Structural and molecular features of the endomyometrium in endometriosis and adenomyosis

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BACKGROUND: Adenomyosis and endometriosis were initially described as ‘adenomyoma’. When the retrograde menstruation theory became widely accepted to explain the pathogenesis of endometriosis, since it does not explain adenomyosis, the two conditions came to be seen as distinct entities. However, emerging evidence suggests that both diseases may be linked to changes in the inner portion of the myometrium. In addition, similar anomalies were found in the eutopic endometrium of both conditions and the debate has re-opened. A common origin for both adenomyosis and endometriosis would have relevance not only for understanding uterine function and pathophysiology, but also for clinical management and treatment.

METHODS: The Scopus and Medline databases were searched for all original articles published in English up to the end of 2012. Search terms included ‘adenomyosis’; ‘endometriosis’; ‘endometrium’; ‘eutopic endometrium’; ‘inner myometrium’; ‘junctional zone’. Special attention was paid to articles comparing features of eutopic endometrium in the two conditions.

RESULTS: A number of similarities exist between adenomyosis and endometriosis and, by using magnetic resonance and laparoscopy, it was found that, at least in some subgroups, the two conditions often coexist. In both situations the inner myometrium (or junctional zone) is altered, although alterations are much more marked in adenomyosis where a thickness > 12 mm is today considered sufficient for diagnosis. Research has shown differences between the eutopic endometrium of women with both diseases when compared with controls. There is an immune dysfunction and there are alterations of adhesion molecules, cell proliferation and apoptosis. An increase in cytokines and inflammatory mediators has also been observed. Finally, the presence of oxidative stress and anomalies in free-radical metabolism may alter uterine receptivity. When the two conditions were compared, dissimilarities were also observed in the extent of apoptosis inhibition and in the expression of some inflammatory mediators. It is not clear if observed differences are primarily related to presenting symptoms. Finally, both conditions are steroid dependent and...
research suggests a role for epigenetic mechanisms. The analysis indicates that much of the published research may have been influenced by the method of diagnosis and/or has not been controlled for the presenting symptoms, the concomitant presence of both diseases or full consideration of fluctuations within cycle phase.

**CONCLUSIONS:** It is difficult to draw firm conclusions from existing evidence since major diagnostic limitations still exist and there is a systematic bias in clinical presentation. In addition, scanty information is available on the natural history of endometriosis and no studies exist on the natural history of adenomyosis. Notwithstanding these limitations, a number of similarities, but also some differences have been found between the eutopic endometrium in the two diseases. These findings need to be taken with considerable caution as the few instances where the research was repeated yielded conflicting results.

**Key words:** adenomyosis / endometriosis / eutopic endometrium / myometrium junctional zone

**Introduction**

Although uterine adenomyosis and endometriosis were described around the turn of the XIX century, their pathogenesis and role in reproduction remain uncertain. Endometriosis and adenomyosis are defined as the presence of endometrial glands and stroma outside the uterus and within the myometrium, respectively. The prevailing view of the conditions has changed over time, but by the end of the first quarter of last century they came to be seen as separate entities (Benagiano and Brosens, 2011; Benagiano et al., 2009). Both diseases were originally described as ‘adenomyoma’. In 1925, Frankl coined the word ‘adenomyosis’ to describe the mucosal invasion of the myometrium and made the first clear distinction between adenomyosis and adenomyoma, the former being characterized by the direct connection of the endometrium with islands of mucosa located in the musculature, which in adenomyosis are accompanied by stromal and smooth muscle hyperplasia. Two years later, Sampson (1927) proposed retrograde menstruation as a mechanism for what he coined ‘peritoneal endometriosis’. This theory became widely accepted, but, as it does not explain adenomyosis, the two conditions came to be seen as distinct. More recent evidence suggests that both conditions may be linked to changes that occur in the inner portion of the myometrium or the junctional zone (JZ) and to molecular abnormalities in the eutopic endometrium (Templeman et al., 2010a; Benagiano and Brosens, 2012; Brosens et al., 2012).

Here we describe the evolution of the modern concepts of endometriosis and adenomyosis and discuss the implications of the observed features of the myometrial JZ and of the eutopic endometrium in the two conditions.

**Methods**

This review is based on a literature search for research on eutopic endometrium and on the JZ in women with adenomyosis to compare identified features with those of eutopic endometrium in endometriosis. The Scopus and Medline databases were searched for all original articles published in English up to the end of 2012. Search terms were ‘adenomyosis’; ‘endometriosis’; ‘eutopic endometrium’; ‘inner myometrium’ and ‘junctional zone myometrium’. Our focus was on the human endometrium. Reference was made to animal studies for added clarity.

**Results**

**Modern concepts of endometriosis and adenomyosis**

Forty-five years after Frankl (1925) coined the name ‘adenomyosis’, Bird (1972) provided the following definition: ‘Adenomyosis may be defined as the benign invasion of endometrium into the myometrium, producing a diffusely enlarged uterus which microscopically exhibits ectopic non-neoplastic, endometrial glands and stroma surrounded by the hypertrophic and hyperplastic myometrium’. More recently, Levy et al. (2013) have pointed out that new imaging techniques have shown that the condition is asymptomatic in one-third of cases and—when there are clinical signs—they remain non-specific. Endometriosis, on the other hand, is defined as “the presence of functional endometriallike tissue outside the uterus, but in the pelvic cavity”, a definition criticised for including asymptomatic disease and excluding extra peritoneal locations. In 1991, a European group suggested that additionally, there should be evidence that the lesions be cellularly active, or have an effect on normal physiology (Audebert et al., 1992).

Today, the dominant view of endometriosis and adenomyosis seems influenced by the observation that they are diseases with different clinical profiles (Templeman et al., 2008). A woman with endometriosis is often nulligravid, is significantly younger and may have an affected mother or sister. Endometriosis is frequently diagnosed by laparoscopy in the course of infertility investigations. In contrast, a woman with adenomyosis is often parous and relatively older. Adenomyosis has also been linked to a history of induced abortion. Women with adenomyosis have a history of early menarche and shorter cycles (Parazzini et al., 2009). These observations, however, may be a function of the method of diagnosis since, until recently, adenomyosis was only diagnosed through histological examination of uterine specimens. This can significantly impact patient profile.

The introduction of laparoscopy in the 1960s (Palmer, 1964) caused a veritable rise in interest in endometriosis and better characterization of the patient profile, although limitations still exist because of the invasive nature of the test, which is only justifiable in symptomatic patients. Despite the availability of high-definition ultrasonography (HD-US), interest in adenomyosis lagged behind well after the introduction of magnetic resonance imaging (MRI) (Hricak et al., 1983). MRI remains expensive and not widely available.

There is lack of agreement on the diagnostic criteria and the significance of ectopic endometrial tissue. In relation to endometriosis, the concept of minimal endometriosis was introduced when laparoscopy revealed the multiple appearances of the peritoneal disease (Vasquez et al., 1984). Soon afterwards, the possibility was raised that subtle types of endometriosis may be a consequence rather than the cause of delayed pregnancy. Cornillie et al. (1990) demonstrated that deep endometriosis defined by a depth of 5 mm or more occurs in a large percentage of women with infertility and/or pain. The observation that a higher percentage of superficial (58%) and deep (68%) but only 25% of intermediate nodules were ‘active’ led some authors to propose that superficial lesions may be a transient or, indeed, a natural...
The JZ myometrium in endometriosis and adenomyosis

Today, imaging techniques allow an accurate, non-invasive evaluation of adenomyosis; the first to evaluate the accuracy of transvaginal ultrasound (TVUS) and MRI in the diagnosis of adenomyosis and to compare them to histological findings were Reinhold et al. (1996). They reported a sensitivity and specificity of 89% for both conditions and concluded that TVUS was as accurate as MRI. Bazot et al. (2001) also correlated the imaging diagnosis (TVUS and MRI) of adenomyosis with histological findings and found no difference in accuracy between TVUS and MRI, although a lower sensitivity was observed in the presence of leiomyomas. Exacoustos et al. (2011) used 2D- and 3D-TVUS in a group with subsequent histological prevalence of adenomyosis of 44.4%. They found the presence of myometrial cysts to be the most specific (98%) and a heterogeneous myometrium the most sensitive (88%) 2D-TVUS feature. The two 3D-TVUS markers: a difference in JZ thickness (JZdiff) ≥ 4 mm and JZ infiltration and distortion, had high sensitivity (88%) and the best accuracy (85 and 82%, respectively).

A number of abnormal JZ features have been observed in women with adenomyosis: usually there is either a diffuse or focal thickening of the JZ, creating an ill-defined area of low signal intensity (smooth muscle hyperplasia), with or without embedded bright foci (islands of ectopic endometrial tissue and cystic dilatation of glands). In general, thickening is more pronounced in the portion of the JZ corresponding to where the adenomyotic foci are located (Tamai et al., 2005). A pictorial review of US, hysterosalpingography and MRI findings in women with adenomyosis has been recently published (Valentini et al., 2011).

In addition to JZ abnormalities, there are also ultra-structural differences between smooth muscle cells from adenomyosis and normal myometrium, with myocytes showing cellular hypertrophy and differences in cytoplasmic organelles, nuclear structures and intercellular junctions (Benagiano et al., 2012). These differences also exist in areas removed from adenomyotic foci, suggesting that they are not a reaction to the presence of ectopic endometrium (Mehasseb et al., 2010a, 2011).

An altered JZ has also been found in women with endometriosis (Fig. 1). Kunz et al. (2000) compared JZ thickness in a group of infertile women who had endometriosis with a group with male factor infertility who did not have endometriosis. They reported that whilst the thickness of the whole myometrium was not different in women with and without endometriosis, those with endometriosis had increased subendometrial halo. They attributed this to the presence of infiltration of endometrium and possible adenomyosis. According to Kunz et al. (2000) focal thickening is more pronounced in older women, although no correlation could be established with the severity of the disease. In a subsequent publication, Kunz et al. (2005) compared the MRI findings in 160 infertile women with, and 67 infertile women without, laparoscopically confirmed endometriosis. The posterior JZ (but not the anterior JZ or the total myometrial thickness) was significantly thicker in women with endometriosis.

There was a positive correlation between the posterior JZ thickness and the stage of endometriosis and patients’ age. Subsequently, the
The eutopic endometrium in adenomyosis and endometriosis

The identification of differences between the eutopic endometrium in adenomyosis (EuEA) and endometriosis (EuEE) can have important implications on our understanding of the aetiology. When comparing the EuEA and endometriosis it is important to remember that, as already stated, both conditions often co-exist especially in infertile women. On the one hand, because MRI is not widely used, studies involving women with endometriosis may have included cases with adenomyosis. On the other, although it is perhaps possible to rule out endometriosis in women whose uterus was removed at hysterectomy, it is not always clear if this was done in research on adenomyosis.

The importance of alterations in the eutopic EuEA (Benagiano and Brosens., 2012) and in endometriosis (Carvalho, 2013) has been recently reviewed. Briefly, in endometriosis there is an abnormal gene expression; a local oestrogen production and altered endometrial response to progesterone; an increased nerve
density and oxidative stress. In adenomyosis metabolic and molecular abnormalities often similar to those observed in endometriosis increase angiogenesis and proliferation, decrease apoptosis, allow local production of oestrogen, create progesterone resistance and impair cytokine expression.

**Immune factors and adhesion molecules**

An immune dysfunction could help endometrial fragments survive outside the uterine cavity. Research on altered immune responses as a possible aetiologic factor in endometriosis has mostly focused on pelvic nodules and the peritoneal fluid. Where it involved the eutopic endometrium, this was because of a possible role in pathogenesis or in infertility (Table 1).

Back in 1990, Mathur et al. (1990) raised the question of autoimmunity in endometriosis when they reported the presence of endogenous IgG in 78% of the endometria and endometriosis implants in affected women, but only in 22% of the endometria in unaffected controls. They also identified serum and/or peritoneal fluid IgG directed against endometrial antigens in women with the disease. Additional work has shown that stromal leukocyte populations in eutopic EuEA did not differ significantly from those in either control endometrium (CE) or EuEE (Jones et al., 1998a). But there may be some differences between the intraepithelial leukocytes (IEL) when comparing EuEE and EuEA. CD45+ cells increased from the proliferative to the late secretory phase in CE and in the EuEE, but not in EuEA (Bullmer et al., 1998).

During the proliferative phase the glandular epithelium of EuEE (but not EuEA) exhibited an increased number of CD45+ and CD43+ IEL compared with controls. CD56+, CD68+, CD4+ and CD8+ cells did not differ significantly, but CD3+ IEL were higher in the proliferative phase of the eutopic endometrium compared with CE in both endometriosis and in adenomyosis (Bullmer et al., 1998). The hypothesis was then formulated that the difference in leukocyte populations between adenomyosis and endometriosis may relate to different disease aetiologies and to the infertility associated with endometriosis. Chiang and Hill (1997) identified no differences in T cells, interferon (IFN)γ and HLA-DR-positive cells in eutopic endometrial samples from adenomyosis, endometriosis or unaffected controls. However, others have presented evidence of increased HLA-DR expression in EuEE (Liu et al., 2002) and also in women with adenomyosis (Ota and Igarashi, 1993), and of increased expression of y6 T cells (gamma-delta T cells) in the stroma and of additional HLA antigens in the glandular cells in both adenomyosis and endometriosis (Ota et al., 1996), but it is unclear if there are differences between the two conditions. It is possible that aberrant expression of HLA-DR in glandular cells of eutopic and ectopic endometria in endometriosis and adenomyosis is involved in various immunological responses.

Wang et al. (2008) examined the expression of HLA-G in eutopic and ectopic endometrium to assess its possible role as a mediator of immune suppression which can confer protection to ectopic endometrial cells. They reported that while virtually no HLA-G was detected in normal endometrium, both eutopic and ectopic EuEA expressed HLA-G. Previous reports had not identified HLA-G in EuEE but there is disagreement over whether it is expressed in endometriosis nodules (Hornung et al., 2001; Barrier et al., 2006).

Ota and Tanaka (1997b) also investigated the expression of the integrins, very late activation antigens (VLA-2, 3, 4, 5, 6) and E-cadherin in the glandular epithelium of EuEE, EuEA and CE. They found that during the proliferative phase, all antigens varied significantly between the three groups. In the secretory phase, there was a significant difference between the three groups in VLA-2–4 and E-cadherin, but not in VLA 5 or 6 expression. However, they did not provide a comparison between adenomyosis and endometriosis. Chen et al. (2010) examined epithelial–mesenchymal transition in the endometrium as possible mechanisms for increased endometrial invasiveness in adenomyosis. Differences (increased vimentin and reduced E-cadherin) were noted in the ectopic endometrium, but not in the eutopic endometrium from affected women.

**Cell proliferation and apoptosis**

Jones et al. (1995) reported no differences in proliferative activity between EuEE and CE. The same group reported that eutopic EuEA displayed similar proliferative activity compared with CE except in the proliferative and early secretory phases. In addition, the expression of Bcl-2 (a protein capable of inhibiting apoptosis) in stromal cells was also statistically significantly higher in the EuEA compared with the EuEE in the proliferative phase. Interestingly, the same authors reported that there were no significant differences in Bcl-2 expression when EuEE or EuEA were compared with CE (Jones et al., 1998b). In another study, the same authors reported that the expression of Bcl-2 in eutopic endometrial stroma in adenomyosis did not vary with the menstrual cycle. This contrasts to the increased levels seen in the late secretory phase in the EuEE. Apoptosis (using dUTP nick-end labelling TUNEL assay) was rare in both tissues (Jones et al., 1998a) (Table 1).

In contrast to the situation in EuEA, Goumenou et al. (2001) reported that Bcl-2 expression in epithelial cells did not vary with the phase of the cycle in the EuEE. Thus, endometrial Bcl-2 expression was significantly higher in adenomyosis in the proliferative phase and significantly lower in the secretory phase compared with the corresponding phase in EuEA. Indeed, all secretory samples were reported as negative for Bcl-2. However, McLaren et al. (1997) and Meresman et al. (2000) found that Bcl-2 expression in the EuEE peaked during the late proliferative phase and virtually disappeared during the late secretory phase. There is controversy over the expression of Bax (Bcl-2-associated X protein), which promotes apoptosis in patients with endometriosis. Meresman et al. (2000) found Bax to be absent during the late proliferative phase with some samples showing positive expression in the late secretory phase, whilst McLaren et al. (1997) reported Bax to be present in both the proliferative and secretory phases. Goumenou et al. (2001) also reported that the expression of Bax did not vary with the phase of the cycle and that it was not statistically different in adenomyosis and endometriosis.

Amongst the most primitive mechanisms of cellular protection is the expression of ‘heat shock’ or ‘stress’ proteins (HSP). They are expressed in response to a variety of stimuli and play a role in the folding and translocation of polypeptides across membranes (De Maio, 1999). Using immunohistochemistry, Ota et al. (1997a) noted higher expression of HSP-27 in the EuEE and EuEA when compared with CE in both phases of the cycle. HSP-70 was higher in EuEE and EuEA compared with CE only in the proliferative phase, whilst there were no differences in HSP-60 expression. There were no differences in HSP-27 or HSP 70 between the EuEE and EuEA. It is hard to interpret these data, although they may indicate a higher protective mechanism against cellular stress. Goteri et al. (2006) evaluated the expression of the cell division control protein analogue, Cdc42 in EuEA eutopic endometrium in
Table I Immune factors and proliferation and apoptosis markers in the EuEE, EuEA and CE.

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<td>Jones et al. (1998a)</td>
<td>No significant difference in stromal leukocytes. No difference in proliferation between EuEA and CE except in the proliferative and early secretory phases. Higher Bcl-2 in stromal cells in EuEA compared with EuEE in the proliferative phase. No difference in stromal Bcl-2 in EuEA with the phase of the menstrual cycle. Higher Bcl-2 in the late secretory phase of the EuEE.</td>
<td>EuEE, EuEA, CE</td>
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<td>Bullmer et al. (1998)</td>
<td>Higher CD45+ IEL in the proliferative, compared with the late secretory, phase in CE and EuEE, but not in EuEA. Higher CD45+ and CD43+ IEL in proliferative glandular epithelium of EuEE (but not EuEA) compared with CE. No difference in CD56+, CD68+, CD4+ and CD8+ cells between EuEA and EuEE. Higher CD3+ IEL in the proliferative phase of the EuEE and the EuEA compared with CE.</td>
<td>EuEE, EuEA, CE (hysterectomy for fibroids or ovarian cysts)</td>
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<td>Chiang and Hill (1997)</td>
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<td>Barrier et al. (2006)</td>
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<td>Ota et al. (1997b)</td>
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<td>Chen et al. (2010)</td>
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<td>Goumenou et al. (2001)</td>
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<td>Mclaren et al. (1997)</td>
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<td>Goteri et al. (2006)</td>
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<td>EuEA, eutopic endometrium of ovarian endometriosis, CE (women with fibroids or ovarian pathology)</td>
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<td>Kim et al. (2010)</td>
<td>Higher p21-activated kinase (Pak1) in the mid-secretory phase of the EuEA compared with CE.</td>
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<td>Kim et al. (2009)</td>
<td>Higher Pak1 in EuEE compared with CE. Significant difference in the secretory phase in the glandular epithelium.</td>
<td>EuEE, CE (women with carcinoma in situ of the cervix)</td>
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CE, control endometrium; EuEA, eutopic endometrium from women with adenomyosis; EuEE, eutopic endometrium from women with endometriosis; IEL, intraepithelial leukocytes; VLA, very late activation antigen.

Women with ovarian endometriotic cysts and CE. The intensity of Cdc42 immunostaining in the eutopic endometrium did not differ significantly in women with adenomyosis compared with controls but was stronger in the eutopic endometrium in women with ovarian endometriosis. The difference between women with adenomyosis and ovarian endometriosis was statistically significant only in the proliferative phase of the cycle.
Kim et al. (2010) demonstrated that p21-activated kinase (Pak1) immunoreactivity was higher in the mid-secretory phase of the cycle in both the glandular and the stromal components of the EuEA compared with unaffected women. Higher Pak1 has also been demonstrated in EuEE (Kim et al., 2009a).

**Cytokine and inflammatory mediators**

Comparing only patients with endometriosis and those with adenomyosis but not including normal controls, Gateri et al. (2009) reported increased vascular endothelial growth factor (VEGF) but not HIF-1α (hypoxia inducible factor) or microvessel density (MVD) expression in EuEA. This is in agreement with Li et al. (2006) who reported increased VEGF, the matrix metalloproteinases (MMP)-2 and -9 in EuEA compared with unaffected women. Schindl et al. (2001) reported no increase in MVD in EuEA compared with control. Tokyol et al. (2009) reported that there were no significant differences in MMP-2 expression in the glandular epithelium or in MVD in the stroma and that there was no significant difference in MMP-2 expression in the luminal epithelium during either the proliferative or the secretory phase of the menstrual cycle when comparing the EuEA to CE (Table II).

Kang et al. (2009) reported that the 22578A or 21154A alleles of VEGF gene could significantly decrease the risk of adenomyosis and might be potential protective factors for adenomyosis development. Furthermore, the haplotypes of VEGF -460/-1154/-2578 polymorphisms may have an effect on adenomyosis development. A different polymorphism (+936TC) is linked to the development of endometriosis, but polymorphisms -460CT, +405CG, -2578AC or -1154GA are not linked to the disease (Liang et al., 2012). These findings should be treated with caution given the known difficulties with gene association studies.

Ota et al. (2001b) investigated the expression of cyclooxygenase (COX-2). They reported that COX-2 is higher during the secretory compared with the proliferative phase in both the luminal and the glandular epithelium. This is at variance with a previous study by Jones et al. (1997) who reported COX-2 to peak during menstruation, and to be at its lowest around ovulation. When comparing the EuEE, EuEA and CE, the only statistically significant difference in COX-2 expression found by Ota et al. (2001b) was in the luminal epithelium during the late proliferative phase and in the glandular epithelium during the mid- and late-proliferative phases. Differences were noted in stromal cells during the early- and mid-secretory phases, but published results do not allow a direct group-to-group comparison. However, Tokyol et al. (2009) found no significant differences in COX-2 expression in luminal epithelium between EuEA and CE. It is important to note that, similar to most publications reviewed, this study did not include tests for enzymatic activity and did not use blocking peptides. Matsuzaki et al. (2004) observed that the level of COX-2 was significantly higher in the stromal cells of eutopic endometrium in women with deep infiltrating endometriosis compared with controls, and the levels seemed to correlate with the severity of pain.

Interleukin-18 (IL-18), a major regulator of immune responses, is expressed in a number of situations including the site of chronic inflammation, autoimmune diseases, a variety of cancers and numerous infectious pathologies. Luo et al. (2006) reported that IL-18 mRNA level was lower in both eutopic and ectopic endometrium in endometriosis. Huang et al. (2010) examined the expression of IL-18, its receptor (IL-18R), and IL-18 binding protein (IL-18BP) mRNA, and protein expression in the EuEA. The eutopic endometrial IL-18R mRNA and the IL-18BP to IL-18 ratio were significantly increased in adenomyosis compared with CE, but the level of IL-18 mRNA was not significantly different. Huang et al. (2011) assessed the expression of tyrosine kinase receptor TrkB in the same group of patients. TrkB mediates the effects of a number of neurotrophins including brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4, which are involved in neuronal function. They reported increased expression of TrkB mRNA and protein in the secretory phase in the EuEA. Higher TrkB protein expression was noted in EuEE compared with CE (Anger et al., 2007).

IL-10 influences multiple traits of immunoregulation and inflammation and enhances B cell survival, proliferation and antibody production. Wang et al. (2009) using immunohistochemical H-scores, reported increased IL-10 expression in the epithelial cells but not in the stroma of the EuEA compared with CE. The expression scores are not provided, and no comment is made about the cell type localization of stromal immunostaining. The same group (Qin et al., 2012) used similar methodology and reported that interleukin-10 receptors (IL-10R1 and IL-10R2) were mainly expressed in epithelial cells in the endometrium. They reported increased IL-10R1 in EuEA compared with CE. On the other hand, studies of cytokine expression in endometriosis have focused on peritoneal fluid, peritoneal macrophages, leukocytes from peripheral blood or the endometrium as the primary source of cytokines; this is in accord with current knowledge of the source of IL-10 from monocytes, lymphocytes and T-Cells. Antsiferova et al. (2005) found that the lymphocytes from EuEE expressed low levels of IL-10 mRNA but the level was not statistically significantly different compared with controls. The percentage of leukocytes expressing intracellular IL-10 protein in the EuEE was not statistically significantly different compared with controls. Antsiferova et al. (2005) also found that the lymphocytes from EuEE possessed slightly reduced levels of IL-2 mRNA and no expression of IL-4 mRNA but the level was not statistically significantly different compared with controls.

Sotnikova et al. (2002) demonstrated altered cytokine production in mononuclear cells obtained from the EuEA. There was a statistically significant increase in INFγ, INFα, IL-1β, tumour necrosis factor (TNF)α and epidermal growth factor and a reduction in IL-8 compared with controls. This suggests a high level of T lymphocyte activation. Increased activity of immune-competent cells may create the conditions that favour cell infiltration and proliferation leading to the development of adenomyosis. On the other hand, Martínez-Roman et al. (1997) reported that a reduction in T cells in the peritoneal fluid was predominantly noted in endometriosis patients who also have infertility. Thus, it remains unclear if the expression of cytokines varies in endometriosis and adenomyosis in relation to the presenting symptoms.

Today, several possible markers of endometriosis have been identified: Gagné et al. (2003) found an altered proportion of CD3+, CD16+, CD3-HLADR-, CD3-CD45RA-, CD3+CD16-, CD3+CD56-, CD56-CD16+ and CD16b+ leukocytes in the endometrium of women with endometriosis, and utilised this in a predictive model to identify women with a high likelihood of suffering with the condition. Agic et al. (2008) investigated the possibility of using combined levels of four molecules as a diagnostic test: serum levels of the C-C chemokine receptor type 1 (CCR1) mRNA, hypoxanthine–guanine phosphoribosyltransferase (HPRT1), an enzyme encoded by the HPRT1 gene,
Table II  Cytokines and inflammatory mediators in the EuEE, EuEA and CE.

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<td>COX-2 peaks during menstruation and is lowest around ovulation</td>
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<td>Anger et al. (2007)</td>
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<td>Ulukus et al. (2009)</td>
<td>Higher epithelial IL-8 and MCP-1 in EuEE in the proliferative phase compared with CE</td>
<td>EuEE, CE (fibroids, voluntary sterilization, myometrial hypertrophy, benign adnexal mass)</td>
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<td>Ulukus et al. (2005a)</td>
<td>EuEE had significantly higher epithelial CXCR2 levels: CXCR1 expression was higher in EuEE during the proliferative phase</td>
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<td>Ulukus et al. (2006)</td>
<td>Higher epithelial CXCR1 and CXCR2 in EuEA compared with CE in the proliferative but not in the secretory phase</td>
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<td>Arici et al. (1998)</td>
<td>No IL-8 in the stroma. No significant cyclical changes in IL-8 expression in the glands or luminal epithelium. Lower IL-8 mRNA in mid-cycle (late proliferative and early secretory phases) compared with other phases of the cycle in CE</td>
<td>CE (endometrium biopsies or hysterectomy for non-endometrial disease)</td>
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<td>Zhang et al. (2009)</td>
<td>PGP 9.5 and NF protein expression in the functional layer of the endometrium only in women with pain. No difference between EuEA, EuEE, CE</td>
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<td>Kang et al. (2009)</td>
<td>The -257BA or -1 154A alleles of the VEGF gene linked to decrease the risk of adenomyosis</td>
<td>Blood from women with adenomyosis, healthy controls voluntary abortion, dysfunctional uterine bleeding, Caesarean section, healthy blood donors</td>
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</table>

CE, control endometrium; COX, cyclooxygenase; EGF, epidermal growth factor; EUAE, eutopic endometrium from women with adenomyosis; EuEE, eutopic endometrium from women with endometriosis; IFN, interferon; MCP, macrophage inhibitory protein; MMP, matrix metalloproteinase; MVD, microvascular density; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.
monocyte chemotactic protein-1 (MCP-1) and the well-known marker CA125. They observed that in patients with endometriosis and adenomyosis the ratio of CCR1/HPRT mRNA in the peripheral blood is significantly elevated compared with healthy subjects.

Ulukus et al. (2005a, b) compared IL-8 and MCP-1 expression in the EuEA and CE. There was immune staining in both the epithelium and stroma, but the expression was more intense in the epithelium. They found that in adenomyosis there was a loss of the rise observed in both factors in the normal secretory endometrium; this caused a statistically significant lower expression in the eutopic endometrium during the secretory phase. In contrast, they reported higher epithelial IL-8 and MCP-1 expression in EuEE in the proliferative phase (Ulukus et al., 2009). In another report, the same group (Ulukus et al., 2006) found that the IL-8 receptors (CXCR1 and CXCR2) showed higher epithelial staining during the proliferative, but not the secretory, phase in the EuEA compared with unaffected controls. In the EuEE there was a significant increase in both the proliferative and secretory phases for epithelial CXCR2 expression, but only in the proliferative phase for CXCR1 expression (Ulukus et al., 2005a). In EuEA, Ulukus et al. (2006) reported higher epithelial CXCR1 and CXCR2 staining intensity compared with CE in the proliferative but not in the secretory phase. The distribution of IL-8 contrasts with that reported by Arici et al. (1998), who found negative staining in the stroma, and no significant difference in the glandular expression between early proliferative, mid-proliferative and late secretory phases of the cycle in the eutopic endometrium in women with no endometriopathy. The levels of IL-8 mRNA were statistically significantly lower in mid-cycle (late proliferative and early secretory phases) compared with other phases of the cycle. The discrepancy may be related to the fact that the studies by Ulukus et al. (2005a, b) did not take into account fluctuations within the same cycle phase. On the other hand, Arici et al. (1998) may not have excluded women with adenomyosis or endometriosis. Zhang et al. (2009) studied the expression of protein gene product (PGP) 9.5 and neurofilament (NF) protein in the functional layer of the endometrium in women with adenomyosis and endometriosis. They found that the expression was negative in all except samples from women with pain symptoms, and that in patients with pain the expression did not vary in relation to the presence of adenomyosis or endometriosis.

Ding et al. (2010a) examined mitochondrial protein expression using SELDI-TOF-mass spectrophotometry. They reported the finding of one significantly different peak m/z 3366 between the samples from women with adenomyosis and control, whereas there were 10 significantly different peaks between samples from EuEE and CE. There was one significantly different peak m/z 3499 when the EuEE and EuEA were compared. This led the authors to suggest that adenomyosis may be a special type of endometriosis that has its own features (Ding et al., 2010b). The m/z ratios corresponding to the peaks in endometriosis and adenomyosis point to different proteins (Kim et al., 2009; Ding et al., 2010b). Mehasseb et al. (2010b) reported 38 different protein peaks between adenomyosis and normal endometrial stromal cells grown in culture, 51 peak differences when the stromal cells were co-cultured with normal myometrial cells and 46 peak differences when co-cultured on adenomyosis myometrium. Of these peak differences, 28 were common between the three culture conditions. This shows that adenomyosis stromal cells are different compared with normal cells. The exact role of proteomic research in this area remains to be determined.

There is accumulating evidence of the role of NF-κB (nuclear factor kappaB), a transcription factor with a role in modulating genes involved in inflammation, proliferation, apoptosis, invasion, angiogenesis and other cellular functions, in the pathogenesis of endometriosis (González-Ramos et al., 2012), and adenomyosis (Li et al., 2013). But whilst NF-κB-DNA-binding activity was present in all phases of the menstrual cycle in women with and without endometriosis, it varied from strong binding to very low or undetectable levels. DNA binding of the p65 subunit of NF-κB was higher in the proliferative endometrium compared with the menstrual or secretory endometrium in unaffected controls, but was lower during the menstrual phase in the EuEE. However, there were no significant differences when respective cycle phases were compared between the EuEE and controls (González-Ramos et al., 2012). Li et al. (2013) reported that the level of NF-κB subunits p65 and p50 and NF-κB-DNA-binding activity in endometrial stromal cells was significantly higher in adenomyosis than in controls. Binding activity was positively correlated with dysmenorrhea. This seems to support evidence derived from an animal model by Mao et al. (2011) who demonstrated reduced myometrial infiltration, reduced amplitude of uterine contractions and improved hyperalgesia using a hotplate pad test in a mouse model of adenomyosis when the animals were treated with androgapholide (aNF-κB) inhibitor.

NF-κB is a pivotal transcription factor that can be activated by various growth factors and oxidative stress, and in turn may regulate enzymes and growth factors such as COX-2, VEGF and tissue factor (TF). Li et al. (2013) recently reported that mRNA and protein levels of COX-2, VEGF and TF in stromal cells in adenomyosis were significantly higher compared with controls.

Oxidative stress and free radical metabolism

Some investigations of eutopic endometria from women with adenomyosis and endometriosis focused on free radical metabolism, and when differences were found, they were assumed to have a role in pathogenesis or in altering endometrial receptivity leading to infertility. Endothelial nitric acid synthase (eNOS) was detected in the luminal and glandular epithelium of the endometrium and in the endothelium of vascular culture. Intense immunoreactivity was detected in the secretory but not in the proliferative phase in EuEA and CE; treatment with GnRH down-regulated eNOS in the EuEA (González-Ramos et al., 2012). But whilst eNOS was higher in adenomyosis compared with healthy subjects.

Integrins are trans-membrane receptors mediating the attachment between adjacent cells. Khorram et al. (2002) reported up-regulation of eNOS and down-regulation of α5β3 integrin in the glandular and luminal epithelium of EuEE in the secretory phase of the cycle compared with unaffected controls. A similar pattern of distribution of eNOS was noted in EuEA (Ota et al., 1998), but these authors did not report different levels of expression when compared with the normal controls. Ota et al. (1998) found the expression of eNOS to be persistently higher throughout the cycle in both EuEA and EuEE compared with unaffected controls.

Van Langendonckt et al. (2002a) summarized evidence linking oxidative stress to the inflammatory reaction in endometriosis. This includes an increased release of reactive oxygen species by macrophages; increased peritoneal levels of oxidized low-density lipoproteins; altered expression of endometrial pro-oxidant and antioxidant enzymes and consumption of peritoneal fluid vitamin E. They believe that retrograde menstruation can carry highly pro-oxidant heme and
Oxidative stress and free radical metabolism in the EuEE, EuEA and CE.

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<td>Kamada et al. (2000)</td>
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<td>EuEA, CE (hysterectomy for carcinoma in situ of the cervix)</td>
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<td>Khorram et al. (2002)</td>
<td>Higher eNOS and lower α-, β-integrin in the glandular and luminal epithelium of secretory EuEE compared with CE</td>
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<td>Ota et al. (1998)</td>
<td>Peak eNOS in glandular and luminal epithelium in the mid-secretory phase in CE. eNOS persistently higher throughout the cycle in EuEA and EuEE compared with CE</td>
<td>EuEE, EuEA, CE (fertile women with male factor infertility)</td>
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<td>Iwahara et al. (2012)</td>
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<td>EuEA, CE (detail not available)</td>
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<td>Ota et al. (1999)</td>
<td>Higher SOD in EuEA and EuEE compared with CE</td>
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<td>Ota et al. (2000)</td>
<td>Loss of cyclicity of GPx in the EuEE. Expression of GPx in the glandular epithelium of EuEA was higher and in EuEE was lower compared with CE</td>
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<td>Ota et al. (2001a)</td>
<td>Loss of cyclic changes of XO in the glandular epithelium in EuEE. Different cyclic pattern of XO in EuEA compared with CE. Higher XO during the late secretory phase in luminal epithelium in EuEA compared with CE. No statistically significant differences between EuEA and EuEE.</td>
<td>EuEE, EuEA, CE (fertile women with male factor infertility)</td>
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<tr>
<td>Ota et al. (2002)</td>
<td>Cyclical changes in catalase in CE, but not in EuEE or EuEA. Higher catalase in EuEE compared with CE and highest in EuEA</td>
<td>EuEE, EuEA, CE (fertile women)</td>
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</table>

CE, control endometrium; EuEA, eutopic endometrium from women with adenomyosis; EuEE, eutopic endometrium from women with endometriosis; GPx, glutathione peroxidise; HO, hemoxygenase eNOS; endothelial nitric oxide synthase; XO, xanthine oxidase; SOD, superoxide dismutase.

Iron and indeed they found higher levels of haemoglobin in the peritoneal fluid of patients with endometriosis, with no concomitant increase in bilirubin concentrations and a poor expression of heme oxygenase (HO)-1, one of the enzymes that catalyse the degradation of heme into iron, carbon monoxide and biliverdin. In contrast, HO-1 and HO-2 were higher in EuEA compared with Ce. Iwahara et al. (2012) studied HO in women with adenomyosis. They confirmed the expression of HO-1 and HO-2 in both eutopic and ectopic endometrium. There were no differences between EuEA and CE. They concluded that both HO-1 and HO-2 contribute little to the pathophysiology of adenomyosis.

Ota et al. (1999) used immunohistochemistry to assess the expression of copper, zinc and manganese-dependent superoxide dismutase which protects cells from free radical damage. They reported that expression was cycle dependent and consistently higher in the EuEA and EuEE, but they did not look for differences between the two pathologies. Glutathione peroxidase, a co-enzyme of glutathione, which acts by reducing peroxides such as those of hydrogen and lipid into water and alcohol, is expressed in the luminal and the glandular epithelium in the endometrium. Using immunohistochemistry, Ota et al. (2000) reported that expression varied with the phase of the cycle in the normal endometrium, being highest in the early secretory phase. There was loss of cyclicity in the EuEE, mainly because of the loss of the secretory phase peak. Expression in the secretory glandular epithelium of EuEA was higher, and in endometriosis was lower compared with the CE. There were no other statistically significant differences between the groups in either the glandular or the luminal epithelium.

The enzyme xanthine oxidase (XO) produces superoxide leading to the accumulation of free radicals within the cell. Ota et al. (2001a) reported that the expression in the glandular epithelium varied according to the menstrual phase in normal controls, but not in patients with endometriosis and that the variation in women with adenomyosis differed from that in controls. However, there were no statistically significant differences between XO expression in the glandular epithelium of EuEE and EuEA. In addition, whilst the expression in the luminal epithelium in the EuEA was statistically significantly higher compared with CE during the late secretory phase, there were no statistically significant differences when compared with the EuEE.

Ota et al. (2002) reported on the expression of catalase, an enzyme involved in the conversion of hydrogen peroxide into water and oxygen. They reported that catalase expression fluctuated in the endometrium during the normal menstrual cycle, but not in the EuEE or EuEA. They also reported that enzyme expression as determined by immunohistochemical score was higher in EuEE compared with CE and was highest in the case of EuEA. However, no statistical analyses were provided.

The role of steroids and epigenetic factors

There are reports that both endometriosis and adenomyosis are associated with increased local oestrogen production. Increased P450 aromatase RNA was reported in EuEA and EuEE, but not in endometrial samples from women with cervical pathology, which were used as control. In EuEA and EuEE samples, aromatase cytochrome P-450 was immunolocalized exclusively in the cytoplasm of glandular cells and faintly in the stroma (Kitawaki et al., 1999), but samples were not classified based on cycle phase. Brosens et al. (2004) detected aromatase mRNA in all samples in a group of infertile women undergoing IVF, which suggests that aromatase expression may not be confined to endometria from women with oestrogen-dependent abnormalities, but levels did not vary with the phase of the cycle. Ishihara et al. (2003) have shown that the level of aromatase mRNA is reduced in EuEA and EuEE in response to GnRH or danazol (Table IV).
Endometrial aromatase was assessed as a possible marker for endometriosis and adenomyosis. Kitawaki et al. (1999) reported immunostaining for P450 aromatase in endometrial biopsy specimens of women diagnosed with endometriosis, adenomyosis and/or uterine fibroids with 91% sensitivity and 100% specificity. Less encouraging results were obtained by Hatok et al. (2011) who detected aromatase in 21.7% of controls compared with 56% of women with adenomyosis and 73.3% of women with endometriosis and 65.5% of women with both conditions. The level of aromatase mRNA was statistically significantly higher in women with adenomyosis and endometriosis compared with controls. The question of aromatase expression in endometriosis remains controversial. Maia et al. (2012) linked aromatase expression in the eutopic endometrium to the presence of infertility and dysmenorrhea irrespective of the presence of endometriosis. Maia et al. (2006) reported that aromatase was expressed in the stroma in 80% of cases of EuEA. This is comparable to the 72% incidence reported in women with infertility and endometriosis, and the 95% incidence in symptomatic women without endometriosis and contrasts with the lack of expression in asymptomatic endometriosis-free patients (Maia et al., 2012). Colette et al. (2009) could not identify aromatase protein or mRNA expression in the endometrium in women with endometriosis. There were also discrepancies between immunohistochemical studies as to whether aromatase is localized to the epithelium or stroma and methodological issues may have resulted in false-positive mRNA detection.

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<td>Kitawaki et al. (1999)</td>
<td>Higher P450 aromatase RNA in EuEA and EuEE compared with CE. P450 aromatase expressed in EuEE and EuEA with 91% sensitivity and 100% specificity</td>
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<td>Brosens et al. (2004)</td>
<td>Aromatase mRNA detected in all samples. Levels did not vary with the phase of the cycle</td>
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<td>Ishihara et al. (2003)</td>
<td>Lower aromatase mRNA in EuEA and EuEE in response to GnRH or danazol</td>
<td>All patients with infertility: EuEE, EuEA, CE (with fibroids)</td>
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<td>Hatok et al. (2011)</td>
<td>Aromatase expression in 21.7% of CE, in 56% of EuEA and in 73.3% of EuEE</td>
<td>All in early proliferative phase: EuEE, EuEA, CE (pelvic pain)</td>
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<td>Maia et al. (2012)</td>
<td>Aromatase in eutopic endometrium linked to infertility and dysmenorrhea irrespective of endometriosis</td>
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<td>Maia et al. (2006)</td>
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<td>Colette et al. (2009)</td>
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<td>Blood samples from patients with endometriosis, adenomyosis or control (women with cervical carcinoma in situ or with tubal occlusion disease)</td>
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<td>Taylor et al. (1999)</td>
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<td>Matsuzaki et al. (2009)</td>
<td>Lower mid-luteal HOXA10 mRNA and protein in stromal cells in EuEE compared with CE</td>
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<td>Fischer et al. (2011)</td>
<td>Lower HOXA10 protein in endometrial stroma but not glands in EuEA</td>
<td>EuEA, CE (fertile with prolapse or pelvic mass)</td>
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<td>Xiao et al. (2010)</td>
<td>LIF in EuEA and uterine flushing in adenomyosis and infertility but not in adenomyosis and dysmenorrhea</td>
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<td>Wu et al. (2005)</td>
<td>HOXA10 promoter hypermethylation in EuEE</td>
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<td>Wu et al. (2006)</td>
<td>No difference in PR-B promoter hypermethylation between EuEE and CE</td>
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<td>Wu et al. (2007)</td>
<td>Higher mRNA level for DNMT3A in the EuEE compared with CE</td>
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<td>Liu and Guo (2012)</td>
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<td>Nie et al. (2010b)</td>
<td>PR-B hypermethylation in ectopic stromal cells from adenomyosis</td>
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<td>Lu et al. (2013)</td>
<td>Lower HOXA10 mRNA and protein in EuEE compared with CE during the secretory phase</td>
<td>Fertile women: EuEE, CE</td>
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CE, control endometrium; DNMT, DNA methyltransferase; EuEA, eutopic endometrium from women with adenomyosis; EuEE, eutopic endometrium from women with endometriosis; LIF, leukaemia inhibitory factor.
Discussion

The data reviewed here indicate that the endometrial microenvironment in endometriosis and adenomyosis differs in some aspects of cellular and humoral immunity from the endometrium of unaffected women. The aberrant immune responses in both conditions could suggest immunologic stress and that endometriosis and adenomyosis differ only by the site of the ectopic endometriotic tissue. However, it remains debatable whether these changes are related to the anatomical disease or to any associated infertility. In addition, the clinical significance of the observed morphological, biochemical and molecular anomalies is unclear. These abnormalities may be expected to impact on infertility and IVF outcomes, although the effect of adenomyosis on fertility is controversial. In reviewing the association of adenomyosis with infertility Campo et al. (2012) mentioned that several pathogenic hypotheses have been presented, including JZ disruption with a consequent perturbed uterine peristalsis, as well as biochemical and functional alterations in both eutopic and heterotopic endometrium. They concluded that there is evidence of lower uterine receptivity, and implantation rates. This conclusion, however, seems contradicted by recent results in women to whom oocytes were donated (Martinez-Conjeros et al., 2011).

Many older trials that evaluated outcomes of IVF/ICSI in women with adenomyosis or endometriosis concluded that both conditions decrease fertility. For instance, Simon et al. (1994) compared the outcome of IVF in...
78 women with tubal infertility and patients with endometriosis and found a statistically significant reduced pregnancy rate per cycle and per transfer and a reduced implantation rate. Other studies, however, failed to confirm this finding: pituitary down-regulation resulted in a relatively high delivery rate (38.9% per cycle, 41.9% per retrieval and 43.2% per transfer) and no difference was observed in pregnancy rates according to staging of the disease (Olivennes et al., 1995). Barnhart et al. (2002) undertook a meta-analysis in which cases were stratified according to the stage of endometriosis. The chance of achieving pregnancy was reported to be significantly lower in endometriosis (odds ratio, 0.56; 95% confidence interval, 0.44–0.70) compared with tubal factor controls. Endometriosis was also associated with lower fertilization and implantation rates, and lower number of retrieved oocytes. Ovarian endometriomas were associated with a significant increase in early pregnancy loss (Yanushpolsky et al., 1998). But removal of the endometrioma prior to IVF did not improve outcomes (García-Velasco et al., 2004). The relation between endometriosis and IVF outcomes remains controversial. Surrey (2013) argued that more recent studies and the data from the US Society for Assisted Reproductive Technology do not support a link between endometriosis and poor IVF outcomes, except perhaps in women with ovarian endometriomas.

Results of IVF in women with non-histologically confirmed diagnosis of adenomyosis have yielded conflicting results. A further difficulty is that current practice in IVF does not routinely rule out endometriosis prior to assisted conception. Specifically, the studies by Costello et al. (2011) and Mijatovic et al. (2010) reporting results of IVF/ICSI in patients treated before 2007 found that the presence of adenomyosis diagnosed by TVUS did not affect outcome. On the other hand, the study by Thalluri and Tremellen (2012) reported a statistically significant decline in viable pregnancy rates in adenomyosis after adjusting for maternal age and duration of infertility. Salim et al. (2012) found a statistically significant lower clinical and ongoing pregnancy rates and a higher miscarriage rate in women with adenomyosis. Finally, in a retrospective matched cohort study, Martínez-Conejero et al. (2011) reported no differences in the implantation rate between patients with adenomyosis and controls. However, miscarriage was higher and term pregnancy rates were lower in women with adenomyosis. Mijatovic et al. (2010) evaluated the effect of both adenomyosis and endometriosis on IVF/ICSI and concluded that, when using the long-term pituitary down-regulation protocol, the contemporary presence of both conditions does not produce adverse effects on outcomes.

There are several major limitations in existing investigation of the EuEE and EuEA. In the first place, there are major diagnostic limitations, because the presence or absence of endometriosis in women with adenomyosis is frequently not known or not taken into consideration. Moreover, adenomyosis as diagnosed by MRI may not represent adenomyosis as diagnosed by histopathology.

Secondly, there is a systematic bias in clinical presentations, since biopsies for the study of the EuEE are mainly obtained during work-up of infertile women, while biopsies in women with adenomyosis are obtained at the time of hysterectomy. Clinical features that may be relevant to studies of the endometrium include the presence or absence of pain, bleeding patterns, patient characteristics such as age, parity, infertility and the type and extent of the disease. All of these should be taken into account in comparative studies. Moreover, menstrual cycle phase and concomitant medication can significantly affect the endometrium. There is clear evidence that some of the features noted in the eutopic endometrium may be related to the presenting symptoms, as well as the disease status. All this renders the conduct of well-controlled studies very difficult. A third complicating factor is patients’ age in relation to both parity and the natural history of the disease under consideration. While information is scant on the natural history of endometriosis, no studies are available on the changes in the eutopic endometrium or on the natural history of adenomyosis. Another critical observation is that most studies included women with concomitant pathology such as fibroids in the study or in the control group or in both, and that presenting symptoms are often not stated or controlled for.

Bearing in mind these inherent limitations, this review demonstrated a number of similarities, but also some differences between the EuEA and EuEE, and many authors have taken these to suggest a role in the pathogenesis of the disease. But there is a need for considerable caution as conflicting results were demonstrated on the few instances where the experiments were repeated. In addition, there are many other methodological issues which call for caution. These include lack of clarity as to the presenting symptoms, absent demographic detail, method of statistical presentation and the use of post hoc statistics. In addition, most of the published literature has taken no account of the effect of the menstrual cycle beyond the broad definition into proliferative and secretory phases, and has paid little or no attention to the site of the biopsy within the uterus or to whether basal or functional layers were assessed. It is thus possible that noted differences may be related to one of the above factors or to other micro-environmental factors such as alterations in blood supply consequent to the presence of ectopic endometrial glands, to myometrial hypertrophy and hyperplasia.

Conclusions

Whilst MRI features such as alterations in JZ thickness and morphology are well recognized features of adenomyosis, there is emerging evidence of uterine changes in women with endometriosis. These include changes in JZ thickness and in contractility. It is interesting that these changes occur in the absence of invading endometrium. Cyclical endometrial change adds an important complexity to research involving the endometrium. The premenopausal endometrium is a dynamic tissue where morphological and molecular features vary considerably with the phase of the cycle. But little of the published literature has taken sufficient account of cyclical changes or of endometrial zonation. Also, it has not always been considered that both adenomyosis and endometriosis can co-exist.

The documented differences between the EuEE and controls were not the focus of this review, but where these existed they were believed to facilitate adhesion or survival of endometrial cells in the peritoneum. There can also be a role for peritoneal factors in the process. An important question is whether the same aberrations in eutopic endometrium can be found in adenomyosis.

This review has collated evidence that the EuEE and in EuEA may differ in some respects such as the leukocyte population and apoptosis markers. There is also some evidence of differences in cytokines and inflammatory mediators including IFNs, ILs and TNFs, but this requires confirmatory studies. Evidence on the roles of oxidative stress and free radicals is inconclusive. There is also inconclusive and some contradictory evidence on the role of aromatase, but some evidence for differences in HOXA10 and DNMT3A.

Besides cycle phase and zonation as described above, future research should take into account that both diseases may coexist and also the
effect of other pathologies, such as fibroids that can have an influence on the endometrium. Few studies have linked molecular alterations to presenting symptoms such as infertility and pain. Differences in bleeding patterns or parity can also be important. Thus, while there is evidence that the two conditions may represent different phenotypes of the same disorder, a ‘unifying’ theory on the pathogenesis cannot be drawn based on current evidence.

**Authors’ roles**

The three authors agreed the topic of the review, they independently undertook literature searches and agreed upon included articles. All authors contributed to all sections of the review. I.B. and B.G. took the lead on the first two sections: Modern concepts of endometriosis and adenomyosis, and the junctional zone myometrium in endometriosis and adenomyosis. M.H. took the writing lead on the section on eutopic endometrium in endometriosis and adenomyosis. The manuscript was developed incrementally with various inputs from each of the three authors. The opinions expressed have been agreed by all three co-authors.

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