Does ovarian suspension during laparoscopic surgery for endometriosis reduce postoperative adhesions?

A randomised controlled trial

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Thesis submitted for the degree of MD(Res),

University College London

March 2017

Declaration

I, Wee-Liak Hoo, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. I was personally involved in the design of the study, applications for ethical approval, recruitment of participants, ultrasound scan assessment and collection of data. None of the data presented is a part of any other thesis. All patients gave informed consent prior to participation in the study.

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Wee-Liak Hoo

Abstract

In this thesis, I have explored complex pathology of endometriosis, described current management strategies and highlighted the common problem of postoperative pelvic adhesions, often associated with the surgical treatment of this condition. Intra-operative suspension of the ovaries to the anterior abdominal wall is a simple method used to facilitate ovarian retraction during surgery.

We found in an observational (pilot) study that the prevalence of ovarian adhesions for each ovary was 56.3% after laparoscopic surgery for severe pelvic endometriosis. A prospective double-blind cross-over comparison randomised controlled trial (RCT) was completed to assess the effect of temporary ovarian suspension following laparoscopic surgery for severe pelvic endometriosis on the prevalence of postoperative ovarian adhesions. Suitable women were randomised to unilateral ovarian suspension for 36 to 48 hours in the postoperative period. A transvaginal ultrasound scan was performed three months after surgery to assess for the prevalence of ovarian adhesions. Our RCT concluded that there was no significant difference (P = 0.23) in the prevalence of postoperative ovarian adhesions between the suspended (20/52) and unsuspended (27/52) side (38.5 versus 51.9%) [odds ratio 0.56 (95% confidence interval 0.22–1.35)].

Using the ovarian suspension RCT as a basis, I have described the detailed journey of an RCT from its conception, protocol design, pilot study, trial management, analysis to publication of results. The rationale for our study design and methodology was discussed. Statistical considerations were made from the outset, which led to a pilot study. Issues surrounding the implementation of our trial including ethical approval, recruitment, consent,

randomisation and details of data management were outlined. Finally, statistical analysis, conclusions, limitations and suggestions for future research were made.

Current Controlled Trials: ISRCTN24242218

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Acknowledgements

I would like to thank a number of people who supported and helped me throughout this work. First, I would like to thank my MD supervisor Mr Davor Jurkovic for all his patience and guidance with this thesis. He was always available for advice and direction when needed, for this I remain ever grateful. Without his support, this work would never have been achieved.

I am very grateful to the University College London Hospital Endometriosis surgical team, Ertan Saridogan, Alfred Curtner, George Pandis and Andreas Stravroulis for their enthusiasm, help with the patient recruitment, randomisation and carrying out the surgical procedures. Also the Endometriosis nurse specialists, Elsa Palmer and Sarah Parker for their support and help with trial participants monitoring.

I wish to thank Naaila Aslam and Rehan Salim for their clinical support during my time as a research fellow. My special thanks to my research fellow colleagues, Joel Naftalin, Michelle Swer, Amna Jamil, Natalie Nunes, Katie Pateman, Tom Holland and Dimitrios Marvelos for their support and flexibility for allowing me to accommodate my research work within my commitments to the Gynaecology Diagnostic Unit.

I also wish to thank all the staff in the Gynaecology Diagnostic Unit at the University College London Hospital for their assistance with chaperoning and arranging patient follow up.

My special thanks also go to Ms Pauline Roger for her help with our sample size calculation, providing the randomisation schedule and statistical advice.

Finally, I wish to thank my parents, my wife Eve and daughter Aerin for their constant words of encouragement and for always taking an interest in my work.

Abbreviations

AIs	Aromatase inhibitors
ASRM	American Society of Reproductive Medicine
CA-125	Cancer antigen 125
CMC	Carboxymethylcellulose
COCP	Combined Oral Contraception
COX	Cyclooxygenase
CTA	Clinical trial authorisation
CTIMPs	Clinical Trials of Investigational Medicinal Products
DCBE	Double-contrast barium enema
DIE	Deep infiltrating endometriosis
DMC	Data Monitoring Committee
ESHRE	European Society of Human Reproduction and Embryology
GCP	Good clinical practice
GnRH	Gonadotropin-releasing hormone
IQR	Interquartile range
IRAS	Integrated Research Application System
ISRCTN	International Standard Randomised Controlled Trial Number
IUS	Intrauterine system
LNG	Levonogestrel
LR+	Positive likelihood ratio
LR-	Negative likelihood ratio
MHRA	Medicines and Healthcare Products Regulatory Agency
MRI	Magnetic resonance imaging
NHS	National Health Service
NK	Natural killer

NPV	Negative predictive values
NRES	National Research Ethics Service
NSAIDs	Non-steroidal anti-inflammatory drugs
OR	Odds ratios
ORC	Oxidised regenerated cellulose
PEG	Polyethylene glycol
PEO	Polyethylene oxide
POD	Pouch of Douglas
PPV	Positive predictive value
PR-A	Progesterone receptors isoform A
PR-B	Progesterone receptors isoform B
PTFE	Polytetrafluorethylene
RCT	Randomised controlled trial
RECs	Research Ethics Committees
RES	Rectal endoscopic sonography
R&D	Research and Development
SAEs	Serious Adverse Events
SSI	Site-Specific Information
SUSARs	Suspected Unexpected Serious Adverse Reactions
TMG	Trial Management Group
TMF	Trial Master File
TSC	Trial Steering Committee
TVS	Transvaginal ultrasound scan
UCLH	University College London Hospital
USMs	Urgent Safety Measures
17β-HSD-2	17β-hydroxysteroid dehydrogenase type 2

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PART I Background

Chapter 1 – History, Epidemiology and Characterisation

Endometriosis is one of the most common benign gynaecological conditions. It is classically defined as the presence of endometrial glands and stroma in ectopic sites outside the uterus. It is variable in both its clinical and surgical manifestations, often with poor correlation between the two. Despite numerous papers on endometriosis, its aetiology and pathogenesis remain elusive and there appears to be a polygenic and multifactorial pattern of inheritance.

1.1 History of endometriosis

The history of medicine is full of controversies and certainly the origin of endometriosis is confounded by the fact that for some time, endometriosis and adenomyosis were considered to be the same condition – 'adenomyoma'. It was not until the mid-1920s that the two conditions were finally separated.

Knapp[1] who performed a historical review of endometriosis, believed that the first descriptions could be found in theses and dissertations published from as early as 1690. Daniel Shroen, a German physician, described in his book, *Disputatio Inauguralis Medica de Ulceribus Ulceri,* ulcers that in their primary form were distributed throughout the 'stomach' (the peritoneum) and were located prominently in the bladder, the intestines, the broad ligament and the outside of the uterus and cervix.

Carl Rokitansky, a German pathologist, in 1860 was the first to provide a detailed pathological description of endometriosis[2]. Rokitansky identified the presence of heterotopic endometrial tissue as three different phenotypes: myometrial, endometrial cavity (a polyp) and ovarian[3]. However, he considered these phenotypes neoplastic and labelled them as 'sarcomas'. Breus used the term "chocolate cyst" for the first time in 1894.

In 1896, Thomas Cullen described for the first time, the morphology and clinical appearance of endometriosis. He described ‘adenomyoma’ of the round ligaments, which he asserted was tissue of Mullerian origin[4]. Further ideas on the pathogenesis were put forward and developed during the early 20th century.

It is perhaps customary to describe John A. Sampson as the originator of endometriosis[5]. In 1927, he formulated a new concept in the article titled “Peritoneal Endometriosis due to the Menstrual Dissemination of Endometrial Tissue into the Peritoneal Cavity[6].” His description of peritoneal endometrium and ovarian endometrioma provided the first theory on the pathogenesis of the disease[6,7]. The hypotheses for the origin of endometriosis from this article dominated the criteria and the scientific literature on endometriosis for the next 80 years.

1.2 Epidemiology

The prevalence of endometriosis in the general population is unknown and varies according to the population studied. It can affect about 6%–10% of women in the reproductive age[8] and has a prevalence rate as high as 35%–50% in women experiencing pain or infertility[9,10].

The incidence of endometriosis is increased with uterine anomalies resulting in obstruction of menstrual outflow, in-utero exposure to diethylstilbestrol, a low birth weight, in women with family history of endometriosis and those with naturally red hair[11–13]. Endometriosis is also associated with prolonged exposure to endogenous oestrogen (early menarche, late menopause or obesity), consumption of red meat and unsaturated fats. Whereas, prolonged lactation, multiple pregnancies and eating fruits, green vegetables, and n−3 long-chain fatty acids are protective[14]. Endometriosis is also associated with an

increased risks of autoimmune diseases and ovarian endometrioid or clear-cell cancers[15].

1.3 Characterisation

Although considered a progressive disease, endometriosis can remain static and even regress without treatment[16]. The extent of endometriosis varies, but three well recognised forms of endometriosis have been described[17]:

1. Peritoneal endometriosis corresponds to minimal or mild endometriosis, has multiple appearances. The classic lesion has a puckered, blue–black powder-burn appearance. Early lesions however, may appear as papular, vesicular or glandular, haemorrhagic or flame-like lesions. Neo-angiogenesis and adhesion formation are typical features of the early active implant. Red lesions are believed to be early and very active lesions, black lesions as advanced and active lesions, whilst white lesions are healed or inactive lesions[18].
2. Ovarian endometriosis is characterized superficial ovarian implants or endometriotic cysts (endometriomas). These are often found adherent to the posterior aspects of the broad ligament. Most endometriomas are pseudocysts formed by invagination of the ovarian cortex, which is sealed off by adhesions. The site of invagination is characterized by fibrosis, retraction of the cortex, islands of glandular endometriotic tissue and organized blood clots. The pseudocysts are completely or partially lined by a thin endometrial-like mucosa consisting of a surface epithelium and highly vascularized stroma[19]. Endometriomas are more commonly found on the left ovary[20].
3. Deep infiltrating endometriosis (DIE) is predominantly glandular and stromal tissue surrounded by hyperplastic smooth muscle cells. DIE

nodules extend >5 mm beneath the peritoneum and typically involve the uterosacral ligaments, vagina, bowel, bladder or ureters. The depth of infiltration is related to the severity of symptoms[21–23]. The inflammatory reaction causes overgrowth and retraction simulating a malignant invasion, however endometriotic infiltrations are not destructive, does not invade fat tissue and does not breach the basal membrane of the bowel[19].

1.4 Location

Endometriotic implants have been found almost anywhere in the female body. More commonly, they occur on the pelvic peritoneum, the ovaries, pouch of Douglas (POD), uterosacral ligament, uterovesical pouch, serosal surface of the uterus, fallopian tubes and round ligament. Endometriosis has also been found in the perineum, along episiotomies scar or in Bartholin glands. Occasionally, the implants can be found at more distant sites, including lung, liver, pleura and pericardium with consequent variations in presenting symptoms.

Chapter 2 – Pathophysiology

The exact aetiology and pathogenesis of endometriosis remains unclear and its variable morphology appears to represent a continuum of individual presentations and progressions. Many theories have been postulated but a unifying theory regarding the origin of endometriosis remains elusive.

2.1 Endometriosis Theories

1. **Vascular and lymphatic metastasis (Halban's theory)**

   The theory of benign metastasis suggests that ectopic endometrial implants occur via vascular or lymphatic spread of viable endometrial cells[24,25]. This theory could explain the rare occurrences of endometriotic lesions found in extra pelvic sites such as the brain, bone and lungs[17].

2. **Coelomic metaplasia (Meyer's theory) and induction theory**

   Coelomic metaplasia is based on the fact that cells from the peritoneum, the ovarian surface and endometrium arise from a common embryological precursor, the coelomic cell. It assumes that a transformation occurs of normal peritoneal tissue into ectopic endometrial tissue[26,27].

   A closely related induction theory holds that an endogenous inductive stimulus, such as a hormonal or immunologic factor, promotes the differentiation of cells in the peritoneal lining to endometrial cells[26].

   Agents responsible for such transformation remain poorly defined and investigators have not been able to show that peritoneal cells can be differentiated experimentally into endometrial cell types.

3. **Müllerianosis**

   The theory of embryonic Müllerian rests or müllerianosis, proposes that residual cells from the embryologic Müllerian duct migration maintain the capacity to develop into endometriotic lesions under the influence of

oestrogen[28]. This theory find support in epidemiological studies reporting a twofold-increase in the risk of developing endometriosis in women exposed to diethylstilbestrol in utero[11].

4. **Retrograde menstruation and implantation theory (Sampson's theory)**

   Sampson's theory from the 1920s on retrograde or reflux menstruation model has been the most widely accepted theory explaining the development of endometriosis and is supported by multiple lines of evidence[6]. This theory suggests that the origin of endometriosis is a consequence of the reflux of endometrial fragments through the fallopian tubes during menstruation, with subsequent implantation and growth on or into the peritoneum and ovary.

   Sampson based his theory on observations of menstrual blood exiting the tubal ostea in menstruating women during pelvic surgery. Reflux menstruation occurs in up to 90% of women with patent fallopian tubes undergoing laparoscopy during menstruation[29]. Further support for this theory can be found in cases of uterine anomalies where outflow obstruction increases the prevalence of endometriosis[13]. A higher prevalence of endometriosis is also seen in cases of compromised antegrade menstruation such as septate uteri and cervical stenosis[30,31]. Menstruations are often longer and heavier in women with endometriosis[32]. The anatomical distribution of endometriotic lesions also supports the retrograde menstruation theory with the tendency of lesions to implant in the pouch of Douglas, the most dependent portion of the peritoneal cavity[33].

Overall, endometriosis is likely the result of a complex interplay of endometrial tissue, the peritoneal environment and the peritoneal lining. When the peritoneal environment cannot remove endometrial tissue in time, the

endometrial tissue will adhere to the peritoneal lining. The innate capacity of the endometrial tissue to invade and acquire a blood supply contributes to the implantation process. After implantation, local production of oestrogens as a result of the expression of aromatase provides a local, continuous, ovary-independent growth stimulus. Changes in the physiology of the endometrium, increasing amounts of retrograde menstruation and/or changes in the contents of the peritoneal fluid, can disturb the defence mechanism against the endometrial tissue and further promote implantation. If endometrial tissue is to implant in the peritoneum, it must be able to adhere to the peritoneal surface, invade the basement membrane and extracellular matrix, acquire a blood supply and survive.

Immunological and inflammatory factors are likely to contribute to the progression from retrograde menstruation to endometriosis. Two theories have been suggested, 1) an intrinsic anomaly of eutopic endometrium that develops resistance from elimination by peritoneal immune cells and 2) a consequence of an altered function of peritoneal macrophages and natural killer (NK) cells that are unable to eliminate the endometriotic implants[34]. The relationship between the two theories is not clear, although they are likely to be interdependent. The peritoneal environment may induce alterations in the ectopic endometrial tissue in those with a genetic predisposition, thus facilitating implantation and invasion. However, an excess of refluxed endometrium may induce a pro-inflammatory and hormonal environment that produces endometrial changes and favours the metaplasia of coelomic epithelium, which is already altered by peritoneal inflammation. Some molecular alterations described in endometriosis are related to disorders of angiogenesis and dysregulation in the apoptosis of immune and ectopic endometrial cells. The serum and peritoneal fluid of infertile

women with endometriosis appears to have higher levels of IL-6, IL-8, and NK cells[35].

Peritoneal endometriotic lesions occur so frequently that they are sometimes considered a physiological and temporal process[34]. Second-look laparoscopy has revealed that spontaneous resolution of peritoneal endometriosis occurred in 42% of affected patients[36]. Hormonal treatments often result in a significant reduction of peritoneal endometriotic lesions, although they subsequently reappeared a few months after the menstrual cycle resumed[37]. Some peritoneal endometriotic lesions progress to mature “black” lesions or white scar lesions[17]. Other implants grow and develop into dense adhesions, endometriomas and DIE. One explanation is that endometriosis is a heterogeneous condition with peritoneal, deep infiltrating and ovarian implants being manifestations of different disease processes[17]. They proposed retrograde menstruation for peritoneal endometriosis, müllerianosis for rectovaginal endometriotic nodules and metaplasia for ovarian endometriotic lesions. Intrinsic to these theories are stimulating factors and genetic susceptibilities whose roles are only now beginning to be delineated, although they are insufficiently established. The developmental timing of action of these factors and their roles in influencing other systems that predispose to endometriosis (endocrine, immune, stem/progenitor cells, epigenetic modifications) must be considered in the context of genetic background[38].

The immunological response triggered to eliminate these implants, could have detrimental effects on fertility. Individuals with genetic predisposition and immunotolerance to endometrial antigens (decreased NK activity and T-cell anergy) could lead to the progression of endometriosis. This progression presents with infiltrating nodular and cystic lesions with characteristic clinical

manifestations with advancing disease. Infertility may be caused by mechanical factors, such as adhesions, tubal distortion, or altered oocyte quality[39,40]. In immunocompetent women, the disease does not progress and a temporary infertility similar to that seen in women with unexplained subclinical infertility may occur.

Tariverdian et al.[41] proposed the concept of endometrial dissemination as a result of neuroendocrine-immune disequilibrium in response to stress caused by cardinal clinical symptoms of endometriosis. This induces a vicious cycle of peritoneal inflammation, angiogenesis resulting in pain and infertility.

The role of steroid hormones in the progression of endometriosis has been highlighted. Normal eutopic endometrium expresses the isoforms A (PR-A) and B (PR-B) of progesterone receptors; in the secretory phase, progesterone indirectly induces the 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD-2), which converts oestradiol to oestrone, leading to the apoptosis of endometrial cells. In ectopic endometrium, low levels of PR-A, no PR-B, and no 17β-HSD-2 are detectable. As a consequence, oestradiol accumulates and likely induces the proliferation of endometrial tissue. Moreover, the enzyme aromatase that is present in ectopic tissue creates oestrone, which is further converted to oestradiol by 17β-HSD type 1, thus contributing to the accumulation of oestradiol. Ectopic endometrium has also been found to express oestrogen receptors, progesterone receptors and occasionally P450 aromatase[42,43]. This enzymatic activity results in the conversion of circulating androstenedione into oestrone in tissue, which is an oestrogen that further promotes the growth of endometriotic implants[44,45]. This explains the use of aromatase inhibitors in combination with Gonadotropin-releasing hormone (GnRH) analogues or oral contraceptives for the treatment of endometriosis[46,47].

A genetic predisposition for endometriosis was illustrated by Simpson et al.[48] who suggested a polygenic or multifactorial inheritance. They reported a 5 – 8% observed risk of endometriosis in first-degree relatives with endometriosis. Severe endometriosis was also most likely when a first-degree relative is affected (61% vs. 24%). Studies found genetic associations with endometriosis for single-nucleotide polymorphisms at different chromosome loci in Caucasian and Japanese populations[49,50]. Genes located in the 1p36 region and 7p15.2 have been associated with endometriosis[51,52].

There is also increasing concern about chemical pollutants mimicking hormonal function, so-called endocrine-disrupting compounds[53]. A substantial number of environmental pollutants, such as polychlorinated biphenyls, dioxins, polycyclic aromatic hydrocarbons, phthalates, bisphenol A, pesticides, alkylphenols, and heavy metals have been shown to disrupt endocrine function[54–57]. Their structural similarity to endogenous hormones causes them to interact with hormone transport proteins or potentially disrupt hormone metabolic pathways, leading to development of endometriosis.

2.2 Mechanisms of Pain in Endometriosis

The cause of endometriosis-associated pain is unknown. It has been suggested that peritoneal inflammation as a result of growth factors and cytokines production by activated macrophages, adhesion formation may be responsible[58]. The active bleeding or direct invasion of pelvic nerves by endometriotic implants has also been suggested[23]. The neural irritation or invasion hypothesis is increasingly popular. Tender nodularity in the pouch of Douglas and uterosacral ligaments has 85% sensitivity and 50% specificity for the diagnosis of DIE[59]. The intensity of pain associated with infiltrative disease has been correlated with the depth of penetration of the lesion and the most

severe pain is seen when the disease extends 6 mm or more below the peritoneal surface[58]. These women are more likely to have deep dyspareunia, severe dysmenorrhea and dyschezia (painful or difficulty in defecating).

Chapter 3 – Signs, Symptoms, Diagnosis and Staging

3.1 Signs & Symptoms of Endometriosis

Endometriosis is frequently associated with dysmenorrhoea, chronic pelvic pain, deep dyspareunia, dyschezia and infertility. The intensity of symptom will range from mild to severe, but the relationship between pain intensity and endometriosis severity is not clear. Ballard et al.[60] found that 83% of women with endometriosis reported one or more of these symptoms when compared with just 29% of controls. Dyschezia during menstruation and deep dyspareunia have been found to be stronger predictors of DIE (sensitivity 74.5%, specificity 68.7%, positive likelihood ratio (LR+) of 2.4 and negative likelihood ratio (LR-) of 0.4)[61]. There is often no clear relationship between the staging of disease and symptom severity, although, many studies have reported an increase in pain symptoms with increasing depth of DIE[62,63].

Pelvic pain can occur unpredictably throughout the menstrual cycle or continuously. It is often described as a dull, throbbing or sharp pain exacerbated by physical activity[16]. Bladder and bowel endometriosis symptoms are typically cyclical.

Despite the growing awareness of endometriosis symptoms, there are often significant delays between the onset of symptoms to definitive diagnosis, with a mean latency of 6.7 years reported in Norway, 8 years in the United Kingdom and 11.7 year in the USA[64]. One explanation for this delay is the significant overlap in symptoms with other conditions such as pelvic inflammatory disease, pelvic adhesions, irritable bowel syndrome, inflammatory bowel disease, interstitial cystitis and depression. Indeed, when the records of general practitioners were reviewed, women with endometriosis were 3.5 times more likely to have had a diagnosis of irritable bowel syndrome and 6.4 times more

likely to have a diagnosis of pelvic inflammatory disease when compared with women without endometriosis[65]. Other than misdiagnosis, factors contributing to a diagnostic delay include the use of contraception causing hormonal suppression of symptoms, stigmata towards menstruation resulting in normalisation of symptoms, higher BMI and healthcare funding[66,67].

3.2 Diagnosis

The diagnosis of endometriosis is first suspected based on the history, then substantiated by physical examination and imaging techniques and is finally confirmed by histological examination of specimens collected during laparoscopy.

When considering laparoscopy to diagnose and treat endometriosis, a balance must be found between the need to avoid the very long diagnostic delays currently experienced, the likelihood of treatment benefits against the cost and risk of laparoscopy. It remains questionable whether the early detection and staging of endometriosis is necessary. As previously discussed, early progression and regression is unpredictable. The reality is that there is currently no cure for endometriosis and it is difficult to justify a diagnostic laparoscopy for minimal or mild endometriosis only. Moreover, even if peritoneal disease is found it might not be the cause of pain. One could argue that empirical treatment can be started without a definitive diagnosis[68]. This may be appropriate in young adolescents or in women that decide not to have a laparoscopy. If medical treatment relieves pain, many women may not be interested in whether or not their pain symptoms were due to peritoneal endometriosis[69].

3.2.1 Clinical Examination

Routine vaginal examination alone is often insufficient to make a diagnosis of endometriosis[70]. However, DIE or nodules can sometimes be visualised or palpated in the posterior fornix of the vagina wall[71]. These appear as dark blue lesions, which are often dense, painful and can increase in size during menstruation[69,71].

3.2.2 Laparoscopy

A definitive diagnosis usually requires the visual inspection of the pelvis at laparoscopy. Diagnosis is ideally accompanied by histological confirmation of both typical and atypical lesions, but a negative histology does not exclude the diagnosis[68]. Although histopathology is not routinely required, selected biopsies are recommended for atypical lesions to exclude malignancy and differentiate from other benign lesions including haemangioma, epithelial inclusions, foreign body reaction (contrast or suture material), inflammatory cystic inclusions, schistosomiasis (gelatinous deposits), Psammoma body reaction, adrenal rest, Walthard's cell rest, ovarian cancer, splenosis, endosalpingiosis, ectopic pregnancy and secondary trophoblast implantation[18].

The indication for laparoscopy needs to be individualised and should include the woman’s choice to have a definitive diagnosis, infertility and/or symptoms and signs of severe disease, such as ovarian endometriomas or DIE or on-going pain symptoms.

A negative diagnostic laparoscopy, where no endometriosis is identified, seems to be highly accurate for excluding endometriosis and is therefore useful to a clinician in making management decisions[72]. However, the experience, skill and knowledge of the surgeon determine whether endometriosis will be diagnosed if

present. DIE and vaginal endometriosis can be easily missed if the patient has not been thoroughly examined, preferably under anaesthesia.

A good quality laparoscopy should include systematic examination of 1) the uterus and adnexa, 2) the peritoneum of ovarian fossae, vesico-uterine fold, pouch of Douglas and pararectal spaces, 3) the rectum and sigmoid, 4) the appendix and caecum and 5) the diaphragm. Under general anaesthesia, there should also be a speculum examination and palpation of the vagina and cervix, to check for 'buried' nodules.

3.2.3 Biomarkers

A diagnostic test without the need for surgery would reduce associated surgical risks and increase accessibility to a diagnostic test. Considerable effort has been invested in searching for less-invasive techniques to diagnose endometriosis. A biomarker is a measurable “biologic marker” that correlates with a specific outcome or state of the disease[73]. Cancer antigen 125 (CA-125), cytokines and angiogenic growth factors all show altered levels in the peripheral blood of women with endometriosis when compared to controls, however their potential as a diagnostic measure for endometriosis, either alone or in combination, has been disappointing[74–77].

Evidence suggesting a significant difference between the eutopic endometrium from women with and without endometriosis has led to proteomic studies searching for a diagnostic test based on the analysis of an endometrial biopsy[78,79].

Circulating microRNAs are also being evaluated as a novel biomarker. As a multifactorial and polygenic disease, the dysregulation of miRNA expression has been implicated in endometriosis[80]. MicroRNAs are a class of regulatory molecules with the ability to control gene expression at the post-transcriptional

level through degradation, repression, and silencing, which could be used as biomarkers or therapeutic tools in endometriosis[81].

3.2.4 Imaging

A non-invasive, preoperative diagnosis of endometriosis has been made possible by recent advances in imaging modalities such as ultrasound and magnetic resonance imaging (MRI)[82].

With regards to endometriomas, transvaginal ultrasound (TVS), with or without the use of Doppler, has been shown to be a highly sensitive tool and is far superior to routine clinical examination alone. Moore et al.[83] concluded in a systematic review that TVS imaging of ovarian endometriomas had a diagnostic sensitivity of 64 to 89%, specificity 89 to 100%, LR+ 7.6 to 29.8 and LR− of 0.1 to 0.4.

Currently, it is not possible to detect peritoneal endometriosis on TVS. MRI was found to only have a sensitivity of 69%, specificity of 75%, LR+ 2.76, and LR− 0.41[84]. Unfortunately, these LRs are too low to justify the routine use of MRI to diagnose peritoneal disease.

Physical examination has limited value for assessing the extent of DIE[85]. Recent studies have shown TVS, rectal endoscopic sonography (RES) and MRI to be useful in the diagnosis of non-ovarian endometriosis, such as uterosacral ligament, rectosigmoid colon, rectovaginal space and the pouch of Douglas. Bazot et al.[71] found that MRI was more accurate than TVS or RES in the diagnosis of uterosacral ligament and vaginal endometriosis, although, TVS appeared to be most accurate in diagnosing bowel endometriosis. The sensitivity, LR+ and LR- values of MRI, TVS and RES were respectively, 84.4%, 7.59 and 0.18, and 78.3%, 2.34 and 0.32, and 48.2%, 0.86 and 1.16 for uterosacral ligament endometriosis; 80%, 5.51 and 0.23, 46.7%, 9.64 and 0.56,

6.7%, - and 0.93 for vaginal endometriosis, and 87.3%, 12.66 and 0.14, 93.6%, - and 0.06, 88.9%, 12.89 and 0.12. In a systematic review of the diagnostic accuracy of TVS for diagnosing DIE with bowel involvement, Hudelist et al.[86] reported a sensitivity of 91%, specificity 98%, LR+ 30.36, LR− 0.09, PPV 98% and NPV 95%.

It has been suggested that a limitation of TVS is its inability to determine the exact distance of the rectal lesions from the anal margin or to evaluate the depth of rectal wall involvement. RES has been suggested in these cases where colorectal involvement is suspected if necessary[87].

TVS is easily accessible, cost and time effective. It is not surprising that it has become the first-line investigation in the diagnosis of endometriosis. However, it should be noted that this level of ultrasonography is operator dependent and requires a very experienced operator. In the clinical setting, excluding DIE is of critical importance since extensive bowel involvement warrants an interdisciplinary approach and may necessitate a referral to a tertiary centre. Accurate preoperative staging could facilitate more effective triaging of women for a more appropriate level of surgical care. Zanardi et al.[88] found a high concordance between MRI and laparoscopy for staging of pelvic endometriosis according to the revised American Society of Reproductive Medicine (ASRM) classification (kappa = 0.913). However, they also found MRI to have a suboptimal depiction of adhesions and complete obliteration of the pouch of Douglas. Holland et al.[89] showed that TVS was a useful test for assessing the severity of pelvic endometriosis and was particularly accurate in detecting severe disease, sensitivity 0.85, specificity 0.98 and LR+ was 43.5, and LR− was 0.15.

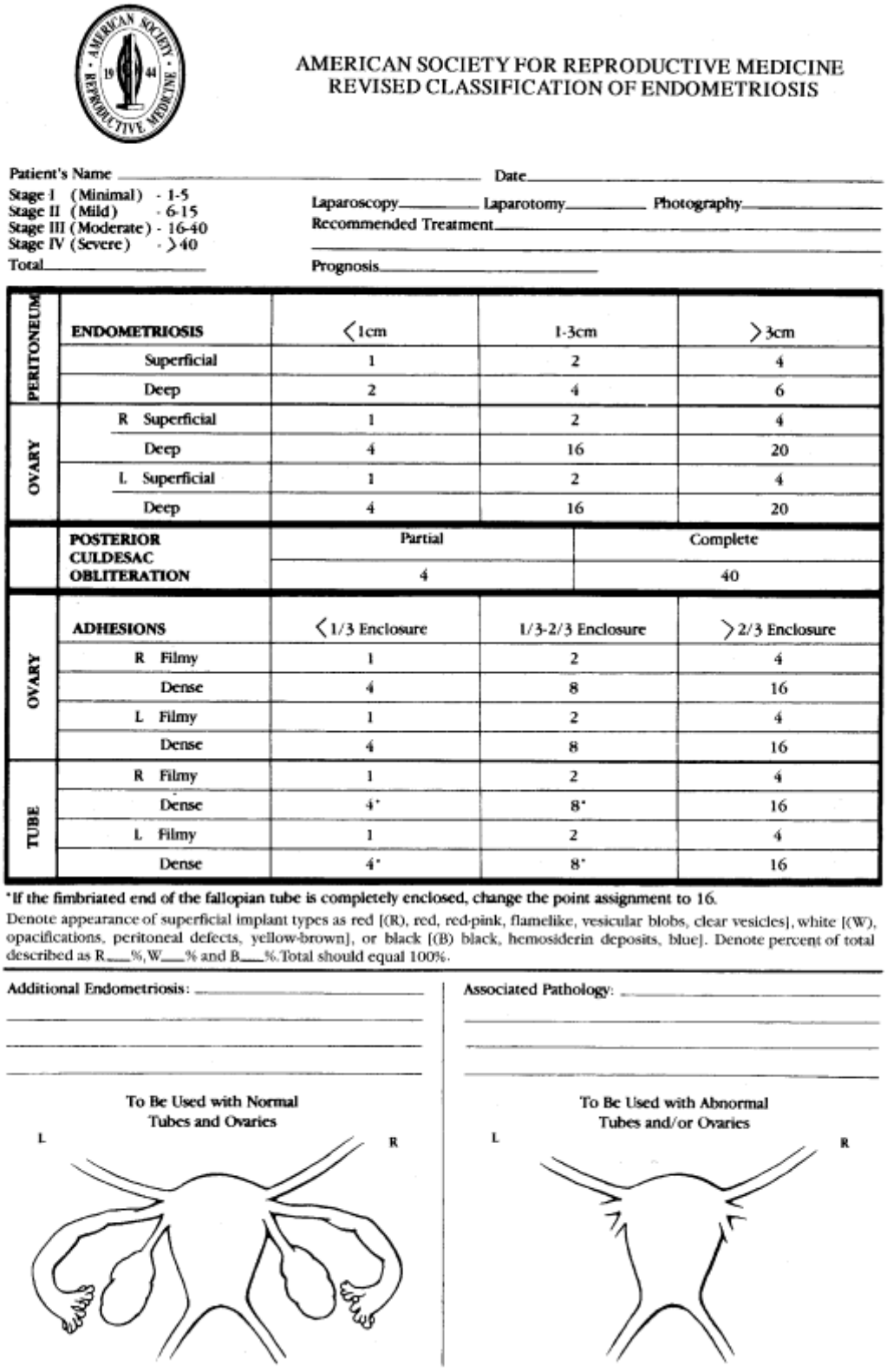
3.3 Staging of Endometriosis

One of the major challenges of making a diagnosis in women with suspected endometriosis is to assess the extent of the disease and its functional consequences for the pelvic or extra-pelvic organs. Although several classification systems have been suggested for endometriosis[90,91], the most widely used staging system for the extent of endometriosis is the revised ASRM classification[92] (Figure 1 and 2).

The ASRM classification system was established to predict fertility outcomes and determine disease burden and management. The extent of endometriosis is graded from stage I, indicating minimal disease, to stage IV, indicating severe disease on the basis of the type, location, appearance, size and depth of peritoneal or ovarian implants and adhesions visualised during laparoscopy and allow uniform documentation of the extent of disease. However, the staging of the disease does not correlate well with the severity of pain symptoms or response to therapies or future infertility or predict disease progression[93,94]. There was also limited reproducibility noted with the ASRM classification system, with the greatest inconsistency noted for endometriosis of the ovary and pouch of Douglas obliteration[93]. An awareness of the subtle appearances of peritoneal endometriosis also increases the likelihood of observational error, which affects staging[95]. Other factors, which can affect staging of endometriosis, include spontaneous or hormone-induced amenorrhoea, previous pelvic surgery and pelvic inflammatory disease.

Other classifications of endometriosis have been suggested, but there is currently no validated system that meets clinical needs[96].

Figure 1 The revised American Society of Reproductive Medicine (ASRM) classification of endometriosis[92]



AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE
REVISED CLASSIFICATION OF ENDOMETRIOSIS

Patient's Name _____ Date _____

Stage I (Minimal) - 1-5
Stage II (Mild) - 6-15
Stage III (Moderate) - 16-40
Stage IV (Severe) - >40
Total _____

Laparoscopy _____ Laparotomy _____ Photography _____
Recommended Treatment _____
_____
Prognosis _____

PERITONEUM	ENDOMETRIOSIS	<1cm	1-3cm	>3cm
	Superficial	1	2	4
	Deep	2	4	6
OVARY	R Superficial	1	2	4
	Deep	4	16	20
	L Superficial	1	2	4
	Deep	4	16	20

	POSTERIOR CULDESAC OBLITERATION	Partial	Complete
		4	40

OVARY	ADHESIONS	<1/3 Enclosure	1/3-2/3 Enclosure	>2/3 Enclosure
	R Filmy	1	2	4
	Dense	4	8	16
	L Filmy	1	2	4
	Dense	4	8	16
TUBE	R Filmy	1	2	4
	Dense	4*	8*	16
	L Filmy	1	2	4
	Dense	4*	8*	16

*If the fimbriated end of the fallopian tube is completely enclosed, change the point assignment to 16.

Denote appearance of superficial implant types as red [(R), red, red-pink, flamelike, vesicular blobs, clear vesicles], white [(W), opacifications, peritoneal defects, yellow-brown], or black [(B) black, hemosiderin deposits, blue]. Denote percent of total described as R___%, W___% and B___%. Total should equal 100%.

Additional Endometriosis: _____

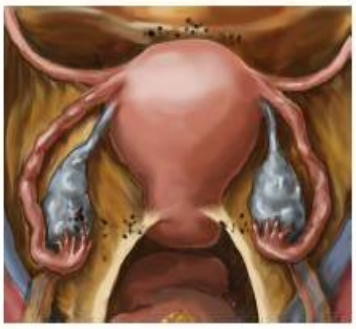
Associated Pathology: _____

To Be Used with Normal Tubes and Ovaries

To Be Used with Abnormal Tubes and/or Ovaries

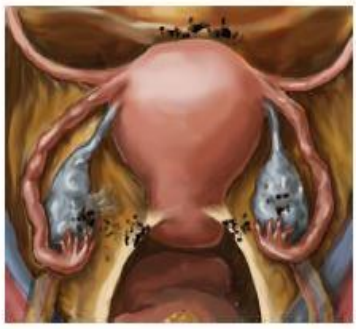
Figure 2 Examples of ASRM classification of endometriosis disease[97]

STAGE I (MINIMAL)



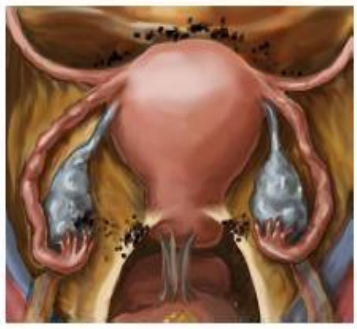
PERITONEUM	
Superficial Endo — 1–3cm	-2
L. OVARY	
Superficial Endo — <1cm	-1
Filmy Adhesions — <1/3	-1
TOTAL POINTS	4

STAGE II (MILD)



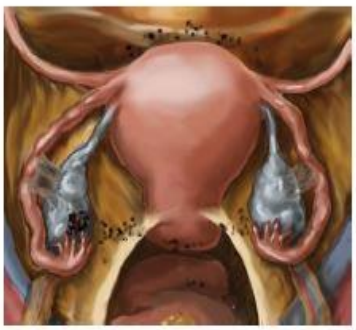
PERITONEUM	
Deep Endo — >3cm	-6
L. OVARY	
Superficial Endo — <1cm	-1
Filmy Adhesions — <1/3	-1
R. OVARY	
Superficial Endo — <1cm	-1
TOTAL POINTS	9

STAGE III (MODERATE)



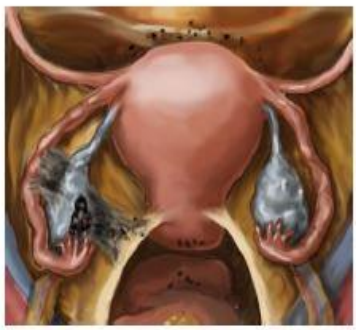
PERITONEUM	
Deep Endo — >3cm	-6
CULDESAC	
Partial Obliteration	-4
L. OVARY	
Deep Endo — 1–3cm	-16
TOTAL POINTS	26

STAGE III (MODERATE)



PERITONEUM	
Superficial Endo — >3cm	-3
L. TUBE	
Dense Adhesions — <1/3	-16*
L. OVARY	
Deep Endo — <1cm	-4
Dense Adhesions — <1/3	-4
R. TUBE	
Filmy Adhesions — <1/3	-1
R. OVARY	
Filmy Adhesions — <1/3	-1
TOTAL POINTS	29

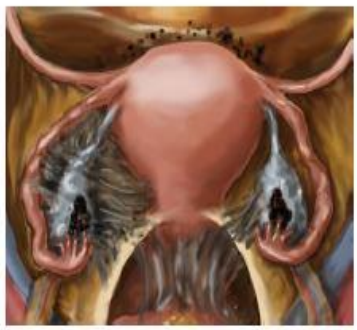
STAGE IV (SEVERE)



PERITONEUM	
Superficial Endo — >3cm	-3
L. OVARY	
Deep Endo — 1–3cm	-32**
Dense Adhesions — <1/3	-8**
L. TUBE	
Dense Adhesions — <1/3	-8**
TOTAL POINTS	51

*Point assignment changed to 16
**Point assignment doubled

STAGE IV (SEVERE)



PERITONEUM	
Deep Endo — >3cm	-6
CULDESAC	
Complete Obliteration	-40
R. OVARY	
Deep Endo — 1–3cm	-16
Dense Adhesions — >1/3cm	-4
L. TUBE	
Dense Adhesions — >2/3cm	-16
L. OVARY	
Deep Endo — 1–3cm	-16
Dense Adhesions — >2/3cm	-16
TOTAL POINTS	114

Chapter 4 – Medical Therapies

4.1 Introduction

The precise pathogenesis of endometriosis remains unclear and it is assumed that these deposits of ectopic endometrium are responsible for the symptoms of endometriosis. Conventional treatments have therefore been directed at the removal of all ectopic tissue. Medical therapies induce atrophy within the hormonally dependent ectopic endometrium so that they shrink in size and number, whilst surgical treatments achieve this by destroying or removing the implant. It is important to note that there is no correlation between laparoscopic findings with symptoms or fertility prognosis or recurrence rate and that the response to hormonal therapy has not been shown to always predict the presence or absence of endometriosis[98,99].

Current approaches for managing endometriosis are aimed mainly at treating symptoms of pain and infertility, while targeting disease progression and preventing recurrence. Treatment must be individualized, taking the clinical problem in its entirety into account, including the age, impact of the disease and the effect of its treatment on quality of life and plans for fertility. Long-term treatment of patients with chronic pelvic pain associated with endometriosis involves repeated courses of medical therapy, surgical therapy, or both. In such circumstances, a multi-disciplinary approach involving a pain clinic and counselling should be considered early in the treatment plan.

4.2 Analgesics

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used as a first line treatment of endometriosis-associated pain even though very limited evidence supports their use. Studies showing elevated prostaglandin levels in peritoneal fluid and endometriotic tissue in women with endometriosis have supported

their use[100]. A Cochrane review on the role of NSAIDs in treating endometriosis related pain analysed one randomised controlled trial (RCT) comparing naproxen sodium (275mg, four times daily) with placebo but found no significance difference[101,102]. Another study that investigated the use of cyclooxygenase (COX)-2 inhibitor (rofecoxib) against a placebo (n=28) reported significant improvement of dysmenorrhea, dyspareunia and chronic pelvic pain in the treatment group[103]. Although, no side effects were reported in this study, rofecoxib has since been withdrawn by its manufacturer Merck because of its cardiovascular toxicity[104]. There were no RCTs found on the use of analgesics (paracetamol, aspirin, ibuprofen, opioids) for treating endometriosis-associated pain[68].

From a clinical perspective, the use of NSAIDs for the management of pain should be discussed with the side effects associated with frequent use of NSAIDs, including inhibition of ovulation, risk of gastric ulceration and cardiovascular disease[105,106].

4.3 Empirical Hormonal Treatment

The European Society of Human Reproduction and Embryology (ESHRE) has suggested that empirical treatment should be started without a definitive diagnosis[69]. This is due to the invasiveness and cost of laparoscopic procedures, but also complimented by the ease of prescribing hormonal contraceptives, which suppress the menstrual cycle and improve pain symptoms, in addition to its contraceptive benefits. This may be more appropriate in young adolescents or in women that decide not to have a laparoscopy. If medical treatment relieves pain, many women may not be interested in whether or not their pain symptoms were due to peritoneal endometriosis[107]. Laparoscopy can then be performed if patients do not

respond favourably to the medical or hormonal treatments, so that endometriosis can be diagnosed and treated or excluded.

4.4 Hormonal therapies for treatment of endometriosis related pains

Endometriosis is a disease of women in their reproductive years associated with cyclical ovarian activity. Therefore, hormonal suppression is considered as a medical approach to treat the disease and its symptoms. Medical treatments theoretically have the ability to treat those implants not visible to the naked eye. Currently, combined oral contraceptives, progestogens, anti-progestogens, GnRH agonists and aromatase inhibitors are in clinical use. There are insufficient data to support the use of selective oestrogen receptor modulators and selective progesterone receptor modulators[68]. Hormonal treatments are also associated with varying side effects and there is a lack of evidence supporting the use of one hormonal treatment over another. Prescribing will therefore depend on the patient's choice and treatment cost.

4.4.1 Combined Oral Contraception (COCP)

Modern low dose COCP is widely used as the first line treatment for patients with presumed endometriosis as it offers many practical advantages, including contraceptive protection and cycle control[108]. COCP has also been observed to reduce menstrual flow and decidualisation of endometriotic implants with decreased cell proliferation and increased apoptosis[109].

Although current guidelines suggest empirical treatment with analgesia, COCP or progestogens, there remains very limited evidence for the efficacy of COCP in treating endometriosis pain[108]. A Cochrane systematic review evaluating the efficacy of COCPs found one small RCT and concluded that COCP (0.02mg ethinyl estradiol with 0.15mg desogestrel daily, taken cyclically) was as effective as GnRH analogues for the relief of dyspareunia, dysmenorrhoea and non-

menstrual pain[108]. Changing from cyclical to continuous treatment may improve symptoms, however, 14% of women on continuous COCP reported moderate to severe side effects[110].

4.4.2 Progestogens and Anti-progestogens

Clinical observation of the apparent resolution of symptoms of endometriosis during pregnancy gave rise to treatment with medication containing a progestogen[111]. A recent Cochrane review concluded that medroxyprogesterone acetate (100 mg daily) was significantly more effective at reducing all symptoms when compared with placebo, however its use was associated with significantly more cases of acne and oedema[112]. There was no evidence of a benefit with depot or oral progestogens over other treatments (COCP or leuprolide acetate) for endometriosis related symptoms, however, progestogens are associated with a better side effects profile than COCP[112].

The levonogestrel-releasing intrauterine system (IUS) releases levonorgestrel (LNG) directly into the uterine cavity at a relatively constant rate of 20 microgram per day for 5 years[113]. LNG exerts strong local progestational activity and renders the endometrium atrophic and inactive, although ovulation is usually not suppressed[114]. RCTs on LNG-IUS showed that it significantly improved endometriosis related pain but this was not significantly different when compared with leuprolide acetate, a GnRH agonist[115,116]. However, LNG-IUS has a significantly better side-effects profile than GnRH.

Danazol has also been shown to reduce endometriosis-associated pain, back pain and dyschezia when compared to placebo[117]. However, significant side effects including acne, non-reversible voice change, oedema, vaginal spotting, weight gain and muscle cramps, greatly reduced its usefulness[68,118].

Anti-progestogen exerts anti-proliferative effects on the endometrium whilst maintaining serum oestradiol levels in the early to mid-follicular phase range thereby offering a better side effect profile by avoiding the bone mass loss and hypoestrogenism associated with progestogen only use[112]. However, in the recent Cochrane review, gestrinone was the only anti-progestogen evaluated in a RCT for endometriosis related symptoms and there was no evidence of significant benefit when compared to danazol or leuprorelin (a GnRH analogue)[112].

4.4.3 GnRH agonists

GnRH agonists effectively deplete the pituitary of endogenous gonadotropins and inhibit further synthesis, thus inducing a hypoestrogenic state resulting in the interruption of the menstrual cycle, endometrial atrophy and amenorrhoea. A systematic Cochrane review of 41 RCTs concluded that GnRH agonist was more effective than placebo but inferior to the LNG-IUS or danazol in the relief of endometriosis-associated pain[68,119]. The review found a worse side-effects profile with the use of GnRH, which should be discussed with the patient when offering this treatment. The hypoestrogenic effects of GnRH agonist include loss of bone mass of up to 13% over a 6 months period (reversible with discontinuation of therapy), therefore the simultaneous use of hormonal add-back therapy (oestrogens and/or progestagens or tibolone) is recommended[120]. The use of hormonal add-back therapy has not been shown to reduce the efficacy of GnRH[121]. This phenomenon may be explained by the oestrogen threshold theory, which suggests that a lower oestrogen levels is needed to protect the bone, cognitive function and avoid/minimise menopausal symptoms such as hot flushes, sleep disturbance, mood swings, than to activate endometriotic tissue[122].

4.4.4 Aromatase inhibitors (AIs)

AIs have been studied as treatment for endometriosis in spite of the controversies surrounding the evidence for increased expression of aromatase P450 in endometriotic tissue. The most common third-generation AIs, letrozole and anastrozole, are reversible inhibitors of the enzyme aromatase, competing with androgens for aromatase binding sites. The adverse effects are mostly hypoestrogenic and include vaginal dryness, hot flushes and diminished bone mineral density. Earlier reports of increased cardiovascular risks have not been substantiated.

Existing evidence for the use of AIs for endometriosis pain are mostly moderate quality non-randomised studies or case reports with a lack of long-term effects evidence[123,124]. Due to their severe adverse effects, current (2014) ESHRE guidelines recommended that AIs should only be prescribed after all other options for medical or surgical treatment have been exhausted[68].

Chapter 5 – Surgical Therapies

5.1 Surgery for Endometriosis Associated Pain

Surgical intervention may be initiated in the diagnosis or treatment of endometriosis after failed medical therapies[125]. A laparoscopic approach provides superior views of the pelvic organs and is associated with a less pain, shorter hospital stays, quicker recovery and better cosmesis. Laparotomy may be necessary for advanced disease with extensive adhesions or when there is involvement of adjoining organs. Surgical procedures include excision, fulguration or laser ablation of peritoneal endometriotic implants; excision, drainage or ablation of endometriomas; resection of rectovaginal nodules and adhesiolysis. Conservative surgery aims to treat all visible endometriotic lesions and restore normal pelvic anatomy.

A Cochrane review of 5 RCTs concluded that there were significant benefits of operative laparoscopy for endometriosis at 6 and 12 months after surgery when compared to diagnostic laparoscopy alone or medical therapies[125]. Most of the patients included in the review did not have severe endometriosis. The excision of lesions would be preferred to obtain a histological specimen, although ablation and excision of peritoneal endometriosis were equally effective for treatment of chronic pelvic pain in women with mild endometriosis[126,127]. In severe endometriosis with DIE, ablative techniques are unlikely to be successful.

An alternative strategy suggested for controlling endometriosis related pain was to interrupt the nerve pathways. A 2005 Cochrane review concluded that laparoscopic uterosacral nerve ablation offered no additional benefit over conservative surgery, while presacral neurectomy combined with laparoscopic ablation of endometriotic tissue significant improved dysmenorrhoea and

reduce severe midline pain at 6 months and 12 months[128]. However, performing presacral neurectomy requires a high degree of surgical skill, which was associated with increased risk of adverse effects such as bleeding, constipation, urinary urgency and painless first stage of labour.

5.2 Surgery for Pain Associated with Endometriomas

A Cochrane review concluded that laparoscopic excision of ovarian endometriotic cyst walls (≥3 cms) was superior to drainage and coagulation with bipolar diathermy in terms of recurrence of dysmenorrhoea, dyspareunia, non-menstrual pain and risks of further surgery[129]. A more recent RCT comparing cystectomy and CO2 laser vaporization found the recurrence rate of endometriomas to be significantly higher at the 12 months postoperative period in the laser vaporization group, although there was no difference 60 months after surgery[130]. Whilst the superiority of excision over drainage and coagulation/ablation can be expected, concerns over excessive resection of ovarian tissue compromising future fertility remains. The risk of ovarian failure after bilateral ovarian cystectomies for endometriomas was reported at 2.4%[131].

5.3 Surgery for Deep Infiltrating Endometriosis (DIE) Associated Pain

Deep infiltrating endometriotic nodules extend more than 5 mm beneath the peritoneum and may involve the uterosacral ligaments, pelvic side walls, rectovaginal septum, vagina, bowel, bladder or ureter. Bowel endometriosis usually affects the rectosigmoid colon and can be associated with symptoms such as bowel cramping, diarrhoea or dyschezia[132]. This has been estimated to occur in about 3.8–37% of the patients[133]. Treatment of colorectal endometriosis is difficult and challenging. Medical management of DIE with colorectal involvement is aimed at suppression of symptoms and often associated with significant side effects[134]. It is unclear whether medical

management in these cases prevents disease progression. Certainly, discontinuation of treatment commonly results in recurrence of symptoms[135].

It is widely agreed that severe endometriosis, especially in symptomatic DIE with colorectal involvement, will require surgical treatment[136]. Surgical strategies include superficial shaving, discoid resection or segmental resection of the involved bowel segments to remove the endometriotic nodules. Although there is an on-going debate about the indication for shaving nodules as opposed to segmental resection, most studies report an improvement in pain outcome, quality of life, gynaecological and digestive symptoms after surgery for colorectal endometriosis[132,137]. In patients who underwent bowel resection with anastomosis (n = 2036), Mueleman et al.[132] reported complication rates of 2.7% (55/2036) for rectovaginal fistulae, 1.5% (30/2036) anastomotic leakages and 0.34% (7/2036) abscesses. In patients who underwent either full thickness disc excision or superficial shaving (n = 1799), complications reported were 0.7% (12/1799) for rectovaginal fistulae, 0.7% (12/1799) anastomotic leakages and 0.3% (6/1799) pelvic abscesses. Although direct comparison of the different surgical techniques was not possible, there was a lower recurrence rate in women who underwent bowel resection anastomosis (5.8%) versus disc or superficial excision (17.6%).

Surgical treatment of bladder endometriosis usually involves excision of the bladder lesion and primary closure of the bladder wall. Ureteral lesions may be excised after stenting the ureter; however, in the presence of intrinsic lesions or significant obstruction, segmental excision with end-to-end anastomosis or re-implantation may be necessary.

5.4 Hysterectomy for Endometriosis

Hysterectomy with bilateral salpingo-oophorectomy is generally reserved for women with debilitating symptoms attributed to endometriosis who have completed childbearing and in whom medical therapies have failed. There were no RCTs found on hysterectomy (with or without oophorectomy) for treating endometriosis-associated pain, but a non-systematic review concluded that hysterectomy for chronic pelvic pain was successful in many, but not all women[68,138]. The success of this surgical approach was attributed to debulking of the disease and the resulting surgical menopause causing atrophy of endometrial tissue. Case series have shown that hysterectomy with ovarian conservation presents a 6-fold greater risk for the development of recurrent pain and an 8.1-times greater risk of reoperation[139].

A Cochrane review found two RCTs investigating recurrence of pain and disease in women with endometriosis who use hormone therapy following bilateral-oophorectomy[120]. In the first RCT, transdermal oestrogen (0.05 mg/day) with cyclical oral medroxyprogesterone acetate (10 mg per day) for 12 days each month was compared with continuous tibolone (2.5 mg/day) in women who had uterine conservation surgery. After 12 months, four (40.0%) women in the first group and one (9.1%) in the second experienced moderate pelvic pain. In the second RCT, continuous transdermal oestrogen with cyclical oral progesterone was compared with no treatment. After 45 months, four (3.5%) patients in the treated arm and none in the non-treated arm reported recurrence of pain. Although there was no statistically significant difference, the authors highlighted residual disease as a risk factor to recurrence.

Considering the physiology of endometrial tissue, it seems sensible that postoperative hormone replacement should include both oestrogen and a

progestogen. The 2014 ESHRE guidelines suggest the use of oestrogen and progestogen therapy or tibolone for the treatment of menopausal symptoms in women with surgically induced menopause because of endometriosis, at least up to the age of natural menopause[68].

5.5 Infertility

Women with endometriosis are confronted with endometriosis associated pain, infertility or both. In women with minimal to mild endometriosis, suppression of ovarian function with medical treatments including danazol, GnRH analogues or COCP, to improve fertility is not effective and should not be offered for this indication alone[140]. In the same groups of women, operative laparoscopy with adhesiolysis is effective in increasing the pregnancy rate when compared to diagnostic laparoscopy alone[125].

In moderate to severe endometriosis, the overall data suggest that laparoscopic surgery is effective for the treatment of infertility[141,142]. The spontaneous pregnancy rate following expectant management was 30% in moderate disease and 0% in severe disease[143]. After operative laparoscopic treatment, including the excision of endometriotic lesions and adhesiolysis, spontaneous pregnancy rate improved to 57–69% in cases of moderate endometriosis and 52–68% in severe disease. Combination of medical and surgical treatment either preoperatively or postoperatively has not been found to improve fertility and may delay fertility treatments[144].

In the presence of ovarian endometriomas, excision of the cyst capsule increased the postoperative spontaneous pregnancy rate, when compared to drainage and electrocoagulation of the cyst wall[129]. However, as discussed previously both techniques can potentially diminish ovarian reserve.

Chapter 6 - Adhesions

6.1 Introduction

Adhesions are connective tissue bridges or internal 'scars' that form after trauma involving the peritoneum. The most common cause of adhesions is previous surgery. Other aetiology includes infection, chemical irritation, trauma, endometriosis and foreign body reactions including sutures. For the majority of women, adhesions do not appear to have any particular consequences, but in some the morbidity in terms of abdominal or pelvic pain, intestinal obstruction and female infertility are severe.

Even when surgery is performed with strict adherence to microsurgical principles, prevalence of pelvic adhesions following laparoscopic surgery for severe pelvic endometriosis varies between 50 and 100%[145,146]. The consequences of adhesion formation include subfertility, development of chronic abdominal pain, dyspareunia and intestinal obstruction[147]. Subsequent surgical procedures become more difficult. The Surgical and Clinical Adhesions Research study found that 5% readmissions 10 years after open gynaecological surgery were due to adhesions[148,149]. It is estimated that in the first year after lower abdominal surgery, the cost of adhesion related readmissions in the UK is £24.2million, which increases to £95.2 million over the subsequent nine years[150]**.** It is estimated that the National Health Service (NHS) could save £700,000 per year if an anti-adhesion agent that reduced adhesions by 25% and cost £110 was used or, at worst, that this approach would be cost-neutral[151]. This significant socioeconomic cost of adhesions has prompted a search for strategies, which may lead to a safe reduction or prevention of adhesions. Strategies have focussed on minimising surgical trauma, the use of

barriers to prevent adhesions and the use of medication both locally and systemically.

6.2 Pathophysiology

Adhesion formation may be considered the result of a normal physiological response to a peritoneal injury that has gone unchecked. Injury to intact peritoneal surfaces creates two raw edges and initiates a complex cascade of events which involves an increase to vessel permeability, release of inflammatory cells, increase in leukotrienes and prostaglandins and decrease in plasminogen activity[152]. The peritoneal defect is initially sealed by a proteinaceous exudate consisting of fibrin deposits, leukocytes and macrophages. Fibrin is a sticky substance and the exudate establishes a bridge between the damaged surfaces[153]. The function of fibrin in the body is to restore injured tissues. This process usually starts within 3 hours of peritoneal injury[154]. Peritoneum would normally contain high levels of plasmin and other fibrinolytic agents, which could completely degrade fibrin during normal healing[155]. However, any abnormity of this process results in an imbalance in the healing pathway, leading to a decrease in the amount of plasminogen and resulting in the organisation of fibrin[152]. The presence of organised fibrin is thought to initiate the activation of an adhesion cascade and within five days, the fibrin mesh is invaded by proliferating fibroblasts, which replace the fibrin with more durable components of the extra-cellular matrix such as collagen[153,156].

General agreement exists between investigators on the time for regeneration of peritoneum[157]. Ellis et al.[158] and Hubbard et al.,[159] reported that healing occurs in 5-6 days in the case of parietal peritoneum. Peritoneal defects 2x2cm and 0.5x0.5cm were both completely covered by a continuous sheet of mesothelium 3 days after peritoneal injury[158].

In terms of adhesion prevention, the minimum postoperative interval required for adhesion prevention was established to be 36 hours[157,160]. Harris et al.,[160] studied the kinetics of peritoneal adhesion prevention and found that the susceptibility to adhesion formation could be eliminated in the first 36 hours after peritoneal injury. In their evaluation of different antiadhesive agents, the magnitude of adhesion prevention was direction proportional to the agent's ability to remain at the site of injury during this critical period of adhesion formation.

6.3 Adhesion Prevention

Adhesiolysis is the only available treatment for adhesions, however, controversy regarding its efficacy continues[161]. The focus of adhesion management is prevention and it is hoped that by eliminating the incidence of adhesions, there should be a benefit noted in pain reduction and improved fertility rates.

Microsurgical technique principles include minimizing serosal trauma, use of atraumatic instruments, inert suture materials, careful tissue handling, prevention of tissue desiccation and ensuring meticulous haemostasis are thought to reduce but not completely prevent the occurrence of adhesions[162]. Laparoscopic surgery embraces the principles of microsurgery, with careful tissue handling, magnification provided by the laparoscope when held close to the tissue and strict attention to haemostasis to coagulate individual bleeding vessels.

Other adhesion prevention modalities target key steps in the fibrin formation cascade or attempts to physically separate raw peritoneal surfaces. These can be divided into fluid, gels, pharmacological and barrier[163].

6.3.1 Fluids

For many years, laparoscopic surgeons have used Ringer's lactate (Hartmann's) as an irrigation fluid and many add 5IU of heparin per litre, which appears to help limit the formation of large blood clots that can be difficult to remove laparoscopically. Reich[164] introduced a concept of hydroflotation at the end of his laparoscopic procedures, often leaving one to two litres of sodium lactate, in an attempt to prevent subsequent adhesion formation. Ultrasound studies shows that this fluid is completely absorbed within 72hours.

A glucose polymer solution, 4% icodextrin (Adept, Baxter, Berkshire, UK) has been used extensively for patients on peritoneal dialysis with little evidence of adhesion formation despite repeated passage of catheters for renal dialysis. Icodextrin is an α-1-4-linked glucose iso-osmolar and non-viscous polymer produced by the hydrolysis of corn starch. It is a substrate for amylase, which is widely distributed throughout the body but is not present in the human peritoneal cavity, therefore, when icodextrin is instilled intraperitoneally, it is largely retained within the peritoneal cavity. Absorption of the polymer occurs gradually via the lymphatic system into the systemic circulation. Studies have shown that icodextrin placed into the peritoneal cavity at the end of surgery will stay in situ for up to 3 to 5 days[165,166].

Hyskon (Pharmacia, Uppsala, Sweden) which consist of 32% dextran 70 (200mls) has been widely used as an intraperitoneal instillant. It works on the principle of drawing fluid into the peritoneal cavity by its osmotic property to produce hydroflotation. Dextran's use has been limited due to a number of complications including pleural effusion, pulmonary oedema, elevated liver enzymes, ascites, labial oedema and rarely, anaphylactic shock[167]. Most

centres have discontinued the use of Hyskon and it is no longer commercially available in the UK.

6.3.2 Gels

Gels are thought to decrease adhesion formation by separating denuded tissues. Derivatives of hyaluronic acid form the basis of a number of antiadhesion gels. Hyaluronic acid is a major component of many body tissues and fluids, where it provides physically supportive and mechanically protective roles[168]. Hyaluronic acid is a linear polysaccharide with repeating disaccharide units composed of sodium D-glucuronate and N-acetyl-D-glucosamine. SepraSpray (Genzyme Corporation, Cambridge, MA, USA) contains hyaluronic acid and carboxymethylcellulose (CMC) powder. It is applied to relevant tissues via a preloaded delivery device. SepraCoat (Genzyme Corporation) is a dilute hyaluronic acid solution that is applied before and after surgery. Hyalobarrier gel (Nordic Pharma, Reading, UK) contains auto-cross-linked hyaluronic acid. Intergel (Gynaecare, Lifecore Biomedical, Chaska, MN, USA) which contains ferrous hyaluronic acid, has been withdrawn from the market because of reports of increased postoperative pain and sclerosing peritonitis.

Polyethylene glycol (PEG) based gels are also available. CoSeal (Baxter) is formed by mixing a powder and a liquid intraoperatively, both of which contains PEG. SprayGel (Confluent Surgical Inc., Waltham, MA, USA) is formed by two PEG containing liquid precursors, which create a cross-linked gel when combined. Intercoat (FzioMed, San Luis Obispo, CA, USA) is an Oxiplex/AP viscoelastic gel composed of polyethylene oxide (PEO), which is very similar to PEG but has a different molecular weight, and CMC.

A recent Cochrane review concluded that hydroflotation agents and gels appear to be effective adhesion prevention agents for use during gynaecological,

compared to no treatment[163]. Participants who received a hydroflotation agent (OR 0.34, 95% CI 0.22-0.55, P < 0.00001) or a gel (OR 0.25, 95%CI 0.11-0.56, P = 0.0006) were significantly less likely to have adhesions at second look laparoscopy (SLL) when compared with those who received no treatment. However, there was a large gap in evidence when clinical outcomes such as pain improvements or live birth rates were considered. One RCT did assess pelvic pain but found no evidence of pain improvement when 4% icodextrin was compared with saline[169].

6.3.3 Pharmacological Agents

Steroids have been used to prevent adhesions and can be administered intraperitoneally during surgery or via hydrotubation postoperatively. Steroids and antihistamines (e.g. promethazine) act as immunomodulating agents and were used in the belief that they may promote fibrinolysis during healing, without hindering the healing process.

As discussed, heparin has often been used as an intraoperative irrigant. Other pharmacological agents used to prevent adhesions include noxytioline (an antibacterial agent), promethazine (antihistamine) and reteplase (thrombolytic drug) have also been instilled intraperitoneally. A nasal GnRH agonist has also been used preoperatively and postoperatively and may work by decreasing oestrogen-related growth factors and promoting fibroblasts.

There was no evidence for adhesions reduction, pain improvements or live birth rates with any pharmacological agent[163].

6.3.4 Barrier Agents

In theory, an inert physical materials placed between traumatised peritoneal surfaces can prevent mechanical contact and allow independent mesothelial healing of each traumatised peritoneal surface. Several synthetic barriers with different characteristics are commercially available, oxidised regenerated cellulose (ORC), expanded polytetrafluorethylene (PTFE) and modified hyaluronic acid with CMC.

ORC is commercially available as Interceed (Johnson & Johnson, Cincinnati, USA) and can be cut as necessary. It does not require suturing, is absorbable and may be applied laparoscopically. It is applied over raw tissue surfaces at the end of surgery after all irrigation fluid has been removed and haemostasis achieved. The most important step to maximise the efficacy of Interceed is via haemostasis, as the presence of bleeding renders it ineffective[170]. It forms a gelatinous protective coat within eight hours of application, and is broken down into its monosaccharide constituents and absorbed within two weeks. The fabric nature of Interceed can make passage through a laparoscopic port cumbersome.

Expanded PTFE (Gore-Tex) is marketed as Preclude (W. L. Gore & Associates, Arizona, USA). It is inert but must be sutured in place and is permanent. Preclude is approved for use for peritoneal repair but not for adhesion reduction. When placed over traumatised tissue, it has been shown to reduce adhesion formation and reformation, regardless of whether haemostasis has been achieved[171]. Preclude has the advantage of being unaffected by the presence of blood, however, it is permanent and unless surgically removed can become engulfed by an adhesion like membrane.

Seprafilm (Genzyme Corporation, Cambridge, USA) an adhesion barrier composed of chemically derived sodium hyaluronate and CMC. It is a nontoxic, non-immunogenic, biocompatible and biodegradable material that has been modified to prolong its intraperitoneal residence time. It is absorbed from the peritoneal cavity within seven days and is completely excreted from the body within 28 days[172]. Unfortunately, the sheets are also rather firm, non-compliant and difficult to place at the required site during laparoscopic surgery[153].

A Cochrane review of barriers agents concluded that Interceed and Preclude appeared to reduce the incidence of postoperative adhesions[162]. Interceed was associated with reduced incidence of both new formation and reformation of pelvic adhesions following laparoscopic surgery or laparotomy (OR 0.39, 95% CI 0.28-0.55). However, there were insufficient data to support its use to improve pregnancy rates. Preclude was more effective than no treatment (OR 0.21, 95% CI 0.05-0.87) or Interceed (OR 0.16, 95% CI 0.03-0.80) in adhesion prevention but its usefulness is limited by the need for suturing and removal at a later date. There was no evidence that Seprafilm was effective in preventing adhesion formation.

6.4 Conclusion

Although gels and 4% icodextrin has shown efficacy in reducing adhesions, there is limited evidence on their effects on clinical outcomes. Ultimately, the search for best anti-adhesion agent or strategy continues and further research is required evaluate existing and newer modalities.

Chapter 7 - Ultrasound

7.1 Introduction

The use of ultrasound has revolutionised the practise of gynaecology in the past three decades. The first published clinical use of ultrasound was in the field of gynaecology when Professor Ian Donald used transabdominal ultrasound to differentiate between solid and cystic abdominal masses[173]. The first clinically useful ultrasound machine was made possible by a combination of Professor Donald's knowledge of SONAR, acquired during the 2nd World War and the technical expertise of an engineer, Tom Brown, who had an interest in the use of ultrasound in metallurgy, to create the first clinically useful ultrasound machine. Its ability to provide instant, clinical information using a safe, non-invasive modality was transformational and has revolutionised clinical practice. TVS is now the default investigational tool for any women presenting with a gynaecological complaint.

7.2 Principles

Medical ultrasound is based on the principle of passing a current through a piezoelectric crystal to create ultrasound wave pulses. When an electric field is placed across a slice of one of these materials, the material contracts or expands. If the electric field is reversed, the effect on the material is also reversed. If the electric field keeps reversing, the crystal alternately contracts and expands. So a rapidly alternating electric field causes the crystal to vibrate. The vibration is largest when the electric field stimulates a natural frequency of the crystal (resonance). The vibrations are then passed through any adjacent materials, or into the air as a longitudinal wave i.e. a sound wave is produced. When these sound waves are applied to the tissue within the body, they encounter an interface between tissues of differing density or acoustic

impedance. As a result, a proportion of the emitted sound waves will be reflected back towards the piezoelectric element. This reflection is called an echo. The piezoelectric effect also works in reverse. If the crystal is squeezed or stretched, an electric field is produced across it. So if ultrasound hits the crystal from outside, it will cause the crystal to vibrate in and out, and this will produce an alternating electric field. The resulting electrical signal can be amplified, processed and converted into an image or visual representation of the varying densities within the body tissue.

7.3 Image formation

7.3.1 Brightness mode image formation

A brightness-mode or B-mode image is a cross-sectional image representing tissues and organ-boundaries within the body. The image is constructed from echoes generated by the reflection of ultrasound waves at tissue boundaries. Each echo is displayed at a point in the image, which corresponds to the position of its origin in the tissue being isolated. The brightness of the image at each point is related to the strength or amplitude of the echo, giving rise to the term brightness mode or B-mode[174].

7.3.2 Transvaginal ultrasound (TVS)

The frequency of ultrasound pulses used is a compromise between image resolution and the depth of penetration required. Higher ultrasound frequency results in better image resolution but there is greater attenuation of the beam within the tissues. Therefore, transabdominal ultrasound probes, which have to pass through multiple tissue layers before reaching the abdominal cavity, tend to use lower frequencies (3.5-5MHz) at the expense of image resolution. The ability of TVS probes to get much closer to the organs of interest enables the use of higher frequencies (8-15MHz), leading to much higher image resolution.

7.4 Doppler

7.4.1 The Doppler effect

When ultrasound is reflected from a moving surface, the frequency of the sound is altered slightly in a manner that depends on the speed of movement of the surface. This is due to the Doppler effect. When the object emitting the waves is stationary the observed frequency is the same as the emitted frequency, however if the sound source is moving towards the observer, the experienced frequency is higher as the sound waves become more compressed and the opposite happens if the sound source is moving away from the observer. This change in frequency is called the Doppler shift and is proportional to the relative velocity of the source to the observer. Evaluation of these Doppler shifts, alongside knowledge of the transmitted ultrasound frequency, the velocity of sound through the tissue and the angle of insonation, allow the calculation of the velocity of blood passing through the vessel[175]. This effect can be applied clinically and is used to assess the velocity of blood flow through blood vessels. This estimated velocity of blood flow over time can be shown on the ultrasound machine as a tracing (Figure 6).

7.4.2 Colour Doppler imaging

Colour Doppler imaging is a technique that combines anatomical information derived using ultrasonic pulse-echo techniques with velocity information derived using ultrasonic Doppler techniques to generate colour-coded maps of tissue velocity superimposed on grey-scale images of tissue anatomy. The velocity signals are presented as a colour coded overlay, superimposed on the real-time B-mode image. This allows production of an angiogram-like map that provides information on the morphological arrangement of the vascular tree in the tissue

of interest. While its sensitivity is good enough to enable visualisation of vessels smaller than one millimetre, it is restricted by its reliance on frequency shifts[176].

7.5 Ultrasound and Adhesions

Improvement in the quality of ultrasound equipment and examination technique has made TVS an accurate and reliable test for detecting pelvic adhesions and assessing the severity of endometriosis. Our group demonstrated that targeted TVS was an accurate test to establish the severity of pelvic endometriosis, and was particularly accurate in detecting severe endometriosis, sensitivity 0.85, specificity 0.98 and LR+ was 43.5, and LR− was 0.15[89]. A subsequent reproducibility study found high level of agreements for the detection of individual features of endometriosis including ovarian adhesions (kappa, 0.751 to 0.837) and pouch of Douglas obliteration (kappa 0.963 to 0.982)[177]. In the diagnosis of ovarian adhesions, TVS was found to be particularly effective in the hands of one of the ultrasound observers who achieved a sensitivity 0.82, specificity 1.00, positive predictive value (PPV) of 1.00 and NPV of 0.84.

Okaro et al.[178] examined women with chronic pelvic pain prior to diagnostic or operative laparoscopy for the presence of ovarian adhesions and classified them as either mobile or fixed. They found a high level of agreement (kappa, 0.80) between ovarian mobility on TVS and laparoscopy. Prior to this, Guerriero et al.[179] used the presence of one of three features to suggestive the likelihood of ovarian adhesions: blurring of the ovarian margins, inability to mobilise the ovary with abdominal palpation (fixation) and an increased distance of the ovary from the transvaginal probe. They found that the presence of a fixed ovary gave a moderate level of agreement (kappa, 0.51) and this was more accurate than blurring of the ovarian margins and detection of increased ovarian distance from the transvaginal probe.

In a more recent study, Guerriero et al.[180] used a combination of applying pressure between the uterus and ovary with the transvaginal probe and gentle abdominal palpation to assess for ovarian mobility. This technique gave a sensitivity of 89%, specificity of 90%, LR+ was 8.92, LR- 0.12 and a high kappa agreement of 0.74.

Two studies have reported lower than expected accuracies with ultrasound in the diagnosis of ovarian adhesions. Ubaldi et al.[181] found that poor definition of pelvic structures at TVS had a relatively low sensitivity of 61% in the detection of pelvic adhesions, and Yazbek et al.[182] suspected pelvic adhesions in 143 women with adnexal masses when the pelvic tumour could not be mobilized by using gentle pressure with the transvaginal probe. The sensitivity obtained was only 44% with a specificity of 98%.

Figure 3 B-mode ultrasound of an ovarian endometrioma with a vascular corpus luteum illustrated by colour Doppler

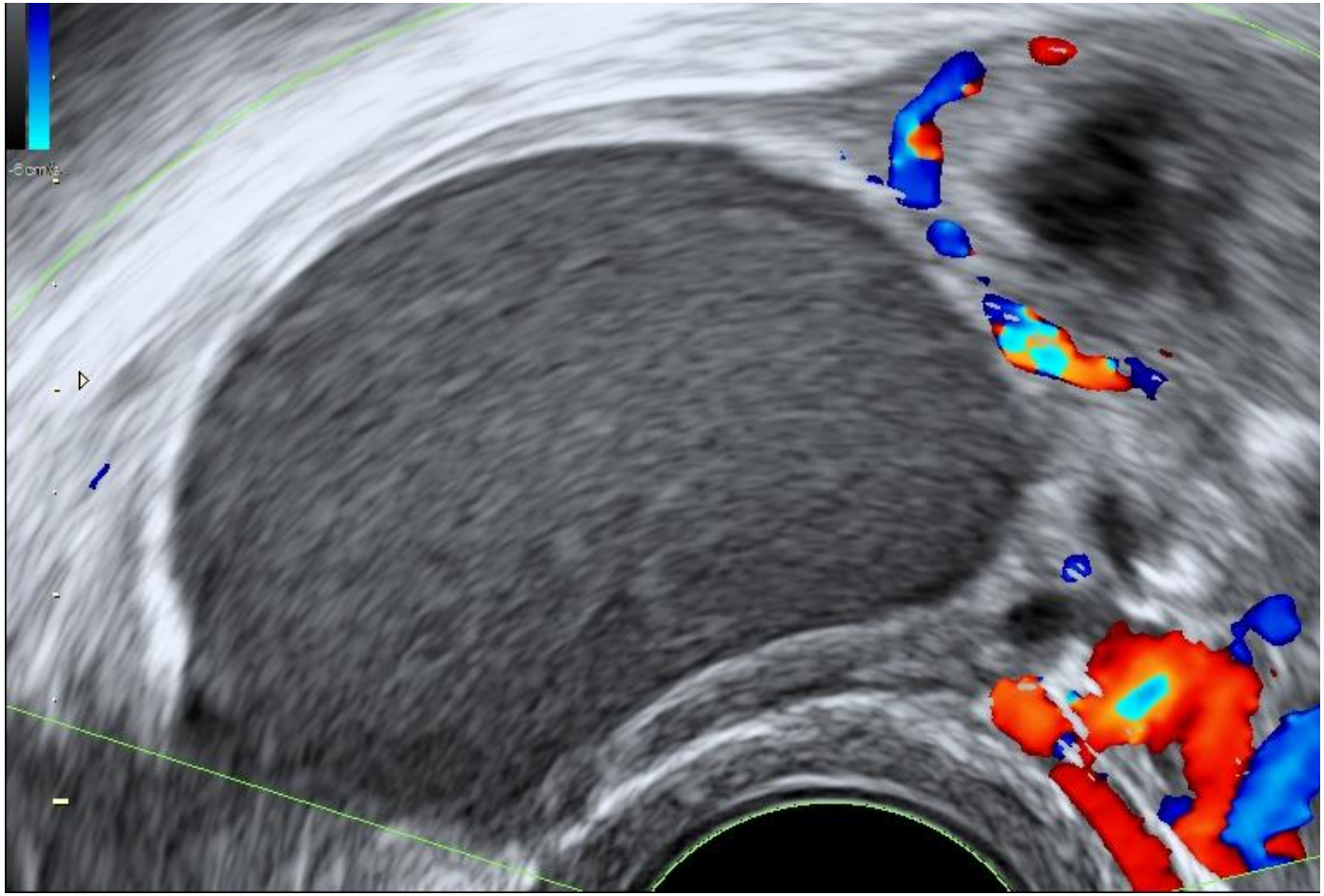


Figure 4 B-mode ultrasound of a rectovaginal endometriotic nodule

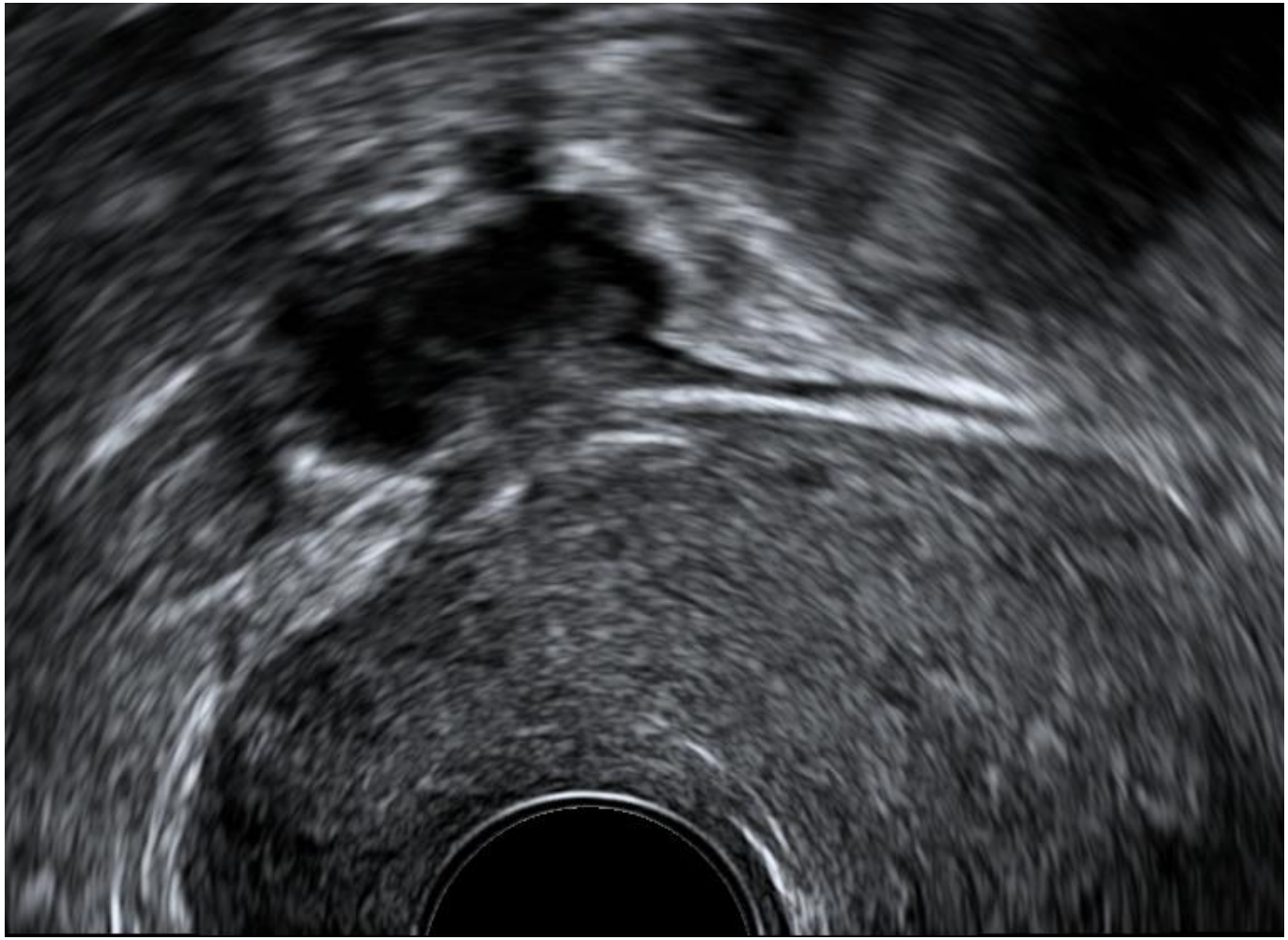


Figure 5 B-mode ultrasound of a bladder endometriotic nodule

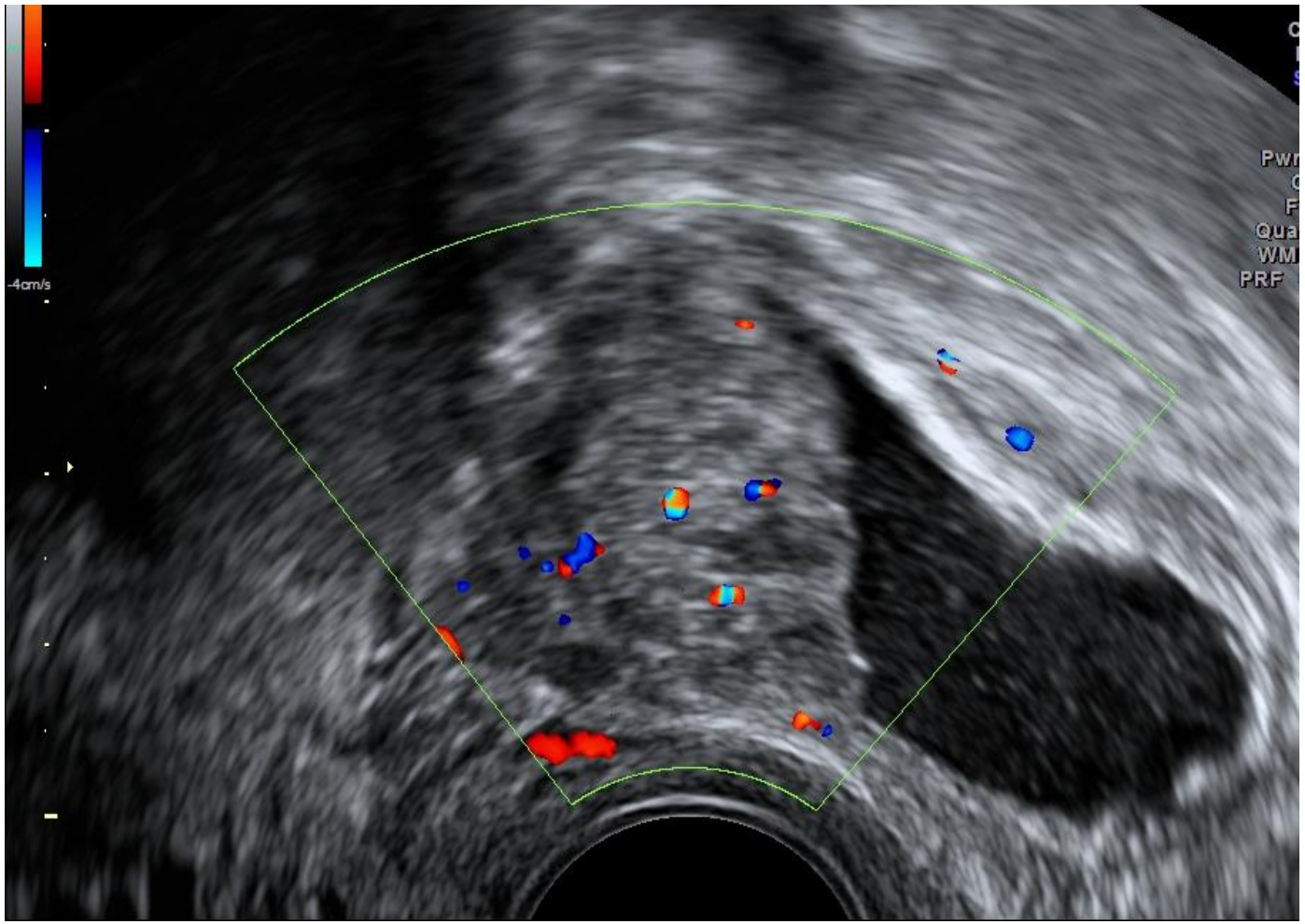
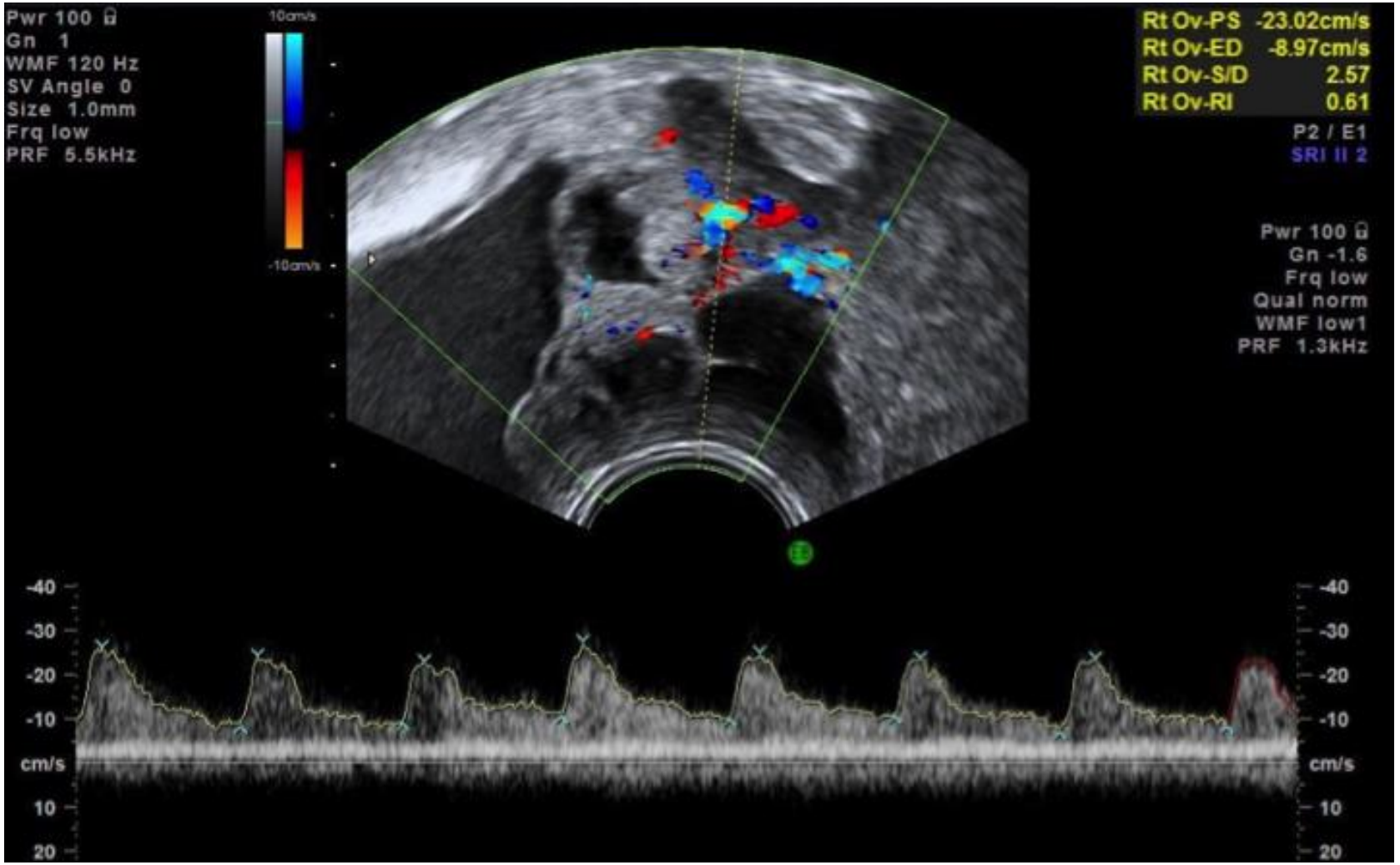


Figure 6 B-mode ultrasound image with Doppler velocimetry of a blood vessel within a malignant ovary



Chapter 8

8.1 Rationale for study

Prevalence of pelvic adhesions following laparoscopic surgery for severe pelvic endometriosis varies between 50 and 100%[183]. Postoperative adhesions most commonly affect the ovaries and the pouch of Douglas[184]. This can result in chronic pelvic pain, dyspareunia, intestinal obstruction and infertility[145]. A wide range of interventions have been tried to reduce postoperative pelvic adhesions, but none has gained wide acceptance or been shown to be clinically effective. Intra-operative suspension of the ovaries to the anterior abdominal wall has been used to facilitate ovarian retraction especially during surgery for severe pelvic endometriosis[185]. Two small observational studies have suggested that temporary ovarian suspension for four to seven days following surgery for severe endometriosis may reduce the frequency of postoperative pelvic adhesions[186,187]. A prospective RCT will be needed to assess effect of postoperative temporary ovarian suspension on the prevalence of postoperative ovarian adhesions.

8.2 Study design

There is a hierarchy of strength of evidence concerning efficacy of treatment. Case reports are weakest and RCTs are strongest, with various observational and retrospective designs in between. Sound scientific clinical investigations almost always demand that a control group be compared against a new intervention. Controls may be on placebo, no treatment, usual or standard care or a specified treatment. Randomisation is the preferred way of assigning participants to control and interventions groups.

A clinical trial is a prospective study comparing the effect and value of interventions against a control. Most trials have a parallel design, where the

intervention and control group are followed simultaneously from the time of allocation to an end point. A modification of the parallel design is the cross-over trial, which uses each participant at least twice, once as a member of the control group and at least once as a member of the intervention group.

8.3 Randomised control trials (RCTs)

RCTs are the most rigorous way of determining whether a cause-effect relation exists between treatment and outcome. The assignment of the subject to a group is determined by the formal procedure of randomisation. Participants in both groups are treated identically except for the experimental treatment. Ideally, participants and clinicians should remain unaware of which treatment was given until the study is completed, although such double blind studies are not always feasible. Analysis is focused on estimating the size of the difference in predefined outcomes between intervention groups. Participants are normally analysed within the group to which they were allocated, irrespective of whether they experienced the intended intervention (intention to treat analysis)

There are three advantages of the randomised design over other methods of selecting controls[188]. Firstly`, randomisation removes the potential of bias in the allocation of participants to the intervention group or the control group. An allocation bias could occur either because the investigator or participant influences the choice of intervention. This influence may be conscious or subconscious. The direction of the allocation bias may go either way and can easily invalidate the comparison. The second advantage is that randomisation tends to produce comparable groups. Prognostic factors and other characteristics of the participants will usually be evenly balanced between the intervention and control groups. Although this does not mean that all baseline variables will be perfectly balanced between the two groups, it does mean that

for independent covariables, the overall magnitude and direction of the differences will tend to be equally divided between the two groups. Often many covariates are strongly associated, therefore any imbalance in one would tend to produce imbalances in others. The third advantage is that the validity of statistical tests of significance is guaranteed. The process of randomisation makes it possible to ascribe a probability distribution to the difference in outcome between treatment groups receiving equally effective treatments and thus to assign significance levels to observed differences. If randomisation is not used, further assumptions concerning the comparability of the groups and the appropriateness of the statistical models must be made before the comparison will be valid.

Not all clinical studies can use randomised controls. If the prevalence of the disease is so rare that a large enough population cannot be obtained, only case-control studies might be possible.

Double blinding ensures that the preconceived views of subjects and clinicians do not bias the assessment of outcomes. While ‘intention to treat analysis’, maintains the advantages of random allocation, which may be lost if subjects were excluded from analysis in cases of withdrawal or failure to comply.

Although RCTs are powerful tools, their use is limited by ethical, emotional and practical concerns[189]. Exposing patients to an intervention believed to be inferior to current treatment is often considered unethical. Many clinicians would feel that they must not deprive a participant from receiving a new therapy or intervention, which they believe to be beneficial, regardless of the validity of the evidence for that claim. On the other hand, failure to perform trials may result in harmful treatments being used.

In some circumstances, a RCT may not be feasible because of difficulties with randomisation or recruitment. Indeed, once an intervention becomes widespread, it can prove impossible to recruit clinicians willing to “experiment” with alternatives. Strong patient preferences may also limit recruitment and bias outcomes if not accommodated within the study design. Another limiting factor is that RCTs are generally more costly and time consuming than other studies.

8.4 Cross-over study design

A cross-over study design is a special case of RCT. The essential feature distinguishing a cross-over trial from a conventional parallel-group trial is that each participant serves as his/her own control. The crossover design thus avoids problems of comparability of study and control groups with regard to confounding variables such as age and sex. Thus, the measured effect of the intervention is the difference in an individual participant’s response to intervention and control.

Moreover, the crossover design is advantageous regarding the power of the statistical test carried out to confirm the existence of a treatment effect. Crossover trials require smaller sample sizes than parallel-group trials to meet the same criteria in terms of type I and type II error risks.

8.5 Trial planning and design

The first trial meeting was held on November 2008. After establishing the aim of trial, the group were alerted to a previous attempt to perform this study in 2003. Unfortunately, the earlier study did not progress beyond protocol development.

All study materials from original study were obtained from the chief investigator who was part of the new trial team. The joint Research and Development (R&D) Office was also contacted to obtain trial details from the original trial.

8.6 The original study

In 2003, a trial was planned to perform temporary ovarian suspension for 3 days post laparoscopic surgery with a repeat diagnostic laparoscopy scheduled 3 months after the primary surgery to assess for pelvic adhesions. A prospective, cross-over design, double blind RCT, where women would act as their own control was intended. It was calculated that 20 women would be needed based on an estimate of the prevalence of adhesion following surgery. The original study received ethics approval and was registered in the R&D department (ID: 003/0279).

8.7 Risk assessment

It was essential for us to examine the trial design, population and procedures to identify specific areas of vulnerability associated with the trial conduct and why the original trial was unsuccessful. This was to allow for risk avoidance strategies, safety monitoring procedures and trial management plans to be included in the study protocol.

Risk assessment were undertaken in parallel with protocol development and reassessed periodically over the lifetime of a trial to account for new information and issues that became apparent after the commencement of the trial. Risk assessments are considered in two parts:

1. Risks of intervention to participant's safety
2. Risks associated with the protocol and study procedures including the clinical procedures, participant rights related to consent, protection of data and reliability of trial results.

Every effort was made to identify potential hazards and consider the appropriate risk avoidance or optimal monitoring strategy. The ability of the trial participants to give fully informed consent needed to be considered. It was

essential that personal data collected during the course of the study were stored securely and accessed only by authorised staff.

The aim was to create a robust study design with simple and relevant eligibility criteria, clear and objective outcome measures, a properly generated randomisation schedule and randomisation method that prevents the prediction of treatment allocation, a simple intervention that is easy to apply, sufficient power to detect realistic effects of the intervention and attempts to minimise risk of missing key data.

In our trial planning, our first concern was with regards to the duration of our intervention. The majority of patients after extensive laparoscopic surgery would require hospital stay of between one to two days after surgery. An ovarian suspension period of three days that was proposed in the original study would have required trial participants to either extend their stay in hospital or be discharged with suspension sutures in situ. Our review of the evidence suggested that although adhesions takes up to seven days to form, susceptibility to adhesion formation may be decreased or eliminated in the first 36 hours after peritoneal injury (Chapter 6.2). After reviewing the evidence on adhesion prevention, we decided to reduce the minimal suspension period to 36 hours.

The original study also received ethical approval for a second diagnostic laparoscopy to assess the outcome of ovarian suspension as an intervention. This repeat surgery may have reduced the uptake of the original trial. To improve our patient acceptability and safety, we decided to use TVS to assess our intervention outcome. TVS would give us a comparable result whilst crucially avoiding the need for repeat surgery.

8.8 Statistical Preparation

A statistician (Pauline Rogers) was recruited from the University College London Biomedical Research Centre at the planning stages of our trial and the first statistical meeting was conducted in February 2009.

Pauline provided us with advice on the development of our study protocol, deciding on appropriate statistical outcomes and later helped generate a randomisation schedule.

However, in our reassessment of the original study protocol, it became apparent that the original sample size calculations performed in the for the 2003 study had to be re-examined.

8.9 Sample Size Preparation

Our RCT plan involved women who received routine laparoscopic treatment for severe pelvic endometriosis to have one ovary randomised to temporary ovarian suspension and the other ovary unsuspended postoperatively. The primary outcome is the binary variable of the prevalence of ovarian adhesion three months after surgery with assessed using TVS.

In order to perform a sample size calculation, the prevalence of ovarian adhesions on TVS, three months after routine laparoscopic treatment of severe pelvic endometriosis, without ovarian suspension had to be determined. As this information was not available, a pilot study was proposed.

8.10 Sample Size Calculation

A pilot study was completed between March 2009 and June 2009 (Chapter 10). The prevalence of ovarian adhesions per ovary, without ovarian suspension, was 18/32 (56.3%). For the sample size calculation, this was approximated to 60%. A clinically significant improvement was defined as a 50% reduction in the prevalence of postoperative ovarian adhesions. The sample size calculation

assumed that at three months' postoperative review, 60% of the non-suspended ovaries will exhibit ovarian adhesions and 30% of the suspended ovaries will exhibit ovarian adhesions.

The software provided by Machin et al.[190] was used to calculate the sample size for paired binary data. The calculation assumed that the response to suspension is independent to the response to non-suspension. The intra-class correlation coefficient for the presence of adhesions three months after surgery was 0.52. Assuming two-sided 5% significance, 80% power and a 54% proportion of discordant pairs, 45 women were required for the study. Allowing for a possible 10% dropout during the follow up period, we had planned to recruit at least 50 patients for the study.

8.11 Randomisation

Opinions regarding the efficacy of a newly proposed intervention will vary among investigators. Randomisation may be a problem for physicians who believe that they must be able to convey to their patients a treatment course of action. A researcher however must accept an uncertainly, because it would be unreasonable to expect that an individual investigator has no preference. The concept of 'clinical equipoise' was proposed, which is the presence of uncertainty to the benefits or harm from an intervention in the medical community that is a justification for a clinical trial[191]. Until an intervention has been proven beneficial, randomisation is the most ethical approach and quickest way to reach this conclusion[192].

Our study participants were randomised into two groups, one group had left ovarian suspension and the other group had right ovarian suspension. Block randomisation was used with three varying block sizes of minimum size four.

The randomisation schedule was produced by our statistician (Pauline Rogers) using the Stata command *ralloc* (StataCorp, College Station, TX, USA).

When a participant was recruited to the trial, the anaesthetist who was not a member of the research team, opened consecutive randomisation envelopes and informed the principal surgeon of which ovary to suspend.

Only the patient's randomisation number was recorded in the patient's operation notes. A label was attached to the operation notes to define (i) the randomisation number, (ii) the operation date and time and (iii) the time to remove the sutures. The principal surgeon was under strict instructions not to discuss the suspension details with other members of the study team or clinical team responsible for the postoperative care or with the patient about which ovary had been suspended. There was no documentation of the randomisation site in the patient's notes.

At the end of the study, the randomisation was unblinded for analysis and details of the ovarian suspension were added to each participant's record.

Pauline Rogers kept a copy of the randomisation schedule on her computer in her personal area. A second copy of the randomisation schedule was kept with the sister in charge of the ward in a sealed envelope. This was in case of the need for emergency unblinding. Unblinding would have only taken place on instruction from the chief investigator or principal investigator.

8.12 Protocol development

A study protocol is often viewed as a written agreement between the investigators, participants and scientific community. It describes the objectives, design, methodology, statistical considerations and organisation of a clinical trial. It also provides information on the background and rationale for a trial and outlines the study plan of the trial. The protocol also serves as a document to

assist communication among those working in the trial and should be made available to others upon request.

A protocol should be developed before the onset of participant recruitments and should remain essentially unchanged except perhaps for minor updates. Careful thought and justification should go into any changes.

The original study protocol from the 2003 study was illustrated in Appendix 1 and current study protocol in Appendix 2.

PART II MATERIAL AND METHODS

Chapter 9

9.1 Setting - University College London Hospital (UCLH)

The studies contained in this thesis was carried out in the UCLH between October 2008 and June 2012. Preoperative and postoperative ultrasound assessments were performed in the Gynaecology Diagnostic and Outpatient Treatment Unit in UCLH.

UCLH is a teaching hospital and the Women's Health Department offers specialist gynaecology services including gynaecological ultrasound scanning, early pregnancy care, urogynaecology, colposcopy, menopause, gynaecological oncology, paediatric gynaecology and a specialist endometriosis centre. The hospital trust has an annual turnover of more than £769 million, employs over 6000 staff and has 665 inpatient beds. It sees over 789,000 outpatients a year and has around 125,000 inpatient admissions a year. The hospital was formed in 1994 and became an NHS foundation Trust in 2004.

9.2 The UCLH Endometriosis Centre

The UCLH Endometriosis Centre is a tertiary referral centre for pelvic endometriosis and consists of a dynamic multidisciplinary team (including nurse specialist, colorectal surgeons, urologists and pain management services) with the aim of providing a high-quality and evidence-based care for the treatment of women with all grades of endometriosis.

The surgical treatment of endometriosis was depended on the abnormalities found. This included mobilisation of adherent ovaries, removal of ovarian cysts, opening the pelvic sidewall peritoneum to dissect the ureters free of endometriosis, dissection of obliterated pouch of Douglas. All this was to excise superficial and deep endometriotic lesions.

9.2.1 Inclusion Criteria:

Premenopausal women who were 19 years or older were invited to participate in our studies. Only women with evidence of severe endometriosis requiring extensive dissection of both pelvic side walls and/or rectovaginal space with preservation of the ovaries and the uterus were included.

9.2.2 Exclusion Criteria:

Women who were unable or unwilling to provide consent and those who were unable to tolerate a transvaginal ultrasound scan were excluded from the study. We also excluded women who had incomplete excision of endometriosis and those who had complicated surgery resulting in unplanned oophorectomy, bowel injury or conversion to open surgery.

9.3 TVS Assessment of Pelvic Endometriosis

All the ultrasound scans were performed by trained gynaecologists using a Voluson E8 ultrasound machine (GE Medical Systems, Milwaukee, WI, USA) with a 4-9 MHz probe. All scans were performed with the women in the dorsal lithotomy position using a standardised and systematic protocol[89]. First, the uterus was assessed in the transverse and sagittal planes. Next, the ovaries were identified and their size was measured in three orthogonal planes.

Ovarian cysts were diagnosed as endometriomas when they appeared as well-circumscribed thick-walled cysts that contained homogeneous low-level internal echoes ('ground glass')[193]. Measurements were recorded from the inside of the cyst wall, again in three orthogonal planes. The average of the three diameters (D1+D2+D3)/3 was used for scoring. The adnexa would then be examined for the presence of tubal dilatation. If tubal dilatation was present, a score of 16 was given, in accordance with the revised ASRM endometriosis classification[92] (Chapter 3).

Ovarian mobility was assessed by a combination of gentle pressure with the vaginal probe and abdominal pressure with the examiner's free hand, mirroring a bimanual examination. The ovary was deemed to be completely free when it could be seen sliding across its surrounding structures without any resistance (adhesion grade 0). Minimal adhesions were considered to be present when less than one third of the surrounding structures could not be separated from the ovary with gentle pressure but the ovary could be mobilized from the majority (greater than two thirds) of the surrounding structures (adhesion grade 1). Moderate adhesions were classified when one-third to two-third of ovarian mobility was reduced as a result of adhesions with the surrounding structures but the structures on one-third of the surface of the ovary slid across it with the application of gentle pressure (grade 2). Severe adhesions were characterized by fixed ovaries, which could not be mobilized at all with gentle pressure or separated from any of the surrounding structures (grade 3).

If the tubes were dilated, the mobility of the dilated tubes was documented in a similar manner. Normal Fallopian tubes are difficult to identify in the absence of background fluid in the pelvis and therefore it was not possible to score non-dilated tubes for adhesions. Filmy adhesions were scored separately from dense adhesions of the tubes and ovaries in the ASRM system, however, it can be difficult to visualise filmy adhesions on TVS unless there is fluid entrapped within the adhesions ('flapping sail sign') or if the mobility of the affected organs is reduced.

The presence of adhesions in the pouch of Douglas was assessed next. The uterus was gently mobilised by a combination of pressure on the cervix with the ultrasound probe alternating with pressure on the fundus from the examiner's free hand on the abdominal wall. The aim was to watch the interface of the

posterior uterine serosa and the bowel behind to ensure that the two structures were sliding easily across one another. If the two surfaces slide completely free of one another, this was assessed as the absence of adhesions. Complete obliteration of the pouch of Douglas was assessed as the absence of any sliding movements between the two surfaces. Partial obliteration was present if there were some adhesions between the bowel and the uterus, but some free sliding was seen. Partial obliteration was also present when adnexal structures were firmly adherent to the posterior aspect of the uterus but the bowel appeared to be free.

Endometriotic nodules or DIE were typically visualised as stellate hypoechoic or isoechogenic solid masses with irregular outer margins[87], which were tender on palpation and fixed to the surrounding pelvic structures. Nodules were usually located in the uterosacral ligaments, adnexa, rectovaginal septum and urinary bladder. Endometriotic nodules of the rectosigmoid colon typically appeared as hypoechoic thickenings of the bowel muscularis propria, which may protrude into the lumen of the bowel[194]. The presence and largest diameter of any deep lesions were documented.

The above features were documented and scored using the ASRM classification[92]. The score was used to grade the disease as absent (ASRM score of 0), minimal (1–5), mild (6–15), moderate (16–40) or severe (>40). In the absence of obliteration of the pouch of Douglas, DIE is given a maximum score of six on the ASRM classification and we have therefore recorded the presence of these lesions separately.

9.4 Statistical Considerations

The background characteristics of patients were presented as means and standard deviations for normally distributed data or medians and inter-quartile ranges for non-normally distributed continuous variables. The Chi square test and Fisher's exact tests were used to test for statistical significance of differences in independent nominal data. The prevalence of ovarian adhesions in our cross-over RCT was analysed with a McNemar test. Statistical significance was defined using a p-value of less than 0.05. Ninety-five percent confidence intervals were calculated for each of the test results to determine the precision of the results.

The level of agreement between ultrasound findings and laparoscopic findings was evaluated with Cohen's kappa, kappa values of 0.81–1.0 being taken to indicate very good agreement, 0.61–0.80 good agreement, 0.41–0.60 moderate agreement, 0.21–0.40 fair agreement and values ≤ 0.20 poor agreement[195].

Sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and positive and negative likelihood ratios (LR+ and LR−) were calculated to determine the ability of the tests to predict the presence or absence of condition. Tests were considered very useful if LR+ was above 10 and LR− was below 0.1; moderately useful if LR+ was between 5 and 10 and LR− was between 0.1 and 0.2; somewhat useful if LR+ was between 2 and 5 and LR− was between 0.2 and 0.5; and useless if LR+ was between 1 and 2 and LR− was between 0.5 and 1[196].

The background characteristics of the patients were age in years, use of hormonal contraception (0 = no hormonal treatment, 1 = hormonal treatment) and the ASRM endometriosis score. The extent of ovarian adhesion ranges from 0 (no adhesions) to 3 (fixed ovaries) and the intensity of pain measured

using the visual analogue scale (range from 0 = no pain to 10 = severe pain). For statistical analysis, pain score 1-3 was described as mild, 4-7 as moderate and 8-10 as severe.

Statistical analysis was performed by using Stata 10.1 (StataCorp, College Station, TX, USA) and IBM SPSS for Macintosh Version 23 (IBM Corp, Armonk, NY).

9.5 Ethical committee approval

Approval for this study was obtained from the Medical Ethical Committees of the University College London Hospital, London, UK. The trial was prospectively registered as an International Standard Randomised Clinical Trial (ISRCTN 24242218).

PART IV RESULTS

Chapter 10 - Pilot Study - Prevalence of Ovarian Adhesions Following Laparoscopic Treatment of Severe Pelvic Endometriosis

10.1 Introduction

Prior to the introduction of any medical or surgical intervention to reduce the prevalence of postoperative ovarian adhesions in women with severe pelvic endometriosis, the prevalence of ovarian adhesions without any intervention needs to be determined.

We carried out this pilot study to determine the prevalence of ovarian adhesions on TVS, three months following laparoscopic treatment for severe pelvic endometriosis.

10.2 Methods

This was a prospective observational study conducted at UCLH and was approved by the UCLH Research and Ethics Committee. Women who had laparoscopic treatment for severe pelvic endometriosis seen at their three months follow up were asked to participate. Suitability for this study was based on the operative findings and extent of surgery performed. Women who had postoperative ovarian suspension were excluded from the study.

A TVS was performed at this appointment and the severity of ovarian adhesions for each ovary was assessed separately. Assessment was based on the mobility of the ovaries on targeted ultrasound palpation. Details of methodology was discussed in Chapter 9.3.

Details of statistical analysis and the criteria used for presentation of data were described in Chapter 9.4.

10.3 Results

Between March 2009 and June 2009, 16 premenopausal women were identified post laparoscopic treatment for severe endometriosis requiring extensive dissection of both pelvic side walls and/or rectovaginal space with preservation of the ovaries and uterus. All 16 women consented to have a TVS to assess for ovarian adhesions at their three months follow up.

The mean age was 34.6 years (range 22 to 51). 15 (93.8%) women were nulliparous and one (6.3%) multiparous.

Prior to taking part in the pilot study, six (37.5%) women had no prior surgery, nine (56.3%) women had one previous laparoscopic treatment for endometriosis and one (6.3%) had two previous surgeries.

At surgery, all 16 women were found to have severe pelvic endometriosis when assessed using the ASRM scoring and their operative findings are summarised in Table 1. Histology confirmed the diagnosis of endometriosis in all 16 women.

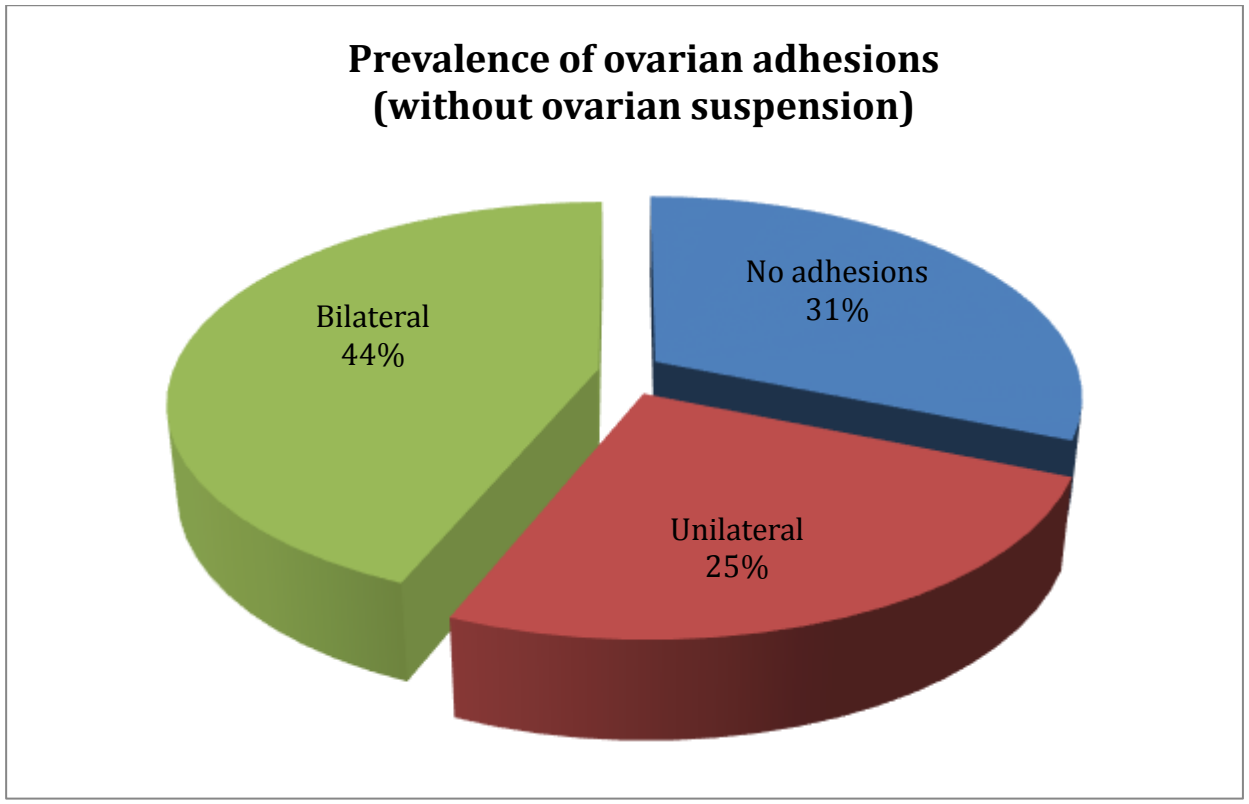
At the three months postoperative follow up, one (6.3%) woman was using the COCP, one (6.3%) woman was being treated with gonadotrophin-releasing hormone agonist, three women had a Mirena IUS in situ and 11 women were not on hormonal treatment.

All postoperative TVS were performed by a single ultrasound observer (WH). 11/16 women (68.8%) were found to have ovarian adhesions on TVS three months post laparoscopic treatment for severe pelvic endometriosis. 4/16 (25.0%) women had unilateral adhesions, while 7/16 (43.8%) women had bilateral adhesions (Figure 7).

Table 1 Operative findings at laparoscopy (Pilot study)

Operative Findings	N	%
Right Ovarian Endometriomas	8	50.0
Left Ovarian Endometriomas	7	43.8
Pouch of Douglas Adhesions		
- None seen	1	6.3
- Partial obliteration	5	31.3
- Complete obliteration	10	62.5
Deep Infiltrating Endometriosis	13	81.3

Figure 7 Prevalence of ovarian adhesion following laparoscopic surgery for severe pelvic endometriosis (without ovarian suspension)



10.4 Discussion

Our pilot study has shown that the prevalence of ovarian adhesion rate per ovary after laparoscopic surgery for severe endometriosis was 18/32 (56.3%). This gave us an approximate adhesion rate of 60% which was used to calculate the sample size required for our RCT (Table 2). The intra-class correlation coefficient for the presence of adhesions three months after surgery was calculated to be 0.52.

Details of the sample size calculation was discussed in Section 8.10.

Table 2 Prevalence of ovarian adhesion on ultrasound following laparoscopic surgery for severe endometriosis without ovarian suspension

		Left ovary		Total
		Adhesions present (n (%))	Adhesions absent (n (%))	
Right ovary	Adhesions present (n (%))	7 (43.8)	2 (12.5)	9 (56.3)
	Adhesions absent (n (%))	2 (12.5)	5 (31.3)	7 (43.8)
Total		9 (56.3)	7 (43.8)	16 (100)

Chapter 11 - Does ovarian suspension following laparoscopic surgery for endometriosis reduce postoperative adhesions? An RCT

11.1 Introduction

A wide range of interventions has been tried in order to reduce postoperative pelvic adhesions, but none has gained wide acceptance. Intra-operative suspension of the ovaries to the anterior abdominal wall is often used to facilitate ovarian retraction during surgery for severe pelvic endometriosis[185]. Observational studies have suggested that temporary postoperative ovarian suspension may reduce the frequency of postoperative pelvic adhesions[186,187].

The aim of this RCT was to assess the effect of temporary ovarian suspension following laparoscopic surgery for severe pelvic endometriosis on the prevalence of postoperative ovarian adhesions.

11.2 Methods

This was a prospective double blind cross-over comparison RCT conducted at UCLH. Premenopausal women diagnosed with severe pelvic endometriosis at their preoperative TVS were invited to participate in the study. Suitability for randomisation was confirmed at surgery. Details of inclusion and exclusion criteria were discussed in Chapter 9.

11.2.1 Intervention

During laparoscopic treatment of severe endometriosis, both ovaries were routinely suspended to the anterior abdominal wall using a 2/0 Prolene suture (Ethicon Inc., Somerville, New Jersey, USA) which was brought out onto the skin and secured using a fine haemostat or ‘mosquito’ clip during surgery. This was performed to facilitate access to the pelvic side walls during operation and a complete excision of the disease (Figure 9 and 10).

Figure 8 Ovarian suspension of both ovaries at laparoscopy to improve access to Pouch of Douglas diseased with endometriosis

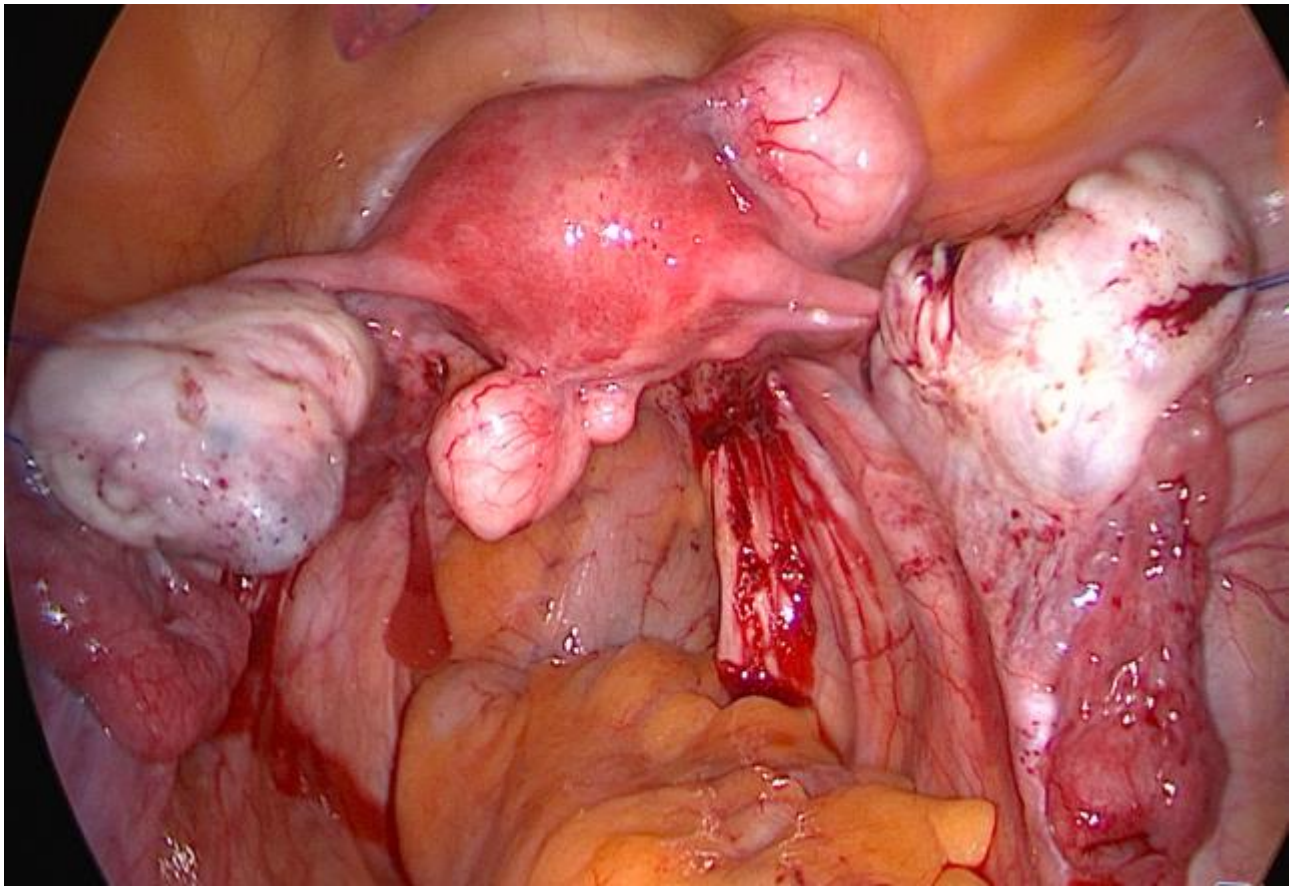
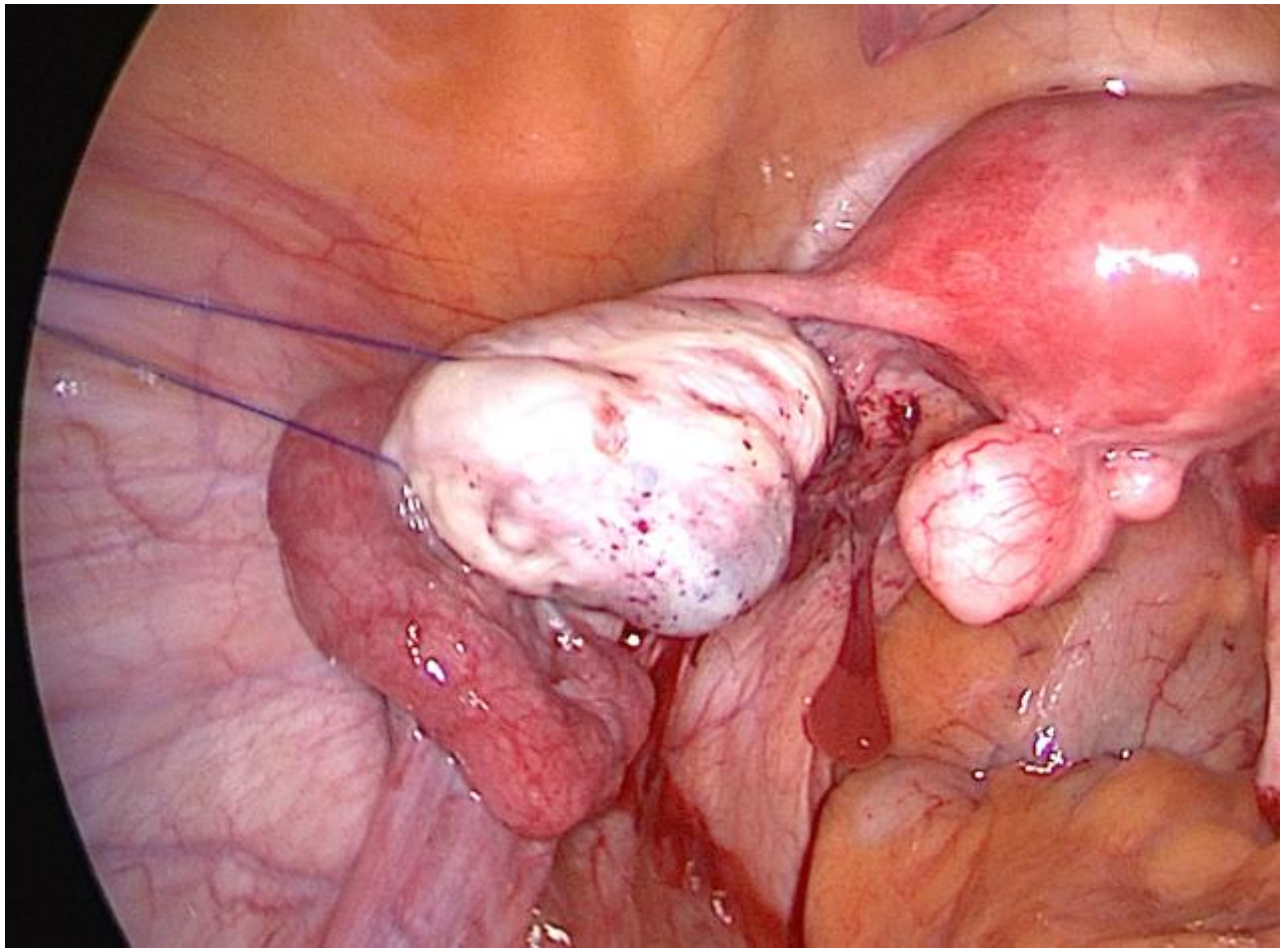


Figure 9 Ovarian suspension stitch through a left ovary

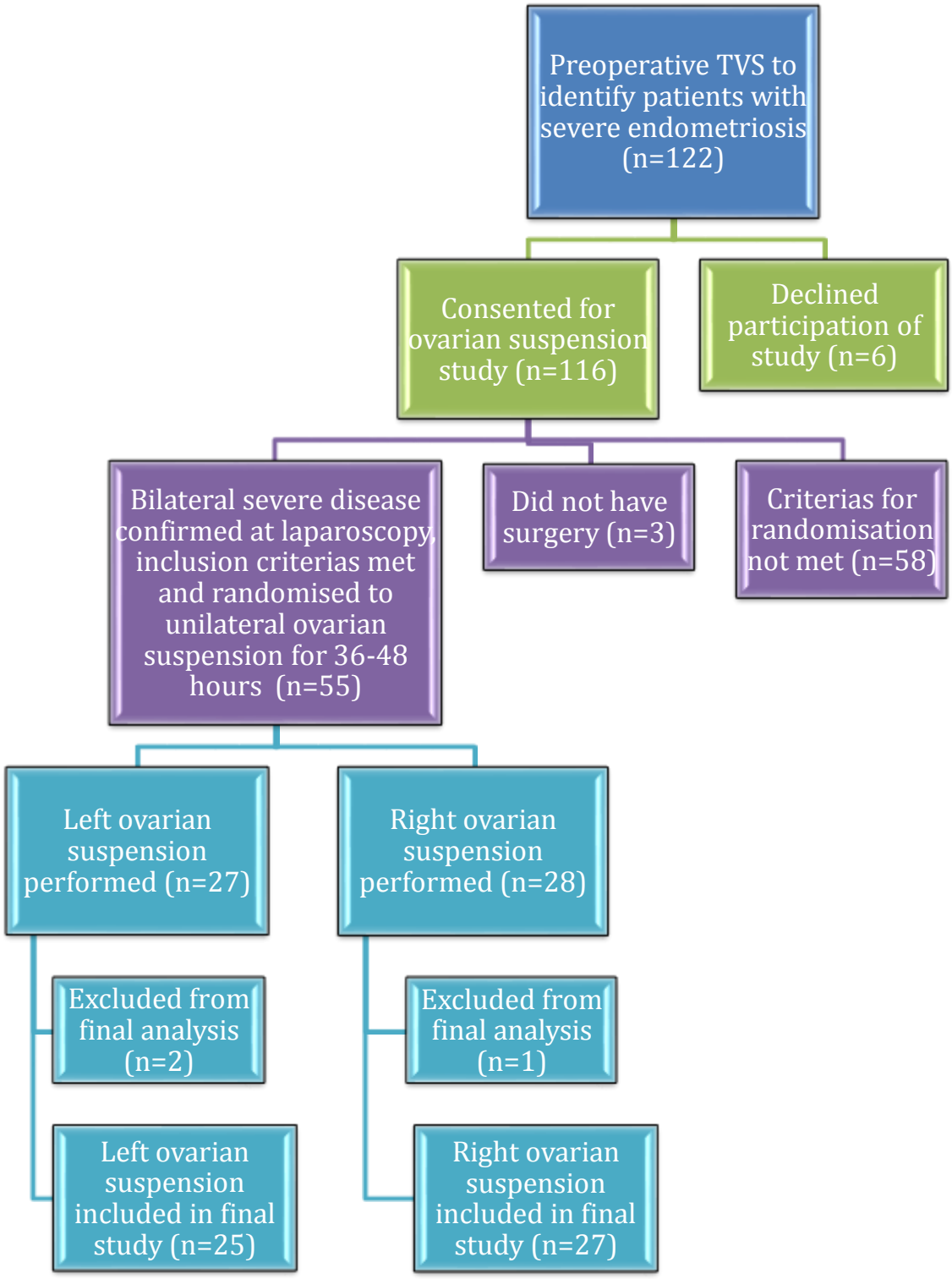


At the end of the operation, women were randomised to have one ovary suspended for 36 to 48 hours postoperatively. One of the ovarian suspension sutures was cut to allow that ovary to fall back into the lesser pelvis. A new transabdominal suture was then re-inserted at the same site to act as a placebo stitch. The pneumoperitoneum was deflated and the Prolene suture of the suspended ovary was tightened with a surgical knot placed over the skin to secure the ovary to the abdominal wall. This was done to ensure that the suspended ovary was lifted as far away from the pelvic side wall as possible. A surgical knot was secured with the space of a straight surgical suture cutting scissors between the skin and the knot to allow easier removal of the suture and reduce patient discomfort. All randomised patients therefore had two abdominal sutures of similar length. The patient and clinical staff were blinded to the randomisation. The only members of staff who were aware of the site of ovarian suspension were the surgeons who were under strict instructions not to discuss individual patient's treatment allocations with the patient or any other members of the clinical and nursing staff. Both sutures were cut 36 to 48 hours after surgery by a ward nurse who was not part of the operating or research team and who was blinded to the ovarian suspension site.

11.2.2 Follow-up

Three months after ovarian suspension, all women were scheduled for a TVS to assess ovarian mobility. Ovarian adhesions were diagnosed by the presence of restricted ovarian mobility on targeted palpation using TVS as described in Chapter 9.3. The ultrasound operators were blinded to the details of the women's randomisation allocation. A CONSORT diagram was produced to show the flow of patients through the RCT (Figure 10).

Figure 10 CONSORT flow diagram of patients through the RCT



RCT, Randomised controlled trial; TVS, transvaginal ultrasound scan

11.2.3 Outcome measures

The primary outcome was the prevalence of ovarian adhesions on TVS after surgery. Secondary outcomes were the extent of ovarian adhesions, the effects of hormonal treatment and cystectomies on adhesion rates and changes in pain symptoms.

11.2.4 Sample size

The sample size calculation was based on the findings of the earlier pilot work. Prevalence of ovarian adhesions for each ovary was 18/32 (56.3%) and this was approximated to 60%. Details of the sample size calculation was discussed in Section 8.10.

11.2.5 Randomisation

Participants were randomised to unilateral suspension of either right or left ovary. Block randomisation was used with three varying block sizes of minimum size four. Details of the randomisation schedule was discussed in Section 8.11.

11.2.6 Statistical analysis

Details of statistical analysis and the criteria used for presentation of data were described earlier (Chapter 9.4).

11.3 Results

Between November 2009 and June 2012, 122 premenopausal women were diagnosed with severe pelvic endometriosis on preoperative transvaginal ultrasound scan and they were invited to join the study. Six (4.9%) women declined the study and three (2.5%) women did not have surgery. 58 women did not fulfil inclusion criteria for the following reasons: 15 (25.9%) women received only partial treatment of endometriosis, 11 (19.0%) women had bowel surgery, nine (15.5%) had ‘two-stage’ procedures, another nine (15.5%) did not have bilateral severe pelvic endometriosis at surgery, five (8.6%) had

oophorectomies, another five (8.6%) had hysterectomies and four (6.9%) had laparotomies.

55 women fulfilled the inclusion criteria for randomisation and underwent unilateral ovarian suspension. Three women were excluded from final data analysis, as they did not attend for a postoperative ultrasound scan: one became pregnant, another was lost to follow up and the third woman suffered a large bowel injury diagnosed postoperatively for which she required further surgery and repair at her local hospital. The research and ethics committee was informed of this complication and a serious adverse event (SAE) notice was filed. Therefore, 52 women were included in the final analysis (Figure 10).

All 52 women were pre-menopausal and their mean age was 32.6 years (range 22-46). 42 (80.8%) women were nulliparous, three (5.8%) were primiparous and seven (13.5%) multiparous.

Prior to taking part in the trial, 21 (40.4%) women had one previous laparoscopic treatment for endometriosis, four (7.7%) had two, two (3.9%) had three and one (1.9%) woman had four previous laparoscopic surgeries.

All participants were asked about symptoms of endometriosis including dysmenorrhoea, deep dyspareunia, chronic pelvic pain and dyschezia. They were also asked about menstrual disorders and history of subfertility. One (1.9%) woman presented with a single symptom. Six (11.5%) women had two symptoms, 12 (23.1%) women had three symptoms, 20 (38.5%) women had four symptoms, 12 (23.1%) women had five symptoms and one (1.9%) woman had six symptoms (Table 3).

At presentation, 38 (73.1%) women were not on hormonal treatment, 6 (11.5%) women were using COCP, four (7.7%) women were being treated with gonadotrophin-releasing hormone agonist, three (5.8%) women were using a

progesterone-only pill and the remaining one (1.9%) woman had a Mirena IUS in situ.

The median interval between preoperative scan assessment and ovarian suspension was 166 [interquartile range (IQR) 117-243] days. The median interval between the first and second stage operation was 161.5 (IQR 108-229) days.

At surgery, all 52 women were found to have severe pelvic endometriosis when assessed using the ASRM scoring and their operative findings were summarised in Table 4. Postoperative TVS was performed to assess ovarian mobility after surgery and ovarian suspension. The median interval between ovarian suspension and postoperative scan was 99 days (IQR 68-114).

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Table 3 Pre and postoperative symptoms

Symptoms of endometriosis	Preoperative symptoms (n (%))	Postoperative symptoms (n (%))	OR (95% CI)	P-value
Dysmenorrhoea	39 (75.0)	11 (21.2)	0.03 (0.00-0.21)	<0.001
Deep dyspareunia	26 (50.0)	7 (13.5)	0.10 (0.01-0.39)	<0.001
Dyschezia	30 (57.7)	5 (9.6)	0.0 (0.00-0.16)	<0.01
Pelvic Pain	43 (82.7)	26 (50.0)	0.06 (0.00-0.35)	<0.001
Mean VAS	5.79	1.98		< 0.001
Pain severity				
None (VAS = 0)	9 (17.3)	26 (50.0)		
Mild (VAS 1-3)	7 (13.5)	16 (30.8)		
Moderate (VAS 4-7)	13 (25.0)	8 (15.4)		
Severe (VAS 8-10)	23 (44.2)	2 (3.9)		

VAS, visual analogue scale; OR, odds ratio

Table 4 Operative findings at laparoscopy (RCT)

Operative Findings	N	%	95% CI
Right Ovarian Endometriomas	25	48.1	34.5-61.7
Left Ovarian Endometriomas	24	46.2	32.6-59.7
Hydrosalpinges	13	25	13.2-36.8
Pouch of Douglas Adhesions			
- None seen	1	1.9	0.0-5.7
- Partial obliteration	8	15.4	5.6-25.2
- Complete obliteration	43	82.7	72.4-93.0
Deep Infiltrating Endometriosis	45	86.5	77.3-95.8

11.3.1 Ultrasound Observers

Ultrasound observer A (WH, candidate) was a clinical fellow in gynaecology who had performed more than 3000 TVS on women with gynaecological conditions in the three years of fellowship. Ultrasound observer B (NA) was a senior consultant in the Gynaecology Diagnostic Unit who had undertaken over 10000 TVS for over 10 years and observer C (KP) another clinical fellow who had performed about 1000 TVS in her first year of fellowship.

Of the 55 women randomised during the trial, four women did not have preoperative ultrasound assessment in our department because a diagnosis of severe endometriosis was made at laparoscopy prior to their referral to our Unit. Five observers performed all the preoperative ultrasound assessments in the remaining 51 women. Observer A assessed 33 women (64.7%), observer B six (11.8%) and observer C five 5/51 (9.8). The remaining scans were completed by two other consultants from the gynaecology unit, DJ four (7.8%) and RS three (5.9%).

Postoperatively, 52 women were followed up for the trial as described. Three observers completed all the postoperative ultrasound assessments. Observer A assessed 33 women (63.5%), observer B 11 (21.2%) and observer C eight (15.4%). A detailed breakdown of the number of scans performed by each observer was summarised in Table 5.

Table 5 Number of scans performed scanned by each observer

Observer	Preoperative Scans (n (%))	Postoperative scans (n (%))
A	33/51 (64.7)	33/52 (63.5)
B	6/51 (11.8)	8/52 (15.4)
C	5/51 (9.8)	11/52 (21.2)
Others	7/51 (13.7)	0

11.3.2 Accuracy of Preoperative TVS for the Diagnosis of Endometriosis

To assess the accuracy of the three postoperative observers, a comparison of their preoperative ultrasound assessments was made to the laparoscopic findings.

Table 6 shows the overall Kappa agreement between the preoperative ultrasound findings and laparoscopic findings during surgery for assessing the individual features of severe endometriosis.

Table 7 shows the inter-observer agreement between each of the observers and laparoscopic findings for the same features of endometriosis. There was a good level of agreement, individually and cumulatively, with findings at surgery. There was no significant difference in the prevalence of endometriomas, ovarian adhesions, pouch of Douglas obliteration and endometriotic nodules assessed by each of the three observers.

Table 6 Preoperative ultrasound Kappa agreement between all observers and laparoscopic findings

Features	Prevalence (n (%))	All observers on TVS and Laparoscopy, Cohen's kappa (SE)
Endometriomas	48/102 (47.1)	0.90 (0.04)
Ovarian adhesions (Any)	81/102 (79.4)	0.76 (0.08)
POD Obliteration (partial or complete)	46/51 (90.2)	0.74 (0.14)
Endometriotic nodules	39/51 (76.5)	0.72 (0.10)

#Total number of patients who had preoperative scans = 51
TVS, transvaginal ultrasound scans

Table 7 Preoperative ultrasound Kappa agreement between observer A, B, C and laparoscopic findings

Features	Prevalence (n (%))	Observer A on TVS and Laparoscopy, Cohen's kappa (SE)	Prevalence (n (%))	Observer B on TVS and Laparoscopy, Cohen's kappa (SE)	Prevalence (n (%))	Observer C on TVS and Laparoscopy, Cohen's kappa (SE)	P - value
Endometriomas	36/66 (54.5)	0.88 (0.06)	4/12 (33.3)	1.00	4/10 (40.0)	1.00	0.32
Ovarian adhesions (any)	55/66 (83.3)	0.70 (0.12)	7/12 (58.3)	0.64 (0.22)	8/10 (80.0)	1.0 (0.00)	0.13
POD obliteration (partial or complete)	30/33 (90.9)	0.84 (0.16)	4/6 (66.7)	0.67 (0.29)	5/5 (100.0)	-	0.22
Endometriotic nodules	25/33 (75.8)	0.72 (0.13)	4/6 (66.7)	0.67 (0.29)	3/5 (60.0)	1.0 (0.00)	0.62

Total number of patients who had preoperative ultrasound by Observer 1, 2 and 3 = 44 (7 patients were scanned by two other observers)
TVS, transvaginal ultrasound scans

Table 8 shows the sensitivity, specificity, positive and negative predictive values, likelihood ratios and area under the receiver–operating characteristics curve for each observers for assessing the individual features of severe endometriosis with respect to the findings on laparoscopy. There were good levels of detection rates for endometriomas, ovarian adhesions, pouch of Douglas obliterations and endometriotic nodules.

Fisher's exact test was used to compare the accuracy of the preoperative ultrasound findings between the three observers. An analysis was considered accurate if adhesions were detection preoperatively and confirmed operatively. There was no statistically significant difference between the three observers in terms of their accuracy in detecting adhesions for either ovary or when data from both ovaries were analysed. A summary of the analysis was illustrated in Table 9.

Table 8 Accuracy of preoperative ultrasound when compared to operative findings

Features	Ob	Prev (%)	Sensitivity (% (95%CI))	Specificity (% (95%CI))	PPV (% (95%CI))	NPV (% (95%CI))	LR+ (% (95%CI))	LR- (% (95%CI))	AUC (% (95%CI))
Endometriomas	All	47.1	91.7 (80.0-97.7)	98.2 (90.1-100.0)	97.8 (88.2-99.9)	93.0 (83.0-98.1)	49.50 (7.09-345.71)	0.08 (0.03-0.22)	0.949 (0.899-1.000)
	A	54.5	91.7 (77.5-98.3)	96.7 (82.8-99.9)	97.1 (84.7-99.9)	90.6 (75.0-98.0)	27.50 (3.99-189.38)	0.09 (0.03-0.26)	0.942 (0.877-1.000)
	B	33.3	100.0 (39.8-100.0)	100.0 (63.1-100.0)	100.0 (39.8-100.0)	100.0 (63.1-100.0)	-	-	1.000
	C	40.0	100.0 (39.8-100.0)	100.0 (54.1-100.0)	100.0 (39.8-100.0)	100.0 (54.1-100.0)	-	-	1.000
Overall Ovarian Adhesions	All	79.4	95.1 (87.8-98.6)	81.0 (58.1-94.6)	95.1 (87.8-98.6)	81.0 (58.1-94.6)	4.99 (2.06-12.07)	0.06 (0.02-0.16)	0.880 (0.777-0.983)
	A	83.3	92.7 (82.4-98.0)	81.8 (48.2-97.7)	96.2 (87.0-99.5)	69.2 (38.6-90.0)	5.1 (1.5-17.9)	0.09 (0.03-0.24)	0.873 (0.733-1.000)
	B	58.3	100.0 (59.0-100.0)	60.0 (14.7-94.7)	77.8 (40.0-97.2)	100.0 (29.2-100.0)	2.50 (0.85-7.31)	-	0.800 (0.509-1.000)
	C	80.0	100.0 (15.8-100.0)	100.0 (63.1-100)	100.0 (15.8-100.0)	100.0 (63.1-100)	-	-	1.000
POD Obliteration (partial and complete)	All	90.2	93.5 (82.1-98.6)	100.0 (47.8-100.0)	100.0 (91.8-100.0)	62.5 (24.5-91.5)	-	0.07 (0.02-0.19)	0.967 (0.921-1.000)
	A	90.9	96.7 (82.8-99.9)	100.0 (29.2-100.0)	100.0 (88.1-100.0)	75.0 (19.4-99.4)	-	0.03 (0.00-0.23)	0.983 (0.942-1.000)
	B	66.7	75.0 (19.4-99.4)	100.0 (15.8-100.0)	100.0 (29.2-100.0)	66.7 (9.4-99.2)	-	0.25 (0.05-1.36)	0.875 (0.571-1.000)
	C	100	100.0 (47.8-100.0)	-	100.0 (47.8-100.0)	-	1.00	-	-
Endometriotic Nodules	All	76.5	84.6 (69.6-94.1)	100.0 (73.5-100.0)	100.0 (89.4-100.0)	66.7 (41.0-86.7)	-	0.15 (0.07-0.32)	0.923 (0.850-0.996)
	A	75.8	84.0 (63.9-95.9)	100.0 (63.1-100.0)	100.0 (83.9-100.0)	66.7 (34.9-90.1)	-	0.16 (0.07-0.39)	0.920 (0.827-1.000)
	B	66.7	75.0 (19.4-99.4)	100.0 (15.8-100.0)	100.0 (29.2-100.0)	66.7 (9.4-99.2)	-	0.25 (0.05-1.36)	0.875 (0.571-1.000)
	C	60.0	100.0 (29.2-100.0)	100.0 (15.8-100.0)	100.0 (29.2-100.0)	100.0 (15.8-100.0)	-	-	1.000

Ob, observers; Prev, prevalence; PPV, positive predictive value; NPV, negative predictive value; LR−, negative likelihood ratio; LR+, positive likelihood ratio; AUC, area under receiver–operating characteristics curve

Table 9 Accuracy of preoperative ultrasound diagnosis of ovarian adhesions per observer

Observers	Accurate preoperative ultrasound diagnosis of ovarian adhesions confirmed at laparoscopy (n (%))	P-value
Observer A	60/66 (90.1)	0.56
Observer B	10/12 (83.3)	
Observer C	10/10 (100.0)	

11.4 Primary Outcome of Ovarian Suspension

The primary outcome was to assess the prevalence of ovarian adhesions on ultrasound after surgery. Of the 52 women who had postoperative follow up scan, 25 women had left ovarian suspension while 27 women had right ovarian suspension. On examination, 38.5% (20/52) of suspended ovaries had postoperative adhesions on scan when compared with 51.9% (27/52) of unsuspended ovaries (Table 10). A McNemar's test found no significant difference between the two groups [OR 0.56 (95% CI 0.22-1.35)] (P=0.23).

11.4.1 Primary Outcome Adjusted for Multiple Observers

A sensitivity analysis was performed using multilevel logistic regression to account for the differences between the three observers who performed the postoperative ultrasound assessment. Two-level models were used with measurements from individual ovaries (Table 11). All three analyses gave similar odds ratios. The regression analysis with and without adjustments for observer differences gave equivalent results (P=0.17), which suggest that the results were not influenced by the presence of more than one ultrasound observer.

Table 10 Absence of adhesions (Grade 0) vs. any adhesions (Grade 1-3) by treatment type

		Unsuspended Ovaries		
		Absence of ovarian adhesion	**Presence of any ovarian adhesions**	**Total**
Suspended Ovaries	**Absence of ovarian adhesion**	16 (30.8%)	16 (30.8%)	32 (61.5%)
	Presence of any ovarian adhesions	9 (17.3%)	11 (21.2%)	20 (38.5%)
	Total	25 (48.1%)	27 (51.9%)	52 (100%)

Table 11 Prevalence of ovarian adhesions after ovarian suspension adjusted for the presence of multiple observers

Analysis method	Adjustments	Odds ratio (95% CI) (*)	P-value
McNemar test	None	0.56 (0.22-1.35)	0.23
Logistic regression	None	0.56 (0.25-1.27)	0.17
	Observer	0.56 (0.25-1.27)	0.17

(*) Odds ratio expressed as odds of adhesions in suspended ovary relative to unsuspended

11.4.2 Primary Outcome for Each Observer

A subgroup analyses was performed to examine the treatment differences (suspended vs. unsuspended ovary) separately for each of the three observers. As with the analysis of all patients combined, the analysis was performed using the McNemar test. The results of the analysis were summarised in Table 12.

The analysis suggested that when the results from observer A were considered alone, there was a significant difference in outcome between the treatment groups. Adhesions were significantly less common in the suspended group, occurring in 33% of patients, when compared to 64% in unsuspended ovaries ($p=0.02$).

There was no difference in adhesion detection for observer B or C. It was noted that the numbers of patients were smaller for these two observers.

Table 12 Prevalence of postoperative ovarian adhesions per observer

	Prevalence of postoperative ovarian adhesions			
Observer	**Unsuspended ovaries (n (%))**	**Suspended ovaries (n (%))**	**Odds ratio (95% CI)**	**P-value**
Observer A	21/33 (63.6)	11/33 (33.3)	0.23 (0.04-0.84)	**0.02**
Observer B	2/8 (25.0)	4/8 (50.0)	0.33 (0.04 – 2.76)	0.5
Observer C	4/11 (36.3)	5/11 (45.5)	1.33 (0.23-9.10)	1

(*) Odds ratio expressed as odds of adhesions in suspended ovary relative to unsuspended ovary

11.4.3 Primary Outcome Between Observers

Additional analyses were performed to examine the differences between the three observers in terms of detection of adhesions on postoperative ultrasound Table 13. There was no significant difference between the three observers for the suspended ovary.

In the unsuspended group, although there appeared to be some suggestion of a difference between observers in terms of detection of adhesions, this difference was not statistically significant.

Table 13 Prevalence of ovarian adhesions for each observer according to treatment groups

Ovary	Observers	Prevalence of ovarian adhesions (n (%))	p-value
Suspended	Observer A	11/33 (33.3)	0.55
	Observer B	4/8 (50.0)	
	Observer C	5/11 (45.5)	
Unsuspended	Observer A	21/33 (63.6)	0.10
	Observer B	2/8 (25.0)	
	Observer C	4/11 (36.4)	

11.5 Secondary Outcomes

11.5.1 Severity of Ovarian Adhesions

The primary outcome was the presence of ovarian adhesions of any degree diagnosed with TVS three months after surgery. In secondary outcomes, two additional analyses were considered, namely the presence of either moderate or severe ovarian adhesions (grade 2 or 3 adhesions) and the presence of severe adhesions only (grade 3 adhesions) at the three months' post-operative ultrasound.

When moderate to severe adhesions were considered, 9.6% (5/52) of suspended ovaries had moderate-severe adhesions when compared with 19.2% (10/52) of unsuspended ovaries [OR 0.38 (95% CI 0.06-1.56)] (P=0.23) (Table 14). When only fixed ovaries or severe adhesions were considered, 7.7% (4/52) of suspended ovaries had fixed ovaries when compared with 13.5% (7/52) of unsuspended ovaries [OR 0.40 (95% CI 0.04-2.44)] (P=0.45) (Table 15). Overall, there was no statistically significant difference in the treatment groups when varying degrees of adhesions were compared.

Table 14 None - mild adhesions (Grade 0 - 1) vs. moderate - severe adhesions (Grade 2-3) by treatment type

		Unsuspended ovaries		
		None to mild adhesions	**Moderate to severe adhesions**	**Total**
Suspended ovaries	**None to mild adhesions**	39 (75.0%)	8 (15.4%)	47 (90.4%)
	Moderate to severe adhesions	3 (5.8%)	2 (3.8%)	5 (9.6%)
	Total	42 (80.8%)	10 (19.2%)	52 (100%)

Table 15 None - moderate adhesions (Grade 0-2) vs. severe adhesions (Grade 3) by treatment type

		Unsuspended ovaries		
		None to moderate adhesions	**Severe adhesions**	**Total**
Suspended ovaries	**None to moderate adhesions**	43 (82.7%)	5 (9.6%)	48 (92.3%)
	Severe adhesions	2 (3.8%)	2 (3.8%)	4 (7.7%)
	Total	45 (86.5%)	7 (13.5%)	52 (100%)

11.5.2 Severity of Ovarian Adhesions Adjusted for Multiple Observers

The original analysis, which was assessed using a McNemar test, was repeated using multilevel logistic regression to evaluate for any multiple observer effects (Table 16). As observer B did not detect any moderate or severe adhesions (grade 2 or 3) in the postoperative scans, the results for observers KP and NA were combined for the purposes of this analysis.

The analysis suggested that for both the presence of moderate or severe adhesions and severe adhesions only, all three analyses gave similar odds ratios with no significant differences. The unadjusted results using logistic regression produced slightly narrower confidence intervals and smaller p-values than those obtained using the McNemar test, which is not uncommon when different methods of analysis were employed. The regression analysis that adjusted for the observer differences produced equivalent results to those without adjustment for observers.

Table 16 The prevalence of moderate-severe adhesions and severe adhesions after ovarian suspension adjusted for the presence of multiple observers

Adhesion type	Analysis method	Adjustments	Odds ratio (95% CI) (*)	P-value
Presence of moderate to severe adhesions	McNemar test	None	0.38 (0.06-1.56)	0.23
	Logistic regression	None	0.37 (0.09-1.41)	0.15
		Observer	0.38 (0.10-1.41)	0.15
Presence of severe adhesions only	McNemar test	None	0.40 (0.04-2.44)	0.45
	Logistic regression	None	0.39 (0.07-2.12)	0.28
		Observer	0.40 (0.08-2.07)	0.28

(*) Odds ratio expressed as odds of adhesions in suspended ovary relative to unsuspended

11.5.3 Severity of Ovarian Adhesions for Each Observer

A subgroup analysis was performed using the McNemar test to examine the prevalence of ovarian adhesions according to the treatment groups, with or without ovarian suspension, for each of the three observers. The results were summarised in Table 17.

The results suggested that there were no significant differences between suspended and unsuspended ovaries for any of the three observers for either treatment groups.

11.5.4 Severity of Ovarian Adhesions Between Observers

Additional analyses were performed using the Fisher's exact test to examine the differences between the three observers in their diagnosis of moderate or severe adhesions and severe adhesions only at the three months' post-operative ultrasound (Table 18).

There was no significant difference between the three observers in their diagnosis of adhesions in either treatment groups.

Table 17 The presence of moderate-severe adhesions and severe adhesions per observer

Severity of adhesions	Observers	Prevalence of ovarian adhesions in unsuspended ovaries (n (%))	Prevalence of ovarian adhesions in suspended ovaries (n (%))	Odds ratios (95% CI) (*)	P-value
Moderate to severe adhesions	Observer A	9/33 (27.3)	5/33 (15.2)	0.43 (0.07, 1.88)	0.34
	Observer B	0/8 (0.0)	0/8 (0.0)	(#)	1
	Observer C	1/11 (9.1)	0/11 (0.0)	(#)	1
Severe adhesions only	Observer A	6/33 (18%)	4/33 (12%)	0.50 (0.05, 3.49)	0.69
	Observer B	0/8 (0.0)	0/8 (0.0)	(#)	1
	Observer C	1/11 (9.1)	0/11 (0.0)	(#)	1

(*) Odds ratio expressed as odds of adhesions in suspended ovary relative to unsuspended
(#) Unable to calculate odds ratios due to the one of the number of patients in one of the discordant pairs being zero

Table 18 The presence of moderate to severe adhesions and severe adhesions per observer

Severity of adhesions	Treatment groups	Observers	Prevalence of ovarian adhesions (n (%))	P-value
Moderate to severe adhesions	Suspended	Observer A	5/33 (15.2)	0.40
		Observer B	0/8 (0.0)	
		Observer C	0/11 (0.0)	
	Unsuspended	Observer A	9/33 (27.3)	0.23
		Observer B	0/8 (0.0)	
		Observer C	1/11 (9.1)	
Severe adhesions only	Suspended	Observer A	4/33 (12.1)	0.45
		Observer B	0/8 (0.0)	
		Observer C	0/0 (0.0)	
	Unsuspended	Observer A	6/33 (18.2)	0.52
		Observer B	0/8 (0.0)	
		Observer C	1/11 (9.1)	

11.5.5 Postoperative Hormonal Treatments

Postoperatively, each woman was also assessed for postoperative symptoms (Table 3) and the use of hormonal treatments. There was a general reduction in the symptoms of endometriosis after surgery. However, an additional 17 (32.7%) women were given hormonal treatment after surgery, which may have contributed to the reduction in symptoms.

At the three months' post-operative follow up, 19 (36.5%) women had a Mirena IUS inserted, five (9.6%) women were taking a COCP, four (7.7%) were treated with gonadotrophin-releasing hormone agonist, three (5.8%) used a progesterone-only pill and 21 (40.4%) women did not use any hormonal treatments.

There was no significant difference in the rates of ovarian adhesion when patients who used postoperative hormonal treatments were compared to patients not on treatment (Fisher's exact p = 0.85). Similar results were obtained when patients were subdivided into their treatment groups (Table 19). A comparison of ovaries exposed to different hormonal treatments did not suggest any difference between the types of hormones used (Fisher's exact p = 0.07) (Table 20).

Table 19 The prevalence of ovarian adhesions according to the use of hormonal treatments by treatment groups

Treatment groups	Postoperative use of hormonal treatment	Prevalence of ovarian adhesions (n (%))	p-value
Suspended ovaries	Hormonal treatment	15/ 31 (48.3)	0.57
	No hormonal treatment	8/ 21 (38.1)	
Unsuspended ovaries	Hormonal treatment	14/ 31 (45.1)	1.00
	No hormonal treatment	10/ 21 (47.6)	

Table 20 Ovarian adhesion rates according to the type of hormonal treatment

Postoperative hormonal treatment	Presence of postoperative adhesions (n (%))	p-value
Mirena IUS	14/38 (36.8)	0.07
POP	5/6 (83.3)	
COCP	6/10 (60.0)	

IUS, intrauterine system

11.5.6 Ovarian Cystectomies

At laparoscopy, nine (17.3%) women had right ovarian cystectomies, seven (13.5%) had left ovarian cystectomies and 16 (30.8%) had bilateral ovarian cystectomies performed.

Additional analysis was performed to assess if having an ovarian cystectomy during their primary surgery had additional effects on the presence of ovarian adhesions at the three months postoperative follow up. There was also no significant difference in the postoperative adhesion rates on the same side when an ovarian cystectomy was performed on the left (Fisher's exact $p = 0.79$) or right ovary (Fisher's exact $p = 0.16$).

11.6 Discussion

Our RCT has shown that temporary unilateral ovarian suspension for 36-48 hours in premenopausal women with stage IV pelvic endometriosis did not result in a significant reduction of postoperative adhesions when compared with the unsuspended side.

There are several possible explanations for our findings. We suspended ovaries for only 36 to 48 hours which may have contributed to the negative result. Some may consider the relatively short period of postoperative ovarian suspension a weakness. We decided on this length of suspension after taking into consideration a methodologically robust study by Harris et al.,[160] who used an animal model to show that susceptibility for adhesion formation was significantly reduced or eliminated when separation of peritoneal surfaces was maintained for at least 36 hours following peritoneal injury. Other authors have suggested that peritoneal healing can take up to five days to complete[158,159]. In addition, it has been hypothesised that the persistent presence of blood in the peritoneal cavity following surgery may stimulate adhesion formation[197]. However the study by Harris et al.[160] was methodologically stronger and its main aim was to evaluate the question of minimal duration of intervention for adhesion prevention. We did consider suspending the ovaries following laparoscopic surgery for longer than 36 hours, but we were concerned about the risk of serious complications such as small bowel strangulation. This complication needs immediate correction and for that reason we decided against discharging patients from hospital with the suspension sutures in situ.

In a retrospective review of 218 patients who had extensive surgery for severe endometriosis with transient ovarian suspension for five days, two complications (0.7%) were reported[198]. One patient had an ovarian abscess drained via a

posterior colpotomy eight days after her primary surgery. The second patient had hemoperitoneum caused by the bleeding from the suspension site on the ovary which required emergency laparoscopy on the first post-operative day.

Our surgical team had previously encountered a case of acute small bowel obstruction following a similar suspension procedure which required immediate release of the suspension suture (data on file). By limiting the duration of ovarian suspension in our study, we shortened the women's postoperative in-patient stay, minimised their social disruption and avoided increasing their treatment costs.

Ouahba el al.[187] suspended 12 ovaries in eight women for four days following extensive surgery for severe pelvic endometriosis. A second-look laparoscopy performed five months after the first procedure found significant ovarian adhesions in 33% of cases. This was only a slight improvement when compared to the 38% adhesions rate in our study. This would suggest that a longer duration of suspension may not actually lead to better surgical outcomes.

Our results are in contrast to a small study by Abuzeid et al.,[186] which suggested a reduction in postoperative ovarian adhesions with temporary ovarian suspension. The authors reported findings at second-look laparoscopy in five women who had ovaries suspended for five to seven days following laparoscopic surgery for stage 3 or 4 pelvic endometriosis. They found mild ovarian adhesions in one woman (20%) whilst the remaining four women were completely free of adhesions. However, the number of patients in this study was very small, whereas, we recruited a sufficient number of patients to detect significant differences between suspended and unsuspended ovaries.

The main strength of our study was our trial design which was a prospective placebo-controlled randomised trial. We opted for a cross-over study design,

which is considered to be particularly powerful and free from disadvantages which may affect the quality of parallel group trials. Although the surgeons were aware of the side of ovarian suspension, the patients and ultrasound operators were blinded to randomisation. A dummy abdominal suture was inserted on the site of the unsuspended ovary and the sutures were removed by a ward nurse who was not part of the trial. Both the surgeons and ward staff were instructed not to discuss possible ovarian suspension site with the patient after surgery or during the time of suture removal. It is therefore very unlikely that our results were influenced by bias.

Ideally, any outcome measure in a clinical trial should be precise and reproducible. Inter-observer variability in clinical trials is a potential source of bias and should be minimised. If possible, all subjective and objective assessments should be performed by the same observer, but this is rarely achievable. In our trial, the postoperative TVS assessments were performed by three observers with Observer A (the candidate) performing the majority of ultrasound assessments (64.7% of preoperative and 63.5% of postoperative scans). The effect of multiple observers was not considered when the sample size was calculated. We assessed the performance of the ultrasound operators in our study by comparing preoperative ultrasound findings with the operative findings. For each observer, the best agreement was with the diagnosis of endometriomas, but there was also a good level of agreement for ovarian adhesions, pouch of Douglas obliteration and endometriotic nodules. There was no significant difference between three observers in the accuracy of preoperative ultrasound diagnosis of ovarian adhesions ($p = 0.56$). It is therefore unlikely that the use of multiple observers was a source of bias.

Further analysis was carried out to evaluate agreement between three observers in the detection and classification of severity of pelvic adhesions using multilevel logistic regression. We found no significant difference between the observers. A subgroup analysis of the primary outcome for each observer did suggest a statistical difference between the suspended and unsuspended ovaries when the results from observer A was considered alone (p=0.02). Adhesions were less common in the suspended group, occurring in only 11 out of 33 ovaries when compared to 21 ovaries in the unsuspended group. This is likely to be a chance occurrence due to the small number evaluated. There was no significant difference in the proportion of adhesions between suspended and unsuspended ovaries in women who were examined by observer B and C. They both reported a slightly higher prevalence of ovarian adhesions in the suspended ovaries. This result is unlikely to be influenced by the experience of the operators as less experienced examiners are more likely to miss rather than over diagnose pelvic adhesions.

The primary outcome in our study was the presence of any ovarian adhesions. However, the presence of severe adhesions may be more clinically relevant than mild or moderate adhesions. We therefore carried out additional analysis to examine differences in the prevalence of moderate and severe adhesions and we found no significant results (Table 16).

In our study, we decided to use ultrasound rather than laparoscopy to assess for pelvic endometriosis preoperatively and diagnose pelvic adhesions postoperatively. Although a second look laparoscopy is commonly perceived by many as a gold standard to assess for the presence of pelvic adhesions or endometriosis, numerous studies have shown significant intra- and

interobserver variability, which is likely to be as operator dependent as non-invasive diagnostic methods.

Bowman et al.[199] investigated the reproducibility of laparoscopy in assessing for pelvic adhesions by using video records of 25 women with pelvic adhesions. The recordings were reviewed by two assessors experienced in tubal surgery. They found a large variation in adhesion scoring between the assessors and poor agreement with endometriosis staging using the ASRM criteria.

In a study by Hornstein et al.,[93] five independent observers reviewed video recordings of 20 laparoscopies in patients with endometriosis and scored them according to the ASRM criteria. They also found a poor level of agreement between the observers in the classification of endometriosis of the ovary, cul-de-sac obliteration and ovarian adhesions.

Similar findings were reported in more recent studies. Weijenborg et al.[200] found only a fair to moderate level of agreement in the intra- and interobserver assessment of ovarian adhesions when 90 video laparoscopic recordings were reviewed by two observers.

All these studies indicate that there is a significant variation between the observers in the assessment of severity of endometriosis and assessment of ovarian adhesions when laparoscopy was used as the primary diagnostic tool. In addition, laparoscopy is costly and carries a significant risk of complication particularly when used to assess the efficacy of previous surgical treatment of endometriosis. In view of this, we decided that ultrasound may be a more appropriate tool to diagnosis endometriosis preoperatively and to assess women following surgery.

Ultrasound had not been routinely used for the diagnosis of pelvic endometriosis in the past due to concerns about possible lack of sensitivity for

the detection of deep infiltrating endometriosis and pelvic adhesions[201]. However, recent studies have shown that continuing improvements in the quality of ultrasound equipment and development of novel examination technique have improved the accuracy and reproducibility of ultrasound diagnosis of pelvic endometriosis[86,89]. Furthermore, subsequent reproducibility studies found good levels of agreements between the operators for individual features of endometriosis including ovarian adhesions and pouch of Douglas obliteration[177].

Moore et al.[83] systematically reviewed the validity of TVS for the detection of pelvic endometriosis and found sensitivities, specificities and positive (LR+) and negative likelihood ratios (LR−) in six studies ranged between 64 and 89%, 89 and 100%, 7.6 and 29.8 and 0.1 and 0.4, respectively.

As discussed in Chapter 7.5, Okaro et al.,[178] found a high level of agreement (kappa, 0.80) between ovarian mobility on TVS and laparoscopy. Our results were slightly better when compared to Guerriero et al.[179] who found only a moderate level of agreement (kappa, 0.51) in detection of ovarian adhesions. A more recent study by the same group assessed ovarian mobility by a combination of applying pressure between the uterus and ovary with the transvaginal probe and gentle abdominal palpation to assess for ovarian mobility[180]. They achieved much better accuracy with this technique which is very similar to our results.

Reid et al.,[202] assessed the reproducibility of TVS by recording the video TVS assessments of 30 women presenting with chronic pelvic pain and assessed for pouch of Douglas obliteration using the TVS ‘sliding sign technique’. Four ultrasound operators demonstrated near-perfect inter- and intraobserver

correlation. Diagnostic accuracy using this technique was sensitivity 93–100%, specificity 91–100%, PPV of 78–100% and NPV of 98–100%.

TVS has also been shown to be a highly accurate and reproducible for the diagnosis of DIE in expert hands[203,204]. Hudelist et al.[86] conducted a meta-analysis and found the sensitivity and specificity of TVS in detecting rectosigmoid endometriosis to be 91% and 98%, respectively. A systematic review found that enhanced TVS (defined as TVS with additional free fluid, saline, water or gel in the rectum or vagina) did not improve the diagnostic accuracy of rectosigmoid DIE[204]. A similar systematic review on the overall diagnostic performance of TVS for detecting DIE in the uterosacral ligaments, rectovaginal septum, vagina and bladder concluded that TVS had high specificities for the diagnosis of DIE at these sites.

Savelli et al.,[205] evaluated 69 women with TVS and double-contrast barium enema (DCBE) to predict posterior compartment DIE preoperatively. With regard to the prediction of bowel DIE, TVS vs DCBE gave accuracy of 91% vs 45%, sensitivity of 91% vs 43%, specificity of 100% vs 100%, PPV of 100% vs 100% and NPV of 29% vs 6%. They concluded that the sensitivity of transvaginal scanning is superior to DCBE and should be used as the method of choice for diagnosing bowel endometriosis.

A recent Cochrane review found that MRI interobserver agreement was variable and a low intraobserver agreement was noted in non-expert MRI observers[206]. A meta-analysis evaluated the overall diagnosis of DIE using MRI found a sensitivity and specificity of 83% and 90%, respectively[207]. These results are comparable to TVS but there was no data on the accuracy of MRI for detecting pelvic adhesions.

The results of all these studies support our decision to utilise TVS rather than laparoscopy for the assessment of endometriosis and pelvic adhesions. It is reassuring that the rate of postoperative adhesions in our study was similar to the previous studies which used second-look laparoscopy to diagnose pelvic adhesions[187].

Our power calculation was based on a 50% reduction in the observed 60% postoperative adhesion rate in women without ovarian suspension after laparoscopic surgery for severe endometriosis. Some researchers may consider a 20% reduction to be clinically significant. However, we felt that a 50% reduction was required to justify the additional hospital stay and the risk of complications following ovarian suspension. Furthermore, a study adequately powered to assess for a 20% reduction would require a sample size of 390 participants, based on a sample size calculation from Machin et al.,[190] which could only be achieved in a large multicentre trial. Recruitment of patients in our study took longer than expected. This was due to a higher than expected rate of open surgery, excision of bowel disease and two stage surgeries. This resulted in the overall recruitment rate of 43% of women with found to have evidence of severe endometriosis on preoperative ultrasound. Despite the increased duration of the trial, the quality of our study was not affected as the surgical treatment and ovarian suspension was completed by the same surgical team throughout the duration of the trial.

It is possible that ovarian suspension may be beneficial for women with less severe endometriosis. In our study, the majority of women had unusually severe endometriosis; 43 (82.7%) had complete obliteration of pouch of Douglas, eight (15.4%) had partial obliteration and 45 (86.5%) had DIE. Temporary ovarian suspension alone may never have been sufficient in this group of patients to

reduce the postoperative ovarian adherence and ovarian suspension in cases of ovarian cystectomies for endometriomas alone may produce a statistically significant result.

Although, we did not find a statistical significant result in outcome, some would argue that the magnitude of effect matters more for clinical relevance than p-values. With an odds reduction of 0.56 for ovarian adhesions with ovarian suspension, these findings may be meaningful. However, others could say that the lack of statistical significance, coupled with publication bias for small studies, would argue against utilisation.

We found a significant improvement in women's pain scores following surgery despite the relatively high prevalence of postoperative pelvic adhesions. Although the proportion of women complaining of pelvic pain was significantly less postoperatively, half of women continued to experience some pain, which was moderate to severe in 19.3% of them. In addition, 13.5% of women continued to complain of deep dyspareunia. This occurred despite successful and complete excision of all endometriotic lesions at laparoscopy. In view of these results, it is possible that postoperative pelvic adhesions are at least partly responsible for the persistent pelvic pain following laparoscopy for endometriosis. Postoperatively, 31 women (59.6%) were taking hormonal therapy compared to 14 (27%) women preoperatively. It is therefore possible that postoperative pain scores could have been worse if the proportion of women on hormone treatment was the same before and after surgery. Statistical comparisons were made between the 31 women who had hormonal treatment postoperatively and the 21 women who did not receive any hormonal treatment, but we found no difference in the adhesion rates between the two groups. Further analysis of the treatment groups and types of hormonal

treatment used did not suggest any difference. There was no suggestion in the literature that hormonal treatment has any effect on the formation of adhesions and our results would support this. However, the number of patients evaluated was small and our study was not sufficiently powered to evaluate this effect. A larger study will be necessary to further evaluate these effect.

Ovarian cystectomies were performed on 61.6% of the participants. One would assume that the rate of adhesions would be naturally be higher when an ovarian cystectomy was performed on the same side. In our group of patients, we did not find this association. Again, this may be explained by our small number of patients and the severity of endometriosis in our patient group.

Chapter 12 - Conclusion and further research

This thesis has explored the complex pathology of endometriosis and highlighted a common problem of postoperative adhesions associated with the surgical treatment of this condition. I have described the detailed journey of an RCT from its conception, protocol design, pilot study, trial management, analysis to publication of results. In agreement with previous publications we found TVS to be an accurate tool in the diagnosis of features of pelvic endometriosis and diagnosis of pelvic adhesions.

We found that temporary ovarian suspension for 36 to 48 hours in the postoperative period did not produce a statistically significant reduction in the prevalence of ovarian adhesion. This finding however, relates to patients with severe (stage 4) pelvic endometriosis. Further studies should be considered to evaluate the role of temporary ovarian suspension in women having surgery for mild to moderate endometriosis.

We opted for shorter length of suspension because we were concerned about the risks with discharging patients home with sutures in situ. We recorded no complications related to the ovarian suspension. Future studies should explore whether longer ovarian suspension may result in significant reduction of postoperative ovarian adhesion. However, prolonged suspension could increase the risk of serious complication which could offset possible benefits of reduced prevalence of adhesions.

Arguments against the use of TVS for the diagnosis of ovarian adhesions have centred on a perceived lack of accuracy with ultrasound and the regard of laparoscopy being the gold standard for diagnosis. Our findings and recent publications have strengthened the case for the use of TVS as a diagnostic tool

for features of endometriosis and adhesions without the need of second look laparoscopies.

We found the prevalence of ovarian adhesion after laparoscopic surgery for severe endometriosis to be 56.3%. We found a significant improvement in women's pain scores following surgery despite the relatively high prevalence of postoperative pelvic adhesions. Although the proportion of women complaining of pelvic pain was significantly less postoperatively, half of women continued to experience some pain, which was moderate to severe in 19.3% of them. Further studies to assess the prevalence of adhesions in varying severity of endometriosis may improve our understanding of the effects of adhesion on symptoms. Larger and longer term studies are also required to assess the long-term impact of adhesions on clinical symptoms and fertility.

We did not find a significant difference in the adhesion rates between women who used hormonal treatment postoperatively and those who did not. A larger study will be necessary to evaluate for the effects of hormonal treatment on the prevalence of adhesions.

Chapter 13 – Contributions by Candidate

The contributions by the candidate has been listed below:

- Pilot Study
    - Design
    - Approval
    - Recruitment
    - All postoperative TVS scanning
    - Data collection & interpretation
- Main RCT – Principal Investigator
    - Trial planning
    - Study protocol development
    - Liaising with statistics department
    - Ethics approval
    - Research and development consultations
    - Trial registration
    - Substantial amendments to trial protocols
    - Trial management and monitoring
        - Patient recruitment and consent
        - 64.7% of preoperative scans were completed by candidate
        - 63.5% of postoperative scans were completed by candidate
        - Data management & collection
    - Reporting of complication
    - Interpretation and publications of results
- Statistical contributions
    - All statistical work and interpretation in this thesis have been completed by the candidate except for the power calculation, randomisation schedule, analysis of RCT primary outcome (Section 11.4) and severity of ovarian adhesion (Section 11.5.1).
    - List of statistical analysis performed by candidate were Cohen's kappa agreement, Chi square/ Fisher's exact test, sensitivity, specificity, positive/ negative predictive value, positive/ negative likelihood ratio, area under receiver-operating characteristic curve, McNemar test and multilevel logistic regression.

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Appendix 1 – Trial protocol from original 2003 study

Study Protocol (Version 1)

Does suspending the ovaries to the anterior abdominal wall reduce the incidence of adhesions found at the second look laparoscopy for endometriosis?

Endometriosis is a common condition causing significant morbidity in women. It is often treated with laparoscopic surgery. Initially, at the first laparoscopy the adhesions are divided, the ovaries freed and the endometriotic cysts treated. The patients are then treated with GnRH analogues, which suppress the ovaries and reduce the activity of the endometriotic tissue. 3 months after treatment a second laparoscopy is performed to excise the remainder of the endometriosis, aided by the previous months of medical treatment. We will have slots allocated to these patients on our operating lists in order to ensure a second operation at 3 months.

We have found that at the second operation the ovaries have often become involved with adhesions again. We propose that by suspending the ovaries to the anterior abdominal wall the incidence of ovarian adhesions will be significantly reduced and thus the second laparoscopy will be less involved and the patient will be symptomatically improved.

In order to see if there is a benefit in suspending the ovaries we propose the following study:

For patients included in the study, at the end of their first laparoscopic treatment to endometriosis both ovaries will be suspended to the anterior abdominal wall with a Prolene suture and the suture brought out onto the skin and tied on the skin surface. The primary operator who will then leave the theatre at the end of the procedure, will grade the endometriosis on the left and right sides of the pelvis. Randomly the suture holding either the most or least affected ovary will be cut allowing that ovary to fall back into the pelvis immediately after the operation so that the principal surgeon is unaware of which side was actually sutured. A Prolene suture will be placed in the skin at the same site in order that the patient remains blinded to which ovary remains suspended. Both sutures are then cut on the third post–operative day prior to the patient going home.

The patient will then receive 3 months of GnRH analogue (either Prostap or Zoladex). At the start of the second laparoscopy the principal surgeon will grade the level of adhesions around each ovary.

These adhesions will be compared with the level of adhesions found at the initial laparoscopy and correlated to whether the ovary was suspended to the abdominal wall or not. Each patient will be asked to complete pain scores using a visual analogue scale after their first procedure in order to assess if the sutures are related to any increase in post-operative pain.

Summary:

1) Information leaflet and consent in clinic likely to need a 2-stage procedure.
2) In theatre
   a) Second procedure not required – NOT entered into study.
   b) Second procedure required– entered into study.
3) Both ovaries suspended to anterior abdominal wall after endometriomas drained and adhesions divided.
4) Principal surgeon grades severity of endometriosis and adhesion formation on each side of the pelvis. Grading is recorded using the same criteria for each patient (namely the revised ASRM along with a written description and a diagram).
5) Randomly one suture is cut from either the most or the least affected ovary after principal surgeon has left the operating theatre.
6) A similar suture is placed in the skin at the same site.
7) Patient fills in postoperative pain scores on day 3.
8) Both sutures are cut prior to the patient going home on day 3.
9) Patient treated with 3 months of GnRH analogue.
10) Second laparoscopy performed. Severity of adhesion formation on each side of the pelvis graded by principal surgeon and any remaining endometriosis treated surgically.

Power calculation

We have calculated that we need 20 patients in the study to detect a 60% vs. 30% difference in adhesion rate with an 80% power and a p value assumed to be 0.05.

State the intended value of the project, giving necessary scientific background.

This study intends to determine whether suspending the ovaries to the abdominal wall will reduce ovarian adhesion formation. This reduction may result in better pain control and higher fertility rates.

What are the outcome measures?

The primary outcome was the grade of ovarian adhesions with and without ovarian suspension.

A secondary outcome measure is the difference in pain between the 2 sides of the abdomen in the first 3 post-op days i.e. relationship of pain scores to ovarian suspension.

Appendix 2a – Final RCT Protocol

Study Protocol Version 3 (Date: 15/03/10)

Randomised study into the benefit of temporarily suturing the ovaries to the abdominal wall (oophoropexy) at laparoscopy for treatment of pelvic endometriosis to reduce the incidence of postoperative ovarian adhesions.

Short title: Effectiveness of ovarian suspension in preventing postoperative ovarian adhesions in women with pelvic endometriosis.

Endometriosis is a common benign condition, which causes a significant morbidity in the population of women of reproductive age. Severe pelvic endometriosis includes the presence of bilateral pelvic side wall and/ or rectovaginal disease. The most effective treatment of severe endometriosis is surgical excision of the disease, which is performed using keyhole surgery (laparoscopy). At the operation, the disease is usually excised completely, however, in a number of women the ovaries become stuck because of postoperative adhesions.

We hypothesise that by suspending the ovaries to the anterior abdominal wall for at least 36 hours following surgery, we would be able to significantly decrease the incidence of postoperative ovarian adhesions, thus providing more effective treatment of pelvic pain and better reproductive outcomes.

In order to see if there is a benefit in suspending the ovaries we propose the following study:

All patients with suspected pelvic endometriosis would attend for a routine transvaginal ultrasound assessment prior to surgical treatment to assess the severity of their endometriosis. Women over the age of 18 with suspected bilateral pelvic endometriosis or endometriosis affecting the pouch of Douglas will be invited to participate in the study.

During laparoscopic treatment for severe endometriosis, both ovaries are routinely suspended to the anterior abdominal wall using a Prolene suture, which is brought out onto the skin and secured using a fine haemostat or 'mosquito' clip during surgery. This is done to facilitate surgical excision of the disease and currently the sutures are removed at the end of the operation. The ovaries will then resume their normal anatomical position within the lesser pelvis.

Women who are included in the ovarian suspension study will be randomised to have one ovary suspended for at least 36 hours. At the end of the operation, one suture will be released allowing that ovary to fall back into the pelvis. A new transabdominal suture will then be placed at the same site (contralateral to the suspended ovary). The air in the abdomen (pneumoperitoneum) will then be deflated and the Prolene stitch of the suspended ovary will be tightened with a surgical knot placed over the skin to secure the ovary to the abdominal wall. This will ensure that the suspended ovary is lifted as far away from the pelvis as possible. The surgical knot will be secured with the space of a Carless scissors between the skin and the surgical knot to allow easier removal of the suture and reduce patient discomfort. All randomised patients will therefore have two abdominal sutures of similar length and both the patient and ward staff will remain unbiased as to which ovary has been suspended.

In the presence of ovarian cysts, there is no planned reconstruction of the ovary after excision of cyst (ovarian cystectomy). Healthy ovarian tissue will be opposed using the

same 2/0 Prolene stitch used for ovarian suspension and haemostasis achieved using diathermy.

A sticker will be attached to the operation notes to define a) the randomization number b) the operation date and time and c) the earliest suture removal time (after 36 hours). There will be no documentation of the randomization site in the operation notes.

Both sutures will be cut between 36 to 48 hours after surgery, prior to the patient being discharged home. Both sutures will be cut by a ward nurse who will not be part of the study and will not be aware of the ovarian suspension site. Instructions will be given to the surgeons and ward staff not to discuss possible ovarian suspension site with the patient after surgery or during the time of suture removal.

In the event of postoperative pain or complication, both abdominal stitches will be cut. The time at which this is performed will be documented in the patient's notes. If the ovarian suspension was performed for less than 36 hours, the patient will be excluded from the study.

Three months after their operation, all patients participating in the study will be invited for a transvaginal ultrasound scan to assess the mobility of the ovaries. Adhesions will be diagnosed in women with evidence of restricted ovarian mobility on targeted palpation using transvaginal ultrasound probe. The ultrasound operators will be blinded to the details of the operative procedure and the site of temporary postoperative ovarian suspension.

Statistical Considerations

Pauline Rogers and Caoimhe O'Sullivan were involved in the trial design. Caoimhe O'Sullivan calculated the original sample size calculations in September 2003 and Pauline Rogers revised these when the protocol was amended in 2009.

1) **Sample size calculation**
   Women with bilateral endometriosis will receive the normal surgical treatment with the difference that one ovary will be randomised to suspension and the other to non-suspension. The primary outcome is the binary variable of the presence of ovarian adhesions three months after surgery.

   The data is paired binary data. The sample size calculation assumes that three months after surgery 60% of the non-suspended ovaries will exhibit ovarian adhesions and 30% of the suspended ovaries will exhibit ovarian adhesions. The calculation follows section 7.3 in 'Sample Size Tables for Clinical Studies' by David Machin, Michael J Campbell, Say Beng Tan, & Sze Heuy Tan, Wiley-Blackwell, third edition 2009. The calculation assumes that the response to suspension is independent to the response to non-suspension. In a pilot study on women undergoing bilateral surgery (unpublished internal data) the intra-class correlation coefficient for the presence of adhesions three months after surgery was calculated to be 0.52, so this assumption may not be true but may be considered reasonable for sample size calculation purposes (Machin page 69). The software provided by Machin et al was used to calculate that 45 women would be required for the study, assuming two-sided 5% significance and 80% power. If it is assumed that there is a 10% dropout over three months, then 50 patients should be recruited to the study.

   36 women had the procedure in a period of 15 months (internal unpublished data): on average 2.4 procedures were carried out per month. Assuming 2.4 women have the procedure per month it will take 21 months to recruit 50 women to the study. Allowing for the 3 months follow up period, data collection will take two years.

2) Statistical analysis

a) The background characteristics of patients recruited to the trial will be described with means and standard deviations (or medians and inter-quartile ranges) for continuous variables and frequency counts and percentages for categorical variables. The background characteristics of the patients are: age in years, use of hormonal contraception (0 = no hormonal treatment, 1 = hormonal treatment) and pre-operative ultrasound assessment endometriosis score (0 = disease absent [score = 0], 1 = minimal disease [score = 1-5], 2 = mild disease [score = 6-15], 3 = moderate disease [score = 16-40] and 4 = severe disease [score = >40]).

b) A CONSORT diagram will be produced to show the flow of patients through the study (Figure 7).

c) The primary outcome, presence of ovarian adhesions, three months after surgery, was recorded for each ovary and analysed with a McNemar test. Statistical significance will be declared at the 5% level. The difference between suspended and unsuspended ovaries in the percentage with adhesions will be reported with 95% confidence limits.

d) Analysis of the secondary outcomes:

   i. The variable, adhesion score, will be analysed in a secondary analysis. The score ranges from 0 (no adhesions) to 3 (fixed ovaries). The adhesion score will be recorded for each ovary and the difference between the suspended and unsuspended ovaries was analysed with a McNemar test. A statistically significant result would only be confirmed in an independent fully powered study. The data from this study could be used for sample size calculations for future studies.

   ii. The presence and intensity of postoperative pain, will be measured using the visual analogue scale, where 0 = no pain and 10 = severe pain, will be summarised with frequency counts and percentages.

e) The frequency and percentage of missing data will be reported for each variable.

f) The frequency and percentage of patients who do not comply with the study protocol will be reported. The reasons for non-compliance will be listed.

g) The time point, frequency and percentage of patient withdrawals will be reported. The reasons for withdrawal will be listed.

h) There were no plans for interim analyses.

i) An independent statistician will carry out the final statistical analysis once trial follow up was complete.

Randomisation:

Subjects will be randomised to two equal groups, one group will have the left ovary suspended and the other group will have the right ovary suspended. Block randomisation will be used with three varying block sizes of minimum size 4.

The randomisation schedule will be produced by our statistician Pauline Rogers using the external Stata command *ralloc*. The randomisation schedule and instructions for producing the randomisation envelopes will be handed to Sian Saw in a sealed envelope. Sian Saw is completely independent from the trial team who will be recruiting to the trial.

When a patient is recruited to the trial, consecutive randomization envelope will be opened and the principal surgeon will be told which ovary to suspend. Only the patient's randomization number will be recorded in the patient's operation notes. The principal surgeon will not inform the study team or clinical team responsible for the postoperative care or the patient of which ovary has been suspended.

At the end of the study, the randomization will be unblinded for analysis and details of the ovarian suspension will be added to each patient's record. A copy of the randomisation schedule will be kept by Pauline Rogers on her computer in her personal area. A second copy of the randomisation schedule will be kept with the sister in charge of ward T13 in a sealed envelope, in case of the need for emergency unblinding. Unblinding will only take place on instruction from the principal investigator or his appointed deputy.

Summary of study protocol:

1) Preoperative transvaginal ultrasound scan is routinely performed to assess the severity of endometriosis.
2) Women with ultrasound features suggestive of severe endometriosis will be given an information leaflet and consented for the study.
3) In theatre:
    - Principal surgeon grades severity of endometriosis and adhesion using the revised American Fertility Society Scoring System for Endometriosis.
    - Patients will have laparoscopic treatment for endometriosis which includes a routine oophoropexy. The Prolene stitch used for suspension will secured using a fine haemostat or "mosquito" clip during surgery.
    - In the presence of ovarian cysts:
        - No planned reconstruction of the ovary.
        - Healthy ovarian tissue will be opposed using the same 2/0 Prolene stitch used for the ovarian suspension.
        - Haemostasis will be achieved with diathermy.
4) Randomization:
    - Only patients with severe endometriosis will be entered into study.
    - After complete laparoscopic treatment, patients included in the study will be randomised to have only one ovary suspended for at least 36 hours.
    - Consecutive randomization envelopes will be opened to obtain the ovarian suspension instruction. The suture holding the contralateral ovary is cut and the ovary is allowed to fall back into the pelvis. A new transabdominal suture will be placed at the same site.
    - At the end of surgery, the air in the abdomen (pneumoperitoneum) will be deflated. The Prolene stitch is tightened and a surgical knot is placed over the skin to secure the ovary to the abdominal wall. The same surgical knot is placed over the skin on the contralateral site. This will ensure that the suspended ovary is lifted as far as possible away from the pelvis as possible.
    - The surgical knot will be secured with the space of a Carless scissors between the skin and the surgical knot. This is to allow easier removal of the suture and reduce patient discomfort.
    - This will ensure that each patient will have two abdominal sutures that are similar in length and remain unbiased as to which ovary was suspended.
    - A sticker will be attached to the operation notes
    - No documentation of the randomization site in the operation notes.
5) Ovaries will be suspended for at least 36 hours and up to 48 hours.
    - A ward nurse who will not be part of the study and will not be aware of the suspension site will cut both sutures.
    - Instructions will be given not to discuss possible suspension site with the patient after surgery or during the time of suture removal.
6) Abandoning of suspension:
    - In the event of postoperative pain or complication, both abdominal stitches will be cut. The time at which this is performed will be documented in the patient's notes.
    - If the ovarian suspension was performed for less than 36 hours, the patient will be excluded from the trial.
7) Three months after operation, a transvaginal ultrasound scan is performed to examine for the presence of adhesions by assessing the mobility of the ovaries.

Appendix 2b – Patient Information Sheets and Consent Form

University College London Hospitals NHS
NHS Foundation Trust

Patient Information Sheet

Title: Effectiveness of ovarian suspension in preventing postoperative ovarian adhesions in women with endometriosis

Researchers:
Dr. W Hoo, Mr. E Saridogan, Mr. G Pandis, Mr. A Cutner and Mr. D Jurkovic.
[redacted]

We suspect that you may have endometriosis and would like to invite you to help us in our research study. This information sheet will provide you with information about the reasons for us wishing to conduct this study and what would be expected of you should you decide to help us.

Background: Endometriosis is a common gynaecological condition, which typically presents with pelvic pain and fertility problems. It is caused by tissues, which are similar to the lining of the womb growing inside women's pelvis. This usually affects the ovaries, bowel and the thin membrane covering the pelvic organs. Endometriosis can lead to the formation of ovarian cysts and extensive scarring within the pelvis, which causes pelvic pain. Severe endometriosis is most effectively treated using keyhole surgery. During operation, endometriosis tissue is excised and scarring is cleared to free the ovaries and other pelvic organs from the disease. However, following successful excision of endometriosis, women may still experience pelvic pain because the ovaries sometimes become stuck to the bottom of the pelvis due to postoperative scarring.

What does the study involve? A surgical technique to reduce the chance of ovaries being stuck to the scar tissue has been proposed. This technique involves suspending the ovaries to the abdominal wall for at least 36 hours after the operation to clear endometriosis. We do not know how effective this technique is and this is the reason why we are conducting this study. In order to find the answer to this question, we are planning to keep one ovary stitched to the abdominal wall for 36 to 48 hours after the operation, whilst the other ovary would be allowed to fall back into the pelvis. By performing an ultrasound scan three months after the operation, we will try to find out whether the ovary, which was stitched to the abdominal wall, is less likely to be stuck to the bottom of the pelvis.

Who can take part in the study? We will only invite women over the age of 18 years of age with confirmed diagnosis of severe endometriosis (affecting both ovaries and/or pouch of Douglas) to help us with this study.

Do I have to take part in the study? It is up to you to decide whether or not to participate. If you do decide to take part, you will be given this information leaflet to keep and asked to sign a consent form. If you decide to take part you are still free to withdraw from the study at any time. A decision to withdraw will not affect your future medical care.

What will happen if I take part? You will have an ultrasound scan and be booked for the operation as normal. If diagnosis of severe endometriosis is confirmed at surgery, your ovaries would routinely be suspended to the anterior abdominal wall to facilitate removal of disease. Once the operation is completed, one of the ovaries will be let free while the other ovary will be kept suspended for 36 to 48 hours. You will not be able to tell which ovary is suspended as we will leave stitches on both sides of the abdomen. Both stitches will be cut before you go home.

Three months after the operation, you will be offered an ultrasound scan during your routine postoperative review to determine whether your ovaries are stuck with adhesions.

What are the risks of the study? This study will not in any way interfere with your treatment of your endometriosis or postoperative care. Suspending an ovary for 36 hours is in addition to the usual operation and may add benefit in terms of long-term outcome. There are no known additional risks involved as a result of this procedure. There may be a very small chance that adhesions may form around the suspended ovary although this has not been our experience to date.

Will my taking part in this study be kept confidential? All information collected about you during the research will be kept strictly confidential and anonymous.

What will happen to the results of the research study? The results will be analysed, presented in scientific meetings and published in peer reviewed journals. Your identity will not be revealed in any report or publications.

The local Research Ethics Committee has reviewed this study and given its approval.

For further information, please contact [redacted]

Thank you for your time and consideration.

University College London Hospitals NHS
NHS Foundation Trust

Study: Effectiveness of ovarian suspension in preventing postoperative ovarian adhesions in women with endometriosis.

Patient Identification:

CONSENT FORM

Please tick box

1) I confirm that I have read and understood the information sheet for the above study and I have had the opportunity to ask questions.
2) I confirm that I have had sufficient time to consider whether or not you want to be included in the study.
3) I understand that this is in addition to my usual procedures of treatment and that my participation is voluntary. I have the right to withdraw from the study at any time without giving any reason and without my medical care or legal rights being affected.
4) I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from UCLH, from regulatory authorities, from the NHS Trust or representatives of the sponsor for purposes of monitoring and auditing, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.
5) I agree to take part in the above study.

_____	_____	_____
Patient name	Signature	Date
_____	_____	_____
Person taking Consent	Signature	Date

Researcher to be contacted if there are any problems: Dr William Hoo

Comments or concerns during the study:
If you have any comments or concerns you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should write or get in touch with the Complaints Manager, UCL hospitals. Please quote the UCLH project number at the top this consent form.

Appendix 2c - Ethical Approval for Substantial Amendment

The Joint UCL/UCLH Committees on the Ethics of Human Research (Committee A)
ICH Research & Development Directorate Office,
[redacted]

09A 180

08 April 2009

Mr Ertan Saridogan
[redacted]

Dear Mr Saridogan

Study title:	Effectiveness of ovarian suspension in preventing post-operative ovarian adhesions in women with endometriosis – Mr Ertan Saridogan
REC reference:	003/0279
Amendment date:	6 March 2009

Thank you for submitting the above amendment, which was received on 10 March 2009. The amendment was considered at the meeting of the Sub-Committee of the REC held on 02 April 2009.

Ethical opinion

I am pleased to confirm that the Committee has given a favourable ethical opinion of the modified amendment on the basis described in the notice of amendment form and supporting documentation.
Please note your patient information sheet is dated 4 Feb 2008 although in the list of submitted documents it is listed as 2009, I have made this change in pen (I assume this was an oversight) please change this in your version. Also version numbers and dates are not listed on the consent form although I believe the intention is to submit as a combined document, please add these on the consent forms also.

Approved Documents

The documents reviewed and approved are:

Document	Version	Date
Protocol	2	4 February 2009
Participant Information Sheet & Consent	2	4 February 2009
Notice of Substantial Amendment	1	6 March 2009

Membership of the Committee

The members of the Committee who were present at the meeting were Prof Raymond MacAllister and Dr Robert Urquhart.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of Compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

003/0279: Please quote this number on all correspondence

Yours sincerely

A Mittu
Committee Co-ordinator

Appendix 3 – Trial Preparations

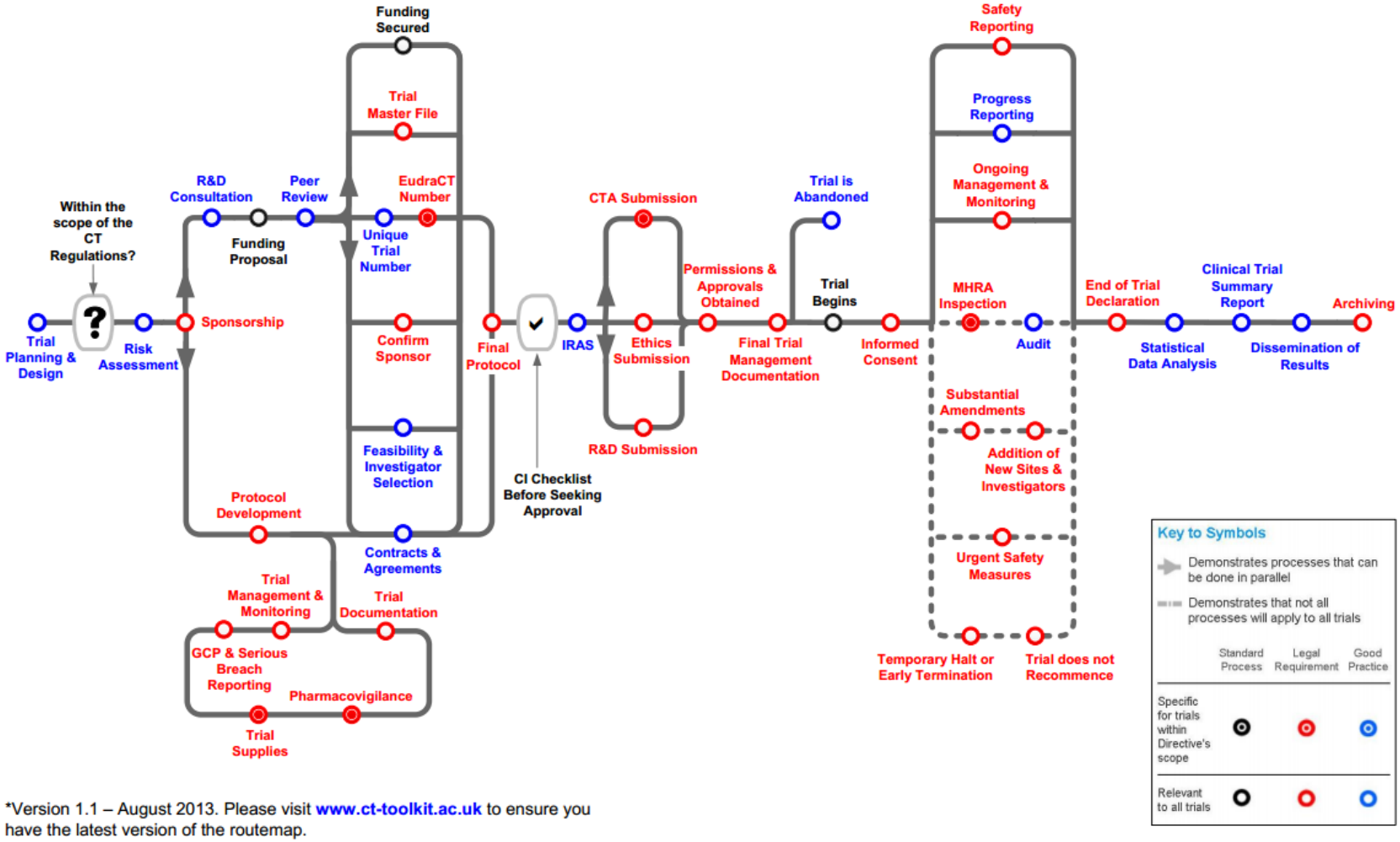
3.1 Regulations

Clinical trials in the UK are regulated by The Medicines for Human Use (Clinical Trials) Regulations 2004. These regulations implement the European Directive 2001/20/EC ('The Clinical Trials Directive'). Clinical trials of medicinal products in human subjects are termed, Clinical Trials of Investigational Medicinal Products (CTIMPs) and require authorisation of the Medicines and Healthcare Products Regulatory Agency (MHRA) in the UK. This authorisation is granted in the form of a clinical trial authorisation (CTA). As our trial did not involve the use of a 'medicinal product', we were exempted from this authorisation.

All clinical trials in the United Kingdom have to be conducted in accordance with the principles of good clinical practice (GCP) to ensure that all trials are conducted with high standards and minimal risks to patient volunteers.

A robust trial design is essential to ensure a successful outcome and will help ensure that all necessary practical requirements are identified early. The Clinical Trials Toolkit (http://www.ct-toolkit.ac.uk/) provides practical advice to all researchers in designing and conducting publicly funded clinical trials. An interactive route map is available which provides information on best practice and outlines the current legal and practical requirements for conducting clinical trials (Figure 12). Although primarily aimed at CTIMPs, non-CTIMPs researchers will find useful information and guidance to the trial environment.

Figure 11 Clinical trials toolkit route map



*Version 1.1 – August 2013. Please visit **www.ct-toolkit.ac.uk** to ensure you have the latest version of the routemap.

3.2 Sponsorship

The Research Governance Framework requires all health-related research to have a formal sponsor. The sponsor is the individual or institution that takes responsibility for the initiation, management and finance of a study. A sponsor must ensure that a study meets the relevant standards and ensure that arrangements are put and kept in place for management, monitoring and reporting. Sponsors can formally delegate one or more of the elements of sponsorship to the chief investigator. Institutions are expected to review individual studies for sponsorship on a case-by-case basis, usually through a formal application process initiated by the chief investigator.

Our study was undertaken at the University College London Hospital (UCLH), which acted as our sponsor. A formal application process was obtained via ethical review and registration with joint Research and Development (R&D) Office.

3.3 Trial Management and Monitoring

Appropriate planning before the trial and adequate oversight and monitoring during the trial will help ensure that trial subjects safety is maintained throughout the trial and that there is accurate reporting of results at its conclusion. The sponsor maintains responsibility for ensuring that robust trial management systems are put in place, although as mentioned previously, these management activities can be delegated to the Chief Investigator or contracted out to third parties.

Trial monitoring is not a standardised activity that must be implemented in an identical way in all trials. The purpose of trial monitoring is to provide oversight during the conduct of a trial to give reassurance that the study protocol and procedures are being followed, that and legal/governance requirements are

being complied with, and that the critical data collected are reliable. The extent and nature of monitoring would normally be determined prior to the start of the trial and be re-assessed during the course of a trial. Clinical risk assessment may be used to determine the intensity and the focus of the monitoring activity, whilst the trial design would inform the methods used for monitoring.

Documentation should be in place to describe all key processes, to ensure that those performing tasks have a clear plan of what, when and how trial activities are undertaken.

Key details and responsibilities that should be described in trial management documentation include:

1. The trial protocol
2. Organisational structure, including relevant details of the identity and responsibilities of all involved (sponsor, chief investigator, trial management team, host institution as applicable).
3. Details of care organisations, participating sites and investigators.
4. Details of the relevant regulatory approvals (e.g. ethics committee, clinical trial authorisation)
5. The name of the individual who should be the first point of contact in the event of questions about the conduct of the trial (e.g. for audit/inspection purposes).

3.4 Trial Documentations

GCP requires that all clinical trial information be recorded, handled and stored in such a way that it can be accurately reported, interpreted and verified. Essential documents are those, which enable both the conduct of the clinical trial and the quality of the data produced to be evaluated. Many essential documents are filed in a Trial Master File / Investigator Site File.

It should be clear who is responsible for overseeing the preparation of key trial documentation and details of the review and sign-off process. The procedure and responsibility for assessing the substantiality of amendments to key documents such as the regulatory approvals, the protocol and patient information documentation should be documented.

As the principal investigator of our RCT, I was responsible for obtaining and maintaining all the essential documents required for the trial master file.

3.5 Trial Master File (TMF)

A TMF consists of essential documents by which both the conduct of a clinical trial and the quality of the data produced can be evaluated. The TMF should be commenced from the beginning of a trial and maintained throughout the course of the trial in a well-kept manner to facilitate the reconstruction of a trial's conduct during the audit/inspection process. Throughout the trial, the TMF should be kept in a secure but accessible manner. In cases where sponsor responsibilities have been transferred to the investigator, the TMF and Investigator Site Files are often combined.

The European Commission has produced a detailed guidance on the list of essential documents for CTIMP trials. For non-CTIMP research, it would be good practice to file any document that meets the definition of an essential document on this list, although sponsors and host organisations may provide specific guidance on this in their policies. A list of essential documents for CTIMP research at various trial stages include:

- Before commencement of trial
    i. Investigator's brochure with all relevant and current scientific information about the investigational product
    ii. Signed protocol and amendments

iii. Patient information including the informed trial consent form
iv. Financial aspects of the trial
v. Insurance statement (where required)
vi. Signed agreement between investigators, institutions and sponsors
vii. Dated, documented favourable opinion of Ethics Committee
viii. Certificate(s) of analysis of investigational medical product(s)
ix. Decoding procedures for blinded trials in cases of emergency
x. Master randomisation list
xi. Pre-Trial Monitoring Report to document site suitability
xii. Trial Initiation Monitoring Report to document that trial procedures were reviewed with the investigator and the investigator's trial staff

- During the conduct of the trial
    i. Document updates including any revision of the protocol, informed consent forms or patient information
    ii. Curriculum vitae for new investigators and supporting trial staff to whom investigator tasks are delegated
    iii. Updates of medical/laboratory/technical procedures/tests including normal ranges for medical/ laboratory/ technical procedures included in the protocol
    iv. Certification or accreditation or established quality control and/or external quality assessment or other validation
    v. Documentation of the distribution of investigational medicinal products and trial related materials

vi. Certificate(s) of analysis for new batches of investigational products

vii. Monitoring visit reports

viii. Signed informed consent forms in accordance with GCP and protocol and dated prior to participation of each subject

ix. Signed, dated and completed case report forms

x. Notification by originating investigator to sponsor of serious adverse events and related reports

xi. Notification by sponsor and/or investigator, where applicable, to regulatory authority and Ethics Committees of suspected unexpected serious adverse reactions and of other safety information

xii. Notification by sponsor to investigators of safety information arising from clinical trials on medicinal products for human use'

xiii. Interim or annual reports to Ethics Committees and authorities

xiv. Subject screening log to identify trial subjects who entered pre-trial screening to allow investigators and institutional identification of any trial subjects

xv. Subject enrolment log to document the chronological enrolment of subjects by trial number

xvi. Investigational medicinal product accountability at each site

xvii. Signature sheet to document signatures and initials of all authorised personnel making entries or corrections to case report forms

- After trial completion
    - Investigational medicinal products accountability and destruction at each site
    - Completed subject identification code list to identification of all subjects enrolled in the trial in case follow-up is required
    - Audit certificate
    - Final trial close-out monitoring report
    - Treatment allocation and decoding documentation
    - Final report by investigator to Ethics Committees and regulatory authorities
    - Clinical study report to document results and interpretation of trial.

3.6 Contracts and Financial Management

The contractual framework and budget management should be clearly defined. In many circumstances this role is undertaken by the host institution.

In our RCT, the contractual framework was undertaken by our host sponsor, UCLH NHS trust.

3.7 Insurance and Indemnity Arrangements

Arrangements for insurance and indemnity, including arrangements to address negligent harm to the participant and adverse consequences of the interventions or trial procedures that are not due to clinical negligence should be stated. Our RCT was covered by the NHS indemnity procedures.

3.8 Monitoring

Compliance with GCP is often interpreted as requiring active site monitoring, however the extent and nature of monitoring should be based on the objective, design, complexity, size and endpoints of the trial. In general, there is usually a need for on-site monitoring, before, during and after the trial with particular emphasis given to consent, eligibility, documenting safety information and study endpoints. There is now a consensus towards a more flexible and targeted monitoring process.

The 'Risk-adapted Approaches to the Management of CTIMPs' has been published to help sponsors undertake the process of risk assessment. This document outlines a scheme for defining the risks associated with each clinical trial by a simple IMP risk categorisation (Type A, B and C) based on marketing status and standard medical care. This monitoring was not required in our non-IMP trial.

3.8.1 Trial Oversight Committees

The funding body or sponsor may specify particular oversight arrangements. Commonly employed oversight committees include, a Trial Management Group, Trial Steering Committee and Data Monitoring Committee.

3.8.1.1 Trial Management Group (TMG)

Most trials have a TMG. In a small trial such as ours, the Chief or Principal Investigator may perform the functions of the trial management group. The TMG should include individuals involved in day-to-day management of a trial, such as the Chief or Principal investigator, research nurse and statistician. This group should keep a close eye on all aspects of the conduct and progress of the trial. They need to ensure that the trial protocol is adhered to and take necessary actions to safeguard participants and the trial itself. Trials with increasing

complexity will require a more formal structure and in larger trials, a Trial Steering Committee is recommended.

3.8.1.2 Trial Steering Committee (TSC)

The role of a TSC is to provide overall supervision of a trial and to ensure that the trial conduct is in accordance with the principles of GCP and the relevant regulations. Formalised procedures should be in place directing its formation and membership as well as its agreed responsibilities. The TSC should approve the trial protocol, any protocol amendments and provide advice to investigators on all aspects of the trial. The TSC monitors the progress of a trial including the recruitment, data completeness, and losses to follow-up and ensures that there are no major deviations from the trial protocol. A TSC will usually have members who are independent of the investigators, as well as two other independent members.

3.8.1.3 Data Monitoring Committee (DMC)

The role of a DMC is to review the accruing trial data and to assess whether there are any safety issues that should be brought to the attention of the TSC or any ethical reasons why the trial should not continue. A DMC should be considered for every trial, although one may not always be necessary. The decision as to whether or not a DMC would be useful should be based on the potential risks and benefits to subjects associated with the trial and the trial design. In the course of a blinded trial, it is the only body that has access to unblinded data. The DMC should be independent of both the investigators and the funder/sponsor. It should report to the TSC (or TMG if there is no TSC).

Due to the small size of our trial, we decided against a DMC. The chief and principal investigators were responsible for the trial data and safety issues, whilst the statistician held the unblinded data.

3.9 Training

Before a trial can begin, it is customary for an initiation meeting to be held to ensure that all trial staff are adequately trained and ready to start the trial. Investigator's meetings, both before and during a trial, play an important role both in providing trial specific training, reviewing knowledge and understanding of trial procedures. Where training is required, the methods used to deliver training should be described with competency assessments detailed where necessary. The training delivered should be specific to their role and should be an on-going process. The trial team would also need to ensure that new staff joining the trial team receive the appropriate training before they undertake trial specific activities.

The method of training and any on-going knowledge assessment will be influenced by the size of the group and geographical location of centres. For more complex trials where requirements differ markedly from routine care or where the use of novel procedures or specialised equipment are required, a site visit to each participating centre may be required to reassure the trial team that an adequate level of training has been achieved.

In our trial, individual roles did not diverge from the routine care of a patient with severe endometriosis. The surgical team performing the ovarian suspension performed the procedure routinely to aid ovarian retraction during such cases. The ovarian suspension and placebo stitches were cut by a trained nurse before discharge. Trained research fellows and consultants performed the ultrasound assessments both before and after surgery. Both the chief and principal investigator attended a GCP course to familiarise ourselves with the basic principles of GCP.

Appendix 4 - Study Approvals

4.1 Research and Development (R&D) Consultation

Within NHS organisations, there are often R&D Departments or Clinical Research Offices. When organisations work closely together such as a local University and its local NHS Trust, as in the case of University College London, a joint R&D office is formed. These offices act on behalf of its organisation to facilitate the local management of all research within that organisation. They need to ensure that appropriate arrangements are put in place to support the research and that risk management measures including appropriate insurance and indemnity provision are in place.

An R&D Office must give formal permission before a research project can take place within their organisation. When acting as the sponsor, NHS R&D offices will be involved in the oversight of the trial by guiding the Chief Investigator and managing the risks associated with any trial initiated. It is advisable to contact the local R&D Office in the early stages of study development so that they can help identify facilities that can provide valuable support.

It will be important when consulting R&D to define how costs are allocated. A recent guidance has been published to provide a framework for the NHS and its partners to identify and attribute the costs of health and social care R&D in a transparent and consistent manner by differentiating between research costs, NHS service support costs and treatment costs in relation to activities specified in the protocol.

4.2 Funding Proposal

Securing funding can be a lengthy process, therefore the time required to secure funding should be included in the wider development and planning of trial activity. Funders will need to be assured that the proposed research is

important and addresses a clear need, well designed, feasible and scientifically valid, and offers value for money. The funding schemes will have varying eligibility requirements and many funders offer resources to enable researchers to confirm suitability 'in principle' at an early stage.

In our small RCT, formal funding was not sought. However, the principal investigator (WH) was supported by the research fund provided by the Gynaecology Ultrasound Centre, UK.

4.3 Peer Review

Peer review is an opportunity for expert examination of the proposed trial to consider aspects including the design quality, feasibility, acceptability and importance of the topic. Experts in this context will include views from relevant clinicians, allied health professionals and other professional groups, patients and members of the public. Peer reviews will usually be undertaken as part of an external funding application process. However, if an external funding was not required, then the sponsoring organisation will be able to assist with this in the form of an ethical approval process.

After completing our ethical approval, our trial protocol was published in a peer reviewed literature[208]. This not only shores up the transparency of reporting of the trial results, but also allows critical comments from the scientific community at the design and initial phase of the trial.

4.4 Unique Trial Number

The registration of clinical trials is now advocated and each trial must have a unique trial number. Trial registration is regarded as the publication of an internationally agreed standard dataset about a clinical trial on a publicly accessible database managed by a registry conforming to the World Health Organization standards. This requirement is quoted in a number of publications

including the Declaration of Helsinki of the World Medical Association and the Research Governance Framework.

From the 1st of July 2005, the International Committee of Medical Journal Editors has agreed that trial results will not be published unless the study has been included on a clinical trials registry. This is to allow a trial to be tracked from initial protocol through to publication.

The International Standard Randomised Controlled Trial Number (ISRCTN) is a simple numeric identification system that can be used to track all publications and reports resulting from each trial. Alternatively, trials may be registered at clinicaltrials.gov. For CTIMPs, there is an additional mandatory reference, EudraCT number, allocated by the European Medicines Agency.

In England, trials where all costs are met fully by an NHS Trust, there is an automated ISRCTN registration on the NHS Clinical Trial Register. Our trial reference is ISRCTN24242218.

4.5 Confirm Sponsor

For UK trials, the chief investigator will be required to approach a potential sponsor who will assess the operational risk of the proposed trial before confirming sponsorship. For NHS sponsors, the NHS R&D office in their respective organization usually performs confirmation of sponsorship. Sponsorship will only be granted once issues raised by the risk assessment have been addressed. A letter confirming sponsorship must be retained in the Trial Master File.

4.6 Feasibility Assessment

Trials that fail to reach their study targets may not achieve a statistically significant result or require further funding. It is therefore worth considering a feasibility study or as in our case, a pilot study, ideally during the funding

process. This process may help identify possible barriers to the recruitment process or allow for an assessment of the expected recruitment rate.

For larger multi-site trials, careful selection and evaluation of investigator sites is critical for the successful completion of a trial within budget, deadlines and to ensure the generation of high quality data.

4.7 Final Protocol

Before seeking approvals to start a trial, the protocol must be finalised and endorsed by the sponsor, chief investigator and trial statistician. The sponsor will usually specify the signatory requirements. In multi-site trials, it is good practice to ensure the Principal Investigator signs a protocol signature page to confirm receipt and also their agreement to work to the current version of the protocol. Our final protocol is illustrated in Appendix 2 including a new patient information leaflet and consent form (Appendix 3).

4.8 IRAS (Integrated Research Application System)

The IRAS is a single system for applying for the permissions and approvals for health and social care research in the UK. This includes applications for Ethics Approval, Clinical Trial Authorisation, R&D Management approval and 'Notice of Substantial Amendment'. Users of the system will need to register for an IRAS account and there is a free e-learning module, which illustrates the system and its functionality.

Completion of the project filter will enable the required permissions and approvals applications to be created for the specified project. Questions that are not relevant to the type of project will be disabled in the project dataset.

4.9 Clinical Trial Authorisation (CTA) Submission

CTIMP trials in the UK will require a CTA from the Medicine and Healthcare Products Regulatory Agency (MHRA). Prior to this application, each trial must

also be registered on the European Clinical Trials Database by obtaining a EudraCT number. Our non- CTIMP trial was exempted from this process.

4.10 Ethics Submission

A well-designed trial should answer important public health questions without impairing on the welfare of individuals. There may, at times, be conflicts between a physician's perception of what is good for his or her patient and the design and the conduct of the trial. In such instances, the needs of the patient must predominate. The National Research Ethics Service (NRES) exists to protect the rights, safety, dignity and wellbeing of research participants whilst facilitating ethical research that is of potential benefit to participants, science and society. This is achieved through the review of research taking place within the NHS by NRES Research Ethics Committees (RECs). Application to NHS RECs should now be made using IRAS as described above. RECs will give their opinion about the proposed participant involvement and whether the research is ethical.

Although the majority of research conducted within the NHS will require ethical review, some projects are more appropriately classified under clinical audit or service evaluation. If a researcher is unsure as to whether their project will require ethical approval, a Health Research Authority algorithm is available to help determine this.

An ethics approval for ovarian suspension was approved for the original study 2003. As there were no intended changes to the study population or intervention, we were advised following consultation with the ethics department to apply for a substantial amendment. A successful application was obtained for our substantial amendment in April 2009 (Appendix 2c).

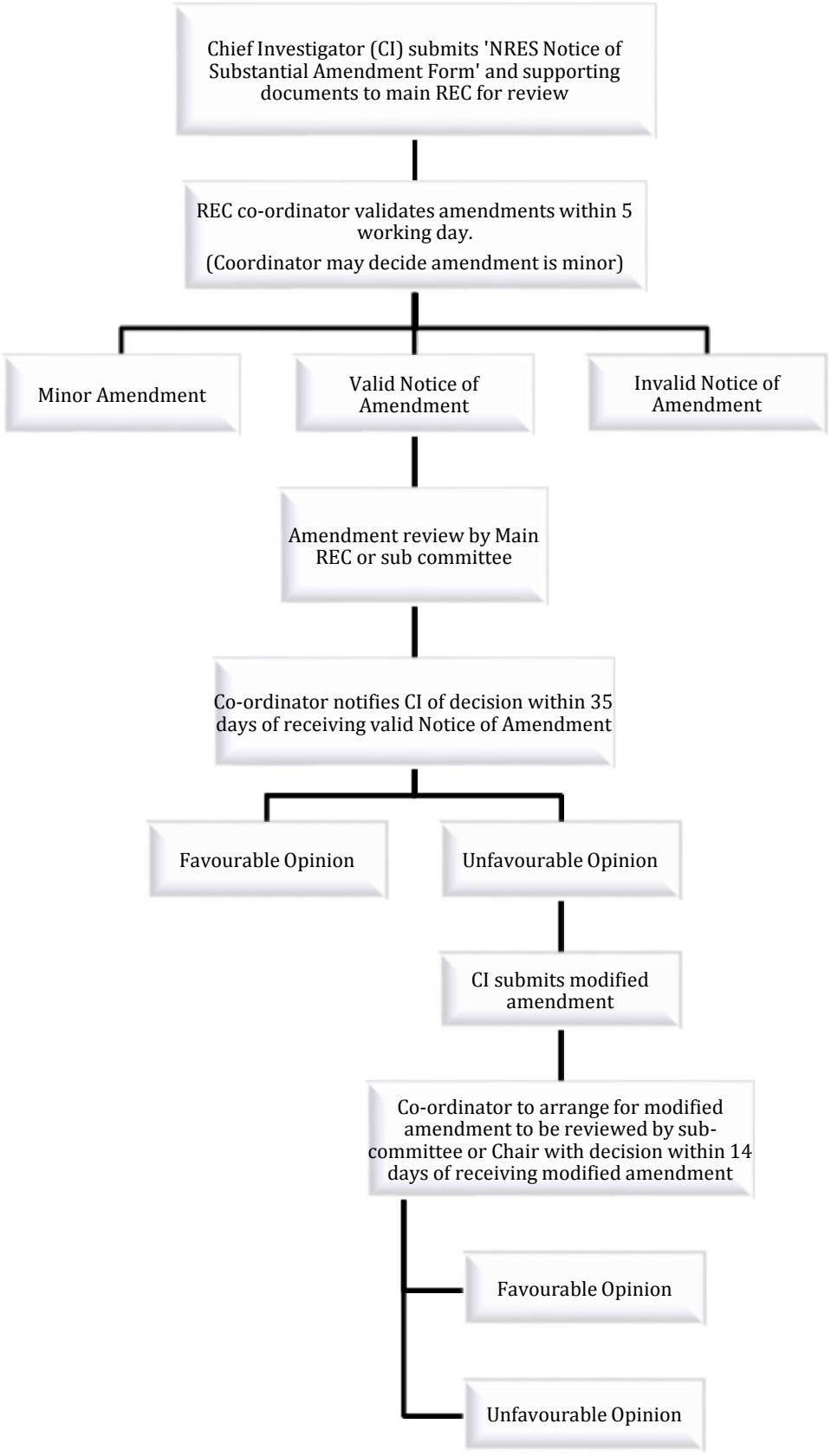
4.11 Substantial Amendments

Amendments are changes made to the research after review body approval has been given. Amendments requiring approval cannot be implemented until the relevant approvals are in place, except in the case of urgent safety measures. A substantial amendment is defined as an amendment to the terms of the application or protocol or any other supporting documentation, that is likely to affect to a significant degree the:

- safety or physical or mental integrity of the trial participants
- scientific value of the study
- conduct or management of the study or
- quality or safety of any IMPs used in the trial.

It is the responsibility of the sponsor to determine whether an amendment is substantial or non-substantial. For both CTIMPs and non-CTIMP research, 'Notice of Substantial Amendment' forms can be created in IRAS. NHS R&D offices sponsoring research will need to be notified of all amendments so that they may assess for any impact on governance arrangements or resources.

Figure 12 Flowchart illustrating the process of ethical review of substantial amendments to approved research



4.12 R&D Submission

Research conducted within NHS organisations must obtain permission from the R&D department of each organisation taking part in the trial. Without this approval in writing, indemnity and insurance cannot be assumed to be in place to cover the proposed research activity. The R&D permission process is two-fold with general trial information captured in the NHS R&D form and local site information on the NHS Site-Specific Information (SSI) form. Each of the SSI forms will need to be completed separately with local information. NHS R&D offices assess the suitability of the local research site and investigator. Applications for NHS permission in England, Wales, Scotland and Northern Ireland should now be made using IRAS, which will include guidance on the completion and submission of the NHS R&D form and specific SSI forms. NHS permission will only be issued after all other approvals required for the trial have been granted.

4.13 Permissions & Approvals Obtained

A trial cannot begin until all the relevant permissions and approvals have been obtained and reviewed by the chief investigator and sponsor. Clear evidence of the documents submitted to the approval bodies and the approval letters need to be retained in the TMF. For multi-site trials, the Chief Investigator will ensure that each Principal Investigator is provided with all relevant, version-controlled documents before commencing recruitment.

Appendix 5 – Study Begins

5.1 Trial Begins

Trial commencement is often accomplished by holding a start-up meeting. This will allow the Chief Investigator to satisfy him or herself with all the technical aspects and to ensure that the protocol requirements are fully understood by all relevant site staff. It is also a great opportunity to ask questions and clarify misunderstandings.

Trial specific training, as well as training on aspects of trial conduct and safety reporting requirements are often undertaken at this stage. For CTIMPs, this communication should include a pharmacist, so that all requirements can be confirmed before dispensing IMPs.

A start-up meeting was held in October 2009, prior to the commencement of our trial and was attended by all the investigators.

5.2 Informed Consent

With the exception of certain emergency trials involving incapacitated adults or minors, all participants must give their informed consent before being entered into a trial. For CTIMPs, Schedule 1 of The Medicines for Human Use (Clinical Trials) Regulations 2004 describes the requirements for consent. For non-CTIMPs conducted in England and Wales, it is the Mental Capacity Act that regulates inclusion into research. This is to ensure that all UK trials are conducted to the appropriate high standard and that risks to patient volunteers are minimised.

For each trial, specific consent documents consisting of a participant information sheet and consent form must be developed and approved by the ethics committee. The ethics committees usually encourage the involvement of patient groups in the development of these documentations.

It is imperative that informed consent is given freely, which may be challenging in trials involving complex interventions, potentially toxic treatments or invasive procedures. Training and competency assessment of investigators obtaining informed consent may be helpful to ensure that the trial is presented in a balanced manner. These techniques may reveal deficiencies in the level of understanding, style of presentation, or extent of discussion in the consent process. If training is required, all those who may request consent from subjects participating in the trial should be included. For simple or low risk trials, it is often sufficient to check that the consent form has been signed and dated and that there is a record of the information provided to subjects.

Throughout the trial, the subject's willingness to continue participation should be reaffirmed periodically. If significant new information becomes available during the course of the trial, participants will need to be provided with revised and re-approved consent documentation so that written consent can be formally documented if the subject is willing to continue.

5.3 Progress Reporting

During the course of a trial, there is a requirement to send regular progress reports. The ethics committee, trial sponsor and R&D Offices where the trial is conducted usually require an annual report. This was completed yearly during the course of our trial. For CTIMPs, this progress report is in addition to an annual safety report.

5.4 Trial Communication

Details relating to the communication of key trial information should be in place. This should include the contents, frequency or timing and mode of communication used. Regular project meetings to review trial progress should be recorded so that all actions, key decisions and timelines are clear.

5.5 Good Clinical Practice (GCP) Inspections

The MHRA are required under European law to inspect all CTIMPs. The sponsors themselves would evaluate the efficiency of their quality control by selecting a number of trials to review their compliance with their relevant legislation and guidance. GCP inspections will include an element of risk assessment within them and will consider the nature of the trial conducted, the extent and vulnerability to the population studied and any prior inspection history. Where possible, the focus is on more complex trials, although trials equivalent to standard care have been included to evaluate the system. Findings that could result an inspection varies from inadequate documentation to concerns regarding participant safety.

We were not subjected to a GCP inspection during the course of our trial.

5.6 GCP & Serious Breach Reporting

The Research Governance Framework requires that all research are conducted in accordance with the principles of GCP. This is to ensure that the rights, safety and wellbeing of trial participants are protected. For CTIMPs, Part 5 of the Medicines for Human Use (Clinical Trials) Regulations SI 1031 (sections 32-35) defines the responsibilities for safety reporting of both the sponsor and the investigational site. For non-CTIMP research, serious breaches of GCP or the protocol should be reported to the relevant ethics committee so implementation of corrective and preventative actions can be made.

Any serious breach of the conditions and principles of GCP or the protocol relating to the trial will need to be reported. A “serious breach” is a breach that is likely to affect to a significant degree the safety or physical or mental integrity of the subjects of the trial, or the scientific value of the trial.

The judgement on whether a breach is likely to have a significant impact on the scientific value of the trial will vary depending on the design of the trial, the type and extent of the data affected and the overall contribution of the data to analysis. It is the responsibility of the sponsor to assess the impact of the breach on the scientific value of the trial and should be documented.

Deviations from clinical trial protocols and GCP occur commonly in clinical trials. The majority of these instances are technical deviations that do not result in harm to the trial subjects or significantly affect the scientific value of the reported results of the trial. These cases should be documented in order for appropriate corrective and preventative actions to be taken. In addition, these deviations should be included and considered when the clinical study report is produced, as they may have an impact on the analysis of the data. The sponsor or responsible person should make notification of serious breaches within 7 days of being aware of the breach.

Serious Adverse Events (SAEs) and Suspected Unexpected Serious Adverse Reactions (SUSARs) resulting from a breach of the conditions and principles of GCP or a breach of the protocol will constitute a serious breach. However, not every SAE or SUSAR would routinely be classified as a serious breach.

5.7 Urgent Safety Measures

The Clinical Trials Regulations make provision for the sponsor and investigator to take appropriate Urgent Safety Measures (USMs) to protect a research participant from an immediate hazard to their health and safety. This measure can be taken before seeking approval from the competent authorities including MHRA or ethics committees.

The Chief Investigator must notify the main REC immediately of any USMs and in any event within three days. NHS R&D offices will also require notification in

accordance with local policies/procedures. Any USMs relating to a CTIMP must also be communicated to the MHRA immediately via telephone with a written notification within three days of the action being taken. The notification should be in the form of a substantial amendment and should describe the event, the measures taken and justification for the measures taken.

There was no requirement to undertake a USM during our trial.

5.8 End of Trial Declaration

The definition of the end of the trial should be provided in the protocol. In most cases this will be the date of the last visit of the last patient undergoing the trial or the date of the final data capture where follow up monitoring is required.

For CTIMP research, the Clinical Trials Regulations require the sponsor to notify the MHRA and ethics committees of the end of a trial within 90 days. A EudraCT Declaration of End of Trial Form should be completed. NHS R&D offices will also require notification in accordance with local policies/procedures.

For non-CTIMP research, notification to the relevant ethics committee and R&D offices is required. The 'NRES Declaration of the End of Trial Form' should be used. This was done at the end of our trial.

5.9 Statistical Data Analysis

In the majority of trials, sponsors would require appropriate arrangements to be specified during the trial design phase and the services of an appropriately trained statistician to be secured. This is to ensure that the analyses to evaluate all planned study hypotheses are conducted in a scientifically valid manner and that all decisions are documented. Support for trial data management and statistical analysis is available from a range of sources including local R&D departments and UKCRC registered Clinical Trials Units.

The statistical analysis should include the:

- Primary and key secondary outcome measures stated in the protocol
- Methods for handling missing data and multiplicity of data
- Justification for any non-standard statistical techniques
- Details of any subsequent post hoc analysis to be justified and reported in any publication

The trial results should be discussed with the Chief Investigator and other relevant oversight groups including the DMC, to assist interpretation and implications of the findings. Other important considerations include practicalities relating to the blinding of the trial statistician and documentations of all data manipulations and analyses performed on the original data to allow replication of analysis. After analysis, all relevant documentation in the possession of the statistician should be filed in the TMF.

5.10 Clinical Trial Summary Report

The investigators must provide a clinical trial summary report to the REC (and MHRA for CTIMPs) within 12 months of the end of the study. Although there is no standardized format for such reports, as a minimum, the report should include details of whether the trial achieved its objectives, main trial findings and arrangements for publication or dissemination of the research. For CTIMPs research, the final report should be formatted according to the ICH E3 guideline for structure and content.

RCTs should be reported in compliance with the CONSORT Statement. This initiative was developed to improve the reporting of RCTs, enabling readers to understand a trial's design, conduct, analysis and interpretation, and to assess the validity of its results. Similar initiatives have been developed for other study designs, STARD for reporting diagnostic accuracy studies, STROBE for

reporting observational studies in epidemiology and PRISMA for reporting systematic reviews.

5.11 Dissemination of Results

It is important that the results of clinical research are disseminated to the research community, trial participants and general public. The most obvious route to inform the research community is through publication in peer-reviewed scientific journals.

The dissemination of research findings promotes research participation and demonstrates that findings have improved current clinical practice. Informing participants of results acknowledges their contribution to the trial. It is important to establish whether a participant will want to be actively informed of trial results, or whether they would like the onus to be left with them to obtain the results. Organisations may also employ different other strategies for informing the public of their trial findings. This may include publication of trial results in open access journals, trust websites or employing the use of leaflets in hospital waiting rooms. It is good practice for investigators to check whether the NHS R&D offices that gave approval require a copy of any publications or reports.

5.12 Conflict of interest

A widely expressed concern in clinical research is the potential for conflict of interest on the part of the investigators. Ideally, no investigator would have any interests other than the wellbeing of the study participants and the generation of new knowledge, however, financial or intellectual conflicts may occur. In the ethical contest, conflict of interest can lead to bias in design, conduct, analysis, interpretation and communication of findings.

Most investigators manage conflict of interest by disclosing financial relationships to participants, although it may not be fully apparent what impact

financial relationships might have on the research design, conduct and analysis. Any investigators with economic interests in the outcome should either not participate or not have opportunities to affect and publish the trial outcome. Completely openness and data analysis by an independent group is crucial. Ultimately, a clinical trial results must be believed and accepted by the clinical communities and if the extent of conflict of interest lessens that acceptance, then a study is impaired.

Possible conflicts of interest in our study were declared in our publications. Ertan Saridogan received honoraria from Ethicon for provision of training to healthcare professionals and consultancy fees from Bayer. Alfred Cutner was on the advisory board for surgical innovations for which he received annual honorarium. He also received support for courses and education from Storz and Johnson and Johnson and support for clinical nursing from Covidien and Lotus. Other authors declared no competing interests.

5.13 Archiving

All study documentations including the TMF, case report forms and other essential documents must be archived. Consideration should be given for the archive of both paper and electronic data. For CTIMP research, the sponsor and chief investigator must ensure that the medical files of trial subjects are retained for at least 5 years after the conclusion of the trial. Clinical Trials Regulations require the sponsor to appoint ‘named individuals’ within its organisation to be responsible for archiving and setting up systems to track and retrieve archived documents. Named individuals should also ensure that archive facilities are secure with appropriate environmental control and adequate protection from physical damage.

For non-CTIMP research, the archive time period is usually stipulated by the local sponsor, although NHS Research Ethics Committees should retain all relevant records for a period of at least three years after completion of a research project.

Appendix 6 –Publications List from this MD

Hoo WL, Saridogan E, Cutner A, et al. Effectiveness of ovarian suspension in preventing post-operative ovarian adhesions in women with pelvic endometriosis: A randomised controlled trial. *BMC Womens Health* 2011; 11: 14.

Hoo WL, Stavroulis A, Pateman K, et al. Does ovarian suspension following laparoscopic surgery for endometriosis reduce postoperative adhesions? An RCT. *Hum Reprod* 2014; 29: 670–676.

Hoo WL, Stavroulis A, Pateman K, et al. Reply: Criticizing the effect of ovarian suspension on adhesions in laparoscopic surgery for endometriosis. *Hum Reprod* 2014; 29(7): 1597-1598.

Hoo WL, Stavroulis A, Pateman K, et al. Reply: Ovarian suspension for longer than 36 h is necessary for temporary ovarian suspension to fulfil its remit. *Hum Reprod* 2014; 29(8): 1832.

Appendix 6a – Study Protocol- Effectiveness of ovarian suspension in preventing post-operative ovarian adhesions in women with pelvic endometriosis: A RCT

Hoo et al. BMC Women's Health 2011, 11:14
http://www.biomedcentral.com/1472-6874/11/14

BMC Women's Health

STUDY PROTOCOL Open Access

Effectiveness of ovarian suspension in preventing post-operative ovarian adhesions in women with pelvic endometriosis: A randomised controlled trial

Wee-Liak Hoo*, Ertan Saridogan, Alfred Cutner, George Pandis and Davor Jurkovic

Abstract

Background: Endometriosis is a common benign condition, which is characterized by the growth of endometrial-like tissue in ectopic sites outside the uterus. Laparoscopic excision of the disease is frequently carried out for the treatment of severe endometriosis. Pelvic adhesions often develop following surgery and they can compromise the success of treatment. Ovarian suspension (elevating both ovaries to the anterior abdominal wall using a Prolene suture) is a simple procedure which has been used to facilitate ovarian retraction during surgery for severe pelvic endometriosis. The study aims to assess the effect of temporary ovarian suspension following laparoscopic surgery for severe pelvic endometriosis on the prevalence of post-operative ovarian adhesions.

Methods: A prospective double blind randomised controlled trial for patients with severe pelvic endometriosis requiring extensive laparoscopic dissection with preservation of the uterus and ovaries. Severity of the disease and eligibility for inclusion will be confirmed at surgery. Patients unable to provide written consent, inability to tolerate a transvaginal ultrasound scan, unsuccessful surgeries or suffer complications leading to oophorectomies, bowel injuries or open surgery will be excluded.

Both ovaries are routinely suspended to the anterior abdominal wall during surgery. At the end of the operation, each participant will be randomised to having only one ovary suspended post-operatively. A new transabdominal suture will be reinserted to act as a placebo. Both sutures will be cut 36 to 48 hours after surgery before the woman is discharged home. Three months after surgery, all randomised patients will have a transvaginal ultrasound scan to assess for ovarian mobility. Both the patients and the person performing the scan will be blinded to the randomisation process.

The primary outcome is the prevalence of ovarian adhesions on ultrasound examination. Secondary outcomes are the presence, intensity and site of post-operative pain.

Discussion: This controlled trial will provide evidence as to whether temporary ovarian suspension should be included into the routine surgical treatment of women with severe pelvic endometriosis.

Trial registration: ISRCTN: ISRCTN24242218

Background

Endometriosis is a common benign condition, which is characterized by the growth of endometrium-like tissue in ectopic sites outside the uterus. The condition is a significant cause of morbidity in women of the reproductive age [1]. Symptoms of endometriosis include dysmenorrhoea, dyspareunia, chronic pelvic pain and subfertility. The revised American Society for Reproductive Medicine (ARSM) classification is the most widely accepted staging system for endometriosis, where a score is used to grade the disease as absent (0), minimal (1-5), mild (6-15), moderate (16-40) or severe (> 40) [2].

Surgical excision of the disease is frequently carried out for the treatment of severe endometriosis. Laparoscopic approach to surgery provides superior views of the pelvis and facilitates complete excision of endometriotic lesions [3]. Although laparoscopic surgery is considered to be less traumatic to pelvic tissues than open surgery, a significant

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number of women develop post-operative pelvic adhesions even after this form of surgery. The most common site of post-operative adhesions formation is the ovary [4]. Formation of severe post-operative adhesions can compromise the success of surgery for endometriosis by causing chronic pelvic pain, infertility, dysparaeunia and intestinal obstruction [5]. Adhesions formation after laparoscopic endometriosis surgery has been reported in the range of 50 to 100 percent [5-8].

The high incidence of post-operative adhesions in endometriosis patients and their clinical significance underlines the importance of modifying surgical techniques in order to reduce potential adhesion formation. Intra-peritoneal administration of anti-adhesive solutions (e.g. icodextrin, hyaluronic acid) and drugs (e.g. steroids, heparin) at the time of surgery has been advocated as a way of reducing the incidence of post operative adhesions. Currently, only anti-adhesive fluids containing hyaluronic acid have showed evidence of reduction of adhesions however, more studies will be needed to confirm this [9]. Adjuvants have also been suggested to further improve the adhesion reduction, however to date, the most effective product for prevention of postoperative adhesion is yet to be discovered [10].

Ovarian suspension

Ovarian suspension is a simple procedure which has been used to facilitate ovarian retraction during surgery for severe pelvic endometriosis without any reported complications [11]. A study by Abuzeid et al. showed that temporary ovarian suspension following surgery for severe endometriosis may lead to a reduction in post-operative adhesions [12]. A small retrospective study also found significant reductions in the number of women with post-operative adhesions following ovarian suspension in comparison with data from the literature [13].

Objective

The aim of this study is to assess the effect of temporary ovarian suspension following laparoscopic surgery for severe pelvic endometriosis on the prevalence of post-operative ovarian adhesions.

Methods

This is a prospective double blind randomised controlled trial which will be conducted at the University College London Hospital Endometriosis Centre. This centre is a tertiary referral unit for women with severe pelvic endometriosis and receives patients from a wide area of south east England.

Inclusion criteria

Women age 19 years or older who are diagnosed with severe pelvic endometriosis on pre-operative transvaginal ultrasound scan would be invited to join the study. Suitability for randomisation will be determined at surgery. Only women with severe endometriosis requiring extensive dissection of both pelvic side walls and/or rectovaginal space with preservation of the ovaries and uterus will be included in the study.

Exclusion criteria are inability or unwillingness to provide written consent, inability to tolerate a transvaginal ultrasound scan, unsuccessful surgeries and in cases of complications such as unplanned oophorectomies, bowel injuries or conversions to open surgery.

Interventions

During laparoscopic treatment for severe endometriosis, both ovaries are routinely suspended to the anterior abdominal wall using a Prolene suture, which is brought out onto the skin and secured using a fine haemostat or 'mosquito' clip during surgery. This is performed to facilitate access to the pelvic side walls during surgery and a complete excision of the disease.

At the end of the operation, women will be randomised to have one ovary suspended for 36 to 48 hours post-operatively. One of the two ovarian suspension sutures will be cut to allow that ovary to fall back into the lesser pelvis. A new transabdominal suture will be reinserted at the same site to act as a placebo. The pneumoperitoneum will then be deflated and the Prolene stitch of the suspended ovary will be tightened with a surgical knot placed over the skin to secure the ovary to the abdominal wall. This will ensure that the suspended ovary is lifted as far away from the pelvis as possible. The surgical knots will be secured with the space of a Carless scissor between the skin and the knot to allow easier removal of the suture and reduce patient discomfort. All randomized patients will therefore have two abdominal sutures of similar length and both the patient and clinical staff - apart from the surgical team - will be blinded to the randomisation process.

A label will be attached to the operation notes to define a) the randomisation number b) the operation date and time and c) the time to remove the sutures. There will be no documentation of the randomisation site in the operation notes.

Both sutures will be cut 36 to 48 hours after surgery by a ward nurse who will not be part of the study and will be blinded to the ovarian suspension site. The only members of staff who will be aware of the site of ovarian suspension will be the surgeons who will be under strict instructions not to discuss individual patient's treatment allocations with the patient or any other members of the clinical and nursing staff.

Randomisation

Participants will be randomised to unilateral suspension of either the right or left ovary. Block randomisation

will be used with three varying block sizes of minimum size 4. The randomisation schedule will be produced by our statistician using the external Stata command *ralloc*.

When a participant is recruited to the trial, consecutive randomisation envelopes will be opened and the principal surgeon will be told which ovary to suspend. Only the patient's randomisation number will be recorded in the patient's operation notes. No other member of staff will be aware of women's treatment allocations.

At the end of the study, the randomisation will be unblinded for analysis and details of the ovarian suspension will be added to each patient's record.

Incident Reporting & unblinding procedure

Adverse events will be recorded from the time of ovarian suspension until three months after surgery when the suspension results can be determined. The principal investigator will be responsible for the reporting of all serious adverse events (SAE) or suspected unexpected serious adverse reactions (SUSAR) immediately as the trial personnel become aware of an event to the chief investigator. The chief investigator should report all fatal or life-threatening events as soon as possible to the joint biomedical research unit (JBRU). This needs to be done not later than seven days after the chief investigator was first aware of the reaction. All events which are not fatal or life-threatening are also reported as soon as possible and not later than 15 days after the chief investigator was first aware of the reaction. The research and ethics committee (REA) also requires a report of all SAEs and SUSARs. The principal investigator will also follow all SAEs and SUSARs through to outcome.

In cases where patients experience SUSAR, both suspension sutures will be cut and the randomisation process will be unblinded by contacting the principal investigator or the ward sister who has a sealed randomisation envelope.

Ethical considerations

Approval for this study was obtained from the Medical Ethical Committees of the University College Hospital, London, UK. In each patient fulfilling the inclusion criteria, written informed consent is obtained before randomisation. Women refusing participation are registered.

Follow-up

Three months after ovarian suspension, all patients in the study will be invited for a transvaginal ultrasound scan to assess ovarian mobility (Figure 1). Ovarian adhesions will be diagnosed in women with evidence of restricted ovarian mobility on targeted palpation using transvaginal ultrasound probe [14]. The ultrasound operators will be blinded to the details of the operative procedure and women's randomisation allocation.

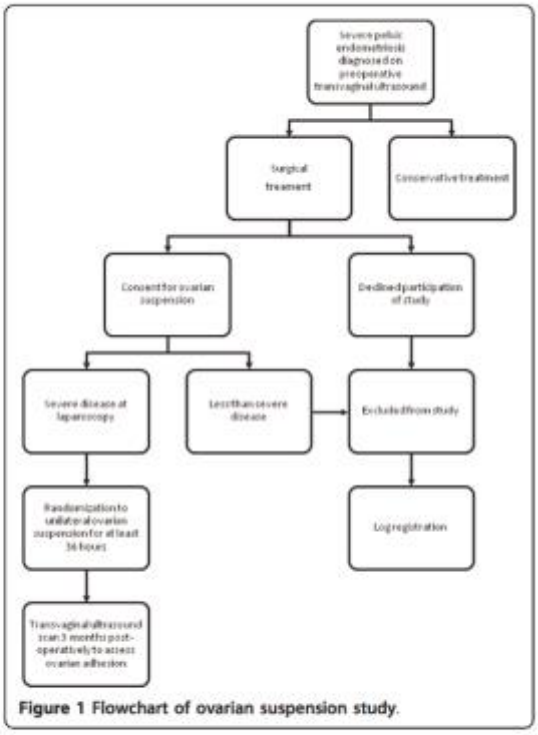

Figure 1 Flowchart of ovarian suspension study.

Outcome measures

Primary outcome measure The primary outcome is the prevalence of ovarian adhesion on ultrasound after surgery. The presence of ovarian adhesions will be assessed by a combination of gentle pressure with the vaginal probe and abdominal pressure with the examiners free hand as in a bimanual examination. The presence of ovarian adhesions will be diagnosed when the ovarian mobility is restricted and the ovary cannot be separated from the peritoneum of the lateral pelvic wall and/or pouch of Douglas.

Secondary outcome measures Secondary outcomes are the presence, intensity and site of post-operative pain.

Statistical analysis

Women with bilateral endometriosis will receive the normal surgical treatment with the difference that one ovary will be randomised to ovarian suspension and the other to non-suspension. The primary outcome is the binary variable of the presence of ovarian adhesions three months after laparoscopic surgery. The data will be paired binary data.

Pilot study

A pilot study was conducted to determine the prevalence of ovarian adhesion on transvaginal ultrasound three months after routine laparoscopic treatment of severe pelvic endometriosis (without ovarian suspension).

Between 1st of March 2009 and 1st of September 2009, 16 women post laparoscopic treatment for severe endometriosis were recruited. The mean age was 34.6 years (range 22 to 51). Histology confirmed the diagnosis of endometriosis in all 16 women. Post-operatively, 11/16 (68.8% 95% CI 46.0 to 91.5) women had evidence of ovarian adhesions on TVS. 4/16 (25.0%, 95% CI 3.8 to 46.2) women had unilateral adhesions, while 7/16 (43.8%, 95% CI 19.4 to 68.1) women had adhesions involving both ovaries. The ovarian adhesion rate for each ovary was 18/32 (56.3%, 95% CI 39.1 to 73.4)

Sample size

The prevalence of ovarian adhesion for each ovary from our pilot study was approximately 60% and this figure was used to calculate the sample size. Clinically significant improvement with ovarian suspension would be defined as a 50% reduction in the prevalence of post-operative ovarian adhesion.

The software provided by Machin et al. was used to calculate the sample size [15]. Assuming two-sided 5% significance and 80% power, 45 women would be required for the study. This calculation assumes that the response to suspension is independent to the response to non-suspension. Allowing for a possible 10% dropout during the follow up period, we plan to recruit 50 patients for the study.

Discussion

Ovarian adhesions after surgery for pelvic endometriosis are common problems with its associated morbidity. Numerous adhesion prevention strategies, mostly based on microsurgical principles, have been reported. These include minimalising tissue trauma, meticulous hemostasis, irrigation and the use of low reactive sutures. Suspending the ovaries post-operatively has been suggested as an easy and possibly effective way of reducing ovarian adhesions. To the best of our knowledge, a larger prospective study has not been done to evaluate this. Our randomised controlled trial will provide evidence as to whether this simple surgical procedure should be included into the routine surgical treatment of women with severe pelvic endometriosis.

There is a potential risk of injury to the bowels and blood vessels during insertion of the suspension sutures. However, the chance of this occurring is likely to be very small. There has also been no reported complication as a result of ovarian suspension in previous studies.

The original study protocol was to perform a diagnostic laparoscopy to assess for ovarian adhesions three months after surgical treatment for endometriosis. However, due to problems with patient recruitment, the study protocol was modified to the current study.

Traditionally, post-operative adhesions can only be assessed by laparoscopy, but recent studies have shown that targeted palpation with ultrasound probe could be used as an alternative method to diagnose adhesion. Guerriero et al. found that the fixation of the ovary to the uterus evaluated by transvaginal ultrasonography, had a good specificity (86%) and high positive predictive value (PPV) of 81% [16]. More recently, Okaro et al. found good correlation between ovarian mobility on transvaginal ultrasound and at laparoscopy (kappa 0.81) [17]. These positive results were also shared by Holland et al [14]. Transvaginal ultrasound has proven to be an indispensible, non-invasive and inexpensive way of assessing ovarian adhesion.

In our study, a decision was made to perform the post-operative ovarian suspension for 36 to 48 hours. This was different from the duration of suspension of 4 days by Ouahba et al [13]. Our decision was based on an animal study by Harris et al. looking at the kinetics of adhesion formation of injured peritoneal surfaces [18]. They showed that a reduction of adhesion formation is achieved when separation of injured peritoneal surfaces occurs for at least 36 hours. This was in accordance with the duration of hospital stay after laparoscopic surgery for severe endometriosis. Therefore all suspension stitches can be removed before surgical discharge.

Acknowledgements and Funding

WH is supported by the research fund provided by the Gynaecology Ultrasound Centre, London UK.

Authors' contributions

All authors were responsible for the development of the study protocol. WH drafted the paper and has the responsibility for the logistical aspects of the trial. DJ provides supervision and writing of the draft paper. AC, ES, GP are responsible for the surgical treatment of endometriosis and executing the randomisation instructions in theatres. All authors have read and approved the final draft of this paper.

Competing interests

ES received honoraria from Ethicon for provision of training to healthcare professionals and consultancy fees from Bayer. AC is on the advisory board for surgical innovations for which he receives an annual honorarium. AC also received support for courses and education from Storz and Johnson and Johnson and support for clinical nursing from Covidien and Lotus. The other authors declared no competing interests.

Received: 12 January 2011 Accepted: 11 May 2011
Published: 11 May 2011

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Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1472-6874/11/14/prepub

doi:10.1186/1472-6874-11-14
Cite this article as: Hoo *et al.*: Effectiveness of ovarian suspension in preventing post-operative ovarian adhesions in women with pelvic endometriosis: A randomised controlled trial. *BMC Women's Health* 2011 **11**:14.

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Appendix 6b - Does ovarian suspension following laparoscopic surgery for endometriosis reduce postoperative adhesions? An RCT.

Human Reproduction, Vol.29, No.4 pp. 670–676, 2014
Advanced Access publication on February 2, 2014 doi:10.1093/humrep/deu007

human reproduction

ORIGINAL ARTICLE *Gynaecology*

Does ovarian suspension following laparoscopic surgery for endometriosis reduce postoperative adhesions? An RCT

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Submitted on August 26, 2013; resubmitted on December 28, 2013; accepted on January 6, 2014

STUDY QUESTION: Is temporary ovarian suspension following laparoscopic surgery for severe pelvic endometriosis an effective method for reducing the prevalence of postoperative ovarian adhesions?

SUMMARY ANSWER: Temporary ovarian suspension for 36–48 h following laparoscopic treatment of severe endometriosis does not result in a significant reduction of postoperative ovarian adhesions.

WHAT IS KNOWN ALREADY: Pelvic adhesions often develop following laparoscopic surgery for severe pelvic endometriosis. Adhesions can cause chronic pelvic pain and fertility problems compromising the success of treatment. Small observational studies suggested that temporary postoperative ovarian suspension to the abdominal wall may significantly reduce the prevalence of postoperative ovarian adhesions.

STUDY DESIGN, SIZE, DURATION: This was a prospective within group comparison double-blind RCT. We recruited premenopausal women with severe pelvic endometriosis who required extensive laparoscopic surgery with preservation of the uterus and ovaries. Severity of the disease and eligibility for inclusion were determined at surgery. A total of 55 women were randomized to unilateral ovarian suspension for 36–48 h, 52 of which were included in the final analysis. Both ovaries were routinely suspended to the anterior abdominal wall during surgery. At the end of the operation, each woman was randomized to having only one ovary suspended postoperatively. The suture suspending the contralateral ovary was cut and a new transabdominal suture was inserted to act as a placebo. Both sutures were removed 36–48 h after surgery prior to discharge. Three months after surgery, all women attended for a detailed transvaginal ultrasound scan to assess ovarian mobility. Both the women and the ultrasound operators were blinded as to the side of postoperative ovarian suspension. The primary outcome was the prevalence of ovarian adhesions as described on ultrasound examination. Secondary outcomes were the severity of adhesions and the presence and intensity of postoperative pain.

PARTICIPANTS/MATERIALS, SETTING, METHODS: All 55 participants had severe pelvic endometriosis confirmed at laparoscopy. As each participant had only one of their ovaries suspended at the end of surgery, they acted as their own control.

MAIN RESULTS AND THE ROLE OF CHANCE: The median interval between ovarian suspension and postoperative scan was 99 days (interquartile range 68–114). There was no significant difference ($P = 0.23$) in the prevalence of postoperative ovarian adhesions between the suspended (20/52) and unsuspended (27/52) side (38.5 versus 51.9%) [odds ratio 0.56 (95% confidence interval 0.22–1.35)].

LIMITATIONS, REASONS FOR CAUTION: Ovaries were suspended postoperatively for 36–48 h. Longer suspension could result in lower prevalence of postoperative adhesions.

WIDER IMPLICATIONS OF THE FINDINGS: The value of temporary ovarian suspension in women having surgery for mild-to-moderate endometriosis should be investigated further. The potential benefits of other adhesion prevention strategies, such as surgical barrier agents, in women undergoing surgical treatment for severe pelvic endometriosis should also be explored.

STUDY FUNDING/COMPETING INTERESTS: E.S. received honoraria from Ethicon for provision of training to healthcare professionals and consultancy fees from Bayer. W.H. was supported by the research fund provided by the Gynaecology Ultrasound Centre, London UK. A.C. is

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on the advisory board for surgical innovations for which he receives an annual honorarium. A.C. also received support for courses and education from Storz and Johnson and Johnson and support for clinical nursing from Covidien and Lotus. The other authors declared no competing interests.

TRIAL REGISTRATION NUMBER: Current Controlled Trials ISRCTN24242218.

Key words: endometriosis / treatment / laparoscopy / pelvic adhesions / ovarian suspension

Introduction

Endometriosis is an important cause of morbidity in premenopausal women. Typical symptoms of endometriosis are dysmenorrhoea, dyspareunia, chronic pelvic pain and subfertility which often have significant negative impact on women's quality of life. Surgical excision is the most effective treatment for severe pelvic endometriosis. Currently, endometriosis surgery tends to be performed using a laparoscopic approach which provides superior views of the pelvic organs and facilitates complete excision of endometriotic lesions (RCOG Greentop Guideline No. 24, 2006). Although laparoscopic surgery is considered to be less traumatic to pelvic tissues than open surgery, a significant number of women develop postoperative pelvic adhesions even after this form of surgery. Postoperative adhesions most commonly affect the ovaries and the pouch of Douglas (Diamond, 1991) and they can cause chronic pelvic pain, dyspareunia, intestinal obstruction and infertility (diZerega, 1994). Reported prevalence of pelvic adhesions following laparoscopic surgery for severe pelvic endometriosis varies between 50 and 100% (Operative Laparoscopy Study Group, 1991; Redwine, 1991; Canis *et al.*, 1992; diZerega, 1994).

A wide range of interventions has been tried in the past in order to reduce postoperative pelvic adhesions. They include intra-peritoneal administration of anti-adhesive solutions such as icodextrin and hyaluronic acid. Various drugs including steroids and heparin have also been tried in the past but none of them has gained wide acceptance (Metwally *et al.*, 2006; Kamel, 2010). Oxidized regenerated cellulose was found to reduce postoperative adhesion in two very small RCTs (Ahmad *et al.*, 2008).

Intra-operative suspension of the ovaries to the anterior abdominal wall is sometimes used to facilitate ovarian retraction during surgery for severe pelvic endometriosis (Cutner *et al.*, 2004). Two small observational studies suggested that temporary ovarian suspension for 4–7 days following surgery for severe endometriosis may reduce the frequency of postoperative pelvic adhesions (Abuzeid *et al.*, 2002; Ouahba *et al.*, 2004).

The aim of this prospective RCT was to assess the effect of temporary ovarian suspension following laparoscopic surgery for severe pelvic endometriosis on the prevalence of postoperative ovarian adhesions.

Materials and Methods

This was a prospective within group comparison double-blind RCT which was conducted at the University College London Hospital Endometriosis Centre. The Centre is a tertiary referral unit for women with severe pelvic endometriosis and receives patients from a wide area of South East England.

Participants

Premenopausal women diagnosed with severe pelvic endometriosis on a preoperative transvaginal ultrasound scan who were 19 years or older were invited to join the study. Suitability for randomization was determined at surgery. Women with evidence of severe endometriosis requiring extensive dissection of both pelvic side walls and/or rectovaginal space with preservation of the ovaries and the uterus were included in the study.

Exclusion criteria were inability or unwillingness to provide written consent, inability to tolerate a transvaginal ultrasound scan, failed or incomplete excision of endometriosis and surgical complications leading to unplanned oophorectomy, bowel injury or conversion to open surgery.

Interventions

During laparoscopic treatment for severe endometriosis, both ovaries were routinely suspended to the anterior abdominal wall using a Prolene suture (Ethicon Inc., Somerville, NJ, USA) which was brought out onto the skin and secured using a fine haemostat or 'mosquito' clip during surgery. This was performed to facilitate access to the pelvic side walls during the operation and a complete excision of the disease. Depending on the abnormalities present, the surgical procedures included mobilization of adherent ovaries, removal of ovarian cysts, opening the pelvic side wall peritoneum and dissecting the ureters, dissection of obliterated pouch of Douglas and excision of superficial and deep endometriotic lesions.

At the end of the operation, women were randomized to have one ovary suspended for 36–48 h postoperatively. One of the two ovarian suspension sutures was cut to allow that ovary to fall back into the lesser pelvis. A new transabdominal suture was then re-inserted at the same site to act as a placebo. The pneumoperitoneum was deflated and the Prolene suture of the suspended ovary was tightened with a surgical knot placed over the skin to secure the ovary to the abdominal wall. This was done to ensure that the suspended ovary was lifted as far away from the pelvic side wall as possible. A surgical knot was secured with the space of a straight surgical suture cutting scissors between the skin and the knot to allow easier removal of the suture and reduce patient discomfort. All randomized patients therefore had two abdominal sutures of similar length. The patient and clinical staff were blinded to the randomization. The only staff members who were aware of the site of ovarian suspension were the surgeons who were under strict instructions not to discuss individual patient's treatment allocations with the patient or any other members of the clinical and nursing staff. Both sutures were cut 36–48 h after surgery by a ward nurse who was not part of the operating or research team and who was blinded to the ovarian suspension site.

Follow-up

Three months after ovarian suspension, all women were scheduled for a transvaginal ultrasound scan to assess ovarian mobility. Ovarian adhesions were diagnosed in women with evidence of restricted ovarian mobility on targeted palpation using a transvaginal ultrasound probe (Holland *et al.*, 2010). The ultrasound operators were blinded to the details of the operative procedure and women's randomization allocation.

Outcome measures

Primary outcome measure

The primary outcome was the prevalence of ovarian adhesions on ultrasound 3 months after surgery. The presence of ovarian adhesions was assessed by a

combination of gentle pressure with the vaginal probe and abdominal pressure with the examiners free hand, as on bimanual examination. The presence of ovarian adhesions was diagnosed when the ovarian mobility was restricted and the ovary could not be separated from the peritoneum of the lateral pelvic wall and/or pouch of Douglas.

Secondary outcome measures

Secondary outcomes were changes in clinical symptoms and severity of ovarian adhesions. The severity of pelvic pain was assessed using a visual analogue scale with no pain classified as 0 and worst imaginable pain as 10. For the purpose of statistical analysis a pain score of 1–3 was described as mild, 4–7 as moderate and 8–10 as severe.

In addition, the severity of ovarian adhesions was assessed as follows. Minimal adhesions were considered to be present when some (<1/3) of the surrounding structures could not be separated from the ovary with gentle pressure but the ovary could be mobilized from the majority (>2/3) of the surrounding structures. Moderate adhesions were classified when one-third to two-third of ovarian mobility was reduced as a result of adhesions with the surrounding structures but the structures on one-third of the surface of the ovary slid across it with the application of gentle pressure. Severe adhesions were characterized by fixed ovaries which could not be mobilized at all with gentle pressure or separated from any of the surrounding structures.

Pilot study

A pilot study was conducted on a sample of 16 women to determine the prevalence of ovarian adhesions on transvaginal ultrasound 3 months after routine laparoscopic treatment of severe pelvic endometriosis (without ovarian suspension).

Postoperatively, 11/16 women [68.8% (95% confidence interval (CI) 46.0–91.5)] had evidence of ovarian adhesions on transvaginal ultrasound. Four out of 16 [25.0% (95% CI 3.8–46.2)] women had unilateral adhesions, while 7/16 [43.8% (95% CI 19.4–68.1)] women had adhesions involving both ovaries. The adhesion rate per ovary operated on was 18/32 [56.3% (95% CI 31.9–80.6)].

Sample size

The prevalence of ovarian adhesions for each ovary from our pilot study was ~60% and this figure was used to calculate the sample size. Clinically significant improvement with ovarian suspension was defined as a 50% reduction in the prevalence of postoperative ovarian adhesion.

The software provided by Machin *et al.* (2009) was used to calculate the sample size for paired binary data. Assuming two-sided 5% significance, 80% power and a 54% proportion of discordant pairs, 45 women were required for the study. This calculation assumed that the response to suspension is independent of the response to non-suspension. Allowing for a possible 10% dropout during the follow-up period, we had planned to recruit at least 50 patients for the study.

Randomization

Participants were randomized to unilateral suspension of either right or left ovary. Block randomization was used with three varying block sizes of minimum size four. The randomization schedule was produced by our statistician using the external Stata command *ralloc*.

When a participant was recruited to the trial, consecutive randomization envelopes were opened by the anaesthetist who was not a member of the research team and the principal surgeon was told which ovary to suspend. Only the patient's randomization number was recorded in the patient's operation notes. A label was attached to the operation notes to define (i) the randomization number, (ii) the operation date and time and (iii) the time to remove the sutures. There was no documentation of the randomization site in the patient's notes.

At the end of the study, the randomization was unblinded for analysis and details of the ovarian suspension were added to each participant's record.

Ethical considerations

Approval for this study was obtained from the Medical Ethical Committees of the University College Hospital, London, UK. Each patient fulfilling the inclusion criteria was asked to sign a written informed consent. Women who refused participation were also registered.

Statistical analysis

Women with bilateral endometriosis received standard surgical treatment with the difference that, at the end of surgery, one ovary was randomized to ovarian suspension and the other to non-suspension. The primary outcome was the binary variable of the presence of ovarian adhesions 3 months after laparoscopic surgery. The outcomes were paired binary data and analyzed with the exact McNemar test. Statistical significance was set at the 5% α-level. The difference between suspended and unsuspended ovaries in the percentage with adhesions was reported with 95% CIs.

The severity of ovarian adhesions was analyzed as a secondary analysis. The adhesion score was recorded for each ovary and the difference between the suspended and unsuspended ovaries was analyzed with a Wilcoxon signed rank test.

At the end of the study, an independent statistician (E.N.C.T.) analyzed the results. Statistical analysis was performed using Stata 10.1 software (StataCorp, College Station, TX, USA).

Results

Between November 2009 and June 2012, 122 premenopausal women were diagnosed with severe pelvic endometriosis on a preoperative transvaginal ultrasound scan and they were invited to join the study. A total of 67 women were excluded from randomization with the majority [15/67; 22.4% (95% CI 12.4–32.4)] excluded because they had only received partial treatment of their endometriosis, 11 [16.4% (95% CI 7.6–25.3)] women had bowel surgery, nine [13.4% (95% CI 5.3–21.6)] had 'two-stage' procedures, another nine did not have severe pelvic endometriosis following preoperative treatment with GnRH agonist, six [9.0% (95% CI 2.1–15.8)] declined the study, five [7.5% (95% CI 1.2–13.8)] had oophorectomies, another five had hysterectomies, four [6.0% (95% CI 0.3–11.6)] had laparotomies and three [4.5% (95% CI 0.0–9.4)] cancelled their operation, of which one [1.5% (95% CI 0.0–4.4)] woman became pregnant before surgery.

Fifty-five women fulfilled the inclusion criteria for randomization and they underwent ovarian suspension at the end of their surgery. Three women were excluded from final data analysis as they did not attend for a postoperative ultrasound scan: one became pregnant, another was lost to follow-up and the third woman suffered a large bowel injury which required a laparotomy to repair. A suspected unexpected serious adverse reaction (SUSAR) report was made to the research and ethics committee for this unexpected bowel complication. Therefore, 52 women were included in the final analysis (Fig. 1).

All 52 women were premenopausal and their mean age was 32.6 years (range 22–46). Of these, 42 [80.8% (95% CI 70.1–91.5)] women were nulliparous, three [5.8% (95% CI 0.0–12.1)] were primiparous and seven [13.5% (95% CI 4.2–22.7)] multiparous.

At presentation, 38 [73.1% (95% CI 61.0–85.1)] women were not on hormonal treatment, 6 [11.5% (95% CI 2.9–20.1)] women were using combined oral contraceptives, four [7.7% (95% CI 0.5–14.9)] women were being treated with gonadotropin-releasing hormone (GnRH) agonist, three [5.8% (95% CI 0.0–12.1)] women were using a progesterone-only pill and the remaining one [1.9% (95% CI 0.0–5.7)] woman had a Mirena intrauterine system *in situ*.

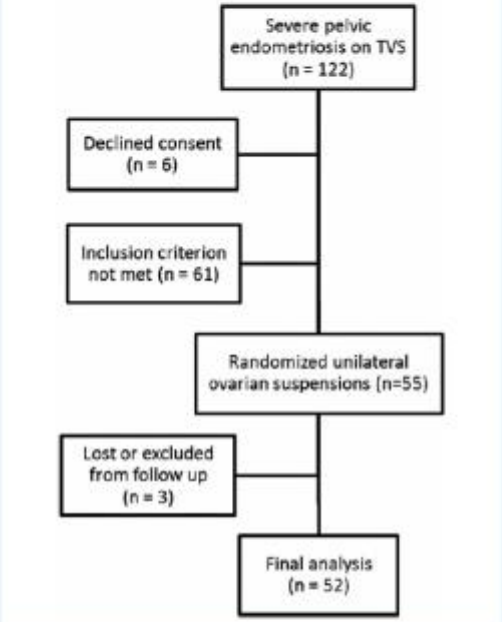

Figure 1 Flowchart of patients in RCT to determine if temporary ovarian suspension following laparoscopic surgery for endometriosis reduces postoperative adhesions. TVS = transvaginal ultrasound scan.

Prior to taking part in the trial, 21 [40.4% (95% CI 27.0–53.7)] women had one previous laparoscopic treatment for endometriosis, four [7.7% (95% CI 0.5–14.9)] had two, two [3.9% (95% CI 0.0–9.1)] had three and one [1.9% (95% CI 0.0–5.7)] woman had four previous laparoscopic surgeries.

All participants were asked about symptoms of endometriosis including dysmenorrhoea, deep dyspareunia, chronic pelvic pain and dyschezia. They were also asked about menstrual disorders and history of subfertility. One [1.9% (95% CI 0.0–5.7)] woman presented with a single symptom, six [11.5% (95% CI 2.9–20.1)] women had two symptoms, 12 [23.1% (95% CI 11.6–34.5)] women had three symptoms, 20 [38.5% (95% CI 25.2–51.7)] women had four symptoms, 12 [23.1% (95% CI 11.6–34.5)] women had five symptoms and one [1.9% (95% CI 0.0–5.7)] woman had six symptoms (Table I).

The median interval between preoperative scan assessment and ovarian suspension was 166 [interquartile range (IQR) 117–243] days. Six women [11.5% (95% CI 2.9–20.2)] required a two-stage procedure to complete their laparoscopic treatment of endometriosis. In these cases ovarian suspension was only performed following the second operation. The median interval between the first and second stage operation was 161.5 (IQR 108–229) days.

At surgery, all 52 women were found to have severe pelvic endometriosis when assessed using the revised American Fertility Society scoring and their operative findings are summarized in Table II.

Postoperative ultrasound was performed to assess ovarian mobility after surgery and ovarian suspension. The median interval between ovarian suspension and postoperative scan was 99 days (IQR 68–114).

Primary outcome

On examination, 38.5% (20/52) of suspended ovaries had postoperative adhesions on scan when compared with 51.9% (27/52) of unsuspended ovaries [OR 0.56 (95% CI 0.22–1.35)] ($P = 0.23$) (Table III).

Secondary outcomes

Of note, 9.6% (5/52) of suspended ovaries had moderate–severe adhesions when compared with 19.2% (10/52) of unsuspended [OR 0.38 (95% CI 0.06–1.56)] ($P = 0.23$) (Table IV). At the follow-up appointment, each woman was also assessed for postoperative symptoms

Table I Pre and postoperative symptoms in women ($n = 52$) undergoing laparoscopic surgery for endometriosis.

Symptoms of endometriosis	Preoperative *N* (%)	Postoperative *N* (%)	OR (95% CI)	*P*-value
Dysmenorrhoea	39 (75.0)	11 (21.2)	0.03 (0.00–0.21)	<0.001
Deep dyspareunia	26 (50.0)	7 (13.5)	0.10 (0.01–0.39)	<0.001
Dyschezia	30 (57.7)	5 (9.6)	0.0 (0.00–0.16)	<0.01
Chronic pelvic pain	43 (82.7)	26 (50.0)	0.06 (0.00–0.35)	<0.001
Mean VAS	5.79	1.98		<0.001
Pain severity				
Zero (VAS = 0)	9 (17.3)	26 (50.0)		
Mild (VAS 1–3)	7 (13.5)	16 (30.8)		
Moderate (VAS 4–7)	13 (25.0)	8 (15.4)		
Severe (VAS 8–10)	23 (44.2)	2 (3.9)		

VAS, visual analogue score; OR, odds ratio; CI, confidence interval.

Table II Operative findings.

Operative findings	N	%	95% CI
Right endometrioma	25	48.1	34.5–61.7
Left endometrioma	24	46.2	32.6–59.7
Hydrosalpinges	13	25	13.2–36.8
Pouch of Douglas adhesions			
None seen	1	1.9	0.0–5.7
Partial obliteration	8	15.4	5.6–25.2
Complete obliteration	43	82.7	72.4–93.0
Deep infiltrating endometriosis	45	86.5	77.3–95.8

Table III Absence of adhesions (Grade 0) versus any adhesions (Grades1–3) by treatment type.

Suspended ovaries	Unsuspended ovaries		
	No adhesions	Adhesions	Total
No adhesions	16 (30.8%)	16 (30.8%)	32 (61.5%)
Adhesions	9 (17.3%)	11 (21.2%)	20 (38.5%)
Total	25 (48.1%)	27 (51.9%)	52 (100%)

(Table I) and use of hormonal treatments. There was a general reduction in symptoms of endometriosis after surgery; however, an additional 17 [32.7% (95% CI 19.9–45.4)] women were given hormonal treatment after surgery which may have contributed to the reduction in symptoms. Postoperatively, 21 [40.4% (95% CI 27.0–53.7)] women were not on hormonal treatment, 19 [36.5% (95% CI 23.5–49.6)] had a Mirena intrauterine system inserted postoperatively, five [9.6% (95% CI 1.6–17.6)] were using combined oral contraception, four [7.7% (95% CI 0.5–14.9)] were being treated with GnRH agonist and three [5.8% (95% CI 0.0–12.1)] women were using a progesterone-only pill. There was no significant difference in the adhesion rates, when any postoperative hormonal treatment was used [OR 0.88 (95% CI 0.27–2.76)] ($P = 1.00$) or when no hormonal treatment was used [OR 0.25 (95% CI 0.03–1.25)] ($P = 0.11$). There was also no significant difference in the adhesion rates on the same side when an ovarian cystectomy was performed on the left ovary (Fisher's exact $P = 0.79$) or right ovary (Fisher's exact $P = 0.16$).

Discussion

Our study showed that temporary unilateral ovarian suspension for 36–48 h following surgery does not result in a significant reduction of postoperative ovarian adhesions compared with the unsuspended side. The result was the same when comparisons were made taking into account the severity of adhesions.

The study was a placebo-controlled prospective RCT which is its main strength. The patients and ultrasound operators were blinded to randomization and therefore it is unlikely that the results were influenced by bias. We opted for the within group (paired) comparisons design, which is considered to be particularly powerful and free from disadvantages which may affect quality of parallel group trials.

Table IV Mild adhesions (Grades 0–1) versus moderate–severe adhesions (Grades 2–3) by treatment type.

Suspended ovaries	Unsuspended ovaries		
	None-to-mild adhesions	Moderate–severe adhesions	Total
None-to-mild adhesions	39 (75.0%)	8 (15.4%)	47 (90.4%)
Moderate–severe adhesions	3 (5.8%)	2 (3.8%)	5 (9.6%)
Total	42 (80.8%)	10 (19.2%)	52 (100%)

Ovaries were suspended for a relatively short period of time which may be considered a weakness. We decided on the length of suspension taking into consideration the findings of animal studies which showed a reduction in adhesion formation when separation of injured peritoneal surfaces was maintained for at least 36 h (Harris *et al.*, 1995). Another reason against longer duration of suspension was our desire to minimize the risk of complications such as small bowel strangulation. Early discharge with sutures *in situ* was not considered to be safe and, by limiting the length of time that the ovaries were suspended, we tried to shorten women's postoperative in-patient stay, minimize their social disruption and avoid increasing treatment costs.

Ouahba *et al.* (2004) suspended 12 ovaries in eight women for 4 days following extensive surgery for severe pelvic endometriosis. They found significant ovarian adhesions in 33% of cases, which was only a slight improvement when compared with the 38% adhesions rate in our study. This indicates that longer duration of suspension may not actually lead to better surgical outcomes.

Our results are in contrast to a very small previous study which suggested a reduction in postoperative ovarian adhesions with temporary ovarian suspension (Abuzeid *et al.*, 2002). The authors reported findings at second-look laparoscopy in five women who had ovaries suspended for 5–7 days following laparoscopic surgery for Stage 3–4 pelvic endometriosis. They found mild ovarian adhesions in one woman (20%) whilst the remaining four women were completely free of adhesions.

It is possible that the ovarian suspension procedure may be beneficial for women who have less severe endometriosis. In our study, the majority of women had unusually severe endometriosis; 43 [82.7% (95% CI 72.4–93.0)] had complete obliteration of the pouch of Douglas, eight [15.4% (95% CI 5.6–25.2)] had partial obliteration and 45 [86.5% (95% CI 77.3–95.8)] had deep infiltrating endometriosis. Temporary ovarian suspension alone may not be enough in these situations to reduce the postoperative ovarian adherence.

Only 43% of women who were found on preoperative ultrasound to have severe pelvic endometriosis were randomized for the trial. A large proportion of this was because of our strict inclusion criteria in order to ensure that all of our trial patients had similar surgical treatments prior to randomization. In our study we decided to use ultrasound rather than laparoscopy to diagnose pelvic adhesions both pre and postoperatively. Ultrasound had not been routinely used for the diagnosis of pelvic endometriosis in the past due to concerns about possible lack of sensitivity for

the detection of pelvic adhesions. Recent improvement in the quality of ultrasound equipment and the examination technique showed that transvaginal ultrasound examination is an accurate and reproducible test to diagnose pelvic adhesions and to assess their severity (Holland *et al.*, 2010, 2013; Hudelist *et al.*, 2011). This approach enabled us to complete the trial without performing a second-look laparoscopy. The rate of postoperative adhesions in our study was comparable to the previous studies, which used second-look laparoscopy to diagnose pelvic adhesions. This finding is reassuring and it supports our view that transvaginal ultrasound is a sensitive test to diagnose pelvic adhesions.

We found a significant improvement in women's pain scores following surgery despite the relatively high prevalence of postoperative pelvic adhesions. Although the proportion of women complaining of pelvic pain was significantly less postoperatively, half of the women continued to experience some pain which was moderate to severe in 19.3% of them. In addition, 13.5% of women continued to complain of deep dyspareunia. This occurred despite successful and complete excision of all endometriotic lesions at laparoscopy. In view of these results, it is possible that postoperative pelvic adhesions are at least partly responsible for the persistent pelvic pain following laparoscopy for endometriosis (Kresch *et al.*, 1984; Duffy and diZerega, 1996). Postoperatively, 60% of women were taking hormonal therapy for endometriosis compared with 27% preoperatively. It is therefore possible that postoperative pain scores could have been worse if the proportion of women on hormone treatment was the same before and after surgery.

This study was powered on an anticipated adhesion proportion of 60% in the unsuspended side and 30% in the suspended side, corresponding to a 50% relative reduction. Some researchers may consider a 20% relative reduction with a 50 and 40% rate for each respective side clinically meaningful. Such a view would increase the sample size to 390 pairs based on calculations from Machin *et al.* (2009). With a larger sample size, further analysis could be considered with conditional logistic regression models to compute adjusted odds ratios. Baseline covariates that may be considered for adjustment include age, pain severity, number of symptoms of endometriosis and observed suspension duration. Further work is required to assess the potential value of other adhesion-prevention strategies, such as surgical barrier agents, in women undergoing surgical treatment for severe pelvic endometriosis. Another study should also be considered to assess the value of temporary ovarian suspension in women having surgery for mild to moderate endometriosis.

Authors' roles

All authors were responsible for the development of the study protocol. W.H. drafted the paper and had the responsibility for the logistical aspects of the trial. D.J. provided supervision and writing of the draft paper. A.S., A.C., E.S. and G.P. were responsible for the surgical treatment of endometriosis and executed the randomization instructions in theatres. E.N.C.T. was the independent statistician who analyzed the results. All authors have read and approved the final draft of this paper.

Funding

W.H. is supported by the research fund provided by the Gynaecology Ultrasound Centre, London UK. Trial registration: Current Controlled Trials ISRCTN24242218.

Conflict of interest

E.S. received honoraria from Ethicon for provision of training to healthcare professionals and consultancy fees from Bayer. A.C. is on the advisory board for surgical innovations for which he receives an annual honorarium. A.C. also received support for courses and education from Storz and Johnson and Johnson and support for clinical nursing from Covidien and Lotus. The other authors declared no competing interests.

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Appendix 6c - Reply: Criticizing the effect of ovarian suspension on adhesions in laparoscopic surgery for endometriosis.

taken to prove (or disprove) that the peritoneum lacked endometriosis at the conclusion of therapy. The results were based on visualization alone.

Dr Roman switches from the concept of OME to proper therapy for endometriosis. There is no evidence to support his statement that it is impossible to remove all endometriosis in the pelvis. This opinion is held by many surgeons, but is too often used to rationalize incomplete surgery or as a reason not to attempt surgery at all. Nothing we wrote implied that symptom response is synonymous with response of endometriosis to aggressive excisional surgery. Women can have multiple causes of pelvic pain. Our hope is that better identification and surgical removal of endometriosis will help remove this diagnosis from consideration and break the cycle of repetitive surgeries and medical therapies that are the unfortunate modern hallmark of endometriosis treatment.

Dr Roman's proposed study would have predictable results: inducing amenorrhea with ovarian suppression will improve uterine dysmenorrhea, which will add to the pain relief of excision of endometriosis alone. While overall symptom improvement is important, ovarian suppression may result in improvement of any estrogen-dependent symptom produced by a variety of diseases. Given the difficulty of detecting adenomyosis without hysterectomy, how can one reliably identify study candidates who do not have adenomyosis, a potential silent confounding variable? In addition to symptom response it would be important to check robust measurements of ovarian function before and ≥ 6 months after cessation of ovarian suppression. This would help to determine if symptom improvement might be due to prolonged or permanently reduced ovarian function.

The concept of OME has important implications for the surgical cure of endometriosis. While it is true that endometriosis requires microscopy for accurate histologic diagnosis, this does not render its sometimes extremely subtle morphologies invisible during surgery. Clinicians applying the Redwine criteria of normal peritoneum have recently documented lack of recurrent disease in reoperated adolescents following excision of endometriosis, (Yeung et al., (2011) For a disease, which has long been considered incurable, these results underscore the previous studies: most patients undergoing aggressive excision of endometriosis do not have endometriosis at reoperation. If the absence of a disease after treatment is defined as cure, it can be said that most patients undergoing excision of endometriosis by experienced surgeons are cured by surgery (Meigs, 1953; Wheeler & Malinak, 1987; Redwine, 1991; Abbott et al., 2004; Yeung et al., 2011). This fact should be embraced and all means available should be used to identify endometriosis accurately at surgery, thus improving surgical outcomes in all endometriosis patients.

Note: In a proprietary study from the 1980s which helped bring Lupron to market and which is now under a USA federal court seal, the sponsor of Lupron found prolonged or permanent impairment to ovarian function in the majority of follow-up participants in the experimental arm of the study.

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doi:10.1093/humrep/deu098
Advanced Access publication on May 16, 2014

Criticizing the effect of ovarian suspension on adhesions in laparoscopic surgery for endometriosis

Sir,

We enjoyed studying the recent paper by Dr Hoo et al. (2014). Despite containing many strong points, there are some limitations, or perhaps misunderstandings on our part, which are discussed here. First of all, it seems that the ovaries, and not the women (as was claimed), underwent randomization. The authors mentioned that the cases had bilateral endometriosis, which is the main prerequisite for ovarian randomization; however, Table II shows that only 25 (48.1%) and 24 (46.2%) cases had right and left endometrioma, respectively. These are not consistent with each other; albeit, they mean bilateral endometrioma. On the other hand, if they mean bilateral endometriosis, the study needed to be randomized in such a way that each side of endometriosis involvement (that is more affected) was equal in both the suspended and non-suspended groups, which was not considered. As an additional point, a joint study has previously shown that left side endometrioma is more common (Matalliotakis et al., 2009). In addition, we are not aware of the equality of ovarian involvement, which is another prerequisite for randomization. This could also be another reason for the similar results found between the suspended and placebo groups.

In one place, the authors mentioned that 17 cases received hormonal treatment (HT), while in the next sentence the number of cases receiving HT reached 31 cases. It seems reasonable that the patients were advised to withdraw from using any hormonal substance, and that is the third prerequisite for randomization. In addition, the use of estrogenic compounds should cease before any elective surgery in order to avoid thromboembolic accidents. More usefully, they could have added up all cases that were on HT after surgery and compared their symptoms with the other 21 be ceased that were not. Such a comparison could more accurately specify the role of HT on changes in the symptoms.

If randomization had been done correctly, we would have expected that the number of suspended and unsuspended ovaries (which were 20 and 27 ovaries, respectively), and suspended ovaries on each side (which are not mentioned in this study) would be equal.

The authors stated that the comparable rate of post-operative adhesions examined by transvaginal ultrasound in their study with previous studies which used laparoscopy indicated that their findings are reassuring. Such judgment would be true if they had used both diagnostic procedures (transvaginal ultrasound and laparoscopy) on the same cases or on a similar study population. Comparable percentages of cases with adhesions using both diagnostic procedures prove nothing. Furthermore, if we conducted a diagnostic study on the same cases and considered laparoscopy as the gold standard, the percentages of cases with false-negative and false-positive adhesions by vaginal ultrasound may be nearly equal and this could result in a comparable percentage of adhesions by the two methods. Alternatively, maybe the cases with adhesions based on laparoscopy are healthy according to a transvaginal ultrasound and vice versa.

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doi:10.1093/humrep/deu097
Advanced Access publication on May 9, 2014

Reply: Criticizing the effect of ovarian suspension on adhesions in laparoscopic surgery for endometriosis

Sir,

We thank Mehdizadehkashi and colleagues for their interest in our study (Hoo *et al.*, 2014). Our study was a prospective, within-group comparison, double-blind randomized control trial. This implies that comparisons were made within and not between individuals. We stated in the discussion that we decided to use this type of trial as it is considered methodologically sound and free of disadvantages that may affect the quality of parallel group trials.

We stated very clearly in our paper that the inclusion criteria were the presence of severe bilateral disease (as scored using the revised AFS staging) requiring extensive dissection of both pelvic side walls and/or rectovaginal space with preservation of the ovaries and the uterus. As severe endometriosis often occurs without endometriomas being present in the ovaries, the presence of endometrioma was not a part of the inclusion criteria. We did perform a secondary analysis, however, which showed that ovarian cystectomy did not have a significant effect on the ipsilateral adhesion rates.

With regard to hormonal treatment we made it clear that 14 women had hormonal treatment pre-operatively and an additional 17 women were treated with hormones post-operatively. Statistical comparisons were made between 31 women who had hormonal treatment (either pre- or post-operatively) and 21 women who did not receive any hormonal treatment. We found no significant difference in the adhesion rates between the two groups. We also made it clear, however, that our study was not powered to evaluate the effect of post-operative hormonal treatment on the presence of adhesions.

The number of suspended and unsuspended ovaries was the same, i.e. 52. There was evidence of ovarian adhesions in 20/52 suspended ovaries compared with 27/52 in unsuspended ovaries. These numbers therefore refer to the primary findings in our study and not to the number of ovaries randomized to intervention and non-intervention.

We accept that a second-look laparoscopy is still perceived by many as a gold standard to assess for the presence of pelvic adhesions. However, little is known about the reproducibility of laparoscopic diagnosis of pelvic adhesions, which is likely to be as operator dependent as in non-invasive diagnostic methods. Second-look laparoscopy is costly, it is associated with surgical risks and many asymptomatic and minimally symptomatic women are reluctant to undergo further surgery which confers no obvious benefit to them. As mentioned in the discussion, recent improvements in the quality of ultrasound equipment and the examination technique showed that transvaginal ultrasound examination is an accurate and reproducible test to diagnose pelvic adhesions and to assess their severity (Holland *et al.*, 2010; Hudelist *et al.*, 2011; Holland *et al.*, 2013).

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doi:10.1093/humrep/deu099
Advanced Access publication on May 9, 2014

GnRH agonist triggering in high-risk patients

Sir,

We were interested in the article published in one of the recent issues of Human Reproduction, entitled 'Consistent high clinical pregnancy rates and low ovarian hyperstimulation syndrome rates in high-risk patients after GnRH agonist triggering and modified luteal support: a retrospective multicentre study' written by Iliodromiti *et al.* (2013). Here, we would like to discuss some important points concerning the article.

Iliodromiti *et al.* (2013) in their three-centre retrospective analysis included 275 patients, at high risk of developing ovarian hyperstimulation syndrome (OHSS), who received a GnRH agonist trigger followed by a bolus of 1500 IU hCG 1 h after oocyte retrieval. GnRH agonist trigger followed by a modified luteal phase support with one bolus of 1500 IU hCG was developed by Humaidan *et al.* (2005, 2010). As stated in the article, within an hour of oocyte retrieval, centres one and three (UK, Australia) administered 1500 IU recombinant hCG (Merck Serono) for luteal support. There is only one form of recombinant hCG which consists of 250 microgram /0.5 ml choriogonadotropin alfa and equals to 6500 IU. We would like to ask how these centres administered 1500 IU recombinant hCG?

The second point we question is the discordance between baseline characteristics and ovarian response of the study population. The study population was presented as having increased risk of an excessive ovarian response, but the total number of patients with polycystic ovary syndrome was 16.7%. Total FSH, peak estradiol levels, and the number of oocytes collected do not represent high-risk patients. In our opinion, the 0.72% incidence of severe OHSS ratio represents for moderate-risk patients.

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doi:10.1093/humrep/deu086
Advanced Access publication on April 29, 2014

Reply: GnRH agonist triggering in high-risk patients

Sir

We are grateful for the interest shown by Gurbuz and colleagues in our manuscript reporting our multicentre experience of modified luteal support after GnRH agonist trigger (Iliodromiti *et al.*, 2013). Rather than as reported in both the Australian and Belgian centre 1500 IU urinary hCG (Pregnyl) was used. In the UK centre, as 1500 IU urinary hCG was not available, this was approximated by using a fractional dose of the Ovitrelle prefilled pen (Merck Serono). Specifically 25% of the total number of clicks to deliver the full dose was dialled prior to injection (8/32) and a dose approximating 1625 IU hCG was administered.

We acknowledge that there was discordance between baseline characteristics (antral follicle count and anti-Müllerian hormone (AMH)) that provide an estimate of potential ovarian response and the actual ovarian response. We would however respectfully suggest that the observed ovarian response does not reflect the inherent risk profile of the patients, but rather successful modification of the stimulation strategy. We have previously shown the utility of antral follicle count and AMH in predicting the range of ovarian responses (Nelson *et al.*, 2007; Broer *et al.*, 2013a,b) and that the chosen stimulation strategy of a GnRH antagonist with a low dose of exogenous FSH can modify oocyte yield, particularly in potential high responders (Macklon *et al.*, 2006; Nelson *et al.*, 2009). The ability to use ovarian biomarkers to identify high risk patients, stratify their treatment to less aggressive stimulation strategies, modify their response and associated risk while still maintaining excellent

Appendix 6d - Reply: ovarian suspension for longer than 36 h is necessary for temporary ovarian suspension to fulfil its remit.

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doi:10.1093/humrep/deu010
Advanced Access publication on February 2, 2014

Ovarian suspension for longer than 36 h is necessary for temporary ovarian suspension to fulfil its remit

Sir,

We read with great interest the recent study published in Human Reproduction which assessed the effectiveness of temporary ovarian suspension after endometriosis surgery (Hoo *et al.*, 2014). The study failed to find a statistically significant reduction in adhesion formation associated with ovarian suspension; this finding is unsurprising and could have easily been predicted from the outset.

The aim of temporary ovarian suspension is to suspend the ovaries whilst the regions of excised peritoneum heal in order to prevent contact between the ovaries and the regions of excised peritoneum. Following healing of the peritoneum in these regions, the sutures suspending the ovaries are cut; as the peritoneum will now have mostly healed, in theory, ovarian adhesions are now less likely to form (Trehan, 2002). It has been known for decades that peritoneal healing takes 5–8 days to occur (Ellis *et al.*, 1965; Eskeland, 1966; Glucksman, 1966; Hubbard *et al.*, 1967; Raftery, 1976), and further, blood in the pelvic cavity may be involved in adhesion pathogenesis (Ryan *et al.*, 1971), which has been shown to take up to 8 days for absorption from the pelvic cavity (Jackson, 1958). In light of this, is 36 h really long enough to allow for peritoneal healing before cutting the ovarian sutures? Hoo *et al.* state that they chose their suspension time of 36 h based on a single rodent study in which a laparotomy was made and a region of anterior abdominal wall parietal peritoneum and a layer of underlying muscle was excised; the caecum was then elevated to be in close proximity to the anterior abdominal wall, and the anterior abdominal wall and caecum were then abraded with a scalpel, left exposed to air for 10 min, and a silastic sheet placed between the two surfaces, and the laparotomy then closed (Harris *et al.*, 1995). The silastic sheet was then removed at various time intervals, and the incidence of adhesions was noted after 7 days. The study found that adhesions between the abdominal wall and the caecum only formed if the silastic sheet was removed prior to 36 h. Whilst an interesting study, it would be dangerous to assume that based on these findings in a single artificial animal model, no ovarian adhesions form in women >36 h after surgery for deep endometriosis, and that therefore suspension for longer than 36 h would not be beneficial.

Hoo *et al.* also note that they chose this short length of ovarian suspension partially based on an apparent risk of small bowel strangulation and wishing not to discharge patients with suspended ovaries. None of the published studies on temporary ovarian suspension with longer suspension times have ever noted this complication (Abuzeid *et al.*, 2002; Trehan, 2002; Ouahba *et al.*, 2004; Poncelet *et al.*, 2012), and so we question whether this purely theoretical concern is borne out in clinical practice. Hoo *et al.* also note the cost of suture removal at a later date as a further disincentive for longer periods of suspension; yet keeping patients, who could be discharged after an overnight hospital stay, in hospital for two nights for not wishing to discharge patients with the ovarian sutures *in situ*, in fact represents a much larger cost than having sutures removed in the community at a later date. In our current practice, the ovarian suspension sutures are removed in the community by 7–9 days post-operatively, usually at their general practice surgery by a practice nurse.

There is also the obvious limitation associated with assessing adhesion formation via ultrasound rather than second-look laparoscopy; clearly ultrasound is cheaper and less time consuming; however, recent studies assessing the impact of certain interventions on adhesion formation in other contexts have used repeat laparoscopy (Trew *et al.*, 2011).

Temporary ovarian suspension is a theoretically compelling means of reducing ovarian adhesions; we hope that readers of Human Reproduction will not be deterred from performing temporary ovarian suspension on the basis of a study in which the ovaries were not suspended for an appropriate length of time. In order to gain a definitive answer on the effectiveness of temporary ovarian suspension, future studies should assess the impact of ovarian suspension for at least 5 days (perhaps even for a minimum of 7 days in order to ensure complete healing before the ovarian sutures are cut), use larger patient numbers and utilize second-look laparoscopy in order to assess adhesion formation.

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doi:10.1093/humrep/deu140
Advanced Access publication on June 7, 2014

Reply: Ovarian suspension for longer than 36 h is necessary for temporary ovarian suspension to fulfil its remit

Sir,

We are grateful to Trehan and Trehan for their comments. We did consider suspending the ovaries following laparoscopic surgery for longer than 36 h. However, we had experienced a case of acute small bowel obstruction following a similar suspension procedure which required immediate release of the suspension suture (data on file). This is a very distressing and potentially serious complication and we did not feel that it would have been safe to discharge women home with the ovarian suspension suture *in situ*. We have decided to adopt a 36 h interval based on a methodologically robust study which used an animal model to show that susceptibility for adhesion formation was significantly reduced or eliminated after the first 36 h following a peritoneal injury. This was a relatively recent study, whilst all the other animal studies quoted by Trehan and Trehan were published almost four decades ago.

We were interested to learn that Trehan and Trehan have introduced post-operative ovarian suspension for 5–7 days into their routine clinical practice. We could not find any publications authored by them or by anyone else describing the efficacy of this approach. In view of that we would urge our colleagues to consider publishing their data in order to help others to learn from their unique experience.

A second look laparoscopy for the outcome evaluation of the trial has its benefits; however, even in their own publication, they did not report on outcomes because a repeat laparoscopy could not be justified for ethical reasons (Trehan, 2002).

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doi:10.1093/humrep/deu141
Advanced Access publication on June 7, 2014

Uterine artery embolization for severe symptomatic fibroids: effects on fertility and symptoms

Sir,

We read with interest the paper by Torre *et al.* (2014) but would like to raise a number of concerns. They conclude that uterine artery embolization (UAE) should not be offered to women of reproductive age as it could be detrimental to their fertility prospects. We strongly contend that the only legitimate conclusion they could reach from the data obtained from their study cohort is that *UAE does not improve fertility prospects* in women whose prospects were, at best, dismal, while the treatment significantly improved their symptoms.

Torre *et al.* studied a relatively small (for purposes of fertility outcomes) cohort of women with extensive fibroid disease, the majority of whom had had previous surgical interventions, and for whom further, or *de novo*, surgical intervention was either refused or advised against. These women were symptomatic and for that alone they needed some form of intervention. Implicit in the way the paper is