A Study of the Histopathological Changes within Ectopic Endometrial Tissue, in Subjects with Known Pelvic Endometriosis Following Treatment with Ulipristal Acetate, a Selective Progesterone Receptor Modulator (SPRM).

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A thesis for the degree of Doctor of Medicine (MD), submitted to the University of East Anglia (Norwich Medical School), relating to a study performed at the Norfolk and Norwich University Hospital.

February 2018.

ABSTRACT

Endometriosis is a common disease, affecting 10% of women of reproductive age. Pharmacological agents play an important role in control of symptoms as well as disease suppression but are limited by systemic side effects. Although surgical management is helpful for some, there is an increasing reliance on medical treatment and the exploration of new treatments such as selective progesterone receptor modulators (SPRMs).

The aim of this interventional descriptive cohort study was to assess the changes in the ectopic endometrial deposits of twenty patients with pelvic endometriosis after a three-month treatment course of ulipristal acetate (Esmya®). Post treatment histological and immunohistochemistry changes were correlated to changes in the macroscopic appearance of the disease and changes in symptom severity.

Features of progesterone receptor modulator associate endometrial changes (PAEC) were seen within eutopic endometrial samples, as expected, but no single specimen exhibited the full features of PAEC. The ectopic endometrium exhibited a different pattern of features with cystic dilatation, ciliated metaplasia and infrequent mitoses a more common finding. The presence of these features correlated with the clinical impact of the drug and may offer some insight into the drugs mechanism of action.

A good clinical response to ulipristal acetate was seen in 56% of the cohort with statistically significant improvements in pain scores and overall quality of life relating to endometriosis, which was maintained after stopping treatment. Ulipristal acetate was considered acceptable by the cohort with the median change described as 'better, and a definite improvement that has made a real and worthwhile difference'.

Ulipristal acetate appears to offer an effective treatment for endometriosis with histological changes in keeping with the known experience of PAEC. The safety of this compound remains to be elucidated but the results from this pilot study are encouraging and should prompt further exploration.

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PREFACE

Endometriosis is a common disease, affecting 10% of women of reproductive age. The clinical symptoms are painful menstruation, persistent pelvic pain, pain during intercourse and infertility. Pharmacological agents play an important role in control of symptoms as well as disease suppression, especially in young women who wish to retain their fertility. This currently involves non-steroidal anti-inflammatory drugs (NSAIDs) or hormonal agents, such as progestogens or gonadotrophin releasing hormone (GnRH) analogues. Systemic side effects limit the effective use of many such hormonal agents.

Ulipristal acetate (UPA) (Esmya®) is a selective progesterone receptor modulator that acts mainly on the progesterone receptors of the reproductive tract. It has recently been introduced to clinical practice as a pre-operative treatment of fibroids. Studies have demonstrated histological changes in the endometrial lining of the uterus after treatment with ulipristal acetate. However, whether similar changes occur in the ectopic endometrium in pelvic endometriosis remains to be elucidated. Dependent on the nature of any such changes, ulipristal acetate may form the basis of a new treatment modality.

The study aim was to assess the changes in the ectopic endometrial deposits of patients with pelvic endometriosis after a three-month treatment course of ulipristal acetate. Post treatment histological changes were described and then correlated with changes in the macroscopic appearance of the disease and symptom severity.

Patients with laparoscopically diagnosed endometriosis, requiring further surgical treatment were given a three-month course of 5mg ulipristal acetate daily, prior to any planned surgery. No other modification to the planned clinical management was made. At the treatment laparoscopy, the disease was re-classified, and samples obtained for histological analysis of both the ectopic deposits and the eutopic endometrium. In addition, symptoms and quality of life were assessed at various time points throughout the study to assess effectiveness and tolerability. The study was carried out over a period of two years to achieve a recruitment target of 20 subjects.

ACKNOWLEDGEMENTS

I am indebted to Mr Eddie Morris for his teaching, wisdom and support throughout this study. His guidance during the set-up of the study and surgical training during my research fellowship have been invaluable. I am extremely grateful for the opportunity to work with him at the Endometriosis Centre of the Norfolk and Norwich University Hospital.

My thanks also go to Dr Ray Lonsdale, whose teaching and support have allowed me to explore a new area of medicine and gain new insight into the histology of the endometrium. I am particularly grateful to the Pathology department of the Norfolk and Norwich University Hospital for their facilities and training.

Professor Alistair Williams at the University of Edinburgh has provided expertise and validation of our study findings. His guidance on study design and results interpretation have been instrumental in the success of this study, for which I am incredibly grateful.

I am also grateful to Julie Dawson, Commercial Research Coordinator (Research and Development, Norfolk and Norwich University Hospital) for her advice and guidance on Good Clinical Practice and study set up. In addition, I am thankful to the R&D department for taking on the sponsorship and monitoring responsibilities for the study.

Finally, my thanks go to Emma Browning who provided vital clerical support to the study. Her willingness to help and enthusiasm for the study were a real help and facilitated the smooth delivery of all the study visits.

Financial support was provided by Preglem SA, part of the Gedeon Richter group. In addition to financial support, scientific guidance and advice was provided by Helen Saunders (Medical Affairs Director), Pablo Arriagada (Vice President of Medical Affairs) and the Preglem scientific advisory board (SAB).

I dedicate this thesis to my wife and family. I am extremely grateful for their support and understanding through this phase of my career. In particular, I am thankful for their help over the last six months, without whom this work would not have been possible.

Chapter 1: ENDOMETRIOSIS

1.1 THE DISEASE

Endometriosis was originally described by Sampson in 1927 but has more recently been defined by Giudice, in 2004, as the presence of tissue resembling the endometrium in locations outside the uterus.(1)(2) A consensus definition by the European Society of Human Reproduction and Embryology (ESHRE) is 'the presence of endometrial-like tissue outside the uterus, which induces a chronic, inflammatory reaction'.(3) It is a poorly understood condition with considerable variability in clinical presentation; some patients experience severe and debilitating symptoms whilst others have only mild symptoms that have little impact on their quality of life. A lack of non-invasive diagnostic tools means it can only be diagnosed at laparoscopy or through invasive biopsy often resulting in a diagnostic delay. As such it is difficult to define the exact prevalence but is it considered to be common - affecting 5-10% of women of reproductive age.(4)

However, the prevalence is much higher in certain groups – for instance, it is diagnosed in up to 25% of women presenting with gynaecological symptoms in the UK and USA, with a peak incidence between 30 and 45 years of age.(5) Despite this high prevalence, there is limited understanding about the condition with regard to aetiology, pathogenesis and management. The current range of conservative, medical and surgical management options each have their own unique limitations; meaning there is significant interest in novel therapeutic agents for the treatment of endometriosis.

1.2 PATHOGENESIS

In endometriosis, ectopic endometrial tissue deposits, which are morphologically and biologically similar to eutopic endometrium, are found in aberrant locations. These deposits contain functional endometrial glands and stroma and can respond to endogenous hormonal stimulation, in a similar way to the eutopic endometrium, leading to a cycle of proliferation and menstrual type bleeding at the site of these implants.(5) Local inflammatory processes occur in response to these deposits leading to the deposition of fibrin and the generation of adhesions. As this process progresses, deep infiltrating lesions develop and endometriomas can form within the ovaries.

The precise aetiology of endometriosis is not known. There are a number of theories with regard to the origin of the ectopic endometrial tissue: retrograde menstruation causing peritoneal endometriosis, coelomic metaplasia leading to ovarian endometriosis and abnormalities of persistent Mullerian duct remnants explaining the development of rectovaginal endometriosis.(5,6) However, these deposits of abnormal cells should be identified and destroyed by the cell-mediated arm of the immune system. The lack of cell clearance by macrophages, irrespective of their origin, contributes to the persistence of these abnormally located cells in patients with endometriosis. The reason behind this persistence in some individuals and not in others is poorly understood but there is a proven familial component to the condition – either with respect to the origin of the ectopic endometrial cells or the subsequent persistence of those cells in that ectopic location. The inheritance is polygenic and leads to a seven times higher incidence in patients with a family history of endometriosis.(4)

Although the aetiology of the disease is unknown some aspects of pathogenesis are becoming clearer, which has resulted in some models of pathogenesis becoming widely accepted in the literature. As such there are three key aspects to consider when exploring the pathological processes behind the establishment of ectopic endometrial lesions in endometriosis:

1) how the lesions become established at the ectopic location,
2) how the cells behave, once they are established to promote their persistence,
3) why the immune system fails to clear them from their ectopic location.

Unfortunately, there is no clear single mechanism that explains all the known features and experimental data, which means that each of the models described have either exceptions or flaws.

1.2.1 Establishment of ectopic endometrial lesions

Retrograde menstruation

The concept of endometrial cells and fragments reaching the pelvic peritoneum by a process of reflux though the Fallopian tubes during menstruation was originally suggested by Sampson in 1929 (1) and is now the most accepted theory.(7,8) This theory is supported by the fact that conditions such as cervical stenosis that promote retrograde menstruation are associated with an increased incidence of

endometriosis.(9) However, cases of endometriosis are seen in prepubescent girls and the fact that retrograde menstruation is far more common (76-90%) in healthy subjects than the accepted prevalence of endometriosis (10%), both suggest other factors are involved.(10)

Another criticism is that this model fails to explain the fact that disease is found outside the peritoneal cavity in locations such as the lung and brain. This was also considered by Sampson at the time of his initial observation when he also described vascular and lymphatic spread of endometrial cells(11), suggesting that endometrial cells can reach ectopic locations via metastatic spread through vessels not just via retrograde menstruation.

Metastatic or embolic theory

Work by Javert in 1949 and Aoki in 1967, highlighted the importance of haematogenous metastasis and suggested that endometriotic inclusions within glands resulted from transportation of endometrial cells from the uterine body through lymphatic channels.(12,13) In patients with deeply invasive rectovaginal disease and bowel endometriosis, lymph node inclusions are more common and their presence is linked to disease burden. In contrast, those with peritoneal or isolated ovarian disease are much less likely to have lymph node involvement. A comprehensive review by Jerman in 2015(14), concluded that lymphatic spread is involved in the spread of eutopic endometrium from the uterus but may also be involved in the spread of endometriotic cells to draining lymph nodes and so may contribute to the dissemination and persistence of the disease.

A case report describing a rapidly growing ovarian endometrioma that was managed surgically revealing a large endometriotic cyst growing within a pelvic lymph node, highlights some of the features of this model of pathogenesis.(15) The systematic review of the published cases of lymph node involvement discussed in the report demonstrates that whilst endometrial tissue can be found in both pelvic and para-aortic lymph nodes, it is quite rare and when it does occur it is usually glandular inclusions consistent with endometriosis rather than formed endometriotic cysts. The current focus of research in this area is centered around the importance of sentinel lymph nodes and their role in disease recurrence. It has been suggested that residual cells in the lymphatics could be a target for hormonal suppression post excision and so they may

play a prognostic and predictive role in the impact and longevity of surgical management of endometriosis.(16,17)

Endometrial stem cell implantation

More recently the retrograde menstruation model has been refined to suggest that it is endometrial progenitor cells and bone marrow-derived mesenchymal stem cells that differentiate into endometrial cells at ectopic locations - having reached that location either as a result of retrograde menstruation or by transportation in the vascular or lymphatic circulation. The ability of such stem cells to differentiate and proliferate mean they are ideally placed to establish themselves in ectopic locations. The work by Leyendecker *et al.*(18) found that receptor expression is paralleled in the basalis layer of the eutopic endometrium and the ectopic endometrial lesions, suggesting ectopic cells may originate in the basalis layer. They also demonstrated that endometrial shedding of the basalis layer in women with endometriosis occurs at a higher rate compared to healthy women, highlighting this as a possible source of ectopic endometrial cells.

Coelomic origins

An alternative theory is that endometriotic cells have a coelomic origin. Embryological studies have demonstrated that the peritoneum, Mullerian ducts and germinal epithelium of the ovary are all derived from the coelomic epithelium. One theory is that coelomic metaplasia occurs, such that mesothelial cells differentiate into endometrium-like cells, which then respond to increasing levels of sex steroids after puberty.(19) Under conditions of hormonal, environmental or infective stress these epithelial cells may differentiate into endometrial epithelium in an ectopic location such as the peritoneum(7) or they may be cells left over from Mullerian duct migration.(20) This theory elegantly explains why deposits of endometriosis can occur outside the abdominal cavity in the peritoneum of the lung but the fact that metaplasia occurs more frequently with age is inconsistent with the pattern of incidence of endometriosis.

1.2.2 Ectopic endometrial persistence

Once the endometrial cells have reached the ectopic location they must attach to a surface to persist at that ectopic location. Eutopic epithelial cells in subjects with endometriosis are known to have an increased proliferative capacity(21) but for that capacity to be realised in the ectopic location the cells must adhere to the extracellular

matrix and potentially modify the local environment to promote persistence. It appears that the stromal cells are the key to adherence and the epithelial cells allow invasion and growth of the deposits.(7)

The persistence of ectopic deposits is multifactorial, but alteration of cell cycle control seems to play a role. The upregulation of the anti-apoptotic gene BCL-2 has been demonstrated in eutopic and ectopic endometrium(22) and mutation of the tumor suppressor gene PTEN has been demonstrated in 21% of endometriotic cysts of the ovary.(23) In women with endometriosis these alterations in cell cycle control occur in conjunction with changes in the local extracellular environment to promote continued cell survival.

Extracellular matrix

The proteolytic capacity of the epithelial cells of women with endometriosis also appears to be increased. This is achieved by altered expression of plasminogen activators and matrix metalloproteinases (MMPs).(8) In endometriosis, urokinase type plasminogen activator (uPA) and MMP-3 expression is increased in the eutopic endometrium when compared to controls and the increased expression is even more marked in the endometriotic tissue and peritoneal fluid.(24,25) The increase of these activators promotes plasmin deposition, which in turn promotes degradation of extracellular matrix and synthesis of growth factors. As such this altered expression within those subjects with endometriosis helps the lesions to establish.

Adhesion of endometrial cells at ectopic sites appears to be triggered by an injury process at the peritoneal surface. Whether this change is heritable or acquired is unclear but up-regulation of matrix metalloproteinase (MMP) -3, intercellular adhesion molecule 1 (ICAM-1), transforming growth factor-beta (TGF-β), and interleukin-6 (IL-6) are involved in the influencing the interaction with the extracellular matrix and ability of the immune system to clear ectopically sited cells.(20)

Local oestrogen production

Oestrogen plays an important role in the repair of the endometrium after menstruation. The proliferation of the basalis layer and re-establishment of the vascular supply is dependent on the oestrogen, which is synthesised by the ovary, adrenal glands and adipocytes. Initially, androstenedione is converted to estrone by the aromatase P450

enzyme before being converted to oestradiol. The activity of the aromatase enzyme P450 is controlled by a cAMP cell signalling cascade by the prostaglandin E2, which is itself synthesised by cyclooxygenase-2 (COX-) from arachidonic acid.(7)

Usually, aromatase activity in the endometrium is undetectable but in women with endometriosis the activity is increased in both the eutopic and ectopic endometrium. This means oestradiol can be produced locally and so promotes the growth and persistence of ectopic deposits, irrespective of the endogenous ovarian cycle of oestradiol production. This may explain why pharmaceutical treatments aimed at lowering oestrogen levels may not be effective in all patients with endometriosis.(26,27)

Progesterone resistance

An abnormal response to progesterone or "progesterone resistance" has been described and is based on the observation that some patients with endometriosis do not respond to progesterone therapy.(28) In normal eutopic endometrium, progesterone promotes paracrine signalling that induces the enzyme 17β-hydroxysteriod dehydrogenase type 2 (17β-HSD-2) in stromal cells to metabolise oestradiol to the less biologically active estrone. This does not appear to occur in endometriosis, which may be as a result of altered progesterone receptor expression. Work by Attia *et al.* has demonstrated that progesterone receptor B (PR-B) is not expressed in endometriosis and progesterone receptor A (PR-A) is only expressed at low levels.(29) This results in a significantly reduced capacity to metabolise oestradiol and this couple with increased local production, as outlined above, results in high local concentrations. The impact of this change is impaired differentiation and decreased apoptosis, promoting cell survival.

Progesterone resistance has also been demonstrated in the luteal phase of the menstrual cycle of those with endometriosis.(30) In a gene expression analysis an incomplete transition from the proliferative to early secretory phase was demonstrated, resulting in a phenotype more suited to cellular survival. A blunting of the progesterone induced reduction of mitosis was also seen. The impact of these changes is impaired differentiation and decreased apoptosis; promoting cell survival and implantation of any refluxed endometrial cells.

Angiogenesis

The pelvic peritoneum is one of the most common sites for endometriotic lesions but is relatively avascular. For an endometrial deposit to thrive and proliferate at an ectopic site, such as the peritoneum, new blood vessel growth must be promoted. It is this new growth that creates the typical red lesions within the peritoneum. This process of angiogenesis is often accompanied by neuro-angiogenesis at those peritoneal lesions resulting in increased nerve supply, which contributes to the pain symptoms associated with the disease.(20)

The inflammatory cytokines IL-8 and tumor necrosis factor-α (TNF-α) promote adhesion, proliferation and angiogenesis. In addition vascular endothelial growth factor (VEGF) is consistently detected at high concentrations in the peritoneal fluid of women with endometriosis.(31) The level of expression appears to correlate with the stage of disease and is abundant in the glandular tissue of peritoneal implants and endometriomas. VEGF expression appears to be under the control of oestradiol(32) so the aberrant local oestradiol levels described above are likely to promote angiogenesis through VEGF upregulation.

1.2.3 Immune dysfunction

Immune dysfunction appears to play a role in endometriosis and may help to explain why the disease becomes established in some women and not in others and may also be the key to why the disease is cleared by some individuals but in others rapid progression is seen. The exact involvement of the immune system remains incompletely understood but aspects of both the innate and adaptive systems are involved.

Inflammation

Endometriosis is an inflammatory condition and neutrophils and macrophages are recruited in response to this inflammation.(33) The inflammatory response is key to many of the clinical features of the disease and the subversion of the normal response to inflammation appears to play a role in the pathogenesis of endometriosis. The recruitment of macrophages promotes the production of pro-inflammatory and chemotactic cytokines, as well as the angiogenic factors IL-8, TNF-α and VEGF, described above.(7)

In addition to macrophages, neutrophils appear to be important. The life span of neutrophils in endometriosis seem to be extended and anti-apoptotic factors in the peritoneal fluid of endometriosis patients appear to contribute to that longevity. This contributes to the pro-inflammatory environment and promotes the establishment and persistence of ectopic foci of endometrium.(34)

Cytokine dysregulation is also a component part of immune dysfunction in endometriosis. The pro-inflammatory cytokines - interleukin-1 (IL-1), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), granulocyte- macrophage colony-stimulating factor (GM-CSF), and the anti-inflammatory cytokines: IL-4, IL-6, and IL-10, are all upregulated in individuals with endometriosis. The innate immune system is in a very fine balance and disturbing the inflammatory signalling mechanisms tends to promote a pro-inflammatory state and enables ectopic endometrial cells to persist.(7)

NK cell dysfunction

Refluxed endometrial tissue that reaches the pelvis should be cleared by the immune system, but this clearance appears to be impaired in endometriosis. Studies looking at the eutopic endometrium in endometriosis have shown that the endometrial cells are more resistant to NK-cell lysis.(35) The release of secretory factors from the endometrial stromal cells appears to be behind this dysfunction and is likely to be contributed to by macrophage dysfunction.(36) One factor that appears to be implicated in this process is intercellular adhesion molecule 1 (ICAM-1) that is secreted by endometrial cells to create an immune privileged environment at ectopic endometrial sites.(37)

Adaptive immune dysfunction

Cell mediated immunity also appears to be altered in endometriosis. Ordinarily, cytotoxic T-cells are responsible for clearance of abnormal cells and cells containing intra-cellular parasites. Their action is promoted by Th1 T-helper cells, whereas Th2 T-helper cells promote B-cell activation and antibody production in response to extra-cellular pathogens. However, in endometriosis the balance of T-helper cells favours Th2 T-helper cells, in response to high levels of IL-4. This causes high levels of antibodies to endometrial cells – a response that is ineffectual against ectopic endometrial cells. This antibody response may explain why women with endometriosis have higher rates of miscarriage and implantation failure.(7)

1.3 CLINICAL FEATURES

The clinical features of endometriosis are painful menstruation (dysmenorrhoea), persistent pelvic pain (non-menstrual pelvic pain), pain during intercourse (dyspareunia), ovulation pain, pain and difficulty passing stool (dyschezia), pain on passing urine (dysuria), chronic fatigue, and subfertility.(6) The cyclical response of the ectopic endometrium to endogenous sex steroids and paracrine signalling causes the chronic inflammatory response, described above. The inflammatory milieu of cytokines and prostaglandins are responsible for stimulating nerves within the pelvis and explain the localised pain and dysmenorrhea, as well as promoting a neuropathic component to the pain symptoms. As this inflammatory process progresses the development of adhesions and peritoneal scarring cause further pain by limiting the free movement of the intra-abdominal viscera. It is not completely clear whether the development of deeply infiltrating lesions and endometriomas are a progression of this chronic inflammatory process or are a separate biological entity.

Unfortunately, the combination of chronic inflammation, adhesion formation, uterine and Fallopian tube dysfunction and iatrogenic damage during surgical management of endometriosis can lead to either primary or secondary infertility. This problem is exacerbated by the fact that endometriosis is often found with concomitant adenomyosis making assisted reproductive techniques less effective.(38,39)

Endometriosis is considered to exist in three clinically distinct forms: peritoneal disease, endometriomas and deeply invasive disease.(4) Peritoneal endometriosis presents as deposits or implants over the peritoneal surfaces of the pelvis and abdomen as well as the surface of the ovaries. Whereas endometriomas are cysts within the ovarian tissue, lined with endometrioid mucosa that develop a pseudo-capsule of compressed ovarian tissue around them. The final subtype of endometriosis presents as a solid mass of fibromuscular tissue, often found between the vagina and rectum (recto-vaginal nodules), and tends to be identified on magnetic resonance imaging (MRI) of the pelvis. Whether these three types of disease are separate entities or represent a continuum of the same pathological process remains to be fully elucidated. However, current thinking is that deeply invasive endometriosis (DIE) and recto-vaginal disease may represent a separate subtype that can be completely eradicated with extensive excisional surgery -

although such surgery carries with it greater risks, it often produces better symptom resolution.

1.4 DIAGNOSTIC PATHWAY

1.4.1 History and clinical examination

The presence of the three classic symptoms of dysmenorrhoea [OR 8.1 (7.2–9.3)], cyclical abdominopelvic pain [OR 5.2 (4.7–5.7)] and dyspareunia/post-coital bleeding [OR 6.8 (5.7– 8.2)] are common in endometriosis, although the lack of these symptoms does not exclude the diagnosis. Other frequently found symptoms are heavy menstrual bleeding [OR 4.0 (3.5–4.5)], infertility [OR 8.2 (6.9–9.9)], and urinary tract symptoms [OR 1.2 (1.0–1.3)]. The ESHRE (European Society of Human Reproduction and Embryology) guidance on the management of endometriosis recommend that the diagnosis should be considered in those presenting with non-gynaecological symptoms such as dyschezia, dysuria, haematuria, rectal bleeding, shoulder pain, and also that a previous diagnosis of irritable bowel syndrome [OR 3.5 (3.1–3.9)] or pelvic inflammatory disease [OR 5.9 (5.1–6.9)] is suggestive of disease.(3,40)

Clinical examination can be useful to aid the diagnosis but the history taken from the patient is far more valuable. In some circumstances, pelvic examination is not appropriate but where possible it should be performed for two reasons. It is useful for localising disease, particularly nodules over the uterosacral ligaments and in the rectovaginal septum, and it can help guide the most appropriate imaging investigation. The diagnostic and management pathway recommended by NICE is illustrated in Figure 1.1.(41,42)

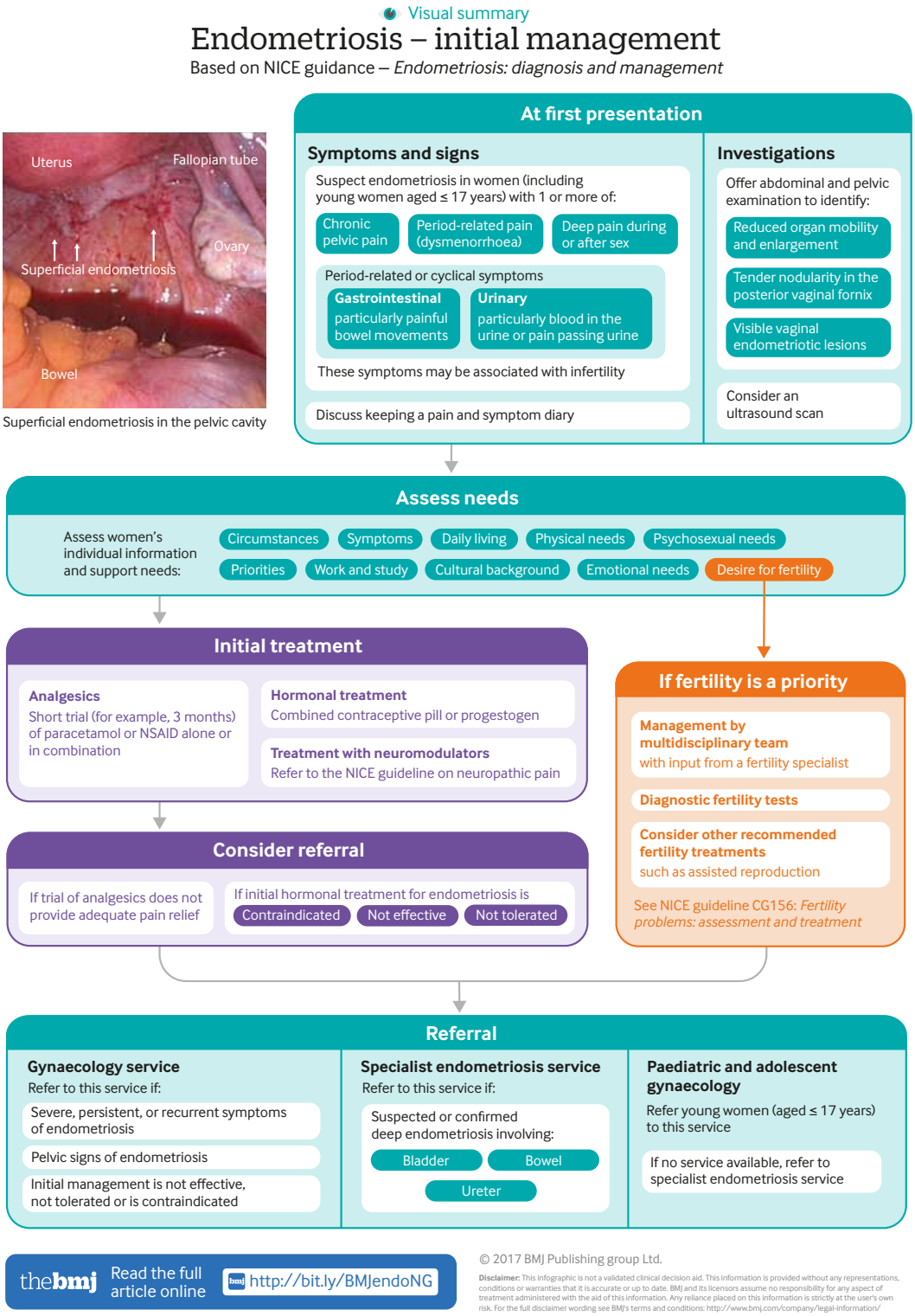


Figure 1.1 **Endometriosis management pathway**

The initial management pathway based on the 2017 NICE Guidance on the diagnosis and management of endometriosis (reproduced with permission).(41)

1.4.2 Imaging & pre-surgical investigation

The use of investigations in the management of endometriosis must be considered carefully, as the tools available each have limitations and should be arranged based on the clinical suspicion. The most commonly used investigation is ultrasound but in some cases MRI, sigmoidoscopy and cystoscopy are needed. These are vitally important as part of the pre-operative planning, particularly when considering excisional management of disease.

USS

Any patient with a suspected adnexal mass on clinical examination should have a transvaginal ultrasound (TvUSS) and its use is recommended by ESHRE as the tool for excluding endometriomas if there is uncertainty.(3) Unfortunately TvUSS cannot be used reliably to identify peritoneal or deeply invasive endometriosis (DIE) within the Pouch of Douglas (POD) or recto-vaginal septum. Depending on the skills of the operator, some information can be obtained about adhesions and fluid filled pockets within the peritoneum, but these features are not unique to endometriosis and so are not diagnostic.

MRI

Magnetic resonance imaging (MRI) is increasingly used in the evaluation of deeply invasive endometriosis (DIE). Current guidance states that MRI is not of value for assessing peritoneal disease location and severity (diagnostic sensitivity of 38% and specificity of 74%)(43) but it is useful in assessing the extent and depth of DIE, particularly in the recto-vaginal septum and para-rectal space, or when ureteric involvement is suspected. MRI is also of value in assessing ovarian endometriomas or haematosalpinx if TvUSS imaging is unclear or suspicious features are identified that require further evaluation prior to surgery.

Sigmoidoscopy & Cystoscopy

Evaluation of the bladder and bowel is necessary if a patient presents with dysuria or dyschezia symptoms. The depth of invasion can be assessed using MRI but visualisation of the lesions by the operating surgeon prior to excision allows appropriate pre-operative planning and ensures the appropriate multidisciplinary team and equipment are available at the time of surgery. This maximises the extent of surgery that can be offered whilst minimising the risks to the patient as much as possible.

1.4.3 Diagnostic laparoscopy

The gold standard investigation is a diagnostic laparoscopy with biopsies to obtain a histological diagnosis. At the procedure, the peritoneum should be thoroughly inspected, the gynaecological organs assessed and the appendix visualised. Unfortunately, this is an invasive investigation and carries with it risks of serious harm. As such it is common practice to treat the disease empirically with medical suppression rather than performing surgery.(3)(6) There is limited evidence to ascertain the importance of histological confirmation of disease. The ESHRE guidance suggests that as a good practice point visual inspection should be accompanied by histological confirmation. However, they concluded that whilst positive histology confirms the disease, negative histology does not exclude it.

Laparoscopic appearance

Peritoneal disease is described as either clear, red, white or blue/black. Initially, microscopic deposits of cells cause a tissue reaction that leads to the accumulation of inflammatory exudate and peritoneal fluid within a clear bleb on the peritoneal surface (clear endometriosis). In response to progesterone withdrawal during the ovarian cycle, endometrial cells within a deposit can prompt an inflammatory response and bleeding at the ectopic site, and the deposit becomes red in colour (red endometriosis). This appearance is usually considered to be active endometriosis, which is more likely to be causing symptoms. Some of these deposits appear to *burn out* and resolve to become an area of white scar tissue (white endometriosis), which is often inactive and is thought to be less likely to be a cause for pain. However, this scarring reaction is often seen in association with adhesion formation, peritoneal pocketing and tethering of the pelvic anatomy, which can often hide more active disease and may be associated with pain symptoms in patients with endometriosis.

Unfortunately, some deposits seem to become more persistent and deeply invasive. Whether this is proliferation of refluxed endometrial cells and a progression of peritoneal disease or cells of a different embryological origin is unknown, as discussed previously. These deposits tend to take on a blue or black colour (blue/black endometriosis) and can often contain small collections of haemosiderin and altered blood. There tends to be a greater tissue reaction around these deposits and more

distortion of the peritoneum. As such, excision of such deposits rather than tissue destruction with laser or electrocautery is considered more appropriate.

Endometriotic cysts within the ovary (endometriomas) have a typical bluish appearance and contain dark altered blood that has a deep brown colour, as such they are often referred to as *chocolate cysts*. These can occur bilaterally and prompt a significant inflammatory response that promotes adhesion formation to the pelvic sidewall, uterus and to the contralateral ovary, resulting in complete obliteration of the Pouch of Douglas. These findings are referred to as *kissing ovaries* and, as a severe form of the disease, are likely to require surgical management to achieve good symptom relief.

Surgical risks

A diagnostic laparoscopy may be considered by some as an intermediate procedure, particularly if no surgical excision is being planned. However, it does require entry into abdominal cavity, establishment of a pneumoperitoneum and general anaesthesia with muscle relaxation. As such, it can be considered a major operation and carries with it risks. These are discussed in detail in Section 5.5.2.

Minimising reliance on diagnostic laparoscopy

The current consensus opinion from international groups such as the World Endometriosis Society (WES) and Society for Endometriosis and Uterine Disorders (SEUD) is that surgical management should be used sparingly and that diagnostic laparoscopies should become less necessary by relying more on clinical evaluation and appropriate imaging.(6)

1.4.4 Clinical staging and classification

The American Society for Reproductive Medicine (ASRM) revised classification of endometriosis is widely used in both a research and clinical setting. It provides a standardised method for recording endometriotic disease and assigns scalar values to disease status. The scoring and staging of disease was intended for use as a fertility prediction tool and to assess the probability of pregnancy following treatment of disease.(44) Unfortunately, it is not designed to assess pain and correlates poorly to the symptoms experienced by patients.

An international meeting of scientists and clinicians was arranged by the National Institutes of Health (NIH), in collaboration with the American Society for Reproductive

Medicine (ASRM) to discuss classification, entry criteria and outcome measure in clinical trials in endometriosis- the "Art and Science of Endometriosis" meeting. They focused on pain assessment and the tools used to objectively record changes in endometriosis symptoms.(45) The consensus statement – 'Pain scoring in endometriosis: entry criteria and outcome measures for clinical trials', produced by this group was approved by the Special Interest Group on Endometriosis of the ASRM and European Society for Human Reproduction and Embryology (ESHRE). Such consideration in the design of future clinical trials is becoming accepted amongst endometriosis researchers.(46)

Biberoglu and Behrman (B&B) scale

The Biberoglu and Behrman (B&B) scale(47) is used in an inconsistent manner in research studies, has never been validated or been shown to be reproducible. It is also of limited use clinically and the component parts of the scale are often included in a standard clinical assessment. However, it is a well-established assessment tool and is commonly used in a research setting.

ASRM classification

The ASRM classification was presented by Paolo Vercellini at the Art and Science of Endometriosis meeting, including his published meta-analysis of 1054 consecutive patients undergoing surgery for endometriosis between 1996 and 2002. He concluded that the classification stage is not predictive of post-operative results or symptom recurrence. However, the conclusion of the group was that the ASRM classification should still be used at baseline for the classification of disease.

1.5 CLINICAL MANAGEMENT

1.5.1 Conservative management

Non-steroidal anti-inflammatory drug (NSAID) therapy with the addition of paracetamol and weak opiates such as codeine, can be very effective and are used to good effect in primary care.(48) This approach is effective for symptom control but is unlikely to have a significant effect on disease progression. The disadvantages of NSAID use are side effects such as peptic ulceration and ovulation inhibition as well as the fact that their use is contraindicated in patients with poorly controlled asthma. The use of opiate analgesia, even weak opiate drugs such as codeine, carries with it a risk of dependence and side effects such as constipation.

Although not a popular choice and one that may increase the risk of disease progression, some patients choose to manage their symptoms conservatively using analgesia either regularly or on an *as needed* basis. This approach tends to be favoured by those who do not tolerate hormonal treatments and those wishing to start a family. It is often the patients in whom fertility is the primary concern that struggle with the difficult decision between managing pain symptoms versus the desire for children.

1.5.2 Medical management

As endometriosis is an oestrogen-dependent and oestrogen driven disease, hormonal manipulation and suppression of oestrogen production form the basis of much of the medical treatments. Initially, hormonal manipulation is trialed with a simple contraceptive agent such as the combined oral contraceptive pill (COCP) (as licensed or tri-cycled to minimise menstrual bleeding time) or a progesterone only pill (POP), which inhibits ovulation. Although concerns have been expressed that oestrogen within the COCP could aggravate the disease by suppressing progesterone production or opposing its action, this has not been supported by studies or indeed in clinical practice. In addition to improving dysmenorrhoea, non-menstrual pelvic pain and dyspareunia(3); the COCP is also particularly valuable for minimising endometrioma recurrence rates.(49)

This first line management can be initiated empirically without a confirmed diagnosis, which minimises any delay between treatment and diagnosis and helps to avoid the cost and potential morbidity of invasive surgical investigation. If a patient fails to respond or their symptoms progress after a period of effective symptom control, laparoscopic confirmation is required before pursuing second line medical therapy. This approach is promoted by the European Society of Human Reproduction and Embryology (ESHRE)(3), reflecting the findings of the 2009 Cochrane review showing there was no difference in pain management outcome between the oral contraceptive pill and GnRH analogues.(50)

Second line medical therapy usually consists of continuous progesterone provision either as the levonorgestrel intra-uterine system (LNG-IUS (Mirena®)), continuous oral norethisterone (NET), an etonogestrel implant or depot injections of medroxyprogesterone acetate (MPA).(51) These tend to be used following confirmation of the diagnosis at laparoscopy but in certain circumstances they can be used

empirically. The Mirena® has quite variable effectiveness but is very successful for certain groups of patients, such as those with dysmenorrhoea predominant endometriosis or concurrent adenomyosis.

Although not all these forms of continuous progesterone have been considered, two Cochrane reviews have demonstrated that medroxyprogesterone acetate is more effective for symptom control than placebo; with the most recent review reporting a Peto OR of 3.00 (99% CI 1.70 to 5.31, two studies, n= 204, I^2 = 22%) for the number or women achieving a greater than 50% reduction in visual analogue scale pain score immediately after MPA treatment.(52,53)

If these second line treatments are ineffective, treatment may progress to complete suppression of the hypothalamic-pituitary-ovary (HPO) axis with a GnRH agonist.(54) This approach has been compared head-to head with progestogen and been shown to be associated with a greater improvement in pelvic pain score (MD 3, 95% CI 2.08 to 3.92, one study, n=47).(53) The removal of oestrogenic stimulation using GnRH analogues is often effective but carries a high treatment burden. The associated menopausal symptoms and osteoporosis risk mean that long term use is limited. A Cochrane review on the use of GnRH analogues in 2009 suggested the use of add back hormone replacement therapy (HRT) as a routine to counter these side effects.(55)

The ESHRE guidelines recommend the use of hormonal contraceptives, progestogens, anti-progestogens, or GnRH agonists as they reduce endometriosis pain.(3) There is no specific guidance as to the sequence in which they should be trialed or the duration of treatment, and no clear evidence exists to demonstrate which is more effective. This stepwise, three-tiered approach to medical therapy is useful but it is important to remain flexible when planning treatment and consider patient preference, side effect profiles and costs.

Hormonal therapies are not suitable for every patient and often a combination of ineffective symptom control, progressive symptoms, intolerance of side effects or contraindications limit their use. It is quite common for patients to find they have exhausted the medical options and so are left considering a surgical solution.

1.5.3 Surgical management

Surgical management is considered when first line medical therapy is ineffective, disease appears to have progressed despite medical therapy or clinical assessment indicates severe disease such as deeply invasive endometriosis (DIE) of the rectovaginal septum and uterosacral complex or large endometriomas that are unlikely to respond to medical treatment. In situations where surgery is required to make a diagnosis laparoscopic removal of endometriosis should be considered at that surgery.(56) A Cochrane review in 2014 highlighted that laparoscopic surgery resulted in decreased pain when compared to diagnostic laparoscopy; both at six months (OR 6.58, 95% CI 3.31 to 13.10, 3 RCTs, 171 participants, $I^2 = 0\%$) and at twelve months (OR 10.00, 95% CI 3.21 to 31.17, 1 RCT, 69 participants).(57)

There is worldwide consensus that excision is preferable to ablation but RCTs have not demonstrated a benefit from excision.(58) Overall, laparoscopic excision of endometriosis is more effective than placebo at reducing pain and improving quality of life but surgery is ineffective in 20% of patients and there is a 30% placebo response rate from surgery so it has its limitations.(59) It should also be noted that any surgery carries with it operative risks and patients must be fully informed of those risks prior to making a decision about surgical management.

The ESHRE guidelines suggest that complete excision of disease is important when managing deeply invasive disease and endometriomas as it results in better pain relief. Performing an ovarian cystectomy also helps reduce the risk of recurrence when compared to drainage and fulguration. In the case of DIE, surgery should be completed by a surgeon with the appropriate laparoscopic skills as complete excision offers better pain control than less extensive surgery coupled with post-operative hormonal suppression using GnRHa therapy.(3,60) The surgical expertise of the operator is key to successful surgical management as there is a growing acceptance amongst endometriosis specialists that a single extensive operation to remove all DIE is curative.

Unfortunately, it should also be noted that there is a significant post-operative recurrence rate - 10 to 55% within 12 months.(61) The risk of recurrence can be ameliorated using simple medical treatment such as the progesterone only pill in the immediate post-operative period, which can be continued longer-term, dependent on the patient's fertility wishes. In situations of recurrence any repeat surgery is likely to

be less effective at managing pain and comes with greater risk of morbidity and so with diminishing returns from repeated surgery a greater reliance on medical treatment is needed.(59) Consequently, there is a clear need for more research into medical treatments, in particular ones in which the side effect profile is more tolerable so that longer term medical treatment can be considered.

When all the available data was considered in an overview of Cochrane reviews relating to endometriosis there was low and moderate quality evidence for GnRH analogues and the levonorgestrel-releasing intrauterine system (LNG-IUS), respectively, when compared to placebo. However, they concluded there was no consistent evidence with respect to comparing, oral contraceptives and goserelin, estrogen plus progestogen and placebo, or progestogens and placebo.(62) This lack of evidence needs addressing with head to head trials of medical and surgical treatments but it also suggests that there may be a place for new treatments, as considered below.

1.6 NEW MEDICAL TREATMENTS/EVALUATIONS

Pre-empt

The ESHRE guidelines do not support the use of post-operative medical treatment as an adjunct to surgery to improve pain as it is ineffective, but they do suggest long-term use (>6 months) can be helpful for secondary prevention of disease. The guidelines do not distinguish between the COCP and LNG-IUS.

The PRE-EMPT Trial (Preventing Recurrence of Endometriosis by Means of long acting Progestogen Therapy is a large randomised controlled clinical trial of women undergoing surgery for endometriosis in which subjects will be given long acting progestogens (either as three-monthly injections (depo Provera) or as a progesterone releasing intra-uterine device, or long-term treatment with the oral contraceptive pill post-operatively. The aim of the study is to provide information on which treatment is the most effective in terms of symptom relief, side-effects, acceptability and costs. This study is eagerly awaited as it will provide a clearer strategy for the management of post-operative patients.

GnRH antagonists

The use of GnRH analogues to establish a hypo-oestrogenic state is common place in the management of endometriosis. The issue of menopausal type side effects is partially

resolved using add-back HRT but some patients still find this treatment intolerable. The *on-off* nature of these drugs makes fine adjustment, dependent on side effects, impossible. Therefore, research has been undertaken to explore GnRH antagonists, which suppress the hypothalamic-pituitary-ovary (HPO) axis sufficiently to relieve symptoms without giving hypo-oestrogenic side effects. The basis behind this strategy is the oestrogen threshold hypothesis, which suggests promotion of endometriosis requires a high oestrogen concentration whereas only a low concentration is required to ensure normal bone metabolism and ameliorate menopausal side effect symptoms.(63,64) It is hoped that by dose adjustment of GnRH antagonists, clinically useful suppression of the HPO axis will be achieved without dropping below the oestrogen threshold.

Two, Phase 3 clinical trials have been completed to evaluate the efficacy and safety of Elagolix in premenopausal women with endometriosis. The results were presented at the 72nd American Society for Reproductive Medicine Scientific Congress & Expo (ASRM) in Salt Lake City in October 2016 and are encouraging. At both three and six months, there is a dose dependent, statistically significant reduction in dysmenorrhea and non-menstrual pelvic pain when compared to placebo.

Chapter 2: HISTOPATHOLOGY OF THE ENDOMETRIUM

The endometrium is an important part of the functionality of the uterus and is a key component of the female reproductive system. The response of the endometrium to the hormonal milieu produced by the hypothalamic-pituitary-ovarian axis determines the menstrual cycle, provides a receptive environment for an early developing embryo and forms a central part of the pathophysiology of endometriosis. An understanding of the anatomy, physiology and histopathology of the endometrium is vital when exploring the pathological processes involved with endometriosis and the action of any pharmacological interventions.

2.1 EMBRYOLOGY

The embryonic development of the uterus from intermediate mesodermal tissue begins at about nine weeks of life. As the ovary descends from the genital ridge to just below the rim of the true pelvis, the cranial portions of the paramesonephric ducts develop into the uterine tubes and the caudal parts fuse to form the uterine canal. The fused paramesonephric ducts give rise to the corpus and cervix of the uterus and then also fuse with the sinovaginal bulbs to create the upper vagina. The corpus of the uterus is surrounded by mesenchymal tissue that goes on to form the myometrium and peritoneal covering.(65)

2.2 ANATOMY

The uterus is a hollow, pear-shaped organ, measuring approximately 9cm long, 6cm wide and 4cm thick. It is made up of three layers: the outer serous layer (peritoneum), the middle layer of smooth muscle (myometrium) and the inner glandular layer (endometrium). The myometrium makes up the bulk of the uterine tissue and consist of interlacing smooth muscle fibres, connective tissue, blood vessels, nerves and lymphatics. The endometrium forms the inner layer but is not sharply separated from the myometrium because the tubular glands and associated stromal tissue dip down into the muscle layer.(66)(67)

The endometrium is made up of abundant endometrial glands which are surrounded by stromal tissue. The density of this stromal tissue is determined by the depth from the endometrial surface. The deepest tissue, closest to the myometrium, is called the *stratum basalis* and contains dense stromal tissue. This layer is not shed during menstruation and allows regeneration of the endometrium with each cycle. The outer two thirds of the endometrium contain less dense stromal tissue (*stratum functionalis*). It is this layer that demonstrates morphological differences throughout the menstrual cycle in response to cyclical production of sex steroids and is then lost at menstruation. There is no anatomical border between these layers but they are differentiated and defined by their function (Fig. 2.1). The surface of the endometrium and the crypts of the individual glands are covered by a single layer of continuous columnar epithelium.

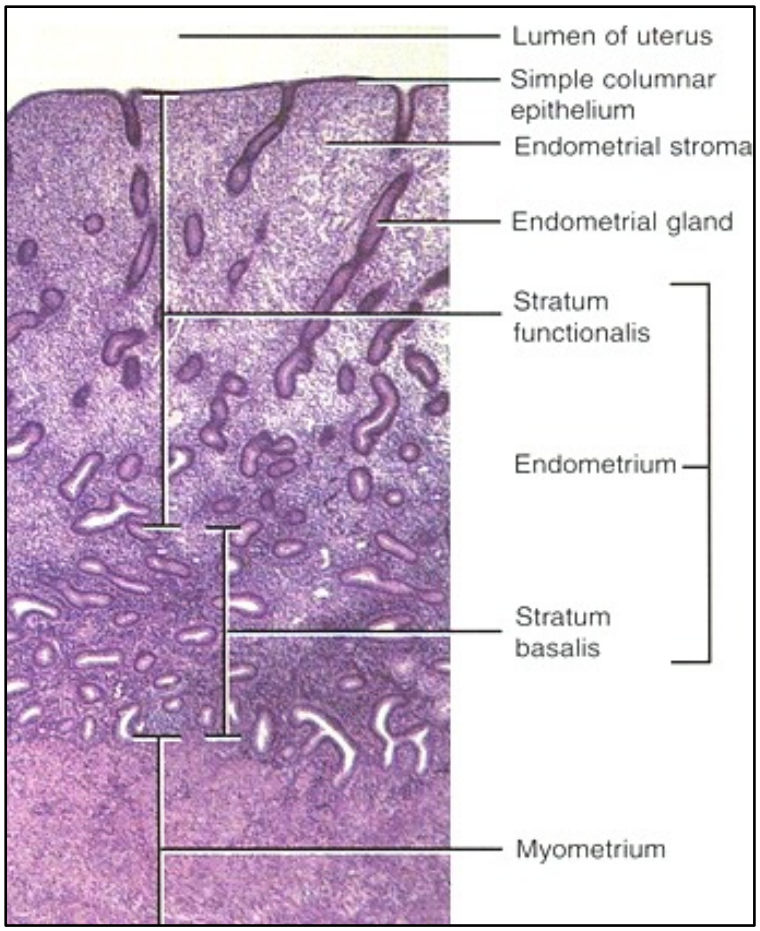


Figure 2.1 **Histology of the uterine wall**

A low power view of the endometrium demonstrating abundant endometrial glands surrounded by stromal tissue in two morphologically distinct zones- stratum basalis and stratum functionalis (H&E) (reproduced with permission).(68)

2.3 ENDOMETRIAL PHYSIOLOGY

The cycle of proliferation, secretion and shedding seen in the *stratum functionalis* is driven by the hormonal changes of the hypothalamic-pituitary-ovarian (HPO) axis. It is this cycle that controls menstruation throughout a woman's reproductive life and is often the focus of managing benign gynaecological conditions such as endometriosis.

2.3.1 Endocrine control

Control of the HPO axis is initiated by pulsatile production of gonadotrophin releasing hormone (GnRH). GnRH is a decapeptide that is released by 2000-3000 neurons found in the pre-optic nuclei, arcuate nucleus and periventricular nucleus of the hypothalamus in response to neural input from higher centres in the brain. The frequency of this release is key to its downstream effect and it is through alterations in pulsatility that negative feedback is achieved. The natural frequency of GnRH release is once every 60 minutes but this is influenced by gonadotrophins, oestrogen and progesterone. At different points in the cycle the frequency varies between >1 pulse/hour and <1 pulse every 2-3 hours, with these frequencies favouring LH (luteinising hormone) secretion and FSH (follicle stimulating hormone) secretion, respectively.(69)(70)

The GnRH neurones release their hormone into the portal vessels supplying the anterior pituitary (adenohypophysis). In the anterior pituitary gland GnRH binds to a GTP (guanosine triphosphate) linked transmembrane receptor resulting in increased intracellular Ca^{2+} and activation of protein kinase C and cAMP (cyclic adenosine monophosphate). These intracellular changes promote protein phosphorylation and result in the following cellular responses:

- synthesis of gonadotrophins ready for storage
- mobilisation of stored FSH and LH ready for release
- immediate release of FSH and LH.

In-vitro studies of pituitary cells suggest that GnRH is not required for the release of stored LH and FSH but it is needed to maintain the synthesis of gonadotrophins. As such, repeated exposure in a pulsatile manner, is needed to ensure adequate pituitary stores. Evidence suggests that there are two pools of gonadotrophins within adenohypophyseal cells; a pool for immediate release which respond to pulsatile GnRH and a reserve pool which require a more continued stimulus to be released. The relative size of these two

pools represent the pituitary sensitivity and reserve, respectively, and they vary throughout the cycle. It is the balance of these two pools that allow GnRH to have a self-priming action, as it promotes transfer of gonadotrophins from the reserve pool to the immediately releasable pool. This 'self-priming' is most active around the mid-cycle.(69)

In the early follicular phase the pulsatile nature of GnRH release leads to a similarly pulsatile release of FSH and LH from the anterior pituitary. Follicle stimulating hormone acts on the ovary to promote follicle recruitment and development. As follicles continue to develop oestrogen levels rise and this has negative feedback action on the hypothalamus and so suppresses the release of further FSH and LH from the anterior pituitary. As a consequence the levels of gonadotrophins within the pituitary increase but this appears to have the greatest effect on the reserve pool.(69)

During the late follicular phase, there is a further increase in oestradiol secreted by the ovary as the dominant follicle begins to grow preferentially in response to paracrine signalling. This results in maximum sensitivity of the adenohypophysis to the self-priming action of GnRH, which is contributed to by a slight rise in progesterone as the granulosa cells of the dominant follicle begin progesterone secretion. This heightened sensitivity results in a gonadotrophin surge, which results in ovulation 35-44 hours after the onset of that LH release.(69)

The luteal phase of the cycle is characterised by an increasing level of progesterone. In the late luteal phase, there is progressive reduction in sensitivity and reserve of the adenohypophysis, which persists into the early follicular phase of the next cycle. This is likely to be related to decreasing levels of oestrogen and progesterone but inhibin, released by the ovary, may also play a role. This pattern of hormonal release is illustrated in Figure 2.2.

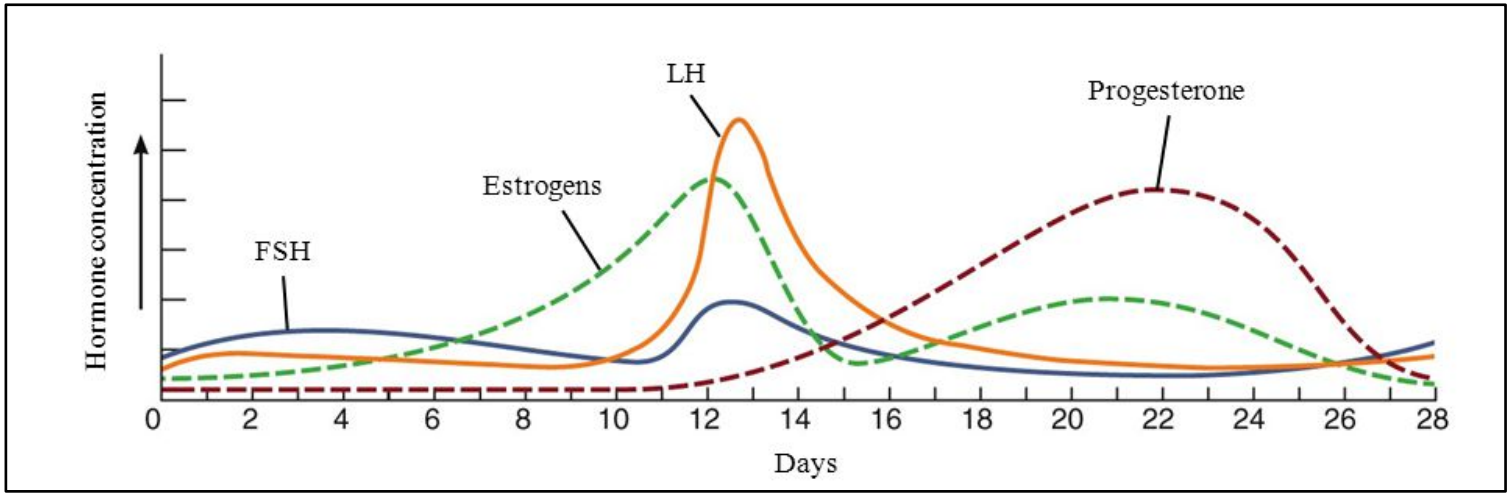


Figure 2.2 **Endocrine changes through menstrual cycle**

Changes in concentration of anterior pituitary and ovarian hormones through menstrual cycle (reproduced with permission).(68)

2.3.2 Action of the hormones on the endometrium

Hormonal control of the endometrial, epithelial, stromal, and presumably the endothelial cells is mediated by estrogen receptors and progesterone receptors.(71) The levels of oestrogen and progesterone and the relative balance of these two hormones determine the endometrial response throughout the ovarian cycle. Oestrogen is the dominant hormone in the follicular phase of the ovarian cycle and it has a proliferative effect on the endometrium. In the luteal phase, progesterone production increases significantly and oestrogen production is lower, relatively, so the balance of hormones is towards progesterone. This causes a secretory change within the endometrium in preparation for implantation.

2.4 ENDOMETRIAL HISTOLOGY

The histopathological appearance of the endometrium is influenced by the above cyclical changes in ovarian hormones. The balance of sex steroids influences both the glandular epithelium and stroma of the *stratum functionalis*. As such the histological appearance can be used as a guide for dating human endometrium but there is low inter-observer agreement and so this cannot be completely relied upon.(72)(73) Seven morphologic criteria are described in the book 'Pathologic Basis of Disease'(67) to allow this dating process to occur:

- *Gland mitoses* - indicate proliferation and are increasingly common in the proliferative phase of the cycle;
- *Tortuosity of glands* - the thickness of the endometrium reflects gland development; in the proliferative phase tortuosity is increased gradually in response to oestrogen but it becomes most pronounced during the secretory phase;
- *Basal vacuolation* - is taken as morphological evidence of ovulation and occurs 36 to 48 hours after ovulation;
- *Secretion* - visible secretion is seen within the lumen of the glands in the mid portion of the secretory phase and towards the later stages becomes inspissated;
- *Stromal oedema* - this varies between individuals in the proliferative phase and may be absent, whereas the oedema seen in the secretory phase is more consistent and uniform;

- *Predecidual reaction* - this is first evident around arterioles in the late secretory phase and progresses until just before menstruation;
- *Leucocyte infiltration* - there are a few lymphocytes within the endometrium throughout the cycle, but neutrophil infiltration begins about two days before the onset of menstruation.

2.4.1 Phases of development and histological features

Menstrual

The cycle begins with the menstrual phase when the *stratum functionalis* is breaking down and being shed. Disintegration of the *stratum functionalis* and escape of blood into the stroma marks the beginning of this process at the end of the secretory phase of the previous cycle. The histological features of this phase are glandular exhaustion leading to gland collapse with prominent necrosis, haemorrhage and intravascular fibrin thrombi.(73)

Proliferative

This phase of the cycle is variable, and its duration is the main determinant of the menstrual cycle length. Under the influence of FSH and oestrogen there is rapid growth of glands and stroma from the *stratum basalis* to regenerate the *stratum functionalis*. This rapid cell division and migration from the *stratum basalis* layer explains the extremely rapid and efficient regenerative properties of the human endometrium seen after the necrosis and tissue breakdown of menstruation.(71) This proliferation is spilt into early, mid and late phases with morphological differences seen as the phases progress.

In the early proliferative phase, there are multiple small, regular glands (Figure 2.3). These are short and straight in appearance and are evenly distributed through quite compact stroma. The glandular epithelium is thin at the surface with occasional areas of pseudo-stratification within the glands. The nuclei are round to ovoid in shape and there are occasional mitotic figures. This proliferation seems to be independent of oestradiol, and immunohistochemical staining indicates minimal oestrogen receptor (ER) and progesterone receptor (PR) expression.(71) However, the deeper tissues of the *stratum functionalis* are more likely to be positive suggesting receptor expression and the dependence on oestrogen changes as proliferation progresses.

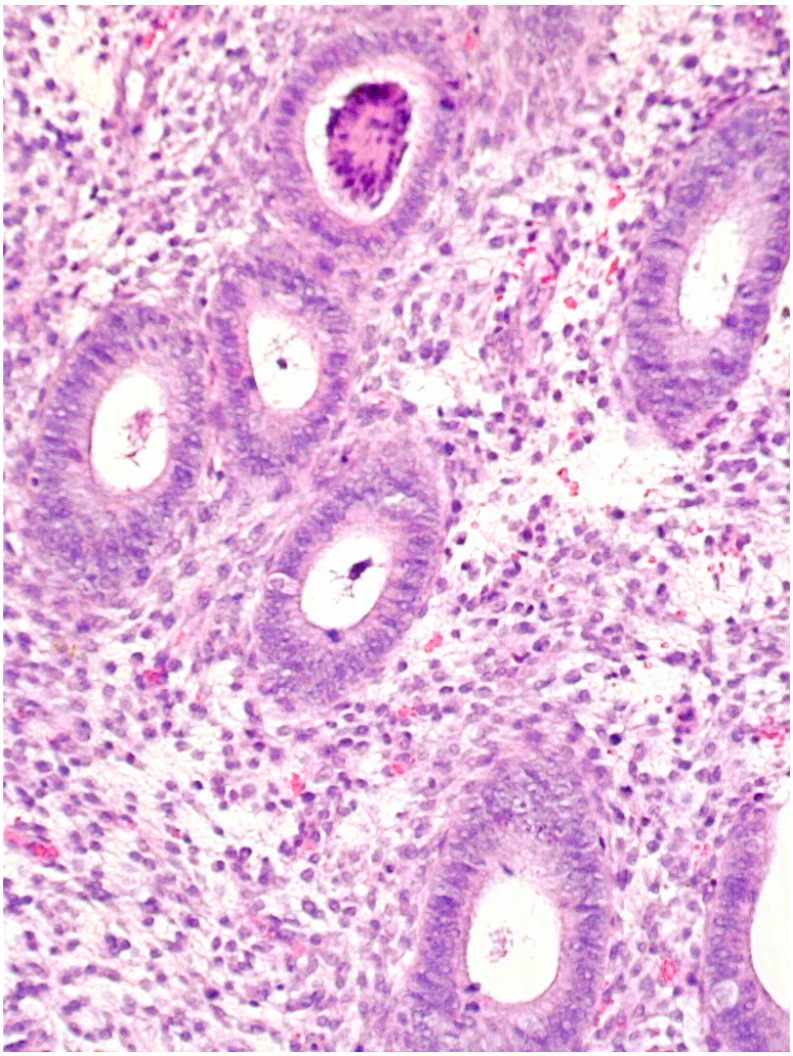


Figure 2.3 **Early proliferative phase endometrium (H&E)**

Early proliferative phase endometrium showing slight pseudo-stratification of nuclei and occasional mitotic figures (H&E) (courtesy of PathologyOutlines.com & The Global Library of Women's Medicine).(73)

Progress into the mid proliferative phase is associated with gland growth to a more elongated form and greater variation in the associated stroma. Now, there are numerous mitotic figures in both the epithelium and stroma reflecting the significant proliferation of the tissues. This is demonstrated using immunohistochemical staining for Ki67, a proliferation marker, which shows widespread staining throughout the epithelium and stroma (Figure 2.4). In addition, ER (oestrogen receptor) and PR (progesterone receptor) expression is increased and more uniform in the mid to late proliferative phase, with ER expression reaching a peak in the mid-proliferative phase.(71) At this stage no vacuolation or secretion is seen.

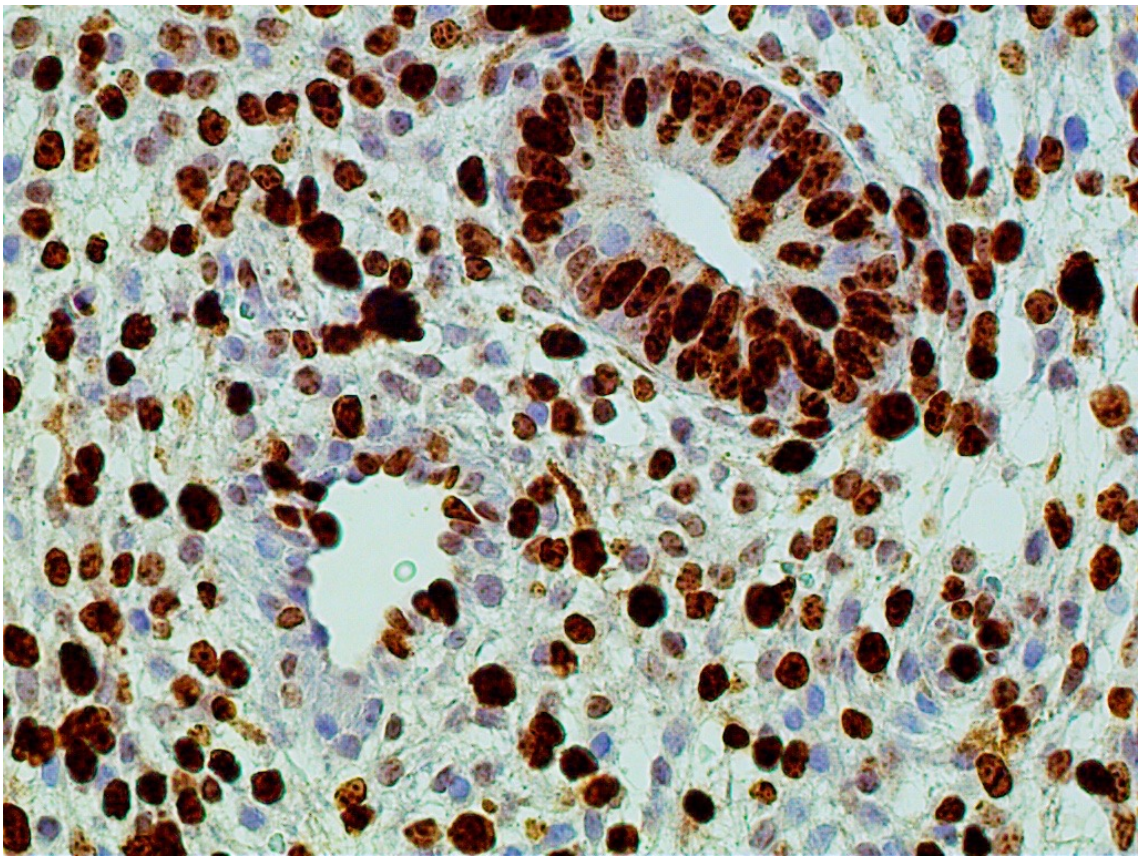


Figure 2.4 **Mid proliferative phase endometrium (IHC: Ki67)**

Mid proliferative endometrium stained for Ki-67 (proliferation marker) showing the intensity of proliferative activity, particularly within the glandular epithelium (courtesy of PathologyOutlines.com & The Global Library of Women's Medicine).(73)

In the late proliferative phase subnuclear vacuoles begin to form but are still seen in less than 50% of the glands. The glands become more tortuous and the pseudo-stratification of the epithelium is more apparent. Mild oedema can be seen within the stroma and the surface epithelium becomes more undulant in nature.

Ovulation

Ovulation occurs at the mid-point of a 28-day cycle and does so in response to a surge of LH. Proliferation of the endometrium ceases and the secretory phase begins. The presence of subnuclear vacuoles in more than 50% of the glandular epithelial cells is taken as morphological evidence of ovulation.

Secretory phase

The secretory phase is much more uniform in length at 14 days but does show some variation. During this phase progesterone inhibits further proliferation and induces secretory activity within the endometrial glands. Similar to the proliferative phase, secretion is spilt into early, mid and late phases with morphological differences seen with each phase.

The early phase is associated with the progression of the subnuclear vacuoles towards the luminal surface of the epithelial cells. The subnuclear positon of the vacuoles give the epithelium a 'piano key' appearance around day 16 (Figure 2.5) and the glands show minimal proliferative activity as evidenced by reduced Ki67 staining (Figure 2.6). By day 17 the vacuoles are level with the nuclei and then by day 18 have reached the luminal aspect of the cell, such that the nuclei are pushed towards the base.

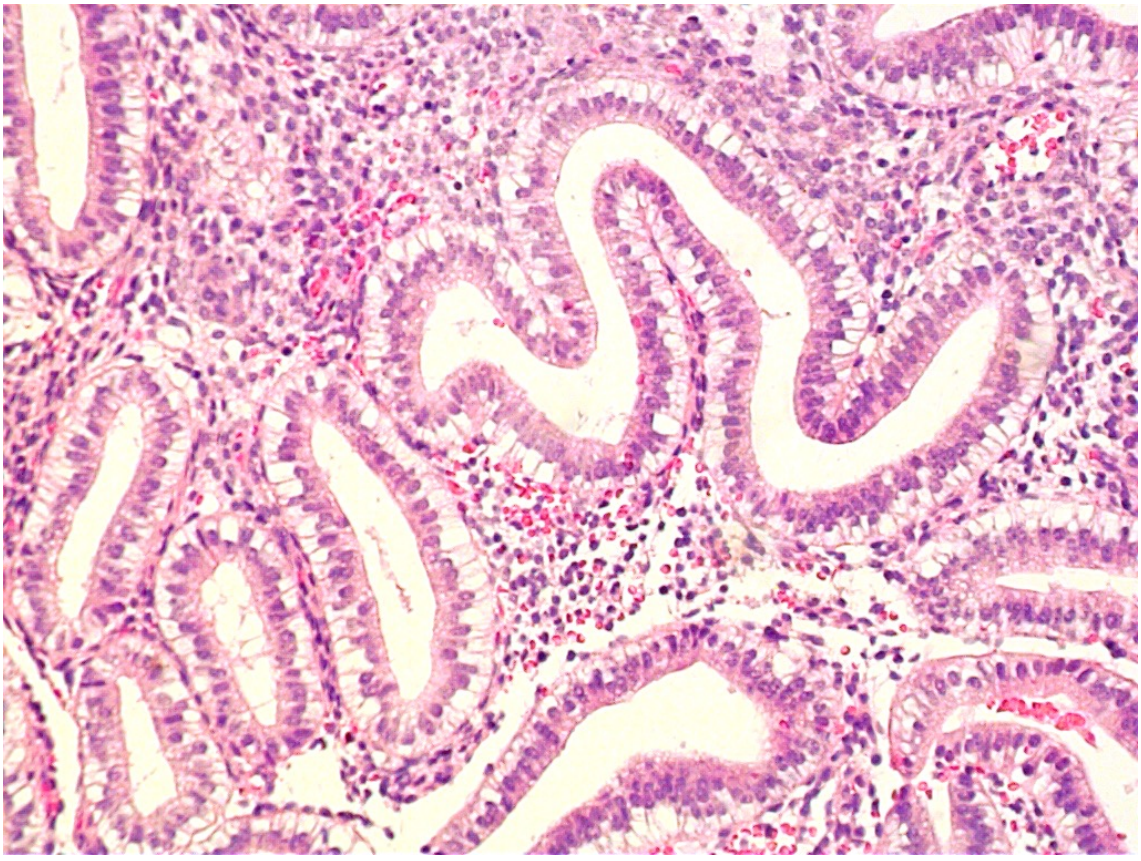


Figure 2.5 **Secretory endometrium (H&E)**

Subnuclear vacuoles (day 16) giving a typical 'piano key' appearance to the glandular epithelium (courtesy of PathologyOutlines.com & The Global Library of Women's Medicine).(73)

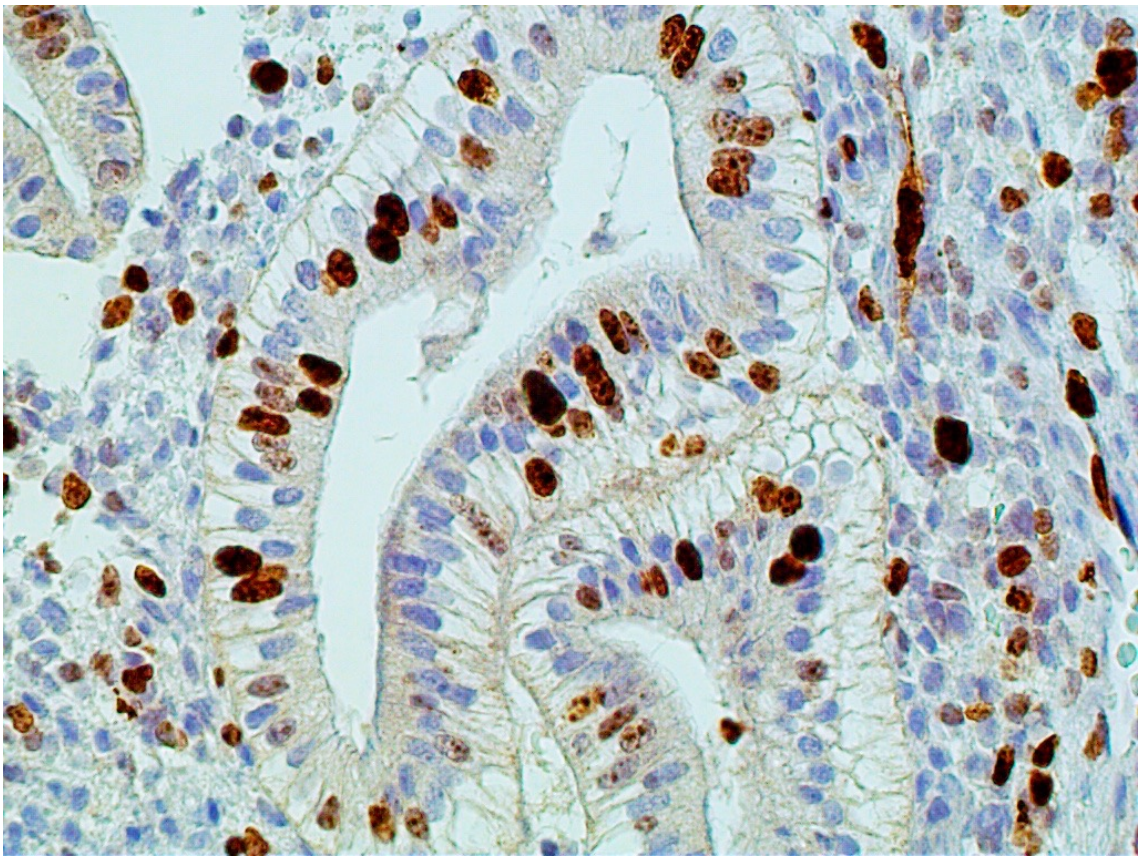


Figure 2.6 **Secretory endometrium (IHC: Ki67)**

Day 17 endometrium in which the vacuoles reach the level of the nuclei (courtesy of PathologyOutlines.com & The Global Library of Women's Medicine).(73)

In the mid secretory phase secretions are discharged into the gland and this is typically maximal at day 20-21. The glands become increasingly tortuous and dilated with mucus. Stromal oedema increases throughout this phase in preparation for implantation, with the endometrium at its most receptive on day 22. Towards the end of this phase the arterioles in the stroma become more prominent.

Immunostaining during the mid-secretory phase shows stromal staining for the proliferation marker Ki67 showing that cell cycling has been downregulated in the glandular epithelium. The epithelial and stromal cells are both positive for ER staining, but the intensity is reduced compared to the proliferative endometrium. The PR pattern is similar to Ki67, with staining confined to only predecidual stromal cells.

On reaching the end of the secretory phase the glands become exhausted, the mucus become inspissated and the glands begin to shrink. This creates a serrated pattern to the glands. At day 23-24 pre-decidual changes begin, typically in the perivascular

stroma; increased stromal oedema, stromal cell hypertrophy (with Ki67 immunostaining confined to stromal cells only) and accumulation of cytoplasmic eosinophilia mark this change. Associated with this there is a non-inflammatory infiltration of granulocytes, of which most are lymphocytes.

At day 27 the infiltration of granulocytes is more prominent, and areas of focal necrosis develop. This progresses to glandular and stromal breakdown with prominent necrosis and haemorrhage on day 28.

These changes are summarised in Table 2.1.

DAY	PHASE		ENDOMETRIAL FEATURES
1	***Menstrual***		▪ Disintegration of the *stratum functionalis* and escape of blood into the stroma marks the beginning of menstruation
2			
3			
4	***Proliferative***	*Early*	▪ Thin surface epithelium ▪ Small, straight, short and regular glands ▪ Glands separated widely by compact stroma ▪ Slight pseudo-stratification ▪ Occasional mitotic figures ▪ Round-to-ovoid nuclei
5			
6	*(FSH & oestrogen dependent)*		
7			
8		*Mid*	▪ Tall columnar epithelial cells ▪ Longer curving glands with more variable stroma ▪ Numerous mitotic figures
9			
10			
11		*Late*	▪ Undulant surface epithelium ▪ Tortuous glands with prominent mitotic activity and pseudo-stratification ▪ Dense stroma with mild oedema and mitotic figures ▪ Subnuclear vacuoles in <50% of glands
12			
13			
14			
15	***Secretory***	***Ovulation*** marks the beginning of the secretory phase - subnuclear vacuoles in ≥ 50% of glands is morphological evidence of ovulation	
16		*Early*	▪ Prominent subnuclear vacuolation
17	*(oestrogen & progesterone dependent)*		▪ Vacuoles reach level of nuclei
18			▪ Vacuoles reach the luminal surface and so the nuclei approach base of cell
19		*Mid*	▪ Intraluminal secretion begins and becomes maximal at day 20-21
20			
21			
22			▪ Stromal oedema is maximal in preparation for implantation
23			▪ Spiral arterioles become prominent
24		*Late*	▪ Perivascular pre-decidualisation occurs - stromal cell hypertrophy with accumulation of cytoplasmic eosinophilia ▪ Tortuous glands become serrated in appearance as they begin to collapse and regress
25			
26			▪ Stromal granulocytes (mostly lymphocytes) begin to appear
27			▪ Prominent stromal granulocytes with focal necrosis
28			▪ Glandular and stromal breakdown with prominent necrosis and haemorrhage

Table 2.1 Histological features of endometrium through menstrual cycle

Number of days in each phase of the cycle based on a standard cycle length of 28 days.

2.5 HISTOLOGY OF ENDOMETRIAL PATHOLOGY

There are several conditions where the usual cyclical changes and classic proliferative and secretory patterns of the endometrium are disturbed. These are described below, along with their histological features.

2.5.1 Disordered proliferation

Disordered proliferation is associated with anovulatory cycles and is common in the perimenopause. It results from unopposed oestrogen stimulation- either from an exogenous source or is the result of a cycle in which progesterone stimulation is lacking in the second half of the cycle.

The histological features are a lack of uniformity within the proliferative endometrium with some glands being cystically dilated and others having shallow budding but a normal gland to stroma ratio. The epithelial cells show metaplastic changes and become ciliated. There is associated endometrial breakdown and haemorrhage despite being in the proliferative phase. These features are the earliest histological changes on a continuum with endometrial hyperplasia.(74)

2.5.2 Endometrial hyperplasia

Endometrial hyperplasia is the presence of irregular endometrial glands and an increase in the gland to stroma ratio. It is the result of prolonged oestrogenic stimulation and can be classified into 3 main types; simple, complex and atypical.(75)(76) Although most women with this condition do not develop carcinoma, atypical hyperplasia is considered a precursor to endometrial carcinoma, particularly in younger women and those developing well differentiated endometrioid adenocarcinoma.(77)

The peak incidence is seen in women in their early 50s for simple hyperplasia (142/100,00 woman-years) and complex hyperplasia (213/100, 000 woman-years); and then in their early 60s for atypical hyperplasia (56/100,00 woman-years).(75) The risk factors for development of hyperplasia are related to oestrogen exposure without appropriate or sufficient progestin to counterbalance that oestrogen. Endogenous oestrogen exposure results from conditions such as chronic anovulation, obesity, diabetes mellitus, polycystic ovarian syndrome (PCOS) and oestrogen secreting ovarian tumours.(78) Alternatively, hormone replacement therapy (HRT) and Tamoxifen use provide exogenous oestrogen in some women.

The classification of endometrial hyperplasia has been the focus of considerable debate within the published literature. There are four different systems in existence but the WHO classification and the endometrial intraepithelial hyperplasia system are the most commonly considered.(76)(79)(78) The World Health Organisation (WHO) system classifies endometrial hyperplasia according to four combinations of glandular crowding and nuclear atypia: simple hyperplasia (SH), complex hyperplasia (CH), simple atypical hyperplasia (SAH), or complex atypical hyperplasia (CAH), although the two forms of atypical hyperplasia (AH) are often considered as one category as cellular atypia is more sinister than changes in glandular architecture.(76) The problem with this system is high intra- and inter observer variability and no consensus on the cytological features of atypia, making reproducibility difficult.(80) Another concern is that there does not appear to be a definite stepwise progression from simple to atypical hyperplasia. As such it does not fully meet the criteria for a classification scheme. The endometrial intraepithelial hyperplasia system is an alternative – using morphometric data from computer image analysis to classify lesions as either endometrial hyperplasia (EH), endometrial intraepithelial neoplasia (EIN) or cancer.(78) This system shows greater reproducibility amongst pathologists and a closer correlation to clinical outcome. However, either of these two classification systems can be used and considered safe when an experienced pathologist is available.(79)

Simple hyperplasia

Simple hyperplasia is characterised by minimal endometrial glandular crowding and no cytological atypia. The glands are usually round but can be irregular and cystically dilated. The epithelium is pseudostratified or mildly stratified with occasional mitotic figures and often shows ciliated cell metaplasia. The stroma shows variable mitotic activity and uniformly distributed blood vessels. As such, it can have a similar appearance to disordered proliferative endometrium leading to a low rate of diagnostic agreement between pathologists.(77)(80)

The risk of progression to endometrial carcinoma is consider to be low (0.7-1.5%)(78). These changes will often regress, particularly if the oestrogenic stimulus that promoted them remits.

Complex hyperplasia

Complex hyperplasia is characterised by greater endometrial glandular crowding, architectural irregularity and bud-like projections seen in the glands (Figure 2.7). By definition, normal stromal cells are present between the glands. The nuclei are uniform in size and shape and the cells have normal polarity- i.e. there is no cytological atypia.(77)

This condition is associated with an intermediate risk of progression to endometrial carcinoma (3-9%). It is managed with progesterone therapy in pre-menopausal patients with hysterectomy and bilateral salpingo-oophorectomy considered in those who are post-menopausal.(78)

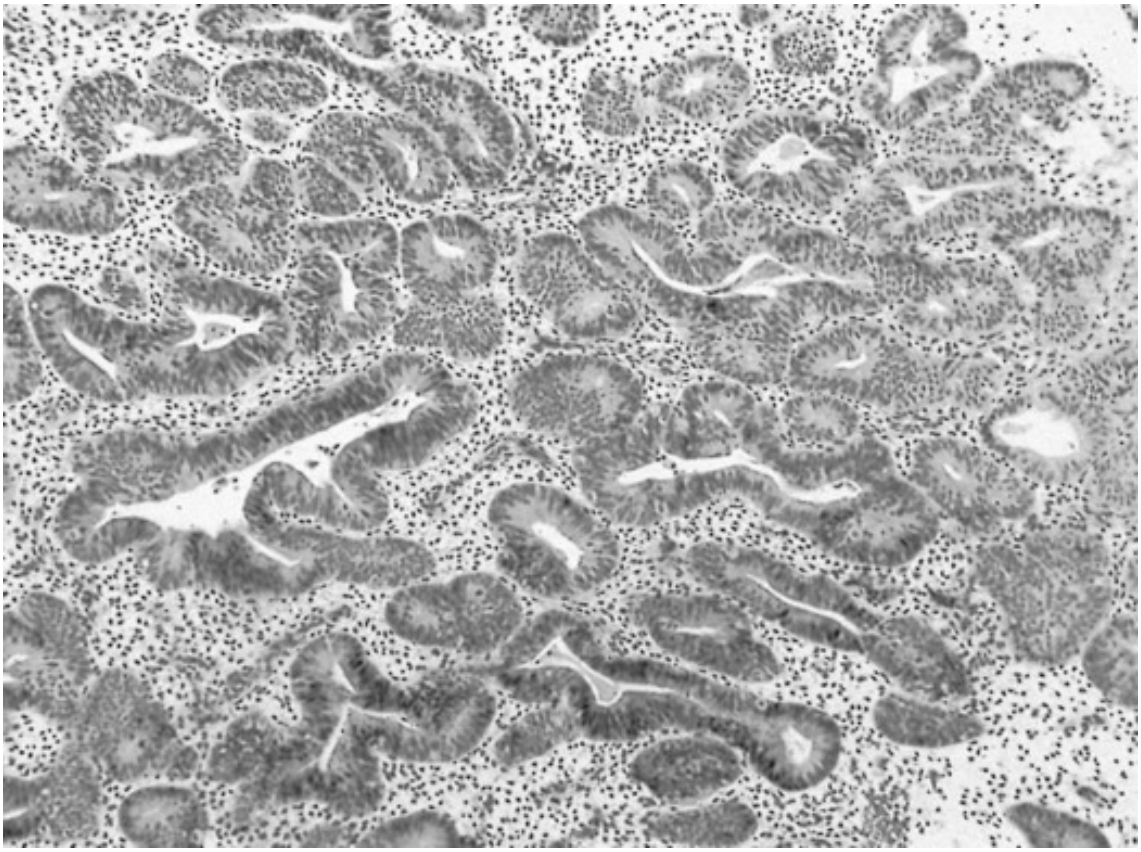


Figure 2.7 **Complex hyperplasia (H&E)**

Closely packed glands with architectural irregularity and numerous bud-like projections (courtesy of PathologyOutlines.com & The Global Library of Women's Medicine).(77)

Atypical hyperplasia

Atypical hyperplasia is the presence of cellular atypia, usually focal, in tissue showing a pattern of complex glandular crowding. Whilst cellular atypia can occur in simple hyperplasia, it is rare (Figure 2.8). The atypical cellular changes are: enlarged, round

nuclei with hyperchromasia and the formation of nucleoli, cellular dyspolarity, irregular nuclear stratification, and cytoplasmic eosinophilia with necrotic debris.

There is 20-30% risk of progression to endometrial carcinoma and also a significant risk of concurrent endometrial carcinoma if the diagnosis is made using an endometrial biopsy alone.(77)(78) As such, the management is often hysterectomy with bilateral salpingo-oophorectomy; although treatment with progestogen or hysterectomy alone can be considered in the pre-menopausal patient.

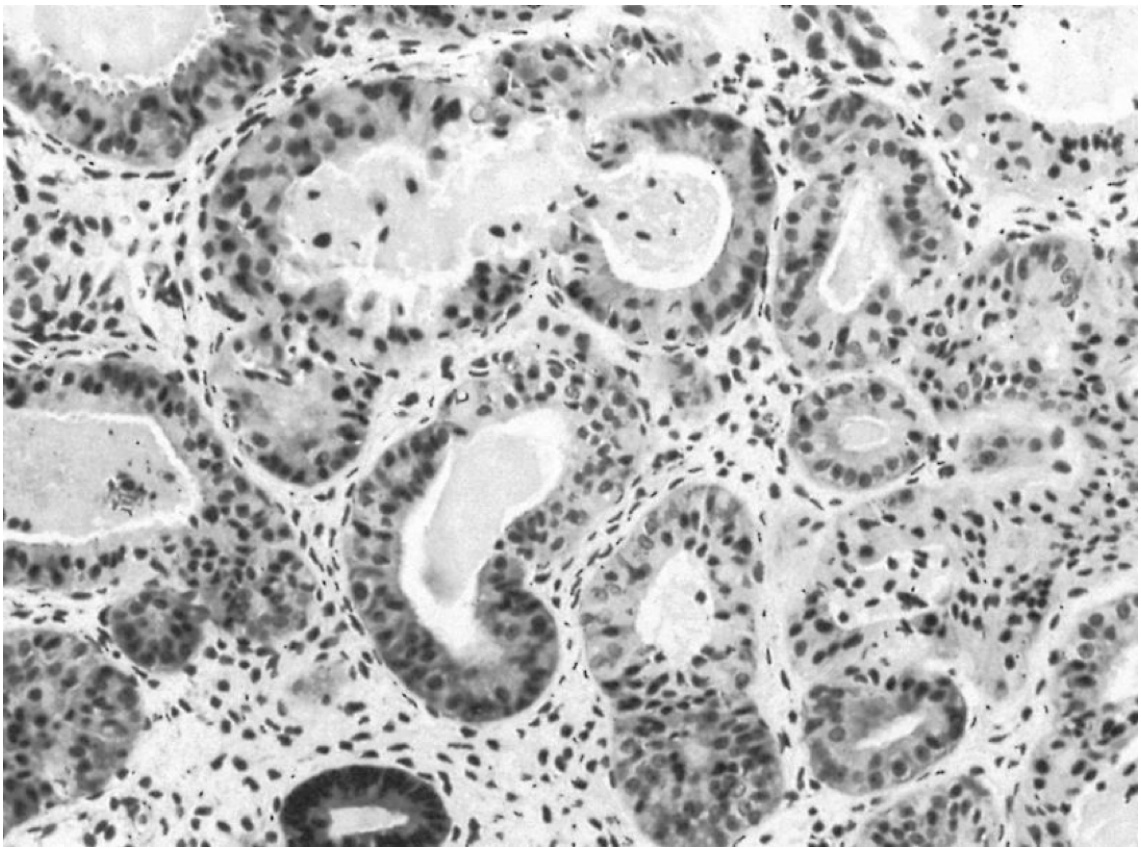


Figure 2.8 **Atypical simple hyperplasia (H&E)**

Glands lined by cells with eosinophilic cytoplasm and dyspolaric, stratified nuclei with moderate anisonucleosis and hyperchromasia. Prominent nucleoli are seen in many of the nuclei. Necrotic debris is present in some gland lumina (courtesy of PathologyOutlines.com & The Global Library of Women's Medicine).(77)

2.5.3 Endometrial polyps

An endometrial polyp is defined as a 'grossly pedunculated mass composed of cystically dilated glands with fibrous stroma and thick walled blood vessels'(81). These can be associated with normal microscopic findings but in up to one third they are associated

with endometrial hyperplasia. The glands within a polyp tend to be unresponsive to progesterone and so are associated with abnormal uterine bleeding (AUB).

The histological features of polyps are: attenuated endometrium on three sides, cystic change within the endometrial glands, large thick-walled blood vessels, fibrous stroma containing fibroblast like cells and abundant extracellular connective tissue. The endometrial glands tend to be proliferative or inactive reflecting their unresponsiveness to progesterone and the associated stromal cells very rarely show atypia.

2.5.4 Endometrial carcinoma

Endometrial carcinoma is the most common gynaecological malignancy, with an estimated incidence of 15-20 per 100,000 women per year.(82) In the UK, the incidence is increasing and went up by 29.3% between 1993 and 2005.(69) This increase has been attributed to increased obesity, the use of tamoxifen for breast cancer and increased life expectancy.

The classification of endometrial cancer separates it into two broad categories based on oestrogen dependence, tumorigenesis and clinical outcome. Type I (endometrioid adenocarcinoma) endometrial cancers are oestrogen dependent and often arise on a background of endometrial hyperplasia.(83) As such there is significant overlap in the risk factors of these two conditions. Factors such as obesity, diabetes, poor diet & lifestyle, early menarche, late menopause, nulliparity, PCOS, functional ovarian tumours, tamoxifen use and unopposed oestrogen therapy, are known to increase the risk. These cancers tend to occur in younger patients, have a less aggressive clinical course and better 5-year survival. Type II (serous adenocarcinoma) endometrial cancers are not oestrogen dependent but tend to occur in the atrophic endometrium of older patients. They tend to be of a higher grade and have a poorer prognosis.(69)

In cancers, the stepwise accumulation of alterations and errors in cell cycle regulation lead to dysfunctional cell growth. However, the genetic mechanisms by which these two types of endometrial cancer develop is very different. In Type I cancers PTEN (phosphatase and tensin homolog deleted on chromosome ten) gene silencing in conjunction with defects in DNA mismatch repair genes lead to microsatellite instability.(82) PTEN is a tumour suppressor gene and its dysfunction leads to activation of multiple signalling pathways, including the PI3K/Akt pathway; affecting cell proliferation, rates of

apoptosis and cell migration.(82) As such it is a promoter of carcinogenesis but is also seen in endometrial hyperplasia. The frequency of loss of PTEN expression has been shown to be related to the type of hyperplasia with the highest frequency seen in complex atypical hyperplasia, suggesting a link to progression of hyperplasia to endometrial intraepithelial neoplasia (EIN) and then on to endometrial carcinoma.(84)

An additional factor in Type I cancers are mutations in the K-ras oncogene, which has been detected in 10-30% of endometrioid endometrial cancers. The frequency of this mutation is proportional to the complexity of any hyperplasia. As such the presence of K-ras mutation in pre-malignant biopsy samples may be a marker of progression to malignancy.(82)

The steroid receptors, and in particular the ratio of the two isoforms of the progesterone receptor (PR): PR-A/PR-B may play a role in pathogenesis of endometrioid adenocarcinoma. Progesterone acts through the PR to negatively regulate the effect oestrogen has on the endometrium. This action is controlled by the ratio of the two isoforms as PR-B acts as an endometrial oestrogen agonist and PR-A acts as a transcription repressor of PR-B. As such an alteration of the ratio to a more PR-B predominant one can increase the chance of endometrial hyperplasia and play a role in the impact of unopposed oestrogen on endometrial cancer risk. Progesterone therapy is effective for reducing risk in such patients as it leads to a downregulation of PR and so reduces the agonistic effect of any endogenous or exogenous oestrogen.

However, Type II endometrial carcinomas arise on a background of atrophy and are associated with mutations in the TP53 tumour suppressor gene in 71-85% of cases and over-expression of the HER-2/neu oncoprotein in 9-30% of all endometrial adenocarcinomas.(82) As such they arise independently of hormonal changes and tend to be seen later in life when more genetic mutations have been accumulated.

Endometrioid adenocarcinoma

Macroscopically, endometrial carcinoma appears as a raised, rough, papillary area with a disrupted and ulcerated surface; usually occupying at least half of the endometrial cavity.(85) The tumours often arise at the fundus or the posterior wall and the degree of the tumour growth within the cavity does not correlate with the risk of myometrial invasion.

The glandular pattern and cellular features resemble that of the proliferative phase endometrium but have a more complex architecture that may include solid growth, maze-like interconnected lumens, a villoglandular appearance, or cribriform growth (Figure 2.9). The epithelial cells tend to be larger than expected in the proliferative phase, but the cytological changes are very variable between different individuals and also within areas of the same tumour. The presence of mitotic figures can also be quite variable. Stromal changes tend to be less useful when characterising adenocarcinoma but the presence of foam cells (lipid laden stromal histiocytes) are a common finding in adenocarcinoma and represents a reactive response to necrotic tumour cells.(85)

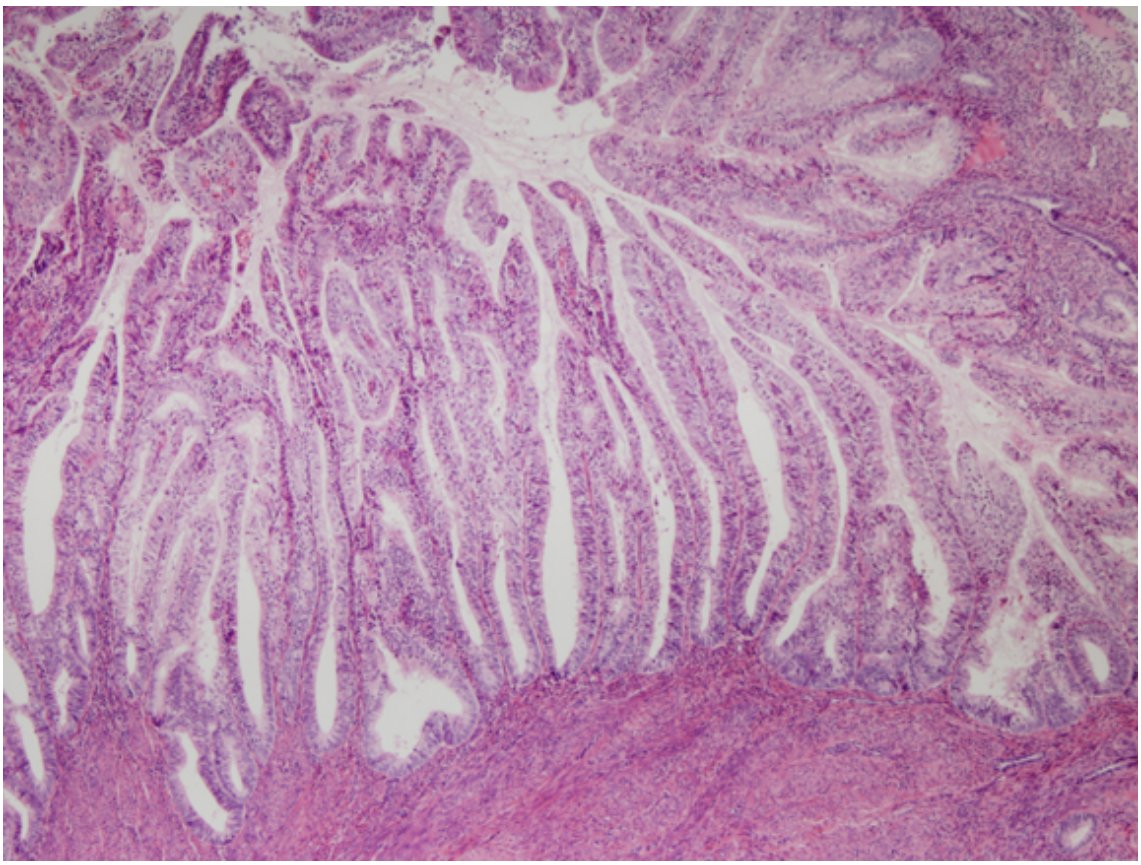


Figure 2.9 **Well differentiated endometrioid endometrial adenocarcinoma (H&E)** *Unlike the EIN lesion above, the glands are no longer visible individually, but are arranged as folded villoglandular sheets (courtesy of PathologyOutlines.com & The Global Library of Women's Medicine).* (86)

2.5.5 Endometriosis

Endometriosis is characterised by the presence of uterine endometrial tissue outside of the normal location and so histologically both endometrial glands and stromal tissue can be seen in ectopic endometrial deposits (Figure 2.10).(2) In addition, fibrosis and

hemorrhage are often associated with endometriotic deposits, but these features are very much less specific. Observation of them adds weight to the diagnosis if they occur in the presence of either glands or stroma.(87) These tissues can respond to ovarian sex steroids, leading to cyclical changes within the tissues, but overall they tend to have a more proliferative pattern than the normal endometrium.(88)

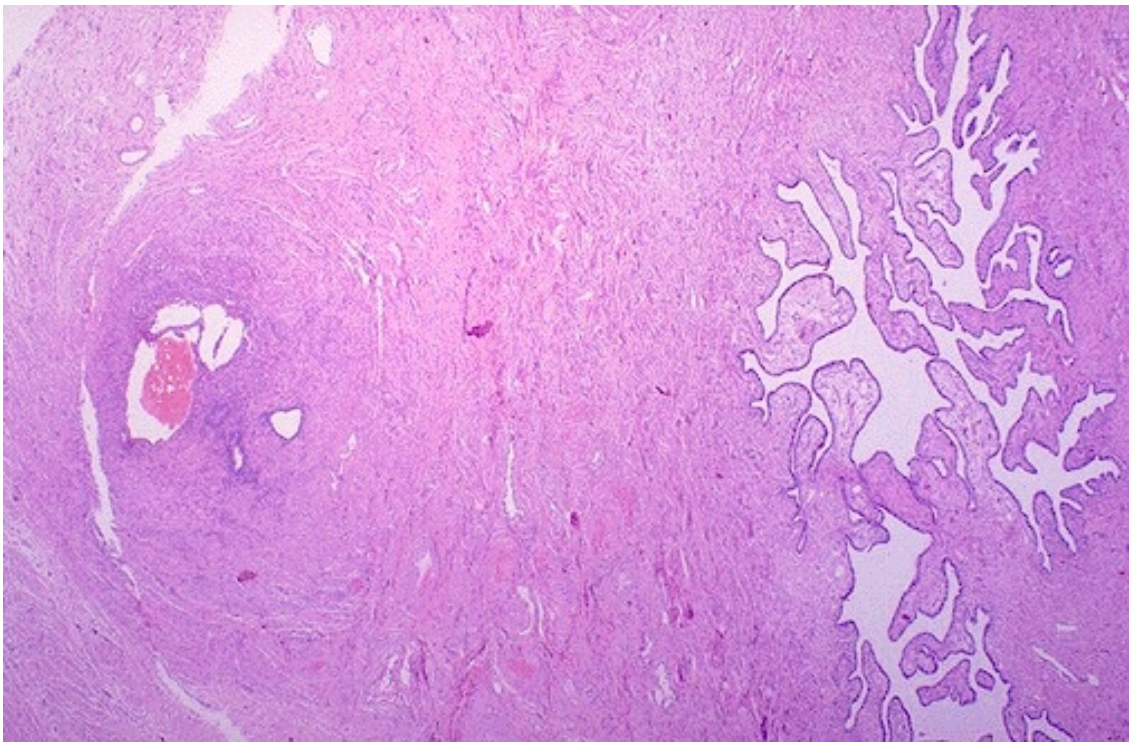


Figure 2.10 **Endometriosis (H&E)**

A small cluster of endometrial glands and stroma with hemorrhage are seen to the left of the panel, near the surface of the fallopian tube (courtesy of PathologyOutlines.com & http://library.med.utah.edu).(88)

The glandular epithelial layer is one cell thick and is made up of tall columnar cells with eosinophilic cytoplasm and ovoid nuclei showing regular vertical orientation. In addition, cilia are sometimes observed on the luminal surface of these cells. There tends to be few mitoses and the overall appearance is that of an inactive or irregular proliferative pattern. However, a more active proliferative pattern and some secretory changes can also be observed. As such, there is not a classic glandular appearance of endometriosis.(89)

The stromal cells are small spindle-shaped cells with minimal cytoplasm. They appear very similar to the stromal cells of the eutopic endometrium. Associated with these

stromal cells is a delicate reticulin network in which hyperemic small vessels are observed in some cases.(90) In the presence of cyclically functioning endometriosis varying degrees of decidualisation can be seen within the stroma; glycogen accumulation within the cytoplasm, increased vascularity and stromal oedema are examples of such changes. The exogenous provision of progestins can also prompt a similar pseudo-decidualisation response. Additional stromal features that are sometimes observed include the presence of haemosiderin laden macrophages, inflammatory infiltrates and collections of smooth muscle fibers.(89)

It is important to recognise that not all these features are seen in all cases and sometimes the epithelial or stromal component is missing, making a firm diagnosis difficult. This is particularly true with disease found in the ovary as the stromal features of endometriosis, such as haemosiderin laden macrophages, can be confused with a haemorrhagic cyst in the ovary or a mature corpus luteum. In non-ovarian endometriosis this confusion is less likely and so a diagnosis can be made based on suggestive histopathological features and the clinical descriptors observed at laparoscopy.(89)

Although endometriosis is hormonally responsive and sometimes the histological features seen in the endometriotic foci are congruent with histological changes seen in the eutopic endometrium this is often not the case.(89) Commonly there are no cyclical changes in the ectopic endometrium but there are some morphological features that are associated with a higher chance of congruency: minimal associated fibrosis, greater numbers of stromal cells and greater associated vascularity. The association between fibrosis and a reduced degree of responsiveness may explain why more established lesions, such as deeply invasive endometriosis (DIE) and endometriomas, are less amenable to hormonal therapy and often require surgery to achieve symptom control.

The chance of seeing cyclical changes will also be influenced by the expression of various steroid receptors within each lesion - with reduced expression of ER and PR being associated with less synchronicity to the eutopic endometrium. As such immunohistochemistry to explore that expression may provide useful information about the character and hormone responsiveness of any lesion. As that hormone responsiveness appears to influence the likelihood of a medical treatment being effective it is sensible to explore this when considering a novel pharmacological

intervention. A study published in 2007 looking at ureteral endometriosis causing ureteric obstruction illustrated that ER and PR expression is seen in both the epithelial and stromal cells, but CD10 expression was limited to only the stromal cells.(91) However, a complete understanding of receptor expression in endometriosis is still lacking.

2.6 ENDOMETRIAL RECEPTOR EXPRESSION & ENDOMETRIOSIS

The use of immunohistochemistry to explore receptor expression within tissues is a key part of histopathological assessment. The expression of either cell surface receptors, cell surface proteins or nuclear apparatus can be used to identify the functionality of the cells being viewed. The anatomical location, histological appearance together with information about cell functionality allows a histopathologist to fully assess the specimen and therefore make a diagnosis.

Assessing cell functionality in both eutopic and ectopic endometrial samples can be achieved by various immunohistochemical stains. Perhaps, unsurprisingly the three main steroid receptors - oestrogen, progesterone and androgen, can be looked at but there are other markers that may also be of value when exploring endometrial function. An understanding of these immunohistochemical stains is needed to assess endometrial response to any pharmacological agent.

2.6.1 ER, PR & AR

Steroid hormones interact with their target cells via specific nuclear receptors. Receptor binding initiates gene transcription and a cascade of downstream molecular and cellular events.(92) Oestrogen receptor (ER) and progesterone receptor (PR) expression within the endometrium is controlled by oestrogen and progesterone, and along with the androgen receptor (AR) is varied through the menstrual cycle. These three receptors are key to endometrial function and may be influenced by pharmacological agents that exert a clinical effect on the endometrium.

Progesterone Receptor

There are two isoforms of the human PR, which are encoded for by a single gene. The PR-A form is 164 amino acids shorter than PR-B and acts as a co-repressor of PR-B and other steroid receptors.(93) PR-B, however, acts as a strong transcription activator. There is a significant decline in PR expression in the glandular epithelium of the *stratum*

functionalis, as the endometrium transitions from a proliferative to a secretory state but the expression in the stromal cells is maintained. Localisation studies have shown that although stromal expression is maintained, the balance of PR-A:PR-B changes to favour PR-A. In the *stratum basalis*, the expression is more uniform in both cell types, throughout the cycle.(92)

The PR is expressed on peri-vascular stromal cells and not on vascular endothelial cells of the human uterus. Therefore, changes in progesterone levels are likely to impact on the balance of vasoconstriction and endometrial bleeding by an indirect action mediated by those perivascular stromal cells.(92)

Wöfler *et al.* conducted a study to observe the pattern of PR-A and PR-B expression in patients with endometriosis. Whilst they confirmed the findings described above in their control group they noted no cycle dependent pattern and a huge variety of inter- and intra-individual variation in PR expression in endometriosis patient group. They suggest this dysregulation might contribute to the pathophysiology of endometriosis. (94) However, this finding suggests using specific PR-A and PR-B immunohistochemical antibodies is unlikely to yield useful results with regards to ectopic endometrium.

Oestrogen Receptor

There are two isoforms of human ER- ERα and ERβ. In the *stratum functionalis* ERα expression in both glandular and stromal cells increases throughout the proliferative phase and then declines, secondary to progesterone suppression, in the secretory phase. Similar, to PR, ERα expression in the *stratum basalis* is more uniform in both cell types, throughout the cycle.(92) However, if an average of all phases are taken into account the expression of ERα in glandular epithelial cells shows a steady gradient from strong expression at the basal layer through to weak expression at the surface epithelial cells.(95)

ERβ appears to regulate the action of oestrogen on endometrial vessels. ERβ is the only sex steroid receptor expressed on endothelial cells.(92)

Androgen Receptor

Androgens can suppress oestrogen and progesterone receptors and have an inhibitory effect on the endometrium leading to endometrial atrophy. AR expression is predominantly in the stromal cells and immunostaining for AR tends to be less intense

than for ER and PR.(95) Expression is maximal in the early proliferative phase and then decreases through the remaining cycle with the changes most notable in the *stratum basalis*.(95) These changes are explained by the observation that expression can be upregulated by oestradiol (E_2) and down regulated by progesterone (P) administration.(96) The use of progesterone antagonists has been shown to increase AR expression in the stroma and induce AR expression in the glandular epithelium.(97) As such, it is thought that the changes in AR expression may be the mechanism by which anti-progestins exert their anti-proliferative effect on the endometrium.(92)

2.6.2 CD10

Enkephalinase or CD10 is a human membrane-associated neutral peptidase that is used widely in lymphoma phenotyping.(98) It has been shown to be a marker for endometrial stromal cells in eutopic and ectopic endometrium as well as neoplastic lesions.(99) The study by Croisman and Meir in 2003, demonstrated the use of CD10 immunohistochemistry as a diagnostic tool in samples of ectopic endometrium where stromal cells are obscured by haemorrhage, inflammation, cystic dilatation, and fibrosis, to distinguish endometriosis from mimickers such as endosalpingiosis and mesothelial hyperplasia.(98)

2.6.3 Ki67

Ki-67 is a nuclear antigen present only in cells undergoing the cycle of changes associated with mitosis and has been used to evaluate tumours to provide prognostic information and guidance on risk of recurrence.(100)(101)(102) The use of Ki-67 immunohistochemistry in the endometrium has been explored with respect to the cyclical endometrial changes. Expression of Ki67 increases through the proliferative phase in response to rising oestrogen levels and then tends to decrease in the secretory phase when mitosis is less.(103) In proliferative samples the pattern of staining reflects the activity of the tissues- Ki67 staining is largely confined to the *stratum basalis* as proliferation is driven from this layer.(71)

As endometriosis can behave in an invasive manner, the use of such markers has been explored to see if it would provide information that would allow the prediction of severity for patients with endometriosis. The case control study by Kahyaoglu *et al.* did show a moderate correlation between the Ki-67 proliferation marker and stage of disease, such that Ki-67 staining can be viewed as a marker for disease severity.(100) Ki-

67 staining has been used in the assessment of suppression of the AKT pathway in a mouse model, where a reduction in pathway activation was associated in less ectopic disease establishment and reduced Ki-67 expression.(104)

2.6.4 PTEN

The *PTEN* (phosphatase and tensin homolog deleted on chromosome ten) gene is a tumor suppressor gene localised on chromosome 10 (10q23-24), which encodes a 403-amino acid protein. The protein it encodes- PTEN, is a lipid phosphatase that modulates cell signal transduction pathways by acting on phospholipid phosphatidylinositol-(3,4,5)-triphosphate (PIP3) signalling.(82) As such it negatively regulates the serine/threonine kinase Akt pathway by phosphorylating downstream signalling proteins; inhibiting cell survival, cell proliferation, angiogenesis and cellular metabolism.(105)

Abnormalities of the PTEN gene have been found in a number of tumours and, in particular in up to 80% of cases of endometrioid carcinoma.(82) A study by Mutter *et al.* in 2000 used immunohistochemistry staining to compare PTEN expression in endometrioid adenocarcinoma and endometria altered by unopposed oestrogen.(84) This demonstrated a progressive and increasingly contiguous accumulation of PTEN-null glands suggesting a role for PTEN in tumourigenesis. In 2006, Kapucuoglu *et al.* also highlighted the association with PTEN loss and the development of endometrioid endometrial carcinomas; including a link between that association and the expression of the anti-apoptotic bcl-2 protein.(106) However, loss of PTEN expression has poor sensitivity (44%) and specificity (51%) as a marker of progression from atypical endometrial hyperplasia to endometrial carcinoma.(107)

There is mounting evidence for a relationship between cancer and endometriosis with respect to the cellular pathways involved and the regulation of the cell cycle. The persistence and proliferation of endometrial cells in ectopic locations maybe promoted through similar pathways to those seen in endometrial carcinoma. As such, studies of tumour suppressors and oncogenes in endometrial samples of patients with endometriosis have been undertaken to explore the Akt pathway and the role PTEN loss may play in the promotion of endometriosis.(108)

Zhang *et al.* used immunochemistry to demonstrate that PTEN expression is reduced in eutopic endometrium of patients with endometriosis when compared to controls. Their work on ectopic endometrial cells in culture also showed significant loss of PTEN expression; with Western blot analysis showing this reduction in expression was much greater than that seen for eutopic cells from patients with endometriosis.(109) Their analysis showed this process was oestrogen dependent, which is in keeping with our understanding of the pathogenesis of endometriosis. Similar work has been done by Govatati *et al.*, who undertook a case-controlled study that showed reduced PTEN expression in eutopic endometria of patients with Stage IV endometriosis and that the greatest effect was in the glandular epithelium.(110) The use of PTEN immunohistochemical assessment on ectopic foci of endometrium (endometriosis) has not previously been published.

2.6.5 p63

There is a 63-kDa membrane protein (p63) that plays a role in regulating epithelial proliferation and differentiation. A study by Neto *et al.* in 2007, investigated expression of this protein in peritoneal deposits, endometriomas and endometriotic nodules. They found that expression varied between the types of endometriosis with peritoneal deposits expressing the highest positivity (93%). However, the significance of this finding and the fact that the immunohistochemistry target is only found on the epithelial cells make this marker of limited value.

2.6.6 VEGF

Angiogenesis in the endometrium is regulated and controlled by a multitude of promoters and inhibitors, all of which are believed to be under the influence of estradiol and progesterone during the menstrual cycle. Vascular endothelial growth factor (VEGF) is a key local mediator of the cyclical neovascularisation seen in the functional layer of the endometrium. It appears to be key to the revascularisation of the endometrium as it repairs and begins to proliferate, as demonstrated by increased levels of VEGF mRNA in the mid proliferative phase.(92)

VEGF concentrations have also been shown to be higher in the peritoneal fluid of patients with endometriosis and VEGF expression is higher in patients with deeply invasive endometriosis.(31)(111) This suggests that VEGF may play a role in the establishment of the endometriotic deposits or the progression of such deposits to

deeply invasive plaques of disease. There is limited published data on the use of VEGF immunohistochemistry in assessment of endometriosis but the highly vascular nature of endometriotic deposits may render VEGF immunohistochemistry staining very homogeneous and so unhelpful in the assessment of endometriosis. However, a uniform change of expression might be associated with pharmacological treatment of the disease and so VEGF IHC may yield meaningful results.

2.7 BEHAVIOUR OF ECTOPIC ENDOMETRIUM

When considering endometriosis, the histopathological features of that disease and whether pharmacological intervention might influence those features, a key question to ask is: does the eutopic and ectopic endometrium behave in the same way. This is a complex consideration and can be broken down into two more specific questions:

1) Does eutopic endometrium within the uterus behave in the same way in both patients with endometriosis and in those without endometriosis? Or does the endometrium behave differently in patients with endometriosis irrespective of the location of that endometrium?
2) Does a pharmacological treatment for endometriosis have the same action on the eutopic and ectopic endometrium of individuals with endometriosis?

Unfortunately, neither of these questions is easy to answer and our limited understanding of the pathogenesis of endometriosis means that we do not have all the tools we might want to explore this area of women's health.(112)

Chapter 3: SELECTIVE PROGESTERONE RECEPTOR MODULATORS (SPRMS)

The progesterone receptor (PR) is an intracellular protein that regulates transcription and is activated by the steroid hormone progesterone. Binding of progesterone causes receptor dimerisation, nuclear entry, DNA binding and subsequently DNA transcription regulation. Each progesterone receptor ligand influences protein expression and cellular responses via several mechanisms that result in a unique response within each type of cell that expresses the progesterone receptor. The factors that influence protein expression include: the variability of PR expression within target tissues, the relative concentrations of co-activators and corepressors, the response of any other sex steroids acting on those tissues, and the type of activating ligand; making the physiological response to PR binding complex and variable.

In addition to this genomic mechanism, progesterone also exerts rapid effects on diverse signalling pathways that are independent of transcription and translation. This action appears to be mediated through the cytoplasmic fraction of the nuclear PR (nPR) and adds a further layer of complexity to the physiological response seen to progesterone.

To explore selective progesterone receptor modulators (SPRMs) use for the management of endometriosis we need to consider the progesterone receptor itself, the physiological action of progesterone and its interaction with the progesterone receptor, and finally the SPRMs themselves, which exhibit quite variable ligand and tissue responses. Although other SPRMs do exist, the discussion will be restricted to Mifepristone, Asoprisnil, Onapristone, Telapristone and Ulipristal acetate as these compounds have either previously been studied with respect to endometriosis or they exhibit a more antagonistic profile, making them more suited to treating endometriosis.

3.1 IDENTIFICATION AND ASSESSMENT OF EVIDENCE

A PubMed and Cochrane database search for relevant publications was performed using the following search terms: 'progesterone receptor modulator', 'SPRM OR SPRMs', 'ulipristal acetate OR Esmya OR CDB-2914', 'asoprisnil OR J-867', 'mifepristone OR RU-486', 'onapristone OR ZK98299', 'telapristone OR CDB-4124', 'endometriosis', 'adenomyosis', 'fibroids OR leiomyoma', 'progesterone', 'endometrium', 'steroid receptor expression', 'endometrial histology', 'immunohistochemistry'. The abstracts for relevant basic science research & reviews, randomised trials, cohort studies, case series and systematic reviews were manually screened to identify those publications containing relevant information. Further relevant articles were also identified from citations within these original publications.

All reviews on SPRMs were included and all studies of the named SPRM compounds conducted in human subjects were included. Animal data were included if considered relevant to the management of endometriosis, in particular relating to ulipristal acetate and mifepristone. As the amount of published literature on progesterone receptor modulators was found to be small (<500 abstracts) no exclusions were necessary. This allowed a broad assessment of the current knowledge on the subject area as well as an understanding on the most appropriate way to study ulipristal acetate.

3.2 PROGESTERONE

Progesterone plays a central role in female reproductive function as well as influencing other body systems like the cardiovascular and central nervous systems.(113) The action of progesterone on its receptor has three main physiological functions within the reproductive system(114):

1) uterine and ovarian function; facilitating oocyte maturation, uterine preparation for implantation and maintenance of pregnancy by promoting uterine growth, suppressing uterine contractility and ensuring cervical closure (parturition is initiated at the end of pregnancy by withdrawal of progesterone and these suppressive functions),
2) mammary function; breast development in pregnancy and suppression of lactation prior to birth,
3) sexual response.

In the proliferative phase of the menstrual cycle oestrogen predominates and this promotes proliferation of the epithelial and stromal cells. In addition, it promotes PR expression to maximise the receptiveness of the endometrium to progesterone after ovulation occurs. The production of progesterone by the corpus luteum ensures that progesterone is the predominant hormone in the secretory phase of the cycle and it promotes the following endometrial effects: growth and coiling of the spiral arterioles, secretory transformation of the glands, infiltration of uterine NK cells, transformation of stromal fibroblasts to promote decidualisation, and increased vascular permeability.(115)

This central role means the action of progesterone and abnormalities in response to progesterone are implicated in many reproductive disorders. In particular, abnormal uterine bleeding and fibroids but also endometriosis, adenomyosis, breast and endometrial cancer, as well as miscarriage and pre-term labour.(116)

3.4 PROGESTERONE RECEPTOR

Progesterone exerts its action on a cell though the Progesterone Receptor (PR). Two different mechanisms have been identified to be responsible for mediating that cellular response- the genomic response and the non-genomic response. Nuclear progesterone receptors (nPR) exert a direct genomic effect by acting as ligand-activated transcription factors to influence transcription (117) but also through cell surface receptors (membrane progesterone receptors (mPR)) that are structurally similar to G-protein coupled receptors in a more indirect manner.(118)

3.4.1 Nuclear superfamily receptors

The nuclear hormone receptor gene superfamily encodes structurally related intracellular receptors for glucocorticoids, mineralocorticoids, and the sex hormones oestrogen, progestin and androgen.(119) As a group they are known as ligand-activated transcription factors and are structurally similar.(120)

Each of the receptors contain three major domains – a ligand-binding domain (LBD) at the carboxyl-terminal end, a variable domain at the amino-terminal end and the DNA-binding domain (DBD) in the central portion of the protein (Figure 3.1). The LBD is moderately conserved between receptors and facilitates the binding of receptor specific ligands. This ligand binding results in the dissociation of the receptor from plasma proteins such as heat-shock protein, which allows the receptors to form homodimers or heterodimers. These then bind to palindromic hormone response elements (HREs) within the DNA separated by three base pairs, e.g. HRE NNN HRE. The DNA-binding domain is highly conserved between receptors and contains a sequence of 70 amino acids that fold into two zinc-fingers motifs that bind to the major groove of the DNA strand to facilitate the recruitment of the transcription machinery (Figure 3.2). Once bound, both the LBD and the highly variable amino-terminus determine the resultant transcription and so are key to the ligand-receptor response seen within each cell.(119)

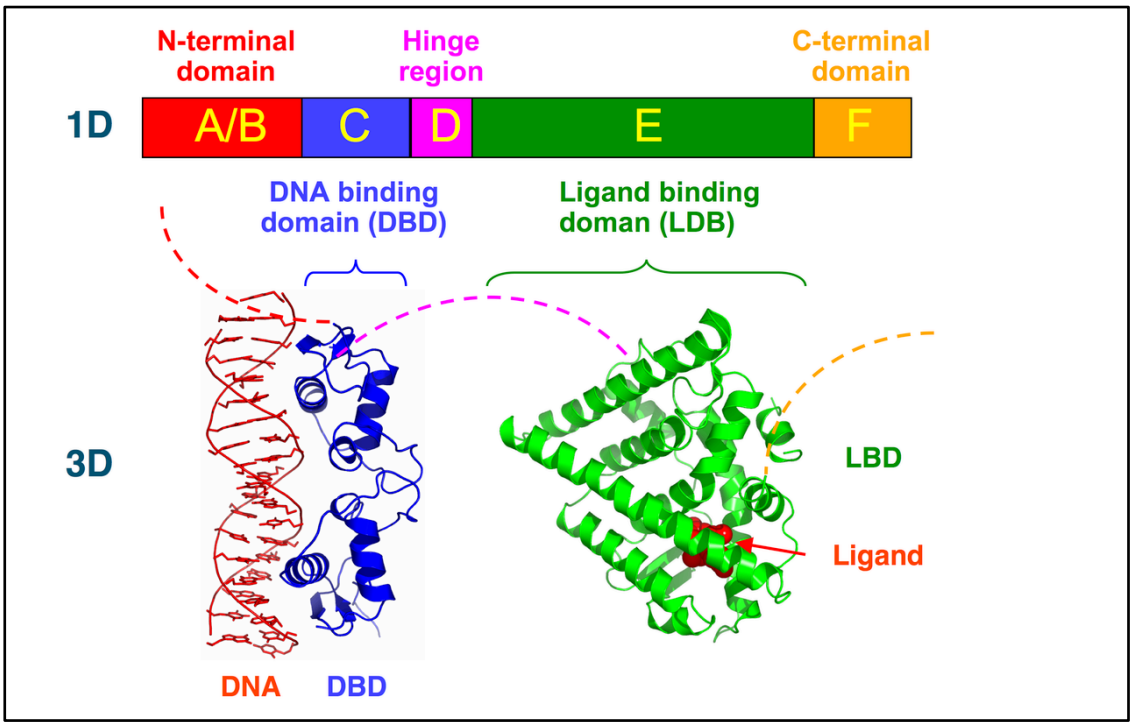


Figure 3.1 **Structural organisation of the nuclear receptor**

The N-terminal regulatory domain (A-B) is highly variable and contains the activation function 1 (AF-1) region which acts as a weak transcription activator independent of ligand binding and acts synergistically with AF-2 in the E-domain. The DNA binding domain or DBD (C) contains two zinc finger portions that allow it to bind to the hormone response elements (HRE) in the DNA (see Figure 3.2). The hinge region (D) links the DBD with the ligand binding domain (LBD) (E) and aids intracellular trafficking. Although the amino acid sequence of the LBD is only moderately conserved the structure of 8 alpha-helices is highly conserved. This structure allows ligand binding and along with the DBD creates the dimerisation interface between receptors. The LBD is the site of co-activator and corepressor binding and contains the ligand dependent activation function (AF-2) region, making it central to transcription activation. The C-terminal domain (F) is a highly variable portion of the receptor (reproduced with permission).(121,122)

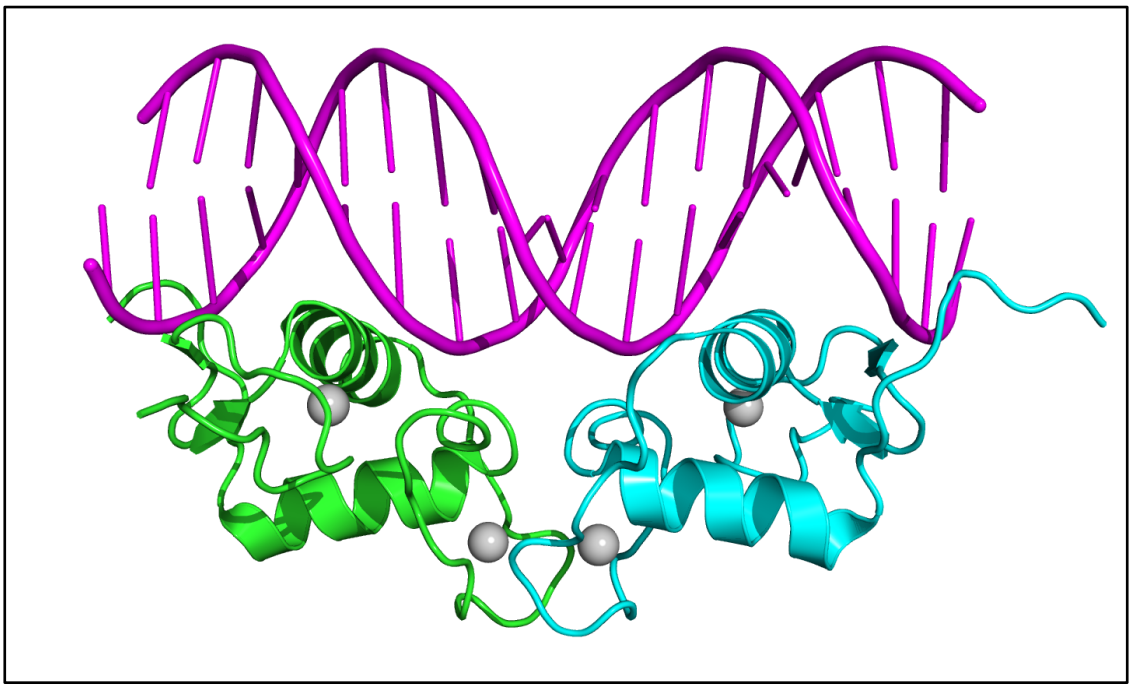


Figure 3.2 **Structure of the human progesterone receptor DNA binding domain**

The DNA binding domain (DBD) is represented by the green and cyan ribbon diagram with the zinc atoms represented by grey spheres. The alpha helices, associated with zinc atoms, produce 'finger' like structure that allows binding into the major groove of the DNA (in magenta) (reproduced with permission).(121,122)

3.4.2 Progesterone receptor isoforms

The progesterone receptor is coded for by the *PGR* gene, which is found on the long arm of chromosome 11 (11q22-q23).(123) This gene codes for both of the main isoforms; PR-A and PR-B. Consequently, they are very similar in structure with a common ligand-binding domain and DNA-binding domain. PR-B is longer with a 164-amino acid sequence at the N-terminus that is not present in PR-A, resulting in PR-B having three transcription domains (AF-1, AF-2 and AF-3) compared to the two domains present in PRA (AF-1 and AF-2) (See Figure 3.3).(124)(125)

A third translational start site has been identified at amino acid position 595 and results in a truncated PR isoform, PR-C. As this isoform lacks the N-terminus and one zinc finger present in PR-A and PR-B it is transcriptionally inactive. *In vitro* studies suggest it may influence the activities of PR-A and PR-B but expression is not seen *in vivo* and so it plays no real physiological role, and will not be considered further.(116,118,126)

The two main isoforms have distinct transcriptional activity due to the additional activation domain (AF-3) found at the amino-terminal end of the PR-B protein. In general, PR-B is a stronger activator of transcription than PR-A. However, under certain circumstances PR-A can act as a strong activator and in alternative circumstances is completely inactive. When inactive, PR-A acts more as a transdominant repressor of other steroid receptors such as PR-B and the oestrogen receptor (ER). This feature of the PR-A protein has been mapped to the first 140 amino acids of the amino-terminus and is called the inhibitory domain (ID) (See Figure 3.3). Although this domain exists in the PR-B protein it is suppressed by the longer N-terminal segment and so PR-B acts solely as an activator of transcription.(120)

The action of progesterone on a cell is determined by the balance of expression of the two isoforms and the co-receptors involved once DNA binding has occurred. These are both influenced by the type of cell and the physiological environment of that cell, making the mechanism of action of progesterone complex. If the possibility of alternative receptor ligands is considered- i.e. progesterone antagonists or SPRMs, the layers of complexity multiply. Therefore, predicting the action of such compounds in human tissue is difficult and tissue/compound specific animal and human studies are required.

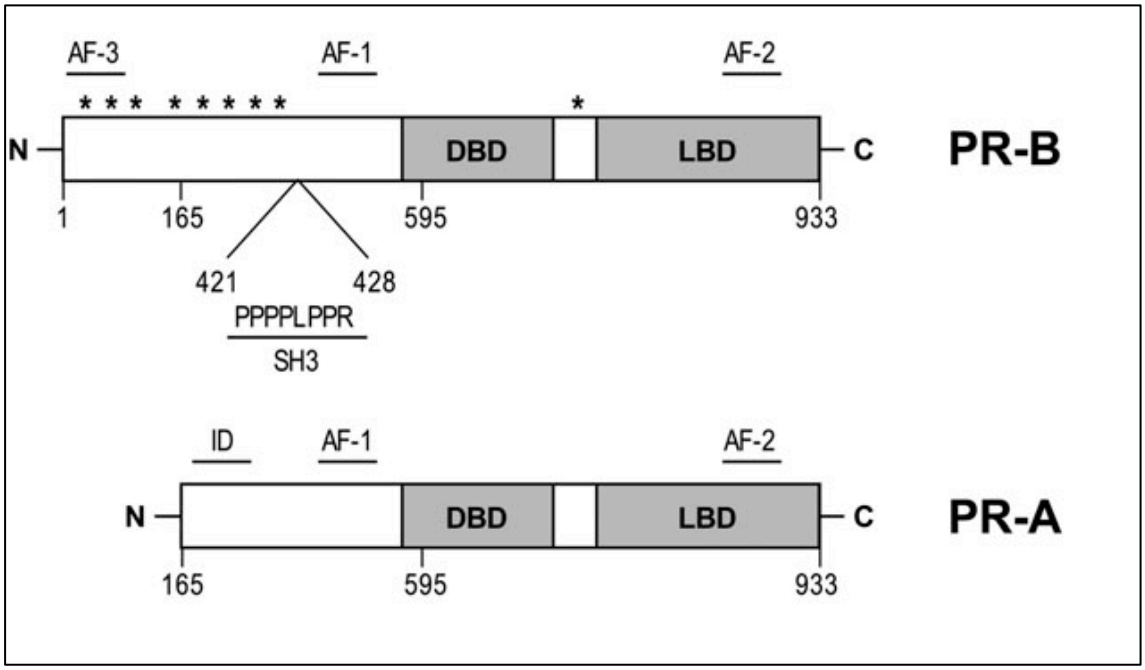


Figure 3.3 **Modular structure of PR-A & PR-B**

*Functional domains of PR-B and PR-A, including the DBD (DNA-binding domain) and LBD (ligand binding domain), are indicated. AF-3, -1 and -2 are transcription activation domains, ID is an inhibitory domain operative in PR-A only and phosphorylation sites are marked with *. The proline-rich region of PR-B that interacts with the SH3 domain of Src tyrosine kinase is underlined (reproduced with permission*).(116,120,127)

3.4.3 Transcription control

Ligand binding to either PR-A or PR-B influences transcription control and thus protein expression by two different mechanisms. Each of these facilitate binding of the basal transcriptional apparatus and co-regulators that allow the synthesis of mRNA ready for translation. The isoform of PR, the ligand that has bound the receptor and the co-regulators that the ligand bound receptor attracts all influence transcription.

Direct

Progesterone binding to PR leads to a conformational change in the protein structure which result in the PR dissociating from chaperone proteins, such as hsp90, and forming homodimers or heterodimers. Once dimerisation has occurred binding to the progesterone response element (PRE) takes place and this influences the recruitment of RNA polymerase to the initiation site to facilitate transcription.(124)

However, the resultant transcription is controlled predominantly by the co-regulators that also bind to the PR dimer DNA complex. Over 300 co-regulators interact with the progesterone receptor to influence transcription leading to activation or repression of specific target genes.(126) It is these co-regulators that influence the tissue and physiological context-specific actions of progesterone and are the mechanism through which different progesterone receptor modulators exert their differing actions (Figure 3.4).(113)

One example of a co-activator is the SRC-1 (steroid receptor activator which recruits histone acetyl transferases and methyltransferases that alter the chromatin structure to facilitate transcription.(118)(128) This has been demonstrated to be the primary cofactor of PR regulation in the uterus through knockout mouse studies.(129)

Co-repressors, on the other hand, tend to interact with the PR when a more antagonist ligand is bound. Studies on the action of the SPRM, Asoprisnil, have demonstrated that co-repressors such as NCoR (nuclear receptor corepressor) and SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) bind to both PR-A and PR-B and exert an inhibitory effect.(130)

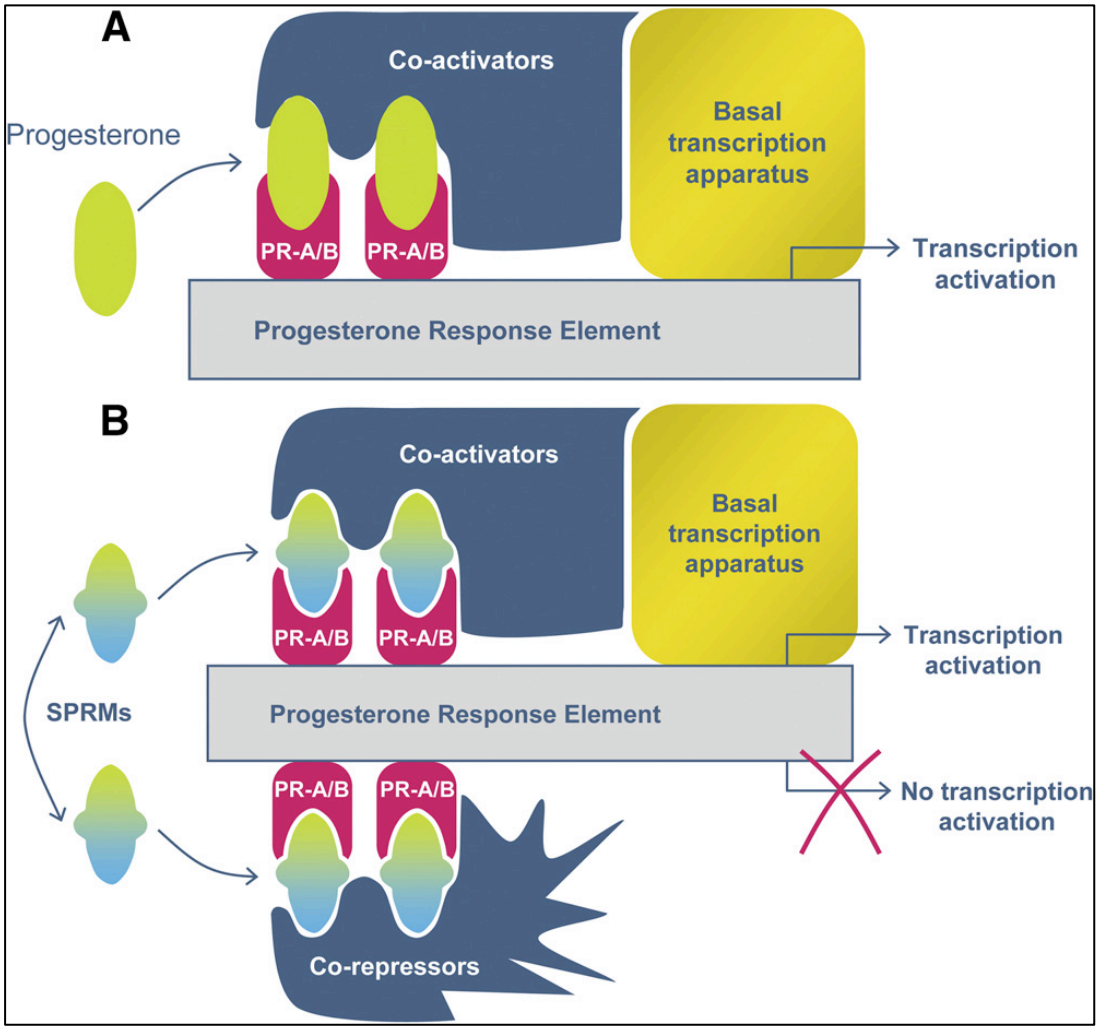


Figure 3.4 **Progesterone and SPRM action on transcription**

(A) Progesterone binding leads to receptor dimerisation and binding of the receptor complex to the progesterone response elements in the promoters of target genes. The different PR isoforms, PR-A & PR-B, exert different biological activities by interacting with different co-activators. The associated co-activators influence the basal transcription apparatus and this determines the resultant gene expression. (B) SPRM binding to PR-A/B and resultant dimerisation allows greater recruitment of co-repressors such as NCoR (Nuclear Receptor Corepressor) and SMRT (Silencing Mediator of Retinoid and Thyroid hormone receptor). However, the conformational change generated by SPRM binding also allows a greater interaction with coactivators such as SRC-1 (Steroid Receptor Coactivator-1) and AIB1 (Amplified in Breast Cancer-1). It is this balance that determines the action of each SPRM- as the conformational change caused by each SPRM is slightly different. This is further influenced by the fact that the action of a SPRM within a specific tissue will also be influenced by the balance of co-activators and co-repressors (reproduced with permission).(113)

Indirect

The extra-nuclear and indirect transcription mechanism involves ligand binding and dissociation from chaperone proteins but dimerisation is not required. Ligand bound progesterone receptors interact with Src tyrosine kinases in the cytoplasm and activate mitogen-activated protein kinase (MAPK) cascade allowing transcription activation and resultant gene expression.[74, 76, 82] It is through this pathway that progesterone influences endometrial stromal cells to promote proliferation in the follicular phase and during the peri-implantation period by activation of the ERK/AKT pathway.(131)

3.4.4 Non-genomic action of progesterone

Some of the actions of progesterone in certain tissues are too rapid to be compatible with transcriptional activation, are not abolished by transcription inhibitors and are observed in anuclear cells like erythrocytes; indicating a non-genomic pathway of progesterone action. The fact that membrane impermeable progesterone derivatives can elicit rapid cellular responses has been taken as evidence of an alternative pathway but should be viewed with caution as the synthesised membrane impermeable progesterone used often contains small quantities of free progesterone and so firm conclusions from such studies are difficult. Many different receptors, intracellular pathways and animal models have been explored.

The membrane bound PR (mPR) is a classic 7 transmembrane domain G-protein coupled receptor, which responds to progesterone binding by initiating a phosphorylation cascade. This mechanism appears to play a role in activation of murine macrophages in response to progesterone.(132) A further example is intracellular kinase activation and Ca^{2+} signalling that has been shown in the VMN of the rat brain in response to progesterone.(133,134)

The physiological impact of the non-genomic pathway is unclear and a review on the subject by Gellersen *et al.* on the subject in 2009 concluded that although nPRs are the main regulators in female reproduction, the diverse tissue and cell specific responses to progesterone cannot simply be explained by a single mechanism of transcription regulation at the promoter regions of specific genes.(116) Whether these pathways are relevant clinically and whether SPRMs influence these mechanisms remains to be elucidated.

3.5 PROGESTERONE ACTION IN EUTOPIC ENDOMETRIUM

The PR-A and PR-B isoforms are both found in the epithelial and stromal cells of the endometrium and progesterone exerts specific actions on each of the cell types at different time points in the cycle. In the *stratum functionalis* proliferation occurs in response to oestrogen during the proliferative phase of the cycle and is further promoted in the stromal cells by the action of progesterone. In the luteal phase, however, progesterone concentration is greater, and it has an inhibitory effect on oestrogen driven proliferation, promotes secretory changes in the epithelial cells and decidualisation of the stromal cells.

Decidualisation involves some stromal proliferation, rounding of nuclear structures and accumulation of cytoplasmic glycogen; and is also associated with the recruitment of various immune cells and angiogenesis.(135,136) The decidualised stromal cells undergo apoptosis on progesterone withdrawal but they can also resist oxidative stress, regulate local immune responses and behave in an invasive manner – all properties associated with ectopic endometrial cells in endometriosis.(137)

3.5.1 PR-A:PR-B ratio

The ratio of the PR isoforms varies between tissues and the balance of these isoforms may contribute to the variable tissue response resulting from progesterone exposure. When PR-A:PR-B is greater than one, PR-A acts more as an inhibitor rather than promoter; so in any tissues, pathophysiological environment or pharmacological modification where PR-A predominates the PR-A driven cellular responses will be strongly expressed.(118) As a transcription suppressor it inhibits endometrial proliferation and mediates the anti-oestrogen activity of progestins. Mimicking or altering this balance of PR expression may be the key to influencing proliferation and explain the endometrial effects seen following exposure to progesterone receptor modulators.

The balance between these isoforms may also be key to certain pathophysiological conditions. However, interpretation of studies looking at receptor localisation must be undertaken with caution as the receptors share a significant proportion of common sequence. Antibodies can be synthesised for PR-A and PR-B only but shared amino acid sequence and structural differences that potentially mask epitopes make this complex.

Ensuring the antibody used is isotope specific and does not also bind the alternative receptor is vital for interpretation and can make IHC less valuable when exploring the different isoforms and the balance of expression.(115)(138)(139)

3.5.2 Isoform specific actions

PR-A and PR-B increase concordantly with oestrogen concentration during the proliferative phase. As no change in the ratio between the PR isoforms occurs the action on the epithelial and stromal cells is similar and is centred around proliferation. PR-A levels in epithelial cells decline in the late secretory phase, whilst PR-B remains constant, suggesting PR-A may be involved in the control of glandular secretion. PR-A appears to be the predominant isoform seen in stromal cells but with less late-secretory decline in expression.(118)

Knockout mouse models have been used to explore the function of the progesterone receptor. PR-null homozygous mice develop normally to adulthood but defects in uterine growth, ovulation defects and impaired implantation make reproduction impossible. These changes were reversed by replacing only PR-A (i.e. PR-B K.O. mice), in which ovarian function, implantation and fertility all returned to normal.(140) However, PR-B acting alone (PR-A K.O mice) promotes endometrial hyperplasia and does not allow stromal decidualisation.(141) This demonstrates that PR-A exerts most of the physiological functions of progesterone and helps inhibit the PR-B driven endometrial hyperplasia. However, the balance between the two isoforms seems to be important as overexpression of PR-A relative to PR-B seems to also cause hyperplasia, atypical changes in the endometrium and inflammatory changes. These findings may give some indication as to the mechanism behind the PAEC (PRM-associated endometrial changes) that SPRMs can promote, which are discussed in Chapter 4.

3.6 PROGESTERONE ACTION IN ECTOPIC ENDOMETRIUM (ENDOMETRIOSIS)

Ectopic endometrial implants undergo growth and morphological change in response to oestrogen and progesterone. Oestrogen is known to promote a proliferative response in eutopic epithelial cells and in keeping with that oestrogen exposure appears to act as an endocrine risk factor for promotion and progression of disease. Progesterone exposure, however, has an inhibitory effect, promotes disease regression and forms the basis of one of the key approaches to medical management, discussed in Chapter 1.

3.6.1 PR balance of expression

The involvement of PR signalling and alterations in the PR-A:PR-B balance in endometriosis is unclear. Decreased progesterone responsiveness (progesterone resistance) in endometrial stromal cells appears to play a role in endometriosis but the role of PR-expression is less clear.(28) Most studies evaluating PR expression in endometriosis have shown reduced levels in ectopic endometrium compared to eutopic endometrium, with five showing a reduction and one study showing no difference.(118) When the difference between PR-A and PR-B has been explored no consistent finding has been demonstrated. In peritoneal lesions PR-B is not expressed and PR-A expression is reduced, but in ovarian disease PR-B shows dominant expression.(142,143)

The most popular pathogenic mechanism to explain endometriosis is retrograde menstruation proposed by Sampson.(1) The often cited flaw in this theory is that endometriosis occurs in only 5-10% of women but retrograde menstruation is almost universal. Based on the assumption that ectopic endometrial deposits originate as cells from the eutopic endometrium that persist at the ectopic location, studies have been undertaken to investigate differences in the functionality of eutopic endometrial cells in patients with endometriosis to help explain this disparity.

Wölfer *et al.* undertook IHC staining of eutopic endometrial samples obtained during diagnostic laparoscopy for patients with pelvic pain & infertility symptoms. They demonstrated that in patients without endometriosis expression of PR-A & PR-B increased during the proliferative and early secretory phase of the cycle and then decreased in the mid to late secretory phase in epithelial cells, as would be expected. A similar pattern was seen in stromal cells but the decline in PR-A and PR-B was imbalanced such that by the late secretory phase only PR-A persisted.(94)

In patients with endometriosis this cycle dependent pattern was not seen and huge levels of inter-individual and intra-individual differences in PR-A and PR-B expression were seen. PR-A expression was more consistent with the receptor identified in epithelial and stromal cells during proliferative and secretory phases of the cycle. PR-B, however, was not detectable in some of the proliferative and mid-secretory samples of both cell types and then strongly expressed in the late secretory phase in the epithelium and stroma. Overall the expression of PR was quite heterogeneous and disrupted. (94)

3.6.2 Progesterone resistance

In patients with endometriosis, the dysregulation of PR expression seen in the study by Wölfer *et al.,* the work by Jones *et al.*(144) showing delay in endometrial maturation, and the transcriptome analysis by Burney *et al.*(30) showing molecular dysregulation of the proliferative-to-secretory transition, are all in keeping with the "progesterone resistance" theory. Whether these changes are just at the isoform level or whether the co-regulators are involved as well is unknown.(137) It is also not clear whether any of the observed changes are seen in ectopic deposits of endometrium in endometriotic deposits as very little work has been published on histological analysis and PR-expression assessment in such tissues.

3.7 PROGESTERONE RECEPTOR MODULATORS

The selective progesterone receptor modulators are steroids derived from norethindrone.(125) These compounds have shown various clinical effects and the therapeutic applications that have been studied are shown in Table 1. Mifepristone, Asoprisnil, Telapristone acetate and Onapristone have been considered for use in Endometriosis but are yet to provide a licensed product. The specific chemical structures of these SPRMs and that of ulipristal acetate are shown in Figure 3.5.

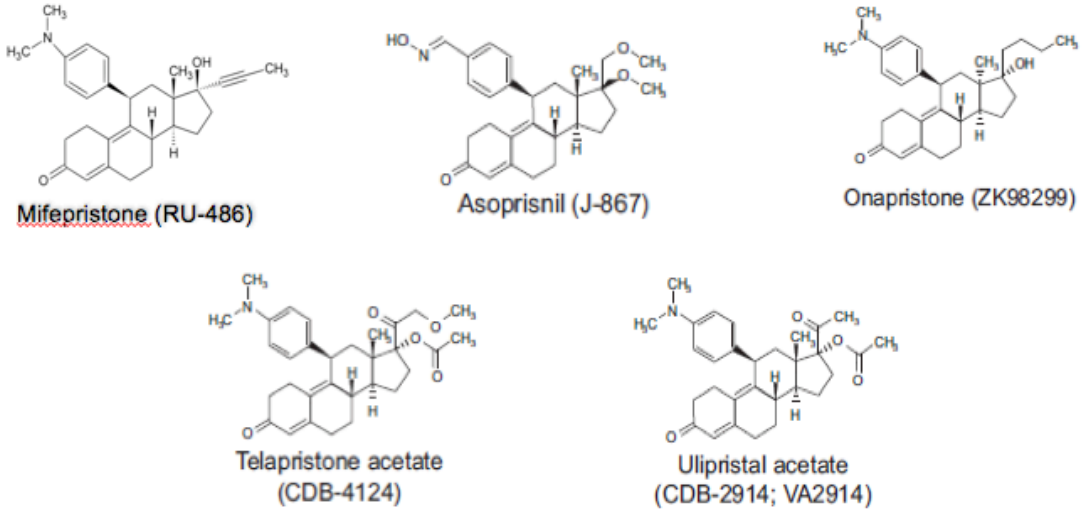


Figure 3.5 **Chemical structures of SPRMs**

Compound numbers shown in brackets (reproduced from company literature with permission from Gedeon Richter).

COMPOUND	THERAPEUTIC APPLICATION
Mifepristone (RU-486)	Termination of pregnancy§
	Emergency contraception§
	Psychosis
	Cushing disease§
	Long-term contraception
	Uterine fibroids
	Endometriosis
	Alzheimer disease
	Endometrial cancer
Ulipristal acetate (CDB-2914; VA2914)	Emergency contraception§
	Uterine fibroids§
	Long-term contraception
Asoprisnil (J-867)	Uterine fibroids
	Endometriosis
	Long-term contraception
Telapristone acetate (CDB-4124)	Uterine fibroids
	Anemia
	Endometriosis
Lonaprisan (ZK230211)	Cancer
CP8816 and CP8863	Gynecologic disorders
WAY-255348	Contraception
Onapristone (ZK98299)	Endometriosis
	Cancer
ORG-31710 and ORG-31806*	Contraception
	Cancer
PF-2413873*	Endometriosis
ZK137316*	Contraception

Table 3.1 **Selective progesterone receptor modulators (SPRMs)**

Selective progesterone receptor modulators (SPRMs) with current or past development noted in the literature (those licensed are indicated, §). Studies of the compounds marked (*) have either been discontinued or suspended since the 1990s (reproduced with permission).(113)

3.7.2 Origins

The first progesterone receptor modulator or anti-progestin was discovered in the 1982. Some of the five types of steroid hormones were known to have antagonists; clomiphene inhibits oestrogen action, spironolactone inhibits mineralocorticoids and cyproterone acetate inhibits the action of androgens. However, no such compound existed for action against glucocorticoids and in the search for one RU486 or Mifepristone was discovered as a potent anti-progestin.(145,146) Healey *et al.* explored the functions of progesterone using this compound and were able to demonstrate that its administration to an appropriately primed endometrium in primates induces menstruation.(147,148)

Mifepristone was evaluated through the 1980s as an abortion-inducing agent but the development was slow due to political pressure and the emotive nature of its intended use. Mouse studies demonstrated the potent anti-progesterone properties of RU486 and this was backed up by work on female monkeys (*Macaca fasicularis*) in 1982.(149) A license for use in France was granted in 1988 and this prompted the first human trial, in which Mifepristone induced a pregnancy termination in 9 out of 11 volunteers.(125,150)

The use of Mifepristone in pregnancy termination has since developed and now offers a safe, non-surgical method to those who wish to use it. This is particularly important in areas of the world where healthcare services are limited and access to alternative methods or preventative methods may be limited. A Cochrane review demonstrated that the combination of Mifepristone and PGE1 analogues was highly effective and safe.(151) This is supported by the Royal College of Obstetricians and Gynaecologists.(152)

3.7.3 Mechanism of action

The exact mechanism of action of progesterone receptor modulators remains incompletely understood but appears to be a complex interplay of factors rather than a single inhibitory feature. The anti-proliferative effects these compounds have on the endometrium is of huge potential in endometriosis but the way in which each compound regulates oestrogen-driven mitosis in the uterine glands and stroma is uncertain.(153) The areas that have been considered are action at the receptor, control of transcription through cofactors and upregulation of the expression of the androgen receptor.(154)

Receptor binding

Studies looking at the action of Mifepristone demonstrated three features. Firstly, competitive inhibition of PR occurs as a result of RU486 having higher binding affinity for PR as compared to that of progesterone. Secondly, RU486 binding has no impact on receptor dissociation from HSP, receptor dimerisation or progesterone response element (PRE) binding; highlighting that the antagonistic result of RU486 binding is achieved by disturbing DNA transcription. The final feature noted is that RU486 binding leads to an alternative conformational change when compared to progesterone that leads to inactivation of AF-2 and prevents coactivator binding and hence renders the receptor transcriptionally inactive.(120)

Transcription cofactors

The balance of agonist and antagonist properties of each SPRM appear to be because of the balance of co-activators and co-repressors that interact with the ligand bound PR-dimer, as previously discussed (Figure 3.4). This may be as a result of the specific conformational changes induced by the ligand in question allowing the co-receptors to be bound or by influencing the rate of co-receptor shuttling and degradation within the endoplasmic reticulum.(155–157)

Liu *et al.* demonstrated that progesterone bound PR interacts only with coactivators such as steroid receptor coactivator-1 (SRC-1) whereas Mifepristone bound PR interacts weakly with SRC-1 but strongly with the corepressor, silencing mediator for retinoid and thyroid hormone receptor (SMRT) *in vitro*.(158) This feature has also been demonstrated with Asoprisnil. However, in this study both Mifepristone and Asoprisnil were shown to recruit the corepressor, nuclear receptor corepressor (NCoR), but with lower affinity in response to Asoprisnil suggesting a less antagonistic profile.(130)

Androgen receptor upregulation

Studies on the rhesus macaque endometrium have shown upregulation of ER, PR and AR in response to chronic administration of Mifepristone, as well as a suppression of glandular secretory function.(96,97,159) As androgens result in an anti-oestrogen action on the endometrium, upregulation of the AR may explain why SPRMs have an anti-proliferative action on the endometrium. This upregulation of AR has also recently been demonstrated in response to ulipristal acetate.(160)

3.7.4 Endometrial effects

The use of progesterone antagonists and SPRMs is associated with reduced menstrual bleeding and amenorrhoea. There are several proposed mechanisms to explain this: suppression of oestrogen-dependent endometrial proliferation and mitosis, direct effects on endometrial vasculature, altered glucocorticoid receptor expression (reduced VEGF expression) and ovulation inhibition.(161–163) In truth, it may be a combination of all these mechanisms but the suppression of proliferation seen in response to PRMs is encouraging with respect to managing endometriosis. The known actions of PRMs on the endometrium and the histological entity of PAEC have been thoroughly reviewed(164), and are discussed in more detail in Chapter 4.

3.8 SPRMS AND ENDOMETRIOSIS

Clinical trials of the use of SPRMs in the treatment of endometriosis have been undertaken for Mifepristone, Asoprisnil and Telapristone, with some encouraging results. These trials show clinical improvement of symptoms and the macroscopic appearance of the disease in response to drug exposure. The most promising of these agents was expected to be Asoprisnil but work with this compound was abandoned due to concerns relating to PAEC though these findings have yet to be formally published.(165) However, ulipristal acetate has not been studied in the context of endometriosis to date.

3.8.1 Mifepristone

Kettel *et al.* have undertaken three small pilot studies of Mifepristone use in endometriosis. Initially a dose of 100mg/day was given to six patients for 3 months, resulting in improved pain symptoms but no significant effect on the extent of disease at follow up laparotomy. Subsequently, a dose of 50mg/day of Mifepristone was administered to nine women with endometriosis for 6 months. In this pilot study, a 55% regression of disease was seen and improvement in symptoms in all patients. Finally, a dose of 5mg/day was used for 6 months. Although this lower dose did result in symptomatic improvement it failed to stablise the endometrium and induce the anovulatory amenorrhoea without hypo-oestrogenism seen with the higher doses.(166–168)

Mifepristone has also been shown to induce apoptosis in an endometrial cell line, which may explain the regression seen by Kettel *et al.* Han & Sidell, demonstrated increased cellular binding of nuclear transcription factor nuclear factor-κB (NF- κB) in cell lines treated with Mifepristone. In addition, they also demonstrated increased *bax* and reduced *bcl-2* expression; both apoptosis regulators whose transcription is regulated by NF-κB.(169)

However, this apoptosis and reduction in ectopic endometrial size has not been demonstrated in a mouse model, in which endometriosis was induced in mice by injecting a suspension of endometriotic cells into the peritoneal cavity. These animals were administered mifepristone and reduced expression of COX-2 and PGE2 was seen, although no significant change in disease volume was demonstrated. As COX-2 and

prostaglandin E2 are both important in the mediation of pain it suggests Mifepristone may also act through an anti-inflammatory mechanism.(170)

Consequently, a Mifepristone-loaded implant is currently in development for the long-term treatment of endometriosis. It has shown promise from both a safety and efficacy point of view in a Wister rat model of endometriosis, with a dose dependent inhibitory effect on the growth of endometrial explants.(171)

3.8.2 Asoprisnil

Asoprisnil, like all selective progesterone receptor modulators, has both partial agonist and antagonistic properties. The balance of these properties is specific to each compound and appears to be related to the balance of coactivators and corepressors. Studies in rats, guinea pigs, rabbits and cynomolgus monkeys have demonstrated that Asoprisnil can abolish menstrual cyclicity, induce endometrial atrophy and inhibit COX-2 production. As such it has been considered for the treatment of fibroids and endometriosis.(130,172,173)

Initially, it was studied for the treatment of fibroids and for cycle control. In the Phase I study, 60 regularly cycling premenopausal women took Asoprisnil for 28-days leading to suppressed menstruation but with oestrogen levels maintained.(174) The Phase II study that followed this involved administering 5, 10, or 25mg, or placebo to 129 women for 12 weeks. The outcomes were controlled uterine bleeding and a reduction in leiomyoma volume and consequently, pressure symptoms.(175)

This was followed up with a small study of patients who were given Asoprisnil for 3 months prior to hysterectomy. This study identified the 'non-physiological secretory effect' or PRM- associated endometrial changes (PAEC), as described below.(163) Immunohistochemical analysis of the data from this study showed no loss of PTEN (phosphatase and tensin homologue) expression, but a reduction in the expression of Ki67 in endometrial changes.(176)

3.8.3 Onapristone

Onapristone is largely an antagonist like Mifepristone and has been tested against a rat model of endometriosis. Remission of the surgically induced ectopic deposits of disease was seen in 40% of cases with a reduction in epithelial cells within ectopic endometrium

but not eutopic endometrium. This work suggests that the two cell populations behave differently in response to SPRMs.(177)

3.8.4 Telapristone (Proellex™)

Use of Telapristone in the management of women with endometriosis has been evaluated. This study also highlighted the features of PAEC in the subjects who took the Telapristone for either fibroids or endometriosis. Unfortunately, evidence of hepatic dysfunction as a result of the study drug was demonstrated and so it is not licensed for use in human subjects.(178)

3.8.5 Ulipristal acetate

The details of ulipristal acetate with respect to the management of endometriosis will be discussed in more detail in Chapter 4.

However, it should be noted that for all SPRMs there is a lack of any good quality research into the histological changes in ectopic endometrial deposits seen in endometriosis following exposure and their use remains largely theoretical. It should be noted that in the few studies looking at ectopic endometrium in animal models the *endometriosis* is induced by injecting endometrial cells into the peritoneal cavity or by surgical implantation of portions of eutopic endometrium at ectopic locations. Although both these methods are successful, they are non-physiological and the lesions they create may not behave in the same manner as true endometriosis. As such inference from such models is difficult and may lead to incorrect conclusions about both efficacy and safety.

An understanding of the histological changes and receptor expression within the ectopic endometrium of human subjects, after treatment with ulipristal acetate, may give useful information about the potential efficacy of this SPRM in the management of endometriosis. Reassurance about safety is still required as selective antagonism of the progesterone receptor in premenopausal women who are actively producing oestrogens is still of some concern with respect to endometrial hyperplasia and carcinoma and is completely unknown with respect to ectopic endometrial deposits that persist even after menstrual shedding in endometriosis.

3.9 SPRMS AND ADENOMYOSIS

The endometrium is abnormally located in adenomyosis, leading to a diffusely enlarged uterus with ectopic foci of endometrial glands and stroma within a hypertrophic and hyperplastic myometrium. The abnormal uterine bleeding and dysmenorrhoea result in chronic pelvic pain and significant symptom overlap with endometriosis, which can occur concurrently. This overlap of clinical presentation make distinguishing the two conditions difficult but the joint prevalence is estimated to be between 1.8% and 3.3%.(179,180)

A mouse study in 2000, suggested Mifepristone could inhibit the genesis of adenomyosis.(181) Then in 2014 a study in humans showed caspase 3 expression within adenomyosis tissue was increased by treatment with Mifepristone, resulting in cell apoptosis and inhibition of the disease.(182) Beyond these studies and the small number of studies exploring SPRM use in endometriosis described above there is little published literature to consider. However, the potential use and safety of SPRMs in adenomyosis remain an important area of research.

Chapter 4: ULIPRISTAL ACETATE

4.1 INTRODUCTION

Ulipristal acetate is a selective progesterone receptor modulator currently marketed for emergency contraception (ellaOne®) and the treatment of uterine fibroids (Esmya®). The anti-proliferative action it exerts on uterine fibroids causes a reduction in fibroid size, a reduction in fibroid related symptoms and helps minimise surgical complications.

As an SPRM, ulipristal acetate has a unique action on the endometrium. This action has been defined as a new histopathological phenomenon and is referred to as PRM-Associated Endometrial Changes (PAEC). Whilst, these changes are reversible once treatment stops and they are not considered a precursor to endometrial hyperplasia, caution about the nature of these changes remains.

Given the anti-proliferative action of ulipristal acetate on eutopic endometrium the action on ectopic endometrium and endometriosis should also be considered. The CHUTE study was designed to explore the use of ulipristal acetate for endometriosis in the context of clinical safety and possible occurrence of PAEC within ectopic endometrium.

4.2 CLINICAL USE

4.2.1 Emergency contraception

Ulipristal acetate was first licensed for use as an emergency contraception in May 2009.(183) A dose of 30mg can be given up to 120 hours after unprotected intercourse to either prevent or delay ovulation in order to prevent conception. The data from Phase II & II studies show a 0.9-2.1% pregnancy rate but no significant difference in efficacy up to 120 hours.(184) However, it is maximally effective if taken as soon as possible after intercourse and is generally considered to be 95% effective.

4.2.2 Fibroids

Uterine leiomyomas (fibroids) are benign smooth muscle tumours of the myometrium, whose growth in promoted by oestrogen and progesterone. Their presence within the uterus is increasingly common with advancing age, such that 20-25% of women over 35

years and 70% of women over 50 have fibroids.(185,186) The majority of women are asymptomatic but 20-50% have symptoms significant enough to require intervention.(187) The use of medical therapy and interventional radiological have reduced the need for surgical intervention but fibroids still remain the leading indication for hysterectomy in the UK.(188)

SPRMs & fibroids

Currently the only SPRM licensed for use in the UK is ulipristal acetate (UPA) (Esmya®). It has an anti-proliferative, anti-fibrotic and pro-apoptotic effect on leiomyoma cells and is consequently used for the medical treatment of moderate to severe fibroid related symptoms. The original marketing approval in the EU was granted on 23rd February 2012 with the therapeutic indication of 'pre-operative treatment of moderate to severe symptoms of uterine fibroids in adult women of reproductive age'. However, evolving safety and efficacy information resulted in a license extension on 23rd April 2015 to include 'intermittent treatment'. As a consequence multiple three month courses for the medical management of moderate to severe uterine fibroids can now be prescribed.(189)

Mechanism of action

To understand the action of SPRMs on fibroids it is important to consider the response of fibroids to the sex steroids. Leiomyoma cells grow in response to exposure to oestrogen. This is explained by the fact that the ratio of oestrogen receptors (ER) to progesterone receptors (PR) is higher in leiomyomas when compared to normal myometrium.(190) Hence why medical treatments that suppress oestrogen levels have a positive impact on fibroid growth.

The action of progesterone on leiomyoma cells as compared to myometrial cells is more complex and was explored by Maruo *et al.*, in 2003.(191) Leiomyoma cells in culture were assessed after exposure to progesterone and quantified the expression of two growth factors (epidermal growth factor (EGF), insulin-like growth factor (IGF-I)) and two apoptosis factors (tumour necrosis factor-a (TNF-a), Bcl-2 protein). The results demonstrated increased levels of EGF and Bcl-2, which promote cell growth and survival, and decreased levels of IGF-I and TNF-a, which inhibit growth and promote apoptosis. This balance of effects helps to explain why progesterone (in the LNG-IUS, for instance)

sometimes improves symptoms and sometimes promotes tumour growth and worsening symptoms.

Ulipristal acetate exerts a tissue-specific partial progesterone antagonist effect, which has a direct action on fibroids reducing their size.(189) The action of the drug on leiomyoma cells results in increased expression of cleaved caspase-3 and decreased expression of Bcl-2; as well as downregulation of angiogenic growth factors, such as vascular endothelial growth factor (VEGF) and it's receptor.(192–194) As a consequence, neovascularisation, cell proliferation and cell survival are all inhibited in leiomyoma cells but not normal myometrium.

Collagen synthesis and deposition are also influenced by ulipristal acetate. Expression of matrix metalloproteinases (MMPs) is increased and expression of tissue inhibitor of metalloproteinases (TIMPs) is reduced in cultured fibroid cells but not in normal myometrial cells. This reduction in collagen deposition within the extracellular spaces impairs tissue integrity and explains the consistency of fibroid tissue seen at myomectomy following pre-operative treatment with ulipristal acetate.(195)

The ratio of progesterone receptor isoforms (PR-A & PR-B) in cultured leiomyoma cells are modulated by ulipristal acetate. This modulation results in decreased cell viability; suppressed expression of growth and angiogenic factors (as discussed above); and elicits endoplasmic reticulum stress and activation of the mitochondrial and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) pathways, resulting in apoptosis.(196)

In addition, ulipristal acetate also acts centrally influencing the hypothalamic-pituitary-ovarian axis resulting in delayed or inhibited ovulation. Despite this action it does not change the basic levels of luteinising hormone (LH) or follicular stimulating hormone (FSH) such that oestradiol levels are maintained in the mid-follicular physiological range (60-150pg/mL). As a consequence oestrogen deficiency and vasomotor symptoms that complication GnRH agonist use are less of a problem with ulipristal acetate.(124)

The inhibition of ovulation and stable oestrogen levels tends to promote amenorrhoea but ulipristal acetate also has a direct action on the endometrial progesterone receptors that may influence this.(190)

4.3 CLINICAL RESEARCH STUDIES INVOLVING ULIPRISTAL ACETATE

Four randomised, double-blind, multinational, Phase 3 clinical trials have been conducted to explore the therapeutic efficacy and tolerability profile. They are collectively known as the PEARL (PGL4001 (Ulipristal Acetate) Efficacy Assessment in Reduction of Symptoms Due to Uterine Leiomyomata) studies. The first two trials provided the evidence to secure the initial pre-operative licence and then the data from the second two allowed the licence extension to include intermittent treatment:

1) **PEARL I** (Ulipristal Acetate versus Placebo for Fibroid Treatment before Surgery), compared oral UPA (5 or 10mg/day) with placebo;(197)
2) **PEARL II** (Ulipristal Acetate versus Leuprolide Acetate for Uterine Fibroids), compared UPA with a GnRH analogue, Leuprolide acetate (LA) (3.75mg once per month);(198)
3) **PEARL III** (Long-term treatment of uterine fibroids with ulipristal acetate), involved four separate 3-month courses of UPA (open label), each followed by randomised double –blind norethisterone acetate (NETA) or placebo;(199)
4) **PEARL IV** (Efficacy and safety of repeated use of ulipristal acetate in uterine fibroid), evaluated the administration of two 3-month courses of UPA (5 or 10mg daily).(200)

4.3.1 PEARL I

Symptomatic women with heavy menstrual bleeding (pictorial blood-loss assessment chart (PBAC) score >100) and anaemia (haemoglobin level of ≤10.2 g/L) were randomly assigned to 13 weeks of oral treatment: 96 women received 5mg ulipristal acetate daily, 98 women received 10mg ulipristal acetate daily, and 48 women received placebo. The primary end points were control of bleeding (PBAC <75) and a reduction of fibroid volume as assessed on MRI.(197)

The trial demonstrated effective control of menorrhagia with ulipristal acetate. At 13 weeks bleeding control was achieved in 91% (P<0.001) and 92% (P<0.001) of the 5mg/daily and 10mg/daily groups, respectively; and was achieved within 10 days in 73% and 82%, respectively. This compares to only 19% in the placebo group achieving bleeding control, and only 6% achieving this at 8 days.(197)

A clinically significant reduction in fibroid size was achieved with ulipristal acetate compared to placebo. The median change in total fibroid volume was -21% (p=0.002, for comparison of 5mg ulipristal acetate with placebo) and -12% (p=0.006, for comparison of 10mg ulipristal acetate with placebo), whereas the total fibroid volume increased by 3% in the placebo group.(197)

4.3.2 PEARL II

This was a double-blind noninferiority trial in which 307 symptomatic patients were randomly assigned to either 5mg or 10mg of oral ulipristal acetate daily (97 and 103 patients, respectively) or a monthly intra-muscular injection of 3.75mg leuprolide acetate (101 patients) for a period of 3 months. The primary outcome was the proportion of patients with controlled bleeding at 13 weeks.(198)

The results showed that heavy menstrual bleeding was controlled in 90%, 98%, and 89% of the women receiving 5mg UPA, 10mg UPA and leuprolide acetate (LA), respectively. All treatments reduced the fibroid volume with 5mg ulipristal acetate causing a 36% reduction, 10mg ulipristal acetate causing 42% reduction and LA the most effective, causing a 53% reduction. However, the ulipristal acetate was quicker to achieve bleeding control with the median time to amenorrhoea of 7 days (5mg UPA group) compared to the 21 days taken with leuprolide acetate.(198)

The study also highlighted two distinct benefits over GnRHa therapy - the effect of ulipristal acetate is sustained for six months after completing the course of treatment and patients on ulipristal acetate experience fewer hot flushes (11% in the 5mg group, 10% in the 10 mg group and 40% in the LA group), due to higher oestradiol concentrations, when compared to leuprolide acetate ($p<0.001$).(198)

4.3.3 PEARL III

To explore the possibility of long-term treatment of symptomatic fibroids with ulipristal acetate, 209 women with symptomatic fibroids were recruited. Each patient received three months of open label ulipristal acetate at 10mg daily followed by 10-day double-blind treatment with 10mg NETA (norethisterone acetate) daily or placebo. After 13 weeks, each patient was given the option of enrolling in the PEARL III extension study to have a further three courses of treatment, which was taken up by 132 patients with 107 receiving 4 courses.

The first course of treatment induced amenorrhoea in 79%, with a median onset of 4 days, and caused a median fibroid volume change of -45%. The control of bleeding with multiple courses was greater and faster than the initial reduction; with amenorrhoea rates of 89%, 88%, and 90% and median times to amenorrhoea of 2, 3 and 3 days for courses 2, 3 and 4, respectively. Multiple courses also had a beneficial effect on the reduction of myoma volume with the median change from baseline of -63%, -67%, and -72% after treatment courses 2, 3, and 4, respectively, resulting in 82% of those taking 4 courses having a >25% reduction in fibroid volume.

4.3.4 PEARL IV

This randomised, double-blind, parallel group, long-term study was undertaken to investigate the efficacy and safety of 5mg and 10mg doses of ulipristal acetate. A total of 451 patients across 46 sites were recruited and 62% in the 5mg treatment group and 73% in the 10mg treatment group, achieved amenorrhoea during both treatment courses. Menstruation after treatment was reduced in both groups and the median reduction in fibroid volume was 54% and 58% for the 5mg and 10mg group, respectively.

4.3.5 Summary of PEARL studies

Overall the PEARL I and PEARL II studies demonstrated an improvement in quality of life for symptomatic women treated with ulipristal acetate. The increased dose of 10mg did not confer any significant benefits and as such 5mg daily was defined as the minimum effective dose.(190,194) The PEARL III and PEARL IV studies have helped to demonstrate that multiple courses are effective and their impact is cumulative in terms of bleeding control and fibroid shrinkage with no concerns about safety (see section 4.4.3).

4.4 PHARMACOLOGICAL PROPERTIES

4.4.1 Pharmacodynamics

Ulipristal acetate ((11β)-11-[4-(Dimethylamino)phenyl]-3,20-dioxo-19-norpregna-4,9-dien-17-yl acetate) is a 19 norprogesterone derivative and belongs to the pharmacotherapeutic group: Sex hormones and modulators of the genital system, progesterone receptor modulators (ATC code: G03XB02).(189,201)

It has two therapeutic indications:

- pre-operative treatment of moderate to severe symptoms of uterine fibroids in adult women of reproductive age
- intermittent treatment of moderate to severe symptoms of uterine fibroids in adult women of reproductive age;

and treatment consists of one tablet of 5 mg to be taken orally once daily for up to 3 months each. Ovulation is inhibited such that progesterone levels are maintained at 0.3ng/ml, and FSH production by the pituitary is partially suppressed to maintain serum oestradiol levels in the mid-follicular range, as previously discussed.(189)

Ulipristal acetate exerts a direct effect on the endometrium and should be initiated after menstruation has occurred. In most subjects (80%), further menstruation will be prevented until after treatment cessation but once the treatment is stopped or completed, menstruation usually resumes within 4 weeks. The drug action on the endometrium leads to endometrial thickening (>16mm) in 10-15% of users and PRM associated endometrial changes (PAEC) in 60% after 3 months. These changes are reversible and are discussed in detail in section 4.5.

4.4.2 Pharmacokinetics

Absorption

Ulipristal acetate (5mg or 10mg) is absorbed rapidly, with a C_{max} of 23.5 ± 14.2 ng/ml and 50.0 ± 34.4 ng/ml occurring approximately 1 h after ingestion, and with an $AUC_{0-\infty}$ of 61.3 ± 31.7 ng.h/ml and 134.0 ± 83.8 ng.h/ml, respectively. It is converted quickly to an active mono-N-demethylated metabolite, with a C_{max} of 9.0 ± 4.4 ng/ml and 20.6 ± 10.9 ng/ml also occurring approximately 1 h after ingestion, and with an $AUC_{0-\infty}$ of 26.0 ± 12.0 ng.h/ml and 63.6 ± 30.1 ng.h/ml respectively.(189)

Distribution & Elimination

Ulipristal acetate is highly (>98%) bound to plasma proteins such as albumin. It is readily converted to a mono-N-demethylated metabolite and then its di-N-demethylated metabolites by the cytochrome P450 3A4 isoform. These metabolites are excreted in the faeces and urine (10%). The terminal half-life of the 5mg dose is estimated at 38 hours.(189)

4.4.3 Safety data

Pre-clinical

The non-clinical data collected on ulipristal acetate has not highlighted any concerns for use in humans with regards to safety pharmacology, repeated dose toxicity and genotoxicity. There are no specific safety concerns with respect to bone health, hepato-renal function or lipid metabolism, however caution should be exercised in users with hepatic dysfunction as this may cause a reduction in drug elimination.(189)

Ulipristal acetate has been shown to be embryo lethal in rats, rabbits, guinea pigs and monkeys but the safety for the human embryo is unknown. At doses, low enough to allow a gestation to continue, no teratogenic potential has been observed. In addition, no impaired fertility has been seen in rats or their offspring following treatment with ulipristal acetate. There is also no evidence of carcinogenic potential with ulipristal acetate exposure.(189)

Adverse events

The most common side effect of amenorrhoea is a desirable outcome for most patients taking ulipristal acetate (79.2%). The most frequent adverse event after this is hot flushes (8.1%) with varied rates across the PEARL studies (1-24%). Headache was reported by 5.8% of patients and ovarian cyst formation seen in about 1% of users. The other common (≥1/100 to <1/10) adverse reactions noted were vertigo, abdominal pain, nausea, acne, musculoskeletal pain, pelvic pain, breast tenderness, fatigue, and weight gain. The reported adverse events were mild to moderate in the main (95%), did not require cessation of medicinal product (98%) and resolved spontaneously.(189)

4.5 HISTOLOGICAL IMPACT

Ulipristal acetate (UPA) treatment, results in novel endometrial changes, which are unique to this group of drugs. The histological features are collectively known as PRM (Progesterone Receptor Modulator)-Associated Endometrial Changes (PAEC) and 'include endometrial thickening and cystic features resembling cystic hyperplasia, yet without the glandular proliferation characteristic of endometrial hyperplasia'.(202) Initially these changes were thought to be simple hyperplasia, raising concerns about the safety of ulipristal acetate, particularly with prolonged use, but they have now been recognised a unique histological response specific to SPRMs.(203)

The PAEC changes seen in response to SPRMs were reviewed at a US National Institute for Health (NIH) workshop on 'Progesterone Receptor Modulators and the Endometrium' in April 2006 (Bethesda, Maryland (USA)). The aim of the meeting was to discuss the clinical applications of PRMs, the pharmacokinetics and endocrine effects of PRMs but also to formulate a consensus opinion about the histological changes that were becoming evident.(154,164)

Eighty-Four H&E (Haematoxylin and Eosin) slides of endometrial biopsies from women taking an SPRM were supplied by pharmaceutical companies. The agents under investigation were mifepristone, ulipristal acetate (CDB-2914), JNJ-17072341 and Asoprisnil. At the meeting, a panel of seven gynaecological pathologists reviewed the slides having been blinded to the agent, dose and exposure interval. Following a microscopic review of the slides, an interactive examination and discussion of the results was completed to establish a consensus summary. It is that consensus, which now defines the features of PAEC.(154)

Since then the PEARL studies have significantly increased the scientific understanding of this histological entity such that it can confidently be concluded that PAEC is distinct from the proliferative response seen secondary to unopposed estrogen exposure or endometrial hyperplasia, the observed changes are reversible once treatment is stopped (by 6 months), and the changes do not carry any known clinical significance.(203)

4.5.1 PRM- Associated Endometrial Changes (PAEC)

The commonest finding seen following exposure to ulipristal acetate for three months is that of cystically dilated endometrial glands; with moderately dilated glands scattered amongst normal appearing glands. This finding is comparable to what is seen in the disordered proliferative pattern (DPP) that occurs with anovulation. However, rather than proliferative epithelial cells as you might expect the lining of the glands is only weakly mitotic with evidence of secretory changes. As such it can be classified as neither proliferative or secretory and is defined as a new histopathological entity- PAEC. (154)

It is defined as a 'benign, inactive and weakly proliferating epithelium associated with asymmetry of stromal and epithelial growth resulting in prominent cystically dilated glands with admixed oestrogen (mitotic) and progestin (secretory) epithelial cells in the endometrium.'

4.5.2 PAEC observed in ulipristal acetate phase III clinical program

The occurrence of PAEC in the PEARL studies of the phase III clinical program have been monitored closely. The reported figures for non-physiological changes consistent with PAEC seen in those studies are summarised in Table 4.1.

In the PEARL I & II studies endometrial biopsies were taken at screening, after 3 months of ulipristal acetate treatment (13 weeks) and 6 months after treatment had finished (38 weeks). The detailed histological data was reported by Williams *et al.* in 2012 highlighting that 50-60% of patients exposed to ulipristal acetate had features of PAEC at the end of treatment. These changes had resolved by 6 months such that the incidence of non-physiological changes in the endometrium at 38 weeks was similar (6-7%) across both treatment groups and the comparator group for each study. In PEARL III, biopsies were taken 6 weeks after treatment course 1 and treatment course 4. The occurrence of PAEC was similar at 25-26% at both time points indicating PAEC is not cumulative. In PEARL IV, biopsies were taken 6 weeks after the 2nd course had been completed and 15-20% had PAEC features. (190,203)

As typical changes related to unopposed oestrogen effect are not seen, endometrial hyperplasia is not seen and the PAEC appears to resolve the risk of malignancy and pre-malignant change appears to be low.

Study	5mg UPA daily (%)	10mg UPA daily (%)	Comparator (%)
PEARL I	62	57	6
PEARL II	58	59	12
PEARL III	-	26 (single course)	11
	-	25 (four courses)	11
PEARL IV	16	19	8

Table 4.1 Non-physiological changes consistent with PAEC seen in PEARL studies. *Biopsies were taken whilst taking UPA in PEARL I & II and 6 weeks after completing treatment in PEARL III & IV (UPA= Ulipristal acetate; Comparator= Placebo (PEARL I), Leuprolide acetate (PEARL II), Screening (PEARL III) & Screening (PEARL IV)).*(197–200)

The pathophysiology underlying PAEC is unknown. Ulipristal acetate is a predominant antagonist at the progesterone receptor but with some agonist action. This mixture of agonist/antagonist action explains the poorly developed secretory differentiation in the endometrial glands- tortuous mid-secretory phase type glands with only focal cytoplasmic vacuolisation. Also, the antagonist action explains the lack of pre-decidual change in the stroma. However, the cystic dilatation and the rapid induction of amenorrhoea are less easily explained.

The antagonistic action of SPRMs led to speculation that these drugs would cause the same histological changes seen with unopposed oestrogen; but with ulipristal acetate, mifepristone and asoprisnil this has not been the case.(203) However, the epithelial cells appear inactive rather than proliferative as a result of selective inhibition of oestrogen dependent endometrial proliferation.(153)

4.5.3 Morphological appearance of PAEC

In order to facilitate appropriate histopathological endometrial assessment in patients exposed to ulipristal acetate, a pathologist's guide has been developed and the details of this guide have been modified and described below with permission from Gedeon Richter.(202)

Architectural irregularity and cystic dilatation

The most common feature of PAEC is cystic dilatation of the endometrial glands. This can be either focal dilatation associated with simple tubular glands or quite diffuse

dilatation within a sample. Those glands which are dilated tend to be lined by a secretory or ciliated metaplastic epithelium (as described below). In areas demonstrating this unusual architectural pattern there tends to be crowding of the glands resulting in quite compact stromal cells (Figure 4.1).(202)

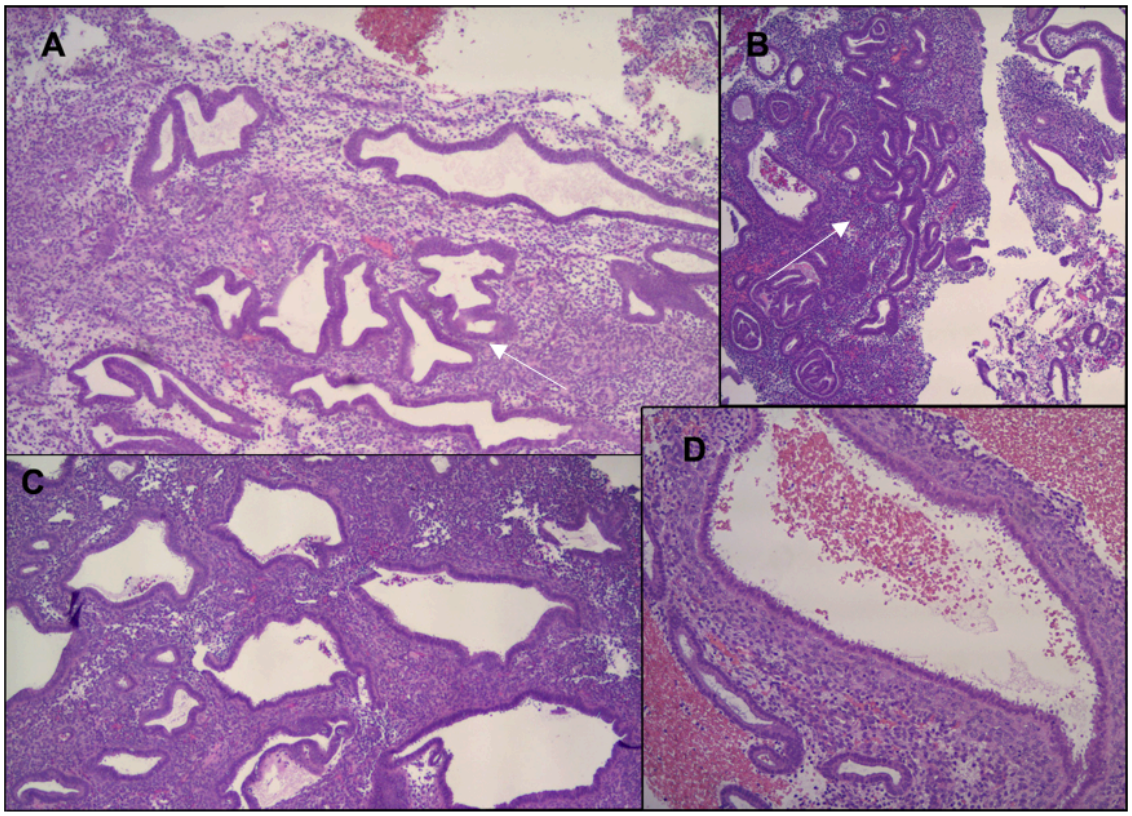


Figure 4.1 **Architectural features of PAEC.**

Cystic dilatation is shown in image A-C, with areas of gland crowding demonstrated in images A&B (white arrow). Image D contains a dilated gland lined by secretory epithelium with a compact layer of stromal cells surrounding it (reproduced with permission).(202)

Inactive glandular endometrium

The glandular epithelium usually shows an inactive appearance with each gland lined by a single layer of cells with no nuclear stratification and infrequent mitoses. The cells lining the dilated glands have a cuboidal or flattened columnar shape with ciliated metaplasia often seen.(202) This ciliated metaplasia is usually an oestrogen associated change seen a proliferative endometrium but in PAEC the epithelium is either inactive or demonstrates some secretory features (Figure 4.2).(154)

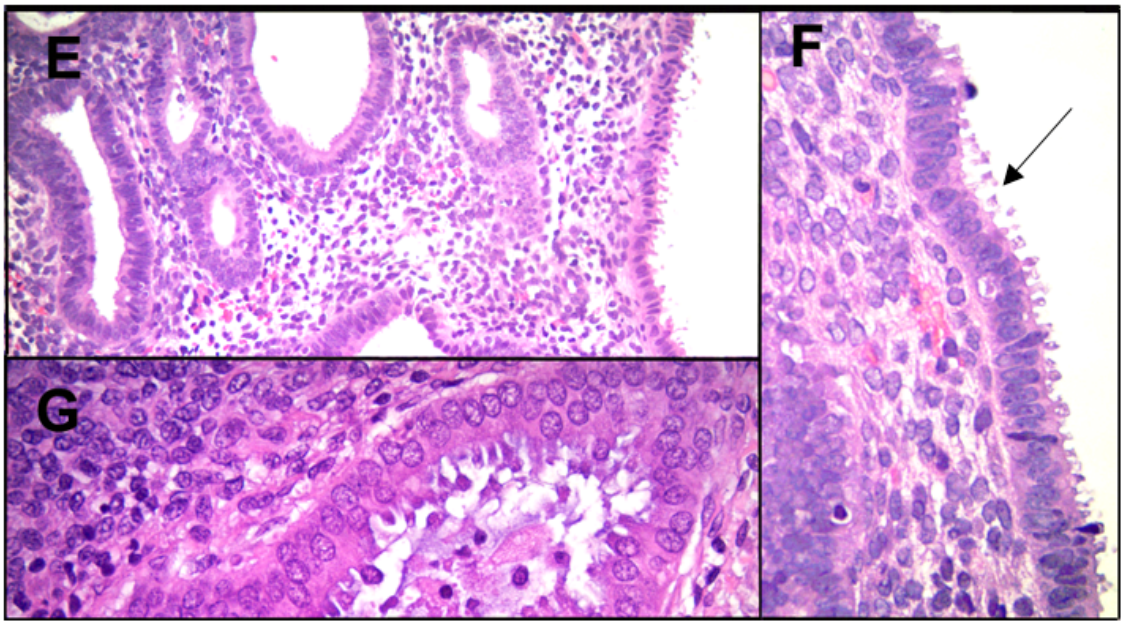


Figure 4.2 **Inactive or weakly secretory epithelium seen in PAEC.**

Low columnar epithelial cells with inactive to weakly secretory epithelium. Image E&F show surface apocrine changes (black arrow) but with no sub-nuclear vacuolation. Image G shows flattened columnar cells and cuboidal cells with small ovoid nuclei, in keeping with PAEC (reproduced with permission).(202)

Stromal vascular changes

The endometrial stroma tends to be compact without evidence of pre-decidual change. Prominent networks of anastomosing capillaries in a 'chicken wire' pattern are sometimes seen.(154) However, aggregates of arterioles with thickened walls are more commonly seen within the stroma much like vasculature seen in polyps. In other samples, occasional ectatic thin-walled vessels are seen but not with fibrin thrombi, as are seen in unopposed oestrogen states (Figure 4.3).(202)

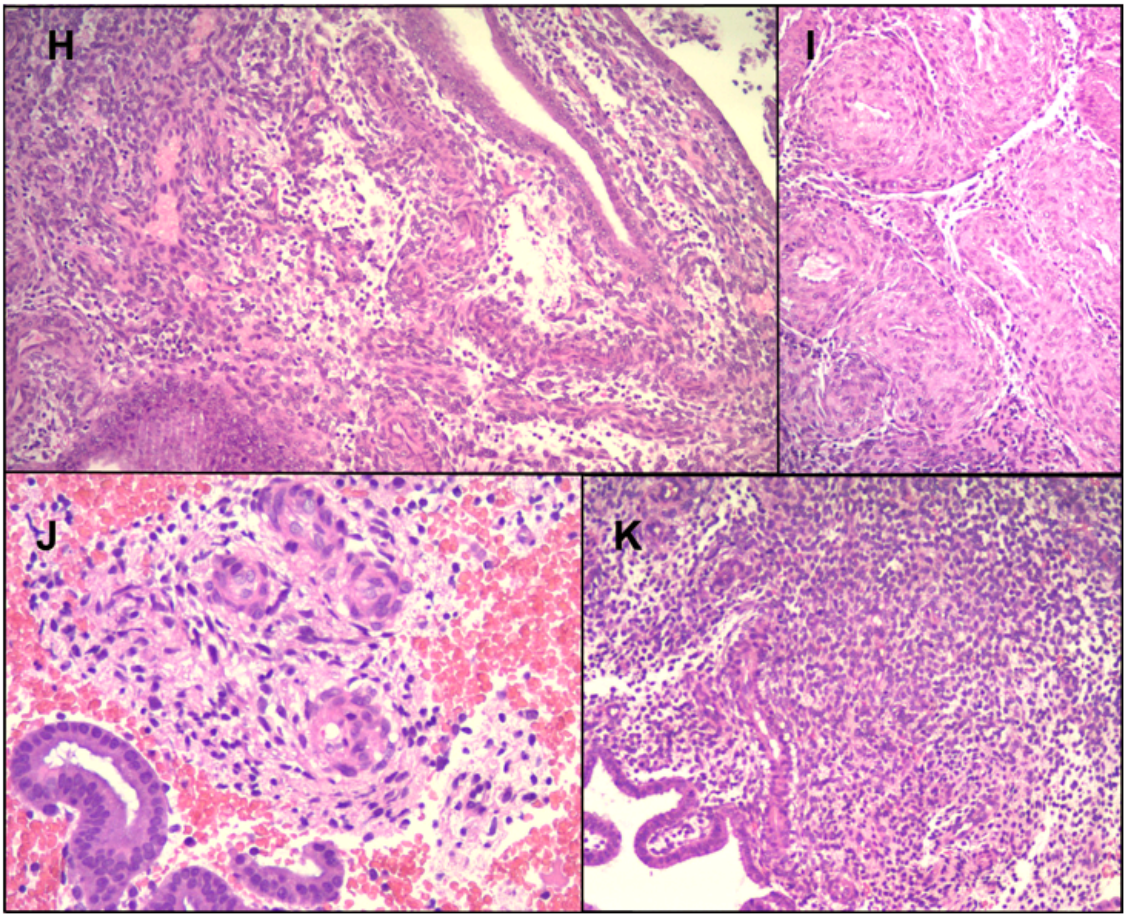


Figure 4.3 **Stromal vascular changes seen in PAEC.**

Branching clusters of capillaries (H & K) and small arterioles (J) are often seen with occasional larger thick walled small arterioles (I) (reproduced with permission).(202)

Non-physiological secretory appearance

A mismatch between architecture and glandular epithelial cells is a prominent feature of PAEC. The glands can be coiled or tortuous in nature as would be expected in the secretory phase but with a poorly-developed secretory changes. Mitotic activity and apoptosis are seen together; cytoplasmic vacuolation and surface apocrine-type changes are seen in some areas but most glands have a non-vacuolated appearance.(154) Overall this is considered non-physiological as the features do not match any specific phase of the menstrual cycle (Figure 4.4).(202)

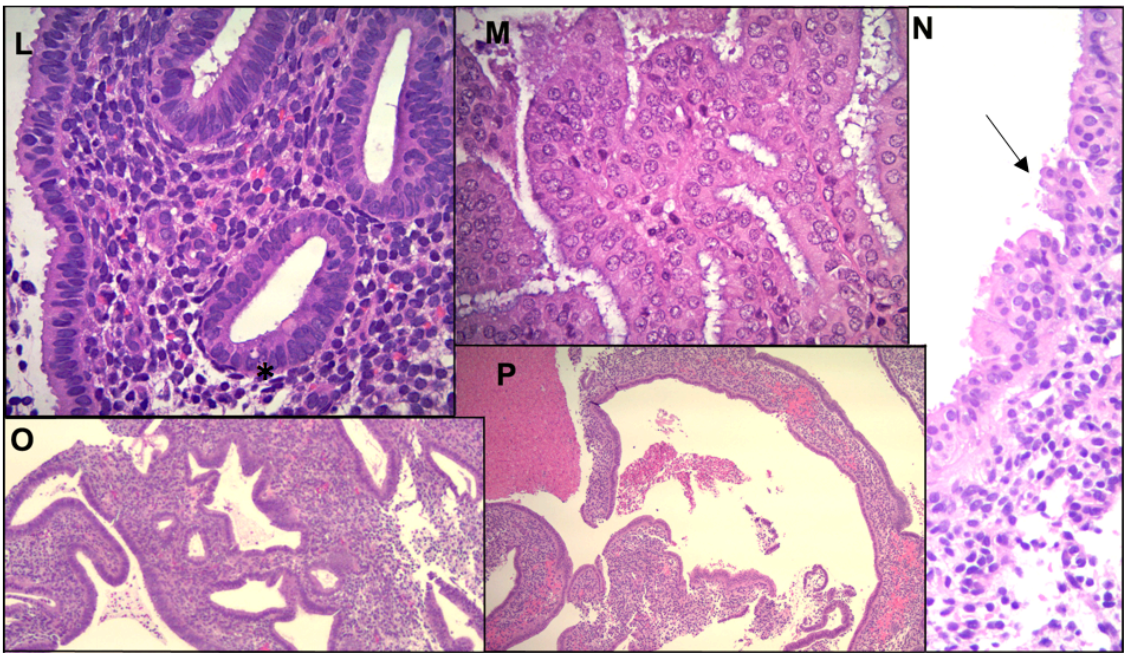


Figure 4.4 **Weakly proliferative and unusual secretory features typical of PAEC.** *Inactive to weakly secretory epithelium (few mitoses and apoptosis, L) with ciliated metaplasia (N, black arrow) and unusual secretory changes (M). Stellate glands are seen (O). Stromal pillars lined on both sides by epithelium are indicative of large cystic glands (P) (reproduced with permission).*(202)

4.5.4 Comparison of PAEC with unopposed oestrogen effect and hyperplasia

As discussed above the most striking feature of PAEC is the cystic dilatation of the glands. It is this dilatation that results in the endometrial thickening reported after exposure to ulipristal acetate. The dilated glands of PAEC can easily be confused with a disordered proliferative pattern (DPP), seen with unopposed oestrogen, except for the following features:

1) low mitotic activity in both the glands and stroma,
2) abortive sub-nuclear vacuoles,
3) apoptosis,
4) occasional stroma pseudodecidual change,
5) absent fibrin thrombi,
6) absent stromal breakdown with glandular crowding.(154)

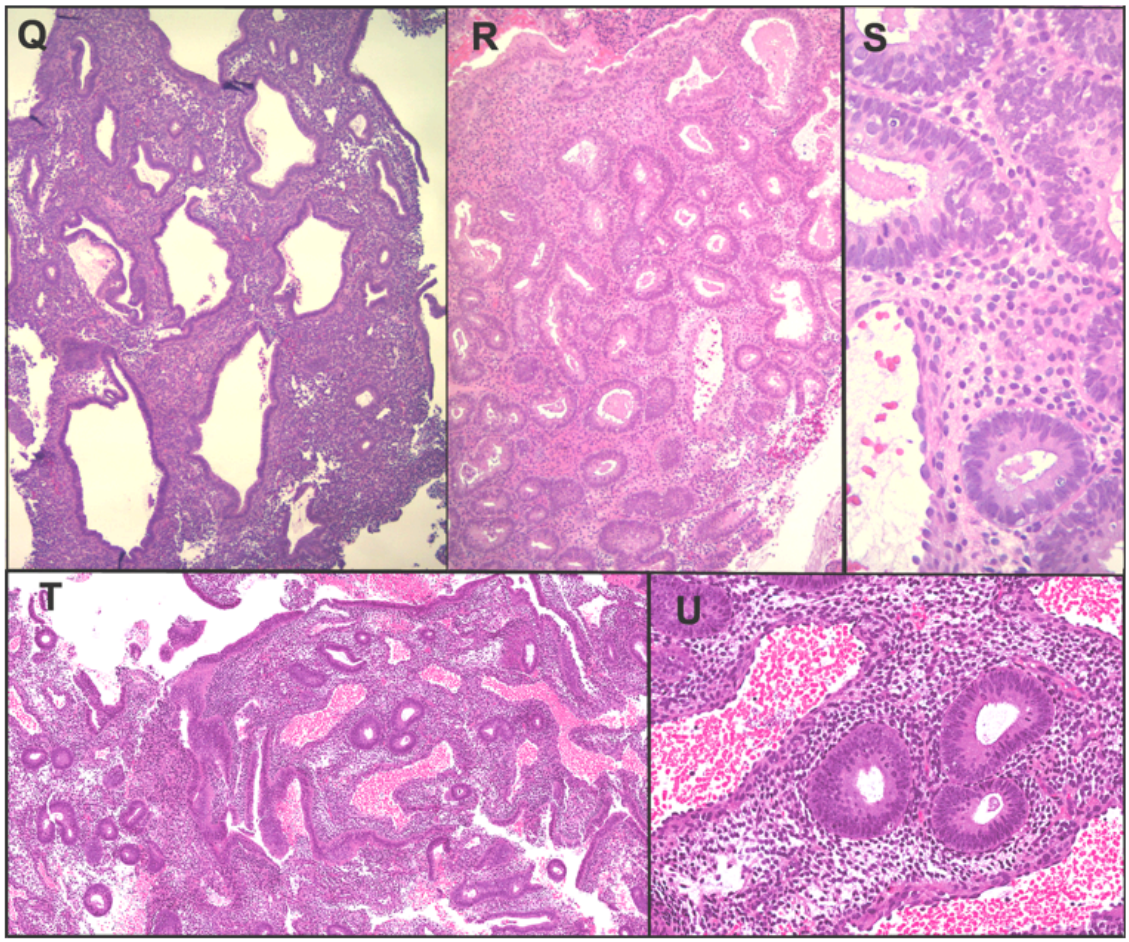


Figure 4.5 **Comparison of PAEC with unopposed oestrogen effect and hyperplasia.** *An example of PAEC (Q) with scattered cystic glands and inactive epithelium. By comparison complex hyperplasia (R, S) has very crowded glands with an active proliferative epithelium. Whereas, disordered proliferative pattern (DPP) (T, U) shows less crowding with dilated venules and proliferative epithelium (reproduced with permission).* (202)

4.6 ENDOMETRIAL IMPACT & USE IN ENDOMETRIOSIS

Consideration of progesterone receptor modulators for other clinical indications has been discussed in chapter 3, including the possibility of using ulipristal acetate. In the majority of reviews into the possible clinical uses for SPRMs endometriosis is often highlighted as a possible target.(113,125,204) Given the encouraging tolerability and safety profile for ulipristal acetate it must be considered a good candidate for further study.

4.6.1 Progesterone receptor modulation & endometriosis

The rationale for using SPRMs in the treatment of endometriosis has been discussed in detail in Chapter 3 and was clearly set out in a review by Chwalisz *et al.* in 2002.(205) They highlighted five key pharmacodynamic properties that justify SPRM use:

1) reversible suppression of menstruation via a direct effect on endometrial blood vessels;
2) selective inhibition of endometrial proliferation without the systemic effects of estrogen deprivation;
3) inhibition of uterine prostaglandins and potential for pain relief;
4) inhibition of ovarian progesterone secretion in the absence of estrogen deprivation;
5) no stimulatory effects on the mammary gland.

Given this combination of endometrial suppression without adversely effecting oestrogen levels, an SPRM should make an ideal treatment for endometriosis. We know many endometriosis patients get some symptom relief from GnRH agonists, but the hypo-oestrogenic side effects limit its tolerability and safety. As such SPRMs may offer the key to counteracting this problem. However, to date there is no SPRM licensed for use in endometriosis.

Ulipristal acetate has a current licence for use in humans, has a good safety record and is well tolerated. As each of the SPRMs have slightly different actions to each other in human tissues a trial of use in humans is needed to explore their potential. Although Asoprisnil has been studied in the context of endometriosis, safety concerns specific to that compound have limited any further investigation. As such, ulipristal acetate appears to be the most appropriate candidate for use in endometriosis.

4.6.2 Response to ulipristal acetate seen in a rat model of endometriosis

Although much of the work looking at SPRMs and ulipristal acetate is theoretical some work animal work has been done suggesting it may be effective in treating endometriosis. Huniadi *et al.* have shown that ulipristal acetate contributed to the regression and atrophy of endometriotic lesions in rats.(206)

Endometriosis was surgically induced in 40 female Wistar albino rats and after a 4-week induction period randomised into two groups. The first group were exposed to ulipristal

(UPA+) for 8 weeks and the second group exposed to vehicle only (UPA-) for 8 weeks. The size of the endometriotic lesions, histological features and immunostaining for Bax/Bcl-2, cytochrome C, Ki-67 and cyclooxygenase-2 (COX-2), were assessed.

Treatment with ulipristal acetate caused regression and atrophy of the endometriotic lesions. Although encouraging it should be noted that the method for inducing endometriosis involves a direct transplant of tissue, including its extracellular matrix, from the eutopic location to an ectopic location. This is not representative of our understanding of how ectopic endometrium is established in endometriosis and so the action of any treatment must be viewed with caution.

However, the immunohistochemical expression findings within the endometrial implants following ulipristal acetate treatment were also encouraging. They suggest a pro-apoptotic and anti-proliferative action within endometrial tissue, as is seen in leiomyoma tissue. Bax and cytochrome C expression is increased, and Bcl-2 expression is decreased demonstrating a pro-apoptotic action. A reduction in cellular proliferation was reflected by a reduction in Ki-67 expression and an anti-inflammatory action indicated by a reduction of COX-2 expression. This is an exciting prospect as it opens the possibility of SPRMs becoming an effective long-term treatment for endometriosis.

4.6.3 Case report on inadvertent use in endometriosis

In January 2017, Bateman *et al.* reported a case of PAEC changes seen in a patient with concurrent leiomyoma and endometriosis. The patient took three 90-day courses of ulipristal acetate to control her heavy menstrual bleeding prior to definitive surgical management for uterine fibroids. At her total abdominal hysterectomy and bilateral salpingo-oophorectomy a focus of endometriosis was noted. On histological examination cystically dilated glands lined by secretory type epithelial cells and occasional apoptotic bodies were noted. In addition, closely packed stromal cells with thick walled blood vessels were observed near the dilated glands. This is the first reported case of PAEC features in ectopic endometrium in humans.(207)

4.6.4 Return of endometrial function allowing pregnancy

Several case reports have been published relating to pregnancy after treatment for fibroids with ulipristal acetate. In 2013, Wdowiak reported the use of ulipristal acetate for 3-months prior to a successful ICSI (intracytoplasmic sperm injection) procedure in a

35-year-old patient with two intramural fibroids (2.26 cm by 2.53cm fibroid in the anterior wall and 1.2cm diameter fibroid in the posterior wall). The pregnancy outcome come was good.(208)

In 2014, Monleon *et al.* reported the use of ulipristal acetate in a 37-year old patient who remained symptomatic eight months after myomectomy. She had a good response to ulipristal acetate and three months after completing treatment she conceived naturally and had an uneventful pregnancy until preterm delivery at 34 weeks gestation by caesarean section due to regular uterine contractions.(209)

Murad *et al.* recently described the use of ulipristal acetate in a patient with a single submucosal fibroid of 102.4cm^2, which reduced by 30% to 72.1cm3 after a three-month course. Two months after completing treatment she conceived, had an uneventful pregnancy and delivered vaginally following an induction of labour at 38 weeks gestation.(210)

In 2014, Luyckx *et al.* reported the first series of pregnancies achieved after ulipristal treatment for fibroids. At one of the institution recruiting to the PEARL studies they looked at a series of patients who wished to conceive after completion of the trial. Twenty-one patients wanted to get pregnant and underwent myomectomy as planned with two no-longer requiring surgery due to the action of the ulipristal acetate. Fifteen patients (71%) conceived, resulting in 18 pregnancies, 13 live births and 6 (33%) miscarriages. This miscarriage rate may be explained by the underlying leiomyoma diagnosis, subfertility need for IVF (3 of these 6 cases were IVF pregnancies) or the median maternal age (38 years). The outcome for the remaining 12 pregnancies was good with no maternal complications secondary to the fibroids or fibroid re-growth. The majority (11/12) were delivered by Caesarean section due to their previous surgery or other obstetric indications. A single congenital anomaly was identified but concluded to be unrelated to the ulipristal acetate.(211)

These data indicate that the endometrial changes caused by ulipristal acetate are reversible and that its use does not adversely impact on the functionality of the endometrium with relation to implantation, in the long term. They also highlight the possible use of ulipristal acetate to shrink fibroids prior to attempting spontaneous

pregnancy or assisted reproductive techniques, particularly in those with submucosal or intramural fibroids.

4.6.5 CHUTE study

The expectation of an anti-proliferative effect in response to ulipristal and the work by Huniadi *et al.* prompted us to consider a study to explore ulipristal acetate in endometriosis. The key question was that of safety and what impact a course of ulipristal would have on ectopic endometrium. We know the ectopic endometrium is not shed in the same way the eutopic endometrium so would any PAEC changes be persistent or cumulative? The case report from Bateman *et al.* indicates that PAEC type changes can occur in ectopic endometrium but there is no indication on the time between treatment cessation and surgery, or if indeed surgery was completed whilst taking ulipristal.

As such the histological examination of ectopic endometrium following treatment formed the basis of the CHUTE study and our research question. Ulipristal acetate was used as the investigational medicinal product (IMP) in the form described in the SmPC, as outlined in the study protocol (Appendix 28) and discussed in detail in Chapter 6.

Chapter 5: AIMS, OBJECTIVES, ETHICAL CONSIDERATION & RISK ASSESSMENT

5.1 RESEARCH QUESTION

What impact do selective progesterone receptor modulators have on ectopic endometrial deposits and can they be used as a treatment option for patients with endometriosis?

5.2 RESEARCH AIM

When investigating a new treatment for endometriosis the first consideration should be whether the treatment is safe to use for the new indication. This safety question had been answered in part by the PEARL studies, which demonstrated the safety of ulipristal acetate in the management of uterine fibroids. The current licensed indication for pre-operative and long-term medical management of leiomyomata means we could be relatively certain about the risks associated with the drug.

However, the PAEC (progesterone receptor modulator associated endometrial changes) changes seen within the endometrium of patients exposed to ulipristal acetate, raises concern about whether:

a) PAEC changes occur in ectopic endometrial deposits (endometriosis);
b) PAEC changes promote a proliferative action on ectopic endometrium (endometriosis);
c) and, if PAEC changes do occur, do they persist after menstruation as typical menstrual shedding does not occur at ectopic sites.

As such our study aim was to explore the histological action of ulipristal acetate on ectopic endometrium as well as determining the clinical impact on endometriosis symptoms.

5.3 RESEARCH OBJECTIVES

5.3.1 Primary objectives

- To describe the post-treatment histological appearance in ectopic endometrial deposits found in patients with pelvic endometriosis after a course of ulipristal acetate.

5.3.2 Secondary objectives

- To study the change in disease severity (symptomatic and laparoscopic appearance) following treatment with ulipristal acetate.
- To correlate any symptom changes with the post treatment histological changes in the ectopic endometrium.
- To assess the histological changes in the eutopic endometrium following treatment with ulipristal acetate.

5.4 ETHICAL CONSIDERATIONS

5.4.1 Diagnosis

All patients considered for this study had a known diagnosis of endometriosis. This was either a recently made diagnosis, from a recent diagnostic laparoscopy, or a long-standing diagnosis. The nature of the disease and the implications of the diagnosis had been made clear to each patient as part of their previous care.

5.4.2 Treatment

In the recruitment phase, it was ensured that all patients had information about the standard approaches to endometriosis care, as outlined in Chapter 1. It was explained that a conservative approach using analgesia & lifestyle modification, hormonal suppression with progesterone or GnRHa, or surgical excision could be considered. However, it was highlighted to the study subjects that definitive surgical management was considered to be the most appropriate step in their disease management. It was also made clear to each potential recruit how the study, with pre-operative medical treatment and then surgical excision, compared to standard management. This discussion was supplemented with information provided in the Patient Information Sheet (PIS).

The potential side effects of each approach to management were also discussed, as would normally occur during a tailored review of the treatment of endometriosis for a particular patient. Progestogenic side effects such as mood disturbance, skin changes, bloating, fluid retention, breast tenderness and headache were highlighted. If GnRHa therapy was considered an appropriate option for the patient then the potential menopausal side effects of this treatment were explained as well as the need to consider add-back HRT, usually in the form of Tibolone. This was an important step in the recruitment phase to ensure subjects were fully informed about their treatment options, knew how the CHUTE study compared to standard care and what the risks were in relation to both standard management and the study medication.

5.4.3 Fertility plans

As part of the pre-screening discussion the subject's fertility wishes were discussed. This is important in any endometriosis consultation as the medical treatment options are not compatible with plans for pregnancy and surgical treatment would be postponed if conception occurred before the surgical date. Those wishing to get pregnant in the near future were excluded from the study and the implications of their chosen management on their fertility wishes were highlighted.

5.4.4 Study design

The key ethical consideration in this study was the fact that the impact the study drug has on ectopic endometrial deposits and the consequent change in macroscopic and histological appearance was unknown. The expectation was that the study drug would suppress the ectopic endometrium and symptoms would improve but this was not proven. It was also important to consider the potential for adverse events related to the study drug. The drug being used had been thoroughly tested and currently has a marketing authorisation for use in another gynaecological condition - heavy menstrual bleeding related to fibroids. As such it had a known safety and side effect profile. This was fully explained in the Patient Information Sheet (PIS) and the participants had the opportunity to discuss this with the study team prior to signing the consent form. The enrolled patients were monitored closely and were provided with telephone contact information for both routine office hours and out of hours for advice and additional information.

Progesterone receptor modulator associate endometrial changes (PAEC)

Previous studies into this study drug have shown some abnormalities within the eutopic endometrial tissue after treatment. The changes (progesterone receptor modulator associated endometrial changes or PAEC) are a novel histological variant that are specific to this group of drugs. These changes are completely reversible after a short (3 month) course and further work is awaited on longer term therapy. The significance of these changes is not known but they are not thought to be pre-cancerous.

Additional visits and procedures

The study required patients to undergo examinations and procedures that would not normally be part of their routine care. This included two additional pelvic examinations, a breast examination, two endometrial biopsies and a serum assessment of renal and liver function (blood test). Some of these procedures can be invasive and uncomfortable. This was documented in the Participant Information Sheet and the reasons for the extra procedures were explained to the participant. The discomfort was minimised by keeping the patient fully informed and reassured at all times. Each procedure was performed by an experienced member of medical staff to minimise any discomfort or complications.

Participants were required to attend the hospital for two additional clinic visits that would not have been part of their routine care. The expectations of the study protocol and level of time commitment were described in the Participant Information Sheet and patients were compensated for any additional travel expenses they incurred. If the number of visits or any other aspect of the study became too onerous the patient was free to withdraw at any time.

Surgical delay

There were no other deviations from the usual care pathway a patient with newly diagnosed endometriosis would follow. The study required a delay between diagnosis and definitive surgical treatment for three months whilst completing the course of study medication. This was comparable to the delay between a diagnostic laparoscopy and definitive surgical treatment during standard care at the time the study was undertaken. This delay was not considered clinically significant as clinical progression would not be expected over that time scale. During the treatment period the patient was fully supported and provided with adequate analgesia as and when they needed it.

Questionnaires

In addition to the extra hospital visits, the participants were also asked to complete a number of questionnaires about their symptoms during screening, the pre-operative visit and at the post-operative follow up visit. These questionnaires were all validated for this purpose and quick to complete. The patients were able to complete them as part of the study visits allowing the study team to offer explanation or clarify the requirements with the participant.

Confidentiality

Confidentiality was maintained at all times with only authorised personnel having access to study and patient data. This data was maintained in the patient's case file, a study file in a locked office or on the local, password protected, computer network.

5.5 RISK ASSESSMENT

5.5.1 Potential risks and burdens for research participants

The side effects of the ulipristal acetate (UA) are (as listed in the BNF): nausea, abdominal pain, oedema, hot flushes, headache, dizziness, malaise, menstrual disturbances, uterine haemorrhage, endometrial thickening, ovarian cysts, breast pain, pelvic pain, myalgia, acne, hyperhidrosis, dyspepsia, dry mouth, flatulence, constipation, epistaxis, anxiety, urinary incontinence. These were clearly documented in the Patient Information Sheet (PIS) and discussed with each participant prior to commencing the study drug. At the time of the first dose the common side effects were re-iterated and the points of contact for medical advice confirmed with the participant.

There was a risk that the participants may experience increased pain symptoms for the duration of the study as a result of coming off any hormonal contraception or medical treatment they had been taking prior to recruitment. This risk and the length of the study protocol was highlighted to all participants prior to enrollment. We minimised the impact of symptom flares by offering close medical supervision and giving advice on analgesic strategies throughout the study period.

We asked the patients to utilise a non-hormonal method of contraception during the study. This was made clear at the start of the study and re-iterated throughout the study. It was made clear that the impact of ulipristal acetate on an early developing embryo was unknown and contraception was paramount to ensure no exposure

occurred in early pregnancy. We offered advice and support for this change over as part of the study protocol and again after the study was complete to ensure her contraceptive needs were met to her satisfaction and to minimise the risk of contraceptive failures.

5.5.2 Surgical risks

The risks related to surgical management, either as part of standard care or following a course of ulipristal acetate as part of the CHUTE study were highlighted to all potential patients. These included; frequent risks like bruising, post-op pain, wound dehiscence, wound infection; as well as more serious but less frequent risks of injury to abdominal viscera (2 in 1000), delayed recognition of bowel injury (15% of cases), failure to complete surgery, hernia and death (3-8 per 100 000).

In addition to or as part of the planned surgical management, three peritoneal biopsies were taken. They were taken by a skilled gynaecological surgeon- either directly by or supervised by a Consultant Gynaecologist with specialist skills in laparoscopic surgery for endometriosis. Taking the biopsies carried a risk of bleeding and damage to underlying structures. However, these risks exist as part of the standard surgical management for endometriosis and all steps were taken to minimise the risk. The size and site of the biopsy was carefully planned to minimise risk and maximise the chance of obtaining an appropriate histological sample.

5.5.3 Potential for benefit to research participants

Our understanding of the way the Selective Progesterone Receptor Modulators (SPRMs) work prior to the study led us to the conclusion that ulipristal acetate would have a positive effect on the patients' endometriosis symptoms. However, there was no guarantee of this and patients were made aware of this prior to consent.

Taking part in the study had no other direct benefits to the patient over the standard treatment offered to patients with endometriosis. However, being involved in research is often a positive experience and provides more contact time with specialists who are knowledgeable about endometriosis. As such the patients involved in the CHUTE study may have develop a better understanding of their condition as a result of being involved in the study.

Chapter 6: STUDY DESIGN & METHODS

6.1 STUDY OVERVIEW

Study design: An interventional descriptive cohort study.

Study setting: Single center pilot study at the Norfolk and Norwich University Hospital.

Study subjects: Women with pelvic endometriosis confirmed by diagnostic laparoscopy, on no medical treatment for endometriosis and not planning for a pregnancy in the 12 months following recruitment.

Sample size and study duration: A sample of 20 subjects was studied over a period of 12 months.

Study layout: All women who had undergone diagnostic laparoscopy and were found to have pelvic endometriosis were staged according to the revised American society of Reproductive Medicine classification of endometriosis (ASRM 1997).(44) Those requiring surgical treatment such as adhesiolysis or laparoscopic excision/diathermy of lesions were recruited to the study and were treated with 5mg of ulipristal acetate daily for three months prior to elective surgery. At the time of that surgical management, their disease was staged according to ASRM 1997 and specimens were obtained from the ectopic endometrium for histological evaluation. There was no other pre-operative modification to the clinical management of the study subjects.

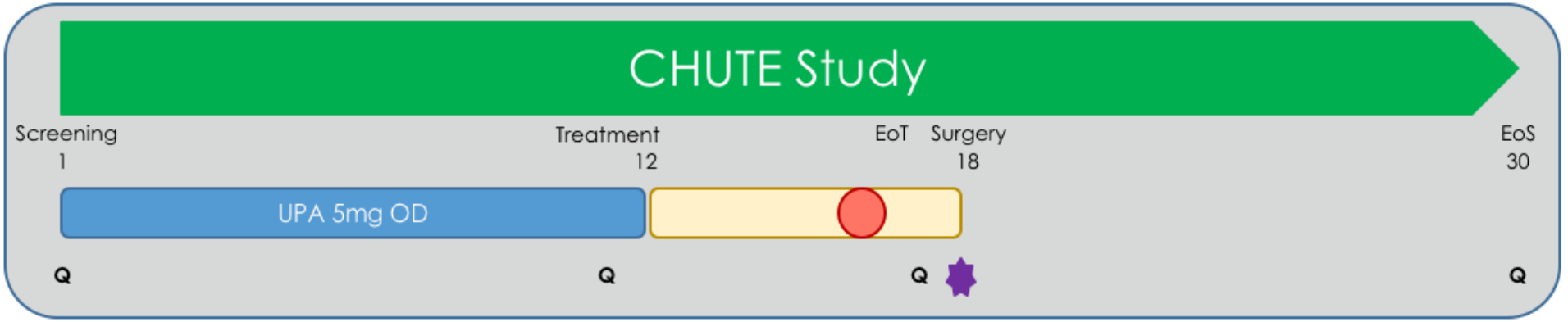
An endometrial biopsy was performed with a Pipelle® aspirator in all subjects following treatment as a safety measure. Subjects were advised to use a non-hormonal method of contraception during the study period.

Data analysis: The histological appearance of the ectopic endometrium has been described for each of the subjects and any features consistent with PAEC highlighted. Changes in the disease stage (from the laparoscopic appearance) and the associated symptoms (from disease specific questionnaires) following treatment with ulipristal acetate have been collected and attempts made to correlate these with changes in histology.

The histological analysis was performed following standard tissue fixation, mounting and staining with Haematoxylin & Eosin. Immunohistochemical staining was also

undertaken to explore the expression of key nuclear receptors (ER, PR, AR), proliferation markers (Ki67 and VEGF) and PTEN (tumour suppressor).

6.1.1 Study plan



Screening	On Treatment (week 11)	Off Treatment/EoT (week 18 +/-2)	End of Study (week 30)
BSGE-Qu EHP-30 CPSSS	EHP-30 CPSSS (sym domain) PGIC	EHP-30 CPSSS PGIC	BSGE-Qu

Menses Surgical specimen obtained

Figure 6.1 **Graphic representation of CHUTE study timeline.**

(UPA 5mg OD= Ulipristal acetate (5mg once per day) taken for a duration of 12 weeks, EoT = end of treatment, EoS= end of study, Integers= whole weeks, Q= Questionnaires, BSGE-Qu= British Society of Gynaecological Endoscopy Pelvic Pain Questionnaire, EHP-30= Endometriosis Health Profile Questionnaire, CPSSS= Composite Pelvic Signs & Symptoms Score, PGIC= Patients' Global Impression of Change Score)

Graphic representation of CHUTE study timeline (Figure 6.1)- continued

The study was conducted over 30 weeks with 5 phases: screening, on-treatment (12 weeks), off treatment (6 +/- 2 weeks), surgery, and post-operative recovery (12 weeks). Questionnaire data was obtained from each subject at four different time points: at screening, during week 11 of treatment, one week prior to surgery and at the end of the study. The BSGE-Qu pain scores at EoS were compared to baseline to evaluate the impact of the entire intervention (ulipristal acetate treatment and surgical excision) on pain symptoms. The EHP-30 and CPSSS scores during treatment and following treatment were compared with baseline and linked to the PGIC scores reported by each subject.

6.2 STUDY DESIGN & JUSTIFICATION

6.2.1 Hypotheses

Null hypothesis:

Administration of the selective progesterone receptor modulator, ulipristal acetate (Esmya), would have no effect in patients with endometriosis with no improvement in symptoms and no histological changes within ectopic endometrial deposits.

Alternate hypothesis:

Administration of the selective progesterone receptor modulator, ulipristal acetate (Esmya), would suppress endometriosis leading to improved symptoms and histological changes within ectopic endometrial deposits.

6.2.2 Outcome measures

The primary outcome measures were the changes in histological appearance in ectopic endometrial deposits after a course of ulipristal acetate. These changes have been described in terms of the architecture and cellular features of both the glands and stroma. This approach allowed the direct comparison of our histological findings with the PAEC findings published following the PEARL studies.(203)

In addition, the expression of nuclear receptors and other cellular markers were also explored using immunohistochemistry and have been described semi quantitatively.

Secondary outcome measures were changes in disease severity (symptomatic and laparoscopic appearance), histological changes in eutopic endometrium, and correlation between symptom change and histological change, following treatment with ulipristal acetate.

6.2.3 Study population

A population size of twenty patients was chosen to maximise the chance of identifying changes within the ectopic endometrium whilst minimising patient exposure to the study drug; as is common practice with pilot studies exploring the action and impact of a new treatment.

6.2.4 Descriptive cohort design

At the time of designing the study there were no published data on the use of ulipristal acetate in endometriosis. Although multiple data existed about PAEC and the

histological response of the eutopic endometrium to ulipristal acetate there were no data on histological changes within ectopic endometrium.

An interventional descriptive cohort study design was decided upon as the most appropriate way to investigate the potential benefit of ulipristal acetate. The CHUTE study was designed as a pilot to explore the possible use of ulipristal acetate in endometriosis and to identify any significant histological changes within the ectopic endometrium that might cause safety concerns. The use of SPRMs in endometriosis has been suggested in the literature for the last 10-15 years but their use has not been thoroughly investigated. Our hope was that the CHUTE study would provide reassurance about the safety of ulipristal acetate, identify the potential mechanisms behind how selective progesterone receptor modulators may exert a positive effect and facilitate the commissioning of a larger study to clarify the role SPRMs may have in the management of endometriosis.

A decision was taken to expose all participants in our cohort to the study drug and not to have a placebo group as a comparator. This decision was taken for three reasons: firstly, our primary objective was to identify histological changes rather than compare ulipristal acetate to placebo or existing treatment; secondly, it would be unusual to see significant clinical change of endometriosis over a period of three months in a placebo group; and lastly, some data about the expected histological appearance of ectopic endometrium is known and can act as a comparator.

6.2.5 Treatment duration and timing of surgery

The treatment period of 12 weeks was chosen to maximise the effect of the IMP on the ectopic endometrial histology and clinical symptoms. This treatment duration is also in keeping with the current marketing authorisation for the IMP.

We considered two possible time points for surgery:

- At the end of the 12-week treatment period with contingency for an additional two weeks of study drug to ensure the patient was taking IMP at the time of surgery

- After a 12-week course of IMP and menstruation to allow assessment of residual PAEC changes after endometrial shedding of the eutopic endometrium.

The initial design would have possibly led to a variable duration of ulipristal acetate treatment across the study population. It was decided that a definite duration of treatment was important but waiting lists and fixed operating lists within the NHS meant that we could not guarantee to perform surgery immediately after the treatment course had been completed.

Also, the objective of the study was to explore safety and the presence of PAEC within the eutopic and ectopic endometrium. The presence of PAEC in the eutopic endometrium is as high as 60% after a single 12 week course of ulipristal acetate but this decreases to 16%, 6 weeks after the end of treatment as a result of menstrual shedding.(198,200) What was unknown was whether the same would be true for ectopic endometrium that does not appear to shed as eutopic endometrium does, or whether the ectopic location of the endometrium would make the PAEC more or less likely.

As such, the design was changed to surgery 6 weeks after the course of ulipristal acetate had been completed. This important change allowed us to explore PAEC with a point of comparison from previous studies and to obtain questionnaire data from the subjects whilst taking ulipristal acetate and once they had stopped pre-operatively. The disadvantages being that we were not able to obtain our histological samples whilst the patients were still taking ulipristal acetate so subtle signs of PAEC may have been missed and any changes in cellular expression caused by ulipristal acetate may have reverted by the time of surgery.

6.2.6 Histological assessment

In addition to descriptive data relating to changes seen with standard H&E staining, expression of the oestrogen receptor, progesterone receptor and androgen receptor were explored, as these are all key receptors in normal endometrial functioning and any changes may have impacted on clinical symptoms.

As active endometriosis is a proliferative disease and PAEC is a proliferative response to ulipristal acetate, Ki67 (a proliferation marker) was also used to assess whether ulipristal acetate had an influence on cellular proliferation. The original method included these four immunohistochemical stains with plans to add other markers, such as CD10, PTEN and VEGF dependent on the results that were obtained.

The use of CD10 was excluded as it was not considered to be of value. The condition of the ectopic tissues was of sufficient quality for us to be confident in the diagnosis and to allow us to distinguish between the epithelium and stroma of the endometriotic deposits.

A decision was taken to explore VEGF expression as it is key mediator of neovascularisation in the *stratum functionalis* of eutopic endometrium. In endometriosis, the ectopic foci of disease promote local vascular changes and in PAEC one of the key features described are the vascular changes. Given these facts we wanted to see if ulipristal acetate was having any influence on the vasculature and VEGF expression was chosen as a marker of this.

The expression of the tumour suppressor gene (PTEN) was assessed to see if there was any suggestion of decreased expression in response to ulipristal acetate. As progressive loss of expression is seen in un-opposed oestrogen use, is part of the tumourigenesis of endometrioid adenocarcinoma and may play a role in the promotion of endometriosis, a PTEN antibody was used.(82,84) Although, data existed on expression in endometrial cells in culture and in eutopic endometrium, there were no published data on PTEN immunohistochemistry in ectopic foci of endometrium in human studies at the time the study was designed.(109,110)

6.3 ETHICAL APPROVAL

The study design, protocol, patient information sheets and study documents were approved by the Office for Research Ethics Committees Northern Ireland (ORECNI) on 11th August 2014. The substantial amendments to the research protocol (Substantial amendment 2 & Substantial amendment 3) were approved on 30th October 2014 and 11th February 2015, respectively. The details of the protocol amendments can be found in Section 6.13.

6.4 TRIAL SETUP & REGISTRATION

6.4.1 Clinical Trials of Investigational Medicinal Products (CTIMPs)

The CHUTE study required the administration of ulipristal acetate to the study patients and so fell under the EU Clinical Trials Directive. Using the MHRA's on-line algorithm, it was clear that clinic trial authorisation (CTA) was needed and that as a CTIMP it would also require additional regulatory requirements.(212,213) The CHUTE study team and local R&D department had limited experience of such a study setup and consequently this phase of the study setup required additional learning and time to ensure all aspects had been appropriately considered prior to start of the study.

To setup and appropriately register the study we made use of the NIHR Clinical Trials Toolkit, associated Routemap document and the joint guidance from the Medical Research Council/Department of Health/MHRA on Risk-adapted Approaches to the Management of Clinical Trial of Investigational Medicinal Products.(214–216) These documents highlighted the need for the following:

- A Protocol
- Peer review
- Ethical approval
- Clinical Trials Authorisation from the MHRA
- Pharmacy review for supply and control of the IMP
- The Investigators Brochure (IB) or Summary of Product Characteristics (SmPC) for the IMP
- Sponsorship and liability cover
- Human Tissue Act consideration
- Contracts
- Safety reporting and monitoring

In October 2013, the research idea had been formulated and an agreement in principal existed for funding from Gedeon Richter Plc. The first step in the process was to involve our local R&D department, who registered the CHUTE trial locally with a protocol ID 2013O&G04L. The application process was initiated through the Integrated Research Application System (IRAS) with the project ID number 137405.

To determine the protocol design requirements, patient document content, sponsorship arrangements and advice on running the study; we used the European Medicine Agency International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guideline on Good Clinical Practice E6 (R1) to guide us.(217)

6.4.2 CTN

The CHUTE protocol was registered with the European Union Clinical Trials Register with the study start date of 16th October 2014. The EudraCT Number is 2013-005494-53.

6.4.3 Sponsorship

Although, funding from Gedeon Richter Plc. had been confirmed under an Investigator Initiated Trial (IIT) agreement with the company, the trial needed a sponsor to ensure appropriate liability cover. The Norfolk and Norwich University Hospitals NHS Foundation Trust had not previously acted as a sponsor for a CTIMP and so agreement was sought from the Director of the Research and Development department for approval. The costing, invoice schedule and funding contract were arranged through the R&D department and all agreements signed by the Trust legal representative prior to study commencement.

The R&D Director (Professor Flather), Research Services Manager (Lisa Chalkley), Commercial Research Coordinator (Julie Dawson) and Research Accountant (Julie Mercer) were all instrumental in achieving local support and approval for the study. The designated sponsors representative was Lisa Chalkley, the Research Services Manager.

6.4.4 MHRA

An application for approval was made to the Medicines and Healthcare Products Regulatory Agency on the 19th August 2014. Following some minor amendments to the exclusion criteria and wording of the study documents, to reflect the content of the SmPC, authorisation was granted on the 16th October 2014.

In line with the MHRA requirements two annual Development Safety Update Reports (DSUR) have been compiled and submitted to the MHRA; DSUR (CHUTE Oct 2014 – Oct 2015) v1.1 and DSUR 9CHUTE Oct 2015 – Oct 2016) v1.1. These were submitted on 21st December 2015 and 20th December 2016, respectively.

6.4.5 Peer review

The application for funding from Gedeon Richter through an Investigator Initiated Trial agreement (IIT) required high quality peer review. The initial step was review by an internal company committee of the Chief Medical Officer, Chief Development Officer, Chief Commercial Officer, Head of Scientific Affairs and the Scientific Manager. Subsequently, the study proposal was reviewed by Dr I. Osterloh BSc MSc MBBS MRCP before approval was granted.

6.4.6 NIHR adoption

Adoption onto the NIHR (National Institute for Health Research) CRN (Clinical Research Network) Portfolio was requested to ensure access to funding and a research nurse to assist with the delivery of the CHUTE study. An application was submitted on 02nd July 2014. To process the application, the details of the funding source and peer review process within Gedeon Richter were requested by the portfolio team. These were provided on 19th September 2014 and progress through the NIHR Coordinated System for Gaining NHS Permission (CSP) was confirmed on 22nd September 2014.

The CHUTE study was uploaded to the portfolio on 07th October 2014 with a UKCRN ID 17552. Our recruitment data was uploaded to the UKCRN Portfolio Database monthly, as per the requirements of adoption.

6.5 TRIAL MONITORING

Monitoring of the study was undertaken by the Clinical Research & Trials Monitor of the Research & Development Department of the Norfolk and Norwich University Hospitals NHS Foundation Trust. The monitoring plan was developed in line with the EMA ICH Guideline on Good Clinical Practice E6 (R1).(217) The following monitoring visits were completed and monitoring reports were produced:

1) Site Initiation Visit (SIV) & initial monitoring visit- 13th November 2014
2) Routine Monitoring Visit (10 days after date of First Patient First Visit)- 23rd April 2015
3) Routine Monitoring Visit (5-10 days after date of First Patient Pre-op Visit)- 25th August 2015
4) Routine Monitoring Visit (5-10 days after date of First Patient Surgical Visit or as appropriate)- 16th September 2015

5) Routine Monitoring Visit- 4th February 2016
6) Routine Monitoring Visit- 11th August 2016
7) Data Verification Visit- 18th May 2017
8) Close Down Monitoring Visit (End of Trial as defined by Protocol)- 18th July 2017

No major concerns were raised by the monitor. A number of minor corrections and updates to the study files were required at various time points but overall the monitor recognised 'the high quality of the study records, oversight and management of the trial'.

6.6 INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

6.6.1 Study drug

Ulipristal acetate (Esmya) 5mg tablets were manufactured by Gedeon Richter Plc as per the details of the SmPC and in line with the marketing authorisation (Marketing Authorisation Number EU/1/12/750/001).(218) The white to off-white, round biconvex tablets of 7mm engraved with "ES5" on one face were packaged into Alu-PVC/PE/PVDC blister packs of 28, as per usual manufacturing.

These were then stored at room temperature in their original outer carton to protect them from light, giving them a shelf life of three years. The original labelling was not changed as this was an open label study but additional study specific labelling (Appendix 2) took place on-site in accordance with Annex 13 of the EU Guideline on Good Manufacturing Practice (GMP). (219)

6.6.2 Dosing regimen & prescription

Participants were required to complete all the screening investigations and for the results to be checked prior to commencing the study drug. At the Treatment visit the prescription (Appendix 3) was completed and three, 28-tablet packs were dispensed.

Participants took their 1st dose between day 1 and day 5 of the cycle, to minimise the risk of irregular vaginal bleeding. The timing of the Treatment visit was arranged to allow the participant to start the drug on the day of the visit. Once start each participant took 1 tablet of ulipristal acetate (UPA) once a day for 12 weeks. They were instructed that the tablets may be taken with or without food.

6.6.3 Dose changes

No dose changes were necessary as part of the protocol. If a dose was missed, the participant was instructed to take the ulipristal acetate as soon as possible, as long as this was not more than 12 hours late. If the dose was missed by more than 12 hours, the participant was asked not to take the missed dose and simply resume the usual dosing schedule. They were asked to contact the study team if that occurred so that missed doses could be recorded.

6.6.4 Participant compliance

Study drug compliance was monitored during the study by documentation of self-reported usage at the End of Treatment (EoT) visit. Missed doses, total number of tablets taken and any returned tablets within the blister packs were documented on a participant's CRF. Although all participants were asked to return the blister packs and cardboard packaging, some forgot to do this. As such the package returns to the pharmacy department were not complete.

6.7 RECRUITMENT

Patients were recruited from the tertiary referral gynecology clinic at the Norfolk and Norwich University Hospital. Those patients with a laparoscopically confirmed diagnosis of endometriosis, requiring surgical management were considered. Many of the participants had undergone a diagnostic laparoscopy at the Norfolk and Norwich University Hospital but patients referred from other centres were also considered.

Once a potential participant was identified by the clinical team, the management options were discussed, and the outline details of the CHUTE study explained. The patient information sheet was provided, and any questions answered. Consent was obtained at that first contact for future contact by a member of the research team.

Those enrolled in the study were managed by the study team and the participants GP was informed about the details of the CHUTE study, the outcome at surgery and the future endometriosis management that was agreed at the End of Study visit. Those who chose not to participate or were excluded were offered standard care.

The details of potential participants and of enrolled subjects was stored in a secure database- an Excel spreadsheet stored on the hospital network. The password access to this database was restricted to the study team only.

6.8 SELECTION OF PATIENTS

6.8.1 Eligibility

All women with pelvic endometriosis confirmed by diagnostic laparoscopy, on no medical treatment for endometriosis and not planning for a pregnancy in the 12 months following recruitment were considered. No limits were placed on the time between diagnostic laparoscopy and inclusion in the study but a surgical diagnosis (inspection +/- biopsy) was required.

6.8.2 Inclusion criteria

To be eligible for the study, patients were required to meet the following inclusion criteria:

1) Provision of written informed consent prior to any study related procedures
2) Pre-menopausal women between 18 and 50 years inclusive
3) Subject with a Body Mass Index ≥18 and ≤40
4) Regular menstrual pattern with cycle length 22-35 days
5) Surgically (laparoscopic) diagnosed endometriosis requiring further surgical treatment
6) If sexually active, agrees to use of adequate non-hormonal contraceptive method(s) to prevent pregnancy for the duration of study and 12 weeks after the last dose:
    a) subject has undergone surgical sterilisation
    b) subjects partner has undergone surgical sterilisation (>12 weeks before consent signed)
    c) condoms
    d) non-hormonal intra-uterine device
    e) abstinence
7) Normal screening breast examination

8) Subject is willing to take part in study and understands definitive surgery will be delayed until course of ulipristal acetate is completed and menstruation has returned

6.8.3 Exclusion criteria

The following criteria prompted exclusion of the patient from the study:

1) Genital bleeding of unknown aetiology or for reasons other than uterine fibroids
2) History of or current uterus, cervix, ovarian or breast cancer
3) Significant and persisting finding on cervical screening (liquid based cytology) within the past 12 months
4) History of endometrial hyperplasia or abnormalities detected on first endometrial biopsy
5) History of medical treatment for leiomyoma, including with a SPRM
6) Taking prohibited medication:
    a) Treatments with progestins (systemic or progestin releasing intra-uterine system) or an oral contraceptive: within the month before the screening visit
    b) Acetylsalicylic acid, mefenamic acid, anticoagulants such as cumarins and/or antifibrinolytic drugs such as tranexamic acid within one week before the screening visit
    c) Systemic glucocorticoid treatments and/or systemic depot glucocorticoid treatments within one week or two months before the screening visit, respectively
    d) GnRH agonist and antagonist:
        i) Immediate or monthly sustained release depot preparation or immediate release form within 6 months of screening visit
        ii) 3 or 6 months sustained release depot preparation within 12 months before the screening visit
7) Treatment or likely treatment during the study with drugs that are not permitted by the study protocol: progestins (systemic or progestin releasing intra-uterine system), hormonal contraceptives, systemic glucocorticoids (oral and injectable), GnRH agonist and GnRH antagonists

8) Treatment with a medication which includes potent inhibitors of CYP3A4 (such as ketoconazole)
9) Treatment with a medication which includes potent inducers of CYP3A4 (such as rifampicin)
10) Abnormal hepatic function at study entry (defined as alanine transaminase [ALT], hepatic alkaline phosphatase, or total bilirubin above twice the upper limit of normal)
11) Positive pregnancy test at baseline, is nursing or planning a pregnancy during the course of the study
12) Current (within last 12 months) problem with alcohol or drug abuse
13) A mental condition rendering her unable to understand the nature, scope and possible consequences of the study, and/or evidence of an uncooperative attitude
14) Abnormal baseline findings, any other medical condition(s) or laboratory findings that, in the opinion of the investigator, might jeopardise the subject's safety or interfere with study evaluations
15) An allergy to SPRMs or progestins or any of the ingredients of the study drug tablet (Microcrystalline cellulose, Mannitol, Croscarmellose sodium, Talc, Magnesium Stearate (vegetable origin))
16) Current enrollment in an investigational drug or device study or participation in such a study within the previous 30 days and is still in the exclusion period

These criteria were chosen to exclude patients with known or suspected conditions that would impact on the safety of taking the study drug for a period of three months or would adversely affect interpretation of the research outcomes.

6.8.4 Information provided to the patients on selection

All patients considering the CHUTE trial or enrolled on the CHUTE trial were given a copy of the up to date Patient Information Sheet (PIS). Any changes to the PIS resulting from protocol amendments were highlighted and an updated version provided. A copy of the last iteration of the PIS is shown in Appendix 4.

6.8.5 Withdrawal

Participants would have been withdrawn from the study if they had requested withdrawal. No pressure to provide a reason would have been made and it would have

been made clear that any decision to cease involvement in the study would have had no impact on the care provided. No patients enrolled in the CHUTE study requested withdrawal.

A plan was also made for withdrawal of a participant if the investigation team felt continuing would be harmful for the patient, the patient became pregnant or there was significant progression of symptoms requiring a change to the management plan prior to completion of the study. Two withdrawals from the study occurred on this basis- CHUTE 17 due to pregnancy and CHUTE 20 due to a change in management plan.

6.9 SCHEDULE OF VISITS

There was a total of five study visits after the consent was signed. Two of these were additional visits when compared to standard care for a patient having surgical management of endometriosis. Compensation to cover time and travel as well as re-imbursement of parking costs was offered to each participant.

A flow chart of the study visits is shown in Section 6.9.2 (Figure 6.2).

6.9.1 Recruitment

Pre-screening

It was important to ensure potential participants were clear about their diagnosis, the nature of the disease, their management options, the implications for their future health and where the CHUTE study fitted into the standard pattern of care. This was achieved through a verbal discussion with a member of the study team as well as written communication in the form of the patient information sheet. Subjects were given sufficient time (>2 weeks) to consider this information and ask questions before scheduling a consent visit. A total of 66 participants were approached with 25 being recruited to the study

Pre-screening involved taking a focused gynaecological history concentrating on endometriosis symptoms and previous endometriosis management. The benefits, risks implications of conservative, medical and surgical management were all explained to any potential participants. They were then asked to decide if surgical management was something they wished to consider and if so a full explanation of the CHUTE study was offered.

There were four key areas that were discussed: the unknown impact of ulipristal acetate on endometriosis, the need for adequate contraception, the extra hospital visits & study procedures, and the delay in surgical management whilst completing the course of study drug. Our expectation that ulipristal acetate would improve the symptoms of endometriosis was explained but it was made clear that this was not known or proven. The impact of the study on any plans for pregnancy, the need for adequate contraception and the potential risks of conceiving whilst taking an SPRM were all explained to highlight the importance of this aspect of the study protocol. Other negative aspects, such as additional intimate examinations, endometrial biopsies and delays to surgical management were all made clear.

In addition, the inclusion and exclusion criteria were discussed and checked to ensure the patient was eligible for the study. An opportunity for asking any questions was made and further outpatient appointments offered if needed.

Informed Consent

Those patients willing to proceed with the study were asked to sign a consent form (Participant Consent Form (CHUTE)- Appendix 5). Entry into washout or screening did not occur until this consent form was completed.

Washout

Patients taking oral contraceptives (i.e. the combined oral contraceptive pill (COCP) or the progesterone only pill (POP)) were instructed to stop these. Those patients using other prohibited hormonal medication such as the Levonorgestrel IUS (Mirena®) or monthly GnRHa were advised about stopping these. The washout period varied between 4-26 dependent on the agent. The resumption of a normal regular menstruation pattern was required prior to entering screening.

6.9.2 Study flow chart

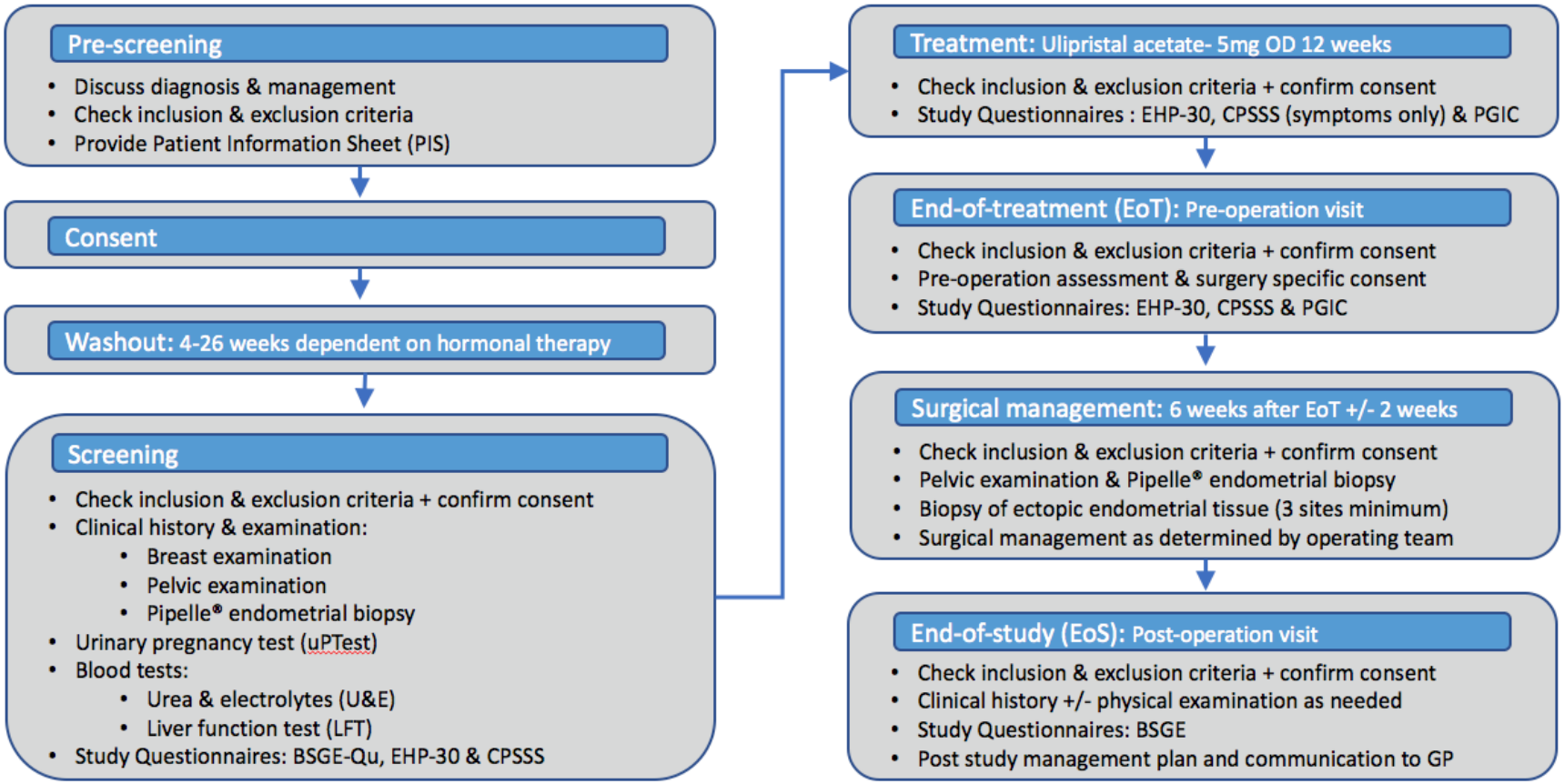


Figure 6.2 Study Flow Chart showing schedule & requirements of visits.

(BSGE-Qu= British Society of Gynaecological Endoscopy Pelvic Pain Questionnaire, EHP-30= Endometriosis Health Profile Questionnaire, CPSSS= Composite Pelvic Signs & Symptoms Score, PGIC= Patients' Global Impression of Change Score, IHC= immunohistochemistry)

6.9.3 Screening

Screening was arranged for day 7-12 of the participants menstrual cycle to ensure she was in the mid proliferative phase. Consent was confirmed, the inclusion/exclusion criteria were checked, and any adverse events recorded. A full medical history was taken to cover the following areas:

- demographic data- year of birth, age, ethnicity and occupation
- endometriosis history- current symptoms (dysmenorrhoea, non-menstrual pelvic pain, dyspareunia, bladder symptoms, dyschezia), age of onset, family history
- reproductive and menstrual history- menarche, current cycle pattern, pregnancies & outcomes, subfertility, inter-menstrual or post-coital bleeding
- past medical history- previous diagnoses or on-going conditions, and a full list of previous surgery, including endometriosis surgery and ASRM classification
- drug history- including all current medicines being used and documentation of any drug allergies or intolerances
- smoking habits, documented as daily usage and alcohol consumption, documented as weekly usage

Each participant was also asked to complete the three baseline questionnaires about their symptoms: the BSGE Pelvic Pain Questionnaire, the Endometriosis Health Profile Questionnaire (EHP-30) and the Composite Pelvic Signs and Symptoms Score (CPSSS) (see Section 6.12.1 for details).

A full physical examination was performed, based on the clinical history, to include body weight, height, breast examination and pelvic examination. The results of the pelvic examination were recorded and the CPSSS questionnaire completed. A urinary pregnancy test was taken, and an endometrial biopsy obtained using a Pipelle® endometrial sampler. A single sample of blood was taken and sent for biochemical analysis- creatinine, potassium, sodium, urea, total protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase and total bilirubin were all measured using standard analysis methodology using the standard operating procedure (SOP) at the Norfolk and Norwich University Hospital. The results of the blood biochemistry and endometrial biopsy were reviewed within 2-5 working days to ensure no exclusion criteria had been met.

6.9.4 Treatment

The treatment visit was arranged for day 1-5 of the participant's menstrual cycle to ensure she could commence study drug on the day of the visit. Consent was confirmed, the inclusion/exclusion criteria were re-checked, any adverse events were noted, and the screening results were recorded in the CRF.

Ulipristal acetate was dispensed in standard packaging (as described above) and advice given on when to start, what to do in case of missed pills, possible side effects and when to seek medical advice. Each participant was asked to keep the empty packaging (foil & cardboard) and return it at the End-of-treatment visit.

The study questionnaires were provided- EHP-30, CPSSS (symptom domains only) and Patients' Global Impression of Change (PGIC) scale. Instructions were given about completing the questionnaires in the final week of taking the study drug and a convenient data agreed and documented on each questionnaire.

6.9.5 End-of-treatment

Surgery was planned for 6 weeks (+/- 2 weeks) after the cessation of IMP to allow the resumption of the ovarian cycle and the occurrence of at least one menstrual period. As such the End-of-treatment visit occurred at the time of the standard pre-operation assessment appointment, usually the week before surgery.

Patients were seen by the pre-operative team- pre-op doctor, pre-op nurse and pre-op anaesthetist as required. Pre-operative clerking was completed as per usual departmental protocols and consent obtained for surgery explaining the risks and benefits of the surgical intervention. Clear discussion and documentation of the following risks took place:

Serious Risks:

- risk of serious complications (e.g. injury to bowel, bladder, uterus or major blood vessels- uncommon (2 in 1000)
- 15% of bowel injuries might not be immediately recognised
- failure to gain entry into the abdomen and complete the intended procedure
- risk of death- very rare (3-8 in 100,000)
- hernia formation at the port sites

Frequent Risks:

- bruising
- shoulder tip pain
- wound dehiscence
- wound infection

Additional procedures:

- blood transfusion
- laparotomy, repair of damage to bowel, bladder, uterus or blood vessel

Study consent was also confirmed, the inclusion/exclusion criteria were re-checked, and any adverse events recorded. The participants were asked to complete the study questionnaires - EHP-30, CPSSS and PGIC, and a pelvic examination was completed.

6.9.6 Surgical management

On admission for surgery the study and surgical consent were confirmed, the inclusion/exclusion criteria were re-checked, and any adverse events recorded. In theatre, a pelvic examination (under anaesthetic) was performed and a repeat Pipelle® endometrial biopsy obtained. The surgical management was individualised and determined by the operating team during the operative laparoscopy. The sites and severity of disease were recorded as per the revised ASRM classification and the details of the surgery was recorded as per usual departmental practice with the use of printed surgical notes, photographs and video as appropriate. At least three sites of ectopic endometrial tissue were biopsied as part of the excisional surgery. All samples were placed in Formalin, labelled and sent for histological assessment.

All post-operative care was routine, and the participants discharge from hospital as clinically indicated. Prior to discharge a study follow up date was arranged.

6.9.7 End-of study visit

Three months after surgery the patient was seen again for post-op follow-up. At the visit, any adverse events were recorded, a menstrual history was taken to ensure a return to normal menses and endometriosis symptoms determined. The clinical response to the surgical management was used to guide further treatment or discharge.

6.10 SAFETY

6.10.1 Definition of adverse events (AEs)

An adverse event (AE) was defined, as per the EMA guidelines on Good Clinical Practice, as any untoward medical occurrence in a subject administered the study drug and which does not necessarily have a causal relationship with the treatment.(217) As such any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug or associated with the participation in this clinical study was considered from the moment of signing to study consent form until the end of treatment (EoT).

Any abnormality identified during a medical test (e.g., laboratory parameter, vital sign, physical examination) was only defined as an AE if the abnormality met one of the following criteria:

- Induced clinical signs or symptoms
- Required active intervention,
- Required interruption or discontinuation of study medication
- The abnormality or investigational value was clinically significant.

6.10.2 Definition of serious adverse events (SAEs)

An AE was considered serious in any of the following circumstances:

- caused death
- placed the subject at immediate risk of death
- resulted in persistent or significant disability/incapacity or substantial disruption of the ability of the individual to conduct normal life functions
- resulted in congenital anomaly, or birth defect
- required inpatient hospitalisation or led to prolongation of hospitalisation
- pregnancy during the study

All SAEs that became apparent after the EoT up to 42 days after the last intake of the study drug were reported. However, hospitalisation for surgery, planned prior to the subject signing the study consent did not need to be reported as an SAE.

6.10.3 Criteria for causal relationship to the study drug

The criteria used to define a causal relationship were:

- **Not Related** - A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which made a causal relationship improbable, and/or in which other drugs, chemicals or underlying disease provided a plausible alternative explanation.
- **Possible** - A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also have been explained by concurrent disease or other drugs or chemicals.
- **Probable** - A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which followed a clinically reasonable response on re- administration (rechallenge) or withdrawal (dechallenge).

6.10.4 Criteria for defining the severity of an adverse event

The criteria used to define the severity of any adverse event were:

- **Mild** - No disruption of normal daily activities.
- **Moderate** - Affect normal daily activities.
- **Severe** - Inability to perform daily activities.

6.10.5 Reporting of serious adverse events

All SAEs were reported to the sponsor (Norfolk and Norwich University Hospitals NHS Foundation Trust) within 24 hours of the event. This was followed by a written report to the sponsor. The sponsor provided an assessment of the SAE and agreed the action plan instigated by the research team.

6.10.6 Follow-up to adverse events

All adverse events occurring during the study were followed up until resolved or judged to be no longer clinically significant. Otherwise follow-up of ongoing adverse events was recorded up to End of Trial visit or until completion of the last follow-up visit(s), whichever is last. The follow up of SAEs was dependent on the nature of the event and was agreed with the sponsor on an individualised basis.

6.11 INVESTIGATIONS/STUDY TOOLS

6.11.1 Questionnaires

Four different questionnaires (Appendix 7-10) were used to collect information from patients about signs & symptoms, pain management, quality of life features and change in disease severity following treatment with ulipristal acetate. These were chosen as they are either disease specific or are commonly used in studies evaluating the management of endometriosis.

BSGE (British Society of Gynaecological Endoscopy) Pelvic Pain Questionnaire

This questionnaire was developed by the BSGE to facilitate data collection as part of the Endometriosis Centre project. The Endometriosis Centre Database was developed to standardise data collection and investigate the morbidity and efficacy of surgical management for rectovaginal endometriosis. As an Endometriosis Centre, the study team were collecting this data routinely and included this in the data set for each study patient.

The questionnaire consists of seven different questions about pain, symptoms, previous treatment and general health. The areas we were interested in for the CHUTE study were pain (question 1), which has nine parts, and the general health score in question 5.

Endometriosis Health Profile Questionnaire (EHP-30)

The Endometriosis Health Profile Questionnaire (EHP-30) is a 30-question patient reported tool, which was developed to provide psychometrically based measures of the Health Related Quality of Life (HRQoL) of women with endometriosis. It was developed from interviews with women with the condition and has been validated to ensure it is responsive to changes in disease status.(220)

The published evidence concludes that the EHP is a reliable and valid instrument for assessing areas of concern to women with endometriosis that are not addressed by other condition-specific and generic questionnaires. The impact of pain symptoms and interplay of psycho-social wellbeing contribute to the reduced QoL (Quality of Life) in patients with endometriosis has been recognised and EHP-30 was developed to measure this. It is particularly appropriate for use in clinical trials to assess the

effectiveness of medical or surgical therapies for endometriosis on the HRQoL of affected women.(45,221)

The EHP-30 consists of a core questionnaire and a modular questionnaire. Only the core questionnaire was used as the modular questionnaire contained domains that may not have been relevant to the study patients or open to bias because of the study itself - e.g. subfertility or interaction with the medical profession. The core questionnaire covered five areas:

- Pain (Question 1-11)
- Control & powerlessness (Question 12-17)
- Emotional well-being (Question 18-23)
- Social support (Question 24-27)
- Self-image (Question 28-30)

Each question required an answer of never (score =0), rarely (score =1), sometimes (score =2), often (score =3) or always (score =4). These raw scores were then used to calculate a score for each domain and a total for the core questionnaire using the formula:

$$\text{Computed score} = \frac{\text{Sum of the scores for each item in the dimension}}{\text{4 (maximum score per item) x nos. of items in the dimension}}$$

Composite Pelvic Signs & Symptoms Score (CPSSS) or Biberoglu and Behrman (B&B)

The Composite Pelvic Signs & Symptoms Score (CPSSS) or Biberoglu and Behrman (B&B) Scale is a commonly used standard for assessing endometriosis symptoms in both a clinical and research setting.(45,47) This tool was chosen to provide some standardisation to the assessment of disease severity and examination findings made by the study team. However, it is limited by both patient recall bias and inter- and intra-rater variability.(222)

The questionnaire consists of two sections- the first is a patient assessment of symptoms and the second a blinded investigator assessment of clinical signs determined by a pelvic examination. The patient was asked to score (absent =0, mild= 1, moderate =2 or severe= 3) their symptoms of dysmenorrhea, dyspareunia and non-menstrual pelvic pain over the proceeding 28 days. A pelvic examination was then performed before

reviewing the symptom scores allowing both pelvic tenderness and induration to be assessed using the same 0-3 scoring system.

Patients' Global Impression of Change (PGIC) scale.

The PGIC is a commonly used assessment tool that provides a standard definition of clinically important improvement in clinical trials investigating chronic pain.(45,223) This tool was included to assess & quantify any change in disease severity experienced by the study patients.

The scale consists of two sections. Section 1 asks the patient to describe any change in activity, limitations, symptoms, emotions and overall quality of life, related to their endometriosis. The options range from 'no change' (score 1) to 'a great deal better, and a considerable improvement that has made all the difference' (score 7). Section II asks them to indicate the degree of change since the beginning of treatment on a visual analogue scale.

6.11.2 Surgery

The surgery was undertaken at the Norfolk and Norwich University Hospital. All surgery was planned and completed laparoscopically using high definition laparoscopic equipment provided by Storz & Olympus. The optical trocars and disposable instruments were provided by Covidien. Surgery was completed using monopolar diathermy, bipolar diathermy and the harmonic devices such as the Lotus ultrasonic scalpel (SRA Developments) and the Thunderbeat device (Olympus).

6.11.3 Histological specimens

The eutopic endometrial samples obtained at screening and surgery as well as the ectopic endometrial samples obtained by excising the disease were processed by the histopathology department of the Norfolk & Norwich University Hospital using the pathology departments standard operating procedures (Appendix 11-17) and the CHUTE Histopathology Plan (Appendix 18). At the time the sample was obtained it was placed into a fixative solution (10% formalin).

To allow the microscopic analysis of the tissues, very thin slices of tissue were mounted on glass slides to allow sufficient light through the specimen for the structures to be identified. To achieve this fixation occurred to prevent tissue degradation; the tissue was then dehydrated and infiltrated with paraffin wax. Once infiltrated with wax it was

embedded in a wax block to provide support to the sample as slices of the tissue were obtained for mounting, staining & immunohistochemistry.

Fixation

The fixation process began once the sample was placed in the fixative solution (10% formalin) in clinic or theatre. The samples remained in fixative until processing occurred and the length of time varied from approximately 18-72 hours dependent on when the sample was obtained. Prior to processing all samples were transferred to processing cassettes.

Processing

Processing occurred using a five-hour (xylene free) programme on a Peloris (fluid-transfer) processor (Leica Biosystems) (Appendix 11). As paraffin wax is hydrophobic the first step in processing was dehydration to replace the water in the sample with industrial denatured alcohol (IDA). This was achieved using a series of IDA solutions to prevent tissue distortion. The IDA was then replaced with the solvent Isopropyl alcohol before finally being infiltrated with histology grade paraffin wax.

Embedding

Once processed the sample was embedded in histology grade paraffin at 63°C before being allowed to cool to create a paraffin block (Appendix 12).

Sectioning

The paraffin blocks were sectioned at 4 μm using a microtome (Thermo Shandon Finesse) and floated out on a 45°C water bath to flatten the slice before being placed onto a standard microscope slide. The slides were then baked on a hotplate for 10-30 minutes dependent on whether they were for staining or immunohistochemistry (Appendix 13 & 14).

6.11.4 Haematoxylin & eosin staining

A technique of regressive staining was used- the haematoxylin was over stained, the excess stain was removed by acid alcohol and then eosin staining of the acidophilic elements of the tissue followed. This was done as an automated process on a Leica Biosystems Autostainer using Protocol 1 of the SOP HIS.SL.P. 3 (Appendix 15). The reagents used were per the protocol and utilised Harris haematoxylin and 1% aqueous eosin.

6.11.5 Immunohistochemistry (IHC)

All immunohistochemistry staining was performed on a Leica BOND™ (Leica Biosystems) using commercially available antibodies and a proprietary polymer detection system with 3,3'-Diaminobenzidine (DAB) as the chromagen. The Compact Polymer™ system (Bond Polymer Refine) from Leica Biosystems was used as it is extremely sensitive, highly specific and can be optimised for automated IHC systems like Leica Bond™ (See Figure 6.3). This is an indirect method where the primary antibody binds to the target epitope and then a secondary antibody binds to the first antibody. The second antibody is bound to an enzyme, peroxidase in this case, which catalyses the conversion of DAB to a brown precipitate that is insoluble in alcohol and xylene and so indicates the presence of the epitope of interest under light microscopy. This method is more sensitive because of signal amplification due to the binding of several secondary antibodies to each primary antibody.

Protocol

The first step in the process is epitope recovery in which the paraffin from the sections of tissue must be completely removed and methylene bridges crosslinking proteins, caused by formaldehyde fixation, broken down. This is usually achieved with an organic solvent and heat (heat induced epitope retrieval or HIER). Three different pre-treatment protocols were used dependent on the epitope of interest. Each uses a specific epitope retrieval (ER) solution and length of heat treatment- see Table 6.1, Table 6.2 & appendix 19-21 for details.

The next step is to block the action of any endogenous peroxidase within the tissues as this would cause non-specific reduction of the DAB substrate and so non-specific staining of the tissues. After that step, the tissue is exposed to the primary and secondary antibodies diluted in a stabilising buffer before the chromagen substrate is added. The dilution, rinsing steps and exposure times are all optimised for each antibody used. The two protocols used are shown in Appendix 22 & 23.

Initial antibodies

Initially immunohistochemistry was performed to identify the following epitopes: Oestrogen Receptor (ER), Progesterone Receptor (PR), Androgen receptor (AR) and the Ki67 proliferation marker. The details of the antibodies, associated dilutions, pre-treatment and protocols are shown in Table 6.1.

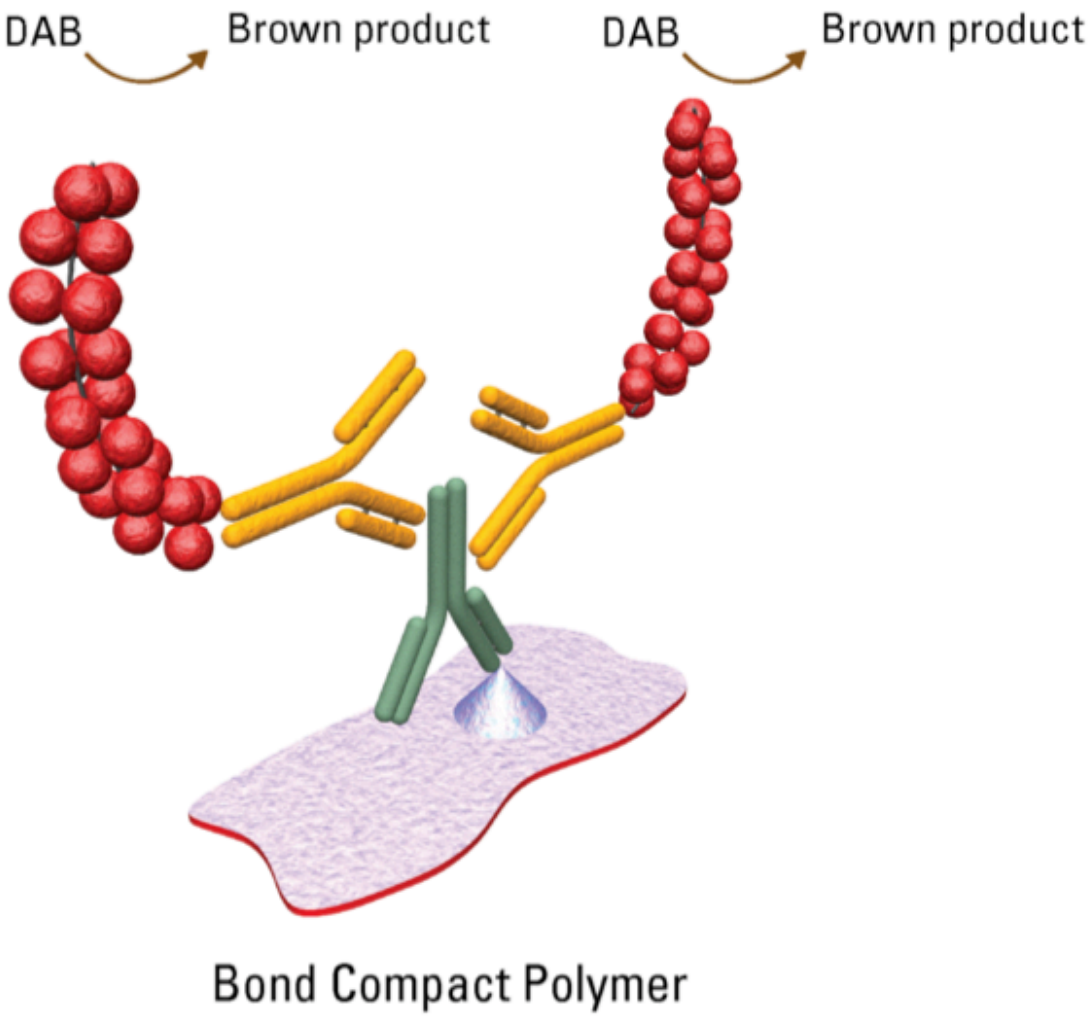


Figure 6.3 Bond Compact Polymer system used for immunohistochemistry.

(Primary antibody= green 'Y' shaped structure, Secondary antibody= yellow 'Y' shaped structure, Peroxidase= red spheres, DAB= 3,3'-Diaminobenzidine (reproduced from promotional material provided by Leica Biosystems))

Additional antibodies

Following interim assessment of the initial immunohistochemistry a decision was taken to complete further immunohistochemistry to identify the following epitopes: VEGF (vascular endothelial growth factor) and PTEN (phosphatase and tensin homolog deleted on chromosome ten). The details of the antibodies, associated dilutions, pre-treatment and protocols are shown in Table 6.2.

MARKER	CLONE	MANUFACTURER	PRODUCT CODE	DILUTION	PRE-TREATMENT	PROTOCOL
ER	EP1	Dako (Agilent)	M3643	1:120	H1(30)	R-LP-E
PR	16	Leica Biosystems	NCL-L-PGR-312	1:200	H1(20)	R-LP-E
AR	EP120	Epitomics	AC-0071	1:50	H2(20)	R-LP-E
Ki-67	EP5	Epitomics	AC-0009	1:100	H2(20)	Rout

Table 6.1 Initial antibodies, dilutions and protocols as per SOP HIS.IM.P.10.

(H1(30) = Heat Induced Epitope Retrieval 30 minutes with Epitope Retrieval solution 1, H1(20) = Heat Induced Epitope Retrieval 20 minutes with Epitope Retrieval solution 1, H2(20) = Heat Induced Epitope Retrieval 20 minutes with Epitope Retrieval solution 2, R-LP-E= Routine long protocol, Rout= Routine protocol)

MARKER	CLONE	MANUFACTURER	PRODUCT CODE	DILUTION	PRE-TREATMENT	PROTOCOL
PTEN	SP218	Spring Bioscience	M5180	1:200	H2(20)	Rout
VEGF	EP1176Y	Menarini Diagnostics	MP-356-CMK01	-	-	-

Table 6.2 Additional antibodies, dilutions and protocols as per SOP HIS.IM.P.10.

(H2(20) = Heat Induced Epitope Retrieval 20 minutes with Epitope Retrieval solution 2, Rout= Routine protocol)

6.12 ANALYTICAL METHODS

6.12.1 Data recording

Following enrolment into the study, each patient was assigned a study number (CHUTE 01 – CHUTE 25). The name and hospital number of the participants were recorded in the Patient ID Log, which was kept in the site file at the Norfolk and Norwich University Hospital. This data, along with a contact phone number, e-mail address and log of visit dates was also recorded in an excel spreadsheet on the hospital network. All study data relating to the participants was recorded under their study number to maintain confidentiality.

Once the consent form was signed, all subsequent study related patient data was recorded in both the medical records and the Case Report Form (CRF) (Case Report Form (CRF) (CHUTE) v3.0 (Appendix 6)). Data from the CRF was transcribed from the paper CRF to an electronic database 'CRF & Questionnaire data' (in Microsoft Excel for Mac 2011), which was password protected.

Clinical data

This was recorded in the medical records as for any clinical interaction with a patient. This data was then transferred to the CRF at the time of the appointment or immediately afterwards to ensure data was correctly recorded.

Patient questionnaire data

Each questionnaire was completed in paper format and secured with the paper CRF. The responses to the questions were then transcribed to the 'CRF & Questionnaire data' password protected database.

Surgical data

Findings from the primary laparoscopy were obtained from the operation notes in the medical record. The surgical stage of the disease was classified per the revised American Society for Reproductive Medicine (ASRM) classification of endometriosis, based on the information available- either written description or photographic records.

At the post treatment surgery, the distribution of disease was again described and classified per the revised American Society for Reproductive Medicine classification. The extent of the disease, location of the disease, sites of the ectopic endometrial biopsies

and surgical steps were all recorded as written notes in the medical record. This was supplemented by intra-operative photographs & video as appropriate and in-line with usual departmental practice. Anonymised copies of the written notes and photos were added to the paper CRF and an electronic version stored on the hospital network ('Operation Images' folder).

Histological data

The H&E slides for the eutopic and ectopic endometrial biopsies were reported by an experienced gynaecological histopathologist in accordance with local protocol. A written report was produced and communicated to the study team as per standard practice. An anonymised copy of this report was filed in the study file.

The H&E and IHC slides were then reviewed by the study team on a batch basis. The features and immunohistochemical staining were described as outlined in the Histopathology Plan (Appendix 24), with the findings being recorded on the Histopathology Report Proforma (Appendix 25). Any features consistent with PAEC in the eutopic endometrium were described with reference to:

1) Gland architecture:
    a) Cystic dilatation (present/absent, focal/widespread)
    b) Disordered architecture (absent/focal/diffuse)
    c) Complex architecture (absent/focal/diffuse)
    d) Budding into stroma (present/absent)
    e) Papillation into lumen (present/absent)
    f) Gland crowding (present/absent)
    g) Gland-stroma ratio (decreased/unchanged/increased)
2) Glandular epithelium:
    a) Cell type (flat cuboidal/tall columnar)
    b) Stratification of nuclei (present/absent)
    c) Mitoses (infrequent/frequent)
    d) Cytoplasmic vacuolation (common/uncommon)
    e) Secretion in lumen (present/absent)
    f) Nuclear size (small/medium/large)
    g) Nuclear shape (ovoid/rounded)
    h) Nucleoli (present/absent)

i) Nuclear atypia (present/absent)

j) Squamous metaplasia (absent/occasional/frequent)

3) Stroma:

a) Stromal density (moderately cellular/densely cellular)

b) Foam cells (absent/infrequent/present)

c) Stromal breakdown (present/absent)

d) Intravascular fibrin thrombi (present/absent)

These features were chosen as they were used by the PEARL study histopathologists to distinguishing between PAEC, unopposed oestrogen effect and endometrial hyperplasia.(203)

The immunohistochemistry slides for detection of ER, PR & AR were assessed using the Quick (Allred) score as outlined in Table 6.3. The proportion score and intensity score are added together to give a maximum score of eight. The Ki67 immunohistochemistry was recorded as an estimated percentage of nuclear staining.

Score	PROPORTION	INTENSITY
0	no staining	no staining
1	< 1% nuclei staining	weak staining
2	1–10% nuclei staining	moderate staining
3	11–33% nuclei staining	strong staining
4	34–66% nuclei staining	
5	67–100% nuclei staining	

Table 6.3 Quick (Allred) score for immunohistochemistry scoring.

(Reproduced from Immunohistochemical detection of steroid receptors in breast cancer: a working protocol, as per the local departmental policy for IHC reporting.(224)*)*

The same reporting structure was used for the ectopic endometrial samples except only cystic dilatation could be assessed in the gland architecture due to the ectopic location of the sample.

The H&E and initial IHC slides were also assessed by a second histopathologist, familiar with PAEC and familiar with the primary studies investigating the IMP- Professor Alistair R.W. Williams. This was required for the purposes of external review and quality

control. It necessitated the temporary transfer of the histology slides to the Department of Pathology at the University of Edinburgh in keeping with the terms of the material transfer agreement (Appendix 26).

The data for each study patient contained in both copies of the Histopathology Report Proforma was then transcribed to the 'CRF & Questionnaire data' password protected database.

Histopathology images

All slides were viewed using an Olympus BX51 Microscope and images captured using Olympus cellSens Entry 1.14 software. All the representative slide images and an image log for each participant were stored on the hospital network ('Histopathology Images' folder).

Results summaries

The surgical notes and histological image data for each study participant was summarised into a single document to facilitate data analysis (Appendix 27).

6.12.2 Data analysis

Primary Outcome Measures

As an interventional descriptive cohort study the primary outcome data was qualitative and descriptive. The histological data for the ectopic endometrium has been described as detailed above but the most important determinants of PAEC were assessed to be cystic dilatation, the epithelial features of cell type, nuclear stratification, mitoses and cytoplasmic vacuolation, and the stromal density. These features have been focused on primarily during the analysis of the H&E slides.

Secondary Outcome Measures

Changes in disease severity were assessed by comparing the laparoscopic appearance of endometriotic lesions between the diagnostic surgery and the second definitive surgery and the ASRM scoring. These changes were correlated with the quantitative changes identified in the patient reported outcome questionnaires as detailed in Table 6.4.

Questionnaire	Screening vs. EoS	Screening vs. Treatment	Screening vs. EoT	Treatment vs. EoT
BSGE-Qu	Pain Score & Health Score			
EHP-30		Mean Pain & Total Score	Mean Pain & Total Score	Mean Pain & Total Score
CPSSS		Symptom Score	Total Score	
PGIC		Total Score	Total Score	

Table 6.4 Questionnaire score comparisons

(The details of the sections of each questionnaire used for the comparison are also highlighted.)

These comparisons were chosen as the best representation of clinical improvement over the course of the study, improvement in QoL in response to ulipristal acetate use and the tolerability of the treatment.

Statistical analysis

The nature of the study meant there was limited statistical analysis of the data. The differences between questionnaire scores has been analysed - the details of the methods used are discussed in the results chapter. The analysis was performed using Microsoft Excel for Mac 2016 & IBM SPSS Statistics 22.

6.12.3 Data handling

Source data

All source data was stored in either the medical record or a paper study file, which was kept in a locked office or as an electronic record stored in a secure folder on the hospital network.

Data protection

Data collected during the research trial was kept strictly confidential and accessed only by members of the trial team. The participant's details were stored on a secure database

under the guidelines of the 1988 Data Protection Act and regular checks and monitoring were undertaken to ensure compliance. The senior IT manager (in collaboration with the Chief Investigator) managed access rights to the data set.

The study data will be archived to a secure data storage facility and not be kept for longer than is required as per the local R&D department SOP.

6.13 CHANGES TO STUDY DESIGN

6.13.1 Substantial amendments

The study went through two substantial amendments, which each requiring a new version of the protocol- first to version 2.0 and then to version 3.0. The first amendment occurred because of the MHRA review of the protocol and the second occurred after the study had opened to recruitment necessitating notification and re-consenting of our first recruited patient- CHUTE 01.

Research Protocol (CHUTE) v2.0

The addition of exclusion criteria 1- 'the subject has genital bleeding of unknown aetiology or for reasons other than uterine fibroids', was requested by the MHRA and so the protocol and associated study documents were updated. In addition, the eligibility criteria were clarified, and the risks of surgery added to the study documents.

The plan to transfer the histology slides to Professor A.R.W. Williams at the University of Edinburgh had been formulated and the protocol, PIS and consent form were adjusted to reflect this. The formulation and signing of the material transfer agreement was also arranged at this time.

Research Protocol (CHUTE) v3.0

The decision to complete the surgical intervention 6 weeks after stopping the study drug rather than whilst still taking it required a substantial amendment to the protocol. The details of the changes have already been discussed and the final protocol represented in Figure 6.1. This major change in the design was accompanied by further clarification of the eligibility criteria, inclusion and exclusion criteria to ensure maximal recruitment to the study.

6.13.2 Minor amendments

The timing for starting study drug needed clarification to ensure that if a patient attended the treatment visit between day 1-5 of her cycle they were permitted to start the study drug immediately rather than wait another month off treatment. The patient information sheet indicated that this was acceptable, but the wording of the protocol needed adjusting for clarity taking the protocol to version 3.1.

A finalised version of the study protocol can be found in Appendix 28- Research Protocol (CHUTE) v3.1.

6.14 CANDIDATE CONTRIBUTION TO STUDY

6.14.1 Study design

An outline proposal and funding agreement to evaluate Ulipristal acetate in 20 patients existed when I began work on the study but the original study design and iterative changes to achieve the final study protocol were completed by the candidate with support from Mr E. Morris and other experts in the field.

6.14.2 Study set-up, registration, regulatory approval and NIHR Portfolio adoption

The candidate was responsible for close liaison with the local R&D department and negotiation of which clinical biochemistry and histopathology tests were possible within the agreed budget. The completion of the IRAS application, CTA registration, ethical approval and MHRA approval, including the appropriate amendments were all undertaken by the candidate.

In addition, the candidate presented the proposed study to the local R&D director and the scientific advisory board of the funding body to ensure adoption onto the NIHR Portfolio, local research nurse support and continued funding.

6.14.3 Principal Investigator (PI) & study coordinator role

The candidate undertook the PI and study co-ordinator role to ensure the smooth running of the study and to complete their educational objectives within a UEA PPD plan. This included independent involvement in all monitoring visits and responsibility for resolving all associated queries.

6.14.4 Recruitment, study visits and patient management

The recruitment, selection and enrolment of study patients was all undertaken by the candidate with advice and support from Mr E. Morris. Following inclusion in the study; all study visits, associated procedures, questionnaire administration, adverse event recording and SAE management was undertaken by the candidate under the indirect supervision of Mr E. Morris.

The candidate was involved in the surgical management of each patient, including the assessment of the patient intra-operatively and the surgical excision of disease. In the early stages of the study this was as an assistant, but the candidate took on the role of the primary surgeon in the final few cases, as their surgical skills developed.

6.14.5 Histopathology and immunohistochemistry

Although the candidate undertook visits to the histopathology laboratory to gain an understanding of the steps involved the processing of the specimens was undertaken by the laboratory technicians. As a working NHS laboratory there was no facility to allow the candidate to be more closely involved.

The evaluation of the histopathology slides and IHC slides was undertaken jointly with Dr R. Lonsdale to ensure correct interpretation. The use of the camera microscope and selection of representative images was undertaken by the candidate following each reporting episode, under the indirect supervision of Dr Lonsdale.

6.14.6 Data recording, analysis and thesis presentation

These aspects of the study were undertaken by the candidate with advice and support from those outlined in the Contributions section.

Chapter 7: RESULTS

7.1 COHORT DATA

7.1.1 Recruitment

Recruitment started on the 14th November 2014, following the completion of the Site Initiation Visit (SIV) on 13th November 2014. The first patient was recruited and signed their consent form on 12th December 2014, ensuring we met our first patient-first visit (FFV) target. Recruitment continued over a period of 18 months and the last patient-first visit was conducted on 5th May 2016.

During recruitment, a total of 66 patients were pre-screened to recruit a total of 25 patients to the study (CHUTE 01 - CHUTE 25), who signed the consent form and formally entered screening. The final number of patients completing the CHUTE study was 18, due to either screen fails or premature discontinuation because of meeting exclusion criteria, as outline in Figure 7.1.

7.1.2 Screen fails

There was a total of five screen fails during washout from previous medical treatments for endometriosis. CHUTE 03 was using GnRHa and Tibolone at the time of recruitment and went into washout as per protocol. During the six months in washout her periods did not return, she developed vasomotor symptoms and her endometriosis improved significantly. Having reached the menopause, she decided surgical management was no longer suitable for her and so was excluded.

CHUTE 07 had her LNG-IUS (Mirena®) removed to enter washout and felt her symptoms subsequently improved and so decided to post-pone a decision on surgical management of her disease. CHUTE 16 moved to a new area and was unable to keep her study appointments at the study site. She requested a transfer of her care to another hospital and this was facilitated. CHUTE 18 did not establish a regular menstrual pattern after stopping the progesterone only pill and so was excluded.

CHUTE 22 went into washout following stopping the progesterone only pill. She did not attend several study appointments and was difficult to reach by telephone or e-mail. It was considered inappropriate for her to continue in the study, in view of these

communication difficulties, and so she was excluded. This was discussed with her and she went on to have standard surgical management of her disease to good effect.

7.1.3 Premature discontinuation

CHUTE 17

This patient was recruited on 02nd October 2015 and went into washout from the progesterone only pill she was taking. Screening was undertaken on 12th November 2015, study drug was commenced on 7th December 2016 and surgery was planned for 18 April 2016. However, a urinary pregnancy test followed by an ultrasound scan confirmed a pregnancy of seven weeks' gestation (see Section 7.1.5 for further details). This resulted in her premature discontinuation from the study as surgery was no longer clinically appropriate.

CHUTE 20

Recruitment and signing of consent took place for this patient on 10th December 2015, based on a previous diagnostic laparoscopy and a worsening of pelvic symptoms. Study drug was then taken from the 16th February 2016 to the 9th May 2016, and surgery was performed on 21st June 2016. At her operative laparoscopy, no significant endometriosis was seen, which was later confirmed by the histological evaluation of the biopsies obtained. However, significantly dilated pelvic veins were noted and a diagnosis of pelvic congestion was made, and an appropriate management plan instigated. Since endometriosis was not confirmed, by biopsy, at the original diagnostic procedure it cast doubt on the appropriateness of her initial inclusion and so it was considered inappropriate to keep her in the study.

7.1.4 Adherence

All study patients attended all the study visits set out in the study protocol. The timing of each visit was in accordance with the protocol, with one exception (detailed below). No visits were missed, and all the data required for each visit was recorded in the CRF, patient study folder and hospital notes, as appropriate.

A protocol deviation occurred for CHUTE 14 as her surgery was performed 27 days later than planned. Her operation had to be postponed on the day of surgery (29th January 2016) due to a theatre overrun because of a complex case. The earliest availability of both surgeons meant her surgery did not occur until 23rd Feb 2016.

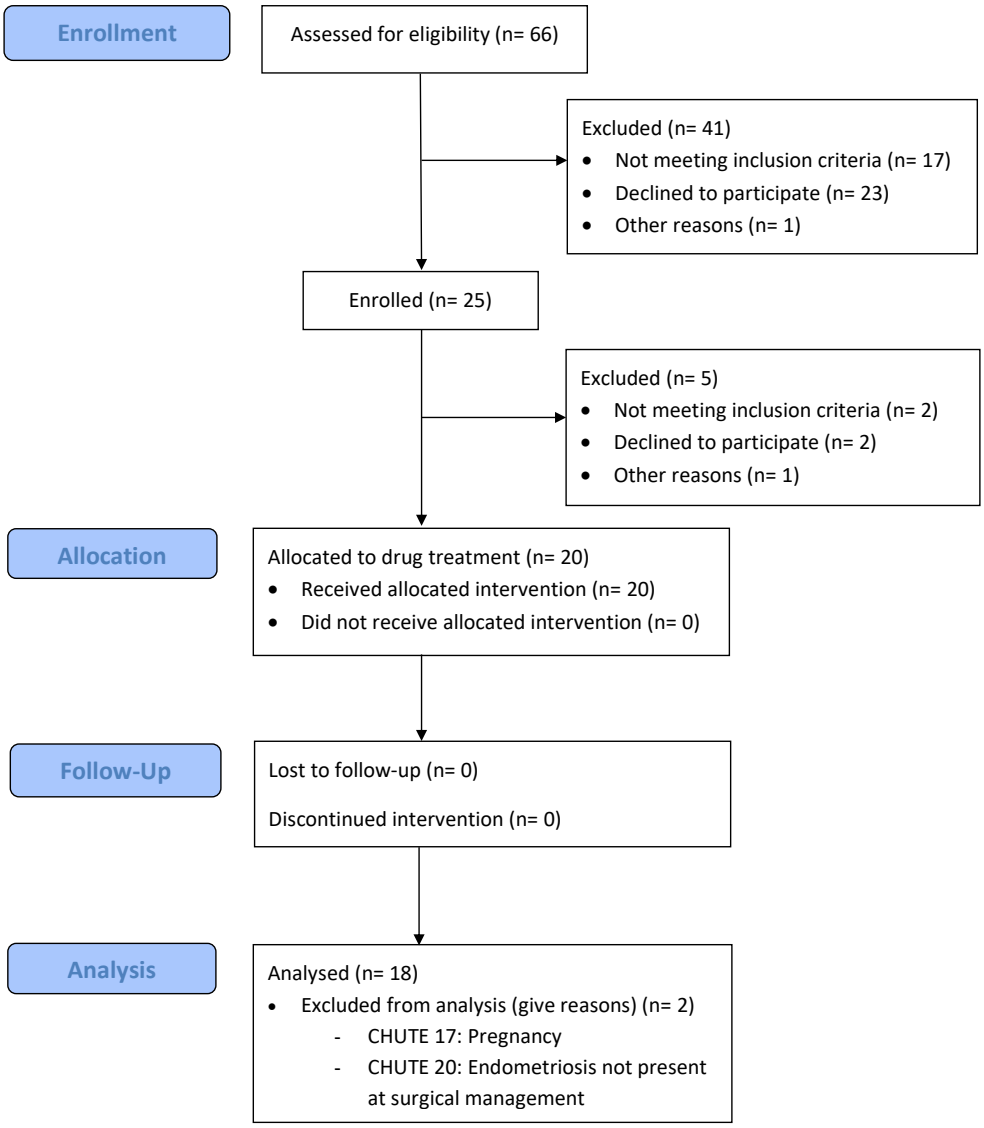


Figure 7.1 Enrollment & outcomes

A total of 25 subjects were consented and enrolled in the study, of whom 5 were excluded during screening. Treatment was completed by 20 subjects but CHUTE 17 was excluded due to pregnancy and CHUTE 20 was excluded as there was no evidence of endometriosis at the time of her surgical management. This left 18 subjects for the final analysis.

7.1.5 Serious adverse events (SAEs)

CHUTE 17

This study subject completed study drug on 28th Feb 2016 and her elective planned surgery was scheduled for 18th April 2016. On 15th April 2016 at 06.17 she notified us of two positive urinary pregnancy tests via e-mail. She had undertaken these tests due to the lightness of her period and two weeks of "morning sickness" symptoms.

An urgent appointment was arranged for 09.00 the same day to assess the situation. A urinary pregnancy test was found to be positive and a plasma βhCG (130292 IU/L) confirmed the result. An ultrasound scan demonstrated an intra-uterine pregnancy (CRL (Crown Rump Length) 9.8mm, 7w1d gestation) with a fetal heartbeat detected. Two follow up scans were arranged in the early pregnancy assessment unit (EPAU) – on the 29th April 2016 the CRL measured 20.0mm (8 weeks, 4 days gestation) with a detectable fetal heartbeat, and on 13th May 2016 the CRL 50.2mm, 11w5d with a fetal heartbeat detected. She was then discharged from EPAU to routine antenatal care and an antenatal appointment was scheduled for 21st June 2016.

Her antenatal care included progesterone supplementation in the first trimester and a plan for additional growth scans in the second trimester. This was arranged because of uncertainty about the action of ulipristal acetate on the endometrium prior to implantation and whether poor implantation might contribute to an increased risk of miscarriage or growth restriction.

A fetal anomaly scan was performed on 13th August 2016, which revealed normal anatomy, a posterior placenta clear of the internal os and a normal amniotic fluid volume. In the third trimester, she was admitted on two separate occasions with nausea and vomiting of pregnancy, but this settled quickly with anti-emetics and intravenous fluid. There were no concerns relating to fetal growth during the antenatal period.

Following an episode of reduced fetal movements on 21st November 2016 at 39^{+2} weeks gestation she was admitted for an induction of labour on 25th November 2016. This was achieved using 10mg dinoprostone, followed by artificial rupture of membranes and oxytocin augmentation of labour; resulting in a normal vaginal delivery of a baby boy at 10.19 on 27th November 2016. He weighed 3575 grams and there were no concerns raised at his new born assessment by the paediatric team.

CHUTE 23

This study subject was enrolled on 16th February 2016 but was admitted on 29th February with abdominal and pelvic pain, prior to her screening appointment. The pain she was experiencing was in keeping with her typical endometriosis symptoms but was not responding to analgesia. Her period was due 6 days later so the clinical impression was one of a typical pre-menstrual flare of pain.

An ultrasound scan of the pelvis was arranged during her inpatient stay, which was reported as normal with no evidence of ovarian pathology. Her pain resolved by 18.00 on 2nd March 2016 and she was discharged. This event was recorded as an SAE in view of the admission to hospital but was considered to be unrelated to the study as no changes to her management had occurred at that time.

7.1.6 Adverse events (AEs)

A total of 63 adverse events were recorded during the study. Many of these were not considered to be related to the study. Those that were either *possibly related* or *definitely related* to the study, and the frequency with which they occurred are described in Table 7.1. A total of 41 events were recorded- 23 as study related adverse events and 18 as perceived side effects recorded by the study subjects.

The most commonly noted symptom was headache, in line with the known safety data related to ulipristal acetate. The other commonly mentioned symptoms of constipation, mood disturbance, skin changes and vaginal discharge are typical progesterone related effects.

7.1.7 Demographics & characteristics

The mean age of the study subjects was 35 (range 22-49) and the mean average BMI was 25 (range 20-39); with 20% being current smokers (4/18). All the subjects (18/18, 100%) described themselves as white British. Most were in full time employment (15/18) but one was unemployed, one was a student and one subject described herself as a housewife.

The mean self-reported age at onset of endometriosis was 23 (range 11-46), giving a mean disease duration of 12 years (range 1-26). As all the subjects had a diagnosis of endometriosis they consequently had a surgical history that included a diagnostic laparoscopy. In addition, eight had also had previous laparoscopic treatment of their

Adverse Events or Side Effects	Frequency
Headache	6
Constipation	4
Mood disturbance	4
Skin changes/spots	3
Vaginal discharge	3
Abdominal/pelvic pain	2
Alopecia	2
Hot flushes	2
Joint ache	2
Urinary incontinence	2
Weight gain	2
Back pain	1
Dark stool	1
Lymphadenopathy	1
Migraine	1
Neuropathic leg pain	1
Post-op nausea	1
Uterine cramps	1
Vaginal dryness	1
Vertigo	1

Table 7.1 Adverse Events (AEs) or study related side effects

The adverse events recorded during the study that were considered by the investigators to be either possibly related or definitely related to the study, and the side effects recorded by the study subjects are shown.

endometriosis, one had a history of a laparoscopic cholecystectomy and one had been sterilised laparoscopically. Five of the subjects had also previously had a laparotomy - each for a lower segment Caesarean section.

Three subjects had been treated for sexually transmitted infections in the past, but none had a history of pelvic inflammatory disease. The median parity of the cohort was one with two subjects having had fertility treatment.

The demographics and baseline patient characteristics are described in Table 7.2.

Characteristic	Study Cohort (n =18)
Age (yr)*	35.0 +/- 8.2
BMI (kg/m²)*	24.8 +/- 4.9
Parity§	1.0 (0, 2.0)
Age at onset (yr)*	22.7 +/- 10.8
Disease duration (yr)*	11.6 +/- 7.7
BSGE Pelvic Pain Questionnaire scores§:	
- **Pre-menstrual pain**	8.0 (6.3, 9.0)
- **Menstrual pain**	8.0 (7.0, 9.0)
- **Non-cyclical pelvic pain**	6.5 (4.5, 7.0)
- **Pain during sexual intercourse**	6.5 (3.0, 9.0)
- **Pain opening bowels during period**	7.0 (1.5, 9.0)
- **Pain opening bowels at other times**	3.0 (0, 6.0)
- **Lower back pain**	7.0 (4.5, 8.0)
- **Bladder Pain**	2.0 (0, 5.8)
American Society for Reproductive Medicine (ASRM) staging:	
- **Score***	20.4 +/- 19.1
- **Stage§**	2.0 (2.0, 3.0)
Endometriosis Health Profile Questionnaire (EHP-30) scores*:	
- **Pain**	50.9 +/- 16.8
- **Control & powerlessness**	63.2 +/- 25.8
- **Emotional well-being**	43.1 +/- 22.6
- **Social support**	49.3 +/- 25.0
- **Self-image**	51.4 +/- 30.6
- **Total EHP-30 score**	257.8 +/- 101.7

Table 7.2 Demographics and baseline characteristics of CHUTE cohort

*The demographics and baseline characteristics of the eighteen CHUTE study subjects is shown, with the onset age being the age the patient was first aware of symptoms rather than the age at which the diagnosis was made (*Mean +/- SD, §Median (IQR), yr= years).*

7.2 CLINICAL DATA

The clinical response to ulipristal acetate was considered based on patient reported symptoms and the results of the EHP-30 and PGIC questionnaires. The results of these two questionnaires allowed the cohort to be divided into responders (10/18, 56%) and non-responders (8/18, 44%); and are discussed in detail in Section 7.5. This distinction between responders and non-responders has been used throughout the analysis of the results to aid in the recognition of patterns that might indicate the mechanism behind the clinical impact seen.

For details of questionnaires at each visit and time points for comparisons, please see Figure 6.1.

7.2.1 Disease status

All the subjects within the cohort had a diagnosis of endometriosis and the symptoms related to the disease were evaluated at screening, during treatment and prior to surgery. The BSGE Pelvic Pain Questionnaire (BSGE- Qu) allowed quantification of these symptoms at each visit and the Composite Pelvic Signs and Symptoms Score (CPSSS) provided a framework for describing clinical signs associated with the disease.

Symptoms

At screening the five classic endometriosis symptoms were enquired about: dysmenorrhoea (14/18), non-menstrual pelvic pain (14/18), dyspareunia (14/18), bladder symptoms (6/18) and dyschezia during menstruation (14/18) were reported by the 18 subjects. The severity of each symptom was quantified in the BSGE-Qu using a 0-10 scale, with 10 being the maximum. The median scores for dysmenorrhoea (8.0), non-menstrual pelvic pain (6.5), dyspareunia (6.5) and dyschezia during menstruation (7.0) indicated severe symptoms but the median score for bladder symptoms was only 2.0, suggesting this was a much less bothersome symptom (See Table 7.2).

Signs

The clinical signs of endometriosis were evaluated at screening to aid clinical assessment and planning of pre-operative investigations. Both pelvic tenderness and induration were rated as moderate with both having median scores of 2 (interquartile range 1-2).

7.2.2 Clinical response

Ulipristal acetate

All the study subjects completed the three-month course of ulipristal acetate, with fourteen taking all 84 doses as instructed and four subjects missing some doses. The total doses missed was 8 (range 1-5) giving a mean average of 0.44 missed doses per patient.

At the end of treatment (EoT) the patient reported symptoms of the cohort were as follows: dysmenorrhoea (2/18), non-menstrual pelvic pain (9/18), dyspareunia (8/18), bladder symptoms (3/18) and dyschezia during menstruation (5/18). There was marked improvement with regards to prevalence across all five of these symptoms when compared to screening. This was reflected by the reduction in analgesia usage reported by the subjects; 10 subjects reported a decrease, 8 reported no change and no subjects reported an increase.

Amenorrhoea was reported in 15/18 (83%) of the study subjects. A lack of menstrual bleeding did not always equate to improvement of endometriosis symptoms- seven of the eight subjects who were considered to be non-responders were amenorrhoeic. However, those subjects who continued to menstruate whilst taking the drug still reported a good clinical response, with two responders having a regular cycle throughout.

Ulipristal acetate and surgical management

The results obtained at the end of study (EoS) visit represent the combination of the pre-operative drug treatment and the surgical excision of disease. The patient reported symptoms of the cohort were: dysmenorrhoea (13/18), non-menstrual pelvic pain (11/18), dyspareunia (4/18), bladder symptoms (0/18) and dyschezia during menstruation (3/18). The prevalence of all the main five symptoms had reduced by the end of the study but dysmenorrhoea remained a common problem within the cohort even following surgical management. However, analgesia usage at the EoS visit compared to the screening visit was reduced in 8 of the 18 subjects (44%).

The patient reported severity data from the BSGE-Qu is shown in Table 7.3. It demonstrates that a statistically significant reduction in median symptom severity was achieved between screening and the end of the study for pre-menstrual pain (-3.0,

p=0.0004), menstrual pain (-2.0, p=0.006), non-cyclical pelvic pain (-2.5, p=0.0034), pain during sexual intercourse (-4.0, p=0.002) and lower back pain (-2.0, p=0.0068).

BSGE Pain Questions	Subjects	End of Study (EoS)		EoS – Screening Difference		
	(n=…)	Median	IQ Range	Median	IQ Range	P Value§
Pre-menstrual pain	17	4.0	(3,6)	-3.0	(-5,-1)	0.0004*
Menstrual pain	17	6.0	(3,8)	-2.0	(-4,0)	0.0060*
Non-cyclical pelvic pain	18	4.0	(0,6)	-2.5	(-4,0)	0.0034*
Pain during sexual intercourse	12	1.5	(0,4.5)	-4.0	(-5.5,-2)	0.0020*
Pain opening bowels during period	16	2.5	(0,6)	-1.5	(-5.5,0)	0.0518
Pain opening bowels at other times	18	0.0	(0,5)	0.0	(-4,1)	0.2319
Lower back pain	18	3.5	(0,5)	-2.0	(-6,0)	0.0068*

Table 7.3 BSGE-Qu end of study data

*The median average and interquartile range (IQ Range) are shown for each domain of the pain section of the BSGE-Qu questionnaire completed by study subjects at the EoS visit. The symptom of 'Bladder Pain' was excluded from this analysis as the median score at baseline was low, indicating that this was not a clinically significant symptom for this cohort. The median average and IQ Range for the difference between screening and EoS is also shown allowing a Wilcoxon signed rank test (§) to be performed. (*p<0.05)*

7.2.3 Surgical data

The descriptive data for each study subject including the operation notes, annotated surgical photographs, annotated histology (H&E) photographs and annotated IHC photographs are shown in appendix 27. Figure 7.2 gives an example of the surgical management undertaken for the CHUTE study subjects and the descriptive data contained within appendix 27 for each subject.

CHUTE 02

This subject was 30 years of age and struggling with her endometriosis symptoms. She was experiencing debilitating pelvic pain but her most bothersome symptoms were dyspareunia and dyschezia during her periods. On pelvic examination nodules were palpable within the posterior fornix and over the left utero-sacral ligament.

At laparoscopy, a dense and active lesion of deeply invasive endometriosis was seen overlying the left uterosacral ligament (Figure 7.2a & 7.2c). In addition, there was rectal tethering in the midline (Figure 7.2b), which was considered to be the likely cause for her symptoms. Stripping of the peritoneum and partial transection of the left uterosacral ligament was required to excise the first nodule. Following that the para-rectal space was opened bilaterally to allow excision of the vaginal nodule.

She had an uneventful post-operative course and was reviewed again three months after surgery. At that review, she reported complete resolution of the majority of her symptoms although her dysmenorrhoea persisted.

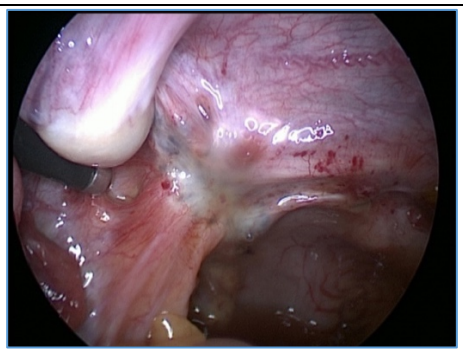
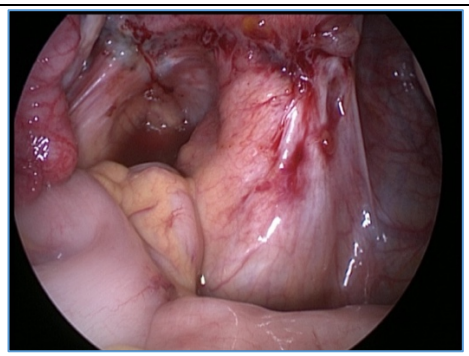
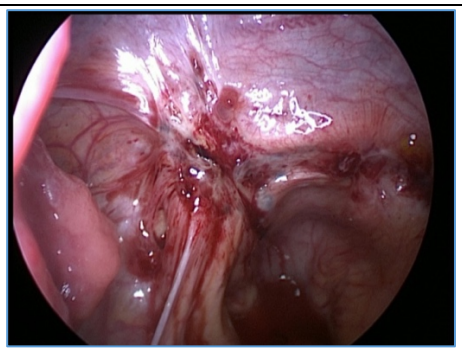
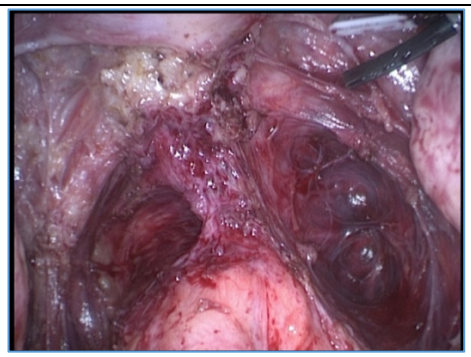
Left uterosacral ligament (a)	
	Left uterosacral disease clear of ureter on left Dense and active disease seen overlying extensive portion of left uterosacral ligament.
Pouch of Douglas (b)	
	Rectal tethering in midline at site of nodule.
Left uterosacral ligament with rectovaginal nodule (c)	
	Midline nodule palpable laparoscopically.
Para rectal dissection revealing nodule (d)	
	Peritoneum opened and stripped from uterosacral ligament on left in two separate portions with a partial transection of left uterosacral ligament. Peritoneal stripping continued over midline to excise peritoneum from right uterosacral ligament. Para-rectal space opened bilaterally and rectovaginal disease separated from rectum to allow excision of vaginal nodule in mid line.

Figure 7.2 CHUTE 02 surgical images

Annotated photographs demonstrating the surgical excision undertaken for CHUTE 02 (Extract from the surgical data record- shown in appendix 27).

7.2.4 ASRM classification

The revised American Society for Reproductive Medicine classification of endometriosis was used to quantify the disease seen at both the diagnostic laparoscopy and the subsequent surgical management of each subject. These data are shown in Table 7.4.

At the diagnostic procedure (baseline) the mean score was 20 and median stage was 2 (range 1-4). Whereas at the surgical procedure for the treatment of endometriosis the mean score had increased to 29 and the median stage had increased to 3 (range 2-4), over a median interval of 12 months (range 7-62).

There were three cases where the ASRM score was lower at the time of the surgical management with an interval between surgical procedures of 20 to 60 months. However, despite this reduced score they each required surgical management for their mild (score 7), moderate (score 28) and severe (score 56) disease. Eight subjects had only a minimal increase in ASRM score (score range 7-48, score difference 0-9, interval 7-33 month) and five had a more moderate increase (score range 16-38, score difference 12-26, interval 7-58 months) with only a single subject having a severe increase in ASRM from 34 to 129 (score difference = 95) over 7 months.

	Diagnostic Laparoscopy		Surgical Management		Score Difference	Interval *(Months)*
	Score	***Stage***	***Score***	***Stage***		
CHUTE 01	6	2	11	2	+5	15
CHUTE 02	6	2	7	2	+1	12
CHUTE 04	10	2	7	2	-3	53
CHUTE 05	46	4	48	4	+2	7
CHUTE 06	22	3	24	3	+2	10
CHUTE 08	76	4	56	4	-20	60
CHUTE 09	26	3	38	3	+12	7
CHUTE 10	6	2	32	3	+26	12
CHUTE 11	38	3	28	3	-10	20
CHUTE 12	17	3	34	3	+17	11
CHUTE 13	UK	2	16	3	-	44
CHUTE 14	10	2	10	2	0	8
CHUTE 15	34	3	129	4	+95	7
CHUTE 19	20	3	36	3	+16	58
CHUTE 21	4	1	16	3	+12	62
CHUTE 23	6	2	9	2	+3	33
CHUTE 24	10	2	19	3	+9	8
CHUTE 25	10	2	10	2	0	7
Mean	20	-	29	-	+10	24
Median	-	2	-	3	-	12
Range	-	1-4	-	2-4		7-62

Table 7.4 ASRM staging data

The ASRM scores and staging at both the diagnostic procedure and the subsequent surgical management. The Score difference is the calculated difference in scores between the two surgical procedures. The interval, in months, between the two surgeries is shown in the final column. (UK = unknown, - = data not applicable)

7.2.5 Biopsy data

A total of 111 different surgical specimens were obtained from the abdomen and pelvis as part of excisional surgery for the study cohort. The anatomical locations these specimens were obtained from are shown in Table 7.5. The disease was not confined to any particular anatomical compartment and even disease in more complex sites (rectal/pararectal) was excised to achieve maximum symptom relief for the patient.

Anatomical Site	**Frequency (n=111)**
Rectal/pararectal disease	22
Left ovary disease/adhesions	12
Left uterosacral	11
Left pelvic sidewall peritoneum	10
Uterovesical fold	10
Right uterosacral	10
Right pelvic sidewall peritoneum	8
Right ovary disease/adhesions	7
Left tubal disease/adhesions	6
Other (POD, uterus, adhesions)	13

Table 7.5 Anatomical location of disease

The anatomical site of each deposit of endometriosis excised from the CHUTE cohort. The frequency with which an excisional biopsy was obtained at each location is shown. A total of 111 excisional biopsies were obtained, all of which underwent H&E staining prior to review.

Although, all due care was taken to minimise tissue damage during the excision of the specimens, patient safety and the needs of surgery were the first priority. As such some specimens were distorted by the surgical process. However, the majority of specimen distortion was the result of the disease process. In view of this all specimens were viewed following H&E staining to determine the most appropriate sample to perform the immunohistochemistry staining on. This created a subset of 18 'biopsies', one for each study subject, which were used for the IHC analysis. The anatomical location of each of those representative biopsies is shown in Figure 7.3 and Table 7.6.

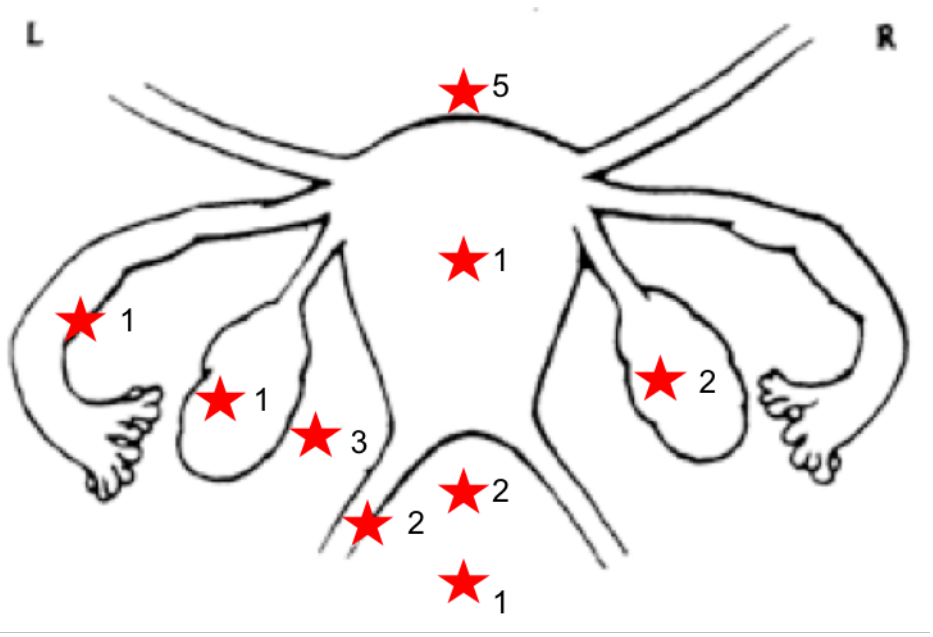


Figure 7.3 Anatomical location of biopsies

Graphic representation of the anatomical site of each biopsy used for IHC analysis within the CHUTE cohort (Red star = biopsy site, associated number = frequency, n = 18).

Subject	Anatomical Site
CHUTE 01	Peritoneum overlying left pelvic sidewall
CHUTE 02	Upper left uterosacral ligament, medial to left ureter
CHUTE 04	Peritoneum overlying right uterovesical fold
CHUTE 05	Peritoneum overlying bladder- right
CHUTE 06	Posterior uterine surface biopsy
CHUTE 08	Left fallopian tube and ovary
CHUTE 09	Uterovesical peritoneum
CHUTE 10	Left pelvic side wall
CHUTE 11	Left ovary
CHUTE 12	Right uterosacral endometriotic cyst
CHUTE 13	Left uterovesical fold peritoneum
CHUTE 14	Peritoneum from right side of uterovesical fold
CHUTE 15	Right ovarian tissue
CHUTE 19	Left pelvic side wall peritoneum and left uterosacral ligament
CHUTE 21	Left uterosacral ligament peritoneum
CHUTE 23	Pouch of Douglas peritoneum
CHUTE 24	Right para-rectal peritoneum
CHUTE 25	Left pelvic side wall & Pouch of Douglas peritoneum

Table 7.6 Anatomical location of biopsies

The anatomical site of each biopsy used for IHC analysis within the CHUTE cohort is shown.

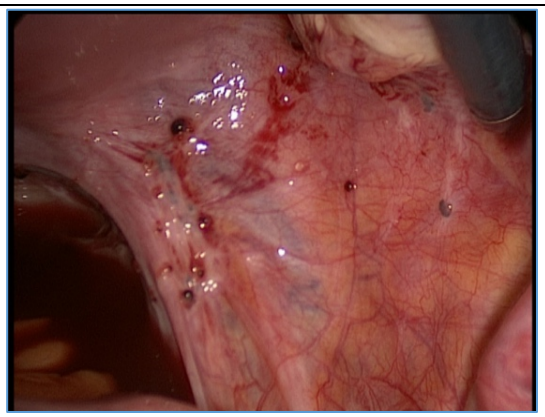
7.2.6 Surgical observations

Widespread & active endometriosis

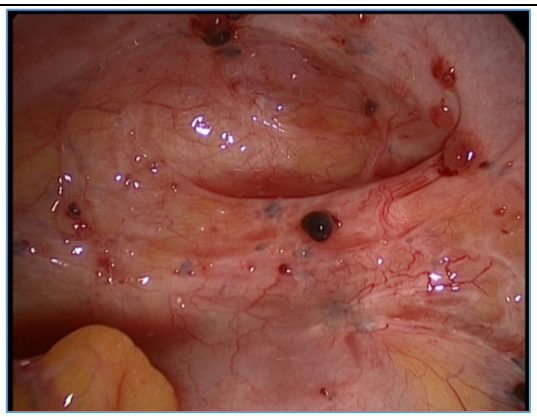
An active and "angry" appearance of the peritoneal endometriosis was noted in four subjects. The appearance was thought to be suggestive of recently developed endometriosis and so, given the recent course of ulipristal acetate, was photographed prior to excision. CHUTE 05 had a stable ASRM score varying by 2 points (46 to 48) over the 7 months between operations. At surgery multiple, red and black lesions were noted over the peritoneum of the right pelvic side wall and ovarian fossa (Figure 7.4 images a & b). Some of these lesions had a 'stuck on' appearance suggesting they had recently developed.

A similar but more florid appearance was seen over the surface of the uterus and in the Pouch of Douglas for CHUTE 06 (Figure 7.4 image c) and CHUTE 11 (Figure 7.4 image d). There were multiple clear and red deposits forming a confluent band of endometriosis extending down from the posterior surface of the uterus to the uterosacral complex. Both of these subjects had a similar ASRM score- 24 and 28 respectively; with a two-point increase in score (22 to 24) over an interval of 10 months for CHUTE 06 and a 10-point decrease in score (38 to 28) over an interval of 20 months for CHUTE 11.

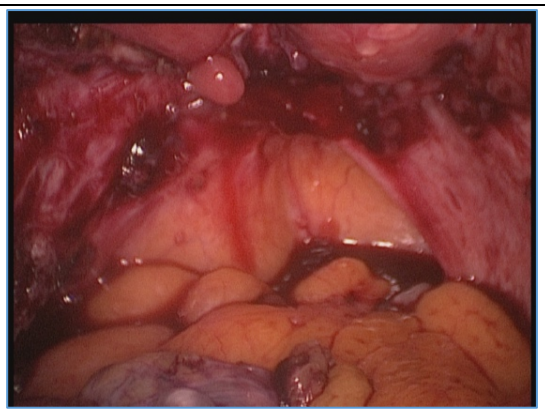
The final patient showing these features was CHUTE 25 with an ASRM score of 10 that remained unchanged over the 7 months between procedures. This subject's disease appeared to be a combination of active red deposits overlying a background of deeper more well established endometriotic deposits (Figure 7.4 images e & f). There was associated white scar tissue overlying both uterosacral ligaments and peritoneal pocketing within the Pouch of Douglas.



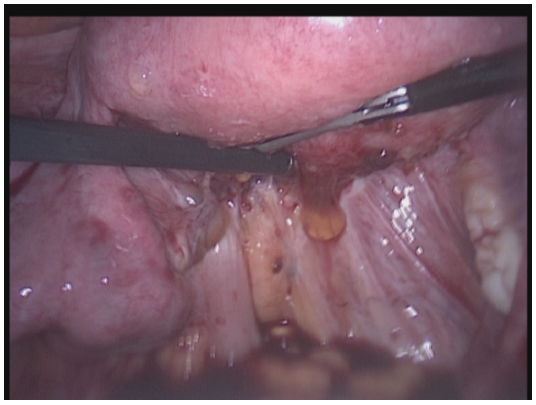
a. CHUTE 05 right pelvic sidewall and ovarian fossa



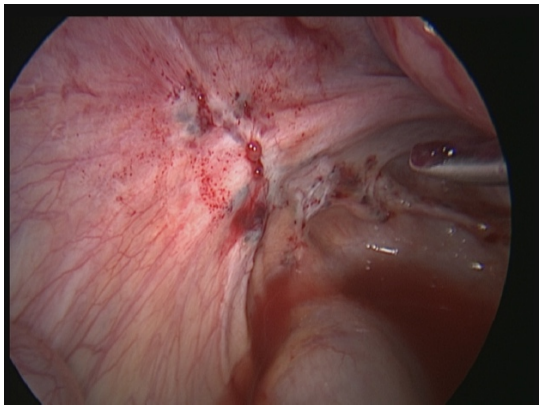
b. CHUTE 05 Pouch of Douglas



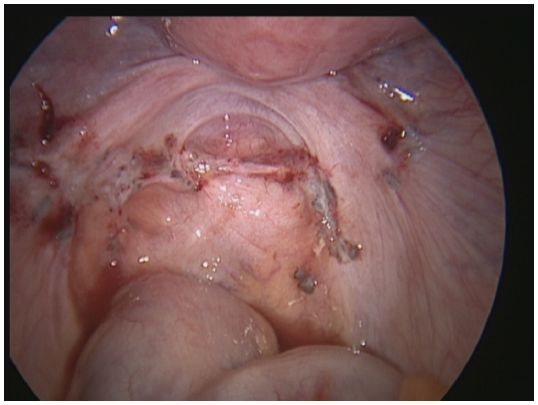
c. CHUTE 06 Pouch of Douglas



d. CHUTE 11 Pouch of Douglas



e. CHUTE 25 left pelvic sidewall and uterosacral ligament



f. CHUTE 25 Pouch of Douglas

Figure 7.4 Active endometriosis following ulipristal acetate treatment

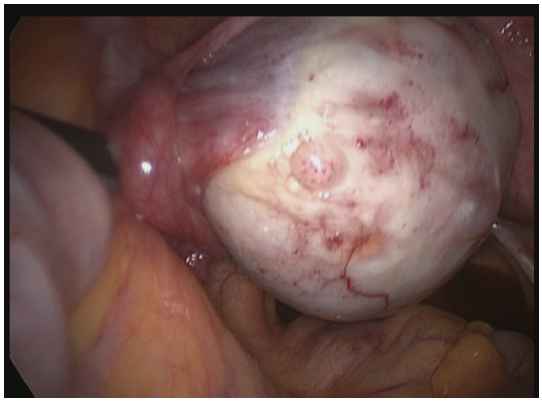
Surgical photographs for CHUTE 05, 06, 11 & 25 demonstrating the highly active appearance of the peritoneal endometriosis seen in these four study subjects. The exact anatomical location is described with each image.

Endometriomas

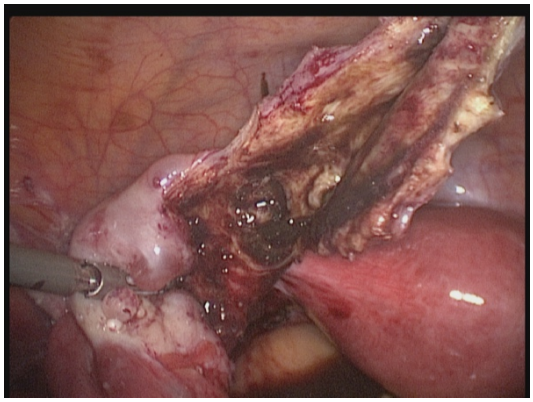
Three subjects had endometriomas, which were managed by total or partial ovarian cystectomy, dependent on the condition of the tissues, disease related adhesions, and patient wishes. Subjects CHUTE 09 and CHUTE 19 had quite similar disease with 6cm & 3cm left ovarian endometriomas respectively (Figure 7.5 images a & f). For CHUTE 09 a partial ovarian cystectomy was performed (Figure 7.5 image b) and the remaining portion of the pseudo capsule was heat treated with monopolar diathermy. In addition, uterovesical deposits and a rectovaginal nodule were excised. Similarly, for CHUTE 19 an ovarian cystectomy was performed as well as excision of a dense endometriotic nodule within the left pelvic sidewall and uterovesical disease. Each of these subjects had moderate to severe endometriosis with a ASRM score of 38 (score difference 12) for CHUTE 9 and 36 (score difference 16) for CHUTE 19; although the time interval was much greater for CHUTE 19 (58 months) compared to CHUTE 09 (7 months).

However, CHUTE 15 had a much greater change in the ASRM score over only 7 months. The score difference was 95 (34 to 129) resulting in bilateral kissing ovaries obliterating the Pouch of Douglas (Figure 7.5 image c & Figure 7.6 image c). The right ovary was enlarged to 8cm with two endometrioma containing chocolate material (Figure 7.5 image d). There were multiple peritoneal pockets around the right ovary containing clear and endometriotic fluid with filmy adhesions forming pockets over posterior surface of uterus containing altered blood or peritoneal fluid. This appearance was similar to the active endometriosis seen in other CHUTE subjects and described above. A smaller (3cm) endometrioma was also seen within the left ovary and this was adherent to the left pelvic sidewall (Figure 7.5 image e).

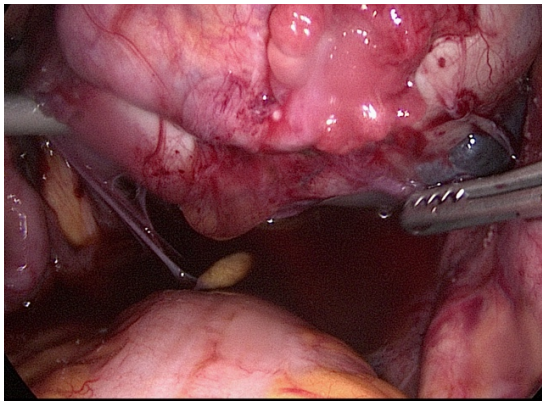
Surgical management included extensive adhesiolysis to normalise anatomy as much as possible. Bilateral ovarian cystectomies were performed, and the widespread active endometriosis excised where possible and treated with monopolar diathermy where excision was not feasible.



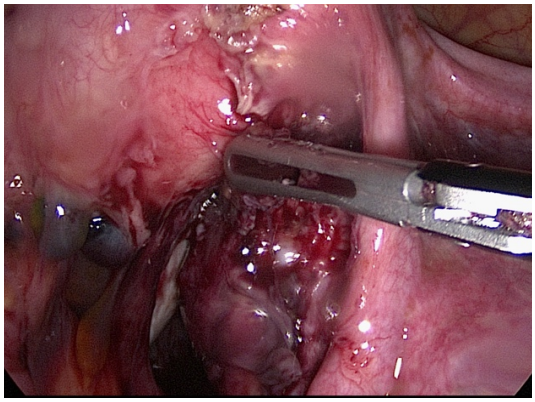
a. CHUTE 09 endometrioma (6cm) within left ovary and adherent to pelvic side wall



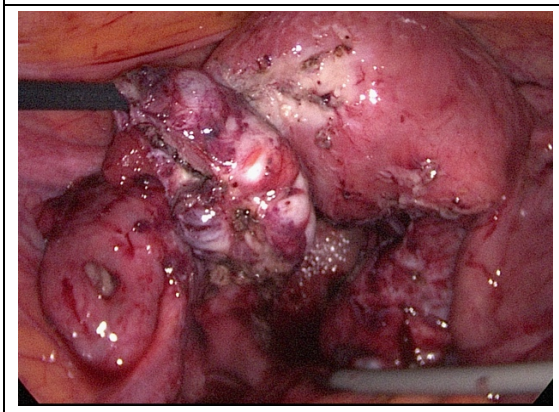
b. CHUTE 09 partial ovarian cystectomy with monopolar diathermy to ovary



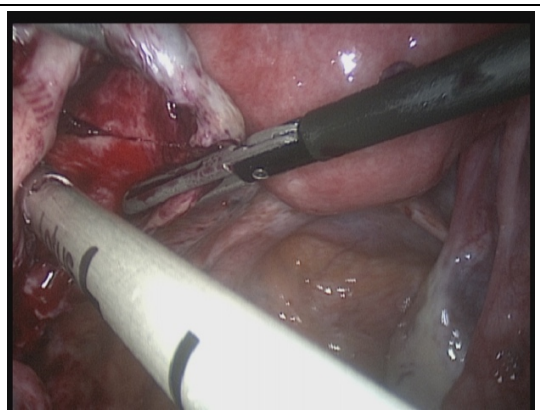
c. CHUTE 15 bilateral kissing ovaries obliterating Pouch of Douglas



d. CHUTE 15 two endometrioma within right ovary enlarged to 8cm diameter



e. CHUTE 15 endometrioma (3cm) within left ovary and adherent to pelvic side wall



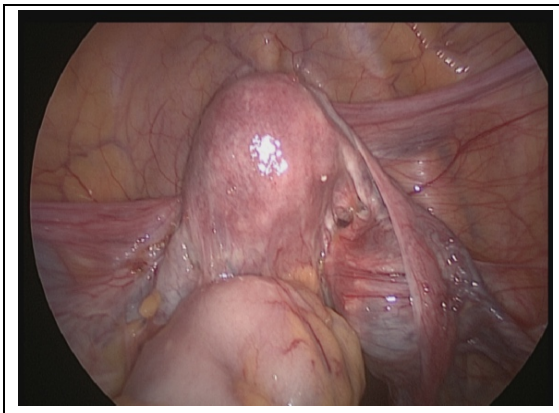
f. CHUTE 19 endometrioma (2-3cm) within left ovary and adherent to pelvic side wall

Figure 7.5 Endometriomas

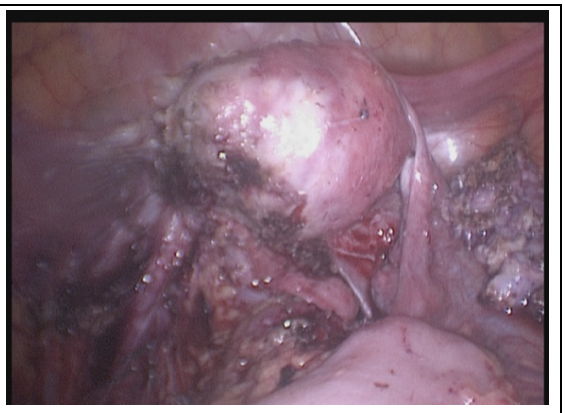
Surgical photographs for CHUTE 09, 15 & 19 demonstrating the endometriomas that were managed surgically with total or partial ovarian cystectomy and monopolar diathermy.

Dense adhesions & frozen pelvis

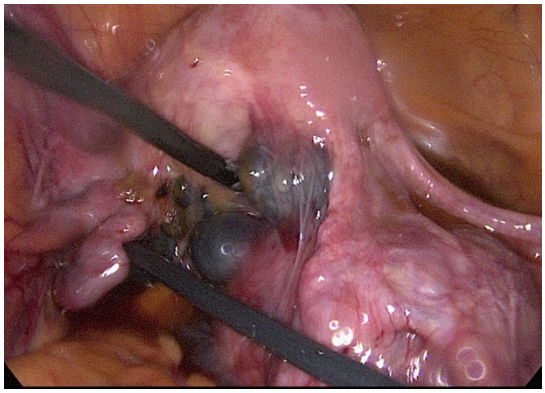
Two subjects, CHUTE 08 and CHUTE 15, had severe disease causing significant anatomical distortion and a frozen pelvis (Figure 7.6). Although the ASRM score for CHUTE 08 was lower (56 points) than the score for CHUTE 15 the excision of disease was equally challenging for each subject.



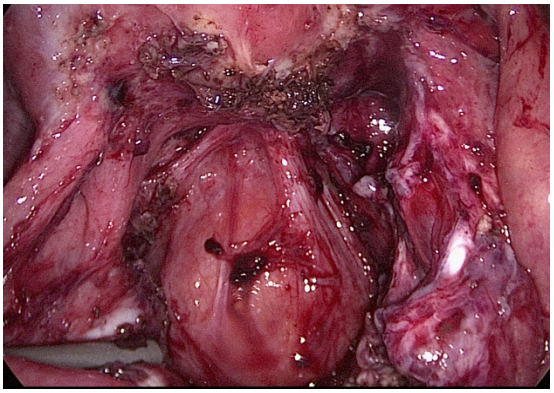
a. CHUTE 08 left ovary and tube buried within dense adhesions with proximal sigmoid colon adherent to ovary & the posterior surface of the uterus, and the distal sigmoid colon partially adherent to the pelvic sidewall



b. CHUTE 08 post excision



c. CHUTE 15 extensive filmy and dense inflammatory adhesions secondary to endometriosis with bilateral endometriomas obliterating the Pouch of Douglas



d. CHUTE 15 post excision

Figure 7.6 Severe disease with 'Frozen Pelvis'

Surgical photographs for CHUTE 08 & 15 demonstrating the more severe spectrum of disease managed within the CHUTE cohort.

7.3 HISTOLOGY DATA

7.3.1 Histopathology reporting

All 18 subjects had an endometrial biopsy obtained, processed and reported at screening. These biopsies were obtained between day 7-12 and revealed healthy endometrium with mid to late proliferative features in keeping with the menstrual cycle day.

At surgery, a single eutopic sample and multiple ectopic samples were obtained for each subject. These were processed and reported as previously described, using the 21 domains to describe the architecture and features of the epithelial and stromal cells. Through the process of reviewing the histopathology slides and evaluating the presence of any PAEC type changes it became clear that six features were key to that assessment: cystic dilatation of glands, cuboidal epithelial cells, ciliated metaplasia, infrequent epithelial mitoses and cytoplasmic vacuolation of epithelial cells as well as stromal vascular changes. The data described below focus on these important features in both the eutopic and ectopic samples.

It should be noted that all the ectopic samples were viewed and considered when producing the official histopathology report and any conclusions for an individual subject. However, for the purposes of CHUTE reporting with respect to the 21 domains described in the Histopathology Plan (Appendix 24) and the immunohistochemistry the single highest quality sample was selected for each subject.

7.3.2 PAEC

The features of PAEC were assessed in both the eutopic and ectopic samples to establish whether similar patterns were seen in both tissue types. The conclusion by both histopathologists was that no subject in the study exhibited enough PAEC features in the eutopic endometrium to confirm the diagnosis.

The data presented in Table 7.7 show the features reported by each of the histopathologists and the similar data from the PEARL I and II as a comparison. Cystic dilatation was identified in 6-22% of the samples with minimal ciliated metaplasia (6%) and infrequent (11%) stromal vascular changes. The CHUTE subjects appeared to have quite inactive endometrium with infrequent mitoses being identified in a high proportion (61-78%).

When using the PEARL I & II data as a comparison it should be noted that the eutopic samples in these studies were obtained whilst the subjects were taking ulipristal acetate, whereas the samples obtained in this study were six weeks after subjects had stopped taking ulipristal acetate. Hence the reduced frequency of PAEC features may be the result of a different response in the CHUTE subjects or a consequence of features resolving and endometrium normalising.

Features	**PAEC**	**Eutopic Endometrium**		**PEARL Data (Week 13)**	
		RL (n=18)	**AW** (n=18)	**I** (n=282)	**II** (n=288)
Cystic Dilatation	Present	4 (22%)	1 (6%)	105 (45%)	96 (38%)
Cell type	Cuboidal	0 (0%)	6 (33%)	-	-
Ciliated metaplasia	Present	1 (6%)	1 (6%)	-	-
Infrequent Mitoses	Present	14 (78%)	11 (61%)	80 (34%)	74 (29%)
Cytoplasmic vacuolation	Common	4 (22%)	4 (22%)	162 (70%)	161 (63%)
Stromal vascular changes	Present	2 (11%)	0 (0%)	107 (46%)	87 (34%)

Table 7.7 PAEC features within eutopic endometrium

The histological features and the expected findings(203) *seen in PAEC are shown in the first two columns. The frequency and percentage as determined by two independent histopathologists is shown and the PAEC data from the PEARL I and PEARL II studies are shown for comparison.*(197,198) *(RL= Dr Ray Lonsdale, AW= Professor Alistair Williams)*

However, despite this delay of six weeks there were still quite significant histological changes seen in the ectopic samples. The comparison between eutopic and ectopic samples is shown in Table 7.8, highlighting high levels of cystic dilatation within ectopic endometrial glands - 83-89%, with ciliated metaplasia seen in 56% of samples. The ectopic glands within the endometriosis were inactive with cuboidal epithelial cells (33-89%) and infrequent mitoses (89-94%). There were no examples of stromal vascular changes identified but the volume of stromal tissue with each sample of endometriosis was small making assessment of vascular patterns difficult.

Features	PAEC	Eutopic Endometrium				Ectopic Endometrium			
		RL (n=18)	**AW** (n=18)	**Kappa**	**Overall agreement**	**RL** (n=18)	**AW** (n=18)	**Kappa**	**Overall agreement**
Cystic Dilatation	Present	4 (22%)	1 (6%)	0.34	0.83**	15 (83%)	16 (89%)	0.17	0.44
Cell type	Cuboidal	0 (0%)	6 (33%)	0	0.67	6 (33%)	16 (89%)	0.12	0.44
Ciliated metaplasia	Present	1 (6%)	1 (6%)	1	1**	10 (56%)	-	-	-
Infrequent Mitoses	Present	14 (78%)	11 (61%)	0.53	0.78*	17 (94%)	16 (89%)	0.34	0.83**
Cytoplasmic vacuolation	Common	4 (22%)	4 (22%)	0.49	0.78*	0 (0%)	1 (0%)	-0.03	0.89**
Stromal vascular changes	Present	2 (11%)	0 (0%)	0	0.89**	0 (0%)	0 (0%)	1	1**

Table 7.8 PAEC features comparing eutopic and ectopic endometrium

The histological features and the expected findings(203) *seen in PAEC are shown in the first two columns. The frequency and percentage as determined by two independent histopathologists are shown for both the eutopic and ectopic endometrial samples from the CHUTE cohort. An assessment of the inter-observer agreement for each histological feature is shown as both a Kappa coefficient and the overall level of agreement. The Kappa statistic is not reliable for this data, a problem called 'High Agreement But Low Kappa' and so the more simple level of agreement is also shown.*(225–227) *(RL= Dr Ray Lonsdale, AW= Professor Alistair Williams, **= strong agreement, *= moderate agreement))*

7.3.3 Eutopic endometrial features

Cystic dilatation

Although cystic dilatation was not seen commonly within the eutopic samples there were some examples. Dr R. Lonsdale considered CHUTE 08, 12, 13 & 14 to have evidence of glandular dilatation but Professor A. Williams felt that CHUTE 08 was the only clear example. The changes noted by Dr R. Lonsdale for subjects 12 &13 were considered to be subtle and possibly in keeping with natural variation. The focal cystic dilatation and associated ciliated cell metaplasia of CHUTE 08 is shown in Figure 7.7.a. In addition, muscularised arterioles and ectatic vessels were also noted (Figure 7.7.b), which together were considered to be consistent with PAEC but not diagnostic.

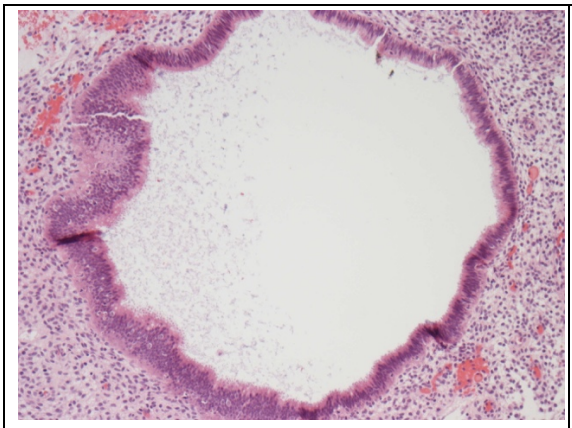
Metaplastic changes

Subject CHUTE 14 had unusual focal changes within the eutopic endometrium, which were noted by both pathologists (Figure 7.8.a). This subject had Grade II endometriosis with an ASRM score of 10. Her disease was mostly concentrated over the right uterosacral ligament with a small nodule of disease over the right side of the uterovesical fold. She had only a poor response to the study drug and her eutopic endometrium showed mildly dilated glands lined by cuboidal epithelium and a hobnail pattern protruding into the lumen. In parts the epithelial cells showed micropapillation but there was no evidence of cellular atypia and this was not considered to be a neoplastic process.

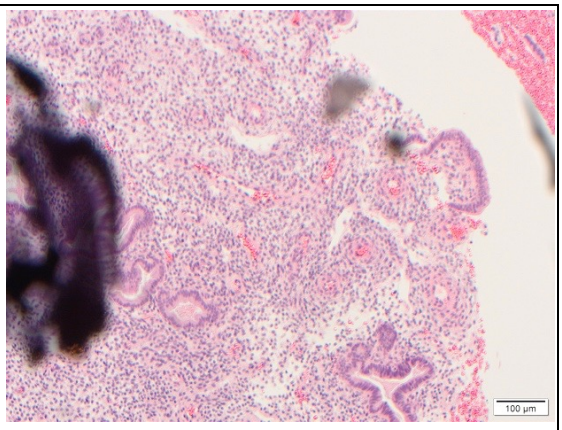
Vascular changes

In addition to the focal metaplastic changes for CHUTE 14 there were also stromal vascular changes with some ectatic stromal vessels seen (Figure 7.8.b). These were different to the perivascular eosinophilia and increased muscularisation seen round the vessels for CHUTE 21 (Figure 7.8.d). This was on a background of normal mid-to-late secretory phase endometrium (Figure 7.8.c).

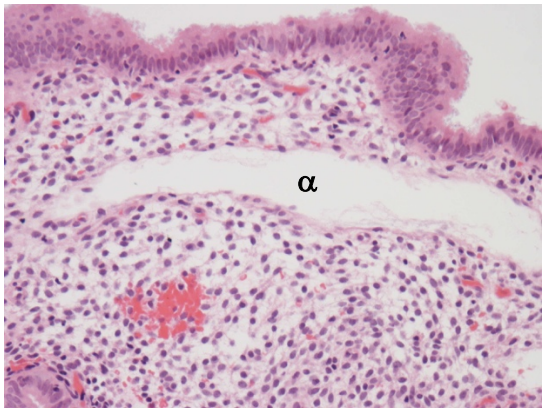
It should be noted that neither the metaplastic changes or the vascular changes described for these eutopic samples of these subjects were mirrored within the ectopic samples. Conversely, cystic dilatation is seen within the ectopic samples but at much higher frequency suggesting that the eutopic and ectopic endometrium are behaving differently. These are discussed in more detail below.



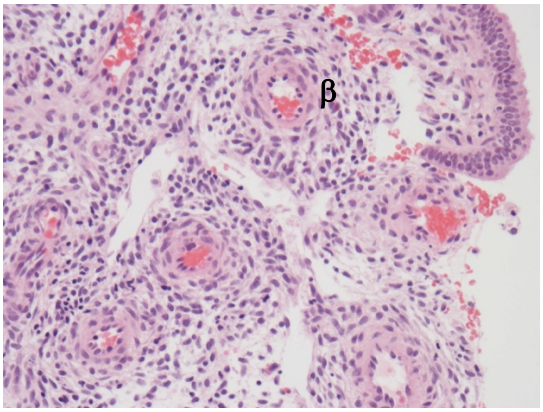
a. CHUTE 08: 'There are focal areas of cystic dilatation and within those dilated glands there is ciliated cell metaplasia and pseudo-stratification of the nuclei'



b. CHUTE 08: 'Muscularised arterioles and ectatic vessels within the stroma (consistent with PAEC but not diagnostic)'



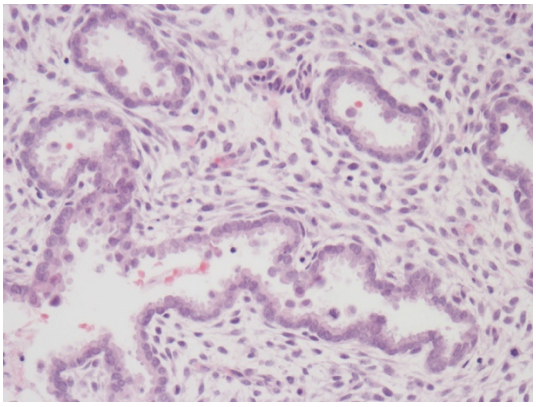
c. CHUTE 08: ectatic vessels (α)



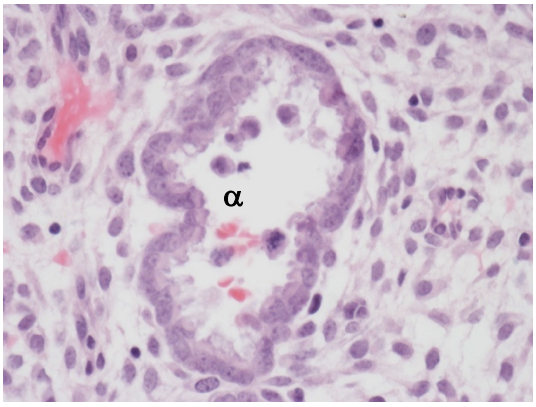
d. CHUTE 08: muscularised arterioles (β)

Figure 7.7 PAEC like features within eutopic endometrium

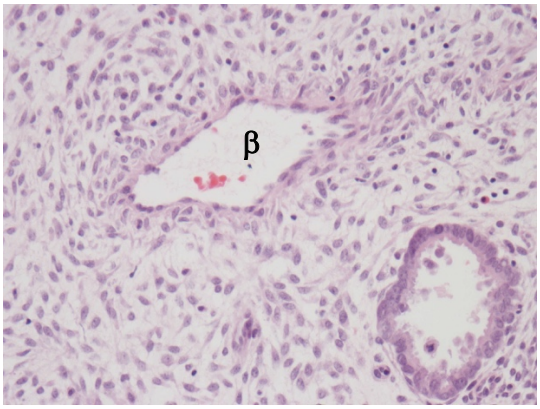
Histological features consistent with PAEC that were noted in eutopic endometrial specimens for CHUTE 08, as described. (H&E x 100 magnification- a & b; H&E x 200 magnification- c & d))



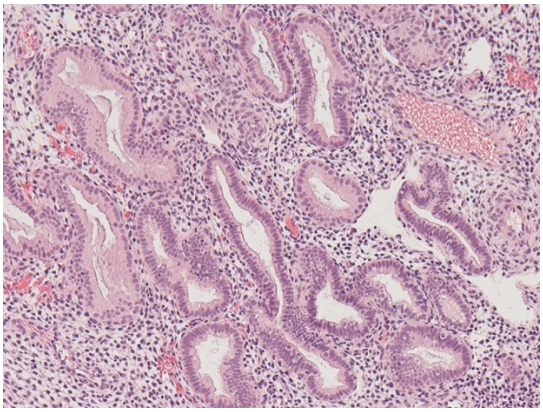
a. CHUTE 14: 'Some glands are dilated and others are lined by cuboidal epithelium with a hobnail pattern protruding into the lumen'



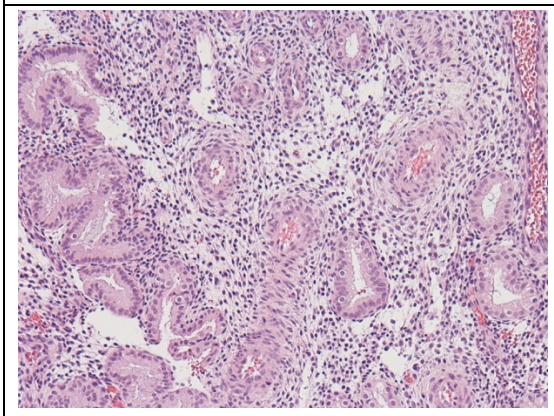
b. CHUTE 14: hobnail pattern protruding into the lumen of gland with surface micropapillation (α)



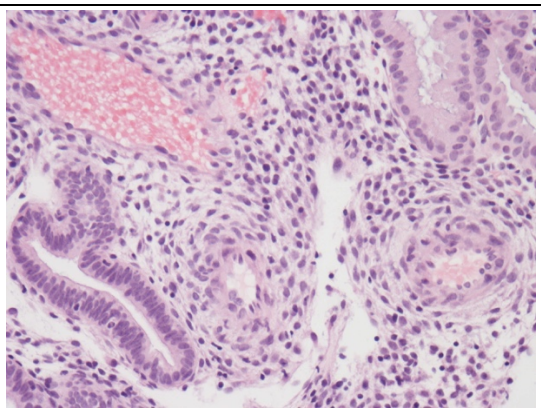
c. CHUTE 14: Ectatic stromal vessels (β)



d. CHUTE 21: 'Mid-to-late secretory phase endometrium with some stromal oedema consistent with phase'



e. CHUTE 21: 'Vascular changes… consistent with PAEC'



f. CHUTE 21: Perivascular eosinophilia & increased muscularisation of the vessels

Figure 7.8 Metaplastic and vascular changes within eutopic endometrium

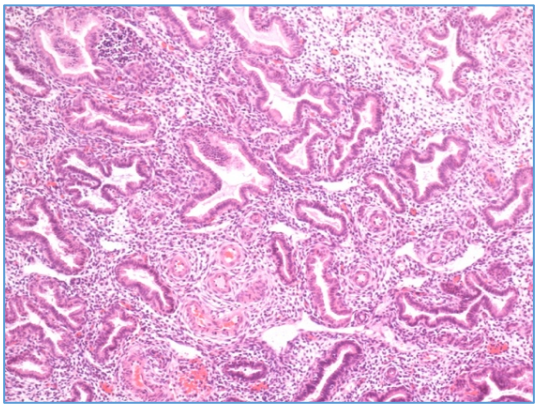
Histological features, consistent with PAEC, which were noted in eutopic endometrial specimens for CHUTE 14 & 21. (H&E Magnification x 200 magnification for a, c & f; x400 for b; x100 for d & e))

7.3.4 Ectopic endometrial features

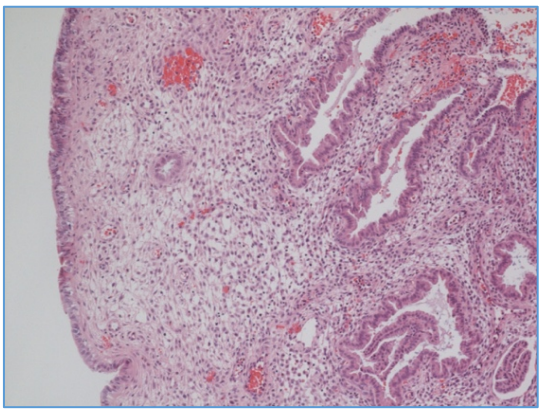
The three different surgical appearances of endometriosis have been described in Section 7.2.6. The following examples demonstrate the corresponding histological findings in those subjects.

Active endometriosis

CHUTE 06 had a florid and active appearance (Figure 7.4.c) at surgery raising concern as to whether the study drug had adversely influenced clinical progress. The eutopic endometrium for this subject was unremarkable (Figure 7.9.a) and the ectopic sample from the posterior surface of the uterus showed no atypical or PAEC features but was noted to demonstrate very well formed endometrial structures (Figure 7.9.b). Histologically the glandular structure of the endometriosis was much more similar to eutopic endometrium than would be expected. There was cystic glandular dilatation seen within the specimen, but the epithelial cells were columnar with no ciliated metaplasia. There were no stromal vascular changes.



a. CHUTE 06 (eutopic): 'Late secretory phase endometrium (Day 27)'



b. CHUTE 06 (ectopic): 'Very well formed endometrial structures with no atypical features '

Figure 7.9 Comparison of eutopic and ectopic endometrium for CHUTE 06

Histological examples for CHUTE 06 with ectopic sample from florid and active looking disease from the Pouch of Douglas. (H&E x 100 magnification)

A similarly active appearance to the disease was seen for CHUTE 25, albeit with more associated scarring (Figure 7.4.e & f). The eutopic and ectopic samples were obtained on day 4 of the cycle and show changes consistent with menstruation. There was no

cystic dilatation or atypical changes noted for the eutopic sample (Figure 7.10.a) but cystic dilation was seen widely in the ectopic sample (Figure 7.10.b). It is of note that the ectopic sample showed evidence of menstrual breakdown histologically.

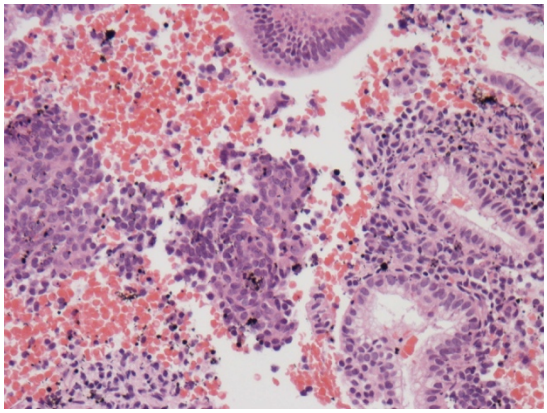
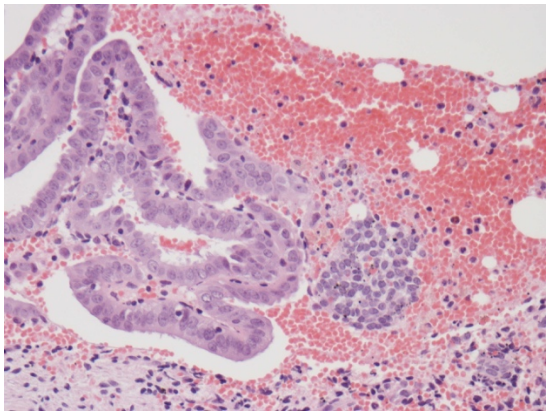
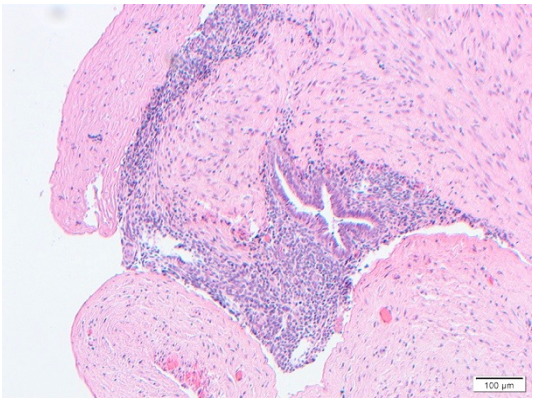
	
a. CHUTE 25 (eutopic): 'epithelial cells have supranuclear vacuoles that have not released their secretion, which is more in keeping with mid secretory phase, but they have begun to breakdown and appear menstrual (Day 4)'	b. CHUTE 25 (ectopic): 'stromal balling & breakdown (suggestive of menstrual breakdown)- in phase with eutopic sample '

Figure 7.10 Comparison of eutopic and ectopic endometrium for CHUTE 25

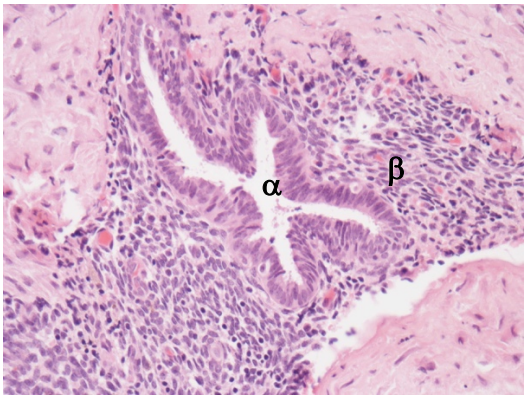
Histological examples for CHUTE 25 with ectopic sample from florid and active looking disease over the left pelvic sidewall and uterosacral ligament. (H&E x 200 magnification- a; x100 magnification- b)

Endometriomas & frozen pelvis

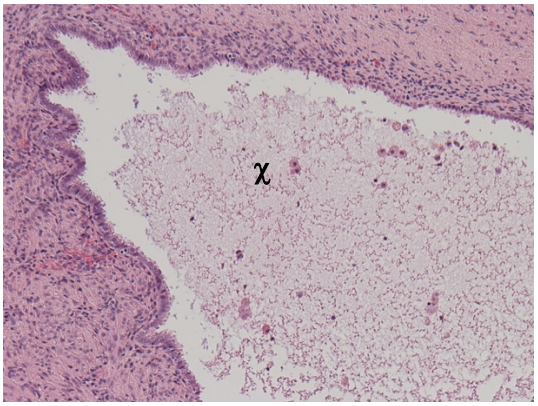
The subjects with more advanced disease with large endometriomas (CHUTE 15, Figure 7.11.a) and associated scarring (CHUTE 08, Figure 7.11.b) appear to have quite similar appearances histologically. There is widespread cystic dilation with evidence of ciliated metaplasia and an inactive epithelium. Although there appears to be a clinical difference at surgery across the CHUTE subjects and different clinical response to study drug there does not appear to be large differences histologically.



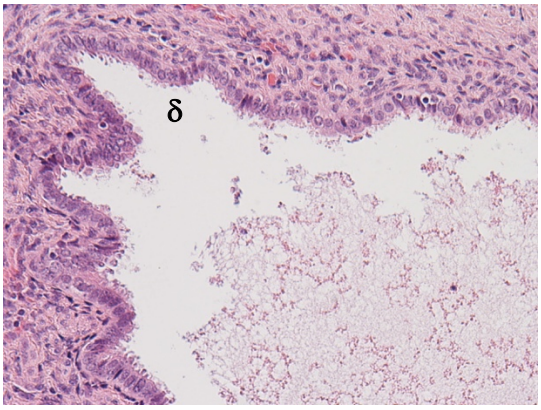
a. CHUTE 15 (ectopic): 'endometriosis is confirmed with no atypical features'



b. CHUTE 15 (ectopic): endometrial gland (α) and surrounding stroma (β) embedded within dense fibrous tissue (light pink)



c. CHUTE 08 (ectopic): 'ciliated cell metaplasia within a dilated gland (χ)'



d. CHUTE 08 (ectopic): ciliated cell metaplasia on cuboidal epithelial cells (δ)

Figure 7.11 Ectopic endometrium in advanced disease

Histological examples for CHUTE 15 & CHUTE 08 with ectopic sample from florid and active looking disease. (H&E x 100 magnification- a & c; H&E x200 magnification- b & d)

7.3.5 Cystic dilatation

Cystic dilatation within ectopic endometrium has been assessed as part of investigating PAEC, as discussed above. Whether dilatation within the ectopic glands is a feature of endometriosis or the result of exposure to study drug is unclear but it does seem to correlate with clinical response. The data shown in Table 7.9 gives the number of biopsies obtained for each subject, how many biopsies confirmed endometriosis histologically and how many of those demonstrated cystic dilatation with or without an inactive epithelium.

There appears to be a correlation between the proportion of endometriosis samples showing cystic dilatation and the clinical response observed. There is no significant difference in the cystic dilatation frequency for responders (N=10, mean= 0.66, SD=0.22) and non-responders (N=8, mean= 0.44, SD= 0.33); t(16)= 1.74, p= 0.10 (Independent Samples T-Test). However, there is a trend towards significance, which may be clinically relevant.

7.3.6 Histological features and ulipristal acetate

Although there are histological features consistent with PAEC no sample of eutopic or ectopic endometrium exhibited enough features to be consistent with that diagnosis. However, within the ectopic samples cystic dilatation and ciliated metaplasia was more consistently demonstrated. Despite this, there does not appear to be a correlation between these features and the severity or surgical appearance of the disease. There may be a weak correlation between the presence of cystic dilatation in the ectopic endometrium and clinical response to ulipristal acetate; this may be a tissue response to the drug or natural variation within the histology of endometriosis that makes the disease more susceptible to progesterone receptor modulation.

	Clinical Response	Number of biopsies	Endometriosis Confirmed	Cystic dilatation +/- inactive epithelium	Proportion
CHUTE 01	Good	3	3	3	1.00
CHUTE 02	Good	5	4	4	1.00
CHUTE 04	Poor	3	3	2	0.67
CHUTE 05	Good	6	6	4	0.67
CHUTE 06	Good	5	4	2	0.50
CHUTE 08	Poor	3	2	1	0.50
CHUTE 09	Poor	4	4	1	0.25
CHUTE 10	Poor	6	6	4	0.67
CHUTE 11	Poor	4	2	0	0.00
CHUTE 12	Poor	6	6	1	0.17
CHUTE 13	Good	6	6	3	0.50
CHUTE 14	Poor	4	4	1	0.25
CHUTE 15	Good	6	5	2	0.40
CHUTE 19	Good	4	4	3	0.75
CHUTE 21	Good	4	3	2	0.67
CHUTE 23	Good	5	4	3	0.75
CHUTE 24	Good	5	5	2	0.40
CHUTE 25	Poor	5	4	4	1.00

Table 7.9 Cystic dilatation in ectopic endometrium

The frequency of histologically confirmed endometriosis within the biopsies obtained at surgery with the frequency of cystic dilatation and the proportion of endometriosis samples showing cystic dilatation. These data are also correlated to whether the subject had a good or poor clinical response to the study drug.

7.4 IMMUNOHISTOCHEMISTRY DATA

The immunohistochemistry (IHC) staining was performed on a batch basis throughout the study with the eutopic biopsy and a single ectopic sample for each subject being processed. After initial reporting of the H&E slides, Dr Lonsdale made an assessment of the highest quality ectopic sample for each CHUTE subject before the lab technicians ran the IHC staining, as per local protocol.

For each CHUTE subject the slide for each receptor or marker was assessed to produce an Allred score and describe any notable features. A representative portion of each slide was also photographed to facilitate comparison across the different tissues and IHC antibodies. No pattern of expression was observed based on clinical response to study drug, so the data are displayed and described according to the menstrual cycle day on which the specimen was obtained. The initial data set for CHUTE 25 is shown in Figure 7.12 as an example.

7.4.1 Oestrogen and progesterone receptor

The oestrogen receptor (ER) and progesterone receptor (PR) results for the epithelial cells and the stromal cells are shown according to menstrual cycle day to aid interpretation (see Figure 7.13 and Figure 7.14, respectively). However, the ER and PR staining was strong and largely uniform throughout the cohort, irrespective of cycle day. Interestingly, the ER and PR epithelial expression and ER stromal expression in the eutopic endometrium was slightly reduced for those samples obtained within the first three days of the cycle; and there may be slightly reduced PR expression in the ectopic stromal cells, but overall no clear pattern of expression was observed.

7.4.2 Androgen receptor

The androgen receptor (AR) does appear to show a pattern of expression in the epithelial cells of the eutopic endometrium dependent on the cycle day (see Figure 7.15). The Allred scores appear to be lower in these cells during the secretory phase of the cycle as compared to the proliferative phase. This pattern does not seem to exist for the ectopic endometrium, in which expression is more heterogeneous across the cohort.

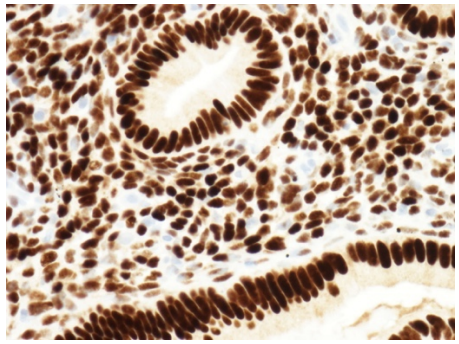
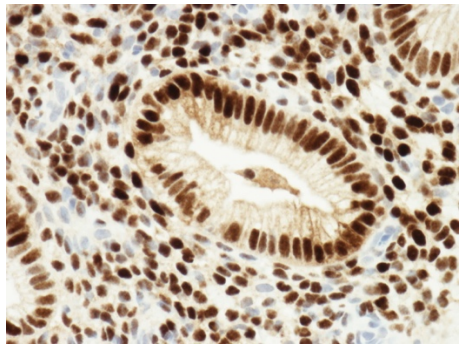
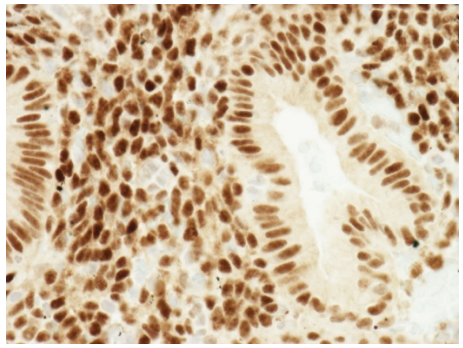
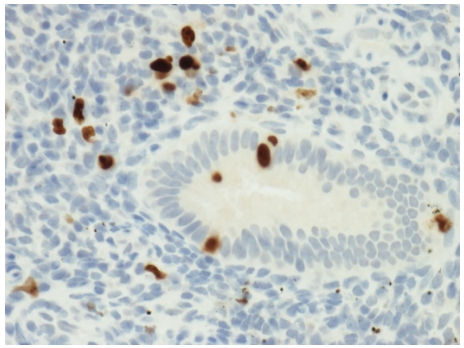
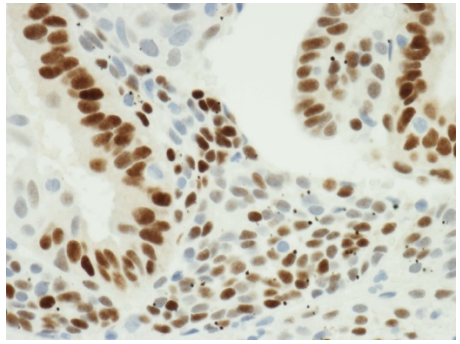
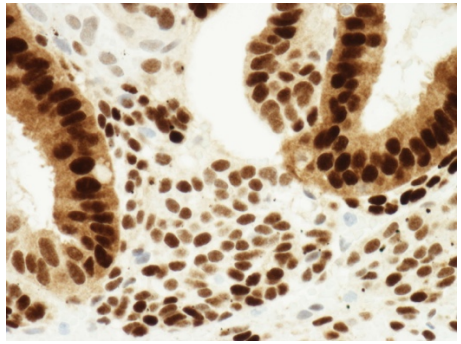
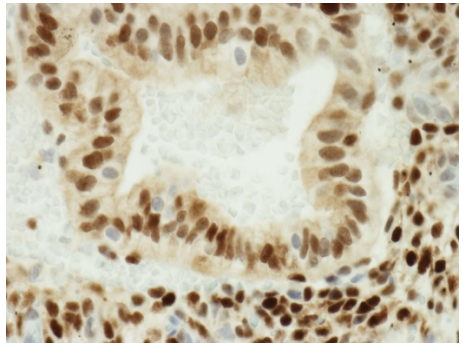
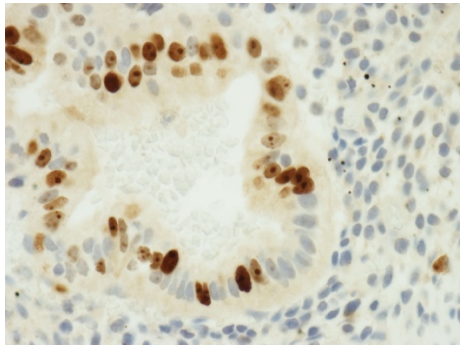
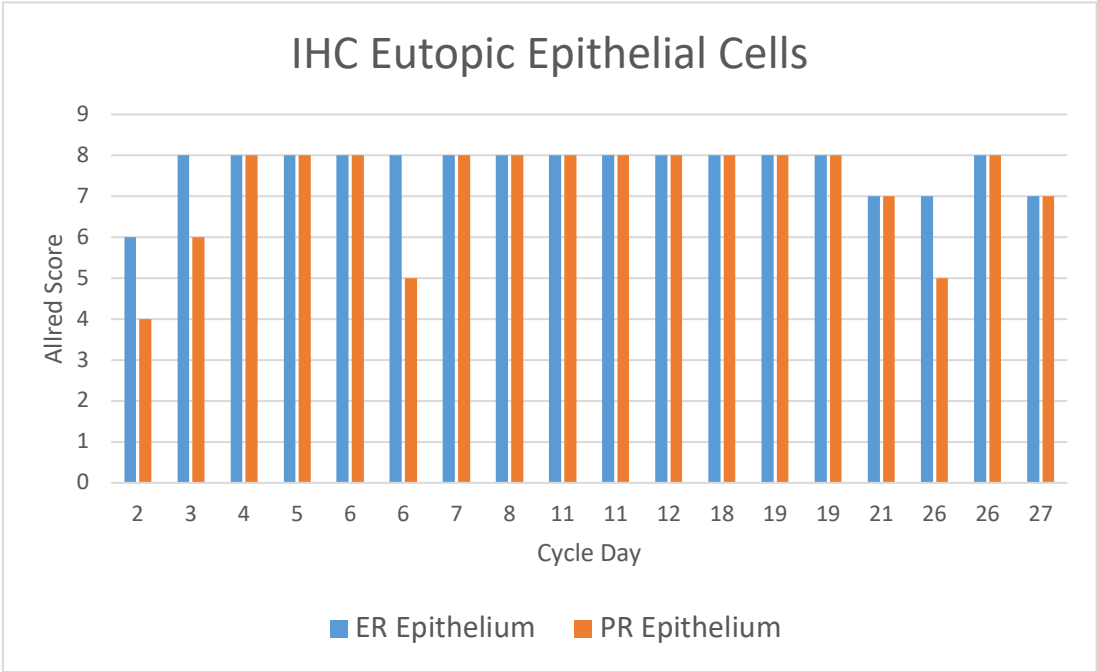
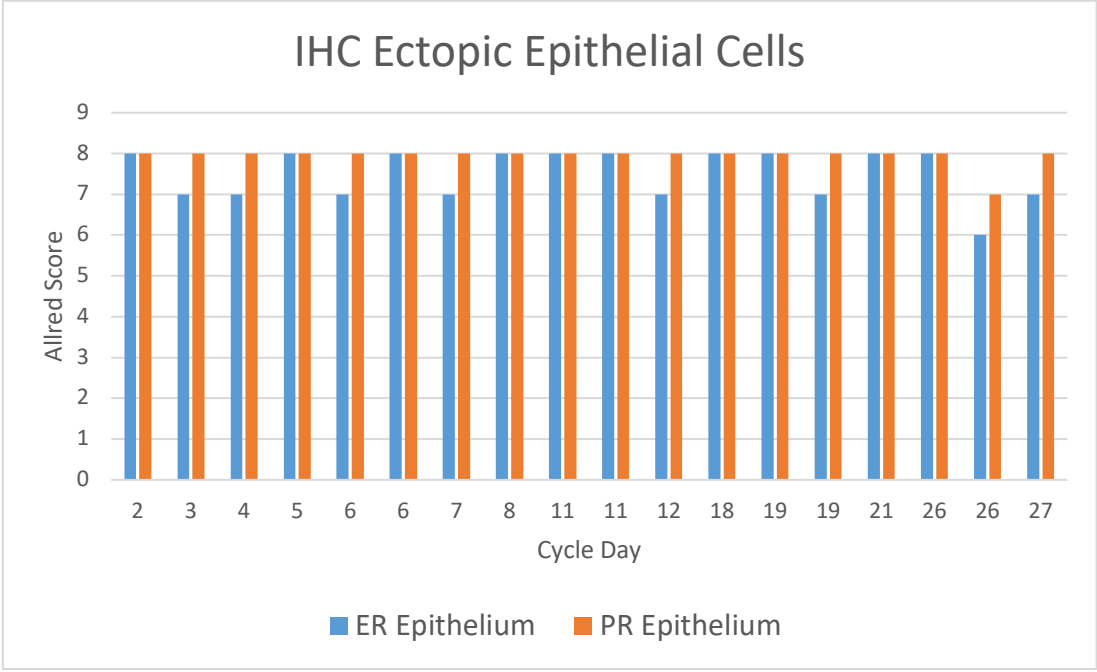
			
a. ER(Eu): epithelium= 8, stroma= 8	b. PR(Eu): epithelium= 8, stroma= 8	c. AR(Eu): epithelium= 7, stroma= 7	d. Ki67(Eu): epi. <1%, stroma <1%
			
e. ER(Ec): epithelium= 7, stroma= 7	f. PR(Ec): epithelium= 8, stroma= 8	g. AR(Ec): epithelium= 6, stroma= 7	h. Ki67(Ec): epi. =50%, stroma <1%

Figure 7.12 Immunohistochemistry results: CHUTE 25 (Day 4)

Results for eutopic (a-d) and ectopic (e-h) samples with Allred scores for epithelial cells (epi.) and stromal cells (strom.) are shown. The oestrogen receptor (ER) (a & e), the progesterone receptor (PR) (b & f), the androgen receptor (AR) (c & g), and Ki67 marker (d& h) panels are a x 400 magnification.



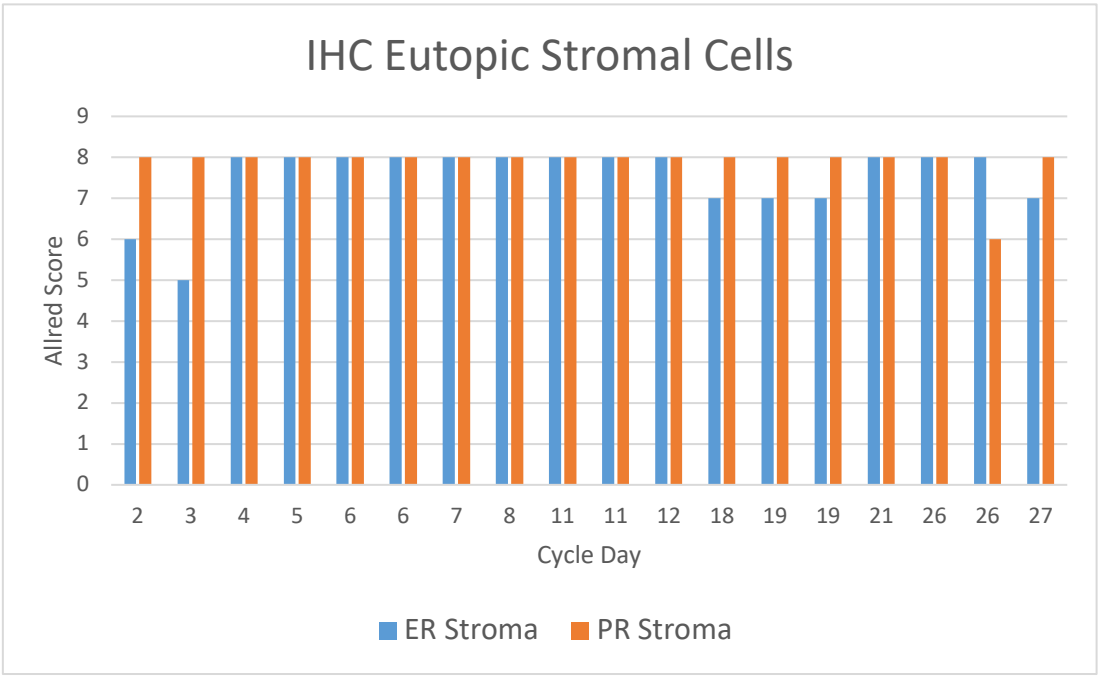
a. Oestrogen & progesterone receptor Allred scores for eutopic epithelial cells



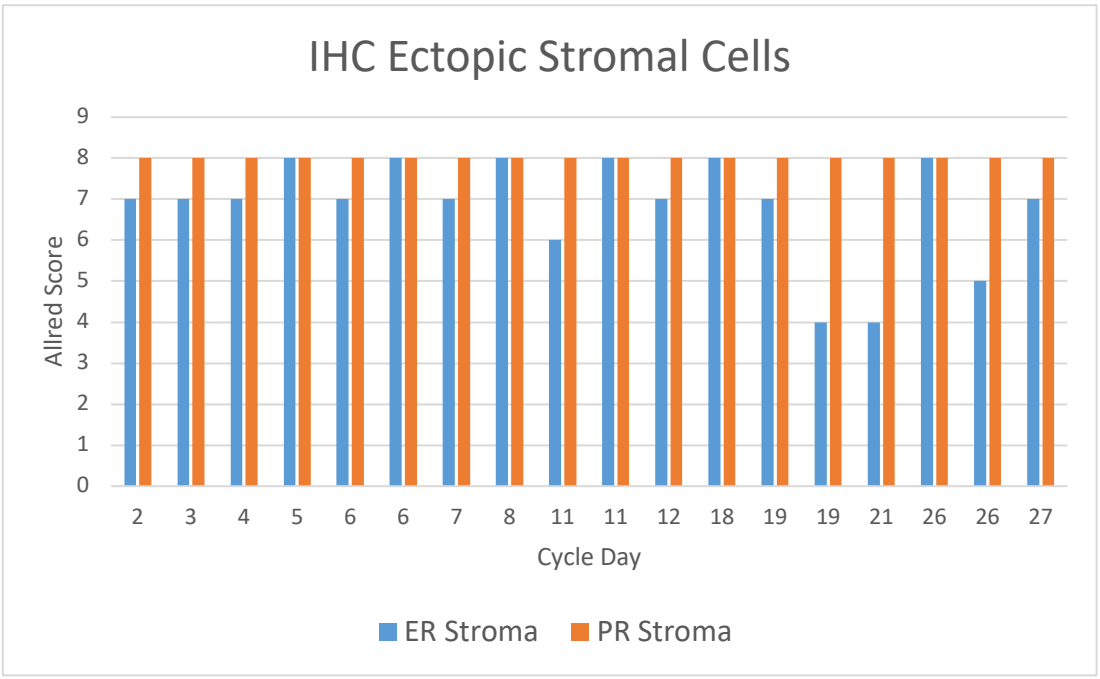
b. Oestrogen & progesterone receptor Allred scores for ectopic epithelial cells

Figure 7.13 Oestrogen and progesterone receptor immunohistochemistry (epithelial cells)

Immunohistochemistry scores for the CHUTE subjects are shown according to cycle day. Epithelial cells for eutopic (a) and ectopic (b) endometrial samples are shown for comparison (ER= oestrogen receptor, PR= progesterone receptor).



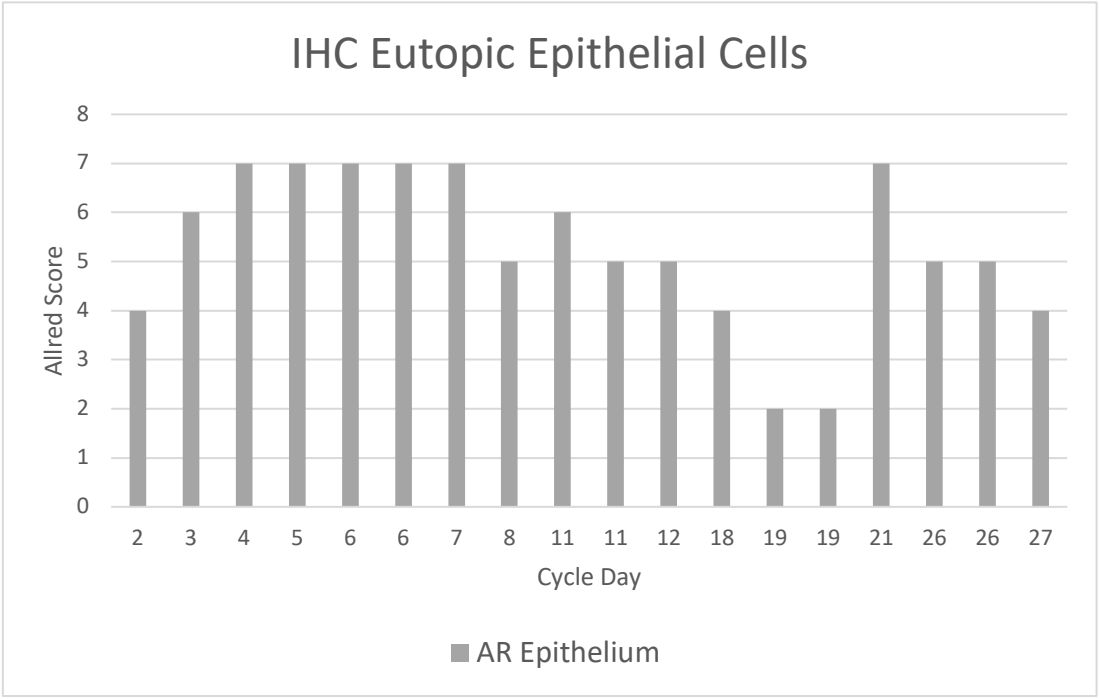
a. Oestrogen & progesterone receptor Allred scores for eutopic stromal cells



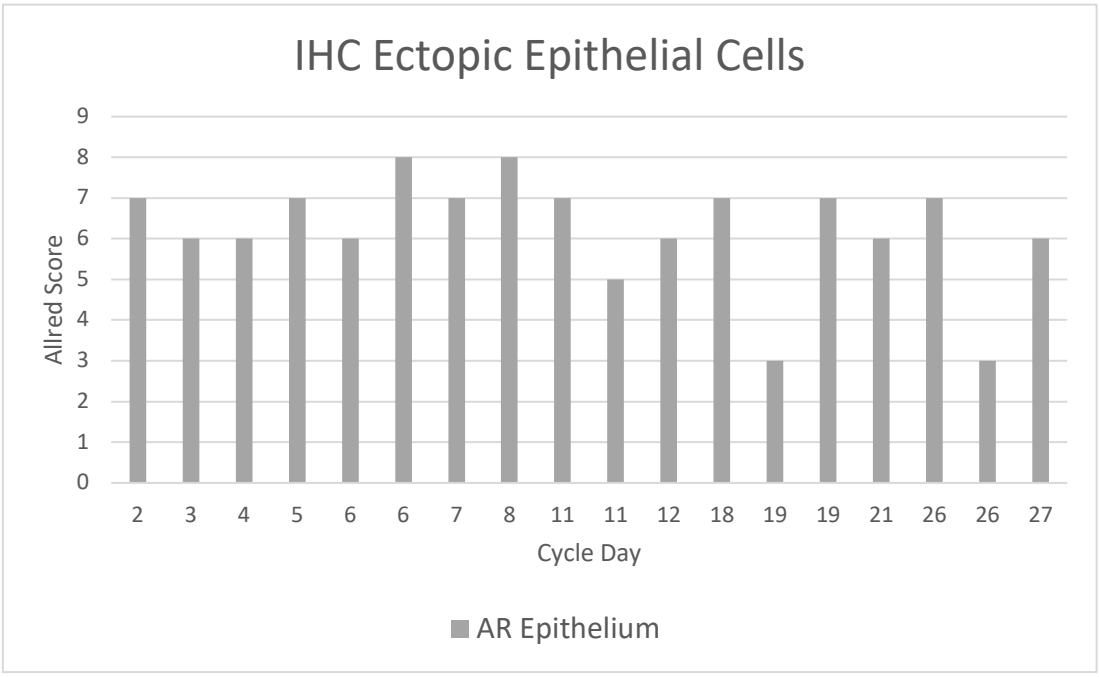
b. Oestrogen & progesterone receptor Allred scores for ectopic stromal cells

Figure 7.14 Oestrogen and progesterone receptor immunohistochemistry (stromal cells)

Immunohistochemistry scores for the CHUTE subjects are shown according to cycle day. Stromal cells for eutopic (a) and ectopic (b) endometrial samples are shown for comparison (ER= oestrogen receptor, PR= progesterone receptor).



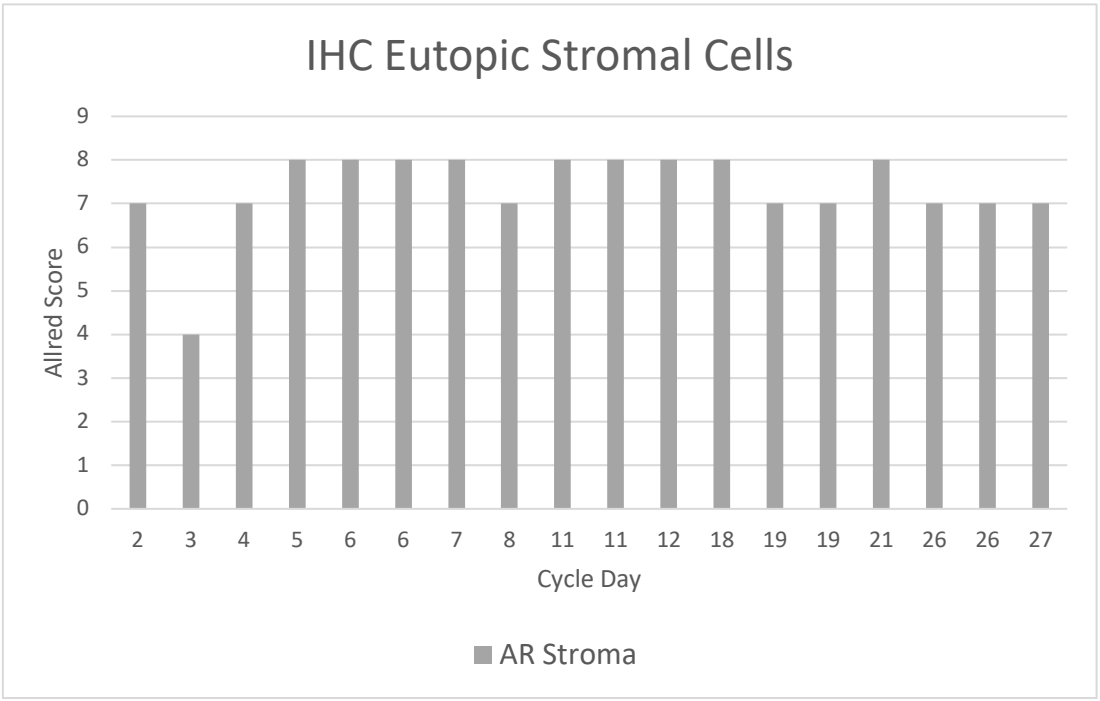
a. Androgen receptor Allred scores for eutopic epithelial cells



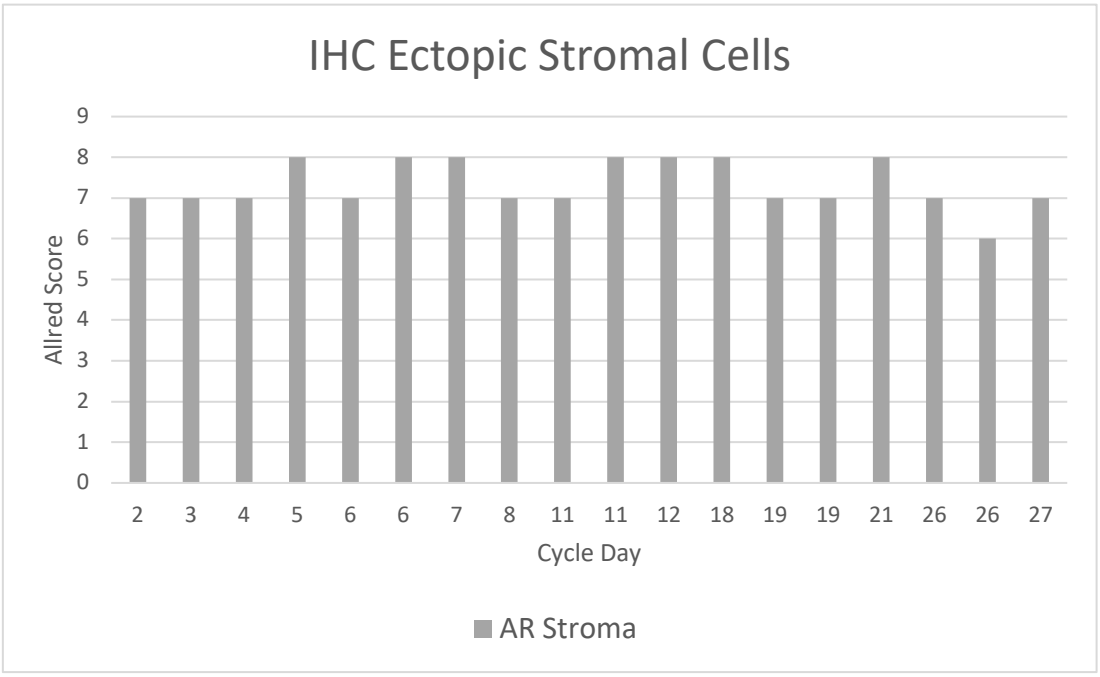
b. Androgen receptor Allred scores for ectopic epithelial cells

Figure 7.15 Androgen receptor immunohistochemistry (epithelial cells)

Immunohistochemistry scores for the CHUTE subjects are shown according to cycle day. Epithelial cells for eutopic (a) and ectopic (b) endometrial samples are shown for comparison (AR= androgen receptor).



a. Androgen receptor Allred scores for eutopic stromal cells



b. Androgen receptor Allred scores for ectopic stromal cells

Figure 7.16 Androgen receptor immunohistochemistry (stromal cells)

Immunohistochemistry scores for the CHUTE subjects are shown according to cycle day. Epithelial cells for eutopic (a) and ectopic (b) endometrial samples are shown for comparison (AR= androgen receptor).

Similarly, the expression of AR in both the eutopic and ectopic stromal cells does not appear to be cycle dependent. The results for the stromal cells are shown in Figure 7.16 and indicate uniform expression throughout the cycle in both tissue types.

7.4.3 Ki-67 proliferation marker

The proliferation marker Ki-67 demonstrates a cyclical pattern within the eutopic endometrium for both epithelial and stromal cells. For the stromal cells, there appears to be a peak in the mid proliferative phase, whereas the percentage expression for the epithelial cells appears to continue to rise until the mid-point of the cycle. These data are shown in Figure 7.17a.

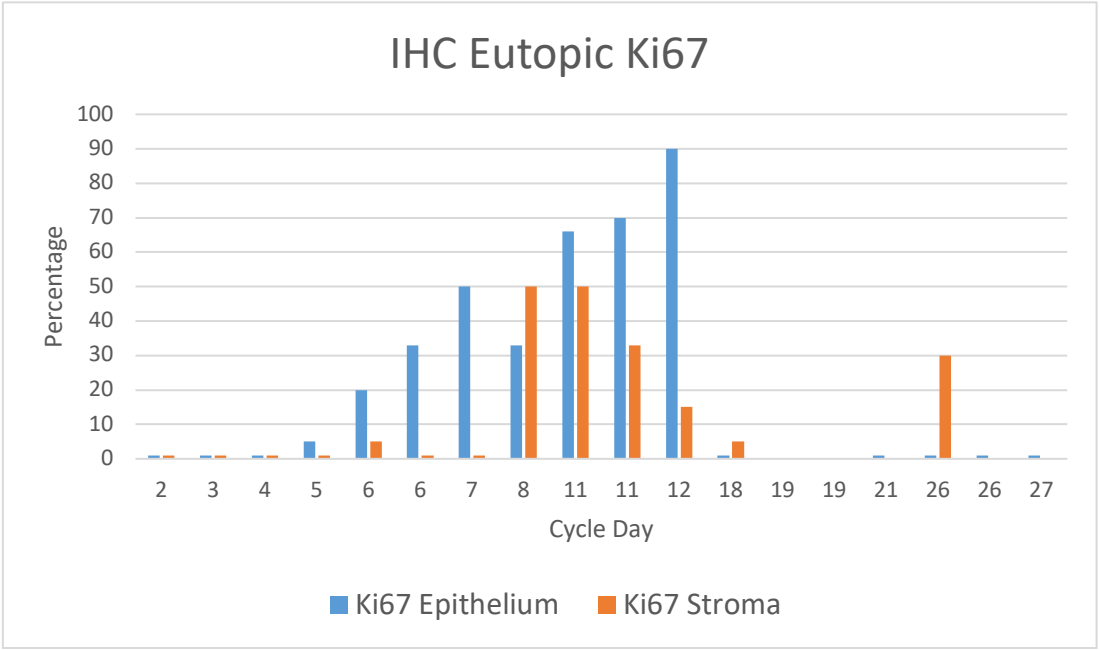
The ectopic cells show much less cyclicity (Figure 7.17b) with very little expression of Ki-67 within the stromal cells and variable expression within the epithelial cells of the cohort. Overall, there appears to be slightly less Ki-67 expression amongst the ectopic cells as compared to the eutopic cells.

7.4.4 Proliferative versus secretory phase observations

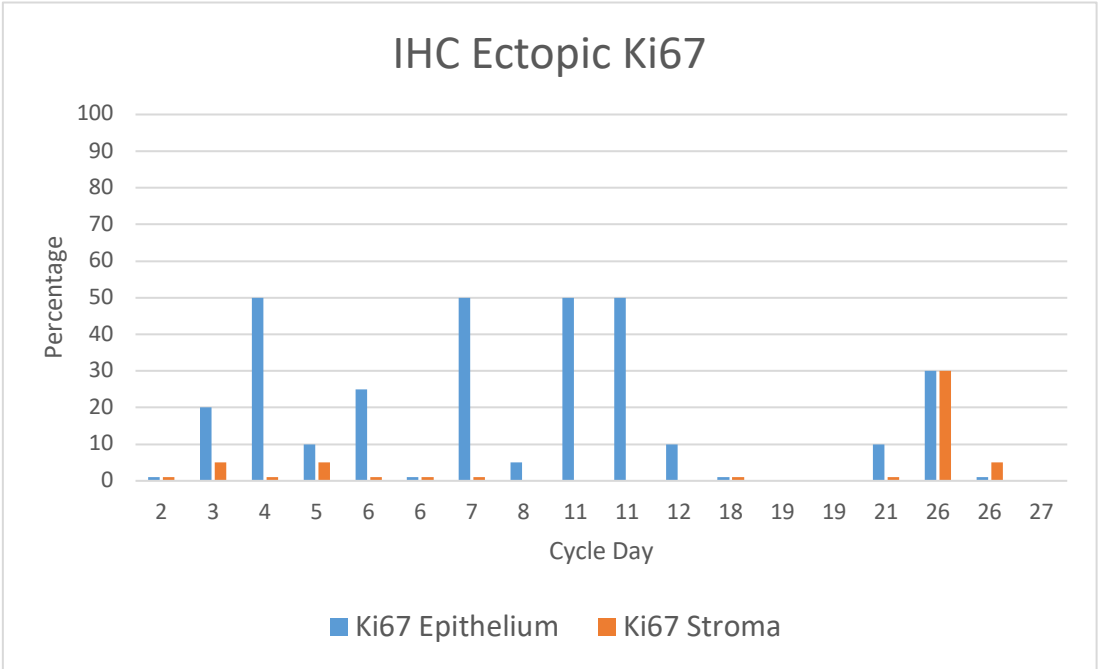
Proliferative phase

The subjects CHUTE 25 and CHUTE 14 had their samples obtained in the proliferative phase of the menstrual cycle, day four and day eight respectively. Clinically they had similar outcomes with a poor response to treatment. A comparison of their AR and Ki-67 IHC is shown in Figure 7.18.

In the eutopic samples AR expression in the stroma is consistent but expression in the epithelial cells is reduced in the later sample (CHUTE 14). Conversely, the expression of epithelial and stromal Ki-67 is greater in the later sample (CHUTE 14). In the ectopic samples stromal expression of AR and Ki-67 is consistent, whereas in the epithelial cells AR and Ki-67 expression is greater in the early proliferative sample of CHUTE 25.



a. Ki67 percentage staining for eutopic epithelial cells



b. Ki67 percentage staining for ectopic epithelial cells

Figure 7.17 Ki67 immunohistochemistry (epithelial & stromal cells)

Immunohistochemistry scores for the CHUTE subjects using the Ki67 proliferation marker are shown according to cycle day. Epithelial & stromal cells are shown together for comparison with eutopic and ectopic endometrial samples shown in a & b, respectively (ER= oestrogen receptor, PR= progesterone receptor).

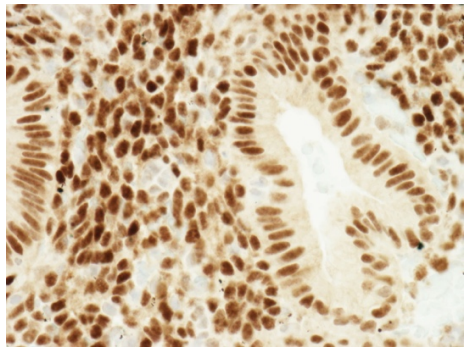
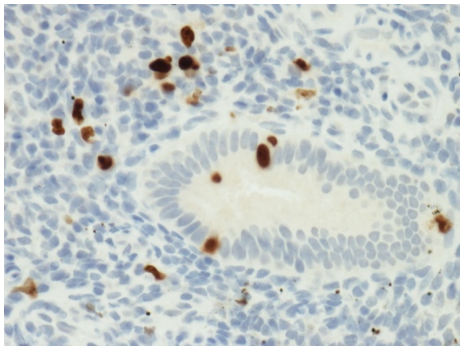
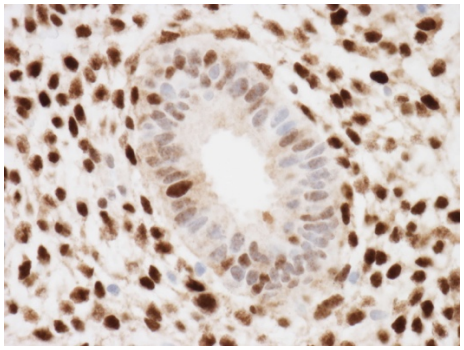
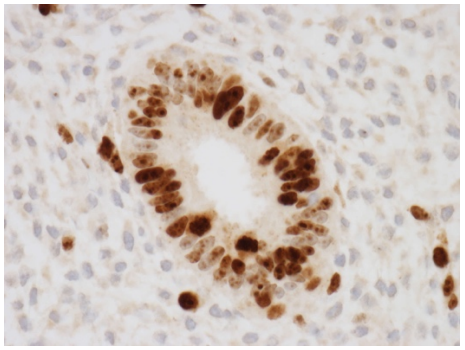
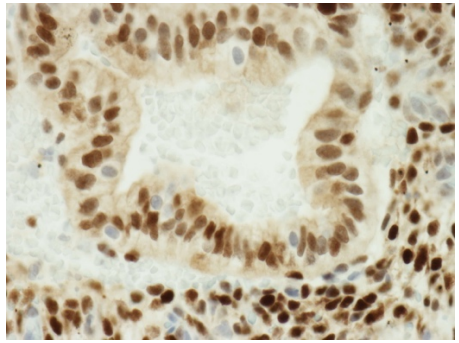
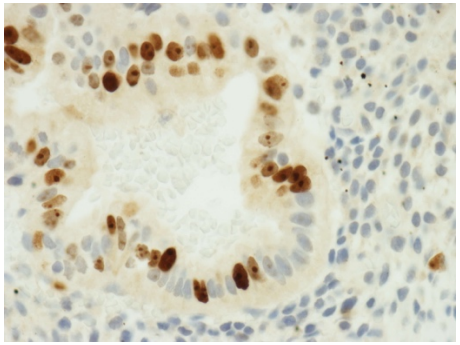
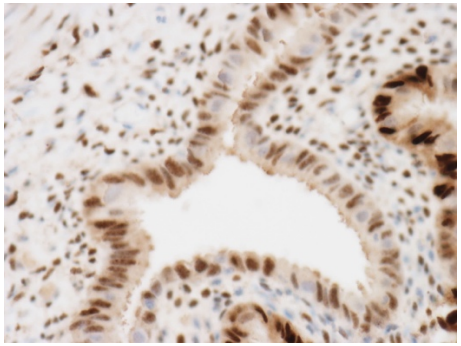
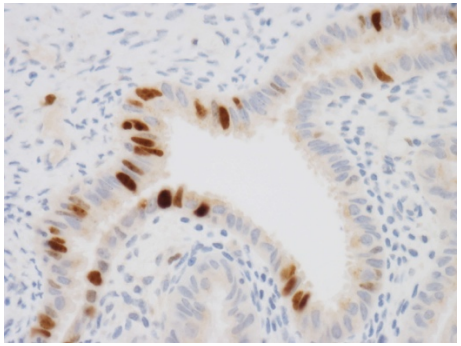
			
a. AR(Eu): epithelium=7, stroma=7	b. Ki67(Eu): epi. 1%, stroma 1%	c. AR(Eu): epithelium=5, stroma=7	d. Ki67(Eu): epi. 33%, stroma 50%
			
e. AR(Ec): epithelium= 6, stroma= 7	f. Ki67(Ec): epi. 50%, stroma 1%	g. AR(Ec): epithelium= 8, stroma= 7	h. Ki67(Ec): epi. 5%, stroma 1%

Figure 7.18 Immunohistochemistry results: CHUTE 25 (Day 4) & CHUTE 14 (Day 8)- proliferative phase

The androgen receptor (AR) (a, c, e & g) and Ki67 marker (b, d, f & h) results for CHUTE 25 (a, b, e & f) and CHUTE 14 (c, d, g & h) are shown. Results for eutopic (a-d) and ectopic (e-h) samples with Allred scores for epithelial cells (epi.) and stromal cells (stroma) are shown (x 400 magnification).

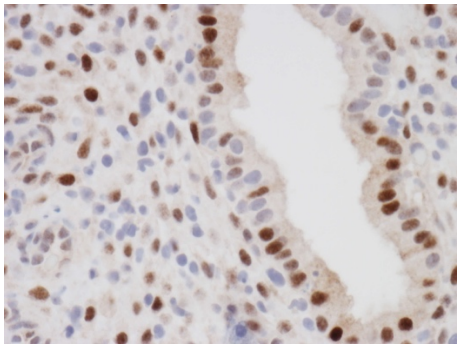
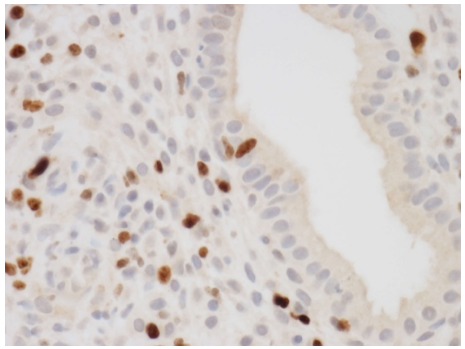
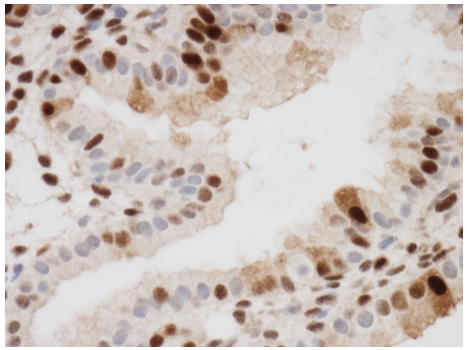
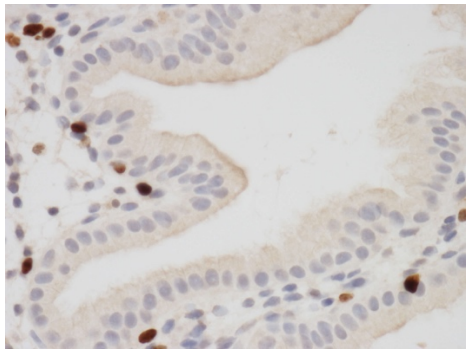
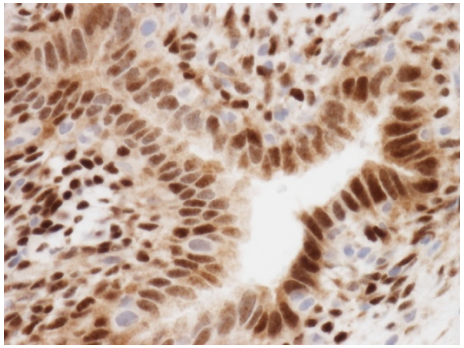
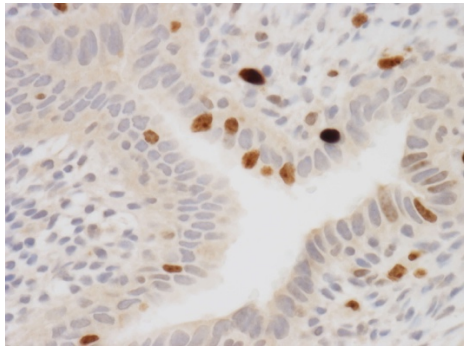
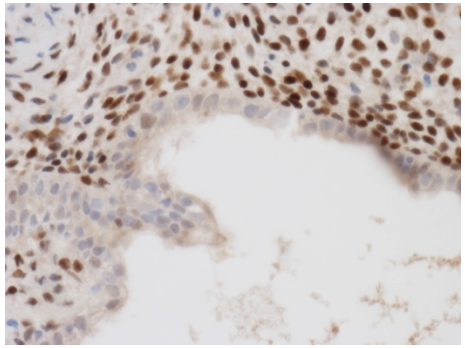
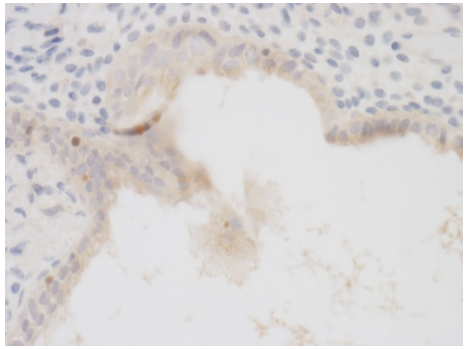
			
a. AR(Eu): epithelium=5, stroma=7	b. Ki67(Eu): epi. 1%, stroma 30%	c. AR(Eu): epithelium=5, stroma=7	d. Ki67(Eu): epi. 1%, stroma 10%
			
e. AR(Ec): epithelium=7, stroma=7	f. Ki67(Ec): epi. 30%, stroma 30%	g. AR(Ec): epithelium=3, stroma=6	h. Ki67(Ec): epi. 1%, stroma 5%

Figure 7.19 Immunohistochemistry results: CHUTE 19 (Day 26) & CHUTE 21 (Day 26)- secretory phase

The androgen receptor (AR) (a, c, e & g) and Ki67 marker (b, d, f & h) results for CHUTE 19 (a, b, e & f) and CHUTE 21 (c, d, g & h) are shown. Results for eutopic (a-d) and ectopic (e-h) samples with Allred scores for epithelial cells (epi.) and stromal cells (stroma) are shown (x 400 magnification).

Secretory phase

As a comparison CHUTE 19 and CHUTE 21 had their samples obtained on the same menstrual day (day 26) and both exhibited a good response to treatment. A comparison of their IHC data is shown in Figure 7.19. For CHUTE 19 expression of AR and Ki-67 is higher in the ectopic epithelium compared to the eutopic epithelium, yet the stromal expression is identical for both tissues. Conversely for CHUTE 21, expression of AR is lower in the ectopic epithelium, but Ki-67 epithelium expression is similar in both tissue types.

7.4.5 PTEN

In general, the stromal cells of both the eutopic and ectopic endometrium stain strong to moderate and the epithelial cells moderate to weak. Some null glands were noted in the epithelial cells of the eutopic endometrium for CHUTE 09, 13, 15 and 23. The most striking example of this is for CHUTE 09 and is shown in Figure 7.20. In panel a there are both positive (*) and negative (§) glands with uniform stromal staining. A comparison is shown in panel b, which is ectopic tissue is which uniform positivity is seen.

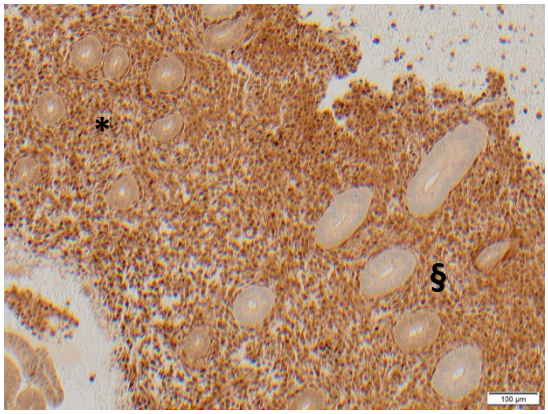
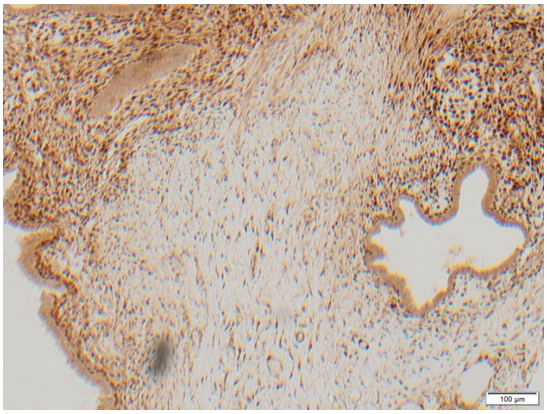
	
a. Eutopic endometrium: Heterogeneous pattern of epithelial staining with clearly defined positive (*) and negative glands (§). Stromal staining is more uniformly positive.	b. Ectopic endometrium: Uniform positive staining of both epithelial and stromal cells.

Figure 7.20 Immunohistochemistry results: CHUTE 09 (Day 11)- PTEN

Immunohistochemistry results for CHUTE 09 using PTEN antibody. This subject had noticeably reduced epithelial staining within the epithelium of the eutopic endometrium with a number of null glands and stretches of negative surface epithelium (Magnification- x 100).

7.4.6 VEGF

A uniform pattern of epithelial and stromal staining was seen across all subjects for both tissue types.

7.4.7 Immunohistochemistry and ulipristal acetate

The immunohistochemistry data obtained for the CHUTE subjects are not consistent with any pattern with respect to response to ulipristal acetate. The aim of the study was to establish the effect of ulipristal acetate on the histological appearance of ectopic endometrium. The immunohistochemical data for AR and Ki-67 expression in the ectopic endometrium do not demonstrate a correlation with either the phase of the cycle or the clinical response to ulipristal acetate.

7.5 QUALITY OF LIFE DATA

7.5.1 The EHP-30 questionnaire

The EHP-30 questionnaire was completed by the subjects at screening, in the final week of taking study drug and six weeks after the study drug had been completed, just prior to surgery. It is a validated questionnaire containing physical, psychological and social domains, in which the higher the recorded score the worse the disease is perceived to be by the subject. As such it has provided reliable quality of life data relating to endometriosis throughout the study, which has been used as an indicator of the clinical impact of the study drug.

EHP-30 total scores

The total scores and score differences for the CHUTE cohort are shown in Table 7.10. The scores at each time point show a large spread of values as indicated by high standard deviation. Overall the pattern of change across the cohort is for a reduction in the mean total score between screening and treatment, indicating the perceived clinical impact of ulipristal acetate. This effect then appears to regress once the drug is stopped. However, when the screening and end of treatment mean score differences are compared there still appears to be a significant reduction of the EHP-30 score indicating there remains some residual effect.

The response from each individual subject was quite variable. For instance, CHUTE 25 scored 125, 38 & 72, in keeping with the mean score results but CHUTE 02 had a profound and maintained reduction (77, 0 & 2), whereas CHUTE 12 had high and very uniform scores (316, 333 & 340) throughout, showing a deterioration in her scores through the study rather than an improvement.

The mean total scores and standard deviation are shown in Figure 7.21 together with the results of the paired T-tests that were undertaken. A paired T-test was chosen as the variables are dependent. The results show a significant score decrease between screening and treatment, $t(17)= -4.47$, $p<0.001$; and a significant score decrease between screening and end of treatment, $t(17)= -3.30$, $p=0.004$; but a non-significant score increase between treatment and endo of treatment, $t(17)= 1.40$, $p=0.180$. This highlights that although the effect appears to diminish after the drug is stopped the rise

in the total score is not statistically significant over this six-week timeframe such that a significant improvement is maintained at the end of treatment.

	Screening	Treatment	Screening - treatment difference (%)	EoT	Screening – EoT difference (%)
CHUTE 01	199	13	-186 (-93)	170	-29 (-15)
CHUTE 02	77	0	-77 (-100)	2	-75 (-97)
CHUTE 04	390	362	-28 (-7)	255	-135 (-35)
CHUTE 05	291	126	-165 (-57)	133	-158 (-54)
CHUTE 06	332	227	-105 (-32)	418	86 (26)
CHUTE 08	268	247	-21 (-8)	150	-118 (-44)
CHUTE 09	8	21	13 (163)	21	13 (163)
CHUTE 10	273	256	-17 (-6)	79	-194 (-71)
CHUTE 11	298	286	-12 (-4)	256	-42 (-14)
CHUTE 12	316	333	17 (5)	340	24 (8)
CHUTE 13	265	24	-241 (-91)	271	6 (2)
CHUTE 14	302	321	19 (6)	302	0 (0)
CHUTE 15	349	197	-152 (-44)	262	-87 (-25)
CHUTE 19	240	130	-110 (-46)	236	-4 (-2)
CHUTE 21	335	123	-212 (-63)	280	-55 (-16)
CHUTE 23	364	141	-223 (-61)	290	-74 (-20)
CHUTE 24	209	141	-68 (-33)	116	-93 (-44)
CHUTE 25	125	38	-87 (-70)	72	-53 (-42)
Mean score	257.8	165.8	-91.9	202.9	-54.9
Standard deviation	101.7	118.5	87.2	115.1	70.5

Table 7.10 Endometriosis Health Profile-30 (EHP-30) (Total) scores

The total EHP-30 scores for each CHUTE subject at screening, during treatment and at the end of treatment (EoT). The score changes between screening & treatment and screening and end of treatment (EoT) are also shown.

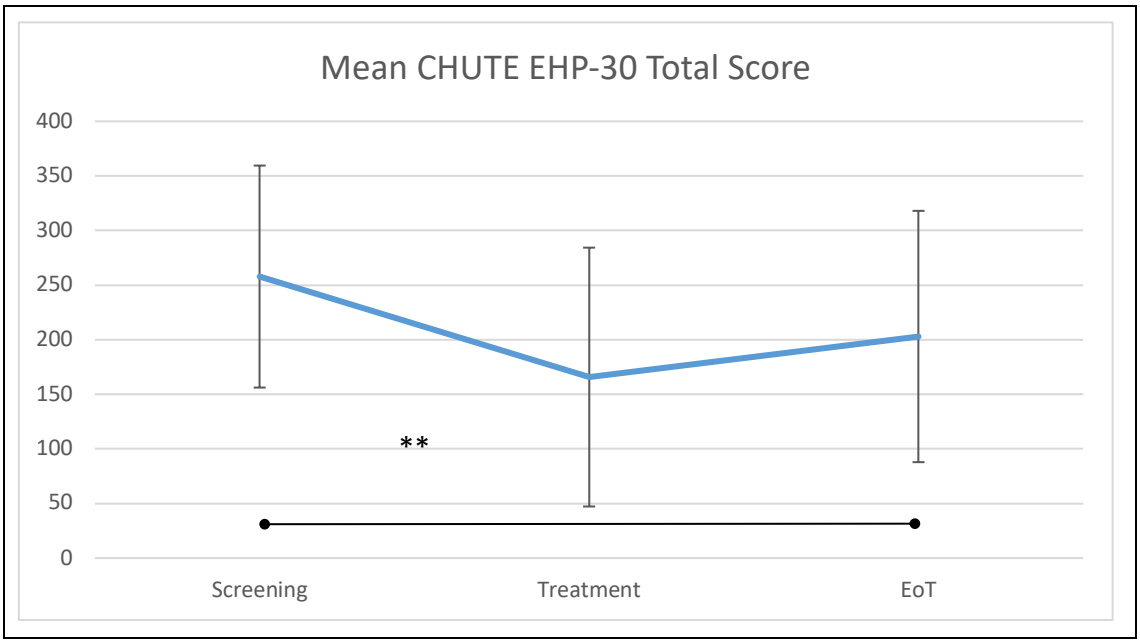


Figure 7.21 Mean Endometriosis Health Profile-30 (EHP-30) (Total) scores

*EHP-30 results for the CHUTE cohort showing the mean total score (blue line) and standard deviation (vertical bars) at screening, treatment and end of treatment (EoT) (Paired T-test: **p<0.001 between screening and treatment, *p=0.004 between screening and EoT, §p=0.180 between treatment and EoT).*

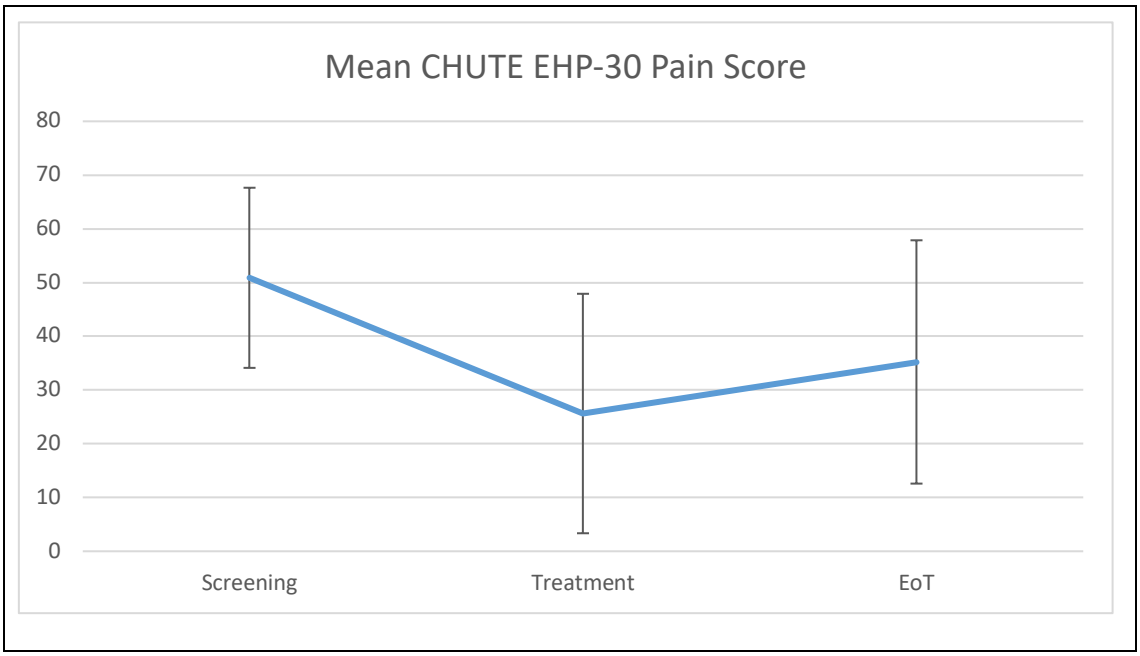


Figure 7.22 Mean Endometriosis Health Profile-30 (EHP-30) (Pain) scores

*EHP-30 results for the CHUTE cohort showing the mean pain score (blue line) and standard deviation (vertical bars) at screening, treatment and end of treatment (EoT) (Paired T-test: **p<0.001 between screening and treatment, *p=0.005 between screening and EoT, §p=0.095 between treatment and EoT).*

EHP-30 pain scores

The mean pain scores (51, 26 & 35) are illustrated in Table 7.11 and show a very similar pattern to the mean total scores but the initial reduction from screening to treatment is more profound reducing the mean score by 50% between screening the treatment. But, the spread of data remains similarly broad with the standard deviation 17, 22 & 23, respectively.

The mean pain scores and standard deviation are shown in Figure 7.22 together with the results of the paired T-tests. There is significant score decrease between screening and treatment, $t(17)= -4.75$, $p<0.001$; and a significant score decrease between screening and end of treatment, $t(17)= -3.27$, $p=0.005$; but again a non-significant score increase between treatment and endo of treatment, $t(17)= 1.77$, $p=0.095$.

EHP-30 domains

The data shown in Table 7.12 indicate how each of the five EHP-30 domains contribute to the mean difference of the total score. The statistically significant reduction (-91.94, $t(17)= -4.47$,$p<0.001$) in total EHP-30 score between screening and treatment is contributed to by a statistically significant reduction in the pain, control & powerlessness, emotional well-being and social support domains. Of these, the pain (-25.17) and control & powerlessness (-28.56) domains make the greatest contribution. The decrease in self-image score is just outside statistical significance at $p=0.051$.

When considering the non-significant increase in total EHP-30 score seen between treatment and end of treatment (mean difference 37.06) we see that the control & powerlessness domain makes the biggest, and statistically significant, contribution (mean difference 13.17, $p= 0.041$) to that change. All the other domains show either increases or decreases but these were not significant when tested.

The mean difference of the total EHP-30 score between screening and end of treatment remains statistically significant (-54.89, $t(17)= -3.30$, $p=0.004$). Again, this is contributed to by significant reductions in all the domains, except self-image; with pain and control & powerlessness making the biggest difference.

	Screening	Treatment	Screening - treatment difference (%)	EoT	Screening – EoT difference (%)
CHUTE 01	41	0	-41 (-100)	36	-5 (-12)
CHUTE 02	45	0	-45 (-100)	2	-43 (-96)
CHUTE 04	59	43	-16 (-27)	41	-18 (-31)
CHUTE 05	45	9	-36 (-80)	23	-22 (-49)
CHUTE 06	55	30	-25 (-45)	61	6 (11)
CHUTE 08	66	34	-32 (-48)	25	-41 (-62)
CHUTE 09	0	0	0 (0)	5	5 (-)
CHUTE 10	50	50	0 (0)	0	-50 (-100)
CHUTE 11	48	55	7 (15)	48	0 (0)
CHUTE 12	68	73	5 (7)	77	9 (13)
CHUTE 13	59	9	-50 (-85)	50	-9 (-15)
CHUTE 14	45	52	7 (15)	50	5 (11)
CHUTE 15	64	34	-30 (-47)	41	-23 (-34)
CHUTE 19	50	32	-18 (-36)	57	7 (14)
CHUTE 21	68	2	-66 (-97)	34	-34 (-50)
CHUTE 23	66	14	-52 (-78)	59	-7 (-11)
CHUTE 24	59	16	-43 (-72)	11	-48 (-81)
CHUTE 25	27	9	-18 (-67)	14	-13 (-48)
Mean score	50.9	25.2	-25.3	35.2	-15.6
Standard deviation	16.8	22.3	22.4	22.6	20.2

Table 7.11 Endometriosis Health Profile-30 (EHP-30) (Pain) scores

The EHP-30 pain scores for each CHUTE subject at screening, during treatment and at the end of treatment (EoT). The score changes between screening & treatment and screening and end of treatment (EoT) are also shown. The mean and standard deviation of the cohort are shown and represented in Figure 7.22.

EHP-30 Domains	Treatment-Screening Difference			EOT -Treatment Difference			EOT - Screening Difference		
	Mean	95% CI	P value*	Mean	95% CI	P value*	Mean	95% CI	P value*
Pain	-25.17	-36.34, -13.99	<0.001§	9.56	-1.85, 20.96	0.095	-15.61	-25.69, -5.53	0.005§
Control & powerlessness	-28.56	-40.60, -16.52	<0.001§	13.17	0.57, 25.76	0.041§	-15.39	-28.11, -2.66	0.021§
Emotional well-being	-15.06	-24.81, -5.30	0.005§	6.89	-6.07, 19.85	0.278	-8.17	-16.30, -0.04	0.049§
Social support	-10.83	-21.36, -0.31	0.044§	-2.72	-17.56, 12.11	0.703	-13.56	-22.66, -4.45	0.006§
Self-image	-12.44	-24.94, 0.05	0.051	10.06	-2.58, 22.69	0.111	-2.39	-13.16, 8.38	0.646
EHP-30 (Total)	-91.94	-135.30, -48.59	<0.001§	37.06	-18.88, 92.99	0.180	-54.89	-89.94, -19.84	0.004§

Table 7.12 Endometriosis Health Profile-30 (EHP-30) score analysis

*The mean difference between screening and treatment, treatment and end of treatment (EOT), and screening and EOT for the pain, control & powerlessness, emotional well-being, social support, and self-image domains are shown as well as the results for the total EHP-30 scores. A paired T-test was performed to compare the means at each time point to explore the impact of ulipristal acetate treatment on the EHP-30 scores of the CHUTE cohort. The mean, 95% confidence interval (95% CI) and P value is shown for each comparison (n= 18, *Paired T-test, §= significant result (p<0.05)).*

7.5.2 Patients global impression of change (PGIC)

The PGIC measured the cohort's views on the clinical impact of the ulipristal acetate treatment directly rather than through QoL indicators. It also provided a tool to define good and poor clinical response, which has been referred to throughout the results section. Section I asked the subjects to describe their change in activity, limitations, symptoms, emotions and overall quality of life, related to endometriosis as compared to beginning of study on a scale of 1 to 7 (see appendix 10). Those giving a score of 6 or 7 were considered to have a good clinical response. In section II each subject rated the degree of that change, with a score of 2 or less being taken as a good response.

The results for section I and section II are shown in Figures 7.23 and 7.24, respectively. In both sections, there are a range of responses with CHUTE 01, 06, 13 and 21 all reporting a dramatic change as a result of the treatment and then others such as CHUTE 09 & 12 reporting no change in section I and qualifying that in section II as a worsening of their condition. Overall the median (IQ) score for section I was 5.5 (3, 7) during treatment and this increased to 6.0 (5, 7) at the end of treatment; and for section II the median score was 2.5 (1.5, 3.5) during treatment but this increased to 3.0 (1, 3.5) at the end of treatment.

The dashed lines on each bar chart indicated the division between good and poor clinical response. Using this division, a total of nine subjects (CHUTE 01, 02, 05, 06, 13, 15, 19, 21, 23 & 24) were defined as good responders at the end of treatment. They all had a section I score greater than or equal to 6 and all but CHUTE 23 had a section II score less than or equal to 2. Alternatively, using a section I score of ≥ 5 and section II score <5 (no change) to include moderate responders in the group adds a further four subjects (CHUTE 04, 08, 10, 11), taking the clinical response to 14/18 (78%).

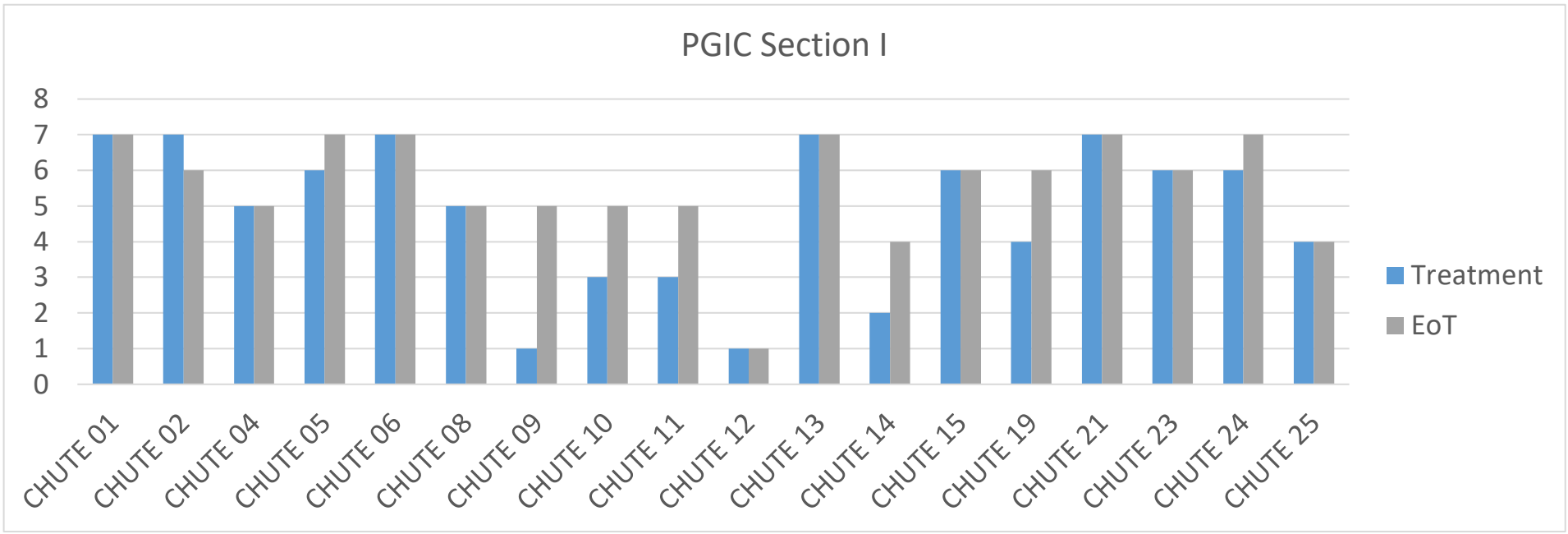
PGIC score analysis

A Wilcoxon Signed Rank test was performed, the results of which are shown in Table 7.13. The PGIC scores for both sections at both time points are highly statistically significant with p values ≤ 0.0026.

	Treatment			End of Treatment		
	Median	**IQ**	**P value*,±**	**Median**	**IQ**	**P value*,±**
Section 1	5.50	3,7	< 0.0001§	6.00	5,7	<0.0001§
Section 2	2.50	1.5,3.5	<0.0001§	3.00	1,3.5	0.0026§

Table 7.13 Patient Global Impression of Change (PGIC) score analysis

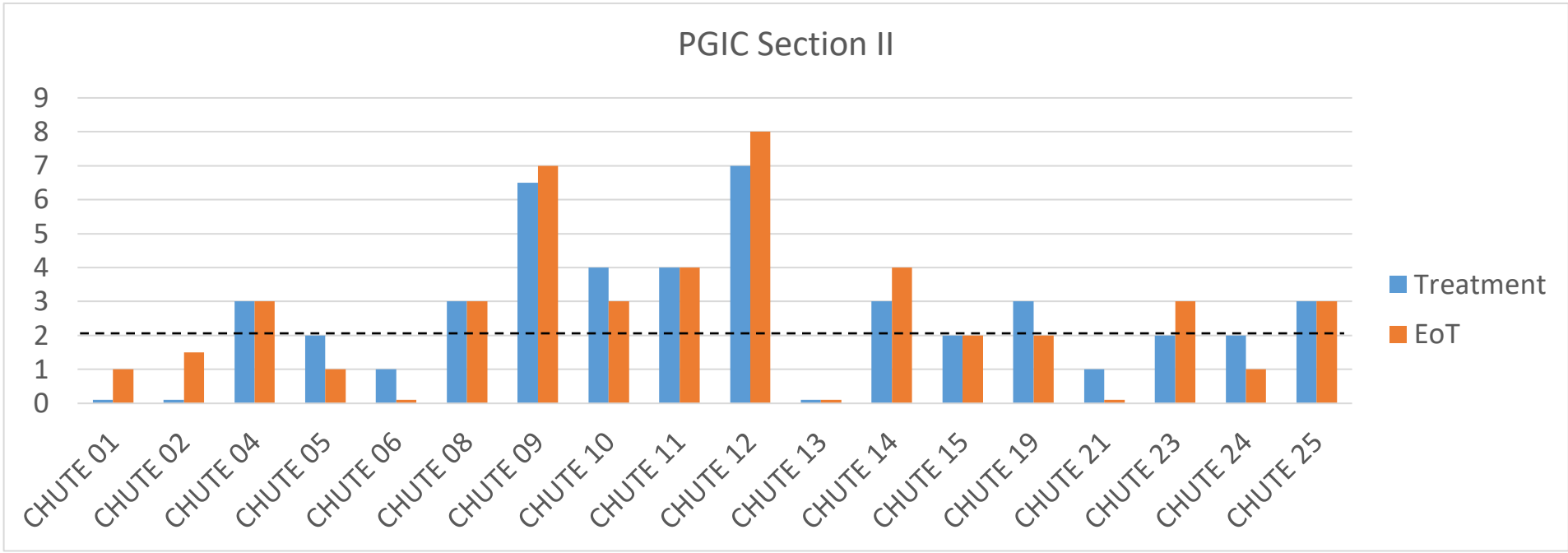
*The PGIC median scores showing significant change during treatment and at end of treatment for both sections of PGIC questionnaire. A Wilcoxon Signed Rank test was performed to explore the PGIC results reported by the CHUTE cohort. The median, interquartile range (IQ) and P value is shown for each section at both time points (*Sign Test for value=1 (No Change) for Section I, ±Sign Test for value=5 (No Change) for Section II), §= significant result (p<0.05)).*



a. Change in activity, limitations, symptoms, emotions and overall quality of life, related to endometriosis as compared to beginning of study. The blue bars represent the patient views during treatment and the grey bars represent the patient views after treatment had discontinued. The dashed line is the cut off between good and poor clinical response (Scale of 1-7 with 1= no change, … 7= a great deal better, and a considerable improvement that has made all the difference (See appendix 10)).

Figure 7.23 Patient Global Impression of Change (PGIC) scores- Section I

The patients' global impression of change (Section I) compared to the beginning of the study, during treatment and after treatment had discontinued.



b. Degree of change since beginning the treatment as part of the study. The blue bars represent the patient views during treatment and the orange bars represent the patient views after treatment had discontinued. The dashed line is the cut off between good and poor clinical response (Scale of 0-10 with 0= much better,…5= no change,…10= much worse (See appendix 10)).

Figure 7.24 Patient Global Impression of Change (PGIC) scores- Section II

The patients' global impression of change (Section II) compared to the beginning of the study, during treatment and after treatment had discontinued.

Chapter 8: DISCUSSION

8.1 STUDY OBJECTIVES AND DESIGN

The pharmacological treatment of endometriosis is becoming the mainstay of management as specialists are realising that surgical management has limitations, as discussed in Chapter 1. Although conventional medical treatments are effective for some there remains a significant number of patients in whom symptom control is inadequate. As such the evaluation of new treatments that may offer more effective symptom control and broaden the range of treatment options available must be a priority for the future of endometriosis management.

Selective progesterone receptor modulators, such as ulipristal acetate, may be effective in the treatment of endometriosis but the primary concern for any evaluation of these drugs must be patient safety and the long-term implications of drug treatment. The PEARL studies that are discussed in Chapter 4 highlight the progesterone receptor modulator-associate endometrial changes (PAEC) associated with ulipristal acetate. Whether these changes occur in ectopic endometrium, whether they have a proliferative effect at that ectopic location and whether any PAEC type changes persist after menstrual shedding are all important safety questions with respect to ulipristal acetate use in endometriosis.

The aim of this pilot study was to explore the safety question whilst also considering the clinical utility of ulipristal acetate for the treatment of endometriosis. The primary outcome measure was histological changes within the ectopic endometrium and the decisions taken with regard to study design have been described in Section 6.2. The most difficult of these was the decision as to whether to undertake surgical management whilst on study drug or after the first menstrual loss following stopping study drug.

The initial design with surgery being completed whilst on study drug may have provided a clearer picture as to the histological impact of ulipristal acetate but would have provided no useful information about the persistence of any changes after stopping treatment. Whereas the six-week delay incorporated in the final design allowed the

assessment of both the eutopic and ectopic endometrium following the cessation of treatment. As the expected prevalence of PAEC in eutopic endometrium is known from the PEARL IV study, the eutopic changes could be compared and then any ectopic endometrial changes evaluated against this baseline.

This decision was taken following a full and open discussion between the study team and the scientific advisory board of Gedeon Richter, including Professor Alistair Williams (lead histopathologist on the PEARL studies). During that discussion, it was also highlighted that a six-week delay would also allow an evaluation of the persistence of any clinical improvement seen in the study subjects after stopping treatment. Although the clinical impact of ulipristal acetate on endometriosis symptoms was only a secondary outcome measure it was still considered a very important aim of this pilot study and so the final study design was agreed.

The disadvantage of this decision is that our samples of ectopic endometrium were not obtained whilst the study subjects were still exposed to study drug. This has made the interpretation of the patterns of histopathology particularly challenging and may also have rendered the immunohistochemical results unusable.

A further consideration when designing the study was the level of secured funding and how to maximise the scientific data with the resources available. The decision not to include a control group in the study was largely influenced by this restriction. In an ideal study, we would have had a control group as a comparator. However, this would have necessitated further recruitment to keep the treatment group at 20 subjects and the prospect of 12 weeks of no treatment prior to surgical management in patients with severe symptoms may have negatively impacted on recruitment.

It was felt that each subject could act as their own control with respect to eutopic endometrial changes and that we should maximise the number of subjects exposed to study drug to explore the ectopic changes. This has meant we have had to rely on the known histological features of endometriosis as a comparator, which has offered some challenges to interpretation of the data.

8.2 PRINCIPAL FINDINGS

The principle findings of the study are as follows:

1) the majority of subjects showed an improvement of their perceived disease as a result of involvement in the study with a statistically significant reduction in severity of pre-menstrual pain, menstrual pain, non-cyclical pelvic pain, dyspareunia and lower back pain between screening and the end of the study;
2) histological assessment of ectopic endometrium was possible due to the high quality of surgical specimens, which were obtained without significant mechanical or thermal disturbance to the tissues resulting in minimal histological artefact;
3) features of PAEC were seen within eutopic endometrial samples but no single specimen exhibited enough features to be described as confirming PAEC;
4) cystic dilatation with ciliated metaplasia and infrequent mitoses was a more common finding within the ectopic endometrium;
5) no clear abnormalities of ectopic receptor expression were seen six weeks after stopping study drug and the eutopic samples exhibited typical cyclical changes in keeping with healthy endometrium;
6) clinical response to ulipristal acetate was seen in the cohort as evidenced by statistically significant improvements in the validated EHP-30 QoL questionnaire results through the drug treatment phase of the study;
7) overall, ulipristal acetate treatment was considered acceptable by the cohort with the median change at the end of treatment being assessed as 'Better, and a definite improvement that has made a real and worthwhile difference'.

These findings suggest that whilst questions remain with regards to safety & efficacy ulipristal acetate appears to be worth pursuing as a medical treatment for endometriosis.

8.3 STUDY COHORT

Recruitment of the cohort took six months longer than expected due to a lower than expected numbers of suitable candidates to approach for the study and higher than expected numbers of screen fails. The commonest reason for failing screening was that surgical management was no longer indicated or desired by the patient. Although the

exclusion criteria were quite strict their impact on recruitment was minimal- the main barrier to inclusion being failure to establish a regular menstrual pattern after discontinuing hormonal medication, which is a known complication of such medicines.

The planned cohort of twenty patients was reduced to eighteen by two early discontinuations that were described in Section 7.1.3. This resultant cohort of patients was representative of the endometriosis population in Norfolk and tolerated the study well with excellent adherence to the study protocol and study procedures. The only protocol deviation occurred as a result of bed pressures within the Trust at the time of planned surgery for CHUTE 14. This could not have been predicted or avoided and is a feature of healthcare provision within the NHS.

The mean age of the study subjects was 35, which is in keeping with the peak incidence of endometriosis between 30 and 45 years of age.(5) The average BMI was just within the normal range and the median parity was one. Twenty percent of the cohort were smokers. This smoking frequency is in keeping with published data for this age group at 24%.(228) The mean duration of endometriosis symptoms was 12 years suggesting this population had either early diagnosis or earlier than average onset of disease at 23 years of age. Unsurprisingly, some of the subjects had previously undergone surgery for the management of their disease.

8.4 ADVERSE EVENTS

In any study, adverse events are to be expected and these were recorded throughout the study as per good clinical practice (GCP). The most notable of these was the SAE (serious adverse event) recorded for CHUTE 17 as a result of conceiving between stopping the study drug and admission for surgical management of her disease. This occurred due to inadequate contraceptive precautions on the part of the patient despite the need for reliable contraception being discussed in detail at recruitment and reiterated at each study visit.

The implications of conceiving immediately following a course of ulipristal acetate were discussed once the pregnancy was confirmed. Unfortunately, there are little data to guide such a discussion, but the information contained in Section 4.6.4 was explained to the patient and her partner. The only data pertaining to conception whilst taking ulipristal acetate was within a review of 18 pregnancies in one of the recruitment

centres for PEARL II and III.(211) A single birth defect (ectopic kidney) was noted in the series, which was explained to the subject and termination of pregnancy offered. This was declined and a decision to prescribe progesterone support during the first trimester was taken in view of the theoretical increased risk of miscarriage.

As surgical management was no longer appropriate, and the data collected with respect to quality of life at the end of treatment may have been influenced by her conception a decision was taken to exclude all her data from the study.

The other adverse events (AEs) and side effects recorded are described in section 7.1.6. They were all mild to moderate, in keeping with the frequency recorded in the summary of product characteristics (SmPC) (95.0%).(189) Headache was reported most frequently as is seen with patients using ulipristal acetate for the management of fibroids but this resolved within the first seven days for all the subjects in the study. In addition, the following common (<1/10) adverse reactions recorded in the SmPC, such as skin changes, abdominal pain, hot flushes, weight gain and vertigo, were experienced by the cohort. And, some uncommon (<1/100) reactions were also seen – constipation, mood disturbance, vaginal discharge, alopecia, urinary incontinence and dizziness. None of the recorded AEs were considered serious or raised significant safety concerns.

8.5 CLINICAL

The management of endometriosis is centred round symptom control, be that through simple analgesia, hormonal suppression or surgical excision of disease. We know that the clinical appearance of disease does not always correlate with the pain symptoms experienced by patients; and that staging and classification systems such as ASRM are imperfect when used as clinical indicators. However, visual inspection of disease remains an important part of clinical assessment and the ASRM classification is widely accepted amongst researchers.(45) These were both used to describe and evaluate the CHUTE cohort and ascertain the impact of ulipristal acetate.

8.5.1 Symptom control

A good clinical response was seen in 56% of the cohort following treatment with ulipristal acetate; with ten of the eighteen giving a score of 6 or 7 in section I of the PGIC questionnaire. However, the symptom control seen as a result of the CHUTE study was

a combination of the drug treatment and the surgical clearance of disease. The results of the BSGE Pelvic Pain Questionnaire (BSGE-Qu) were used to demonstrate this.

The five classic symptoms of endometriosis- dysmenorrhoea, non-menstrual pelvic pain, dyspareunia, dysuria and dyschezia, were assessed at the outset and all but dysuria had high median scores for severity. When these symptoms were enquired about at the end of treatment they had become less frequent, but they were not formally assessed with the BSGE-Qu until the post-operative review, 3 months after surgery. This showed that although the frequency of dysmenorrhoea and non-menstrual pelvic pain had not altered a great deal the frequency of the other symptoms had decreased markedly, with dyspareunia reducing from 14 to 6 subjects within the cohort. This was not a surprise as it is these symptoms that often respond to surgical management.

Interestingly, whilst dysmenorrhoea and non-menstrual pelvic pain remained common within the cohort at the end of the study the median scores for severity were significantly improved. Taken together these data suggest that the combination of ulipristal acetate treatment and surgery had a positive impact on symptom control but without a control group it is not possible to assess whether such improvement would be seen with surgery alone. The observed improvement in clinical symptoms is further supported by the fact that analgesia use decreased by 44% within the cohort.

As the study progressed it was clear that there were some subjects in whom the study drug had very little impact. The PGIC questionnaire allowed a distinction to be made between good responders and those who had little or no improvement. Using this division, it was possible to see if the initiation of amenorrhoea was related – i.e. are those whom are not amenorrhoeic less likely to show improvement of symptoms. Amenorrhoea is expected in 79% of users(189), and occurred in 83% of the CHUTE subjects. However, three subjects continued to cycle throughout the study, two of whom showed an excellent clinical response to the drug. This suggests that amenorrhoea cannot be relied upon as a marker of biological action in endometriosis and that the action of the drug is doing more than just preventing menstruation and so improving dysmenorrhoea by default.

8.5.2 Disease status

The ASRM classification of disease was used to define the disease at the diagnostic procedure prior to recruitment and compare this to the disease status at the time of surgical excision. The mean score of 20 (median stage II) did increase by nine points to a mean score of 29 (median stage III) suggesting a worsening of disease. However, it should be noted that there was a mean time interval of 24 months (range 7-62 months) during which time progress of disease would be expected. As such, the design of the CHUTE study does not allow a causative assessment of this progression of disease; it may have been secondary to drug therapy, indicating a proliferative impact of ulipristal acetate or it may represent the known deterioration of disease over time.

Disease type

As endometriosis exists in three clinically distinct forms: peritoneal disease, endometriomas and deeply invasive disease(4), and peritoneal disease can have a different histological appearance to more deeply invasive tissue, it was important to ensure a broad range of surgical specimens were obtained and evaluated. This was achieved, with a total of 111 specimens and a good anatomical spread of biopsy sites. In addition, the frequency of occurrence at each location was in keeping with that expected of patients with severe endometriosis, giving further reassurance about how representative the cohort was.

The subset of biopsies used for the immunohistochemistry was similarly broad with no anatomical location overlooked and a range of tissue types included. The peritoneum over the uterovesical fold often contains more superficial disease, which was utilised for the IHC in five out of the eighteen cases due to the higher quality of this tissue. The ease of surgical access may explain this but it may have resulted in the data being skewed towards a more superficial type of peritoneal disease.

8.5.3 Surgical findings

The three different types of disease were seen represented in the CHUTE cohort and are described in section 7.2.6. The most interesting finding was the active, “angry” appearance of the peritoneal disease seen in four of the cohort. It was most notable in CHUTE 06 where it appeared to be stuck to the posterior surface of the uterus. When examined under the microscope this tissue was largely indistinguishable from the eutopic endometrium with similar immunohistochemistry for both tissue types.

Concern about the impact of SPRMs on ectopic endometrium and the possibility of a proliferative effect, particularly in the Pouch of Douglas have been raised through various personal communications with the author. Whether the changes seen in these four subjects support these concerns is difficult to assess. Using the ASRM classification there appears to be minimal progression in score over a time period of 7-20 months but the disease in these subjects does appear to be more active and erythematous. It is not possible to say if this is a variant of endometriosis or as a result of drug therapy, but it does raise the suspicion that ulipristal acetate is associated with proliferation within the ectopic endometrium. The histological and histochemical assessment of the biopsy material from these subjects was unremarkable with respect to the rest of the cohort and so offered no further reassurance or cause for concern.

Further evidence of possible proliferation was seen in CHUTE 15. Although this subject exhibited less of the angry erythematous peritoneal disease, she still showed a 95-point score increase over the course of seven months. This was the result of the rapid growth of three endometriomas - two in the right ovary and one in the left ovary resulting in obliteration in the Pouch of Douglas.

The questions this raises are:

1) Are these proliferative changes related to ulipristal acetate?
2) If they are, do they persist after stopping ulipristal acetate?
3) If they persist, are the changes cumulative?
4) What impact do these changes have on the symptoms experienced by the patient?

However, it should be noted that in the two most notable cases described above a good clinical response to study drug was noted with the symptoms of endometriosis improving through treatment. If the underlying disease changed as a result of 3-months of ulipristal acetate this did not translate to a deterioration of endometriosis symptoms or a failure to control baseline symptoms.

8.6 HISTOPATHOLOGY

The argument to support the use of SPRMs in endometriosis has been set out in section 4.6.1. In summary, these drugs have the potential to suppress the endometrium & inhibit ovarian progesterone production without leading to the side effects of oestrogen deprivation. Consequently, they may offer a more tolerable management option for patients with endometriosis as compared to GnRH analogues. However, the key to this potential is understanding and exploring the histological impact of such compounds with respect to PRM-associated endometrial changes (PAEC).

Unfortunately, there is no classic histopathological appearance of endometriosis so evaluating the histological impact of a treatment such as ulipristal acetate is difficult. The disease is defined as epithelial and stromal endometrial cells in an ectopic location with inflammatory infiltrates and associated fibrosis.(2) The histological features are variable between patients, can be variable within the same specimen and are sometimes congruous with the eutopic endometrium, but not always. Overall, they tend to have an irregular proliferative rather than secretory pattern and often contain dilated glands with inactive epithelial cells demonstrating ciliated metaplasia.

Given this heterogeneity of appearance the opinion of the two consultant histopathologists involved has become paramount. The system of utilising more than one histopathologist and obtaining a consensus opinion was employed in the PEARL studies. Similar to those studies the sample processing and reporting criteria were defined before any samples were viewed (see Histopathology Report Proforma (Appendix 25)) and each histopathologist viewed and reported the slides in a blinded and independent fashion.

A decision about whether certain observations were significant or not and whether the ulipristal acetate had affected the histology of the endometrium was heavily influenced by their expert opinion. The use of standardised reporting and defined features of PAEC helped in the analysis but histopathology is often about pattern recognition based on years of experience, so their overall impression was also viewed as scientifically valid.

There were high levels of agreement between Dr Lonsdale and Professor Williams throughout the histological analysis. The reporting of PAEC features for eutopic and ectopic endometrium are shown in Table 7.8, with Kappa and overall agreement

statistics to illustrate levels of agreement. The Kappa statistic was found to be unreliable for this data set and so the overall level of agreement across the whole data set was used. It demonstrated strong or moderate agreement for five of the six PAEC domains for eutopic endometrium reporting and three out of five PAEC domains for ectopic endometrium reporting. This level of agreement between two experts adds further weight to the conclusions drawn about histopathological changes.

Some subtle differences existed between their reports, but their overall impression was that no specimen of either eutopic or ectopic endometrium exhibited all the features of PAEC; or showed features of hyperplasia, endometritis or neoplasia. This is a key conclusion for the on-going investigation of ulipristal acetate with respect to safety.

8.6.1 PAEC type changes

The PAEC features seen in the eutopic endometrium were compared to the published data from PEARL I and II (197,198) to put them into context. The pattern of features seen in the CHUTE cohort was clearly different with cystic dilatation, cytoplasmic vacuolation and stromal vascular changes all being less common than was seen in PEARL I&II. However, it should be noted that the PAEC features reported in the PEARL I&II studies were assessed whilst the study subjects were taking ulipristal acetate. Whereas the CHUTE samples were obtained six weeks after completion of study drug.

A more accurate comparison would be the data from PEARL III & PEARL IV, where non-physiological changes were seen in 26% & 16% of the subjects 10-18 days after first menstruation following treatment cessation.(199,200) Our data for PAEC type changes are much more in keeping with this frequency and suggest that any changes that occur in response to ulipristal acetate normalise quickly after menstrual shedding. This recovery of functionality would be supported by the unremarkable nature of the immunohistochemistry data in the cohort and the successful implantation of an embryo for CHUTE 17 within 10 days of completing the study drug.

Although no subject had all the histological features consistent with PAEC, CHUTE 08 showed clear cystic dilatation and vascular changes that were consistent with it. However, the most unusual changes seen were the metaplastic changes exhibited by CHUTE 14. Whether these observations are secondary to drug action is impossible to know. The subject showed vascular changes, typical of PAEC, suggesting some drug

related action on the histology but attributing the unique epithelial changes seen in this subject to ulipristal acetate may not be appropriate, as it is a single observation. Interestingly, no metaplastic changes were seen in the ectopic samples and cystic dilatation was only noted in one of the four specimens.

When the PAEC type features were used to compare the eutopic and ectopic samples there was a marked difference. Cystic dilatation with ciliated metaplasia of the epithelial cells is much more prevalent in the ectopic samples. The epithelial cells appear inactive with cuboidal shaped cells, infrequent mitoses and no evidence of secretory activity. This finding may just represent the typical appearance of endometriosis or may be a response to ulipristal acetate. The impression from both histopathologist was that the ectopic samples were in keeping with their expectations for endometriosis but they each noted that the cystic dilatation was a more prominent feature within the samples than they were expecting. It is difficult to assess this feature in isolation as samples of endometriosis in normal clinical practice tend to contain more surgery artefact and are evaluated in a less detailed manner, making the known 'control' difficult to define for this tissue type.

Four of the subjects showed florid disease at surgical inspection. The active appearance of the disease raised concern about whether the ulipristal acetate had caused a proliferative type change in the ectopic endometrium. The ectopic samples did demonstrate cystic dilatation but no other PAEC type features and they did not differ significantly from other samples obtained from more standard looking endometriosis. Some of the subjects exhibiting these florid changes reported a good response to the study drug with respect to symptoms and EHP-30 scores, and others did not. Interestingly, the Ki67 IHC in the epithelial cells for these subjects was prominent in some cases and minimal in others. This pattern did not correlate to whether the subject showed a good clinical response. As such the active appearance of the disease does not appear to be associated with either a histological or clinical worsening of disease, over the time course of the study.

Of note, the florid peritoneal disease described above and the samples from those subjects with severe invasive disease resulting in a frozen pelvis did not appear to differ significantly. Although we consider peritoneal disease, ovarian endometriomas and deeply invasive disease as three different forms of the disease that behave differently

with respect to our conventional management options, histologically they appeared quite similar within this cohort. This uniformity may be helpful when assessing the clinical impact and safety of ulipristal acetate for endometriosis in future studies as the surgical sub-types may not need to be considered separately.

8.6.2 Cystic dilatation, PAEC and safety

Cystic dilatation is an easily identifiable histological feature but as it is a feature of both PAEC and untreated endometriosis, using it to evaluate PAEC within ectopic endometrium is challenging. However, the prevalence of these changes was noted to be higher in the CHUTE subjects than the histopathologists were expecting suggesting this might be the result of drug action. This raises the interesting question as to whether such changes relate to the clinical impact of the drug or are just a consequence of ulipristal acetate exposure.

The proportion of ectopic endometrium samples demonstrating cystic dilatation was compared between the responders and non-responders. There was no significant difference between the groups using an independent T test ($t(16)= 1.74$, $p= 0.10$) but there appears to be a trend towards significance. As the sample size is only small this finding may suggest an association between cystic dilatation and a positive clinical response to ulipristal acetate that the study is underpowered to demonstrate.

If we assume that the observation of the histopathologists is accurate and ulipristal acetate is causing a degree of cystic dilatation. The association between clinical response and that dilatation suggests cystic dilatation of the glands may pay a part in the drug action in endometriosis. As such the establishment of PAEC within the ectopic endometrium could be viewed as a positive rather than a cause for concern. However, safety must be a priority and the potential for such PAEC type changes to evolve into proliferative or suspicious changes within endometriotic deposits needs much greater evaluation.

The key consideration for safety is the fact that menstrual shedding does not occur in the same way it does within the eutopic endometrium. Some menstrual breakdown of the tissues can occur within ectopic deposits, but those tissues are not completely lost as they are within menstrual flow. As such, any changes with the tissue related to drug action could accumulate during drug treatment and then persist after drug cessation. If

multiple courses of ulipristal acetate are taken as per the current medial licence those PAEC type changes may become cumulative and result in hyperplastic type changes. However, some reassurance can be taken from the PEARL III data. During that study multiple courses of ulipristal acetate were given and no cumulative effect in the eutopic endometrium was demonstrated. In fact the frequency of PAEC decreased slightly following four repeated doses.(199)

Although confidence is building that PAEC is not a pre-cursor to endometrial hyperplasia or endometrial cancer in eutopic endometrium it is difficult to be as reassured about the safety in ectopic endometrium without further evidence. The PAEC type changes observed in the eutopic endometrium of the CHUTE subjects has been consistent with the expected changes from previous studies, whereas the changes seen in ectopic endometrium have shown less secretory and stromal vascular changes but a much higher frequency of cystic dilatation with ciliated metaplasia. As such we cannot conclude that the eutopic and ectopic endometrium behave in the same manner under the influence of ulipristal acetate. Consequently, there is insufficient evidence to support the use of eutopic endometrial sampling as a safety assessment in those subjects with endometriosis.

It must be noted that the heterogeneity of the histopathological appearance of ectopic endometrium together with the design of the study have hampered interpretation. The histological features of endometriosis are not well defined and there is huge inter- and intra-subject variation, preventing the establishment of a normal baseline in a study such as CHUTE. When combined with the six-week lag between drug treatment and obtaining the ectopic endometrial biopsies there are too many variables to be certain about any conclusions with respect to histopathological changes in response to ulipristal acetate. This confounder must be addressed in any future studies.

8.7 IMMUNOHISTOCHEMISTRY

Immunohistochemistry is not extensively utilised during routine assessment of endometriosis. The diagnosis tends to be dependent on the histological features rather than expression of receptors, but when doubt exists the stromal marker CD10 is used to aid the diagnosis.

Normal eutopic endometrium tends to show minimal ER and PR expression in the early proliferative phase, which is largely oestrogen dependent. Once in the mid proliferative phase ER and PR expression increases as does Ki-67.(71) The expression of all of these then decreases in the secretory phase. However, this pattern does not seem to be true in the eutopic endometrium of endometriosis patients, who have demonstrated significant variation in PR expression with no cycle dependent pattern.(94)

Endometriosis and endometrial hyperplasia are both oestrogen dependent & proliferative conditions that can be treated with continuous progesterone therapy. This treatment leads to a reduction in PR expression and promotes an increase in the PR-A:PR-B ratio, helping to counter oestrogen driven proliferation of the endometrium. As such an increase in PR expression within tissues would raise the suspicion of proliferative changes within the tissues being evaluated.(82) Whereas a decrease in PR expression within a sample may be indicative of endometrial suppression.

There is also evidence that anti-progestins such as ulipristal acetate may exert their anti-proliferative effect on the endometrium by influencing AR expression, which in turn causes a reduction in PR expression. (92,97) As such a reduction in PR expression & Ki-67, together with an increase in AR expression within the tissue could be taken as evidence of drug action. Whether such changes would also occur within ectopic endometrium was uncertain but high levels of variability were expected.

8.7.1 Oestrogen and progesterone receptors

There was strong positive staining across both cell types, in both tissue types. There was no clear cycle dependent expression other than a suggestion of slightly reduced expression of ER and PR, particularly in the eutopic tissue, in the first three days of the cycle. This reduced expression in the early proliferative phase was in keeping with expectations but the reduced expression that would also be expected in the secretory phase was not demonstrated. It is unclear whether this is an upregulation of expression, resulting from drug action, or the result of strong antibody binding that prevented subtle differences in expression being identified.

8.7.2 Androgen receptor & Ki-67 proliferation marker

The expression of the AR in the endometrium is known to be reduced when compared to ER and PR and tends to be more marked in stromal cells. This was seen in the CHUTE

subjects where both eutopic and ectopic stromal expression was strong and did not appear to be influenced by the phase of the cycle. A clearer cyclical pattern of expression was seen in the eutopic epithelial cells; with increasing AR expression, up to the mid-proliferative phase of the cycle followed by decreasing expression through to the mid-secretory phase. After this the AR expression began to increase again. The possible link to ulipristal acetate and the clinical significance of this is unknown.

The expression of Ki67 should increase through the proliferative phase in response to rising oestrogen levels and then decrease in the secretory phase when mitosis is less.(103) This is the pattern that was observed within the eutopic endometrium with the peak of expression occurring slightly later in the epithelial cells as compared to the stromal cells. A similar pattern of cyclical expression was not seen in the ectopic cells. In those, there appeared to be more uniform expression of Ki-67, particularly in the epithelial cells; in keeping with known data on the association between Ki-67 expression and stage of disease. (100)

There did not appear to be a correlation between AR and Ki-67 expression, and clinical response to ulipristal acetate. The comparison of responders and non-responders at different points in the menstrual cycle did not reveal an interpretable pattern that could be used to comment on study drug action. As with the H&E observations the six weeks off study drug before obtaining tissue samples has hampered interpretation.

8.7.3 PTEN & VEGF

The expression of PTEN within endometrial cells should be uniform. Although the occasional null gland can be seen any significant loss of PTEN expression would be a marker of early hyperplastic changes. Within the CHUTE cohort no significant pattern of loss of PTEN expression was seen. However, a heterogeneous pattern of positive and negative glands was noted in the eutopic endometrium of CHUTE 09. This did not correlate with similar changes in the ectopic endometrium of this subject. The ectopic sample showed uniform staining in keeping with all the other subjects. This pattern of expression in the eutopic endometrium of CHUTE 09 is of uncertain significance.

Similarly, the VEGF immunohistochemistry staining was unremarkable. The eutopic and ectopic endometrial tissues showed uniform epithelial and stromal staining irrespective of cycle day.

Overall, the significant reductions in PR and Ki-67 expression expected as a result of ulipristal acetate were not seen but evidence of significant proliferation was also not demonstrated. The eutopic tissues, in particular, showed typical cyclical changes and there was little to suggest any residual drug effect. This may have been because no changes occurred but more probably is a consequence of the six-week delay between completing the medication and the samples being obtained, allowing the normalisation of any drug related changes in receptor expression.

8.8 QUALITY OF LIFE

One of the secondary objectives of the study was to assess the change in disease severity as a result of treatment. The EHP-30 questionnaire was used to provide an assessment of the impact of the disease at three different time points, thus illustrating changes in quality of life as a result of ulipristal acetate treatment.

The total scores given by the subjects suggested the impact of the disease improved significantly ($t(17)= -4.47$, $p<0.001$) by the end of the treatment course. Following the cessation of treatment some of that improvement was lost but the increase in total score was not statistically significant over that 6-week period. As such the score decrease between baseline and end of treatment, just prior to surgery, was still significant ($t(17)= -3.30$, $p=0.004$). Given the small sample size these data are very encouraging.

The breakdown of all five domains of the EHP-30 (shown in Table 7.13) questionnaire shows that the study drug is having a positive impact across a range of domains. Pain and, control & powerlessness make the biggest contribution but the emotional well-being and social support domains also significant contributions to the final result. However, an assessment of effective pain control when evaluating a new treatment for endometriosis is paramount. Happily, the pain domain scores reported by the cohort were very much in line with the total scores; demonstrating a significant drop in the pain domain scores between screening and treatment, which was then maintained after the cessation of treatment.

The IMMPACT recommendations for chronic pain clinical trials suggest a reduction in pain scores of 30% should be taken as significant.(229) The data from the pain domain of the EHP-30 questionnaire shows that 12 subjects have a >30% reduction in their pain

score whilst on the study drug and that 9 subjects have a >30% reduction in their pain scores in the six weeks following drug cessation.

The patient global impression of change (PGIC) median scores during the final week of treatment and 6-weeks after treatment suggested a significant change in 'activity, limitations, symptoms, emotions and overall quality of life' as a result of ulipristal acetate. The degree of that change was reported to be an improvement for all but 2 subjects. The scores in both sections for both time points were statistically significant and also allowed a distinction between good and poor/no clinical response. Using this division, 14 showed some response to treatment and a total of 10 were classified as showing a strong response. Whereas four showed little or no response. For those who showed little clinical response there did not appear to be any predictors of this; be it disease location, depth of disease, histological make up of tissues or receptor expression. For those who experienced improvement, it was often dramatic with subjects reporting that they "have their life back" or "feel normal again". One subject explained that ulipristal acetate was the first treatment she had tried "that actually worked" and was desperate to continue the drug even after the study was complete.

The questionnaire data also suggest that subjects experienced sustained pain relief, even after the study drug was discontinued. This could suggest some residual endocrine suppression or altered inflammatory response as a result of treatment but the design of the study does not allow any further assessment of these factors.

8.9 LIMITATIONS

As a small pilot study, the size of the cohort is an obvious limitation. The small numbers make spotting patterns of histological features difficult and limit the validity of any statistical test performed to explore questionnaire date. The original cohort size of twenty subjects was chosen to be in line with other pilot studies in this area; and was considered appropriate given the study objectives.

The decision to proceed without a placebo group was well considered but did lead to significant difficulties when interpreting the data. The primary objective was histological assessment of endometriosis in response to ulipristal acetate, so a decision was taken to maximise numbers exposed to study drug and use published data on eutopic

response to ulipristal acetate as a comparator. The assessment of endometrial samples at screening also allowed each subject to act as her own eutopic endometrial control.

The argument for ectopic endometrial controls, either as pre-treatment biopsies or a non-treatment group is a valid one. However, the study team felt it was unethical to design a study in which a mandatory diagnostic laparoscopy was planned to confirm the diagnosis and obtain a biopsy as part of screening. It was also considered unethical to map and partially excise a portion of disease with a view to treating it medically for three months before returning for further laparoscopic excision.

Unfortunately, the lack of existing knowledge on the histopathological appearance of endometriosis, combined with a decision not to have a non-treatment group as a comparator and the six-week lag between drug therapy and surgery made interpretation of the histopathological features seen very challenging. These factors also compromised the validity of the immunohistochemistry data to such an extent that no safe conclusions can be drawn on this data.

A further consequence of not including a control group is that it prevents any blinding to treatment on behalf of the subjects or investigators. This combined with the small numbers and semi-subjective nature of the histopathological interpretation has the potential to introduce significant bias to the study interpretation.

The distinction between patients requiring medical treatment of endometriosis and those requiring surgical excision has been described in chapter 1. The subjects included in the CHUTE study were by the nature of the inclusion criteria, patients needing surgery. As such our study was conducted on subjects where medical treatment may have been less effective. This has the potential to have diminished the clinical impact observed and should be taken into account in future study design.

When considering the observed clinical impact, it must be highlighted that the time between the diagnostic procedure and recruitment was not defined in the inclusion criteria. This led to a broad range of intervals resulting in reduced accuracy of staging data at screening. This may have influenced the apparent increase in ASRM score and stage seen between screening and excision. As such there is insufficient evidence to support the conclusion that clinical progression of the disease is seen as a result of ulipristal acetate treatment.

The relatively short duration of treatment, the fact that all subjects received study drug and all subjects knew surgical management of disease would occur at the end of the study were all significant confounding factors. The Hawthorne effect (230) resulting from being observed may well have influenced the scores given in the questionnaires. This bias may have also been compounded by the extra clinical visits, access to medical expertise and research nurse support- all factors that can contribute to patient perception of improvement in disease.

This placebo effect is seen in blinded endometriosis trials. The two studies using GnRH analogues evaluated by J Brown, *et al.*, in a recent overview of treatments for endometriosis demonstrated an 18% and 16% improvement of disease in the placebo arms of the studies.(231) The lack of placebo or blinding, combined with the questionnaire based patient evaluation of disease has the potential to compromise the quality of life data completely.

Chapter 9: CONCLUSIONS & FUTURE DIRECTION

The histopathology of the ectopic endometrium is very variable, and this makes interpreting the results of the study difficult. However, despite this confounding factor, possible PAEC type changes were observed within the ectopic endometrium. Cystic dilatation with ciliated metaplasia was a more prominent feature than expected suggesting this may be the result of drug action. This observation also appears to correlate with the clinical response of subjects to the drug, although the study was not suitably powered to demonstrate a statistically significant association. This finding is particularly interesting as it may suggest that PAEC type changes within the ectopic endometrium and possible alterations in the balance of PR-A and PR-B expression are key to the clinical impact of the drug on endometriosis.

Concern exists amongst endometriosis specialists that the use of SPRMs such as ulipristal acetate may have a proliferative effect on ectopic endometrium and that prolonged use may cause a hyperplastic type effect within endometriotic deposits. Such reservations should be noted, and studies conducted to help answer that question. The florid appearance of the disease in some CHUTE subjects does highlight concerns about proliferation, however it is reassuring that the histological appearance and immunohistochemical staining was not markedly different for those subjects with florid disease.

The most encouraging result is the clinical impact of ulipristal acetate over the three-month course- a number of the subjects describing it as “the best treatment” they have ever taken. Analgesia use decreased in 44% of subjects and there was a statistically significant reduction in symptom severity for pre-menstrual pain, menstrual pain, non-cyclical pelvic pain and pain during sex. These data were also backed up with statistically significant improvements in quality of life scores and *global impression of change* data showing high tolerability.

Overall, the study has demonstrated some encouraging evidence for those clinicians already using ulipristal acetate *off licence* to treat endometriosis but concern about long-term safety has not been resolved and caution should still be exercised.

9.1 FUTURE WORK

The priority for any future research would be to answer two broad questions, which remain unanswered by the CHUTE study; is ulipristal acetate safe to use in endometriosis from a PAEC point of view & what efficacy does it demonstrate in controlling symptoms. Further studies are required to answer these questions and those studies should involve greater numbers, irrespective of design, to ensure robust & reliable data are obtained.

The development of PAEC is key to the safety question and it must be considered for both eutopic endometrium and ectopic endometrium. There is evidence to suggest that the eutopic endometrium in endometriosis patients behaves differently to the eutopic endometrium of healthy individuals. However, the key area of interest is whether PAEC occurs in ectopic endometrium. A clearer answer to that question would be achieved if endometrial samples were obtained whilst the study subjects were still taking the study drug.

Amongst researchers familiar with ulipristal acetate there remains concern that it may have a proliferative effect on ectopic endometrium. This needs further exploration through surgical inspection & histopathological analysis. This must be conducted whilst the subjects are taking study drug in a future study. The histopathology data should be assessed by three independent histopathologists and receptor expression assessed using microbiology techniques such as Western blotting or DNA microarrays to give quantitative results.

The majority of current medical therapy for endometriosis is used for a period of six months to assess its effectiveness. Given the current medical licence for taking multiple course of ulipristal acetate, consideration should be given to the length of treatment. A longer course would allow an assessment of the longevity of any clinical response and would help minimise some of the Hawthorn effect seen in the current CHUTE design.

The study population in a future study should also be considered. The design of the CHUTE study included subjects considered suitable for surgical management of their disease. As such their disease may not have been suitable for medical treatment. Once the safety of ulipristal acetate in endometriosis is more established a study looking at

medical management without recourse to surgery should be considered either with a placebo arm or with an existing treatment as a comparator.

9.1.1 Study features

Placebo arm

Given the uncertainty about the histopathological features of ectopic endometrium and any overlap with PAEC a future study should contain a placebo arm. In addition to providing a comparator for histopathology it would add weight to any clinical or questionnaire-based disease assessment following drug treatment.

Blinding

The bias introduced by not blinding the subjects or the study team to the treatment received in the CHUTE study should be corrected. This would add much greater weight to any conclusions and give a clear picture of the placebo effect generated as a result of a subject being enrolled in a study.

Surgical timing

Surgical specimens and visual inspection of either disease suppression or staging would need to be made whilst subjects were still taking the study drug. The problems associated with a treatment gap have been highlighted in the CHUTE study. This would be particularly important if quantitative receptor expression assessment were being undertaken.

Head to head

A further assessment of efficacy of ulipristal acetate in endometriosis management would be to compare it head-to-head with an existing treatment such as a GnRH analogue.

9.1.2 CHUTE 2

A number of possible study designs have been considered, to incorporate as many of the desired features as possible. The current design of CHUTE 2 is shown in Figure 9.1. There will be two treatment arms as shown with a placebo group running alongside. The study will be double blinded with dummy injections and placebo tablets. All samples will be obtained whilst subjects are still in treatment and the EHP-30 questionnaire and PGIC will be used at all time points for consistency.

9.2 CONCLUDING REMARKS

The results of the CHUTE study support the largely theoretical conclusion that SPRMs can successfully treat endometriosis. Ulipristal acetate potentially offers an effective treatment for endometriosis with histological changes of the eutopic endometrium in keeping with the known experience of PAEC. The safety of this compound remains to be elucidated but the results from this pilot study are encouraging and should prompt further exploration.

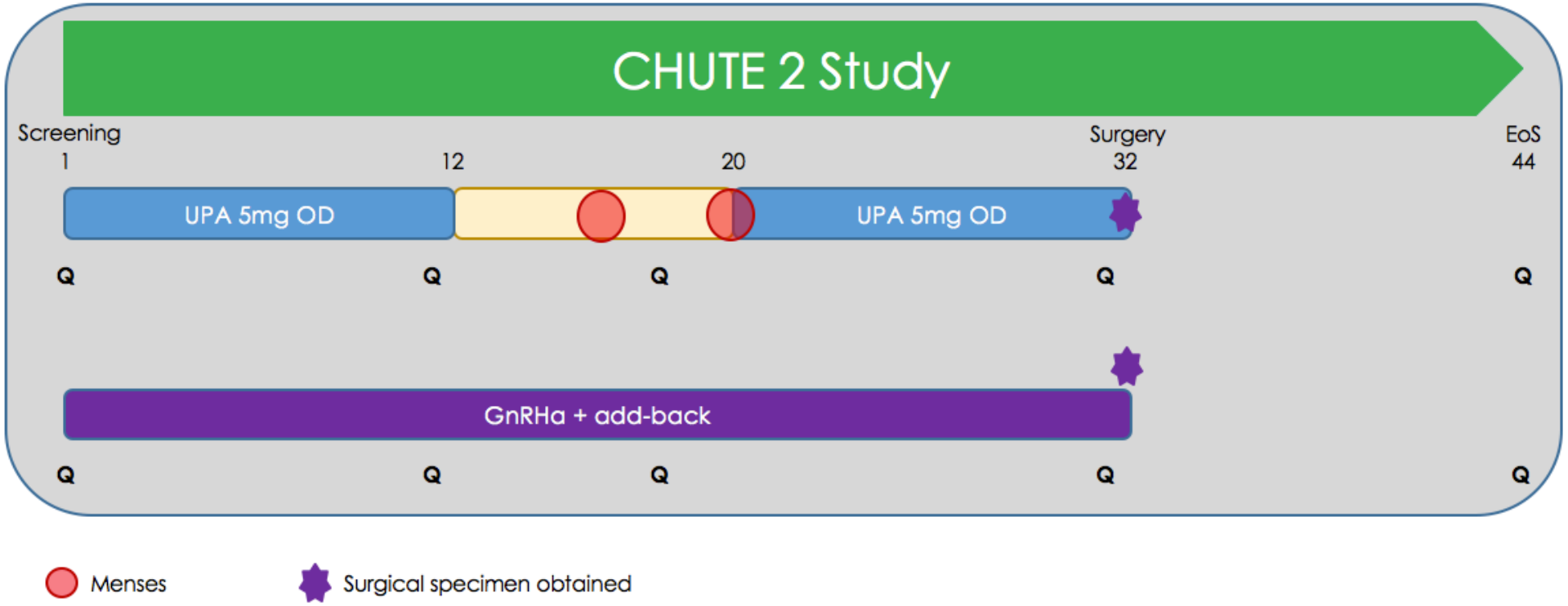


Figure 9.1 **Graphic representation of CHUTE 2 study timeline.**

(UPA 5mg OD= Ulipristal acetate (5mg once per day) taken for a duration of 12 weeks, EoS= end of study, Integers= whole weeks, Q= Questionnaires)

CONTRIBUTIONS

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GLOSSARY

AR	Androgen Receptor
ASRM	American Society for Reproductive Medicine
βhCG	Beta Human Chorionic Gonadotrophin
B&B	Biberoglu and Behrman
BSGE- Qu	BSGE pelvic pain Questionnaire
COCP	Combined Oral Contraceptive Pill
COX-2	Cyclooxygenase-2
CPSSS	Composite Pelvic Signs and Symptoms Score
CRF	Case Report Form
CRN	Clinical Research Network
CRL	Crown Rump Length
CTA	Clinical Trials Authorisation
DIE	Deeply Invasive Endometriosis
DBD	DNA-Binding Domain
DPP	Disordered Proliferative Pattern
EPAU	Early Pregnancy Assessment Unit
EHP-30	Endometriosis Health Profile questionnaire
ER	Oestrogen Receptor
ESHRE	European Society of Human Reproduction and Embryology
FSH	Follicle Stimulating Hormone
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GnRHa	Gonadotrophin Releasing Hormone agonist

H&E	Haematoxylin and Eosin
HIER	Heat Induced Epitope Retrieval
HPO	Hypothalamic-Pituitary-Ovarian
HRE	Hormone Response Element
HRT	Hormone Replacement Therapy
17β-HSD-2	17β-Hydroxy-Steroid Dehydrogenase type 2
ICAM	Inter-Cellular Adhesion Molecule
IHC	Immunohistochemistry
IMP	Investigational Medicinal Product
IFN-γ	Inter-Feron Gamma
IQ	Interquartile range
LBD	Ligand-Binding Domain
LNG-IUS	Levonorgestrel Intra-Uterine System (Mirena®)
LH	Luteinising Hormone
MAPK	Mitogen-Activated Protein Kinase
MHRA	Medicine and Healthcare products Regulatory Agency
MMP	Matrix Metallo-Proteinase
MPA	Medroxy-Progesterone Acetate
MRI	Magnetic Resonance Imaging
NCoR	Nuclear Receptor Corepressor
NET	Norethisterone
NIHR	National Institute for Health Research
NSAID	Non-Steroidal Anti-Inflammatory Drug

PAEC	PRM Associated Endometrial Changes
PCOS	Polycystic Ovarian Syndrome
PGIC	Patients' Global Impression of Change
POD	Pouch of Douglas
POP	Progesterone Only Pill
PR	Progesterone Receptor
PRM	Progesterone Receptor Modulator
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
QoL	Quality of Life
RCT	Randomised Controlled Trial
SMRT	Silencing Mediator of Retinoic acid and Thyroid hormone receptor
SPRM	Selective Progesterone Receptor Modulator
SRC-1	Steroid Receptor activator
TGF-β	Transforming Growth Factor-Beta
TNF-α	Tumor Necrosis Factor-α
TvUSS	Transvaginal Ultra-Sound Scan
uPA	Urokinase type Plasminogen Activator
UPA	Ulipristal Acetate
VEGF	Vascular Endothelial Growth Factor

REFERENCES

1. Sampson JA. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol. 1927;14(4):422–69.

2. Giudice LC, Kao LC. Endometriosis. Lancet (London, England). 2004 Jan;364(9447):1789–99.

3. Dunselman GAJ, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, et al. ESHRE guideline: management of women with endometriosis. Hum Reprod. 2014 Mar 15;29(3):400–12.

4. Bulun SE. Endometriosis. N Engl J Med. Massachusetts Medical Society; 2009 Jan 15;360(3):268–79.

5. Shaw RW. Gynaecology: Endometriosis. 4th ed. Shaw RW, Luesley D, Monga A, editors. Churchill Livingstone, Elsevier; 2011. 488-508 p.

6. Johnson NP, Hummelshoj L. Consensus on current management of endometriosis. Hum Reprod. 2013 Jun;28(6):1552–68.

7. Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R, Tayade C. Pathophysiology and Immune Dysfunction in Endometriosis. Biomed Res Int. 2015;2015:795976.

8. Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol. 2014;10(5):261–75.

9. Barbieri RL. Stenosis of the external cervical os: an association with endometriosis in women with chronic pelvic pain. Fertil Steril. 1998 Sep;70(3):571–3.

10. Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM. Retrograde menstruation in healthy women and in patients with endometriosis. Obstet Gynecol. 1984 Aug;64(2):151–4.

11. Sampson JA. Metastatic or Embolic Endometriosis, due to the Menstrual Dissemination of Endometrial Tissue into the Venous Circulation. Am J Pathol. 1927 Mar;3(2):93–110.43.

12. Aoki M. ENDOMETRIOSIS OF THE PELVIC LYMPH NODES. Pathol Int. 1967 May;17(2):217–34.

13. JAVERT CT. Pathogenesis of endometriosis based on endometrial homeoplasia, direct extension, exfoliation and implantation, lymphatic and hematogenous metastasis, including five case reports of endometrial tissue in pelvic lymph nodes. Cancer. 1949 May;2(3):399–410.

14. Jerman LF, Hey-Cunningham AJ. The role of the lymphatic system in endometriosis: a comprehensive review of the literature. Biol Reprod. 2015 Mar;92(3):64.

15. Simpson P, Tyler X, Morris E. An unusual clinical presentation and histological findings provide insight into the pathogenesis of endometriosis. Gynecol Surg. Springer Berlin Heidelberg; 2016 Nov 14;13(4):479–84.

16. Gong Y, Tempfer CB. Regional lymphatic spread in women with pelvic endometriosis. Med Hypotheses. 2011 Apr;76(4):560–3.

17. Tempfer CB, Wenzl R, Horvat R, Grimm C, Polterauer S, Buerkle B, et al. Lymphatic spread of endometriosis to pelvic sentinel lymph nodes: a prospective clinical study. Fertil Steril. 2011 Sep;96(3):692–6.

18. Leyendecker G, Herbertz M, Kunz G, Mall G. Endometriosis results from the dislocation of basal endometrium. Hum Reprod. Oxford University Press; 2002 Oct 1;17(10):2725–36.

19. Ferguson BR, Bennington JL, Haber SL. Histochemistry of mucosubstances and histology of mixed müllerian pelvic lymph node glandular inclusions. Evidence for histogenesis by müllerian metaplasia of coelomic epithelium. Obstet Gynecol. 1969 May;33(5):617–25.

20. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril. 2012 Sep;98(3):511–9.

21. Wingfield M, Macpherson A, Healy DL, Rogers PA. Cell proliferation is increased in the endometrium of women with endometriosis. Fertil Steril. 1995 Aug;64(2):340–6.

22. Jones RK, Searle RF, Bulmer JN. Apoptosis and bcl-2 expression in normal human endometrium, endometriosis and adenomyosis. Hum Reprod. 1998 Dec;13(12):3496–502.

23. Sato N, Tsunoda H, Nishida M, Morishita Y, Takimoto Y, Kubo T, et al. Loss of heterozygosity on 10q23.3 and mutation of the tumor suppressor gene PTEN in benign endometrial cyst of the ovary: possible sequence progression from benign endometrial cyst to endometrioid carcinoma and clear cell carcinoma of the ovary. Cancer Res. 2000 Dec 15;60(24):7052–6.

24. Bruse C, Bergqvist A, Carlström K, Fianu-Jonasson A, Lecander I, Astedt B. Fibrinolytic factors in endometriotic tissue, endometrium, peritoneal fluid, and plasma from women with endometriosis and in endometrium and peritoneal fluid from healthy women. Fertil Steril. 1998 Nov;70(5):821–6.

25. Gilabert-Estelles J, Estelles A, Gilabert J, Castello R, Espana F, Falco C, et al. Expression of several components of the plasminogen activator and matrix metalloproteinase systems in endometriosis. Hum Reprod. 2003;18(7):1516–22.

26. Attar E, Bulun SE. Aromatase and other steroidogenic genes in endometriosis: translational aspects. Hum Reprod Update. 2006;12(1):49–56.

27. Noble LS, Takayama K, Zeitoun KM, Putman JM, Johns DA, Hinshelwood MM, et al. Prostaglandin E 2 Stimulates Aromatase Expression in Endometriosis-Derived Stromal Cells 1. J Clin Endocrinol Metab. 1997 Feb;82(2):600–6.

28. Bulun SE, Cheng Y-H, Yin P, Imir G, Utsunomiya H, Attar E, et al. Progesterone resistance in endometriosis: link to failure to metabolize estradiol. Mol Cell Endocrinol. 2006 Mar 27;248(1–2):94–103.

29. Attia GR, Zeitoun K, Edwards D, Johns A, Carr BR, Bulun SE. Progesterone receptor isoform A but not B is expressed in endometriosis. J Clin Endocrinol Metab. 2000 Aug;85(8):2897–902.

30. Burney RO, Talbi S, Hamilton AE, Vo KC, Nyegaard M, Nezhat CR, et al. Gene Expression Analysis of Endometrium Reveals Progesterone Resistance and Candidate Susceptibility Genes in Women with Endometriosis. Endocrinology. 2007 Aug;148(8):3814–26.

31. McLaren J, Prentice A, Charnock-Jones DS, Smith SK. Vascular endothelial growth factor (VEGF) concentrations are elevated in peritoneal fluid of women with endometriosis. Hum Reprod. 1996 Jan;11(1):220–3.

32. Shifren JL, Tseng JF, Zaloudek CJ, Ryan IP, Meng YG, Ferrara N, et al. Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis. J Clin Endocrinol Metab. 1996 Aug;81(8):3112–8.

33. Beste MT, Pfäffle-Doyle N, Prentice EA, Morris SN, Lauffenburger DA, Isaacson KB, et al. Molecular network analysis of endometriosis reveals a role for c-Jun-regulated macrophage activation. Sci Transl Med. 2014 Feb 5;6(222):222ra16.

34. Kwak J-Y, Park S-W, Kim K-H, Na Y-J, Lee K-S. Modulation of neutrophil apoptosis by plasma and peritoneal fluid from patients with advanced endometriosis. Hum Reprod. 2002 Mar;17(3):595–600.

35. Oosterlynck DJ, Cornillie FJ, Waer M, Vandeputte M, Koninckx PR. Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. Fertil Steril. 1991 Jul;56(1):45–51.

36. Somigliana E, Viganò P, Gaffuri B, Candiani M, Busacca M, Di Blasio AM, et al. Modulation of NK cell lytic function by endometrial secretory factors: potential role in endometriosis. Am J Reprod Immunol. 1996 Nov;36(5):295–300.

37. Somigliana E, Viganò P, Gaffuri B, Guarneri D, Busacca M, Vignali M. Human endometrial stromal cells as a source of soluble intercellular adhesion molecule (ICAM)-1 molecules. Hum Reprod. 1996 Jun;11(6):1190–4.

38. Barnhart K, Dunsmoor-Su R, Coutifaris C. Effect of endometriosis on in vitro fertilization. Fertil Steril. 2002 Jun;77(6):1148–55.

39. Maubon A, Faury A, Kapella M, Pouquet M, Piver P. Uterine junctional zone at magnetic resonance imaging: A predictor of in vitro fertilization implantation failure. J Obstet Gynaecol Res. 2010 Jun;36(3):611–8.

40. Ballard K, Seaman H, de Vries C, Wright J. Can symptomatology help in the diagnosis of endometriosis? Findings from a national case-control study-Part 1.

BJOG An Int J Obstet Gynaecol. 2008 Oct;115(11):1382–91.

41. Kuznetsov L, Dworzynski K, Davies M, Overton C, Guideline Committee. Diagnosis and management of endometriosis: summary of NICE guidance. BMJ. British Medical Journal Publishing Group; 2017 Sep 6;358:j3935.

42. NICE. Endometriosis: diagnosis and management | Guidance and guidelines | NICE. NICE; 2017.

43. Stratton P, Winkel C, Premkumar A, Chow C, Wilson J, Hearns-Stokes R, et al. Diagnostic accuracy of laparoscopy, magnetic resonance imaging, and histopathologic examination for the detection of endometriosis. Fertil Steril. 2003 May;79(5):1078–85.

44. American Society for Reproductive Medicine. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril. 1997 May;67(5):817–21.

45. Vincent K, Kennedy S, Stratton P. Pain scoring in endometriosis: entry criteria and outcome measures for clinical trials. Report from the Art and Science of Endometriosis meeting. Fertil Steril. NIH Public Access; 2010 Jan;93(1):62–7.

46. Duffy J, Bhattacharya S, Herman M, Mol B, Vail A, Wilkinson J, et al. Reducing research waste in benign gynaecology and fertility research. BJOG An Int J Obstet Gynaecol. 2017 Feb 1;124(3):366–9.

47. Biberoglu KO, Behrman SJ. Dosage aspects of danazol therapy in endometriosis: short-term and long-term effectiveness. Am J Obstet Gynecol. 1981 Mar 15;139(6):645–54.

48. Allen C, Hopewell S, Prentice A, Gregory D. Nonsteroidal anti-inflammatory drugs for pain in women with endometriosis. In: Allen C, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2009. p. CD004753.

49. Seracchioli R, Mabrouk M, Frascà C, Manuzzi L, Montanari G, Keramyda A, et al. Long-term cyclic and continuous oral contraceptive therapy and endometrioma recurrence: a randomized controlled trial. Fertil Steril. 2010 Jan;93(1):52–6.

50. Davis L-J, Kennedy SS, Moore J, Prentice A. Oral contraceptives for pain associated

with endometriosis. In: Davis L-J, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2007.

51. Abou-Setta AM, Houston B, Al-Inany HG, Farquhar C. Levonorgestrel-releasing intrauterine device (LNG-IUD) for symptomatic endometriosis following surgery. In: Abou-Setta AM, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2013. p. CD005072.

52. Brown J, Kives S, Akhtar M. Progestagens and anti-progestagens for pain associated with endometriosis. In: Brown J, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2012.

53. Cheong YC, Smotra G, Williams AC de C. Non-surgical interventions for the management of chronic pelvic pain. Cochrane Database Syst Rev. John Wiley & Sons, Ltd; 2014 Mar 5;

54. Brown J, Pan A, Hart RJ. Gonadotrophin-releasing hormone analogues for pain associated with endometriosis. In: Brown J, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2010. p. CD008475.

55. Farmer JE, Prentice A, Breeze A, Ahmad G, Duffy JM, Watson A, et al. Gonadotrophin-releasing hormone analogues for endometriosis: bone mineral density. In: Prentice A, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2003. p. CD001297.

56. Jacobson TZ, Duffy JM, Barlow DH, Koninckx PR, Garry R. Laparoscopic surgery for pelvic pain associated with endometriosis. In: Jacobson TZ, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2009. p. CD001300.

57. Duffy JM, Arambage K, Correa FJ, Olive D, Farquhar C, Garry R, et al. Laparoscopic surgery for endometriosis. In: Duffy JM, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2014.

58. Healey M, Ang WC, Cheng C. Surgical treatment of endometriosis: a prospective randomized double-blinded trial comparing excision and ablation. Fertil Steril. 2010 Dec;94(7):2536–40.

59. ABBOTT J, HAWE J, HUNTER D, HOLMES M, FINN P, GARRY R. Laparoscopic excision of endometriosis: A randomized, placebo-controlled trial. Fertil Steril. 2004 Oct;82(4):878–84.

60. Angioni S, Pontis A, Dessole M, Surico D, De Cicco Nardone C, Melis I. Pain control and quality of life after laparoscopic en-block resection of deep infiltrating endometriosis (DIE) vs. incomplete surgical treatment with or without GnRHa administration after surgery. Arch Gynecol Obstet. 2015 Feb;291(2):363–70.

61. Vercellini P, Somigliana E, Viganò P, De Matteis S, Barbara G, Fedele L. The effect of second-line surgery on reproductive performance of women with recurrent endometriosis: A systematic review. Acta Obstet Gynecol Scand. 2009 Jan;88(10):1074–82.

62. Brown J, Farquhar C. Endometriosis: an overview of Cochrane Reviews. Cochrane Database Syst Rev. John Wiley & Sons, Ltd; 2014 Mar 10;

63. Barbieri RL. Hormone treatment of endometriosis: the estrogen threshold hypothesis. Am J Obstet Gynecol. 1992 Mar;166(2):740–5.

64. Barbieri RL. Endometriosis and the estrogen threshold theory. Relation to surgical and medical treatment. J Reprod Med. 1998 Mar;43(3 Suppl):287–92.

65. Sadler TW. Langman's Medical Embryology. Lippincott Williams Wilkins, Inc.; 2006.

66. Sujan S, Lumsden M-A. Menstruation and menstrual disorders. In: Shaw RW, Luesley D, Monga A, editors. Gynaecology. Fourth. Churchill Livingstone, Elsevier; 2011. p. 448–72.

67. Kumar V (The U of C, Abbas AK (University of C, Fausto N (University of WS of M. Robbins and Cotran Pathologic Basis of Disease. 7th ed. Philadelphia: Elsevier Saunders; 2005. 1079-91 p.

68. Tortora G, Grabowski S. Principles of Anatomy & Physiology. 10th Editi. Wiley; 2003.

69. Shaw RW. Control of hypothalamic-pituitary-ovarian function. In: Shaw RW, Luesley D, Monga A, editors. Gynaecology. Fourth. Churchill Livingstone, Elsevier;

2011. p. 197–211.

70. Tsutsumi R, Webster NJG. GnRH pulsatility, the pituitary response and reproductive dysfunction. Endocr J. 2009;56(6):729–37.

71. Ferenczy A, Mutter GL. The Endometrial Cycle. Glob Libr Women's Med. 2009;

72. Murray MJ, Meyer WR, Zaino RJ, Lessey BA, Novotny DB, Ireland K, et al. A critical analysis of the accuracy, reproducibility, and clinical utility of histologic endometrial dating in fertile women. Fertil Steril. 2004 May;81(5):1333–43.

73. Pernick N. Dating of Endometrium [Internet]. www.pathologyoutlines.com. 2012. Available from: http://pathologyoutlines.com/topic/uterusdating.html

74. Pernick N. Disordered proliferative hyperplasia [Internet]. www.pathologyoutlines.com. 2011. Available from: http://pathologyoutlines.com/topic/uterusdisorderedproliferative.html

75. Reed SD, Newton KM, Clinton WL, Epplein M, Garcia R, Allison K, et al. Incidence of endometrial hyperplasia. Am J Obstet Gynecol. Elsevier; 2009 Jun;200(6):678.e1-678.e6.

76. Lacey J V, Chia VM. Endometrial hyperplasia and the risk of progression to carcinoma. Maturitas. 2009;63:39–44.

77. Pernick N. Endometrial Hyperplasia [Internet]. www.pathologyoutlines.com. 2011. Available from: http://pathologyoutlines.com/topic/uterusendometrialhyperplasiageneral.html

78. Moore E, Shafi M. Endometrial hyperplasia. Obstet Gynaecol Reprod Med. 2013;23(3):88–93.

79. Salman MC, Usubutun A, Boynukalin K, Yuce K. Comparison of WHO and endometrial intraepithelial neoplasia classifications in predicting the presence of coexistent malignancy in endometrial hyperplasia. J Gynecol Oncol. 2010 Jun;21(2):97–101.

80. Allison KH, Reed SD, Voigt LF, Jordan CD, Newton KM, Garcia RL. Diagnosing endometrial hyperplasia: why is it so difficult to agree? Am J Surg Pathol. 2008

May;32(5):691–8.

81. Shutter J. Endometrial Polyp [Internet]. www.pathologyoutlines.com. 2012. Available from: http://pathologyoutlines.com/topic/uterusendopolyp.html

82. Doll A, Abal M, Rigau M, Monge M, Gonzalez M, Demajo S, et al. Novel molecular profiles of endometrial cancer-new light through old windows. J Steroid Biochem Mol Biol. 2008 Feb;108(3–5):221–9.

83. Lacey J V, Sherman ME, Rush BB, Ronnett BM, Ioffe OB, Duggan MA, et al. Absolute risk of endometrial carcinoma during 20-year follow-up among women with endometrial hyperplasia. J Clin Oncol. 2010 Feb 10;28(5):788–92.

84. Mutter GL, Lin MC, Fitzgerald JT, Kum JB, Baak JP, Lees JA, et al. Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. J Natl Cancer Inst. 2000 Jun 7;92(11):924–30.

85. Mutter GL, Matius-Guiu X, Lax SF. Endometrial adenocarcinoma. In: Robboy SJ, Mutter GL, Prat J, Bentley RC, Russel P, Anderson MC, editors. Robboy's Pathology of the Female Reproductive Tract. Second. Churchill Livingstone, Elsevier; 2009. p. 393–426.

86. Mutter G, Ferenczy A. Endometrial Hyperplasia and Neoplasia: Definition, Diagnosis, and Management Principles. Glob Libr Women's Med. 2009;

87. Kim AH, Adamson GD. Endometriosis. Glob Libr Women's Med. 2009;

88. Pernick N. Endometriosis [Internet]. www.pathologyoutlines.com. 2011 [cited 2016 Jun 15]. Available from: http://pathologyoutlines.com/topic/uterusendometriosis.html

89. Robboy SJ, Haney A, Russel P. Endometriosis. In: Robboy SJ, Mutter GL, Prat J, Bentley RC, Russel P, Anderson MC, editors. Robboy's Pathology of the Female Reproductive Tract. Second. Churchill Livingstone, Elsevier; 2009. p. 515–41.

90. Kondi-Pafitis A. Pathological Aspects of Endometriosis. In: Chaudhury K, editor. Endometriosis- Basic Concepts and Current Research Trends. InTech; 2012. p. 101–12.

91. Al-Khawaja M, Tan P-H, MacLennan GT, Lopez-Beltran A, Montironi R, Cheng L. Ureteral endometriosis: clinicopathological and immunohistochemical study of 7 cases. Hum Pathol. 2008 Jun;39(6):954–9.

92. Critchley HOD, Kelly RW, Baird DT, Brenner RM. Regulation of human endometrial function: mechanisms relevant to uterine bleeding. Reprod Biol Endocrinol. 2006;4 Suppl 1:S5.

93. Conneely OM, Lydon JP. Progesterone receptors in reproduction: functional impact of the A and B isoforms. Steroids. 2000;65(10):571–7.

94. Wölfler MM, Küppers M, Rath W, Buck VU, Meinhold-Heerlein I, Classen-Linke I. Altered expression of progesterone receptor isoforms A and B in human eutopic endometrium in endometriosis patients. Ann Anat. 2016 Apr 2;206:1–6.

95. Mertens HJMM, Heineman MJ, Theunissen PHMH, de Jong FH, Evers JLH, Muechler EK, et al. Androgen, estrogen and progesterone receptor expression in the human uterus during the menstrual cycle. Eur J Obstet Gynecol Reprod Biol. Elsevier; 2001 Sep;98(1):58–65.

96. Slayden OD, Brenner RM. Hormonal regulation and localization of estrogen, progestin and androgen receptors in the endometrium of nonhuman primates: effects of progesterone receptor antagonists. Arch Histol Cytol. 2004 Dec;67(5):393–409.

97. Narvekar N, Cameron S, Critchley HOD, Lin S, Cheng L, Baird DT. Low-dose mifepristone inhibits endometrial proliferation and up-regulates androgen receptor. J Clin Endocrinol Metab. 2004 May;89(5):2491–7.

98. Groisman GM, Meir A. CD10 is helpful in detecting occult or inconspicuous endometrial stromal cells in cases of presumptive endometriosis. Arch Pathol Lab Med. 2003 Aug;127(8):1003–6.

99. Wei J-J, William J, Bulun S. Endometriosis and ovarian cancer: a review of clinical, pathologic, and molecular aspects. Int J Gynecol Pathol. NIH Public Access; 2011 Nov;30(6):553–68.

100. Kahyaoglu I, Kahyaoglu S, Moraloglu O, Zergeroglu S, Sut N, Batioglu S, et al.

Comparison of Ki-67 proliferative index between eutopic and ectopic endometrium: a case control study. Taiwan J Obstet Gynecol. Elsevier; 2012 Sep;51(3):393–6.

101. Brown DC, Gatter KC. Monoclonal antibody Ki-67: its use in histopathology. Histopathology. 1990 Dec;17(6):489–503.

102. Jonat W, Arnold N. Is the Ki-67 labelling index ready for clinical use? Ann Oncol. 2011 Mar 1;22(3):500–2.

103. Mertens HJMM, Heineman MJ, Evers JLH. The expression of apoptosis-related proteins Bcl-2 and Ki67 in endometrium of ovulatory menstrual cycles. Gynecol Obstet Invest. 2002;53(4):224–30.

104. Kim TH, Yu Y, Luo L, Lydon JP, Jeong J-W, Kim JJ. Activated AKT pathway promotes establishment of endometriosis. Endocrinology. 2014 May;155(5):1921–30.

105. Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol. 2012 May;13(5):283–96.

106. Kapucuoglu N, Aktepe F, Kaya H, Bircan S, Karahan N, Ciriş M. Immunohistochemical expression of PTEN in normal, hyperplastic and malignant endometrium and its correlation with hormone receptors, bcl-2, bax, and apoptotic index. Pathol Res Pract. 2007;203(3):153–62.

107. Lacey J V., Mutter GL, Ronnett BM, Ioffe OB, Duggan MA, Rush BB, et al. PTEN Expression in Endometrial Biopsies as a Marker of Progression to Endometrial Carcinoma. Cancer Res. 2008 Jul 15;68(14):6014–20.

108. Laudanski P, Szamatowicz J, Kowalczuk O, Kuźmicki M, Grabowicz M, Chyczewski L. Expression of selected tumor suppressor and oncogenes in endometrium of women with endometriosis. Hum Reprod. 2009 Aug;24(8):1880–90.

109. Zhang H, Zhao X, Liu S, Li J, Wen Z, Li M. 17betaE2 promotes cell proliferation in endometriosis by decreasing PTEN via NFkappaB-dependent pathway. Mol Cell Endocrinol. 2010 Apr 12;317(1–2):31–43.

110. Govatati S, Kodati VL, Deenadayal M, Chakravarty B, Shivaji S, Bhanoori M. Mutations in the PTEN tumor gene and risk of endometriosis: a case-control

study. Hum Reprod. 2014 Feb;29(2):324–36.

111. Machado DE, Abrao MS, Berardo PT, Takiya CM, Nasciutti LE. Vascular density and distribution of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (Flk-1) are significantly higher in patients with deeply infiltrating endometriosis affecting the rectum. Fertil Steril. 2008 Jul;90(1):148–55.

112. Liu H, Lang JH. Is abnormal eutopic endometrium the cause of endometriosis? The role of eutopic endometrium in pathogenesis of endometriosis. Med Sci Monit. 2011 Apr;17(4):RA92-9.

113. Bouchard P, Chabbert-Buffet N, Fauser BCJM. Selective progesterone receptor modulators in reproductive medicine: pharmacology, clinical efficacy and safety. Fertil Steril. 2011 Nov;96(5):1175–89.

114. Graham JD, Clarke CL. Physiological Action of Progesterone in Target Tissues [1]. Endocr Rev. Endocrine Society; 1997 Aug;18(4):502–19.

115. Wagenfeld A, Saunders PTK, Whitaker L, Critchley HOD. Selective progesterone receptor modulators (SPRMs): progesterone receptor action, mode of action on the endometrium and treatment options in gynecological therapies. Expert Opin Ther Targets. 2016 May 14;1–10.

116. Gellersen B, Fernandes MS, Brosens JJ. Non-genomic progesterone actions in female reproduction. Hum Reprod Update. 2009;15(1):119–38.

117. Evans RM. The steroid and thyroid hormone receptor superfamily. Science. 1988 May 13;240(4854):889–95.

118. Patel B, Elguero S, Thakore S, Dahoud W, Bedaiwy M, Mesiano S. Role of nuclear progesterone receptor isoforms in uterine pathophysiology. Hum Reprod Update. 2015 Jan;21(2):155–73.

119. Ribeiro RC, Kushner PJ, Baxter JD. The nuclear hormone receptor gene superfamily. Annu Rev Med. 1995;46:443–53.

120. Leonhardt SA, Boonyaratanakornkit V, Edwards DP. Progesterone receptor transcription and non-transcription signaling mechanisms. Steroids. 2003;68:761–70.

121. Nuclear receptor. Wikipedia. 2016.

122. Roemer SC, Donham DC, Sherman L, Pon VH, Edwards DP, Churchill MEA. Structure of the Progesterone Receptor-Deoxyribonucleic Acid Complex: Novel Interactions Required for Binding to Half-Site Response Elements. Mol Endocrinol. Endocrine Society ; 2006 Dec;20(12):3042–52.

123. Rousseau-Merck MF, Misrahi M, Loosfelt H, Milgrom E, Berger R. Localization of the human progesterone receptor gene to chromosome 11q22-q23. Hum Genet. 1987 Nov;77(3):280–2.

124. Chabbert-Buffet N, Meduri G, Bouchard P, Spitz IM. Selective progesterone receptor modulators and progesterone antagonists: mechanisms of action and clinical applications. Hum Reprod Update. Oxford University Press; 2005 Jan 1;11(3):293–307.

125. Chabbert-Buffet N, Pintiaux A, Bouchard P. The immninent dawn of SPRMs in obstetrics and gynecology. Mol Cell Endocrinol. 2012 Jul 25;358(2):232–43.

126. Scarpin KM, Graham JD, Mote PA, Clarke CL. Progesterone action in human tissues: regulation by progesterone receptor (PR) isoform expression, nuclear positioning and coregulator expression. Nucl Recept Signal. Nuclear Receptor Signaling Atlas (NURSA); 2009 Dec 31;7:e009.

127. Boonyaratanakornkit V, Scott MP, Ribon V, Sherman L, Anderson SM, Maller JL, et al. Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases. Mol Cell. 2001 Aug;8(2):269–80.

128. Xu J, Li Q. Review of the *in Vivo* Functions of the p160 Steroid Receptor Coactivator Family. Mol Endocrinol. 2003 Sep;17(9):1681–92.

129. Han SJ, DeMayo FJ, Xu J, Tsai SY, Tsai M-J, O'Malley BW. Steroid Receptor Coactivator (SRC)-1 and SRC-3 Differentially Modulate Tissue-Specific Activation Functions of the Progesterone Receptor. Mol Endocrinol. 2006 Jan;20(1):45–55.

130. Madauss KP, Grygielko ET, Deng S-J, Sulpizio AC, Stanley TB, Wu C, et al. A structural and in vitro characterization of asoprisnil: a selective progesterone

receptor modulator. Mol Endocrinol. 2007 May;21(5):1066–81.

131. Vallejo G, La Greca AD, Tarifa-Reischle IC, Mestre-Citrinovitz AC, Ballaré C, Beato M, et al. CDC2 Mediates Progestin Initiated Endometrial Stromal Cell Proliferation: A PR Signaling to Gene Expression Independently of Its Binding to Chromatin. Lydon JP, editor. PLoS One. Public Library of Science; 2014 May 23;9(5):e97311.

132. Lu J, Reese J, Zhou Y, Hirsch E. Progesterone-induced activation of membrane-bound progesterone receptors in murine macrophage cells. J Endocrinol. NIH Public Access; 2015 Feb;224(2):183–94.

133. Balasubramanian B, Portillo W, Reyna A, Chen JZ, Moore AN, Dash PK, et al. Nonclassical Mechanisms of Progesterone Action in the Brain: I. Protein Kinase C Activation in the Hypothalamus of Female Rats. Endocrinology. Oxford University Press; 2008 Nov;149(11):5509–17.

134. Balasubramanian B, Portillo W, Reyna A, Chen JZ, Moore AN, Dash PK, et al. Nonclassical Mechanisms of Progesterone Action in the Brain: II. Role of Calmodulin-Dependent Protein Kinase II in Progesterone-Mediated Signaling in the Hypothalamus of Female Rats. Endocrinology. Oxford University Press; 2008 Nov;149(11):5518–26.

135. Mote PA, Balleine RL, McGowan EM, Clarke CL. Colocalization of progesterone receptors A and B by dual immunofluorescent histochemistry in human endometrium during the menstrual cycle. J Clin Endocrinol Metab. 1999 Aug;84(8):2963–71.

136. Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL, McCarty KS. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. J Clin Endocrinol Metab. 1988 Aug;67(2):334–40.

137. Al-Sabbagh M, Lam EW-F, Brosens JJ. Mechanisms of endometrial progesterone resistance. Mol Cell Endocrinol. 2012 Jul 25;358(2):208–15.

138. Clarke CL, Zaino RJ, Feil PD, Miller J V, Steck ME, Ohlsson-Wilhelm BM, et al. Monoclonal antibodies to human progesterone receptor: characterization by

biochemical and immunohistochemical techniques. Endocrinology. 1987 Sep;121(3):1123–32.

139. Mote PA, Johnston JF, Manninen T, Tuohimaa P, Clarke CL. Detection of progesterone receptor forms A and B by immunohistochemical analysis. J Clin Pathol. 2001 Aug;54(8):624–30.

140. Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM. Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. Proc Natl Acad Sci U S A. National Academy of Sciences; 2003 Aug 19;100(17):9744–9.

141. Mulac-Jericevic B, Mullinax RA, DeMayo FJ, Lydon JP, Conneely OM. Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform. Science. 2000 Sep 8;289(5485):1751–4.

142. Misao R, Iwagaki S, Fujimoto J, Sun W, Tamaya T. Dominant expression of progesterone receptor form B mRNA in ovarian endometriosis. Horm Res. 1999;52(1):30–4.

143. Attia GR, Zeitoun K, Edwards D, Johns A, Carr BR, Bulun SE. Progesterone Receptor Isoform A But Not B Is Expressed in Endometriosis [1]. J Clin Endocrinol Metab. 2000 Aug;85(8):2897–902.

144. Jones CJP, Inuwa IM, Nardo LG, Litta P, Fazleabas AT. Eutopic endometrium from women with endometriosis shows altered ultrastructure and glycosylation compared to that from healthy controls--a pilot observational study. Reprod Sci. NIH Public Access; 2009 Jun;16(6):559–72.

145. Moguilewsky M, Deraedt R, Teutsch S, Philibert D. 203. RU 38486 — An antiglucocorticoid with a new mechanism of antihormonal activity. J Steroid Biochem. Pergamon; 1982;17(3):lxviii.

146. Philibert D, Deraedt R, Tournemine C, Mary I, Teutsch G. 204. RU 38486 — A potent antiprogesterone. J Steroid Biochem. Pergamon; 1982;17(3):lxviii.

147. Healy DL, Fraser HM. The antiprogesterones are coming: menses induction, abortion, and labour? Br Med J (Clin Res Ed). BMJ Group; 1985 Feb 23;290(6468):580–1.

148. Healy DL, Baulieu EE, Hodgen GD. Induction of menstruation by an antiprogesterone steroid (RU 486) in primates: site of action, dose-response relationships, and hormonal effects. Fertil Steril. 1983 Aug;40(2):253–7.

149. Herrmann W, Wyss R, Riondel A, Philibert D, Teutsch G, Sakiz E, et al. [The effects of an antiprogesterone steroid in women: interruption of the menstrual cycle and of early pregnancy]. C R Seances Acad Sci III. 1982 May 17;294(18):933–8.

150. Ulmann A, Silvestre L. RU486: The French experience. Hum Reprod. Oxford University Press; 1994 Jun 1;9(suppl 1):126–30.

151. Kapp N, Lohr PA, Ngo TD, Hayes JL. Cervical preparation for first trimester surgical abortion. Cochrane database Syst Rev. 2010;(2):CD007207.

152. RCOG. The Care of Women Requesting Induced Abortion The Care of Women Requesting Induced Abortion Evidence-based Clinical Guideline Number 7. 2011;

153. Chwalisz K, Brenner RM, Fuhrmann UU, Hess-Stumpp H, Elger W. Antiproliferative effects of progesterone antagonists and progesterone receptor modulators on the endometrium. Steroids. 2000;65(10):741–51.

154. Mutter GL, Bergeron C, Deligdisch L, Ferenczy A, Glant M, Merino M, et al. The spectrum of endometrial pathology induced by progesterone receptor modulators. Mod Pathol. 2008 May;21(5):591–8.

155. Afhüppe W, Sommer A, Müller J, Schwede W, Fuhrmann U, Möller C. Global gene expression profiling of progesterone receptor modulators in T47D cells provides a new classification system. J Steroid Biochem Mol Biol. 2009 Jan;113(1–2):105–15.

156. Bouchard P. Selective progesterone receptor modulators: a class with multiple actions and applications in reproductive endocrinology, and gynecology. Gynecol Endocrinol. 2014 Oct;30(10):683–4.

157. Giangrande PH, Kimbrel EA, Edwards DP, McDonnell DP. The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. Mol Cell Biol. 2000 May;20(9):3102–15.

158. Liu Z, Auboeuf D, Wong J, Chen JD, Tsai SY, Tsai M-J, et al. Coactivator/corepressor

ratios modulate PR-mediated transcription by the selective receptor modulator RU486.

159. Slayden OD, Nayak NR, Burton KA, Chwalisz K, Cameron ST, Critchley HO, et al. Progesterone antagonists increase androgen receptor expression in the rhesus macaque and human endometrium. J Clin Endocrinol Metab. 2001 Jun;86(6):2668–79.

160. Whitaker LHR, Murray AA, Matthews R, Shaw G, Williams ARW, Saunders PTK, et al. Selective progesterone receptor modulator (SPRM) ulipristal acetate (UPA) and its effects on the human endometrium. Hum Reprod. 2017 Jan 26;

161. Cameron ST, Critchley HO, Thong KJ, Buckley CH, Williams AR, Baird DT. Effects of daily low dose mifepristone on endometrial maturation and proliferation. Hum Reprod. 1996 Nov;11(11):2518–26.

162. Murphy AA, Zhou MH, Malkapuram S, Santanam N, Parthasarathy S, Sidell N. RU486-induced growth inhibition of human endometrial cells. Fertil Steril. 2000 Nov;74(5):1014–9.

163. Williams ARW, Critchley HOD, Osei J, Ingamells S, Cameron IT, Han C, et al. The effects of the selective progesterone receptor modulator asoprisnil on the morphology of uterine tissues after 3 months treatment in patients with symptomatic uterine leiomyomata. Hum Reprod. 2007 Jun;22(6):1696–704.

164. Horne FM, Blithe DL. Progesterone receptor modulators and the endometrium: changes and consequences. Hum Reprod Update. 2007 Jan;13(6):567–80.

165. Chwalisz K, Perez MC, Demanno D, Winkel C, Schubert G, Elger W. Selective progesterone receptor modulator development and use in the treatment of leiomyomata and endometriosis. Endocr Rev. 2005 May;26(3):423–38.

166. Kettel LM, Murphy AA, Mortola JF, Liu JH, Ulmann A, Yen SS. Endocrine responses to long-term administration of the antiprogesterone RU486 in patients with pelvic endometriosis. Fertil Steril. 1991 Sep;56(3):402–7.

167. Kettel LM, Murphy AA, Morales AJ, Ulmann A, Baulieu EE, Yen SS. Treatment of endometriosis with the antiprogesterone mifepristone (RU486). Fertil Steril. 1996

Jan;65(1):23–8.

168. Kettel LM, Murphy AA, Morales AJ, Yen SS. Preliminary report on the treatment of endometriosis with low-dose mifepristone (RU 486). Am J Obstet Gynecol. 1998 Jun;178(6):1151–6.

169. Han S, Sidell N. RU486-induced growth inhibition of human endometrial cells involves the nuclear factor-kappa B signaling pathway. J Clin Endocrinol Metab. 2003 Feb;88(2):713–9.

170. Li X, Bao Y, Fang P, Chen Y, Qiao Z. Effect of mifepristone on COX-2 both in eutopic and ectopic endometrium in mouse endometriotic model. Arch Gynecol Obstet. Springer-Verlag; 2012 Oct 31;286(4):939–46.

171. Mei L, Bao J, Tang L, Zhang C, Wang H, Sun L, et al. A novel mifepristone-loaded implant for long-term treatment of endometriosis: in vitro and in vivo studies. Eur J Pharm Sci. 2010 Mar 18;39(5):421–7.

172. Schubert G, Elger W, Kaufmann G, Schneider B, Reddersen G, Chwalisz K. Discovery, Chemistry, and Reproductive Pharmacology of Asoprisnil and Related 11β-Benzaldoxime Substituted Selective Progesterone Receptor Modulators (SPRMs). Semin Reprod Med. 2005 Feb;23(1):58–73.

173. DeManno D, Elger W, Garg R, Lee R, Schneider B, Hess-Stumpp H, et al. Asoprisnil (J867): a selective progesterone receptor modulator for gynecological therapy. Steroids. 2003;68(10):1019–32.

174. Chwalisz K, Elger W, Stickler T, Mattia-Goldberg C, Larsen L. The effects of 1-month administration of asoprisnil (J867), a selective progesterone receptor modulator, in healthy premenopausal women. Hum Reprod. 2005 Apr;20(4):1090–9.

175. Chwalisz K, Larsen L, Mattia-Goldberg C, Edmonds A, Elger W, Winkel CA. A randomized, controlled trial of asoprisnil, a novel selective progesterone receptor modulator, in women with uterine leiomyomata. Fertil Steril. 2007 Jun;87(6):1399–412.

176. Wilkens J, Williams ARW, Chwalisz K, Han C, Cameron IT, Critchley HOD. Effect of

asoprisnil on uterine proliferation markers and endometrial expression of the tumour suppressor gene, PTEN. Hum Reprod. 2009 May;24(5):1036–44.

177. Stoeckemann K, Hegele-Hartung C, Chwalisz K. Effects of the progesterone antagonists onapristone (ZK 98 299) and ZK 136 799 on surgically induced endometriosis in intact rats. Hum Reprod. 1995 Dec;10(12):3264–71.

178. Ioffe OB, Zaino RJ, Mutter GL. Endometrial changes from short-term therapy with CDB-4124, a selective progesterone receptor modulator. Mod Pathol. 2009 Mar;22(3):450–9.

179. Pontis A, D'Alterio MN, Pirarba S, de Angelis C, Tinelli R, Angioni S. Adenomyosis: a systematic review of medical treatment. Gynecol Endocrinol. 2016 Sep 5;32(9):696–700.

180. Morassutto C, Monasta L, Ricci G, Barbone F, Ronfani L. Incidence and Estimated Prevalence of Endometriosis and Adenomyosis in Northeast Italy: A Data Linkage Study. Shen R, editor. PLoS One. Public Library of Science; 2016 Apr 21;11(4):e0154227.

181. Benagiano G, Bastianelli C, Farris M, Brosens I. Selective progesterone receptor modulators: an update. Expert Opin Pharmacother. 2014 Jul 3;15(10):1403–15.

182. Wang Y, Jiang X, Wang S. The influence of mifepristone to caspase 3 expression in adenomyosis. Clin Exp Obstet Gynecol. 2014;41(2):154–7.

183. European Medicines Agency. ellaOne 30 mg Tablets (ulipristal acetate) - Summary of Product Characteristics (SPC) - (eMC) [Internet]. 2009. p. 1–29. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/001027/human_med_000758.jsp&mid=WC0b01ac058001d124

184. McKeage K, Croxtall JD. Ulipristal Acetate. Drugs. 2011 May 7;71(7):935–45.

185. Lethaby A, Vollenhoven B, Sowter MC. Pre-operative GnRH analogue therapy before hysterectomy or myomectomy for uterine fibroids. In: Lethaby A, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2001. p. CD000547.

186. Baird DD, Dunson DB, Hill MC, Cousins D, Schectman JM. High cumulative

incidence of uterine leiomyoma in black and white women: ultrasound evidence. Am J Obstet Gynecol. 2003 Jan;188(1):100–7.

187. Buttram VC, Reiter RC. Uterine leiomyomata: etiology, symptomatology, and management. Fertil Steril. 1981 Oct;36(4):433–45.

188. Weston G, Healy DL. Gynaecology: Uterine fibroids. 4th ed. Shaw RW, Luesley D, Monga A, editors. Churchill Livingstone, Elsevier; 2011. 473-487 p.

189. European Medicines Agency. Esmya 5 mg Tablets (ulipristal acetate) - Summary of Product Characteristics (SPC) - (eMC) [Internet]. 2013 [cited 2015 Sep 22]. p. 1–28. Available from: http://www.medicines.org.uk/EMC/medicine/26068/SPC/

190. Trefoux Bourdet A, Luton D, Koskas M. Clinical utility of ulipristal acetate for the treatment of uterine fibroids: current evidence. Int J Womens Health. Dove Press; 2015 Jan 26;7:321–30.

191. Maruo T, Matsuo H, Shimomura Y, Kurachi O, Gao Z, Nakago S, et al. Effects of progesterone on growth factor expression in human uterine leiomyoma. Steroids. 2003 Nov;68(10–13):817–24.

192. Horak P, Mara M, Dundr P, Kubinova K, Kuzel D, Hudecek R, et al. Effect of a Selective Progesterone Receptor Modulator on Induction of Apoptosis in Uterine Fibroids In Vivo. Int J Endocrinol. Hindawi Publishing Corporation; 2012;2012:1–6.

193. Maruo T, Matsuo H, Samoto T, Shimomura Y, Kurachi O, Gao Z, et al. Effects of progesterone on uterine leiomyoma growth and apoptosis. Steroids. 2000;65(10–11):585–92.

194. Biglia N, Carinelli S, Maiorana A, D'Alonzo M, Lo Monte G, Marci R. Ulipristal acetate: a novel pharmacological approach for the treatment of uterine fibroids. Drug Des Devel Ther. Dove Press; 2014;8:285–92.

195. Talaulikar VS, Manyonda I. Progesterone and progesterone receptor modulators in the management of symptomatic uterine fibroids. Eur J Obstet Gynecol Reprod Biol. 2012 Dec;165(2):135–40.

196. Yoshida S, Ohara N, Xu Q, Chen W, Wang J, Nakabayashi K, et al. Cell-Type Specific

Actions of Progesterone Receptor Modulators in the Regulation of Uterine Leiomyoma Growth. Semin Reprod Med. 2010 May 22;28(3):260–73.

197. Donnez J, Tatarchuk TF, Bouchard P, Puscasiu L, Zakharenko NF, Ivanova T, et al. Ulipristal Acetate versus Placebo for Fibroid Treatment before Surgery. N Engl J Med. Massachusetts Medical Society; 2012 Feb 1;366(5):409–20.

198. Donnez J, Tomaszewski J, Vázquez F, Bouchard P, Lemieszczuk B, Baró F, et al. Ulipristal Acetate versus Leuprolide Acetate for Uterine Fibroids. N Engl J Med. Massachusetts Medical Society; 2012 Feb 1;366(5):421–32.

199. Donnez J, Vázquez F, Tomaszewski J, Nouri K, Bouchard P, Fauser BCJM, et al. Long-term treatment of uterine fibroids with ulipristal acetate ☆. Fertil Steril. 2014 Jun;101(6):1565-73-18.

200. Donnez J, Hudecek R, Donnez O, Matule D, Arhendt H-J, Zatik J, et al. Efficacy and safety of repeated use of ulipristal acetate in uterine fibroids. Fertil Steril. Elsevier; 2015 Sep 22;103(2):519–527.e3.

201. National Centre for Biotechnology Information. Ulipristal Acetate. PubChem Compound Database. 2017.

202. Williams ARW. ESMYA (ulipristal acetate): Pathologist's guide. PRM-Associated Endometrial Changes (PAEC). 2013.

203. Williams ARW, Bergeron C, Barlow DH, Ferenczy A. Endometrial Morphology After Treatment of Uterine Fibroids With the Selective Progesterone Receptor Modulator, Ulipristal Acetate. Int J Gynecol Pathol. 2012 Nov;31(6):556–69.

204. Spitz. Clinical utility of progesterone receptor modulators and their effect on the endometrium. Curr Opin Obstet Gynecol. Lippincott Williams Wilkins, Inc.; 2009;21(4):318–24.

205. Chwalisz K, Garg R, Brenner RM, Schubert G, Elger W. Selective progesterone receptor modulators (SPRMs): a novel therapeutic concept in endometriosis. Ann N Y Acad Sci. 2002 Mar;955:373-88-93, 396–406.

206. Huniadi CA, Pop OL, Antal TA, Stamatian F. The effects of ulipristal on Bax/Bcl-2, cytochrome C, Ki-67 and cyclooxygenase-2 expression in a rat model with

surgically induced endometriosis. Eur J Obstet Gynaecol Reprod Biol. 2013;169:360–5.

207. Bateman J, Bougie O, Singh S, Islam S. Histomorphological changes in endometriosis in a patient treated with ulipristal: A case report. Pathol - Res Pract. 2017 Jan;213(1):79–81.

208. Wdowiak A. Pre-treatment with ulipristal acetate before ICSI procedure: a. Menopausal Rev. 2013;6:496–500.

209. Monleón J, Martínez-Varea A, Galliano D, Pellicer A. Successful pregnancy after treatment with ulipristal acetate for uterine fibroids. Case Rep Obstet Gynecol. 2014 Jan;2014:314587.

210. Murad K. Spontaneous Pregnancy Following Ulipristal Acetate Treatment in a Woman with a Symptomatic Uterine Fibroid. J Obstet Gynaecol Canada JOGC = J d'obstétrique gynécologie du Canada JOGC. Elsevier; 2016 Jan 1;38(1):75–9.

211. Luyckx M, Squifflet J-L, Jadoul P, Votino R, Dolmans M-M, Donnez J. First series of 18 pregnancies after ulipristal acetate treatment for uterine fibroids. Fertil Steril. 2014 Nov;102(5):1404–9.

212. MHRA. Medicines & Healthcare products Regulatory Agency. www.gov.uk.

213. MHRA. IS IT A CLINICAL TRIAL OF A MEDICINAL PRODUCT? www.gov.uk. 2014.

214. MRC/DH/MHRA. Risk-adapted Approaches to the Management of Clinical Trials of Investigational Medicinal Products. 2011.

215. NIHR. The Clinical Trials Toolkit - Routemap. www.ct-toolkit.ac.uk. http://www.ct-toolkit.ac.uk; 2013. p. 1.

216. NIHR. Clinical Trials Toolkit [Internet]. www.ct-toolkit.ac.uk. Available from: http://www.ct-toolkit.ac.uk

217. European Medicines Agency. ICH E6 (R1) Good clinical practice guideline. European Medicines Agency; 2002. p. 1–48.

218. European Medicines Agency. UPA- Summary of Product Characteristics (Original 18-12_2013). 2013.

219. European Commission. EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use Annex 13. European Commission; 2010. p. 1–19.

220. Jones G, Jenkinson C, Kennedy S. The Endometriosis Health Profile User Manual. 2001. p. 1–104.

221. Jones G, Jenkinson C, Kennedy S. Evaluating the responsiveness of the endometriosis health profile questionnaire: The EHP-30. Qual Life Res. 2004 Apr;13(3):705–13.

222. Deal LS, DiBenedetti D, Williams VS, Fehnel SE. The development and validation of the daily electronic Endometriosis Pain and Bleeding Diary. Health Qual Life Outcomes. 2010 Jul 2;8(1):64.

223. Hurst H, Bolton J. Assessing the clinical significance of change scores recorded on subjective outcome measures. J Manipulative Physiol Ther. 2004 Jan;27(1):26–35.

224. Leake R, Barnes D, Pinder S, Ellis I, Anderson L, Anderson T, et al. Immunohistochemical detection of steroid receptors in breast cancer: a working protocol. UK Receptor Group, UK NEQAS, The Scottish Breast Cancer Pathology Group, and The Receptor and Biomarker Study Group of the EORTC. J Clin Pathol. 2000 Aug;53(8):634–5.

225. Viera AJ, Garrett JM. Understanding Interobserver Agreement: The Kappa Statistic. Fam Med. 2005;37(5):360–3.

226. Feinstein AR, Cicchetti D V. High agreement but low kappa: I. The problems of two paradoxes. J Clin Epidemiol. 1990;43(6):543–9.

227. Cicchetti D V, Feinstein AR. High agreement but low kappa: II. Resolving the paradoxes. J Clin Epidemiol. 1990;43(6):551–8.

228. Health and Social Care Information Centre. Statistics on Smoking. 2016.

229. Dworkin RH, Turk DC, Farrar JT, Haythornthwaite JA, Jensen MP, Katz NP, et al. Topical Review and Recommendations Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. Pain. 2005;113:9–19.

230. McCambridge J, Witton J, Elbourne DR. Systematic review of the Hawthorne effect: new concepts are needed to study research participation effects. J Clin Epidemiol. Elsevier; 2014 Mar;67(3):267–77.

231. Brown J, Farquhar C. An Overview of Treatments for Endometriosis. JAMA. 2015;313(3).

APPENDIX

1 SmPC- Ulipristal Acetate (Appendix 1)

2 CHUTE Study Labels V1.0 (Appendix 2)

3 CHUTE Study Prescription V2.0 (Appendix 3)

4 Patient Information Sheet (CHUTE) v3.0 (Appendix 4)

5 Participant Consent Form (CHUTE) v3.0 (Appendix 5)

6 Case Report Form (CRF) (CHUTE) v3.0 (Appendix 6)

7 BSGE Pelvic Pain Questionnaire v1.1 (Appendix 7)

8 Endometriosis Health Profile Questionnaire (EHP-30) v1.1 (Appendix 8)

9 Composite Pelvic Signs & Symptoms Score (CPSSS) v1.1 (Appendix 9)

10 Patients Global Impression of Change (PGIC) scale v1.1 (Appendix 10)

11 HIS.PR.P.01 Selecting Processing Programs (Appendix 11)

12 HIS.BL.P.1 Embedding Procedures (Appendix 12)

13 HIS.BL.P.2 Cutting Procedures and Guidelines (Appendix 13)

14 HIS.IM.P.02 Section Cutting for IHC (Appendix 14)

15 HIS.SL.P.3 Leica Autostainer Protocols (Appendix 15)

16 HIS.IM.P.01 Setting Up A Staining Run (Appendix 16)

17 HIS.IM.P.03 Antibody & Reagent Preparation (Appendix 17)

18 HIS.IM.P.10 Antibody Verification Validation (Appendix 18)

19 H1(30) (Appendix 19)

20 H1(20) (Appendix 20)

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24 Histopathology Plan (CHUTE) v1.2 (Appendix 24)

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26 Material Transfer Agreement (CHUTE) (11-03-15) (Appendix 26)

27 Results Summaries (Appendix 27)

28 Research Protocol (CHUTE) v3.1 (Appendix 28)