strength of rescue analgesia and type of TBEA (**Table 2.11**). In the LA cohort, there were no postoperative complications in 44 (86%) women but 7 (14%) stayed overnight; 2 (4%) due to excessive vomiting and 5 (10%) due to pain. In the GA cohort, there were no postoperative complications in 36 (72%) patients but 19 (38%) stayed overnight; 2 (4%) due to excessive vomiting, 4 (8%) due to pain, 3 (6%) due to urinary retention, 4 (8%) due to dizziness and 6 (12%) due to medical reasons unrelated to the ablation procedure (such as hypotension, hypertension, transient oxygen requirement).

Table 2.9. Baseline and procedural characteristics of LA vs GA TBEA

	LA TBEA N=51	GA TBEA N=50	Overall N=101
Mean age years (Range)	44.1 (30-54)	42.6 (29-55)	43.4 (29-55)
Mean BMI (Range)	30.3 (19-55)	27.7 (14-45)	28.9 (14-55)
Presenting complaint			
Menorrhagia	46	40	86
Menorrhagia & dysmenorrhoea	5	10	15
Phase of cycle			
Menstrual	9	1	10
Proliferative	12	19	31
Mid-cycle	5	14	19
Secretory	25	16	41
Uterine Scan findings			
Normal	43	41	84
Polyp or fibroid	8	9	17
Uterine axis			
Anteverted	36	37	73
Retroverted	7	13	20
Axial	8	0	8
Hysteroscopic Uterine findings			
Normal	44	47	91
Polyp or fibroid	7	3	10
Intrauterine Ablation pressures (mmHg)	170	171	170
(95% CI intervals)	(164-175)	(168-174)	(168-173)

Table 2.10. Outcomes of LA vs. GA TBEA

	LA-TBEA N=51	GA-TBEA N=50	Overall N=101	Difference between LA and GA P value
Mean duration of stay (hours)	11	17	14	**0.001**
(95% CI intervals)	(9-13)	(14-20)	(12-16)	
Strength of analgesia				
None	1	0	1	**0.001**
Mild	42	3	45	
Moderate	7	7	14	
Strong	1	33	34	
Very strong	0	7	7	
Paracetamol Used (mean dose, mg)	25 (617)	42 (1760)	67 (1206)	**0.001**
Not used	26	8	34	
Diclofenac Used (mean dose, mg)	0	44 (101)	44 (52)	**0.001**
Not used	51	6	57	
Morphine Mean Equiv.Dose (mg)	13.8	14.2	14.0	0.940
(95% CI intervals)	(11.5-16.1)	(11.0-17.3)	(12.0-15.9)	

Footnotes

Statistical tests include Chi-square and Mann-Whitney U test.

Table 2.11. Regression analysis

	Duration of Hospital Stay $
LA vs. GA Thermachoice	
Univariate ^	0.001
Multivariate*	0.786
Strength of Rescue analgesia	
Univariate ^	0.001
Multivariate*	0.303

Footnotes

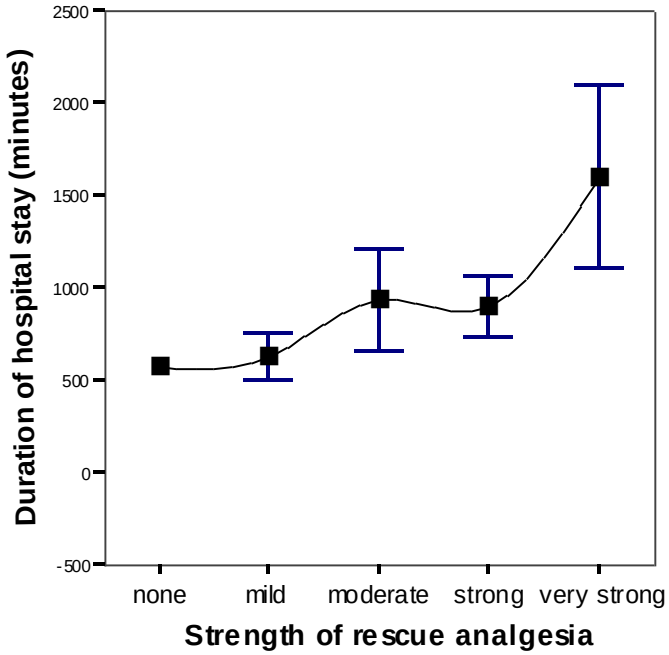
$ Univariate Linear Regression modelling

* Multivariate Regression corrected for the presence of fixed categorical factors [LA or GA; presenting complaint; uterine axis; scan findings; hysteroscopic findings; menstrual phase] and covariates [strength of rescue analgesia; intrauterine ablation pressure; uterine length; age; BMI].

^ All statistical models were statistically significant (P<0.001) apart from final multivariate regression model.

Figure 2.3. Correlation of duration of stay with strength of analgesia for combined LA and GA TBEA cohort

Statistically significant correlation (Pearson P=0.001; Kendall P=0.001)



Footnotes Central box dot shows Mean. Error Bars show 95% Confidence Interval of Mean.

DISCUSSION:

This preliminary study suggests that duration of hospital stay is independent of setting (outpatient or daycase) of endometrial ablation or amount of rescue analgesia administered. Even though on direct observation it appears that there may be shorter post-recovery times and lower rescue analgesia with outpatient compared to daycase ablation. This information may be useful for preoperative counselling, but its unexpected result deserves to be validated in future confirmatory trials.

To date, there is a dearth of evidence comparing outpatient LA and GA daycase hysteroscopic based treatments, including endometrial ablation [55;57-61]. We believe our study is the largest sized comparison of LA and GA endometrial ablation, and exceeds the size of the recently published RCT comparison of outpatient and daycase Thermachoice [65]. Introduction of study bias was minimized by the prospective continuously recruited cohort study design and adopting standardized regimens for perioperative analgesia and post-operative care. The study was conducted in a pragmatic manner and therefore our findings are applicable to current practice.

However, we accept there may be limitations that may make our conclusions less reliable. We did not utilise any specific method of reliably identifying women's individual pain thresholds (e.g. able to either tolerate outpatient endometrial Pipelle or outpatient hysteroscopy procedure) prior to ablation and so are uncertain to the prevalence of women with low-to-high pain thresholds in our two cohorts. Women who opted for LA TBEA may have an inherently higher pain threshold, received more detailed pre-procedure counselling, and be more motivated to successfully complete and recover from this procedure, than

women who opted for GA TBEA. Likewise, higher analgesia in the GA cohort may relate to the higher prevalence of reported dysmenorrhoea and retroverted uterus (possibly indicating more significant pathology such as endometriosis) compared to the LA cohort. Alternatively, it is conceivable that the local anaesthetic itself induces highly effective peri-operative analgesia and its effects are sustained over several hours. We attempted to correct for this confounding using multivariate regression. However, overall, our study is non-randomised and likely to be underpowered; the use of regression methodology in such circumstances may have led to spurious interpretation. It would have been useful to record patient satisfaction with pre-procedure counselling, as well as their original preferences for TBEA setting (even if they ultimately had a different TBEA setting) prior to the procedure and explore how these factors could impact on both short (post procedure analgesia and recovery time) and long-term outcomes (e.g. surgical re-intervention rates).

We achieved successful completion of outpatient TBEA in all our cases [100%, 51/51], which exceeds that reported by the recently published RCT [87%, 34/39] [65]. Our mean outpatient recovery time of 11 hours (which includes 7/51 overnight admissions) is considerably greater than the trial's 1 hour 40 minutes [65]. We believe these differences arise due to fundamentally differing patient selection criteria and protocols for perioperative analgesia and nurse-led discharge.

At first glance, and in agreement with a recent RCT [65], we showed that LA may result in a lower analgesia requirement and shorter recovery time period, indicating from both a cost-effective and patient's perspective that TBEA should be preferentially performed in the outpatient LA rather than currently favoured daycase GA setting. However, our "multivariate" regression, which corrected for all potential confounders and was not

undertaken by the previous trial [65], showed that there was no statistically significant association between setting (outpatient LA or daycase GA) or amount of rescue analgesia upon duration of hospital stay. This contradicts the earlier stated hypotheses that there may be inherent differences between LA and GA groups in relation to women's pain thresholds or of a "superior" analgesic effect induced through use of LA compared to GA technique. In order to define the optimum role for outpatient ablation, we recommend further RCTs directly comparing outpatient against daycase treatments. It is important that these trials are sufficiently powered, and are able to correct for the confounding influences we have discussed earlier.

2.4. Outpatient Thermachoice endometrial balloon ablation: long-term, prognostic and quality of life measures

STUDY OBJECTIVE: To compare short and long term treatment outcomes of outpatient local anaesthetic thermal balloon endometrial ablation (LA-TBEA) and identify any prognostic factors.

DESIGN: Prospective observational study

DESIGN CLASSIFICATION: II-2

SETTING: U.K. teaching hospital.

PATIENTS: 102 menorrhagic women undergoing LA-TBEA between 2001-2005.

INTERVENTIONS: Thermachoice I (n=51) and Thermachoice III (n=51) TBEA performed under local anaesthesia without conscious sedation.

MEASUREMENTS: Treatment completion, pain and analgesia, duration of stay (from admission to discharge), duration of follow up, need for secondary treatment (repeat ablation, hysterectomy or LNG-IUS), menstrual symptoms and amenorrhoea, patient satisfaction, and quality of life.

RESULTS: TBEA was completed in 97.1% of women. Mean duration of stay was 8.0 hours (95% CI 6.6-9.3). Mean follow up was 29 months (95% CI 26-32). Secondary treatment occurred in 19/102 (19%) and was more likely in Thermachoice I (15/51, 29%) than Thermachoice III (4/51, 8%). Overall, 50% of surgical re-interventions occurred by 19 months. There were high rates of amenorrhoea (29%) and treatment satisfaction (76%). Higher mean intrauterine ablation pressure was associated with increased treatment satisfaction.

CONCLUSION: Endometrial ablation can be successfully performed in the outpatient setting with better success rates achieved with Thermachoice III. Higher ablation pressures improve long term outcomes.

INTRODUCTION

There has been considerable expansion in the establishment of Outpatient 'One Stop' 'See and Treat' ambulatory clinics in the management of women with abnormal uterine bleeding [72]. Endometrial ablation is being increasingly used as a treatment option [56] and is endorsed by National Institute for Health and Clinical Excellence, NICE [55]. Outpatient therapy has obvious advantages to the patient in terms of safety, convenience and short discharge time after treatment. The health provider gains by avoidance of costs associated with in patient admission and general anaesthesia. There is wide variation in the preferred endometrial ablation device [73;74] and whether treatment should be performed in the outpatient (using local anaesthetic and/or sedation) or daycase general anaesthesia setting [69;75].

We [64], along with other groups [65-68], have had considerable experience and success in performing outpatient thermal balloon endometrial ablation (TBEA). We perform local anaesthetic thermal balloon endometrial ablation (LA-TBEA) in the conscious patient without sedation at any time in the menstrual cycle and without prior endometrial preparation.

In relation to TBEA, there are particular prognostic factors associated with favourable outcome following ablation; these include: anteverted compared to retroverted uterus, older age, shorter uterine length, lower (<10ml) intrauterine balloon volumes and higher intrauterine pressures [68;76-79]. Our aim was to compare the short and long term (minimum 12 months follow-up) treatment outcomes for outpatient LA-TBEA using Thermachoice I and Thermachoice III devices and identify any prognostic factors that may influence treatment outcome.

MATERIALS AND METHODS

Study population Recruitment for the study occurred prospectively, in a continuous manner, between February 2001 and August 2005. During this time period, we upgraded our Thermachoice device: at study commencement we used Thermachoice I (Gynecare®, Menlo Park, California, USA) and this was replaced with Thermachoice III (Gynecare®, Menlo Park, California, USA) from August 2003 onwards. Thermachoice III contained an impeller fan that provided a more even temperature gradient within the balloon and on its surface. Accepting that there may be differences in the treatment outcomes between the different devices, we have compared outcomes between Thermachoice I and III, as well as reported overall combined outcomes.

Pre-menopausal women with subjectively defined heavy menstrual bleeding were referred by primary care (GP) and / or by secondary care physicians for assessment in our menstrual disorders clinic. In the clinic, all patients are assessed on the need for treatment based on the impact of heavy menstrual bleeding (HMB) on the patient's quality of life, reported menstrual symptoms, presence of gynaecological pathology (all women routinely had pelvic ultrasound), fertility requirements, and proven anaemia. Our routine practice was to offer a first line trial of medical treatments for at least 6 months if there was no clinical suspicion of underlying pathology. The medical treatments included Levonorgestrel-releasing intrauterine hormone system (LNG-IUS; Mirena®, Schering Health Care), combined oral contraceptive, progestogens (oral and long-acting), tranexamic acid and / or mefenamic acid. This method of practice has been endorsed by the National Institute of Clinical Excellence (NICE) guideline on HMB [55].All women were investigated by transvaginal pelvic sonography, outpatient endometrial Pipelle (Laboratoire C.C.D, Paris, France) and outpatient

hysteroscopy. Women with normal sized uteri (less than 10cm cavity size), no underlying structural uterine pathology and unresponsive to medical therapy commenced by their GP or secondary care, were offered endometrial ablation (either under general anaesthetic or local anaesthetic), or hysterectomy as second-line treatments. Those women who opted for LA-TBEA were invited to participate and included in this study. This population included women with regular and irregular menstrual cycles who expressed a desire for further treatment. No specific screening test (e.g. able or unable to tolerate endometrial Pipelle® biopsy without local anaesthesia) was undertaken prior to LA-TBEA in order to minimise potential bias in patient selection and maintain the pragmatic nature of the study.

Intervention LA-TBEA was undertaken in our ambulatory gynaecological clinic according to our previously described treatment protocol [64]. Essential elements of the protocol include:-

Timing of TBEA: Ablation was performed at any time during the menstrual cycle and without any prior endometrial preparation.

Pre-medication: All women received diclofenac 100mg rectally, oral co-dydramol 10/500 (two tablets) and oral cyclizine 50 mg. Tramadol hydrochloride 100mg was used if non steroidal analgesia was contra-indicated.

- *Conscious patient*: no intravenous cannulation was present. There was no use of sedation.

- *Local anaesthetic*: The cervix was directly injected in a circumferential manner with three 2mL cartridges containing 3% prilocaine hydrochloride (30 mg/mL) and felypressin 0.03 unit/mL (citanest with octapressin®, Dentslply, UK) using a 27G dental syringe.

- *Dedicated patient nurse:* A particular nurse was allocated to provide continuous supportive care to the patient during the procedure. The nurse engaged the patient in conversation ('distraction' analgesia effect termed "vocal local") and often held the patient's hand throughout the procedure.

- *Pre-ablation hysteroscopy*: All women underwent an outpatient hysteroscopy check prior to LA-TBEA. An endometrial biopsy had usually been carried out prior to the scheduled TBEA. A zero degree microhysteroscope with a 2.5-mm rigid outer sheath (Karl Storz, Tuttlingen, Germany) was used. Between 10-100mL of Normal Saline via a nurse controlled syringe was used as intrauterine distension medium. Any significantly sized intrauterine polyps (greater than 2cm in size) were excised by either blind polyp forcep avulsion or Versapoint [Gynecare, Ethicon Inc., Somerville, NJ, USA]) resection prior to LA-TBEA (**Table 2.12**). Women were excluded from the study if there were significantly sized uterine fibroids (fibroids greater than 3cm size), enlarged uterine size (uterine cavity length greater than 10cm), abnormally shaped uterine cavity (e.g. bicornuate uterus), endometrial hyperplasia or cancer, or active pelvic infection.

- *Type of Thermachoice device:* Thermachoice I (February 2001-July 2003) and Thermachoice III (August 2003-August 2005) devices were used.

- *Intrauterine ablation pressure*: The manufacturer recommends this is maintained between 160mmHg and 180mmHg. However at the discretion of the operator, the upper limit of pressure was controlled in manner so that it did not exceed 195mmHg. This was consistently applied in both Thermachoice I and III groups (**Table 2.13**).

Table 2.12. Baseline demographic data for outpatient TBEA

	Thermachoice I (N=51)	**Thermachoice III (N=51)**	**Thermachoice I and III N=102**	**P-value (Thermachoice I vs. III)***
Mean Age (95% CI; St Dev)	43.4 (41.9-44.8;4.7)	44.1 (42.4-45.7;5.6)	43.7 (42.6-44.8;5.2)	0.118
Mean BMI (95% CI; St Dev)	28.8 (26.5-31.1;7.4)	30.3 (28.0-32.6;7.8)	29.6 (28.0-31.2;7.6)	0.318
Indication for Ablation				
Menorrhagia alone	43	46	89	0.373
Menorrhagia & severe dysmenorrhoea	8	5	13	
Cycle phase^				0.167
Proliferative	18	11	29	
Mid-cycle	11	15	26	
Secretory	17	25	42	
Uterine axis^				0.539
Anteverted	20	36	56	
Axial	2	8	10	
Retroverted	5	7	12	
Ultrasound scan findings				**0.029**
Normal	34	45	79	
Polyp	5	1	6	
Fibroid $	12	5	17	
Hysteroscopy findings				
Normal	44	44	88	**0.020**
Polyp	0	5	5	
Fibroid $	7	2	9	
Median uterine size cm And (Range)	8.0 (7-10)	8.0 (7-13)	8.0 (7-13)	0.964

Footnotes

* Statistical tests include Chi-square, Mann-Whitney U

^ Data not reported in all cases, calculation based on cases that were reported

$ Fibroid corresponds to identification of any submucous, intramural or subserosal fibroids by either ultrasound or hysteroscopy that are less than 3cm in size

The comparisons in **BOLD** are those that are statistically significant with a P value <0.05 .

Table 2.13. Peri-procedure outcomes of outpatient TBEA

	Thermachoice I	**Thermachoice III**	**Thermachoice I+III**	**P-value (Thermachoice I vs. III)***
Mean Volume of fluid in mL (95% CI; SD)	24.1 (16.5-31.8;13.3)	19.2 (15.8-22.7 ; 11.6)	20.4 (17.3-23.5 ; 12.1)	0.07
Average Intrauterine pressure (95% CI ; SD)	157 (147-166 ; 16)	169 (164-176 ; 19)	167 (162-172; 19)	**0.004**
Mean hospital stay minutes (hours)$ (95% CI; SD in minutes) (95% CI; SD in hours)	433 (7.2h) (318-547; 382) (5.3-9.1; 6.4)	522 (8.7h) (405-639; 398) (6.8-10.7; 6.6)	478 (8.0h) (397-559; 391) (6.6-9.3; 6.5)	0.277
Mean Visual Analogue Pain (95% CI; SD)	5.6 (4.7-6.6; 1.6)	5.9 (5.1-6.8; 2.9)	5.8 (5.2-6.5; 2.7)	0.541
Rescue Analgesia ^				
Paracetamol (frequency)	32	25	57	0.412
Diclofenac (frequency)	3	0	3	0.074
Mean Morphine Equivalent Dose (mg)$$ (95% CI; SD)	4.8 (3.0-6.5; 6.2)	13.0 (10.7-15.3; 8.0)	8.9 (7.3-10.5; 8.3)	**0.001**
Overall strength of analgesia $				
No rescue analgesia	12	3	15	**0.005**
Mild	30	25	55	
Moderate	4	6	10	
Strong	5	17	22	

Footnotes

* Statistical tests include Chi-square, Chi-Square trend, Mann-Whitney U

^ Data not reported in all cases, calculation based on cases that were reported

$ Excluding 13/102 cases that stayed overnight, the mean duration of hospital stay (minutes) with 95% Confidence limits are: 294 [257-330], 364 [326-402] and 329 [303-357; standard deviation 120] for Thermachoice I, III and overall combined I and III respectively.

The comparisons in **BOLD** are those that are statistically significant with a P value <0.05 .

Post ablation day case bed stay: All women recovered in a day case bed and were allowed home after a minimum stay of 2 hours. A strict protocol of post-procedure pain relief was adhered to: supplementary analgesia (termed *Rescue Analgesia*) was provided according to the patient's pain relief scores and patient request from a standardised 'as required' written drug prescription. A patient information leaflet was provided detailing expected symptoms and analgesic advice post LA-TBEA. All women were contacted by telephone at home the following day to check on their progress.

Strength of rescue analgesia. To quantify the amount of rescue analgesia utilised a numerical (morphine equivalent dose) and ordinal (mild, moderate, severe) scale was created according to the following methods:

a) Morphine Equivalent Dose. This is the estimated bioequivalent dose of morphine sulphate (in milligrams) that corresponds to the oral analgesic preparations (such as codeine phosphate, dihydrocodeine) given according to an accepted validated conversion scale [71].

b) An ordinal ranking of mild, moderate, severe rescue analgesia. This scale was created by the study authors, and recorded as mild (paracetamol or diclofenac only), moderate (paracetamol and diclofenac or low morphine equivalent dose) or strong (paracetamol /diclofenac / morphine, or high morphine [>15mg] dose) grading for strength of analgesia usage.

Outcome measures Initial baseline data recorded were: age, body mass index, menorrhagia alone or combined with dysmenorrhoea, menstrual cycle phase, ultrasound and hysteroscopy findings, uterine axis and uterine size. Procedure-related data recorded were: types of Thermachoice device, total fluid volume used, mean intrauterine ablation pressure, completion of TBEA procedure and any complications (e.g. vasovagal episodes).

All women were asked to record the pain they experienced immediately following LA-TBEA on a graduated Visual Analogue Scale, ranging from 0 (no pain) to 10 (worst imaginable pain), which had been validated in our previous study [64]. Amount and type of patient-initiated post procedure analgesia and duration of hospital stay (from initial admission to hospital and actual discharge) were also recorded.

A postal questionnaire was sent to all women post LA-TBEA to determine the effectiveness of therapy between July-September 2006. Questionnaire response was maximised by re-contacting women (by phone and letter) with non-returned forms in accordance with accepted practice [80]. Patient completed data recorded were: menstrual improvement (amenorrhoea, lighter periods), menstrual worsening or no change; satisfaction with treatment result or dissatisfaction; need for secondary treatment and type (e.g. LNG-IUS, repeat TBEA or hysterectomy); usage of HRT; Menorrhagia-specific and generic quality of life measures. Both menorrhagia disease-specific (Shaw) [81] and generic (EuroQol-5D) [82] Quality of Life tools were utilised to improve the sensitivity and accuracy in determining this outcome; both these tools had been validated in previous related studies [83;84]. The clinical case records of women undergoing hysterectomy secondary treatment were accessed to determine uterine histology. Similarly, the case records for women with missing questionnaires were accessed to determine if any secondary treatment had been necessary.

Statistical analysis All statistical analysis was performed using SPSS 13.0 statistical software (release 1 Sept 2004, ©SPSS Inc., USA). Categorical data was analysed by Chi-square and Chi-square trend testing. Continuous data was analysed by Mann-Whitney U test. A P-value less than 0.05 was considered statistically significant. Multivariate regression (binary logistic, ordinal and linear) was used to explore the significance of various baseline

and procedure related factors (i.e. prognostic factors or independent variables) on key outcome measures (duration of hospital stay, satisfaction, amenorrhoea and quality of life i.e. dependent variables). We accept the risk of increased overall Type I error (the probability of incorrectly rejecting a null hypothesis) when performing multiple hypothesis tests in multivariate regression. The Bonferroni method lowers the observed significance level because of multiple testing and provides a method to achieve an overall study error rate of 0.05 using a corrected p-value derived by $1- (1-\alpha)^{1/n}$, where $\alpha=0.05$ and n=number of hypothesis tests. However, given this was an exploratory statistical analysis, rather than a formal confirmatory study, then correcting for multiple testing procedures is not always considered necessary [85;86]. We have therefore reported both uncorrected and Bonferroni corrected P-values to enable readers to interpret the true significance of any $p<0.05$ result in line with other factors (e.g. consistency of finding, biological plausibility and clinical relevance) [85;86].

Ethics A formal application to a Research Ethics Committee was made and they recommended that ethics approval was not required as the study was classified as service evaluation according to established Central Office for Research Ethics Committees (COREC) guidelines. The study was conducted in accordance with basic ethical principles and complying with the Data Protection Act 2000 (e.g. informed consent, maintaining patient confidentiality, anonymizing patient held data, secure electronic storage of data).

RESULTS

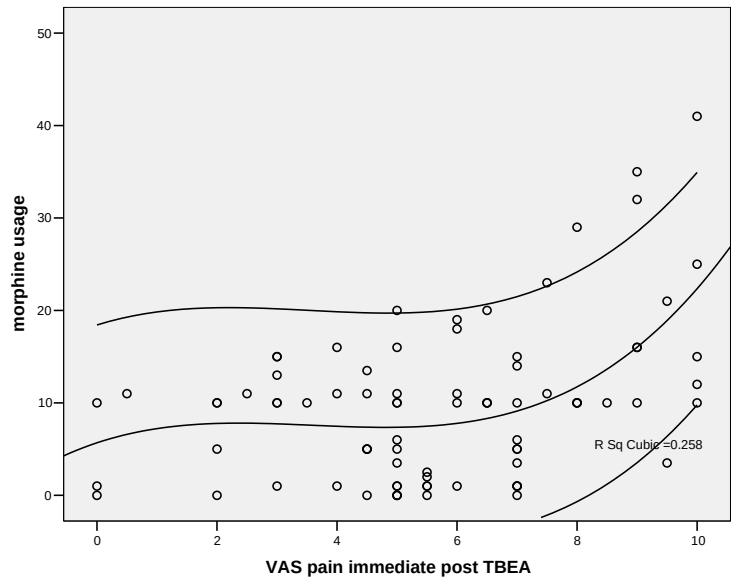
Baseline and peri-procedure outcomes There were no significant differences in the baseline characteristics between Thermachoice I (n=51) and Thermachoice III (n=51) cohorts apart from differences in ultrasound and hysteroscopic findings (**Table 2.12**). Of 105 consecutively recruited women that underwent planned LA-TBEA, the procedure was successfully completed in 102 (97%). Of the 3 failures, two were technical failures (one equipment breakdown, one severe cervical stenosis), and due to abandonment of the LA-TBEA at 3 minutes due to severe patient discomfort. These 3 failures all occurred in the first 10 cases of Thermachoice I cohort and may therefore be related to a learning curve effect of the operator and nursing team (i.e. develop better patient reassurance and analgesic regimes). Other complications included: 1 case of severe vasovagal syncope (not requiring atropine), 1 case of endometritis, 3 cases of severe vomiting, and 9 cases of severe pain requiring overnight admission. Fully completed questionnaires were returned by 88/102 participants (86%), and partially completed in a further 7 participants (95/102; 93%).

All peri-procedure outcomes are depicted in **Table 2.13**. The overall (n=102) mean duration of hospital stay following Thermachoice I and III was 8.0 hours (95% CI 6.6 to 9.3 hours; Standard Deviation 6.5 hours) **[Table 2.13].** However, this mean has been skewed due to the inclusion of a small proportion of women (n=13/102; 12.8%) who required overnight admission. Exclusion of this subgroup (6/51 Thermachoice I, 7/51 Thermachoice III) leads to an overall mean duration of stay of 5.5 hours (95% CI 5.1 to 6.0 hours; Standard Deviation 2.0 hours) **[Table 2.13].** Thus, outpatient LA-TBEA was successfully completed as an ambulatory day case (under 6 hours hospital stay) procedure in the vast majority.

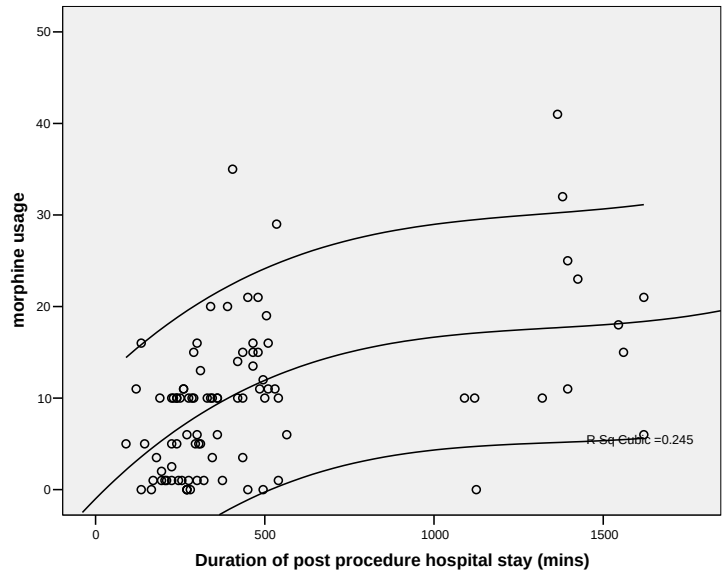
The amount of morphine rescue analgesia used directly correlated to the post ablation VAS score and duration of hospital stay (**Figure 2.4**). Univariate analysis showed that Thermachoice III was associated with greater use of rescue analgesia, but was also performed at higher mean intrauterine pressure than Thermachoice I (**Table 2.13**).

Long term outcomes: **Table 2.14** shows the long term outcomes in Thermachoice I and III procedures (performed between February 2001-July 2003, follow up range 26-54 months and August 2003-August 2005, follow up range 12-29 months respectively). Overall, despite the majority of women reporting improvement in their menstrual symptoms (amenorrhoea 29%, lighter periods 55%; total 84%), not all of these were satisfied (overall satisfaction rate 78%). Further treatment (repeat TBEA, hysterectomy or LNG-IUS) was required in 19/102 cases (19%). Of the 14 hysterectomies performed as secondary treatment around two-thirds had adenomyosis or fibroids on uterine histopathology. Satisfied compared to dissatisfied women reported higher levels of quality of life and menstrual improvement (**Table 2.15**). Overall, 50% of surgical re-interventions (n=16, 14 hysterectomies, 2 repeat ablations) occurred by 19 months (Range 10-46)(**Figure 2.5**). Kaplan-Meier survival analysis suggested a statistically significant trend to earlier surgical re-intervention with Thermachoice III than Thermachoice I (Log Rank Mantel-Cox p=0.024). However, Cox regression showed that this was a non-significant (p=0.056) trend when corrected for identified confounders (duration of follow up; intrauterine pressures; morphine equivalent dose) (**Figure 2.5**).

Figure 2.4. Correlation of morphine usage to post ablation VAS Score and duration of hospital stay



Footnote: Morphine rescue analgesia directly correlates to VAS score immediately post TBEA (Pearson p=0.001, Kendall's tau p=0.006).



Footnote: Morphine rescue analgesia directly correlates to duration of hospital stay post TBEA (Pearson p=0.001, Kendall's tau p=0.001), and this relationship remains statistically significant after multivariate analysis (**see Figure 2.5**).

Table 2.14. Long-term outcomes of outpatient TBEA

	Thermachoice I	Thermachoice III	Thermachoice I+III	P-value (Thermachoice I vs. III)*
Mean follow up time (months)	41	18	29	**0.001**
(95% CI ; SD)	(38-43; 8)	(16-19; 5)	(26-32; 13)	
Further treatment (Repeat ablation, Hysterectomy or LNG-IUS)				
No	36 (70.6%)	47 (92.2%)	83 (81%)	**0.005**
Yes	15 (29.4%)	4 (7.8%)	19 (19%)	
All Types of further treatment				
No further treatment	30	44	74 (73%)	**0.024**
LNG-IUS	3	0	3	
Drugs (including HRT)	6	3	9	
Repeat Endometrial Ablation	1	1	2	
Hysterectomy ($$ histology)	11	3	14 (14%)	
Periods at review ^				
Amenorrhoea	11 (23%)	16 (35%)	27 (29%)	
Lighter	23 (49%)	28 (61%)	51 (55%)	**0.009**
No change or worse	13 (28%)	2 (4%)	15 (16%)	
Dysmenorrhoea at review ^				
Pain free or Less	27	37	64	**0.027**
No change	6	5	11	
Worsening	14	4	18	
Satisfaction				
Satisfied	35(69%)	43 (84%)	78 (76%)	0.062
Dissatisfied	16 (31%)	8 (16%)	24 (24%)	
Mean EuroQoL VAS score (95% CI; SD)	76 (69-84; 19)	80 (73-87; 19)	78 (73-83; 19)	0.420
Mean EuroQoL Index (95% CI; SD)	0.81(0.74-0.88; 0.19)	0.87 (0.80-0.96; 0.21)	0.84 (0.79-0.90; 0.20)	**0.022**
Mean Shaw QOL (95% CI; SD)	83 (73-92; 25)	87 (78-96; 24)	84 (78-91; 25)	0.504

Footnotes

* Statistical tests include Chi-square, Chi-Square trend, Mann-Whitney U

^ Data not reported in all cases, calculation based on cases that were reported

$$ Histology of the 14 hysterectomies reported adenomyosis, fibroids and normal uterus in 4, 5 and 5 cases respectively

The comparisons in **BOLD** are those that are statistically significant with a P value <0.05 .

Table 2.15. Patient satisfaction and its relationship to quality of life and other treatment outcomes following endometrial ablation

Outcomes	**Satisfied** **N=78**	**Dissatisfied** **N=24**	**P Value** **Statistical significance $**
No further treatment	69	5	**0.001**
Further treatment	9	19	
LNG-IUS	0	3	
Drugs (including HRT)	9	0	
Repeat Endometrial Ablation	0	2	
Hysterectomy	0	14	
Periods now			
Amenorrhoea	25	2	**0.001**
Lighter	43	8	
No change or worsening	1	14	
Quality of life			
Mean EuroQOL VAS score (95% CI ; SD)	80.5 (75.8-85.2, 16.8)	63.1 (35.5-84.5, 26.5)	0.077
Mean EuroQoL Index (95% CI ; SD)	0.89 (0.86-0.92, 0.13)	0.50 (0.22-0.79, 0.31)	**0.001**
Mean Shaw QOL (95% CI ; SD)	87.0 (80.6-93.3, 22.6)	68.3 (37.6-99.0, 33.2)	**0.009**

Footnotes

* Statistical tests include Chi-square, Mann-Whitney U

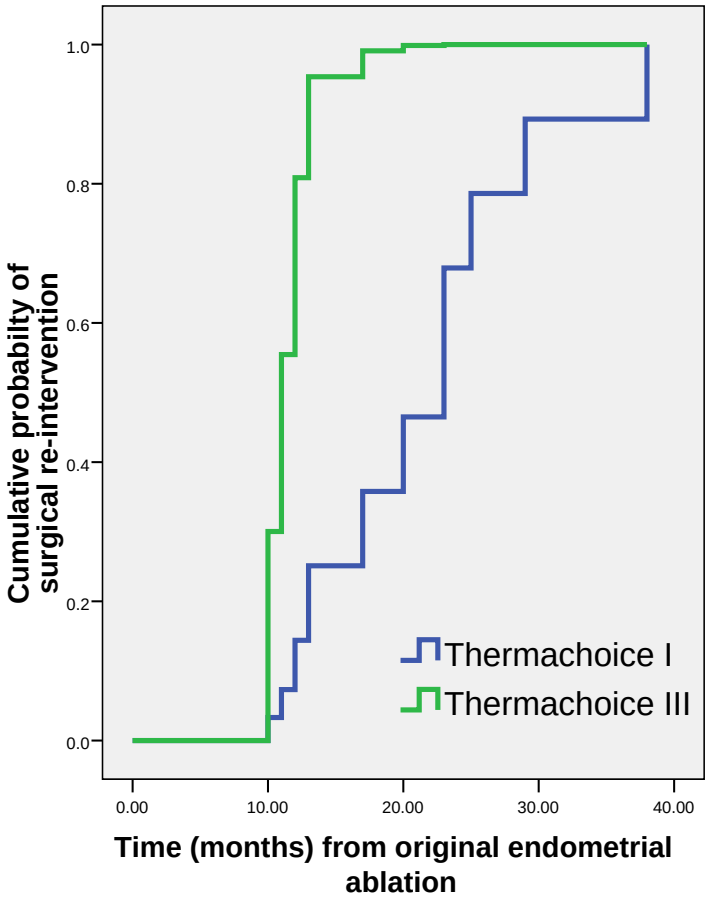
The comparisons in **BOLD** are those that are statistically significant with a P value <0.05.

Figure 2.5. Survival analysis for likelihood of surgical re-intervention post TBEA

	Mean (95% CI; SD)	Median (Range)	P value comparison
All surgical re-interventions (n=16)	21.6 (15.8-27.5; 11.0)	19.0 (10-46)	Not applicable
Hysterectomy(n=14)	20.8 (14.5-27.1)	18.5 (10-46)	*0.721
vs.	vs.	vs.	
Repeat ablation (n=2)	27.5	27.5 (18-37)	
Thermachoice I (n= 12)	24.3 (17.9-30.8)	23.0 (18.0-28.0)	*0.024
vs.	vs.	vs.	
Thermachoice III (n=4)	13.5 (10.5-16.5)	12.0 (10.0 vs. 14.0)	
			** 0.056

* Log Rank (Mantel-Cox)

** Cox Regression analysis (corrected for duration of follow up; intrauterine pressures; morphine equivalent dose)



Regression analysis for prognostic factors

Univariate analysis showed that Thermachoice III compared to Thermachoice I was more likely to be associated with primary treatment success, menstrual improvement, dysmenorrhoea improvement and improved generic Quality of Life (EuroQol-5D index) (**Table 2.14**). However, multivariate regression analysis (**Table 2.16;** corrected for all baseline and peri-procedure characteristics that utilized a P value less than 0.05 as indicative of statistical significance) showed:-

- Morphine dosage in rescue analgesia, but not the overall strength of analgesia (combining non-steroidal, Paracetamol and opiates) was independently associated with a longer duration of hospital stay.
- Thermachoice III compared to Thermachoice I increased the likelihood for amenorrhoea, but was not associated with increased hospital stay, satisfaction or quality of life.
- Regardless of the type of Thermachoice device, higher mean intrauterine ablation pressures and/or higher morphine rescue analgesia correlated to better long term patient satisfaction.
- Neither uterine axis, age nor uterine length was associated with any of the outcomes.

As previously stated in our methods section, there is an increased risk of identifying a falsely positive statistical finding due to multiple testing. For the analysis shown in **Table 2.16** the Bonferroni corrected p value for a significant factor is 0.001. This meant that only type of Thermachoice would be considered statistically significant (p=0.001) amongst all factors tested if using Bonferroni correction.

Table 2.16. Prognostic outcomes for endometrial ablation (using multivariate regression analysis)

Indicator variable	Multivariate statistical P values of indicator variable in predicting outcome			
	Duration of Hospital Stay$	**Amenorrhoea~**	**Satisfaction***	**EuroQoL Index $**
Age	0.604	0.571	0.078	0.675
BMI	0.825	0.322	0.778	0.489
Indication (prior dysmenorrhoea)	0.925	0.526	0.315	0.487
Cycle phase	0.958	0.982	0.522	0.274
Uterine axis	0.614	0.234	0.146	0.942
Ultrasound findings	0.754	0.267	0.169	0.331
Hysteroscopy findings	0.479	0.742	0.593	0.864
Uterine size	0.155	0.342	0.918	0.415
Intrauterine pressure	0.956	0.350	**0.014**	0.070
Volume of fluid	0.402	0.425	0.682	0.621
Post procedure pain VAS	0.792	0.335	0.369	0.064
Strength of analgesia	0.820	0.791	0.486	0.205
Morphine dose	**0.042**	0.595	**0.030**	0.394
Type of Thermachoice	0.591	**0.001**	0.697	0.387
Is model statistically significant	No	Yes	Yes	No

Footnotes

* Binary Logistic Regression; $ Univariate Linear Regression ; ~ Ordinal Logistic Regression

The Bonferroni method lowers the observed significance level because of multiple testing and provides a method to achieve an overall study error rate of 0.05 using a corrected p-value derived by $1- (1-\alpha)^{1/n}$, where $\alpha=0.05$ and n=number of hypothesis tests. For the entire table (14 by 4 tests, giving n=56 and $\alpha=0.05$) the Bonferroni corrected p value that is 0.001.This means that only type of Thermachoice is statistically significant (p=0.001) if using Bonferroni corrected interpretation.

The comparisons in **BOLD** are those that are statistically significant with a P value <0.05.

DISCUSSION

Local anaesthetic TBEA can be carried out as an outpatient daycase procedure and is an effective treatment option. The vast majority (76%) were satisfied with their treatment at a mean 2½ years follow up. Upgrading to Thermachoice III, compared to Thermachoice I was associated with improved rates of amenorrhoea, although overall, both devices achieved similar rates of patient satisfaction and quality of life. We found higher intrauterine ablation pressures to be associated with improved long term treatment satisfaction. Overall, 50% of surgical re-interventions occurred by around 1½ years.

This study is an important advancement to the published knowledge in outpatient TBEA [64-68;87]. This study's principal attribute is that it is of pragmatic design and reflects actual clinical management of menorrhagia. We believe this study to be the largest published cohort of outpatient TBEA under local anaesthetic without sedation. Apart from two other studies of 4-6 year follow up [67;88], this study represents the longest follow up of outpatient TBEAs (mean of 30 months, range of 12 to 54 months). Importantly, this study is the first to utilize both menorrhagia-specific [81] and generic [82] quality of life tools which has been advocated as the preferred way to measure these outcomes [83;84]. Furthermore, our study's long term outcomes are derived from a high response rate (86%) which improves the accuracy of our data collection.

We agree there may be caveats when interpreting the results from our prospective study, which may lessen the reliability of our conclusions. Our study population may be heterogeneous, as we did not use objective criteria to define heavy menstrual bleeding or stratify according to differing bleeding patterns. Because of temporal differences between Thermachoice I and III cohorts, this has inevitably led to differences in follow up between Thermachoice I (mean 41 months) and III cohorts (mean 18 months) at our time of

questionnaire enquiry. This precluded the reliability of any survival regression analytical techniques, although by opting to use regression analysis we have attempted to correct for confounding influences. Intrauterine polyp or fibroid removal may have exerted an independent curative effect, although their combined proportions were similar in both Thermachoice I and III cohorts and thus any distinguishing influence minimized. Our study did not collect baseline quality of life data, and therefore we were unable to quantify a change in quality of life following LA-TBEA at specified time intervals. Finally, our study is likely to be under-powered (Type II error). Based on unpaired student's T-test and a minimal difference of interest between means of 0.3 Standard Deviations, we estimate a sample of size of 178 for each group would be required to show any significant difference in quality of life outcomes.

The mean duration of stay in our study was around 5.5 hours, which is significantly longer than an analogous outpatient Thermachoice LA-TBEA study that reported a mean total time spent in hospital of 1 hour 40 minutes [65]. This discrepancy may be partly explained by differences in pre- and post-operative analgesia and nursing-led or physician-led discharge practices. However, duration of stay is likely to be even more multifactorial than this (see **Table 2.16**), and any attempt to explain such differences would be frank conjecture. We feel that a mean duration of stay shown by our study represents a realistic recovery period before discharge.

For some outcome measures, the extent of incomplete questionnaire responses or prolonged time interval from original treatment may have had a greater effect in over-estimating or under-estimating their frequency. This may be particularly pertinent to our reported rates of amenorrhoea; if we assume those with missing responses were not truly amenorrhoeic then the rates of amenorrhoea for Thermachoice I and III would be 23% and 31% instead of the reported 23% and 35%. However, our rates of amenorrhoea are consistent to those reported

by other studies [66-68;88]. Nonetheless, we accept there may be a tendency to under-report satisfaction and amenorrhoea rates in Thermachoice I because assessment at longer follow up may have enabled women to have regeneration of the endometrial lining and symptomatic recurrence. Several studies have explored prognostic factors on TBEA success [68;76-79]. Unlike previous studies, we have shown no adverse prognostic effects due to a retroverted uterus, large uterine size or young age [68;76-79]. However, it is notable that our association of increased intrauterine pressure and improved outcome has been consistently identified in other studies [76-79]. Furthermore, this study showed associations for amenorrhoea (influenced by type of Thermachoice), satisfaction (influenced by intrauterine pressure, rescue morphine usage and/or post procedure VAS) and Quality of Life (influenced by intrauterine pressure) outcomes. This would reinforce the logical notion that the higher the intrauterine ablation pressure, and/or the more painful the TBEA procedure is, perhaps by inducing a greater depth uterine ablation, the more likely it is to achieve a successful long term outcome.

This study reports on the safety and effectiveness of outpatient LA-TBEA which is clinically relevant to improve patient selection and preoperative counselling. Furthermore, there is continued expansion in this area, as evidenced by a growing body of literature which includes a randomised trial comparing outpatient and general anaesthetic TBEA [65]. In order to determine the optimum role for outpatient endometrial ablation in treating women with heavy periods, further trials are needed to determine the clinical and cost-effectiveness of second generation ablation techniques (e.g. microwave, TBEA and radiofrequency ablation devices) against each other, against general anaesthetic and local anaesthetic settings, and against appropriate treatment alternatives (e.g. LNG-IUS).

2.5. Long term outcomes following hysteroscopic myomectomy for abnormal uterine bleeding

OBJECTIVE: To evaluate the long term effectiveness of hysteroscopic submucous myomectomy for women with abnormal uterine bleeding and explore any prognostic factors associated with treatment success.

DESIGN: Prospective observational study.

SETTING: University teaching hospital in U.K.

PATIENT(S): 92 women symptomatic of abnormal uterine bleeding with submucous myomas.

INTERVENTION(S): Hysteroscopic myomectomy performed as outpatient local anaesthetic (38%) or daycase general anaesthesia (62%) using Versapoint™ .

MAIN OUTCOME MEASURES: Need for secondary surgical or medical re-intervention, menstrual improvement and patient satisfaction over a minimum 12 month period. Other outcome measures include: successful completion of primary resection, type of secondary treatment and any prognostic factors.

RESULT(S): Mean follow up was 2.6 years (95% CI 2.3-2.9). Complete fibroid excision and removal was achieved in 66%. Secondary surgical re-intervention was required in 27 (29%) of which 11 (12%) were repeat hysteroscopic myomectomy and 10 (11%) were hysterectomy procedures. Multiple uterine fibroids and adenomyosis were identified in 80% of hysterectomies. Overall, improved menstrual symptoms and patient satisfaction were reported by 91% and 86% at follow up. Size of the submucous fibroid or presence of any intramural or subserosal fibroids were not related to treatment success.

CONCLUSION(S): Women with abnormal uterine bleeding diagnosed with submucous myomas may be successfully treated by removing the submucous myoma component, irrespective of co-existent intramural or subserosal fibroids. This effect is sustained over the long term.

INTRODUCTION

Uterine fibroids are present in 25-40% of women presenting with abnormal uterine bleeding [89]. Although a direct cause-effect relationship has not been completely established, there is sufficient observational data to suggest that shrinkage or removal of any identified uterine fibroids is beneficial in alleviating menstrual bleeding abnormalities in most symptomatic women.

Hysteroscopic myomectomy is considered the first-line conservative surgical therapy for the management of symptomatic submucous fibroids[89-93]. Data, from mainly observational studies, has suggested beneficial effects in treating both menstrual abnormalities and infertility with this procedure. The few studies that have reported on long term outcomes for fibroid-related menstrual abnormalities, indicate that hysteroscopic myomectomy is associated with a 10-35% risk of surgical re-intervention, including repeat myomectomy, open myomectomy or hysterectomy[90;92;93]. However, such a high re-intervention rate may alter the cost effectiveness of hysteroscopic myomectomy compared to other uterus-conserving treatment options and hysterectomy.

Presently, there is insufficient evidence on reliable selection criteria and long term outcomes for women with symptomatic fibroids who opt for hysteroscopic myomectomy. This knowledge would be particularly important for preoperative counselling and appropriate patient selection. We therefore wished to evaluate long term efficacy of this treatment, and identify whether there were any adverse (e.g. co-existence of intramural or subserosal fibroids) or favourable (e.g. submucous myoma less than 5cm size, completeness of lesion excision) peri-operative prognostic factors.

MATERIALS AND METHODS:

Patient Population Women symptomatic of abnormal uterine bleeding (i.e. mainly with heavy menstrual bleeding [HMB]) were referred by primary care (GPs) or secondary care to our "One Stop" "See and Treat" menstrual disorders clinic. All women underwent transvaginal pelvic sonography, outpatient hysteroscopy and endometrial Pipelle biopsy (Laboratoire C.C.D, Paris, France) investigations. Women who were considered suitable for hysteroscopic myomectomy were included in this study. Women were excluded from the study if an abnormally shaped uterine cavity (e.g. bicornuate uterus), endometrial hyperplasia, cancer or active pelvic infection were present.

Study design Prospective patients presenting between June 2003 and November 2006 were included in this study.

Intervention All hysteroscopic myomectomies were performed using Versapoint [Gynecare, Ethicon Inc. USA] according to the manufacturer's recommended guidance and as previously reported by our group [94;95]. We defined a submucous intracavity fibroid at hysteroscopy as having characteristic appearances (sessile or pedunculated, superficial large blood vessels) and non-mobility with intrauterine fluid or gentle hysteroscopic tapping of the lesion. In all cases, our preoperative suspicion of intracavity fibroid was confirmed on histological analysis of the excised lesion. All women were offered to have the intervention under local anaesthetic (LA) outpatient setting or general anaesthetic (GA) daycase setting. Factors that influenced the final decision included: patient preference, how she tolerated outpatient hysteroscopy, intrauterine location and size of intracavity fibroids. Preoperative preparation with a 3 month course of GnRHa prior to myomectomy was deemed necessary in women with intracavity fibroids greater than 5cm in size. A patient information leaflet was provided

detailing the procedure, expected symptoms and analgesic advice post hysteroscopic myomectomy.

LA hysteroscopic myomectomy This was performed on a "See and Treat" basis with no fasting prior to the procedure. Other elements of the treatment included:-

- *Local anaesthetic*: The cervix was directly injected in a circumferential manner with three 2mL cartridges containing 3% prilocaine hydrochloride (30 mg/mL) and felypressin 0.03 unit/mL (Dentsply, UK) using a 27G dental syringe.

- *Dedicated patient nurse:* A particular nurse was allocated to provide continuous supportive care to the patient during the procedure. The nurse engaged the patient in conversation ('distraction' analgesia effect termed "vocal local") and often held the patient's hand throughout the procedure.

- *Post procedure analgesic regimen:* All women received diclofenac 100mg rectally and oral co-dydramol 10/500 (two tablets). All women were recovered in a dedicated patient waiting area and allowed home after a minimum 30 minute stay. A strict protocol of post-procedure pain relief was adhered to.

GA hysteroscopic myomectomy: Women, fasted for at least 6 hours, were admitted to hospital on the day of the procedure. In a minority of cases, women with high risk medical disorders (e.g. diabetes) were admitted the day before the planned procedure. Hysteroscopic myomectomy was carried out in gynaecology theatres after induction of general anaesthesia. All women received diclofenac 100mg and 1g paracetamol rectally (or paracetamol alone if diclofenac was contraindicated) just prior to the procedure. Infiltration of the cervix with a local anaesthetic was not done in these women. The hysteroscopic myomectomy surgical

procedure, post-procedure analgesia regimen and day case bed stay for GA was identical to the LA hysteroscopic myomectomy procedure described above.

Complete excision, partial excision and devascularisation at hysteroscopic myomectomy

In all cases, a standardised technique was adopted in order to completely excise and remove the fibroid. The submucous fibroid was resected at the junction between the fibroid and uterine wall using a shearing technique. To facilitate this it was occasionally necessary to bisect, trisect or quadrisect the fibroid lesion to access this fibroid-uterine wall interface. Complete excision was achievable in most pedunculated (Type 0) and in those superficially myometrially invading (type 1) intracavity fibroids. Occasionally, where the hysteroscopic view became obscured following commencement of the procedure, one of two modified procedures was performed:

- *Partial excision and removal of the fibroid* was performed. The percentage of the fibroid removed relative to entire intracavity lesion was clinically estimated and recorded.
- *Devascularisation of the intracavity without its excision*. This entailed multiple scoring of the fibroid lesion (e.g. trisecting the lesion in a "hot cross bun" technique) at or near its vascular attachment base. The percentage of the fibroid devascularised relative to the entire intracavity lesion was clinically estimated and recorded.

Outcome measures Initial baseline data recorded were: age, body mass index, parity, menstrual bleeding abnormality, ultrasound and hysteroscopy findings, and use of pre-procedure GnRHa. The size of the intracavity uterine fibroid selected for myomectomy was determined using ultrasound (objective) data in most cases. Where ultrasound had failed to identify the intracavity fibroid prior to myomectomy the practitioner recorded their clinical

estimate of intracavity fibroid size. In relationship to the hysteroscopic myomectomy procedure, the following data were recorded: LA or GA setting, completeness of excision, operation length, procedure related complications (e.g. vasovagal episodes) and duration of hospital stay.

A postal questionnaire was sent to all women post procedure between June-November 2007, ensuring there was a minimum 12 month follow up period. Questionnaire response was maximised by re-contacting women (by phone and letter) with non-returned forms. Patient completed data recorded were: need for and nature of any secondary treatment, improvement in their menstrual bleeding pattern and dysmenorrhoea (ordinal Likert scales), and patient satisfaction at that time (ordinal Likert scale). Secondary treatments were categorised according to medical (LNG-IUS, oral progestins, combined oral contraceptive, tranexamic acid) and surgical (repeat hysteroscopic myomectomy, open myomectomy, endometrial ablation, hysterectomy) interventions. Primary treatment success was defined as the absence of any type of medical or surgical secondary treatment following the primary treatment of hysteroscopic myomectomy. The case records and histology results of all study participants were reviewed and recorded.

Statistical analysis: Dichotomous data were presented as simple proportions. SPSS version 13 was used to undertake multivariate regression analysis and to conduct Chi-square tests. $P < 0.05$ was considered statistically significant.

RESULTS

Ninety-two women participated in the study and their baseline characteristics are depicted in **Table 2.17**. The characteristics associated with hysteroscopic myomectomy procedure are depicted in **Table 2.18.** Of the 35 (38%) women undergoing LA procedure, none were admitted for overnight stay. Of the 57 (62%) women undergoing GA procedure, 20 (35%) were admitted for overnight stay.

In relation to menstrual symptom improvement and patient satisfaction outcomes, only 2 women (2%) failed to return their questionnaire, representing a 98% follow-up. Examination of the clinical case notes and contacting their GPs confirmed that neither of these two women had undergone secondary treatment following hysteroscopic myomectomy. The mean follow up was 2.6 years (95% CI 2.3-2.9; Range 1-7.3 years; St Dev 1.5). Overall, greater than, or equal to, 12 months, 24 months and 36 months outcome data were available for 90 (98%), 52 (57%) and 31 (34%) women.

The menstrual and secondary treatment outcomes are depicted in **Table 2.19.** Surgical re-intervention was necessary in 27 (29%) women, and this involved hysterectomy in 10 cases and their characteristics are depicted in **Table 2.20**. Seven hysterectomies (70%) were performed by 12 months of the primary hysteroscopic myomectomy, and of these, 2 hysterectomies were performed for unexpectedly identified gynaecological pathology (one case complex hyperplasia, one case leiomyosarcoma). Adenomyosis and multiple fibroids were the commonest histological findings at hysterectomy (8/10 cases). Multivariate analysis of the need for secondary treatment identified no statistically significant prognostic factor (**Table 2.21**).

Table 2.17. Baseline characteristics for 92 women undergoing hysteroscopic myomectomy

Patient characteristics	**N=92** **Frequency (Percentage)**
Age	
20-30 years	4 (4)
30-40 years	33 (36)
40-50 years	42 (46)
>50 years	13 (14)
BMI	Mean 28.0 (95% CI 26.4-29.7) Range 20-52; St Dev 6.9
Menopausal status at presentation	
Pre-menopausal Post-menopausal	84 (91) 8 (9)
Menstrual Bleeding abnormality	
Heavy Menstrual Bleeding (HMB) Unscheduled bleeding on HRT	84 (91) 8 (9)
Scan findings	
Submucous Submucous & Intramural Submucous & Intramural & Subserosal	41 (45) 47 (51) 4 (5)

Table 2.18. Characteristics associated with hysteroscopic myomectomy procedure

Procedure setting LA Local anaesthetic outpatient GA General anaesthetic daycase	 35 (38) 57 (62)
Preop GnRHa Yes No	 20 (22) 72 (78)
Length of operation* <30 minutes 30-60 minutes > 60 minutes	 77 (84) 14 (16) 1 (1)
Size of uterine fibroid (u/s and by clinical estimation) <3cm 3-5 cm >5cm	 22 (24) 53 (58) 17 (19)
Primary treatment performed Complete excision and removal >50% excision and removal >50% devascularised and left in situ Complete excision and removal and endometrial ablation Complete excision and removal and insertion of Mirena	 48 (52) 13 (14) 18 (20) 11 (12) 2 (2)
Complications None Bleeding requiring balloon tamponade Cervical trauma	 83 (90) 8 (9) 1 (1)
Length of hospital stay Daycase Overnight	 72 (78) 20 (22)

Table 2.19 Outcomes after hysteroscopic myomectomy

Outcome measure	Entire cohort, including 10 women who had hysterectomy (n=92)	Excluding 10 women who had hysterectomy (n=82)
Menstrual bleeding characteristics at enquiry		
Amenorrhoea	28 (30)	18 (22)
Brown discharge	3 (3)	3 (4)
Much lighter	40 (43)	40 (49)
Marginally lighter	13 (14)	13 (16)
No change	4 (4)	4 (5)
Heavier	2 (2)	2 (3)
Unknown	2 (2)	2 (2)
Overall menstrual symptoms improved	**84 (91)**	**74 (90)**
Dysmenorrhoea characteristics at enquiry		
None	50 (54)	40 (49)
Less	26 (28)	26 (31)
No change	11 (12)	11 (13)
Worse	3 (3)	3 (4)
Unknown	2 (2)	2 (2)
Overall dysmenorrhoea symptoms improved	**76 (83)**	**66 (81)**
Degree of satisfaction at enquiry		
Very satisfied	55 (60)	54 (66)
Satisfied	24 (26)	20 (24)
Dissatisfied	6 (7)	4 (5)
Very Dissatisfied	5 (5)	2 (2)
Unknown	2 (2)	2 (2)
Overall satisfied	**79 (86)**	**74 (90)**
Secondary treatment		
Myomectomy (open)	4 (4)	
Thermal Balloon Endometrial Ablation	2 (2)	
LNG-IUS (Mirena)	7 (8)	
Hysterectomy	10 (11)	
Repeat hysteroscopic myomectomy	8 (9)	
Repeat hysteroscopic myomectomy and ablation	1(1)	
Repeat hysteroscopic myomectomy and Mirena	2 (2)	
Oral progestins	2 (2)	
No secondary treatment	**56/92 (61)**	
Secondary treatment (all types) required	**36/92 (39)**	
No surgical re-intervention	**65/92 (71)**	
Overall repeat surgical treatment required	**27/92 (29)**	

Footnotes for Table 2.19 * Missing questionnaire responses for menstrual (2) and dysmenorrhoea (2) characteristics and patient satisfaction (2). Case notes and GPs were contacted and no secondary treatments were undertaken in the 2 non-returned questionnaire responses.

Table 2.20 Women (n=10) undergoing hysterectomy following hysteroscopic myomectomy

Characteristic	Value
Number of hysterectomies	10
Average time to Hysterectomy	Mean 14.4 months (95% CI 4.5-24.3) Median 9.5 months; Range 1-41 months; St Dev 13.9
Time from procedure and cumulative rate of hysterectomy	By 6 months: 4/10 cases [one for leiomyosarcoma] By 12 months: 7/10 cases [one for complex hyperplasia] By 24 months: 8/10 cases By 48 months: 10/10 cases
Very satisfied **Satisfied** **Dissatisfied** **Very dissatisfied** **Overall satisfied** **Overall dissatisfied**	1 4 2 3 5 (50%) 5 (50%)
Histology **Adenomyosis and fibroids (multiple)** **Fibroids (multiple)** **Leiomyosarcoma** **Complex Hyperplasia**	 6 2 1 (identified on resection histology and reason for TAH) 1 (identified at resection histology and reason for TAH)

Table 2.21. Multivariate analysis of prognostic factors

	Need for secondary treatment (all types)		Need for secondary surgical re-intervention	
Prognostic factor and its significance (p-value)	Menstrual pattern	[p=0.90]	Menstrual pattern	[p=0.09]
	Primary treatment	[p=0.10]	Primary treatment	[p=0.12]
	Scan findings	[p=0.61]	Scan findings	[p=0.66]
	Size of fibroid	[p=0.35]	Size of fibroid	[p=0.84]

Footnotes

1. Multivariate regression corrected for the following confounding factors, including: age, BMI, parity, menopausal status, type of menstrual bleeding abnormality, scan findings, preoperative GnRHa, size of uterine fibroid, type of primary myomectomy treatment.

2. Binary logisitic regression models for secondary treatment and secondary surgical re-intervention were statistically significant ($p<0.001$)

DISCUSSION

Women with abnormal uterine bleeding diagnosed with submucous myomas may be successfully treated by removing the submucous myoma component, irrespective of co-existent intramural or subserosal fibroids or size of fibroid that has been resected. The beneficial effects of hysteroscopic myomectomy persist long term (mean follow up around 2½ years), and with the secondary surgical re-intervention rate of 29% this suggests that the removal of the submucous component can avoid hysterectomy in 70% of cases. The majority of women who underwent hysterectomy as secondary treatment were identified to have adenomyosis and multiple uterine fibroids.

To date, studies published on hysteroscopic myomectomy have utilised various technical approaches, been mainly performed under GA in daycase settings, have mixed retrospective and prospective observational designs, and have minimal data on long term follow up, particularly patient satisfaction and surgical re-intervention rates[94;96-104]. Our study adds to this published literature by exclusively utilising a modern Versapoint bipolar system; has been successfully undertaken in both outpatient and daycase setting; has a prospective design; has long term follow up incorporating patient satisfaction; has evaluated peri-operative features that may have prognostic value; and expands on our previously published work[94]. Our surgical re-intervention rate of 29% (over mean 2½ years) was lower than that reported by a previous study of 35% (over mean 5 years)[101]. This study has been pragmatic in design, ensuring our results are applicable to actual clinical practice.

However, we accept our study may have limitations that may make our conclusions less reliable. Our study sample size, although at 92 with a low dropout rate (2%), may be underpowered to identify all peri-operative prognostic factors. Because our follow up intervals varied between patients, there may be a tendency to overestimate or underestimate the beneficial effects of hysteroscopic myomectomy at the extremes of follow up. The variation in follow up outcome data also precluded our use of survival analysis techniques to asses both efficacy and durability of the hysteroscopic procedure.

Given the 29% risk of surgical re-intervention following submucous myomectomy, there is a need to identify significant peri-operative prognostic factors that could be usefully employed during preoperative counselling. Even though our study did not identify any specific prognostic factor previous studies have identified enlarged uterine size, three or more intracavity myomas, fibroid size>3cm and increased depth of myometrial penetration to be adverse prognostic factors [100;101] . In fact, our study reinforces the widely held opinion that it is only the presence of the submucous fibroid itself that appears to be responsible for the heavy menstrual bleeding [92;93]. Furthermore, our study showed that adenomyosis was frequently identified in those women who required hysterectomy as secondary treatment. There is insufficient evidence on the ultrasonographic criteria predictive of adenomyosis and whether adenomyosis should be routinely screened for in women with menstrual disorders[105;106]. Future studies are needed to identify the clinical efficacy and optimal patient selection criteria for submucous myomectomy, and whether preoperative imaging suspicion of adenomyosis may be usefully employed in the treatment decision making process.

Chapter 3. SYSTEMATIC REVIEWS AND SYSTEMATIC LITERATURE APPRAISAL

Systematic reviews performed for two clinical queries:

- Do screening-preventative interventions in asymptomatic pregnancies reduce the risk of preterm delivery.
- Is Levonorgestrel-releasing hormone system (LNG-IUS) effective therapy for a variety of non-contraceptive indications .

Publications arising from the chapter

Chapter	Manuscript title	Reference
3.1	Do screening-preventative interventions in asymptomatic pregnancies reduce the risk of preterm delivery--a critical appraisal of the literature.	[1]
	Antibiotic treatment of bacterial vaginosis in pregnancy: Multiple meta-analyses and dilemmas in interpretation	[2]
3.2	Non-contraceptive uses of levonorgestrel releasing hormone system (LNG-IUS)- a systematic enquiry and overview	[3]

Introduction

Background

Systematic reviews are considered to provide the definitive evidence-based answer as to whether a particular intervention or test is clinically effective and to quantify the strength of this effect. The term "systematic review" is widely considered to be synonymous to the highest level of evidence-based medicine available for that cited topic. The key strength of the research methodology is its ability to produce a more reliable measure of effectiveness through mathematically pooling outcomes of clinical trials rather than using an outcome ascertained from an individual trial. The methodology is further underpinned through a rigorous systematic search, with strict quality control of studies that are eligible to be included or excluded in the final meta-analysis stage.

The methodology conforms to established standards which are, by convention, explicity stated prior to the systematic review being accepted by peer-reviewed publications or the Cochrane collaboration[4;5]. Hence, systematic reviews, analyzing the same clinical question, ought to be consistent and reproducible. Importantly, inherent to the transparency of the methods and trial selection process, systematic reviews are relatively easily updated as newer trials are published; the process of periodic update is mandatory for all Cochrane reviews and ensures the review evidence is continually up-to-date and reliable. Despite a multitude of published systematic reviews, mostly presented through the Cochrane collaboration, there remains several unanswered clinical questions within our specialty of obstetrics and gynaecology.

Aims

Undertake a systematic review, incorporating the elements of : systematic literature search ; appraisal of studies for rejection or inclusion and meta-analyses. This will be performed in accordance to standardized methodology as set out by the Cochrane collaboration and others[4;5].

For each therapeutic intervention, appraise the quality of supporting evidence and assign a grade to the strength of recommendation that can be derived according to the evidence by using established evidence appraisal tools (Royal College of Obstetricians and Gynaecologists guideline development criteria (**Table 3i** and GRADE evaluation of evidence **Table 3ii**) [6;7]. *These appraisal tools will be discussed at greater length in Chapter 4 (Clinical Guideline Development).*

The specific clinical queries that will be used as examples of the systematic review research methodology process are:

1. Do screening-preventative interventions in asymptomatic pregnancies reduce the risk of preterm delivery.
2. Is Levonorgestrel-releasing hormone system (LNG-IUS) effective therapy for a variety of non-contraceptive indications .

Table 3i. Classification of evidence used by RCOG Guideline development (originate from US Agency for Health Care Research and Quality)

Classification of Evidence Levels

Ia Evidence obtained from meta-analysis of randomised controlled trials.

Ib Evidence obtained from at least one randomised controlled trial.

IIa Evidence obtained from at least one well-designed controlled study without randomisation.

IIb Evidence obtained from at least one other type of well-designed quasi-experimental study.

III Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case studies.

IV Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities.

Grades of Recommendations

A	Requires at least one randomised controlled trial as part of a body of literature of overall good quality and consistency addressing the specific recommendation. (Evidence levels Ia, Ib)
B	Requires the availability of well controlled clinical studies but no randomised clinical trials on the topic of recommendations. (Evidence levels IIa, IIb, III)
C	Requires evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities. Indicates an absence of directly applicable clinical studies of good quality. (Evidence level IV)

Good Practice Point

✓	Recommended best practice based on the clinical experience of the guideline development group

Table 3ii. GRADE approach (http://www.gradeworkinggroup.org/index.htm)

The Grading of Recommendations Assessment, Development and Evaluation (GRADE)

GRADE: Quality of evidence

The GRADE system classifies the quality of evidence in one of four levels:

High quality— Further research is very unlikely to change our confidence in the estimate of effect

Moderate quality— Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate

Low quality— Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate

Very low quality— Any estimate of effect is very uncertain

Evidence based on randomised controlled trials begins as high quality evidence, but our confidence in the evidence may be decreased for several reasons, including:

Study limitations
Inconsistency of results
Indirectness of evidence
Imprecision
Reporting bias.

Although observational studies (for example, cohort and case-control studies) start with a "low quality" rating, grading upwards may be warranted if the magnitude of the treatment effect is very large, if there is evidence of a dose-response relation or if all plausible biases would decrease the magnitude of an apparent treatment effect.

GRADE: Strength of recommendation

The GRADE system offers two grades of recommendations: "strong" and "weak" depending on whether effects of intervention clearly outweigh the undesirable effects, or clearly do not. If trade-offs are less certain—either because of low quality evidence or because evidence suggests that desirable and undesirable effects are closely balanced—weak recommendations become mandatory.

Factors that affect the strength of a recommendation

Factor	Examples of strong recommendations	Examples of weak recommendations
Quality of evidence	Many high quality randomised trials have shown the benefit of inhaled steroids in asthma	Only case series have examined the utility of pleurodesis in pneumothorax
Uncertainty about the balance	Aspirin in myocardial infarction reduces mortality with minimal	Warfarin in low risk patients with atrial fibrillation results in small

between desirable and undesirable effects	toxicity, inconvenience, and cost	stroke reduction but increased bleeding risk and substantial inconvenience
Uncertainty or variability in values and preferences	Young patients with lymphoma will invariably place a higher value on the life prolonging effects of chemotherapy than on treatment toxicity	Older patients with lymphoma may not place a higher value on the life prolonging effects of chemotherapy than on treatment toxicity
Uncertainty about whether the intervention represents a wise use of resources	The low cost of aspirin as prophylaxis against stroke in patients with transient ischemic attacks	The high cost of clopidogrel and of combination dipyridamole and aspirin as prophylaxis against stroke in patients with transient ischaemic attacks

3.1. Do screening-preventative interventions in asymptomatic pregnancies reduce the risk of preterm delivery: a systematic review and meta-analysis

Background: Recent research has suggested that women who experience preterm delivery (PTD) may be identified earlier in pregnancy and before onset of symptoms. Interventions commenced at this earlier asymptomatic stage may offer an opportunity to prevent PTD or lengthen gestation sufficiently to reduce adverse perinatal outcome.

Objectives: To **e**xamine the evidence that supports or refutes interventions that prevent PTD**.** To examine whether interventions are effective in all women or only women at high risk of PTD. To generate clinical recommendations for each intervention according to evidence appraisal tools [6;7].

Methods: A systematic search, meta-analysis and evidence-based appraisal of the identified literature.

Results: There is evidence that introducing screening-preventative strategies for asymptomatic pregnancies may reduce the rate of PTD. Evidence for screening and selective treatment exists for : asymptomatic bacteriuria in all women**;** bacterial vaginosis in low-risk population groups**;** elective cervical cerclage in high-risk pregnancies; indicated cervical cerclage in women with short cervical length on ultrasound; prophylactic progesterone supplementation in high-risk pregnancies; Smoking cessation in all women. However, for most other strategies, such as increased antenatal attendance, or routine administration of prophylactic micronutrients, the evidence is inconsistent and conflicting.

Conclusion: The review suggests an antenatal management plan designed to prevent PTD based on current practice and the evidence identified. Data on neonatal outcomes apart from PTD (such as serious neonatal morbidity and mortality) were lacking in most studies. It was therefore not possible to establish whether preventing PTD or prolonging gestation would correlate to improved perinatal outcome. This lessened the potential clinical usefulness of any proposed preventative strategy. Furthermore, no studies were found that evaluated the effectiveness of combining screening-preventative strategies.

INTRODUCTION

Preterm delivery (PTD) is defined in the UK as delivery after 24 completed weeks' gestation and before the onset of 37 weeks' gestation. In the United States, the lower limit of PTD is 20 weeks, which is used for all US Perinatal statistics. PTD affects 6%-15% of deliveries and represents a major worldwide health concern [8]. PTD has multifactorial aetiology. The causes and subgroups associated with PTD include : spontaneous preterm labour (PTL), 31-50%; multiple pregnancy, 12-28%; preterm premature rupture of membranes (PPROM), 6%-40%; medically indicated (e.g. hypertensive disorders of pregnancy, intrauterine growth restriction, antepartum haemorrhage and chorioamnionitis), 20%-25%; miscellaneous (cervical incompetence, uterine malformation), 10% [9]. Some clinicians believe that cervical insufficiency and PPROM/chorioamnionitis have moreover similar origins and have combined such subgroups when reporting studies of PTD. PTD accounts for 50%-70% of all neonatal morbidity and mortality. Importantly, the earlier the gestation at delivery, the greater the risk of adverse perinatal outcome (**Table 3.1**) [10]. Consequently, there is a need to prevent PTD and any proposed strategy should ideally aim to target PTDs that occur before 34 weeks gestation, as this group contributes most to perinatal morbidity and mortality.

Table 3.1. Gestation-specific perinatal mortality

Gestational age (weeks')	Survival (%)
22-24	5-40
25-27	55-75
28-30	80-85
31-33	95-100
34-36	100

In trying to reduce rates of PTD, the emphasis has been on applying screening-preventative interventions to women symptomatic of PTL or PPROM. However, these have had limited efficacy [11]. Recent research has suggested that women who experience PTD, PTL and PPROM may be identified earlier in pregnancy and before onset of symptoms[12;13]. It has thus been suggested that prophylactic and therapeutic interventions commenced at an earlier asymptomatic stage of pregnancy, either as specific measures or general measures, may offer an opportunity to prevent PTD or lengthen gestation sufficiently to reduce adverse perinatal outcome. We have therefore conducted a systematic search and critical appraisal of the literature to identify the evidence that supports or refutes this approach to reducing the rate of PTD and related perinatal morbidity and mortality. In particular, this review considers health approaches that address all risk factors that affect the entire population of pregnant women, as well as those screening-preventative strategies directed only at high-risk asymptomatic women. The review concludes with a suggested an antenatal management plan designed to prevent PTD based on current practice and the evidence presented in this article.

METHODS An electronic search of MEDLINE (1966- October 2005), EMBASE (1980-October 2005), and the Cochrane library (2005) was conducted using combinations of principle MeSH terms and text words: “preterm labour”, “preterm birth”, “preterm labor”, “labor, premature”, “infant mortality”, “infant premature”, “infant, premature, diseases”, “cerclage, cervix”, “cervical incompetence”, “vaginosis, bacterial”, “fibronectins”, “glucocorticoids” and “tocolysis”. The reference lists of all known primary, review and clinical evidence-based guidelines were also examined to identify cited articles not captured by electronic searches. Articles cited frequently were used in the Science Citation Index to identify additional citations. A meta-analysis was conducted to examine whether differences in outcome occur when the intervention is applied to high or low risk of PTD study populations.

DEFINITIONS

Several studies, albeit based on varying population groups and competing risks, have consistently shown that women with a previous history of PTD, PPROM, medically indicated PTD, delivery of a small-for-gestational-age infant, second trimester pregnancy loss, congenital uterine anomalies, or suspected cervical incompetence are at increased risk of subsequent PTD [14-17]. A selection of these and other risk factors for PTD, with supporting odds ratios is depicted in **Table 3.2.** Associations for a particular risk factor are supported with a single reference citation of a high quality study.

Unless indicated by the presence of another risk factor, 'high-risk' groups are defined as those asymptomatic pregnancies who are deemed to be at increased risk of PTD because of experiencing previous PTD. The pregnancy is asymptomatic if symptoms of PPROM, PTL are absent and there are no overt manifestations of obstetric complications (e.g. multiple pregnancy, hypertension, antepartum haemorrhage). The review is structured by considering screening-preventative interventions that may be commenced following routine antenatal care or antenatal care combined with specialist investigations, and then elaborate on the evidence for the value of strategies in specific high-risk groups as well as population wide health strategies.

Table 3.2. Risk factors associated with increased risk of preterm delivery.

RISK FACTOR	Preterm delivery under study	Odds Ratio	Reference
Routine Screening			
Women aged <18 years at delivery compared to 18-34 years	<32 weeks'	1.41 (1.02-1.90)	18
Second birth in women aged 15-19 years compared to 20-29 years	24-32 weeks'	2.5 (1.5-4.3)	19
Previous singleton PTD < 35 weeks' compared to >35 weeks'	<35 weeks'	5.6 (4.5-7.0)	20
Body mass index<20	<37 weeks'	3.96 (2.61-7.09)	21
Elevated (>90th centile) maternal serum alpha- fetoprotein (AFP)	<35 weeks'	3.9 (1.7-8.7)	22
Singletons following *in vitro* fertilisation	<37 weeks'	2.0 (1.7-2.2)	23
Genital bleeding below 24 weeks'	<37 weeks'	2.5 (1.6-3.8)	15
Placenta praevia	24-27 weeks'	2.90 (2.46-3.42)	24
Loop electrosurgical excision of cervix (matched for smoking status)	<37 weeks'	2.53 (1.42-4.49)	25
Urinary tract infection	<37 weeks'	4.4 (1.47-13.34)	21
Short inter-pregnancy interval (<6 months)	24-32 weeks'	4.1 (3.2 -5.3)	26
Ethnicity- Black Afro-Caribbean Asian vs. White Europeans (UK Study)	<37 weeks'	1.33 (1.15-1.56) 1.45 (1.33-1.56)	27
Alcohol (>7 drinks/week)	<32 weeks'	3.26 (0.8-13.24)	28
Smoking	27-32 weeks'	1.7 (1.3-2.2)	29
Specialist Screening			
Bacterial vaginosis< 16 weeks'	<37 weeks'	7.6 (1.8-31.7)	30
Positive cervico-vaginal fetal fibronectin	<35 weeks'	6.6 (1.7-25.5)	22
Cervical length ≤25mm	<35 weeks'	3.9 (1.7-9.2)	22
Bilateral uterine artery notching	<37 weeks'	2.38 (1.19-4.75)	31
Serum granulocyte colony-stimulating factor (>75th centile)	<35 weeks'	3.1 (1.4-6.9)	22

Evidence for the value of screening-preventative interventions on routine antenatal population screening

The components of routine antenatal care will vary according to country and local resources. We have used UK's NICE guideline as a model for recommended routine antenatal care practice [32].

1. **Early pregnancy booking and ultrasound dating (10-13 weeks)** This provides an opportunity to accurately date the pregnancy, identify multiple pregnancies, and categorise the pregnancy risk based on obstetric history and routine investigations. There is no direct evidence that this care would decrease PTD.

2. **Psychosocial, work and lifestyle factors** There is epidemiological evidence that shows that PTD is associated with low maternal weight, poor weight gain during pregnancy, and low birth weight [33] **(Table 3.2).** Two meta-analyses [34;35] have shown there is insufficient evidence of a beneficial reduction in PTD following increased psychosocial support and home visits, preterm delivery education, bed rest, hydration, reducing excess manual labour and psychological stress, and ensuring that BMI is greater than 20 before conception. Similar results were found for interventions undertaken in both high-risk and low-risk pregnancies [34;35].

3. **Smoking and drugs avoidance**The association between smoking or illicit drugs (such as heroin or cocaine) and adverse perinatal outcomes is well established. A Cochrane meta-analysis of 16 trials showed a reduction in low birthweight (RR 0.81, 95% CI 0.70 to 0.94), a reduction in PTD (RR 0.84, 95% CI 0.72 to 0.98), and an increase in mean birthweight of 33 g (95% CI 11 g to 55 g) with smoking cessation programs[36]. Three non-randomised comparative studies have shown improved neonatal outcomes (but not neonatal

mortality) with antenatal drug avoidance programmes [37-39], with two of these studies suggesting a modest reduction in PTD[38;39].

4. Screening and treatment of anaemia There is epidemiological evidence to support an association between low maternal hemoglobin concentration and low birth weight, as well as between low maternal haemoglobin concentration and PTD[40;41]. However, a meta-analysis [42], and two recent RCTs [43;44] have shown that supplementation of anaemic or non-anaemic pregnant women with iron, folic acid, or both, does not appear to increase either birth weight or the duration of gestation.

5. Screening and treatment of asymptomatic bacteriuria A meta-analysis has shown that antibiotic treatment in pregnant women with asymptomatic bacteriuria found on antenatal screening is effective in reducing the risk of pyelonephritis (OR 0.24; 95% CI 0.19 - 0.32), and PTD or low birthweight (OR 0.60; 95% CI 0.45-0.80)[45], and is thus advocated as part of routine antenatal care [32] .

6. Elective prophylactic cervical cerclage for cervical incompetence A history that comprises any combination of: second trimester miscarriage, painless and progressive dilatation of the cervix, bulging membranes through the cervix prior to onset of labour, or previous cervical surgery (e.g. cone biopsy), may suggest cervical incompetence and an increased risk of PTD in the current pregnancy. This information would normally be identified through routine antenatal screening. Presently, overall evidence suggests that elective cervical cerclage (defined in **Table 3.3**) compared to no cerclage or bed rest is likely to reduce the risk of PTD in women considered to be 'at very high-risk' of a second trimester miscarriage due to a cervical factor [46-48]". There is no consensus on defining this "very high-risk" group, but subgroup analyses suggest this mainly comprises of women with three or more prior preterm births or second trimester losses.

Table 3.3. Defining elective and indicated types of cervical cerclage

Elective cerclage	*Cerclage is performed before clinical or ultrasonographic evidence of cervical dilatation, using McDonald or Shirodkar techniques. Usually performed at 12-16w and based on reproductive history, or other criteria suggestive of cervical incompetence. Also termed as primary cerclage.*
Indicated (emergency) cerclage	*Cerclage is performed following clinical or ultrasonographic evidence of cervical dilatation, funneling or shortening. Also uses McDonald or Shirodkar techniques. Theoretically may be performed at any preterm gestation below 32 weeks', but most often undertaken at midtrimester (18-22 weeks') period. Also termed as secondary cerclage (if scan evidence of cervical dilatation) or tertiary cerclage (if clinical evidence of cervical dilatation)*

Of the three meta-analyses that have clearly distinguished between emergency and elective cervical cerclage, one [47] has shown no statistically significant reduction in rates of PTD, whereas the other two meta-analyses [46;48]have showed elective cervical cerclage to be effective at preventing PTD . Heterogeneity in defining the risk of PTD due to a "cervical factor" has contributed to differences in the findings of the meta-analyses.

The largest cervical cerclage trial [49] showed that elective cervical cerclage performed between 12-16 weeks gestation, in women at risk of cervical incompetence based on clinical history, reduced the risk of PTD (<34 weeks') but did not reduce perinatal mortality. It found that 24 women (95% CI 10-61) would need to undergo elective cervical cerclage to prevent one additional PTD before 34 weeks'. Importantly, a quasi-randomisation method was adopted, which allowed clinicians to allocate cerclage or no cerclage according to the perceived risk of cervical incompetence and only when the clinician felt unsure if such cerclage would be beneficial or non-beneficial.

Evidence for screening-preventative interventions based on routine antenatal care plus specialist investigations

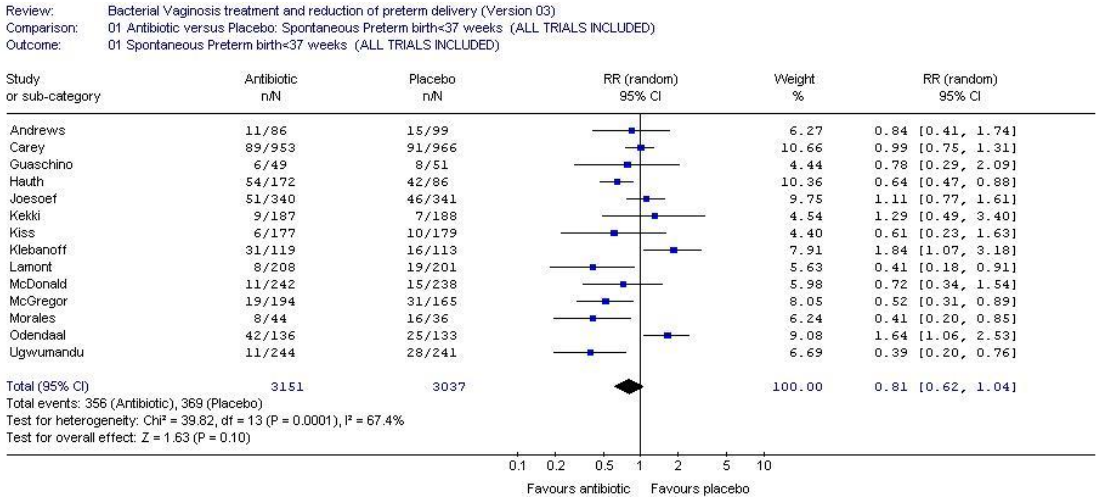
1. Microbiological screening and treatment of the genital tract

- ***Bacterial vaginosis (BV) and trichomonas vaginalis (TV)*:** Bacterial vaginosis (BV) is found in 9%-23% of pregnant women. The presence of BV or trichomonas vaginalis (TV) in asymptomatic women in the second trimester is associated with PTD independent of other known risk factors[50-52]. Importantly, the earlier in gestation BV is detected, the greater is the risk of an adverse outcome. For example, BV at 26-32 weeks' is associated with PTD odds ratio (OR) of 1.4 to 2 whereas BV at 7-16 weeks' carries an OR of 5 to 7.5 [52].

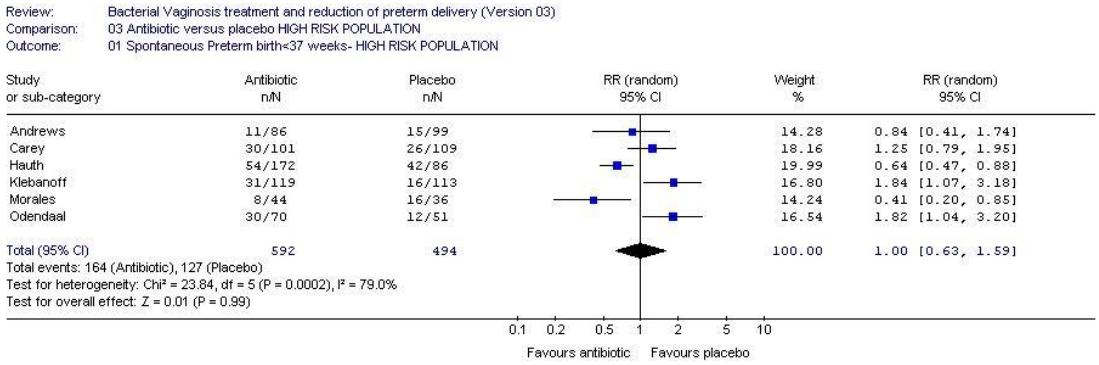
There is evidence that screening and treating BV in unselected low-risk population groups, rather than a heterogeneous combination of high-risk population groups, is effective at reducing the rate of PTD. When considering the combined screening of both low and high-risk populations, six meta-analyses [53-58]have shown that screening and treating asymptomatic BV, using either oral metronidazole (majority of trials) or vaginal/oral clindamycin, does not reduce the risk of PTD. However, our recently published meta-analysis (Figure 3.1) [58] has shown that screening and treating BV from a low-risk population does result in a statistically significant reduction in PTD (nine trials, RR 0.73; 95% CI 0.55-0.98).

Figure 3.1 Meta-analyses for treatment of bacterial vaginosis and reduction in preterm delivery

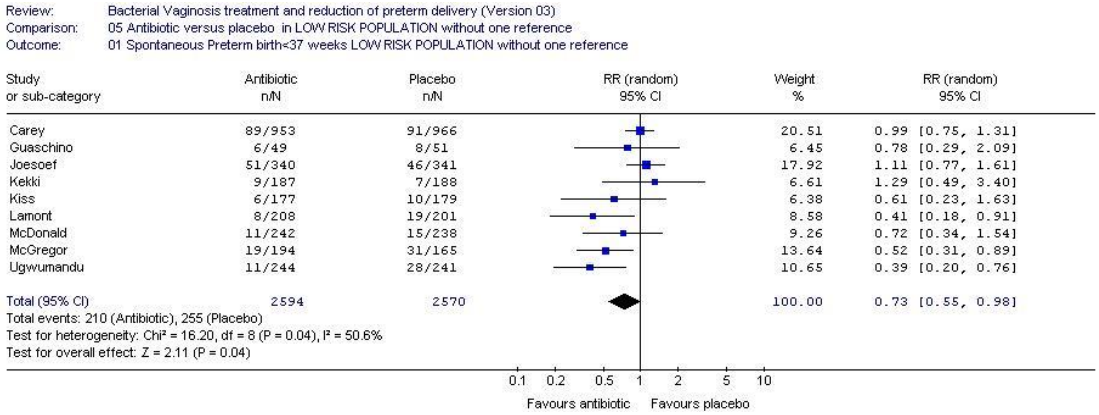
Screening and treatment of bacterial vaginosis in all population groups (both high and low risk) and reduction in preterm delivery



Screening and treatment of bacterial vaginosis in high-risk population and reduction in preterm delivery



Screening and treatment of bacterial vaginosis in low risk population and reduction in preterm delivery



In addition to pregnancy risk stratification, other factors contribute to heterogeneity of the trials and methods used by the meta-analyses of screening and treating BV in pregnancy. These factors have been highlighted by two recent commentaries[58;59] and include: antibiotic types, dosaging and gestation-specific efficacies; criteria for diagnosing BV; and unexplained high therapeutic responses observed from both placebo [60] and the screening process itself without any antibiotics being administered [61]. A randomized trial showed that screening and treating with metronidazole asymptomatic pregnant women for TV at 16 to 23 weeks' did not reduce PTD, and rather worryingly increased the risk of PTD (RR 1.8; 95% CI 1.2 to 2.7; P=0.004) [62]. This is the only trial included in the corresponding Cochrane systematic review[63].

- ***Chlamydia trachomatis:*** Chlamydia trachomatis is estimated to infect 2%-37% of pregnant women. Data from the NIH Preterm Prediction Study showed that women with Chlamydia trachomatis infection at 24 weeks'' gestation were twice as likely as uninfected women for PTD <37 weeks' (OR 2.2; 95% CI 1.03-4.78) and 3 times as likely to have PTD <35 weeks'' gestation (OR, 3.2; 95% CI 1.08-9.57)[64]. Only one trial [65] has examined screening and treatment for Chlamydia to prevent PTD, and this showed no statistically significant reduction. Information from on-going national opportunistic chlaymdia screening programmes may provide further evidence in this area.

- ***Ureaplasma*:** Ureaplasma genital tract infection is associated with PTD and PPROM. A Cochrane 'meta-analysis' including only one trial, concluded there was insufficient evidence to show whether screening and treating women with ureaplasma in the vagina would prevent PTD[66].

- ***Group B streptococcus (GBS)*:** Around 20% of pregnant women have Group B streptococcus (GBS) urogenital colonization. This is associated with an increased risk of urinary tract infection, PTL, PPROM, and infectious perinatal transmission[67]. Only one trial was identified, and this showed no reduction in PTD when pregnant women were routinely screened and treated (using erythromycin) for GBS in the third trimester [68]. Based on this trial, and other observational studies, both the RCOG and CDC have stated that routine screening and antenatal treatment of women with GBS does not reduce the risk of PTD [67;69]. However, screening of high-risk pregnancy groups is recommended by the UK [67], and is universally undertaken during the third trimester in United States[69] and Canada[70]. This facilitates the policy of prophylactic antibiotic treatment to carriers of GBS in labour, which has been shown to reduce the incidence of GBS-related neonatal morbidity and mortality [67;69].

2. **Screening for cervical length by ultrasound or clinically and subsequent indicated cervical cerclage** There is considerable evidence to show that in the absence of uterine contractions transvaginal sonographic measurement of cervical length is an effective way of identifying pregnancies at high-risk of PTD, and has greater predictive value than other ultrasonographic measurements of the cervix such as dilatation of the internal os or funneling of the internal os [71-78]. A systematic review showed for asymptomatic women at or below 20 weeks gestation, a cervical length of 25 mm or less had a test positive likelihood ratio of 6.29 (95% CI, 3.29-12.02) and negative test likelihood ratio of 0.79 (95% CI, 0.65-0.95) for predicting spontaneous PTD before 34 weeks gestation [76].

Cervical cerclage may be performed **electively** (prophylactic procedure discussed earlier) or as an **indicated (emergency) procedure** (defined in **Table 3.3**) following clinical or ultrasonographic evidence of cervical dilatation, funneling or shortening.

- ***Cerclage vs. no cerclage for short cervical length by ultrasound*** A meta-analysis has showed no statistically significant effect of midtrimester indicated cerclage compared to no cerclage on the rates of PTD (four studies) or neonatal mortality (three studies) in women with shortened cervical length on transvaginal ultrasound scanning [79]. However, a meta-analysis of four trials using individual patient-level data has shown that indicated midtrimester cervical cerclage prevents PTD before 35 weeks in women with singleton pregnancies and a short cervical length (RR 0.74, 95% CI 0.57-0.96), and this risk reduction is greater in singleton gestations with prior PTD or prior second-trimester loss [80]. This meta-analysis included two recently published trials [81;82] that had individually shown no beneficial effect of cerclage, as well as the CIPRACT trial [83]which was the only trial to show any beneficial effect of cerclage on preventing PTD.

- **Emergency cerclage in women with cervical incompetence on physical examination** In women with cervical incompetence on physical examination, with membranes at or beyond a dilated external cervical os, a small RCT (n=23) showed that a combination of emergency cerclage, bed rest, antibiotics and indomethacin was more effective at reducing PTD <34 weeks than bed rest with antibiotics alone [84].

3. **Elective first trimester cerclage vs. cervical ultrasound surveillance and indicated emergency cerclage** Four retrospective comparative studies [85-88] have shown no difference in obstetric outcomes with either strategy, whereas a recent prospective study showed better outcome with cervical surveillance and indicated cerclage [89]. Reliable interpretation of these studies, as well as comparison with the trials of cerclage (elective and indicated) discussed earlier, is markedly hampered due to variation in the definition and magnitude of the risk in the population under study. Ultrasonographic cervical length, combined with previous obstetric and reproductive history, has been successfully incorporated into a risk scoring system for predicting PTD [90]. However, there is no evidence from any robust studies that indicates whether such a pregnancy risk stratification strategy followed by indicated cervical cerclage in those at most risk would reduce the rate of PTD. The accumulated evidence therefore suggests that a combination of assessment of risk factors, obstetric history and serial follow-up of cervical length is more likely to identify the group of women who would benefit most from cervical cerclage.

4. **Positive fetal fibronectin testing followed by antibiotic treatment** Fetal fibronectin (fFN) is a basement membrane protein produced by fetal membranes which functions as an adhesive factor of the placenta and membranes to the decidua. It is normally present in cervical secretions until 16-20 weeks gestation. Before testing for the presence of cervicovaginal fFN the following criteria must be met: intact amniotic membranes; minimal cervical dilatation (<3cm); sampling between 22 and 34 weeks gestation. A meta-analysis of cervicovaginal fFN testing in asymptomatic pregnancies to predict PTD before 34 weeks showed a test positive likelihood ratio of 4.01, and a test negative likelihood ratio of 0.78 [91]. Similar results were found by another meta-analysis[92], where for the prediction of outcomes of delivery <37 and <34 weeks'' gestation, a positive fFN had overall sensitivity rates of 52%

and 53%, and overall specificity rates of 85% and 89%, respectively. For the outcomes of delivery within 7, 14, and 21 days, the sensitivities were 71%, 67%, 59% and specificities were 89%, 89% and 92% respectively.

A positive midtrimester fFN test has been associated with an increased risk of subsequently diagnosed maternal and fetal infection. A primary analysis of the trial conducted by MFMU showed that metronidazole plus erythromycin treatment of asymptomatic women with a positive midtrimester fFN (screened between 21 and 26 weeks') did not reduce the risk of PTD as hypothesized, but caused a non-statistically significant increase in PTD <37 weeks and <32 weeks'[93]. Furthermore, a subgroup analysis in women with previous PTD showed a statistically significant increased risk of PTD when the treated group was compared to placebo (46.7% versus 23.9%, P =0.039). Whereas, a secondary analysis of the MFMU study showed that women with both BV or TV and a positive fFN, who were treated with metronidazole, had a non-significant reduction in spontaneous PTD from 14.6% to 8.3% [94].

If the detection of fFN does not alter the natural history of PTD through earlier antibiotic treatment, could there still be a beneficial role for fFN testing in the asymptomatic 'low' or 'high' risk woman? A positive fFN may have clinical benefit by lowering the threshold for admission, or in utero transfer, or administering antenatal corticosteroids. Conversely, a negative fFN may have clinical value in avoiding unnecessary, costly and potentially harmful interventions. However, clinical trials examining improvements in perinatal outcomes following such risk assessment with fFN were not identified in the literature.

Evidence for the value of screening-preventative strategies in specific high risk groups

Multiple Pregnancies Overall, there is a paucity of RCTs that have evaluated screening-preventative interventions in women with multiple pregnancies. A retrospective study showed no difference in perinatal outcomes between multiple weekly prophylactic administration and single course antenatal corticosteroids in women with twin pregnancies [95]. Prophylactic corticosteroids have no proven benefit in twin or higher order multiple pregnancies, and may in fact be associated with increased harm such as decreased birthweight and increased risk of infection [96]. A short cervical length (less than or equal to 25mm), with or without funneling, at midtrimester screening is predictive for PTD in twin pregnancy, albeit at lower sensitivity than when the same test is applied to singleton pregnancies[97-100]. A meta-analysis of trials using individual patient data [80]showed a significant increase in PTD (RR 2.15, 95% CI 1.15-4.01) at less than 35 weeks when indicated cervical cerclage was performed in twin gestations with short cervical length. However, a non-randomized prospective trial showed that indicated midtrimester cerclage in multiple pregnancies does not alter the risks of PTD, PPROM or low birth weight[101]. One retrospective study showed that prophylactic elective cerclage in triplet and higher order multiple pregnancies had no beneficial effect on obstetric or perinatal outcome[102], although this was contradicted by another retrospective study[103].

Antiphospholipid syndrome (APLS Antiphospholipid syndrome (APLS) in pregnancy is characterized by the presence of autoantibodies (anticardiolipin and/or lupus anticoagulant) in association with recurrent fetal loss, maternal thrombocytopenia and other pregnancy complications. Systemic lupus erythematosus (SLE), APLS, and thrombophilias have been associated with similar pregnancy complications of early and late fetal loss, abruption, pre-eclampsia and intrauterine growth restriction in three meta-analyses [104-106]. Evidence from observational studies of rates of PTD in women with SLE, APLS, or thrombophilias is conflicting, and the analysis is complicated by complex co-morbidities of maternal disease (hypertension, renal impairment), fetal compromise (growth restriction), spontaneous PTL, PPROM and medically-indicated PTD. A retrospective study suggested that actively treated SLE (requiring prednisone or other immunosuppresants), or the presence of anticardiolipin antibodies, are predictive of a higher risk of PTD than inactive disease[107]; implying a potential beneficial role in suppressing active SLE disease in pregnancy to reduce the risk of PTD.

Systematic reviews of therapeutic trials for treating APLS in pregnancy conclude that there is currently only weak evidence for a role of low dose aspirin and low-molecular-weight heparin in preventing adverse outcomes [108;109], despite this being the recommended treatment. However, recent preliminary pilot studies suggests the beneficial effect of such prophylaxis has been underestimated, and further research in this area is currently being actively pursued [105;110;111]. Current consensus is that thrombophilia screening is recommended for women with the following previous complications: fetal loss including three or more first trimester loss, two or more second trimester loss, or any stillbirth; early, severe or recurrent preeclampsia and severe intrauterine growth restriction [112].

Pre-eclampsia and uterine artery Doppler There is evidence that women with a previous history of pre-eclampsia-related PTD have a greater risk of pre-eclampsia-related PTD in a subsequent pregnancy as compared with women with a previous PTD[113;114]. A systematic review showed that low dose aspirin (150mg) reduces the risk of perinatal death, pre-eclampsia and PTD in women with a history of previous pre-eclampsia, and should therefore be strongly advocated [115]. The reduction of recurrent pre-eclampsia and perinatal death was greater in women with previous severe early-onset (second trimester) pre-eclampsia. There is evidence showing an association between impaired midtrimester uterine artery Doppler velocimetry and/or uterine artery notching and subsequent pre-eclampsia[116]. However, there are no data from any individual trial or meta-analysis demonstrating any direct reduction in PTD following low dose aspirin administration in women with impaired with impaired uterine artery Doppler characteristics that have been identified by either selective or unselective population Doppler screening[117-121].

Gestational Diabetes and Impaired Glucose Tolerance Overall, both gestational diabetes (GDM) and impaired glucose tolerance (IGT) affect 3%- 6% of pregnancies, and are associated with PTD, PTL, PPROM, and numerous other pregnancy complications. Once identified, women are usually intensively managed with increased obstetric surveillance, dietary regulation, insulin therapy and instructed to maintain tight glycaemic control. However, evidence to support this intensive treatment is lacking. Cochrane meta-analyses have concluded there is insufficient evidence to determine any beneficial or non-beneficial effect of dietary therapy, tight glycaemic control, or other treatments for GDM and IGT, upon pregnancy outcomes[122-124]. A non-randomised comparative study has suggested that universal glucose tolerance screening performed at the first antenatal visit compared to later screening (24-28 weeks') resulted in a reduced risk of PTD and polyhydramnios [125].

Evidence for the value of population-wide preventative strategies in high and low risk groups

Increased antenatal care and attendance There is conflicting opinion whether increased antenatal attendance reduces rates of PTD, and robust RCTs in this area are lacking. Nonetheless, lack of antenatal care has been associated with increased rates of PTD in the presence, as well as absence, of high-risk conditions [126]. In an attempt to reduce PTD, many health organizations such as Canada [127] and France[128] have adopted a population wide health strategy that integrates disease prevention, health promotion, improvements in socioeconomic standards and increased attendance to antenatal care. Observational studies examining variations of this approach have shown modest reduction in rates of PTD when applied to the general pregnant population [128;129]. However, two meta-analyses [130;131] have shown that increased antenatal attendance without specific specialist investigations (such as fetal biophysical or microbiological surveillance) does not reduce the risk of PTD, low birth weight or perinatal mortality in low-risk women.

Prophylactic micronutrients e.g. fish oil, magnesium, vitamins

An overview of trials and systematic reviews concluded that there was insufficient evidence to show that antenatal prophylactic micronutrient supplementation reduced the risk of PTD in either low or high-risk pregnancy groups[42]. Small studies have shown limited reductions in PTD when using fish oil, omega-3 fatty acids, calcium, zinc, magnesium, or multivitamin combinations[132-139], although vitamin C supplementation might even increase the risk of PTD [140]. These interventions need to be further explored in larger RCTs along with other important perinatal outcomes such as growth restriction and pre-eclampsia.

Prophylactic tocolytics Three meta-analyses evaluating prophylactic or maintenance oral tocolytics (mainly beta-mimetics) in high-risk pregnancies (women with threatened PTL or previous PTD) have not shown any reduction in PTD, PTL, perinatal morbidity or perinatal mortality[141-143]. Importantly, newer tocolytics such as nifedipine and atosiban have not undergone evaluation in this manner[144]. A recent trial showed no beneficial effect on PTD, and a potential harmful effect on fetal renal function and the ductus arteriosus, when rofecoxib (a COX-2-specific prostaglandin inhibitor) was administered prophylactically to women at high-risk of PTD between 16-32 weeks gestation [145].

Prophylactic corticosteroids Meta-analysis has shown maternal antenatal administration of a single course of corticosteroids is associated with a significant reduction in perinatal mortality (OR 0.60, 95% CI 0.48 to 0.75), respiratory distress syndrome (OR 0.53, 95% CI 0.44 to 0.63) and intraventricular haemorrhage in preterm infants [146]. Consequently, a single course of antenatal corticosteroids is recommended in women symptomatic of PTL or PPROM or threatening to deliver preterm because of an obstetric disorder [147]. No beneficial effect has been reported following corticosteroids given before 28 weeks' or if infants are delivered more than seven days after initiation of treatment. However, there are no prospective trials on the prophylactic use of corticosteroids (single or multiple courses) in high-risk asymptomatic pregnancies (e.g. growth restricted fetuses, pre-eclampsia, multiple pregnancies, previous recurrent PTD) not at imminent risk of PTD. Their use in these circumstances remains controversial and unproven [147-150]. In particular, many of these women may remain at risk of PTD seven days after the first course, which creates the clinical dilemma of whether to administer a repeat course of antenatal corticosteroids. Repeated courses of antenatal corticosteroids may have a lower rate of neonatal lung disease according to one meta-analysis [148]. However, an extensive review performed by the NIH [151] reported

that there was insufficient evidence to conclusively show any marked adverse or beneficial change with repeated courses of corticosteroids for important neonatal outcomes like small-for-gestational-age at birth, perinatal death, periventricular haemorrhage, periventricular leucomalacia, infectious morbidity, and neonatal lung disease. Absence of beneficial effect of repeated weekly vs. single course antenatal corticosteroids in women at risk of PTD was shown in a recently published trial [152]. Notably, a subgroup analysis of the trial in women with PPROM[153] showed that there was no difference in neonatal morbidity but an increased risk of chorioamnionitis in women who received weekly courses of corticosteroids.

Prophylactic antibiotics A meta-analysis has shown that prophylactic antibiotics given during the second and third trimester of pregnancy in unselected pregnancies reduces the risk of PPROM (OR 0.32, 95% CI 0.14-0.73) [154]. There was a risk reduction in PTD in pregnant women with previous PTD associated with bacterial vaginosis (BV) but there was no risk reduction of PTD in pregnant women with previous PTD unrelated to BV. This observation complements our meta-analysis [58]discussed earlier, that showed screening and treating BV in unselected low-risk populations was beneficial in reducing PTD.

Prophylactic progesterone Two recently published meta-analyses have shown a beneficial role for prophylactic progesterone supplementation in the prevention of PTD [155;156]. Despite differences in the pregnancy risk status of the population included, and the number of included trials [seven trials [155] and ten trials [156]] both meta-analyses have reported similar rates of risk reduction of PTD: OR 0.58, 95% CI 0.48-0.70 [155] and OR 0.45, 95% CI 0.25-0.80 [156] . Based on increasing research in this area, a supportive but cautionary statement was released by the ACOG [157] in 2004, which recommended that prophylactic progesterone to be

used only in women with a history of previous PTD. Two recently published RCTs are included in the meta-analysis. One RCT [158]. (n=142) showed that daily administration of prophylactic vaginal progesterone (100mg) compared to placebo between 24 and 34 weeks' in high-risk pregnancies (women with previous PTD) reduced the frequency of uterine contractions and the rate of PTD (OR 0.40, 95% CI 0.17-0.94). The other RCT (n=463) [159] showed that women with a history of previous PTD, who received weekly injections of 17 alpha-hydroxyprogesterone caproate (17P) from recruitment (16-20weeks') to 36 weeks' gestation, had a reduced risk of PTD before 37 weeks' (OR 0.66, 95% CI 0.54 to 0.81), necrotizing enterocolitis, intraventricular hemorrhage, and need for supplemental oxygen. A secondary analysis of this study showed the risk reduction in PTD is greatest in the subgroup of women whose previous PTD was before 34 weeks [160]. Further research on the correct progesterone formulation, mechanism of action, efficacy, and risk-benefit profile is needed before prophylactic progesterone may become an accepted clinical intervention in high-risk asymptomatic pregnancies.

Antenatal management plan and role of specialist antenatal prematurity clinics

Specialist antenatal clinics for women with multiple pregnancy, diabetes, epilepsy, and haematological disorders are widespread and well established. Likewise, women at high-risk of prematurity may also benefit from such specialised antenatal care with individualised risk assessment and application of general and specific screening-preventative measures to prevent PTD or reduce adverse perinatal outcome. These clinics are common in many university teaching hospitals[161], although rigorous evaluation of their exact beneficial role in reducing PTD is pending. The exact antenatal design, resources needed, and timing of screening interventions remain a controversial issue and have little supporting evidence. Nevertheless, we suggest an antenatal management plan (**Table 3.4)** that may prevent PTD based on established practice and evidence presented in this review that may be considered a basis for further modification and research.

Table 3.4. Suggested antenatal strategy to prevent preterm delivery

ANTENATAL VISIT AND PURPOSE	**Infection (Screen and treat BV, UTI)**	**Cervico-vaginal fFN**	**Ultrasound Abdominal and Transvaginal**	**Other interventions to be considered**
Pre-pregnancy Counselling on recurrence risk and any modifiable predisposing factors	Yes	No	No	Cessation smoking and illicit drugs Improve BMI>25 Thrombophilia screen if history suggests Optimise control of diabetes, high BP Change anticoagulation or antihypertensive drugs
8 weeks' Routine booking bloods	Yes	No	Dating pregnancy	Thrombophilia screen and commence aspirin & LMWH if positive. Low dose aspirin if previous pre-eclampsia (consider use if previous stillbirth, abruption, severe IUGR) Prophylactic progesterone General preterm birth education, support, and risk factor avoidance. Screen and treat BV, UTIs Low threshold for GTT testing
12, 16, 20, 24, 28 weeks' *Nuchal Translucency(12w) and/or Triple Test or msAFP (15-18w)*	No	No	Serial Cervical assessments in women at high risk of PTD	Emergency or elective (12-16w) cervical cerclage based on ultrasound findings and/or reproductive history Emergency cervical cerclage is not indicated if above 32 weeks' Low threshold for GTT testing
22 weeks'	Yes	No	Detailed fetal survey Uterine artery Doppler	Low dose aspirin if suspect pre-eclampsia or IUGR due to uterine artery notching and/or previous history Screen and treat BV and UTIs

24, 28, 32, 36 weeks' GTT at 28 weeks'	No	Only if symptomatic	Fetal growth and umbilical artery Doppler	Prophylactic corticosteroids, antibiotics if symptomatic of PTL or PPROM. In utero transfer to unit with NICU if symptomatic with positive fFN
Labour **Spontaneous or induced**	Yes	Helps confirm Likelihood of PTL, PPROM	Asses fetal well-being, and presentation	Prophylactic corticosteroids, antibiotics (especially GBS prophylaxis). Tocolytics if in utero transfer to unit with NICU is needed.
Post-partum **6 week antenatal check**	No	No	No	Review antenatal events and delivery Identify modifiable factors for future prevention of PTD

FootNote: Bacterial vaginosis, BV; BMI, body mass index; BP, blood pressure; fFN, fetal fibronectin; GBS, Group B streptococcus; GTT, glucose tolerance test; LMWH, IUGR, intrauterine growth restriction; low-molecular weight heparin; msAFP, maternal serum alpha-fetoprotein; NICU, neonatal intensive care unit; PPROM, preterm premature rupture of membranes; PTL, preterm labour; UA, uterine artery; UTI, urinary tract infection.

Discussion

There is evidence that introducing screening-preventative strategies for asymptomatic pregnancies may reduce the rate of PTD. Evidence for screening and selective treatment exists for: asymptomatic bacteriuria (meta-analysis: OR 0.60; 95% CI 0.45-0.80); bacterial vaginosis in low-risk population groups (meta-analysis: RR 0.73; 95% CI 0.55-0.98, **figure 3.1**); elective cervical cerclage in high-risk pregnancies; indicated cervical cerclage in women with short cervical length on ultrasound (meta-analysis: RR 0.74, 95% CI 0.57-0.96); prophylactic progesterone supplementation in high-risk pregnancies (meta-analysis: OR 0.45, 95% CI 0.25-0.80). A summary of the quality of evidence and grading of recommendation for these interventions are depicted in **Table 3.5.**

However, for most other strategies, such as increased antenatal attendance, or routine administration of prophylactic micronutrients, the evidence is inconsistent and conflicting. Information on neonatal outcomes apart from PTD (such as serious neonatal morbidity and mortality) was found to be lacking in most studies. It was therefore not possible to establish whether preventing PTD or prolonging gestation would correlate to improved perinatal outcome, and so lessened the potential clinical usefulness of any proposed preventative strategy. No studies were found that evaluated the effectiveness of combining screening-preventative strategies.

Table 3.5 Summary of screening and preventative strategies that may reduce the risk of preterm delivery

Strategy for preventing preterm delivery	RCOG Level of Evidence	GRADE Quality of Evidence	GRADE Strength of Recommendation
Asymptomatic bacteriuria in all women	Ia	High	Strong
Bacterial vaginosis in low-risk population groups	Ia, Ib	Moderate	Weak
Elective cervical cerclage in high-risk pregnancies	Ib, IIa, IIb	Moderate	Strong
Indicated cervical cerclage in women with short cervical length on ultrasound	Ib, IIa, IIb	Moderate	Strong
Prophylactic progesterone supplementation in high-risk pregnancies	Ia, Ib	High	Strong
Smoking cessation in all women	IIb, III	Very Low	Weak

Reviews discussing screening-preventative interventions for preventing PTD often consider both symptomatic (symptoms of PTL or PPROM) and asymptomatic pregnancies. We have focused solely on asymptomatic pregnancies and adopted a rigorous systematic review methodology to provide the best possible analysis of the data available. The review is weakened by over-reliance on conclusions drawn from meta-analyses and underpowered RCTs. We have identified considerable heterogeneity in the studies and methodologies adopted by the meta-analyses, in particular, the groups of women considered to be 'high' and low' risk of PTD, the magnitude of their risk of PTD, and gestation-specific timing of the intervention differs considerably for each trial and meta-analysis (e.g differences in types of antibiotic, dosage, method of administration, and gestation when given). This heterogeneity would propagate any potential omission, de-emphasis or misinterpretation of the results of RCTs.

The poor clinical efficacy of the proposed screening-preventative strategies is not unexpected. Firstly, current routine antenatal screening is relatively ineffective at identifying the majority of pregnancies at risk for PTD, even if combined with specialist investigations. Secondly, most of the preventative interventions discussed appear to have, at best, only mild efficacy at preventing PTD. Importantly, adverse effects of increasing the risk of PTD were noted for some of the interventions. Examples include antibiotic treatment for women screened to be positive for fFN or trichomonas vaginalis, and inherent surgical risks associated with cervical cerclage.

Further trials are needed to identify the optimum gestation and subgroups that may benefit most from such screening and therapeutic interventions. Currently on-going meta-analyses of individual patient data [162;163] may provided further evidence for the roles of elective and indicated cerclage on preventing PTD, and aspirin on the prevention of pre-eclampsia related consequences.

It was surprising to show a reduction in PTD following screening and treating BV in the low-risk (RR 0.73; 95% CI 0.55-0.98) **(Figure 3.1)** but not the high-risk group, as one would normally expect an opposite relationship and treatment to exert greater risk reduction in the higher risk group. The differences in antibiotic sensitivity between high and low risk groups may suggest differing causal contributions of the infectious process to PTD. The evidence, along with prior knowledge of differing predisposing factors and prognosis between these risk groups [52;164], supports the hypothesis that **PTD in high and low risk pregnant women are different entities and not linear extremes of the same syndrome;** a view shared by others [8], and deserving of further confirmatory research.

This review has provided a structured approach to addressing the complex issue of preventing PTD. By elaborating on the use of both specific and general measures this review should appeal to all health care professionals (General Practitioners, Health Visitors, Midwives, Obstetricians) involved in the care of pregnant women, as well as colleagues involved in delivering public health care strategies. We have proposed an antenatal care strategy that adopts a gestation-specific approach to assessing risk and intervening as needed (**Table 3.4**) that may be commenced at initial antenatal booking. However, the efficacy and cost-effectiveness of these approaches (**Tables 3.4 & Table 3.5**) needs to be rigorously evaluated before routine clinical implementation. Differences in the prevalence of infection and other obstetric and reproductive factors means that any proposed preterm prevention strategy should be individualised to the population and health care setting. Specialist antenatal clinics for women deemed at high-risk of PTD may provide an opportunity to carry out this research and perform this clinical role.

The recent NICE UK antenatal care guideline [32] has stated pregnant women should not be offered routine screening for BV, Chlamydia, group B streptococcus, cervical ultrasonography, or cervical fFN [32]. Our review has presented preliminary evidence that some of these strategies may actually be beneficial, and as such, adds to the current debate in this important clinical area.

Discussion on bacterial vaginosis meta-analyses

Meta-analyses are liable to numerous biases despite quality control measures, and their results may not necessarily be trusted [165;166]. Concerning screening and treating BV in pregnancy, five meta-analyses [Cochrane [53], 13 trials; Riggs [54], 11 trials; Leitich [55], 10 trials; Guise [56], 7 trials, and Okun [57], 11 trials] have been published in the last four years. All have showed no reduction in PTD. The authors of all five meta-analyses have reported significant clinical, methodological and statistical heterogeneity of the included studies, and have utilised different fixed or random effects pooling. Only two meta-analyses [53;57] undertook a comprehensive investigation of the reasons for heterogeneity or attempted strategies to counter this effect. Nevertheless, without undertaking this process, the summary estimate produced by each meta-analysis may not be valid. Our "repeat" meta-analysis , which considered the classification of population risk and therefore addressed the issue of heterogeneity to some extent, showed an unexpected beneficial effect of screening in women that were at low-risk rather than high risk of PTD .

In summary, we wish to emphasise why it is important to consider the individual primary study as well as the methodology criteria adopted by meta-analyses, particularly when included trials are underpowered, few in number, and exhibit marked heterogeneity. These factors may contribute to why meta-analyses to date have reported evidence of lack of effectiveness, but in fact may be subject to the bias of varying study methodologies, mixing high and low risk pregnancies groups, and a confounding effect introduced by the screening process itself that is difficult to distinguish from antibiotic treatment of bacterial vaginosis.

3.2. Non-contraceptive uses of levonorgestrel releasing hormone system (LNG-IUS)- a systematic enquiry and overview

Abstract

Levonorgestrel releasing intrauterine systems (LNG-IUS) were originally developed as a method of contraception in the mid 1970's. The only LNG-IUS approved for general public use is the Mirena® LNG-IUS, which releases 20mcg of levonorgestrel per day directly in to the uterine cavity. However, new lower dose (10mcg and 14mcg per day) and smaller sized LNG-IUS (MLS, FibroPlant-LNG) are currently under clinical development and investigation. Research into the non-contraceptive uses of LNG-IUS is rapidly expanding. In the UK, LNG-IUS is licensed for use in menorrhagia and to provide endometrial protection to perimenopausal and postmenopausal women on estrogen replacement therapy. There is limited evidence to suggest that LNG-IUS may also be beneficial in women with endometriosis, adenomyosis, fibroids, endometrial hyperplasia and early stage endometrial cancer (where the patient is deemed unfit for primary surgical therapy). This systematic enquiry and overview evaluates the quality of evidence relating to the non-contraceptive therapeutic uses of LNG-IUS in gynaecology.

Additional point relating to date listed in tables: For all studies listed in tables, we have reported the sample sizes originally recruited by the studies. Where the study drop out rate exceeds 10% we have stated this rate to provide the reader with an impression of the number of subjects actually evaluated by the study where this drop out rate is exceeded.

Introduction

The only levonorgestrel-releasing intrauterine system (LNG-IUS) approved for general public use is the Mirena® (Schering AG), which is a T-shaped plastic intrauterine device (IUD) that releases levonorgestrel (20mcg per day) directly into the uterine cavity. The mean systemic levels of levonorgestrel with this LNG-IUS (425pg/mL at 1 month, 330 pg/mL at 6 months, mean age of subjects was 31 years (range 18-42) [1] are less than those achieved with therapeutic oral or parenteral doses of progestogens (hence minimizing systemic side effects) and exceeds the critical value of 200 pg/mL below which ovulation occurs [2]. Mirena was first launched in Finland in 1990 and has been marketed in the UK since 1995 as a contraceptive device. Two new lower levonorgestrel dose and smaller sized LNG-IUS devices are currently under clinical development and investigation: FibroPlant™-LNG (frameless device, Contrel Research, Belgium) and MLS system, releasing 14mcg and 10mcg levonorgestrel per day respectively[3;4].

Mirena® LNG-IUS is currently licensed in the UK as a 5-year contraceptive agent (license awarded 1995), treatment for idiopathic menorrhagia (license awarded 2001), and to provide uterine protection during estrogen replacement therapy in peri- and postmenopausal women (license awarded 2005). The latter two applications for Mirena® LNG-IUS are not licensed in USA or Canada. The fertility control provided by LNG-IUS is comparable with that of female sterilisation, and is completely reversible [5]. There are many other non-contraceptive beneficial effects of LNG-IUS that have important public health implications. These have been summarized by several reviews [6-9] and policy statements [10], and incorporated within one systematic review examining all types of intrauterine device [11]. However, there has since been a considerable expansion of publications in this area, many of which have contrasting methodological quality and results. This article expands on past reviews by incorporating

these recent advances and performs an up-to-date systematic review focused entirely on LNG-IUS. Furthermore, this review evaluates the quality of supporting evidence, and where available, presents information relating to adverse effects, cost-effectiveness, and health related quality of life (HRQL) issues.

Materials and Methods All observational and experimental studies examining the use of LNG-IUS in Gynaecology were retrieved from MEDLINE (1996-2005), EMBASE (1996-2005 week 08), Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects (DARE), The National Research Register NRR (http://www.update-software.com/National/), Medical Research Council's Clinical Trials Register, and details on reviews in progress collected by the NHS Centre for Reviews and Dissemination were searched. Schering HealthCare (UK) were also contacted for further information on licensing and any unpublished controlled clinical trials. The following search terms and word variants were used: 'exp Intrauterine Devices, Medicated/', 'levonorgestrel releasing', 'levonorgestrel-releasing','LNG-IUS', 'LN-IUS', 'LN-IUD', 'LNG-IUD', 'mirena.tw.' 'Levonorgestrel adj5 (intrauterine or device or coil or system). tw, 'progest$ adj5 (intrauterine or device or coil or system).tw', 'intra-uterine progestogen' combined with "AND" to 'gyne$', 'therapy' 'endometriosis', 'endometrio$.mp', 'genital neoplasms, female', 'dysmenorrhoea', 'pelvic pain', 'estrogen replacement therapy', 'hormone replacement therapy', or 'genital diseases, female'. The search was completed in March 2005. Obtained data were qualitatively and quantitatively analysed. If trials are deemed suitable (similar population groups, trial methodology and outcome measures) meta-analysis will be performed.

Results

A summary of the studies identified describing the non-contraceptive therapeutic use of LNG-IUS according to the therapeutic indication is shown in **Table 3.6**. The associated level of evidence and strength of recommendation for each indication is also indicated according to accepted criteria [12].

Table 3.6. Summary of studies that assess LNG-IUS use in various non-contraceptive therapeutic indications as primary study outcome measures

Therapeutic use of LNG-IUS	**RCTs**		**Cohort Studies**	**Prospective or Retrospective Observational Studies**	**Case Report or small case series**	****Level of evidence**	***** Strength of recommendation**
	More than 50 women in LNG-IUS arm of trial	**Less than 50 women in LNG-IUS arm of trial**					
Menorrhagia	1	9	2	5	0	I, II, III	A
Fibroids/Fibroid related menorrhagia	1#	2#	1	6	1	II, III	B
Endometriosis	0	2	0	3	0	I, III	C
Adenomyosis	1	0	0	1	1	I, III, III	C
Uterine protection with estrogen replacement therapy in per- and postmenopausal women	3	4	3	7	0	I, II, III	A
Uterine protection with tamoxifen in postmenopausal women	1	0	0	1	0	I, III	A
Endometrial hyperplasia	0	0	1	3	2	II, III	C

Footnotes to Table 3.6

[#] Trial(s) exist, but therapeutic outcome was not assessed as a priori primary outcome measure in the RCT comparison

****Classification of Evidence Levels**

Ia Evidence obtained from meta-analysis of randomised controlled trials.

Ib Evidence obtained from at least one randomised controlled trial.

IIa Evidence obtained from at least one well-designed controlled study without randomisation.

IIb Evidence obtained from at least one other type of well-designed quasi-experimental study.

III Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case studies.

IV Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities.

*****Strength of Recommendation**

A Directly based on category I evidence

B Directly based on category II evidence or extrapolated recommendation from category I evidence

C Directly based on category III evidence, or extrapolated recommendation from category I or II evidence

GPP Directly based on category IV evidence, or extrapolated recommendation from category I, II or III evidence

Menorrhagia

Early RCTs and cohort studies evaluating the contraceptive efficacy of LNG-IUS against Cu-IUCD showed women who received LNG-IUS reported less dysmenorrhoea and menstrual blood loss (MBL)[35;36]. This provided a basis to examine whether LNG-IUS would also decrease menstrual blood loss in women with idiopathic menorrhagia (dysfunctional uterine bleeding DUB) and compare its efficacy against established medical and surgical treatments for menorrhagia. In total, approximately 670 women with menorrhagia have used LNG-IUS as part of a comparative or non-comparative study (**Table 3.7**) evaluating the efficacy of LNG-IUS in treating menorrhagia. Women using the frameless FibroPlant-LNG™ or Femilstrade LNG-IUS (20mcg/24hr) devices for contraception [33;37] or treatment of menorrhagia [30-33] also reported decreased MBL, however study sample sizes were limited (n=76 menorrhagia cases) and the devices remain under clinical development.

Two incomplete trials were identified in the search, SMART (Satisfaction with Mirena and Ablation: a Randomised Trial) [38] and TALIS (Thermo-Ablation versus the Levonorgestrel Intrauterine System)[39]. Furthermore, our unit is about to commence the ECLIPSE trial (Effectiveness and Cost-effectiveness of Levonorgestrel containing Intrauterine system in Primary care against Standard treatment, ISRCTN 86566246) in the UK.

Overall, for all listed studies, LNG-IUS use in women with menorrhagia reduces menstrual blood loss by 79% to 97%. No RCTs have compared LNG-IUS with placebo or no treatment in women with menorrhagia. Importantly, studies have used various outcome measures, which precludes pooled meta-analysis. These include: indirect (pictorial blood loss

assessment chart, PBAC) or direct (alkaline haematin method) measures of menstrual blood loss (MBL); patient willingness to continue with treatment; or patient preference to abandon LNG-IUS treatment in favour of hysterectomy or endometrial resection. There are insufficient participants to show long term therapeutic effect with LNG-IUS, as most studies did not extend beyond one year follow up. The total number of participants continuing with LNG-IUS by 3-year [19;34] and 5-year follow up[14] was 96 cases. Of the ten trials depicted in **Table 3.7,** seven [13;16-18;20;23;24] have been incorporated in two Cochrane reviews [40;41] and one systematic review [42]. Three recent RCTs [15;21;22] and two quality cohort studies [25;26] not included in the prior published meta-analyses have been listed in **Table 3.7**. The high patient satisfaction (72-94%) and overall continuation rates (65- 88%) obtained in these RCTs are consistent with those identified in observational studies of LNG-IUS use for treating menorrhagia [29;43;44]. Interpreting the evidence from **Table 3.7**, LNG-IUS system is at least comparable or more effective than oral progestogens. Similar rates of patient satisfaction and quality of life are reported when comparing LNG-IUS against transcervical endometrial resection or balloon ablation. However, surgical methods are significantly more effective in reducing menstrual bleeding or inducing amenorrhoea within one year follow up. However, one trial with longer follow up of three years [19] showed no significant difference between the LNG-IUS and TCRE in the reduction of menstrual blood loss.

Table 3.7. LNG-IUS studies assessing therapeutic effect in women with menorrhagia

Author	**Year of Publication**	**Study Type**	**Sample Size of women with menorrhagia**	**Comparison**	**Outcomes (within one year follow up unless stated otherwise)**
Hurskainen [13;14]	2001, 2004	RCT	236	119 LNG-IUS vs. 117 hysterectomy For the LNG-IUS group: at one year 81/119 and at five year 57/119 continued to have LNG-IUS in situ	**5 year follow up** Of the LNG-IUS group by one year 68% continued with LNG-IUS and 20% had TAH. Both treatments had comparable improvements in HRQL
Soysal [15]	2002	RCT	72	36 LNG-IUS vs. 36 thermal balloon ablation 14% drop out from LNG-IUS	Greater reductions in PBAC with ablation than LNG-IUS. Comparable improvements in haemoglobin Ablation group perceived greater improved HRQL than LNG-IUS
Crosignani [16]	1997	RCT	70	35 LNG-IUS vs. 35 TCRE 14% drop out from LNG-IUS	Marginally greater reductions in PBAC with TCRE Comparable satisfaction rates
Kittelsen [17]	1998	RCT	60	30 LNG-IUS vs. 30 TCRE 12% drop out rate	Comparable reductions in PBAC Comparable satisfaction rates
Istre [18] Rauramo [19]	2001,2004	RCT	59	30 LNG-IUS vs. 29 TCRE 31% drop out rate	**3 year follow up** Greater reductions in PBAC with TCRE than LNG-IUS (90% cure vs. 67% cure) at one year , but

					comparable reductions of MBL noted at 3 years. Increased haemoglobin and ferritin with both treatments
Lahteenmaki [20]	1998	RCT	56	28 LNG-IUS vs. 28 medical treatment whilst awaiting hysterectomy 25% drop out from LNG-IUS	At 6m, 64% LNG-IUS cancelled TAH whilst 14% cancelled TAH in medical treatment group
Reid [21]	2005	RCT	51	25 LNG-IUS vs 26 mefenamic acid 16% drop out from LNG-IUS	Greater reductions in MBL, PBAC and total menstrual fluid loss with LNG-IUS (90% vs 23%) at 6 months.
Barrington [22]	2003	RCT	50	25 LNG-IUS vs. 23 balloon ablation 12% drop out rate	Comparable reductions in PBAC
Irvine [23]	1998	RCT	44	22 LNG-IUS vs. 22 oral norethisterone No drop out rate	Comparable reductions in MBL (>90%). Greater satisfaction with LNG-IUS
Milson [24]	1991	RCT	35	20 LNG-IUS vs. 15 transexamic acid 20% drop out from LNG-IUS	Greater reduction in MBL with LNG-IUS (>90%)
Romer [25]	2000	Prospective cohort	30	LNG-IUS vs. roller ball endometrial ablation	Comparable reductions in MBL and rates of amenorrhoea
Henshaw [26]	2002	Retrospective cohort	62	LNG-IUS vs. microwave endometrial	**mean 14 month follow up** Comparable reductions in MBL

				ablation	and dysmenorrhoea Comparable patient satisfaction rates
Mansour [27]	1998	Prospective	52	No comparison LNG-IUS	91% of women had improved dysmenorrhoea and menorrhagia 83% continued with treatment beyond one year
Barrington [28]	1997	Prospective	50	LNG-IUS No comparison. Women were awaiting TCRE or hysterectomy	Reduced PBAC in 82% 8% amenorrhoea No change in haemoglobin or ferritin Decreased premenstrual symptoms in 56% Reduced dysmenorrhoea in 80%
Monteiro [29]	2002	Prospective	44	LNG-IUS No comparison	Decreased MBL and increased haemoglobin 80% continuation rate at one year
Wildemeersch [30] [31;32]	2004	Prospective	12 in 2004, 32 in 2001	No comparison. FibroPlant-LNG	Decreased PBAC (median MBL decreased by 90%) Decreased dysmenorrhoea
Wildemeersch [33]	2005	Prospective	60 women: 28 normal periods, 32 menorrhagia	No comparison Femilstrade LNG-IUS 20mcg/24 hr	Similar reductions in MBL (96-99%) for both groups 33% developed amenorrhoea (10 women in each group)
Xiao [34]	2003	Prospective	34	LNG-IUS No comparison	**3 year follow up** Decreased MBL at one year (84%) and three (85%) years. 33% amenorrhoea at 6 months. Increased Hemoglobin and serum ferritin.

Footnotes FibroPlant-LNG is a frameless low-dose (releasing 14mcg levonorgestrel/day) frameless LNG-IUS; HRQL, Health related quality of life assessments; LNG-IUS releasing 20mcg levonorgestrel /day; MBL menstrual blood loss; PBAC pictorial blood loss assessment chart; TCRE transcervical endometrial resection

Meta-analyses and RCTs have shown that a significant proportion of women with menorrhagia initially treated with either conservative surgery [45] or LNG-IUS [42] are likely to require hysterectomy as a definitive treatment. However, an RCT (n=236) with five year follow up has shown hysterectomy does not improve overall health related quality of life significantly more than LNG-IUS and it can cause serious complications [14]. Furthermore, the same trial showed that LNG-IUS was more cost-effective than hysterectomy at one-year [13] (US $ 1530 vs. US $ 4222) and five-years [14] follow up (US $ 2817 vs. US $ 4660 per participant). This estimate includes the direct (e.g. operative, costs) and indirect costs (e.g. sick leave days) associated with the 42% of the women assigned to the LNG-IUS group who eventually underwent hysterectomy. Menorrhagia may arise from inherited bleeding disorders (e.g. von Willebrand's Disease). A prospective study (n=16) has shown reduction in menstrual blood loss, improvement in quality of life in women with menorrhagia due to an inherited bleeding disorder when treated with LNG-IUS [46].

Uterine fibroids and fibroid related menorrhagia One cohort study, five prospective observational studies, and one case report have directly assessed the use of LNG-IUS in treating fibroids and fibroid related menorrhagia or dysmenorrhoea. Three RCTs, undertaken for other indications, have described decreased incidence of fibroids following LNG-IUS insertion [35;54;55]. All these studies are depicted in **Table 3.8**. Apart from one study[35], study duration and follow up did not exceed one year. Inclusion criteria were clearly stated in two studies: women with fibroid uterus below 12 weeks gestational size on pelvic examination or 380ml uterine volume on pelvic ultrasound [47;48].

Table 3.8. LNG-IUS studies directly or indirectly assessing therapeutic effect on fibroids or fibroid related menorrhagia

Author	**Year of Publication**	**Study Type**	**Sample** Size	**Comparison**	**Outcomes (within one year follow up unless stated otherwise)**
DIRECT STUDIES					
Soysal [47]	2005	Prospective and retrospective cohort	64	32 LNG-IUS vs. 32 thermal balloon ablation (historical matched group)	Comparable effective reductions in PBAC (around 90%) Comparable increases in haemoglobin Fibroid size change not assessed
Grigorieva [48]	2003	Prospective and retrospective	67	No comparison	Effective reductions in PBAC. Improved ferritin and haemoglobin 40% amenorrhoea at 12 months Decrease in fibroid size (33%)
Mercorio [49]	2003	Prospective	19	No comparison	Reduced PBAC, but 14/19 still had persistent menorrhagia
Wildemeersch [50]	2002	Prospective	14	No comparison FibroPlant-LNG	Reduction in MBL in 13/14 No reduction in fibroid size
Starczewski [51]	2000	Prospective	12	No comparison	Reduction in MBL 11/12 cases. Amenorrhoea 50% cases Improved Haemoglobin No change in fibroid size
Singer [52]	1994	Prospective	5	No comparison	Reduction in MBL Reduction in fibroid size Follow up to 18 months
Fong [53]	1999	Case report	1	No comparison	Reduction in MBL and fibroid size
INDIRECT STUDIES					
Gardner [54]	2000	RCT	122	64 LNG-IUS and tamoxifen against 58 tamoxifen	13% reduction in fibroids from baseline in LNG-IUS group

				27% drop out rate from LNG-IUS group	
Inki [55]	2002	Prospective study (examine one arm of RCT)	38	117 had LNG-IUS for menorrhagia, of this 38/119 (32%) had uterine fibroids	No ultrasonographic change in uterine fibroids, but decreased endometrial thickness. Increased risk of ovarian cysts compared to hysterectomy
Sivin [35]	1994	RCT	2226 recruited, 1125 had LNG-IUS, 1121 had Cu-IUCD. Baseline fibroid incidence: unclear. Identified 15 fibroids at end of study	LNG-IUS vs. Cu-IUCD (TCu 380Ag) Parous women aged 18-38, all desiring contraception. 7 year study follow up (3416 women years in LNG-IUS and 3975 women years in Cu-IUCD) 11.4% drop out rate from LNG-IUS	**7 year follow up** LNG-IUS compared to Cu-IUCD has decreased incidence of dysmenorrhoea, vaginitis, fibroids, but higher rates of amenorrhoea, follicular ovarian cysts, acne, mastalgia, weight gain, and headache. LNG-IUS: 50% amenorrhoea or oligoamenorrhoea by end of study, compared to 9% with Cu-IUCD

All studies directly assessing LNG-IUS in women with fibroids reported decreased menstrual blood loss (84-90%) and similar increases in haemoglobin of 2-3 g/dl [47;48;51]. However, there was inconsistency on whether LNG-IUS is associated with decreased fibroid size [48;52;53] or no change in fibroid size [50;51;55]. Fibroid size following LNG-IUS was not assessed in one cohort study [47]. Regarding the indirect studies, one large RCT suggested there may be decreased incidence of uterine fibroids with LNG-IUS compared to Cu-IUCD [35]. A similar observation of 13% decreased incidence of fibroids was observed in a RCT comparing LNG-IUS and tamoxifen against tamoxifen alone [54].

Endometriosis

Two RCTs and three prospective observational studies were identified. All studies had limited sample sizes (range 11 to 39 participants in LNG-IUS arm of study), and their features are shown in **Table 3.9**. Population groups differed considerably between studies and included women with early stage and late stage endometriosis, rectovaginal endometriosis, immediately surgically treated endometriosis, prior history of endometriosis diagnosis, chronic pelvic pain and/or dysmenorrhoea. This heterogeneity of population, combined with small sample size, limits the strength and validity of the findings. Two studies from the same group [57;60] report approximately 40% absolute risk reduction in dysmenorrhoea by one year with LNG-IUS use. This is consistent with a three year prospective study[59] and a one year RCT [56] that reported similar magnitude reductions in dysmenorrhoea and chronic pelvic pain . A prospective study reported decreasing severity of endometriosis on AFS staging following LNG-IUS insertion [58].

Table 3.9. LNG-IUS studies assessing therapeutic effect in women with endometriosis

Author	Year of Publication	Study Type	Sample Size	Comparison	Outcomes (within one year follow up unless stated otherwise)
*Petta [56] *electronic publication ahead of written publication	2005	RCT	82 with endometriosis, dysmenorrhoea and chronic pelvic pain	39 LNG-IUS vs 43 GnRH analogue	**6 months follow up** Comparable reductions in pelvic pain and improved quality of life measures. Greater amenorrhoea with GnRH than LNG-IUS (98% vs 70%)
Vercellini [57]	2003	RCT	40 parous women , not desiring fertility, with endometriosis associated dysmenorrhoea and receiving conservative surgical treatment of endometriosis	20 Post operative LNG-IUS and endometriotic surgery vs. 20 endometriotic surgery alone 10% drop out from LNG-IUS group	Decreased recurrence of dysmenorrhoea in LNG-IUS vs. surgery alone group (10% vs. 45%, p=0.03) 28% or 50% LNG-IUS users had amenorrhoea or oligoamenorrhoea Comparable levels of patient satisfaction (75% and 50%)
Lockhat [58;59]	2004,2005	Prospective	34 with symptomatic mild-moderate endometriosis	No comparison (1 yr and 3yr follow up)	Decreased dysmenorrhoea and/or non-cyclical pelvic pain and AFS staging of endometriosis. 68% continuation rate at one year 56% continuation rate at 3 years.
Vercellini [60]	1999	Prospective	18 Parous women who had history of previous endometriotic surgery and had recurrent dysmenorrhoea	No comparison	Amenorrhoea in 24% Oligoamenorrhoea in 47% Decreased dysmenorrhoea by 45% Decreased menstrual blood loss by 76% 75% Satisfaction rates

Fedele [61]	2001	Prospective	11 symptomatic women with rectovaginal endometriosis	No comparison	Decreased pelvic pain, dyspareunia, dysmenorrhoea related to endometriosis Decreased size of endometriosis lesions (ultrasound)

Adenomyosis

One non-blinded RCT (n=95), one prospective observational study and one case report were identified. The features of the studies are listed in **Table 3.10**. All studies showed a reduction in adenomyosis related dysmenorrhoea and menorrhagia, and this effect was statistically significant in the RCT [62] that compared LNG-IUS against expectant treatment in women following TCRE for adenomyosis. However, dysmenorrhoea and menorrhagia observed in the trial may not necessarily be due to adenomyosis.

Table 3.10. LNG-IUS studies assessing therapeutic effect in women with adenomyosis

Author	Year of Publication	Study Type	Sample Size	Comparison	Outcomes within one year follow up
Maia [62]	2003	RCT Non-blinded	95 women post TCRE for adenomyosis	53 LNG-IUS vs. 42 expectant No drop out reported.	19% of expectant group needed second treatment for uterine bleeding and pain compared to none in LNG-IUS Significantly lower rate of dysmenorrhoea in LNG-IUS (10%) than expectant (80%) group Significantly higher rate of amenorrhoea in LNG-IUS group (100% vs. 9%) at one year
Fedele [63]	1997	Prospective	25 with adenomyosis related menorrhagia	No comparison	For all cases, reduction in PBAC, dysmenorrhoea. Improved haemoglobin and ferritin
Fong [64]	1999	Case report	1 enlarged adenomyosis uterus	No comparison	Reduction in uterine size, dysmenorrhoea, MBL

Endometrial protection during oestrogen replacement therapy or tamoxifen in peri-menopausal women

Seven RCTs, three cohort studies, and seven observational studies have described the use of LNG-IUS to protect the endometrium from endometrial hyperplasia or malignant transformation during exogenous estrogen replacement therapy (ERT) in peri- and postmenopausal women. One RCT [54] and one observational study (n=6)[65] have examined the endometrial protective effect of LNG-IUS during tamoxifen therapy in postmenopausal women. The characteristics of these studies are summarised in **Table 3.11**. The tamoxifen RCT [54] showed that 91% women had endometrial suppression (histological decidual or atrophic response) in the LNG-IUS and tamoxifen group (n=47) compared to 75% in the tamoxifen only group (n=52) [54].

RCTs differed in population subgroups (peri-menopausal and post-menopausal women), methods of ERT administration (such as implant, oral, transdermal gel, vaginal ring) comparisons (cyclic oral estrogen/progestogen HRT, continuous combined estrogen/progestogen HRT, vaginal progestogen, subdermal progestogen, low dose LNG-IUS [10mcg or 14mcg systems] vs. higher does LNG-IUS [20mcg]) and methods of assessing endometrial suppression outcome (clinical, histological, ultrasonographic, MRI). A meta-analysis of discrete groups of studies may be less informative than individually listing the study design and outcomes, and was therefore not performed.

Table 3.11. LNG-IUS studies assessing use to provide uterine protection during oestrogen replacement or tamoxifen therapy

Author	Year of Publication	Study Type	Sample Size	Comparison group	Outcomes (within one year of follow up unless stated otherwise)
TAMOXIFEN STUDIES					
Gardner [54]	2000	RCT	Initial recruitment of 122 Postmenopausal breast cancer women	64 LNG-IUS and tamoxifen group vs. 58 tamoxifen group only 27% drop out rate from LNG-IUS arm	All LNG-IUS had endometrial suppression (histological decidual response) Decreased endometrial polyps and submucous fibroids in LNG-IUS group
Turnbull [65]	1998	Prospective	6 postmenopausal breast cancer women with irregular thickened endometrium on tamoxifen therapy	No comparison. Inserted LNG-IUS	No change in endometrial thickness with TV ultrasound A reduction in sub-endometrial cysts and endometrial volume with MRI by 6 months
ESTROGEN REPLACEMENT STUDIES					
Boon [66]	2003	RCT	200 perimenopausal women	100 LNG-IUS and 100 oral estradiol vs. cyclic/combined oral estrogen and progestogen HRT (Trisequens)	**2 year follow up** endometrial suppression

				18% drop out rate from LNG-IUS group	(atrophic or inactive) greater with LNG-IUS than oral HRT: 100% vs 6% LNG-IUS: initial erratic bleeding, 62% amenorrhoeic by 2 years. Cyclic HRT: normal regular monthly bleeds in 70-80%
Wolter-Sven. [67]	1997	RCT	112 Perimenopausal women symptomatic of menopause	51 LNG-IUS 10 mcg/24hr plus estrogen (oral/transdermal) vs. 45 LNG-IUS 5cmg/24hr plus estrogen (oral/transdermal) 11% drop out rate	95/96 cases had histological endometrial suppression Amenorrhoea in most cases (62% for 5mcg and 61% for 10mcg groups) Satisfactory relief of menopausal vasomotor symptoms
Raudaskoski [68]	2002	RCT	163 postmenopausal women Oral estrogen	Different progestogen formulations of HRT combining oral estradiol with High or low dose	Endometrial suppression (histologically) and amenorrhoea in >98% of

			with: 54 10mcg/24hr LNG-IUS (MLS) or 56 20mcg/24hr LNG-IUS or 53 oral progestogen.	LNG-IUS or cyclical oral progestogen 7% drop out from combined LNG-IUS 10mcg and 20mcg groups	LNG-IUS cases. Proliferative endometrium and regular withdrawal bleeds with oral progestogen
Raudaskoski [69]	1995	RCT	40 postmenopausal	20 LNG-IUS plus transdermal estrogen vs 20. continuous oral estrogen and progestogen 12% drop out from LNG-IUS group	Comparable endometrial suppression (histological and ultrasound) Comparable improvement of menopausal symptoms
Andersson [70]	1992	RCT	40 perimenopausal	20 LNG-IUS and oral estrogen vs. 20 Cyclic HRT (oral estrogen 3 weeks, oral progestogen 1 week)	83%of LNG-IUS became amenorrhoeic, but cyclic HRT had regular withdrawal bleeds. Both groups had endometrial suppression
Suhonen [71]	1995	RCT	36 postmenopausal	16 LNG-IUS and one subdermal estrogen implant vs 20 LNG-IUS and three subdermal estrogen	Endometrial suppression in all cases 72% had

				implants No drop out reported	amenorrhoea or spotting by three months
Suhonen [72]	1995	RCT	19 postmenopausal	10 oral estrogen and LNG-IUS vs. 9 oral estrogen and subdermal levonorgestrel-releasing implant No drop out reported	Comparable endometrial suppression
Suvanto-Luuk. [73-75]	1997, 1998, 1999	Prospective cohort	60 postmenopausal women 20 received LNG-IUS 21 oral progesterone 19 vaginal progesterone	All received transdermal estrogen gel 25% drop out rate of LNG-IUS group at 5 years	**5 year follow up for 20 cases in LNG-IUS group** At one year varying degrees of amenorrhoea: 80%, LNG-IUS; 67%, oral progesterone; 53% in the vaginal progesterone. At five years 80% amenorrhoea in LNG-IUS Endometrial suppression (histological, ultrasound) in all LNG-IUS cases
Antoniou [76]	1997	Prospective cohort	56 postmenopausal women	28 women with LNG-IUS plus daily transdermal estrogen	Comparable endometrial suppression

			with urogenital symptoms	vs. 28 women with estradiol-releasing vaginal ring plus vaginal progesterone	(ultrasound)
Kalogirou [77]	1996	Prospective cohort	56 postmenopausal	LNG-IUS and transdermal estrogen vs. Estrogen releasing vaginal ring and oral progestogen	Comparable endometrial suppression (ultrasound and histological)
Sturdee [3]	2004	Prospective	294 postmenopausal	No comparison LNG-IUS 10mcg/24hr (MLS device) and transdermal estrogen	Interim 1 year results from 3 yr study 67% amenorrheic at one year. 9/294 discontinued because of bleeding.
Wildemeersch [78]	2003	Prospective	83 perimenopausal and 58 postmenopausal * Mixed group of women-contraception needs, menorrhagia, vasomotor symptoms, fibroids	No comparison Used FibroPlant-LNG with transdermal estrogen gel	***Up to 3 year follow up*** All effective endometrial suppression (ultrasound) 64% amenorrhoea in perimenopausal group and 100% in postmenopausal group 5 cases of fibroid related menorrhagia

					improved
Hampton [79]	2005	Prospective	82 perimenopausal	No comparison Use LNG-IUS with oral estrogen	**5 year follow up** 96-98% non-proliferative endometrium 55% amenorrhoea at one year 93% amenorrhoea by fifth year 80 per 100 women continuation rate at 5 years
Varila [80]	2001	Prospective	40 postmenopausal	No comparison Used LNG-IUS with oral or transdermal estrogen	**5 year follow up** 39 completed 12 mths 29 completed 5 years All cases had endometrial suppression (histological and ultrasound) 51% amenorrhoea or only spotting at 5 years
Wildemeersch [81]	2000	Prospective	22 perimenopausal, 8 postmenopau	19 cases had FibroPlant LNG 14mcg/24hr and 11 cases had	Up to 2½ years follow up

			sal	10mcg/24hr doses All with transdermal estrogen gel	All effective endometrial suppression (ultrasound) 77% amenorrhoea in perimenopausal group and 100% in postmenopausal group
Suhonen [82]	1997	**Prospective**	29 peri- and postmenopausal women	No comparison LNG-IUS and transdermal/subdermal/ oral estrogen	**3 year follow up** All cases had endometrial suppression (ultrasound, histology) 79% amenorrhoea at 3 years
Wildemeersch [83]	2004	Prospective	24 postmenopausal women	No comparison Used FibroPlant-LNG with oral estradiol or estrogen patches	**3 year follow up** All effective endometrial suppression (histologically and ultrasound) and clinical amenorrhoea

Footnotes

FibroPlant-LNG is a frameless low-dose LNG-IUS (releasing 14mcg levonorgestrel/day)

MLS is a low dose smaller sized LNG-IUS (releasing 10mcg levonorgestrel/day)

Endometrial suppression and symptomatic improvement of menopausal symptoms (e.g. hot flushes) was achieved in all studies examining LNG-IUS use in women receiving ERT. From the study outcomes, amenorrhoea appeared to be more common in postmenopausal women receiving LNG-IUS (studies ranging from 61% to 100% of subjects) than peri-menopausal women (studies ranging from 38% to 83% of subjects), although this was not formally statistically tested due to study heterogeneity. Seven studies have reported follow up beyond one year [66;75;78-83], three reported up to a maximum of five-years [75;79;80], and one study published its interim one year results from a proposed three year study duration [3]. There was no statistically significant difference between LNG-IUS 10mcg and LNG-IUS 5mcg in one RCT (n=108)[67]. Participants in three separate publications [78,81,83] are likely to be from the same study cohort.

Endometrial hyperplasia

No RCTs were identified. Characteristics of the one cohort, three prospective observational studies, and two case reports/case series are shown in **Table 3.12.** Most studies examined women with non-aypical endometrial hyperplasia, but three studies have included women with atypical hyperplasia [86;87;89]. Hyperplasia of all types was regressed in all cases treated with LNG-IUS.

Table 3.12. LNG-IUS studies assessing therapeutic effect in women with endometrial hyperplasia

Author	Year of Publication	Study Type	Sample Size	Comparison	Outcomes within one year follow up
Vereide[84]	2003	Retrospective cohort	57 endometrial hyperplasia	LNG-IUS vs. oral progestogen	Greater regression with LNG-IUS that with oral progestogens (100% vs. 55%) at 3 months
Scarselli [85]	1988	Prospective	31 (4 atypical types)	No comparison	Endometrial regression in all cases
Perino [86]	1987	Prospective	14 (1 case atypical type)	No comparison	Endometrial regression in 29/31 cases at 16 months follow up
Wildemeersch [87]	2003	Prospective	12 (non-atypical and atypical types)	No comparison	Endometrial regression in all cases by three years
Rose [88]	2001	Case report	1	No comparison	Endometrial regression
Bahamondes [89]	2003	Case report	1	No comparison	Endometrial regression

Endometrial cancer

The preferred primary treatment for early stage endometrial cancer is surgical hysterectomy, with systemic progestins used palliatively or as adjuvant treatments for higher stage cancers. A literature review of limited sized case series and cohort studies (n=81 cases, 27 articles) has shown safe and effective treatment (overall 76% cure) with systemic progestin therapy in women with well differentiated stage 1 endometrial cancer [90]. This evidence, although limited in quality, establishes a plausible role for LNG-IUS in early stage disease, particularly in those women medically unfit for surgical therapy. One case report describes successful reversion of the cancer on endometrial biopsy when using a combination of oral progestogens and LNG-IUS in such an indication [91]. However, another case series (2 patients) showed no regression of the endometrial cancer when treated with LNG-IUS alone in patients awaiting definitive surgical treatment [89]. A comparative study performed in 14 women with early

stage endometrial cancer considered high risk for surgery showed successful reversion of cancer on endometrial biopsy in 75% of cases at 12 months [92]. However, a case series has identified two cases of endometrial carcinoma that were diagnosed following insertion of LNG-IUS [93]. Clearly, further cases, controlled trials, and longer follow up are required in order to obtain more valid conclusions.

Dysmenorrhoea and pain Only one observational study has formally examined the therapeutic use of LNG-IUS in women with primary and secondary dysmenorrhoea [32]. The study is of poor quality (limited sample size, n=18, and non-comparative) which makes interpretation of the observed beneficial response difficult. However, reductions in dysmenorrhoea have been reported in numerous LNG-IUS trials [26;35;94-97] and observational studies [28;98;99], albeit not being an a priori primary outcome measure in the vast majority.

An RCT (n=236) that compared LNG-IUS with hysterectomy for women with menorrhagia evaluated pain as an outcome using a RAND-36 health survey [13;14]. The trial showed greater improvement in pain by the hysterectomy group than LNG-IUS at one year. However, by five years, both LNG-IUS and hysterectomy groups had achieved almost identical reductions in pain. Most studies have failed to distinguish dysmenorrhoea from co-existent pelvic pain disorders (e.g. endometriosis, chronic pelvic pain, chronic pelvic inflammatory disease) in their subgroup analyses. This may cause confounding. However, the fact the association is reproducible in so many studies suggests the effect is real even though the magnitude cannot be accurately ascertained.

LNG-IUS and effect on pelvic inflammatory disease

No RCTs have examined whether the incidence of pelvic inflammatory disease (PID) is modified following introduction of LNG-IUS as a primary outcome measure. One RCT [100] and reviews of the early LNG-IUS trials [101;102] has suggested a lowering of PID rates when using LNG-IUS compared to Cu-IUCD. Whereas, two early RCTs [35;103], a recent 5-year study [104], and a systematic review [105] of all the contraceptive trials have shown comparable rates of PID during the use of the LNG-IUS or a copper IUD.

Other non-contraceptive therapeutic indications Large multicentre studies have not detected differences in cervical cytology or breast cancer incidence between copper IUD and LNG-IUS users, and non-users [35;101;102]. Long-term epidemiological studies are needed to confirm this finding, and whether these may represent alternative therapeutic indications.

Adverse effects Irrespective of study design and indication all studies have reported adverse side effects following insertion of LNG-IUS, although a direct causal relationship to LNG-IUS cannot always be confirmed. Around 15-20% of LNG-IUS users experience at least one or more unwanted side effects[5;106;107]. The most frequent (around 10-15% of users) is unscheduled erratic menstrual bleeding, which usually occurs during the first 3-4 months following LNG-IUS insertion but tends to subside thereafter. Erratic irregular menstrual bleeding is cited by women as the most common reason for discontinuing LNG-IUS treatment. During LNG-IUS use, 17.5% of women had a cyst at 6 months (diameter over 3cm) and 21.5% at 12 months [55]. The vast majority of these were asymptomatic and functional, and exhibited a high rate (94%) of spontaneous resolution by six months[55]. Other

less common side effects include mastalgia, migraine, acne, weight gain, oedema, labile mood, abdominal pain, pelvic pain, nausea and coil-related (infection, perforation, spontaneous expulsion) complications [35;101;102]. Nevertheless, the continuation and patient satisfaction rates in women using LNG-IUS for contraception remains over 75% [98;108-111].

Studies conflict on whether the induction of amenorrhoea is considered a desired effect [98] or an unwanted side effect [5;35] that may lead to LNG-IUS discontinuation. This determination is based on the individual's clinical symptomology pre-LNG-IUS insertion. Amenorrhoea occurs following LNG-IUS insertion in 20-60% of normally menstruating women using the device for contraception, between 50-75% in women with menorrhagia, and 61%-100% in postmenopausal women using the device to protect the uterus during estrogen replacement therapy[9;35;42;75;82;97;107;108;112].

Discussion

Our systematic review has shown strong evidence that LNG-IUS is effective in treating women with idiopathic menorrhagia and in providing uterine protection for women receiving estrogen replacement therapy or tamoxifen. There is preliminary evidence that shows LNG-IUS may be therapeutic in women with fibroids, endometriosis, adenomyosis, endometrial hyperplasia, early stage endometrial cancer and dysmenorrhoea, and may reduce the risk of pelvic inflammatory disease. The grading of evidence is depicted in **Table 3.6**. The incidence of adverse effects, in particular initial period of erratic menstrual bleeding, is unaffected by the indication for the use of LNG-IUS. The incidence of amenorrhoea following LNG-IUS insertion appears to be influenced by age and independent of underlying gynaecological pathology: the incidence is greater as the woman approaches her menopause.

This review has been original in systematically collecting and presenting the data relating to LNG-IUS use in HRT, tamoxifen, endometrial hyperplasia, endometrial cancer, endometriosis and adenomyosis. The systematic search strategy employed was comprehensive and methodological analysis followed standardized criteria. This review has updated and expanded on studies listed in the Cochrane database [40;41;113] and a previous related systematic review [11]. Our findings complement the recently published Cochrane protocol on post-operative LNG-IUS in endometriotic surgery [113], and supplements the evidence reported in a Cochrane review of pre- and post-operative medical therapy for endometriotic surgery which had excluded LNG-IUS usage [114]. Our review has included recent developments such as data from lower dose LNG-IUS devices currently under development (e.g. FibroPlant™-LNG) and health related quality of life assessments for women using LNG-IUS[14].

We observed a general paucity of RCTs, varying study methodologies and outcome measures, which made the interpretation of study data difficult and prevented us from performing a meta-analysis. We had intended to perform a systematic review of LNG-IUS, and instead this review is a narrative assimilation of the available literature. Furthermore, our systematic search strategy may have missed relevant studies. However, by maintaining a sensitive keyword search, contacting the manufacturer Schering for unpublished studies, and checking registered clinical trials databases, we believe this loss has been minimized. Apart from menorrhagia and HRT therapeutic indications, the published literature mainly consists of limited sample-sized (below 50 participants in LNG-IUS arm of study) non-controlled observational studies with less than one year follow up, which although showing consistent trends, are likely to be subject to information and selection biases. Consequently, no firm conclusions can be inferred from these studies (evidence grading C). However, these studies may provide a basis to estimate minimum numbers needed to be recruited to demonstrate clinically significant results in future therapeutic trials using LNG-IUS.

There is strong evidence demonstrating the efficacy, cost-effectiveness, and safety of LNG-IUS in menorrhagia. This evidence has been translated to clinical practice through recent licensing (2001) of LNG-IUS for women with menorrhagia. A similar abundance of RCTs, cohort and observational evidence, demonstrating efficacy and endometrial safety, exists for the use of LNG-IUS in providing endometrial protection during estrogen replacement therapy. Research in to this modality of HRT has been abundant since its inception in the late 1980s[115;116]. However, unlike menorrhagia, the license for HRT use has not been forthcoming in many countries, and was only awarded in 2005 by the UK.

The Women's Health Initiative and Million Women Study, showed HRT use increased the

risk of stroke, pulmonary embolism, and breast cancer, but decreased risk of hip fracture, with no effect on coronary heart disease incidence [117-120]. Incidence of breast cancer was significantly increased for users of hormone replacement therapy containing estrogen only (1.30 [1.21-1.40]), estrogen-progestogen (2.00 [1.88-2.12]), and Tibolone (1.45 [1.25-1.68]), but the magnitude of the associated risk was substantially greater for estrogen-progestogen than for other types of HRT. The reluctance to use LNG-IUS may be based on concerns that stable systemic levels of levonorgestrel (330-350 pg/mL)[1] may be sufficient through its progestogenic effect to promote tumourigenesis in the breast (particularly if given with exogenous estrogen) or blunt the anti-tumour effect of tamoxifen on the breast. Similarly, it is plausible to extrapolate the endometrial suppression data observed in the perimenopausal hormone replacement therapy, tamoxifen and endometrial hyperplasia studies, and hypothesize that the risk of endometrial cancer may be reduced in long-term users of LNG-IUS. However, we found no data relating LNG-IUS use to an increased or decreased risk of breast or endometrial cancer risk. However, absence of publications showing association does not necessarily indicate a lack of association between LNG-IUS and cancer. We believe this to be an important safety issue that remains to be addressed, either through long-term follow up and re-analysis of published studies or further prospective trials.

Despite promising findings, further trials are needed to establish efficacy, safety, cost-effectiveness, and quality of life measures before recommending LNG-IUS in most of the non-contraceptive indications discussed. Studies need to identify which population groups benefit most from LNG-IUS use, and this is made difficult due to the varying spectrum of disease, co-existence of multiple gynaecological pathology, and whether LNG-IUS is being tested as a first line or second line treatment following failed medical or surgical intervention. For example, subgroup analysis of trial data has shown that the magnitude of baseline

menstrual blood loss was negatively predictive of successful treatment with LNG-IUS[121]. The authors and manufacturers of the newer lower-dose and smaller sized LNG-IUS devices assert they are easier to insert, have less adverse side effects and greater patient acceptability [3;67;67;68;83] than conventional 20mcg/24hr LNG-IUS. However, there is little supporting evidence for this assertion, and these devices need to be rigorously evaluated in robust head-to-head comparisons with conventional LNG-IUS to validate this viewpoint.

There is a paucity of data on patient preference and decision analysis strategies in the use of LNG-IUS [8]. This research should accompany future trials, particularly given the number of competing similar efficacy therapeutic medical and surgical interventions. A recent questionnaire study highlighted how patient's choice of treatment is influenced by several factors. These may include the likelihood of whether the treatment will be completely successful, prolonged hospital stay and convalescence, and preservation of future fertility. The majority of women scheduled for an endometrial ablation or LNG-IUS for menorrhagia were inclined to take a risk of 50% likelihood of treatment failure to avoid a hysterectomy [122]. LNG-IUS can no longer just be considered suitable for women with menorrhagia who wish reversible contraception. The fact that so many conditions in Gynaecology are likely to be amenable to LNG-IUS underlies the importance of progestogens in the normal and pathological female genital tract. This review's findings complement the current resurgence of basic science research interest in this area and clinical trials evaluating potential therapeutic use of selective progesterone receptor modulators in the conditions discussed in this review[123]. This review has provided a foundation to undertake robust research trials in this area that could potentially show greater therapeutic benefit and lesser patient harm when using LNG-IUS compared to currently available medical and surgical therapies.

Chapter 4: CLINICAL GUIDELINE DEVELOPMENT

Contents

Introduction Aims of clinical guidelines and their value in clinical practice

Methodology Development and appraisal of clinical guideline methodology:

as illustrated through case studies.

Results Clinical guidelines for four topics (**Table 4.1**)-

Conclusion Considerations that may improve guideline development process

Table 4.1 Guideline publications arising from chapter 4:

Chapter	Manuscript title	Reference
4.1	Varma, R, Gupta, J.K., Smith, G.C. Birth after previous caesarean section. Royal College of Obstetricians and Gynaecologists Clinical Green top guideline No.45. February 2007. http://www.rcog.org.uk/index.asp?PageID=1913	1
	Varma R, Smith GC. Management of women with previous caesarean section. In Press. In: Warren R, Arulkumaran S, editors. Best Practice in Labour and Delivery. Cambridge University Press, Cambridge, UK.; 2008.	2
4.2	Varma R, Gupta JK. Ectopic Pregnancy. http://clinicalevidence.bmj.com/ceweb/conditions/pac/1406/1406_background.jsp . BMJ Clinical Evidence . 2006.	3
4.3	Varma R, Gupta JK. Laparoscopic entry techniques: clinical guideline, national survey, and medicolegal ramifications. Surg Endosc 2008; 22(12):2686-2697.	4
4.4	Varma R, Gupta JK. Failed sterilisation: evidence-based review and medico-legal ramifications. BJOG 2004; 111(12):1322-1332.	5
	Varma R, Gupta JK. Minimizing the risk of sterilization faliure: An evidence based approach. In: Complications in Gynecological Surgery. Editor: O'Donovan P. Spinger-Verlag, London 2008. Chapter 12; pages 106-126	6

Introduction

Clinical guidelines are designed to be educational aids that will promote Good Clinical Practice. Guideline development and practice has become widespread in modern healthcare. In the UK, both national bodies (National Institute of Clinical Excellence, Scottish Intercollegiate Guideline Network) and specialty based professional organisations (Royal College of Obstetricians and Gynaecologists) have active programmes of clinical guideline development and publication. There are many clinical topics that lend themselves to guideline development, although topics that have the greatest '*clinical impact*' are prioritised by guideline development bodies (**Table 4i**). The impetus for the continued proliferation of guidelines is the drive to ensure best clinical practice is achieved for both the patient (such as desired clinical outcomes, reduction of clinical risk) and health care provider (optimum use of healthcare resources and consideration of costs). Furthermore, although not tested, there are likely to be medico-legal ramifications in cases where clinical harm has occurred and the clinician has not followed established clinical guidelines (either national or at local Trust level) or has not clearly justified their rationale for adopting alternative clinical decision making. There are established methodologies utilised in the production of clinical guidelines; **four essential criteria** have been defined by the Appraisal of Guidelines for Research and Evaluation in Europe (AGREE) guidelines [7] and include:

1. Systematic review of the literature
2. Graded recommendations with explicit links to the evidence
3. Input of a multidisciplinary working group
4. Quality control; for example, input by an independent advisory board or by independent peer review.

Table 4i. Assessment criteria for selecting topics for clinical guideline development: high clinical impact topics

Assessment criteria

- Areas where there are high rates of mortality, morbidity or disability.
- Areas where improved standards of care would reduce rates of mortality, morbidity or disability.
- Areas where there is uncertainty, as evidenced by a wide variation in clinical practice and service delivery.
- Areas where new high-quality clinical evidence has been published.
- Areas where there are resource implications: either high cost and low turnover or low cost and high turnover.
- Areas where there are implications across the primary–secondary care interface.
- Areas where there is a frequent chance of litigation

However, not all clinical guidelines incorporate all of these criteria. Furthermore, concerns have been raised on the 'practical' value of clinical guidelines to real life clinical practice. Guidelines place considerable weight on the evidence originating from randomised controlled trials. Nevertheless, in practice, there is considerable patient heterogeneity, the clinical environment is less well controlled, patient compliance is less reliable and resources are more restricted. than the trial setting. Consequently, the anticipated benefits of the guideline may not be fully realised in an everyday setting. There has been no robust research that has demonstrated clear superiority of clinical guideline direct practice over conventional practice.

Aims of chapter

- To undertake systematic reviews and develop clinical guidelines in topics in obstetrics and gynaecology that are assessed to be of high clinical importance and impact (see earlier definition). Case examples selected are: Vaginal Birth after caesarean, Ectopic Pregnancy, Laparoscopic entry (**Table 4.1**).

- To explore the value of utilising differing methodological approaches to clinical guideline development (RCOG, SIGN, GRADE approaches) (**Tables 4ii, 4iii, 4iv**). [7-9]

- To identify if there are any potential improvements to the guideline development process based on appraisal of the guideline methodology. Evidence to justify improvements to be acquired through 1) the methodological and practical problems encountered during the case examples and 2) any published evidence.

Table 4ii. Classification of evidence used by RCOG Guideline development (originate from US Agency for Health Care Research and Quality) [7]

Classification of Evidence Levels

Ia Evidence obtained from meta-analysis of randomised controlled trials.

Ib Evidence obtained from at least one randomised controlled trial.

IIa Evidence obtained from at least one well-designed controlled study without randomisation.

IIb Evidence obtained from at least one other type of well-designed quasi-experimental study.

III Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case studies.

IV Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities

Grades of Recommendations

A	Requires at least one randomised controlled trial as part of a body of literature of overall good quality and consistency addressing the specific recommendation. (Evidence levels Ia, Ib)
B	Requires the availability of well controlled clinical studies but no randomised clinical trials on the topic of recommendations. (Evidence levels IIa, IIb, III)
C	Requires evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities. Indicates an absence of directly applicable clinical studies of good quality. (Evidence level IV)

Good Practice Point

✓	Recommended best practice based on the clinical experience of the guideline development group

Table 4iii. Classification of evidence used by Scottish Intercollegiate Guidelines Network (SIGN) Grading System [9]

Levels of evidence	
1++	High quality meta analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias
1+	Well conducted meta analyses, systematic reviews of RCTs, or RCTs with a low risk of bias
1 -	Meta analyses, systematic reviews of RCTs, or RCTs with a high risk of bias
2++	High quality systematic reviews of case-control or cohort studies High quality case-control or cohort studies with a very low risk of confounding, bias, or chance and a high probability that the relationship is causal
2+	Well conducted case control or cohort studies with a low risk of confounding, bias, or chance and a moderate probability that the relationship is causal
2 -	Case control or cohort studies with a high risk of confounding, bias, or chance and a significant risk that the relationship is not causal
3	Non-analytic studies, e.g. case reports, case series
4	Expert opinion
Grades of recommendation	
A	At least one meta analysis, systematic review, or RCT rated as 1++, and directly applicable to the target population; or A systematic review of RCTs or a body of evidence consisting principally of studies rated as 1+, directly applicable to the target population, and demonstrating consistency of results
B	A body of evidence including studies rated as 2++, directly applicable to the target population, and demonstrating overall consistency of results; or Extrapolated evidence from studies rated as 1++ or 1+
C	A body of evidence including studies rated as 2+, directly applicable to the target population and demonstrating overall consistency of results; or extrapolated evidence from studies 2++
D	Evidence level 3 or 4; or Extrapolated evidence from studies 2+
GPP	Good practice points: Recommended best practice based on the clinical experience of the guideline development group

Table 4.iv. GRADE approach [8] (http://www.gradeworkinggroup.org/index.htm)

The Grading of Recommendations Assessment, Development and Evaluation (GRADE)

GRADE: Quality of evidence

- The GRADE system classifies the quality of evidence in one of four levels:
- *High quality*— Further research is very unlikely to change our confidence in the estimate of effect
- *Moderate quality*— Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate
- *Low quality*— Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate
- *Very low quality*— Any estimate of effect is very uncertain

Evidence based on randomised controlled trials begins as high quality evidence, but our confidence in the evidence may be decreased for several reasons, including:

- Study limitations
- Inconsistency of results
- Indirectness of evidence
- Imprecision
- Reporting bias.

Although observational studies (for example, cohort and case-control studies) start with a "low quality" rating, grading upwards may be warranted if the magnitude of the treatment effect is very large, if there is evidence of a dose-response relation or if all plausible biases would decrease the magnitude of an apparent treatment effect.

GRADE: Strength of recommendation

The GRADE system offers two grades of recommendations: "strong" and "weak" depending on whether effects of intervention clearly outweigh the undesirable effects, or clearly do not. If trade-offs are less certain—either because of low quality evidence or because evidence suggests that desirable and undesirable effects are closely balanced—weak recommendations become mandatory.

Factors that affect the strength of a recommendation

Factor	Examples of strong recommendations	Examples of weak recommendations
Quality of evidence	Many high quality randomised trials have shown the benefit of inhaled steroids in asthma	Only case series have examined the utility of pleurodesis in pneumothorax

Uncertainty about the balance between desirable and undesirable effects	Aspirin in myocardial infarction reduces mortality with minimal toxicity, inconvenience, and cost	Warfarin in low risk patients with atrial fibrillation results in small stroke reduction but increased bleeding risk and substantial inconvenience
Uncertainty or variability in values and preferences	Young patients with lymphoma will invariably place a higher value on the life prolonging effects of chemotherapy than on treatment toxicity	Older patients with lymphoma may not place a higher value on the life prolonging effects of chemotherapy than on treatment toxicity
Uncertainty about whether the intervention represents a wise use of resources	The low cost of aspirin as prophylaxis against stroke in patients with transient ischemic attacks	The high cost of clopidogrel and of combination dipyridamole and aspirin as prophylaxis against stroke in patients with transient ischaemic attacks

Tables 4v

Summary of evidence for each clinical guideline according to RCOG and GRADE guideline development tools [7; 8;9]

All tables exclude recommendations that have been generated from Level IV Evidence (absence of directly applicable clinical studies of good quality; evidence generated from committee reports or expert opinion).

Chapter 4.1 Birth after previous caesarean	RCOG Level of Evidence	GRADE Quality of Evidence	GRADE Strength of Recommendation
Women with a single previous caesarean section and uncomplicated pregnancy may be offered VBAC	IIb, III	Moderate	Weak
Women with previous uterine rupture, classical caesarean, two previous caesarean sections, should not be offered VBAC	III	Very Low	Weak
The probability of successful planned VBAC is around 75%	IIb	Moderate	Strong
The probability of uterine scar rupture during planned VBAC labour is around 0.5%	IIb	Moderate	Strong
Planned VBAC may increase the risk of uterine endometritis and requirement for blood transfusion	IIb	Low	Weak
Planned VBAC is associated with a 10 per 10,000 risk of antepartum stillbirth beyond 39 weeks and a 4 per 10,000 risk of delivery related perinatal death	IIb, III	Moderate	Weak
Planned VBAC carries an 8 per 10,000 risk of the infant developing hypoxic ischaemic encephalopathy (HIE)	IIb	Low	Weak
Planned VBAC reduces the risk of neonatal respiratory after birth: rates are 2 to 3% with planned VBAC and 3 to 4% with ERCS.	IIb, III	Low	Weak
The risk of subsequent placenta praevia and accreta is linearly associated with the number of previous caesarean deliveries	IIb, III	Moderate	Strong
In women with previous caesarean delivery, there is a 2 to 3-fold increased risk of uterine rupture and around 1.5-fold increased risk of caesarean section in induced and/or augmented labours compared to spontaneous labours	IIb, III	Very Low	Weak

Chapter 4.2 Ectopic pregnancy	RCOG Level of Evidence	GRADE Quality of Evidence	GRADE Strength of Recommendation
Salpingectomy in women not desiring future fertility is beneficial compared to salpingotomy or methotrexate in achieving primary treatment success	IIa, IIb	Moderate	Strong
Prophylactic methotrexate (systemic) following salpingotomy compared to salpingotomy alone is beneficial in reducing the risk of persistent trophoblast	Ib, IIa	Moderate	Strong
In women desiring future fertility, systemic methotrexate (single or multiple dose) and salpingotomy achieve similar primary treatment success and subsequent fertility outcomes	Ia, Ib, IIa	Moderate	Strong
In women desiring future fertility, there is marginally improved subsequent fertility rate by performing salpingotomy compared to salpingectomy	III	Very Low	Weak
Single dose methotrexate may result in higher rates of treatment failure in women with ectopic pregnancies compared with multiple dose regimens.	Ia, Ib, IIb	Low	Weak
In selected cases, expectant management has similar primary treatment success and future fertility outcomes to salpingectomy or salpingotomy	III	Very Low	Weak
Methotrexate plus mifepristone is no more effective at increasing treatment success rates compared with methotrexate alone but it seems this combination may be more effective in increasing treatment success rates in women with high levels of progesterone.	Ib	Moderate	Weak

Chapter 4.3 Safe Laparoscopic Entry	RCOG Level of Evidence	GRADE Quality of Evidence	GRADE Strength of Recommendation
In high risk women (previous abdominal surgery; obesity, extremely thin or known abdominal adhesions), an alternative to close umbilical entry (e.g. Palmer's point or open (Hasson) technique) may reduce the risk of laparoscopic entry related injury	IIb,III	Very Low	Weak
The Veress needle should be inserted at the deep umbilical pit, at 90º to the skin, with or without stabilising or elevating the umbilical sheath/fascia or anterior abdominal wall.	IIb, III	Low	Weak
A safety check of correct Veress placement is most reliably achieved by using a Veress Intra-Abdominal Pressure (IAP) of less than 10mmHg.	IIa	Moderate	Weak
A safety check of intra-abdominal pressure of at least 25mmHg should preceded vertical insertion of the primary trocar	IIa, IIb	Moderate	Weak
Secondary trocars should be inserted under direct visualisation	III	Moderate	Strong

Chapter 4.4 Preventing sterilisation failure	RCOG Level of Evidence	GRADE Quality of Evidence	GRADE Strength of Recommendation
Pre-sterilisation pregnancy testing and ensuring the woman has taken adequate contraceptive precautions prior to the procedure	III	Very low	Strong
Sterilisation performed at the time of abortion or immediate post-partum period is associated with increased risk of failure and regret compared to interval sterilisation	III	Very low	Weak
Sterilisation performed by laparoscopy is equivalent to mini-laparotomy in terms of primary treatment success but is superior in terms of patient recovery and shorter operative time	IIb, III	Low	Weak
Laparoscopic tubal occlusion using mechanical devices have the lowest risk of sterilisation failure.	Ib, IIa	Low	Weak
A second operating surgeon that counter checks the sterilisation procedure has been correctly performed may reduce the risk of sterilisation failure	IIb	Very Low	Weak
Sterilisation failure occurred significantly earlier in negligent than non-negligent failure mechanisms	IIb	Low	Weak

4.1. Birth after previous caesarean section

Aim To provide evidence-based information to inform the care of women undergoing either planned vaginal birth after previous caesarean section (VBAC) or elective repeat caesarean section (ERCS).

Introduction and background There is widespread public and professional concern about the increasing proportion of births by caesarean section [10]. Increasing rates of primary caesarean section have led to an increased proportion of the obstetric population who have a history of prior caesarean delivery. Pregnant women with a previous section may be offered either planned VBAC or ERCS. The proportion of women who decline VBAC is, in turn, a significant determinant of overall rates of caesarean delivery [11-14] . New evidence is emerging to indicate that VBAC is not as safe as originally thought [15;16]. These factors, along with medico-legal fears, have led to a recent decline in clinicians offering, and women accepting, planned VBAC in the UK and North America [11-14] . This guideline presents the best available evidence to facilitate antenatal counselling in women with prior caesarean delivery and intrapartum management of women undergoing planned VBAC. Prior to this guideline, the NICE/RCOG Caesarean Section guideline (April 2004) provided the only UK generated guidance on the management of childbirth after caesarean [17]. Our guideline supports the recommendations made in the NICE/RCOG Caesarean Section guideline but addresses VBAC in more detail.

Identification and assessment of evidence Electronic searches were performed in MEDLINE (Ovid version 1996-October 2006), EMBASE (Ovid version 1996-October 2006) using relevant medical subject headings and text words. Evidence based reviews and guidance from ACOG [18;19], SOGC [20] , ARHQ USA [21], New Zealand Guidelines Group [22] and

The Cochrane Library (2006) [23] were identified and used in the development of this guideline. The definitions of the types of evidence used in this guideline originate from the US Agency for Health Care Research and Quality (**Table 4.ii)**[7]. Where possible, recommendations are based on and explicitly linked to the evidence that supports them. Areas lacking evidence are highlighted and annotated as 'Good Practice Points'. The definition of the terms used in this guideline is shown in **Tables 4.2** and **4.3.**

Limitations of data used in guideline Presently, there are no published randomised controlled trials (RCTs) comparing planned VBAC against planned ERCS. Evidence for these interventions is obtained mainly from retrospective non-randomised studies [1] making their conclusions less reliable. However, a study by the National Institute of Child Health and Human Development (NICHD) Maternal–Fetal Medicine Units Network[15] has overcome some of the shortcomings of previous studies by combining a large sample size, a prospective cohort design and utilisation of standardised definitions for assessing outcomes. Where possible, data on various risks and benefits of VBAC and ERCS reported in this chapter originate from this study. Further robust data on maternal and infant health outcomes will become available following completion of the BAC trial (Birth After Caesarean) [24].

Options for Delivery: VBAC or ERCS Pregnant women with a history of previous caesarean section may be offered either planned VBAC (vaginal birth after caesarean) or ERCS (elective repeat caesarean section) for their delivery. Such women would have consultant-led antenatal care and typically would follow an antenatal strategy that is depicted in **Figure 4.1.**

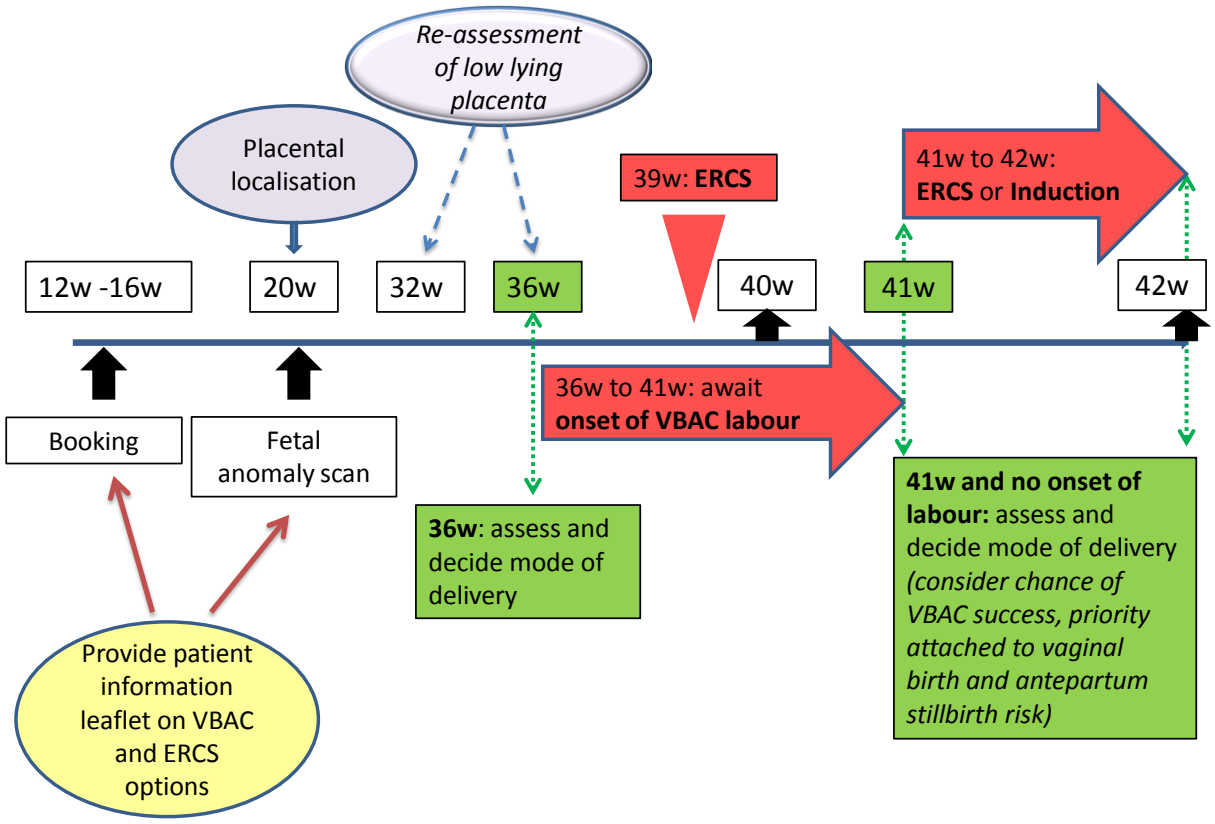
Table 4.2. Definition of obstetric terms

Planned VBAC	Planned VBAC (vaginal birth after caesarean) refers to any woman who has experienced a prior caesarean birth who plans to deliver vaginally rather than by elective repeat caesarean section (ERCS).
Successful and unsuccessful planned VBAC	A vaginal delivery (spontaneous or assisted) in a woman undergoing planned VBAC indicates a **successful** VBAC. Delivery by emergency caesarean section during the labour indicates an **unsuccessful** VBAC.
Uterine rupture	Disruption of the uterine muscle extending to and involving the uterine serosa or disruption of the uterine muscle with extension to the bladder or broad ligament.
Uterine dehiscence	Disruption of the uterine muscle with intact uterine serosa

Table 4.3. Definition of perinatal terms

Term perinatal mortality	Combined number of stillbirths (antepartum and intrapartum) and neonatal deaths (death of a live born infant from birth to age 28 days) per 10,000 live births and stillbirths at or beyond 37 weeks gestation. Term perinatal mortality rates exclude deaths due to fetal malformation unless otherwise stated.
Term delivery-related perinatal death	Combined number of intrapartum stillbirths and neonatal deaths per 10,000 live births and stillbirths at or beyond 37 weeks gestation. Delivery-related perinatal mortality rates exclude antepartum stillbirths and deaths due to fetal malformation unless otherwise stated.
Neonatal respiratory morbidity	Combined rate of transient tachypnoea of the newborn (TTN) and respiratory distress syndrome (RDS).

Figure 4.1 Plan of care for singleton uncomplicated pregnancy with previous LSCS



Determining the mode of delivery For some women, the decision to attempt VBAC may be very clear on the basis of their first antenatal visit. In such cases, it may be acceptable, following thorough counselling, to have their next review in the consultant clinic post-dates, to discuss elective delivery in the event that they do not go into labour spontaneously. For all other women, it has been suggested that the final decision on mode of delivery should be established at a 36 week gestation antenatal visit. However, it would be prudent to at least document an initial preference by the woman at her hospital booking visit (12-16 weeks) together with provision of a patient information leaflet detailing VBAC and ERCS options. This approach would provide her with the opportunity to consider her options and help guide decision making should she go into labour prior to her 36 week review (**Figure 4.1**).

Suitability for planned VBAC:

Women with a prior history of one uncomplicated lower segment transverse caesarean section, in an otherwise uncomplicated pregnancy at term, with no contraindication for vaginal delivery should be able to discuss her options for planned VBAC, and should also be offered information about the alternative of a repeat caesarean section (ERCS).

There is limited evidence on whether maternal or neonatal outcomes are significantly influenced by the number of prior caesarean deliveries or type of prior uterine scar [15;25-29]. Nonetheless, due to higher absolute risks of uterine rupture or unknown risks, planned VBAC is contraindicated in women with:-

- Previous uterine rupture- risk of recurrent rupture is unknown [27;30].
- Previous high vertical classical caesarean section (200-900 per 10,000 risk of uterine rupture)- where the uterine incision has involved the whole length of the uterine corpus [27;30].
- More than two previous caesarean deliveries (reliable estimate of risks of rupture unknown)

Evidence Levels IIIb, III and IV

However, it is recognised that in certain extreme circumstances (e.g. miscarriage, intrauterine fetal death), for some women in the above groups, the vaginal route (although risky) may not necessarily be contraindicated. A number of other variants are associated with an increased risk of uterine rupture. These include: women with a prior inverted T or J incision (190 per 10,000 rupture risk) [15] and women with prior low vertical incision (200 per 10,000 rupture risk) [15]. **Evidence Level IIa**

There is insufficient and conflicting information on whether the risk of uterine rupture is

increased in women with previous myomectomy or prior complex uterine surgery[31-33].

Evidence Level III

Therefore, women with a previous uterine incision other than an uncomplicated low transverse caesarean section incision who wish to consider vaginal birth should be assessed by a consultant with full access to the details of the previous surgery. **Evidence Level IV**

Women with a prior history of two uncomplicated low transverse caesarean sections, in an otherwise uncomplicated pregnancy at term, with no contraindication for vaginal delivery who have been carefully counselled and selected, may be considered suitable for planned VBAC. This should be a Consultant-led decision.

A multivariable analysis of the NICHD study, showed that there was no significant difference in the rates of uterine rupture in VBAC with two or more previous caesarean sections (9/975, 92 per 10,000) compared to women with a single previous caesarean section (115/16,915, 68 per 10,000) [34]. However, the rates of hysterectomy (60 per 10,000 vs. 20 per 10,000) and transfusion (3.2% vs.1.6%) were increased in the former group [34]. These findings concur with other observational studies, which overall, have shown similar rates of VBAC success with two previous caesarean deliveries (VBAC success rates of 62%-75%) and single prior caesarean delivery [26;35-37] . Therefore, provided the woman has been adequately counselled regarding these increased risks and a comprehensive individualised risk analysis of the indication for - and the nature of - the previous caesarean sections has been undertaken, then planned VBAC may be allowed in women with two previous low transverse caesarean deliveries. This counselling process should be Consultant-led. **Evidence Levels IIa,IIb and III**

Antenatal counselling:

The antenatal counselling of women with a prior caesarean delivery should be documented in the notes. There should be provision of a patient information leaflet with the consultation.

All women who have experienced a prior caesarean birth should be counselled about the maternal and perinatal risks and benefits of planned VBAC and ERCS when deciding the mode of delivery. The trade off between risks and benefits for VBAC and ERCS is highly individualised. Women differ in the magnitude of risks they are willing to expose either themselves or their unborn child to during delivery [38]. For example, women who wish to minimize the risk of rare, but severe adverse outcome for their child may prefer ERCS in preference to VBAC. Conversely, there are many reasons why a woman might prefer to attempt vaginal birth and these may lead them to accept a small degree of risk to both themselves and their infant during labour and to choose VBAC in preference to ERCS.

Evidence Level IV

The risks and benefits should be discussed in the context of the woman's individual circumstances, including her personal motivation and preferences to achieve vaginal birth or ERCS, her attitudes towards the risk of rare but serious adverse outcomes, her plans for future pregnancies and her chance of a successful VBAC (principally whether she has previously had a vaginal birth - see below). In addition, where possible, there should be review of the operative notes of the previous caesarean to identify the indication, type of uterine incision and any peri-operative complications. Decision making should be a shared

process between the woman and her obstetrician. Items that should be discussed and documented during the consultation are listed in **Tables 4.4 and 4.5**, and are expanded on below. Decision aids and specific patient information literature may facilitate this process [39].

Evidence Levels II and IV

A final decision for mode of delivery should be agreed between the woman and her obstetrician before the expected/planned delivery date (ideally by 36 weeks gestation). However, as up to 10% of women scheduled for ERCS go into labour before the 39th week, it is good practice to have a plan for the event of labour starting prior to the scheduled date[15].

Evidence Level IIa

Table 4.4. Items to be discussed when determining mode of delivery

Items		Special considerations
1	**Her understanding of the maternal and perinatal risks and benefits of VBAC compared to ERCS**	Particularly her attitude towards the risk of rare but serious adverse outcomes.
2	**Any contraindications to VBAC**	Any complicating obstetric factors *e.g. placenta praevia, fetal malpresentation, obstructing cervical fibroid, maternal medical disorders*. Assessment of previous caesarean delivery and any peri-operative complications. *A classical scar or more than two previous lower segment incisions or previous uterine rupture would be absolute contraindications to VBAC.*
3	**The likelihood of a successful VBAC**	Particularly if she has had a previous vaginal birth or successful VBAC
4	**Her plans for future pregnancies**	
5	**Her personal preference and motivation to achieve vaginal birth or ERCS**	

Table 4.5 Risks and Benefits of opting for VBAC or ERCS

	^Planned VBAC	ERCS at 39 weeks
Mother Benefits	72%-76% chance of successful VBAC If successful, shorter hospital stay and convalescence Increases likelihood that future pregnancies may be delivered vaginally	Able to plan to known delivery date **Lower risk of blood transfusion (1%) and endometritis (1.8%) *Essentially zero risk of uterine scar rupture No risk of vaginal tears and no worsening of pelvic floor support and continence mechanisms Able to be surgically sterilised at the same time
Mother Risks	*Around 50 per 10,000 (0.5%) risk of uterine scar rupture-if occurs associated with maternal morbidity and fetal morbidity/mortality 24-28% chance of emergency caesarean 10-15% chance of instrumental delivery and/or perineal tear requiring suturing **Higher risk of blood transfusion (1.7%) and endometritis (2.9%)	0.1%-2% risk of serious surgical complications such as injury to bladder Longer stay and convalescence Future pregnancies would require caesarean delivery Increased risk of surgical complications with each subsequent caesarean delivery due to adhesions, placental praevia/accreta
Infant Benefits	1% risk of transient respiratory morbidity	Avoids the 10 per 10,000 prospective risk of antepartum stillbirth as delivery is undertaken at commencement of 39th week 1 per 10,000 (0.01%) risk of delivery-related perinatal death or hypoxic ischaemic encephalopathy (HIE) at delivery
Infant Risks	10 per 10,000 (0.1%) prospective risk of antepartum stillbirth beyond 39 weeks whilst awaiting spontaneous labour 4 per 10,000 (0.04%) risk of delivery-related perinatal death $8 per 10,000 (0.08%) risk of hypoxic ischaemic encephalopathy (HIE) during labour	1-3% risk of transient respiratory morbidity [6% risk if delivery performed at 38 instead of 39 weeks]

Footnotes Table 4.5

^ The estimates of risk for adverse maternal or fetal events in VBAC are based on women receiving continuous electronic monitoring during their labour. The relative and absolute risks of such events in the absence of continuous electronic fetal monitoring are unknown.

*Uterine rupture in an unscarred uterus is extremely rare at 0.5 to 2 per 10,000 deliveries, and this risk is mainly confined to multiparous women in labour [60].

**In the NICHD study there was no statistically significant difference between planned VBAC and ERCS groups in relation to hysterectomy (23 per 10,000 vs. 30 per 10,000), thromboembolic disease (4 per 10,000 vs. 6 per 10,000) or maternal death (17/100,000 vs. 44/100,000)[15]

[$]Approximately half of the increased risk of HIE in planned VBAC arises due to the additional risk of HIE caused by uterine rupture (4.6 per 10,000)[15]

Women considering their options for birth after a single previous caesarean should be counselled that overall, the chances of successful planned VBAC are 72%-76%

Individual studies report success rates of 72%-76% [15;16;40] for planned VBAC after a single previous caesarean, which concurs with pooled rates derived by systematic and summative reviews [**Table 4.5]** [41-43]. **Evidence Levels IIa and IIb**

A number of factors are associated with successful VBAC. Previous vaginal delivery, particularly previous VBAC, is the single best predictor for successful VBAC and is associated with an approximately 87%-90% planned VBAC success rate [29;44;45]. Risk factors for unsuccessful VBAC are induced labour, no previous vaginal delivery, body mass index greater than 30 [46-48] and previous caesarean for dystocia [29]. When all these factors are present, successful VBAC is achieved in only 40% of cases [29]. There are numerous other factors associated with a decreased likelihood of planned VBAC success [29;44;49-52]: VBAC at or after 41 weeks gestation; birth weight >4000g; no epidural anaesthesia; previous preterm caesarean delivery; cervical dilatation at admission less than 4cm; less than 2 years from previous caesarean delivery; advanced maternal age, non-Caucasian ethnicity, short stature and a male infant. Where relevant to the woman's circumstances, this information should be shared during the antenatal counselling process to enable the woman to make the best informed choice. **Evidence Levels IIa, IIb and III**

There is limited and conflicting evidence on whether the cervical dilatation achieved at the primary caesarean for dystocia impacts on the subsequent VBAC success rate[53;54]. Unfortunately, the NICHD study was unable to address this concern as data relating to the labour of the primary caesarean were not collected during the study[29]

Evidence Levels IIb and III

Several pre-admission and admission based multivariate models have been developed to predict the likelihood of VBAC success [44;53;55-58] or uterine rupture [59] in planned VBAC. However, their usefulness in assisting women to make the decision about whether VBAC or ERCS is the best choice in their personal situation remains to be determined.

Evidence Level IIb

Women considering their options for birth after a previous caesarean should be counselled that planned VBAC carries a risk of uterine rupture of 22 to 74 per 10,000. There is virtually no risk of uterine rupture in women undergoing ERCS.

B

Uterine rupture in an unscarred uterus is extremely rare at 0.5 to 2 per 10,000 deliveries, and this risk is mainly confined to multiparous women in labour [60]. The NICHD study reported the overall risk for symptomatic uterine rupture at term was 74 per 10,000 planned VBACs [15]. There was zero risk in women undergoing ERCS [15]. Studies with differing methodological designs and definitions of scar rupture report similar estimates for risk of uterine rupture per 10,000 planned VBACs: systematic and non-systematic reviews of 39 [43], 43 [61] and 62 [41] ; retrospective studies of 22 [62] , 33 [63] , 35 [64] and 65 [40] per 10,000. For counselling purposes a mean uterine rupture risk of 50 per 10,000 may be utilised (as depicted in **Table 4.5**). Although a rare outcome, uterine rupture is associated with significant maternal and perinatal morbidity and perinatal mortality (see below).

Evidence Levels IIa and IIb

There is limited evidence from a case control study that women who experienced both intrapartum and postpartum fever in their prior caesarean delivery were at increased risk of uterine rupture in their subsequent planned VBAC labour (OR 4.02; 95% CI 1.04-15.5)[65] . There is conflicting evidence on whether single-layer compared with double-layer uterine closure may increase the risk of uterine rupture in subsequent planned VBAC [17;66].

Evidence levels IIb and III

Women considering their options for birth after a previous caesarean should be counselled that planned VBAC compared to ERCS carries around 1% additional risk of either blood transfusion or endometritis.

B

Women undergoing planned VBAC compared to ERCS are at greater risk of blood transfusion requirement (170 per 10,000 vs. 100 per 10,000) and endometritis (289 per 10,000 vs. 180 per 10,000))[**Table 4.5]** [15]. There was no statistically significant difference between planned VBAC and ERCS groups in relation to hysterectomy (23 per 10,000 vs. 30 per 10,000), thromboembolic disease (4 per 10,000 vs. 6 per 10,000) or maternal death (17/100,000 vs. 44/100,000)[15]. The vast majority of cases of maternal death in women with prior caesarean section arise due to medical disorders (such as thromboembolism, amniotic fluid embolism, pre-eclampsia and surgical complications). **Evidence Level IIa**

Maternal death due to uterine rupture in planned VBAC occurs in less than 1 in 100,000 cases in the developed world, and this estimate is based on information from case reports [40;67]. **Evidence Level III**

The increased risk of morbidity overall among women attempting VBAC is due to higher rates among women who attempt VBAC and are unsuccessful. The NICHD study [15] showed that unsuccessful planned VBAC compared to successful VBAC is associated with an increased risk of uterine rupture (231 per 10,000 vs. 11 per 10,000), uterine dehiscence (210 per 10,000 vs. 14.5 per 10,000), hysterectomy (46 per 10,000 vs. 14.5 per 10,000), transfusion (319 per 10,000 vs. 116 per 10,000) and endometritis (767 per 10,000 vs. 116 per 10,000). Similar trends were identified in a retrospective study from a Canadian dataset [40].

Evidence Level IIa

Women considering planned VBAC should be counselled that this decision carries a 2 to 3 per 10,000 additional risk of delivery-related perinatal death compared to ERCS, but that the absolute risk of such delivery-related perinatal loss is comparable to the risk for women having their first birth.

In the NICHD study [15], perinatal mortality at term was significantly greater among women having a planned VBAC than ERCS. Overall perinatal mortalities for planned VBAC vs. ERCS respectively were 32 per 10,000 vs. 13 per 10,000 (RR 2.40, 95% CI 1.43 to 4.01) and perinatal mortalities after excluding fetal malformation were 24 per 10,000 vs. 9.3 per 10,000 (RR 2.52, 95% CI 1.37-4.62). The increased risk of perinatal mortality is largely attributable to the statistically significantly increased risk of antepartum stillbirth beyond 37 weeks in planned VBAC compared to ERCS (19.6 per 10,000 vs. 8.0 per 10,000; RR 2.45, 95% CI 1.27-4.72) in infants without fetal malformation. Approximately 43% of such stillbirths in planned VBAC were at or after 39 weeks gestation (approximately 9 per 10,000 women delivering at or after 39 weeks), and may have been prevented by ERCS at 39 weeks. A similar estimate was identified from analysis of a Scottish data set which showed that the absolute risk of antepartum stillbirth at or after 39 weeks among women with one prior caesarean section was 10.6 per 10,000 [68].

Evidence Level IIa

In the NICHD study, rates of delivery-related perinatal death were 4 per 10,000 for planned VBAC and 1.4 per 10,000 for ERCS [15]. A report of data for the whole of Scotland demonstrated higher overall rates of delivery-related perinatal death associated with attempted VBAC of 12.9 per 10,000 whereas the risk of death associated with ERCS was

comparable to the US study at 1.1 per 10,000 [16]. The reason for the higher rate of delivery-related deaths among women attempting VBAC in Scotland may reflect the fact that these were population-based data whereas the US data were exclusively from tertiary centres. Consistent with this interpretation, a further study of data from Scotland demonstrated a lower risk of perinatal death due to uterine rupture in larger centres [64]. **Evidence Level IIa**

Accepting the limitations of using these observational data, a reasonable summary is that **planned VBAC is associated with a 10 per 10,000 risk of antepartum stillbirth beyond 39 weeks and a 4 per 10,000 risk of delivery related perinatal death (if conducted in a large centre)** [**Table 4.5]**. It is likely that these risks can be reduced by ERCS at the start of the 39th week, but direct evidence to support this is lacking. It may be helpful to emphasise to women that the absolute risks of delivery-related perinatal death associated with VBAC are comparable to the risks for nulliparous women [16;69]. **Evidence Level IIa**

Women considering their options for birth after a previous caesarean should be counselled that planned VBAC carries an 8 per 10,000 risk of the infant developing hypoxic ischaemic encephalopathy (HIE). The effect on the long term outcome of the infant upon experiencing HIE is unknown.

The incidence of intrapartum hypoxic ischaemic encephalopathy (HIE) at term is significantly greater in planned VBAC (7.8 per 10,000) compared to ERCS (zero rate)[**Table 4.5]** [15]. Approximately half of the increased risk in planned VBAC arises due to the additional risk of HIE caused by uterine rupture (4.6 per 10,000)[15]. The definition used and distribution of severity of HIE is not stated in the NICHD study [15]. Severe neonatal metabolic acidosis (pH<7.00) occurred in 33% of term uterine ruptures [15]. There is no information

comparing long term outcome, such as cerebral palsy, associated with VBAC and ERCS. Given that cerebral palsy following term birth is very rare (approximately 10 per 10,000) and only 10% of cases are thought to be related to intrapartum events [70], appropriate analysis of this question would require a scale involving hundreds of thousands of women. No adequate study has currently been reported. **Evidence Level IIa**

Women considering their options for birth after a previous caesarean should be counselled that attempting VBAC reduces the risk that their baby will have respiratory problems after birth: rates are 2 to 3% with planned VBAC and 3 to 4% with ERCS.

Three observational studies, pooling data from around 90,000 deliveries, have shown an increased risk of neonatal respiratory morbidity (defined earlier) among term infants delivered by elective caesarean (3.5%-3.7%) compared to vaginal delivery (0.5%-1.4%) [71-73]. The NICHD study [15] (n=30,352 deliveries) reported a similar trend in women with prior caesarean section, where the incidence of TTN in ERCS vs. planned VBAC was 3.6% vs. 2.6% (RR 1.40, 95% CI 1.23-1.59; NNT -98)**[Table 4.5]**. These rates concur with rates of TTN derived from a smaller data set that examined women with prior caesarean section (2 studies, n=4,478 deliveries) of 2.4%-6% vs. 1.3%-3% [73;74] for ERCS vs. planned VBAC respectively. The NICHD study did not report rates of RDS, however the smaller data set reported RDS as 0.4%-0.6% vs. 0%-0.05% for ERCS vs. planned VBAC respectively [73;74].

Evidence Level IIa

Women considering ERCS should be counselled that delaying delivery by one week from 38 to 39 weeks reduces the risk of respiratory morbidity, but this delay may be associated with a 5 per 10,000 risk of antepartum stillbirth.

Evidence from observational studies [71-73] and a recently published trial [75] has shown a beneficial effect on reducing respiratory morbidity by delaying elective caesarean section to at least 39 weeks. The trial reported respiratory morbidity was 11.4%, 6.2% and 1.5% at 37, 38 and 39 weeks gestation respectively [75]. Thus, delaying delivery by one week from 38 to 39 weeks enables around a 5 per 100 reduction in the incidence of respiratory morbidity, but this delay may be associated with a 5 per 10,000 increase in the risk of antepartum stillbirth [68;69] .

Evidence Levels Ib and IIa

Furthermore, the trial [75] demonstrated an approximate 50% reduction in respiratory morbidity (for both TTN and RDS components) by administering prophylactic Betamethasone to women having elective caesarean deliveries beyond 37 weeks (steroid vs. control; 2.4% vs. 5.1%; RR 0.46, 95% CI 0.23-0.93), and this treatment effect was still apparent at 39 weeks (steroid vs. control; 0.6% vs. 1.5%). However, it has been suggested that even a single course of antenatal steroids may have long term consequences for the baby [76] and therefore it may be safer to delay ERCS until 39 weeks rather than give steroids and deliver at 38 weeks. The routine use of prophylactic Betamethasone in ERCS is beyond the scope of this guideline.

Evidence Level Ib

Women considering their options for birth after a previous caesarean should be counselled that the risk of anaesthetic complications is extremely low, irrespective of whether they opt for planned VBAC or ERCS.

Anaesthetic procedure-related complications are extremely rare [77]. Of the women undergoing caesarean section (emergency and elective) in the NICHD study (n=37,142), 93% received a regional anaesthetic and only 3% of regional procedures failed. There was one maternal death (2.7 per 100,000) attributed to an anaesthetic problem (failed intubation) [78].

Evidence Level IIa

Women considering their options for birth after a previous caesarean should be counselled that ERCS may increase the risk of serious complications in future pregnancies.

Evidence Levels IIa, IIb & III

When considering mode of delivery, women should be advised about the effect of their decision on future pregnancies. The following risks significantly increase with increasing number of previous caesarean deliveries:

- **Placenta praevia.** Overall placenta praevia occurs in 0.5% of deliveries. However, praevia is present in 0.38%, 0.63% and 0.72% after single vaginal delivery, single caesarean, and two consecutive caesareans, respectively [79].

- **Placenta accreta**. Overall placenta accreta between 0.25-2 per 1000 deliveries[80]. However, accreta is present in 0.24%, 0.31%, 0.57%, 2.13%, 2.33% and 6.74% of women

undergoing their first, second, third, fourth, fifth, and sixth or more caesarean deliveries, respectively [81]. The risk that placenta accreta coexists with placenta praevia is 3%, 11%, 40%, 61%, and 67% for first, second, third, fourth, and fifth or more repeat caesarean deliveries, diagnosed to have placenta praevia [81].

- **Placental abruption**. Overall placenta abruption occurs in 1% of deliveries. However, abruption is present in 0.74%, 0.95% and 1.06% after single vaginal delivery, single caesarean, and two consecutive caesareans, respectively [79].

- **Injury to bladder, bowel or ureter**. A retrospective study of approximately 3000 women from Saudi Arabia showed a linear increase in the risk of bladder injury (0.3%, 0.8%, 2.4%), with a history of two, three and five caesarean sections, respectively [82].

- **Ileus**

- **Need for postoperative ventilation**

- **Intensive care unit admission**

- **Hysterectomy**-required in 0.65%, 0.42%, 0.90%, 2.41%, 3.49% and 8.99% of women undergoing their first, second, third, fourth, fifth, and sixth or more caesarean deliveries.

- **Blood transfusion** (requiring 4 or more units)

- **Duration** of operative time and hospital stay.

Given the high absolute risks of serious complications, caesarean delivery of women with high numbers of previous caesarean sections requires the immediate availability of senior surgical staff.

Evidence levels IIb and III

Placenta praevia and accreta: preoperative investigations

It is widespread practice in the UK, and endorsed by a RCOG guideline[83], that women identified to have low lying placentas at the routine mid-pregnancy fetal anomaly scan should be re-scanned in the third trimester. Provided the woman is asymptomatic (not bled), it is suggested that re-scan be conducted at 32 or 36 weeks gestation depending on whether the mid-pregnancy scan suggested major or minor praevia, respectively (30). However, given the strong association between placenta praevia, placenta accreta and prior caesarean birth, and the importance of their pre-operative identification, then re-scan and placental localisation assessment should commence at 32 weeks (and repeated at 36 weeks) for women with prior caesarean delivery. Furthermore, those women identified to have praevia (especially anterior placenta praevia) should undergo further antenatal imaging (such as power amplitude ultrasonic angiography, MRI or colour flow Doppler) to help clarify the risk of accreta [83;84].

Identification of placenta accreta prior to delivery enables instigation of specific management strategies to minimise adverse outcome at delivery. These include: consultant anaesthetist and obstetrician conducting the delivery; access to crossed matched blood; colleagues from other specialties/subspecialties to be on standby to attend as needed; discussing the risk of haemorrhage, transfusion and hysterectomy with the women as part of the consent procedure. In addition, advance planning and consideration could be given to: prophylactic or therapeutic uterine artery embolisation; internal iliac artery ligation at the same time as initial surgery; methotrexate treatment following delivery, and expectant management (placenta left in place at the end of the caesarean section)[83;84].

Evidence Level III and IV

Planned VBAC in special circumstances

Women who are preterm and considering their options for birth after a previous caesarean should be counselled that planned preterm VBAC has similar success rates to planned term VBAC but with a lower risk of uterine rupture.

A retrospective cohort study showed women who were preterm (24-36 weeks gestation) and undergoing planned VBAC had higher success rates when compared with term patients undergoing planned VBAC (82% vs. 74%) and non-significantly lower risks of uterine rupture [85]. The prospective NICHD study showed planned VBAC success rates for preterm and term pregnancies were similar (72.8% vs. 73.3%), however, the rates of uterine rupture (34 per 10,000 vs. 74 per 10,000, respectively) and dehiscence (26 per 10,000 vs. 67 per 10,000, respectively) were significantly lower in preterm compared with term VBAC [86]. Thromboembolic disease, coagulopathy and transfusion were more common in women undergoing preterm than term VBAC, although overall combined absolute risks were less than 3% in the preterm VBAC group. Perinatal outcomes were similar with preterm VBAC and preterm ERCS [86]. Therefore, following appropriate counselling and in a carefully selected population, planned VBAC may be offered as an option to women undergoing preterm delivery with a history of prior caesarean delivery. **Evidence Levels IIa and IIb**

A cautious approach should be adopted when considering planned VBAC in women with twin gestation, fetal macrosomia and short inter-delivery interval as there is uncertainty in the safety and efficacy of planned VBAC in such situations.

Study sample sizes are underpowered to provide reliable evidence suitable for any clinical practice recommendation in relation to twin gestation, fetal macrosomia and short inter-delivery interval.

- **Twin Gestation:** The NICHD study [87] (n=186 twins), US retrospective study [88] (n=535 twins) and a review [27] (7 studies, n=233 twins) have reported similar successful rates of VBAC in twin pregnancies to that in singleton pregnancies (65%-84%). However, a population based study reported a lower VBAC success rate (45%) but a comparable risk of uterine rupture (90 per 10,000) [89] **Evidence Levels IIa, IIb and III**

- **Fetal Macrosomia:** A review [27] of four retrospective studies, and the NICHD study [29], has reported a significantly decreased likelihood of successful trial of VBAC for pregnancies with infants weighing 4000g or more (55-67%) compared to smaller infants (75-83%). The risk of uterine rupture was reported in one of the retrospective studies to be only increased in those who did not have previous vaginal delivery (relative risk, 2.3; P <.001)[90] . A subgroup analysis of the NICHD study showed that women with previous caesarean delivery for dystocia, greater birth weight in the subsequent planned VBAC labour relative to the first birth weight decreased the likelihood of VBAC success [91]. However, in reality, birth weight cannot be accurately predicted by antenatal ultrasound which limits the clinical usefulness of discussing these observations when counselling women for planned VBAC and ERCS. **Evidence Levels IIa, IIb and III**

- **Short inter-delivery interval:** Three observational studies of limited size [92-94] have shown a two-to-three fold increased risk of uterine scar rupture for women with a short inter-delivery interval (below 12-24 months) from their previous caesarean section. In the NICHD study, women undergoing planned VBAC whose previous caesarean delivery was within 2 years of their labour had an increased risk of caesarean delivery compared to women

whose labour was more than 2 years from their previous caesarean (32% vs. 25% respectively)[29] . Although this information is useful antenatally, it should also be shared with women postnatally to enable them to plan their preferred spacing intervals for subsequent pregnancies. **Evidence Levels IIa and III**

Intrapartum support and intervention during planned VBAC

Planned VBAC should be conducted in a suitably staffed and equipped delivery suite, with continuous intrapartum care and monitoring, and available resources for immediate caesarean section and neonatal resuscitation.

Obstetric, midwifery, anaesthetic, operating theatre, neonatal and haematological support should be continuously available throughout planned VBAC and ERCS. **Evidence Level IV**

A retrospective study of Canadian data showed that the relative risk of uterine rupture when comparing planned VBAC with ERCS increased two fold in low-volume obstetric units (<500 births per year) than high-volume (>500 births per year) units, even though lower volume units had lower-risk obstetric population [40]. A retrospective study of Scottish data showed that planned VBAC in low-volume hospitals (<3000 births/year) was not associated with an increased risk of uterine rupture overall but was associated with an increased risk of uterine rupture that led to perinatal death [64]. It is likely that the availability of resources for immediate delivery and neonatal resuscitation may reduce the risk of infant morbidity and mortality due to uterine rupture. **Evidence Level IIa**

Epidural anaesthesia is not contraindicated in planned VBAC.

In the NICHD study, planned VBAC success rates were higher among women receiving epidural analgesia than those not receiving epidural analgesia (73.4% vs. 50.4%) [29]. The authors suggested that this difference may relate to the disproportionate use of spinal anaesthesia in short planned VBAC labours or opting for non-epidural analgesia in cases with non-reassuring fetal well being. **Evidence Level IIa**

A smaller observational study showed comparable rates of unsuccessful VBAC and operative delivery in those women receiving epidural analgesia compared to those not receiving epidural, even when correcting for oxytocin usage [95]. **Evidence Level III**

Furthermore, concerns that epidural analgesia might mask the signs and symptoms associated with uterine rupture were based on a single case report [96], and VBAC is not a contraindication for epidural analgesia [77]. A retrospective comparative study showed that within the planned VBAC group, infants of mothers who received epidural analgesia were more likely to be subjected to diagnostic tests and therapeutic interventions (including sepsis evaluation and antibiotic treatment) compared to infants from a matched no-epidural analgesia group [97]. **Evidence Levels III and IV**

Monitoring in Labour

Women should be advised to have continuous electronic fetal monitoring following onset of uterine contractions for the duration of planned VBAC.

B

An abnormal CTG is the most consistent finding in uterine rupture and is present in 55%-87% of these events **[Table 4.6]** [61]. **Evidence Level IIb**

Table 4.6 Clinical features associated with uterine scar rupture

Abnormal CTG
Severe abdominal pain, especially if persisting between contractions
Acute onset scar tenderness
Abnormal vaginal bleeding or haematuria
Cessation of previously efficient uterine activity
Maternal tachycardia, hypotension or shock
Loss of station of the presenting part

Footnotes Table 4.6. An abnormal CTG is the most consistent finding in uterine scar rupture and is present in 55%-87% of these events[61].

Moreover, continuous CTG is generally used among women during planned VBAC and thus the estimates of risk of both lethal and non-lethal perinatal asphyxia associated with VBAC are in this context. The relative and absolute risks of severe adverse events in the absence of continuous electronic fetal monitoring are unknown. **Evidence Level IV**

Continuous intrapartum care is necessary to enable prompt identification and management of uterine scar rupture.

Early diagnosis of uterine scar rupture followed by expeditious laparotomy and resuscitation is essential to reduce associated morbidity and mortality in mother and infant. There is no single pathognomic clinical feature that is indicative of uterine rupture but the presence of any of the factors listed in **Table 4.6** occurring in the peripartum period should raise the concern of the possibility of this event [30]. The diagnosis is ultimately confirmed at emergency caesarean section or postpartum laparotomy. **Evidence Levels III and IV**

There is insufficient evidence to support the use of intrauterine pressure catheters in the early detection of uterine scar rupture

Observational studies, with varying methodology and case mix, have shown intrauterine pressure catheters may not always be reliable and are unlikely to add significant additional ability to predict uterine rupture over clinical and CTG surveillance [98-100]. Furthermore, intrauterine catheter insertion may be associated with risk [101]. However, some clinicians may prefer to use intrauterine pressure catheters in special circumstances (e.g. in obese women to limit the risk of uterine hyper-stimulation) - this should be a Consultant-led decision.

Evidence Level III

<u>Induction and Augmentation</u>

Particular caution should be applied to women requiring induction or augmentation with prior caesarean delivery.

Women should be informed of the 2 to 3-fold increased risk of uterine rupture and around 1.5-fold increased risk of caesarean section in induced and/or augmented labours compared to spontaneous labours.

The risks of induction and/or augmentation should be weighed against the advantages of a successful VBAC, avoiding the risks that may occur whilst awaiting spontaneous labour and avoiding the short and long-term risks of repeat caesarean delivery.

There should be careful serial cervical assessments, preferably by the same person, for both augmented and non-augmented labours, to ensure there is adequate cervicometric progress thereby allowing the planned VBAC to continue.

The decision to induce, the method chosen, the decision to augment with oxytocin, the time intervals for serial vaginal examination, and the selected parameters of progress that would necessitate discontinuing VBAC labour, should be Consultant-led decisions.

The risk of adverse maternal and perinatal outcomes are lower among women in spontaneous VBAC labour not requiring induction or augmentation (**Table 4.7**). Although augmentation and induction are not contraindicated in women with prior caesarean delivery, there remains considerable disagreement amongst clinicians on their use. Systematic reviews [102-105] examining induction and augmentation of labour for women with previous caesarean birth have found no RCTs comparing induction/augmentation in planned VBAC against ERCS. In the NICHD study, the risks of uterine rupture per 10,000 planned VBACs were 102, 87 and 36 per 10,000 for induced, augmented and spontaneous labour groups, respectively (**Table 4.7**) [15]. This compares to an overall risk of uterine rupture of 2 per 10,000 in women with

unscarred uteri, and this risk includes the combined risks of women undergoing induction, augmentation and spontaneous labour [60]. In the NICHD study, the increased risk of uterine rupture after labour induction was found only in women with no prior vaginal delivery[106]. In the NICHD study the rates of caesarean section in women undergoing planned VBAC were 33%, 26% and 19% for induced, augmented and spontaneous labour groups respectively (**Table 4.7**) [29]. **Evidence Level IIa**

Prostaglandin vs. Non-Prostaglandin induction methods

Two studies have expanded on the differences in adverse outcomes between PG and non-PG based induction regimens [15;64]. In the NICHD study, PG induction compared to non-PG induction incurred a non-significantly higher rupture risk of uterine (140 per 10,000 vs. 89 per 10,000; p=0.22) [15]. In an analysis of nationally collected data from Scotland, PG induction compared to non-PG induction was associated with a statistically significantly higher uterine rupture risk (87 per 10,000 vs. 29 per 10,000) and a higher risk of perinatal death due to uterine rupture (11.2 per 10,000 vs. 4.5 per 10,000) [64]. This compares to 6 per 10,000 risk of perinatal death in women with an unscarred uterus induced by prostaglandin identified by a Cochrane review [107]. **Evidence Level IIa**

Given these risks, and the absence of direct robust evidence, it is important not to exceed the safe recommended limit for prostaglandin priming in women with prior caesarean delivery [102]. Moreover, due consideration could be given to restricting the dosaging and adopting a lower threshold of total prostaglandin dose exposure. Importantly, the decision to induce and the method chosen (e.g. prostaglandin or non-prostaglandin methods such as intracervical Foley catheter) should be Consultant-led. **Evidence Level IV**

Table 4.7. Risks of planned VBAC labours from NICHD study (N=17,898 planned VBACs) [15;29]

	Induced	Augmented	Spontaneous	Overall All Planned VBAC s
Uterine rupture	**Overall** 102 per 10,000 (1.0%) **PG method** 140 per 10,000 1.4% **Non-PG method** 89 per 10,000 0.9%	87 per 10,000 0.9%	36 per 10,000 0.4%	69 per 10,000 0.7%
Caesarean section	33%	26%	19%	27%

Table 4.8. Management of augmentation in established VBAC labour

	Clinical management issues
1	The decision for augmentation should follow careful obstetric assessment, maternal counselling and be Consultant-led.
2	Oxytocin augmentation should be titrated such that it should not exceed the maximum rate of contractions of 4 in 10 minutes. Particular caution is necessary when using high oxytocin augmentation doses as there is a “"dose response" for maximum oxytocin amount and uterine rupture.
3	Careful serial cervical assessments, preferably by the same person, are necessary to show adequate cervicometric progress, thereby allowing augmentation to continue. These intervals should not exceed 4 hours.
4	If there was less than 2 cm progress after 4 hours of oxytocin then caesarean section should be considered. A more conservative threshold of inadequate progress after 2 hours of augmentation may also justify consideration for caesarean section depending on the woman’s individual circumstances.
5	If there was 2 cm or more progress, augmentation could be continued and vaginal examinations performed 4-hourly.

Post dates induction

The RCOG Induction of labour guideline suggests induction for post dates be offered from 41weeks as this reduces perinatal mortality without an increase in caesarean section rates. There are no adequate data which directly address this issue among women with a previous caesarean section. However, there are some specific issues about women with a previous caesarean delivery which may influence the decision making process. First, these women are at increased risk of antepartum stillbirth [68;108]. Hence, the reduction in risk of perinatal death associated with post-dates elective delivery may be even greater among women with a previous caesarean. However, it is also possible that the effect of routine post-dates induction on the risk of emergency caesarean section may be different among women with a previous caesarean delivery. These women have a higher background risk of emergency intrapartum caesarean section and the risk of a failed VBAC is increased both post-dates and with induction of labour. These issues lead some women to decide to attempt VBAC if they labour spontaneously prior to 41 weeks but to have a planned caesarean section if their pregnancy proceeds post-dates. The choice about the method of elective delivery post-dates will also be informed by other factors determining the likelihood of a successful VBAC (favourable cervix and previous vaginal birth) and by the priority attached to achieving vaginal birth (such as plans for many future pregnancies).

There is no direct evidence to recommend what is acceptable or unacceptable cervicometric progress in women being augmented with a previous caesarean section [109-113]. Amongst women with unscarred uteri, the NICE Intrapartum guideline defines delay in the established first stage of labour as cervical dilatation of less than 2 cm in 4 hours [114]. For women with intact membranes, an amniotomy would then be recommended and repeat vaginal

examination performed 2 hours later: if progress was still less than 1cm then diagnosis of delay would be confirmed. If there was less than 2 cm progress after 4 hours of oxytocin, further obstetric review would be required to consider caesarean section. If there was 2 cm or more progress, augmentation could be continued and vaginal examinations performed 4-hourly.

If, in the presence of adequate (strength and frequency) uterine contractions, there is a slowing down of a previously normally progressing labour, augmentation may increase the risk of uterine rupture. A small sized retrospective study suggested that early recognition and intervention for labour dystocia (specifically, not exceeding two hours of static cervicometric progress) may have prevented a proportion of uterine ruptures among women attempting VBAC[113]. Awareness of the increased risk of uterine rupture in scarred uteri, particularly if there is labour dystocia, implies that a more conservative threshold to the upper time limit (such as 2 hours instead of 4 hours) of oxytocin augmentation without progress may be justified. Furthermore, a retrospective multicentre study showed a "dose response" for maximum oxytocin amount and uterine rupture, with a uterine rupture rate of 2.07% at the highest dosages [115]. Therefore, particular caution is necessary when using high oxytocin augmentation doses. A summary of the key management issues relating to augmented VBAC is shown in **Table 4.8**.

Evidence Level III

The key management issues relating to augmented VBAC labour are listed in **Table 4.8**, and although not based on robust evidence, are considered to be helpful in minimising additional harmful risks that are consequent to augmentation. When counselling women for induction (prostaglandin or non-prostaglandin methods) and/or augmentation clear information should be provided on all potential risks and benefits of such a decision and how this may impact on

her long term health. For example, women who are contemplating many future pregnancies may be prepared to accept the short-term additional risks associated with induction and/or augmentation in view of the reduced risk of serious complications in future pregnancies if they have a successful VBAC.

Evidence Level IV

Auditable standards

Standards for audit of practice should include the following:

- Use of continuous electronic fetal monitoring during VBAC labour.

Standards for audit of documentation could include the following:

- Documented discussion of risks and benefits of VBAC and ERCS
- Documentation of Consultant involvement in:

Deciding to induce or augment labour
Establishing a plan for induction or augmentation (e.g. preferred vaginal examination interval, expected minimal cervicometric progress, and the criteria needed to discontinue labour and proceed to emergency caesarean section).

Future research

1. Development, validation and pragmatic clinical evaluation of a scoring system to identify women at high or low risk of unsuccessful VBAC that is antenatally and/or intrapartum based.
2. The clinical effectiveness of differing induction and augmentation regimens, perhaps individualised according to clinical features rather than standardised strategies.
3. Identify if there are differences in long-term maternal and infant outcomes between planned VBAC and ERCS e.g. subfertility, depression, pelvic floor dysfunction, incontinence, psychosexual problems, respiratory illness, and neurodevelopmental disorders (…*this list is not exhaustive).*

4. Investigate the aetiology and prevention (e.g. specific antenatal monitoring strategies) of the increased risk of stillbirth in women with previous caesarean delivery, in the presence or absence of other previous complications (e.g. pre-eclampsia, preterm delivery, small for gestational age) [68;116].

5. Research in to factors that may explain the regional and unit-based variation in uptake of VBAC, and which factors impact most on women accepting or declining VBAC (e.g. patient information leaflet, previous childbirth experiences, desired family size, understanding the risk analysis during counselling, how to reduce any decisional conflict, variation in case mix)[117-127].

6. Assess maternal satisfaction [128-130], quality of life measures and health-state utilities in women following VBAC and ERCS to undertake robust economic modelling assessments.

Pending relevant trials

- BAC Birth After Caesarean - Planned vaginal birth or planned caesarean section for women at term with a single previous caesarean birth. ISRCTN 53974531, Prof C Crowther, University of Adelaide, Australia[24].

- The Twin Birth Study- a multicentre RCT comparing planned caesarean section with planned vaginal birth for twins at 32-38 weeks gestation, ISRCTN 74420086, Dr J Barrett, Toronto, Canada

- DiAMOND-Decision Aids for Mode Of Next Delivery, ISRCTN 84367722, Dr A Montgomery, Bristol, UK

- CAESAR-Caesarean Section Surgical Techniques, ISRCTN 11849611, Dr P Brocklehurst, National Perinatal Epidemiology Unit, Oxford, UK

4. 2. What treatments improve outcomes in women with unruptured tubal ectopic pregnancy?

SUMMARY OF TREATMENT OPTIONS (see also Tables 4v)

Beneficial	Salpingectomy in women not desiring future fertility
Likely to be beneficial	Prophylactic methotrexate (systemic) following salpingotomy Systemic methotrexate (single or multiple dose)
Unknown effectiveness	Expectant management of a subgroup of unruptured ectopic pregnancies Salpingotomy compared to salpingectomy in the presence of a healthy contralateral tube for those women desiring future fertility Salpingotomy in women with contralateral tubal disease who desire future fertility
Unlikely to be beneficial	Systemic methotrexate combined with mifepristone versus systemic methotrexate alone

KEY POINTS Approximately one in a hundred pregnancies are ectopic, with the conceptus usually implanting in the fallopian tube. Some ectopic pregnancies can resolve spontaneously, but others continue to grow and lead to rupture of the tube.

Risks are higher in women with damage to the fallopian tubes due to pelvic infections, surgery, or previous ectopic pregnancy or abortion, and in smokers. The intrauterine contraceptive device does not increase the absolute risk, but a pregnancy that does occur with IUD use is more likely to be ectopic than intrauterine.

Expectant management of unruptured ectopic pregnancies may lead to similar subsequent intrauterine pregnancy rates compared with surgery, but few studies have been done. Ongoing surveillance is required as part of expectant management, but tubal rupture can occur despite falling beta hCG levels.

Methotrexate , as single or multiple dose regimens, seems to be as likely as salpingotomy to remove trophoblast material and leave a patent fallopian tube in women with non-invasive, small ectopic pregnancies with no tubal rupture or bleeding, no sign of fetal cardiac activity and low beta human chorionic gonadotrophin (hCG) levels. About 15–40% of ectopic pregnancies may be suitable for such non-surgical management.

Systemic or intratubal methotrexate may also reduce persistent trophoblast after salpingotomy.Adding mifepristone to systemic methotrexate seems unlikely to increase treatment success compared with methotrexate alone, other than in women with higher progesterone levels.

DEFINITION Ectopic pregnancy is defined as a conceptus implanting outside the uterine endometrium. The most common implantation site is within the fallopian tube (95.5%), followed by ovarian (3.2%) and abdominal (1.3%) sites. The sites of tubal implantation in descending order of frequency are ampulla (73.3%), isthmus (12.5%), fimbrial (11.6%), and interstitial (2.6%).[131] **Population:** In this systematic review, we will consider haemodynamically stable women with unruptured tubal ectopic pregnancy, diagnosed by non-invasive or invasive techniques. All terms used in this chapter are defined and listed in **Table 4.9**

Table 4.9 Glossary of terms used in ectopic pregnancy guideline

βhCG is the pregnancy hormone beta-human chorionic gonadotrophin**.**

Contralateral tube denotes the opposite tube to that affected by the ectopic pregnancy. Compare with homolateral or ipsilateral tube.

Discriminatory zone denotes a serum βhCG level at which it is assumed that all intrauterine pregnancies will be visualised by transvaginal ultrasound. This may vary according to sonographic expertise but is often between 1000 and 1500 IU/L.

Expectant management is where ectopic pregnancy treatment involves a watch and wait policy in conjunction with close clinical, ultrasonographic, and serum βhCG surveillance.

Fecundity rate ratio (FRR) The fecundity rate represents the probability of spontaneous intrauterine pregnancy (IUP) per time unit elapsed derived from analysing the cumulative probability of pregnancy over the study duration. Only women trying to conceive are included in the calculation, and women who have conceived using additional treatments (e.g. IVF) are excluded up and till the start of their additional treatment. The fecundity rate ratio (FRR) is the ratio of fecundity between the test treatment (e.g. salpingotomy) against the reference treatment (e.g. salpingectomy). A significant treatment difference between salpingotomy compared to salpingectomy is indicated if 1 is not included in the 95% CI for the FRR of salpingotomy compared to salpingectomy. Thus a FRR of 1.9 for intrauterine pregnancy indicates that the probability of intrauterine pregnancy is 90% higher with salpingotomy than salpingectomy.

Fertility outcome reports the rates of subsequent intrauterine pregnancy, repeat ectopic pregnancy, and live birth rate. Such pregnancies may either be spontaneous or achieved through assisted reproductive technology, and this should be stated clearly in the fertility outcome. Furthermore, fertility outcome rates differ according to the ectopic pregnancy associated reproductive and pathological characteristics, and treatment method chosen. The denominator will differ in those women who desire future fertility and who are trying to conceive compared to those women taking contraceptive measures.

Homolateral or ipsilateral tube denotes the tube that is affected by the ectopic pregnancy. Compare with contralateral tube.

Persistent trophoblast is defined as suboptimal falling, increasing, or plateauing serum βhCG concentrations following initial ectopic pregnancy treatment for which additional treatment (surgical or medical) is needed. This rarely occurs following salpingectomy, but may arise following salpingotomy, methotrexate, or expectant management.

Pregnancy of unknown location is defined as absence of pregnancy localisation (either intrauterine or extrauterine) by transvaginal sonography when serum βhCG levels are below the discriminatory zone (1000–1500 IU/L). If there is an absence of pregnancy localisation with the serum βhCG above the discriminatory zone then this, along with other clinical, ultrasonographic, and serum βhCG features increases the likelihood of ectopic pregnancy.

Primary treatment success is defined as progressive decline of serum βhCG to undetectable levels following initial treatment without reintervention (surgical or medical) for persistent trophoblast or supervening clinical sequelae (e.g. tubal rupture or worsening clinical

symptoms).

Salpingotomy is where the ectopic conceptus is removed from the affected tube through a linear incision of the tube overlying the ectopic. This incision is not surgically closed and is allowed to heal through secondary intention. This surgical treatment conserves the affected tube.

Treatment failure denotes the sum of the reintervention rates for persistent trophoblast and supervening clinical sequelae (e.g. tubal rupture or worsening clinical symptoms).

Tubal excision or salpingectomy is defined as the surgical removal of the tube affected by the ectopic pregnancy.

Tubal preservation is a treatment approach designed to preserve the tube affected by the ectopic. This involves expectant, medical (e.g. systemic methotrexate) or salpingotomy treatment approaches.

Tubal patency examines the homolateral tube for the passage of dye at hysterosalpingogram, or at second look laparoscopy, or the passage of contrast media at transvaginal ultrasound. Only those cases that have been managed by tubal preservation, rather than salpingectomy, are eligible for tubal patency testing.

INCIDENCE/PREVALENCE Around 10,000 ectopic pregnancies are diagnosed annually in the UK. The incidence of ectopic pregnancy in the UK (11.0 per 1000 pregnancies) is similar to other countries like Norway (14.9 per 1000) and Australia (16.2 per 1000).[132-134] Since 1994, the overall rate of ectopic pregnancy and mortality rate (0.4 per 1000 ectopic pregnancies) has been static in the UK. [134] Until recently, most epidemiological studies have failed to distinguish between ectopic pregnancies occurring in women who did not use contraception (**reproductive failure**) and women who used contraception (**contraceptive failure**). [135;136] A French population study undertaken from 1992 to 2002 found that, over the duration of the study, the rate of reproductive failure ectopic pregnancies increased by 17%, whilst the rate of contraceptive failure ectopic pregnancies decreased by 29%.[136] Increasing rates of Chlamydia infection, smoking, and assisted reproductive technology usage may have contributed to the disproportionate increase in reproductive failure ectopic pregnancy rate over contraceptive failure ectopic pregnancy rate. Widespread use of dedicated early pregnancy assessment units and non-invasive diagnostic algorithms are likely to have contributed to increasing rates of ectopic pregnancy diagnosis.[137;138]

AETIOLOGY/RISK FACTORS The aetiology of ectopic pregnancy is unclear. Ectopic pregnancy arising from reproductive failure or contraceptive failure should be considered separate entities with differing aetiology, risk factors and reproductive outcomes. [135;136;139]The main risk factors for reproductive failure are a history of pelvic inflammatory disease, previous ectopic pregnancy, pelvic and tubal surgery, infertility, smoking, and assisted conception. [135;140]The main risk factor for contraceptive failure ectopic is intrauterine contraceptive device (IUD) failure. IUDs do not increase the absolute risk of ectopic pregnancy, but a pregnancy occurring with IUD is more likely to be ectopic than intrauterine.

Other risk factors for ectopic include prior spontaneous abortion, prior induced abortion, endometriosis, uterotubal anomalies, and prior in utero exposure to diethylstilbestrol. However, less than half of the ectopic pregnancies diagnosed are associated with risk factors.[141]

PROGNOSIS OF ECTOPIC PREGNANCIES As the pregnancy advances, tubal pregnancies may either diminish in size and spontaneously resolve, or increase in size and eventually lead to tubal rupture with consequent maternal morbidity and mortality. There are no reliable clinical, sonographic or biological markers (e.g. serum βhCG or serum progesterone) that can predict rupture of tubal ectopic pregnancy.[142;143] Maternal mortality following ectopic pregnancy is an uncommon short-term outcome in developed countries. The recent UK Confidential Enquiry into Maternal Deaths cited ectopic pregnancy as a cause of 11 maternal deaths (0.4 per 1000 ectopic pregnancies). [134] Short-term maternal morbidity relates to pain, transfusion requirement and operative complications.

Primary treatment success and long-term fertility outcomes depend on the clinical characteristics of the ectopic pregnancy (e.g. whether the ectopic occurred in a woman using contraception or not, tubal rupture or not, contralateral tubal disease) and the type of surgical or medical treatment chosen. A ten-year follow up of ectopic pregnancies showed the rate of repeat ectopic pregnancy was much higher in women who had a IUD at the time of the index ectopic pregnancy compared to women whose ectopic was not associated with IUD use. In contrast, the rate of intrauterine pregnancy was 1.7-fold higher (Fecundity Rate Ratio 1.7, 95% CI 1.3-2.3) in women who had a IUD at the time of the index EP compared to women whose index ectopic was not associated with IUD use.[139]Short and long-term consequences on health-related quality of life and psychological issues (e.g. bereavement) are also important, but are rarely quantified.

PREGNANCIES OF UNKNOWN LOCATION Pregnancy of unknown location is defined as the absence of pregnancy localisation (either intrauterine or extrauterine) by transvaginal sonography when serum βhCG levels are below the discriminatory zone (1000–1500IU/L). An observational study of pregnancies of unknown location has shown 55% spontaneously resolve, 34% are subsequently diagnosed as viable, and 11% are subsequently diagnosed as ectopic pregnancies. [144]

AIMS OF INTERVENTION

Short-term: primary treatment success; to reduce maternal morbidity and mortality related to ectopic pregnancy (tubal rupture and haemorrhage) and/or treatment method used (e.g. surgical complications, medical drug toxicity).

Long-term (all women): to reduce risk of recurrent ectopic pregnancy.

Long-term (for subgroup of women desiring future fertility): to maximise chance of future intrauterine pregnancy and live birth rate from unassisted spontaneous conception, or following use of assisted reproductive technology techniques (e.g. *in vitro* fertilisation).

OUTCOMES

Primary outcomes: primary treatment success (eradication of the ectopic pregnancy without the need for secondary treatment arising from persisting trophoblast and/or tubal rupture and/or worsening clinical symptoms and signs); persistent trophoblast.

Secondary outcomes: future fertility-spontaneous intrauterine pregnancy, live birth rate, and repeat ectopic pregnancy in women desiring future fertility (this should ideally be expressed

as fecundity rate ratios over specific time intervals corrected for known confounders [e.g. history of infertility and contraception usage at time of index ectopic pregnancy]).

Other outcome measures: tubal rupture; ipsilateral tubal patency following tubal preserving treatment (salpingotomy, methotrexate, or expectant management); maternal morbidity and mortality (prior to ectopic treatment [natural history of ectopic pregnancy] and following treatment alternatives); harms of treatment alternatives; complications of surgery [injury, infection, thromboembolism]; drug toxicity; health-related quality of life assessments.

METHODS

Clinical Evidence search and appraisal June 2006. Given that there are limitations in performing RCTs comparing medical and surgical ectopic pregnancy treatments, and limited trial numbers, the search was extended to incorporate large sample sized quality cohort studies (either prospective or retrospective, with control or comparison treatment groups). Where appropriate, evidence from quality observational studies is utilised, when RCT evidence is lacking. The following databases were used to identify studies for this chapter: Medline 1966 to June 2006; Embase 1980 to June 2006; and The Cochrane Library 2006, issue 2. Additional searches were carried out using the

following websites: NHS Centre for Reviews and Dissemination (CRD), Database of Abstracts of Reviews of Effects (DARE), Health Technology Assessment (HTA), Turning Research into Practice (TRIP), and National Institute of Health and Clinical Excellence (NICE) guidance. Abstracts of the studies retrieved were assessed independently by two information specialists using predetermined criteria to identify relevant studies. Design criteria included: study types — published systematic reviews, meta-analysis, RCTs,

controlled clinical trials, cohort studies with a control or comparison group, or case-control studies in any language; open or blinded studies acceptable; studies had to contain 20 or more individuals. There was no maximum loss to follow up or minimum length of follow up. Fecundity rate ratios have been calculated by the Clinical Evidence author, except where indicated. A GRADE (Grading of Recommendations Assessment, Development and Evaluation) [8] approach and evaluation of the quality of evidence for interventions is included in this review (see **Introduction to Chapter 4 ; Table 4.iv; Table 4v**).

TREATMENT OPTION: SALPINGECTOMY

Treatment failure (persistent trophoblast)

Compared with salpingotomy: salpingectomy may be more effective at reducing initial treatment failure rates compared with salpingotomy. (**moderate quality evidence**)

Compared with methotrexate: Salpingectomy may be more effective at reducing initial treatment failure rates compared with methotrexate . **(moderate quality evidence)**

Subsequent pregnancy rates

Compared with salpingotomy: we don't know whether salpingectomy may result in lower rates of subsequent intrauterine pregnancies or recurrent ectopic pregnancy rates compared with salpingotomy. **(very low quality evidence)**

Compared with expectant management: Salpingectomy may be no more effective at increasing subsequent pregnancy rates in women with ectopic pregnancies compared with expectant management. **(very low quality evidence)**

SALPINGECTOMY BENEFITS

Salpingectomy versus salpingotomy: We found no systematic review or RCTs. We found one non-systematic review and four cohort studies (and related single follow up publication) that compared salpingectomy versus salpingotomy (**see Table 4.10**). [139;145-149]

Table. 4.10. Comparison of fertility outcomes of salpingotomy versus salpingectomy

Study	**Sample size** **(sum of salpingectomy and salpingotomy cases unless otherwise stated)**	**Salpingotomy compared with salpingectomy as the reference treatment**	
		Crude spontaneous intrauterine pregnancy (IUP) rates and/or **Fecundity Rate Ratios* (FRR) (95% CI)**	**Crude repeat ectopic pregnancy (REP) rates and/or** **Fecundity Rate Ratios* (FRR) (95% CI)**
Non-systematic review [149]	1774 women (in 9 cohort studies) undergoing salpingotomy or salpingectomy for ectopic pregnancy and desiring future fertility 176 women (in 18 cohort studies) with cTD after salpingotomy (corresponding results for salpingectomy not reported)	280/528 (53%) with salpingotomy v 614/1246 (49%) with salpingectomy *Crude FRRs* [$] *1.08 (0.97 to 1.19)* Salpingotomy in women with cTD then IUP is 96/176 (55%)	78/528 (15%) v 123/1246 (10%) *Crude FRRs* [$] *1.50 (1.15 to 1.95)* Salpingotomy in women with cTD then REP is 36/176 (21%)
Prospective cohort [148]	86 women undergoing laparoscopic surgery for ectopic pregnancy and were attempting conception. cTD present in 33/60 salpingotomy and 15/26 salpingectomy cases	36/60 (60%) with salpingotomy v 14/26 with salpingectomy (53.9%) FRR 1.11 (0.77 to 1.76)* *Irrespective of the type of surgery performed if cTD then crude FRR 0.53 (0.36 to 0.75)* *(based on 20/50, 40% pregnant with cTD vs. 27/34, 79.4% not pregnant with cTD)*	11/60 (18.3%) v 2/26 (7.7%) FRR 2.38 (0.67 to 9.30)
Retrospective cohort [147]	135 women undergoing laparoscopy or laparotomy for	62% v 38% at 18 months (numbers not	28% v 23% at 3 years (numbers not reported)

	ectopic pregnancy. cTD present in 15/56 salpingotomy and 38/79 salpingectomy cases	reported) FRR 1.9 (0.91 to 3.8) If cTD, FRR 0.80 (0.13 to 4.9) If bilateral tubal pathology, FRR 1.4 (0.13 to 16) *Irrespective of the type of surgery performed:* *If cTD then FRR 0.48 (0.18 to 1.2)*	FRR 2.4 (0.57 to 11) *Irrespective of the type of surgery performed:* *If cTD, FRR 0.79 (0.18 to 3.4)*
Retrospective cohort [145]	276 women undergoing salpingotomy or salpingectomy (by laparoscopy or laparotomy) for their first ectopic pregnancy. cTD present in 30/208 salpingotomy and 17/68 salpingectomy cases	89% v 66% at 7 years (numbers not reported); P<0.05 FRR 1.58 (1.06 to 2.38)* *Irrespective of the type of surgery performed:* *If cTD, FRR 0.46 (0.26 to 0.82)* *If previous fertility surgery,* *FRR 0.74 (0.34 to 1.60)*	17% v 16% at 2 years (numbers not reported; reported as not significant) FRR 1.28 (0.57 to 2.87)* *Irrespective of the type of surgery performed:* *If cTD, FRR 2.25 (1.11 to 4.531)* *If previous fertility surgery,* *FRR 2.51 (1.002 to 6.31)*

Cohort [139;146]	476 women with tubal ectopic pregnancy who were not using contraception at conception. Salpingotomy (262, cTD in 236/262 cases); Salpingectomy (178, cTD in 159/178 cases); Methotrexate (36, cTD in 8/36 cases)	Salpingotomy vs. Salpingectomy vs. Methotrexate 73% vs. 57% vs. 80%. *Irrespective of the type of surgery performed:* *If cTD, FRR 0.53 (0.33 to 0.83)* Women with infertility factors: Salpingotomy vs. Salpingectomy FRR 1.67 (1 to 2.78) Methotrexate vs. Salpingectomy FRR 2.5 (1.95 to 8.33) Women with no infertility factors: Salpingotomy vs. salpingectomy FRR 1.18 (0.63 to 2.22) Methotrexate vs. Salpingectomy FRR 2.12 (0.49 to 9.78)	Salpingotomy vs. Salpingectomy vs. Methotrexate 25% vs. 27% vs. 41% (no significant difference between groups, p=0.55) Salpingotomy vs. Salpingectomy FRR 0.93 (0.76 to 3.5) Methotrexate vs. Salpingectomy FRR 1.51 (0.25 to 7.08)
	[139] 1595 women with ectopic pregnancy. Salpingotomy (798); Salpingectomy (654); Methotrexate (143) Numbers of cases with cTD for each treatment is unstated	Salpingotomy vs. Salpingectomy FRR 1.25 (1 to 1.67) Methotrexate vs. Salpingectomy FRR 1.25 (0.7 to 2.33) *Irrespective of the type of surgery performed:* *If cTD, FRR 0.83 (0.67 to 1.0)*	Salpingotomy vs. Salpingectomy FRR 1.25 (0.67 to 2) Methotrexate vs. Salpingectomy FRR 2.25 (0.6 to 7.4) *Irrespective of the type of surgery performed:* *If cTD, FRR 1 (0.5 to 2.0)*

Footnotes

cTD contralateral tubal disease. This may be absent or occluded or distorted by pathology (hydrosalpinges, adhesions)

Asterisked FRRs – calculated by author

***Fecundity Rate Ratio**-See glossary.

FRR are stated for salpingotomy compared to salpingectomy as the reference unless otherwise stated. FRRs are also stated for the presence relative to absence of confounding factors (e.g. cTD or infertility) disregarding the type of surgery (either salpingotomy or salpingectomy) that was performed. Where studies have calculated FRR using salpingotomy as the reference standard the reciprocal of this FRR has been quoted as this provides the FRR of salpingotomy compared to salpingectomy as the reference standard.

Crude FRRs[$] We report an FRR based on the results reported in the meta-analysis. However, due to study heterogeneity and non-adoption of survival analysis techniques by included studies within the meta-analysis, a pooled FRR as we have reported is likely to be crude and subject to bias.

Primary treatment success: Salpingectomy compared to salpingotomy has higher primary treatment success and a lower risk of persistent trophoblast. Primary treatment success rate of salpingectomy is almost 100%, with the risk of persistent trophoblast less than 1%. [146;149] Primary treatment success rate of salpingotomy ranges from 72%-98% [150;151], with a 3-20% risk of persistent trophoblast.[145;146;149;152;153]

Subsequent pregnancy rates in salpingectomy versus salpingotomy (**see Table 4.10**): Subsequent spontaneous intrauterine pregnancy rates following salpingotomy (range 53%-89%) did not significantly differ from those following salpingectomy (range 38-66%) apart from one cohort study [145] that showed an improved fecundity with salpingotomy [89% vs. 66%, FRR 1.58 (1.06-2.38)]. A similar trend of no difference between salpingotomy (range 10%-28%) and salpingectomy (range 8%-23%) was observed for rates of repeat ectopic pregnancy.

Contralateral tubal disease and infertility factors (**see Table 4.10**): In the presence of contralateral tubal disease (hydrosalpinx, peritubal adhesions or absent tube) or infertility factors (e.g. previous ectopic, previous tubal surgery, previous pelvic inflammatory disease, infertility factors) there is a trend to decreased subsequent intrauterine pregnancy and increased risk of ectopic pregnancy irrespective of whether salpingotomy or salpingectomy is performed. [145-148;154-156]. In the presence of such factors, salpingotomy provides a greater probability of subsequent intrauterine pregnancy than salpingectomy; however this does not achieve statistical significance.

Salpingectomy versus methotrexate: We found no RCTs or observational studies of sufficient quality.

Salpingectomy versus methotrexate (systemic): One cohort study, and its follow up publication, compared three interventions: salpingotomy, salpingectomy, and methotrexate. [139;146] It found that the rate of treatment failure with salpingectomy was similar to salpingotomy, but less than methotrexate. The study showed no significant difference between salpingectomy and salpingotomy in rates of subsequent intrauterine pregnancy or subsequent ectopic pregnancy**.**

Salpingectomy versus expectant management: see benefits of expectant management.

SALPINGECTOMY HARMS

Salpingectomy versus salpingotomy: The non-systematic review and cohort studies listed in (**see Table 4.10**) did not report on harms**.**

Salpingectomy versus methotrexate: We found no RCTs or observational studies of sufficient quality.

Salpingectomy versus salpingotomy or methotrexate management (systemic): We found no RCTs or observational studies of sufficient quality. The cohort study gave no information on adverse effects. [139;146] One cost-effectiveness meta-analysis found rates of 0–22% (mean 10%) for minor complications (e.g. drug side effects), and 0–11% (mean 7%) for serious complications (e.g. ruptured ectopic, or other symptoms of persistent trophoblast) in women who had methotrexate. [151] It also found intraoperative complications of 0–8% (mean 2%) and postoperative complications of 0–15% (mean 9%) for laparoscopy (either salpingectomy or salpingotomy).

COMMENTS ON SALPINGECTOMY EVIDENCE: All comparisons included here have been based on retrospective or prospective observational cohort designs in women with unruptured tubal ectopic pregnancies (**see Table 4.10**). Few studies have considered the impact of infertility factors (known infertility, contralateral tubal disease) on treatment choice (conservative salpingotomy or radical salpingectomy) and future fertility outcome. Differences in such prognostic factors may not be adequately clarified when comparing salpingotomy with salpingectomy, even when adopting multivariate analysis techniques. However, further information may be provided by an RCT comparing salpingotomy with salpingectomy that is currently recruiting. This is the ESEP (European Surgery in Ectopic Pregnancy) study, which represents an international multi-centre Dutch–Swedish–British collaboration. Importantly, any potential benefits of improved intrauterine pregnancy rate with salpingotomy compared with salpingectomy appear to be small, and possibly restricted to subgroups with contralateral tubal disease. This effect and its magnitude should be verified by RCTs comparing salpingotomy with salpingectomy.

TREATMENT OPTION: PROPHYLACTIC METHOTREXATE AFTER SALPINGOTOMY

Treatment failure (persistent trophoblast)

Compared with no salpingotomy alone: A single prophylactic dose of methotrexate after salpingotomy is more effective at reducing persistent trophoblast compared with salpingotomy alone. **(moderate quality evidence)**

BENEFITS PROPHYLACTIC METHOTREXATE AFTER SALPINGOTOMY

Salpingotomy plus single systemic dose methotrexate versus salpingotomy alone: One RCT found that adding a single prophylactic dose of systemic methotrexate (1 mg/kg im) after salpingotomy (by laparoscopy or laparotomy) significantly reduced the incidence of persistent trophoblast compared with salpingotomy alone (1/54 [2%] v 9/62 [15%]; RR 0.13, 95% CI 0.02 to 0.74; NNT 8, 95% CI 4 to 33). [153]

HARMS AND COMMENTS ON PROPHYLACTIC METHOTREXATE AFTER SALPINGOTOMY.

See under methotrexate section.

TREATMENT OPTION: SYSTEMIC METHOTREXATE

Treatment failure

Single dose methotrexate compared with multiple dose regimens: Single dose methotrexate may result in higher rates of treatment failure in women with ectopic pregnancies compared with multiple dose regimens. **(low quality evidence)**

Compared with salpingectomy: Methotrexate may be less effective at reducing initial treatment failure rates compared with salpingectomy. **(moderate quality evidence)**

Single dose methotrexate compared with salpingotomy: Single dose methotrexate is no different at increasing primary treatment success rates in women with small unruptured tubal pregnancies compared with salpingotomy by laparoscopy. **(moderate quality evidence)**

Multiple dose methotrexate compared with salpingotomy: Multiple dose methotrexate is no different at increasing primary treatment success rates in women with confirmed unruptured tubal pregnancy compared with salpingotomy by laparoscopy. **(moderate quality evidence)**

Subsequent pregnancy rates

Single or multiple dose methotrexate compared with salpingotomy: Single dose methotrexate is no different at increasing tubal patency, subsequent intrauterine or ectopic pregancy rates in women with small unruptured tubal pregnancies compared with salpingotomy. **(moderate quality evidence)**

SYSTEMIC METHOTREXATE BENEFITS

Systemic single dose versus multiple dose methotrexate regimens: We found one systematic review (3 RCTs, 23 observational studies, 1327 women with ectopic pregnancy)[157], one RCT (108 women)[158] and one cohort study (643 women). [159] The systematic review found that single dose methotrexate had significantly higher primary treatment failure than multiple dose methotrexate (absolute numbers not reported; OR 1.71, 95% CI 1.04 to 2.82). The review also found a significant difference for the studies considered high quality (high quality according to the authors' own rating system; absolute numbers not reported; OR 1.96, 95% CI 1.07 to 3.60) and for studies that controlled confounding factors (βhCG and fetal cardiac activity: OR 4.74, 95% CI 1.77 to 12.62)

Systemic methotrexate (single or multiple dose) versus salpingotomy: We found one systematic review (search date 2004, 4 RCTs, 307 women; see **Table 4.11**).[150] The review (3 RCTs, 207 haemodynamically stable women with a small unruptured tubal pregnancy) found that single dose methotrexate was significantly less effective than salpingotomy (by laparoscopy) in primary treatment success (elimination of tubal pregnancy), but found no significant difference in tubal patency, subsequent intrauterine pregnancy, and repeat ectopic pregnancy rates. The systematic review also found no significant difference between multiple dose methotrexate (1 RCT, 100 haemodynamically stable women with a laparoscopically confirmed unruptured tubal pregnancy) compared with salpingotomy (by laparoscopy) in primary treatment success or tubal patency (see **Table 4.11**). One RCT identified by the review found that physical functioning (measured as part of SF-36, 0 = worst, 1 = best) was significantly better with single dose methotrexate compared with salpingotomy at 4 and 10 days (4 days: 73 with methotrexate v 43 with salpingotomy, P = 0.001; 10 days: 93 v 70; P = 0.006). [160] Another RCT identified by the review found that a variety of scores of quality of

life were significantly lower with multiple dose methotrexate compared with salpingotomy at 2 weeks (Medical Outcomes Study, 0 = worst, 100 = best; role function: 29 v 51; social function: 45 v 68; health perceptions: 52 v 63; P < 0.05 for all comparisons). [161]

Methotrexate (systemic) versus salpingectomy or salpingotomy: See **Table 4.11**

Systemic methotrexate versus expectant management: We found no RCTs.

Table 4.11. RCTs and meta-analyses of surgical and surgical versus medical treatments in the management of ectopic pregnancy. [150]

Type of comparison	No Of RCTs	Sample size	Trial / including longer follow up of trial in separate publication	**Meta-analysis of trials as reported by Cochrane review [150]**			
				Primary treatment success Relative risk (95% CI)	Tubal patency in those desiring future fertility Relative risk (95% CI)	Subsequent intrauterine pregnancy rate Relative risk (95% CI)	Repeat ectopic pregnancy rate Relative risk (95% CI)
Laparoscopic salpingotomy vs. laparotomy salpingotomy[194;200-202].	3	105 63 60	Lundorff 1991/1992 Murphy 1992 Vermesh 1989	91/104 (88%) v 121.124 (98%) (RR 0.90, 95% CI 0.83 to 0.97; NNT 10, 95% CI 6 to 27)	78% v 87% (0.89, 0.74 to 1.1)	61% v 53% (1.20, 0.88 to 1.15)	6% v 15% (0.43, 0.15 to 1.2)
Systemic MTX multiple dose i.m. vs. laparoscopic salpingotomy[203;204]	1	100	Hajenius 1997/ Dias Pereira 1999	82% v 72% (1.15, 0.93 to 1.4)	55% v 59% (0.93, 0.64 to 1.4)	36% v 43% (0.89, 0.42 to 1.9)	9% v 10% (0.77, 0.17 to 3.4)
Systemic MTX single-dose i.m vs. laparoscopic salpingotomy.[160;205-207]	3	71 74 62	Fernandez 1998 Saraj 1998 Sowter 2001/ Sowter 2001	71% v 88% (0.83, 0.71 to 0.97)	60% v 57% (1.1, 0.74 to 1.5)	37% v 47% (0.99, 0.55 to 1.8)	0% v 17% (0.27, 0.02 to 4.5)

SYSTEMIC METHOTREXATE HARMS

Systemic single dose versus multiple dose methotrexate regimens: One systematic review found significantly lower rates of adverse effects (including nausea, vomiting, alopecia) in women who had single dose compared with multiple dose methotrexate (31% v 41% [absolute numbers not reported]; OR 0.44, 95% CI 0.31 to 0.63). However, it found no significant difference between regimens when it adjusted for serum βhCG (OR 0.79, 95% CI 0.21 to 3.01). [157] It also found no significant difference between regimens for abdominal pain or hospital admission (abdominal pain: 22% v 26%; OR 0.80, 95% CI 0.53 to 1.19; hospital admission: 12% v 11%; OR 1.11, 95% CI 0.83 to 1.47).

Systemic methotrexate (single or multiple dose) versus salpingotomy:

The systematic review gave no information on adverse effects. [150] The first RCT found that women who received single dose methotrexate had significantly longer vaginal bleeding than did those who underwent salpingotomy (7.5 days v 3 days; $P < 0.001$). [160] The second RCT found that pain was greater with multiple dose methotrexate over 16 weeks compared with salpingotomy (results presented graphically; significance assessment not reported). [161]

Salpingotomy plus single systemic dose methotrexate versus salpingotomy alone: The RCT reported that there were no "clinically significant" adverse effects in people who had methotrexate. It also reported that there was no significant difference in laboratory values (white blood cell count, haemoglobin, haematocrit, serum creatinine, and transaminase) between groups at 7 days after surgery (reported at non-significant; P value not reported). [153]

Systemic methotrexate versus expectant management: We found no RCTs or observational studies of sufficient quality.

COMMENTS ON SYSTEMIC METHOTREXATE EVIDENCE: The primary treatment success rate of systemic methotrexate (single or multiple dose regimens) in treating ectopic pregnancies has been reported by meta-analyses as 87% (range 75%-90%) [151], 84%[162]and 89%. [157] The risk of persistent trophoblast is 18% (range 6%-31%). [150] Despite the term single dose methotrexate regimen, repeat doses are permitted every 7 days if there is inadequate hCG fall and a meta-analysis has shown two or more doses are required in 13.5% women undergoing single dose regimens. [157]A retrospective study (n=93) has shown two-year subsequent cumulative intrauterine pregnancy rates of 67% and repeat ectopic pregnancy rates of 24%[163], which correlates to fertility outcomes obtained by RCTs included in the meta-analysis (see **Table 4.11**). [150]

Clinical guide: Multiple dose systemic methotrexate involves a regime of once daily methotrexate 1mg/kg i.m on alternate days (days 1, 3, 5, 7), and leucovorin 0.1mg/kg i.m.on alternate days (days 2, 4, 6, 8). The regimen is continued unless βhCG falls by more than 15% in 48 hours or until four doses of methotrexate are given. A repeat course can be given if βhCG is not less than 40% of its initial value by day 14.Single dose systemic methotrexate regime involves a single dose of methotrexate i.m. (50mg/m2). The dose is repeated if βhCG has not fallen by at least 15% between day 4 and day 7 of treatment. Up to four doses can be given if βhCG does not decline by 15% every week. Prospective studies suggest around 25-40% of non-invasively diagnosed ectopic pregnancies are suitable for non-surgical (expectant or methotrexate) management [160;164-166].The criteria necessary for methotrexate treatment have been agreed by the RCOG and includes: non-invasive diagnosis of ectopic pregnancy; haemodynamically stability with no signs of tubal rupture; ectopic mass is less than 3.5cm in diameter and there is no fetal cardiac activity; hCG does not exceed 3000 IU/L; no medical contraindications to methotrexate usage; woman consents to frequent outpatient follow up. [167]

Observational (prospective and retrospective) studies have shown higher primary treatment success of methotrexate with ectopics that have: low pre-treatment βhCG (preferably < 1000 IU/l); [160;168-174]absent fetal cardiac activity [170;175] or absent yolk sac identified by sonography; [176;177];no prior history of treated ectopic; [175]no pelvic pain; [172];no previous history of infertility.[163] Therefore, treatment outcomes of methotrexate should be compared against the other tubal conserving methods (salpingotomy and expectant management).

Adverse effects: The frequency of methotrexate complications is similar to that associated with laparoscopy.[151] However, the nature of the complications differs, with serious complications of laparoscopy having greater morbidity and mortality than those related to methotrexate. Women who experienced side effects were more likely to have successful treatment regardless of single or multiple dose methotrexate regimen.[157] Drug adverse effects, although prevalent, are usually self-limiting and relatively minor and include: nausea, vomiting, gastritis, diarrhoea, abdominal pain, oral mucositis, pneumonitis, bone marrow suppression and abnormal liver function. Case reports have described other rare but serious complications: life-threatening neutropenia and fever,[178]anaphylaxis, [179] haematosalpinx and pelvic haematocoele, [180]and death due to multi-organ failure.[181] A meta-analysis of single dose methotrexate showed side-effects in 24% (95% CI 9% to 47%) and 10% (95% CI 7% to 14%) had a ruptured ectopic pregnancy. [162]

TREATMENT OPTION: EXPECTANT MANAGEMENT

Subsequent pregnancy rates

Compared with surgery: Expectant management may lead to similar subsequent pregnancy rates in women with nonviable embryos (non-invasive with declining hCG levels) compared with salpingectomy or salpingotomy. **(very low quality evidence)**

Note: We found no clinically important results about expectant management compared with methotrexate in women with ectopic pregnancies.

EXPECTANT MANAGEMENT BENEFITS

We found no systematic review or RCTs.

Expectant management versus salpingectomy or salpingotomy:

One retrospective cohort study (180 women with ectopic pregnancy) found similar rates of expectant management and salpingectomy or salpingotomy in subsequent intrauterine conception rate (19/37 [51%] with expectant management v 31/49 [63%] with surgery). [182] The study did not report on success of treatment or report separate data by type of surgery. We found one small prospective observational study that compared expectant (16 women) versus systemic methotrexate (26 women) versus salpingotomy/salpingectomy (46 women); interpretation of outcomes was biased by case selection. [183] The study also only reported treatment success for women who had methotrexate.

Expectant management versus methotrexate: We found no RCTs or observational studies of sufficient quality.

Expectant management in studies with no control group: We found one non-systematic review (15 prospective cohort studies, 482 women with ectopic pregnancy who were described as "stable" or "well") which found a mean rate of 67% (range 47–82%) for

successful management of ectopic pregnancy by expectant management. [184] The review also reported that rates of tubal patency were 57/74 (77%), subsequent intrauterine pregnancy 42/62 (68%), and repeat ectopic pregnancy 6/47 (13%). One prospective cohort study (107 women who were clinically stable with non-viable pregnancies and no signs of haematoperitoneum) found that 75/107 (70%) of ectopic pregnancies resolved spontaneously. [166] Another prospective cohort study (30 women who wanted to become pregnant again) found tubal patency in 28/30 (93%) of women, subsequent intrauterine pregnancy in 21/24 (88%), and repeat ectopic pregnancy in 1/24 (4%).[185]

EXPECTANT MANAGEMENT HARMS

Expectant management versus salpingectomy or salpingotomy: The retrospective cohort study did not report on harms. [182]

Expectant management versus methotrexate: We found no RCTs or observational studies of sufficient quality.

Expectant management in studies with no control group: The meta-analysis reported that 2.5% of women had a tubal rupture in one of the cohort studies. [184] The two cohort studies did not report on harms. [166;185]

COMMENTS ON EXPECTANT MANAGEMENT EVIDENCE: Expectant management was confined to a selected subgroup of unruptured ectopic pregnancies. We found no RCTs that compared expectant management with laparoscopic surgery or systemic methotrexate. Data for expectant management are derived from retrospective studies with different inclusion criteria (e.g. ectopic size, serum βhCG, presence of fetal cardiac activity) that contribute to methodological bias and preclude effective statistical comparison. There is limited evidence that expectant management has similar primary treatment success and future fertility outcomes to surgically treated ectopic pregnancy.

Clinical guide on expectant management Cases considered suitable for expectant management should conform to strict criteria: non-invasive diagnosis of ectopic pregnancy, unruptured ectopic, woman is haemodynamically stable, less than 100 mL of fluid in Pouch of Douglas, initial βhCG is below 1000 IU/L (when the success rate increases to 80% [184]), consecutive serial serum hCG levels show spontaneous decline, no worsening of symptoms (especially abdominal pain, vaginal bleeding) during this interval, and woman understands the need for ongoing surveillance. [167]These factors have been verified as favourable prognostic signs in observational studies. [184]Prospective and retrospective observational studies have shown that low serum progesterone (< 20 nmol/L) and increased rate of βhCG decline to be important predictor of successful expectant management in pregnancies of unknown location. [144;186-189] **.** There is no quantifiable harm in expectant management as intervention is absent. However, harm would arise should primary treatment fail or tubal rupture ensues. Expectant management necessitates regular surveillance until normalisation of clinical, ultrasound, and βhCG variables. The risks of tubal rupture and persistent trophoblast remain despite adequately declining serum βhCG concentrations. Tubal rupture has been reported with serum βhCG levels below 50 IU/L.[190;191]

TREATMENT OPTION: SALPINGOTOMY

Treatment failure (persistent trophoblast)

Salpingotomy by laparoscopy compared with salpingotomy by laparotomy: Salpingotomy by laparoscopy is less effective at increasing primary treatment success rates compared with salpingotomy by laparotomy. **(high quality evidence)**

Compared with salpingectomy: Salpingotomy may be less effective at reducing initial treatment failure rates compared with salpingectomy **(moderate quality evidence)**

Compared with single or multiple dose methotrexate Salpingotomy is no different at increasing primary treatment success rates compared with single or multiple dose methotrexate **(moderate quality evidence)**

Subsequent pregnancy rates

Salpingotomy by laparoscopy compared with salpingotomy by laparotomy: Salpingotomy by laparoscopy is as effective at increasing tubal patencies, subsequent intrauterine pregnancy rates, and decreasing subsequent ectopic pregnancies compared with salpingotomy by laparotomy. **(high quality evidence)**

Compared with salpingectomy: We don't know whether salpingotomy may result in lower rates of subsequent intrauterine pregnancies or recurrent ectopic pregnancies compared with salpingectomy. **(very low quality evidence)**

Compared with expectant management: Salpingotomy may be no more effective at increasing subsequent pregnancy rates in women with ectopic pregnancies compared with expectant management. **(very low quality evidence)**

Compared with single or multiple dose methotrexate: Salpingotomy by laparoscopy is no different at increasing tubal patency, subsequent intrauterine or ectopic pregancy rates in women with small unruptured tubal pregnancies compared with single dose methotrexate. **(high quality evidence)**

SALPINGOTOMY BENEFITS

Salpingotomy (via laparoscopy) versus salpingotomy (via laparotomy):

We found one systematic review (search date 2004, 3 RCTs, 228 women haemodynamically stable women with a small unruptured tubal pregnancy) that compared laparoscopic salpingotomy with laparotomy salpingotomy (see **Table 4.11).** [150] It found that significantly fewer women have primary treatment success with salpingotomy by laparoscopy compared

with salpingotomy by laparotomy (see **Table 4.11**) due to a higher rate of persistent trophoblast (RR 3.6, 95% CI 0.63 to 21), but found no difference in tubal patency (see **Table 4.11**). In those women desiring future fertility (145/228 [64%]), there was no significant difference between salpingotomy by laparoscopy and salpingotomy by laparotomy in intrauterine pregnancy rate and rate of repeat ectopic pregnancies (see **Table 4.11**).

Salpingotomy versus salpingectomy: See benefits of salpingectomy.

Salpingotomy versus expectant management: See benefits of expectant management.

SALPINGOTOMY HARMS

Salpingotomy (by laparoscopy) versus salpingotomy (by laparotomy): The systematic review gave no information on adverse effects.[150]

Salpingotomy versus salpingectomy: See harms of salpingectomy.

Salpingotomy versus expectant management: We found no RCTs or observational studies of sufficient quality.

COMMENTS ON SALPINGOTOMY EVIDENCE:

The surgeon's preference and operative experience, as well as patient related factors (e.g. obesity, previous abdominal surgery, known pelvic adhesions, haemodynamic instability) dictates whether laparoscopy or laparotomy is preferred. These confounding factors may lead to an overestimation of laparotomy related complications in high operative risk groups.[192] See also comment on salpingectomy.

Laparoscopy or laparotomy surgical treatment of ectopic pregnancy Evaluation of these trials showed that laparoscopy compared with laparotomy treatment of ectopic pregnancy

incurs less blood loss and analgesic requirement, and has a shorter duration of operation time, hospital stay, and convalescence time.[150] A reduced prevalence of pelvic adhesions has been suggested as a mechanism for the potential higher future fertility rate observed with laparoscopy compared to laparotomy. [193;194] A multicentre observational study reported major surgical complication rates of 2.7/1000 for diagnostic laparoscopic procedures, and 17.9/1000 for operative laparoscopy.[195] The major complications arise following laparoscopic bowel (0.4-0.7/1000 cases) and major vessel (0.2/1000 cases) injury.[196] Apart from possible differences in primary treatment success and future fertility, there are no additional maternal harms between laparoscopic salpingotomy and laparoscopic salpingectomy.

TREATMENT OPTION: METHOTREXATE (SYSTEMIC) PLUS MIFEPRISTONE

Treatment failure

Compared with methotrexate alone: Methotrexate plus mifepristone is no more effective at increasing treatment success rates compared with methotrexate alone but it seems this combination may be more effective in increasing treatment success rates in women with high levels of progesterone. **(moderate quality evidence)**

METHOTREXATE (SYSTEMIC) PLUS MIFEPRISTONE BENEFITS

Systemic methotrexate plus mifepristone versus systemic methotrexate alone:

One RCT found no significant difference between methotrexate plus mifepristone and methotrexate in the number of women who had initial treatment success (22/25 [88.0%] v 18/25 [72.0%]; OR 2.85, 95% CI 0.54 to 19.17). [197] However, the median time to resolution of the ectopic pregnancy was quicker with the combined treatment (14 days v 21 days; significance assessment not reported). A second RCT also found no significant difference between methotrexate plus mifepristone and methotrexate in the number of women who had initial treatment success (90/113 [79.7%] v 72/97 [74.0%]; RR 1.07, 95% CI 0.92 to 1.25). [198]

For women with higher levels of progesterone (greater than or equal to 10nmol/L), it found that treatment success was significantly more successful with the combined treatment than with methotrexate alone (15/18 [83.3%] v 5/13 [38.5%]; RR 2.16, 95% CI 1.06 to 4.44). One prospective cohort study found that there were less treatment failures with methotrexate plus mifepristone compared with methotrexate alone (1/30 [3.3%] with methotrexate plus mifepristone v 11/42 [26.2%] with methotrexate). [199]

METHOTREXATE (SYSTEMIC) PLUS MIFEPRISTONE HARMS

Systemic methotrexate plus mifepristone versus systemic methotrexate alone:

The first RCT found that two women in each group reported mild nausea. [197] The second RCT found the same rate of gastritis in both groups (34/113 [30.1%] v 30/99 [30.3%]; P = 1.00). [198] The cohort study gave no information on adverse effects. [199]

COMMENTS ON EVIDENCE: See comment under methotrexate.

4. 3. Laparoscopic entry techniques: clinical guideline, national survey and medicolegal ramifications

INTRODUCTION Although complications associated with laparoscopic surgery are fortunately rare, a significant proportion of these occur at the time of laparoscopic abdominal wall entry [208-233].Meta-analyses and large multicentre centre studies have provided pooled risks of vascular and bowel injury at the time of laparoscopic entry as 0.2 per 1000 and 0.4 per 1000 respectively [208;226;234-245]. Such complications may incur serious morbidity and mortality, and this is compounded if such injuries are not detected at the time of original surgery, particularly in the case of bowel injury [208;212;246-254].

Two laparoscopic entry methods are principally used in gynaecology and general surgery respectively:

- Closed entry and creation of a pneumoperitoneum at the umbilicus (or Palmer's point).
- Open laparoscopy (Hasson) [255-257].

Other techniques, used less frequently and with limited supporting evidence[213], are direct entry [258-266], optical access trocars [267-277] and radially expanding trocars [278-283].

On current evidence, mainly based on observational studies, no one laparoscopic entry method has demonstrated clear superiority over another. This has led to wide variation amongst clinicians as to which entry method should be recommended [213;284;285]. It has been suggested that open (Hasson) entry is superior as vascular injury is less likely to occur compared to closed entry techniques [235;239;256;257;264;284;286-296], although this viewpoint has been challenged [235].

There is significant variation in laparoscopic entry practice in the UK [297-300] and International locations [234;301;302]. In an attempt to minimise the risks of laparoscopy and unify clinical practice, several international bodies [International Middlesbrough Consensus [303], RCOG (draft version only)[304] , SOGC [285], RANZCOG [305], EAES [306], SAGES [307], French society of Endoscopic Gynecology [308], Netherlands [309]] and experts [310-312] have recommended specific "safe laparoscopic entry" principles. In fact, several small-sized studies [313-315] have shown that adopting a recommended technique [285;303] can reduce the incidence of laparoscopic entry related complications.

We wanted to evaluate the status of gynaecological laparoscopic entry in the UK, bearing in mind that litigated cases normally consider both what should be (published recommendations) and what is (questionnaire enquiries) occurring in clinical practice. To achieve this we planned to:

1. Establish evidence based criteria for safe laparoscopic entry through a systematic literature search and critical appraisal of the literature.

2. Currently identify what laparoscopic entry techniques are used in the UK, and explore any factors that may influence the preference for a particular technique. This was determined through a UK wide questionnaire survey.

3. Identify the current judicial viewpoint on laparoscopic entry injuries from the published literature.

METHODS

Establish criteria for safe laparoscopic entry: Electronic searches were performed in MEDLINE (Ovid version 1996-December 2007), EMBASE (Ovid version 1996-December 2007) using relevant combinations of medical subject headings (laparoscopy; gynecological surgical procedures; intraoperative complications; postoperative complications; pneumoperitoneum, artificial; malpractice; risk assessment; legal liability; judicial role; jurisprudence) and text words. International guidelines were identified by interrogating specialised electronic repositories (e.g. national guideline clearinghouse, national electronic library for health, OMNI, TRIP database, E guidelines and GFMER databases) and searching national Collegiate (e.g. RCOG, ACOG, RANZCOG, SOGC) and specialist international laparoscopy organisation websites (e.g. AAGL, SLS, ISGE, BSGE, SAGES, EAES, ASERNIP-S). Literature was critically appraised according to established evidence-based criteria [see Scottish Intercollegiate Guidelines Network (SIGN) recommendations Table 4.ii-4iv [7;316]] to generate a list of key steps necessary for safe laparoscopic entry (see **Table 4.v and 4.12**). For each step, we denoted a level of evidence and grade of recommendation and discussed their derivation from the supporting literature.

Table 4.12: Evidence-based criteria for safe laparoscopic entry: 10 steps

Step	Intervention	Level of evidence and Grade of recommendation	Supporting references
1	**Suitability criteria:** Consider alternative to close umbilical entry (e.g. Palmer's point or open (Hasson) technique) in patients with risk factors such as: previous abdominal surgery; obesity, extremely thin or known abdominal adhesions.	2++, **B**	**Adhesion risks:** 324-344
2	**Safety Criteria:** Patient should be lying flat with an empty bladder. Palpation for the abdominal aorta, any masses and check Veress needle for spring action and gas patency.	4, **GPP**	
3	**Incision:** 10mm vertical intra-umbilical incision starting deep inside the umbilicus pit extending caudally.	4, **GPP**	
4	**Insertion of Veress:** At the deep umbilical pit, at 90º to the skin, with or without stabilising or elevating the umbilical sheath/fascia or anterior abdominal wall, and in a controlled manner with insertion of less than 2cm of the Veress needle tip	2+, 2-, **C** *(Indirect evidence from knowledge of abdominal anatomy)*	258;317-319;351-355
5	**No movement of the Veress needle** following insertion - avoid converting a possible needlepoint injury into a large complex tear	4, **GPP**	
6	**Safety Abdominal pressure check of Veress placement: Most reliably achieved by using a Veress Intra-Abdominal Pressure (IAP) of less than 10mmHg.**	2+, **C**	315;356-358
7	**Safety Abdominal pressure check for Primary trocar:** The intra-abdominal pressure should be 25mmHg to achieve the maximum safe distance between anterior abdominal wall and underlying abdominal contents.	2+, **C**	315;359-363
8	**Vertical Primary Trocar insertion:** Inserted in a controlled two-handed screwing manner, vertically at 90° to the skin, with only the tip of the trocar inserted through the abdominal wall.	2+, **C**	317-319;351-353
9	**Injury check:** An initial 360º laparoscopic check for intra-peritoneal organ injury is performed	4, **GPP**	
10	**No *Epigastric* for Secondary trocar(s) insertion** Inserted under direct vision in a controlled two-handed manner at 90° to the skin, avoiding inferior *Epigastric* vessels.	2+, **C** *(Indirect evidence from knowledge of abdominal anatomy)*	364-368.

Footnotes: We suggest an acronym, **SCIIN SAVE SAVING**, for the 10 steps: Suitability, Criteria, Incision, Insertion, No movement, Safety Abdominal VEress, Safety Abdominal pressure (Trocar), Vertical trocar, Injury check, No epiGastrics.

Questionnaire survey: The questionnaire was developed in collaboration with the British Society of Gynaecological Endoscopy (BSGE) which recorded:

1. Clinician grade
2. Method of entry in the uncomplicated woman and the high-risk woman (defined as any woman with previous suprapubic or midline laparotomy, very thin or obese)
3. Angle of entry for Veress needle and primary trocar
4. Criteria used to test for correct placement of Veress and adequacy of CO_2 pneumoperitoneum prior to primary trocar insertion.
5. Whether the clinician routinely inspected the abdomen for laparoscopic injury at the beginning or end of the laparoscopy procedure
6. Whether the clinician had experienced (personally or through witnessing) any laparoscopic entry-related bowel or vascular injury.
7. Awareness of Middlesbrough Consensus and RCOG sourced information on recommended laparoscopic entry practice.

In contrast to previous questionnaire studies, we wished to compare practice amongst trainee grades as well as consultant specialists. The study population comprised of three groups.

1. *Registered BSGE members at May 2006***.** The questionnaire (and pre-paid postage reply envelope) was included in the BSGE May 2006 quarterly newsletter, which was sent to all 180 registered BSGE members.

2. *Specialist Registrar trainees*. The questionnaire was distributed to all trainees who attended regional study days at Birmingham Women's Hospital, UK.

3. Attendees at the joint RCOG/BSGE conference held on Friday 8th December 2006 at Royal College of Obstetricians and Gynaecologists, London, UK where a questionnaire through an electronic-audience participation format was used. Audience members responded through handheld devices and instantaneous feedback on the entire audience was electronically displayed after each question.

RESULTS

A. Evidence based criteria for safe laparoscopic entry

The original systematic literature review identified 276 primary studies relating to laparoscopic techniques and complications, 21 secondary studies (13 meta-analyses and 8 clinical guidelines) and 12 citations relating to medicolegal aspects of laparoscopy entry related complications. A further 17 relevant citations were identified through the bibliography of primary and secondary (clinical guidelines, reviews) studies. Through a process of critical appraisal of the literature a **10 step evidence-based criteria for safe closed umbilical laparoscopic entry** was constructed and shown in **Table 4.12**. The level of evidence justifying each step is outlined below.

Suitability criteria (Step 1): Women who are extremely thin [317-319] or obese [320-323] or known to have abdominal adhesions are at increased risk of laparoscopic entry related injury at the umbilical entry point. The estimated risks of umbilical and/or anterior abdominal wall adhesions in women with no prior laparoscopic surgery, previous suprapubic laparotomy and previous midline laparotomy are 0-5%, 20%-30% and 50-65%, respectively [324-344]. Prospective observational studies suggest the risk of laparoscopic entry related injury may be considerably reduced by adopting alternative entry (e.g. left upper quadrant Palmer's point or open Hasson technique) in women with such risk factors. However, the actual relative risk

reduction is not quantified as the studies have no comparator. Left upper quadrant Palmer's laparoscopic entry could also be considered if there has been failure to achieve pneumoperitoneum at the umbilicus. Of significance, there is limited evidence that testing for reduced (<1cm) visceral slide (ultrasound-visualised movement of the underlying bowel or omentum) may be helpful in detecting sub-umbilical adhesions, thereby allowing consideration of an alternative laparoscopic entry strategy [345-350].

Supine patient positioning, safety checks and umbilical incision (Steps 2 and 3): Reliable data on appropriate patient positioning and location/type of umbilical incision were not identified. Consequently, we suggest the patient should be laid flat at commencement of laparoscopy to avoid the theoretical risk that "pelvic" bowel being displaced towards the umbilicus, thereby exposing the bowel to entry related injury. On a similar stance, adopting an alternative entry technique is advisable if a prominent abdominal aorta pulsation is identified in close proximity to the undersurface of the umbilicus. Current consensus among clinicians is for a 10mm vertical intra-umbilical incision extending caudally.

Controlled vertical (90 degree) Veress needle entry (Steps 4 and 5): There are no comparative studies assessing the optimum angle of Veress needle entry. The fusion of the parietal peritoneum and *linea alba* at the pit of the umbilicus logically dictates that a vertical (90 degree to the horizontal abdomen) Veress insertion represents the shortest skin-to-peritoneum anatomical distance to enable direct peritoneal entry. According to CT abdominal mapping [317;318;351] and actual laparoscopy [319;352;353], this skin-to-peritoneum distance at the umbilical pit is consistently no greater than 2cm, irrespective of abdominal obesity. Nevertheless, it has been suggested that the Veress angle of entry should vary (45 degrees in non-obese women and 90 degrees in obese women) as CT abdominal imaging [318], and

visualisation at laparoscopy [319], has shown that the location of the underlying aortic bifurcation (which may be prone to Veress injury) tends to be directly under or 2-3cm caudal to the umbilicus in non-obese and obese women, respectively. The umbilicus pit (and underlying parietal peritoneum) may also be stabilised and/or successfully elevated away (either by grasping the lower abdominal wall or by applying tissue forceps/towel clips within 2cm from the umbilicus) from underlying abdominal viscera during Veress insertion [258;353-355]. However, a reasonable summary of the indirect evidence stated is that, traversing the thinnest portion of the abdomen by controlled 90 degree vertical entry, with insertion of no greater than 2cm of the Veress needle tip, with selective umbilical stabilisation/elevation, is likely to be safest route of Veress insertion for the vast majority of women, regardless of any caudal displacement of their umbilicus.

Less than 10mmHg IAP safety test for correct Veress placement (Step 6): A variety of safety tests for correct intra-peritoneal placement of the Veress needle are employed in clinical practice, and include: double-click, aspiration, and hanging drop tests**.** Prospective studies in women undergoing laparoscopy have shown that a Veress intra-abdominal pressure (IAP) less than, or equal to, 10 mm Hg, reliably indicates correct Veress placement at umbilical [315;356;357] and Palmer's point entry [358] locations. The Veress IAP pressure correlates positively with the weight and BMI and negatively with the parity of women [357].

Controlled vertical (90 degree) primary trocar insertion at 25mmHg IAP (Steps 7, 8, 9): Prospective observational studies have shown higher intra-abdominal CO_2 insufflated pressures achieve greater anterior abdominal wall splinting and intra-abdominal CO_2 gas

bubble space [315;359-361]. An IAP of 25mmHg has been shown to achieve a maximum safe distance between anterior abdominal wall and underlying abdominal contents, without compromising cardio-respiratory function [362;363]. A two-handed screwing manner controlled vertical (90 degree) entry of only the primary trocar tip utilises the safe CO_2 bubble depth afforded through an IAP of 25mmHg and is highly unlikely to injure underlying vessels based on actual laparoscopy [319;352;353] and abdominal vasculature CT mapping studies [317;318;351]. Although there is no direct supporting evidence, an initial check for bowel and vascular injury, immediately after primary trocar insertion, is recommended to avoid missing this complication and exposing the women to serious morbidity.

Controlled insertion of secondary trocars under direct vision (Step 10): *Epigastric* vessels can be reliably identified through a combination of direct visualisation [vessels lie 1-2 cm lateral to the medial umbilical ligaments (obliterated umbilical arteries)], transillumination and external anatomical landmarks [364-368]. In most women, a useful and safe point of insertion is 2 cm from the anterior superior iliac crest along an imaginary line connecting the iliac crest to the umbilicus. The controlled insertion, at a 90 degree angle to the skin, using a two-handed screwing manner of the secondary trocar (analogous to that used to insert the primary trocar), should be observed under direct vision to ensure no inadvertent injury of abdominal organs.

B. Questionnaire survey

There was a 62% (n=112) response rate from the postal questionnaire, and 100% response rates from SpR registrars (n=82) and attendees at the RCOG/BSGE meeting (n=32). Analysis was performed on all 226 total respondents.

Entry technique in uncomplicated vs. high risk women: The vast majority would perform a closed umbilical laparoscopic entry in uncomplicated women and a Hasson or Palmer's point entry in women with previous midline laparotomy (**Table 4.13)**. However, there was inconsistency when selecting entry technique in women with previous suprapubic laparotomy, obesity, or who were extremely thin **(Table 4.13**).

Veress and primary trocar entry: Only 18% would use the recommended 90º/90º Veress and primary trocar entry method **(Table 4.14)**. Safety checks performed to ensure correct Veress placement and prior to primary trocar insertion are depicted in **Tables 4.15 and 4.16,** respectively. The proportion of respondents aware of evidence-based guidance, or who have previous experience of laparoscopic injury, is depicted in **Table 4.17**.

Table 4.13 Laparoscopic entry technique in uncomplicated vs. high-risk women

Veress entry technique	Uncomplicated women	High risk women			
		Women with Previous Suprapubic laparotomy	Women with previous midline laparotomy	Women with obesity	Women who are extremely thin
Closed umbilical (umb.)	**213 (94%)**	**193 (85%)**	**37 (16%)**	**179 (79%)**	**189 (84%)**
Open (Hasson)	**5 (2%)**	**14 (6%)**	**49 (22%)**	**13 (6%)**	**15 (7%)**
Palmer's point	1 (<1%)	**8 (4%)**	**102 (45%)**	4 (2%)	2 (<1%)
Suprapubic point	**3 (1%)**	1 (<1%)	4 (2%)	**9 (4%)**	**6 (3%)**
Direct entry	**3 (1%)**	2 (<1%)	0	3 (1%)	2 (<1%)
transvaginal culdoscopy	1 (<1%)	1 (<1%)	1 (<1%)	1 (<1%)	1 (<1%)
Closed umb. or suprapubic	0	0	1 (<1%)	4 (2%)	1 (<1%)
Hasson or Palmer's	0	**3 (1%)**	**23 (10%)**	0	1 (<1%)
Closed umb. or Palmer's	0	2 (<1%)	5 (2%)	0	4 (2%)
Closed umb. or Hasson or Palmer's	0	0	3 (1%)	1 (<1%)	0
Closed umb. or Hasson	0	2 (<1%)	1 (<1%)	**9 (4%)**	**6 (3%)**

Footnotes

Umb. Refers to umbilical

Direct entry would be gasless direct primary trocar abdominal entry and would not utilise Veress.

Table 4.14 Frequency of angle of entry for Veress and Primary Trocar

		Angle of Primary Trocar entry [Count]					Total
		90 °	**60 °**	**45 °**	**30 °**	**Z angle [b]**	
Angle of Veress entry [Count]	**90 °**	40 (18%)	28 (12%)	24 (11%)	1	1	94
	60 °	6	34 (15%)	9	0	2	51
	45 °	1	11	57 (25%)	1	3	73
	30 °	0	1	1	0	0	2
	Not used [a]	3	1	1	1	0	6
Total		50	75	92	3	6	226

Footnotes

[a] Veress angle not determined as practitioner prefers to use either Hasson or direct entry method for insertion of primary trocar.

[b] Z angle system corresponds to initial shallow angle <30° then a steeper angle >60°.

The five most frequent Veress/Primary trocar combinations are shaded in grey and bolded font.

Table 4.15. Safety checks performed to ensure correct Veress placement

TESTS	SpR 1-3 n=63 Count	SpR 4-5 n=41 Count	Consultant n=122 Count	Total Count (%)
Pressure & saline aspiration & two Veress clicks	14	11	28	53 (23%)
Pressure & saline aspiration	13	11	24	48 (21%)
Saline aspiration	20	6	10	36 (16%)
Pressure & two Veress clicks	6	5	21	32 (14%)
Pressure	3	4	14	21 (9%)
Saline aspiration & two Veress clicks	6	1	4	11 (5%)
Pressure & freely moving Veress & two Veress clicks	0	0	7	7 (3%)
Two Veress clicks	1	1	3	5 (2%)
Freely moving Veress & two Veress clicks	0	1	2	3 (1%)
Pressure and freely moving Veress	0	0	3	3 (1%)
Freely moving Veress	0	0	2	2 (<1%)
Not use Veress	*0*	*1*	*4*	*5 (2%)*

Footnotes

Pressure refers to pre-insufflation intra-abdominal pressure recorded as below 8mmHg

Two Veress clicks refers to the audible or tactile impression of two Veress clicks on abdominal insertion

Saline aspiration refers to the four-component saline aspiration, injection, aspiration, drop test commonly known as Palmer's test.

Table 4.16. Safety checks performed prior to primary trocar insertion

TESTS	SpR 1-3	SpR 4-5	Consultant	Total Count (%)
IAP 25mmHg	29	22	48	99 (44%)
Distension and IAP 25mmHg	20	6	14	40 (18%)
Distension and IAP 12-15mmHg	4	0	21	25 (11%)
Distension	2	4	13	19 (8%)
IAP 12-15mmHg	4	4	8	16 (7%)
Distension, >3L CO_2 , IAP 12-15mmHg	1	3	6	10 (4%)
Distension, >3L CO_2, IAP 25mmHg	2	2	6	10 (4%)
Distension, >3L CO_2	0	0	5	5 (2%)
CO_2 >3 litres	1	0	1	2 (<1%)

Footnotes

IAP refers to intra-abdominal pressure

Distension refers to clinical abdominal wall distension

Table 4.17. Awareness of evidence-based guidance and previous experience of laparoscopic injury

	SpR 1-3 n=63 Count	SpR 4-5 n=41 Count	[a] Consultant n=122 Count	Total n=226 Count (%)
Awareness of Middlesbrough Consensus				
Yes	27	22	100	149 (66%)
No	36	19	22	77 (34%)
Awareness of RCOG Guidance				
Yes	55	33	90	178 (79%)
No	8	8	32	48 (21%)
Previous experience laparoscopic injury				
Yes, bowel injury	14	13	57	84 (37%)
Yes, vascular injury	7	4	7	18 (8%)
Yes, both vascular and bowel injury	4	5	32	41 (18%)
No	38	19	26	83 (37%)
Routine Inspection of abdomen				
Yes	48	38	110	196 (87%)
No	15	3	12	30 (13%)

Footnotes

[a] Consultant category includes 4 Staff Grades, 5 Associate Specialists and 113 Consultants.

C. Medico-legal ramifications

The civil standard of law is used in UK medico-legal litigation. This means it is the responsibility of the claimant (woman patient) to prove that, it is more likely than not (greater than 51% probability), that the injury she incurred arose through a negligently performed rather than non-negligently performed surgical technique by the defendant (Surgeon).

Laparoscopic entry related complications have contributed significantly to medical litigation in gynaecological surgery [233;247;248;369-376]. Until recently, there had been inconsistency in the judicial viewpoint in awarding negligent or non-negligent verdicts. However, the case of Palmer v Cardiff & Vale NHS Trust [377] has now set judicial guidance in this area. The court ruled that the likelihood of laparoscopic related bowel injury occurring in an uncomplicated case if there had been good surgical technique, was highly unlikely. If there was no alternative plausible non-negligent explanation for the complication then the defendant was liable - complying with the legal maxim *res ipsa loquitir* ("the thing speaks for itself"). This overruled the defendant's viewpoint that injury was a recognized complication of laparoscopy and therefore its occurrence was not proof of negligence per se. The judicial guidance accepted that given a woman without risk factors, and a surgeon following a safe technique (i.e. correctly inserting Veress needle, it's position checked, insufflation of the peritoneal cavity to 25mmHg, controlled insertion of the primary trocar with penetration of the cavity by just the trocar tip), then the risk of injury was highly improbable. Thus the occurrence of any injury under these circumstances would imply a negligent technique.

Discussion

Based on our systematic literature search, critical appraisal of the published literature and available guidelines, we have constructed a ten step evidence based guideline necessary for safe closed laparoscopic entry. Our findings are analogous to Semm's original 11 safety steps [312]. However, we have updated these steps in line with current evidence-based literature and have ascribed the level of evidence to each step supported by the literature citation(s) for that step. We feel that these 10 steps represent the current most up to date evidence to enable clinicians to practice safe closed laparoscopic entry **(Table 4.12).**

Our national questionnaire study revealed considerable heterogeneity in laparoscopic entry practice despite widespread awareness of the Middlesbrough Consensus or RCOG sourced guidance. The inconsistency was inherent throughout every step of the laparoscopic entry procedure, and has been identified by previous UK based surveys [297-300]. Fundamentally, there was a failure to appreciate risk factors that would justify a change in entry technique, as well as not adopting the correct safety checks following Veress insertion and prior to primary trocar insertion. Even if there was authoritative guidance on safe laparoscopic entry technique it is unclear how many practitioners would actually change their clinical practice accordingly. However an Australian based questionnaire study suggests that this would be supported by the majority of minimally invasive surgeons [301].

We acknowledge that we have a limited sample size and have surveyed a highly selected group. On one hand it is reassuring that we have shown no real differences between trainees and specialists. However, it is of great concern that even in the "expert" specialist group there is such a wide variation in entry technique. It is possible that a survey of general gynaecologists may identify an even wider and more alarming variation in practice.

We strongly feel that safe laparoscopic entry guidance should be disseminated widely such as the 10 steps shown in **Table 4.12.** However, we accept that following such guidance would not necessarily negate the risk of laparoscopic entry related injury nor would it protect the clinician against any negligent ruling should a complication occur. We believe that written guidance should be reinforced through simulated training [378;379], structured formal assessment and consistent clinical direction by specialists. Unless practice concurs with recommended guidance, women undergoing laparoscopy will be exposed to increased unnecessary operative risk.

4.4. Minimising the Risk of Sterilisation Failure-an evidence-based approach

Introduction Female sterilization is one of the commonest procedures performed worldwide. In 1999 around 50,000 female sterilisations were performed in England in the NHS and charitable sectors [380] . The procedure is performed on mainly healthy women at their request, and the intention is to occlude each fallopian tube. This may be achieved through tubal surgical excision, application of a mechanical device or electrocautery coagulation (**Table 4.18**). Where resources permit, the preference, and most widely established technique, is laparoscopic tubal occlusion, which has moreover replaced the earlier technique of performing female sterilisation via mini-laparotomy. In the UK, the RCOG [380] recommends that laparoscopic sterilisation should be performed using either Filshie clip or ring. Tubal excision and separation and related techniques (e.g. Pomeroy procedure) are preferred if sterilisation is performed at caesarean delivery.

Hysteroscopic sterilisation may be considered a non-incisional, non-surgical form of permanent contraception, and is a promising alternative to laparoscopic tubal occlusion. The procedure involves the insertion of a small flexible titanium microinsert into each of the fallopian tubes through the cervix using a guidewire and a hysteroscope (ESSURE®, Conceptus Inc.). The procedure is usually performed under local anaesthesia and/or intravenous sedation. Despite being licensed in the UK, NICE considers hysteroscopic sterilisation to still be under evaluation and should only be performed in accordance with specific NICE guidance (particularly on patient consent and coordinated follow up.[381] This is mainly because there is insufficient evidence on long term efficacy (single case report of failure[382] and tubal perforation[383]) and safety of hysteroscopic sterilisation, with the manufacturer reporting 99.8% effectiveness at preventing pregnancy at 2 year follow up

(http://www.essure.co.uk). [384-386]Furthermore, there are no published randomised controlled trials comparing ESSURE directly with commonly used female tubal occlusion methods.[387]

Table 4.18: Female surgical sterilisation techniques

Method	Techniques	Comments
Ligating tube with partial or complete tubal excision	Pomeroy Fimbriectomy Salpingectomy	Preferred option at mini-laparotomy, but laparoscopic salpingectomy is an alternative
Mechanical occlusion of the tubal lumen	Filshie clip Hulka-Clemens clip Falope ring Silastic ring	Less of the tube is damaged increasing the chance of reversibility
Coagulation induced tubal closure	Unipolar diathermy Bipolar diathermy	Not recommended as the first line method in the UK by the RCOG
Hysteroscopic tubal occlusion	Expanding metal tubal micro-insert implant (ESSURE)	Licensed in UK and under evaluation. Guidance for usage in accordance to NICE. Virtually no possibility of reversal. Contraceptive precautions to continue for at least 3 months post procedure and X-Ray HSG confirmation of tubal occlusion

Rates of sterilisation failure Conception occurring after sterilisation is termed sterilisation failure, and can occur several years after the procedure. Publications have reported differences in rates in sterilisation failure rates, even amongst the same sterilisation method. Such variation is due to differences in: the characteristics of the women undergoing sterilisation; operator experience; operating centre workload; sterilisation method chosen, and the time interval to resuming sexual activity post sterilisation and its frequency.[5]

The two largest studies that have examined failed sterilisation have reported the ten-year cumulative probability of pregnancy of 18.5 per 1000 procedures (US CREST study) [388] and 8 per 1000 procedures (Canada) [389] . The reason for the lower sterilisation failure rate in the Canadian study compared to the US CREST study may be due to predominant use of the Filshie clip and incorporation of non-teaching hospitals in the Canadian dataset. However, both studies were also significant in:

- Utilizing the superior and preferred life table analysis method (cumulative probability of pregnancy at serial time intervals since sterilisation) for reporting sterilisation failure, rather than the less accurate crude failure or Pearl index outcomes that were reported by previous studies.

- Obtaining follow up data for at least 5 to 15 years following the sterilisation

This concept of cumulative risk of pregnancy is particularly important for those women sterilized at a young age (who will be exposed to a risk of pregnancy for a greater time period) and who have been sterilisation by methods of low short and long term efficacy (because such methods, over certain time frames, will acquire a greater percentage of total failures than other more effective methods).

Both US and Canadian dataset studies [388;389] validated this concept of cumulative risk of pregnancy. In the Canadian dataset [389] the cumulative probability of pregnancy increased from 0.3% at 1 year, to 0.7% by 5 years and 0.9% by 15 years.[389] This is depicted in Figure 1. It is therefore important to quote women a 10 year risk of sterilisation failure, individualised to each method and patient age, when counselling them for the sterilisation procedure. Bearing in mind that as long as a woman is fertile, and sexually active, she may continue to be at risk for sterilization failure.The RCOG has recommended a 10-year sterilisation failure rate of 2-3 per 1000 procedures be used for the Filshie clip method. However, this rate is predominantly drawn from a retrospective questionnaire study, of 5 year follow up, with an exaggerated denominator.[390] Given this information, and considering the other reported Filshie clip studies (listed in **Table 4.19**), 2-3 per 1000 risk is more likely to correspond to the first year or even annual non-cumulated absolute risk of sterilisation failure.

Table 4.19. Filshie Clip: reported sterilisation failure rates

Study	Period data are collected from	Sterilisations Performed	Sterilisation method	Outcome	Type of study
Peterson[388] US Collaborative review of Sterilisation (CREST)	1978-1986	10,685 Filshie clip was not used- as it was not licensed in USA until 1996 [392]	Various methods. Hulka spring clip (1595) Silicone Rubber band (3329)	Overall 18.5 per 1000 over 10 years Hulka 36.5 per 1000 Silicone rubber band 17.7 per 1000	Prospective cohort multicentre
Trussell [389]	1980-1999	311,960	Mainly Laparoscopic Filshie clip	8 per 1000 [2496 failures]	Retrospective multicentre
Kovacs [390]	1994-1998	30,000 (estimate)	All Filshie	2.4 per 1000 [73 failures][a]	Retrospective multicentre
Filshie [424]	1982-1992	First 202 responders from a series of 434	All Filshie	2.3 per 1000 [1 failure at 6 months]	Case series
Birdsall [415]	1988-1989	1094	Mainly Laparoscopic Filshie clip	12 per 1000 at 12 months [b]	Case series
Sokal [423]	1984-1990	2746	Filshie clips vs. Rings [2 in each group became pregnant]	1.7 per 1000 for both Ring and Filshie clip groups at 12 months	RCT
Dominik [422]	1984-1990	2126	Filshie clips vs. Hulka clips [11 pregnancies occurred: 9 Hulka, 2 Filshie]	At 12 months 1.1 per 1000 for Filshie Clip 6.9 per 1000 for Hulka Clip group. At 24 months, 9.7 per 1000 for Filshie and 28.1 per 1000 for Hulka	RCT

Footnotes

[a] Kovacs: Of the 73 failures, 14 cases were due to operator error, 29 were properly applied clips and 30 cases had unknown reason for failure

[b] Birdsall: Registrars had a 1.3% failure rate, consultants 1.9% and when both a consultant and registrar performed the procedure a failure rate was 0.7%. Eighty-six percent (6/7) of failed sterilisations were due to operator error (wrong structure, initial non-occlusion).

Key factors [excluding operator error] identified to alter cumulative probability of pregnancy

The failure rate for each sterilisation method tends to stabilize over the long term and may thus be represented as a constant lifetime risk of sterilisation failure (1 in 200 is quoted for the Filshie clip[380]). However, a more precise estimate would also be based upon her age at sterilisation and the subsequent number of fertile years during which she is at risk of pregnancy. The Canadian dataset [389] showed that sterilisation of young women (< 30years of age) compared to older women (>35 years age) was associated with an overall increased absolute risk of pregnancy after sterilisation (1.5% vs. 0.4%), and that this cumulative risk stabilized later in the younger age group. This is depicted in **Figure 4.2**. Multivariate regression analysis of the CREST study [388] showed the following factors were associated with an increased risk of sterilisation failure:

- **Sterilisation method used**. Most effective were postpartum partial salpingectomy and laparoscopic unipolar coagulation at 7.5 pregnancies per 1000 procedures, but laparoscopic spring clip application had the highest risk of failure at 36.5 pregnancies per 1000 procedures (see **Figure 4.3**)

- **Age at sterilisation**. The probability of failure for women sterilized at ages <28 years is greater than that for women sterilized at ages >34 years for all methods of sterilization except interval partial salpingectomy.

- **Race-ethnicity**. Black, non-Hispanic women were at significantly greater risk for sterilization failure than were white, non-Hispanic women.

- **Operating centre**. Substantial differences in procedure specific failure rates between sites, likely representing variation in operator experience, requirements to teach juniors and volume of sterilisation operations.

Figure 4.2: Clinico-pathological mechanisms proposed in sterilisation failure based on Canadian dataset [389]

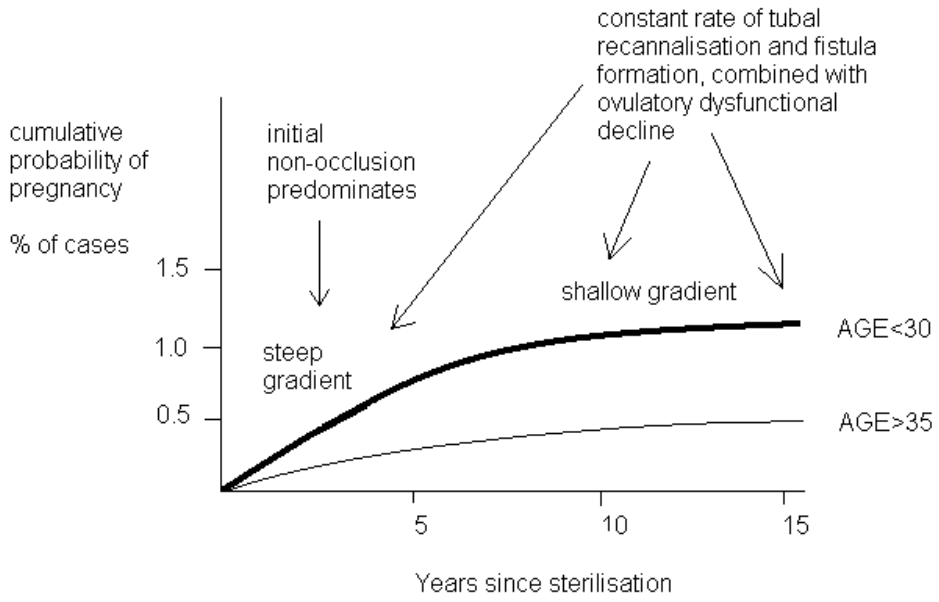
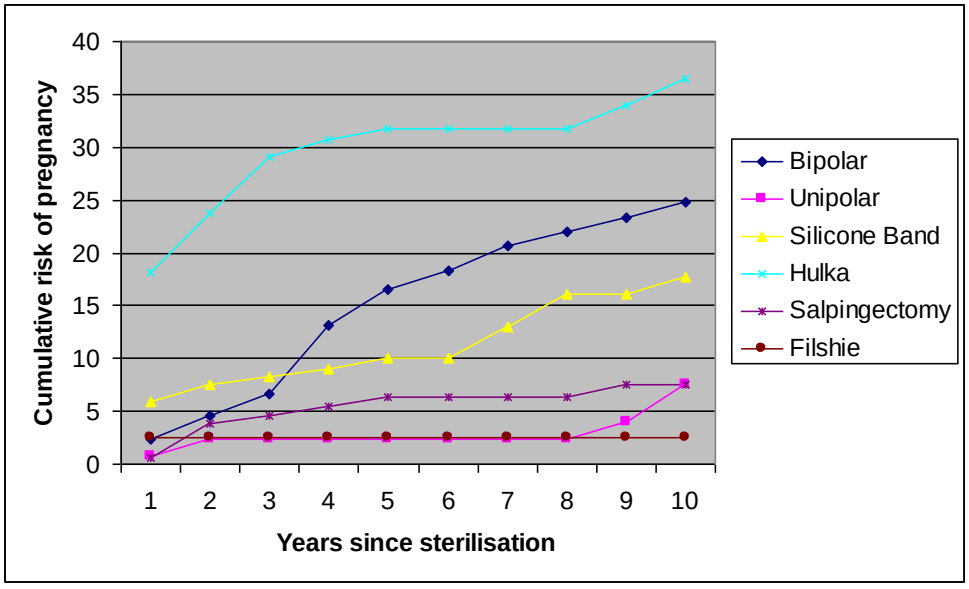


Figure 4.3. Cumulative risk of pregnancy by method from US CREST study [388] and Filshie clip references

Years since sterilisation	Cumulative risk of pregnancy per 1000 sterilisation procedures					
	Bipolar	Unipolar	Silicone Band	Hulka clip	post partum salpingectomy	Filshie clip (estimate only)
1	2.3	0.7	5.9	18.2	0.6	2.5
2	4.6	2.3	7.6	23.8	3.9	2.5
3	6.7	2.3	8.3	29.1	4.6	2.5
4	13.1	2.3	9	30.7	5.4	2.5
5	16.5	2.3	10	31.7	6.3	2.5
6	18.3	2.3	10	31.7	6.3	2.5
7	20.7	2.3	13	31.7	6.3	2.5
8	22	2.3	16.1	31.7	6.3	2.5
9	23.3	4	16.1	34	7.5	2.5
10	24.8	7.5	17.7	36.5	7.5	2.5



Notably, the US Crest study showed no statistically significant associations between risk of sterilisation failure and a history of pelvic inflammatory disease, history of previous abdominal or pelvic surgery, or presence of any adhesions recorded at sterilization. Although these factors have been assumed empirically by practitioners to affect the risk of sterilisation failure.

Sterilisation failure and subsequent intrauterine or ectopic pregnancy

Overall, for all sterilisation methods, studies have shown ectopic pregnancy may occur in 4.3–76.0% of failed sterilisations.[380] The relative risk of intrauterine to ectopic pregnancy occurrence in failed sterilisation varies according to the sterilisation method and time interval from the sterilisation procedure. Women who have been sterilized have a considerably lower absolute risk of an ectopic pregnancy compared to non-sterilised fertile women (as sterilisation protects against both intrauterine and ectopic pregnancies). However, should pregnancy occur, the relative risk of it being ectopic rather than intrauterine is higher in pregnant women who have been sterilized. Women should be counselled about such risks when deciding the method of sterilisation.

There were 47 ectopic pregnancies in the 10,685 sterilised women in the US CREST study, which equates to a 10-year cumulative probability of ectopic pregnancy for all sterilisation methods combined of 7.3 per 1000 procedures. [391] Women sterilized by bipolar tubal coagulation before the age of 30 years had a probability of ectopic pregnancy that was 27 times as high as that among women of similar age who underwent postpartum partial salpingectomy (31.9 vs. 1.2 ectopic pregnancies per 1000 procedures).[391]

Classification of causes of sterilisation failures: the role of operator error [negligent mechanism]

The mechanism of failure should be identified through a systematic assessment of fallopian tube histology, X-ray hysterosalpingography and direct pelvic visual inspection. Neither of the major observational studies on sterilisation failure reported on the underlying mechanism of sterilisation failure.[388;389] Our systematic review identified only 81 cases in the world literature where the mechanism of sterilisation failure had been confirmed by such systematic methodology. [5] Sterilisation failure may be classified as arising from negligent or non-negligent mechanisms, which may be dependent or independent of the sterilisation method utilised (**Table 4.20**). If the mechanism of failure is due to 'tubal non-occlusion' or 'wrong structure sterilisation ', these are considered negligent mechanisms, whereas 'spontaneous tubal recanalisation' or 'fistula formation' mechanisms of failure are considered non-negligent.

Several studies have shown operator error to represent a significant (if not the major) cause of sterilisation failure. One summative review showed that the overall ten year failure rate for worldwide Filshie clip sterilisations was 0.56% in 10,000 women, but fell significantly to 0.2% when cases caused by operator error were excluded.[392] A questionnaire based study examining Filshie clip use in Australia showed of the 73 sterilisation failures from 30,000 procedures, 14 were due to operator error, 30 unknown reason and 29 occurred in the presence of a 'properly applied clip'.[390] Another study, which incorporated participants of the US CREST study, reported that all 20 sterilisation failures using spring clip and silicone rubber band arose to improper application of the occlusive devices.[393;394] Of the 81 sterilisation failures reported in our systematic review of published literature [5], 57 cases were due to operator error (wrong structured 'sterilised' and initial tubal non-occlusion,) and 24 not due to operator error (fistula formation or recanalisation). We have recently published an

analysis of 131 cases of sterilisation failure, incorporating our systematic review, where 88 were negligent and 43 non-negligent sterilisation failures (**see Chapter 2 and reference** [395]).

Table 4.20. Classification system for mechanism of sterilisation failure

DEPENDENT ON THE STERILISATION METHOD **Negligent** Initial tubal non-occlusion (**poor operator technique**) e.g. slippage or overclosure of Filshie clip (**see Figure 3**). Wrong structured 'sterilised' Improperly maintained equipment (e.g. non-calibrated/serviced Filshie clip applicator) which contributed to initial tubal non-occlusion. **Non negligent** Initial tubal non-occlusion (**true method failure**)- this is reported extremely rarely and occurs despite correctly applied technique the ends of the fallopian tube can reconnect spontaneously (recanalisation) a fistula can develop at the occluded portion of the tube
INDEPENDENT OF THE STERILISATION METHOD* Already conceived in the cycle prior to sterilisation Or in the case of Filshie clip, conceives following sterilisation in the remainder of the menstrual cycle because the ovulatory ovum is proximal to tubal sterilisation point **(luteal-phase pregnancy)** Or in the case of Hysteroscopic sterilisation, conceives within the 3 month interval post sterilisation and/or prior to confirmation of effective sterilisation by HSG or ultrasound.
**Most studies on sterilisation failure have excluded such pregnancies from their reported final analysis*

Mechanical tubal occlusive methods have lower rates of tuboperitoneal fistula formation than coagulation based techniques.[396-398] This may be because mechanical occlusion methods destroy much less tube (approximately 4 mm for clips and 2 cm for rings) than electrocoagulation methods (3-4 cm). However the exact aetiology of tubal lumen regeneration remains unclear. Other factors such as individual's tubal 'healing' response, pre-

existing proliferative tubal disease (e.g. endosalpingiosis), degree of tubal avascularity and interval from operation are likely to modify tubal lumen regeneration ability.[396;399-403] Presently there is no evidence to suggest that operator fault in sterilisation technique predisposes to tubal lumen regeneration, and therefore this mechanism of sterilisation failure would be considered to be non-negligent and independent of operator error.

Medico legal consequences The psychological and physical morbidity following failed sterilisation often leads to litigation.[404] A gynaecologist has a duty to inform women of the risk of failure, to carry out the operation in accordance with accepted good medical practice and to avoid foreseeable complications. Women who have undergone *sterilisation performed negligently* are entitled to recover damages according to:

- Wrongful conception: In addition, an action in contract may also arise if the sterilisation procedure was performed outside the NHS in the private sector.

- Negligence: A breach of duty arises when an operation is not carried out in accordance with practice accepted as proper by a reasonable body of gynaecologists (Bolam test). Negligence also occurs when there is omission in appropriate pre-operative counselling.

- Wrongful birth: The negligent act deprived the mother of the possibility to prevent the conception of a disabled child or to have a lawful abortion.

Women are entitled to recover general damages for pain and suffering during pregnancy and delivery, and loss of earnings during pregnancy. A recent judgment in the Australian High Court [405] led the Australian government to amend the Civil Liberty Act to restrict the amount of damages that could be awarded in such situations. In the majority of failed sterilisation cases, even those in the advanced stages of litigation, the mechanism of failure remains unknown as there is no uniform requirement for such cases to undergo systematic enquiry or

to be reported to any supervisory national registry. The RCOG should consider this requirement at the time of the sterilisation guideline review in 2006. [380] Thus, a common scenario in the legal setting is to cast judgment on the likelihood of negligence or non-negligence in cases with unknown mechanism of sterilisation failure. Based on pooling the 81 cases of sterilisation failure with documented interval to pregnancy and mechanism of failure we proposed:

That a greater proportion of early (within 12 months from operation) than late (after 12 months from operation) sterilisation failures occurred by a negligent mechanism. Thus, the time interval to sterilisation failure may be predictive of negligence. In our recent publication of 131 cases of sterilisation failure[395], we showed sterilisation failure occurred significantly earlier in negligent than non-negligent failure mechanisms (mean failure intervals 7.5 vs. 14.2 months; Hazard Ratio 2.35 [95% CI 1.31-4.21]).

Initial tubal non-occlusion is more likely to lead to early sterilisation failure (within one year), and as it is less likely to damage the tube, the resulting pregnancy is more likely to be intrauterine than ectopic. Conversely, late sterilisation failure arising from tubal re-canalisation or fistula formation is more likely to result in an abnormal lumen predisposing to a decreased risk of pregnancy, but should it occur there would be an increased risk of ectopic pregnancy. This is graphically illustrated in **figure 1.**

Identification and assessment of evidence

MEDLINE 1966-2006, the Cochrane library, 2006, Royal College of Obstetricians and Gynaecologists (RCOG, UK) were searched for relevant randomised controlled trials, systematic reviews, meta-analyses, and evidence-based guidelines relating to sterilisation. The searches were performed using the relevant MeSH terms including: sterilization, tubal;

sterilization; sterilization sexual; surgical instruments; electrocautery; cautery; liability, legal; jurisprudence; malpractice; medical errors; treatment failure; risk factors. The majority of publications were retrospective observational studies, case reports and reviews, with a paucity of prospective controlled trials or meta-analyses.[5;380;406] The definitions of the types of evidence used in this chapter are as denoted in the RCOG Clinical Governance advice.[407] Where possible, recommendations on strategies to minimise sterilisation failure are annotated with the level of evidence that supports them (A, B, C or GPP) as indicated (**Table 4.2ii**). Data generated was incorporated in our recently published systematic review in failed sterilisation [5] and utilised for this chapter to generate a best evidence based guideline [6].

Clinical Guideline: Minimising the risks of sterilisation failure

1. Patient Selection **Level GPP**

There is limited evidence that pre-existing gynaecological pathology, in addition to increasing the technical difficulty of performing the sterilisation procedure, independently predisposes to sterilisation failure. Factors such as pre-existing tubal disease, history of abdominal or pelvic surgery, history of pelvic inflammatory disease previous ectopic pregnancy, pregnancy or post-partum state,, obesity, prior use of an intrauterine contraceptive device, previous induced abortion, congenital uterine anomalies, fibroids, endometriosis, endosalpingoblastosis and adenomyosis.[388;389;397;408-412] The myth that sterilisation protects against pelvic inflammatory disease has recently been challenged.[413]

2. Pre-sterilisation pregnancy testing and timing of sterilisation **Level C**

Both hysteroscopic and laparoscopic tubal occlusion may be performed at any time during the menstrual cycle provided that the clinician is certain that the woman has used effective contraception up until the day of the operation. It is recommended practice that all women

should have a urine pregnancy test prior to sterilisation. Routine pre-operative same day pregnancy testing has been shown to reduce the incidence of pregnancies discovered after the sterilisation procedure that have been falsely attributed to presumed negligent sterilisation procedure.[414] However, such a test may still be falsely negative in a very early pregnancy. A serum hCG pre-operatively may be considered, however, if there is any doubt, then the sterilisation should be deferred until the follicular phase of a subsequent cycle.

3. Pre-procedure contraception and the need to continue until onset of next menstrual cycle (reduce risk of luteal pregnancy) **Level GPP**

Contraception is immediately effective if using the combined pill (if commenced between day 1 and day 5 of period) and Mirena IUS. However, with laparoscopic tubal occlusion, contraception is only likely to be completely effective by the onset of the next menses. Therefore, for this method, pre-procedure contraception measures should be continued until the onset of next menses to prevent "luteal phase" pregnancy failure (**Table 4.20**). This is where sterilisation has occurred just after ovulation, and the ovum is already 'proximal' to the tubal occlusion, enabling pregnancy to occur in this luteal phase through post sterilisation 'unprotected' intercourse. Studies have identified luteal pregnancy occurring in 0.32% to 0.6% of sterilisation cases.[388;415;416] Women selecting hysteroscopic sterilisation (ESSURE) need to continue with contraceptive precautions for at least three months post procedure and may resume 'unprotected' sexual intercourse only after there is confirmation of satisfactory tubal occlusion (e.g. by X Ray hysterosalpingogram).

4. Timing the operation - Interval preferred **Level B**

Wherever possible, tubal occlusion should be performed at an appropriate interval following pregnancy. Sterilisation can be performed in the immedate post-partum period (combined

with caesarean section or via minilaparotomy) or post-abortion. However this period is associated with higher rates of failure and regret by the woman[416 417], and this should be incorporated into the counselling and documentation prior to the procedure. In terms of post-partum sterilisation, salpingectomy and Filshie clip have similar rates of failure (7.5 and 8.8 per 1000 respectively).[388 416]

5. Selection of technique- Laparoscopy preferred over laparotomy **Level B**

Each sterilisation method has specific advantages, disadvantages and individualised failure rates according to the sterilisation method and patient characteristics. This information should be conveyed during the counseling process. A meta-analysis [418], and large population study [419], has shown no significant difference in failure rate or major operative morbidity between mini-laparotomy and laparoscopy methods of sterilisation. However, laparoscopic methods have lower minor operative morbidity and are preferred for interval sterilisations as it offers obvious advantages in terms of shorter operative time, same day hospital discharge and shorter convalescence period.

6. Selection of technique- Modified Pomeroy at caesarean section **Level B**

A modified Pomeroy procedure rather than Filshie clip application may be preferable for postpartum sterilisation performed by mini-laparotomy or at the time of caesarean section, as this leads to lower failure rates.[388;416;420], although both procedures are equally popular choices with surgeons.[421]

7. Selection of technique- Filshie clip sterilisation is preferred method **Level B**

Two small RCTs [422;423] and observational studies[390;424] have shown Filshie clip to have the lowest failure rate for interval sterilisation failure and has therefore been recommended by the RCOG [380] as the preferred method at laparoscopic tubal occlusion (**Table 4.19**). Ring

methods have also been recommended by the RCOG, and appear to have equal contraceptive efficacy to Filshie clip. However, ring methods tend to be technically more difficult to apply to the fallopian tubes and have gradually become less popular in UK clinical practice.

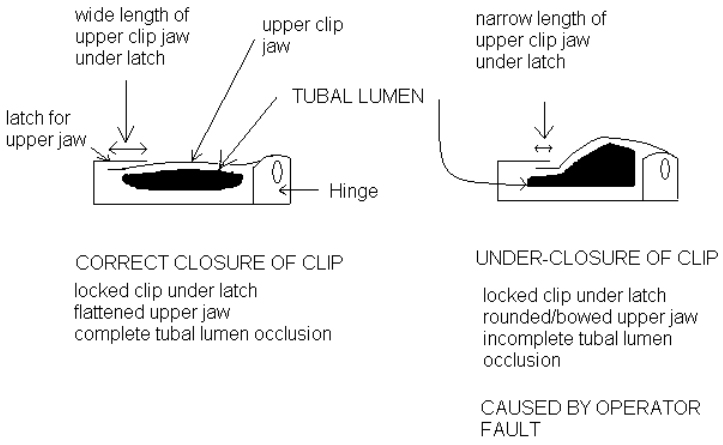
8. Operative technique for Filshie clip: **Levels C and GPP**

a) Care should be taken to ensure the Filshie clip is applied to the **optimal mid-isthmic tubal position** (1cm to 3cm from the uterine cornu) and this structure not be mistaken for an adjacent structure e.g. the round ligament or a fold of peritoneum.[425]

b) The Filshie clip should be applied in a manner to **completely encapsulate the tube** and lumen, be fully locked with the upper jaw compressed, completely flattened and its end adequately secured under the under the latch which 'locks' the clip jaw (**Figure 4.4).** The clip should flatten the whole tube portion within the clip without leaving any unflattened tubal 'knuckles' without transecting the tube. Finally, the clip should sit perpendicular to the long axis of the tube. [425] facilitated by stretching the isthmic portion with hinge placed on the antimesenteric aspect of the tube.

c) **Excessive forceful clip applicator overclosure (Figure 4.4) or underclosure** may lead to tubal transection and subsequent sterilisation failure through luminal regeneration (i.e. tubal fistula or re-canalisation) or incomplete tubal occlusion. [425]

Figure 4.4: Filshie clip under-closure due to operator fault



Despite the clip appearing locked, on closer inspection the upper jaw of the clip will be noted to be incompletely compressed, rounded rather than flattened, and the end insufficiently secured under the under the latch for the upper jaw. Most causes of clip under-closure are due to operator fault.

A predisposing factor to improper closure is a 'faulty' Filshie clip applicator. However, this is rare, as it is a legal requirement that device applicators are well maintained and adequately checked to ensure optimum function. In the case of the Filshie clip, both the manufacturer (Femcare, UK www.femcare.co.uk) and MDA strongly recommend that all single Filshie clip applicators are serviced and re-adjusted at least once a year or after every 100 procedures. Furthermore, a closing checking gauge should be used prior to every sterilisation procedure to ensure the applicator functions correctly. There is only one published case of failed sterilisation, which proposes Filshie clip under-closure as the most likely mechanism of sterilisation failure. Therefore this cause of failure should be considered rare.[426]

d) **Applying two mechanical clips** adjacent to each other on the tube **does not decrease the failure rate**, but may even increase it if they are applied too closely together.[425;427-429]

e) Following clip application there should be a **systematic checking procedure** to ensure the correct structure and both sides of the tube have been satisfactorily occluded, and this should be documented. Although not a legal requirement in the UK, we recommend:

e) **Taking clinical photographs** or operative videos of the sterilised structures identifying them as fallopian tubes. However photographs may be unhelpful in confidently excluding other negligent causes of incomplete tubal occlusion e.g. protruding knuckle of tube inadequate locking of clip jaws, clip under-closure, or tubal transection (partial or complete)

f) **Presence of second operating surgeon for counter-checking**. A recent study involving 1094 sterilisations from 1988-1989 showed that Registrars had a 1.3% failure rate, Consultants 1.9% and when both a Consultant and Registrar performed the procedure a

failure rate of 0.7% was recorded.[415] A medical witness to concur the sterilisation procedure is a legal requirement in some countries.[430]

9. **True method failure**. There is evidence that anatomical tubal patency can occur following a correctly undertaken sterilisation (true method failure), and has been reported following correctly applied Filshie clips in three cases of Filshie clip failure (**table 4.19**)[431] and is implied to have occurred in the 29/73 correctly applied clip sterilisation failures reported by an observational study. [390]However, persisting anatomical tubal patency does not necessarily imply sterilisation failure, as tubal patency rates of 1-2% at three months and 16% at five years have been noted following correctly applied tubal ligation, with the actual pregnancy occurrence of 1-2% over this time period. [400]. Even so, true method failure is rare and difficult to prove; nonetheless three possible mechanisms of true method failure are suggested:

- *A partially non-occluded segment of tubal lumen has formed within the clip*. This tubal 'knuckle', with a patent lumen, can exist within the completely flattened tube portion inside the clip identifiable only at microscopy.

- *Pre-existing utero-tubal structural abnormalities* such as accessory fallopian tube, uterine didelphys [432], and utero-tubal fistulas

Mechanical failure of the Filshie clip. Manufacturers for Filshie clip have not reported spontaneous mechanical failure as a possibility for sterilisation failure, and this concurs with an absence of such cases in the published literature. Nevertheless, there remains at least a theoretical possibility of mechanical material failure, and manufacturers like FEMCARE® offer an examination of the Filshie clips in failed sterilisation to exclude the possibility of this failure mechanism (Femcare - personal communication).

10. **Operator experience and training** **Levels C and GPP**

Improper application of tubal occlusive devices by inexperienced surgeons is frequently reported in cases of sterilisation failure.[394;433;434] Furthermore, operator preference is likely to have an impact on method related failure rates. The CREST study showed failure rates of 7.1 to 78.0 per 1000 for the Hulka clip and 0 to 42.5 per 1000 for the silicone ring - dependent on the operating centres being surveyed.[388] Higher failure rates were more common in centres with performing fewer annual procedures. RCOG recommends that trainees should perform at least 25 supervised laparoscopic tubal occlusions before operating without supervision. [380]

11. Follow up required if uncertainty in tubal occlusion **Level GPP**

Following a complicated sterilisation good clinical practice (rather than a legal requirement) dictates testing of tubal patency.[394;435-438] However, a negative dye spill post sterilisation HSG does not completely preclude the possibility of pregnancy at a later stage.[439]

12. Other issues: Clip Migration and dropped 'lost' Filshie clips **Level C**

Good clinical practice dictates that proof of tubal occlusion (X-ray HSG or tubal dye insufflation or histology of salpingectomy) should be undertaken once missing clips are identified, not only when examining failed sterilisation cases, but also at laparoscopy or laparotomy for other reasons.[425;440] However, missing clips do not necessarily indicate failed application or imminent pregnancy failure, as over time there is a tendency for clips to migrate and even be expelled without resulting in clinical morbidity.[390;423;441-449] There are no reports of this leading to sterilisation failures.[441] It is estimated that over 25% of women will experience a migration of one or more Filshie clips.[441] The tissue between the Filshie clip jaws normally undergoes avascular necrosis and fibrosis, leaving two healed stumps, which tend to separate, permitting clip displacement. Filshie clips may be inadvertently dropped

during laparoscopic sterilisation. If possible the clip should be laparoscopically removed upon completion of the sterilisation procedure. However, if the clip is irretrievable, either open or closed, it should be left. Performing a laparotomy would subject the woman to greater operative morbidity risk than leaving the lost clip in the abdomen. To date, there have been no reports of any serious morbidity or mortality consequent to a lost clip. Women should be informed of the lost clip and reassured accordingly.[425]

Conclusion and Further research

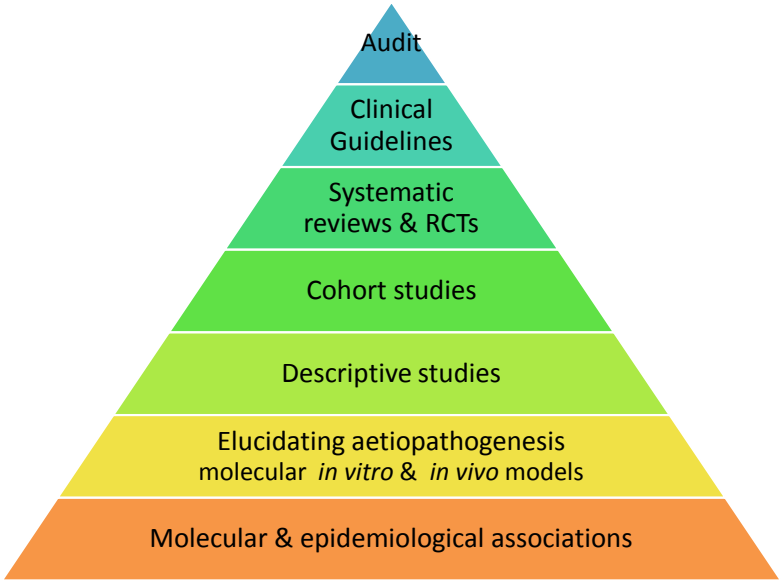
Overall, the level of evidence supporting any screening-preventative measures to reduce the risk of sterilisation failure remains poor. There appears to be a propensity for negligent rather than non-negligent sterilisation failures. However, this can only be verified by establishment of a national register of failed sterilisations (as recommended by the RCOG[380]) that have been subjected to systematic enquiry to establish the mechanism of failure. Like other Confidential Enquiries, such a registry could identify areas of substandard care that could be used as an impetus to improve research and medical training in sterilisation procedures and help design effective clinical risk prevention strategies. The introduction of an operative checklist or proforma, similar to the pre-operative counseling checklist recommended by the RCOG[380] and used in another study [450], may result in reduced numbers of negligently performed sterilisations.

CHAPTER 5. THESIS CONCLUSION

Thesis Précis

The central aim of this PhD thesis was to produce research that could inform and benefit clinical practice. Each chapter of this thesis has achieved this aim, within the limits of the research methodology applied The chapters have been ordered to follow a stepwise ascension of a research methodological pyramid (**Figure 5.1**).

Figure 5.1. Ascension of a research pyramid of research methodologies to benefit clinical practice



Footnotes
RCT randomised controlled clinical trials
Audit refers to clinical audit to assess impact of clinical guidelines

5.1. Experimental investigation of endometriosis

The experimental data explored whether there was a causal link for endometriosis as a neoplastic precursor to ovarian cancer. The chapter was original in adopting distinctive, yet complimentary, approaches to testing this hypothesis through clinical epidemiology (Bradford Hill causality criteria[1]), histopathology, immunohistochemistry, genetic and molecular approaches. The chapter reported the largest EAOC series to be subjected to clinico-epidemiological and LOH mapping for the entire length of chromosome 9 (20 markers) and chromosome 11 (27 markers). Furthermore, this chapter includes the first study to apply SNP 100K genome wide genotyping to endometriosis[2].

Epidemiological and causality literature analysis showed:

- No strong evidence to support a causal link between endometriosis and ovarian cancer[3-5].
- However, there was moderate quality evidence that endometriosis may display similar properties to a cancer cell (Hanahan's Hallmarks of cancer[6]). There are numerous anti-cancer therapies that target the specific molecular properties of the 'cancer cell'. Hence it is conceivable, that anti-endometriosis therapies may also be designed around such cancer cell-like molecular targets (**Table 5.1**).

Experimental analysis showed:

- A prognostic association of the LOH (loss of heterozygosity) identified at 9q34.3 and 11q23.3 chromosomal regions suggesting that these regions may harbour genes that impact on malignant transformation and progression of cancer.

- Decreased glycodelin immunohistochemical expression in endometriosis adjacent to ovarian cancer compared to endometriosis distant from ovarian cancer- hence a possible role for glycodelin as a TSG responsible for development for "malignant" endometriosis.

- Ovarian endometriosis harbours micro-regions of LOH through Affymetrix genome-wide 100k SNP microarray. However, to confirm and validate the location of these multiple micro-regions of LOH analysis further customised microsatellite markers analysis is required. [2;5]

Future directions

High throughput molecular technologies (as used in this chapter) allow parallel genomic, transcriptomic and proteomic evaluation of diseases, at the genome-wide level. Such approaches could be used to elucidate the multigene pathways involved in aetiopathogenesis of endometriosis (**Figure 5.2, Table 5.2**), as well as numerous other diseases. Application of techniques in cancer biology may also facilitate the research and development of therapies for endometriosis (**Tables 5.1 and 5.3).** A key future goal would be the identification of characteristic endometriotic 'genetic' or 'proteomic' signatures that could form the basis of an early screening-preventative testing strategy from women's urine, menstrual endometrium or blood. Furthermore, a similar genomic/transcriptomic/proteomic approach may be considered for the investigation of analogous gynaecological disorders (such as adenomyosis and fibroids) and explore whether genetic alterations are focal or widespread in a diseased reproductive tract.

Table. 5.1. Individualise therapeutic approach to endometriosis according to cancer cell hallmarks [6]

	CANCER CELL HALLMARKS	TARGETS UNDER EVALUATION IN ENDOMETRIOSIS BASED ON AGENTS USED IN CANCER TRIALS	TARGETS/ PUTATIVE TARGETS UNDER INVESTIGATION IN CANCER
1	Self-sufficiency in growth signals	Aromatase inhibitors, Selective oestrogen (e.g. Arzoxifene) and progesterone receptor modulators, Mirena Coil, Gonadotrophin releasing hormone antagonists (e.g. Cetrorelix)	Inhibitors of: Mitogen-activated protein kinase inhibitors, HER-2 receptor (trastuzumab), IGF-1 receptor, EGFR (erbitux), EGFR tyrosine kinase (gefitinib) farneysl transferase, Bcr-Abl tyrosine kinase (imatinib mesylate)
2	Insensitivity to antiproliferative signals		Proteasome inhibitors (bortezomib), cdk inhibitors (flavopiridol)
3	Resistance to Apoptosis	Angiostatin gene transfer; transfection with pro-apoptotic (e.g. BAX) gene COX-2 inhibitors	COX-2 inhibitor (celecoxib), thalidomide, apoptosis inducers (exisulind inhibits cGMP). Immunotherapy by genetically modified tumour vaccines (e.g. HER-2 peptide vaccination) or humoral factors (e.g. immunokines like IL-12, TNF antagonists; monoclonal antibody to CA-125 [ovarex], recombinant immunotoxin to mesothelin)
4	Limitless replicative potential		Telomerase modifiers
5	Sustained angiogenesis	Anti-VEGF monoclonal antibody (bevacizumab), VEGF receptor tyrosine kinase inhibitors	Angiozyme (cleaves mRNA for Flt-1, the main receptor for VEGF), Protein kinase C-beta inhibitor (LY317615), COX-2 inhibitor, thalidomide, lysophosphatidic acid inhibitors
6	Tissue invasion and metastasis		Inhibit/modify Catenin/Cadherin signaling, Selective MMP inhibitors
7	Genomic instability		Gene therapy to deliver therapeutic or corrective gene to alter oncogenes/TSG balance. Genes may be delivered by infectious (adenovirus) or non-infectious (liposome) vectors. Examples: Adenoviral E1A (oppose HE-2/neu oncogene), Adenovirus transfection of wild-type p53 (restore TSG) Transfect viral suicide genes like HSV-thymidine kinase (sensitizes to ganciclovir cytotoxicity) Antisense oligonucletoides (targeting proto-oncogenes, oncogenes like c-myc, protein kinase C-alpha [affinitak]) Tribozymes (cleave oncogenes transcripts)

Footnotes Consequent to endometriosis heterogeneity, the exact contribution of each hallmark component may vary between individuals and clinical symptoms. Nevertheless, therapies can be designed to target predominant categories following endometriosis molecular classification (*expression signature*).

Figure 5.2. Evaluating, in parallel, differences between genomic, transcriptomic and proteomic array platforms to identify candidate molecular pathways

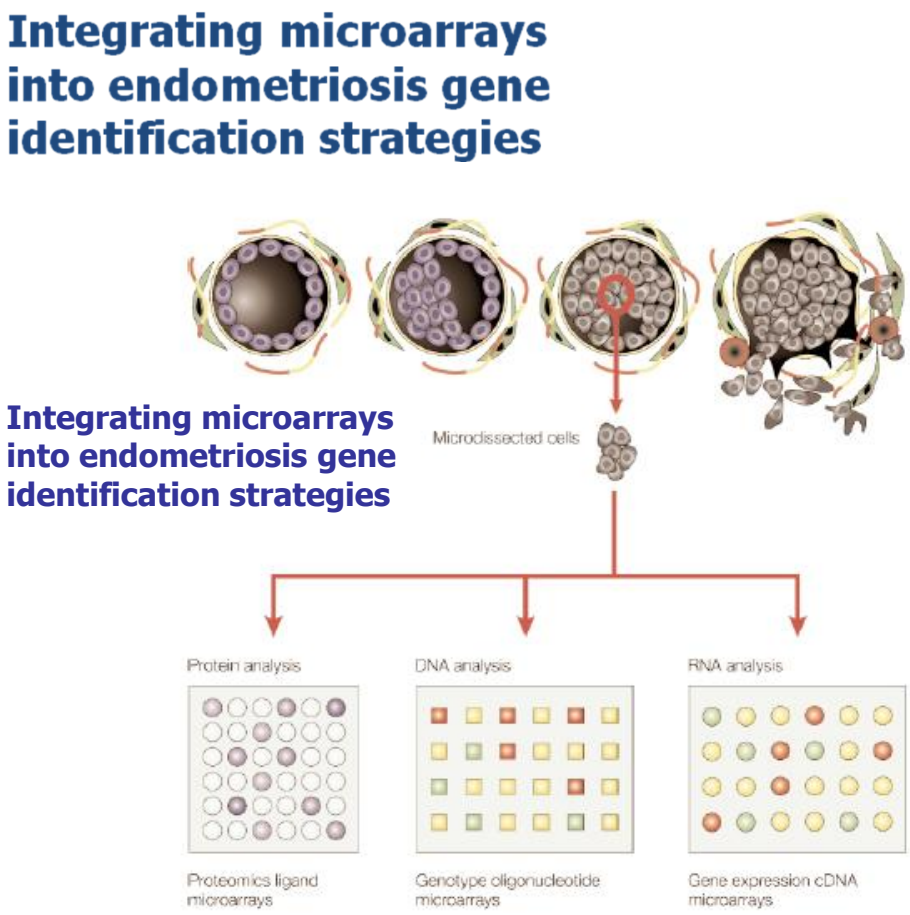


Table 5.2. Summary of studies comparing genomic, transcriptomic and proteomic profiling of endometriosis using high-through put microarray technology

GENOMIC	TRANSCRIPTOMIC	PROTEOMIC
Comparative Genomic Hybridisation (CGH) [7-9]	**Human endometriosis** (ectopic vs. eutopic endometrium) [10-24]	Endometriosis: **protein tissue microarrays** [28;29]
Single nucleotide polymorphisms (SNP) microarray based chips **None identified for endometriosis (apart from data presented in this thesis)**	**DNA originating from blood** (lymphocyte sourced) of women with endometriosis [25] **Animal model endometriosis** [26;27]	**Endometriosis: surface-enhanced laser desorption/ionization time-of-flight mass spectrometry protein chip array** [30]
SNP microarrays in other disorders **Adenomyosis** [31] **Bladder cancer** [32] **Prostate cancer** [33]	**Expression microarrays in other disorders** Endometrial cancer Normal endometrium[34]	**Proteomics in other related disorders** Adenomyosis and leiomyomas: **protein tissue microarrays** [35]

Footnotes

Most studies have compared endometriosis (ectopic endometrium) with matched eutopic endometrium.

CGH) studies have been undertaken in endometriosis, but could only detect relatively large-scale deletions or duplications.

Table 5.3. Implications of thesis findings and future research directions for endometriosis

Key overall themes Establish biological tissue bank- biological samples (e.g. endometriosis, endometriosis associated ovarian cancer, matched eutopic endometrium and ovarian surface epithelium and blood) should be considered in conjunction with originating patient clinical epidemiological outcome data Establish international coordinating body to pursue biological tissue bank, basic science and clinical endometriosis research
Implications for genetic epidemiology Meta-analysis of genetic association studies Meta-analysis of multiple gene expression analyses to validate candidate genes Interrogate accessible bioinformatic tissue expression databases to cross tabulate and identify candidate genes-combine this with above meta-analyses.
Implications for basic science research Compare and contrast molecular profiles from endometriosis and matched eutopic endometrium/ovarian surface/peritoneum/blood using genomic, transcriptomic and proteomic technology IN parallel. Evaluate clinical prognostic value of nuclear morphometry in evaluation of endometriosis Utilise animal models of endometriosis (e.g. baboon) to study molecular profiling (compare with human studies) and evaluate novel medical therapies
Implications for clinical trials Standardize methodology (e.g. inclusion, exclusion and diagnostic criteria, fertility and endometriosis-specific quality of life outcomes) Introduce temporality in studies (i.e. long term follow up) as cost-effectiveness between medical and surgical treatments may depend on rates of re-treatment or persistence of impaired quality of life outcomes. Consider assessing from duration of onset of symptoms rather than from time of diagnosis of endometriosis (delayed onset of presentation)-factor this in to epidemiological associations and quality of life outcomes of new endometriosis cases or individualised retrospective analysis of cases with known endometriosis onset and duration Aim to identify early biomarkers (urine, blood, menstrual flow) which correlate to disease onset to enable early therapy (medical or surgical). RCTs to evaluate medical vs. surgical treatments for specific anatomical or clinical presentations of endometriosis i.e. consider endometriosis as multiple differing clinical entities Consider (or evaluate the need) for long term follow up of early onset severe or atypical endometriosis in view of increased risk of ovarian cancer

5.2. Observational Analytical Studies

The observational analytical cohort studies have ascertained the incidence, natural history and treatment outcomes of common encountered gynaecological disorders such as heavy menstrual bleeding and endometrial hyperplasia [36-39] **(Table 5.4).** Furthermore, the cohort design has been employed to identify prognostic factors associated with the rare outcome of failed female sterilisation[40].

The results of each study are immediately transferable to clinical practice and are likely to improve health care outcomes as outlined in **Table 5.4** Improvements will mainly arise through improved pre-operative patient counselling, better patient selection, consideration of outpatient rather than inpatient treatment modalities and adopting treatments that would reduce rates of hysterectomy for menorrhagia.

Although observational analytical studies (for example, cohort and case-control studies) start with a "low quality" rating of evidence (**Table 5.5** *Level of Evidence*; **Table 5.6** GRADE quality of evidence), grading upwards may be warranted if the magnitude of the treatment effect is very large, if there is evidence of a dose-response relation or if all plausible biases would decrease the magnitude of an apparent treatment effect. Further confirmatory randomised controlled trials (RCTs) would be needed to validate the observations noted in the menorrhagia studies cited in this chapter. However, a larger prospective data set(perhaps multicentre or national), with defined inclusion/exclusion criteria, may be provide a study population that minimises biases, and be of sufficient power to generate data of a standard that approaches a RCT. Hence a future goal would be the creation of large linked prospectively collected patient datasets that could be flexibly used by both clinical and research organisations e.g. NHS electronic patient record.

Table 5.4. Improved health care resulting from analytical observational studies.

Chapter and Reference	Study	Beneficial health care outcome
2.1 [40]	Predicting negligence in female sterilization failure	Early sterilisation failure is suggestive of a negligent rather than non-negligent mechanism of failure. Result increases awareness of need for adequate surgical training in sterilisation procedure Result also has medico-legal implications
2.2 [37]	Effectiveness of a Mirena in the treatment of endometrial hyperplasia	Mirena is highly effective in treating non-atypical endometrial hyperplasia Use of Mirena will reduce the rate of hysterectomy for women with non-atypical endometrial hyperplasia
2.3 [38]	Outpatient vs. Daycase Thermachoice ablation	Thermachoice balloon ablation may be successfully carried out in outpatient local anaesthetic setting Outpatient Thermachoice population utilise less analgesia than day case Thermachoice population Duration of hospital stay is not entirely dependent on whether outpatient or daycase endometrial ablation is considered Consider patient suitability criteria (e.g. pain thresholds) when offering outpatient vs daycase ablation
2.4 [39]	Long term outcome of outpatient Thermachoice endometrial balloon ablation	Thermachoice balloon ablation may be successfully carried out in outpatient local anaesthetic setting Ablation reduces the rate of hysterectomy for women with menorrhagia that is unresponsive to medical therapy Higher intrauterine ablation pressures correlate to better long term outcome
2.5 [36]	Long term outcomes following hysteroscopic myomectomy	Removal of the intracavity component of the fibroid is effective in reducing abnormal uterine bleeding. This effect is independent of the size of the fibroid removed, uterine cavity size and presence of other intramural/subserous fibroids Widespread adoption of this minimally invasive surgical technique will improve patient quality of life and reduce the need for hysterectomy

Table 5.5. Classification of evidence used by RCOG Guideline development (originate from US Agency for Health Care Research and Quality) [41]

Classification of Evidence Levels

Ia Evidence obtained from meta-analysis of randomised controlled trials.

Ib Evidence obtained from at least one randomised controlled trial.

IIa Evidence obtained from at least one well-designed controlled study without randomisation.

IIb Evidence obtained from at least one other type of well-designed quasi-experimental study.

III Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case studies.

IV Evidence obtained from expert committee reports or opinions and/or clinical experience of respected authorities.

Grades of Recommendations

A	Requires at least one randomised controlled trial as part of a body of literature of overall good quality and consistency addressing the specific recommendation. (Evidence levels Ia, Ib)
B	Requires the availability of well controlled clinical studies but no randomised clinical trials on the topic of recommendations. (Evidence levels IIa, IIb, III)
C	Requires evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities. Indicates an absence of directly applicable clinical studies of good quality. (Evidence level IV)

Good Practice Point

✓	Recommended best practice based on the clinical experience of the guideline development group

Table 5.6. GRADE approach [42] (http://www.gradeworkinggroup.org/index.htm)

The Grading of Recommendations Assessment, Development and Evaluation (GRADE)

GRADE: Quality of evidence

The GRADE system classifies the quality of evidence in one of four levels:

High quality— Further research is very unlikely to change our confidence in the estimate of effect

Moderate quality— Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate

Low quality— Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate

Very low quality— Any estimate of effect is very uncertain

Evidence based on randomised controlled trials begins as high quality evidence, but our confidence in the evidence may be decreased for several reasons, including:

- Study limitations
- Inconsistency of results
- Indirectness of evidence
- Imprecision
- Reporting bias.

Although observational studies (for example, cohort and case-control studies) start with a "low quality" rating, grading upwards may be warranted if the magnitude of the treatment effect is very large, if there is evidence of a dose-response relation or if all plausible biases would decrease the magnitude of an apparent treatment effect.

GRADE: Strength of recommendation

The GRADE system offers two grades of recommendations: "strong" and "weak" depending on whether effects of intervention clearly outweigh the undesirable effects, or clearly do not. If trade-offs are less certain—either because of low quality evidence or because evidence suggests that desirable and undesirable effects are closely balanced—weak recommendations become mandatory.

Factors that affect the strength of a recommendation

Factor	Examples of strong recommendations	Examples of weak recommendations
Quality of evidence	Many high quality randomised trials have shown the benefit of inhaled steroids in asthma	Only case series have examined the utility of pleurodesis in pneumothorax

Uncertainty about the balance between desirable and undesirable effects	Aspirin in myocardial infarction reduces mortality with minimal toxicity, inconvenience, and cost	Warfarin in low risk patients with atrial fibrillation results in small stroke reduction but increased bleeding risk and substantial inconvenience
Uncertainty or variability in values and preferences	Young patients with lymphoma will invariably place a higher value on the life prolonging effects of chemotherapy than on treatment toxicity	Older patients with lymphoma may not place a higher value on the life prolonging effects of chemotherapy than on treatment toxicity
Uncertainty about whether the intervention represents a wise use of resources	The low cost of aspirin as prophylaxis against stroke in patients with transient ischemic attacks	The high cost of clopidogrel and of combination dipyridamole and aspirin as prophylaxis against stroke in patients with transient ischaemic attacks

5.3. Systematic reviews

The systematic review in chapter 3 has utilised robust methodology (systematic search, meta-analysis, grading of evidence) to assimilate the published literature relating to the screening and prevention of preterm labour[43;44]. A similar approach has been applied to the systematic review of the clinical use of levonorgestrel-releasing intrauterine system (LNG-IUS) [45]. However, for the LNG-IUS study, meta-analysis was precluded due to extensive study heterogeneity and paucity of suitable clinical trials.

Included in each review is an appraisal of the quality of evidence for each therapeutic intervention according to standardised criteria (RCOG, GRADE; **Table 5.5**, **Table 5.6**). In relation to the screening-prevention of preterm labour, tables listing the evidence appraisal (**Table 5.7**) and resulting care algorithm (**Table 5.8)** are shown below. The algorithm for managing a women at high risk of preterm labour exemplifies how this evaluated research evidence may be effectively applied in the clinical setting.

Furthermore, both reviews have identified areas where future research is likely to be clinically advantageous, but where the evidence is currently lacking. The generation of hypotheses that require further confirmatory validation is another important end-product of systematic reviews, and has been considered an essential pre-requisite by most research funding councils when seeking funding to conduct the confirmatory clinical trials [46].

Table 5.7 Screening and preventative strategies that may reduce the risk of preterm delivery

Strategy for preventing preterm delivery	RCOG Level of Evidence	GRADE Quality of Evidence	GRADE Strength of Recommendation
Asymptomatic bacteriuria in all women	Ia	High	Strong
Bacterial vaginosis in low-risk population groups	Ia, Ib	Moderate	Weak
Elective cervical cerclage in high-risk pregnancies	Ib, IIa, IIb	Moderate	Strong
Indicated cervical cerclage in women with short cervical length on ultrasound	Ib, IIa, IIb	Moderate	Strong
Prophylactic progesterone supplementation in high-risk pregnancies	Ia, Ib	High	Strong
Smoking cessation in all women	IIb, III	Very Low	Weak

Table 5.8 Suggested antenatal strategy to prevent preterm delivery

ANTENATAL VISIT AND PURPOSE	Infection (Screen and treat BV, UTI)	Cervico-vaginal fFN	Ultrasound Abdominal and Transvaginal	Other interventions to be considered
Pre-pregnancy Counselling on recurrence risk and any modifiable predisposing factors	Yes	No	No	Cessation smoking and illicit drugs Improve BMI>25 Thrombophilia screen if history suggests Optimise control of diabetes, high BP Change anticoagulation or antihypertensive drugs
8 weeks' Routine booking bloods	Yes	No	Dating pregnancy	Thrombophilia screen and commence aspirin & LMWH if positive. Low dose aspirin if previous pre-eclampsia (consider use if previous stillbirth, abruption, severe IUGR) Prophylactic progesterone General preterm birth education, support, and risk factor avoidance. Screen and treat BV, UTIs Low threshold for GTT testing
12, 16, 20, 24, 28 weeks' *Nuchal Translucency(12w) and/or Triple Test or msAFP (15-18w)*	No	No	Serial Cervical assessments in women at high risk of PTD	Emergency or elective (12-16w) cervical cerclage based on ultrasound findings and/or reproductive history Emergency cervical cerclage is not indicated if above 32 weeks' Low threshold for GTT testing
22 weeks'	Yes	No	Detailed fetal survey Uterine artery Doppler	Low dose aspirin if suspect pre-eclampsia or IUGR due to uterine artery notching and/or previous history Screen and treat BV and UTIs
24, 28, 32, 36 weeks' GTT at 28 weeks'	No	Only if symptomatic	Fetal growth and umbilical artery Doppler	Prophylactic corticosteroids, antibiotics if symptomatic of PTL or PPROM. In utero transfer to unit with NICU if symptomatic with positive fFN
Labour **Spontaneous or induced**	Yes	Helps confirm Likelihood of PTL, PPROM	Asses fetal well-being, and presentation	Prophylactic corticosteroids, antibiotics (especially GBS prophylaxis). Tocolytics if in utero transfer to unit with NICU is needed.
Post-partum **6 week antenatal check**	No	No	No	Review antenatal events and delivery Identify modifiable factors for future prevention of PTD

FootNote: Bacterial vaginosis, BV; BMI, body mass index; BP, blood pressure; fFN, fetal fibronectin; GBS, Group B streptococcus; GTT, glucose tolerance test; LMWH, IUGR, intrauterine growth restriction; low-molecular weight heparin; msAFP, maternal serum alpha-fetoprotein; NICU, neonatal intensive care unit; PPROM, preterm premature rupture of membranes; PTL, preterm labour; UA, uterine artery; UTI, urinary tract infection.

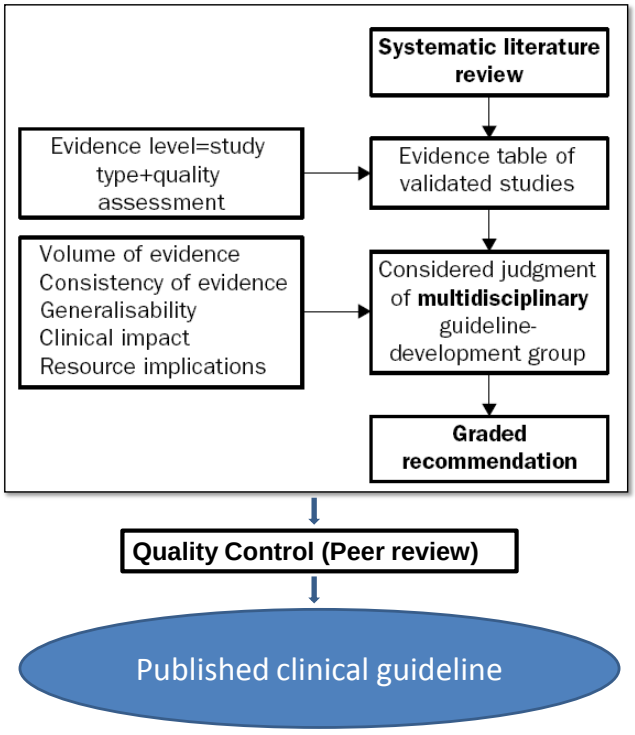
5.4. Clinical guideline development

There are established methodologies utilised in the production of clinical guidelines (depicted in **Figure 5.3**). Four essential criteria have been defined by the Appraisal of Guidelines for Research and Evaluation in Europe (AGREE) guidelines [41] and include:

- Systematic review of the literature
- Graded recommendations with explicit links to the evidence (**Tables 4v in Chapter 4**)
- Input of a multidisciplinary working group
- Quality control; for example, input by an independent advisory board or by independent peer review.

All guidelines developed in this chapter comply with the four essential AGREE criteria [41].

Figure 5.3 Derivation of clinical guidelines



The publications of the clinical guidelines in chapter 4 are likely to have immediate and maximal benefit on clinical practice. Hospital clinical guidelines are normally developed from guidelines published by professional bodies (*e.g. RCOG[47]; chapter 4*), specialist evidence-based resources (e.*g. BMJ Clinical evidence[48]; chapter 4*) and peer-reviewed publications (e.*g. Surgical Endoscopy[49]; chapter 4*). Clinical governance demands the utilisation of best up-to-date evidence, as indicated by such clinical guidelines, to ensure patient receive excellence in their clinical care[50].

The work performed in the production of this research thesis has also identified potential drawbacks in the research methodologies utilised. Given that the aim of research is to inform and benefit clinical practice, due consideration should be given to strategies that may augment the research methodological approaches considered in this thesis in order to achieve this goal. These include:

Quality control. For example, input by an independent advisory board or by independent peer review. In relation to the VBAC guideline [47], the RCOG Guidelines Development Group invited peer review from British Maternal Fetal Medicine Society, National Childbirth Trust, Obstetric anaesthetists, Midwifery supervisors, Obstetricians, and Neonatologists; this served as a the multidisciplinary component of the guideline development process. No specific multidisciplinary working groups were employed by the other chapter guidelines[48;49;51], although all had at least three external peer reviewers in addition to the journal's editor in chief.

Grading the evidence base of the recommendation. The guidelines produced in this chapter have highlighted the significant change in how evidence is now valued for guideline development. The traditional and most common approach has been to value the research study evidence alone using the SIGN classification [52] or amendments from this (**Table 5.5)**;

the SIGN classification had been derived from earlier work by U.S. Preventive Services Task Force [53;54]. This was used by RCOG VBAC, safe laparoscopic entry and failed sterilisation guidelines. However, this system neglects: the study's relevance for particular patients and settings; inconsistencies amongst studies examining the same interventions; potential impact of health care resource limitations. The newer approach, using the GRADE system[42], applied in the ectopic pregnancy guideline[48], considers all these components when evaluating the study by using a strict framework (**Table 5.6**). Grading judgments are expressed in a clear and simple manner as either *high, moderate, low, or very low levels* of supporting evidence and these are incorporated in the allocation of either *strong or weak recommendations* for each intervention (**Table 5.9** provides an example for how ectopic pregnancy guideline was appraised). However, it has not been proven that this grading system is significantly superior to the traditional grading system in pragmatic clinical decision-making. Nonetheless, the GRADE system[42] is advantageous in clearly identifying areas where there is uncertainty in the level of evidence.

Evaluating the quality of clinical guidelines. Concern has been raised that guidelines published by both professional medical bodies and peer-reviewed journals :lack sufficient reliability; are scientifically inaccurate; fail to clarify what influence the level of health care resources may have on guideline practice; and should be critically reviewed by 'experts' in guideline development prior to publication if not produced by 'experts '(such as SIGN or NICE)[55-59]. Such criticism is harsh, but it is true that no guideline can ever achieve complete coverage and applicability in every health care setting.

Table 5.9.Ectopic pregnancy evidence appraised using RCOG and GRADE criteria

Chapter 4.2 Ectopic pregnancy	RCOG Level of Evidence	GRADE Quality of Evidence	GRADE Strength of Recommendation
Salpingectomy in women not desiring future fertility is beneficial compared to salpingotomy or methotrexate in achieving primary treatment success	IIa, IIb	Moderate	Strong
Prophylactic methotrexate (systemic) following salpingotomy compared to salpingotomy alone is beneficial in reducing the risk of persistent trophoblast	Ib, IIa	Moderate	Strong
In women desiring future fertility, systemic methotrexate (single or multiple dose) and salpingotomy achieve similar primary treatment success and subsequent fertility outcomes	Ia, Ib, IIa	Moderate	Strong
In women desiring future fertility, there is marginally improved subsequent fertility rate by performing salpingotomy compared to salpingectomy	III	Very Low	Weak
Single dose methotrexate may result in higher rates of treatment failure in women with ectopic pregnancies compared with multiple dose regimens.	Ia, Ib, IIb	Low	Weak
In selected cases, expectant management has similar primary treatment success and future fertility outcomes to salpingectomy or salpingotomy	III	Very Low	Weak
Methotrexate plus mifepristone is no more effective at increasing treatment success rates compared with methotrexate alone but it seems this combination may be more effective in increasing treatment success rates in women with high levels of progesterone.	Ib	Moderate	Weak

Future directions in guideline development

It is clear that strategies are needed, above and beyond new developments in grading evidence, in order to maintain guideline quality, consistency and applicability across all health care settings for all health care interventions. Apart from adopting the GRADE criteria [42] to grade the level of evidence, one possibility is that guidelines should go through a formal appraisal process using validated tools, such as AGREE tool (Appraisal of Guidelines, Research, and Evaluation in Europe (AGREE)[60] based on the Cluzeau instrument [59]prior to publication. Alternatively, a "second" expert consensus panel peer review should be undertaken prior to publication; these could be representatives of SIGN, NICE or Cochrane group. Importantly, consideration should be given to producing a more time efficient means of guideline development, with fewer group meetings and a shorter time frame; the RCOG VBAC guideline took the author nine months to complete from commencement to final peer-reviewed amended draft submission. Finally, there needs to be periodic review of the clinical guideline (such as every 2 years) to ensure the guideline remains valid and up-to-date with the latest research evidence.

5.5. Future research themes arising from Thesis

The remainder of this chapter suggests future research themes that may augment the research methodological approaches evaluated in this thesis in order to benefit clinical practice.

Integrating genomic, transcriptomic and proteomic high throughput technology

The Affymetrix Single Nucleotide Polymorphism (SNP) DNA microarray chip technology has been validated [61]and its raison d'etre of obtaining genome wide association data are now becoming fulfilled across many health conditions[62]. The Affymetrix SNP microarray chip technology has been successfully applied to the investigation of endometriosis (in this thesis), bladder cancer [32], and prostate cancer [33]. The integration of genomic, transcriptomic and proteomic microarray technology is likely to yield greater discovery of genetic and molecular aetiopathology [63-65]. **Table 5.2** lists the published studies that utilised either genomic, transcriptomic or proteomic approaches to investigate endometriosis (and related adenomyosis). Given this consensus opinion, and the established knowledge relating to the difficulty of identifying endometriosis polymorphisms (that are likely to be multiple and highly variably expressed across the population) using traditional candidate gene investigative approaches[66], future research should now be focused on adopting this high throughput combinatorial approach (**Table 5.2, Figure 5.2**).

Anatomical and molecular re-classification of endometriosis

Genomic, transcriptomic and proteomic profiling (**Table 5.2, Figure 5.2**) should provide a better understanding of the temporo-spatial relationship of endometriosis in relation to location within the female reproductive tract, nature during menstrual cycle phase, nature during pelvic pain and infertility. Novel therapies should target the aberrant process at the molecular level, rather than focussing solely on endometriotic lesion eradication. Examples of strategies

using the cancer hallmark model (**Table 5.1)** are other approaches (**Table 5.3**) are discussed earlier.

Tissue banks for endometriosis (and other important diseases) Our host institution provided a unique tissue bank of endometriosis-associated ovarian cancer, but shared biological tissue banks, matched with clinical epidemiological outcome data, would enable greater research throughput and encourage collaboration. These aims are postulated bthey UK [67;68] and European bodies[69], but various restrictions (particularly Human Tissue Act 2004, implemented September 2006) have hindered the creation of biological libraries. Given the significant burden of endometriosis on worldwide health, a central international body to coordinate endometriosis tissue banking and scientific research is justified and urgently needed.

Developing and utilising animal models of disease There may be a significant difference between a pharmacological agent that is effective *in vtiro* on a fundamental cellular process and one that is disease-specific and safe *in vivo*. Animal models could be useful experimental tools, although opinion is divided as to their correlation to human disease[70]. Nonetheless, several animal models for endometriosis exist (mouse[71;72], rat[73], baboon[74]) , and these have been successfully used to test pathophysiology (such as molecular profiling [26] and capacity for malignant transformation[75]) and novel pharmacological therapies[76].

Improved basic science and clinical science collaboration There appears to be virtually no collaboration between clinical trialists and basic scientists. This is undoubtedly a missed opportunity, as clinical trials could be designed to recover both biological specimens and clinical outcome measures. The effort and expense in generating a specific patient population may thus be used for greater benefit, particularly as quality biological sample

resources are scarce. However, when designing the clinical trial, due consideration should be given to ensure that the trial is adequately powered for both primary clinical and translational research endpoints.

The need to increase translational potential of basic science research Translational research has been defined as research that considers and supports the transition of findings from research setting to benefit clinical practice. Importantly, the concept also includes feedback of findings from the practice to research setting to ensure that the originating research remains applicable and appropriate; a concept that is frequently neglected. There has been concern that academic basic science research has not achieved its full potential. Consequently, there has been renewed impetus to support and create translational research partnerships, with significant financial grants tendered by National Institute for Health Research (NIHR) and Medical Research Council (joint strategy), Wellcome Trust and Cancer Research UK.

Co-ordinated research programmes and commitment from Government funded research bodies Endometriosis research has been relatively neglected despite having a major burden of disease worldwide. It is entirely justified that a central worldwide coordinating body of endometriosis research should be created given the importance of the condition. Funding should be at government, European Parliament [69]or international (such as WHO) level, particularly given the expense involved in high throughput molecular technology . In contrast, integrated clinical care and a common research strategy are fundamental components of the successful National Cancer Institute (NCI), the Gynecologic Oncology Group (GOG) and the Specialized Programs of Research Excellence (SPOREs) collaboration in the North American approach to ovarian cancer[77].

Utilising and developing high quality clinical datasets The "gold standard" research methodology that provides the highest level of evidence on interventions is the randomised controlled clinical trial (RCT). However, it is unrealistic to assume the RCT design may be achievable for all disorders. Particularly, as many of the main outcomes of interest are relatively uncommon or tend to occur over several years. Furthermore, randomisation may be considered unethical in certain conditions, such as randomising between planned VBAC and elective caesarean as discussed in chapter 4[78].

The alternative is to utilise a lower level of evidence in the form of the non-randomised cohort study. The optimum components of such a cohort study would be large sample size, long term follow up, standardised definitions for recruiting and assessing outcome and ability to compare against matched non-intervention participants. The Scottish Linked Maternity dataset represents such a cohort dataset based on routinely collected obstetric and neonatal data; the interrogation of the dataset yielded publications [79;80] utilised by the VBAC clinical guideline (**Chapter 4**). However, there is considerable heterogeneity in which disorders are being monitored through such data repositories [81]. Inevitably, most clinical guidelines conclude a need to establish national database registries for important outcomes (such as hypoxic ischaemic encephalopathy by NICE Intrapartum guideline[82]), but there is no agreement on which body should coordinate the data collection.

Fortunately, there appears to be a realisation that such datasets are urgently needed, both by Department of Health (NHS Connecting for Health-NHS Care record service is one of many components[83]) and medical research organisations (e.g. MRC[67]). However, there needs to be stringent quality assurance to ensure that the electronic health record contains accurate data, a concern that has been raised by those establishing the analogous United States National Health Information Infrastructure (NHII) project[84].

Nevertheless, there are immense clinical and biomedical research opportunities by creating such datasets and sharing these amongst interested public and commercial stakeholders, that have thus far, been relatively underplayed by the UK National Programme for Information Technology[85]. It is feasible that given the large sample size within the dataset and utilisation of individual patient data meta-analysis techniques, the clinical results generated are at least as reliable (if not more robust) than RCT derived data, even though the primary data are non-randomised. Furthermore, the exchange of biological clinical samples, linked with patient epidemiological outcome, facilitates translational basic science research.

Caution with over-reliance on meta-analyses: value according to the quality and methodology of the RCTs included Despite adherence to rigorous quality standards for RCTs[86], systematic reviews and meta-analyses may provide misleading results or be of poor quality[87]. Greater bias is likely in meta-analyses that pool underpowered trials, fewer trials, or exhibit marked heterogeneity[86;88;89]. The latter factor was identified by the meta-analysis in Chapter 3 which demonstrated a statistically significant pooled result if the study population were divided into high or low risk of preterm delivery[43]. Methods for assessment of methodological quality by systematic reviews are still in their infancy, but there is likely to be substantial room for improvement[86]. When interpreting meta-analysis, careful consideration should be given to the relevance, quality and robustness of the individual primary study in addition to the methodology used by the systematic review[90].

Ensure that clinical guidelines and their utilisation adds value to clinical practice

There is a concerted development to expand the programme of guideline development though professional medical bodies (such as RCOG) and national policy drivers (principally National Institute of Clinical Excellence, UK) to fulfil the remit of delivering excellence in clinical care. However, clinical guidelines are not necessarily transferable to current clinical practice[91;92]. Guidelines are mainly based on data derived from the "perfect" population and health care resource setting because of the weighting it affords to RCTs. In contrast, the actual clinical population and environment tends to be heterogeneous and "imperfect". Furthermore, such evidence-based health care policy will be practiced in a mainly non-evidence-based health care system.

Importantly, there is a dearth of evidence that has conclusively shown that the introduction of clinical guidelines has actually improved clinical practice[93;94]. This research is vitally important, as it not only justifies continuing with the guideline but also provides feedback to guideline developers to enable improvements to content to maintain guideline validity, applicability and overall effectiveness[95;96]. A national audit represents the best study design for evaluation and feedback of clinical guideline-led practice; examples of such audits are the long-established perinatal and maternal mortality reports produced by CEMACH (Confidential Enquiry into Maternal and Child Health, www.cemach.org.uk). Alternatively, instead of establishing numerous discrete national audits, data on multiple health outcomes could be retrieved and processed from the proposed national electronic linked patient records system[83]. Again, the problem lies in creating a national coordinating audit body that could validate the effectiveness of guidelines, perhaps allied to the professional medical colleges.

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Chapter 2

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Chapter 3.2

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Chapter 4

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