The Endometrium in Adenomyosis

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Abstract

The observation has long been reported of direct continuity between the eutopic and the ectopic endometrial glands in adenomyosis. Recent evidence point to the existence of differences between the endometrium in adenomyosis compared to control endometrium including increased 'invasiveness' in adenomyosis. Differences were also reported between the eutopic endometrium in adenomyosis and endometriosis. However, almost all published literature suffers from methodological weaknesses including inadequate control for cycle phase and symptoms. This calls for caution when interpreting the findings or drawing conclusions relevant to the pathophysiology of adenomyosis.

Keywords

Ectopic endometrium • Eutopic endometrium • Innervations • Proliferation • Apoptosis • Angiogenesis • Extracellular matrix • Steroid receptors • Cytokines • Immunomodulators • Oxidative stress • Free radicals • Molecular signalling

Although its pathophysiology is poorly understood, uterine adenomyosis is traditionally defined in terms of endometrial invasion within the myometrium. Ectopic glands are also surrounded by stroma. Observations were published of direct continuity between the ectopic and the eutopic endometrium. An important question is whether the eutopic endometrium in adenomyosis differs from the endometrium in unaffected women and whether any observed differences may predispose to adenomyosis. Also relevant is the question of whether the eutopic endometrium in adenomyosis and

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endometriosis share properties that predispose to ectopic implantation.

There has been considerable advance in our understanding of the structural and the functional properties of the endometrium including factors that may be relevant to implantation and menstruation, but less attention has been paid to the endometrium in adenomyosis. Far less is understood about mechanisms that may be involved in abnormal uterine bleeding, dysmenorrhoea or infertility which may be linked to adenomyosis.

A number of hypotheses have been proposed to explain the genesis of adenomyosis these have been discussed previously (Chap. 3). This chapter will focus on the properties of the endometrium and on whether there is evidence of increased 'invasiveness'.

It is notable that most studies of the endometrium in adenomyosis have relied on hysterectomy specimens possibly because adenomyosis remains a post-hysterectomy diagnosis. One of the difficulties arises because it becomes difficult to determine whether deviations from normal endometrium can be attributed to adenomyosis *per se* or to the genesis of the symptoms that necessitated the hysterectomy. Published studies on adenomyosis have not controlled for the specific symptom or symptoms that existed prior to surgery. This adds to the difficulty referred to elsewhere in this volume about the cut-off point for diagnosis. The complexity does not end here as the presence of concomitant pathology, for example endometriosis, can be associated with changes in the eutopic endometrium even in the presence of histologically normal endometrium.

Although it may be 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. Ruling out adenomyosis in women with endometriosis prior to assessment of the eutopic endometrium can be more challenging. The alterations in the eutopic endometrium in adenomyosis [1, 2] and in endometriosis [3, 4] have been recently reviewed. In endometriosis there is abnormal gene expression; a local estrogen 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 estrogens, create progesterone resistance, and impair cytokine expression.

The endometrium is a highly dynamic tissue and endometrial changes linked to steroids and the menstrual cycle have long been recognised; still many studies have not included classification beyond dividing the cycle into the proliferative and the secretory phases. Another important source of variation is the regional zonation of the endometrium. This may be affected by the exact site from which the biopsy is taken as well as in relation to the depth from the lumen with distinction being made between the functional and the basal endometrium. It is recognised that the endometrium from the isthmic portion of the uterus is not suitable for functional diagnosis as the glands may not show normal cyclical changes [5] and that fragments from different regions of the endometrium (cornua, fundus, isthmus) normally show variations in their development [6]. It is notable that despite the availability of full thickness endometrial samples, a large number of publications did not take into account the regional zonation of the endometrium and, as such, did not specifically report on the basalis and functionalis. Clearly, the resource implications of the large sources of variation in studies of the endometrium are considerable and can, arguably, be overwhelming.

There are no unique histological features that distinguish the eutopic endometrium in women with adenomyosis but, because of its accessibility, the eutopic endometrium became a target for research aiming at diagnosing adenomyosis. This includes hysteroscopic visualisation and hysteroscopic biopsy [7–9]. The finding of irregular endometrium with endometrial defects, hypervascularisation, strawberry pattern vasculature or of cystic haemorrhagic lesions has been linked to the presence of uterine adenomyosis. The report by Ota and Tanaka linked increased endometrial vascularity to the presence of adenomyosis in both the proliferative and the secretory phases of the cycle. In their study, 23 out of the 40 patients

with adenomyosis had abnormal vascularisation on hysteroscopy. Some had markedly dilated vessels and the number of vessels also varied. The vascular distribution was irregular presumably because of underlying lesions [10]. Other features of adenomyosis may include cystic areas particularly those featuring blue discoloration [11], irregular endometrium with superficial opening, irregular subendometrial myometrium which may be described as whorled or fibrotic. Abnormally wide glandular openings have also been described on hysterosalpingography. However, the diagnostic value of these features is not clear. Related to finding of irregular endometrium and the visualisation of superficial 'glandular' openings on hysteroscopy is the irregular branching and outpouching radiating from the uterine cavity or a honeycomb appearance of the uterine cavity that were noted on hysterosalpingography [12, 13]. But the diagnostic value of this technique is recognised as low because of the possibility of false negative in cases where adenomyosis does not communicate with the uterine cavity or where communicating glands do not fill with contrast or false positive diagnosis in cases of intravascular or endolymphatic intravasation [14]. Obtaining a hysteroscopic myometrial biopsy offers the possibility of tissue diagnosis [7, 9], but the technique is again hampered because of the possibility of a false negative diagnosis.

There are different mechanisms whereby the eutopic endometrium in adenomyosis can differ from that of unaffected women: (1) it is possible that the eutopic endometrium may exhibit distinct features because of the presence of underlying disease, a possible route of action could be an effect on the vasculature and the blood supply to the endometrium; (2) local hyperestorgensim or unidentified systemic factors that are relevant to the aetiology of adenomyosis. The increased incidence of endometrial hyperplasia or of polyps in women with adenomyosis may be secondary to this mechanism; (3) It is possible that the assumed increased invasiveness is an innate property of the endometrium itself; (4) It is possible that differences exist in the endometrium in adenomyosis that could be related to the presenting symptoms e.g. increased pain, bleeding or infertility rather than being relevant to adenomyosis *per se*; (5) It is also possible for differences to exist in eutopic endometrium that are related to associated pelvic pathology such as endometriosis. This complex array of possibilities emphasises the need for thorough clinical characterisation of both disease and control samples in comparative studies.

The following section will explore the main features of the eutopic endometrium in women with adenomyosis in comparison with unaffected endometrium and with the eutopic endometrium in women with endometriosis.

Endometrial Innervation

Interest in uterine and endometrial innervation in adenomyosis stems from a possible role of relevant growth factors in the pathogenesis of adenomyosis or in relation to dysmenorrhoea. Nerve growth factor has been linked to adenomyosis in the mouse model [15–17].

Quinn and Kirk (2002) compared the patterns of uterine innervations in uteri removed by hysterectomy for a variety of clinical conditions, including 8 uteri from nulliparous women (Group I), 21 uteri with no reported histological abnormality from multiparous women (Group II), 31 uteri reported with adenomyosis (Group III), and 17 uteri from women with pelvic pain (Group IV). Sections were taken from the isthmus of stored uteri (in the majority of cases) and stained with protein gene product 9.5 (PGP 9.5) as a marker of nerve distribution. Group (I) had significant nerve bundles at the endometrial-myometrial interface and in the subserosal layers, with nerve fibres noted in intervening neurovascular bundles supplying the myometrial stroma. Group (II) exhibited patterns of innervation similar to those of group (I) with the exception that 6 uteri demonstrated areas of nerve fibre proliferation. The vast majority of uteri with adenomyosis (Group III) exhibited large areas of myometrium without nerves and the absence of nerves in the neurovascular bundles supplying these areas. Five uteri exhibited areas of nerve

fibre proliferation at the margins of the adenomyosis with subserosal nerves present in the majority. In group (IV), eleven uteri demonstrated proliferation of small-diameter nerve fibres throughout the myometrium and in 6 uteri, there was asymmetry of nerve fibre proliferation. The absence of innervations in uteri with adenomyosis and their presence in uteri from women with pain is interesting because pain is commonly reported in women with adenomyosis [18]. Quinn published similar observations in 2007 [19]. However, it is interesting to note that the study focused on sections obtained from the uterine isthmus. The reason for this is unclear.

Zhang et al. reported on the endometrial and myometrial innervations in adenomyosis compared to women with fibroids. Nerves were identified by immunostaining using antibody for protein gene product 9.5 (PGP9.5). PGP9.5-immunoactive nerve fibres were identified in the functional layer of the endometrium in women with but not in women without pain. PGP9.5-immunoactive nerve fibre density in the basal layer of the endometrium or the myometrium significantly increased in women with pain. However, there was no statistically significant difference in PGP9.5-immunoactive nerve fibre density between women with adenomyosis and uterine fibroids [20]. The same authors reported the detection of nerve fibres in the endometrium and myometrium in women with endometriosis in the presence of pain but not in pain free disease [21]. Zhang et al. reported the expression of PGP9.5 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 the expression did not vary in relation to the presence of adenomyosis or endometriosis [22].

Barcena de Arellano et al. compared PGP 9.5, substance P, and tyrosine hydroxylase positive nerve fibres in women with and without adenomyosis and compared innervations in relation to the expression of aromatase cytochrome P450 and estrogen receptor in uterine nerve fibres. They reported that adenomyotic lesions were not innervated and that the density of sympathetic nerve fibres in the myometrium of women with adenomyosis was reduced when compared with the group without adenomyosis. There was a higher ERα/ERβ ratio in adenomyosis compared to the controls. The authors concluded that estrogen may play a role in the disruption of uterine sympathetic innervation which may impact the pathogenesis of adenomyosis [23].

Barcena de Arellano et al. compared the expression of nerve growth factor (NGF), neurotrophin 3 (NT-3), the high-affinity NGF receptor (TrkA), the low-affinity neurotrophin receptor p75 (NTR), the neuronal marker S100 (for myelinated nerve fibres) and protein gene product 9.5 (PGP9.5) in the uterus in women with and without adenomyosis. There was no significant difference in the NGF, NT-3 and p75 (NTR) expression in the myometrium or endometrium between the adenomyosis and the control group. But in contrast to the studies by Quinn and by Zhang, nerve fibre density S100, PGP9.5 and p75(NTR) did not differ significantly between the adenomyosis and controls [24]. There is evidence that the expression of nerve growth factor (NGF) and its receptors, NGFRp75 and TrkA in both the endometrium and the myometrium in women with adenomyosis is reduced in response to the levonorgestrel intrauterine device (Mirena®) [25]. Tyrosine kinase receptor TrKB protein and TrKB mRNA were reported to be higher in the secretory endometrium in women with adenomyosis compared to controls [26]. Higher TrKB protein expression was noted in the eutopic endometrium in endometriosis compared to control endometrium [27].

There are three SLIT [1–3] proteins that bind to and activate each of the four ROBO [1–4] receptors [28]. The SLIT/ROBO pathway is expressed during development and is involved in regulating cell migration [29], the development of the nervous system [30], and angiogenesis [31]. SLIT/ROBO interaction can promote apoptosis [32]. Compared with normal endometrium, SLIT expression was statistically significantly higher in the ectopic endometrium from women with adenomyosis, while roundabout 1 (ROBO1) immune-reactivity and microvessel density (MVD)

level were statistically significantly higher in both eutopic and ectopic endometrium compared to the normal endometrium. Both SLIT immune-reactivity in ectopic endometrium and MVD in eutopic endometrium were positively correlated with the severity of dysmenorrhea in women with adenomyosis [33].

Chen et al. reported increased expression of the adult stem cell marker Musashi-1 in the ectopic endometrium in adenomyosis. Musashi-1is a protein that in humans is encoded by the *MSI1* gene which is an evolutionally conserved marker for CNS progenitor cells including neural stem cells. Musashi-1 was expressed throughout the menstrual cycle. In the secretory phase epithelial, expression in adenomyosis was higher compared to the normal endometrium [34].

Extracellular Matrix and Blood Vessels

The endometrium is a dynamic tissue that undergoes cycles of rapid growth and shedding during the menstrual cycle. This involves active angiogenesis and the development of arterioles and a capillary network as well as mechanisms to control menstrual blood loss. Adenomyosis has been linked to excessive menstrual bleeding, but the effects of adenomyosis on vascular regulatory mechanisms within the endometrium are unclear. Of special interest is the role of matrix metalloproteinases (MMPs) because of their putative role in the degradation of extracellular matrix which can facilitate endometrial and stromal cell invasion and the role of vascular endothelial growth factor in relation to angiogenesis.

MMPs are steroid regulated and their modulation has been shown to affect ectopic endometrial implantation in endometriosis [35, 36]. Li et al. reported that the expression of MMP-2, MMP-9 and VEGF (vascular endothelial growth factor: a major mediator of angiogenesis and vascular permeability) was higher in the eutopic and the ectopic endometrium in women with adenomyosis compared to normal controls. Microvessel density (MVD) was higher in ectopic endometrium compared to eutopic endometrium. In adenomyosis, there was a positive correlation between VEGF and MMP-2 and MMP-9 [37]. Using a matrigel invasion assay, Yang et al. reported no difference in invasion between stromal cells derived from the endometrium in adenomyosis compared to controls despite a higher expression of MMP-2. The apparent lack of effect of increased MMP-2 on invasiveness may be attributed to the concomitant increase in the metalloproteinase inhibitor TIMP-1. Neither MMP-9 nor TIMP-2 was increased in stromal cells in adenomyosis [38]. Tokyol et al. [39] studies the expression of cyclo-oxygenase-2 (COX-2) and matrix metalloproteinase-2 (MMP-2) and microvessel density (MVD) in patients with adenomyosis. COX-2 expression in endometrium did not vary during the menstrual cycle in the control group but was higher in the secretory phase in patients with adenomyosis. There was no statistically significant difference in MMP-2 expression in stromal cells between the eutopic endometrium in adenomyosis and controls but there was no significant correlation between MVD and the expression of MMP-2 or COX-2 [39]. VEGF expression was higher in the eutopic endometrium in patients with adenomyosis compared to controls but this was not associated with a significant increase in hypoxia inducible factor-1α HIF-1α and MVD [40].

Comparing only patients with endometriosis and those with adenomyosis but not including normal controls, Goteri et al. reported increased vascular endothelial growth factor (VEGF) but not HIF-1 or microvessel density expression in the eutopic endometrium in adenomyosis [40]. This is in partial agreement with Li et al. who reported increased VEGF, the matrix metalloproteinases (MMP)-2 and -9 and MVD in the eutopic endometrium in adenomyosis compared to unaffected women [37]. Schindl et al. reported no increase in MVD in eutopic endometrium in adenomyosis compared to control [41]. Tokyol et al. reported that there were no significant differences in MMP-2 expression and MVD in the glandular epithelium or the stroma or in cyclooxygenase-2 (COX-2) or MMP expression in the luminal epithelium during either the proliferative or the

secretory phases of the menstrual cycle when comparing the eutopic endometrium in adenomyosis to control endometrium [39].

Cyclooxygenase-2 or COX-2, is an enzyme that in humans is encoded by the *PTGS2* gene. COX-2 antagonizes apoptosis, increases invasiveness and promotes angiogenesis. COX-2 expression in surface and glandular epithelia of the control group varied markedly during the menstrual cycle. It was lowest in the early proliferative phase and gradually increased and remained high throughout the secretory phase. In patients with endometriosis, expression of COX-2 in glandular epithelium was higher than that in the control group, though it varied throughout the menstrual cycle. On the other hand, there was no variation in expression of COX-2 in patients with adenomyosis during the menstrual cycle, and expression was lower compared to women with endometriosis in all phases of the cycle [42].

This is at variance with a previous study by Jones et al. who reported COX-2 to peak during menstruation, and to be at its lowest around ovulation [43]. However, when comparing the eutopic endometrium in endometriosis, adenomyosis and controls, the only statistically significant difference in COX-2 expression 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. Matsuzaki et al. observed that the level of COX-2 was significantly higher in the stromal cells of eutopic endometrium in women with deep infiltrating endometriosis compared to controls, and the levels seemed to correlate with the severity of pain [44].

Kang et al. reported that the −2578A or −1154A alleles of VEGF gene could significantly decrease the risk of adenomyosis and might be potentially protective factors for adenomyosis development and that the haplotypes of VEGF −460/−1154/−2578 polymorphisms may have an effect on the adenomyosis development [45]. A different polymorphism (+936TC) is linked to the development of endometriosis, but polymorphisms −460CT, +405CG, −2578 AC or −1154GA are not linked to the disease [46]. These findings should be treated with caution given the known difficulties with gene association studies.

Integrins are trans-membrane receptors mediating the attachment between adjacent cells. Khorram et al. reported up-regulation of endothelial nitric acid synthase (eNOS) and down regulation of $\alpha_v\beta_3$ integrin in the glandular and luminal epithelium of eutopic endometrium in endometriosis in the secretory phase of the cycle compared to unaffected controls [47]. A similar pattern of distribution of eNOS was noted in eutopic endometrium in adenomyosis [48], but these authors did not report different level of expression when compared to the normal controls. Ota et al. found the expression of eNOS to be persistently higher throughout the cycle in both eutopic endometrium in adenomyosis and eutopic endometrium in endometriosis compared to unaffected controls [48].

Ota et al. reported significant variations in the expression of the integrins: Very Late Activation antigens (VLA-2, 3, 4, 5, 6) and E-cadherin in the glandular epithelium during the proliferative phase when comparing the eutopic endometrium in endometriosis, adenomyosis and controls [49]. 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 [49]. Chen et al. 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 ectopic endometrium, but not in eutopic endometrium from affected women [50].

Cellular Proliferation and Apoptosis

Increased proliferation and reduced cell death has the potential to contribute to ectopic cell implantation. Jones et al. examined bcl-2 (a protein capable of inhibiting apoptosis) expression using immunohistochemistry and apoptosis using TdT-mediated dUTP nick-end labelling (TUNEL)

in 5 proliferative, 5 early- and 5 late-secretory samples of adenomyosis. They demonstrated rare apoptosis in the eutopic endometrium from women with adenomyosis as well as from control [51]. They reported that eutopic endometrium in adenomyosis displayed similar proliferative activity compared to control endometrium except in the proliferative and early secretory phases. The expression of bcl-2 in stromal cells was statistically significantly higher in the eutopic endometrium in adenomyosis compared to the eutopic endometrium in endometriosis in the proliferative phase. Interestingly, the same authors reported that there were no significant differences in bcl-2 expression when the eutopic endometrium in adenomyosis or endometriosis were compared to control endometrium [51]. 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 eutopic endometrium in endometriosis. Apoptosis (using dUTP nick-end labelling TUNEL assay) was rare in both tissues [52]. Most stromal bcl-2 positive cells were identified as leukocytes [51]. Yang et al. studied the expression of annexin V, 7-amino-actinomycin, caspase-3, Ki-67 and bcl-2 in isolated endometrial stromal cells from the eutopic endometrium in adenomyosis and control samples of women with fibroids or cervical intraepithelial neoplasia. All samples were obtained from the early- and mid-proliferative phases of the cycle. They reported reduced apoptosis and increased proliferation in endometrial stromal cells obtained from adenomyosis compared to controls and concluded that alteration in stromal cell proliferative and cells death may be relevant to the occurrence of adenomyosis [53].

In contrast to the case in eutopic endometrium in adenomyosis, Goumenou et al. reported that bcl-2 expression in epithelial cells did not vary with the phase of the cycle in the eutopic endometrium in endometriosis [54]. Thus bcl-2 expression was significantly higher in adenomyosis in the proliferative phase and significantly lower in the secretory phase. Indeed all secretory samples were reported as negative for bcl-2. However, Mclaren et al. and Meresman et al. found that bcl-2 expression in the eutopic endometrium in endometriosis 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 [55, 56]. Meresman et al. found Bax to be absent during the late proliferative phase with some samples showing positive expression in the late secretory phase [56], whilst McLaren et al. reported Bax to be present in both the proliferative and secretory phases [55]. Goumenou et al. 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 [54].

Matsumoto et al. compared the expression of TdT-mediated dUTP nick-end labelling (TUNEL), Ki-67 and bcl-2 in the eutopic and ectopic endometrium in women with adenomyosis. In the eutopic endometrium, Ki-67 was up-regulated and bcl-2 was down-regulated in the proliferative phase. Ectopic endometrium, on the other hand, was rarely influenced by progesterone and the induction of apoptosis and bcl-2 expression showed no cyclical changes. Apoptosis, however, was more frequent in stromal cells of the ectopic endometrium in all menstrual cycle phases. Ki-67 was constantly expressed in the glandular epithelium of the ectopic endometrium irrespective of the phase of the cycle [57]. They concluded that the observed differences in proliferation and apoptosis strongly suggest that adenomyotic lesions do not originate in the basal endometrium. However, the sample size per phase of the cycle was very small and the authors did not consistently examine the basal endometrium. It is to be considered that the presence of differences between the ectopic and the eutopic endometrium does not – in itself – preclude the ectopic endometrium being derives from the eutopic endometrium.

The apoptotic ratio of eutopic endometrial cell from adenomyosis was lower than that in control group and the apoptotic ratio increased after gonadotrophin releasing hormone agonist (GnRHa) addition in both groups and apoptotic ratio in

adenomyosis was significantly higher than that in control endometrium [58]. VEGF was significantly higher in the eutopic endometrium in adenomyosis compared to the normal endometrium and VEGF was down-regulated in both adenomyosis and controls by GnRHa in a dose-dependent manner. Difference in apoptotic ratio and higher VEGF in adenomyosis may be associated with the pathogenesis of adenomyosis [58] GnRHa may increase the apoptotic ratio of cultured endometrial cells by autocrine or paracrine mechanisms. GnRHa can directly suppress the survival and growth of ectopic endometrial by decreasing the release of VEGF which was related to the adenomyosis angiogenesis [58]. GnRHa therapy was shown to reduce cell proliferation possibly through a direct anti-proliferative effect [59].

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 [60]. Using immunohistochemistry, Ota et al. noted higher expression of HSP-27 in the eutopic endometrium in endometriosis and adenomyosis as compared to control endometrium in both phases of the cycle [61]. HSP-70 was higher only in the proliferative phase, whilst there were no differences in HSP-60 expression, with no differences between the eutopic endometrium in endometriosis and the eutopic endometrium in adenomyosis. It is hard to interpret these data, although it may indicate a higher protective mechanism against cellular stress.

Goteri et al. evaluated the expression of the cell division control protein analogue, Cdc42 in eutopic and ectopic endometrial tissue in patients with adenomyosis or ovarian endometriotic cysts compared with patients without either condition [62]. The intensity of Cdc42 immunostaining in eutopic endometrium did not differ significantly in women with adenomyosis compared to controls but was stronger in women with ovarian endometriosis. The difference between women with adenomyosis and endometriosis was statistically significant only in the proliferative phase of the cycle.

Kim et al. 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 eutopic endometrium in adenomyosis compared to unaffected women [63]. Higher Pak1 has also been demonstrated in the eutopic endometrium in endometriosis [64]. Tissue Factor is a member of the family of cytokine receptor class II involved in angiogenesis and apoptosis and has a role in the coagulation pathway. Its expression was reported to be higher in the eutopic and ectopic endometrium of women with adenomyosis compared to control [65]. Nie et al. (2010) reported that the expression of oxytocin receptor and of the transient receptor potential vanilloid type-1 (TRPV1) were higher in the ectopic compared to the eutopic endometrium in adenomyosis and controls [65].

Steroid Receptors

It is often stated that local rather than systemic hyperestrogensim is a factor in the pathopysiology of adenomyosis [66]. Aromatase and estrone sulfatase activity were detected in ectopic endometrium and aromatase was detected in glandular cells of eutopic and ectopic endometrial tissues in women with adenomyosis [67]. The activity of these enzymes was suppressed by danazol [67]. Hatok et al. reported increased aromatase mRNA expression in eutopic endometrium in women with endometriosis and adenomyosis compared to healthy controls [68]. Invading endometrial glands but not the stroma in adenomyosis express more human chorionic gonadotrophin/luteinizing hormone receptor (hCG/LH) mRNA and immune-reactive receptor protein than non-invading glands but the difference was not consistent as it varied among patients [69]. GnRH agonist or danazol were shown to decrease the expression of aromatase cytochrome P450 in the eutopic endometrium in adenomyosis [70], although the mechanisms of action of these agents differ. GnRH agonist reduced aromatase cytochrome P450 expression mainly by promoting a hypoestrogenic state,

whereas danazol reduced aromatase cytochrome P450 in part by direct action on the eutopic endometrium [70]. Interestingly, Maia et al. reported that aromatase was detected in the majority of eutopic endometrium in adenomyosis but that most ectopic endometrium was negative [71]. The observation by Chen et al. of a negative correlation between serum estradiol and E-cadherin expression in the epithelial components of the eutopic and the ectopic endometrium in adenomyosis – if confirmed – would suggest a role for systemic steroid levels in the pathogenesis of adenomyosis [50].

There are reports that both endometriosis and adenomyosis are associated with increased local estrogen production. Increased P450 aromatase RNA was reported in eutopic endometrium in adenomyosis and eutopic endometrium in endometriosis, but not in endometrial samples from women with cervical pathology. In Eutopic endometrium in adenomyosis and eutopic endometrium in endometriosis samples, aromatase cytochrome P-450 was immune-localised exclusively in the cytoplasm of glandular cells and faintly in the stroma [72, 73], but samples were not classified based on cycle phase. Brosens et al. detected aromatase mRNA in all samples in a group of infertile women undergoing IVF [74], which suggests that aromatase expression may not be confined to endometria from women with estrogen dependent abnormalities, but levels did not vary with the phase of the cycle.

The question of aromatase expression in endometriosis remains controversial. Maia et al. linked aromatase expression in the eutopic endometrium to the presence of infertility and dysmenorrhoea irrespective of the presence of endometriosis [75]. Maia et al. reported that aromatase was expressed in the stroma in 80 % of eutopic endometrium in women with adenomyosis [71]. 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 to the lack of expression in asymptomatic endometriosis free patients [75]. Colette et al. could not identify aromatase protein or mRNA expression in the endometrium in women with endometriosis [76]. There were also discrepancies between immunohistochemical studies as to whether aromatase is localised to the epithelium or stroma and methodological issues may have resulted in false positive mRNA detection.

During the proliferative phase, mRNA level and the activity of 17β-hydroxysteroid dehydrogenase-2 (HSD2), the enzyme responsible for the conversion of estradiol to estrone were comparable in the eutopic endometrium in women with endometriosis, adenomyosis and controls [77]. In the secretory phase, mRNA and HSD2 activity increased four- to six-fold in the eutopic endometrium in adenomyosis or endometriosis [77]. The findings suggest that locally active estrogens are higher in the normal compared to diseased endometrium during the secretory phase secondary to increased conversion [78], but no differences were reported between the eutopic endometrium in adenomyosis and the eutopic endometrium in endometriosis.

Takahashi et al. reported that estradiol levels in menstrual blood were highest in adenomyosis, followed by endometriosis, and lowest in normal menstrual blood. But these findings have not been confirmed [79]. It is to be noted that the differences in HSD2 and aromatase levels and activity between diseased and normal endometria do not explain the reported differences in estradiol levels in menstrual blood. Whilst *PvuII* polymorphism of the *ER-α* gene and in intron 4 of the *CYP19* gene encoding P450 were associated with risks of estrogen-dependent disease, no differences were reported between endometriosis and adenomyosis, and leiomyomas [80, 81]. The role of steroids is also demonstrated by the induction of adenomyosis in the animal model, but the effect is dose and strain dependent [82, 83].

Ueki et al. reported that estrogen receptor (ER) expression was more intense in ectopic compared to eutopic endometrium during the secretory phase in women with adenomyosis and that bcl-2 was constantly expressed throughout the menstrual cycle [84]. Danazol administration resulted in weaker expression of ER and bcl-2 in the ectopic endometrium in adenomyosis compared to expression following the administration of GnRH agonists. The number of apoptotic

(TUNEL-positive) cells increased in the ectopic adenomyotic endometrium treated with danazol or GnRH agonist [84].

Nie et al. reported lower progesterone receptor B (PR-B) in the eutopic and the ectopic endometrium in women with adenomyosis compared to control endometrium. There was a statistically significant difference in the expression of PR-B in the normal endometrium and eutopic and ectopic endometrium from women with adenomyosis. PR-B was reduced in the ectopic and eutopic endometrium in adenomyosis compared to controls [85]. ER-α expression in the adenomyotic endometrium was different from that of the normal endometrium in the mid-secretory phase of the cycle. The ER-β expression was reported to be statistically significantly elevated in the adenomyotic functionalis gland during the proliferative phase across the entire menstrual cycle. Expression of PR-A was similar to that of PR-B, with reduced expression in the basalis stroma in the adenomyotic samples. The pattern of ER-β, PR-A, and PR-B expression was similar in the endometrial basalis and adenomyotic foci [86].

Cytokine and Immune Components

An immune dysfunction could enable the survival of endometrial fragments outside the uterine cavity. There is considerable literature on altered immune response as a possible aetiological factor in endometriosis. This has mostly focussed on pelvic nodules and on factors detected in peritoneal fluid. Research involving the eutopic endometrium has largely been concerned with a possible role in the pathogenesis or the impact on fertility.

Mathur et al. reported the presence of endogenous IgG in 78 % of the endometrium and endometriosis implants in women with endometriosis compared to 22 % of controls. They also reported the presence of serum and/or peritoneal fluid IgG against endometrial antigen in affected women [87].

Stromal leukocyte populations in eutopic endometrium in adenomyosis do not differ significantly from those in either control endometrium or eutopic endometrium from women with endometriosis [52]. But there may be some differences between the intraepithelial leukocytes (IEL) when comparing eutopic endometrium in endometriosis and adenomyosis. In one study CD45+ cells increased from the proliferative to the late secretory phase in control endometrium and in the eutopic endometrium in endometriosis, but not in the eutopic endometrium in adenomyosis [88]. But the samples size was too small to reach firm conclusions in relation to adenomyosis.

During the proliferative phase the glandular epithelium of eutopic endometrium in endometriosis, but not eutopic endometrium in adenomyosis exhibited an increased number of CD45+ and CD43+ IEL compared to 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 to control endometrium in both endometriosis and in adenomyosis [88]. Chiang and Hill identified no differences in T cells, IFNγ and HLA-DR-positive cells in eutopic endometrial samples from adenomyosis, endometriosis or unaffected controls [89]. The study by Chiang and Hill included 7 women with endometriosis, compared with hysterectomy specimens from 7 women with adenomyosis and 10 women without endometriosis or endometrial pathology. They reported that T cells, IFNγ and HLA-DR-positive cells were present in eutopic endometrial samples throughout the menstrual cycle and that differences exists between the eutopic endometrium and the ectopic endometrium in endometriosis [89]. Gagné et al. reported that the proportion of CD3+, CD16+, CD3-HLADR−, CD3-CD45RA−, CD3+CD16−, CD3+CD56−, CD56-CD16+, and CD16b+ leukocytes in the endometrium of women with endometriosis is different compared to controls [90].

Much of recent research on the role of immunological abnormalities and free radicals in adenomyosis is based on the work of Ota and colleagues [41–52]. These publications include differential expression of HLA-DR antigen [91], immune cells [92], integrins and adhesion molecules [49] and heat shock proteins [61]. These

observations are interesting, but it is often difficult to ascertain the significance of reported differences or the possible impact of methodological issues with the studies. In addition, it is not possible to determine whether the reported differences are related to the aetiology of the disease as the authors suggest or, whether they are a consequence of the existing uterine pathology.

Interleukin-18 (IL-18), a major regulator of immune responses, is expressed in a number of situations including sites of chronic inflammation, autoimmune diseases, some cancers, and numerous infections. Luo et al. reported that IL-18 mRNA level was lower in both the eutopic and ectopic endometrium in endometriosis [93]. Huang et al. examined the expression of IL-18, its receptor (IL-18R), and IL-18 binding protein (IL-18BP) mRNA, and protein expression in the eutopic endometrium in adenomyosis [94]. The eutopic endometrial IL-18R mRNA and the IL-18BP to IL-18 ratio were significantly increased in adenomyosis compared to control endometrium, but the level of IL-18 mRNA was not significantly different.

Differences were also reported between endometrial stromal cells from adenomyosis and controls. Yang et al, reported that the addition of medroxyprogesterone acetate or danazol to cultures results in a significant reduction in IL-6 concentration in culture supernatant of endometrial stromal cells obtained from control samples but not from samples obtained from adenomyosis. IL-6 mRNA in stromal cells and IL-6 in culture supernatant were higher in adenomyosis compared to the controls after 8 days in culture after the addition of medroxyprogesterone acetate or danazol [95]. Whereas Mehasseb et al. reported increased invasiveness of endometrial stromal cells from adenomyosis [96], and also on differences in proteomic profiles of culture supernatants [96].

The expression of leukaemia inhibitory factor (LIF), a member of the IL-6 family linked to implantation, has been related to the fertility status rather than to adenomyosis. LIF was found to be down-regulated in the endometrium and uterine flushing of women with adenomyosis and infertility but not in those with adenomyosis and dysmenorrhoea. Levels of LIF in the latter group were comparable to fertile controls that did not have adenomyosis [97].

Interleukin 10 (IL-10) influences many features of immunoregulation and inflammation and enhances B cell survival, proliferation, and antibody production. Wang et al. used immunohistochemistry and H-scores and reported increased IL-10 expression in the epithelial cells but not in the stroma of the eutopic endometrium in adenomyosis compared to control endometrium [98]. But the expression scores are not provided and no comment is made about cellular localization of stromal immunostaining. The same group, used similar methodology and reported that interleukin-10 receptors (IL-10R1 and IL-10R2) were mainly expressed in epithelial cells in the endometrium [99]. They reported increased IL-10R1 in the eutopic endometrium in adenomyosis compared to control endometrium. In contrast, studies of cytokine expression in endometriosis have focussed on peritoneal fluid, peritoneal macrophages, leukocytes from peripheral blood or the endometrium as the primary source of cytokines. Sotnikova et al. demonstrated altered cytokine production in mononuclear cells obtained from the eutopic endometrium in adenomyosis [100]. There was a statistically significant increase in interferon-γ (IFNγ), INFα, IL-1β, tumour necrosis factor-α (TNFα), and epidermal growth factor (EGF), and a reduction in IL-8 compared to controls. This suggests a high level of lymphocyte activation including T cells. Increased activity of immune-competent cells may create the conditions that favour cell infiltration and proliferation leading to the development of adenomyosis. Antsiferova et al. reported that the lymphocytes from eutopic endometrium in endometriosis expressed lower levels of IL-2 mRNA, absent IL-4 mRNA, and low IL-10 mRNA but the levels were not statistically significantly different compared to controls [101]. The percentage of leucocytes expressing intracellular IL-10 in the eutopic endometrium in endometriosis was not statistically significantly different compared to controls. Martinez-Roman et al. reported a reduction in T cell population in endometriosis patients who also have infertility.

This is a significant observation as it links endometrial abnormality to a functional state rather than to endometriosis *per se* and it remains unclear if other observed differences in the expression of cytokines between endometriosis, adenomyosis and controls varies in relation to the presenting symptoms [102].

Ulukus et al. compared IL-8 and monocyte chemotactic protein expression in the eutopic endometrium in adenomyosis and control endometrium using immunohisochemistry [103]. Both the epithelium and stroma were positive, but expression was more intense in the epithelium. In control tissue but not in adenomyosis there was a rise in both factors in the secretory phase. IL-8 and monocyte chemo-attractant protein-1 (MCP-1) were higher in the epithelium in eutopic endometrium in endometriosis during the proliferative phase [104]. The same group reported that the IL-8 receptors (CXCR1 and CXCR2) showed higher epithelial staining during the proliferative, but not the secretory, phase in the eutopic endometrium in adenomyosis compared to controls [105]. In the eutopic endometrium in endometriosis there was a significant increase in epithelial CXCR2 expression in both the proliferative and secretory phases, but CXCR1 expression was higher only in the proliferative phase. Ulukus et al. reported higher epithelial CXCR1 and CXCR2 staining in the eutopic endometrium in adenomyosis compared to control endometrium in the proliferative but not in the secretory phase [103]. The distribution of IL-8 contrasts with that reported by Arici et al. who found negative staining in the stroma, and no significant difference between early-proliferative, mid-proliferative and late- secretory phases of the cycle and that the levels of IL-8 mRNA were statistically significantly lower in mid-cycle (late-proliferative and early-secretory phases) compared to other cycle phases [106, 107]. This discrepancy may be related to methodological issues as some studies did not take into account fluctuations within the cycle phase.

The levels of IFNγ, IFNα, TNFα, IL-1β and EGF were significantly increased and the level of IL-8 was reduced in culture supernatant of mononuclear cells obtained from the eutopic endometrium of women with adenomyosis compared to the control endometrium. Mononuclear cells from ectopic endometrium in adenomyosis produced higher levels of IFNγ, IFNα and TNFα compared to mononuclear cells from normal endometrium. The production of IL-1β, IL-8 and EGF by ectopic endometrial mononuclear cells was significantly reduced [100]. The authors suggested a significant role of local cytokine production in the development of adenomyosis [100].

There was a statistically significant difference in the expression of nuclear and cytoplasmic p65 and IκBα, and nuclear p50 and p52 in the normal endometrium and the eutopic and ectopic endometrium from women with adenomyosis. Cytoplasmic IκBα expression was reduced while nuclear p65, p50 and p52 expressions were all increased in ectopic endometrium [85].

IκBα (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha) is a member of the family of cellular proteins that inhibit NF-κB transcription factor. IκBα inhibits NF-κB by masking the nuclear localization signals (NLS) of NF-κB proteins and keeping them sequestered in an inactive state in the cytoplasm. In addition, IκBα blocks the ability of NF-κB transcription factors to bind to DNA, which is required for NF-κB functioning.

NF-κB (Nuclear factor KappaB) is a transcription factor with a role in modulating genes involved in inflammation, proliferation, apoptosis, invasion, angiogenesis and other cellular functions. It may regulate enzymes and growth factors such as Cyclooxygenase-2 (COX-2), VEGF, and Tissue Factor (TF). NF-κB may have a role in the pathogenesis of endometriosis [108], and adenomyosis [109]. NF-κB-DNA binding was present in all phases of the menstrual cycle in women with and without endometriosis, but this varied from strong binding to very low or undetectable. DNA binding of the p65 subunit of NF-κB was higher in the proliferative compared to the secretory or menstrual phase endometrium in controls, but was lower during the menstrual phase in the eutopic endometrium in endometriosis. There were no significant differences when cycle phases were compared between the eutopic endometrium in endometriosis and controls [108]. NF-κB subunits p65 and p50 and NF-κB-DNA

binding activity in endometrial stromal cells was significantly higher in adenomyosis compared to controls [109]. Li et al. reported that mRNA and protein levels of COX-2, VEGF, and tissue factor (TF) in stromal cells in adenomyosis were significantly higher compared to controls [109].

GnRHa therapy reduced the infiltration with CD68-positive macrophages, angiogenesis (von Willebrand positive vessels) and increased apoptosis (TdT- mediated dUTP-biotin nick end-labelling – TUNEL assay) in the endometrium of women with adenomyosis [110].

There is some evidence of increased HLA-DR (a major histocompatibility complex class II cell surface receptor) expression in eutopic endometrium in endometriosis [111] and also in women with adenomyosis [91], and of increased expression of γδT cells (gamma-delta T cells) in the stroma and of HLA antigens in the glandular cells in both adenomyosis and endometriosis [92], but it is unclear if there are differences between the two conditions. It is possible that aberrant expression of HLA-DR antigen in glandular cells of eutopic and ectopic endometria in endometriosis and adenomyosis is involved in various immunological responses.

Wang et al. examined the expression of Human Leukocyte Antigen-G (HLA-G) in eutopic and ectopic endometrium to assess its possible role as mediator of immune suppression which can confer protection to ectopic endometrial cells [112]. They reported that virtually no HLA-G was detected in normal endometrium, but both eutopic and ectopic endometrium in adenomyosis expressed HLA-G. It is notable that previous reports had not identified HLA-G in eutopic endometrium in endometriosis but there is disagreement over whether it is expressed in endometriosis nodules [113, 114].

In contrast to control endometrium from women with fibroids, both the eutopic and the ectopic endometrium in adenomyosis expressed HLA-G. Expression was higher in the glands compared to the stoma [112]. Human leukocyte antigen-G (HLA-G) is a non-classical major histocompatibility complex class I antigen. The presence of HLA-G has been proposed as a mechanism by which cells can escape immunosurveillance. Thus the Wang et al. proposed this as a mechanism for the persistence of ectopic endometrium without being eliminated by the immune system [112].

Propst et al. reported that immunohistochemical staining for Granulocyte macrophase colony-stimulating factor (GM-CSF) ligand was significantly higher in adenomyotic glands compared with autologous endometrial glands especially during the secretory phase of the menstrual cycle but that there was no statistically significant differences in the amount and intensity of staining of the granulocyte macrophage-colony stimulating factor (GM-CSF) receptor [115].

Oxidative Stress and Free Radical Metabolism

Free radicals are involved in the physiology of reproduction and enzymes that produce and eliminate various free radicals are believed to modulate the concentrations of free radicals within the uterus and the endometrium. Reactive oxygen species may be modulated in the presence of adenomyosis.

Van Langendonckt et al. summarized evidence linking oxidative stress to the inflammatory reaction in endometriosis. This includes an increased release by macrophages of reactive oxygen species; 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 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 [116]. In contrast, HO-1 and HO-2 were strongly expressed in the ectopic endometrium, especially in red lesions [117]. Iwahara et al. studied HO in women with adenomyosis. They confirmed the expression of HO-1 and HO-2 in both eutopic and ectopic

endometrium, but found lower levels in the ectopic compared to eutopic endometrium. They concluded that both HO-1 and HO-2 contribute little to the pathophysiology of adenomyosis [118].

Endothelial nitric acid synthase (eNOS) was detected in the luminal and glandular epithelium of the endometrium and in the endothelium of vasculature. Intense immunoreactivity was detected in the secretory but not in the proliferative phase in eutopic endometrium in adenomyosis and control endometrium; treatment with GnRH down regulated eNOS in the eutopic endometrium in adenomyosis [119].

Glutathione peroxidase (GPx), a coenzyme 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. The expression of glutathione peroxidise on the surface of glandular epithelia during the menstrual cycle in fertile controls is weak in the early proliferative phase and gradually increases to become most marked in the early secretory phase, decreasing thereafter [120]. There was loss of cyclicity in the eutopic endometrium in endometriosis; mainly because of the loss of the secretory phase peak. Expression in the glandular epithelium of eutopic endometrium in adenomyosis was higher, and in endometriosis was lower compared to the control endometrium. There were no other statistically significant differences between the groups in either the glandular or the luminal epithelium [120].

The enzyme Xanthine Oxidase (XO) produces superoxide leading to the accumulation of free radicals within the cell. Xanthine oxidase expression in the glandular epithelium exhibits variation with the menstrual cycle in controls but not in ectopic endometrial tissue in adenomyosis [121]. The expression of XO 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 eutopic endometrium in endometriosis and eutopic endometrium in adenomyosis. The expression in the luminal epithelium in the eutopic endometrium in adenomyosis was statistically significantly higher compared to control endometrium during the late secretory phase but there were no statistically significant differences when compared to the eutopic endometrium in endometriosis.

Superoxide Dismutase (SOD) protects cells from free radical damage. Ota et al. reported the expression of SOD in the endometrium of women with adenomyosis or endometriosis [122] The expression of copper, zinc and manganese -SOD in controls varied with the stages of the menstrual cycle. But SOD was persistently over-expressed in all phases of the menstrual cycle in patients with endometriosis and adenomyosis.

Catalase enzyme is involved in the conversion of hydrogen peroxide into water and oxygen. Ota et al, reported the expression of catalase, in eutopic and ectopic endometria in patients with endometriosis or adenomyosis [123]. In the control endometrium, catalase expression was lowest in the early proliferative and peaking in the late secretory phase of the menstrual cycle, but the expression was persistently elevated in women with adenomyosis and endometriosis and showed no cyclical variation. They also reported that enzyme expression as determined by immunohistochemical score was higher in eutopic endometrium in endometriosis compared to control endometrium and was highest in the eutopic endometrium in adenomyosis. But no statistical analyses were provided [123].

Molecular Signalling and Epigenetic Factors

HOXA10 is a homeobox-containing transcription factor that is essential for embryonic uterine development and for normal endometrial development during the menstrual cycle. HOXA10 in endometrial glandular and stromal cells is upregulated in response to estrogen and progesterone. It was reported that the mid-luteal rise in

HOXA10 which was linked to the implantation window was absent in women with endometriosis [124]. Matsuzaki et al. [125] reported that mid-luteal HOXA10 mRNA and protein were lower in stromal cells in women with endometriosis and infertility compared to controls. The expression of HOXA10 protein was shown to be down-regulated in endometrial stroma, but not in the glands of women with adenomyosis [126].

Decrease in HOXA10 mRNA was observed in the eutopic endometrium of baboons with induced endometriosis and this was associated with a decreased expression of β3-integrin and down regulation of Homeobox protein EMX2 [127]. Hoxa10 and Hoxa11 were also decreased in the eutopic endometrium in the experimental mouse model of endometriosis but there were no changes in β3-integrin mRNA expression [128].

Epigenetic factors may have a role in the pathogenesis of both endometriosis [129] and adenomyosis [33, 130–132]. This is supported by the finding of HOXA10 [133] and PR-B promoter hypermethylation in the endometrium in endometriosis [134], and the existence of a link between the aberrant expression of the deoxyribonucleic acid methyltransferases (DNMT) -1, -3A and -3B in endometriosis and DNA methylation. mRNA for DNMT1, DNMT3A and DNMT3B were statistically significantly higher in ectopic endometrium compared to controls, but only mRNA level for DNMT3A was statistically significantly higher in the eutopic endometrium in endometriosis compared to controls [135]. In adenomyosis, DNMT3A was significantly reduced in eutopic endometrium compared to controls and DNMT1 was positively correlated with heavy bleeding [131]. Ectopic stromal cells from adenomyosis exhibit PR-B hypermethylation [136], but no studies have addressed methylation status in adenomyosis eutopic endometrium.

Conclusion

Endometrial micro-environment in adenomyosis differs in some aspects of cellular and humoral immunity from the normal endometrium. The aberrant immune responses could suggest immunological stress or immunological tolerance. Alternatively, the observed differences may be the result of structural alterations. An interesting hypothesis that has not been adequately explored is that endometrial differences may be linked to clinical symptoms rather than to the presence of adenomyosis. The clinical significance of the observed morphological, biochemical and molecular differences is unclear and whilst some of these abnormalities might be expected to impact on fertility and on the outcomes of IVF, it is the case that the effect of adenomyosis on fertility remains controversial.

There are several major limitations in existing research on the eutopic endometrium in women with adenomyosis. Firstly, there are major diagnostic limitations inherent in the definition of the disease, and the issue of control for co-morbidities. The presence or absence of endometriosis or fibroids in women with adenomyosis is frequently not taken into consideration. Secondly, there is a systematic bias because of the clinical presentations, since biopsies used for the study of the eutopic endometrium are obtained from patients with clinical presentations that warrant a biopsy. Thirdly, there are a large number of clinical features that may be relevant to studies of the endometrium including 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. No studies are available on the changes in eutopic endometrium or on the natural history of adenomyosis. This is compounded by methodological weaknesses particular to the laboratory experiment themselves which may account for the limited reproducibility and contradictory findings. All this hinders the ability to reach firm conclusions in connection with the findings discussed above.

References

1. Benagiano G, Brosens I. The endometrium in adenomyosis. Womens Health (Lond Engl). 2012;8: 301–12.
2. Benagiano G, Brosens I, Habiba M. Structural and molecular features of the endomyometrium in endometriosis and adenomyosis. Hum Reprod Update. 2014;20:386–402.
3. Carvalho L, Podgaec S, Bellodi-Privato M, Falcone T, Abrao MS. Role of eutopic endometrium in pelvic endometriosis. J Minim Invasive Gynecol. 2011; 18:419–27.
4. Brosens I, Brosens JJ, Benagiano G. The eutopic endometrium in endometriosis: are the changes of clinical significance? Reprod Biomed Online. 2012;24:496–502.
5. Danforth DN, Chapman JC. The isthmic mucous membrane of the human uterus. Science. 1949; 109:383.
6. Dallenbach-Hellweg G. Histopathology of the endometrium. 4th ed. Berlin: Springer; 1987.
7. al-Azzawi F, Habiba M, Bell SC. The Leicester Endometrial Needle Sampler: a novel device for endometrial and myometrial junctional zone biopsy. Obstet Gynecol. 1997;90:470–2.
8. McCausland AM. Hysteroscopic myometrial biopsy to diagnose adenomyosis and its clinical application. Surg Technol Int. 1995;IV:216–9.
9. McCausland AM. Hysteroscopic myometrial biopsy: its use in diagnosing adenomyosis and its clinical application. Am J Obstet Gynecol. 1992;166:1619–26. discussion 26–8.
10. Ota H, Tanaka T. Stromal vascularization in the endometrium during adenomyosis. Microsc Res Tech. 2003;60:445–9.
11. Puttemans P, Molinas R, Gordts S, Peperstraete L, Campo R, Brosens I. Hysteroscopic images of an isolated lesion of unknown origin in a young infertile patient. J Minim Invasive Gynecol. 2005;12:104–5.
12. Eng CW, Tang PH, Ong CL. Hysterosalpingography: current applications. Singapore Med J. 2007;48: 368–73. quiz 74.
13. Valentini AL, Speca S, Gui B, Soglia G, Micco M, Bonomo L. Adenomyosis: from the sign to the diagnosis. Imaging, diagnostic pitfalls and differential diagnosis: a pictorial review. Radiol Med. 2011; 116:1267–87.
14. Reinhold C, Tafazoli F, Wang L. Imaging features of adenomyosis. Hum Reprod Update. 1998;4:337–49.
15. Parrott E, Butterworth M, Green A, White IN, Greaves P. Adenomyosis – a result of disordered stromal differentiation. Am J Pathol. 2001;159:623–30.
16. Green AR, Edwards RE, Greaves P, White IN. Comparison of the effect of oestradiol, tamoxifen and raloxifene on nerve growth factor-alpha expression in specific neonatal mouse uterine cell types using laser capture microdissection. J Mol Endocrinol. 2003; 30:1–11.
17. Green AR, Styles JA, Parrott EL, Gray D, Edwards RE, Smith AG, et al. Neonatal tamoxifen treatment of mice leads to adenomyosis but not uterine cancer. Exp Toxicol Pathol. 2005;56:255–63.
18. Quinn MJ, Kirk N. Differences in uterine innervation at hysterectomy. Am J Obstet Gynecol. 2002;187: 1515–9. discussion 9–20.
19. Quinn M. Uterine innervation in adenomyosis. J Obstet Gynaecol. 2007;27:287–91.
20. Zhang X, Lu B, Huang X, Xu H, Zhou C, Lin J. Innervation of endometrium and myometrium in women with painful adenomyosis and uterine fibroids. Fertil Steril. 2009;94:730–7.
21. Zhang X, Lu B, Huang X, Xu H, Zhou C, Lin J. Endometrial nerve fibers in women with endometriosis, adenomyosis, and uterine fibroids. Fertil Steril. 2009;92:1799–801.
22. Zhang X, Lu B, Huang X, Xu H, Zhou C, Lin J. Innervation of endometrium and myometrium in women with painful adenomyosis and uterine fibroids. Fertil Steril. 2010;94:730–7.
23. Barcena de Arellano ML, Oldeweme J, Arnold J, Schneider A, Mechsner S. Remodeling of estrogen-dependent sympathetic nerve fibers seems to be disturbed in adenomyosis. Fertil Steril. 2013;100:801–9.
24. Barcena de Arellano ML, Wagner MF, Oldeweme J, Arnold J, Ebert A, Schneider A, et al. Neurotrophin expression is not affected in uteri of women with adenomyosis. J Mol Neurosci. 2012;47:495–504.
25. Choi YS, Cho S, Lim KJ, Jeon YE, Yang HI, Lee KE, et al. Effects of LNG-IUS on nerve growth factor and its receptors expression in patients with adenomyosis. Growth Factors. 2010;28:452–60.
26. Huang Y, Zheng W, Mu L, Ren Y, Chen X, Liu F. Expression of tyrosine kinase receptor B in eutopic endometrium of women with adenomyosis. Arch Gynecol Obstet. 2010;283:775–80.
27. Anger DL, Zhang B, Boutross-Tadross O, Foster WG. Tyrosine receptor kinase B (TrkB) protein expression in the human endometrium. Endocrine. 2007;31:167–73.
28. Legg JA, Herbert JM, Clissold P, Bicknell R. Slits and Roundabouts in cancer, tumour angiogenesis and endothelial cell migration. Angiogenesis. 2008;11:13–21.
29. Dickinson RE, Duncan WC. The SLIT-ROBO pathway: a regulator of cell function with implications for the reproductive system. Reproduction. 2010;139:697–704.
30. Hinck L. The versatile roles of “axon guidance” cues in tissue morphogenesis. Dev Cell. 2004;7:783–93.
31. Jones CA, London NR, Chen H, Park KW, Sauvaget D, Stockton RA, et al. Robo4 stabilizes the vascular network by inhibiting pathologic angiogenesis and endothelial hyperpermeability. Nat Med. 2008;14:448–53.
32. Dallol A, Morton D, Maher ER, Latif F. SLIT2 axon guidance molecule is frequently inactivated in colorectal cancer and suppresses growth of colorectal carcinoma cells. Cancer Res. 2003;63:1054–8.

33. Nie J, Liu X, Zheng Y, Geng JG, Guo SW. Increased immunoreactivity to SLIT/ROBO1 and its correlation with severity of dysmenorrhea in adenomyosis. Fertil Steril. 2010;95:1164–7.
34. Chen YZ, Wang JH, Yan J, Liang Y, Zhang XF, Zhou F. Increased expression of the adult stem cell marker Musashi-1 in the ectopic endometrium of adenomyosis does not correlate with serum estradiol and progesterone levels. Eur J Obstet Gynecol Reprod Biol. 2013;173:88–93.
35. Bruner KL, Eisenberg E, Gorstein F, Osteen KG. Progesterone and transforming growth factor-beta coordinately regulate suppression of endometrial matrix metalloproteinases in a model of experimental endometriosis. Steroids. 1999;64:648–53.
36. Braundmeier AG, Fazleabas AT, Lessey BA, Guo H, Toole BP, Nowak RA. Extracellular matrix metalloproteinase inducer regulates metalloproteinases in human uterine endometrium. J Clin Endocrinol Metab. 2006;91:2358–65.
37. Li T, Li YG, Pu DM. Matrix metalloproteinase-2 and -9 expression correlated with angiogenesis in human adenomyosis. Gynecol Obstet Invest. 2006;62:229–35.
38. Yang JH, Wu MY, Chen MJ, Chen SU, Yang YS, Ho HN. Increased matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-1 secretion but unaffected invasiveness of endometrial stromal cells in adenomyosis. Fertil Steril. 2009;91:2193–8.
39. Tokyol C, Aktepe F, Dilek FH, Sahin O, Arioz DT. Expression of cyclooxygenase-2 and matrix metalloproteinase-2 in adenomyosis and endometrial polyps and its correlation with angiogenesis. Int J Gynecol Pathol. 2009;28:148–56.
40. Goteri G, Lucarini G, Montik N, Zizzi A, Stramazzotti D, Fabris G, et al. Expression of vascular endothelial growth factor (VEGF), hypoxia inducible factor-1alpha (HIF-1alpha), and microvessel density in endometrial tissue in women with adenomyosis. Int J Gynecol Pathol. 2009;28:157–63.
41. Schindl M, Birner P, Obermair A, Kiesel L, Wenzl R. Increased microvessel density in adenomyosis uteri. Fertil Steril. 2001;75:131–5.
42. Ota H, Igarashi S, Sasaki M, Tanaka T. Distribution of cyclooxygenase-2 in eutopic and ectopic endometrium in endometriosis and adenomyosis. Hum Reprod. 2001;16:561–6.
43. Jones RL, Kelly RW, Critchley HO. Chemokine and cyclooxygenase-2 expression in human endometrium coincides with leukocyte accumulation. Hum Reprod. 1997;12:1300–6.
44. Matsuzaki S, Canis M, Pouly JL, Wattiez A, Okamura K, Mage G. Cyclooxygenase-2 expression in deep endometriosis and matched eutopic endometrium. Fertil Steril. 2004;82:1309–15.
45. Kang S, Zhao J, Liu Q, Zhou R, Wang N, Li Y. Vascular endothelial growth factor gene polymorphisms are associated with the risk of developing adenomyosis. Environ Mol Mutagen. 2009;50:361–6.
46. Liang S, Huang Y, Fan Y. Vascular endothelial growth factor gene polymorphisms and endometriosis risk: a meta-analysis. Arch Gynecol Obstet. 2012;286:139–46.
47. Khorram O, Lessey BA. Alterations in expression of endometrial endothelial nitric oxide synthase and alpha(v)beta(3) integrin in women with endometriosis. Fertil Steril. 2002;78:860–4.
48. Ota H, Igarashi S, Hatazawa J, Tanaka T. Endothelial nitric oxide synthase in the endometrium during the menstrual cycle in patients with endometriosis and adenomyosis. Fertil Steril. 1998;69:303–8.
49. Ota H, Tanaka T. Integrin adhesion molecules in the endometrial glandular epithelium in patients with endometriosis or adenomyosis. J Obstet Gynaecol Res. 1997;23:485–91.
50. Chen YJ, Li HY, Huang CH, Twu NF, Yen MS, Wang PH, et al. Oestrogen-induced epithelial-mesenchymal transition of endometrial epithelial cells contributes to the development of adenomyosis. J Pathol. 2010;222:261–70.
51. Jones RK, Searle RF, Bulmer JN. Apoptosis and bcl-2 expression in normal human endometrium, endometriosis and adenomyosis. Hum Reprod. 1998;13:3496–502.
52. Jones RK, Bulmer JN, Searle RF. Phenotypic and functional studies of leukocytes in human endometrium and endometriosis. Hum Reprod Update. 1998;4:702–9.
53. Yang JH, Wu MY, Chen CD, Chen MJ, Yang YS, Ho HN. Altered apoptosis and proliferation in endometrial stromal cells of women with adenomyosis. Hum Reprod. 2007;22:945–52.
54. Goumenou A, Panayiotides I, Matalliotakis I, Vlachonikolis I, Tzardi M, Koumantakis E. Bcl-2 and Bax expression in human endometriotic and adenomyotic tissues. Eur J Obstet Gynecol Reprod Biol. 2001;99:256–60.
55. McLaren J, Dealtry G, Prentice A, Charnock-Jones DS, Smith SK. Decreased levels of the potent regulator of monocyte/macrophage activation, interleukin-13, in the peritoneal fluid of patients with endometriosis. Hum Reprod. 1997;12:1307–10.
56. Meresman GF, Vighi S, Buquet RA, Contreras-Ortiz O, Tesone M, Rumi LS. Apoptosis and expression of Bcl-2 and Bax in eutopic endometrium from women with endometriosis. Fertil Steril. 2000;74:760–6.
57. Matsumoto Y, Iwasaka T, Yamasaki F, Sugimori H. Apoptosis and Ki-67 expression in adenomyotic lesions and in the corresponding eutopic endometrium. Obstet Gynecol. 1999;94:71–7.
58. Xiao-xia W, Jia-li K, Xue-fei S, Jia Y, Ling-hong D. Effect of GnRHa on apoptosis and release of VEGF in endometrial cell cultures from patients with adenomyosis. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2012;28:72–3.
59. Khan KN, Kitajima M, Hiraki K, Fujishita A, Nakashima M, Ishimaru T, et al. Cell proliferation effect of GnRH agonist on pathological lesions of women with endometriosis, adenomyosis and uterine myoma. Hum Reprod. 2010;25:2878–90.

60. De Maio A. Heat shock proteins: facts, thoughts, and dreams. Shock. 1999;11:1–12.
61. Ota H, Igarashi S, Hatazawa J, Tanaka T. Distribution of heat shock proteins in eutopic and ectopic endometrium in endometriosis and adenomyosis. Fertil Steril. 1997;68:23–8.
62. Goteri G, Ciavattini A, Lucarini G, Montik N, Filosa A, Stramazzotti D, et al. Expression of motility-related molecule Cdc42 in endometrial tissue in women with adenomyosis and ovarian endometriomata. Fertil Steril. 2006;86:559–65.
63. Kim SR, Kim SH, Lee HW, Chae HD, Kim CH, Kang BM. Increased expression of p21-activated kinase in adenomyosis. Fertil Steril. 2010;94:1125–8.
64. Kim YA, Kim JY, Kim MR, Hwang KJ, Chang DY, Jeon MK. Tumor necrosis factor-alpha-induced cyclooxygenase-2 overexpression in eutopic endometrium of women with endometriosis by stromal cell culture through nuclear factor-kappaB activation. J Reprod Med. 2009;54:625–30.
65. Liu X, Nie J, Guo SW. Elevated immunoreactivity to tissue factor and its association with dysmenorrhea severity and the amount of menses in adenomyosis. Hum Reprod. 2010;26:337–45.
66. Kitawaki J. Adenomyosis: the pathophysiology of an oestrogen-dependent disease. Best Pract Res Clin Obstet Gynaecol. 2006;20:493–502.
67. Yamamoto T, Noguchi T, Tamura T, Kitawaki J, Okada H. Evidence for estrogen synthesis in adenomyotic tissues. Am J Obstet Gynecol. 1993;169: 734–8.
68. Hatok J, Zubor P, Galo S, Kirschnerova R, Dobrota D, Danko J, et al. Endometrial aromatase mRNA as a possible screening tool for advanced endometriosis and adenomyosis. Gynecol Endocrinol. 2011;27: 331–6.
69. Lei ZM, Rao CV, Lincoln SR, Ackermann DM. Increased expression of human chorionic gonadotropin/human luteinizing hormone receptors in adenomyosis. J Clin Endocrinol Metab. 1993;76:763–8.
70. Ishihara H, Kitawaki J, Kado N, Koshiba H, Fushiki S, Honjo H. Gonadotropin-releasing hormone agonist and danazol normalize aromatase cytochrome P450 expression in eutopic endometrium from women with endometriosis, adenomyosis, or leiomyomas. Fertil Steril. 2003;79 Suppl 1:735–42.
71. Maia Jr H, Casoy J, Correia T, Freitas L, Pimentel K, Athayde C, et al. Effect of the menstrual cycle and oral contraceptives on aromatase and cyclooxygenase-2 expression in adenomyosis. Gynecol Endocrinol. 2006;22:547–51.
72. Kitawaki J, Kusuki I, Koshiba H, Tsukamoto K, Fushiki S, Honjo H. Detection of aromatase cytochrome P-450 in endometrial biopsy specimens as a diagnostic test for endometriosis. Fertil Steril. 1999;72:1100–6.
73. Kitawaki J, Kusuki I, Koshiba H, Tsukamoto K, Honjo H. Expression of aromatase cytochrome P450 in eutopic endometrium and its application as a diagnostic test for endometriosis. Gynecol Obstet Invest. 1999;48 Suppl 1:21–8.
74. Brosens J, Verhoeven H, Campo R, Gianaroli L, Gordts S, Hazekamp J, et al. High endometrial aromatase P450 mRNA expression is associated with poor IVF outcome. Hum Reprod. 2004;19:352–6.
75. Maia Jr H, Haddad C, Casoy J. Correlation between aromatase expression in the eutopic endometrium of symptomatic patients and the presence of endometriosis. Int J Womens Health. 2012;4:61–5.
76. Colette S, Lousse JC, Defrere S, Curaba M, Heilier JF, Van Langendonckt A, et al. Absence of aromatase protein and mRNA expression in endometriosis. Hum Reprod. 2009;24:2133–41.
77. Kitawaki J, Koshiba H, Ishihara H, Kusuki I, Tsukamoto K, Honjo H. Progesterone induction of 17beta-hydroxysteroid dehydrogenase type 2 during the secretory phase occurs in the endometrium of estrogen-dependent benign diseases but not in normal endometrium. J Clin Endocrinol Metab. 2000;85:3292–6.
78. Kitawaki J, Kado N, Ishihara H, Koshiba H, Kitaoka Y, Honjo H. Endometriosis: the pathophysiology as an estrogen-dependent disease. J Steroid Biochem Mol Biol. 2002;83:149–55.
79. Takahashi K, Nagata H, Kitao M. Clinical usefulness of determination of estradiol level in the menstrual blood for patients with endometriosis. Nihon Sanka Fujinka Gakkai Zasshi. 1989;41:1849–50.
80. Kitawaki J, Obayashi H, Ishihara H, Koshiba H, Kusuki I, Kado N, et al. Oestrogen receptor-alpha gene polymorphism is associated with endometriosis, adenomyosis and leiomyomata. Hum Reprod. 2001;16:51–5.
81. Kado N, Kitawaki J, Obayashi H, Ishihara H, Koshiba H, Kusuki I, et al. Association of the CYP17 gene and CYP19 gene polymorphisms with risk of endometriosis in Japanese women. Hum Reprod. 2002;17: 897–902.
82. Mehasseb MK, Bell SC, Habiba MA. The effects of tamoxifen and estradiol on myometrial differentiation and organization during early uterine development in the CD1 mouse. Reproduction. 2009;138:341–50.
83. Mehasseb MK, Bell SC, Habiba MA. Neonatal administration of tamoxifen causes disruption of myometrial development but not adenomyosis in the C57/BL6J mouse. Reproduction. 2010;139:1067–75.
84. Ueki K, Kumagai K, Yamashita H, Li ZL, Ueki M, Otsuki Y. Expression of apoptosis-related proteins in adenomyotic uteri treated with danazol and GnRH agonists. Int J Gynecol Pathol. 2004;23:248–58.
85. Nie J, Lu Y, Liu X, Guo SW. Immunoreactivity of progesterone receptor isoform B, nuclear factor kappaB, and IkappaBalpha in adenomyosis. Fertil Steril. 2009;92:886–9.
86. Mehasseb MK, Panchal R, Taylor AH, Brown L, Bell SC, Habiba M. Estrogen and progesterone receptor isoform distribution through the menstrual cycle in uteri with and without adenomyosis. Fertil Steril. 2011;95:2228–35. 35 e1.
87. Mathur S, Garza DE, Smith LF. Endometrial autoantigens eliciting immunoglobulin (Ig)G, IgA, and IgM responses in endometriosis. Fertil Steril. 1990;54:56–63.

88. Bulmer JN, Jones RK, Searle RF. Intraepithelial leukocytes in endometriosis and adenomyosis: comparison of eutopic and ectopic endometrium with normal endometrium. Hum Reprod. 1998;13:2910–5.
89. Chiang CM, Hill JA. Localization of T cells, interferon-gamma and HLA-DR in eutopic and ectopic human endometrium. Gynecol Obstet Invest. 1997;43:245–50.
90. Gagne D, Rivard M, Page M, Lepine M, Platon C, Shazand K, et al. Development of a nonsurgical diagnostic tool for endometriosis based on the detection of endometrial leukocyte subsets and serum CA-125 levels. Fertil Steril. 2003;80:876–85.
91. Ota H, Igarashi S. Expression of major histocompatibility complex class II antigen in endometriotic tissue in patients with endometriosis and adenomyosis. Fertil Steril. 1993;60:834–8.
92. Ota H, Igarashi S, Tanaka T. Expression of gamma delta T cells and adhesion molecules in endometriotic tissue in patients with endometriosis and adenomyosis. Am J Reprod Immunol. 1996;35:477–82.
93. Luo Q, Ning W, Wu Y, Zhu X, Jin F, Sheng J, et al. Altered expression of interleukin-18 in the ectopic and eutopic endometrium of women with endometriosis. J Reprod Immunol. 2006;72:108–17.
94. Huang HY, Yu HT, Chan SH, Lee CL, Wang HS, Soong YK. Eutopic endometrial interleukin-18 system mRNA and protein expression at the level of endometrial-myometrial interface in adenomyosis patients. Fertil Steril. 2010;94:33–9.
95. Yang JH, Chen MJ, Wu MY, Chen YC, Yang YS, Ho HN. Decreased suppression of interleukin-6 after treatment with medroxyprogesterone acetate and danazol in endometrial stromal cells of women with adenomyosis. Fertil Steril. 2006;86:1459–65.
96. Mehasseb MK, Taylor AH, Pringle JH, Bell SC, Habiba M. Enhanced invasion of stromal cells from adenomyosis in a three-dimensional coculture model is augmented by the presence of myocytes from affected uteri. Fertil Steril. 2010;94:2547–51.
97. Xiao Y, Sun X, Yang X, Zhang J, Xue Q, Cai B, et al. Leukemia inhibitory factor is dysregulated in the endometrium and uterine flushing fluid of patients with adenomyosis during implantation window. Fertil Steril. 2010;94:85–9.
98. Wang F, Li H, Yang Z, Du X, Cui M, Wen Z. Expression of interleukin-10 in patients with adenomyosis. Fertil Steril. 2009;91:1681–5.
99. Qin X, Zhang H, Wang F, Xue J, Wen Z. Expression and possible role of interleukin-10 receptors in patients with adenomyosis. Eur J Obstet Gynecol Reprod Biol. 2012;161:194–8.
100. Sotnikova N, Antsiferova I, Malyshkina A. Cytokine network of eutopic and ectopic endometrium in women with adenomyosis. Am J Reprod Immunol. 2002;47:251–5.
101. Antsiferova YS, Sotnikova NY, Posiseeva LV, Shor AL. Changes in the T-helper cytokine profile and in lymphocyte activation at the systemic and local levels in women with endometriosis. Fertil Steril. 2005;84:1705–11.
102. Martinez-Roman S, Balasch J, Creus M, Fabregues F, Carmona F, Vilella R, et al. Immunological factors in endometriosis-associated reproductive failure: studies in fertile and infertile women with and without endometriosis. Hum Reprod. 1997;12: 1794–9.
103. Ulukus M, Ulukus EC, Seval Y, Cinar O, Zheng W, Arici A. Expression of interleukin-8 receptors in patients with adenomyosis. Fertil Steril. 2006;85: 714–20.
104. Ulukus M, Ulukus EC, Tavmergen Goker EN, Tavmergen E, Zheng W, Arici A. Expression of interleukin-8 and monocyte chemotactic protein 1 in women with endometriosis. Fertil Steril. 2009;91:687–93.
105. Ulukus M, Ulukus EC, Seval Y, Zheng W, Arici A. Expression of interleukin-8 receptors in endometriosis. Hum Reprod. 2005;20:794–801.
106. Arici A, Seli E, Senturk LM, Gutierrez LS, Oral E, Taylor HS. Interleukin-8 in the human endometrium. J Clin Endocrinol Metab. 1998;83:1783–7.
107. Arici A, Seli E, Zeyneloglu HB, Senturk LM, Oral E, Olive DL. Interleukin-8 induces proliferation of endometrial stromal cells: a potential autocrine growth factor. J Clin Endocrinol Metab. 1998;83:1201–5.
108. Gonzalez-Ramos R, Rocco J, Rojas C, Sovino H, Poch A, Kohen P, et al. Physiologic activation of nuclear factor kappa-B in the endometrium during the menstrual cycle is altered in endometriosis patients. Fertil Steril. 2012;97:645–51.
109. Li B, Chen M, Liu X, Guo SW. Constitutive and tumor necrosis factor-alpha-induced activation of nuclear factor-kappaB in adenomyosis and its inhibition by andrographolide. Fertil Steril. 2013;100:568–77.
110. Khan KN, Kitajima M, Hiraki K, Fujishita A, Sekine I, Ishimaru T, et al. Changes in tissue inflammation, angiogenesis and apoptosis in endometriosis, adenomyosis and uterine myoma after GnRH agonist therapy. Hum Reprod. 2010;25:642–53.
111. Liu Y, Luo L, Zhao H. Immunohistochemical study of HLA-DR antigen in endometrial tissue of patients with endometriosis. J Huazhong Univ Sci Technolog Med Sci. 2002;22:60–1.
112. Wang F, Wen Z, Li H, Yang Z, Zhao X, Yao X. Human leukocyte antigen-G is expressed by the eutopic and ectopic endometrium of adenomyosis. Fertil Steril. 2008;90:1599–604.
113. Barrier BF, Kendall BS, Ryan CE, Sharpe-Timms KL. HLA-G is expressed by the glandular epithelium of peritoneal endometriosis but not in eutopic endometrium. Hum Reprod. 2006;21:864–9.
114. Hornung D, Fujii E, Lim KH, Vigne JL, McMaster MT, Taylor RN. Histocompatibility leukocyte antigen-G is not expressed by endometriosis or endometrial tissue. Fertil Steril. 2001;75:814–7.
115. Propst AM, Quade BJ, Nowak RA, Stewart EA. Granulocyte macrophage colony-stimulating factor in adenomyosis and autologous endometrium. J Soc Gynecol Investig. 2002;9:93–7.

116. Van Langendonckt A, Casanas-Roux F, Donnez J. Oxidative stress and peritoneal endometriosis. Fertil Steril. 2002;77:861–70.
117. Van Langendonckt A, Casanas-Roux F, Donnez J. Iron overload in the peritoneal cavity of women with pelvic endometriosis. Fertil Steril. 2002;78:712–8.
118. Iwahara Y, Nagai A, Yoshiki N, Igarashi K, Yamashita K, Kubota T. Expression of heme oxygenase in the eutopic and ectopic endometrium in patients with adenomyosis. Gynecol Endocrinol. 2012;28(11):892–6.
119. Kamada Y, Nakatsuka M, Asagiri K, Noguchi S, Habara T, Takata M, et al. GnRH agonist-suppressed expression of nitric oxide synthases and generation of peroxynitrite in adenomyosis. Hum Reprod. 2000;15:2512–9.
120. Ota H, Igarashi S, Kato N, Tanaka T. Aberrant expression of glutathione peroxidase in eutopic and ectopic endometrium in endometriosis and adenomyosis. Fertil Steril. 2000;74:313–8.
121. Ota H, Igarashi S, Tanaka T. Xanthine oxidase in eutopic and ectopic endometrium in endometriosis and adenomyosis. Fertil Steril. 2001;75: 785–90.
122. Ota H, Igarashi S, Hatazawa J, Tanaka T. Immunohistochemical assessment of superoxide dismutase expression in the endometrium in endometriosis and adenomyosis. Fertil Steril. 1999;72: 129–34.
123. Ota H, Igarashi S, Sato N, Tanaka H, Tanaka T. Involvement of catalase in the endometrium of patients with endometriosis and adenomyosis. Fertil Steril. 2002;78:804–9.
124. Taylor HS, Bagot C, Kardana A, Olive D, Arici A. HOX gene expression is altered in the endometrium of women with endometriosis. Hum Reprod. 1999;14:1328–31.
125. Matsuzaki S, Canis M, Darcha C, Pouly JL, Mage G. HOXA-10 expression in the mid-secretory endometrium of infertile patients with either endometriosis, uterine fibromas or unexplained infertility. Hum Reprod. 2009;24(12):3180–7.
126. Fischer CP, Kayisili U, Taylor HS. HOXA10 expression is decreased in endometrium of women with adenomyosis. Fertil Steril. 2011;95:1133–6.
127. Kim JJ, Taylor HS, Lu Z, Ladhani O, Hastings JM, Jackson KS, et al. Altered expression of HOXA10 in endometriosis: potential role in decidualization. Mol Hum Reprod. 2007;13:323–32.
128. Lee B, Du H, Taylor HS. Experimental murine endometriosis induces DNA methylation and altered gene expression in eutopic endometrium. Biol Reprod. 2009;80:79–85.
129. Guo SW. Epigenetics of endometriosis. Mol Hum Reprod. 2009;15:587–607.
130. Liu X, Guo SW. Valproic acid alleviates generalized hyperalgesia in mice with induced adenomyosis. J Obstet Gynaecol Res. 2011;37:696–708.
131. Liu X, Guo SW. Aberrant Immunoreactivity of Deoxyribonucleic Acid Methyltransferases in Adenomyosis. Gynecol Obstet Invest. 2012;74(2): 100–8.
132. Liu X, Nie J, Guo SW. Elevated immunoreactivity against class I histone deacetylases in adenomyosis. Gynecol Obstet Invest. 2012;74(1):50–5.
133. Wu Y, Halverson G, Basir Z, Strawn E, Yan P, Guo SW. Aberrant methylation at HOXA10 may be responsible for its aberrant expression in the endometrium of patients with endometriosis. Am J Obstet Gynecol. 2005;193:371–80.
134. Wu Y, Strawn E, Basir Z, Halverson G, Guo SW. Promoter hypermethylation of progesterone receptor isoform B (PR-B) in endometriosis. Epigenetics. 2006;1:106–11.
135. Wu Y, Strawn E, Basir Z, Halverson G, Guo SW. Aberrant expression of deoxyribonucleic acid methyltransferases DNMT1, DNMT3A, and DNMT3B in women with endometriosis. Fertil Steril. 2007;87: 24–32.
136. Nie J, Liu X, Guo SW. Immunoreactivity of oxytocin receptor and transient receptor potential vanilloid type 1 and its correlation with dysmenorrhea in adenomyosis. Am J Obstet Gynecol. 2010;202:346. e1–8.