Chapter 1

General introduction
PURPOSE AND MOTIVATION

Endometriosis prevalence rates in the general population are unknown, because a definitive diagnosis is established only at laparoscopy and histological confirmation, and diagnostic accuracy depends on the surgeon’s skills and experience in the disease. However, based on community prevalence estimates of symptoms (1–3), endometriosis probably affects 10% of all and 30%–50% of symptomatic premenopausal women (4). This represents 176 million affected women worldwide (5). Recently, two prospective, multi-center studies, evaluated thousands of endometriosis patients, from different countries and ethnicities. The investigators demonstrated a significant reduction in health-related quality of life in endometriosis patients compared to women without endometriosis. They underlined the economic burden associated with endometriosis arising from treatment costs and productivity loss (6, 7).

Endometriosis may develop anywhere within the pelvis and on other extrapelvic sites. Macroscopically, three forms of endometriosis are described: 1) superficial (peritoneal), 2) ovarian endometriosis (endometriotic cyst of the ovary or ovarian endometrioma) and 3) deep infiltrating endometriosis (DIE)(8). Deeply infiltrating endometriotic nodules extend > 5mm underneath the peritoneal surface and may involve the utero-sacral ligaments, vagina, bowel, bladder or ureters. The depth of infiltration is related to the type and severity of symptoms. Where present, endometriotic tissues can be associated with extensive fibrosis and adhesion formation causing marked distortion of pelvic anatomy, which is one of the main factors inducing subfertility in those patients (9). The association between disease stage, according to the revised American Fertility Society (AFS) classification, and severity of pain was studied in a large group of women with endometriosis. However, it has been demonstrated that this relationship between pain severity and disease stage was marginal and inconsistent (10).

Recently, there is an emphasis on the “problem-oriented” approach for management of endometriosis. Balancing the effect of the kind of treatment (including medical-, surgical- and/or ART treatments), on resolving the patient’s problems and improving her quality of life are taken into consideration. The choice of treatment is based on various factors such as size, location and extent of the disease, organ dysfunction, type and severity of symptoms, wish for pregnancy, age of the patient and the cost of therapy (11).

Endometriosis surgery is based mainly on removal of visible endometriotic lesions and it significantly reduces painful symptoms and improves quality of life (12). However, complete excision (described by some authors as radical removal), with conservation of reproductive functions, remains a real challenge. Endometriosis surgery can be associated with peri-operative morbidity, pain recurrence and re-operation at long-term follow-up (13,14). On the other hand, medical treatment is not cytoreductive and has two targets: pain control and suppression of disease.
progress. In this context, combined oral contraceptives (COC) can be considered as they are relatively safe, well tolerated and inexpensive. However, the exact role of COC in management of endometriosis sub-types is not well defined (15-17).

The main emphasis of this thesis is to investigate certain aspects in the clinical management of endometriosis. We aim to explore the following:

- The role of long-term COC therapy in the prevention of postoperative pain recurrence and anatomical relapse after ovarian endometriosis surgery.
- The effect of pre-operative COC use in patients with posterior DIE.
- The correlation between histological pattern and clinical outcomes in patients undergoing segmental bowel resection for colorectal endometriosis.
- The impact on sexual function of laparoscopic treatment and subsequent COC therapy in women with deep infiltrating endometriosis.
- The effect of surgical treatment of DIE on Quality of life.
- The performance of peripheral (serum and molecular) blood markers for diagnosis and follow up of endometriosis.
GENERAL INTRODUCTION

Endometriosis is classically defined as the growth of endometrial glands and stroma outside the uterine cavity, most commonly implanted over visceral and peritoneal surfaces within the female pelvis (18). Although numerous studies have investigated into the origins of endometriosis, it remains an enigmatic disorder in that the cause, the natural history and the precise mechanisms of its presentation are still not well known (19). Endometriosis remains, however, a leading cause of disability in women of reproductive age, responsible for pelvic pain, organ dysfunction and/or subfertility; all of them adversely affecting the quality of life (20).

Pathogenesis

The exact etiology of endometriosis is unknown. However, many theories have been proposed to explain the multiple clinical presentations of the disease. Genetic, hormonal, immunological, environmental and angiogenetic factors are suggested to be implicated in the pathogenesis of endometriosis.

Some of the theories tried to explain the histogenesis of endometriosis may be divided in:

a) Theories that assume the transplantation of endometrium on extra-uterine sites.

b) Theories hypothesizing that endometriosis arise on sites of metaplasia of the coelomic peritoneum.

c) Theories that favor the induction of mesenchyme to produce endometriotic tissues under the influence of various factors.

Sampson’s theory of transplantation is the most widely accepted (18). According to this theory the menstrual flow products migrate in a retrograde fashion through the fallopian tubes into the peritoneal cavity. The endometrial products implant, grow and produce tissues with morphology of endometrial glands and stroma (18). Laparoscopic studies show that during menstruation there is blood in the peritoneal cavity of most women and the retrograde menstrual flow is indeed a usual phenomenon which explains the endometriotic implantation in the ovary and the uterosacral ligaments. Experimental findings support the transplantation theory and suggest that endometriosis develops in two distinct phases. In the first phase, the uterine products are invasive and ovarian hormone-dependent and in the second phase, express endogenous estrogen receptors. By the first month after inoculation of primate models with menstrual endometrial tissues these elements attach, grow and invade through the peritoneal mesothelial covering and express estrogen receptor-beta, although aromatase activity is expressed later (21). Other routes of implantation such as vascular and/or lymphatic spread and direct implantation are reported and explain the development of endometriosis in distant and unusual places such as lung or lymph nodes.
The metaplastic theory proposes that the peritoneum adjacent to ovaries is a multipotentential tissue which may obtain characteristics of endometrial tissue (22, 23). This theory is supported by the observation that endometriosis may develop in cases where no retrograde menstrual flow is possible such as in Rokitansky-Kuster-Hauser syndrome (24).

Finally, according to the induction theory which adds information to the transplantation theory, the endometriotic glands and stroma develop de novo from the host tissues, stimulated by the transplanted endometrium. Most pathologists favor a combination of the metastatic and metaplastic theories and support the theory that endometriosis represents a polygenic disorder, with alterations in multiple biological pathways (25, 26) leading to a metaplastic process under the irritating effect of endometrial tissue shed during retrograde menstrual flow.

**Clinical presentation**

Although women with endometriosis may be asymptomatic, symptoms are common and typically include pelvic pain and subfertility. The atypical presentation, the lack of awareness about this disease and many other factors contribute to a considerable delay in diagnosis and management of endometriosis with important consequences on women’s quality of life and their work productivity (27, 28).

The following symptoms can be caused by endometriosis:

- Severe dysmenorrhea;
- Deep dyspareunia;
- Chronic pelvic pain;
- Ovulation pain;
- Cyclical or peri-menstrual symptoms (e.g. bowel or bladder associated) with or without abnormal bleeding;
- Infertility;
- Chronic fatigue.

However, the predictive value of any one symptom or set of symptoms remains uncertain as each of these symptoms can have other causes. A large group of women with endometriosis is completely asymptomatic. In these women, endometriosis remains undiagnosed or is diagnosed at laparoscopy for another indication. A subset of women with more advanced disease, ovarian or DIE, can be asymptomatic as well. (29)

Physical examination of the pelvis is useful for the diagnosis of deep infiltrating lesions or endometriotic cysts. However, the overall accuracy of bimanual pelvic examination alone is not satisfying (30).
Imaging and endometriosis diagnosis:
Many imaging modalities have been described in evaluating endometriosis, the most widely used of them include: Transvaginal Sonography (TVS), Transrectal Sonography (TRS), Rectal Endoscopic Sonography (RES) and magnetic resonance imaging (MRI). TVS with color flow Doppler is usually the first imaging modality for screening of patients with pelvic pain and/or subfertility. In experienced hands it can also be used for diagnosis of adenomyosis, bladder endometriosis as well as rectal endometriosis, with a high degree of accuracy (31-34). TRS and TVS showed similar degrees of accuracy in detecting bowel wall involvement in endometriosis. (34). Compared to TVS, RES showed less accuracy for diagnosis of DIE at specific locations (35) MRI is a valid imaging modality for the evaluation of patients with DIE, showing high accuracy in the diagnosis and prediction of disease extent. The MRI diagnosis of deep pelvic endometriosis is based on the conjoint presence of signal intensity and morphologic abnormalities in the anterior and posterior compartments of the pelvis and the presence of surrounding fibrosis. Nonetheless, an accurate diagnosis therefore resides in clinical awareness and systematic examination and instrumental review via sonographic examination or MRI. (36-38). Imaging modalities, however, are limited in their ability to detect small peritoneal implants and adhesions (39). A more challenging point is the diagnosis of some locations of DIE, such diagnosis can be missed even on laparoscopic inspection because of the poor correlation between extent of retroperitoneal disease and the appearance of the overlying peritoneum. (40)

Management of endometriosis
Endometriosis has several unique biological properties. Despite being a benign pathology, it behaves as a chronic and recurrent disease with some malignant characteristics. Some patients have quiescent disease with rare episodes of pain, while others have frequent, recurrent pain. These differing symptom patterns are often not correlated with lesion size or extent of disease. Current approaches for managing endometriosis are symptom oriented, aimed mainly at treating chronic pelvic pain and/or subfertility. Management protocols may also be targeted at slowing disease progression or preventing recurrence.
Medical, surgical or a combined approach can be chosen. In addition, assisted reproductive techniques (ART) are frequently used to treat endometriosis-associated subfertility.
The following discussion reviews medical treatment of endometriosis, the various surgical approaches for endometriosis management, including conservative procedures, definitive interventions, and adjunct surgical treatments. (28, 41)
Medical treatment

Medical therapeutic drugs may be either non-hormonal or hormonal. Non-hormonal therapeutic options, mainly work on inflammatory and immunologic aspect of endometriosis, and hormonal attempts basically deprive endometriotic implants from hormones. Medical hormonal treatment has always been described for management of mild-moderate disease. However, the effect of medical treatment in terms of pain relief in women with rectovaginal endometriosis appears substantial (42).

The following drug categories are widely used in management of endometriosis:

- Combined Oral Contraceptives (COC)
- Selective progesterone receptor modulators, progestogens and anti progestins
- Gn-RH analogues (Gn-RH-a).
- Androgens.
- Other hormonal regulators and modulators (Aromatase inhibitor, Cox2- inhibitors, Angiogenesis inhibitors, Modulation of Cytokines, inhibition of Matrix Metalloproteinase)
- Gene therapy.

**Combined Oral Contraceptives (COC)**

Combined oral contraceptives have been used for women with endometriosis since the 1950s (43). This medication consists of a low combination of estrogen and progesterone. It has a feed-back blocking mechanism of action leading to inhibition of ovulation and ovarian production of steroid hormones. It has been shown to be effective for patients with mild symptoms of endometriosis (44). The main advantages are that its use is inexpensive and is usually reasonably well tolerated by women. It can also be taken safely for many years if necessary. However, it is not free of side effects. These include irregular vaginal bleeding, fluid retention, abdominal bloating, weight gain, increased appetite, nausea, headaches, breast tenderness and depression (45). These drugs can be used conventionally in a cyclic regimen or may be used continuously, without a break for withdrawal menses. The continuous regimen may be preferable for its decreased frequency of menses for women who fail to achieve pain relief with cyclic COC therapy. Long-term COC therapy can be a reliable adjuvant post-operative measure to prevent or reduce frequency/severity of recurrent dysmenorrhoea and anatomical relapse of endometriosis.

In this thesis, (studies I, II, II) we investigate the potential role of COC in the clinical management of endometriosis.
Selective progesteron receptor modulators, progestogens and anti progestins

a) Progestogens (eg. Medroxyprogesterone acetate, Megestrol acetate, Lynoestrenol, Dydrogestrone, Levonorgestrel)

Progestogens appear to work by both directly inhibiting the functional effects of estrogen on endometrial cell proliferation, and also suppressing ovarian function, to induce anovulatory amenorrhoea, with acceptable control of pain symptoms and disease progression (46). From the clinical practice, it seems that the efficacy of this class of agents in patients with endometriosis, however, is relatively modest and the tolerability (breakthrough bleeding and bloating), as well as, concerns on the long term safety (risk of breast cancer and thromboembolism, effect on bone mineral density) has also limited their broader utility.

A locally acting, **Levonorgestrel**-releasing intrauterine system (LNG-IUS) may be a useful form of prolonged progestogen therapy for endometriosis. The role of the LNG IUS in management of endometriosis has been evaluated by multiple studies. As a postoperative adjunct to surgical ablation for endometriosis, when compared with expectant management, or traditional medical therapies as GnRH analogues, LNG IUS showed effectiveness in the management of pain symptoms, reduction of pelvic pain recurrence and increasing rate of patients’ satisfaction (47-52).

**Dienogest** (DNG), a progestin of 19-nortestosterone derivative, has good oral bioavailability and is highly selective for progesterone receptors. Owing to its anti-ovulatory, anti-proliferative activities in endometrial cells, and its inhibitory effects on the secretion of cytokines, DNG is expected to be an effective treatment for endometriosis. Progesterone receptor-binding affinity is higher for DNG than for progesterone. DNG shows low binding to the androgen receptor and almost negligible binding to the estrogen receptor, glucocorticoid receptor and mineralocorticoid receptor (53).

Treatment with a GnRH-a followed by long-term Dienogest therapy may maintain the relief of endometriosis-associated pelvic pain achieved with GnRH-a therapy for at least 12 months. This regimen might reduce the amount of irregular uterine bleeding that often occurs during the early phase of Dienogest therapy (54, 55).

b) Antiprogestins:

**Gestrinone** (ethynorgestrienone; R2323) is an antiprogestational agent prescribed in Europe for the treatment of endometriosis. Gestrinone equals the effectiveness of Danazol and of GnRH agonists for relief of endometriosis related pain. Mifepristone is a progesterone receptor antagonist, currently approved for use in medication abortion and, in some countries, as an emergency contraceptive, but some trials have demonstrated a reduction in endometriosis symptoms with Mifepristone therapy (56,57). Randomized double-blind placebo-controlled trials have been conducted in the treatment of fibroids with mifepristone, CDB-4124 (Proellex), CDB-2914 (VA 2914, Ulipristal) and asoprisnil (J867). CDB-4124 has shown to be effective in endometriosis as well (58).
Gn-RH analogues

Treatment with GnRH-agonists for 3 months is as effective as the 6-months treatment as far as pain is concerned, and when combined with estro-progestational agents (‘add-back therapy’) up to a maximum of 2 years, is effective for pain control and safe in terms of protecting bone density (41). These drugs act by blocking the GnRH receptor directly and preventing it from activating. This results in a down regulation of the pituitary gland, a reduction of gonadotropin secretion, and a suppression of ovarian steroid production. Unlike GnRH agonists, however, GnRH antagonists do not cause an initial stimulation of gonadotropin and ovarian hormone secretion. At the molecular level, GnRH antagonists interrupt the basic activation process of the GnRH receptor, blocking the receptor dimerization synthesis and secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Given the high binding affinity, relative abundance and long half-life of the antagonist, these molecules monopolize the GnRH receptors. As a result of the above characteristics, the GnRH antagonists offer the theoretical advantage of working faster and more effectively than GnRH agonists, with better patient compliance because of earlier amelioration of symptoms (59).

Androgens

The first medication approved for the treatment of endometriosis in the United States was the androgen Danazol. The predominant mechanism of action appears to be suppression of midcycle luteinizing hormone (LH) surge, creating a chronic anovulatory state as a result, Danazol creates a hypoestrogenic, hyperandrogenic state, inducing endometrial atrophy in endometriotic implants. For endometriosis management, the recommended dosage of Danazol is 600 to 800 mg daily. Significant androgenic side effects develop at this dosage and include acne, hot flashes, hirsutism, adverse serum lipid profiles, voice deepening (possibly irreversible), elevation of liver enzymes, and mood changes (56).

Other hormonal regulators and modulators

Aromatase inhibitors

Large quantities of estrogen can be produced locally within ectopically located endometrium (endometriosis tissue) via an intracrine mechanism, via the expression of the enzyme aromatase. This enzyme, not expressed in normal endometrium, is stimulated by prostaglandin E2 (PGE2); the resulting estrogen production then stimulates PGE2, further enhancing estrogen. An obvious therapeutic target would thus be this aromatase enzyme and aromatase inhibitors were tested in the rodent endometriosis model, with good success (60, 61).
**Cox2- inhibitors**

COX-2 selective inhibitors given at the minimal dosage can be effective against the pelvic pain symptoms (dysmenorrhea, dyspareunia, and chronic pelvic pain) associated to endometriosis. Some data were generated suggesting a potential use of COX-2 selective inhibitors, usually in combination with other hormonal treatments. However, they seem to be not well tolerated and display serious side effects which lower the compliance considerably (62-64).

**Angiogenesis inhibitors**

The most prominently studied among angiogenic factors is the vascular endothelial growth factor (VEGF), which is responsible for inducing early vascular growth. In any event, one logical therapeutic step would be to attempt inhibition of these new vascular structures as a way of deterring the development of endometriosis. A human study conducted with an angiogenesis inhibitor for the treatment of endometriosis associated pain with thalidomide demonstrated a noted pain relief in these patients (65).

**Modulation of Cytokines, inhibition of Matrix Metalloproteinase**

In rats with experimental endometriosis, recombinant human TNF-α-binding protein can reduce 64% of the size of endometriosis-like peritoneal lesions (66). In nude mice, suppression of MMPs by progesterone or by a natural inhibitor slows the establishment of ectopic lesions by human endometrium. (67)

**Gene Therapy**

HOX genes, encoding homeodomain transcription factors, are dynamically expressed in endometrium, where they are necessary for endometrial growth, differentiation, and implantation. In human endometrium, the expression of HOXA10 and HOXA11 is driven by sex steroids, with peak expression occurring at time of implantation in response to rising progesterone levels. However, the maximal HOXA10 and HOXA11 expression fails to occur in women with endometriosis resistance to progesterone which can explain inhospitable implantation environment and medical treatment failures in endometriosis. Alterations in progesterone receptor expression and decreased HOX gene expression secondary to hypermethylation of its promoter region are the possible mechanisms of the progesterone resistance. A gene therapy approach involving the manipulation of HOXA10 expression or by using DNA demethylation agents to restore methylation aberrations can potentially have a role in the future treatment of endometriosis (68-70).
**Surgical treatments**

The goal of surgery is to restore normal anatomy through excision/ablation of all visible endometriotic lesions and associated adhesions. In the literature, there is a debate about which of the two methods (excision or ablation) is better for endometriosis management (12, 71, 72).

For ovarian endometriosis, there is good evidence that excisional surgery for endometriomata (of greater than 3cm in size) provides for a more favourable outcome than drainage and ablation with regard to the recurrence of the endometrioma, recurrence of pain symptoms, and in women who were previously subfertile, subsequent spontaneous pregnancy (73). Considering the approach to surgery, laparoscopic treatment of endometriosis seems to provide a long-term substantial relief of symptoms for a significant percentage of women (74-75).

Surgical treatment of DIE (Deeply Infiltrating Endometriosis) requires a multidisciplinary team, able to perform surgery in the gynecological, urological, gastrointestinal and nervous structures of the pelvis, as the disease ‘knows no boundaries’. Rectal endometriosis management is a matter of debate in the recent literature. The removal of rectal endometriosis is mainly conducted using either of three procedures: colorectal segmental resection, discoid intestinal resection and rectal nodule excision without opening the rectum. The choice of the operative procedure depends on different parameters: size of the nodule, rectal circumference involved in the disease, frequency of multifocal intestinal nodules and of other associated deep lesions and the surgeon’s experience (76, 77).

Bladder endometriosis, once deemed relatively uncommon, is now increasingly recognized. Surgical management of bladder endometriosis includes resection of bladder nodule, with or without full-thickness excision of the bladder wall, with subsequent bladder reconstruction (78).

Ureter endometriosis is usually related with reproductive tract endometriosis. It can be extrinsic (peri-ureteral) or intrinsic (involving the ureteral wall). It has insidious process resulting in difficulty for early diagnosis and may be complicated by renal function loss. The laparoscopic approach for ureteral endometriosis is well accepted and has a reasonable incidence of complications, as well as a low rate of recurrence. Ureterolysis could be used as the initial surgical step for patients with extrinsic ureteral endometriosis, and may be the only treatment if the extension of ureteral involvement is limited in length and there is no residual ureteral damage or dilatation. For patients presenting with extended ureteral involvement, stenosis, or moderate or severe hydronephrosis with a high risk of having intrinsic ureteral disease, ureterolysis is probably insufficient and ureteral resection with end-to-end anastomosis or ureteroneocystostomy represent the surgical solution (79-82).
Recurrence

A frustrating aspect in endometriosis management is the tendency to recur after surgery. The recurrence rate is about 5% to 20% per year, reaching a cumulative rate of 40% after 5 years. Some investigators found an approximately 15-percent rate of recurrence at 2 years following initial surgery. Pain recurs within 5 years in about one in five patients with pelvic pain treated by complete laparoscopic excision of visible endometriotic lesions (83-84). The risk factors for recurrence as reported by different studies are often conflicting. For ovarian endometrioma, it seems that younger age, rAFS stage/score at surgery, size of the largest cyst and previous surgical/ medical treatment for endometriosis can be considered risk factors for recurrence (85).

Non-Invasive markers for early detection and follow-up of endometriosis

Being a chronic, recurrent disease, many studies are focusing on identifying markers for the detection and follow-up of endometriosis. Many reports have suggested that various serum, peritoneal fluid and tissue markers might be associated with endometriosis. The identification of more sensitive and specific markers of endometriosis should facilitate the development of accurate and non-invasive techniques for its diagnosis and prognosis as well as follow-up. The early diagnosis of endometriosis and/or endometriosis recurrence could prevent the possible progression of endometriosis, resulting in less suffering from pain, infertility and deteriorating quality of life. However, there is no agreement about a single, ideal, non-invasive, marker that can be used for screening, diagnosis and/or follow-up of endometriosis (86). Classes of endometriosis markers are summarized in Table 1 (86).
| SERUM AND/OR PERITONEAL FLUID MARKERS | Glycoproteins | CA125  
|                                      |              | CA19-9  
| Growth factors                       | Hepatocyte growth factor (SF/HGF) |  
|                                      | Fibroblast growth factor (FGF)    |  
|                                      | Epidermal growth factor (EGF)     |  
|                                      | Transforming growth factor-alpha (TGF-α) |  
|                                      | Transforming growth factor-beta (TGF-β) |  
|                                      | Vascular endothelial growth factor (VEGF) |  
|                                      | Epidermal growth factor receptor (EGF-R) |  
|                                      | Insulin-like growth factor I (IGF-I) |  
| Cytokines                            | TNF-α        |  
|                                      | IL-1         |  
|                                      | IL-6         |  
|                                      | IL-8         |  
|                                      | Monocyte chemoattractant protein (MCP)-1 |  
|                                      | Interferon-γ |  
| Autoantibodies                       | Antiendometrial antibodies |  
|                                      | Autoantibodies to oxidized lipoproteins |  
|                                      | Thyroid peroxidase antibodies |  
|                                      | IgG anti-laminin-1 antibodies |  
|                                      | Anti-phospholipid antibodies |  
| Hormones                             | Luteinizing hormone (LH) |  
|                                      | Progesterone |  
|                                      | Estradiol    |  
|                                      | Thyroid stimulating hormone (TSH) |  
|                                      | Follicle stimulating hormone (FSH) |  
|                                      | Leptin       |  
| Proteolytic enzymes and their inhibitors | Matrix metalloproteinases (MMPs) |  
|                                      | Tissue inhibitors for MMPs (TIMPs) |  
| Soluble adhesion molecules           | Intercellular adhesions molecule-1 (sICAM-1) |  
|                                      | Human leukocyte class I antigens (sHLA-I) |  
|                                      | E-cadherin   |  
| Environmental contaminant            | Dioxin-like chemicals |  
| ENDOMETRIAL MARKERS                  | Cell adhesion molecules (CAMs) |  
|                                      | Proteolytic enzymes |  
| ENDOMETRIAL TISSUE BIOCHEMICAL MARKERS | Aromatase P450 |  
|                                      | Hormone receptors |  
| GENETIC MARKERS                      | Survivin gene expression |  
|                                      | p53 mutations |  
|                                      | Polymorphisms |  

**Table 1:** Summary of endometriosis markers

In this thesis we aimed to investigate the role of COC in management of ovarian and deep infiltrating endometriosis. We also explored the correlation between clinical/ surgical outcomes of segmental colorectal resection and histological patterns of bowel DIE.

In addition, we tried to examine the impact of DIE surgery only or followed by COC therapy on QOL and quality of sexual function in women treated for DIE. Finally, we sought to evaluate the performance of peripheral blood markers for diagnosis and prognosis of endometriosis.
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