Uterine Adenomyosis

Marwan Habiba
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Editors

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Preface

Over the past few decades, we have witnessed countless advances in bioscience. This is reflected in the range of information published on adenomyosis, as well as in the experimental designs of recent research.

However, despite these advances, fundamental questions about the pathophysiology, clinical significance and impact of adenomyosis – a condition originally described more than a century ago – remain unanswered.

L. Emge in 1962 credited his teacher, Gideon Wells, for recognising that adenomyosis can elude clinical recognition. The term "elusive" is perhaps as applicable to adenomyosis today as it was in these earlier writings; this is perhaps all the more surprising, given the frequency by which it is identified in uteri removed at hysterectomy. The relatively small body of available literature also reflects that the prevalence of the condition has not been matched by corresponding research interest.

Non-invasive diagnosis using modern imaging techniques has fallen short of the expectation that it would enhance clinical management and research and further our understanding of the epidemiology, natural history and pathophysiology of adenomyosis. This may, at least in part, be attributable to the large number of parameters that need to be taken into consideration before well grounded conclusions could be derived from research in the field.

The purpose of this book is to provide the reader with an account of the current understanding of uterine adenomyosis. Many of the chapters attempt to cover the full spectrum of data available, from early to current literature, but discussions on diagnosis and therapy inevitably focus on more recent literature.

As it stands, the conclusion necessarily reached in this book is that much more needs to be done to enable us to better understand adenomyosis. We can, however, be cautiously optimistic, because of a number of promising developments in diagnostics as well as an improved understanding of the role of the myometrium.

We hope that this book will be useful both to the practicing gynaecologist and the postgraduate researcher, and any medical professional or scientist seeking to broaden their understanding of adenomyosis.

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History of Adenomyosis

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Giuseppe Benagiano, Ivo Brosens,
Marwan Habiba, and Donatella Lippi

Abstract

The first description of a condition today recognised to be a form of adenomyosis was published in 1860 by Carl Rokitansky who reported one case of *fibrous polyps of the uterus*, containing nests of endometrial cells. In 1882 von Recklinghausen suggested the name *adenomyoma uteri* and by the end of the nineteenth century the condition was clearly described by several Authors.

In spite of a clear description by Thomas Cullen, the medical community investigating 'mucosal invasions' of abdominal organs in general failed to identify them as being due to the heterotopic presence of uterine mucosa.

It must be stressed that some of the early descriptions of adenomyomas would today be considered as cases of endometriosis,

Cullen was the first to provide a description of the two main symptoms of adenomyosis: *lengthened menstrual periods* and *a great deal of pain*.

In 1925 Sampson led the way to the separation between mucosal invasion of the uterine body and of peritoneal organs and introduced the term *endometriosis* for the extrauterine forms of invasions. The same year Frankl described the anatomical picture of the intramyometrial endometrial invagination and called it *adenomyosis uteri*.

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Introduction

During the last two decades a controversy has developed over who was the first to describe adenomyosis and endometriosis, two conditions that until the 1920s were grouped together under the name "*adenomyoma*" (plural: "*adenomyomata*").

Indeed, the claim has been made that ancient descriptions exist of endometriosis [1] and that several eighteenth and nineteenth century treatises described the condition [2]. This is not the place to address this controversy and only the history of the discovery of adenomyosis (also named endometriosis interna) will be reconstructed.

The Identification of Adenomyoma

According to Emge [3], in 1882 von Recklinghausen suggested to coin this type of pathology "*adenomyoma uteri*", but gives no reference. What we know is that at the end of the nineteenth century, in 1895 and 1896 to be exact, the condition was clearly described by von Recklinghausen [4, 5] and Cullen [6], followed by Pick [7] and Rolly [8] in 1897. In a seminal book on adenomyoma published in 1908, Cullen [9] states that approximately 100 cases were described prior to his first paper of 1896.

In fact, the first description of a condition today recognised to be a form of adenomyosis is contained in an article published in 1860 by Rokitansky [10] in which he reported on three cases of "*fibrous polyps of the uterus*" and stated: "*among them there are some in which glandular tubes are found*". In detailing his findings Rokitansky mentions that "*In some rare cases the extension of the uterine glands occurred in both directions, i.e. both into the uterus cavity, as well as into the uterus parenchyma, such that the sloped bulge represents a plug of longitudinal fibrous appearance driven into, as it were, the uterine mass*".

Rokitansky's description apparently misled later readers, even those almost his contemporaries, on two grounds: First, a polypoid structure protruding inside the uterus would hardly be considered an "adenomyoma". Second, the name attributed to this lesion by Rokitansky was "*cistosarcoma adenoids uterinum*" and later "*sarcoma adenoids uterinum polyposum*". Both names seemed to distract attention from his description as representing what we today call adenomyosis.

At any rate, the link between Rokitansky's description and the discovery of adenomyosis is reiterated in a few recent articles describing "*adenomyomatous polyps*". A literature search [11] identified 13 reports (mostly from Japan) of adenomyomatous polyps; structures that Raghavendra Babu et al. [12] defined as follows: "*in addition to the usual features of endometrial polyps, they also contain a smooth muscle component*". Histologically they are composed of "*endometrial glands intimately mixed with smooth muscle and thick walled blood vessels*". An interesting issue surrounds adenomyomatous polyps, because a Medline search for "polypoid adenomyomas" yields more than 100 articles (mostly of atypical forms); yet the descriptions lead to the clear conclusion that the same pathology is reported under two different titles.

At any rate, Rokitansky specifies: "*The thick-walled uterus of an aged female showed this inter alia. On the left hand side under the mouth of the tuba was a swollen, about 1–2 long smoothly coated polyp of 11/2 diameter in the pedunculus, from 4–5 on the free end. A similar bisecting perpendicular section continuing into the uterine mass shows that the pedunculus penetrates to a depth of 4 into the uterine mass and stores a wedge driven right in to the uterine tissue; on its section along its length it has a fibrous appearance and can be torn into fibres in this direction; the arrangement of the fibres is determined by numerous extremely long glandular tubes held*

together by means of a core-rich connective tissue" [10].

A comprehensive definition of an *adenomyoma* was provided by Cuthbert Lockyer in 1918 [13]: "*the term 'adenomyoma' implies a new formation composed of gland-elements, hyperplastic cellular connective tissue, and smooth muscle*". He added: "*so far as the adenomatous elements are concerned, the same type of tumour-formation can be found also in the digestive tract (bowel and stomach), and some observers claim that analogous conditions can exist in the gall-bladder, in the kidney, and elsewhere*".

In spite of the clarity of Cullen's early description [6], which he expanded in his 1908 book [9], the medical community investigating 'mucosal invasions' of abdominal organs, in general failed to identify them as being due to the heterotopic presence of uterine mucosa and the nature of glands found in adenomyomata remained controversial for a long time [**see also** Chap. 6]: in 1904 Schickele [14] attacked Cullen's theory and argued that the mucosal growth was of mesonephric origin. He wrote "*when I try to take an impartial view of published cases, I am compelled to state that the mucosal theory is not proved*". Indeed, the presence of multiple communications with the lumen of the tube, or with the endometrial cavity constituted no proof to his mind.

During the early part of the twentieth century, the controversy over the origin of the epithelial cells lining the 'cysts' found in adenomyomata continued. Ignoring Rokitansky's conclusions, pathologists of the fame of von Recklinghausen [5] argued that adenomyomata were the result of displacement of Wolffian or mesonephric vestiges. He illustrated his theory of the origin of adenomyomata showing that glands were scattered along Wolffian remnants. The majority of pathologists and gynecologists then rejected the hypothesis that the glands they observed were "endometrial."

As late as 1918, Lockyer [13] in the above-mentioned book "*Fibroids and allied tumours*", in detailing the various theories on the origin of epithelial glands and stroma found in the pelvis outside the uterine cavity, was unable to resolve the question of their origin. He wrote: "*nothing but the topography of the tumour, nothing but laborious research entailing the cutting of serial sections in great numbers, can settle the question as to the starting point of the glandular inclusions for many of the cases of adenomyoma*". Lockyer reviewed a series of cases which he considered of 'serosal' origin. Five cases were labelled 'adenomyoma of the ovarian ligament' and had been published previously [14–18] (with today's knowledge these would be probably considered cases of deep endometriosis); he accepted Frankl's theory [18] that these tumours arose from parts of the Wolffian system (medullary cord or duct). Lockyer [13] also reported that before the name was coined there had been clear descriptions of adenomyomata, the first being those made by Babes [19], who, in 1882, published a case of an intramural myoma containing cysts lined with "*low cubical epithelium derived from embryonic germs*" and by Diesterweg [20] who, the following year, described "*two polypi of the posterior uterine wall containing cysts lined with ciliated epithelium and filled with blood*". At the time it was widely believed that epithelial cells found in adenomyomata (within and outside the uterus) had a Müllerian origin. In his 1908 book Cullen [9] mentions that, by 1884, some 100 cases had been reported by Schröder, Herr and Grosskopf, but provides no reference. Then, in 1893, von Recklinghausen published his first observations on adenomyomata (initially named by him "*adenocysten*" of the uterus) [21], followed by his 1895 publication [4] and his acclaimed book of 1896 [5] and divided adenomyomata into two classes:

1. Those situated at the periphery of the uterus and in the tubes;
2. Those arising centrally (within the uterus).

He believed that the first group derived from a "*numerical increase of the Wolffian tubules*", whereas the second originated from the uterine mucosa. He claimed to have seen three cases in which a malignant change occurred and therefore expressed the view that those arising with the myometrium were prone to undergo cancerous

degeneration. In a way this classification represents the first known attempt at separating what we call adenomyosis from endometriosis; in the latter case, however, von Recklinghausen failed to recognise the 'epithelial colonisation' as being endometrial in nature and provided elaborated descriptions of the similarities between these extra-uterine growths and the mesonephros. The mesonephric origin of what we probably would consider cases of endometriosis, was accepted by most pathologists for two decades and in 1897 Ludwig Pick stated that the "*mesonephric origin of adenomyoma*" had been definitely established by "*fundamental proof*" [7].

Following early descriptions, a number of cases of adenomyoma were published during the first two decades of the twentieth century and various theories were elaborated to explain the presence of epithelial glands in the peritoneal cavity. Probably the first to address the issue was Orloff [22] who, in 1895, described "*glandular spaces under the serosa covering uterine myomata"*, which he considered as arising from 'embryonic cells'.

Some of the early descriptions would today be considered as endometriosis, like the one made by Mayer in 1903 [15] who, in performing a relaparotomy for severe pelvic pains following uterine ventrofixation, found epithelial glands around the silk ligatures. This case, which would today be labelled as "secondary endometriosis", prompted Mayer to elaborate a theory of 'epithelial heterotopy', a phenomenon that he believed could occur both in dystopic (embryonic), as well as orthotopic (mature) epithelia. According to this theory, adenomyomas represent an "*epithelial invasion of inflammatory infiltrated tissue"*.

It is important to stress that, whereas adenomyosis and endometriosis were considered one disease, this condition was believed to be totally separated from the so-called "haemorrhagic ovarian cysts" (or chocolate cysts), the condition today labelled as "ovarian endometrioma". Notwithstanding this situation, already in 1899, Russell [23] had reported the microscopic evaluation of a case where he found "*the right ovary enveloped in adhesion of the posterior face of the broad ligament*". Russell was "*astonished to find areas which were an exact prototype of the uterine glands and interglandular connective tissue*".

The Study of Adenomyomata

As mentioned, the first systematic investigation of what is today known as adenomyosis was carried out by Thomas Stephen Cullen, the often forgotten pioneer who – over the end of the nineteenth and the beginning of the twentieth century – fully researched 'mucosal invasion' already observed by a number of investigators in several parts of the lower abdominal cavity.

According to Emge [3], it was Cullen who in 1892 was the first to suggest the term "*adenomyosis*" to be applied to the diffuse form of adenomyoma. As mentioned, his first observations dated back to 1893–1896 and were published in 1896 [6]. What seems astonishing is his findings that, out of 1283 cases of myomas, he found 73 adenomyomata described as interstitial, sub peritoneal and submucous. In 1908, Cullen [9] published a book dedicated to adenomyomata of the uterus and provided a more detailed and comprehensive classification of the condition than the subdivision proposed by von Recklinghausen [5].

Cullen distinguished three types:

1. Adenomyomata in which the uterus preserves a relatively normal contour;
2. Subperitoneal or intraligamentary adenomyomata;
3. Submucous adenomyomata.

In the preface of his book he provides the following account of his first observation: "*One afternoon in October, 1894, while making the routine examination of the material from the operating room I found a uniformly enlarged uterus about four times the natural size. On opening it I found that the increase in size was due to a diffuse thickening of the anterior wall. Professor William H. Welch, when consulted, said that the*

condition was evidently a most unusual one and suggested that sections be made from the entire thickness of the uterine wall. Examination of these sections showed that the increase in thickness was due to the presence of a diffuse myomatous tumor occupying the inner portion of the uterine wall, and that the uterine mucosa was at many points flowing into the diffuse myomatous tissue". He then proceeded to describe all the cases that came to his attention and provided an astonishing clear iconography of the various types of adenomyoma he found. Although in their vast majority these consisted of myomatous tissue clearly infiltrated by uterine mucosa, occasionally there were no visible myomata.

Being a gynaecologist, Cullen did not limit himself to the anatomical and histological description of his cases: he also provided a description of the clinical profile of the condition. He mentions two main symptoms: "*lengthened menstrual periods*" that – as the disease progresses – "*may be replaced by a continuous haemorrhagic discharge*"; and "*a great deal of pain*". He confessed that "*in the early years of our investigations we also failed to detect it clinically, but in the early and fairly advanced stages of the process so definite are the symptoms that the hospital assistant now frequently comes and says that a given case has all the signs of an adenomyoma and that he feels sure that this is the cause of the bleeding*".

When discussing treatment, Cullen explained that: "*abdominal hysterectomy is indicated*", because "*myomectomy is inapplicable, as the growth is so interwoven with the normal muscle that it cannot be shelled out*".

The following year, Kelly and Cullen [24] published a book dedicated to myomata where they described within some myomata the presence of heterotopic epithelial cells, stroma and glands: "*In cases of adenomyoma of the uterus we usually find a diffuse myomatous thickening of the uterine muscle. This thickening may be confined to the inner layers of the anterior, posterior, or lateral walls, but in other cases the myomatous tissue completely encircles the uterine cavity. This diffuse myomatous tissue contains large or small chinks, and into these the normal uterine mucosa flows. If the chinks are small, there is only room for isolated glands, but where the spaces are goodly in size, large masses of mucosa flow into and fill them. We accordingly have a diffuse myomatous growth with normal mucosa flowing in all directions through it. The mucosa lining the uterine cavity is perfectly normal*".

Going against 'conventional wisdom' Cullen made a clear identification of the epithelial tissue in adenomyomata as 'uterine mucosa'. He also provided a neat description of the fact that the endometrium invades the inner myometrium through the presence in it of 'chinks', or fissures and claimed that "*sometimes its direct connection with the mucosa of the uterine cavity can be traced*". Finally, he identified a 'myomatous' thickening of the uterine muscle as a direct consequence (or a sort of pre-requisite) for the formation of what we call adenomyosis. Identifying this 'thickening' seems a clear anticipation of the identification of a thickened junctional zone myometrium characteristic of adenomyosis [**see** Chap. 6].

Defining Adenomyosis

In the already mentioned book, Lockyer [13] provided the following definition: "*the term 'adenomyoma' implies a new formation composed of gland-elements, hyperplastic cellular connective tissue, and smooth muscle*". There is however a major difference between the definition given by Cullen, who clearly referred to adenomyosis only, and that of Lockyer, who considered adenomyomata as part of a process involving a number of abdominal organs.

The inability of most of the early researchers into the origin of the 'islets' of epithelial tissue observed in various abdominal organs to imagine that they were in fact 'transplants' of uterine mucosa, led to the above-described, long controversy until – as Lockyer expresses it – there was a "*gradual ascendancy of Cullen's mucosal theory*". Among the early supporters of Cullen's

views was von Franqué [25] who believed that epithelial growths found in a number of abdominal organs derived from the "*mature mucous membrane*" of the uterus which became capable of 'infiltrating' other organs as a consequence of an inflammatory process. Other early supporters of this theory were Baldy and Longscope [26], who, not only refused the Wolffian hypothesis of von Recklinghausen, but also rejected that put forward by Kossman [27] of a Müllerian origin.

Then in 1920 Cullen published a comprehensive review [28] in which he mentioned that he had observed the presence of uterine mucosa almost ubiquitously in the lower abdomen, including the ovary. In other words, he had identified both cases of adenomyosis and of peritoneal and ovarian endometriosis. What he failed to do, however, was to realise the differences between the two conditions.

It was only in the nineteen-twenties through the efforts of John Sampson that the "endometrial" origin of the mucosa found infiltrated in the walls of the uterus became accepted by all and that adenomyosis was recognised as an entity different from endometriosis. In 1925 Sampson affirmed that in menstruating women, the endometrium sloughing into uterine veins could cause adenomyoma and disease beyond the pelvis [29]. In the same year he introduced the term "endometriosis" for the first time, but he also utilised the term "implantation adenomyoma" [30]. Then, in 1927 Sampson published his original work on 'retrograde menstruation' as the cause of 'peritoneal endometriosis' [31] a term that describes the presence of islets of uterine mucosa in the peritoneal cavity.

On the other hand, in 1925, Frankl [32] adopted the name first utilised by Cullen to designate the mucosal invasion of the myometrium. He clearly described the anatomical picture and called it "adenomyosis uteri". Frankl provided the following explanation: "*I have chosen the name of* adenomyosis*, which does not suggest any inflammatory genesis as do terms like* adenometritis*,* adenomyositis*,* adenomyometritis*, still employed. My own conception has been verified by the observations of various authors, among whom are Lahxn, Meyer, Schwarz, Schiller, and also supported by my more recent thirteen additional cases. We were never able to find any trace of an inflammatory infiltration, either in the musculature or in the mucosa of this region. In the histories of these patients, we did not find a single symptom suggesting a preceding puerperal or gonorrheal infection*".

Referring to cases he had previously published, Frankl drew a distinction between *adenomyosis* and *adenomyoma*. He wrote: "*In an adenomyoma the glands originate independently within the myoma as an autochthonous growth, while in adenomyosis, even when localized, the direct connection of the endometrium with the islands of mucosa located in the musculature can be established in serial sections. In the majority of cases of genuine adenomyoma, which are extremely rare, the glands are not accompanied by stroma*". Another feature described by Frankl is the different appearance of the mucosa making-up adenomyosis: "*The entire material from thirty cases shows in twelve instances the presence of myomas, mostly of very small size, a fact which should not be overlooked. Eleven times we found the mucosa in a hyperplastic condition. The coincidence of small myomas is not so striking inasmuch as they are quite common in general, but the presence of a hyperplastic mucosa eleven times is noteworthy. The penetration of a hyperplastic mucosa into the myometrium can be readily understood if we assume for it a more marked tendency toward proliferation*".

It is important to recall that Frankl considered that there were many similarities between adenomyosis and endometriosis: "*An observation made only once should be mentioned, namely, the presence of blood in the glands within the myometrium. This finding was made in a woman of fifty years, who still was menstruating regularly. The last menstruation had occurred three weeks previous to operation. In a few glands, which were dilated cystically, we found only slightly changed blood. This observation reminds one of menstruating uterine mucosa on the surface of the ovary, first described by Sampson. By the courtesy of Sampson I had an opportunity of studying the original slides and I confirm that both in his and in my case, misplaced uterine*

glands were seen filled with blood, undoubtedly menstrual blood". He even illustrated these similarities with the words: "*The illustration* (a microphotograph) *will easily convince the reader of the close similarity between my picture and the one shown by Sampson*".

Several features of adenomyosis were identified since the early days: over and above the descriptions made by Cullen, Frankl also pointed to the early (for these days) appearance of menorrhagia: "*With the clinical picture of adenomyosis we must always associate a sudden onset of a very excessive hemorrhage, coincident with or independent of menstruation, and not one which gradually increases as is observed in a hemorrhagic metropathia*". Probably because steroid hormones had not yet been identified except for their general actions, Frankl wrote: "*Not being able to prove or to disprove a hormonal cause for adenomyosis I am unwilling to consider the hemorrhages in this disease as having any connection with endocrine influences*".

A feature of adenomyosis that remains unsettled is a possible familial inheritance. In 1962 Emge [3] mentioned that for 15 years he had tried to collect information on familial occurrence in adenomyosis and that he discovered 7 instances in which mothers and daughters had both been operated upon for the condition. Unfortunately, no additional information on this subject is available.

It is notable that much of the earlier descriptions with their focus on localised lesions are at variance with more modern descriptions. In contemporary literature, the definitions more widely accepted are derived from that proposed by Bird [33] in 1972: "*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*".

Conclusions

Cullen intuition that 'epithelial invasions' observed throughout the reproductive tract were endometrial in nature and Sampson's contribution to the understanding of this 'invasion', which he called 'endometriosis', have been influential in directing research in the search for evidence in support of the 'regurgitation theory'. Throughout the past century, much research was focussed on the characterisation of the ectopic implants. Only in recent years, with the introduction of new imaging techniques and molecular diagnostic tools, has it become clear that adenomyosis and endometriosis may represent different phenotypes of a more fundamental disorder characterised by impaired cellular responses to ovarian sex steroids throughout the reproductive tract. Although advances in our understanding of the cross-talk between steroid hormone receptors and cell surface signalling pathways are of particular relevance to the pathogenesis of endometriosis, the search for a molecular definition of this syndrome has barely started.

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The Incidence and Clinical Significance of Adenomyosis

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Abstract

For more than a century the diagnosis of adenomyosis was only possible through pathological examination of hysterectomy specimens but this has changes with the introduction of transvaginal ultrasound and MRI. Despite the large number of published studies reporting on the incidence and the clinical correlates of adenomyosis, there is no agreement on the definition and cut-off between adenomyosis and normal uteri and most reports still rely on case series of women undergoing hysterectomy. This poses considerable challenge to our understanding of the disease, its impact and of the accuracy of imaging diagnosis.

Keywords

Incidence • Adenomyosis • Adenomyosis sub-basalis • Endometrial myometrial interphase • Transvaginal ultrasound • Magnetic resonance imaging • Junctional zone • Subendometrial myometrium • Abnormal uterine bleeding • Parity • Infertility • Peristalsis • Utero-tubal transport

The Incidence and Clinical Significance of Adenomyosis

For more than a century after adenomyosis was first described, the diagnosis was only possible through pathological examination of hysterectomy specimens. This changed following the advent of high definition transvaginal ultrasound and MRI which enabled non-invasive diagnosis. There are a large number of published studies reporting on the incidence and the clinical correlates of adenomyosis. Mostly, these still rely on case series of women undergoing hysterectomy. The reported incidence in different studies

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varies from 5 to 70 % [7]. The very wide variation is more likely to be related to differences in diagnostic criteria and to methodological issues in case ascertainment. But there are also differences between the study populations.

It is well recognised that the endometrial-myometrial interface in normal uteri is irregular [37]. Classically, pathologists made the diagnosis of adenomyosis based on their subjective assessment of the degree of deviation from what they regarded as normal. More than a century on, there remains lack of agreement on the definition of the appropriate cut-off point.

Defining Adenomyosis

Classically, adenomyosis is defined by the presence of heterotopic endometrial glands and stroma within the myometrium. Relevant diagnostic features are the depth of stromal and glandular presence within the muscle and the presence of myometrial hypertrophy or hyperplasia around adenomyotic glands. Hendrickson and Kempson [37] described myometrial changes as a collar of hypertrophic smooth muscle around adenomyotic foci [37]. But there are no objective definitions for the reported changes in smooth muscles. Irregularity at the endometrial myometrial interface is almost universal [37], thus the identification of adenomyosis has relied on an assessment of the degree of deviation of mucosal presence compared to uteri judged to be normal. Inevitably, this will remain subjective unless defined features can be linked to the genesis of symptoms. In one study the reported incidence of adenomyosis varied almost fivefolds (from 12 to 58 %) between hospitals and by almost ninefolds (from 10 to 88 %) between pathologists. There were also important differences amongst pathologists working at the same hospital [96]. Because of the perceived risk of over diagnosis, many pathologists argued for the adoption of conservative cut-off points [32, 112]. But as mentioned above, this approach remains arbitrary [12] and can clearly result in overlooking early stages of the conditions. Consequently, any possible contribution of less extensive disease to symptoms will not be recognised. Indeed, it likely that in most cases, it is the presence of symptoms that necessitated the hysterectomy in the first instance. Thus it remains unclear how a conservative approach to histological diagnosis can be scientifically justified. The few studies that addressed this point, did find a link between symptoms and minimal depth lesions [12, 94]. The term "adenomyosis sub-basalis" was introduced to denote lesions within one low power field (LPF) [94] or <1 high power field (HPF) [112] below the basal endometrium. Despite this, these uteri would be classified as "normal" using the more prevalent definition of adenomyosis.

It is also unfortunate that definitions used to describe adenomyosis utilise expressions that convey a particular conception of the pathophysiology of the disease especially given the current limitation of our understanding of the condition. It is now common for the term 'invasion' to be used when describing the observation of the mere presence of glands within the myometrium. Vercellini et al. (2006), for example, states that: "it is generally agreed that adenomyosis occurs when the normal boundary between the endometrial basal layer and the myometrium is disrupted" [113]. They add that "as a consequence of this disruption, the endometrial glands *invade* the myometrium". The sequence is thus set as disruption leading to invasion. Uduwela et al. (2000) propose the inverse sequence when they write: "adenomyosis is a disease characterized by deep invasion of the inner myometrium by endometrial glands and stroma thereby disrupting the EMI (Endometrial Myometrial Interphase)" [107]. Whilst both invasion and disruption may have a role in the disease, it is important to consider the framing bias entailed in utilising these terms.

Beside the question of defining the cut-off for identifying adenomyosis, the reported incidence of adenomyosis after hysterectomy is also necessarily affected by the degree of diligence in case ascertainment. The extent of sampling becomes important because uterine affection is not uniform. Yet, some studies have relied on as few as two random sections of the uterus [120]. In this respect, it seems that the rate of diagnosis

increased overtime. In the early series by Dreyfuss (1940), 1807 surgically removed uteri were examined. He reported a combined incidence of "adenomyosis and endometriosis" in 224 instances (12.4 %) and that in 152 cases (8.4 % of the total) the lesion was localised within the myometrium representing cases of adenomyosis [25]. Three decades later, Bird et al. (1972) reported on the incidence of adenomyosis in 200 consecutive hysterectomies [12]. When these cases were examined routinely, adenomyosis was identified in 31 % of instances. The figure rose to 38.5 % when 6 additional blocks were examined and to 61.5 % when sub-basal lesions (within one LPF below the basal endometrium) were included. There is controversy over which of the uterine walls is most affected. Some studies reported more affection in the posterior wall [120], but others disagreed. In a study involving 88 samples, Sammour et al. (2002) reported affection of both the anterior and posterior walls in 76 % of cases, affection in the anterior wall only in 6.8 % of cases and of the posterior wall only in 17 % of cases [94].

The cut-off point for the diagnosis of adenomyosis remains open to interpretation and also to miscommunication. It is sometimes expressed with reference to the microscope optical field but this varies according to microscope design and objective lens used. Attempts at standardisation included the use of cut-off points reported in terms of millimetres below the EMI. But this method may require calibration, or in terms of the percentage of myometrial wall affected. The latter can also be fraught with difficulty because full thickness myometrial biopsy may not be part of routine processing especially for benign disease and also because of the practical difficulty of processing full thickness hypertrophied muscle wall. In this regards it is to be reiterated that there is no objective definition of myometrial hypertrophy and hyperplasia which are often stated as characteristic features of adenomyosis. Whether myometrial hyperplasia is considered essential to the diagnosis or not can also affect the reported incidence. In one study the incidence varied from 18.2 % using 1 mm cut-off to 11.5 % using 5 mm cut-off and the authors reported that the incidence will be lower if myometrial hyperplasia was considered an essential diagnostic requirement [11].

Table 2.1 The depth for endometrium presence within the myometrium that was used as a cut-off point for the histological diagnosis of adenomyosis in various studies

Diagnostic cut off point	References
>1 HPF	[84]
>0.5 LPF (1 mm)	[89, 112, 120]
>1 medium-power field (×100)	[32]
>1 LPF	[85]
>1/4 of total uterine wall thickness	[37]
2.5 mm or more	[56]
3 mm or more	[11]

LPF low power field, *HPF* high power field

Table 2.2 The classification of adenomyosis as proposed by Bird et al. [12] based on depth and extent of involvement

Depth of "invasion"	
Grade I	Sub-basal lesions within one LPF
Grade II	Up to mid myometrium
Grade III	Beyond mid-myometrium
Degree of involvement	
Slight	1–3 glands/LPF
Moderate	4–9 glands/LPF
Marked	10 or more glands/LPF

The classification does not however take into account the overall uterine size or the extent of uterine affection. *LPF* low power field [12]

In terms of the depth at which endometrial gland and stroma should be present within the myometrium for the diagnosis to be made, the adopted cut-off points vary (Table 2.1). Bird et al. (1972) proposed a classification into: Sub-basal lesions which are lesion present within one LPF (grade I); presence to mid myometrium (grade II); presence beyond mid-myometrium (grade III). They also classified the degree of involvement into three degrees: slight (1–3 glands/LPF), moderate (4–9 glands /LPF), and marked (10 or more glands/LPF) disease (Table 2.2) [12].

In the study by Bird et al. (1972), adenomyosis (including sub-basal disease) was the sole pathology (termed 'essential' adenomyosis) in 92 (46 %) out of the study population of 200 cases and was the sole pathology in 75 % of cases of adenomyosis [12]. There were 47 women who had sub-basal (grade I) adenomyosis and no other

pathology. Out of this subgroup, 60 % had significant menorrhagia. The incidence of menorrhagia was higher in women with sub-basal disease when compared to women with grade II and grade III lesions (n = 45) where the incidence of menorrhagia was 42 %. Thus this finding does not support definitions that exclude sub-basal lesions. Dysmenorrhoea, on the other hand, was related to the depth and the degree of involvement. The degree of involvement has rarely been the focus of research and even if reported it has seldom been included in statistical analysis. One possible explanation is that most published studies have relied on routine histology which does not regard the degree of involvement as prognostically relevant. The uncertainty linked to the appropriate cut off point lends considerable support to the suggestion made by McCausland and McCausland (1998) that histopathology should report on the actual depth of glandular presence rather than attempt a dichotomous diagnosis into normal and adenomyosis using arbitrary cut-off points [71].

Imaging Diagnosis

There are no symptoms or physical signs that are specific to adenomyosis. Classically, the uterus with adenomyosis is described as tender and symmetrically enlarged. It is interesting to note that the debate about whether adenomyosis has any characteristic symptoms is longstanding. Cullen, among other early investigators, believed that in contrast with early stage disease which is difficult to detect, fairly advanced disease could be diagnosed with great ease including by the 'hospital assistant'. Lockyer (1918) on the other hand observed that: "it is, however, clear that in many cases, if not in most, the diagnosis is made at the operation or by the microscope" [59]. This led Lockyer (1918) to conclude that "we are therefore obliged to accept the view that an opinion expressed before operation only amounts to a probability" [59]. There is agreement in more recent literature that the specificity of preoperative diagnosis based on clinical features is poor [12], with a reported range of 2–26 % [7, 78, 85].

The introduction of transvaginal ultrasound offered an opportunity to improving the diagnostic accuracy. But earlier attempts at preoperative diagnosis using ultrasound were hampered because of the inability to reliably distinguish these lesions form fibroids [4]. The advent of transvaginal ultrasound provided a breakthrough as it was linked to improved sensitivity and specificity of >80 % (Table 2.3). The ultrasound features linked to adenomyosis include uterine enlargement in the absence of fibroids, asymmetric thickening of the anterior or posterior uterine wall, lack of contour abnormality, lack of mass effect, heterogeneous poorly circumscribed areas within the myometrium, anechoic myometrial blood-filled cysts, increased echogenecity of the endometrium, and subendometrial linear striations. Ultrasound could also detect adenomyosis as localised non-homogenous lesions within the myometrium. There is disagreement in published literature on the diagnostic value of each of these features. Meredith et al. (2009) analysed data from 14 selected published studies on the use of preoperative ultrasound and compared the findings to histological diagnosis [74]. They reported

Table 2.3 The diagnostic accuracy of Transvaginal Ultrasound (TVU) and Magnetic Resonance Imaging (MRI) in various studies

Study	Sensitivity (%)	Specificity (%)
Accuracy of TVU in diagnosis of adenomyosis		
Fedele et al. (1992) [28]	87	99
Ascher et al. (1994) [2]	53	67
Brosens et al. (1995) [15]	86	50
Reinhold et al. (1995) [90]	86	86
Atzori et al. (1996) [5]	87	96
Koçak et al. (1998) [47]	89	88
Bromley et al. (2000) [14]	84	84
Bazot et al. (2001) [8]	65	98
Dueholm et al. (2001) [26]	63	65
Accuracy of MRI in the diagnosis of adenomyosis		
Mark et al. (1987) [65]	61	100
Ascher et al. (1994) [2]	88	66
Reinhold et al. (1996) [91]	89	89
Bazot et al. (2001) [8]	78	93
Dueholm et al. (2001) [26]	70	86

that adenomyosis was more common in women with heavy bleeding (31.9 %) compared to all hysterectomies (25.9 %). The probability of adenomyosis in a woman with heavy bleeding and positive ultrasound features was 68.1 %, compared to 65.1 % probability in a woman with positive ultrasound undergoing a hysterectomy for any symptom. But the probability of adenomyosis after a normal transvaginal ultrasound scan was 10 % in symptomatic patients compared to 8.7 % probability for women undergoing hysterectomy for any reason. The sensitivity and specificity for symptomatic women was 84.3 and 82.3 %, and for all women undergoing hysterectomy was 81.1 and 85.1 % (Table 2.4). The figures lend support to the conclusion that transvaginal ultrasound scan is an accurate test for adenomyosis, but this is necessarily weakened because of the lack of uniform histopathological or ultrasound based diagnostic criteria. This is particularly important given that most studies have used histopathology as the gold standard.

Champaneria et al. (2010) published a systematic review including a meta-analysis of published articles that compared the diagnostic accuracy of transvaginal ultrasound (TVU) or MRI and that used histological diagnosis as the gold standard comparator [17]. The selection criteria for the systematic review were studies that involved premenopausal women (although studies often included both pre- and post- menopausal women) and that used the same individuals for the test and subsequently had a hysterectomy which enabled histological diagnosis. Initially, the systematic search identified 23 articles that met these selection criteria. However, 17 of identified studies were excluded because they were judged to be of poor quality, were partially duplicated with other published research or because published details were insufficient for the construction of comparison 2x2 table. This left only 3 studies that reported on the use of MRI [8, 26, 91] and 6 studies that reported on the use of TVU [8, 9, 26, 42, 91, 111]. In these studies, the pooled sensitivity and specificity of TVU was 72 % (95 % CI 65–79 %) and 81 % (95 % CI 77–85 %) respectively. TVU had a positive likelihood ratio of 3.7 (95 % CI 2.1–6.4) and a negative likelihood ratio of 0.3 (95 % CI 0.1–0.5). The pooled sensitivity and specificity for MRI were 77 % (95 % CI 67–85 %) and 89 % (95 % CI 84–92 %) respectively. MRI had a positive likelihood ratio of 6.5 (95 % CI 4.5–9.3), and a negative likelihood ratio of 0.2 (95 % CI 0.1–0.4) [17].

Despite the apparent favorable diagnostic statistics, there are many important differences between these studies. The first difficulty concerns the point discussed earlier about the cut-off point for histological diagnosis. Histological diagnosis was critical to the inclusion criteria as it was used as the reference point, but it is difficult to establish whether the cut-off points were equivalent. Bazot et al. (2001, 2002) used 2.5 mm as their cut-off point [8, 9], Dueholm et al. (2001) used a medium power field (×100) or 2 mm [26], Vercellini et al. (1998) used half a low power field (or 2.5 mm) [111], and Reinhold et al. (1996) described using one high power field [91]. The number of sections examined also varied between the studies and whilst some studies described assessment of uterine weight and

Table 2.4 The diagnostic accuracy of preoperative ultrasound in women undergoing hysterectomy in relation to presenting symptoms [74]

Variable	All studies, n (95 % CI)	Hysterectomy in symptomatic patients, n (95 % CI)	Hysterectomy for any reason, n (95 % CI)
Sensitivity	82.5 (77.5–87.9)	84.3 (76.3–93.2)	81.1 (74.5–88.2)
Specificity	84.6 (79.8–89.8)	82.3 (72.5–93.5)	85.1 (79.3–91.4)
Likelihood ratio of positive results	4.7 (3.1–7.0)	4.1 (2.0–8.2)	5.1 (2.3–8.7)
Likelihood ratio of negative results	0.26 (0.18–0.39)	0.25 (0.14–0.43)	0.28 (0.17–0.45)

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morphological descriptors such as uterine wall thickness, no account is provided as to whether or how this was taken into consideration. Histological criteria recorded in a number of studies include the grade of lesions based on the depth of presence of glands and stoma within the myometrium, and lesion density [8, 9, 42]. But again, where these were mentioned as part of the methodology, they were not taken into account in the analysis. Also, those studies that made a histological distinction between focal and diffuse adenomyosis [8, 9, 26, 42] did not take this factor into account when the results were analyzed. All the studies included in the meta-analysis by Champaneria et al. (2010) included women who were scheduled for hysterectomy, but there are indicators of differences between the study populations [17]. It should be considered that the threshold for hysterectomy varies based on the population and the health care system and this may have been a factor why the incidence of adenomyosis varied between the studies ranging from 21 [26] to 37.1 % [42]. All of the 6 studies reported positive and negative predictive values which can be affected by the incidence in the population considered. It is notable that the positive predictive value of ultrasound was low (below 55 %) in all three studies included in the meta-analysis [26, 42, 111].

Also, despite the strict exclusion criteria, the two studies by Bazot et al. (2001, 2002) included 120 and 129 women respectively and described different patient profile [8, 9]. Yet both studies reported that they recruited consecutive women from the same hospital during the same time interval. This suggests significant overlap. Bazot et al. (2001) reported that 61 of the 120 patient had menorrhagia and 32 had endometrial cancer compared to the report by Bazot et al. [9] where 92 out of 129 women had menorrhagia and 13 had endometrial cancer [8, 9]. Reinhold et al. (1996) included 26 women with endometrial cancer into the study despite the fact that this can affect the appearance of the subendometrial myometrium [91]. In the study by Kepkep et al. (2007) only 8 out of 70 women had hysterectomies because of premenopausal abnormal uterine bleeding [42]. In the study by Reinhold et al. (1996) almost half the participants were postmenopausal [91]. In the study by Vercellini et al. (1998) the indication for hysterectomy was menorrhagia and/or worsening dysmenorrhoea, but the study excluded women with fibroids that distort the uterus or that were more than 12 weeks size [111]. All of the other studies included women with fibroids without stipulation of any cut off points related to the size of fibroids. A concern is that both Vercellini et al. (1998) and Reinhold et al. (1996) excluded women after investigation if the uterus could not be assessed because of fibroids although this can affect quoted sensitivity and specificity [91, 111].

With regards to image based diagnostic criteria, there was agreement on three of the features used to diagnose adenomyosis: the presence of myometrial cysts, heterogeneous myometrium and focal abnormal echotexture. In addition all studies except Dueholm et al. (2001) and Reinhold et al. (1996) included the presence of globular or asymmetrical uterus [26, 91]. Kepkep et al. (2007) and Bazot et al. (2002) but not the other studies emphasised the diagnostic value of subendometrial linear striations [9, 42]. Only Bazot et al. (2002) utilised colour Doppler [9]. Bazot et al. (2002) reported on the diagnostic value of the individual features used for ultrasound identification of adenomyosis [9]. But here again, it is interesting to note that some of these individual features had a higher sensitivity than the overall ultrasound assessment. Thus it remains unclear what relative weight was assigned by the investigators to each of the identified features. In the earlier study report by Bazot et al. (2001), the combined assessment had a higher sensitivity than the individual features [8]. The reported sensitivity, specificity, positive and negative predictive value for TVU in the study by Kepkep et al. (2007) are identical to those reported for the sonographic feature or "heterogeneous myometrium" [42]. Here again it becomes unclear what impact, if any, the other features had on the final classification.

In contrast to histopathological classification which focuses on the presence of glands and stroma within the myometrium, ultrasound diagnosis seems focussed on the appearance of the

myometrium, the overall shape and size of the uterus or the presence of asymmetry. Ultrasound also emphasises the role of myometrial cysts not all of which can be histologically linked to adenomyosis. There is disagreement between the studies on whether ultrasound correctly identified the grade or degree of adenomyosis. No correlation was found between ultrasound and histopathology in the study by Bazot et al. (2001) where sonography and histopathology concurred in only 57 % of cases when assessing the depth of presence of endometrium within the myometrium and in only 23 % of cases when assessing the degree of involvement and lesion density [8]. On the other hand, Reinhold et al. (1996) reported a Kappa statistic of 0.69 indicating good agreement between TVU and histology in depicting the location of adenomyosis and a Kappa statistic of 0.81 in relation to the maximum depth of involvement [91].

Thus whilst Champaneria et al. (2010) concluded that TVU has a high level of accuracy for the diagnosis of adenomyosis, it should be borne in mind that the studies included in their review were focused on a subset of patients scheduled for hysterectomy [17]. There is lack of clarity as to the exact diagnostic criteria and the relative weight of the various features linked to the condition. In addition, the choice of histological cut off points, and the choice regarding inclusion and exclusion criteria e.g. the inclusion of women with endometrial cancer may have affected the overall assessment and ultimately the judgment in favour of TVU.

A more recent development is the advent of three dimensional ultrasound and its use in relation to adenomyosis. Little research has been published so far. Naftalin et al. (2012) reported on the use of 3D-TVU in 985 consecutive women who attended a general gynaecology clinic in a large teaching hospital in the UK [81]. They reported the prevalence of adenomyosis in the whole group as 20.9 % (95 % CI: 18.5–23.6 %). It was possible to compare histological findings with 3D-TVU in the subgroup of women (n = 45) who subsequently underwent a hysterectomy. After excluding women with cancer (n = 14) and with large fibroids (n = 4), the investigators reported a fair level of agreement between 3D-TVU and histological diagnosis of adenomyosis [$\kappa = 0.62$ ($p = 0.001$), 95 % CI (0.324, 0.912)]. They reported a positive correlation between age and the finding of adenomyosis, but the incidence is not provided divided by age groups or by clinical presentation. Thus although the study population may provide a range of diverse presentations, it will necessarily be affected by specialisation within the clinic and referral criteria therefore caution should be exercised when extrapolating the figures to different populations. Luciano et al. (2013) prospectively evaluated the accuracy of 3D-TVU in 54 symptomatic premenopausal women undergoing hysterectomy for benign conditions [61]. Of these, there were 32 patients who had no previous treatment, 26 of whom had adenomyosis. Features linked to adenomyosis were: (1) Maximum Junctional Zone thickness (JZmax) ≥ 8 mm, (2) myometrial asymmetry and (3) hypoechoic myometrial striations. They reported that when at least 2 of these features were present, 3D-TVU was 90 % accurate (sensitivity = 92 %; specificity = 83 %; PPV = 99 %; and NPV = 71 %). Interestingly the accuracy reduced to 50 % in the subgroup who had undergone endometrial ablation (n = 12), and was 60 % in the group receiving medical treatment (n = 10).

Thus – despite much promise – studies assessing the role of ultrasound and MRI in diagnosing adenomyosis all suffer methodological weaknesses some of which are due to the constraints inherent in the study population. The need for histology as the gold standard means that only a particular cohort could be assessed. Still, a major difficulty has been in the use of retrospective cohorts which suffer from lack of standardisation and incomplete assessment. The indications for hysterectomy are becoming increasingly narrow which add to the need for reliable non-invasive diagnostics.

Biomarkers in Adenomyosis

Despite the breakthrough achieved with the use of MRI and transvaginal ultrasound, reliable diagnosis of adenomyosis remains difficult and

expensive. Thus, the identification of a non-invasive reliable marker for the disease will have significant clinical value. Such a marker may also help monitor disease progression or response to treatment.

CA125 is perhaps one of the earliest biomarkers to be studies in relation to endometriosis and adenomyosis. CA125 is produced by most non-mucinous epithelial ovarian tumours. More research has been directed to assessing its use in endometriosis than to adenomyosis, but meta-analysis of published results concluded that it is of limited utility [77]. More recent research has again demonstrated the limited utility of CA125 in endometriosis without endometrioma, but that accuracy could be improved by using a lower combined cut-off values for CA-125 at 20 and 30 U/mL [46]. Concomitant use of CA125, CA19-9 and IL-6 did not add significantly to the value of CA125 alone [99].

In relation to adenomyosis, Takahasi et al. (1985) examined 11 patients with fibroids, 7 with adenomyosis and 1 with adenomyosis and fibroids and reported that the mean CA125 level (± SD) was 18.3 (±6.1) U/ml in patients with fibroids and 93.3 (±49.4) U/ml in those with adenomyosis [100]. The difference was statistically significant. Seven out of the 8 women with adenomyosis but none of those with fibroids had serum CA125 > 35 U/ml. Following surgery, CA125 level in patients with adenomyosis gradually decreased and returned to normal 1 month postoperatively. But the diagnostic value was disputed by others. Halila et al. (1987) measured serum CA125 in 22 women undergoing a hysterectomy for adenomyosis or fibroids but reported normal CA125 levels (<35 U/ml) in 20 patients including in all those with histologically proven adenomyosis [35]. One complicating factor is the observation that serum levels of CA125 varies with the menstrual cycle. Masahashi et al. (1988) reported a transient rise during menstruation. They also reported that serum levels are higher in patients with adenomyosis and with advanced endometriosis compared to normal controls [67]. Takahashi et al. (1988) reported that after control for cycle phase, CA125 was elevated in patients with adenomyosis (as well as in endometriosis) [101]. Interestingly, Bischof et al. (1992) reported elevated CA125 levels in women with fibroids. They attributed this to increased peritoneal distension secondary to uterine enlargement by the fibroid [13].

Agic et al. (2008) measured chemokine (C-C motif) receptor 1 mRNA (CCR1 mRNA) in peripheral blood leukocytes together with monocyte chemotactic protein-1 (MCP-1) and CA125 protein in serum of women with endometriosis and adenomyosis. The ratio of CCR1/HPRT mRNA (Hypoxanthine-guanine phospho-ribosyltransferase) in peripheral blood of patients with endometriosis was significantly elevated compared to women without endometriosis. No significant difference in CCR1/HPRT mRNA levels was found between women with adenomyosis and the control group. Serum levels of MCP-1 and CA125 were significantly higher in patients with endometriosis. The combined test using the three markers was considered positive if at least one of the markers was above the set threshold. When used to detect endometriosis, this combined test showed sensitivity, specificity, NPV, PPV of 92.2 %, 81.6 %, 83.3 % and 92.3 % respectively. The combined test predicted the presence or absence of adenomyosis to a lesser extent: sensitivity, specificity, NPV, and PPV were 72.7 %, 81.6 %, 93.0 %, 47.1 % respectively [1].

Another approach is the use of proteomic analysis of serum samples. Long et al. (2013) compared serum samples from women with adenomyosis, endometriosis and controls using MALDI-TOF-MS proteomic analysis. They identified 13 protein peaks that were abnormally expressed in endometriosis and 12 in adenomyosis compared with control groups. Five-peak masses were significantly down regulated both in the women with endometriosis and adenomyosis. Two protein peaks with m/z of 2.748 and 5.759 kDa were reported to be of high value in the diagnosis of adenomyosis [60]. However, this approach is fraught with difficulty. Previous studies using this technique in endometriosis have led to the identification of different putative protein markers. Jing et al. (2009) identified two marker proteins with m/z of 5.83 and 8.865 kDa [40]. Kyama et al. (2011) reported that endometriosis was diagnosed with high sensitivity (89.5 %) and specificity (90 %) with use of five down-regulated

mass peaks (1.949, 5.183, 8.650, 8.659, and 13.910 kDa), and minimal-mild endometriosis was diagnosed with four mass peaks (two up-regulated: 35.956 and 90.675 kDa and two down-regulated: 1.924 and 2.504 kDa) with maximal sensitivity (100 %) and specificity (100 %). The 90.675 and 35.956-kDa mass peaks were identified as T-plastin and annexin V [52]. Ding et al. (2010) detected 3 mitochondrial protein peaks as potential biomarkers for endometriosis with m/z of 15.334, 15.128 and 16.069 kDa [24]. The differences may be due to different experimental conditions, different protein chips or technologies used, or to patient related factors.

Xiaoyu et al. (2013) used iTRAQ (isobaric tags for relative and absolute quantitation) technology to compare serum samples from women with and without adenomyosis. They reported that 21 proteins were significantly up-regulated and 4 proteins were significantly down regulated in women with adenomyosis (Table 2.5) [119]. They thus raised the possibility of using the identified proteins as biomarkers for adenomyosis.

Dechaud et al. (2014) performed gene expression array in adenomyosis and reported that the most up regulated genes in the endometrium in adenomyosis were SH2D3A, KLHL31 and ADAMTS16 whilst the most down regulated genes were FOXP2, F2RL2 and DGKB and raised the possibility of these being useful as markers of adenomyosis [23].

Table 2.5 Proteins differentially expressed when comparing serum samples from women with adenomyosis and controls using iTRAQ analysis [119]

Up-regulated proteins	
Fibrinogen α	Fibrinogen β
Fibrinogen γ	CD44
Fibronectin 1	Complement C1r
Apolipoprotein B-100	Complement factor B
Hemoglobin subunit δ	Complement C1s
Complement C3	Complement C5
Antithrombin-III	Vitamin K-dependent protein S
Ceruloplasmin	Serum amyloid P-component
Leucine-rich α-2-glycoprotein	α-1-antichymotrypsin
Inter-α-trypsin inhibitor heavy chain H4 isoform 1	Vitamin D-binding protein
Apolipoprotein C-II	
Down-regulated proteins	
Gelsolin isoforms-a	Apolipoprotein A-IV
Transthyretin	Keratin, type I cytoskeletal 9

Clinical Manifestations of Adenomyosis

As mentioned above, the preoperative diagnosis of adenomyosis is poor. In one study, the diagnosis was suspected preoperatively in only 10 % of cases and recognized at surgery in 35 % of patients [85]. It is perhaps well recognized that there are no symptom or symptoms that are individually or collectively pathognomonic of uterine adenomyosis. Traditionally adenomyosis has been linked to a variety of common gynaecological presentations, most prominently abnormal bleeding, dysmenorrohea and although it is more common in parous women, it has been linked to infertility. It is also recognised that many cases are identified in asymptomatic women. This will be explored further, but it is important to point out that a variety of other gynaecological conditions such as endometriosis and fibroids have also been linked to these presentations as well as being diagnosed in asymptomatic women. Both endometriosis and fibroids are commonly present in association with adenomyosis. The significance of the finding of adenomyosis needs to be considered against the knowledge that the threshold at which women seek medical care for any of these presentations varies and at the same time, the clinical threshold for defining normality is not always clear or agreed.

Symptoms Linked to Adenomyosis (Box)

Abnormal Uterine Bleeding

Heavy menstrual bleeding is one of the more common indications for hysterectomy, and as adenomyosis has been reported in a sizable percentage of surgically removed uteri, it is not surprising that heavy menstrual bleeding has come

to be linked to adenomyosis. In the study by Bird et al. (1972) 200 hysterectomy specimens were assessed for the presence of adenomyosis. Lesions were classified into three grades: Grade (I), sub-basal adenomyosis where the lesions were found within one low power field below the basal endometrium, but no further; Grade (II), where adenomyosis was found up to the mid-myomstrium; and Grade (III) where adenomyosis extended beyond the mid-myometrium [12]. Adenomyosis was identified histologically in 31 % of the 200 specimens examined using routine histopathology, but when additional sections were taken, 38.5 % were identified as having adenomyosis and the figure rose to 61.5 % when Grade I (sub-basal) adenomyosis was included. Adenomyosis was the only uterine lesion in 16.5 % of cases and was the major pathology found in 32.5 % of cases. In 46 % of all cases (92 out of the 200 women included in the study) adenomyosis was either present alone or together with other non-significant pathology, this included 47 Grade I, 33 Grade II, and 12 Grade III cases. Thus all the 47 cases of sub-basal adenomyosis belonged to the group where adenomyosis was the sole significant pathology. Ninety (83.5 %) of the women identified with adenomyosis (n = 123) had associated pathology. These included fibroids (n = 68), endometrial hyperplasia (n = 9), endometriosis (n = 8), or polyps (n = 5). The presence of pathology associated with adenomyosis is well recognised in literature. Of the 92 women who had adenomyosis alone or with no other significant pathology in the report by Bird et al. (1972), 51.2 % had menorrhagia, 10.9 % had metrorrhagia, 28.3 % had dysmenorrhoea, 2.2 % had postmenopausal bleeding and 23.9 % were asymptomatic [12]. Only 18.7 % had both menorrhagia and dysmenorrohea. Of the 47 patients who had adenomyosis sub-basalis, 60 % had significant menorrhagia compared to 19 (42 %) of the 45 women who had grade II or III adenomyosis. Thus the difference between the two is not statistically significant. Two of the 47 patients with Grade I disease had dysmenorrhea, compared to 14 of 33 with Grade II, and 10 of the 12 women with grade III. In terms of the degree of involvement, dysmenorrhea was present in 13.3, 26.7, and 58.8 % of women with slight, moderate, or marked disease. The difference was statistically significant. Although Bird et al. (1972) did not provide information about how symptoms or symptom severity were assessed or a definition of what constituted metrorrhagia, they concluded that adenomyosis "may cause hypermenorrohea and increasingly severe, acquired dysmenorrhea" [12].

Box Symptoms Linked to Adenomyosis

Menorrhagia	Increased
Dysmenorrhoea	Increased
Chronic pelvic pain	Increased
Dyspareunia	Limited data
Infertility	Increased
Spontaneous abortion	Increased

Owolabi and Strickler (1977) used one LPF as a cut-off point and used two random tissue blocks in routine histopathology to diagnose adenomyosis and identified adenomyosis in 161 out of 1619 (10 %) consecutive hysterectomies [85]. In 97 (60.2 %) cases, there was coexistent pathology, mostly fibroids, endometrial hyperplasia and carcinoma, and endometriosis. They reported that 65 % of the group who had adenomyosis as the sole pathology (n = 64) had abnormal bleeding and that there were also symptoms of dysmenorrhea, non-menstrual pelvic pain and/or dyspareunia. It is not possible to understand these figures further as the exact number of patients affected is not provided and the article reports individual symptoms rather than patients affected. None of this group was asymptomatic, but two of those with abnormal bleeding were postmenopausal with atrophic endometrium and their symptoms are thus unlikely to be related to adenomyosis. In addition, there were five asymptomatic women who had associated pathology (three had CIN and two had adnexal masses) and thus no symptoms attributable to the presence of adenomyosis. It remains speculative if non-menstrual pain or dyspareunia that was present in 12 and 6 % of the adenomyosis only group can in fact be attributable to adenomyosis. Although

the study concludes that the presence of adenomyosis is always associated with symptoms it should be considered that the group as a whole were symptomatic, hence the hysterectomy, but also that the study does not provide a comparison with patients who underwent hysterectomy but did not have adenomyosis. Furthermore, it is possible that a significant number of cases with adenomyosis were missed because of the sampling protocol that was followed.

Levgur et al. (2000) assessed 111 uteri all of which were below 280 g for the presence of adenomyosis [56]. When present, the lesions were classed as superficial if they were at a depth of less than 40 % of the uterine wall, intermediate if they were found at a depth between 40 and 80 % of uterine wall and were classed as deep if they were present at more than 80 % of uterine thickness. The authors reported an association between the number of foci and the depth of endometrial presence within the myometrium. The median number of foci was higher in women with dysmenorrhoea compared to those without dysmenorrhoea, but there was no difference in the number of foci in women with or without menorrhagia. In this study, superficial-depth-adenomyosis was not associated with menorrhagia or with dysmenorrhea. However, Levgur et al. (2000) excluded from the definition of adenomyosis lesions that were less than 2.5 mm below the endometrium [56]. Also excluded were 132 women who had a uterus >280 g in weight. The given reason for the exclusion was the difficulty obtaining full thickness myometrial biopsies (this group included 6 women with adenomyosis). In the 111 women included in the study, 17 had adenomyosis alone, 19 had adenomyosis and fibroids and 39 had fibroids but no adenomyosis. No information is provided on other associated pathology, or on the indications for hysterectomy. The authors state that menorrhoagia and dysmenorrhoea were associated with 'degree of myometrial depth' and that menorrhagia occurred in 36.8 % of women with deep foci and 13.3 % with intermediate foci. The corresponding figures for dysmenorrohea were 77.8 % and 12.5 % respectively. However, it is difficult to assess the significance of the findings as the figures were only provided as percentages and it is not stated whether the denominator included all women with adenomyosis or whether that was restricted to the subgroup without fibroids. Also, while the study objective was to correlate symptoms of uterine adenomyosis with histopathologic findings, the number of women with menorrhagia, dysmenorrohea or both and the indications for hysterectomy are not provided. The age ranges suggest that a large percentage were postmenopausal. Other methodological problems include the relatively small number of slides examined per patient, and that lesions were reported as number per sections examined rather than as lesion density.

Sammour et al. (2002) examined 94 uteri from women who underwent a hysterectomy and who were diagnosed with adenomyosis. Twenty five of these women also had fibroids [94]. The indications for hysterectomy are not provided, but the mean ages suggest that a good proportion may have been postmenopausal. The specimens were classified into four groups each corresponding to 25 % of myometrial thickness. Foci less than 2 mm below the endometrium were not included in the definition. The four groups were compared in relation to the symptoms of menorrhagia, dysmenorrhea, dyspareunia or pelvic pain, put no difference was found between the groups. The 'spread' of adenomyosis was assessed by examining the number of foci per slide and the number of slides varied according to the presence or absence of gross disease. The symptoms were not defined beyond the title, thus the distinction between pelvic pain and other pain symptoms is not clear. Comparisons were made based on the main complaints, yet more than one complaint was recorded per patient. The main finding of this study was a lack of correlation between symptoms and the depth of adenomyosis and that there was a significant correlation between pelvic pain or dysmenorrhea but not between menorrhagia or dyspareunia and the 'spread' of adenomyosis. These findings should be interpreted with caution because of lack of standardization in defining disease 'spread' and because the indications for the surgery is not provided. In addition, only three tissue blocks were examined per specimen

leaving the possibility of under diagnosis of adenomyosis.

Ozkan et al. (2011) reviewed the records of 1680 patients who underwent a hysterectomy [86]. Amongst this group, 98 patients were identified with adenomyosis and 106 had fibroids. Most (61 %) of the group with adenomyosis and 48 % of the group with fibroids were >50 years old. The diagnostic cut-off point and the number of tissue blocks assessed is not stated, but the overall incidence of adenomyosis in this group (12 %) was lower than reported in most other recent series. No indication is provided about associated pathology or about the number of patients with concomitant fibroids and adenomyosis. Ozkan et al. (2011) made a distinction between the frequency of dilatation and curettage – which was not statistically significantly different between the two groups – and endometrial sampling and the incidence of adenomyosis [86]. There was a higher incidence of endometrial sampling in the adenomyosis group. Therefore, Ozkan et al. (2011) argued that intrauterine sampling may trigger adenomyosis through deterioration of the endomyometrial junction [86]. But whist it is possible to speculate that deep endometrial sampling through overzealous curettage may disrupt the endomyometrial junction, modern alternative endometrial sampling techniques are designed to obtain more superficial samples of the functionalis endometrium and are unlikely to result in direct injury to deeper tissue. The more frequent resort to endometrial sampling in this group may reflect clinical practice in response to clinical presentation. In support of this is the observation that there was a statistically significant difference between the number of women undergoing hysterectomy for endometrial hyperplasia in the adenomyosis group (n = 32) and in the group with fibroids (n = 20), and a statistically significantly higher number of postmenopausal women in the adenomyosis group (n = 48) compared to the group with fibroids (n = 36). Interestingly, more than half of the patients in both groups were diagnosed with 'tubal inflammation', between 35 and 49 % had ovarian cysts and between 91 and 93 % had 'chronic cervicitis' as coexisting pathology. But there was no mention of other pathologies known to be associated with adenomyosis such as polyps or endometriosis. In their binary logistic regression Ozkan et al. (2011) identified age, menometrorrhagia and endometrial sampling as important covariant associated with adenomyosis [86]. However, the incidence of menometrorrhagia in the adenomyosis group (35 %) was lower than the incidence in the group with fibroids (43 %) and examination of menstrual bleeding was limited to a classification into 4 groups: regular, oligomenorrhea, menometrorrhagia and menopause which may be a reflection of clinical practice where menstrual bleeding patterns and/or quantity are poorly explored.

In a retrospective case control study from the United States, Taran et al. (2010) compared women undergoing hysterectomy with adenomyosis or with fibroids as the sole pathology [102]. They identified 76 cases with adenomyosis which were matched 2:1 by surgeon and by year of surgery to 152 women with fibroids only. The rationale for matching by surgeon is stated as the elimination of confounders of referral patterns and the elimination of bias based on the effect of concomitant procedures on practice style. However, no indication is provided as to what these confounders might be, or of how matching was undertaken within the practice of each surgeon beyond the given time frame (±1 year). Of the patients identified as having had a hysterectomy during the study period (n = 1871), 582 had fibroids, 133 had adenomyosis and 53 had both. This gives a relatively low overall incidence of adenomyosis of 186 (10 %), but the diagnostic criteria used for adenomyosis are not provided. The exact ethnic distribution is not provided, but it is stated that 95.1 % of both study populations were Caucasian. The indications for hysterectomy in 92.1 % of the adenomyosis group and in 94 % of the hysterectomy group were the presence of adenomyosis or leiomyomas or the presence of one or more disease-specific symptoms. The remaining hysterectomies were performed for indications of uterine prolapse, grade II cervical intraepithelial neoplasia, endometriosis and permanent sterilization. Taran et al. (2010) identified differences in the age distribution, the

group with adenomyosis being relatively younger (41 ± 6.4 years) compared to the group with fibroids (44.4 ± 4.8 years) [102]. There were no differences between the groups in the number of children, miscarriages or abortions the women had. The duration of menstrual bleeding was also similar in both groups (7.9 ± 3.6 days in the adenomyosis group and 7.9 ± 4.2 days in the group with fibroids). There was a higher incidence of depression (55.3 % vs. 26.3 %) and of the use of antidepressant in the adenomyosis group (35.5 %) compared to the group with fibroids (19.1 %). The authors put forward the suggestion of a possible aetiological link to antidepressants through an effect on raised prolactin secretion secondary to their use. There was a higher incidence of dysmenorrhea (60.5 % vs. 39.7 %), dyspareunia (17.1 % vs. 6 %) and of the use of NSAID (67.1 % vs. 42.1 %) in the adenomyosis group compared to the group with fibroids. There was also a higher proportion of women with abnormal cervical smears (30.3 % vs. 16.5 %) and of procedures for cervical dysplasia (9.2 % vs. 2 %) in the group with adenomyosis. However, the symptom complex of the two groups is necessarily affected by the indication for hysterectomy. Adenomyosis *per se* is not, and rarely are fibroids, an indication for hysterectomy in the absence of associated symptoms. As such, much of the quoted outcomes including abnormal smears, pain symptoms, abnormal bleeding, surgical intervention for cervical dysplasia, and endometriosis were not independent of the reason why surgery was performed. It is also possible that the presence of chronic pain was associated with the need for antidepressants. As mentioned above, the reliability of the outcome data of this and other retrospective studies will necessarily be affected by the thoroughness by which clinical detail was collected. This is not restricted to random errors, but there can be systematic points emanating from the way diseases are viewed. Whilst documentation in prospective research can be standardized between comparison arms, information available for retrospective research relies on available documentation which may vary from the most thorough to what individual clinicians may regard as sufficient. Thus the absence of documentation of any particular symptom can be open to various interpretations. In addition, the severity of documented symptoms and their clinical impact can vary considerably for a variety of reasons. It is also possible that the threshold for surgery may be lower in the presence of anatomical lesions such as fibroids. Taran et al. (2010) identified a history of infertility to be significantly linked to adenomyosis (14.1 % vs. 4.6 %) mainly because of associated endometriosis [102]. However, endometriosis was one of the quoted indications for surgery. Still, there was no difference between the adenomyosis and the fibroid groups in gravidity (2.7 ± 2.2 vs. 2.4 ± 1.8), parity (1.9 ± 1.4 vs. 1.9 ± 1.3), the number of spontaneous miscarriages (0.7 ± 1.4 vs. 0.4 ± 0.9) or of therapeutic abortions (0.1 ± 0.4 vs. 0.02 ± 0.2). It is notable that Taran et al. (2010) restricted their multivariable regression analysis to patients with symptoms of abnormal bleeding and/or pain which they believed to be 'disease-specific symptoms' [102]. This assumption limits the utility of this study towards addressing the basic question of whether adenomyosis is in fact relevant to these symptoms or whether it is incidental.

A different view-point was presented by Weiss et al. (2009), who reported on the findings of a study involving women who underwent a hysterectomy whilst under follow-up as part of a trial primarily concerned with the health of women during their middle years [118]. There were 3302 eligible women identified from seven centers in the US, but 200 women never completed a follow-up. At the time of recruitment women had to be aged between 42 and 52 years and to have an intact uterus. After 9 years of follow-up, 239 women underwent a hysterectomy (8 %). It was possible to obtain consent and the medical records of 137 women for the purpose of the report by Weiss et al. [118]. These were divided into two groups; one group comprised women reported as having adenomyosis on histological examination (n = 66), the other group comprised all other patients (n = 71). Case notes were obtained retrospectively and examined to compare the characteristics of both groups. The diagnosis of adenomyosis was obtained from the

clinical records based on local hospital practice, but the criteria are not defined. It is notable the while adenomyosis was present in 48 % of all samples, only one patient had adenomyosis with no associated pathology. Women with adenomyosis were more likely to have been pregnant (95 %) compared to those with no adenomyosis (85 %) and the difference was statistically significant. The two groups were not statistically significantly different in factors of ethnicity, educational attainment, income category, smoking, number of pregnancies, BMI, age at hysterectomy or uterine weight. There were no differences between the two groups with regards to their symptoms at the time of hysterectomy. The most common presentations in the adenomyosis group were problems with vaginal bleeding (n = 35), fibroids (n = 34), chronic pelvic pain (n = 15), prolapse (n = 6), stress urinary incontinence (n = 5), acute pelvic pain (n = 3). The most common presentations for the group with no adenomyosis were fibroids (n = 46), problems with vaginal bleeding (n = 43), chronic pelvic pain (n = 19), prolapse (n = 7), stress urinary incontinence (n = 6) and acute pelvic pain (n = 3). As there were no statistically significant differences in the presenting diagnosis for women with or without adenomyosis, Weiss et al. (2009) argued that despite a woman's presenting symptom or indication for hysterectomy, she is equally likely to have or not to have adenomyosis [118]. Weiss et al. (2009) identified three 'associations' with adenomyosis: fibroids, endometriosis and abnormal bleeding [118]. These were present in 51 (37 %), 4 (3 %) and 35 (27 %) of cases with adenomyosis, and in 59 (43 %), 7 (5 %), and 43 (33 %) of the group that did not have adenomyosis. The authors therefore argued that there was no association between the presence of abnormal bleeding or endometriosis and the presence or absence of adenomyosis. The authors also undertook a multivariate logistic regression analysis with fibroids, endometriosis, abnormal bleeding or chronic pain as independent variables to assess whether these conditions were associated with adenomyosis independent of other factors and found no association. Yet again, this study shares many of the weaknesses of the other retrospective studies published to date. There is no indication about how adenomyosis was defined and the symptoms prior to hysterectomy were only superficially described. All uterine bleeding is included under the heading of abnormal bleeding thus overlooking basic distinctions such as that between pre- and post- the menopause. In addition, fundamental problems become apparent when assessing the study design against the hypotheses being tested. Weiss et al. (2009) wrote that their study tested four hypotheses: (1) adenomyosis is associated with the presence of fibroids; (2) adenomyosis is more common in the presence of endometriosis; (3) adenomyosis is associated with abnormal uterine bleeding; (4) symptoms of chronic pain are more likely in uteri with fibroids if adenomyosis is present [118]. They concluded the data generated is their study did not provide evidence in support of these hypotheses. Testing the association with fibroids requires the assessment of uteri identified with adenomyosis for the presence of fibroids compared to a group without adenomyosis. The difficulty here is that fibroids were present as a reason for hysterectomy in the majority (n = 80 or 58 %) of the study population, yet it is included as an outcome measure. In relation to the second hypothesis, the research design does not inform what associated pathology exists in women with endometriosis. In addition endometriosis is not a disease of the fifth or sixth decades. Neither can this study design inform the debate about the symptoms that may be linked to adenomyosis or to uteri with both fibroids and adenomyosis. Thus a main flow in the study is the inclusion of entry criteria (fibroids, bleeding, and pain) as outcome variables in the analysis.

Vercellini et al. (1995) compared the incidence of adenomyosis in 1334 hysterectomy specimens in relation to the indication for hysterectomy. Adenomyosis was identified in 332 (24.9 %) of all cases [112]. The incidence of adenomyosis was 23.3 % in women with fibroids and menorrhagia compared to 25.7 % in women with prolapse, 21.4 % in women with ovarian cysts, 19 % in women with cervical cancer, 28.2 % in women with endometrial cancer, 28.1 % in women with ovarian cancer and in

24.7 % of women with other miscellaneous indications. The difference between the groups was not statistically significant. These findings, if confirmed, suggest a weaker link between adenomyosis and menstrual symptoms. The study by Vercellini et al. (1995) relied on routine histological assessment of removed samples, and used a cut-off point of half a LPF for adenomyosis (estimated to be about 2.5 mm) [112]. But again it has a number of significant weaknesses. For example, the study included some cases with malignancy which may undergo more rigorous sampling; in addition the analysis included fibroids and menorrhagia within the same analysis group without a clear rationale. The retrospective design did not allow adequate assessment of the menstrual history, or an assessment of dysmenorrhea or pelvic pain which are important outcome measures. No definition is provided of what is grouped under the heading 'menorrhagia', and no indication is given of the menstrual history of patients who underwent hysterectomy for other reasons. The study also suffers from incomplete ascertainment of data. For example, information about spontaneous or induced abortion is provided on 134 (40 %) and 105 (32 %) women respectively in the adenomyosis group. The corresponding figures for the group without adenomyosis were 343 (34 %), and 262 (26 %). Some of the two patient groups may have been misclassified in relation to the presence or absence of adenomyosis and the indication for surgery. It is clearly possible that adenomyosis may account for menstrual symptoms in some but not all those affected, or that some women with menstrual symptoms respond to conservative or medical treatment but undergo hysterectomies for other indications later in life.

In a subsequent study, the same group published a report on a group of women (n = 707) who underwent a hysterectomy and who had clinical information collected in advance of the operation [89]. The indications for hysterectomy were fibroids and/or menorrhagia (n = 140, 19.8 %), prolapse (n = 100, 14.1 %), ovarian cyst (n = 81, 11.5 %) or cancer (n = 14, 2 %). About a fifth of the cohort (n = 150, 21.2 %) were identified with adenomyosis using the same cut-off point as per their previous study; half a LPF or about 2.5 mm below the endometrial-myometrial junction. But no indication is provided of the incidence or the type of associated pathology in the group with adenomyosis or of the findings in the control group. Parazzini et al. (1997) reported that women who smoked were at lower risk of adenomyosis, and that the risk seemed inversely related to the number of cigarettes smoked [89]. But the age-adjusted trend in risk was of borderline statistical significance (χ^2 trend 3.57, $p = 0.06$). Adenomyosis was higher in parous women and in relation to number of children compared to nulliparous women (χ^2 trend 20.71, $p < 0.01$) and in those who had spontaneous abortions (odds ratio = 1.7; 95 % CI 1.1–2.6). There was no difference in relation to the use of oral contraception, IUCD or a history of induced abortion. Parazzini et al. (1997) stated that the risk of adenomyosis tended to be lower in more educated women but that the finding was not statistically significant [89]. The study found no difference between the two groups in the incidence of dysmenorrhea, intermenstrual pelvic pain or dyspareunia.

One of the main difficulties with the study is the challenge of controlling for confounders. There is a complex interaction between socioeconomic and demographic factors including factors such as age and parity and symptoms in decisions for hysterectomy. Much of this is now well documented. Indeed the authors attempted to control for these through the use of age and multivariate adjusted models. One analysis included controlling for age and intensity of flow in a comparison involving the menopausal status. This concluded that there was no relation between the menopausal status and the incidence of adenomyosis, although a significantly higher proportion of the group with adenomyosis were postmenopausal (48 % vs. 33.5 %, $p = 0.0016$). There was no difference in the incidence of heavy flow based on the presence (39.7 %) or the absence (35.4 %) of adenomyosis when the two groups were compared, but the difference was statistically significant in the age adjusted (odds ratio 1.7; 95 % CI 1.1–2.6) but not in the multivariate (odds ratio 1.4; 95 % CI 0.9–2.2) model. Indeed it is arguable

that age is relevant to a number of other factors included in the analysis such as a history of dilatation and curettage which used to be a very common procedure in the past but that has now been largely abandoned. Induced abortion is far more common now compared to former years. Because of the more focused effort at recoding the menstrual history, Parazzini et al. (1997) were able to perform a more detailed analysis than was possible in older literature [89]. Despite this, it is difficult to see how the information included in the analysis could be a reflection of patients' presentation. The main categories included in the study are described as: (1) categories based on the 'lifelong menstrual pattern'. Menstrual history is used to categorize women into three categories based on the length of the menstrual cycle (<25, 26–30, and >31 days); (2) categories based on duration of bleeding. Here, 'flow days per month' was used to categorize women into two groups depending on whether their loss lasted 5 or fewer days, or >5 days; (3) categories based on amount of loss. Here, 'intensity of flow' was used to categorize women into two categories as being either regular or heavy. Whilst recognizing the difficulties inherent in providing an accurate description of menstrual cycles, information collected retrospectively but prior to hysterectomy could hardly provide an accurate account of lifelong menstrual patterns. Menstrual patterns are known to change overtime including in women who have no menstrual complaints. In their conclusion, Parazzini et al. (1997) stated that no relationship was found in their study between adenomyosis and several menstrual characteristics including polymenorrhea and pain and that the relationship with heavy cycles disappeared in the analysis after adjustment for potential covariate [89]. They add that the presence of endometriosis was not associated with adenomyosis. But as explored above, the design of this study is not suited to addressing the question of whether there is a relationship between adenomyosis and the symptoms described.

Bergholt et al. (2001) reported on a series of 549 consecutive hysterectomies. The indications for hysterectomy were bleeding disorders in 50.6 % of cases, malignancy in 33.7 %, pelvic pain in 26.6 % and pelvic relaxation in 142 (25.9 %), 22.8 % had both bleeding and pain as indications for surgery [11]. The vast majority had abdominal hysterectomy and only 11 (2 %) had vaginal hysterectomy. When histopathological sections were examined, the incidence of adenomyosis varied depending on the chosen criteria. In the absence of myometrial hyperplasia, the reported incidence was 18.2 % when the cut-off point for adenomyosis was set at >1 mm, 15.8 % when using >3 mm depth as the cut-off point and was lower at 11.5 % when >5 mm was used as a cut-off point. The corresponding figures when myometrial hyperplasia was considered as a prerequisite for diagnosis were 14.3 %, 12.5 % and 10 %, respectively. It is notable that the reported incidence of adenomyosis in this cohort is low. This may be related to the particular patient profile or to local clinical practice. The authors used >3 mm cut-off point for subsequent analysis and reported that the only variable significantly associated with adenomyosis was endometrial hyperplasia, but that other factors included in the analysis (previous caesarean section, endometrial curettage, or surgical evacuation of the uterus) were not linked to adenomyosis. The study did not find a link between adenomyosis and pain-related symptoms (dyspareunia, dysmenorrhea or chronic pelvic pain), the indication for hysterectomy, age, parity or the number of myometrial samples examined [11]. The incidence of caesarean section in this cohort was low at 5.8, 18 % of women were nulliparous and only 25.5 % were <45 years old. The investigators reported that there was no association between the four indication groups: bleeding disorders, pelvic relaxation, pelvic pain and neoplasia of the genital tract and the incidence of adenomyosis. But it remains unclear what is classified under each of the given headings as the age distribution suggests that a large proportion were in fact postmenopausal. On histological examination, only 204 (37 %) had cycling endometrium and out of a total of 185 women with genital cancer, 41 (7.5 % of the whole group) had endometrial cancer. The proportion of women with bleeding disorders who had postmenopausal bleeding is unclear. There is a discrepancy between the

number of cases with pelvic relaxation (n = 142) and the number who had vaginal hysterectomies (n = 11). One interesting observation from that study is that the adjusted odds ratio for adenomyosis in those with neoplasia was 0.6 (95 % CI 0.2–1.4). Whilst it is unclear whether adenomyosis and cancer risks are independent, the figure suggests a tendency to lower adenomyosis compared to the rest of the cohort. This, however, did not reach statistical significance. Firm conclusions will inevitably be hampered because of the difficulty inherent in making a diagnosis of adenomyosis in cases with endometrial cancer because of the possible effect of cancer invasion and also in women with ovarian cancer who are often older with atrophic endometrium and where adenomyosis can be more difficult to detect. In addition, it is questionable if the chosen (>3 mm) cut off point for the diagnosis of adenomyosis is the appropriate diagnostic threshold for this group.

Benson and Sneeden (1958) reported on 2536 abdominal and 740 vaginal hysterectomies from premenopausal women aged <50 years old (age range 18–50 years) [10]. Using a cut-off point of >2 low power fields, they identified 701 cases of adenomyosis in this cohort. Uteri were grouped into four groups according to uterine weight: those >100 g were considered not enlarged; 100–150 g were classed as slightly enlarged; 150–200 g were classed as moderately enlarged; and <250 g were classed as markedly enlarged. Cases were classified by the investigators according to the likelihood that adenomyosis was the cause of symptoms. In the absence of any other lesion, the question of causation was classed a 'likely' (n = 112); in the presence of other conditions (examples given are hypertension, myomas and salipingitis), adenomyosis was considered 'contributory' (n = 344); and in cases where adenomyosis was discovered incidentally such as in cases of prolapse, adenomyosis was considered as 'no cause' (n = 245). Fibroids were present as an associated finding in 56.6 % of cases of adenomyosis, and pelvic endometriosis was present in 13.3 % of cases. In this series the investigators reported that there was no association between adenomyosis and endometrial hyperplasia. Benson and Sneeden (1958) observed that ectopic glands resemble the basalis and that they only occasionally respond to progesterone and that blood is rarely seen within these glands suggesting that bleeding in these lesions is rare [10]. The most frequent menstrual complaint in this group was menorrhagia occurring as a sole complaint in 43/112 (38.4 %) of women who had no associated pathology, and in 98/344 (28.5 %) of those who had pathology associated with adenomyosis. Menorrhagia was also the most frequent complaint amongst women who had multiple presentations. Menometrorrhagia was less common, whilst metrorrhagia was rare. This study may have adopted a higher cut-off point for the diagnosis of adenomyosis which was diagnosed as the only pathology in 3.4 % of the group, and was discovered as an incidental finding in 7.5 % of the cases. A complete list of associated pathology is not provided but it would appear that some of the provided diagnoses may not be relevant to abnormal bleeding. However the incidence of adenomyosis as an associated pathology is 10.5 %. The research methodology cannot provide convincing evidence of a relation between symptoms of adenomyosis.

Given the lack of clarity and the diagnostic difficulties linked to adenomyosis, it is hardly surprising that the incidence of adenomyosis in asymptomatic women is even less known. Lewinski (1931) reported an incidence of 54 % in 54 autopsies [57]. In one series, seven cases were reported in which mothers and daughters were affected [27]. Using MRI criteria Hauth et al. (2007) identified adenomyosis in 12 out of 100 healthy women [36]. In another study, the diagnosis of adenomyosis was suggested by MRI in 19 of 204 (9.1 %) women following term deliveries and in 16 of 104 (15.4 %) women following preterm delivery; the overall incidence was 11.3 % [41].

Fraser et al. (1986) assessed menstrual blood loss in 55 women presenting with subjective menorrhagia including 40 women with 'recognizable' pelvic disease and 15 women with confirmed coagulation disorder [30]. Menstrual blood loss was measured using the alkaline haematin method. They concluded that women with

fibroids always had large volumes of menstrual blood loss and that women with other pathologies such as endometriosis, adenomyosis and myometrial hypertrophy also often exhibited genuine menorrhagia. The study group comprised 18 women with fibroids, 5 with adenomyosis, 11 with endometriosis, 2 with pelvic inflammatory disease, 1 with endometrial polyp, one with myometrial hyperplasia and two women who had a bicornuate uterus. It is not clear how adenomyosis was diagnosed but 3 out of the 5 women with the condition had objective menorrhagia compared to 4 out of the 11 with endometriosis and 15 out of the 18 with fibroids. The measured blood loss in the adenomyosis group was 84.7 ml (SEM = 22.6) and was comparable to the group with endometriosis (83.8 ± 21.5 ml) but lower than the group with fibroids (171.7 ± 31.2 ml). Of interest, is that there was one woman (age 24 years) who was diagnosed with pure myometrial hyperplasia and who had an enlarged uterus to 14 weeks size. The finding was confirmed on full thickness biopsy. She had severed bleeding leading to anaemia.

Idiopathic myometrial hypertrophy has been described in the literature under various names including fibrosis uteri and chronic subinvolution. Uterine size is recognized to vary with age and parity, and is also increased as a result of myometrial hypertrophy in adenomyosis. The question of uterine size can be compounded in the presence of fibroids. Molitor (1971) reported on uterine weight in their series of women with adenomyosis with no fibroids, the largest of these uteri weighed 705 g [78]. However, interpreting these findings require better definition of the size of the normal uterus which is perhaps very surprisingly little reported in literature and remains uncertain. One frequently quoted study in historical literature is that by Langlois (1970) who reported that parity was the primary determinant of uterine weight in women <49 years of age [53]. He suggested the upper limit of normal to be 130 g in nulliparous women, 210 g in women with parity 1–3, and 250 g in women of parity 4 or above. Verguts et al. (2013) reviewed uterine measurements obtained by ultrasound in 5466 non-pregnant uteri with no identifiable pathology on ultrasound [114]. Those with adenomyosis or fibroids were excluded. Increased gravidity was associated with increased uterine length, anterioposterior diameter and width and also with a lower mean length-to-width ratio. Maximum uterine dimensions were recorded between age 35–40. Exact figures are not provided, but the plots suggest a range of variability. Determination of the size of the normal uterus is relevant to discussions about what constitutes myometrial hyperplasia. One other consideration in relation to myometrial hyperplasia and the presence of glands within the myometrium is whether a causative like exists between these features.

Molitor (1971) reported the finding of adenomyosis in 281 (8.8 %) of hysterectomy specimens removed over a 10 year period (Table 2.6) [78]. He stated that 71 % of these patients had symptoms that were due to or contributed to by the presence of adenomyosis; he also argued that functionally active ectopic endometrium does not always produce symptoms. In cases where there was coexistent disease, symptoms where considered to be due to either adenomyosis or the co-existent pathology depending on clinicians' evaluation of the merits of both, e.g. a small fibroid or minimal endometriosis were considered less important than bigger or more extensive diseases. In this series the most common symptom was menorrhagia, followed by metrorrhagia followed by pain and dysmenorrhoea alone and, less frequently, in combination. There were 28.8 % (n = 81) asymptomatic women in this series including 38 women with minimal involvement (confined to the inner third of the myometrium), 33 women with moderate involvement (confined to the inner two thirds of the myometrium) and 10 women with extensive disease involving whole myometrial thickness. Fibroids coexisted in 108 (38.5 %) of

Table 2.6 The size of the uterus in cases of adenomyosis

Size of the uterus (gm)	No (%)
>80	9 (5.2)
81–120	36 (20.8)
121–150	35 (20.3)
151–200	54 (31.2)
<200	39 (22.5)

Data from Molitor [78]

cases, but symptoms were attributable to adenomyosis in 116 (41.3 %) patients. The majority of these had moderate to extensive involvement. Adenomyosis was believed to have contributed to symptoms in 84 women (30 %) who had adenomyosis in association with other pathology. A greater proportion of these cases had minimal and moderate involvement (Table 2.7). The difference between the groups was statistically significant $p<0.001$ (Contingency table X^2). The most common associated pathology was uterine fibroids in 38.4 %, endometriosis in 14.2 % followed by polyps in 1.7 % of cases. The infrequent association with endometrial hyperplasia was considered as evidence of lack of association with hyperestrogenism.

Like most studies of the subject, the report by Molitor (1971) in necessarily influenced by the indications for hysterectomy, which are not provided [78]. The classification adopted in the study was based on the presence or absence of symptoms and associated pathology is interesting, but the study did not include a group where adenomyosis was the sole diagnosis. Overall 181 cases had associated pathology, but no breakdown is provided as to the distribution of these between the three analysis groups. The presenting symptoms comprised abnormal bleeding (menorrhagia or metrorrhagia) and/or pain (pain or dysmenorrhoea). No further description is provided, and there is no break-down of the symptom complex in relation to age groups.

In order to ascertain their symptoms, Kilkku et al. (1984) interviewed 212 women who were scheduled for hysterectomy for benign disorders prior to surgery [43]. All women were below age 60, 28 (13.2 %) women were later diagnosed with adenomyosis and 157 had neither adenomyosis nor endometriosis. The authors found no difference between the two groups in the presenting symptoms including: urinary symptoms, pain and duration of menstrual bleeding. The final diagnosis in the control group was uterine fibroids (n = 131), dysfunctional uterine bleeding (n = 10), endometrial hyperplasia (n = 8), ovarian cyst (n = 4), endometrial polyp (n = 2) and chronic cervicitis (n = 2). But no indication is provided of the presence or type of associated pathology in the adenomyosis group. Kilkku et al. (1984) concluded that there is no symptom profile that is specific to adenomyosis [43]. But no detail is provided about the reasons for the hysterectomy or how patients were selected or of the criteria used for to diagnose adenomyosis.

Vavilis et al. (1997) set to estimate the frequency and risk factors for adenomyosis by studying the clinical records of 594 women undergoing hysterectomy [110]. They identified adenomyosis in 116 (19.4 %) of the cases. Adenomyosis was diagnosed by the presence of glands and stroma one or more low power field below the endometrial myometrial junction. The indication for surgery were fibroids (n = 308), genital prolapse (n = 43), benign ovarian tumors (n = 62), endometrial hyperplasia (n = 44) cervical cancer (n = 11) endometrial cancer (n = 62), ovarian cancer (n = 13) and three cases had leiomyosarcoma (Table 2.8). The incidence of adenomyosis was 20.4 % in the group with fibroids, compared to 25.55 % in the group with prolapse, but there was not statistically significantly different in the incidence of adenomyosis between the groups. Examining the figures provided demonstrates that 63/116 (54.3 %) of the group with adenomyosis are among the group where fibroids is provided as the indication for

Table 2.7 The number and percentage of symptomatic women who had adenomyosis in relation to the depth of adenomyosis within the myometrium compared to asymptomatic women

The depth of endometrium present within the myometrium	Symptomatic		Asymptomatic
	Adenomyosis sole or main pathology (n = 116)	Adenomyosis contributes to symptoms (n = 84)	(n = 81)
Up to the inner third	6 (5.2 %)	24 (28.6 %)	38 (13 %)
Up to the inner two thirds	62 (53.4 %)	41 (48.8 %)	33 (40.7 %)
All thickness	48 (41.3 %)	19 (22.6 %)	10 (12.3 %)

Data from Molitor [78]

Table 2.8 The indications for hysterectomy in the group with and without adenomyosis

Indication for hysterectomy	Adenomyosis	No adenomyosis
1. Fibroids	63	245
2. Prolpase	11	32
3. Benign ovarian cysts	11	51
4. Cervical cancer	2	9
5. Ovarian cancer	13	48
6. Leiomyosarcoma	0	3
All 2–6	37	143
7. Endometrial hyperplasia	6	38
8. Endometrial cancer	10	52
All 2–8	53	233

There was no statistically significant difference in the incidence of adenomyosis when the group with fibroids was compared to group 2–6 ($p=1$), or to groups 2–8 ($p=0.6$). Fisher's exact test, from Vavilis et al. (1996, [110])

hysterectomy. The list of associated pathology in the group with adenomyosis is not provided, but as mentioned above, more than half had fibroids and 25 (21.5 %) women had cancer. It is notable that the traditional indications for hysterectomy e.g. abnormal uterine bleeding and/or pain were absent from the list of surgical indications although it is possible that these indications were subsumed under the title 'fibroids' which is itself more likely to indicate hysterectomy if symptomatic. Comparing the incidence of adenomyosis in the group with fibroids as an indication for hysterectomy against those whose indication is unlikely to be linked to adenomyosis (genital prolapse, benign ovarian tumors, cervical cancer, ovarian cancer, leiomyosarcoma) suggests that adenomyosis may not be linked to symptoms. However, firm conclusions cannot be drawn because of the uncertainty as to the way the indications for surgery were classified and the lack of clarity as to whether additional or overlapping symptoms existed.

In a study from Pakistan, Shaikh and Khan (1990) published a retrospective review of 419 hysterectomy specimens and identified 237 (56.5 %) cases with adenomyosis [97]. The apparent high percentage was noted despite the apparent use of the strict criteria for diagnosis based on the presence of endometrial glands and stroma within at least a third or a fourth of the myometrium, and the use of routine histological sections (at least 3 per specimen). They argued that the high percentage (97.9 %) of parous women and women in the fourth and fifth decade of life (82.8 %) amongst the subset with adenomyosis was significant. The incidence of adenomyosis was at least twice as high in parous compared to nulliparous women. However, it must be kept in mind that nulliparous women were a small minority in this study ($n=18$, 4.3 %) and that the indications for hysterectomy are likely to be different in nulliparous women. The authors provide a list of associated pathology in both groups. They state that there were 54 (22.7 %) cases with adenomyosis but no associated pathology and 48 women (26.3 %) in the group without adenomyosis who did not have any associated pathology. The most common associated findings in women with adenomyosis were fibroids which were present in 32.9 % of cases, cervicitis which was present in 31.6 % of case and endometrial hyperplasia which was present in 12.2 % of cases ($n=29$), but endometrial hyperplasia was the only statistically significant association. The list of "associated pathology" of which more than one may be present in any specimen include cervicitis which is currently seen as an insignificant finding and, curiously, uterovaginal prolapase. Whilst it is not possible to assess the significance of the associated pathologies, it is to be considered that some such as endometrial hyperplasia may not have been independent from the indications for hysterectomy. Indeed the reasons why these women underwent a hysterectomy are not provided or considered and there is no exploration of any of the symptoms traditionally linked

to adenomyosis or fibroids such as abnormal bleeding and pain.

Naftalin et al. (2014) analyzed their series which included women attending a gynecology clinic, the majority of whom had a pelvic ultrasound. In this analysis, they reported on the relation of ultrasound diagnosed adenomyosis and menorrhagia [80]. The study population was women before the menopause (n = 892) who were attending the clinic for a variety of indications including menorrhagia (16.7 %), menorrhagia and dysmenorrhoea (4.3 %), intermenstrual or postcoital bleeding (9.5 %), mild or moderate oligomenorrhoea (9.2 %), pelvic pain (18.1), dysmenorrhoea (2.5 %), dyspareunia (1.7 %), infertility (16.1 %), recurrent miscarriage (1.3 %) and other indications in 20.1 %. Menorrhagia was diagnose subjectively (binary response: yes or no) for all participants and using pictoral charts [38] for the month following the ultrasound assessment. The response rate for those who were given menstrual charts was 57.5 %. Using multivariable analysis, there was no significant association between adenomyosis and menorrhagia when adenomyosis was assessed as a binary outcome. But when severity of adenomyosis was assessed by counting the number of morphological features of adenomyosis as seen by ultrasound in each woman, there was a significant (22 %) increase in menstrual loss for each additional feature of adenomyosis [OR 1.21 (95 % CI: 1.04–1.40)]

The study by Naftalin et al. [80] attempted to assess the association between menorrhagia and adenomyosis in women who are not undergoing a hysterectomy. However, the findings need to be interpreted with caution. First, the definition of menorrhagia in the study is not provided beyond either a subjective binary response or using the semi-quantitative charts but with no attempt at standardisation or to consider bleeding patterns. There was only a moderate level of agreement between the methods of assessment. It is also to be considered that the level of agreement between ultrasound and histology in the diagnosis of adenomyosis in this group was only moderate. Future studies should consider the impact of study population, multiple pathologies and the possibility that adenomyosis can contribute to the other presentations in the study population including pain and infertility.

Table 2.9 The basic classification of the causes of abnormal uterine bleeding (PALM-COIEN) as proposed by FIGO [79]

P	Polyp
A	Adenomyosis
L	Leiomyoma (submucosal or other)
M	Malignancy & hyperplasia
C	Coagulopathys
O	Ovulatory dysfunction
E	Endometrial
I	Iatrogenic
N	Not yet classified

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Recently, FIGO proposed a classification system (PALM-COEIN) for the causes of abnormal uterine bleeding [79]. The expert group, whilst acknowledging that the relationship between adenomyosis and abnormal uterine bleeding (AUB) is unclear, included a category for adenomyosis in the classification (AUB-A) (Table 2.9). The FIGO protocol included adenomyosis in the classification because of the existence of sonographic and MRI based diagnostic criteria. The minimal requirement being the performance of ultrasound to include the minimum sonographic criteria needed for diagnosis as well as ultrasound based distinction between diffuse and focal (or multifocal) disease. The group proposed the inclusion of a metric indicating the volume or extent of the disease. Munro et al. (2011) point out that the investigation into the aetiology of abnormal uterine bleeding (AUB) has been hampered by confusing and inconsistent use of nomenclature and by the lack of standardization for investigation and categorization of the various potential etiologies [79]. They also add that these deficiencies have hampered research and comparisons between studies and metanalysis to the point that some have been made counterproductive because of inaccurate conclusions. As such the FIGO classification can be seen as a step in the right direction, but its utility for addressing the difficulties highlighted is questionable. The difficulty for research addressing

AUB is threefold: (1) there is the question of defining normality and deviations there from, (2) the need for a classification of abnormal bleeding that has relevance to aetiology, (3) the need to explore the link between abnormal bleeding with possible aetiology and pathophysiolgy.

The classification adopted by the FIGO provides for nine main categories arranged according to the acronym PALM-COEIN: polyp; adenomyosis; leiomyoma; malignancy and hyperplasia; coagulopathy; ovulatory dysfunction; endometrial; iatrogenic; and not yet classified. As such, the classification does not address the inconsistent use of nomenclature referred to in the article by Munro et al. (2011), but leaves the definition of what is abnormal open to interpretation with no attempt to introduce the desired consistency [79]. The proposed linkage of 'abnormal' bleeding with the identified or assumed aetiological factors does not take into account the significant uncertainties in current knowledge. It is interesting to note that older publications have attempted to classify bleeding abnormalities according to severity and pattern. Molitor (1971) for example described bleeding in terms of menorrhagia, metrorrhagia or menorrhagia and metrorrhagia [78] and Graves WK (1971) [33] in his discussion of the same article suggested the possibility of a link between adenomyosis and the occurrence of postmenstrual abnormal scant and dark flow and intermenstrual bleeding. These contrast with more recent articles detailed in this chapter which have not made distinctions based on patterns of bleeding and which have often included postmenopausal bleeding and bleeding related to endometrial malignancy in the same analysis.

Lessons from Endometrial Ablation

McCausland and McCausland (1996) studies 50 women who underwent rollerball endometrial ablation for heavy menstrual bleeding that did not respond to medical treatment including cyclical progestogen [70]. A 5 mm biopsy was obtained prior to ablation and assessed histologically for the presence and depth of adenomyosis. They reported that the depth of myometrial involvement correlated with the severity of menorrhagia and also with the likelihood of ablation failure. The majority of patients with no or with minimal endometrial presence within the myometrium had good outcome following rollaball ablation which is assumed to destroy 2–3 mm of the superficial myometrium. McCausland and McCausland (1996) thus proposed the plausible hypothesis that bleeding from adenomyosis is not only due to the additional endometrial glands present but also from dysfunctional hypertrophic smooth muscle that lacks the physiological contractility required for the control of bleeding [70]. Indeed Benson and Sneeden (1958) quoted Meyer R [75] as the first to suggest altered uterine contractility as a mechanism of bleeding in adenomyosis [10].

In an earlier publication McCausland (1992) studied the depth of endometrial presence within the myometrium using hysteroscopic biopsy in 50 women [69]. All patients had no intrauterine lesions (fibroids or polyps) and had menorrhagia that did not respond to non-steroidal anti-inflammatory. The study group either had ovulatory cycles as proven by serum progesterone measurements or by luteal phase biopsies or had anovulatory cycles that did not respond to cyclical progestogens. Biopsies were also obtained from a control group who had no menstrual problems. The study involved assessment of menstrual blood loss by quantification of clot size which correlated with the frequency of change of pads and tampons. Clot size was classed as: dime-sized or + (1.5 cm); quarter size or ++ (2.5 cm); 50-cent piece size or +++ (3 cm); and silver dollar egg or fist size or ++++ (4 cm). Myometrial biopsy was obtained from the posterior wall in all cases and an additional sample was taken from the anterior wall in 15 cases for comparison. The depth of adenomyosis was taken from the deepest point below the endometrial myometrial junction. The average depth of adenomyosis in the posterior wall was 0.8 mm and was almost always greater than the depth in the anterior wall (mean 0.46 mm). The average posterior wall adenomyosis in women with menorrhagia was nearly twice the average depth in the control group, and >1 mm depth of adenomyosis was associated with

very heavy (+++ or ++++) bleeding. McCausland (1992) found a statistically significant correlation between the depth of adenomyosis and the severity of menorrhagia ($p=0.05$) and reported that a woman with a grossly normal endometrial cavity who passed clots the size of a quarter or larger is 2.9 times as likely to have adenomyosis >1 mm deep compared to a woman who has not passed such large clots [69]. However, using 1 mm as cut-off point for the diagnosis of adenomyosis would identify the condition in 14/30 of the control group who had no menstrual problems compared to 33/50 of those with menorrhagia. The difference between the two groups with regards to the incidence of adenomyosis is not statistically significant ($p=0.1$, χ^2 test). The tables provided show that there were 18 patients with depth of endometrial presence within the myometrium at ≥2 mm in the menorrhagia group, but none in the control group. Interestingly, 14 women who had gross polyps and 8 with submucous fibroids were identified with deep adenomyosis following removal of the polyps and fibroids. In these two groups, the amount of bleeding did not correlate with the depth of adenomyosis, suggesting that the intra-cavity lesions were the primary cause of the symptoms. The incidence of significant adenomyosis in the group who had a normal cavity was 33 out of 50 (66 %). Based on the distinction between normal and abnormal bleeding, McCausland (1992) went on to propose 1 mm as a cut-off point for the diagnosis of adenomyosis [69]. It is also interesting to note that the depth of endometrial presence within the myometrium in the control group that had normal periods was also deeper in the posterior (mean 0.8 mm) compared to the anterior wall (0.46 mm). McCausland (1992) recognized that clot size is perhaps only a gross measurement of the amount of bleeding, but the study represents an advance in as far as there was an attempt to quantify menstrual blood loss and to give an account of bleeding pattern [69]. The finding of presence at greater depth in the posterior wall compared to the anterior wall is interesting, but the finding was also repeated in the control group, which suggests a possibility of a 'normal' anatomical variation. McCausland (1992) argued that this is in line with previous research that shows the posterior wall to be most affected in diffuse adenomyosis and that a single myometrial biopsy of the posterior wall in both diagnostic and representative of the area most severely involved in adenomyosis [69]. However, studies that included hysterectomy specimens have demonstrated that adenomyosis can be present solely in the anterior wall in a proportion of case.

McCausland and McCausland (1998) argued that it required 1 mm of endometrial presence within the myometrium together with abnormal smooth muscle hypertrophy to cause menorrhagia [71]. Asymptomatic patients had either no glandular presence or up to an average of 0.8 mm. It is to be noted that smooth muscle affection is always deeper than the depth of gland presence in affected women. McCausland and McCausland (1998) argued that histopathology should report on the actual depth of glandular presence rather than attempt a dichotomous diagnosis into normal and adenomyosis using arbitrary cut-off points [71].

Adenomyosis Response to Steroids

It is interesting to note that correlations between the depth or extent of adenomyosis and symptoms assume a relation between symptoms and the phenomena of glands present within the myometrium but seem to miss the possibility of myometrial disease or role in the genesis of symptoms. A frequently overlooked feature of adenomyosis is that it does not uniformly respond to progestogens. This feature has been recognized for a long time [79]. In the study by Molitor (1971), there was agreement between eutopic and ectopic endometrium in 83 % of instances of pseudodecidualisation in response to exogenous progestins and in 44 % of cases of hyperplasia [79]. In addition, the response to progestogens may not always be uniform. It has been suggested that symptoms may be absent in women whose adenomyosis does not respond to steroids [31]. However, in the series by Molitor (1971), some of the cases that exhibited progestogenic response in adenomyosis were asymptomatic [78]. On the

other hand, there is variation in the reported observation of secretory changes in adenomyosis. Novak and de Lima (1948) found no evidence of secretory change in the ectopic endometrium in 99 cases of adenomyosis [83]. Azziz (1989) reviewed available literature and reported that 30–50 % of adenomyotic foci are able to respond to progesterone, but that endometrial dating may be somewhat delayed with respect to the overlying endometrium [7]. There may also be variation in response depending on the depth of adenomyotic foci. Sandberg and Cohn (1962) reported that the response of ectopic endometrium in caesarean hysterectomy specimens (there were 27 uteri with adenomyosis out of 151 uteri examined) varies regionally as deeper glands showed progestogenic response in about a quarter (26 %) of the samples in contrast to adenomyotic glands nearer to the endometrium (at a depth of 1–2 low power fields) which behaved in a manner similar to the basal layer of the endometrium in the vast majority (89 %) of cases [95]. There can also be a patchy response within the same specimen. Azziz (1986) was able to identify 72 cases published in the literature of adenomyosis identified in gravid uteri removed by caesarean hysterectomy [6]. Of these 29 were associated with obstetric complications, the rest were identified in specimen removed electively such as at the time of caesarean sterilization. He concluded that adenomyosis is rarely associated with obstetrical surgical complications, but the cut-off point for defining adenomyosis in the hypertrophied gravid uterus is unclear.

Adenomyosis and Parity

Adenomyosis diagnosed at hysterectomy has traditionally been linked to multiparity [112, 113], pregnancy termination and to uterine curettage, especially after pregnancy. More recent attention has been paid to possible link between adenomyosis and infertility possibly through adverse endometrial factors interfering with implantation or through effects on junctional zone function that results in impairing sperm transport and fertilization [16].

The root of the link between adenomyosis and parity is longstanding but perhaps less firmly established than is often implied. Bird et al. (1972) reported on the parity distribution of women identified with adenomyosis amongst 200 women who underwent hysterectomy [12]. The average parity of the 123 women with adenomyosis was 3.2 compared to 2.5 for all women undergoing hysterectomy. Also, 89.5 % of women with adenomyosis were parous. Of the women with adenomyosis 123 (89.5 %) were parous and 13 (10.5 %) were nulliparous. Bird et al. (1972) concluded that these findings support existing reports which on average indicated that 80 % of women with adenomyosis have borne at least one child [12]. Molitor (1971) identified adenomyosis in 281 uteri out of 3207 hysterectomies [78]. Out of these 281, 263 (93.6 %) were parous and 18 (6.4 %) were nulliparous. Although the parity distribution of the whole group in the study by Molitor [78] is not provided, the author considered the fact that the overwhelming proportion of women with adenomyosis was parous, as evidence to support the notion that childbearing has a role in the aetiology of adenomyosis. Molitor (1971) added that there was no correlation between the number of pregnancies and the degree of uterine involvement in adenomyosis [78]. Thus he predicted that the trend to smaller family sizes is unlikely to reflect in a lower incidence of adenomyosis. In another study, adenomyosis was diagnosed in 5 out of 18 nulliparous women (27.7 %) compared to 150/264 (56.8 %) of women with parity range 1–4, and in 82/137 (59.8 %) of women with parity >4 [97]. The difference between parous and nulliparous women was statistically significant. However, the same study also reported that there were no cases of adenomyosis in the small group of women (n = 7) who were <29 years old, and that the prevalence in the group aged 30–39 was 30.6 % compared to 70.4 % in the 40–49 age group and 74.4 % in the 50–59 age group. Thus the study did not take into consideration the possible interaction between age and parity or the possible difference in the indication of hysterectomy between the nulliparous and multiparous women. This point is particularly important given

that the overwhelming majority (97.9 %) of the whole group were parous.

In another retrospective study involving 1334 women who underwent a hysterectomy, adenomyosis was identified in 332 patients (24.9 %) using one-half of a low-power field below the endometrial-myometrial junction as a diagnostic cut off [112]. The authors reported that in comparison with nulliparous women, the odds ratio (OR) for adenomyosis was higher in women who had one (OR 1.3) or ≥ two (OR 1.5) births ($p < 0.05$) [112]. But the incidence of adenomyosis reported in this study for nulliparous women 25/113 (22.1 %) was not significantly different compared to women who had one (80/322, 24.8 %) or more than one (188/686, 27.4 %) children. The study reported that no relation was found between age at surgery, age at menarche, indications for surgery, menopausal status at intervention and the presence of endometriosis. But there were 147 women in the adenomyosis group who were <50 years old and 184 women >50 years old compared to 508 women and 488 women in the two age brackets respectively who did not have adenomyosis. This indicates a significant difference ($p = 0.038$) between the two groups. It is notable that in contrast to the study by Vavilis et al. (1997) discussed below, the incidence of adenomyosis in the group aged ≥60 years old (22.7 %) was similar to the incidence in the group <50 (22.7 %), but was statistically significantly lower compared to the 50–59 age group ($p = 0.006$) where the incidence of adenomyosis was 32.1 % [110]. Perhaps a clearer link between adenomyosis and parity is provided in the follow-up study by the same group who reported that after adjusting for age, the OR for adenomyosis in primiparous women was 1.8 (95 % CI = 0.9–3.4), and for multiparous women the odds ratio was 3.1 (95 % CI = 1.7–5.5) compared to nulliparous women [89].

In the study by Vavilis et al. (1997), adenomyosis was identified in 116 out of 594 uteri (19.5 %) removed at hysterectomy [110]. Adenomyosis was present in 61/295 (20.6 %) of women <50 years old, 39/136 (28.7 %) of women aged between 50 and 59 years, and in 16/163 (9.8 %) of women ≥60 years old. The difference between the latter group and the other two groups reached statistical significance. There was also a higher incidence of adenomyosis in parous (116/554, 20.9 %) compared to nulliparous (2/40, 5 %) women and the difference was statistically significant ($p = 0.015$). But no analysis is provided that takes account of confounding factors in relation to parity such as age and presenting symptoms. There is a possibility that the low incidence of adenomyosis in older women, may indicate that it contributes to symptoms in younger women leading to early hysterectomy. However, this finding has not been consistently demonstrated.

Bergholt et al. (2001) performed a retrospective study involving 549 women who underwent a hysterectomy [11]. Factors that may be linked to adenomyosis were introduced into a multiple regression model. They reported that the presence of endometrial hyperplasia was the only factor significantly associated with adenomyosis. They did not find an association with age or with parity. In this study, the adjusted OR (95 % CI) for adenomyosis in primiparous women was 1.2 (95 % CI: 0.5–2.8) and for multiparous women was 1 (95 % CI: 0.5–2.0). In comparison to the <45 age group, women between 45 and 54 years old had an adjusted OR (95 % CI) of 1.2 (95 % CI: 0.6–2.4), and those >54 had an adjusted OR of 2.4 (95 % CI: 1.0–5.8).

Panganamamula et al. (2004) reported the findings from a study involving 873 women who underwent hysterectomy for benign conditions [88]. Of these, 412 (47.1 %) were identified with adenomyosis. They reported a statistically significantly higher gravidity (mean 3.5 ± SD = 1.8) and parity (mean 2.7 ± SD = 1.6) in the group with adenomyosis compared to those without adenomyosis (mean gravidity 3.06 ± SD = 1.92, mean parity 2.4 ± SD = 1.5). But there was no difference in age between the group with (mean 47.1 ± SD = 10.7) and without (mean 47.3 ± SD = 11.3) adenomyosis. But no comparison is provided between parous and nulliparous women. In a later study, Panganamamula et al. (2004) identified adenomyosis in 116/594 (19.5 %) uterine hysterectomy specimens; comprising 61/295 (20.6 %) women <50 years old;

39/136 (28.7 %) women aged 50–59 years old; and in 16/163 (9.8 %) women ≥60 years old [88]. The difference between the latter group and the other two was statistically significant. Adenomyosis was more common in parous (116/554, 20.9 %), compared to nulliparous (2/40, 5 %) women. However, the analysis provided does not account for confounding factors in relation to parity, such as age and presenting symptoms.

In the prospective cohort California Teachers Study of female teachers and school administrators, a total of 133,479 women, ranging in age from 22 to over 90 years, completed a self-administered, baseline questionnaire in 1995–1996 [103]. Amongst these 88,273 women were eligible to be included in analysis related to adenomyosis. Members of the cohort provided health related information including the diagnosis of endometriosis, reproductive history, use of hormones, physical activity, diet and alcohol intake, smoking history, and family history of health conditions. Participants were followed up longitudinally and an eligible cohort member was defined as having adenomyosis if the diagnosis was coded within the first three hospital discharge codes of admitted women between the date they joined the cohort and the end of the year 2003. There were 961 women with surgically confirmed adenomyosis. Templeman et al. (2008) reported a higher incidence of adenomyosis in parous (791/56,502, 1.4 %) compared to nulligravid women (116/16,947, 0.68 %) or compared to women who had previous pregnancies but no term pregnancies (50/5015, 0.99 %) [103]. The very low rate of diagnosis of adenomyosis is a factor of the cohort design as only a small proportion of women undergoing surveillance would be expected to undergo a hysterectomy which was the mainstay of histological diagnosis in 96 % of confirmed cases of adenomyosis. In addition, the authors excluded 419 cases from the analysis because adenomyosis was not classed in the top three on the hospital discharge codes. The comparative group comprised 79,329 women. Whilst these findings may indicate a link with parity, it should be considered that adenomyosis was not ruled out in the control group, and the interplay between symptoms and parity and the desire for children is an important driver that influences the choice of hysterectomy as a treatment.

In a retrospective multinominal regression analysis of the risk factors associated with adenomyosis alone or with a combination of adenomyosis and fibroids that involved 206 women, Jean-Baptiste et al. (2013) reported that dysmenorrhea was the only variable significantly associated with adenomyosis (OR 3.34; 95 % CI, 1.14–9.80) [39]. Variables significantly associated with combined adenomyosis and fibroids were age (OR 1.08; 95 % CI, 1.01–1.15), black ethnicity (OR 2.72; 95 % CI, 1.11–6.68) and parity (OR, 1.44; 95 % CI, 1.08–1.92). However, women included in the study either had fibroids only (n = 148), adenomyosis only (n = 21) or a combination of adenomyosis and fibroids (n = 37).

Adenomyosis and Infertility

Since the introduction of non-invasive imaging for the diagnosis of adenomyosis interest was renewed in the hypothesis that there is a strong association between endometriosis and adenomyosis and that coexisting adenomyosis can play a role in the infertility of women with endometriosis and vice versa. Kunz et al. (2005) performed MRI in women with (n = 160) and without (n = 67) endometriosis, taking into account age, disease stage and partners' sperm count [50]. Adenomyosis was present in 90 % of the subset of women with endometriosis who were <36 years and who had fertile partners. It is notable that 81/160 of this group had minimal or mild endometriosis and 79/160 had moderate or severe disease. The prevalence of adenomyosis in the control group which had infertility but no endometriosis was 19/67 (28 %). The authors concluded that adenomyosis causes infertility. The assumed mechanism was adverse effects that impair sperm transport. In the same cohort Kunz et al. (2005) reported on a secondary analysis of 227 patients with infertility and endometriosis [51]. They demonstrated that junctional zone

thickness in the group with endometriosis was higher compared to the control group in all four age groups (17–24, 25–29, 30–34, and >34). The difference was statistically significant only for the two latter groups. The number of women in each age group with junctional zone thickness consistent with adenomyosis is not provided, but the authors proposed that the process of adenomyosis development had already commenced in the third decade of life and that it progressed steadily during the fourth decade in women with endometriosis. Women without endometriosis showed almost no signs of adenomyosis up to the age of 34 years. Kunz et al. (2005, 2007) have not provided in their articles a breakdown of the incidence of the disease taking the 12, 8–12, <8 cut off points and there is a different age based analysis in the two papers [50, 51]. This brings-up the possibility of an alternative explanation for their observation on the differences in junctional zone thickness between the different age groups. Their findings remain consistent with the more widely held view that the incidence of adenomyosis increases with age. This will necessarily make the average thickness higher in older women.

Using MRI and hysterosalpingo-scintigraphy (HSSG), Kissler et al. (2006) linked endometriosis to hyperperistaltic and dysperistaltic utero-tubal transport. They performed HSSG and MRI on 41 infertile women aged 25–39, who had minimal or mild endometriosis (n = 28) or moderate or severe endometriosis (n = 13) [45]. All women had patent fallopian tubes. Adenomyosis was diagnosed in 29/41 (71 %) of participants using a cut-off JZ thickness of 8 mm and was diagnosed in 6 cases who had JZ thickness of <7 mm but who had localised JZ thickness, poor definition of borders or high signal-intensity foci. Taking all these together, the authors considered adenomyosis to be present in 85 % of cases. Based on MRI findings patients were classified into three groups: (1) Group (I), no adenomyosis (n = 6); (2) Group (II), focal adenomyosis (n = 24); (3) Group (III), diffuse adenomyosis (n = 11). Ipsi-lateral (to the dominant follicle) or bilateral tubal transport on HSSG was reported in 4 (67 %) participants in Group (I), compared to 10 (42 %) in Group (II), and 1 (9 %) in Group (III). The proportion of patients who had contra-lateral transport was 33 %, 33 % and 18 % respectively, while the proportion that showed no transport in the three groups was 0 %, 25 %, and 73 % respectively. Interestingly the failure in transport and contra-lateral transport were not significantly dependent on an increase of JZ thickness. The authors considered both endometriosis and adenomyosis to impact utero-tubal transport, but also that much of the reduced fertility in subjects with patent tubes was related to the presence of adenomyosis. However, the study should be interpreted with caution. First, the criteria on which adenomyosis was made is not clear. There was no statistically significant difference in JZ thickness between the group with diffuse (11.2 ± 2.7 mm) and the group with focal (10.3 ± 3.1 mm) adenomyosis compared to 3.2 ± 1.2 mm for the group without adenomyosis. Second, the reason for the very high incidence of adenomyosis amongst participants is unclear. Thirdly – and perhaps most importantly – because of the use of the controversial technique of HSSG as a test for tubal function. Habiba (1994) argued that many of the images produced by HSSG are artefacts [34]. Other authors have demonstrated the inconsistency of radioactive-labelled particle transport [62, 63, 117].

An alternative hypothesis was proposed by Tocci et al. (2008) who argued that because of the different epidemiological features of thickening of the JZ as identified using MRI on the one hand and histologically proven endometriosis on the other, that MRI should be regards as indicative of a "subendometrial myometrial unit disruption disease", as a distinct entity from adenomyosis [105]. Whilst this view point is interesting, it remains highly likely that the difference in epidemiological features reported is a factor of the method of diagnosis. Thus features linked to histological diagnosis are necessarily linked to the older age group who undergo hysterectomy and in whom MRI is often unnecessary, whilst MRI features are largely derived from cohorts of younger women seeking fertility investigation and treatment [51, 121]. It is to be noted that the reason for the distinct echogenic features attributable to adenomyosis on MRI is

unclear. Distinct zonation has been observed in vitro, suggesting that the features may not be linked to differential blood flow. Studies have demonstrated differences in cell density, total nuclear area and in extracellular matrix components, but in contrast to the clear zonation seen on some MRI images, the transition from the innermost to the outermost myometrial layer was shown to be gradual [73].

The effect of adenomyosis on fertility has been assessed through examining its prevalence in infertility clinics or its impact on outcomes of assisted conception. In the study by Kunz et al. (2005) referred to above, adenomyosis was identified in 79 % of cases with endometriosis, rising to 90 % in the subgroup of women <36 years old who had a fertile partner compared to 19/67 (28 %) in the infertile group who did not have endometriosis [50]. Martinez-Conejero et al. (2011) attempted to examine the relation between adenomyosis and implantation by comparing the outcome of donor IVF cycles in their infertility clinic in relation to the presence or absence of adenomyosis and endometriosis [66]. They compared three groups. The first was a group in whom adenomyosis was diagnosed based on ultrasound (n = 152) and who received 328 ovum donation cycles. These included 23 women who also had endometriosis. The second group comprised women with ovarian endometriosis but no adenomyosis (n = 144) and who received 242 ovum donation cycles. The third control group comprised women who had no visual pathology (n = 147) and who received 331 ovum donation cycles. The study reported that implantation rates in ovum donation cycles did not differ among the three groups. There were 88 term pregnancies in the adenomyosis group, 92 term pregnancies in the endometriosis group and 123 term pregnancies in the control group. The corresponding number of miscarriages in the three groups was 43, 15, and 24. Martinez-Conejero et al. (2011) also performed an RNA micro array comparison using endometrial samples obtained 7 days after the LH surge from women with adenomyosis and a control group of health young women with regular cycles and no uterine or endocrine anomalies and who had proven fertility [66]. They reported that there were no differences between the adenomyosis and the control group when comparing the genes known to be relevant to the window of implantation. However, there was a statistically significant higher incidence of miscarriage in the group with adenomyosis compared to the group with endometriosis and to the control group. The reason for the higher clinical miscarriage rate in the group with adenomyosis is unknown. The authors' interpretation is that adenomyosis does not impair implantation but may affect the function of the junctional zone leading to miscarriage. However, it is possible that implantation defects do contribute to increased pregnancy loss [93], or that the mechanisms active in this process involve factors affecting embryo selection [48]. On the other hand, implantation rates in the group with adenomyosis was marginally lower compared to the group with endometriosis, a condition associated with impaired endometrial receptivity [55]. The term pregnancy rate for the control group in the study by Martinez-Conejero et al. [66]. Martinez-Conejero et al. [66] is remarkably high at 84 %, but the indications for donor oocyte are not clear [115]. Martinez-Conejero et al. (2011) did not identify differences in implantation relevant genes in adenomyosis, but in common with other studies in the field, they did not control for the various down-regulation protocols [66].

In a retrospective study involving 74 patients Mijatovic et al. (2010) reported no significant differences when comparing outcomes of women with and without adenomyosis who were undergoing in-vitro fertilisation (IVF) or intracytoplasmic sperm injection cycles (ICSI) [76]. But all women received long-term GnRH-agonist pretreatment and the possibility should be considered that these drugs may have modified the effect of adenomyosis. Mijatovic et al. (2010) also reported the outcome of 74 infertile women with endometriosis who underwent IVF/ICSI [76]. There was a high (90.4 %) proportion with revised American Society for Reproductive Medicine (rASRM) stage III-IV disease. Based on ultrasound criteria adenomyosis was diagnosed in 20/74 of cases. All women received

GnRH-agonist prior to IVF-ICSI. The implantation rate in the adenomyosis group (31 %) was comparable to the rate in the group without adenomyosis (28.2 %). The authors reported that there were no significant differences in any outcomes between women with (n = 20) and without (n = 54) adenomyosis. In contrast to this, in the study by Thalluri and Tremellen (2012) women with ultrasound diagnosed adenomyosis (n = 38) had a statistically significant lower clinical pregnancy rate compared to controls (n = 175) [104]. All women in the study were undergoing IVF and adenomyosis was diagnosed or excluded based on ultrasound. The study reported a significantly lower clinical pregnancy rate in the adenomyosis group (23.6 % vs. 44.6 %). The lower clinical pregnancy rate in the adenomyosis group was maintained after adjustment for maternal age (OR = 0.408, CI = 0.181–0.922, p = 0.031) and when adjusting for the duration of infertility (OR = 0.417, CI = 0.175–0.989, p = 0.047). The same research group linked the outcomes in IVF cycles to differences in stromal leukocyte population, but again they did not control for exogenous steroids [106].

In agreement with the findings of Martínez-Conejero et al. (2011) of a higher miscarriage rate in women with adenomyosis [66]. Chiang et al. (1999) reported a significantly higher miscarriage rate (66.7 % vs. 21 %, p <0.04) in a small group (n = 19) of women who had ultrasound features suggestive of adenomyosis who were undergoing IVF when compared to a control group (n = 144) [18]. Both groups had comparable clinical pregnancy rates of 31.6 % and 26.4 % respectively. Maubon et al. (2010) conducted a prospective clinical study of 152 infertile women all had a pelvic MRI prior to IVF and the average and the maximal junctional zone thickness were measured [68]. Implantation outcomes were correlated with junctional zone thickness and with the causes of infertility (endometriosis, tubal infertility, anovulation, male infertility and unexplained infertility). They reported higher implantation failure (95.8 %) when the average JZ was >7 mm, compared to 37.5 % in those with JZ <7 mm. They did not directly correlate the findings with adenomyosis, but the highest pregnancy rate was in the group with endometriosis (59.3 %). The proportion with JZ thickness >7 was comparable in the group with endometriosis (14.8 %), male infertility (8.3 %), anovulation (9 %) or tubal factor infertility (13 %), but was lower compared to those with unexplained infertility (32.1 % p = 0.003). This is at variance with the high incidence of JZ thickening in endometriosis reported by Kissler et al. (2006) [45].

Costello et al. (2011) performed a retrospective review of 37 women with adenomyosis compared to 164 women without adenomyosis who were undergoing IVF/ICSI treatment [21]. Adenomyosis was diagnosed based on the findings on TVU and all participants received long down regulation protocols. There were no differences in live birth rates between the two groups (29.7 % Vs. 26.1 %; p = 0.395; OR 1.45 with 95 % CI 0.61–3.43). But the study was retrospective and small and the accuracy of ultrasound diagnosis of adenomyosis cannot be certain and there was heterogeneity in the indications for IVF/ICSI and in the treatment protocols received. Salim et al. (2012) published a prospective controlled study evaluating 275 consecutive women, commencing IVF/ICST for the first time [92]. The control group included 256 women and the adenomyosis group included 16 women. In this study the authors found that the clinical and ongoing pregnancy rates were lower in women with adenomyosis compared to the control group (22.2 % versus 47.2 % and 11.1 % versus 45.9 %, respectively). They concluded that ultrasound evidence of adenomyosis is found in a significant number of women presenting with infertility and that it has a negative impact on the outcome of IVF.

While there was no uniform agreement on the most appropriate therapeutic methods for managing women with uterine adenomyosis and/or adenomyoma who want to preserve their fertility, multiple modalities to restore fertility have been used including hormonal therapy and conservative surgical therapy via laparoscopy or exploratory laparotomy, uterine artery embolization, and magnetic resonance-guided focused ultrasound. The evidence base for these interventions remains poor. The review by Maheshwari

et al. (2012) concluded that there is little data on the epidemiology of adenomyosis associated with subfertility and that most studies on treatment have been uncontrolled and outcomes are usually reported in the form of case series [64]. The conclusion was that there is currently no evidence to support the need to identify or treat adenomyosis in patients who wish to conceive.

Adenomyosis During Pregnancy

Although pregnancy is not rare after spontaneous or assisted conception, there is little data on the epidemiology of adenomyosis in pregnancy. Sandberg and Cohn (1962) analysed 151 caesarean hysterectomies and found adenomyosis in 17 % of the specimen [95]. Azziz (1986) published a comprehensive report of 72 pregnancies in women with adenomyosis; 14 cases where published before 1930 and therefore probably refer to "adenomyoma", a term that was used to encompass both adenomyosis and endometriosis [6]. However, Azziz states that he excluded cases where the distinction was not made. There were 7 ectopic pregnancies. Obstetrical or surgical complications were described in 29 reports and there were 11 cases of uterine perforation or rupture. Today reported complications are rare and may include rapid growth in pregnancy [44], spontaneous rupture of an unscarred uterus [82] and delayed postpartum haemorrhage [116]. Uterine rupture during pregnancy may also occur after adeno-myomectomy [109].

In a case controlled study involving 104 cases and 208 controls, Juang et al. (2007) evaluated the incidence of adenomyosis in women with spontaneous preterm delivery or preterm rupture of membranes [41]. Adenomyosis was identified by ultrasound and/or MRI in 16 (15.4 %) women who delivered <37 weeks compared to 19 (9.1 %) who delivered at term. Whilst the figures do not reach statistical significance ($p=0.13$, Fisher exact test), the odds ratio after adjusting for age, BMI, smoking and previous preterm delivery is reported as 1.96 (95 % CI: 1.23–4.47). Juang et al. (2007) stated that there was a link between adenomyosis and preterm birth [41]. But their study design does not lend itself to this conclusion. The incidence of adenomyosis in women with preterm labour is a distinct question from the incidence of preterm birth in women with adenomyosis. Fernando et al. (2009) found increased risk of preterm birth in infertility patients with ovarian endometrioma, but did not control the study for the presence of adenomyosis [29]. Recently, Shitano et al. (2013) reported on MRI features during pregnancy in three cases with adenomyosis. Low signal intensity areas with embedded bright few millimetre diameter intramyometrial foci were attributed to decidualization [98]. This raises the question about what advice could be given to pregnant women with adenomyosis? Given that the majority will have uneventful pregnancies and that the impact of the disease on the course of pregnancy is unclear, together with the lack of specific interventions, it may best that available information be given to pregnant women in a way that would avoid raising unnecessary anxiety.

Post-menopausal Adenomyosis

The presence of adenomyosis in post-menopausal women is well documented. Lewinski (1931) reported adenomyosis in 26 cases amongst 49 women >50 and in 3 out of the 5 cases >70 years old undergoing autopsy [57]. In the series reported by Dreyfuss (1940) 13 (8.5 %) out of a total of 152 women with adenomyosis were more than 50 years old [25]. Dreyfuss (1940) stated that "*The adenomyotic structures were of the 'resting' type in women who were not menstruating any more*" [25]. There were 55/119 (46 %) postmenopausal women in the study by Reinhold et al. (1996) and 23 % postmenopausal women in the study by Kepkep et al. (2007) [91]. In a series of 1334 consecutive women undergoing hysterectomy, adenomyosis was diagnosed in 332 (24.9 %) of all cases and in 132 (24.3 %) of the postmenopausal cohort (n=544) [112]. In the California Teachers Study, adenomyosis was linked to the pre- or peri-menopause, and to the use of postmenopausal HRT [103]. Contrary to the case in premenopausal women, being overweight

or obese was not associated with increased risk of adenomyosis in postmenopausal women, but case selection may have influenced the conclusions of this study.

Postmenopausal adenomyosis was, however, an incidental finding in most reported cases. As such it seems to have little, if any, clinical significance. Lister et al. (1988) described a case of post-menopausal adenomyosis who had an apparent thickening of the endometrium mimicking a carcinoma [58]. Davies and Oram (1994) described a case where there was flare-up in symptoms and elevated CA125 in response to post-menopausal Tibolone HRT [22]. Özkan et al. (2012) compared women who underwent hysterectomy for fibroids (n = 98) with those who had adenomyosis (n = 106); overall, 40 % were postmenopausal [87]. Women with adenomyosis were statistically significantly older and of higher parity. In a sizable proportion, adenomyosis was an incidental finding. Tamoxifen has been linked to postmenopausal adenomyosis and to an endometrioma in one case report [54] and to adenomyosis and an adenomyomatous endometrial polyp in another [108]; in a small series (n = 8) with endometrial pathology during tamoxifen therapy; one had adenomyosis [49]. Cohen et al. (1995) reported adenomyosis in 8 (57.1 %) out of 14 women who had a hysterectomy whilst receiving tamoxifen [20]. Seven had small microscopic foci and one case had a large fundal adenomyotic lump. Cohen et al. (1997) reported adenomyosis in 15 (54 %) women with breast cancer receiving tamoxifen compared to only 2 of 11 women not receiving tamoxifen, pointing to an association [19]. A comparative histopathologic evaluation concluded that in tamoxifen-associated cases there was more often a cystic dilatation of glands, fibrosis of the stroma and various epithelial metaplasias, indicating a higher proliferation [72]. Tamoxifen also induces distinct MRI patterns in the postmenopausal uterus on tamoxifen. The majority have heterogeneous endometrial signal intensity on T2-weighted images (mean = 1.8 cm) with enhanced endometrial-myometrial interface, coexisting sub-endometrial cysts, nabothian cysts, leiomyoma, and adenomyosis [3].

Conclusion

What is apparent from this review it that there remains considerable uncertainties about adenomyosis including about its clinical presentations and impact. Research into adenomyosis has been hampered by the many methodological challenges posed by the inability to diagnose the condition through non-invasive means and because much of the research has relied on retrospective reviews with little attempt to correlate clinical presentation with gross or macroscopic features. Except in women treated with HRT – adenomyosis becomes silent in the vast majority of cases past the menopause.

Most of the studies reported on adenomyosis are undermined because of classical pitfalls such as selection bias because of the necessity of considering hysterectomy samples, non-blinding, lack of definition of either the disease itself or of the outcome measures and the problem with confounding association and causation.

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The Pathophysiology of Adenomyosis

3

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Abstract

Our understanding of adenomyosis remains hampered because of the lack of clarity of the clinical significance of the finding of aberrant endometrial glands and stoma within the myometrium. Despite the growing number of publications and the renewed interest generated by advances in vaginal ultrasounds and magnetic resonance imaging as non-invasive diagnostic techniques, many basic questions remain. Some of the theories of the pathogenesis stem from observations made in early writings with little supporting evidence. More recent research has highlighted a possible role for the myometrium.

Keywords

Organoid tumour • Histoid tumour • Wolffian duct • Müllerian theory • Endo-Myometrial Junctional Zone • α-SMA and desmin • Ultrasound • Invasion • Estrogen • Estrogen receptor • Progestogen • Progesterone receptor • Tamoxifen • Epithelial-mesenchymal transition

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Introduction

Uterine adenomyosis was recognised more than a century and a half ago. Rokitansky described the condition as cystosarcoma adenoids uterinum [1]. The first theories on the pathogenesis of adenomyosis date back to the end of the nineteenth century. Von Recklinghausen in a well recognised book published in 1896 described two classes of adenomyomas: (1) those situated at the periphery of the uterus and in the tubes and, (2) those arising centrally in the uterus. He viewed the first type to be derived from a numerical increase of the Wolffian tubules and the second type to originate from the uterine mucosa. Von Recklinghausen believed central adenomyosis to be rare and, because of the identification of three cases with malignancy, he concluded that they are prone to malignant change. Von Recklinghausen described cystadenoma as resembling an infiltrating fibroid that has diffused itself in the muscle wall and regarded these masses as 'organoid tumours' arising from a whole organ as distinct from 'histoid tumours' that arise from a particular tissue [2]. Much of the decline of the Wolffian and embryonic origin theories are attributable to the work of Cullen who first published his observations in 1896 [3]. Cullen emphasised the mucosal origin of the glandular component of adenomyosis and demonstrated extension of the endometrium within adenomyosis [4]. The Wolffian duct theory had many advocates who viewed it as established by fundamental proof [5].

The importance of the smooth muscle component of adenomyosis can be seen from earlier controversy about the theories of origin of adenomyosis. One of the arguments made by Von Recklinghausen against the Müllerian theory is that the spread of adenomyoma did not occur along the whole length of Müllerian ducts, but stopped at the internal os. Thus it was argued that the Müllerian component present in the cervix did not cause adenomyosis. Kossmann (1897) proposed that the reason for the rarity of adenomyosis in the cervix is not the lack of mucosal invasion, which is commonly evident, but the lack of myomas in the cervix [6]. The importance of mucosal continuity with the eutopic endometrium was emphasised by Cullen, but even instances where continuity cannot be demonstrated can be explained by the downward growth becoming cut-off from the eutopic mucosa or by the presence of accessory Müllerian glands. Another argument put forward in support of the Wolffian origin of adenomyosis is that adenomyosis is (perhaps) more common in the posterior uterine wall. But whether the incidence is higher in the posterior compared to the anterior uterine wall has long been a matter for debate including in more recent literature. Nevertheless, Kossmann (1897) argued that a higher incidence of adenomyomas in the posterior wall may be a reflection of the observation that fibroids may also be more common in the posterior uterine wall [6]. Based on the work of Kossmann and others, the theory subsequently emerged that adenomyomas are formed from Müllerian duct epithelium and 'inflammation' was suggested as a mechanism relevant to the process. Links to inflammation were mainly hypothesised with reference to tuberculosis. Meyer (1903) described adenomyosis as originating from uterine mucous membrane which sends hyperplastic and hypertrophic glands into the muscle even when the endometrium is not hyperplastic [7]. Meyer described these as arising from different points and spreading mostly to involve the inner and middle muscle layers and the fundus. He thus described 'invading masses of mucosa and branching complex glands that rarely become malignant' [7].

Although adenomyosis is basically a disease of adult women, the process may start during adolescence and become manifest during the third decade of life [8–12]. Meyer examined 100 uteri from foetuses, newborn children and girls up to the age of 14 years. He reported that mucosal invasion was seldom seen and concluded that the disease is one of adult life. Among the observations that favoured 'epithelial invasion' is the well recognised absence of a uterine submucosa. Thus it is hypothesised that a breach in the muscle

interstitial substance or mechanical stress acting on interfascicular connective tissue would facilitate such invasion [13].

Cullen (1908) described cases of diffuse adenomyomas, all of which had no evidence of inflammation [14]. This added support to the view that mucosal invasion is not necessarily linked to inflammation. Another theory advanced in early writings to explain adenomyosis is an origin from the uterine serosa by in-growth of epithelium into the outer layers of the uterus. This was also proposed as a mechanism for adenomyosis of the rectovaginal septum. Erich Opitz proposed that peripheral growth arose from the serosa and that deeper growth arose from the mucous membrane [13].

Forms of mucosal invasion into the myometrium, termed '*adenomyomata*', were thought to represent "a new formation composed of gland-elements, hyperplastic cellular connective tissue, and smooth muscle" [13]. The origin of these "mucosal invasions" was debated for decades and it took half a century before their "endometrial" nature became fully accepted [15]. Typically, focal adenomyosis has poorly defined margins merging with the surrounding normal myometrium, and – in contrast to leiomyomata – cannot be enucleated. 'Focal' lesions can resemble leiomyomata; hence the term adenomyoma which was criticised as it implies neoplasia which is not relevant to the condition [16].

When, in 1925, Frankl coined the term '*adenomyosis*' (alternatively called '*endometriosis interna*') to distinguish it from the forms of endometrial colonization within the peritoneum (also termed 'endometriosis externa') he specified that "*the direct connection of the endometrium with the islands of mucosa located in the musculature can be established in serial sections*". He defended the use of the term adenomyosis because it "does not suggest any inflammatory genesis" [17]. At this point adenomyosis came to be identified as an entity separate from endometriosis.

The link to stromal invasion is maintained in more recent writings. Bird recognises that the lesion is benign, yet he defines adenomyosis in terms of *invasion* of the endometrium into the myometrium [18]. Vercellini et al. (2006) describes stages in the process starting with disruption of the normal boundary between the basal layer and the myometrium and invasion of endometrial glands into the myometrium as a consequence of this disruption. The resulting ectopic intramyometrial glands then cause myometrial hypertrophy and hyperplasia [19].

Until the 1980s, adenomyosis could only be detected by histological examination of hysterectomy specimens. This changed following the introduction of non-invasive diagnosis using Magnetic Resonance (MRI) [20–22], Ultrasound imaging [23], transvaginal ultrasound (TVS) [24, 25] and, more recently, three dimensional transvaginal ultrasound scan (3D-TVS) [26]. The lack of non-invasive diagnostic tests and doubts about the reliability of imaging based diagnostics have for a very long time impeded research and our understanding of the condition. The lack of reliable preoperative diagnostic meant that, in the past, the condition came to clinical attention only as a finding in hysterectomy specimens and consequently, less emphasis was placed on its importance and clinical impact.

Some advances were made following the recognition of the diagnostic value of MRI and TVS, yet at an elemental level, there is still a lack of congruity between the diagnostic criteria for adenomyosis. Histological diagnosis is based on the presence of endometrium beyond the junction of endometrium and myometrium, whilst imaging techniques mostly measure thickness and/or irregularity of the inner portion of the myometrium. Thus imaging diagnoses utilise techniques based on differences in the appearance of smooth muscle, particularly the inner myometrium (the *myosis* component), whereas histology relies on the presence of ectopic endometrial glands within the myometrium (the *adeno* component). The relative contribution of each component varies considerably, and this can account for the discrepancies observed between the diagnostic modalities.

The Endo-myometrial Junctional Zone

The inner myometrial layer immediately underlying the endometrium appears on MRI as a distinct area, named the Junctional Zone (JZ). Despite the absence of distinctive histological features on light microscopy, the distinct features seen on imaging emphasised or perhaps overemphasised the importance of this zone. Some characterisations view this zone as having distinct embryological origin and this seems to generate an impression of anatomical distinction. This portion of the myometrium has been variously labelled '*archimyometrium*', inner myometrium, endometrial-myometrial interphase, transitional zone or subendometrial myometrium and is often distinguished on T2-weighted MR images of the uterus [20]. One important limitation of MRI, however, is the absence of a definable JZ in 20 % of premenopausal women [27] and in a bigger proportion of postmenopausal women. When seen on MRI, the JZ appears as a distinct and regular inner layer of the myometrium, measuring 5 mm or less in thickness. But the reasons for the distinct zonation are unclear [28, 29]. It is still unclear whether the JZ undergoes cyclical changes in thickness mimicking those of the endometrium [30]. Kunz et al. (2000) argued that, like the endometrium, the inner myometrium is of Müllerian origin, while the outer myometrium is of non-Müllerian, mesenchymal derivation and that it has structural and functional differences compared to the outer myometrium [31]. Both EMI components have a common embryological origin from the paramesonephric duct. Konishi et al. (1984) examined autopsy material obtained from human abortuses and stillborn fetuses [32]. At 12 weeks of gestation, the mesenchymal cells were distributed sparsely in the uterine mesenchyme and were mostly round in shape, with high nucleo-cytoplasmic ratios but there were no smooth muscle cells. By 14 weeks, mesenchymal cells formed two layers: the cells near the serosa were the most abundant and elongate, whereas the cells toward the lumen were sparse and round in shape. From 14 to 20 weeks the spindle shaped cells of the outer layer gradually increased in number and at 20 weeks this layer formed the bulk of the uterine thickness. Mitotic figures of the mesenchymal cells were most frequent in the inner layer between 14 and 20 weeks. At 26 weeks the thickness of the outer layer increased markedly and at 31 or 40 weeks bundles of cells resembling smooth muscles became obvious. These observations imply that the outer mesenchymal layer of the body of the fetal uterus gives rise to the myometrium and that the inner layer corresponds to endometrial stroma of the adult uterus [32]. Using electron microscopy, Konishi et al. (1984) were able to demonstrate the stages in smooth muscle differentiations and development of intracellular organelles and myofilaments [32]. The process starts around 16 week gestation with the appearance of cytoplasmic filaments with dense bodies which are characteristic of smooth muscle cells at 16 weeks of gestation. Spindle shaped cells containing a few myofilaments scattered in the cytoplasm and well developed organelles such as mitochondria, free ribosomes, granular endoplasmic reticulum and Golgi membranes appear in the outer layer of the uterus at 18 weeks. These cells resemble immature smooth muscle cells. Other ultrastructural characteristics of smooth muscle cells develop later; surface vesicles of the cell membrane begin to increase at about 20 weeks, dense plaques along the cell membrane appear by 26 weeks and an external lamina is almost complete by 31 weeks. These observations imply that undifferentiated mesenchymal cells which develop into smooth muscle cells may exist in the inner layer of the fetal uterus and that smooth muscle differentiation may occur at the junctional area between the myometrial and endometrial stromal layers [32]. Thus it is possible that disruption of the process can result in loss of orientation leading to the observed presence of stroma within the myometrium. In addition, the developmental sequence resembles that observed in rodents. It casts doubts on theories that view the inner and the outer myometrium as embryologically distinct. The research by Fujii et al. (1989), shows that stromal and myometrial cells in the adult uterus exhibit plasticity as the cells at the stromal-myometrial interface

morphologically resemble myofibroblasts in the follicular phase and differentiate into cells morphologically resembling smooth muscle cells in the luteal phase and early pregnancy. This is perhaps not surprising given the shared enbryological origin. It further opens the possibility that disruption of the mechanisms that control the process can result in the disorganisation noted in adenomyosis [33].

The Müllerian origin of the outer myometrium is also supported by Robboy et al. (1982) who implanted human reproductive track obtained from early embryos into BALB/C athymic nude nice and used the model to describe the normal development of the human female reproductive tract and the alterations resulting from experimental exposure to diethylstilbestrol [34].

In the presence or absence of adenomyosis, JZ thickness is reduced in post-menopausal women and in premenopausal women taking oral contraceptives or gonadotrophin-releasing hormone analogues. This effect results because of the suppression of ovarian activity and is reversed by the administration of hormone replacement therapy which may cause the reappearance of the typical JZ [35]. In women with normal menstruation, variations in myometrial contractility waves suggest that uterine peristaltic activity originates exclusively from the JZ, while the outer myometrium remains quiescent [36, 37]. It appears that uterine peristalsis is hormone dependent, and that it is affected by both estrogen and progesterone. It is possible that inner myometrial peristalsis plays a role in sperm transport, as well as in stabilization of the blastocyst prior to nidation and there is evidence that the "zonal-endometrial" complex may play a role in uterine receptivity in relation to the outcomes of assisted conception. On the other hand, the use of oral contraceptive and intrauterine contraceptive device [38] or sterilization by tubal ligation [39] do not appear to be associated with a risk of adenomyosis.

While MRI may identify the uterine JZ as a distinct hypodense layer [29, 40], the reason(s) for this demarcation is unclear. It may be due to an increase in nuclear area in the inner myometrium [29], to different water content between the inner and outer myometrium [41] or to differences in the distribution of laminin-β2 [42]. Differences in blood flow between the inner and outer myometrium have been proposed as an explanation of the MRI appearance, but against this is the observed zonation in uterine hysterectomy specimens when examined using MRI despite the clear absence of blood flow [29]. MRI features suggestive of adenomyosis are indirect and include increased JZ thickness [40, 43, 44]. Mehasseb et al. (2011) demonstrated that the inner myometrium of normal uteri has higher cell density and total nuclear area compared to the outer myometrium (increased by 1.6–1.8 fold) and importantly in relation to the features seen on MRI, they demonstrated that the change in cell density is gradual throughout the uterine thickness with no distinct point of demarcation [28]. This echoes the finding of gradual increase in elastin expression from the inner to the outer myometrium [30], but contrasts with the notion of a clearly defined JZ as implied by MRI.

The maximum change observed by Mehasseb et al. (2011) in cell density from the inner to the outer myometrium is less than the three-fold change reported by Scoutt et al. (1991) [28, 29]. The difference between the two studies may be related to methodological issues. The study by Mehasseb et al. used true colour image analysis without grey-scale transformation, which allows more accurate definition of cellular structures. Nevertheless, both studies agree on the main findings. It remains possible that the distinct MRI features are due to lower water content in the inner myometrium reflecting a reduction in intercellular space between more tightly packed cells. It has been demonstrated that water content is lower in the subendometrial myometrium compared to the outer myometrium [41]. This implies that the in vivo difference in cell density between the inner myometrium and outer myometrium may in fact be larger than the differences noted in formalin-fixed tissue. It is possible that the subendometrial halo seen on MRI reflects a transitional point of cell count or water content, but it remains unclear why this zone is not seen in all uteri [44]. This also leaves the definition of the JZ dependent on MRI, which raises important question about its very nature. On the other hand, the

finding of similar expression of the intracellular components α-SMA and desmin in the corresponding layers (inner or outer myometrium) in adenomyosis compared to normal myometrium points to a reduced extracellular fluid content in adenomyosis [28], thus the reason for increased JZ thickness in affected uteri remains unclear. The lack of a clear distinction between the inner and outer muscle layers does not rule out a functional distinction as demonstrated in studies using ultrasound [31, 45, 46], because differences may exist in steroid receptors expression [47] or in innervation [48] which can lead to differential functional response.

The extent of adenomyosis varies from simple JZ thickening to more diffuse or nodular lesions involving the entire uterine wall. It can also take the form of an adenomyoma, a focal thickening or a nodular structure arising at the JZ and extending within the myometrium where it is identified with low signal intensity on T2-weighted MRI [35]. A normal JZ is between 5 and 12 mm thick on T2-weighed MRI, and features highly predictive of histological adenomyosis include JZ measuring >12 mm, with hemorrhagic high-signal myometrial spots [36]. Adenomyosis can also be observed utilizing transvaginal sonography where it appears as heterogeneous and hypoechogenic, poorly defined areas in the myometrium [21, 37, 43, 49]. These areas may appear with or without anechoic lacunae or cysts of varying size, and the echo texture of the myometrium may be increased. Moreover, there may be linear striations radiating out from the endometrium into the myometrium and an indistinct endo–myometrial junction with a pseudo-widening of the endometrium. The hypoechogenicity may be caused by the muscular hypertrophy, while the increased echogenicity, the cyst, the linear striations, and the indistinct endo–myometrial junction may be caused by ectopic endometrial tissue. In a meta-analysis of reports published between 1966 and 2007 on the diagnostic accuracy of TVS in women having a hysterectomy, Meredith et al. (2009) reported that TVS has a predictive likelihood ratio of 4.67 (95 % CI: 3.13–6.17) [50]. The overall prevalence of adenomyosis was 27.9 % (95 % CI: 25.5–30.3) and the probability with an abnormal TVS of 66.2 % (95 % CI: 61.6–70.6). The probability of adenomyosis with a normal TVS was 9.1 % (95 % CI: 7.3–11.1). They concluded that TVS is a moderately accurate test for the diagnosis of adenomyosis. The myometrium surrounding the adenomyotic foci, whether diffuse or focal, showed normal immunohistochemical staining for low and high molecular weight cytokeratins, estrogen and progesterone receptors, vimentin, actin and desmin [51].

A recent study compared histopathology to morphological alterations of the myometrium as detected on two-dimensional (2D) and three-dimensional (3D) TVS in 72 premenopausal women. The most specific 2D-TVS feature of adenomyosis (specificity = 98 %; accuracy = 78 %) was the presence of myometrial cysts, whereas the most sensitive feature was the finding of a heterogeneous myometrium (sensitivity = 88 %; accuracy = 75 %). On 3D-TVS the best markers of adenomyosis were related to the JZ myometrium. A difference in its thickness of more than 4 mm and JZ distortion and infiltration had high sensitivity (88 %) and the best accuracy (85 % and 82 %, respectively). Overall, for 2D-TVS and 3D-TVS, respectively, the accuracy was 83 and 89 %; sensitivity was 75 and 91 %; specificity was 90 and 88 %; positive predictive value was 86 and 85 %; and negative predictive value was 82 and 92 % [52]. It seems therefore that a diagnosis of adenomyosis can be made when one or more of the following sonographic findings are present: (1) a globular uterine configuration; (2) poor definition of the endometrial-myometrial interface; (3) sub-endometrial echogenic linear striations; (4) myometrial anterior-posterior asymmetry; (5) myometrial cysts; (6) a heterogeneous myometrial echo texture [53]. Additional preliminary information seems to indicate that diagnosis may be more accurate during the luteal phase [54].

Champaneria et al. (2010) conducted a systematic review with meta-analysis of all published data on test accuracy of ultrasound scans and MRI for the diagnosis of adenomyosis based on studies which also reported histological confirmation of the diagnosis [55]. TVS had a pooled sensitivity of 72 % (95 % CI = 65–79 %),

specificity of 81 % (95 % CI = 77–85 %), positive likelihood ratio of 3.7 (95 % CI = 2.1–6.4) and negative likelihood ratio of 0.3 (95 % CI = 0.1–0.5). MRI had a pooled sensitivity of 77 % (95 % CI = 67–85 %), specificity of 89 % (95 % CI = 84–92 %), positive likelihood ratio of 6.5 (95 % CI = 4.5–9.3), and negative likelihood ratio of 0.2 (95 % CI = 0.1–0.4). The authors concluded that both TVS and MRI showed high levels of accuracy for the non-invasive diagnosis of adenomyosis.

Possible Pathogenetic Mechanisms

Direct Endometrial Invasion

Adenomyosis has been traditionally described in terms of abnormal ingrowths and invagination of the basal endometrium into the subendometrial myometrium [18]. The more widely accepted theory on pathogenesis proposes that during periods of regeneration, healing and re-epithelialisation, the endometrium can invade a predisposed myometrium or a traumatised endometrial–myometrial interface. In support of this theory is the observation of increased incidence following uterine curettage. Parazzini et al. (1997) reported on 707 consecutive women who underwent a hysterectomy. The incidence of adenomyosis was 21.2 % (n = 150) [38]. The risk of adenomyosis was reported to be lower in women who smoked (OR 0.7; 95 % CI 03–1.3) compared to women who never smoked, and the risk decreased in relation to increased number of cigarettes per day. The frequency of adenomyosis was higher in parous women and was higher in women who reported one of more than one spontaneous miscarriage compared to those who did not have a miscarriage (OR = 1.7; 95 % CI, 1.1–2.6). But whether miscarriage is the cause or the result of adenomyosis remains to be ascertained [38, 56–58].

The relation between adenomyosis and parity has also reported in other studies [59, 60] but remains controversial (See Chap. 2). In the study by Parazzini et al. (1997) the odds ratio for adenomyosis in the group who ever had a dilatation and curettage was 2.2 (95 % CI 1.2–4.0) and the authors suggested that the trauma of curettage may favour the inclusion of islands of endometriosis in the myometrium [38]. Levgur et al. (2000) reported on clinical correlations in 111 uteri removed at hysterectomy [58]. All samples weighed less than 280 g. There were 17 uteri with adenomyosis alone, 19 with adenomyosis and fibroids, 39 with fibroids only and 36 samples with no fibroids or adenomyosis. The incidence of pregnancy termination in the group with adenomyosis alone was 58.8 %, and in the group with adenomyosis and fibroids was 47.4 %. This compares to an incidence of 20.5 % in the group with fibroids alone and of 22.2 % in the group with neither. Adenomyosis was assessed based on 5–10 sections per specimen and using a cut-off point for endometrial glands within the myometrium of at least 2.5 mm. Perhaps uncharacteristically in relation to the diagnosis of adenomyosis, they reported that there was no inter-observer variability. Interestingly, during the study period the incidence of adenomyosis in the 111 women in whom the uterine specimens weighed <280 g as 32.4 % compared to an incidence of 6 % amongst the group (n = 132) whose uterus weighed >280 g. The group with larger uteri were excluded from the analysis. The reasons given were uterine distortion and the lack of full-thickness sections which they believed would preclude accurate histological evaluation. However, the indications for the hysterectomy are not provided and the rationale for excluding larger uteri from this analysis is debatable as it should be possible to examine the subendometrial myometrium even in large uteri. Nevertheless, the authors argued that their study provided evidence of a link between adenomyosis and pregnancy termination. Ostrzenski (1998) reported a case which resembled adenomyosis that was identified with endometrial tissue within the myometrium following laparoscopic myomectomy, suggesting a link to myometrial disruption [61]. However, this case does not describe the classic disease, and the presence of inclusion cysts is well recognised in other parts and tissues of the body. The link to pregnancy and pregnancy termination is weakened by evidence that about

20 % of women with adenomyosis have never had a pregnancy [62]. Interestingly, sharp curettage in the non-pregnant status does not seem to increase the risk of adenomyosis [58, 63–65]. The differential effect might be related to the disruption of the EMI by the invading trophoblast. Focal disruption of the EMI in early pregnancy can be observed using MRI, which reverts to normal 2–24 weeks after delivery [66]. It cannot be ruled out that changes occurring in the JZ during pregnancy, such as angiogenesis and trophoblast invasion, may aggravate existing adenomyosis. Parazzini et al. (1997) reported on the risk factors for adenomysis in a series of 707 consecutive women who underwent a hysterectomy [38]. Women reporting one or more spontaneous abortions had an odds ratio of 1.7 (95 % CI 1.1–2.6) for adenomyosis. Women who had a dilatation and curettage for a gynaecological indication (n = 58) had an adjusted odds ratio for adenomyosis of 2.2 (95 % CI: 1.2–4.0). Curtis et al. (2002) examined the relation between pregnancy-related and non-pregnancy-related curettage and the development of adenomyosis in a series of 1850 women [65]. Adenomyosis was diagnosed after hysterectomy in 368 (19.9 %) of the cohort although the diagnostic criteria are not provided. The investigators obtained self reported history of abortion or curettage from participants. Women with adenomyosis reported a history of induced abortion more frequently than did women without adenomyosis (17.1 % and 12.6 %, respectively; $p = 0.02$). The prevalence of caesarean delivery and D&C were similar between the two groups. No association was found between caesarean section or dilatation and curettage and adenomyosis, but induced abortion was associated with a non-significant increased risk.

The process of "invasion" of endometrial glands within the myometrium is said to develop in stages as referred to above [19]. A number of hormonal, genetic, immunological and growth factors were hypothesised to play a role in this sequence of events. Familial predisposition may have a role as documented in one series, of seven cases in which mothers and daughters were affected [67].

In a retrospective study including 200 women who underwent hysterectomy for benign disease, Whitted et al. (2000) observed a higher incidence of caesarean delivery in patients with adenomyosis compared to the group without adenomyosis (30 % vs. 23 %) [68]. But some studies found no statistically significant association between adenomyosis and previous caesarean section, endometrial curettage, or evacuation of the uterus [58, 63, 64, 69]

The hypothesis originally proposed by Cullen centres on an origin of adenomyosis from basalis endometrium invaginating deep within the myometrium. The hypothesis is built on the observed histological continuity between the basal endometrium and underlying inner myometrium and also because of the absence of a separating basal membrane. The hypothesis can be seen as being supported on two further counts: the first is the proven relationship of the disease to factors that favour increased 'invasiveness', either because of external or mechanical forces or as innate properties of the endometrium in adenomyosis; and the second is the presence of similarities between the basalis glands and adenomyosis nodules. But histological continuity is not always demonstrable or present.

There is evidence of increased invasiveness of endometrial cells in adenomyosis, as well as in endometriosis [70, 71] and, at least in some subgroups, the two conditions often coexist. Research has shown differences between the eutopic endometrium of women with both diseases when compared to controls. This suggests an immune dysfunction and 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-radicals 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 differences are primarily related to the presenting symptoms. Finally, both conditions are steroid–dependent and research suggests a role for epigenetic mechanisms.

Gaetje et al. (1995) reported that endometrial cells obtained from endometriosis nodules, but not from normal endometrium, grown on a collagen invasion assay, had invasive potential similar to metastatic bladder carcinoma cell line (EJ28) and exceed those of non-metastatic bladder cell line (RT112) [72]. This needs further corroborative evidence. In a subsequent publication, the same group identified the invasive cells in endometriosis as E-Cadherin negative epithelial cells [73]. Such invasion could be facilitated by the loss of cohesion of myometrial bundles influenced by enzymes such as matrix metalloproteinases [74, 75]. Endometrial stromal fibroblasts produce tenascin, a fibronectin inhibitor that in turn facilitates epithelial migration. Tenascin mediates epithelial-mesenchymal interactions by inhibiting cell attachment to fibronectin, an action stimulated by hormonally regulated epidermal growth factors. But whether this interaction plays a role in the development of uterine adenomyosis is unknown [76].

Thus whilst abnormal stromal cell differentiation and invasion has been proposed in the aetiology of adenomyosis, the features in the microenvironment that limit myometrial penetration by the overlying endometrium and the changes that precede or trigger the development of uterine adenomyosis are unknown. Mehasseb et al. (2010) examined the migration of human endometrial stromal cells in a three-dimensional co-culture in plain collagen or in collagen in which myometrial cells were grown. The in-vitro experiment included crossover between cells from uteri with and without adenomyosis. Stromal cells from adenomyosis exhibited greater invasiveness when grown on a plain collagen matrix or in double culture with myocytes from normal or adenomyosis affected uteri compared to normal stromal cells [77]. Also myocytes from adenomyosis were shown to enhance invasion of stromal cells when compared to normal myocytes. This suggests that both the stromal and the myometrial component have a role in the aetiology of the disease. In a subsequent study the same group reported the effect of estradiol, progestogen and tamoxifen alone or in combination on stromal cell invasion in three dimensional co-cultures [78]. The addition of estradiol or tamoxifen, but not of the estradiol and progesterone combination, increased the depth of invasion of both adenomyotic stromal cells and control stromal cells in all cell combinations. When grown on plain collagen, the depth of invasion for control stromal cells and adenomyotic stromal cells increased by 126 and 93 % with the use of tamoxifen, and by 71 and 50 %, with the use of estradiol. The depth of invasion for adenomyotic stromal cells was statistically significantly higher compared to the control stromal cells whether grown on plain collagen, on collagen containing control or on adenomyotic muscle cells. The addition of estradiol or tamoxifen, but not of the estradiol and progesterone combination, increased the depth of invasion of both adenomyotic stromal cells and control stromal cells in all cell combinations. When grown on plain collagen, the depth of invasion for control stromal cells and for adenomyotic stromal cells increased by 126 and 93 % with the use of tamoxifen, and by 71 and 50 %, with the use of estradiol. Thus both estradiol and tamoxifen enhance stromal cell invasion and the addition of progesterone to estrogen inhibits estrogen effect. This would appear to support a role for estrogen in stromal invasion and in the pathophysiology of adenomyosis. But the greater depth of invasion of adenomyotic stromal cells and the enhancing effect of adenomyotic muscle were maintained under all experimental conditions which suggest an inherent predisposition that is related to the origin of stromal cells rather than an effect of estrogen on the ability of cells to invade within the matrix.

Derivation from Multipotential Perivascular Cells

An alternative theory suggests that the basalis endometrium invagination occurs along the intra-myometrial lymphatic system, rather than by disruption and “invasion” of the muscle bundles. This possibility is supported by the occasional finding of endometrial tissue in the intra-myometrial lymphatics in hysterectomy

specimens. Sahin et al. (1989) identified endometrial tissue in 14 cases that had extensive adenomyosis [79]. They speculated that the intimate relationship between endometrial tissue and the vessels may be explained by an origin of the endometrial tissue from uncommitted or multi-potential perivascular cells. They also reported on the absence of a connection between adenomyosis foci and the eutopic endometrium. In their study, they emphasised that the phenomena is distinct from neoplasia. Intravascular proliferation of adenomyotic stroma was found in 17.5 % of cases in another study involving 200 cases with adenomyosis [80]. A more recent study seems to confirm the phenomenon [81]. Vascular involvement in adenomyosis was noted in 54 (12.4 %) cases out of a large series (n = 434) of uteri affected by adenomyosis. The degree of vascular involvement varied. A single vessel was involved in 19 of 54 cases (35 %), 2–3 vessels were involved in 16 cases (30 %), and multiple vessels were involved in 19 cases (35 %). The intravascular component comprised endometrial stroma only in 34 cases (63 %) and contained glands and stroma in 20 cases (37 %). In most cases, the intravascular component was covered by intact endothelial lining.

Mai et al. (1997) examined cases of adenomyosis and endometriosis using immunohistochemistry for estrogen receptor, vimentin, Ber-EP-4 (Epithelial specific antibody to EpCAM: Epithelial cell adhesion molecule) and cytokeratin [82]. They identified nodes of stromal cells without endometrial glands, located along blood and lymphatic vessels. They termed these type 1 nodules. Stromal cells were positive for vimentin and negative for cytokeratin and were not connected to adjacent adenomyosis. These nodules were often seen at the periphery of adenomyosis and appeared fusiform or satellite, tapering in the myometrium or in the adventitia of blood vessels or in contact with other type 1 nodules. Occasionally, these nodules had projections as polypoid structures into the vessel lumen. The term *type 1 nodules* was used to distinguish these lesions for *type 2 nodules* which also contained mesothelial cysts and *type 3 nodules* which describes nodules containing endometrial glands. Type 2 and 3 nodules were present in endometriosis, but not in adenomyosis. In adenomyosis, there were foci of adenomyosis with sparse glands, characterised by low gland/stromal ratio. Around blood vessels within type 1 nodules, there were cells with embryonic features with hyperchromatic nuclei. Many of the type 1 nodules had no connection with adjacent endometrium. Mai et al. (1997) viewed these as precursors or early stages of adenomyosis [82]. Interestingly, they also identified regions were endometrial glands were present where the surrounding stroma was undergoing fibrosis with replacement of stromal cells by fibromuscular tissue in what they considered to be end stage disease. The proposition put forward is that these nodules develop from perivascular pericytes and that hormonal, immunological or other growth factors induce the transformation into endometrial stromal nodules. These stromal nodules are assumed to later incorporate endometrial epithelial cells either through growth from adjacent nodules or through 'transformation' of the stromal and smooth muscle cells into endometrial stromal cells. Thus, the newly enlarged area of stroma serves as "new soil", facilitating further downward growth of the endometrial glands [82]. An important differential diagnosis is stromatosis or endometrial stromal sarcoma (endolymphatic stromal myosis), both of which are characterized by endometrial stroma without endometrial glands (as opposed to adenomyosis that consists of both endometrial stroma and glands). Goldblum et al. (1995) described the features that can help differentiate adenomyosis with sparse glands from low-grade endometrial stromal sarcoma [83]. These include the small size of the lesions in the absence of grossly evident tumour nodules and the presence of typical adenomyosis with glands elsewhere in the myometrium. Also relevant is the patient's age and menopausal status.

Adenomyosis as a Uterine Disorder

Smooth muscle cells from uteri with adenomyosis are ultra-structurally different from smooth muscle cells of normal uteri [84]. In adenomyosis, myocytes exhibit cellular hypertrophy and

the intermediate filaments are abundant and form cytoplasmic aggregates. The nuclei have a smooth outline with a clear ground substance, prominent nucleoli and peripherally arranged nuclear chromatin. There is an occasional infolding of the nuclear envelope with entrapment of cytoplasmic organelles. The sarcolemmal bands are significantly longer and there are fewer caveolae. The perinuclear cell organelles are more distinct. The rough endoplasmic reticulum and Golgi apparatus are more prominent, denoting active protein synthesis, this is consistent with the observed cellular hypertrophy [84]. The transition from the inner to the outer myometrium is reported to be gradual with no clear demarcation between the two components [28, 30]. The identification of differences between myocytes in the outer myometrium remote from adenomyotic lesions suggests that the changes are not a reaction to the presence of ectopic endometrium. Myocytes obtained from women with adenomyosis and added to the co-cultures resulted in enhanced stromal cell invasion within the matrix when compared to the effect of myocytes obtained from unaffected women. In the same study, Mehasseb et al. (2010) reported the presence of differences in secretory proteins in the culture supernatant of cells depending on their source of origin (adenomyosis or control) [77]. Allowing a 0.1 % margin of error in estimating the m/z ratio for protein clusters, there were 28 common peak clusters among the peaks differentially expressed in culture supernatant between stromal cells from uteri not affected by adenomyosis and stromal cells from uteri affected by adenomyosis. These differences were noted in experiments were stromal cells were grown on plain collagen as well as in experiments were stromal cells were grown on cultures of collagen containing control, or adenomyotic myocytes. There were nine common peak clusters among the peaks differentially expressed when comparing cultures in which muscle cells from controls and adenomyosis when grown on plain collagen, or when co-cultured with control or adenomyotic stromal cells. Interestingly, there were six distinct peaks that were common to both "adenomyotic stromal cells" and "adenomyotic myometrial cells."[77]. This suggests that: (1) both stromal and muscle cells have a role in the aetiology of adenomyosis, (2) that the disease may be a reflection of a pan-uterine abnormality [77, 85]. Steroids have been shown to affect the migration of stromal cells derived from uteri with or without adenomyosis. The observation that myocytes from adenomyosis enhance stromal cell invasion and the presence of similar peak cluster patterns for secreted proteins when adenomyosis stromal and muscle cells grown in culture are compared to normal stromal and muscle cells respectively suggests that both stromal and muscle cells have a role and reflect a pan-uterine abnormality.

Studies that used the neonatal mouse model [86] and the prolactin induced adenomyosis mouse model [87] suggested that disruption and/or "permissiveness" of the inner myometrium could play a role in the development of uterine adenomyosis. Mehasseb et al. (2009) reported the effects on uterine development and myometrial differentiation of the administration of tamoxifen and estradiol to neonatal mice [88]. Female CD1 pups were administered tamoxifen or estradiol from age 1 to 5 days and compared to controls. Uteri were examined on days 2, 5, 10, 15, and 42 of age using image analysis and immunohistochemistry for α-smooth muscle actin (α-SMA), desmin, and estrogen receptor-α (ER-α). Following tamoxifen exposure, all uteri showed adenomyosis by 6 weeks of age. The inner myometrium showed thinning, lack of continuity, disorganization, and bundling but α-SMA expression was comparable to normal controls. In untreated neonatal uteri, desmin expression showed a wave of maturation that was absent in tamoxifen-treated mice. In the group administered estradiol, uterine layers were normally developed but hypertrophied and there was no development of adenomyosis. The inner myometrium retained its circular arrangement. This suggested that the development of the inner myometrium is sensitive to estrogen antagonism and that disruption of the inner myometrium may play a role in the development of uterine adenomyosis.

However, administration of tamoxifen to the neonatal C57/BL6J mice, whilst associated with similar disruption of the inner myometrium, did

not result in the development of adenomyosis [89]. Following tamoxifen exposure, all uteri showed inner myometrial thinning, lack of continuity, disorganisation and bundling. αSMA immunostaining was reduced in the circular muscle layer of tamoxifen treated mice. The temporal pattern of desmin immunostaining found in control mice was absent in tamoxifen-treated mice. Tamoxifen induced similar inner myometrial changes in C57/BL6J and CD-1 neonatal mice. Thus, disruption of the development and differentiation of the inner myometrium cannot be the sole explanation of the development of tamoxifen-associated adenomyosis and adenomyosis must be dependent upon interactions that are strain-dependent.

This supports the hypothesis that adenomyosis is a disease of both the myometrium and the endometrial stroma which is perhaps not surprising given the common paramesonephric duct embryologic origin of the endometrial stroma and the inner myometrium. The research by Fujii et al. (1989) shows that stromal and myometrial cells exhibit some level of plasticity [33]. Fujii et al. (1989) examined the ultrastructure of mesenchymal cells at the endometrial-myometrial junction during the menstrual cycle and early pregnancy and identified cells with features of smooth muscle among the usual endometrial stromal cells in every specimen [33]. In the follicular phase of the menstrual cycle, such cells resembled myofibroblasts, but in the luteal phase and during early pregnancy they had more distinct cytoplasmic filaments with dense bodies and dense plaques and other fairly well developed characteristics of smooth muscle. The identification of smooth muscle-like cells amongst stromal cells in the adult uterus and the finding that their morphology changes into cells having many of the characteristics of smooth muscle cells during the luteal phase and early pregnancy, suggests that smooth muscle differentiation possibly occurs from multi-potential mesenchymal cells in the endometrial stroma. It could be envisaged that disruption of the process can result in the genesis of adenomyosis. Studies in rodents are indicative of a role for neurotrophins such as nerve growth factor (NGF) which was significantly upregulated in endometrial luminal epithelium in the CD-1 mouse model of adenomyosis [90]. Thus neurotrophins may affect myogenic differentiation through paracrine mechanisms. The pattern of neurotrophin (NGF, BDNF) and neurotrophin receptor (trkB, trkC and $p75^{NTR}$) expression in the human myometrium also points to a possible role [85].

Epithelial-Mesenchymal Transition

Whilst the derivation of stromal cells could be explained by derivation from perivascular cells, through mechanisms involved in stromal to smooth muscle differentiation, these do not explain the derivation of the epithelial component. One possibility is that the epithelium develops through direct extension from the basalis or from adjacent adenomyosis foci. Alternatively, this may arise through mechanisms involving epithelial-mesenchymal transition [91]. Epithelial-mesenchymal transition (EMT) is believed to be estrogen dependent and may be important to the acquisition by epithelial cells of invasive properties. Chen et al. (2010) reported increased vimentin and reduced E-Cadherin in ectopic endometrium, but not in eutopic endometrium from women with adenomyosis [91]. This provides evidence for EMT. They also reported that serum estradiol was negatively correlated with E-cadherin expression in the epithelial components of the eutopic endometrium and in adenomyotic lesions suggesting a role of estradiol in the process. Chen et al. (2010) also reported that Ishikawa endometrial epithelial cells undergo morphological changes acquiring a fibroblast-like phenotype in response to estrogen and that these cells also exhibit a shift from epithelial to mesenchymal marker expression, increased migration and invasion, and up-regulation of the EMT regulator Slug [91]. Slug is a zinc finger transcriptional factor and has been recognized as a major EMT inducer through repressing E-cadherin expression. These effects were inhibited by raloxifene, a selective estrogen receptor modulator (SERM). Zhou et al. (2012) utilized a two-dimensional polyacrylamide gel

electrophoresis and Mass Spectrometry based proteomics analysis to compare and identify differentially expressed proteins in matched ectopic and eutopic endometrium of adenomyosis [92]. They identified 93 significantly altered proteins. These included 22 estrogen responsive proteins, estrogen responsive genes, genes involved in cell proliferation, apoptosis, cell adhesion, cell motility, angiogenesis, cell signalling and redox homeostasis.

These included annexin A2, which was upregulated most significantly in the ectopic endometrium of adenomyosis. Over-expression of ANXA2 was tightly correlated with markers of epithelial to mesenchymal transition. Expression of ANXA2 enhanced the proangiogenic capacity of adenomyotic endometrial cells through HIF-1/VEGF-A pathway. ANXA2 inhibition abrogated endometrial tissue growth, metastasis, and angiogenesis in the adenomyosis nude mice model and significantly alleviated hyperalgesia. This suggested a role for ANXA2 in the pathogenesis of human adenomyosis through conferring endometrial cells both metastatic potential and proangiogenic capacity. Angiogenesis is required for the invasion of ectopic endometrial implants and their subsequent proliferation. VEGF, a major angiogenic factor, is an important protein for angiogenesis and thus the development of adenomyosis [92].

Chen et al. (2010) isolated mesenchymal like stem cells from the endometrium (EMSC) and from adenomyosis (AMSC) and demonstrated increased COX-2 expression in AMSC compared to EMSC and that the addition of COX-2 inhibitor suppressed migration and invasion and induced apoptosis in AMSC but not in EMSC [93]. The findings suggested a role for COX-2 in adenomyosis. Cyclooxygenase-2 (COX-2), the enzyme that converts arachidonic acid into prostaglandins (PGs), is over expressed in adenomyotic and endometriotic lesions compared with eutopic endometrium. Also importantly, the isolation of AMSC raises the possibility of a role in the pathophysiology of adenomyosis. The same group (Huang et al. 2014) demonstrated that estradiol elicited a Slug-VEGF axis in endometrial epithelial cells, and also induced proangiogenic activity in vascular endothelial cells [94]. The antagonizing agents against estradiol or VEGF suppressed endothelial cells migration and tubal formation. The authors suggested that these results highlight the importance of estrogen induced angiogenesis in adenomyosis development and provide a potential strategy for treating adenomyosis through intercepting the estradiol-Slug-VEGF pathway.

Tissue Injury and Repair

Adenomyosis and endometriosis have long been argued to share a common pathogenesis [95–97]. Endometrial stroma is in direct contact with the underlying myometrium allowing communication and interaction. This may facilitate endometrial invagination or invasion of either a normal or structurally weakened myometrium During periods of regeneration, healing and re-epithelialisation, the endometrium could invade a normal or a predisposed myometrium possibly as a result of trauma to the epithelio-myometrial interface (EMI) [51]. Mechanical damage or physical disruption of the EMI by dysfunctional uterine hyperperistalsis or by dysfunctional contractility of the sub-endometrial myometrium [31, 95] or by sharp curettage especially if done during pregnancy in relation to pregnancy termination [65] may allow for the dislocation of basal endometrium into the myometrial wall and subsequently leads to the development of adenomyosis. An altered immune response has also been proposed as a mechanism for disturbance of the junctional zone resistance to invasion [98].

Most of the literature describing immunological changes associated with adenomyosis is attributed to Ota and coworkers [98–108]. In these studies, changes in both cellular and humoral immunity were described (e.g. a strong expression of cell surface antigens or adhesion molecules, an increased number of macrophages or immune cells, and deposition of immunoglobulins and complements components). Using immunohistochemistry, increased expression of the major histocompatibility complex class II antigen (HLA-DR) in the gland cells of eutopic

and adenomyotic endometrium has been described [108]. Macrophages in the myometrium of adenomyotic uteri seem to increase, possibly activating helper T-cells and B-cells to produce antibodies [98]. Peripheral blood concentrations of autoantibodies are increased in adenomyosis [101]. The deposition of complement components C3 or C4 in adenomyosis is increased in 74 % and 89 % of patients respectively. The expression of E-cadherin in the endometrial tissue of adenomyosis was found to be significantly higher [98].

Using immunohistochemistry, adenomyotic uteri showed excessive expression of superoxide dismutase throughout the menstrual cycle [107]. Similarly, glandular tissue in adenomyosis showed increased expression of glutathione peroxidase [105], cyclo-oxygenase-2 [104] and xanthine oxidase [103] when compared to eutopic endometrium. The exact significance of these immune phenomena in adenomyosis remains to be elucidated. On occasions there has been a problem with reproducibility of results between laboratories and the statistical analysis presented often does not enable clear comparisons between the various layers or clinical conditions [71]. It is not known whether these immunological changes and biochemical derangements are a consequence or a cause of adenomyosis.

Increased expression of basic fibroblast growth factor (bFGF) and its receptor (FGF-R) are found in the epithelium of adenomyosis compared with eutopic endometrial epithelium in menopausal women. The authors suggested that bFGF might contribute to the pathogenesis of abnormal uterine bleeding associated with adenomyosis [109].

Intraepithelial leukocytes (IEL) are an immunological component of most mucosal surfaces although the function and significance of their presence is not known. IEL in eutopic endometrium of patients with adenomyosis varied during the menstrual cycle, with *CD45+, CD43+ and CD56+* cells increasing from the proliferative to the late secretory phase. IEL were elevated in surface compared with glandular epithelium in the proliferative and early secretory phases. Throughout the menstrual cycle there was no significant differences in IEL between eutopic and ectopic endometrium in adenomyosis [110].

Endometrial stromal fibroblasts produce tenascin, a fibronectin inhibitor which in turn facilitates epithelial migration. Tenascin mediates epithelial-mesenchymal interactions by inhibiting cell attachment to fibronectin, an action stimulated by hormonally regulated epidermal growth factors. Whether this interaction plays a role in the development of uterine adenomyosis or endometriosis is unclear [76].

The EMI is also disturbed by the penetration of trophoblast into the myometrium during early pregnancy and this may underlie the higher incidence of adenomyosis in parous women. This putative mechanism may be supported by the finding that the administration of tamoxifen to neonatal CD1 mice is associated with disruption of the inner myometrial circular layer and the development of adenomyosis. However, as mentioned above, neonatal exposure of C57/BL6J mice to tamoxifen resulted in disruption of the inner circular layer, but not to the development of adenomyosis which suggests that disruption of the myometrium is not sufficient for the development of the disease [88, 89].

Expression of the motility-related molecule Cdc42 in eutopic endometrium was higher in patients with ovarian endometriotic cysts compared with patients with adenomyosis [111], suggesting that Cdc42 may not be involved in the pathogenesis of adenomyosis, but may play a role in the process of endometrial cell migration; this could contribute to the pathogenesis of ovarian endometriosis by supporting the process of adhesion of endometriotic cells on the ovarian surface followed by invagination and pseudocyst formation [111, 112]. On the other hand, Fibroblast Growth Factor (FGF-1) polymorphism has been linked to risk of endometriosis but not to adenomyosis, whilst FGF-2 754C/G polymorphism was associated with a decreased susceptibility to developing endometriosis [OR = 0.575, 95 % confidence interval (CI) = 0.387–0.854] and adenomyosis [OR = 0.577, 95 % CI = 0.367–0.906]. This shows some differences in the risk factors of both diseases [113].

Leyendecker et al. (2009) presented what they described as a unifying concept linking the pathogenesis of adenomyosis to that of endometriosis [114]. According to this theory, circumstantial evidence suggests that these two conditions are caused by trauma, since chronic uterine peristaltic activity added to possible phases of hyperperistalsis may induce micro-traumatisations that, in turn, can activate a mechanism identified as 'tissue injury and repair' with a local production of estrogen. With time, the number of sites affected might increase and, in turn, increase the production of estrogens that, in a paracrine fashion, begin to interfere with the ovarian control over uterine peristaltic activity, resulting in permanent hyperperistalsis and a self-perpetuation of the disease process. The result is an auto-traumatisation of the uterus causing, in the case of endometriosis, the dislocation of fragments of basal endometrium into the peritoneal cavity and in the case of adenomyosis the infiltration of the basal endometrium into the inner myometrial wall. The hypothesis, postulates the existence in most cases of endometriosis and/or adenomyosis, of a causal event rapidly leading to uterine hyperperistalsis. Even if such an event does occur it is postulated that unavoidably, with time, even normoperistalsis will lead to some extent of micro-traumatisation. According to this hypothesis, endometriosis in young women and adenomyosis in premenopausal women, share the same pathogenetic mechanism. Both result from the physiological mechanism of 'tissue injury and repair' which respond to the local estrogens outside the control of the ovary.

The Role of Steroids and Steroid Receptors

It is not surprising that adenomyosis is influenced by steroids and a link between adenomyosis and estrogen has been proposed for almost a century. The role of steroids is supported by the identification of adenomyosis in women receiving tamoxifen as well as by the experimental animal model [115]. If hyperestrogenism is involved in the pathogenesis, it is proposed to operate through local production rather than through systemic hyperestrogenism [116]. Increased local estorgen may also account for the hypertrophy or hyperplasia in the surrounding myometrium and overlying endometrial hyperplasia. Experimental data in rodent models have shown that *in utero* or neonatal exposure to tamoxifen or to diethylstilboestrol can induce adenomyosis and induce marked myometrial disruption [86, 117]. The critical stage for neonatal exposure in rodents is because of the significant uterine development that takes place in the early neonatal period. These experiments raise the hypothetical possibility that the corresponding developmental stages in the humans which occur *in utero* will be sensitive to steroids and that *in utero* exposure may lead to adenomyosis. Studies in animal models also support a role for hyperprolactinemia (either induced by pituitary transplantation or by drug therapy), although it is unclear if a similar mechanism is involved in humans [87].

Whilst adenomyosis occurs spontaneously in many animal species, the first reported experimental animal model was by Lacassagne in 1935 who administered estrogen to mice for more than 6 months [118]. Subsequently, it was demonstrated that prolonged estrogen treatment resulting in adenomyosis, is also associated with elevated serum prolactin in intact animals [119]. Prolonged administration of progesterone either alone or in combination with estrogen also result in the development of adenomyosis [120, 121]. The effect of progesterone may be direct or may be enhanced by elevated levels of prolactin [122]. It has also been proposed that elevated level of progesterone may have a role in the induction of adenomyosis in the pituitary implant model. The observation that the development of adenomyosis was completely eliminated by ovariectomy immediately after pituitary transplantation and that the effect of ovariectomy was reversed by the administration of estrogen and progesterone combination but not by either estrogen or progesterone alone [123] suggested that the development of adenomyosis results from chronic hormonal imbalance involving the three hormones [122]. Spontaneous adenomyosis develops in SHN mice with advancing age, but this is

inhibited by the administration during the critical development window of bromocriptine-mesilate for more than 4 weeks [124, 125]. But whilst this supports the role of prolactin, is it also possible that bromocriptin-mesilate exerts an effect through inhibition of estrogen binding to its receptor.

There is considerable literature on the distribution of estrogen (ER) and progesterone (PR) receptors and their isoforms in the endometrium. Most of these studies have documented fluctuation in steroid receptor expression during the menstrual cycle. Some studies have reported on receptor distribution in the inner but did not examine the outer myometrium [126–128]. The cyclical changes in the uterine junctional zone as seen by magnetic resonance imaging, together with the peristaltic waves seen by videosonography, support the hypothesis that this layer is influenced by steroid hormones [129, 130]. Steroid hormones have also been implicated in the pathogenesis of uterine adenomyosis [116], and local rather than systemic hyperestrogenism may be implicated [131].

Takahashi et al. (1989) [132] [quoted from [116]], demonstrated no significant differences in estradiol levels in peripheral blood in women with adenomyosis. But women with adenomyosis had the highest estrogen level in menstrual blood compared to women with endometriosis who, in turn, had higher levels compared to unaffected women. This suggests that these higher estrogen concentrations are generated locally within the uterus. The mechanism for higher local estrogen production may be through the action of aromatase on androgen precursor [133], or the action of estrone sulphatase which converts estrone-3-sulphate to estrone [134]. Kitawaki et al. (1997) performed immunohistochemistry and examined the catalytic activity, and mRNA expression for aromatase cytochrome P450 ($P450_{arom}$) in tissue specimens [135]. $P450_{arom}$ was immunohistochemically localized in the cytoplasm of glandular cells of endometriotic and adenomyotic tissues and mRNA for aromatase cytochrome P450 was identified in adenomyotic tissue homogenate. However, neither $P450_{arom}$ protein activity nor mRNA was detected in endometrial specimens obtained from normal menstruating women. There is also evidence of altered 17β-hydroxysteroid dehydrogenase type-2 expression in the endometrium in women with adenomyosis and this results in increased conversion of estradiol to estrone during the secretory phase of the cycle [136]. Estrogen receptor alpha (ER-α) isoform expression is reduced in a CD-1 neonatal mouse model fo*r* adenomyosis, but a similar reduction is noted after tamoxifen administration to C57/BL6J mice that did not develop the disease.

Aromatase and estrone sulfatase activities were detected in adenomyosis foci using anion-exchange resin column chromatography, thin-layer chromatography, cocrystallization, and immunohistochemistry [137]. This may also account for the hypertrophy/hyperplasia in the surrounding myometrium. Interestingly, endometrial hyperplasia is often found in women with adenomyosis [51]. In the model proposed by Leyendecker and colleagues for the development of adenomyosis, a key modulator may be an increase in the local production of estrogen secondary to a pathological expression of the P450 aromatase enzyme [114, 138]. The starting event may be hyperactivity of the endometrial inflammatory response or hyperactivity in the endometrial oxytocin receptor system or in the pathological expression of the P450 aromatase system itself. This leads to uterine hyperperistalsis and endometrial hyperproliferation.

Endometrial glands in adenomyotic tissue selectively express more *human chorionic gonadotropin (HCG) – luteinizing hormone (LH) receptor mRNA* and immunoreactive protein than the non-invaginating eutopic glandular epithelium. This increased expression was also found in endometrial carcinoma and in invasive trophoblasts of choriocarcinoma [139]. The increased receptor expression in the invaginating endometrial epithelium may be related to the potential to invaginate into the myometrium and to form adenomyotic foci.

There are also several animal models that support a role for hormonal disturbance in the pathogenesis of adenomyosis. A high rate of uterine adenomyosis in mice was induced by intrauterine

or ectopic anterior pituitary isografts [140, 141]. Fluoxetine (a serotonin uptake inhibitor) was used to induce hyperprolactinaemia in castrated and non-castrated rats. Adenomyosis uteri developed in the non-castrated group, suggesting that high prolactin concentrations can cause myometrial degeneration/weakness and subsequent endometrial invasion, in the presence of ovarian steroids [87]. It is not known if hyperprolactinaemia plays a role in the development of adenomyosis in humans.

Adenomyosis has also been reported in postmenopausal breast cancer patients treated with the selective estrogen receptor modulator (SERM) tamoxifen [142]. The associated risk of adenomyosis with conventional postmenopausal hormone replacement therapy (HRT) is not known. Adenomyosis was incidentally found in some HRT users having endometrial ablation for postmenopausal bleeding [143]. The use of oral contraceptives does not appear to be associated with a risk of adenomyosis [38].

Mehasseb et al. (2011) reported on the distribution of estrogen and progesterone receptor isoforms in adenomyosis [144]. In the adenomyotic functionalis glands and stroma, there was a statistically significant ($p<0.001$) decrease in ER-α expression during the mid-secretory phase of the menstrual cycle, but the expression of ER-α in the inner and outer myometrium was not statistically significantly different. The ER-β expression was statistically significantly elevated in the adenomyotic functionalis gland during the proliferative phase and throughout the myometrium across the entire menstrual cycle. Expression of PR-A was similar to that of PR-B, with reduced expression in the basalis stroma, and the inner and outer myometrium in adenomyosis. The pattern of ER-β, PR-A, and PR-B expression was similar in the endometrial basalis and adenomyotic foci [144]. Higher ER-β expression and the lack of PR expression may be related to the development and/or progression of adenomyosis and might explain the poor response of adenomyosis to progestational agents.

Tamoxifen has been linked to postmenopausal adenomyosis and to an endometrioma in one case report [145] and to adenomyosis and an adenomyomatous endometrial polyp in another [145]. In a small series of eight women with endometrial pathology during tamoxifen therapy; one had adenomyosis [147]. Cohen et al. (1995) reported adenomyosis in 8 (57.1 %) out of 14 women who had a hysterectomy whilst receiving tamoxifen [142]. Seven of these women had small microscopic foci, and one had a large fundal adenomyotic lump. Cohen et al. (1997) reported adenomyosis in 15 (53.6 %) out of 28 postmenopausal women with breast cancer receiving tamoxifen who underwent hysterectomy compared to only 2 (18.2 %) out of 11 postmenopausal women with breast cancer not receiving tamoxifen [148]. This suggested an association between tamoxifen use and adenomyosis, but differences in the incidence of adenomyosis may be related to the indications for hysterectomy in the two groups. A comparative histopathologic evaluation concluded that tamoxifen-associated cases of adenomyosis were more likely to feature cystic dilated glands, stromal fibrosis and various epithelial metaplasias and higher epithelial proliferation [149]. Tamoxifen also induces distinct MRI patterns in the postmenopausal uterus on tamoxifen and the majority have heterogeneous endometrial signal intensity on T2-weighted images (mean = 1.8 cm) with enhanced endometrial-myometrial interface, coexisting sub-endometrial cysts, nabothian cysts, leiomyoma, and adenomyosis [150].

Familial and Genetic Factors

Whether hereditary or familial factors for adenomyosis exist is unknown. We only identified one case series by Emge, who operated on seven cases of adenomyosis in which mothers of the patients were operated upon for the same reason, raising the possibility of a hereditary factor [151]. The same article refers to a description of adenomyosis in a fetus at term. We are not aware of any studies on adenomyosis in twins.

Experimental observations in animals suggest that hereditary factors may be involved in the pathogenesis of adenomyosis. The uteri of recombinant inbred SMXA mice spontaneously

develop histological changes similar to adenomyosis [152]. The uteri of F1 mice strain contain more prominent changes resembling human adenomyosis.

Pandis et al. (1995) reported the finding of *del [7] [q21.2q31.2]* in three short term cultures from human mesenchymal cells of adenomyosis [153]. The deletion was found in myometrial cells of adenomyotic lesions. The karyotypic anomaly was previously reported in uterine leiomyomas. This led the authors to suggest that adenomyosis may be similar to fibroids in that it arises through a neoplastic process.

Goumenou et al. (2000) used 17 microsatellite markers, in four tetraplex and one single PCR assay, to determine the incidence of loss of heterozygosity in 31 cases of adenomyosis [154]. The markers used are located close to tumor suppressor genes, DNA repair genes, and genes which are thought to be involved in endometriosis. The markers were involved in regions on chromosomal arms 1p, 1q, 2p, 2q, 3p, 9p, 9q, 17p and 17q. Nine samples (29.0 %) showed loss of heterozygosity in at least one locus. Loci 2p22.3-p16.1, 3p24.2-p22 and 9p21 exhibited imbalance (19.4 %, 9.7 % and 6.5 % respectively). The study suggested a link between hMSH2, hMLH1, p16Ink4 and GALT genes and the pathogenesis of adenomyosis. Using comparative genomic hybridisation, Wang et al. (2002) were not able to identify positive recurrent gene copy number alterations in 25 cases of pathologically proven adenomyosis [155]. Which suggests that genetic alterations in adenomyosis are either rare of that the technique used was insufficiently sensitive.

Aberrant expression of ERα might be partly involved in the onset or growth of adenomyosis and its poor response to anti-estrogen therapy. Using PCR/single strand conformation polymorphism analysis, Oehler et al. (2004) identified somatic estrogen receptor ERα gene mutations in three out of 55 samples from adenomyosis uteri [156]. Two of the mutant ERα proteins display severely impaired DNA-binding and transactivation properties secondary to an altered response to estrogens or changes in epidermal growth factor-mediated ligand-independent activation. They suggested that such mutations may account for the apparent resistance of endometriotic cells to hypoestrogenic conditions and poor response to estrogen-ablative therapy in adenomyosis [156].

Gene expression profile demonstrated differences between mRNA expression in the inner and the outer myometrium in women with adenomyosis and the corresponding layers in unaffected uteri. WNT5A mRNA was consistently down-regulated in adenomyosis, both in the secretory and the proliferative phases [157]. WNT5A is a conserved homolog of Wingless, a key regulator of *Drosophila melanogaster* embryonic segmentation and patterning. The WNT gene family are critical regulators of cell polarity, motility, differentiation, apoptosis, and carcinogenesis.

The Role of Endometrial Stromal Cells in the Development of Adenomyosis

The development and differentiation of myometrial smooth muscle is affected and directed by the uterine epithelial and stromal cells. Using uteri from BALB/c mice 1–60 days postpartum, uterine mesenchyme produced larger amounts of smooth muscle when co-cultured with epithelium. This suggests that uterine epithelium plays a promotional role in the differentiation and spatial organization of the myometrium [158, 159].

To determine whether myometrial smooth muscle is newly produced at the EMI of the adult uterus, Fujii et al. (1989) examined the ultrastructure of the mesenchymal components of the EMI during the menstrual cycle and early pregnancy [33]. They identified cells that morphologically resembled myofibroblasts in the follicular phase and which differentiated into cells that morphologically resembled smooth muscle cells in the luteal phase and early pregnancy. This adds to the evidence that smooth muscle differentiation may occur from mesenchymal cells in the endometrial stroma.

In neonatal rodents models, the use of tamoxifen produces abnormal and aberrant endometrial tissue growth leading to adenomyosis. This is the

result of the disruption of the mesenchymal layers surrounding the endometrium in the neonatal period, triggering a disordered development of the uterine stroma, smooth muscle, blood vessels and possibly innervation [160]. Decidual stromal cells express α-smooth muscle actin and show ultrastructural similarities with myofibroblasts, supporting the view that metaplasia can occur in the endometrium [161]. Using immunohistochemical staining, isolated nodules of endometrial stromal cells without glands were characterized along blood or lymphatic vessels in the myometrium [82]. Multipotent pericytes are thought to be the origin of these stromal nodules. Due to the proliferative nature of the endometrial glands, the newly enlarged area of stroma could serve as "new soil", facilitating further downward growth of the endometrial glands. Hormonal, genetic, immunological and growth factors possibly play a role in this sequence of events [82].

Adenomyosis and Endometriosis

Whether adenomyosis and endometriosis share a common aetiology has long been debated in literature. Many hypotheses exist that propose common mechanisms including the link to hyperestrogenism, hyperperistalsis and microtraumatisation and the development from multipotential perivascular cells. The advent of MRI has renewed interest in this link because of the possibility of non-invasive diagnosis. Some studies have indicated a possible link. Bazot et al. (2004), for example, found that 27 % of the women with endometriosis concomitantly had adenomyosis [162]. Kunz et al. (2005) reported an even higher association of 70 % in a highly selected subpopulation [163]. Larsen et al. (2011) performed MRI of the uterus in 153 women with suspected deeply infiltrating endometriosis and a reference group of 29 women with cervical cancer and another 100 women without endometriosis who underwent a hysterectomy for other indications [36]. They reported that 53 (34.6 %) of the women in the endometriosis group had adenomyosis, compared to 6 (20.7 %) in the cervical cancer group and 19 (19.4 %) in the hysterectomy group. The difference between the groups was statistically significant ($p<0.05$). Sixty one (39.9 %) of the women in the endometriosis group had an irregular JZ (JZ-dif>2) compared to 6 women (20.75 %) in the cervical cancer group and 23 women (23 %) in the hysterectomy group. The difference was statistically significant ($p<0.01$). In a subgroup analysis, 4 (26.7 %) of women (n=15) with AFS stage I endometriosis had adenomyosis compared to 13 (35.1 %) in women with AFS stage II (n=37), 8 (24.2 %) of women with AFS stage III (n=33) and 24 (42.8 %) of women with AFS stage IV (n=56) endometriosis. The difference was not statistically significant. But more women with AFS stage IV endometriosis had deeper wall invasion with adenomyosis. The presence of deep infiltrating rectovaginal endometriosis and the size of infiltration did not correlate with the presence or the depth of adenomyosis. Thus they concluded that there was only limited correspondence between the invasive potential of endometrial cells in the uterus and in the peritoneum. It is also important to note that there were significant differences between the groups in factors of age and parity which are both relevant to the incidence of adenomyosis.

The mechanism by which endometrial cells might migrate within the myometrium is not known. Factors may involve local imbalance or, as mentioned earlier, factors that are innate to the cells in question. Goteri et al. (2006) evaluated the motility-related molecule Cdc42 expression in eutopic and ectopic endometrial tissue in patients with adenomyosis (n=24) and ovarian endometriotic cysts (n=19) compared with patients without endometriosis (n=9) [111]. In eutopic endometrium of patients with adenomyosis and with fibroids or benign ovarian cysts, the intensity of Cdc42 immunostaining was weaker, especially in the specialised stromal cells, compared with cases with ovarian endometriosis. Expression of Cdc42 in eutopic endometrium showed a trend to be higher in the secretory than in the proliferative phase and in patients with ovarian endometriotic cysts compared to patients with adenomyosis (unpaired t test, $p=0.005$),

especially in the proliferative phase. Cdc42 is a key molecule in intracellular signalling pathways that lead to changes in cellular morphology, cell polarity, motility and migration, gene transcription, cell cycle progression, and programmed cell death, both in normal conditions and in tumours. Cdc42 induces the formation of actin-rich finger-like membrane extensions (filopodia) and regulates anchorage independent cell growth. The differential expression in ovarian endometriosis and adenomyosis let Goteri et al. (2006) to speculate on a role for Cdc42 in endometrial cell migration in ovarian endometriosis but not in adenomyosis, implying that the mechanisms of the two conditions may be different [111].

The question therefore arises whether the pathogenesis of adenomyosis is more associated with deep recto-vaginal endometriosis than cystic ovarian endometriosis. Yang et al. (2009) examined the invasiveness into matrigel matrix of endometrial stromal cells obtained from eutopic endometrium from women with and without adenomyosis and measured the concentration of the matrix metalloproteinases (MMP-2, MMP-9) and the tissue inhibitors of metalloproteinases (TIMPP-1, TIMP-2) in culture supernatants [164]. Using migration assay, they reported that migration of endometrial stromal cell from adenomyosis was not different from that of the control group of endometrial stromal cells cultured alone or with the addition of IL-6, anti-IL-6 or the GM6001 (a synthetic inhibitor of MMP). Furthermore, these agents did not significantly alter cell migration compared to controls. Yet, the concentration of MMP-2, but not of MMP-9, produced by ESCs from women with adenomyosis was higher compared to women without adenomyosis. The level of TIMP-1, but not of TIMP-2 in culture media was also higher in women with adenomyosis compared to controls. This suggests that the concomitant elevation of MMP-2 and TIMP-1 may explain why invasiveness was not increased.

A marked increase in vascularization of the endometrium in adenomyosis was reported with the total surface area of capillaries up to 11.6 times that of the controls in the proliferative phase [165]. This has not been confirmed in a subsequent study, although microvessel density in adenomyotic tissue was increased compared to the endometrium of the same patient [166]. A recent molecular study found an elevation of matrix metalloproteinase (MMP-2 and −9) expressions in eutopic and ectopic endometria with a good correlation with increased microvessel density [167]. The role of the MMPs and TIMPs in the development of adenomyosis was further investigated through genetic studies; there was an association between adenomyosis and MMP-2 -1306C/T polymorphism in North Chinese women [168]. The same investigators also suggested that the presence of the −2578*A* or −1154*A* allele of the vascular endothelial growth factor (*VEGF*) gene might be protective [169], and that polymorphisms of two angiogenic factors, fibroblast growth factor 1 and 2 might play a role in the initiation of angiogenesis in endometriosis or adenomyosis [113]. Caution, however, is needed when interpreting these gene association studies [71].

Benagiano et al. (2014) reviewed studies that involved comparison between the eutopic endometrium in women with endometriosis or with adenomyosis [71]. Many similarities and also some differences were identified between the eutopic endometrium in both conditions. Much of published research in this area may have been influenced by bias consequent to the method of diagnosis. Compared to control endometrium, both endometriosis and adenomyosis exhibited immune dysfunction and there were alterations in adhesion molecules, cell proliferation and apoptosis. There was also an increase in cytokines, inflammatory mediators and in oxidative stress and free-radical metabolism. Dissimilarities were reported in the extent of apoptosis and in the expression of some inflammatory mediators.

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The Myometrium in Heath and Disease

4

Anthony H. Taylor and Marwan Habiba

Abstract

The uterus consists of two major tissue components; the muscular layer (the myometrium) and the inner mucosal lining (the endometrium). Cyclical endometrial changes and its functional role in relation to implantation and subsequent embryo and fetal development have been the focus of research for many decades. Recent evidence suggests that the myometrium has an important supportive role during early pregnancy and that it acquires more significance as the uterus adapts to the growing fetus and in parturition. Postnatally, the myometrium has a role in preventing excessive blood loss. Dysregulation of myometrial function is associated with adverse outcomes.

Keywords

Mammalian • Blastocyst implantation • Steroids • Smooth muscle • Fibroblast • Outer myometrium • Inner myometrium • Parturition • Endocannabinoids • Myocytes

Introduction

The uterus is a critical reproductive organ in that preservation of mammalian species depends on its normal physiological function. It consists of two major tissue components; the muscular layer (the myometrium) surrounding the inner mucosal lining (the endometrium). Much of the research on the human and rodent uterus over the last century centred on the endometrium, which is often viewed as the functional component of the uterus responsible for blastocyst implantation and subsequent embryo and fetal development and thus the endometrium plays a very important role in early pregnancy success. Recent evidence suggests

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that the myometrium has an important supportive role during early pregnancy, a role that acquires more significance as pregnancy advances and the uterus adapts to the growing fetus and in preparation for parturition. Postnatally, the myometrium has a different role when it contracts to prevent blood loss and undergoes involution. Thus, dysregulation of myometrial function is associated with adverse outcomes. This Chapter provides an overview of the myometrium in health and disease, with particular relevance to the mechanisms involved (where known).

Uterine Anatomy and Physiology

The inner lining of the uterus, the endometrium is a highly specialised mucosa that undergoes cyclical changes in response to gonadal steroids, primarily oestrogens and progesterone; such changes during the menstrual cycle have long been recognised [1]. Androgens also appear to have a role, particularly at the end of the menstrual cycle and before menstruation. During menstruation the uppermost layer of the endometrium, or the functionalis layer, is lost, leaving a lower basalis layer intact. The myometrium is also influenced by the actions of oestrogens and progesterone, and although the exact role of androgen is not well understood, it has long been recognised that the myometrium also expresses androgen receptors [2].

It has been demonstrated that androgens induce trophic effects on the uterus and on the myometrium of ovariectomized rats resulting in increased uterine weight to the levels observed in intact rats, where the trophic effects of oestradiol and the synthetic androgen, mibolerone on the myometrium were shown to be additive [3]. Yet, whilst androgen promotes the growth and differentiation of the rat uterus with apparent similarities to the effect of oestrogen, there are also distinct differences that could be demonstrated histologically and using gene expression arrays. Histologically, the myometrium under the influence of androgen has extended interstitial spaces, suggesting oedema and/or differences in adherence or extracellular components [2], whilst during pregnancy, androgen receptor activation initiates anti-apoptosis mechanisms [4]. Androgens may also have a role in smooth muscle relaxation during pregnancy and reduced androgen levels prior to labour have been implicated in increased muscle contractility leading to parturition [5].

Unlike most other organs, the uterus lacks an intervening sub-mucosal layer. The lack of this layer renders the underlying myometrium at risk of 'invasion' by the endometrium, which is a possible mechanism for the development of adenomyosis. The area where the basal endometrium and inner layer of myometrium join is variably referred to as the endometrial-myometrial interface (EMI) or the junctional zone (JZ) [6, 7]. The use of the term EMI is favoured when viewed by ultrasonography and JZ is favoured when identified by magnetic resonance imaging [8]. Some authors regard the inner myometrium and the endometrium to arise embyrologically from Müllerian ducts and the outer myometrium to have a non-Müllerian mesenchymal origin [9]. A difference in tissue architecture between the inner and outer myometrium is readily observed in the rodent uterus because of the different orientation between the inner circular and the outer longitudinal muscle layers (Fig. 4.1). This contrasts with the case in the human where the structure is complex (Fig. 4.2). Description of three layers;

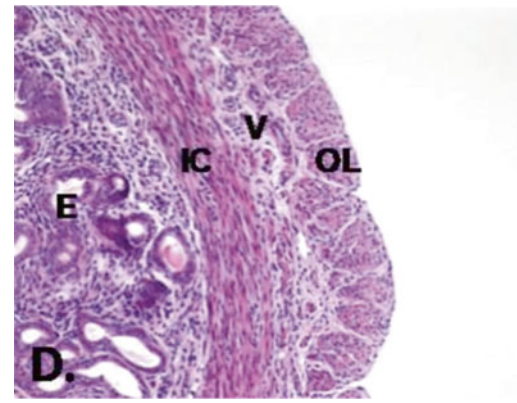

Fig. 4.1 The mouse uterus shows distinct endometrial (*E*), inner circular (*IC*) and outer longitudinal (*OL*) myometria layers that are separated by a loosely packed vascular layer (*V*) (haematoxylin and eosin)

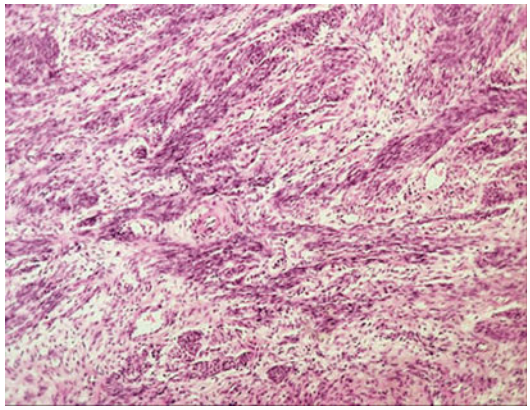

Fig. 4.2 Haematoxylin and eosin section of the human myometrium showing the lack of distinct muscle layers

inner circular, outer longitudinal and intermediate 'criss-cross' [10], is perhaps a simplification. The recognised complex arrangement challenges our understanding of the embryological origins of the myometrium.

There is growing evidence for the role of myometrial stem cells particularly in relation to uterine growth in pregnancy [11]. Clonal smooth muscle and fibroblast proliferation has been shown in relation to fibroids [12] but the relevance of this to regional myometrial zonation or to the development of adenomyosis remains speculative.

The Inner Myometrium or Myometrium Junctional Zone

There is evidence that the inner myometrium develops earlier *in utero* and is visible before week 21 of gestation compared to the outer myometrium which only appears after 24 weeks of gestation [13]. In contrast to the well-defined circular and longitudinal layers found in rodents (Fig. 4.1) and in many other species, the micro-anatomy of the human uterus is complex. Confusion arises from the observation that the subserosal myometrium appears to be different to the inner myometrium. The outer myometrium contains large blood vessels with their own smooth muscle cell coat and sensory neurones that are under hormonal influence. The question arises as to whether there are distinct functions for the inner and outer myometrial layers and how the two layers are differentially regulated or affected by the presence of commonly encountered diseases such as adenomyosis and fibroids. The inner myometrium close to the endometrial junction exhibits higher cellular density compared to the outer myometrium and also higher nuclear area [14], and that the transition from the inner to the outer myometrium is gradual with no clear demarcation [14].

It is thought that the inner myometrium is responsible for supporting sperm transport and the retention of the blastocyst prior to implantation. The inner myometrium may also have a role in aiding the separation of endometrial functionalis [15] and possibly in the regulation of uterine blood loss during menstruation [16].

In rodent pregnancy the two muscle layers have clear and different roles. The circular muscle is responsible for maintaining the fetal units as separate entities and assisting with parturition [17] whilst the longitudinal muscle aids the correct positioning of the fetal units within the uterus and retraction of the cervix towards the oviducts during parturition.

The Myometrium and Sperm Transport

It has been suggested that the inner myometrium of the non-pregnant uterus (in rodents and human) aids sperm transport from the region of the cervix [15, 18]. This may be triggered by prostaglandins and other inflammatory mediators in the ejaculate. The resulting sporadic contractions of the inner myometrium create negative intra-uterine pressures that draws the sperm into the uterine cavity where it binds to pinopodes on endometrial epithelial cells [19]. Sexual stimulation and the release of oxytocin results in more coordinated and forceful uterine contractions creating a peristaltic wave from the cervix towards the oviduct and these contractions position the sperm closer to the oviduct where a relatively small number migrate for fertilisation to take place [20].

An alternative mechanism postulates that, in the female, an intrinsic contractile feedback response during intercourse increases the stimulation of the purinergic and neurotrophin-sensitive neurones that innervate the outer myometrium. This, in turn, stimulates the entire uterus to contract and thus move sperm towards the oviduct. Simultaneously, the release of mediators associated with 'pleasure' stimulates the basal cilial receptors which aid sperm progression in the oviduct from the isthmus to the ampulla. Endocannabinoids, which increase ciliary beat frequency and sperm motility *in vitro*, may have a role in this process [21]. A similar mechanism may be involved in retrograde uterine transport of endometrial tissue during menstruation and this may be a factor in the pathogenesis of endometriosis [22].

The Myometrium During Pregnancy

The uterus is a unique organ in that its primary role of holding and nurturing the developing fetus is concerned with the preservation of the species rather than the survival of the individual. For pregnancy to be successful, the process requires coordinated sequential changes. The changes that occur in the myometrium during the earlier synthetic stage of pregnancy are relatively slow when compared to the changes associated with the contractile stage that occurs at the end of pregnancy and during the perinatal period.

Synthetic Stage

The uterus adapts to all stages of fetal development from implantation to term. In the rodent, fluctuations in muscle tension in the outer longitudinal muscle layer space the blastocysts along the length of the tubular uteri [23], a process that may be influenced by blastocysts. Mediators, such as the lipid-like substance released by blastocysts that inhibits the local expression of the endocannabinoid catalytic enzyme fatty acid amide hydrolase (FAAH) at the implantation site [24] leading to an increase in endocannabinoids at the implantation site are suggested to play a key role [25]. Myometrial receptors for endocannabinoids inhibit myocyte contraction [26, 27], suggesting that focal relaxation of the smooth muscle fibres at the point of implantation but relatively higher levels of contraction between implantation sites assist in the correct positioning of the blastocysts along the length of the uteri. In species that are confined to mainly singleton pregnancies, evidence for the induction of FAAH or decrease of endocannabinoids at the site of implantation is currently lacking [28].

The human uterus enlarges from approximately 75 g before pregnancy to approximately 1300 g at term. The increase in uterine weight occurs through myocyte hypertrophy rather than hyperplasia during the 'synthetic stage' of myometrial development. During this stage, the myometrium remains refractory to contractions and the enzyme protein kinase Cα (PKCα) may have a role in myometrial quiescence [29]. The key hormone involved is progesterone, which binds to progesterone receptors (PR) affecting transcription regulation [30]. PR-B dominates in the human myometrium during pregnancy, whilst the PR-A dominates in the decidua [31]. Progesterone decreases proinflammatory gene expression when the PR-A to PR-B ratio favours PR-B and increases proinflammatory gene expression when the ratio favours PR-A. This mechanism of action has been shown to be mediated by PR-B leading to increase in the expression of inhibitor- κBα, a repressor of the nuclear factor-κB transcription factor, and inhibition of basal and lipopolysaccharide-induced proinflammatory gene expression. At parturition, the rise in PR-A expression promotes labour by inhibiting the anti-inflammatory actions of PR-B and stimulating proinflammatory gene expression in response to progesterone [32].

Contractile Stage

An important function of the myometrium is to respond to contractile signals in labour. This phase is characterised by the up-regulation of contraction-associated proteins, such as the gap

junction proteins, connexin 43 and 45, oxytocin receptor, and the prostaglandin synthesis enzyme cyclooxygenase [33].

Uterine contractions leading to delivery involve coordinated smooth muscle cell contraction and retraction followed by involution to the prepregnancy state. Labour-associated proteins are influenced by biomechanical stretch [34, 35], which during the period of fetal growth, remain low, only increasing in response to a critical 'limit of stretch' that triggers the expression of connexin 43, cyclooxygenase-2 and the oxytocin receptor [36, 37]. It has been shown that biomechanical factors rather than the presence of the fetus are responsible for the production of the contraction-associated proteins [38]. Similar increases in some of these proteins have been demonstrated in cultured human myocytes [39]. One unifying theory for myometrial response is based on the observation that up regulation of the connexin 43 protein could induce a syncytial myometrium that is able to respond to contractile stimuli in such a way that the entire uterus contracts as one.

The final major function of the myometrium during pregnancy occurs after the fetus has been delivered as the uterus undergoes tonic contraction to shear and expel the placenta with the attached fetal membranes. Failure of this stage can lead to post-partum haemorrhage. Subsequent uterine involution occurs over approximately 4–6 weeks, with the initial stages reducing myocyte size and number through both necrosis and apoptosis by mechanisms that include the recruitment of immune cells and autophagy [40]. This is followed by a small increase in new myocytes. Currently, the initial increase in cell number is considered to result from either an expansion of stem cell [41] or existing myocyte populations [40].

The Impact of Adenomyosis

Health information websites report that during the normal menstrual cycle most patients with adenomyosis have no outward symptoms, and can pass their entire reproductive life with asymptomatic adenomyosis, whilst the medical literature reports that only 35–40 % of women are asymptomatic [42]. Adenomyosis can be associated with a range of concomitant pathology including endometriosis and fibroids. The peak incidence of adenomyosis occurs in the fourth decade of life [43] and many women with adenomyosis also have endometriosis, which may itself account for symptoms of dysmenorrhoea, infertility and possibly menorrhagia. There is some evidence of heightened central nervous system sensitivity in some women with adenomyosis, resulting in heightened pain perception, presumably because the myometria of women with adenomyosis have increased numbers of sensory neurones. This increase in neuronal density is dependent upon local production of neurotrophins and their cognate receptors [44] and an altered expression of the neurotrophins NGF and BDNF and their cognate receptors have been reported in the myometrium in women with adenomyosis [45, 46]. This is in line with previous reports of increased nerve growth factor expression and changes in the lower affinity neurotrophin receptor (p75NTR) in the mouse model of adenomyosis [47].

PGP9.5 immunoreactivity was identified in the functional layers of the endometrium in women with adenomyosis and fibroids who also had pain symptoms but not in women without pain [48]. PGP9.5 nerve fibre density in the basal layer of the endometrium and in the myometrium was also significantly increased in women with pain [48]. It was hypothesised that dysmenorrhoea linked to adenomyosis may result from bleeding within the adenomyotic nodules in response to steroid withdrawal at menstruation, although entrapped blood is not commonly observed in affected uteri. Many of the ligands and receptors for the neurotrophin family of proteins have been shown to increase under the influence of oestradiol [49] which has been implicated in the aetiology of adenomyosis [47].

If the propagation of myometrial contractility and function is dependent on the production of a complete syncytium, then the presence of adenomyotic lesions may affect the normal physiology of the myometrium by interfering with the

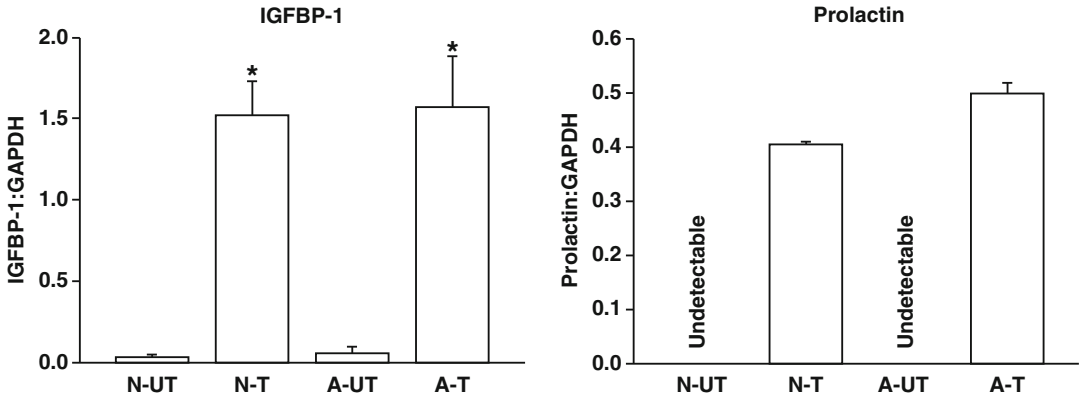

Fig. 4.3 Effect of adenomyosis on *in vitro* decidualisation markers. Stromal cells derived from adenomyotic (*A*) and normal (*N*) endometria were subjected to *in vitro* decidualisation in the presence of 17β-oestradiol (10^{-8} M), 8-Bromo-cyclic AMP (0.5×10^{-3} M) and medroxyprogesterone acetate (10^{-6} M) for 21 days (*T*) or with 0.1 % ethanol (*UT*), with medium changes occurring on alternate days. Cellular mRNA was incubated with AMV-RT and the resultant cDNA subjected to PCR. The levels for IGFBP-1 and prolactin transcript were corrected for that of GAPDH. Data are presented as the mean ± SEM of three biological replicates performed in triplicate. The number of independent data points (n) thus equals nine. Comparison of IGFBP-1 levels in the decidualised adenomyotic and normal stromal cells against the untreated controls was performed with Student's *t*-test; *$p < 0.05$. Prolactin transcript levels in the untreated stromal cells were undetectable

transfer of small molecules through the gap junctions produced by connexin proteins or by interrupting the propagation of membrane depolarisation through the muscle fibres. In women with adenomyosis, this might impair sperm transport [50], reduce fertility [51, 52], increase first trimester miscarriage [52, 53], increase the incidence of preterm labour [54] and precipitate placental abnormalities [55]. Recent research has found that women with adenomyosis undergoing IVF/ICSI treatment for infertility have higher miscarriage rates than women without the condition, with lower pregnancy rates and a much higher risk for early pregnancy loss [54]. Affected women also appear to have an increased risk of premature labour and placental abruption [55]. This suggests a dysfunctional myometrium. Another serious but rare complication of adenomyosis in pregnancy is the risk of uterine rupture during labour [56]. This may be due to aberrant decidualisation of deep lesions resulting in a reduced myometrial mass leading to rupture under tension. However, the majority of women with adenomyosis do become pregnant with normal outcome, suggesting that sperm transport may not be significantly affected by adenomyosis. But fertility rates measured by on-going pregnancy (but not biochemical pregnancy) in assisted reproduction are lower in adenomyosis [57], suggesting that impairment of blastocyst retention rather than in sperm delivery and fertilisation occurs in these women.

Whether there is altered decidualisation in adenomyosis remains uncertain [58]. Preliminary data demonstrated no impairment of *in vivo* decidualisation of stromal cells derived from adenomyosis as evidenced by normal production of prolactin and insulin-like growth factor binding protein-1 (IGFBP-1) (Fig. 4.3). It is possible that lower on-going pregnancy rates results from inner myometrial hyperperistalsis or dysperistalsis [59], resulting in disruption of implantation, perhaps under the influence of the increased production of nitric oxide [60].

Ectopic pregnancy rates may not be increased in women with adenomyosis suggesting normal transport of the zygote through the oviduct, but ectopic pregnancy has been linked to adenomyosis in smokers [61] and a case was reported of an intramural pregnancy linked to adenomyosis [62], although such cases must be extremely rare. Caesarean section rates are not significantly increased in women with adenomyosis and affected women do not experience prolonged

labour [61], however, myometrium affection varies significantly depending on the extent of the disease and further research is needed to correlate disease spread and its impact.

Conclusion

Despite recent technological advances, there remains a paucity of information on many important aspects of uterine physiology and on the impact of adenomyosis. Even though research on the functioning of the myometrium in pregnancy is moving at a steady pace, our understanding of the role of the myometrium during the non-pregnant state remains limited.

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The Role of the Myometrium in Adenomyosis

5

Marwan Habiba and Giuseppe Benagiano

Abstract

Classically adenomyosis is defined by the presence of ectopic endometrial glands surrounded by 'hyperplastic-hypertrophic myometrium'. Whilst there remains disagreement on the definition and diagnostic criteria, the diagnosis of adenomyosis by modern imaging relies on the identification of features that distinguish the inner from the outer myometrium. There are demonstrable differences between the inner and outer layers of the myometrium as well as differences between uteri with and without adenomyosis. Myometrial changes have long been regarded as a response to invasion by the endometrium but more recent literature raises the possibility that the myometrium may have a role in the pathogenesis of adenomyosis, perhaps because of innate predisposition.

Keywords

Hyperplasia • Hypertrophy • Myometrium • Junctional Zone • MRI • Radioisotope scintigraphy • Archimetra • Extracellular matrix • Ultrastructure • Myofilaments

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The classic definition of adenomyosis incorporates the observation that ectopic endometrial glands are surrounded by 'hyperplastic-hypertrophic myometrium' [1]. Myometrial changes contribute to producing a diffusely enlarged uterus. In his article, Cullen (1908) refers to his first observation of adenomyosis when in 1894 he found a uniformly enlarged uterus about four times the natural size [2]. The increase in size was due to diffuse thickening of the anterior wall. Histological examination demonstrated that anterior wall thickness was due to the presence of a diffuse 'myomatous tumour'

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and the description provided is that the uterine mucosa was at many points 'flowing into the myomatous tissue'. Most of the uteri described by Cullen (1908) were considerably larger than normal but he only used the term 'hypertrophy' in relation to the uterine mucosa or the glands within the myometrium and did not use the term 'hyperplasia' [2]. Hypertrophy is the term used to describe an enlargement in size of an organ that results from enlargement of its component cells, while hyperplasia reflects in an increase in size due to an increase in cell number. Characterisation of uterine hypertrophy-hyperplasia requires assumptions about the expected weight or size of the uterus which itself is not well defined.

The importance of myometrial hyperplasia as a diagnostic criterion for adenomyosis is not always emphasised. Novak and Woodruff (1979) refer to myometrial hypertrophy as a feature associated with endometrial invasion within the myometrium [3]. But whilst the myometrial changes are regarded as responsible for the observed uterine enlargement, adenomyosis is diagnosed chiefly upon the finding of endometrial islands deep beneath the mucous surface. Hendrickson and Kempson (1980) state that they are: 'loath to make a diagnosis of adenomyosis in the premenopausal uterus unless there is associated smooth muscle hypertrophy'. Notwithstanding the problem of diagnosing adenomyosis in the premenopausal uterus in the presence of a thin uterine wall, one important difficulty is defining objective criteria for myometrial hypertrophy. Hendrickson and Kempson (1980) describe, as a characteristic feature of adenomyosis, the presence of a collar of hypertrophic smooth muscle around adenomyotic foci [4]. Hypertrophy results in overall enlargement of the uterus. This draws the attention to another difficulty referred to above, namely the determination of the size of a normal uterus. It has long been recognised that determination of the size of the normal uterus is difficult to establish because of the need to control for age and parity and because studies based on surgical material is intrinsically biased. Little systematic study is available to address this question. Langlois (1970) demonstrated that parity is the primary determinant of uterine weight in premenopausal women and that the upper limit of what could be considered normal uterine weight has been greatly underestimated [5]. Langlois (1970) reviewed 1348 uteri removed at hysterectomy [5]. After excluding those with fibroids, endometrial hyperplasia or adenomyosis which might reflect an estrogenic effect, 468 uteri were considered to be "normal". In this study, a further 7 cases were excluded as they were from women of Oriental origin although the relevance of this is unclear. Out of the remaining 461 women, 184 were Caucasian and 277 were Black. Uterine weight was noted to vary depending on parity with the average uterine weight almost doubling in women with parity of 4 or more compared to nulliparous women (Table 5.1).

However, when the analysis was repeated taking age into consideration, the average uterine weight did not vary with advancing age in nulligavidas (Table 5.2) or in women with any other parity, but variation was observed based on age when all patients were considered together. This suggested that parity rather than age was the primary determinant of uterine weight.

The maximum weight of an apparently normal uterus also varied with age and parity and was almost fourfold higher compared to the mean weight of nulliparous women. In addition, the data suggested that parity not gravidity was the primary determinant of uterine weight. Thus pregnancies resulting in a miscarriage prior to viability did not result in permanent enlargement of the uterus. Thus determining the point at which a uterus would be considered abnormally large based on its weight becomes difficult. Based on estimates from the study population, Langlois (1970) proposed 130 g as the cut-off point for nulliparous women, 210 g for parity 1 to 3 and 250 g for higher parities [5]. Another observation made by Langlois (1970) is that uterine weight was lower in Caucasian compared to black women [5]. For example, the uterine weight for Caucasian nulliparous women was 49 ± 5.1 g and for nulliparous Black women was 78.3 ± 5.8 g. This difference was not present after the first pregnancy that reaches the age of viability (90.4 ± 8.7 g and 90.5 ± 13.2 g respectively). Some older writings employed the terms 'subinvolution', 'uterine fibrosis' or myometrial hypertrophy to describe the observation of symmetrically enlarged uteri that contain no gross or microscopic

Table 5.1 The relation between uterine weight, dimensions and parity

Parity	Number of uteri	Mean weight (SD)	Maximum weight (gm)	Vertical length	Transverse	Anteroposterior
0	30	63.2±21.4	110	7.7±0.2	4.7±0.1	2.9±0.2
1	26	90.4±39.8	170	8.6±0.3	5±0.2	3.5±0.2
2,3	173	104.1±36	210	9.2±0.1	5.6±0.1	3.9±0.1
4,5	115	118.5±42.2	243	9.4±0.1	5.8±0.1	4.2±0.1
6+	117	125.7±36.9	242	9.7±0.1	5.9±0.1	4.2±0.1

Data from Langlois (1970) [5]

Table 5.2 The relation between uterine weight, dimensions and parity taking age into account

	All uteri						Nulligravidas
Age range	Number of uteri	Mean weight±SD for all group	Maximum weight	Vertical length	Transverse	Anteroposterior	Mean weight±SD
10–19	3	56±13.9	65	8±0.0	5±0.3	2.8±0.4	63
20–29	68	107±34.5	225	9.2±0.2	5.5±0.1	4.1±0.1	46
30–39	223	114.9±36.1	240	9.4±0.1	5.7±0.1	4.1±0.1	71
40–49	114	118.1±44.7	243	9.5±0.1	5.9±0.1	4.2±0.1	67
50–59	38	84.3±41.9	189	8.1±0.3	5±0.2	3.2±0.2	51
>60	15	56.1±20.3	98	8±0.5	4.5±0.2	2.8±0.2	54

Data from Langlois (1970) [5]

abnormalities. But given the variations in the size of the uterus described here, it becomes unclear how uterine size and myometrial hyperplasia can be considered within the definition of adenomyosis apart – perhaps - in the less frequently encountered extremes.

In a more recent study Esmaelzadeh et al. (2004) assessed uterine size using ultrasound in 231 healthy women [6]. The study group comprised 54 nulliparous and 177 multiparous women. For nulliparous women, the mean (SEM) uterine length, antero-posterior diameter and width were 72.8 mm (±1.3), 32.4 mm (±0.1) and 42.8 mm (±1.2) respectively. The corresponding figures for parous women were 90.8 mm (±1.1), 43.0 mm (±0.8) and 51.7 mm (±0.7) respectively. The measurements are in line with those reported by Longlois (1970) [5].

The Structure of the Myometrium

Myometrial Zones

The idea of myometrial zonation came into prominence with the advent of ultrasound and MRI imaging. Based on ultrasound appearance the normal myometrium can appear as having three distinct sonographic layers. The middle layer is the most echogenic and is separated from the thin outer layer by the arcuate venous and arterial plexus. The inner layer is hypo-echoic relative to the middle and outer layers (subendometrial or myometrial halo). The presence of adenomyosis can alter or distort the sonographic appearance of these zones [7–13].

The junctional zone (JZ) was shown to be hormonally dependent; being indistinct before puberty and after the menopause, and showing maximum increase in thickness in the second half of the proliferative phase [14, 15]. The normal JZ as seen in MRI or ultrasound is defined as being regular and ≤5 mm thick [16], and a JZ ≥12 mm is reported to be highly predictive of adenomyosis [17]. The diffuse homogenous low-signal-intensity seen in adenomyosis was attributed to smooth muscle hyperplasia; a recognised feature of the disease [18].

In women of reproductive age, three different zones may be identified within the uterus using MRI. The normal endometrium and endometrial secretions appear as a high signal-intensity type stripe on T2-weighted sagittal images. Immediately subjacent to this is a band of low

signal intensity that represents the innermost layer of the myometrium: the Junctional Zone (JZ) that forms the outer boundary of the EMI [19]. The outer layer of the myometrium is of intermediate signal intensity. The thickness of the normal JZ varies considerably, ranging from 2 to 8 mm [20, 21]. Diffuse or focal widening of the JZ on MRI is suggestive of adenomyosis. Areas of low signal intensity are taken to correspond to smooth muscle hyperplasia and high signal intensity foci or linear striations are taken to represent ectopic endometrial tissue.

Hricak et al. (1983) published the first study on the use of MRI for the assessment of the female pelvis [22]. The study included 7 volunteers, 12 patients who had non-gynecological pathology and 2 women with gynecological disease. One participant was premenarcheal, 12 were of reproductive age (including two patients who had had a hysterectomy), 2 were postmenopausal and one was 8 weeks pregnant. Hricak et al. (1983) observed a low-intensity line separating the myometrium from the cyclic endometrium in women of reproductive age [22]. Their initial assessment was that this layer may represents the basal layer of the endometrium or that it relates to vascular or physiochemical phenomena at the myometrial-endometrial junction. They went on to suggest that assessment of this layer may allow the recognition of changes in the endometrium in relation to the menstrual cycle. In a following report, Lee at al. (1985) studied twelve uteri removed by hysterectomy [20]. These included 9 cases which did not have cancer or previous radiotherapy. They noted a 2–6 mm band of low-intensity signal between the central area of high intensity and the outer area of intermediate density. This area was clearly visible in 4 specimens, faintly visible in two cases and absent in three cases. Comparing endometrial thickness on histological examination with that measured on MRI, demonstrated that the high intensity central area corresponded to the endometrium, but there were no histological features that could explain the low intensity area and there were no histological differences between specimens which exhibited the low intensity zone and those specimens which did not. McCarthy et al. (1986) concurred with the conclusion that the junctional zone represents an area within the myometrium [23]. The explanation for the presence of the low-intensity area remains puzzling and its presence in uterine specimens suggests that it may not be related to the difference in blood flow. McCarthy et al. (1989) attempted to explain the presence of this area of low intensity by comparing the inner and outer myometrium. No differences were noted in the number of blood vessels, the content and nature of elastin, the amount of extracellular mucin and intracellular glycogen or the amount of collagen when comparing the junctional zone and the remainder of the myometrium [24]. However, there was a statistically significant difference in the water content. This was lower in the junctional zone compared to the endometrium and to the rest of the myometrium. As a percentage of weight, water constituted 82.88 % (SD ± 2.9) of the endometrium, 79.28 % (SD ± 1.4) of the junctional zone and 81.05 % (SD ± 1.8) of the myometrium. McCarthy et al. (1989) attributed the different signal intensity in the uterus to differences in water content as well as to differences in the relaxation phenomena that occur in heterogeneous tissue when imaged by MRI [24]. They also opined that other factors such as differences in blood flow do not need to be invoked to explain the phenomena.

Scoutt et al. (1991) compared the myometrial junctional zone and the outer myometrium after staining tissue section with Feulgen to delineate nuclei [25]. Five sections were examined per region utilizing the 20× objective light microscopy lens and image analysis. Sections were also stained for Collagen type III, IV and V; laminin; and fibronectin. Immunoreactivity was scored on a semi-quantitative score (from 0 to 3). The percentage of the nuclear area per high power field reported in this study was 61.7 % (SD ± 6.7) for the junctional zone and 21.3 % (SD ± 4.4) for the outer myometrium giving a JZ/OM ratio of 3.00 ± 0.61. On the other hand, there were no significant differences in the distribution of laminin; types III, IV, and V collagen; or fibronectin between the junctional zone and the outer myometrium. Scoutt et al. (1991) concluded that the decrease in T2 values observed in the JZ may be

related to increase in cell number per unit volume with resultant decrease in extracellular matrix [25]. The findings are in line with the earlier observation of increased musculature in the inner myometrium compared to the outer layers [26]. Thus differences in composition between the inner and outer layers of the myometrium have been noted for more than half a century. Schwalm and Dubrauszky (1966) examined a total of 40 uteri representing various physiological states (pre- and post- menopausal and postpartum) and uteri removed surgically as well as uteri from cadavers to determine the percentage of musculature in each region of the uterine wall [26]. Uterine wall was divided into three layers (inner, middle and outer thirds). The muscle content was higher depending on the region examined. Thus it was higher in the uterine corpus compared to the isthmus and was lowest in the cervix and in each region the muscle content was higher in the inner followed by the middle and was lowest in the outer layers. The difference in muscle content between the inner and outer layer of the anterior-posterior wall of the uterine corpus was statistically significant.

However, a more recent study demonstrated that the transition from the inner to the outer myometrium is gradual with no line of demarcation that would correspond to the MRI appearance [27]. In addition, it should be borne in mind that the distinction between the myometrial layers is not a consistent finding on MRI and is absent in a good proportion of cases. Mehasseb et al. (2011) examined sequential high-power fields (hpf ×*200*; *124,403 μm*^{2}) of the whole myometrial thickness from the endometrium to the serosa avoiding glandular tissue and large blood vessels [27]. Each field was assessed for the number of nuclei (nuclear count = n/hpf) as a reflection of cell density, the total nuclear area (i.e. area of the image occupied by nuclei, expressed as percentage), and the average nuclear size (μm^2). The percentage area that expressed α-SMA per hpf was examined using image analysis as an index of the muscle mass. In premenopausal women, with or without adenomyosis, cell density, nuclear size, total nuclear area and muscle mass were significantly greater ($p < 0.01$) in the inner compared to the outer myometrium (Table 5.3). The same was noted in postmenopausal women, but the difference in nuclear size was not statistically significant [27]. In premenopausal uteri with adenomyosis, both the inner and outer myometrium featured lower cell density and larger nuclear size compared to controls ($p < 0.05$), and the total nuclear area was lower in adenomyosis compared to controls, but the difference was statistically significant in the inner but not the outer myometrium. Although not statistically significant, similar differences were noted in postmenopausal uteri. Examination of the full myometrial thickness through sequential high power fields showed that the reduction in cell density and total nuclear area starting from

Table 5.3 The characteristics of the inner (IM) and outer myometrium (OM) in control and adenomyotic uteri

		Cell density, nuclear count (n/hpf)	Nuclear size (μm^2)	Total nuclear area (%)	Muscle mass (%/hpf)[c]
Premenopausal control (n = 35)	IM	1171 ± 52[a]	24.81 ± 0.47[a]	23.12 ± 1.17[a]	65.01 ± 0.76[a]
	OM	801 ± 43	23.44 ± 0.52	14.83 ± 0.8	42.6 ± 1.39
Premenopausal Adenomyosis (n = 54)	IM	970 ± 36[a,b]	26.38 ± 0.27[a,b]	20.54 ± 0.77[a,b]	65.01 ± 0.76[a]
	OM	625 ± 25[b]	25.15 ± 0.33[b]	12.76 ± 0.57	46 ± 1.25
Post-menopausal control (n = 10)	IM	1374 ± 53[a]	24.6 ± 0.89	26.7 ± 1.51[a]	57.27 ± 3.85[a]
	OM	813 ± 55	24.5 ± 0.59	16.3 ± 1.23	39.35 ± 1.85
Post-menopausal Adenomyosis (n = 10)	IM	1106 ± 44[a]	26.2 ± 0.72	23.4 ± 0.74[a]	62.78 ± 2.45[a]
	OM	778 ± 61	25.6 ± 0.84	16.2 ± 1.49	44.54 ± 5.61

Values given as mean ± SEM [51]

[a]Statistically significant compared to the outer myometrium ($p < 0.01$) in the same group (control and adenomyosis)

[b]Statistically significant compared to the corresponding zone in the control group ($p < 0.05$)

[c]Expressed as percentage area expressing α-SMA per high power field

the inner through to the outer myometrium in both adenomyosis and unaffected control samples was gradual and there was no distinct zonation that would correspond to the demarcation seen on MRI [27].

Junctional Zone Contractility Function

The junctional zone (JZ) was shown to be hormonally dependent. On MRI, it is indistinct before puberty and after the menopause and showing maximum increase in thickness in the second half of the proliferative phase [14, 15]. Studies using video-sonography have demonstrated peristaltic waves confined to the JZ myometrium. These waves vary during the cycle [28]. JZ contractions during the late proliferative phase may have a role in sperm transport, whilst quiescence during the secretory phase may facilitate implantation [29].

Studies using ultrasound scan and radioisotope scintigraphy reported that the subendometrial myometrium has distinct contractile properties that varied with the phases of the normal menstrual cycle. Using ultrasound identified the contractions as antegrade (from fundus to cervix) during menstruation, and retrograde (from cervix to fundus) during in the rest of the cycle. It was suggested that these contractions have a role initially to facilitated sperm transport and subsequently to support blastocyst implantation [30, 31]. It was also speculated that inner myometrial contractility could help to control menstrual blood flow and that its disturbance might explain the occurrence of menorrhagia [32]. In the study by Fraser et al. (1986) there was one woman (age 24 years) who had severed bleeding leading to anaemia [33]. She had an enlarged uterus to 14 weeks size and was histologically confirmed on full thickness biopsy with pure myometrial hyperplasia. Indeed Benson et al. (1958) quoted Meyer R (1925) as the first to suggest altered uterine contractility as a mechanism of bleeding in adenomyosis [34, 35].

Using MRI and hysterosalpingo-scintigraphy (HSSG), endometriosis and adenomyosis were linked to hyperperistaltic and dysperistaltic utero-tubal transport, but reduced fertility was linked to adenomyosis in women with patent tubes [36]. This study should be interpreted with caution, first because of the unusually high incidence of adenomyosis and the lack of clear diagnostic criteria. Second; and perhaps most importantly, because of the test chosen to assess tubal function. It has been argued that many of the images produced by HSSG may be artefacts [37], and radioactive-labelled particle transport is inconsistent [38–40]. Habiba (1994) demonstrated that HSSG has a false negative rate of 34 % in the control group (n = 13 representing 26 tubes) who had patent tubes on laparoscopy and that the positive predictive value for HSSG for predicting tubal patency was 65 % and the negative predictive value for an obstructed fallopian tube was 42 % [37]. Wånggren et al. (2011) examined ^{99m}Tc-radio-labelled particle transport through the uterus and the tubes in ten women with proven fertility. Transport of radioactive particles could only be seen in some cases and most frequently during the periovulatory period [40].

Embryonic Origins

The endometrial-subendometrial unit has also been described in the literature as the "*archimetra*" (the endometrium of older phylogenetic origin) [41], with reference to *Werth and Grusdew* (1898), who used the term *archimyometrium* to describe the ontogenetically old character of the subendometrial myometrium [42]. A distinctive feature of the EMI is the lack of an intervening connective tissue layer, or a protective submucosa. As a result, the endometrial glands and stroma lie in direct contact with the myometrium, allowing extensive free interaction [43–45]. The EMI is also irregular over its entire surface [43].

Although neither Noe et al. (1999) nor Uduwela et al. (2000) studied the embryological origin of the uterus, they stated that both EMI components (basalis endometrium and subendometrial myometrium) are believed to have a common embryological origin from the paramesonephric ducts whereas the outer myometrium is of non-paramesonephric mesenchymal origin [45, 46]. There are few early studies of the subject. Konishi et al. (1984) examined autopsy material obtained from human abortuses

and stillborn fetuses obtained at different gestational ages (12–40 weeks) [47]. They observed that the outer part of the mesenchyme of the uterus gives rise to the myometrium and that the inner part corresponds to the endometrial stroma [47]. The Mullerian (paramesonephric) origin of the outer myometrium is also supported by Robboy et al. (1982) who described the normal development of the human female reproductive tract and the alterations resulting from experimental exposure to diethylstilbestrol [48].

Noe et al. (1999) argued that the endometrium including both the glandular and stromal component together with the subendometrial myometrium form a unit which they termed the 'archimetra' [46]. They argued that the archimetra is derived from the paramesonephric ducts and the surrounding mesechyme whilst the stratum vasculare and the stratum suravasculare develop later and do not share the same embryonic origin as the archimetra. This view contrasts with electron microscopy studies which suggested that the development of inner myometrial layers from undifferentiated stromal cells at the endometrial-myometrial junction [47]. Whilst there appears to be agreement that the stratum vasculare does not develop from the archemyometrium (Lebedev 1952; quoted from Wetzstein 1965) [49, 50], it is unclear if the layers of the myometrium do have different origins. In the study by Wetzstein (1965), the direction of the muscle bundles in the inner myometrium was predominantly circular [49]. In the largest part of myometrium or the stratum vasculare, muscle bundles were interwoven in random directions forming a meshwork. Bundles were shown not to run the whole length of the uterus but to lose and gain additional bundles along their path and have also been shown to change direction abruptly. The outermost stratum supravasculare was formed of four layers: a longitudinally directed outermost layer followed by a circular layer, an incomplete longitudinal layer and an innermost circular layer. Wetzstein (1965) also noted that there was clear interconnectivity between all the layers of the myometrium [49]. Muscle bundles were also anchored to the vasculature. Experimental observations from early uterine development in mice suggest that uterine layers evolve through a process of differentiation from the undifferentiated mesenchyme that surrounds the endometrial epithelium [51, 52]. The mesenchymal cells that will form the endometrial stroma and both the inner and outer myometrium could be identified in the very early phases.

Mehasseb et al. (2009, 2010) described uterine development in the neonatal mouse [51, 52]. On day 2, the uterine cavity consisted of an oval-shaped lumen, elongated in the mesometrial antimesometrial axis. The luminal epithelium consisted of a monolayer of low columnar cells. This was surrounded by mesenchymal cells that did not form distinct layers or have distinct orientation. The perimetrium was composed of a single layer of epithelium. By day 5, the mesenchymal cells started to segregate into three layers: endometrial stroma, inner circular, and prospective outer longitudinal muscle layers. The endometrial stroma formed the inner half of the uterine wall thickness. Stromal cells retained their undifferentiated shape. The prospective inner circular muscle layer was the most defined layer and was five to six cells thick. This was formed of circularly orientated and tightly packed cells. The prospective outer myometrium was formed of one to two layers of cells retaining their undifferentiated appearance. Vascular spaces started to appear especially between the inner and outer myometrium.

By day 10 all layers were more distinct. The stroma appeared more tightly packed. The inner circular muscle layer was organized into bundles. By day 15 the adult configuration of the uterus became apparent. Stromal cells were randomly orientated except around the individual glands. A distinct loose vascular layer separated the inner and outer myometrial layers. The outer myometrial cells became grouped in bundles connected by loose connective tissue sheaths, and separated from the inner myometrium by a distinct loose vascular layer.

The Expression of ECM Components

McCarthy et al. (1989) compared histological sections of the inner and outer myometrium for the number of blood vessels, smooth muscle

cells, fibroblasts, elastin, iron, collagen, mucin, polysaccharide and amyloid [24]. There were no significant differences between the myometrial layers. In a subsequent study, the same group [25] compared the density of the extracellular matrix components using immunohistochemical staining with antibodies to type III, IV and V collagen; laminin, and fibronectin. They used a semiquantitative score (from 0 to 3+). Immunohistochemical analysis demonstrated a normal distribution of extracellular matrix components and no difference in distribution between the inner myometrium and outer myometrium in women without adenomyosis.

Metaxa-Mariantou et al. (2002) studied elastin distribution in the myometrium during the phases of the menstrual cycle using immunohistochemistry, orcein staining and image analysis [53]. Elastin was noted in arteries and arterioles and within the perivascular tissue in the myometrium. In the endometrium, elastin was present in the basal portions of the spiral arterioles. No elastin was found in the more superficial parts of the vascular tree or in the endometrial stroma. Within the smooth muscle of the inner myometrium, elastin was absent or of low abundance. In the outer myometrium, elastin was also noted within smooth muscles. Thus in contrast to the study by McCarthy et al. (1989), Metaxa-Mariantou et al. (2002) noted a decrease gradient from outer to inner myometrium [24, 53]. There was no distinct demarcation between the inner and outer myometrium. An interesting observation made by Metaxa-Mariantou et al. (2002) is that elastin was expressed in endoemtrial basal layer in 3 out of the 12 samples exposed to the Levonorgestrel Intrauterine Device (LNG-IUS, Mirena®), but not in any samples not exposed to LNG-IUS [53]. This suggests that exposure to progestogens may be associated with the acquisition of a myofibroblastic phenotype by endometrial stromal cells. This seems to support the observation by Kohnen et al. (2000) that some stromal cells in the basal endometrium express α-smooth muscle actin and thus exhibit a myofibroblastic phenotype in respose to progestogens [54]. Leppert and Yu (1991) used scanning electron microscopy and reported that the extracellular matrix of the myometrium contains flat sheets or lamellae within a sponge-like matrix [55]. Uterine elastic fibres were formed into two distinct structures: fibrils and thin sheets of elastic membranes. Isolated fibres and membranes formed thin sheets of elastic membranes and elastic fibrils that may allow the uterus to maintain elasticity without exerting excess pressure on the growing fetus. The elastic tissues in the non-pregnant human uterus had no specific architectural arrangement and formed a sponge-like structure. This contrasts with the elastic fibres of the cervix which formed membranes and fibrils, organized into fishnet-like structures. The concentration of insoluble elastin and collagen in human uterine body was 1.38 % and 38.8 % of dry-defatted tissues, respectively. No similar studies have been conducted focussing on adenomyosis [55].

Zheng et al. (2006) used histochemical staining for elastin (orcein and modified Victoria blue/ethanol solution PPA-VB) in uteri with and without leiomyomas, adenomyosis and adenomyoma [56]. They reported that expression of elastin within the myometrium was largely present in perivascular tissue particularly near larger vessels in the outer myometrium. Scattered elastic fibres were also present between the myometrial fibres in the outer myometrium. There was a trend for higher elastin density in older women. Staining showed a decreasing gradient from the outer to the inner myometrium but interestingly, expression was absent in fibroids, adenomyosis or adenomyomas. There are no studies that examined the effect of parity on the distribution of elastin. These findings contrasts with those of McCarthy et al. (1989) referred to above [24].

Steroid Receptor Expression in the Myometrium with and without Adenomyosis

Scharl et al. (1988) reported on estrogen receptor expression in the uterus including the myometrium through the menstrual cycle [57]. They observed strong estrogen receptor (ER) expression in the majority of nuclei in the myometrium during the proliferative phase. The expression

level did not vary during this phase but ER expression reduced significantly following ovulation. More than 50 % of myometrial cells in mid- and late- luteal phases were ER negative, and the rest were usually weakly stained. They also reported that the distribution of ER positive and ER negative muscle cells was not diffuse but was arranged in receptor positive and receptor negative muscle bundles, and that expression was lower in the subserosal myometrium compared to the subendometrial myometrium. The observations are interesting, but it is important to keep in mind that the number of samples examined per phase of the cycle varied from a maximum of 6 in the early proliferative phase, 5 in the late proliferative, 3 in the early secretory, 3 in the late secretory and only one sample representing the mid-secretory phase.

Mertens et al. (2001) examined the expression of androgen, estrogen and progesterone receptors in the uterus utilising full thickness biopsies [58]. Five sections were examined in each of the phases of the cycle. Immunostaining took into account the percentage of stained cells and staining intensity. In the myometrium, ER expression was at its maximum during the early proliferative phase and decreased markedly in the early secretory phase. The expression of progesterone receptor did not vary with the phase of the cycle (Table 5.4).

Kawaguchi et al. (1991) reported that estrogen receptor is expressed in the myometrium during the proliferative phase of the cycle, but is suppressed during the secretory phase whilst there was strong progesterone receptor expression during both phases of the cycle [59]. The findings are consistent with the observation that estrogen induces the expression of both estrogen and progesterone receptors during the proliferative phase. But, whilst progresterone suppresses its own receptor in the endometrium, the same was not observed in the myometrium. The study by Snijder et al. (1992) reported that estrogen receptor expression in the myometrium fluctuates during the menstrual cycle [60]. Five samples were examined in each phase. Maximum estrogen receptor expression was noted in the late proliferative phase followed by a sharp drop in the early secretory phase. The pattern was similar to that noted in endometrial stroma, but contrasted with expression in endometrial glandular epithelium where the decreased expression from the late proliferative and into the secretory phase was gradual. Progesterone receptor expression in the myometrium was strong throughout the phases of the cycle and showed little fluctuation. Thus the pattern of receptor expression in the stroma of the functionalis, basalis and the myometrium was similar and shows little fluctuation. Neither Kawaguchi et al. (1991) or Snijder et al. (1992) made a distinction based on myometrial zonation [59, 60]. Lessey et al. (1988) used immunohistochemistry to examine the expression of estrogen and progesterone receptors in the uterus in 33 premenopausal women. Samples were classified according to the phases of the menstrual cycle into menstrual (n = 4), early proliferative (n = 9), late proliferative (n = 7), early secretory (n = 5) and late secretory (n = 8) [61]. The expression of estrogen receptor peaked in the late proliferative phase in the endometrial glands and stroma but in the myometrium, estrogen receptor expression peaked in the early proliferative phase. During the secretory phase, expression declined rapidly in all layers. In contrast to estrogen receptor, progesterone receptor expression increased in the

Table 5.4 The distribution of steroid receptors in the myometrium throughout the menstrual cycle [58]

Menstrual cycle phase	Estrogen receptor	Progesterone receptor	Androgen receptor
Menstrual	91 ± 46	199 ± 22	70 ± 5
Early proliferative	132 ± 38	193 ± 37	66 ± 9
Late proliferative	92 ± 27	193 ± 35	56 ± 16
Early secretory	22 ± 5	188 ± 40	49 ± 20
Mid secretory	60 ± 4	175 ± 27	33 ± 7
Late Secretory	18 ± 21	214 ± 9	0

endometrium throughout the proliferative phase but dropped rapidly in the glands but not in the stroma during the secretory phase. Also, progesterone receptor expression in the myometrium paralleled the pattern in the stroma not the glands. Thus, there was a divergence in progesterone receptor expression in the endometrial epithelium on one hand and the stroma and myometrium on the other. However, Lessey et al. (1988) do not provide statistical analyses comparing expression in the various layers [61]. Also, it appears that the analysis was confined to the inner layers of the myometrium.

Amso et al. (1994) reported that the intensity of estrogen receptor expression in the myometrium was moderate throughout the cycle except in the late follicular phase when it was very strong, and that contrary to the slight fluctuations seen in ER, the intensity of progesterone receptor expression was strong throughout the cycle [62]. The study however was of poor quality because of the small sample size as each cycle phase was represented by only one or two samples. In this study only the superficial myometrium was assessed.

A more recent study by Noe et al. (1999) using immunohistochemistry of the whole uterine wall, took into account myometrial zonation [46]. The myometrium was divided into three zones: the stratum subvasculare or subendometrial myometrium adjacent to the endometrium with a predominantly circular muscular fibres; the subserosal stratum supravasculare with a predominantly longitudinal muscular fibres; and the stratum vasculare, which consists of a three-dimensional mesh of short muscular bundles that form the bulk of the uterine muscular wall. The expression of estrogen receptor in the subendometrial myometrium almost completely paralleled the expression in the endometrium. The immunoreactivity in the inner portion (about the inner third) of the stratum vasculare exhibited a reduced cyclical pattern, whilst the outer two thirds of the stratum vasculare and the stratum supravasculare showed no cyclic pattern. It is notable that the outer layers of the myometrium exhibited strong ER expression in all the phases of the cycle. The expression of progesterone receptor was reported as showing cyclical changes in the subendometrial myometrium and in the inner third of the stratum vasculare, but there were no cyclical changes in the outer portion of the stratum vasculare or in the stratum supravasculare. Again, the outer layers of the myometrium exhibited strong PR expression in all the phases of the cycle. Both ER and PR were highly expressed in all layers on the myometrium in postmenopausal samples suggesting that the receptors are constitutive for the myometrium.

Richards and Tiltman (1995) also examined the expression of estrogen receptor in the myometrium [63]. They reported that the number of myometrial cells per high power field (hpf) was lower in the outer myometrium compared to the inner myometrium. In the fundal region, the total number (±SD) of cells per HPF was 111.7 (SD ± 12.66) in the subendometrial myometrium, 60.55 (SD ± 13.51) in the midmyometrium and 36.75 (SD ± 10.61) in the subserosal myometrium. The corresponding figures in the lower uterine segment were 105.27 (SD ± 20.8), 55.53 (SD ± 11.66) and 37.53 (SD ± 6.29) respectively. Whilst the number of estrogen receptor positive cells in the fundal region was 88.15 (SD ± 12.06) in the subendometrial myometrium, 39.7 (SD ± 15.25) in the midmyometrium, and 16.7 (SD ± 7.38) is the subserosal myometrium. The corresponding figures for receptor expression at the level of the lower uterine segment were 94.13 (SD ± 24.37), 32.4 (SD ± 8.77) and 18.67 (SD ± 7.83) respectively. Thus there were fewer estrogen receptor positive myometrial cells both in absolute terms and as a percentage of all myometrial cells in the outer layers of the myometrium. In addition, it is to be taken into account that the values quoted here from the study by Richards and Tiltman (1995) did not divide uteri based on cycle phase [63]. Their observation are thus at variance with those reported by Noe et al. (1999) [46]. This may be explained because the studies reported using different methodologies. Noe et al. (1999) relied on calculating an immunoreactive score that takes into account the percentage of stained cells for each intensity where intensity was graded as 0 = no, 1 = weak, 2 = moderate and 3 = strong [46]. But whilst the semi-

quatitive score has not been shown to reflect a doubling or tripling of receptor expression, it is unclear if the colorimetric measure is of biological significance. Richards and Tiltman (1995) calculated the percentage of estrogen receptor+ve cells and also included assessment of receptor concentration by radioimmunoassay and expressed as femtomoles per milligram of cytosolic protein [63]. The mean (±SD) receptor concentration in the subendometrial myometrium, midmyometrium and subserosal myometrium was 31.64 (SD ± 13.99), 14.01 (SD ± 6.78) and 9.50 (SD ± 4.28) respectively.

More recently, Mehasseb et al. (2011) reported on receptor expression in the myometrium taking into account both estrogen receptors α and β (ER-α and ER-β) and both progesterone receptors A and B (PR-A and PR-B) comparing women with and without adenomyosis and full thickness uterine wall biopsies [64]. All samples were standardised from the anterior wall of the uterus near the fundus and adenomyosis was defined by the presence of endometrial glands and stroma deeper than 2.5 mm below the endometrial-myometrial interface. The included specimens were classified according to the phase of the menstrual cycle into early, mid-, late- proliferative and early, mid-, late-secretory phases.

The results are summarized in Table (5.5). There was no cyclical variation in ER-α expression in the inner or outer myometrium of control or adenomyotic uteri. ER-β expression in the myometrium was weak and there was no-significant cyclical variation in either the inner or outer myometrium of the controls. However, ER-β expression was significantly higher in the mid-proliferative, late-proliferative, and mid-secretory cycle phases in both the inner and outer myometrial layers of adenomyotic uteri. There was no difference between the inner and outer myometrium in the control or in the adenomyosis groups.

Mehasseb et al. (2011) also examined the distribution of PR-A and PR-B using immunohistochemisrty [64]. Reactivity was confined to the nucleus of positive cells. There were minimal cyclical changes in PR-A and PR-B in the inner and the outer myometrium of control uteri. In adenomyosis, PR-A expression was significantly lower in all phases of the cycles (except the early secretory phase) both in the inner and the outer myometrium compared to control uteri. PR-B immunostaining was lower in adenomyosis compared to controls in both the inner and outer myometrium (Table 5.6). The observed lack of cyclicity of steroid receptors in the inner myometrium in the study by Mehasseb et al. (2011) is at variance with the studies by Scharl et al. (1988) and by Mertens et al. (2001) [57, 58]. This may be due to methodological differences as neither of the earlier studies involved receptor isoforms, and both relied on semi-quantitative or H-scores. There are discrepancies between published

Table 5.5 Estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) distribution in the inner and the outer myometrium in adenomyosis and in control uteri during the different phases of the menstrual cycle

		Early proliferative (n=5)	Mid proliferative (n=6)	Late proliferative (n=5)	Early secretory (n=8)	Mid secretory (n=6)	Late secretory (n=5)
ERα							
IM	Control	88.3 ± 1.6	84.5 ± 2.2	66.8 ± 8.6	76.7 ± 1.8	81.3 ± 3.4	83.2 ± 1.9
	Adeno	81.4 ± 4.6	83.7 ± 1.9	89.6 ± 0.5*	84.7 ± 3.9	78.8 ± 4.7	90.9 ± 1.7*
OM	Control	81.6 ± 7.2	80.8 ± 2.9	54.8 ± 15	62.2 ± 7.4	72.9 ± 3.4	75 ± 8.4
	Adeno	71.1 ± 5.6	77 ± 4	77.8 ± 3.4	71.6 ± 7.1	71.2 ± 2.4	76.7 ± 7.7
ERβ							
IM	Control	0.6 ± 0.1	1.4 ± 0.4	7.7± 7.1	2.4 ± 1.8	6.9 ± 5.2	0.9 ± 0.2
	Adeno	4.7 ± 2.8	22 ± 5.7*	20.5 ± 4.9	6.7 ± 4.4	23.6 ± 3.1*	19.2 ± 4.5*
OM	Control	0.2 ± 0.1	0.2 ± 0.1	6.8 ± 6.1	2.9 ± 1.8	5.6 ± 3.8	0.3 ± 0.1
	Adeno	10.4 ± 6.2	33.3 ± 12.2*	45.6 ± 15.5*	12.9 ± 5.1	43 ± 9.4*	20.9 ± 9.4

Immunohistochemical staining is expressed as the percentage of positively stained cells per high power field (mean ± SEM)
*Statistically significantly different between adenomyosis and control uteri

Table 5.6 PR-A and PR-B distribution in different uterine layers in the different phases of the menstrual cycle

		Early proliferative (n = 5)	Mid proliferative (n = 6)	Late proliferative (n = 5)	Early secretory (n = 8)	Mid secretory (n = 6)	Late secretory (n=5)
PR A							
Inner myometrium	Control	91.8 ± 1.2	90 ± 1.1	90 ± 1.5	86.9 ± 2.7	91 ± 0.9	88.2 ± 2
	Adenomyosis	51.9 ± 9.2*	68.1 ± 3.9*	74.1 ± 2.3*	77.7 ± 3.8	69.6 ± 2.3*	78.8 ± 3.8*
Outer myometrium	Control	89.1 ± 3.3	88.4 ± 1.6	84 ± 3.2	86.7 ± 4	88.3 ± 2.3	88 ± 2.4
	Adenomyosis	59.9 ± 8.8*	55.1 ± 7.7*	66.1 ± 5.5*	74.1 ± 4.6	62.1 ± 8.8*	61.8 ± 10.6*
PR B							
Inner myometrium	Control	88 ± 1.9	81.1 ± 2.7	77.9 ± 5.6	84.6 ± 2.9	77.2 ± 4.4	82.1 ± 0.9
	Adenomyosis	62.2 ± 10.3*	66.4 ± 5.4*	62.5 ± 6.3	61.1 ± 14.3	59.4 ± 6.2*	29.4 ± 17.2*
Outer myometrium	Control	85.8 ± 3.3	82.9 ± 2.2	68.6 ± 8.5	73.2 ± 7.6	69.7 ± 4.3	76.7 ± 3.9
	Adenomyosis	66.2 ± 9.9	58.4 ± 4.7*	58.1 ± 13.1	55.8 ± 9	49.6 ± 9.3	57.4 ± 5.1*

Immunohistochemical staining expressed as percentage of positively stained cells per high power field (mean ± SEM)
*Statistically significantly different between adenomyosis and control uteri

reports on ER-β expression in the endometrium [65–69]. The reasons for these differences may be related to sampling or other methodological differences [70].

Differences in steroid receptor distributions is unlikely to be related to disturbances in ovarian function, but could be related to the previously reported differences in local steroid synthesis [71, 72].

The differences in steroid receptor expression may reflect functional significance as it could enable a differential response to normal circulating steroids [73–75]. It is well established that steroid receptor expression in the uterus and the variations during the cycle are sensitive to circulating steroid hormones [76]. But there is very limited data on peripheral steroid levels in women with adenomyosis [72]. Takahashi et al. (1989) reported elevated level of estrogen in menstrual blood in women with adenomyosis [72]. This needs to be confirmed, but such higher levels may reflect the effect of increased P450 aromatase in adenomyotic uteri [71]. The higher ER-β and the lower PR expression in the myometrium in adenomyosis may be related to the presence of the classically described myometrial hyperplasia and the reduced PR expression might explain the poor response to progestogens [77–79]. The lack of progestogen response may be overcome by higher doses administered locally [80].

Mesenchymal Markers in Adenomyosis

Mehasseb et al. (2011) examined the expression of mesenchymal markers: α-smooth muscle actin (α-SMA), desmin, and vimentin in the inner and outer myometrium in women with adenomyosis [27]. The muscle mass was calculated as percentage positive area expressing α-SMA per high power field (hpf) using image analysis. α-SMA expression (muscle mass) did not vary significantly across the menstrual cycle or between adenomyotic and control uteri. Desmin expression was confined to the myocytes and there was no staining in extracellular tissue. Expression of desmin and α-SMA in the inner and the outer myometrium were uniform and did not vary significantly across the menstrual cycle or between adenomyosis and control samples. Mehasseb et al. (2011) reported that there was no distinct point of demarcation between the inner and the outer myometrium using these markers. Vimentin immunostaining was more intense in connective tissue cells surrounding muscle bundles [27]. The expression in muscle cells was weak with no cyclical changes but was higher in the inner myometrium of women with adenomyosis compared to controls in all phases of the menstrual cycle except the early proliferative phase. Vimentin expression in the inner myometrium in adenomyosis exhibited significant cyclical variation,

being higher in the late proliferative and the early secretory phases. Vimentin, desmin and cytokeratin are important intermediate filament proteins in the myometrium. Mehasseb et al. (2011) demonstrated higher staining index of vimentin in the inner, but not in the outer myometrium in women with adenomyosis compared to controls [27]. In the inner myometrium, the expression of vimentin was also lower in the early proliferative phase of the cycle compared to the other cycle phases. However, desmin immunostaining in the inner and outer myometrium did not vary significantly across the menstrual cycle or between adenomyotic and control uteri.

Both desmin and α-SMA expression in adenomyosis were consistent with the muscle layer being organised into thinner, less well-defined bundles. The disruption of normal geometry of the muscle bundles and fascicles observed on desmin and α-SMA staining with widening of the intercellular space and rearrangement of the myocytes may influence uterine function. Myometrial cell density did not vary significantly according to the phases of the cycle in normal uteri. The reduction in cell density in the inner myometrium in adenomyosis during the MS phase suggests increased tissue oedema, perhaps in response to progesterone. The finding of similar expression of the intracellular components α-SMA and desmin supports reduced extracellular fluid content in adenomyosis, thus the reason for increased JZ thickness in affected uteri remains unclear. The lack of a clear distinction between the inner and outer muscle layers does not rule out a functional distinction as demonstrated in studies using ultrasound [81–83]. Also, other differences for example in innervation [84] may exist.

Ultrastructure of the Myometrium

Much of the focus of ultra-structural studies of the human myometrium was on understanding the function of smooth muscle. This included comparing the contractile components to striated muscle, examining the interconnection between muscle fibres and the synchronisation of contractions. Some studies of uterine smooth muscle also examined the changes linked to pregnancy and subsequent involution [85, 86]. Mark (1956) used electron microscopy to study the differences between uterine smooth muscle and striated muscles [87]. The study demonstrated some of the fibrillar structures and cell-cell connections. Jaeger (1963, 1965, 1967) reported on the ultra structure of the myometrial smooth muscle, the changes related to delivery, uterine innervations and connective tissue [88–90]. In a subsequent publication Jaeger, (1971) reported on intracellular contacts between smooth muscle cells [91].

Vertebrate smooth muscle fibres contract more extensively on stimulation compared to skeletal muscles and also have a wider range of length in which they can function. Isolated smooth muscle pieces can shorten to one third of their length during a contraction and can be stretched to more than double their resting length without irreversible damage. Each smooth muscle cells is surrounded by a fine collagen mesh and cells are arranged in bundles separated from adjacent bundles by a collagen matrix. Shoenberg (1977) pointed out that whilst the mechanisms of smooth muscle contraction resemble those of striated muscles, there are many structural aspects where smooth muscle cells more closely resemble fibroblasts [92]. The internal structure of smooth muscle cells comprises a centrally located elongated nucleus. The contractile substance of the sarcoplasm is formed of myofilaments whose diameter is approx 100Å. Unlike the case in striated muscle fibres, the myofilaments are not ordered into repeating sarcomeres. Jaeger (1963) described a fluid transition between the resting, mostly light coloured cells, and active cells that appear darker on electron microscopy [88].

There are three types of filaments within smooth muscle cells: the thick (myosin), the thin (actin) and the intermediate filaments. There is great diversity of intermediate filaments depending on the cell of origin. Desmin is a predominant protein of intermediate filaments of smooth muscles. Vimentin is the major component of intermediate filaments in mesenchymal cells. Leoni et al. (1990) demonstrated that desmin content increased dramatically during pregnancy, whereas vimentin content remains unchanged or

changes only very little [93]. Muscle cells kept in culture demonstrated considerable increase in vimentin but little change in desmin content compared to freshly cultured cells. Immunoreactivity to cytokeratin has also been demonstrated in relation to intermediate filaments in the myometrium [94–100].

Intermediate filaments are attached to cytoplasmic dense bodies and to plasmalemmal dense plaques. Their main role is probably the regulation of cell shape. But the arrangement of intermediate filaments in the myometrium and their precise role remains unclear [94]. The attachment of the intermediate filaments to dense plaques at the plasmalemma and to the dense bodies in the cytoplasm provides the anchoring necessary for these fibres and the structural integrity of the scaffolding structures. Eyden et al. (1992) reported that intermediate filaments in the myometrium are present dispersed amongst organelles as well as forming aggregates that were smaller in normal myometrial cells compared to those from fibroids [94]. The reason for these aggregates remains unclear, but may be related to the pathological conditions that necessitated removal of the uterus or to the aging process. However, Eyden (1992) considered their presence to signify deviation from normal because such aggregates are likely to interfere with the normal function of the cell [94]. The intermediate filament system has been investigated immunohistochemically and by gel electrophoresis in myometrium [95–98, 100] and in leiomyomata [96–98, 101].

In addition, the cytoplasm contains microtubules, sarcoplasmic reticulum, Golgi apparatus, mitochondria and numerous ribosomes. Dense bodies and numerous vesicles are located adjacent or adherent to cell membranes. Microtubules, intermediate filaments and vesicles opening into the cell membranes are features that are present in smooth but not in skeletal muscle cells. The myometrium is perhaps unique amongst vertebrate smooth muscles because of the continuous change in the development and the distribution of its organelles. The biggest changes are those observed during pregnancy where muscle cells enlarge considerably to accommodate the growing fetus. The changes during pregnancy result from increase cell size and the increase in collagen production. Postpartum involution results from decrease in intracellular organelles and resorption of intercellular collagen. Smooth muscle cells can respond to a myriad of external stimuli such as mechanical stretch, local stimulants or inflammatory stimuli by changing their phenotype, changes to intracellular signalling, cell growth, proliferation or contraction [102]. The ability of uterine smooth muscle cells to adapt to the requirements of pregnancy and parturition adds to the evidence that smooth muscles cells are not in a terminally differentiated state [102].

The structure of uterine smooth muscle is similar to other smooth muscle cells in the body. There is a basement membrane, a plasma membrane, and contrary to earlier theories there is no syncytium. The nucleus has a double membrane with one or two nucleoli. The cytoplasm is identified with mitochondria, the Golgi complex, the endoplasmic reticulum, ribosomes, and centrosomes. Pinocytosis vesicles are noted near the cell edge [103]. The individual muscles cells are formed into different size bundles. The number of cells within the bundles is lower in the lower uterine segment and the cervix compared to the body of the uterus. The size of the narrow connective tissue bands between the muscle bundles is not different in different parts of the uterus and the differences between the pregnant and nonpregnant uterus are very small [89]. In contrast to this, connective tissue septae containing blood vessels are significantly enlarged in pregnancy and are larger in the fundus compared to the cervix. The number and form of contracted muscle cells is dependent on the amount of muscle contractile force. The space between muscle cells within individual bundles contains homogenous ground substance with occasional collagen fibres. Between the muscle bundles there are substantial connective tissue septae consisting of loose fibrous connective tissue and fibrocytes. These have a very large nucleus and a narrow cytoplasm strip. Lipoid droplets are more commonly seen in connective tissue cells compared to muscle cells. In the lower uterine segment, particularly after a

long labour, damage is often noted to the internal structure of mitochondria which are left as empty capsules [88]. Neurofilamants that do not form reticulum are noted in studies of the rat and mouse bladder. These are in direct contact with smooth muscle cells with no intervening myelin. The contacts contain multiple vesicles and as such, have the characteristics of the neuromuscular end place. In the human myometrium, both demyelinated and myelinated fibers are present, but there are more demyelinated fibres. In contrast to studies on the bladder, Jaeger (1965) was unable to identify end plates or axon connections with myometrial smooth muscle cells. Because of this, he proposed that the co-ordination of uterine contractions may occur through either remote synapse biochemical transmission or direct cell-to-cell contact [89].

Steroid hormones are known to stimulate the myometrium and to expand the cellular structures for the synethesis of intracellular and extracellular components. Following estrogen treatment, there is an increase in the number of mitochondria, Gogli apparatus, ribosomes and granular endoplasmic reticulum [104, 105]. In general, similar changes accompany the onset of puberty and pregnancy [86, 106]. Gap junctions are believed to link individual myometrial cells into a functional syncytium [107]. These permit the exchange of small ions and molecules between the cells and thus synchronise the metabolic and contractile activities of myometrial cells.

Endometrial stromal cells are spindle-shaped and contain moderately developed organelles. Collagen fibrils are easily seen in the matrix. An interesting observation made by Fujii et al. (1989) is that cells having some features of smooth muscle were found among endometrial stromal cells at the endo-myometrial junction (EMJ) of the adult uterus [108]. In the proliferative phase of the menstrual cycle, these cells resembled myofibroblasts, but during the secretory phase and in early pregnancy they changed morphologically to resemble smooth muscle cells by acquiring more distinct cytoplasmic filaments with dense bodies and dense plaques. This suggests that smooth muscle differentiation may occur from multi-potential mesenchymal cells in the endometrial stroma. Fujii et al. (1989) noted the similarity between the cells exhibiting smooth muscle features within the endometrial stroma and the myofibroblasts that were described in granulation tissue [108–110]. The presence of these cells within the endometrial stroma supports the hypothesis that smooth muscle cells may be newly produced by metaplasia of endometrial stroma [111]. The process appears to be influenced by sex steroids. Thus smooth muscle cells increase their cytoplasm and organelles in response to estrogen and increase their myofilament content in response to progestogens [104, 112–114].

Konishi et al. (1984) reported the development of myometrial smooth muscle cells from undifferentiated stromal cells in the human embryo. They examined human foetuses at various stages of development (weeks: 12, 14, 16, 18, 20, 26, 31, and 40) [47]. At 12 weeks gestation, the mesenchymal cells in the body of the uterus are round or stellate with large, round nuclei. There are scarce mitochondria, granular endoplasmic reticulum and free ribosomes and no intracytoplasmic filaments. There are negligible intercellular junctions and only sparse collagen fibrils. At 14–16 weeks gestation, the outer layer of the uterine wall consists of elongated cells with oval nuclei, poorly developed cytoplasmic organelles; a few filaments but no dense bodies. There are few small vesicles along cell membranes compared to what is observed in smooth muscle cells. Desmosome-like junctions between cells are rare. Cells near the inner mucosa remain unchanged compared to 12 weeks gestation. The cells in the outer layers become more elongated by 18–20 weeks. The nucleus becomes oval and the nucleo-cytoplasmic ratio is reduced. At this stage, intracytoplasmic organelles are well developed and intracytoplasmic filaments and dense bodies can be seen. There is an increase in surface vesicles but no external lamina or dense plaques along the cell membrane. Intercellular contacts and desmosome-like junctions start to appear. These features are consistent with immature smooth muscle cells. At 26 weeks gestation, the number of immature smooth muscle cells increase markedly. Filaments with dense bodies

become more abundant. At 31 weeks gestation the cells of the outer layer of the uterus are even more elongated, and have the ultrastructural characteristics of smooth muscle cells, including abundant filaments, dense bodies, dense membrane plaques, surface vesicles and an external lamina. Intracytoplasmic organelles are limited to the perinuclear region [47].

Thus, uterine smooth muscle originates from undifferentiated mesenchymal cells from the 18th week of gestation. Mitotic figures are mainly seen in the inner layer of the developing uterus. Immature muscle cells appear in the region between the myometrium and the developing endometrial stroma. These observations imply that undifferentiated mesenchymal cells which develop into smooth muscle cells exist in the inner layer of the fetal uterus and that smooth muscle differentiation occurs at the junctional zone. These are the same cells that are able to develop into endometrial stroma [47].

Studies of early uterine development in the neonatal mouse [51, 52] referred to above, also demonstrate the development of myometrial smooth muscle cells from the undifferentiated mesenchyme that gave rise to endometrial stromal cells. Endocrine disruption by tamoxifen in neonatal CD1 mice results in the development of adenomyosis. The expression of desmin is weaker in the myometrium in the uteri which developed adenomyosis following tamoxifen administration. On the other hand, the changes in desmin (and also of ACTA2, ESR1 and laminin) were not associated with the development of adenomyosis in the C57/BL6J mouse.

The Myometrium in Adenomyosis

Mehasseb et al. (2010) studied the ultrastructure of the myometrium in women with adenomyosis [115]. In the junctional zone in the normal myometrium there was a higher proportion of connective tissue-to-myocytes compared to women with adenomyosis.

They reported that myocytes in the JZ of the normal myometrium had sparse cytoplasm. Cell membrane showed the typical tri-laminar appearance and had an even distribution of short dense plaques (sarcolemmal bands or attachment plaques) alternating with prominent caveolae. The cytoplasm contained abundant myofilaments and dense bodies. The nuclei were fusiform and had blunt ends. Nuclei were centrally placed in the myocytes and had a crenated nuclear envelope. The chromatin material was dense and finely dispersed in the nuclear ground substance. In uteri with adenomyosis, the JZ myocytes were widely separated by a loose connective tissue matrix, with less prominent collagen fibrils. Myocytes had abundant cytoplasm consistent with cellular hypertrophy. There were fewer myofilaments arranged in less distinct bundles. Intermediate filaments were abundant but there was a tendency to form cytoplasmic aggregates. Myelin bodies (lipolysosomes) were similarly more frequent.

Compared to the normal JZ, the nuclei in adenomyosis were more round and were significantly enlarged. The nuclear envelope had a smooth outline. The nuclei showed a clear ground substance and prominent nucleoli. The nuclear chromatin was peripherally arranged. There were occasional infoldings in the nuclear envelope with entrapment of cytoplasmic organelles. The sarcolemmal bands were significantly longer with fewer caveolae. The perinuclear cell organelles (rough endoplasmic reticulum and Golgi apparatus) were more distinct and the internal cristae of the mitochondria exhibited unfolding. All this suggested a synthetic rather than a contractile tendency which is consistent with cellular hypertrophy. In the outer myometrium, the muscles cells showed similar features. The bundle structure was observed, but there was an increase in intercellular space and less dense collagen fibrils. An important observation is that the nuclei of both in the inner and outer myometrium in adenomyosis were significantly larger compared to the corresponding nuclei in control myometrium. Also, the sarcolemmal plaque length in adenomyosis was increased in adenomyosis compared to controls (Table 5.7).

Intracellular structures are known to change in response to steroids. The administration of supraphysiologic doses of estrogen to the rat has been

Table 5.7 Myocytes attachment plaques length (mm, mean ± SEM) and nuclear size (μm², mean ± SEM): measurements were based on 10 measurements per patient [115]

		Normal (n = 6)	Adenomyosis (n = 4)
Sarcolemmal plaques length	Inner myometrium	0.81 ± 0.1	1.33 ± 0.14
	Outer myometrium	0.66 ± 0.06	1.3 ± 0.08
Nuclear size	Inner myometrium	24.75 ± 0.41	26.34 ± 0.24[a]
	Outer myometrium	23.66 ± 0.34	24.71 ± 0.32[b]

[a]Significantly greater than normal junctional zone ($P < 0.01$)
[b]Significantly greater than normal outer myometrium ($P < 0.05$)

shown to result in enlargement of endoplasmic reticulum and to an increase in free ribosomes in myometrial smooth muscle cells [105]. Increased myofilament content and hypertrophy were induced with synthetic progestogens [116]. In uteri with adenomyosis, there was a loose connective tissue matrix and less prominent collagen fibrils which may be explained by intercellular space expansion. The presence of myelin bodies (lipolysosomes) have been linked to cell injury, particularly ischemia [117]. Nevertheless, the observation that outer myometrial cells from normal uteri contained an abundance of myelin bodies is unlikely to be explained by ischemia, because of the rich uterine blood supply. Thus, the cause of these myelin bodies in normal uteri remains speculative, but could possibly be related to postpartum involution.

In line with the observations made by light microscopy [118, 119], the nuclei in adenomyosis are enlarged and there is increased cytoplasm which is consistent with cellular hypertrophy. In the presence of adenomyosis, both the inner and the outer myometrial myocytes showed cellular and nuclear hypertrophy, abnormal nuclear and mitochondrial shape, abundant myelin bodies and intermediate filaments aggregates, extensive endoplasmic reticulum, and lengthening of sarcolemmal plaques with reduced caveolae. The findings indicate pan uterine affection rather than a disease solely of the inner myometrium. But it is not clear if these changes represent a primary myometrial defect or are secondary to the presence of adenomyosis.

The changes in nuclear shape are consistent with a change in the contractile function [120]. Thus, the nuclear abnormalities in adenomyosis may be explained by abnormal contractility. On the other hand, the increase in intracellular aggregates suggest an increase in intermediate filaments which may be related to an increased synthetic activity in the myocyte, as evidenced by the observed cellular hypertrophy, expanded cytoplasm, and by the increase in ribosomes and rough endoplasmic reticulum. The net effect can be an imbalance between the production and turnover of the cytoskeletal components.

The sarcolemma of smooth muscle cells is divided into two structurally distinct regions: those bearing submembranous dense plaques and intervening zones which bear many vesicular invaginations or caveolae. The dense bands are junctions of the adherens type, and serve as anchorage sites for actin cytoskeleton and are typically marked by antibodies to vinculin [121].

Caveolae have been implicated in a wide range of cellular functions. Caveolae contain a host of receptors, second messenger generators, G proteins, kinases, and ion channels in close proximity. Caveolae are often in close proximity to sarcoplasmic reticulum or mitochondria, and have been proposed to organize signalling molecules [122, 123]. The increased length of the dense bands that anchor intracellular myofilaments could reflect an increase in cytoskeletal filaments [124]. Abnormally shaped mitochondria with unfolded cristae suggest an abnormality in active cellular processes or the initiation of a degenerative process [117, 124]. These ultrastructural changes suggest a possible defect in myometrial contractility. Dysfunctional contractility could be the result of the presence of adenomyosis or could contribute to its pathogenesis.

Although there is no clear evidence of an impaired systemic hormonal milieu, local hyperestrogenaemia has been suggested to be involved in the development of uterine adenomyosis. Similar to uterine leiomyomata, estrogen was found to be synthesized and secreted by adenomyotic tissue [125]. In normal uteri, estrogen and progesterone receptors were suggested to show cyclic changes in the subendometrial myometrium but not in the overlying basalis endometrium [41]. Immunohistochemical studies of estrogen and progesterone receptors in adenomyosis foci and surrounding myometrium showed that ER was always present but in a reduced quantity when compared to the corresponding normal myometrium. In contrast, progesterone receptors were not always present [126].

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The Endometrium in Adenomyosis

6

Marwan Habiba and Giuseppe Benagiano

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 immunereactivity 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 cyclooxygenase-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 antiproliferative 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.

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The Animal Model of Adenomyosis

7

Marwan Habiba

Abstract

Spontaneous adenomyosis is known to occur in several animal species and experimental models were developed in laboratory animals through hormonal manipulation. The induction of adenomyosis in laboratory animals is species and strain dependent and is also highly dependent on the exact timing of the intervention. Neonatal administration of tamoxifen and altered prolactin production in the mouse are the models most widely reported in literature but the functional significance of the presence of adenomyosis in the mouse model and the relevance of these models to the human disease remain unclear.

Keywords

Spontaneous adenomyosis • Hyperprolactinemia • Pituitary graft • In utero exposure • Neonatal exposure • Steroids • Progestogen • Estrogen • Tamoxifen • Mouse strain

Spontaneous adenomyosis occurs in several animal species including nonhuman primates such as the rhesus monkey (Macaca Mulatta) [1] and baboon [2], dog [3], cat [4], and laboratory rodents and rabbit [5]. But the incidence in these species seems to be low. In the baboon adenomyosis was associated with infertility and with endometriosis [2]. More recently, two cases were reported in fertile chimpanzees that died at advanced age [5]. Spontaneous adenomyosis in CD-1 mice occurs in relation to age starting from about 6 months of age and by 12 months over 80 % are affected by a minimal degree of adenomyosis [6]. The incidence of adenomyosis was also high in the SHN and the SLN mouse strains that develop mammary tumours and in the GR/A and C3H/He strains [7]. Rabbit develops spontaneous adenomyosis and this can be enhanced by prolonged estrogen administration [8].

Animal models were developed through hormonal manipulation. This includes the induction of hyperprolactinemia by ectopic implantation

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of the pituitary gland either in one of the uterine horns or under the renal capsule in adult SHN and SLN mouse strains [9–11]. This is associated with a high success rate. Adenomyosis was also reported following neonatal administration of prolactin or dopamine antagonists [12]. Prolactin could be administered either in the neonatal period (day 1–14 of age) or young adult life (age 40–79) [11]. Pituitary grafting or fluoxetine also induces adenomyosis in the rat [13, 14]. Prolonged estrogenic stimulation [15], prolonged exposure of BALB/c mice to progesterone, norethisterone, or norethynodrel [16] also increase the incidence of adenomyosis. Steroids have also been used in combination. Prolonged treatment with 17β-estradiol and progesterone combination or with progesterone alone to ovariectomized mice that were given diethylstilboestrol in the neonatal period, also induced adenomyosis and the incidence of adenomyosis was comparable in both groups [17]. Induced adenomyosis in primates is more limited, but adenomyosis was reported in one out of six ovariectomised rhesus monkeys that were administered long term unopposed estrogen [18], but the numbers are too small to derive definite conclusions. However, both hyperprolactinemia and diethylstilboestrol result in complex hormonal imbalance which may influence the development of adenomyosis.

More recently, adenomyosis has been demonstrated in CD1 mice following oral administration of tamoxifen to female pups between days 1 and 5 of age. Adenomyosis was reported in all uteri examined at 6 weeks postnatal [19, 20]. Early features of adenomyosis were noted at postnatal day 10 [21]. Adenomyosis was also induced following the administration toremifene but not following the administration of an equivalent uterotropic dose of estradiol, or following the administration of raloxifene that has no uterotropic effect [19].

Features of adenomyosis in mice include marked disorganization of the myometrium and the mesenchyme. The mesenchyme surrounding the developing Mullerian duct gives rise to 90–95 % of the uterine mass and forms both the myometrium and the endometrial stroma [22]. The ovaries of the adult mice develop normally and contain corpora lutea.

Perinatal uterine development represents a critical phase for uterine development [23]. At birth, the uterus of CD1 mice is made of simple low columnar epithelium supported by undifferentiated mesenchyme, and lacks endometrial glands. As the uterus develops endometrial glands form from the luminal epithelium and the inner circular and outer longitudinal layers of muscle develop from the uterine mesenchyme [24–26]. With the development of adenomyosis, there is disruption of the inner myometrial layer and in-growth of endometrial glands and stroma.

Longer term follow-up demonstrated the development of adenomyosis in control CD-1 mice as well as in those administered tamoxifen [20], but disease severity was consistently less prevalent and severe than in treated mice. Advanced adenomyosis in mice exhibited massive uterine enlargement. The myometrium was thickened by bulky, enlarged, disorganised fascicles of smooth muscle with increased interstitial collagen. Extensively down grown endometrial glands were cystic and the endometrial epithelium showed areas of mild focal hyperplasia and squamous metaplasia [20]. Interestingly, tamoxifen treated mice appeared to have a normal estrus cycle.

The earliest uterine change in the pituitary graft model of adenomyosis is the infiltration of endometrial stromal cells into the inner myometrium along vascular and lymphatic network. Stromal cells translocate within the myometrium between the inner and outer smooth muscle layers. Electron microscopy reveals changes in the morphology of smooth muscle cells. These become markedly loosened, irregular in shape and reduced in size creating wider intercellular spaces. The muscle layers appear to disintegrate and the organelles become scant. Pyknotic muscle cells are frequently observed [27]. The findings suggest that invasion is preceded by degeneration of the muscle layer [11]. Yamashita and Mori reported increased cell permeability and increased apoptotic cell death in the myometrium adjacent to blood vessels [28]. The

sequence is thus different than that observed in the tamoxifen model.

The different effects of steroids on early uterine development may be related to differential effect on steroid receptors. In the CD1 mouse, estrogen receptor expression differs in the epithelium and stroma. In the epithelium ER is expressed at postnatal days 6–7 but Mullerian duct mesenchyme expresses ER from day 13 of gestation [29–31]. ESR1 mRNA is expressed at an earlier stage (fetal day 14) compared to ESR2 mRNA which in only detected in low-levels on the first postnatal day. ERα was detected using immunohistochemistry in stromal cells during late gestation and the postnatal period [32, 33], ERβ was not detected before postnatal day 6 [19, 32, 33]. An important observation is that the development of the model is also sensitive to the route of tamoxifen administration. Subcutaneous tamoxifen administration to neonatal mice results in the development of adenocarcinoma but not adenomyosis [34]. Neither adult rats nor mice develop uterine tumours following long-term treatment with tamoxifen [22, 35].

In neonatal mice, cDNA arrays showed that 24 h after cessation of treatment on day 6 after birth, key genes differentially modified by treatment included nerve growth factor alpha (NGFα), preadipocyte factor-1 and insulin like growth factor-2 [19]. A cDNA microarray demonstrated >200 genes that were differentially expressed when adenomyosis was compared to control CD-1 mice uteri. Of these, 12 genes were continuously up-regulated when the uteri were assessed at months 1.5, 3, 6, 9 and 12 [20]. These include NGFα and TGFβ induced. NGF and its low affinity receptor $p75^{NTR}$ are believed to have a role in myogenic differentiation and may have a role in modulating stromal development [20].

The exact genetic and environmental factors regulating cell line differentiation in the uterus are not fully known. Interaction of estrogen agonists with ESR1 plays a key role in the initial uterotrophic effect since immature ESR1 knockout mice when given tamoxifen show no significant increase in uterine weight [36]. The immunohistochemical changes observed following administration of tamoxifen to neonatal mice are due to a direct action on the uterus. Epithelial–mesenchymal interactions are recognized to play an important role in the postnatal development and the spatial organization of the uterus [25, 26, 37]. It has suggested that ESR2 could be involved in the differentiation process of stromal cells and fibroblasts into myofibroblasts in various breast tumours [38]. It is possible that similar mechanisms can be relevant to the pathogenesis of adenomyosis in response to tamoxifen. It is interesting to note that mice carrying latent alleles of active mutant K-ras developed endometriosis but not adenomyosis [39] suggesting different that the diseases have aetiologies [6].

In prolactin-induced adenomyosis, the myometrium becomes loose with increased intercellular space, and muscle cells become small and irregular. Disintegration of the muscle layer, with reduced cell organelles, and pyknosis of the myocytes were frequently seen in adenomyotic areas [27]. Adenomyosis development following tamoxifen treatment in CD1 mice could also be due to an alteration in the composition of the extracellular matrices. Increased matrix metalloproteinase-14 (MMP-14) may play an important role in facilitating the invasion of endometrial tissues into the myometrium in the SHN mouse model [40]. Laminins and fibronectins are important glycoprotein components of the extracellular matrix and basement membranes, and are critically involved in cell differentiation in early development and in tissue formation and maintenance of mature tissues [41, 42].

Despite the promise offered by the animal model, its relevance to human disease remains to be established. The hyperprolactinemia model is primarily applicable to mice strains with high incidence of mammary tumours, which indicate particular genetic tendency. Furthermore, the relation between hyperalgesia in these mice and adenomyosis need to be established. Prolactin (PRL) modulates sensory neurons and is tightly regulated by estrogen. Thus prolactin itself could contribute to the development of certain pain disorders. Prolactin has been shown to lower the activation threshold temperature in neurons, thus increasing sensitivity to noxious stimuli [43]. There are two isoforms of the prolactin receptor

(PRLR) termed the long and short isoforms. Most biological functions of the PRLR are attributed to the long form [44], but the short form may be the main receptor in the decidua and the ovary [45]. The differential expression of these receptors is regulated by estrogen [43, 46, 47] and endogenous prolactin has been shown to contribute to thermal hyperalgesia [48]. The CD-1 mouse model is interesting, but the model is strain specific. The feature of glands present within the myometrium is a response to an endocrine disruptor administered during a narrow window in organogenesis and no data is available about the reproductive performance of affected animals.

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Improving the Preclinical Mouse Efficacy Studies of Adenomyosis

8

Sun-Wei Guo and Marwan Habiba

Abstract

The development of specific therapeutics for patients with adenomyosis is a pressing unmet need. Yet no novel compounds are currently undergoing clinical evaluation. This may be attributable to our limited understanding of the pathophysiology of adenomyosis. Many methodological deficiencies in animal studies are an important cause of translational failure. Systematic review of published preclinical mouse efficacy studies of adenomyosis demonstrates the need for greater attention to rigorous methodology. Besides standard quality criteria that apply to all animal studies, there are issues that are specific to adenomyosis. Addressing these can improve the value of preclinical mouse efficacy studies in translational research. This chapter considers ways of improving mouse efficacy studies of adenomyosis and highlights areas where more research is needed.

Keywords

Adenomyosis • Animal studies • Drug development • Efficacy • Methodological quality • Mouse • Outcome measures

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Introduction

Undisputedly, the ultimate goal of medical research is to translate scientific discoveries into better prevention, and treatment of disease. Consequently, one of the hallmarks of success of research is the development of novel disease specific and patient tailored interventions. This is especially relevant for symptomatic adenomyosis because, currently, the only definitive treatment is by hysterectomy [1]. Hysterectomy is contraindicated in women who wish to preserve their fertility or who do not wish to have their uterus

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removed. Little research exists which focuses on the effect of medical treatments on adenomyosis. Available medical treatment options are not specific or curative, and symptoms can be expected to recur when treatment is discontinued.

Despite the clear unmet clinical need for non-surgical treatment, a search of clinical trials registries produces a disquieting picture. Compared with 2,368 trials on ovarian cancer, 242 trials on uterine fibroids and 243 trials on endometriosis, there are merely 37 registered trials on adenomyosis (www.ClinicalTrials.gov, accessed September 8, 2015). Among these 37 trials, only five are concerned with non-surgical treatments: the first addresses the use of acupuncture (Trial ID: NCT01259180; Estimated primary completion date: September 2011; Current status: Unknown), the second is a Phase I trial of the use of vaginal Bromcriptine, a potent dopamine D2 receptor agonist that is used to treat pituitary tumors and hyperprolactinemia (Trial ID: NCT01821001; Estimated primary completion date: June 2016; Current status: Recruiting), the third is a study of the use of letrozole, an aromatase inhibitor (Trial ID: NCT01218581; Current status: Completed), the fourth is a Phase II trail on the use of levonorgestrel-releasing intrauterine system (LNG-IUS) to treat adenomyosis (Trial ID: NCT01601366; Estimated primary completion date: April 2013; Current status: Unknown), and the fifth is a Phase III trial on the use of dietary supplement to treat adenomyosis (Trial ID: NCT02437175; Estimated primary completion date: March 2016; Current status: Recruiting). No novel compound is currently undergoing more advanced phase trials. A follow-up of the completed trial on letrozole indicates that its efficacy, evaluated 12 weeks after treatment, is comparable to GnRH agonist [2]. Both agents are associated with recognized side-effect profiles. This illustrates that drug development for adenomyosis seems to be at a standstill.

At least some of this undesirable picture can be attributed to our inadequate understanding of the pathophysiology of adenomyosis. A PubMed search using the phrase "adenomyosis not endometriosis" yielded only 631 publications (accessed September 8, 2015), compared to 20,679 entries against the phrase "endometriosis not adenomyosis" (accessed September 8, 2015). In other words, the volume of publications on adenomyosis is ~3 % of published work on endometriosis, even though the two conditions share many similarities including a similar definition, estrogen dependency, symptoms, treatment options, and many documented molecular aberrations [3, 4].

Extensive and often painstaking preclinical studies involving in-vitro and in-vivo experiments are often undertaken before embarking on clinical trials. Prior to clinical trials involving humans, a lead compound, called New Chemical Entity (NCE) identified through the course of drug discovery, requires a critical phase to evaluate its therapeutic potential, safety, toxicity, and pharmacokinetics. There is also a need to determine the physicochemical properties of the NCE, i.e. its chemical makeup, stability, solubility, and the potential for large scale production. Other hurdles must be overcome to satisfy the regulatory requirements before an Investigational New Drug (IND) is granted. Animal studies are often an important component in these processes.

The attrition rate of clinical phase trials is in the neighborhood of 95 % [5]. In endometriosis, the majority of completed phase II/III clinical trials especially of non-hormonal drugs are not published [6, 7] and are presumed to have failed [8]. The vast majority of drugs advanced into phase II/III clinical trials do not replicate the safety and efficacy promise reported in preclinical and animal studies. This results in considerable time and resource loss.

The reasons for translational inefficiency have been extensively discussed [9–12] and include methodological deficiencies in animal studies. But this has only recently received attention [13, 14]. This inefficiency prompted calls from various bodies including the US National Institute of Health for a greater focus on the characterization of suitable animal models [15, 16] and for transparent reporting [17].

A systematic review identified ten studies of drug efficacy in the mouse model of adenomyosis, all of these had methodological deficiencies [18]. None of the studies provided justification for number of mice or included sample size calculations. Eight studies indicated that the animals

were randomly allocated to treatment arms, but none described randomization method, allocation concealment or blinding. Of the ten studies, four were dose ranging and eight evaluated at least two disease-related outcomes but in only two cases were these outcomes related to a function such as pain behavior [18]. These methodological issues have general applicability and will not be discussed further as the focus here is on those issues that are particularly relevant to adenomyosis.

Choice of Induction Methods

Many animal models of adenomyosis have been reported [19]. In the mouse, these include: (1) ectopic grafting of the pituitary glands either directly into a uterine horn or under the renal capsule (EGPG) in the SHN or the ICR strain [20, 21]; (2) neonatal administration of tamoxifen (NAT) to the CD-1 mouse or to its genetically related ICR strain [22–24], and; (3) hormonal manipulation methods such as prolonged estrogen or progesterone administration [25, 26]. The hormonal manipulation methods have not been used in animal efficacy studies, possibly because of the need for a long induction period.

Both EGPG and NAT methods have a high success rate in inducing adenomyosis: over 90 % after EGPG [27] and a full 100 % after NAT [28]. Detailed histological studies have been reported on the outcomes of both methods [28–30]. NAT appears to be simpler and requires less time compared to EGPG which requires 7 weeks. EGPG also involves competent surgical intervention.

Neither method, however, is without limitations. First, both are strain-dependent [31]. In C57/BL6J mouse, NAT causes disruption of myometrial layers but not adenomyosis [32]. In fact, the change of route of neonatal tamoxifen administration alters outcome. At 3 months, neonatal CD-1 pups receiving subcutaneous tamoxifen exhibited derangement of the uterine stroma smooth muscle and mild endometrial glandular hyperplasia but no adenomyosis [33]. For the EGPG method, the induction rate seems to be sensitive to solvent solutions such as Dimethyl sulfoxide (DMSO), which may be presumed to be inert. The success rate was reported to be 40% 6 weeks after treatment with DMSO as controls [72].

It is debatable whether adenomyosis induced by EGPG or NAT truly recapitulates the human disease. The mechanism of adenomyosis induction using EGPG or NAT remains to be elucidated. It is possible that hyperplocatinemia induces adenomyosis through the induction of vascular changes and locally increased vascularity. EGPG has been shown to be inhibited by antiprogestogen (RU486) [34], danazol [21], bromocriptin [35], angiogenesis inhibitor [36] and also by the cholesterol lowering agent probucol [37] which also acts as an antioxidant. NAT may be preferable to EGPG due to its procedural simplicity, slightly shorter induction period and the demonstrated utility as a model for measuring the change of hypersensitivity to noxious thermal stimuli associated with the progression of adenomyosis [24, 38]. In contrast, no difference in food intake was reported in SHN mice with EGPG induced adenomyosis, following the administration of a matrix metalloproteinase inhibitor (ONO-4817) which is known to significantly reduce the incidence of adenomyosis [27]. Since reduced food intake is a pain-depressed behavior [39, 40], this finding appears to suggest that ONO-4817 treatment, while successful in reducing the incidence of adenomyosis, may not affect adenomyosis-induced pain behavior.

Aside from species differences, it is clear that neither EGPG nor NFT is relevant for studies of adenomyosis-related menorrhagia.

Outcome Measures

With a few exceptions, most published mouse preclinical studies of adenomyosis have used disease incidence, number of nodules, graded levels of myometrial infiltration, uterine weight (or weight-to-bodyweight ratio), or histology as outcome measures. However, these measures are *features* but not *symptoms* of adenomyosis. In contrast, women with adenomyosis seek medical attention when they become symptomatic and treatment success is measured by the ability to address the presenting problem(s). Thus, it is arguable that parameters used in animal studies

Table 8.1 Contrast of outcome measures used in mouse (or animal) efficacy studies of adenomyosis and clinical trials

Outcome measure	Animal efficacy studies	Clinical trials
Incidence of adenomyosis	+	–
Number of nodules	+	–
Depth of myometrial infiltration	+	–
Grade of levels of cell invasiveness	+	–
Uterine weight v. bodyweight ratio	+	±[a]
Food intake	+	–
Uterine contractility (ex vivo)	+	–
Evoked hypersensitivity	+	–
Spontaneous cyclic pain	–	+
Chronic pelvic pain	–	+
Dypareunia (presence/absence, severity)	–	+
Menstrual characteristics	–	+
Quality of life	–	+
Sexual satisfaction	–	+
Pain diaries	–	+
Adverse events	–	+
Global impression	–	+

[a]Uterus size by ultrasonographic measurement is frequently used

do not reflect clinical need and are not suitable outcome measures for clinical trials. The different focus is likely to add to barriers of successful translation from the bench to the bedside. Overall, there is little if any overlap between the outcome measures used in animal efficacy studies and clinical trial outcomes (Table 8.1).

There is no well documented correlation between the histological extent of adenomyosis and the severity of dysmenorrhea, the amount of blood loss or infertility in women [41]. Since rodents do not menstruate, the mouse model does not yield itself to the study of adenomyosis-related menorrhagia or dysmenorrhea. However, the induction of adenomyosis and its progression in mice is associated with increased sensitivity to noxious thermal stimuli [24, 38]. This is most likely due to central sensitization. It is possible that, similar to endometriosis [42], adenomyosis may be associated with features of neuropathic pain. As such, patients with adenomyosis may exhibit an array of sensory phenotypes including sensory gain [43]. In endometriosis such sensory gains [44] may be related to molecular changes in the central nervous system particularly in the dorsal root ganglia [45]. Since the severity of adenomyosis-associated pain in the mouse cannot be measured directly, sensitivity to noxious thermal stimuli has been used as a surrogate marker. For example, the hotplate test is performed by placing the mouse on a metal plate that is gradually heated to preset levels. The response latency is measured as the time from placement on the plate to the point when the mouse licks its hind paws. The hotplate test measures response thresholds to high intensity stimulus and is thus an "acute pain test" [46], but the response is mediated by spinal-brain stem-spinal reflexes [47]. Therefore, the test may reflect changes at the *supraspinal* level resulting from adenomyosis-induced pain, which supports its use as a surrogate measure of pain in adenomyosis. It also has the advantage of being simple to perform and of low cost. The tail-flick test is performed by directing a focused high-intensity light to generate a thermal stimulus 1–2 cm distal to the end of the tail. The time from start of stimulation to tail withdrawal determines tail-flick latency. The vaginal distention test was introduced as a non-thermal pain behavior measure [48]. The test is performed using a small latex balloon (10 mm long and 1.5 mm wide when not inflated) tied to a thin catheter. Immediately prior to the testing session, the un-inflated balloon is lubricated by K-Y jelly and inserted into the mid-vaginal canal. The balloon is gradually inflated in situ using a computer-controlled pump. The mouse is trained to respond to the noxious stimulus by interrupting the electric circuit which stops the pump. The test requires sophisticated equipments that are not widely available. Similar to dysmenorrhea, vaginal distention induces a form of visceral pain. Still, the relevance of these tests to women presenting with pelvic pain or dysmenorrhea is debatable. The mechanism of visceral pain is less well understood than somatic pain [49].

Caution should be exercised when using vaginal distention or response to noxious thermal stimuli tests to evaluate adenomyosis-associated pain behavior in mice. As in the case in neuropathic pain, sensory gain may well be a feature that is only

observed in a subset of patients [43, 50]. Vaginal distention test, hotplate test, and other tests that measure the response of mice to noxious stimuli actually evaluate the severity of *evoked* pain, i.e. the hypersensitivity of withdrawal reflexes to sensory stimuli. This contrasts with the primary efficacy measure(s) used in clinical trials of adenomyosis where the focus is on dysmenorrhea or chronic pelvic pain which are forms of *spontaneous* pain. These types of pain have never been evaluated in animal models of adenomyosis or endometriosis, presumably due to the enormous difficulties this entails.

Outside of the field of adenomyosis, various attempts have been made to measure spontaneous pain in rodents. For example, ultrasound vocalization at 22–28 Hz was reported to be associated with chronic pain in rats [51], but the value of this test has been disputed [52, 53]. Two recent studies described the use of facial grimace scale which is used to assess pain in infants, for evaluating pain severity in mice and rats [54, 55]. This model seems promising, but it remains to be determined if it can be applied to the assessment of adenomyosis-related pain in mouse, assuming that adenomyosis in mouse does indeed induce pain.

Abnormal uterine contractility may contribute to adenomyosis-associated dysmenorrhea and pain. Intensified and somewhat deranged uterine contractility have recently been documented and found to correlate with reduced response latency to noxious thermal stimulus in mice with induced adenomyosis [38]. Uterine contractility could also be a functional measurement in the sense that it may be used in efficacy evaluation. There is a need to develop neural biomarkers and objective correlates of adenomyosis-associated pain as these may provide more reproducible measures.

Significant Results: Statistical, Biological or Clinical?

Published mouse efficacy studies almost always report positive findings. But almost always, these studies report *statistically* significant differences in response or outcome measures. Few studies considered biological or clinical significance. Thus questions remain about the clinical significance of a demonstrable statistical difference.

Table 8.2 Suggested descriptors corresponding to the percentage change for the findings of mouse efficacy studies

Percentage of change	Descriptor of improvement/ reduction
<30	Moderate
30–50	Considerable
51–75	Substantial
>75	Profound

It is difficult to draw biologically meaningful conclusions from a statistically significant difference to put it simply, there is no conversion rule that could be applied. However, it would be helpful if studies report relative or percentage change in response to treatment as this enables a more intuitive appreciation of the importance of any reported changes. As a rule of thumb, barring a type I error, a 10 % change in a moderately powered study suggests that the intervention (which may still be efficacious) has not targeted major pathways, whilst an 80 % change may signal that a major pathway has been affected. Table 8.2 provides a proposed descriptor of possible biological significance in mouse studies.

Disease and Symptom Heterogeneity

Mice used in preclinical studies of adenomyosis are often genetically homogeneous (inbred strains such as SHN) or genetically similar (such as ICR or CD-1), and they are also identical in age and disease severity. The situation is different in women as patients are genetically heterogeneous and differ in a large number of important characteristics. In addition, in women, adenomyosis is often associated with co-morbidities such as endometriosis [56] and leiomyomas [57] or general health conditions such as hypertension and obesity which do not feature in animal models. Also important are factors relevant to parity. In NAT induced adenomyosis, affected mice appear to be uniformly hypersensitive to evoked pain [24, 38]. Women with adenomyosis, on the other hand, are symptomatically heterogeneous and a sizeable portion are *asymptomatic* [58]. All of this further complicates extrapolation from animal to human.

Chronicity and Treatment Duration

While the long delay from the onset of symptoms to diagnosis in endometriosis is well-documented [59–62], the symptom-to-diagnosis interval in adenomyosis has not been reported. As mentioned above, a sizeable portion of women with adenomyosis are asymptomatic. Adenomyosis is still more often diagnosed only in hysterectomy specimens of symptomatic women, thus it is likely that delayed identification of adenomyosis may well be the norm. Parker et al. reported that persistent dysmenorrhea following treatment for endometriosis is associated with increased junction zone thickness [63]. The severity of dysmenorrhea was significantly lower in patients with a junctional zone thickness (JZ) ≥11 mm compared with those with JZ <8 mm. There was a positive correlation between the severity of non-menstrual pain and JZ thickness ($r=0.51$, $p=.004$) at 3 months after treatment and a significant decrease in non-menstrual pain only in women with a JZ <8 mm [63]. Similar findings were reported by Ferrero et al. in a group of women who underwent surgical resection of pelvic and colorectal endometriosis [64]. Delayed diagnosis is also suggested from the study of Kissler et al. who demonstrated that dysmenorrhea of long duration in patients who have had endometriosis for over 11 years is significantly related to uterine adenomyosis [65].

The relative lifespan of a laboratory mouse is approximately 37 times shorter than human life. The average estimate of the diagnostic delay for endometriosis is 7 years [59–62], which for a mouse translates into about 9.8 weeks or 69 days. This is still likely to be an underestimate as it does not take into account the time lapse from the *genesis* of endometriosis to it becoming symptomatic. In order to mimic the situation in humans, this time lag may be considered as the minimal induction of adenomyosis to start of treatment interval in the mouse model. Yet only 4 of 11 published drug efficacy studies had an induction period longer than 69 days (Table 8.3). The relevance of the induction period is emphasized by the finding that treatment efficacy in mouse models seems to be negatively correlated with the length of induction [18].

In the 11 published studies referred to above, the longest duration of treatment following EGPG- or NAT was 90 and 60 days, respectively [18] which correspond to treatment in humans for about 9.1 and 6.1 years. This is probably way too long and while prolonged treatment seems to correlate with increased efficacy in mouse (Fig. 8.1) [18], it increases cost, risk of side-effects and non-compliance. It is interesting to note that the question of treatment duration in preclinical studies of adenomyosis is not considered in literature but extrapolation from lifespan suggests that treatment for 1.4–3 weeks can be a good start.

Assessing True Efficacy

The use of positive control drugs, defined as a drug currently in use with documented efficacy, can provide a more useful comparator and improve the predictive value of mouse efficacy studies, but this was included in only three out of the 11 mouse efficacy studies published after 2007 [18].

The use of a single dose, though logistically easier, provides no information on the minimally effective or maximally tolerated doses or on the therapeutic range. Single dose selected for studies risk being above or below the optimal therapeutic range. Therefore, it is recommended that two or three doses be tested. But of the 11 studies, only 5 used two or more dosages [18] (Table 8.3). Of note, the presence of a dose–response relationship is a strong indication that the compound of interest has therapeutic potentials, which provides added assurance of efficacy.

Biomarkers for Monitoring Treatment Response

So far, there are no biomarkers for monitoring treatment response. Although uterine size is often used as an indication for therapeutic efficacy, this does not closely correlate with symptom severity, nor does it indicate the disruption of pathways relevant to adenomyosis. An ideal biomarker is

Table 8.3 The characteristics of published preclinical mouse efficacy studies

ID	Publication	Compound(s) tested	Mouse strain	Method of induction	Length of induction (days)	Treatment duration (days)	Major outcome measures	Randomized?	Include dose-response?	Sample size[a]
D1	Singtripop et al. (1992) [69]	Danazol	SHN	S	NA	35 (+35 days no Tx)	P	Yes	No	8+13
D2*				EGPG	0	35 (+35 days no Tx)	P	Yes	No	9+11
K	Mori et al. (1993) [13]	KBG	SHN	S	NA	95	P, H	Yes	No	11+13
M	Zhou et al. (2000) [15] (2000)	Mifepristone (SPRM)	SHN	EGPG	21	28	P, UWW	Yes	No	10+9
O1	Mori et al. (2001) [17]	ONO-4817 (MMP inhibitor)	SHN	EGPG	7	42	P, FI, GLCI	No	Yes	12+12
O2*					42	28	P, FI, GLCI	No	No	10+10
C1	Mori et al. (2002) [18]	CP8816, CP8863 (SPRM)	SHN	EGPG	0	41	P	No	No	10+10
C2*					7	35	P	No	No	12+10
T	Zhou et al. (2003) [19]	TNP-470 (anti-angiogenic)	SHN	EGPG	1	41	P, BW, OW, EC	Yes	No	15+15
P	Zhou et al. (2004) [20]	Probucol (hypo-cholesterolemic)	SHN	EGPG	1	41	P, BW, OW, SL	Yes	No	10+10
U	Zhang et al. (2008) [22]	Danazol-IUD	ICR	EGPG	120	60	UWW/BW ratio, N, HE	Yes	Yes	5+5
V	Liu and Guo (2011) [23]	VPA (HDACI)	ICR	NFT	84	28	UWW/BW ratio, D, HP, TF	Yes	Yes	12+12
H	Mao et al. (2011) [24]	*l*-THP, Andro, VPA	ICR	NFT	107	21	D, HP, UT, UWW	Yes	Yes	10+8
G	Chen et al. (2013) [76]	EGCG	ICR	NAT	107	21	D, HP, UT, UWW	Yes	Yes	28+12

Revised and updated from Guo [18]

Abbreviations: *KBG* Keishi-Bukuryo-Gan, a Chinese herb concoction, *MMP* matrix metalloproteinase, *SPRM* selective progesterone receptor modulator, *IUD* intrauterine device, *VPA* valproic acid, *HDACI* histone deacetylase inhibitor, *l-THP* levo-tetrahydropalmatine, *Andro* andrographolide, *S* spontaneous, *EGCG* epigallocatechin-3-gallate, *EGPG* ectopic graft of pituitary gland, *NAT* neonatal administration of tamoxifen, *H* histology, *P* prevalence, *FI* food intake, *GLCI* Graded levels of cell invasiveness, *UWW/BW* ratio: uterine wet weight vs. bodyweight ratio, *N* number of nodules, *HE* H-E staining, *D* depth of myometrial infiltration, *HP* hotplate test, *TF* tail-flick test, *UT* uterine contractility, *OW* organ weight, *SL* serum levels of lipids, *EC* estrous cycle

[a]Sample size of the treatment and the control groups combined

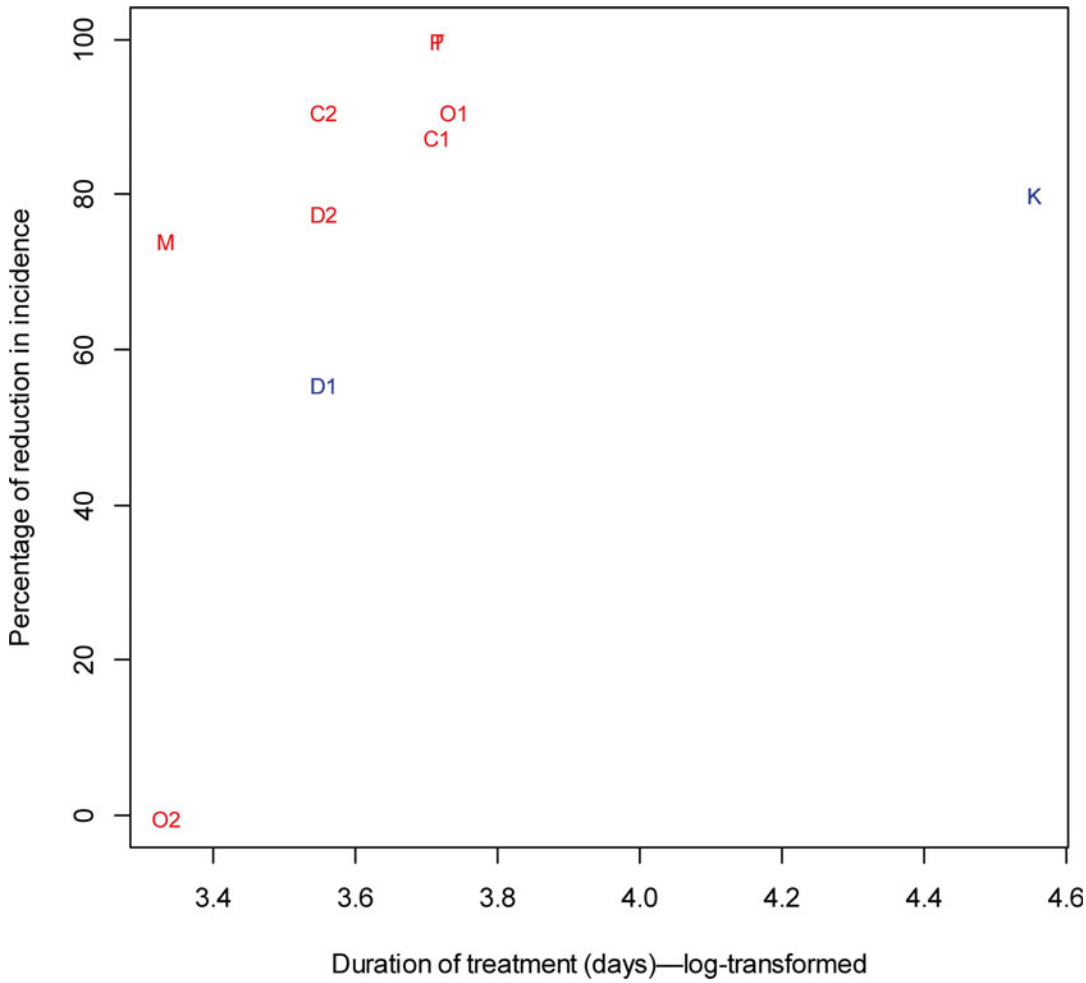

Fig. 8.1 Scatter plot of the difference in incidence of adenomyosis between treatment and control groups vs. duration of treatment (in days). The alphabets were identifications of experiments listed in Table 8.3. The experiments with *blue* alphabets were done with spontaneous adenomyosis in SHN mice, while those with *red* were performed with ectopic graft of pituitary glands

one that indicates disease burden or whether the intended therapeutic target(s) has been achieved. This is an area for future research but the challenges are considerable given the limited understanding of the molecular mechanisms involved and, consequently, the limited understanding of the best molecular targets for therapeutics.

One possible way forward involves the use of the mouse model to identify molecular mechanisms including biomarkers for CNS sensitization. This may enable the exploration of the link between the evoked pain (e.g. hotplate test) response and molecular changes in the CNS and the dorsal root ganglia and their modulation by therapeutics. Such work may provide an opportunity to extrapolate findings to humans. It is also possible that the use of sensory tests in patients with adenomyosis may provide an insight into the mechanisms involved in CNS sensitization.

Conclusions

The management of adenomyosis remains challenging [66]. Developing specific and more efficacious therapeutics is a pressing clinical need. Progress requires better understanding of the pathophysiology of adenomyosis. Despite their limitations which should be

recognized, the small number of available animal models provides an opportunity to further our understanding of the disease. Mouse models [28, 32] may be useful toward characterizing the molecular and cellular aberrations and the mechanisms involved in CNS sensitization. But there is also a need for the design of better mouse efficacy studies

Given the high prevalence, it is surprising that drug development for adenomyosis is still in its infancy. There are many challenges but the introduction of accurate imaging techniques such as transvaginal sonography and MRI [67] provide important opportunities for progress.

As previously reviewed [18], published preclinical mouse efficacy studies of adenomyosis have several deficiencies. There is a need to appreciate the advantages and limitations of the animal model and for a critical evaluation of research methods. The use of at least one functional outcome and greater attention to dosing regimen can enhance the validity of mouse studies. The ARRIVE guidelines [68] and the established core set for reporting standards for experimental design, execution, and reporting [17] provide a useful framework. In addition, the predictive power may be enhanced by careful consideration of methodological factors such as induction methods, outcome measures, and use of multiple doses and by controlling for heterogeneity, chronicity and treatment duration. Outcomes should take into account the distinction between statistical, biological, and clinical significance [8]. Incidentally, one Phase I trial registered at ClinicalTrials.gov examining *vaginal* bromocriptine, seems not to have published preclinical efficacy studies prior to its launch, despite the known link between adenomyosis and dopamine D2 receptor deficiency [69, 70].

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Conflict of Interest Statement None declared.

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9 Adenomyosis and Ultrasound: The Role of Ultrasound and Its Impact on Understanding the Disease

Caterina Exacoustos

Abstract

Transvaginal sonography had a sensitivity of 80–86 %, specificity of 50–96 %, and overall accuracy of 68–86 % for diagnosing diffuse adenomyosis. These figures are poorer in the case of focal adenomyosis or if there are coexistent fibroids. Three dimensional (3D-TVS) transvaginal sonographic signs of adenomyosis are based on the evaluation of the junctional zone on the acquired volume of the uterus in order to obtain the coronal view. Three dimensional transvaginal sonography seems to be more accurate than conventional two dimensional (2D-TVS) ultrasound in detecting adenomyosis.

A strong association is found between deep infiltrating endometriosis and the presence of 2D-TVS/3D-TVS features of adenomyosis. A detailed non-invasive diagnosis of the extent of adenomyosis can facilitate the choice of safe and adequate treatment.

Keywords

Adenomyosis • Junctional zone • 3-dimensional ultrasound • 2-dimensional ultrasound • Two dimensional ultrasound • Three dimensional ultrasound • Transvaginal ultrasound • Magnetic resonance imaging • Junctional zone • Volume contrast imaging • Multi-planar view • Diffuse adenomyosis • Focal adenomyosis • Doppler flow

Introduction

Adenomyosis is a common gynaecologic disease characterized by the migration of endometrial glands and stroma from the basal layer of endometrium into the myometrium, and associated smooth muscle hyperplasia. This generates ultrasound appearance of ill-defined lesions within the myometrium. Adenomyosis may be

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present in one or more sites within the uterine wall or may involve most of the myometrium. Often adenomyosis is dispersed within the myometrium (*diffuse adenomyosis)* rather than being confined to localised lesions. On the other hand, *focal adenomyosis* is the term used to describe adenomyosis present in only one part of the myometrium. Adenomyoma is used to describe a focal lesion with additional compensatory hypertrophy of the surrounding myometrium and in rare cases it may present as a large cyst (*adenomyotic cyst* or *cystic adenomyoma*).

Detection of adenomyosis remains a diagnostic challenge. Transvaginal ultrasound and magnetic resonance imaging (MRI) have high levels of accuracy in the preoperative diagnosis of adenomyosis [1]. Several studies have illustrated that the sensitivity and specificity of two dimensional transvaginal sonography (2D-TVS) in diagnosing adenomyosis are comparable to those of MRI and/or histology ranging from 75–88 % and 67–93 % respectively [2–6]. However, compared to MRI, transvaginal ultrasound is well tolerated by patients, is repeatable, inexpensive and widely available.

The presence of adenomyosis denotes hyperplasia and hypertrophy of myocytes surrounding heterotopic endometrial tissue and can be seen on T2-weighted MRI as diffuse or focal thickening of the junctional zone (JZ). The 2D-TVS features of adenomyosis described in the literature are generally alterations of the outer myometrium. Because optimal sonographic differentiation into inner and outer myometrium is often absent, 2D-TVS transvaginal sonographic evaluation of the junctional zone, including with the use of high-frequency probes (5–10 MHz), is often difficult and imprecise. Recently, it has been observed that it is possible to visualize the junctional zone more clearly with some post-processing using coronal section of the uterus obtained with three dimensional (3D-TVS) transvaginal sonography [7–9].

Table 9.1 Summary of the ultrasound features associated with histological diagnosis of adenomyosis

	Feature
2D-TVS	
Serosal contour of the uterus	Uterus often globally enlarged
Definition of lesion	Ill-defined in diffuse adenomyosis (adenomyoma may be well-defined)
Symmetry of uterine walls	Anterior-posterior myometrial asymmetry
Outline	Ill-defined
Shape	Ill-defined
Contour	Irregular or ill-defined
Rim	No rim
Shadowing	No edge shadows, fan shaped shadowing, linear hypoechoic striation
Echogenicity	Non-uniform Presence of intramyometrial: Mixed echogenicity Cyst Hyper-echogenic islands Subendometrial echogenic lines Buds
Vascularity	Translesional flow Diffuse minimal or few vessels
Endometrial rim	Irregular or ill-defined Distorted or imprinted
3D-TVS	
JZ thickness	Thickened JZ: Maximum JZ thickness (JZ_{max}) > 6–8 mm Ratio of JZ (JZ_{max}/total myometrial wall thickness) ≥50 % Difference (JZ_{max}) − (JZ_{min}) = JZ_{dif} ≥4 mm
JZ regularity	Irregular or ill-defined Distorted
JZ interruption	Interrupted Infiltration of the JZ by hyperechoic endometrial tissue

2D-TVS Features of Adenomyosis

Continuous improvements in the resolution of transvaginal ultrasound have enabled a more detailed assessment of uterine architecture. This has facilitated the detection of myometrial features of adenomyosis which could not have been seen with older ultrasound equipment.

According to several studies, the following 2D-TVS features were considered associated with adenomyosis [1, 5, 10–13] (Table 9.1):

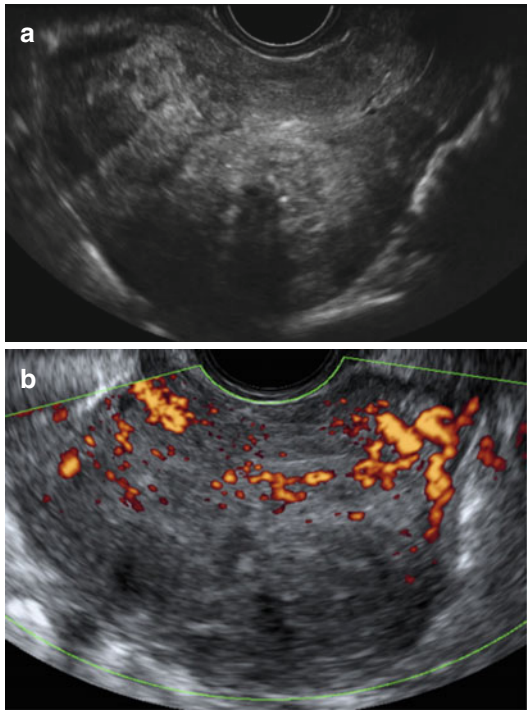

Fig. 9.1 Ultrasound images of a uterus with adenomyosis. (**a**) Gray scale image showing globally enlarged uterus unrelated to leiomyoma with irregular myometrial echotexture with hyperechoic irregular myometrial areas and small anechoic areas. Note the ill-defined endometrial stripe. (**b**) Power Doppler image showing diffusely spread vessels without the circular flow along a capsule that is typical for leiomyoma

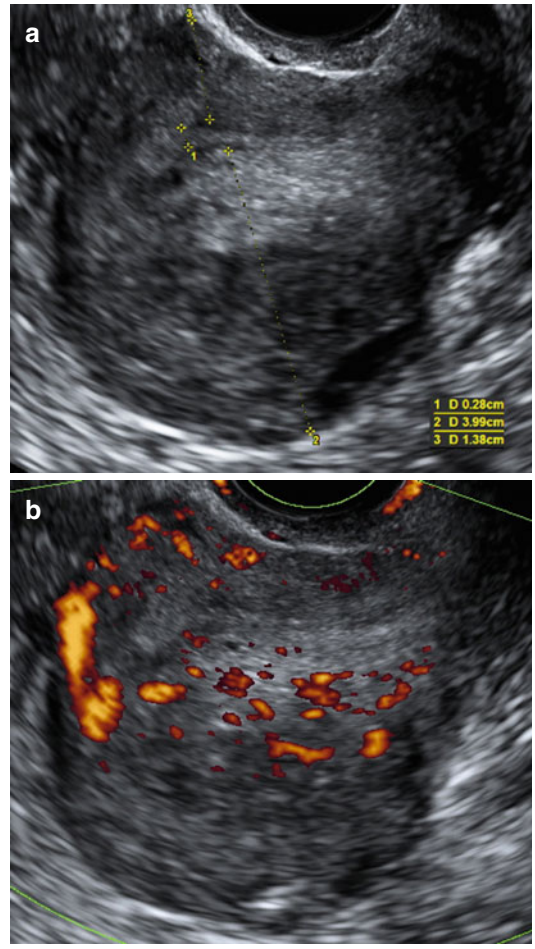

Fig. 9.2 Ultrasound images of a uterus with adenomyosis. (**a**) Gray scale image showing asymmetrically thickened, posterior wall (*2*) is thicker than anterior wall posterior uterine wall 3, with inhomogeneous, irregular myometrial echotexture due to hyperechoic and small cystic anechoic areas. Endometrial thickness (*1*). (**b**) Power Doppler image showing diffusely spread small vessels

- a globally enlarged uterus: the fundus of the uterus appears enlarged (Fig. 9.1)
- asymmetrically enlarged uterus (for example anterior wall thicker than posterior wall or vice versa) unrelated to leiomyoma (Figs. 9.1 and 9.2)
- round cystic area within the myometrium (Fig. 9.3)
- inhomogeneous, irregular myometrial echo texture in an indistinctly defined myometrial area with decreased or increased echogenicity; hyper-echogenic islands, subendometrial lines and buds (Fig. 9.4)
- myometrial hypoechoic linear striations seen as a radiating pattern of thin acoustic shadows not arising from echogenic foci or leiomyoma (fan shaped shadowing) (Fig. 9.3)
- indistinct, fuzzy endometrial-myometrial border (ill-defined endometrial stripe) (Figs. 9.1 and 9.2)
- presence of diffuse minimal vascularity seen as diffusely spread of small vessels which do not have the normal course of the arcuate and radial arteries inside the myometrium (Figs. 9.1, 9.2, 9.5, and 9.6)

Moreover, a new interesting sign called question mark form of uteri was reported recently [14]. This is described when the corpus uterus is flexed backwards, the fundus of uteri is facing the posterior pelvic compartment and the cervix is directed frontally towards the urinary bladder (Fig. 9.7).

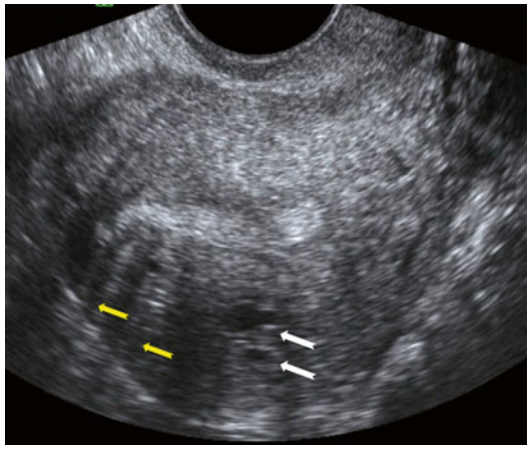

Fig. 9.3 Ultrasound image of a uterus with adenomyosis. Note the round cystic anechoic areas (*white arrows*) in the myometrium below the endometrium and hypoechoic linear striation (*yellow arrows*)

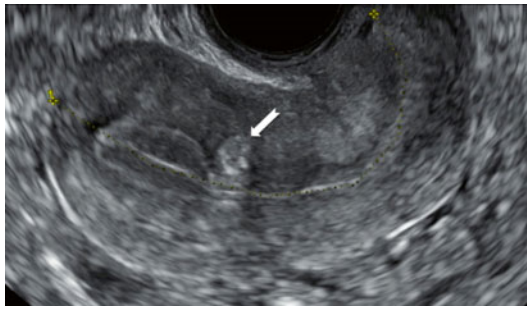

Fig. 9.4 Ultrasound image of a uterus with a hyperechoic buds (*white arrows*) in the myometrium beneath the endometrium

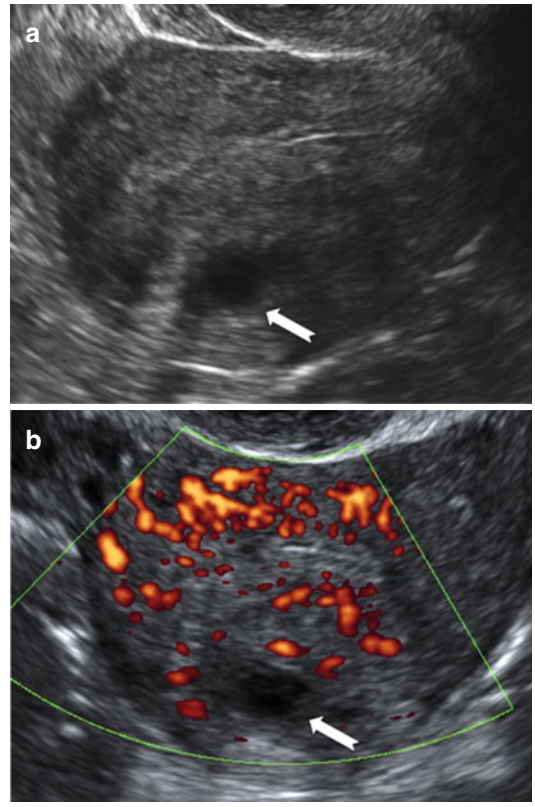

Fig. 9.5 Ultrasound images of a uterus with focal cystic adenomyosis of the posterior wall. (**a**) Gray scale image showing a cystic anechoic area in the myometrium of the posterior wall (*white arrow*). (**b**) Power Doppler image showing diffusely spread vessels around the cystic area (*white arrow*)

Power Doppler can be used to distinguish myometrial cysts from blood vessels and to discriminate between leiomyomas and focal adenomyosis (Figs. 9.5 and 9.6). Uterine leiomyomas feature circular flow along the myoma capsule, while localized adenomyosis and adenomyomas are characterized by diffusely spread vessels inside the lesions (Figs. 9.1, 9.2, and 9.8). Reinhold et al. reported that 2D-TVS had a sensitivity of 80–86 %, specificity of 50–96 %, and overall accuracy of 68–86 % for diagnosing diffuse adenomyosis [2]. However, 2D-TVS can yield equivocal result in the case of focal adenomyosis and in the presence of coexistent fibroids [1, 5]. A recent meta-analysis of 14 trials involving 1985 participants, reported the sensitivity and specificity of ultrasound diagnosed adenomyosis to be as high as 82.5 and 84.6 %, respectively [15].

Usually the diagnosis adenomyosis is made on histological examination of a uterus following a hysterectomy. The histological frequency of adenomyosis ranges from 5 to 70 % according to reported series. The wide variation is affected by the histological criteria and the number of sections examined. The majority of previous studies reporting the accuracy of 2D-TVS diagnosis of adenomyosis have assessed populations of women who underwent hysterectomy [1, 15, 16]. These included mainly women with severe symptoms who were more likely to have adenomyosis compared to the general population, and it is likely that the prevalence of adenomyosis in these studies is an overestimate.

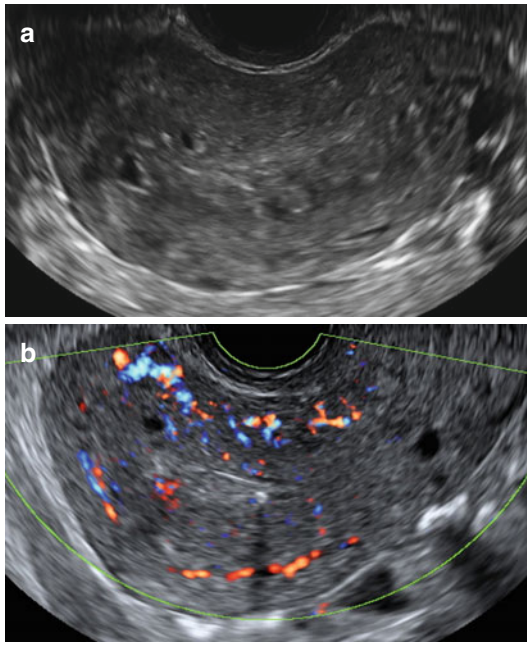

Fig. 9.6 Ultrasound images of a uterus with diffuse cystic adenomyosis of the uterus. (**a**) Gray scale image showing several small cystic anechoic areas and hyperechoic islands in the myometrium, note the ill-defined endometrial stripe. (**b**) Power Doppler image showing diffusely spread vessels in the affected myometrium

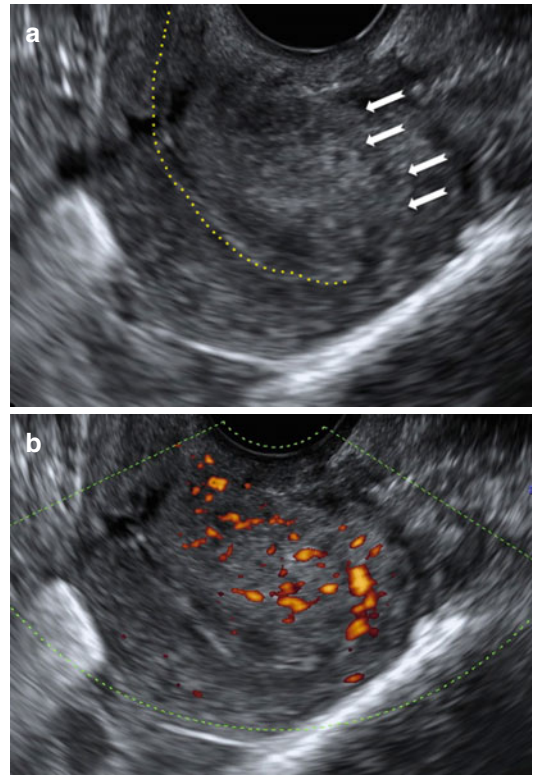

Fig. 9.7 Ultrasound images of a retroverted uterus with adenomyosis with the typical 'question sign'. (**a**) Gray scale image showing asymmetrically thickened posterior uterine wall with abnormal echogenicity (*white arrows*). (**b**) Power Doppler image showing diffusely spread small vessels

Furthermore, the 2D-TVS findings are more likely to appear in advanced stages of the disease. Most of the studies utilising TVS required the presence of at least two or three of ultrasound features for the diagnosis of adenomyosis [8, 9, 17–19]. But the presence of only one of the typical TVS features of adenomyosis raises some concern especially in young women (Fig. 9.9).

Targeted ultrasound guided biopsies have been proposed in an attempt to correlate histological findings to ultrasound features of adenomyosis in those younger women who will not undergo hysterectomy [20–22]. Recently, Nam and Lyu, performed abdominal ultrasound-guided transvaginal myometrial core needle biopsy in 1032 premenopausal women aged 22–53 years who had 2D-TVS findings suggestive for adenomyosis [22]. They reported a 92.26 % concordance rate between the transvaginal myometrial core needle biopsy and ultrasonographic diagnoses of adenomyosis [22].

The severity of adenomyosis is difficult to express in quantitative terms as the lesions are often poorly defined and may be disseminated throughout different parts of the myometrium. The number of different morphological features in an individual woman has been proposed as an indirect semi-quantitative measure of severity of adenomyosis [8, 19].

Tomassetti et al. defined a number of factors encouraging the development of adenomyosis including a history of spontaneous miscarriage, curettage, hysteroscopic resection of the endometrium, uterine myomectomy, caesarean section and the use of tamoxifen [23]. Furthermore, there are a number of studies that reported a correlation between ultrasound findings of adenomyosis and symptoms like menometrorrhagia or dysmenorrhea, infertility and multiparity has

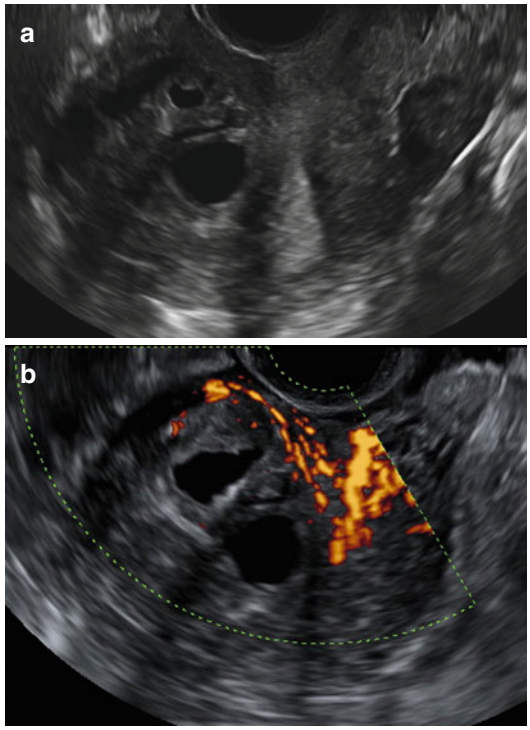

Fig. 9.8 Ultrasound images of a uterus with an anterior cystic adenomyoma. Gray scale image showing inhomogeneous, irregular myometrial echotexture with cystic areas (**a**). Power Doppler image showing more circular vessels in the hypertrophic myometrium surrounding the cystic areas (**b**)

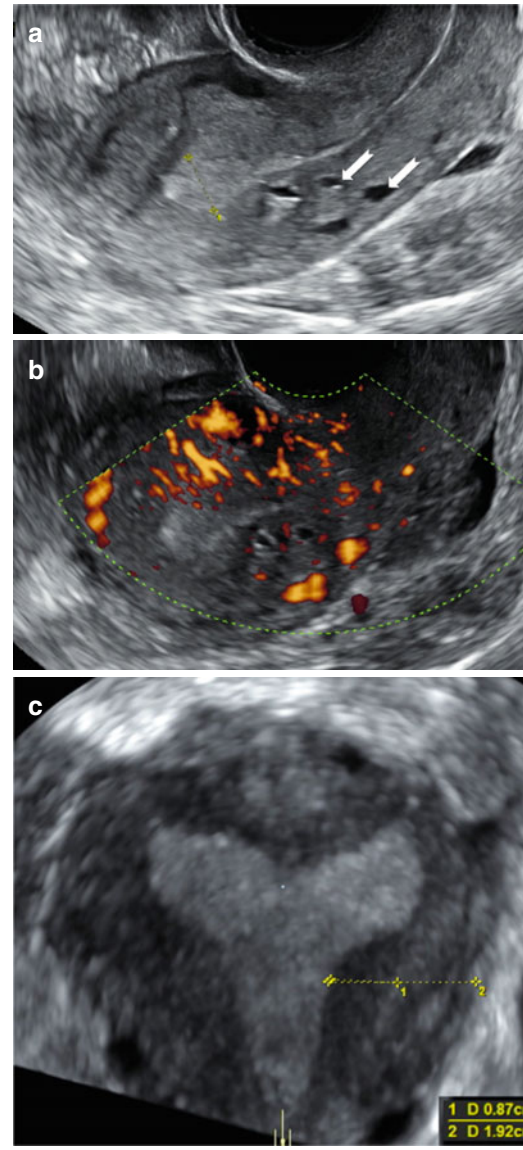

Fig. 9.9 Ultrasound images of a uterus in a 25 year old woman with cystic areas only in the posterior wall (*white arrows*). Gray scale image (**a**) and Power Doppler image (**b**) showing diffusely spread small vessels surrounding the cystic areas. Coronal section (**c**) obtained by 3D ultrasound of the same uterus showing a thickened JZ

been shown [24–26]. Therefore the association with symptoms could improve the diagnostic accuracy of ultrasound features for adenomyosis [8, 19, 24].

Adenomyosis seems also to be associated with endometriosis [17, 27, 28]. According to some authors, adenomyosis seems to share pathogenic mechanisms as well as similar symptoms (menometrorrhagia dysmenorrhea, dyspareunia, abnormal uterine bleeding and infertility), with endometriosis [27, 29, 30]. Knowing the prevalence of adenomyosis in a population with endometriosis helps in evaluating the likely contribution of adenomyosis to their symptoms.

Di Donato et al [2014] reported recently on a series of patients undergoing surgery for endometriosis a prevalence of adenomyosis diagnosed by ultrasound of 21.8 %. This prevalence is slightly higher than the prevalence (20.9 %) reported by Naftalin who evaluated patients attending a general gynecological ultrasound unit [28]. An interesting feature is the strong association between deep infiltrating endometriosis and adenomyosis reported in some recent studies [17, 31]. In a recent study, Lazzeri et al. confirmed the strong association between adenomyosis diagnosed by transvaginal

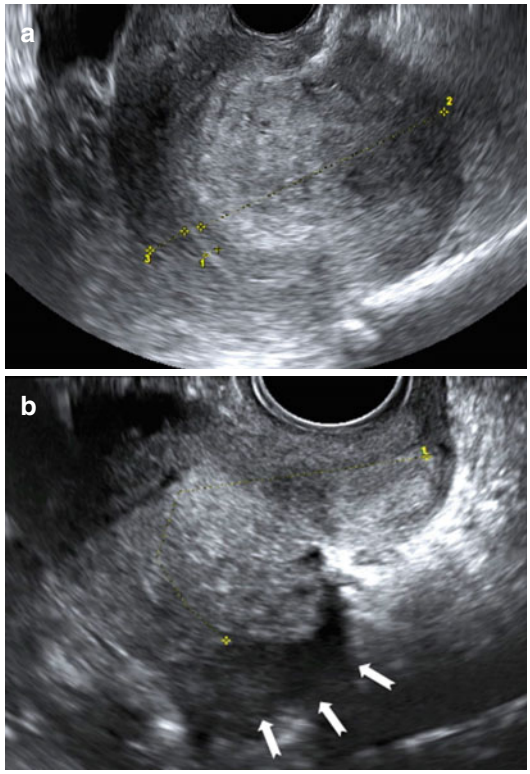

Fig. 9.10 Ultrasound images of a retroverted uterus with adenomyosis with the typical 'question sign'. (**a**) Grayscale image showing asymmetrically thickened posterior uterine wall (*2*) with abnormal echogenicity, (*1* endometrila thickness, *3* thicknes of the anterior uterine wall). (**b**) Posterior deep infiltrating lesions (*white arrows*) involving the adenomyotic myometrium and infiltrating the rectal wall posteriorly

ultrasound and endometriosis. The incidence of adenomyosis in patients affected by deep infiltrating endometriosis was 48.7 % [17]. Di Donato et al. found that the ultrasound 'question mark sign' of the retroverted fixed uterus, was strongly related to posterior deep infiltrating endometriosis (Fig. 9.10) [14].

Two dimensional transvaginal sonography has now achieved a high level of accuracy and many authors have reported high agreement between ultrasound diagnosis of adenomyosis and histological findings. A recent review concluded that transvaginal sonography should be the primary tool for the diagnosis of adenomyosis, with MRI being reserved for cases where TVS is inconclusive or in the presence of large fibroids [1].

3D-TVS Features of Adenomyosis

Although the junctional zone (JZ) can be visualized on 2D-TVS, acquisition of a 3D-TVS-volume enables more complete assessment in the sagittal, transverse and coronal planes as shown in a standardized multi-planar view [7, 9, 13] (Fig. 9.11). 3D-TVS signs of adenomyosis are based on the evaluation of the junctional zone on the acquired volume of the uterus in order to obtain the coronal view. In the coronal view, the junctional zone appears as a hypoechoic zone around the endometrium. Using the volume contrast imaging (VCI) modality with 2–4 mm slices the JZ can be seen clearer in all planes of the multiplanar view, this is also the case in the longitudinal and transverse uterine sections where the anterior and posterior junctional zones could be evaluated [8, 9, 13] (Fig. 9.11). The standardized multi-planar view which may be obtained by the z-rotation technique, is used in clinical practice for the evaluation of the coronal view as it reduces inter-observer variation in measurements. Imaging of the JZ may be optimized by using a post-processing rendering mode, for example VCI. The thickness of the slices or render box may be selected between 1 and 4 mm [8, 9, 13].

The JZ may be regular, irregular, interrupted, not visible, not assessable or may manifest more than one feature (e.g. irregular and interrupted). Any irregularity in the JZ can be described (e.g. cystic areas, hyperechogenic dots, hyperechogenic buds and lines) in each location of the uterus (anterior, posterior, lateral left, lateral right, fundus) [8, 9, 13] (Table 9.1).

In order to avoid reliance on subjective morphological descriptions of the JZ in terms of irregularity and infiltration, objective parameters were proposed. These include measurement of the thickness of the JZ in a manner familiar to the case of MRI evaluation [9, 32, 33]. The JZ and the total myometrial wall thickness can be measured perpendicular to the endometrium in the same section through the uterus. The maximum *thickness* of the junctional zone (JZ_{max}) is measured at the area where the JZ appears to be at its thickest, and the minimum thickness (JZ_{min})

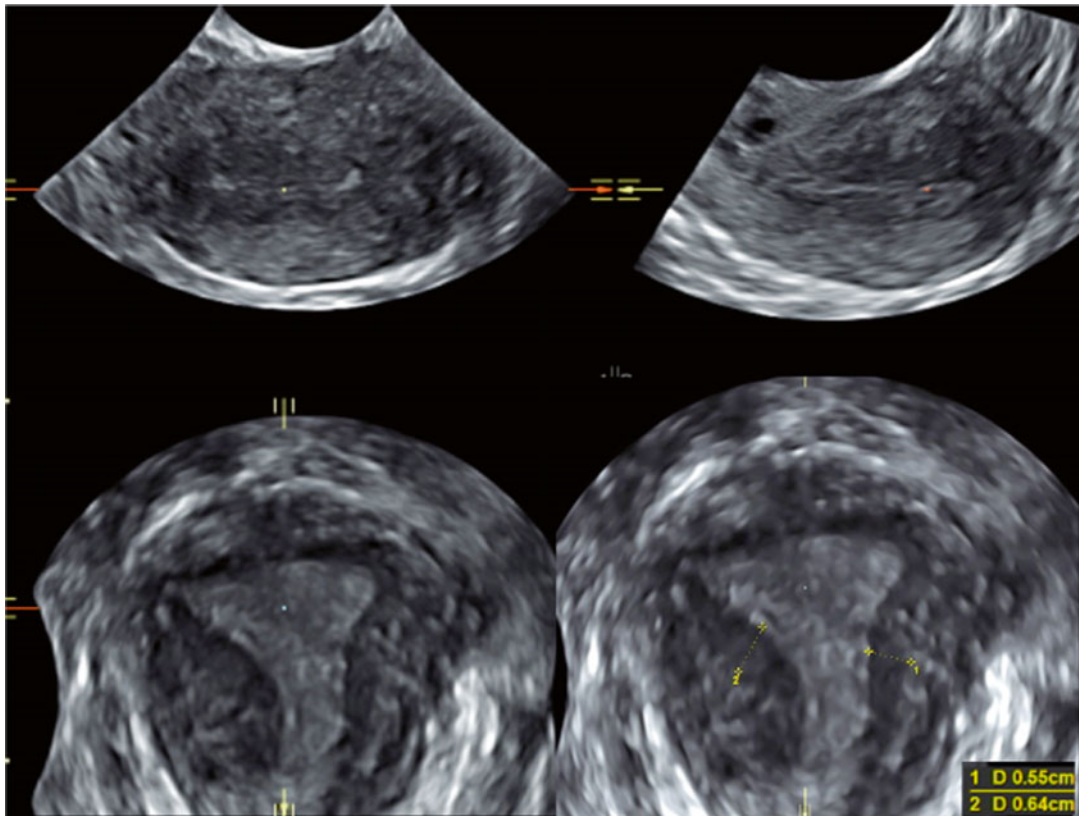

Fig. 9.11 Ultrasound image of the uterus obtained using three-dimensional ultrasound and volume contrast imaging (VCI) with 4 mm slices. A multiplanar view is shown: transverse and coronal sections of the uterus are shown on the *left side* of the image, a longitudinal section is shown on the *right side* of the image. The thickened junctional zone appears as a hypoechoic zone surrounding the endometrium. 2D ultrasound features of adenomyosis are not clearly seen in the longitudinal and transverse sections. However, in the coronal section, a slightly thickened distorted junctional zone is seen on the left

is measured where it appears to be at its thinnest after evaluation of the total three-dimensional volume of the uterus (Fig. 9.6). To define the *ratio* between the JZ and the total uterine wall thickness, both the JZ and the total uterine wall thickness should be measured using the same image [9, 13, 33]. The *magnitude* of JZ irregularity is expressed as the difference between the maximal and minimal JZ thickness: $(JZ_{max}) - (JZ_{min}) = JZ_{dif}$. The *extent* of JZ irregularity can reported as the subjective estimation of the percentage of the JZ that is irregular (<50 % or ≥50 %) [9, 13, 33] (Fig. 9.12). Detailed morphological measurement of the JZ is currently only relevant in the context of research protocols.

Interruption of the JZ may be caused by focal infiltration of the JZ by endometrial tissue, but contractions and changes within the JZ may also give rise to apparent JZ irregularities or influence measurement of JZ thickness. The extent of interruptions is recorded as a subjective estimation of the percentage of the JZ that is interrupted (<50 % or ≥50 %) [13] (Figs. 9.13 and 9.14).

Several studies have illustrated the sensitivity and specificity of 2D-TVS in diagnosing adenomyosis, but 2D-TVS generally describes alterations of the outer myometrium and does not consider alteration of the junctional zone. JZ, however, forms the basis for MRI diagnosis of adenomyosis. The study by Kepkep et al., (2007) was the only report that included poor

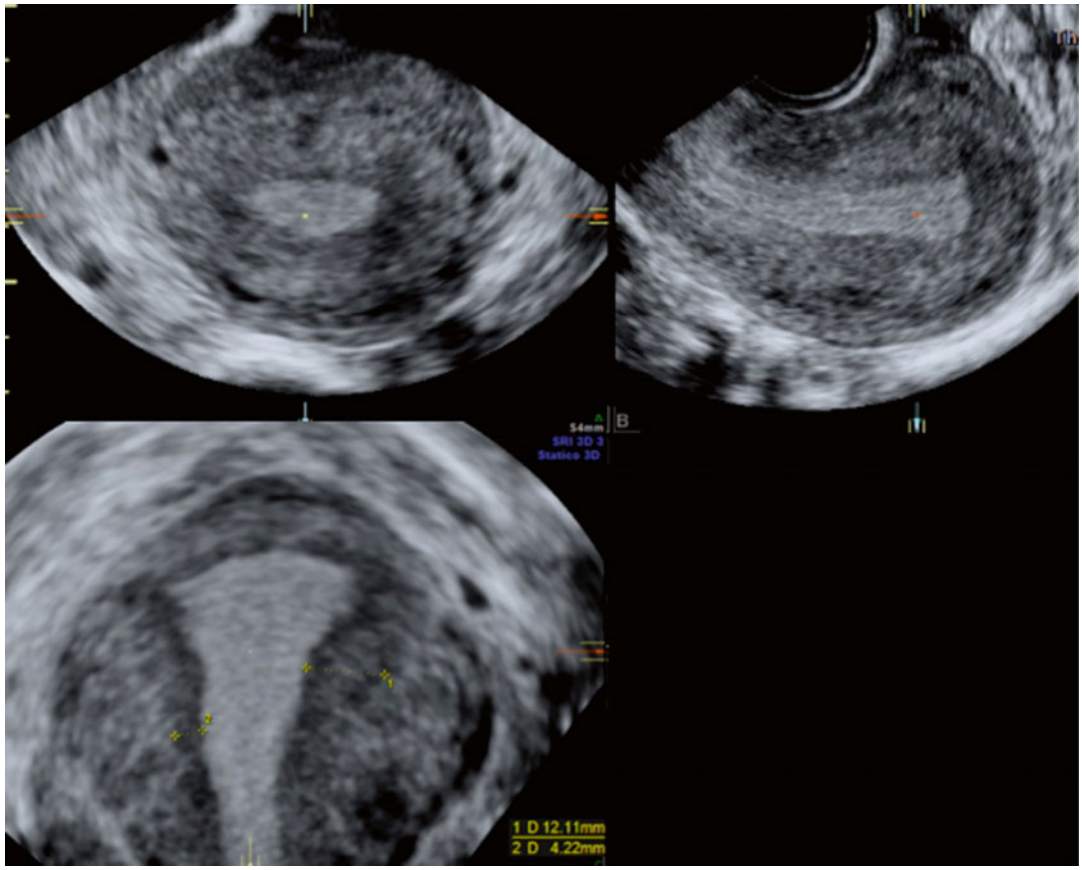

Fig. 9.12 Ultrasound image of the uterus obtained using three-dimensional ultrasound. A multiplanar view is shown: transverse and coronal sections of the uterus are shown on the left side of the image, a longitudinal section is shown on the right side of the image. 2D ultrasound features for adenomyosis are not clearly seen in the longitudinal and transverse section. However in the coronal section a thickened junctional zone is seen on the right side (1 = 12 mm = JZ_{max}) and a normal junctional zone is measured on the left side (2 = 4 mm = JZ_{min}). (JZ_{max}) − (JZ_{min}) = 8 = JZ_{dif}

definition of the JZ as a diagnostic feature in assessment of the accuracy of various 2D-TVS findings in adenomyosis [12]. They found that poorly definition of junctional zone had a high specificity (82 %) but a low sensitivity (46 %) in its diagnosis [12]. Three dimensional reconstruction of uterine anatomy in the coronal plane provides a new view of the junctional zone [7, 9]. Comparing transvaginal sonographic features to histology of the uterus after hysterectomies it was shown that junctional zone thickness $JZ_{max} \geq 6$–8 mm and (JZ_{max}) − (JZ_{min}) ≥4 mm were more significantly associated with adenomyosis compared to other 2D-TVS features [9, 33] (Fig. 9.12). Also, subjective evaluation of infiltration and disruption by endometrial tissue in the junctional zone is an accurate tool for the diagnosis of adenomyosis [7, 9, 33, 34] (Figs. 9.13, 9.14, and 9.15).

Thickening and disruption of the junctional zone are strongly associated with uterine adenomyosis [7, 9, 33] (Figs. 9.13, 9.14, and 9.15). Considering the hypothesis that adenomyosis is more likely to be caused by 'invasion' of endometrial tissue across the junctional zone and into the myometrium [29] 3D-TVS evaluation of junctional zone may be able to detect early adenomyosis [34] (Figs. 9.11 and 9.12). It has been reported that pelvic endometriosis, especially in advanced stages, is also strongly associated with junctional zone thickening and adenomyosis [9, 32, 34, 35]. Alterations of the junctional zone are

correlated with adenomyosis and may be involved in the process that determines pelvic endometriosis [27, 29, 35, 36].

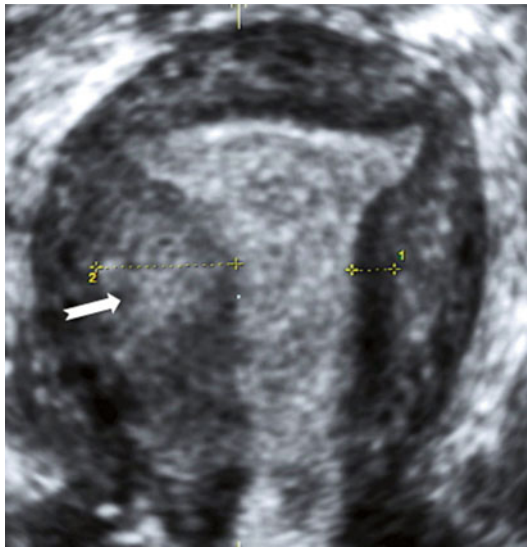

Fig. 9.13 Coronal view of a uterus obtained using three-dimensional ultrasound. A focal hyperechoic infiltration and thickening of junctional zone (*2*) is seen on the left side (*white arrow*) and a normal junctional zone is measured on the right side (*1*)

Therefore the evaluation of junctional zone and its alterations by non-invasive imaging seems very important especially in patients with suspect of pelvic endometriosis and adenomyosis. Three dimensional transvaginal sonography seems to be more accurate than conventional 2D-TVS for the diagnosis of adenomyosis and has the potential to evaluate early stages of the disease. As such, 3D-TVS should be considered in counselling and planning treatment of patients with deep endometriosis [34].

Using 3D ultrasound adenomyosis, junctional zone hyperplasia and adenomyotic cysts can now be detected in younger patients during their reproductive years (Figs. 9.11 and 9.12). The use of 3D transvaginal ultrasound for the diagnosis of junctional zone abnormality or adenomyotic pathology has been extensively described [9, 32, 34] and can now be used in patients seeking fertility treatment. As more women are postponing pregnancy into their third and fourth decades, an increasing proportion of women with fertility problems will have adenomyosis [26].

Recently, the possibility has been proposed of the use of hysteroscopy for the diagnosis and resection or ablation of intramural cystic

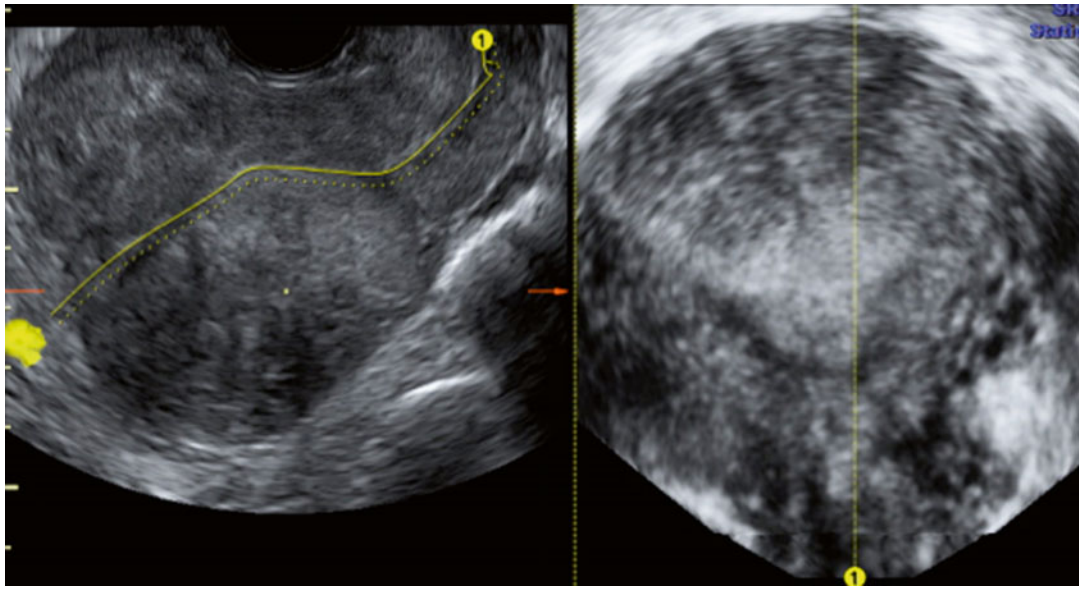

Fig. 9.14 Ultrasound image of the uterus with adenomyosis. On the *left*, a longitudinal section of a globular enlarged uterus with asymmetrically thickened of the uterine wall and with diffuse inhomogeneous, irregular myometrial echotexture and presence of hyperechoic areas. On the *right* the coronal section of the same uterus (*red arrow*) (obtained by a manual cut with Omni view modality of the 3D uterine volume), the junctional zone appears irregular, ill defined and completely infiltrated by the adenomyosis

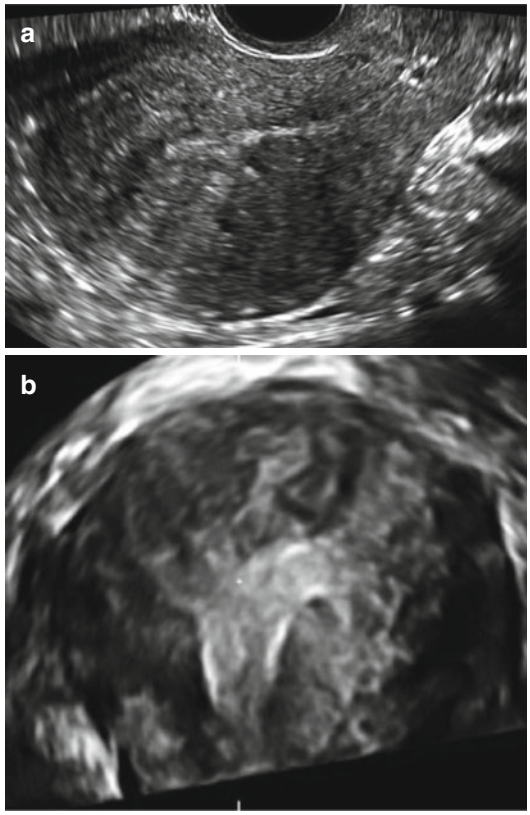

Fig. 9.15 Ultrasound image of the uterus with adenomyosis. (**a**) Gray scale longitudinal section showed an asymmetrically thickened of the uterine wall and with diffuse inhomogeneous, irregular myometrial echotexture, and presence of small hyperechoic and cystic areas. (**b**) Coronal section of the same uterus, the junctional zone is irregular, ill defined and completely infiltrated by adenomyosis

adenomyosis [21]. Treatment can be performed by mechanical dissection or bipolar ablative surgery but careful inspection of the endometrial cavity and evaluation of the uterine wall with special attention to the junctional zone using 2D-TVS and 3D-TVS is mandatory. The uterine spirotome proposed by Gordts allows a direct forward biopsy. Access can be gained to intramural cystic lesions that do not have visible intracavitary components under ultrasound guidance. Ultrasound guided hysteroscopy also offers an alternative for the treatment of subendometrial or junctional zone adenomyosis by localising cystic lesions, JZ hyperplasia or hyperechoic buds and thus minimizing tissue damage (Figs. 9.4, 9.9, 9.11, and 9.12).

Conclusions

Two dimensional transvaginal ultrasound has achieved a high level of accuracy. A high level of agreement has been demonstrated between ultrasound and histological diagnosis of adenomyosis. The presence of different morphological features in an individual woman could be an indirect measure of the severity of disease. The presence of more than one 2D-TVS feature permits a diagnosis of adenomyosis with high level of confidence without the need for histological confirmation. Thus identified however mostly patients with severe disease.

Three dimensional ultrasound evaluation of the junctional zone and its alterations has an important place especially in patients in whom pelvic endometriosis and adenomyosis are suspected. Three dimensional transvaginal sonography seems to be more accurate than conventional 2D-TVS.

The association between more than two 2D-TVS-3D-TVS features of adenomyosis and symptoms (menometrorrhagia dysmenorrhea, dyspareunia, abnormal uterine bleeding), multiparity and endometriosis has been demonstrated. However further studies are needed in young and asymptomatic women and more research is needed to evaluate the diagnostic value of less extensive or isolated features. Especially in cases with infertility or in the presence of endometriosis, isolated 2D-TVS or 3D-TVS features of adenomyosis could be useful in the detection of early disease, monitor its progression and in patient counselling.

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Adenomyosis and Endometrial Carcinoma

10

Marwan Habiba and Giuseppe Benagiano

Abstract

Although adenomyosis has been linked to hyper-estrogenism and with increased endometrial "invasiveness", whether adenomyosis predisposes to endometrial cancer remains a matter of debate. Literature contains few case reports of cancer identified solely in the ectopic endometrium within the myometrium. The origin of cancer detected in both eutopic and ectopic endometrium is difficult to determine and research in this area remains hampered because of the difficulty of early and non-invasive diagnosis. The presence of cancer within adenomyosis does not seem to adversely affect prognosis, but caution should be exercised because of the limited number of published studies.

Keywords

Adenocarcinoma • Serous carcinoma • Endometrial hyperplasia • Myometrium • Lymphovascular space • Prognosis • Diagnostic criteria

Introduction

The existence of a link between adenomyosis and endometrial cancer remains a matter for debate. Determining the origin of cancer present in the myometrium can be difficult. It is possible to envisage an association between the two conditions based on the link to hyper-estrogenism [1], or to increased 'invasiveness'. When cancer is identified in both the eutopic endometrium and adenomyosis, questions could be raised about its site of origin, the mode of spread (surface spread vs. infiltration) and the possibility of a multifocal origin. The presence of stromal cells and of non-malignant components within the lesions identified within

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the myometrium is indicative of adenomyosis, rather than invasive cancer. Relevant to this distinction is the question of whether cancer arising in adenomyosis carries worse prognosis because of its deeper location within the myometrium.

In 1918 Lockyer stated that: "adenomyomata may become malignant, but they do so very rarely" [2]. Although this conclusion seems to be supported by current literature, it was, in part, based on an assumption – that would not gain much support today – of a link between chronic inflammatory processes, such as chronic salpingitis, and malignancy. The reason why cancer within adenomyosis is a rare occurrence is not clear and the possibility must be raised as to whether the infrequent diagnosis is a factor of the mode of presentation. As discussed below, most cases were diagnosed following presentation with abnormal uterine bleeding, which may signal breakdown of the eutopic endometrium. Infrequently, cancer was diagnosed following the identification of abnormal cytology or suspicious lesions on imaging.

The first report of a case of cancer arising in an adenomyoma is perhaps the case of a 54 year old women reported by Rolly in 1897 [3]. In 1921, Andrew presented to the Royal Society in London a case of endometrial carcinoma arising in an "adenomyoma" in a woman with postmenopausal bleeding [4]. The eutopic endometrium was atrophic and cancer was identified together with non-cancerous endometrium in an adenomyoma. Cullen, in discussing whether adenocarcinoma of the body of the uterus can extend to a fibroid stated that only in the very rare instances where glands which conform to the type found in normal uterine tissue may have scattered through a myoma, is it possible for the myoma to become primarily carcinomatous [5]. He stated that he had not found a single instance in which an adenomyoma has become carcinomatous. But this view should not be extrapolated to cases of diffuse adenomyosis. Cullen himself later described a case of a woman with co-existing fibroid, adenomyoma and adenocarcinoma, and another case of cancer arising in a cystic gland [6].

There is debate in early literature about the classification of reported lesions. Borowski in a review of published literature identified 18 cases which had adenomyoma with "adenocarcinomatous changes" and 7 other cases containing sarcoma [7]. He added one other case of his own [7]. Kumar and Anderson proposed that it is necessary to demonstrate either transitional stages or continuity between benign and malignant ectopic endometrium in order to make a convincing cases for the origin of malignancy [8]. They were thus critical of the classification of the cases collated by Borowski [7]. Kumar and Anderson argued that in only 2 out of all the 26 cases identified by Borowski could malignant transformation of an adenomyoma be demonstrated beyond reasonable doubt and that literature till 1958 contained only 9 documented cases of malignant changes in an adenomyoma. They reported one case with multicentric adenocarcinoma in endometriosis interna, adenocarcinoma of the endometrium and an endometrial polyp, but despite the multiple sites, they argued that diagnosis was supported by the presence of benign adenomyosis and of early malignant changes and because there was no direct connection between the sites of cancer [8]. Still, Colman and Rosenthal cast doubt about the origin of all but two cases of cancer in adenomyosis reported till 1959 [9]. They argued that only the cases reported by Cullen in 1906 [6] and that by Polano in 1910 [10] were convincing instances of cancer originating within adenomyosis.

The need for strict diagnostic criteria was also emphasized by Hendrickson and Kempson who argued that it is almost impossible to determine the primary site if adenocarcinoma is present in both the endometrium and adenomyosis and that confidence in the diagnosis can only be assured if the overlying eutopic endometrium is normal [11]. They acknowledged that the use of such strict criterion renders the diagnosis vanishing rare. This is not surprising, given that endometrial cancer is usually identified in women with abnormal uterine bleeding where cancer has affected the eutopic endometrium.

Adenocarcinoma Within Adenomyosis

As mentioned above, the identification of cancer within adenomyosis is rare. In 1940, Dreyfuss identified adenomyosis in 152 cases out of 1807

surgical specimens. He pointed out the possibility of carcinoma given the presence of hyperplastic features but could only identify one or two cases which were highly suggestive of carcinoma arising primarily in adenomyotic lesions [12].

In the recent literature, most reports of cancer primarily arising in adenomyosis remain confined to case reports. Hsu et al. reported one case of endometrioid adenocarcinoma that was diagnosed in a focus of adenomyosis following debulking surgery [13]. The eutopic endometrium was benign with no atypia or hyperplasia. Motohara et al. reported a case that was followed-up over 11 years after the diagnosis of adenomyosis when endometrial cytology revealed the presence of malignant cells. Magnetic resonance imaging (MRI) showed that the adenomyotic lesion was substituted by a poorly demarcated lesion and histology following hysterectomy revealed an endometrioid adenocarcinoma arising from adenomyotic epithelium [14]. The eutopic endometrium was atrophic with no evidence of hyperplasia or malignancy. Takeuchi et al. reported a case of adenocarcinoma arising from adenomyosis involving the recto-sigmoid [15]. Puppa et al. reported one case of adenocarcinoma arising in adenomyosis is an asymptomatic postmenopausal woman who underwent a hysterectomy because of postmenopausal increase in the size of a fibroid and multiple focal myometrial lesions on ultrasound; the endometrium was free of tumour [16]. Boes et al. reported a case of endometrioid adenocarcinoma with squamous differentiation origination in an adenomyotic nodule. The patient presented with postmenopausal bleeding and although the eutopic endometrium was atrophic she had endometrioid adenocarcinoma in what appeared as an endometrial polyp; at hysterectomy, there were nodular adenomyosis zones and the tumour invaded more than half the myometrium and the endometrium was atrophic [17]. Couto et al. described a case of adenocarcinoma in adenomyosis in a woman who presented with postmenopausal bleeding. The endometrium was atrophic and there was a well-differentiated adenocarcinoma in one adenomyotic nodule and transition from benign to malignancy in several foci [18]. Sordia-Hermandez et al., identified adenomyosis in 140 out of 794 women (17.6 %) who underwent a hysterectomy for a variety of indications. There was no statistically significant difference in the proportion of women with adenomyosis in the group who had endometrial hyperplasia (5/36, 13.9 %) compared to the group without hyperplasia (135/758, 17.4 %), but no analysis was provided in relation to endometrial cancer [19]. Kazandi et al. reported a case of grade II adenocarcinoma arising in adenomyosis in a postmenopausal woman who presented with pelvic pain and a large pelvic mass adherent to the sigmoid and containing the tumour [20]. An interesting report by Abushahin et al. included five cases of endometrial intraepithelial carcinoma in adenomyosis. The eutopic endometrium showed invasive endometrial serous carcinoma in one case, serous endometrial intraepithelial carcinoma in two cases, and benign endometrium in two cases. In one of the cases with endometrial serous carcinoma there was no connection between the invasive component of the endometrial cancer and the lesion in adenomyosis and there was a distance of at least 0.5 cm between the endometrial and adenomyosis lesions in cases with intraepithelial cancer. The findings suggested that such lesion can arise within adenomyosis [21].

Adenocarcinoma in Patients with Adenomyosis

A question could be asked as to whether adenomyosis is associated with increased incidence of cancer in the eutopic endometrium. It is difficult to establish the incidence of cancer in adenomyosis as, in most cases, adenomyosis is only identified in uteri following hysterectomy and the reasons for surgery vary and any meaningful assessment of the incidence must take into account the indications for surgery. It is perhaps easier to establish the incidence of adenomyosis in patients with endometrial cancer. In the 1948 series by Novak and de Lima, eutopic endometrial hyperplasia was found in 36.3 % out of 92 cases with adenomyosis, but they noted that the abnormal endometrial growth propensity which forms part of adenomyosis is different to that seen in endometrial hyperplasia and that the

eutopic endometrium in women with adenomyosis may be normal [22].

More recently, Kairi-Vassilatou et al. examined 135 hysterectomy specimens with adenomyosis and 82 cases of endometrial adenocarcinoma and reported that adenomatous hyperplasia was present in 47/135 (34.8 %) and fibroids were present in 86/135 (63.7 %) of cases of adenomyosis. Thirty one cases had both adenocarcinoma and adenomyosis and four cases malignant changes in foci of adenomyosis were found, although in only one case was cancer confined to adenomyosis [23].

Adenomyosis in Patients with Endometrial Carcinoma

More than 50 years ago, Giammalvo and Kaplan reported the presence of adenomyosis in 40 out of 120 (33 %) cases with endometrial cancer compared to 48 out of 264 (18 %) in the controls [24]. In 1961 Marcus reported a series of 100 consecutive cases of adenocarcinoma of the endometrium and a control group of 100 patients who had hysterectomy for benign causes. The depth of invasion of cancer and adenomyosis was classed as slight, moderate or extensive by dividing the myometrium into affected thirds. Adenomyosis was identified in 60 % of cases with endometrial cancer compared to 39 % of controls. Forty percent of women with endometrial cancer had moderate or extensive adenomyosis compared to 14 % of the control group [25]. However, because no details were provided about the characteristics of the control group, little can be deduced from this finding beyond the known relation between endometrial hyperplasia and cancer. In the report by Marcus, adenomyosis and endometrial hyperplasia coexisted in 39 % of cases of endometrial cancer compared to 7 % of the controls. Hyperplasia was present in adenomyotic glands and eutopic endometrium in 15/39 of the cases with endometrial cancer and in 3/7 of controls [25]. This does not support a cause and effect relationship between adenomyosis and endometrial cancer.

Hernandez and Woodruff reported the identification of adenomyosis in 21 (10 %) of 204 patients who underwent a hysterectomy for endometrial cancer at John Hopkins Hospital between 1955 and 1974. Two of the patients with adenomyosis had atypical hyperplasia and 6 had adenocarcinoma in foci of adenomyosis as well as cancer in the eutopic endometrium [26].

Koshiyama et al. reported that postmenopausal women with endometrioid cancer had a higher incidence of fibroids and of adenomyosis compared to postmenopausal women who had a hysterectomy for prolapse [27]. The findings suggest persistent hyper-estrogenism in the group with cancer. In another report, Koshiyama et al. reviewed all cases of endometrial cancer treated at their hospital between 1989 and 2001 (n = 179). Adenomyosis was identified in 29 (16 %) and endometriosis in 12 (7 %) cases. Patients with coexisting adenomyosis or endometriosis were younger and included a higher percentage of premenopausal women compared to those with no concomitant pathology; this group also tended to have less advanced disease [28].

In a retrospective review, Kucera et al. identified adenomyosis in 88 (40 %) uteri out of 219 cases who underwent a hysterectomy for early endometrial cancer. The majority (n = 205) had endometrioid adenocarcinoma, 10 had clear cell carcinoma and 4 had papillary serous carcinoma. In all except one case, adenomyosis was present in women with endometrioid adenocarcinoma and in six of these cases cancer was present in adenomyotic foci. The tumour in adenomyosis was of the same or lower grade compared to the eutopic endometrium. All cancer-positive adenomyotic foci also showed hyperplastic changes. Interestingly, whilst myometrial invasion was deeper than 50 % of the myometrial thickness in five of the six cases, whenever there was cancer in the adenomyotic foci, no stromal invasion was found into the myometrium adjacent to malignant foci. This may be related to increased stromal reaction observed histologically. Atypical hyperplasia was identified in 18 out of the 87 cases with both adenomyosis and endometrial cancer. This was interpreted as suggestive of similar mechanism of carcinogenesis in adenomyotic foci and the eutopic endometrium [29]. Musa et al. reviewed 2346 hysterectomy specimens and

identified endometrioid adenocarcinoma (EAC) in 197 cases (8.4 %) [30]. Adenomyosis was present in 42 % of the 2346 hysterectomy specimens and in 66 % of those with EAC (P = 0.009). Adenomyosis was associated with lower tumour grade, less myometrial invasion, negative lymphovascular space invasion (LVSI) and negative lymph node invasion. This may be because adenomyosis was associated with endometrioid tumours that were hormonally responsive, well differentiated and more likely to be diagnosed early. Adenomyosis had no independent survival impact on women with endometrial cancer. Interestingly, the presence of LVSI was associated with lymph node metastasis in patients without adenomyosis, but the relationship was not significant in patients with adenomyosis. This is in line with previous studies that suggested that adenocarcinoma involving adenomyosis in the absence of myometrial invasion is associated with good prognosis [30].

The higher incidence of adenomyosis in women with endometrial cancer is not universally accepted. Gün et al. identified adenomyosis in 98 out of 472 (20.8 %) hysterectomy specimens and it was the sole pathology in 28 (5.9 %) cases [31]. The incidence of adenomyosis was comparable in the group who had endometrial cancer (n = 57) and those who did not have cancer [19.2 % (n = 11) and 20.9 % (n = 87) respectively]. In a study involving 375 premenopausal and 132 postmenopausal women, Nomelini et al. reported adenomyosis in 79 (21.07 %) of the premenopausal group and in 15 (11.36 %) of the postmenopausal group. The authors concluded that there was a significant association between endometrial cancer and adenomyosis in women with four or more pregnancies and in those aged over 40 years (p < 0.0001) [32]. However, in their study there were no patients with adenomyosis amongst premenopausal (n = 3) or postmenopausal (n = 13) women with endometrial cancer.

Taskin et al. identified adenomyosis in 28 patients out of 84 (34 %) with endometrial cancer. Simple and complex hyperplasia were observed in adenomyotic foci in eight cases [33]. An additional seven patients had atypical complex hyperplasia or carcinoma in situ. But there were no cases of invasive cancer within adenomyosis foci. But in this small study, the authors did not identify a relation between the depth of invasion of endometrial cancer and the depth of adenomyosis.

Origin of Cancer in Adenomyosis

The identification of carcinoma in adenomyotic glands raises the question about their origin. A surface spread theory proposes that extension within the glands is favoured because of the lower resistance to spread compared to invasion through the endometrial-myometrial junction. The other possibility is that cancer in adenomyotic glands arises from within the ectopic epithelium from malignant precursors. Support for this theory comes from the observation of premalignant changes within adenomyosis [9]. The case reported by Couto et al. also suggests a field change with malignant precursors [18].

It is remarkable, given the high incidence of adenomyosis and its assumed relation to hyperestrogenism that the incidence of cancer confined to adenomyosis is limited to individual case reports. Pregnancy, through the presence of elevated circulating progesterone levels, may represent a protective factor for endometrial cancer [34–37]. Alternatively, the protective effect may be through mechanical loss of malignant or precancerous epithelial cells at the time of delivery [36, 38, 39]. Neither of these mechanisms is relevant to adenomyosis as the response to progestogens is less pronounced and the response to menstrual cycle limited. The role of other steroid modulators appears limited and the only reported case of cancer within adenomyosis in a patient receiving tamoxifen was a papillary serous carcinoma [40].

Diagnosis

The majority of instances were diagnosed following investigation for abnormal, mostly postmenopausal, bleeding. Woodruff et al. reported on two cases of post-menopausal adenocarcinoma aris-

ing from adenomyotic foci that were detected through the persistent presence of atypical vaginal cytology even though eutopic endometrium was unaffected by cancer [41]. Others have reported similar findings [42–44] but cases were also reported with negative cytology and no features of malignancy on MRI or ultrasound [45].

Prognosis

It appears from the limited observations available that the presence of adenomyosis does not adversely affect prognosis [46]. Koshiyama et al. reported that there was no difference in prognosis in the presence or absence of adenomyosis in patients with stage I adenocarcinoma [28].

In the study by Hall et al. 52 women were identified with stage I carcinoma of the endometrium with coexisting adenomyosis [46]. In 11 cases, there was also cancer within adenomyosis resulting in invasion to a greater depth than would have been expected in the absence of adenomyosis. In 9 of these cases, there was no myometrial invasion; in 2 cases the depth of invasion was greater than that in the myometrium. The 5 years survival rate for women with cancer in adenomyosis was 100 %, compared to 96 % for the whole group. This suggests excellent prognosis but the group was very small and cancer extended into adenomyosis was confined to the inner myometrium in all except three cases and in only two cases did the depth of adenomyosis involvement exceeded the depth of endometrial invasion within the myometrium. Thus, although the presence of cancer in adenomyosis may be associated with a better prognosis, the series is too small to draw firm conclusions.

In 1990, Jacques and Lawrence reviewed 23 cases of stage I (18 grade 1 and 5 grade 2) endometrial cancers in which myometrial involvement was limited to adenomyosis foci and reported that they were associated with a better 5-year survival than endometrial cancers with myometrial invasion at the corresponding depth [47]. In a similar investigation, Mittal and Barwick, analysed EAC that involved foci of adenomyosis separating them from cancers invading the myometrium and found 18 cases in which adenomyotic foci were affected, without myometrial invasion and 43 cases of cancers invading the myometrium. There were no cancer-related deaths over a minimum of 5 years follow-up in the absence of myometrial invasion, but there were 8 deaths in the group with myometrial invasion [48]. Subsequently, Hirai et al. reported on a group of 286 surgically treated patients with EAC (stage I-III). The presence of EAC and adenomyosis was associated with increased incidence of endometrial hyperplasia, less invasive lesions and more favourable prognosis [49].

Ismail et al. examined histological samples from 93 patients with an endometrial endometrioid adenocarcinoma with grade 1 (FIGO) lesions associated with adenomyosis to assess the depth of myometrial invasion [50]. EAC involved adenomyosis in 46 of the 93 specimens and myometrial invasion was more common in carcinoma-positive adenomyosis cases (n=42, 91.3 %) compared to carcinoma-negative adenomyosis cases (n=30, 63.8 %) and the difference was statistically significant. In addition, invasion of the outer half of the myometrium was observed in a statistically significantly higher proportion in cases where there was involvement of adenomyosis compared to cases where cancer did not extend into adenomyosis (n=16, 34.8 % vs. 3, 6.4 %). The same group also published a comparison of findings for grade 1 (FIGO) cases with or without involvement of adenomyosis. They examined 95 specimens: in 46 cases, cancer involved adenomyosis. Here too, they reported a statistically significant higher incidence of myometrial invasion in the presence of adenomyosis (n=42, 91.3 %) than in its absence (n=38, 77.5 %) [51]. The authors concluded that the involvement of the coexisting adenomyosis probably increases the surface area of the interface between cancer and the adjacent myometrium, making these tumours more likely to invade to greater depth.

Hanley et al. evaluated the significance of low grade endometrial cancer involving adenomyosis in 82 hysterectomies invading to <50 % of the myometrium [52]. Patients were divided into 4 groups: in group 1 (n=38), cancer did not involve adenomyosis; in group 2, cancer involved

adenomyosis and was surrounded by endometrial stroma (n = 31); in group 3, cancer involved adenomyosis with incomplete peripheral endometrial stroma (n = 10); and in group 4, tumour involved adenomyosis with invasion into adjacent smooth muscle (n = 3). Cancer involving adenomyosis was confined to the inner half of the myometrium in 35 cases, but involved the outer half of the myometrium in 9 cases. None of these 9 patients had recurrence of cancer over the 2 year follow-up. Although the number of affected patients was small, the findings suggest a favourable prognosis.

Taneichi et al. compared the outcomes of women with endometrial cancer in two groups: those with (n = 121, 33.4 %) and those without (n = 241, 66.6 %) adenomyosis [53]. The 5-year survival was 100 % and 95.9 % for FIGO stages I and II respectively, and 88.0 % and 73.5 % for stages III and IV with no significant between-group differences. When limiting the results to only FIGO stage I endometrioid adenocarcinoma, despite no grade variance between the two groups, a significant difference was observed in the ratios of outer-half muscle invasion between the adenomyosis and non-adenomyosis groups (19.5 % [17/87] vs. 10.1 % [16/158], $P < 0.05$); however, the prognosis was similar in the two groups. Thus although uterine adenomyosis was associated with deep myometrial invasion in stage I endometrioid adenocarcinoma, this did not affect the recurrence or mortality rates. This is in line with the report by Musa et al. who suggested that the presence of adenomyosis was associated with a lower risk of lymph node metastasis in patients with lymphovascular space invasion [30]. Despite the apparent favourable prognosis, caution should be exercised also because of the reported difficulty in assessing the depth of invasion in the presence of adenomyosis [54].

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Myometrial Cystic Adenomyosis

11

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Abstract

There are many case reports of the rare cystic uterine lesions which are often classed as variants of adenomyosis. The introduction of Ultrasound and Magnetic Resonance Imaging has enabled non-invasive diagnosis. Cystic variant of adenomyosis, which is often amenable to surgical treatment, should be suspected in cases of refractory dysmenorrhoea. More recently, we proposed a standardised classification system to be used when reporting these rare lesions. Uniform reporting may enable better understanding of the condition and can replace the confusion terminology currently reported in literature.

Keywords

Circumscribed adenomyoma • Cavitated adenomyoma • Cystic adenomyoma or adenomyosis • Juvenile adenomyotic cyst • Juvenile cystic adenomyoma • Intra-myometrial • Congenital uterine cyst • Müllerian duct • Gubernaculums • Uterus like mass

Introduction

Cystic uterine lesions are rare and are often classed as variants of adenomyosis. There is lack of agreement on their nomenclature as demonstrated by the many terms used in literature (Box 11.1).

Box 11.1 Nomenclature Used to Describe Myometrial Cystic Lesions

Circumscribed, cavitated, or cystic adenomyoma or adenomyosis
Juvenile adenomyotic cyst
Adenomyotic cyst
Juvenile cystic adenomyoma (JCA)

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Intra-myometrial or congenital uterine cyst
Accessory cavitated uterine mass (ACUM)
Accessory uterine cavity
Uterus or uterine like mass

Cystic lesions can be readily identified by ultrasound and magnetic resonance imaging and need to be distinguished from congenital uterine anomalies and rudimentary, cavitated uterine horns. The fluid content often exhibits high signal intensity on T1-weighted images, and the surrounding solid wall has distinct low signal intensity on T2-weighted images [1].

Cucinella et al. [2] argued against the use of the word 'adenomyoma' in connection with these lesions and in favour of the use of 'adenomyotic cysts' or, depending on age at onset, '*juvenile adenomyotic cyst'* – a designation favoured by Takeda et al. [3]. Acién et al. reviewed published images on the subject [4] and concluded that the majority of published images and descriptions are in fact instances of what they termed '*accessory cavitated uterine masses*' (ACUM). Lesions classified under that category were mostly isolated cysts located in the lateral right or left wall of the uterus at the insertion of the round ligament. One characteristic of uteri with ACUM is that the uterine cavity is itself normal. Cysts similar to those described as JCA identified in adults have been termed adult cystic adenomyosis, which adds to the confusing terminology as they seem to represent the same type of lesion.

Early Descriptions

A very early description of '*submucous adenomyoma with cyst formation*' was made by Cullen in 1908 [5]. The cysts identified in 5 women measured around 10 mm and were lined by cylindrical epithelium. Cullen also described advanced cases forming complex adenomyomas and extending under both the serosa and the endometrium. He speculated that those extending within the uterine cavity remain confined in size by uterine pressure. Cullen proposed that lesions be included within his definition only if they have no communication with the uterine cavity so as to distinguish these from congenital uterine anomalies and if they are lined by endometrium, surrounded by myometrium and filled with haemorrhagic fluid [5]. He described solid and cystic forms of adenomyomata. The cystic forms varied in size from few millimetres to a 'very large' size. One of the cases described by Cullen was a subserosal adenomyoma linked by a narrow canal to the uterine cavity [5]. Similar subserous cysts with connecting canals were described by Keating et al. [6] and by Dobashi et al. [7].

An important differential diagnosis is with the cystic degeneration of myomas, but the distinction can be made because cystic myomas do not have an epithelial lining [8]. Another differential diagnosis is with the congenital cyst of the corpus uteri, and several cases were described in women undergoing hysterectomy during the second half of the 20th century [9, 10].

Adenomyotic Cyst

Cystic uterine lesions include adenomyotic cysts [3, 4, 11–22]. In adolescent girls it can present with early onset dysmenorrhoea that is resistant to medical treatment. Tamura et al. described a case which they coined juvenile cystic adenomyoma (JCA) in a 16-year-old girl who had severe dysmenorrhoea that started 3 years after menarche [23]. Typically the cyst is independent of the uterine cavity and is ≥10 mm in diameter with no other evidence of adenomyosis. The review by Takeuchi et al. included 39 young patients with "cystic adenomyoma" [12]. The diagnostic criteria proposed by Takeuchi et al. are: (1) age ≤30 years; (2) cystic lesion of ≥10 mm in diameter independent of the uterine lumen and surrounded by hypertrophic myometrium on diagnostic imaging; (3) an association with severe dysmenorrhea [12].

Acién et al. and Bedaiwy et al. argued that most published cases of non-communicating accessory uterine cavities and of JCA or of isolated cystic adenomyomas, as well as some cases of uterus-like masses are actually the same

pathology [13, 24]. They recommended the term ACUM proposed by Acién et al. [4] for this type of lesion and expressed the opinion that it originates from duplication and persistence of ductal Müllerian tissue in a critical area related to the attachment of the round ligament and that the location indicates a developmental dysfunction in the gubernaculum [4, 13].

Some reported cysts were very large. In one woman who had two previous myomectomies the cyst measured 17×11×8 cm and contained 1150 ml of serous non-coloured clear fluid; it was lined by endometrial-like epithelia with squamous metaplasia [25]. Ejeckam et al. reported an even larger cyst measuring 20×15.5×12.6 cm located on the posterior side of the uterine fundus [26]. The cyst was lined by endometrial epithelium and loose mesenchymal-like stroma. Very few similar cases have been reported in the literature [6, 27–30].

In older women the adenomyotic type is no longer the dominant form of uterine cyst and should be differentiated from classical congenital uterine cysts that can be large or even giant, have a clear content and are lined by a ciliated surface. The adenomyotic cysts in older women are frequently larger compared to the younger women and are more frequently subserosal, pediculated and part of a large adenomyotic mass. These cysts are not commonly associated with diffuse junctional zone adenomyosis. Malignant transformation of these adenomyotic cysts is rare with only few reported cases [26, 30].

Uterus Like Mass

Oliver described an "accessory uterus distended with menstrual fluid", that he enucleated from the right broad ligament [31]. A similar "Uterus-like mass" in the ovary was reported by Cozzuto [32]. Similar masses have more frequently been described in relation to the ovary, colon and other pelvic structures.

Fukunaga et al. reported a case of a 47 year old woman with a 25-mm mass growing into the endocervical canal and which had superficial cervical endometriosis and florid smooth muscle metaplasia [33]. Other lesions have been described that were related to the lower uterine segment. These masses are lined by endometrium and can reach the size of the uterus [34, 35]. A similar lesion was described with attachment to the uterine fundus [36]. Redman et al. proposed that this lesion originates from a secondary Müllerian system [37].

Diagnostic Issues

The primary condition for diagnosis of adenomyotic cyst is clinical awareness of the condition as a differential diagnosis especially in young women. The typical presentation is severe, progressive dysmenorrhea or pelvic pain that persists before and after menstruation and does not respond to common analgesics. Diagnosis is often only noted at surgery [22]. Differential diagnosis includes uterine malformation [15, 38] and cavitated rudimentary uterine horn where the differentiating feature is the presence of a unicornuate uterus [39] and of congenital uterine cysts [7, 8, 40–42]. Histological diagnostic criteria include the presence of a cavity filled with haemorrhagic fluid that has no communication with the uterine cavity. The cyst is lined by endometrium and is surrounded by myometrium; the haemorrhagic contents can be evaluated using imaging techniques. In addition, the surrounding myometrium and the myometrial junctional zone should be investigated to confirm absence of other adenomyotic lesions. Assessments to rule out uterine anomalies include TVS, MRI, hysterosalpingography and hysteroscopy.

Classification of Adenomyotic Cysts

Uterine cystic lesions do not constitute a homogenous group and may have a different pathophysiology. A precise knowledge of the spectrum of MRI features can greatly help in comparative studies and in establishing an accurate diagnosis and determining appropriate treatment [43].

We have therefore proposed [44] that all case-reports of cystic structures surrounded by myometrial smooth muscle should include a

Table 11.1 M.U.S.C.L.E

1. Myometrial location: Intramural, submucous, subserous
2. Uterine site: Midline, paramedian, lateral
3. Structure: Cystic, mixed solid and cystic, cyst with polypoid growth
4. Content: Clear fluid or hemorrhagic fluid
5. Level: Fundus, body, cervix
6. Endometrial or inner lining: Endometrium, endosalpinx, metaplastic, other, unknown

description allowing the lesion to be classified as follows:

A. ***Adenomyotic cyst***: including all forms that, at least in part, are lined by endometrial epithelium and stroma.
B. ***Non-adenomyotic cyst***: if the cyst is lined by a different type of epithelium.
C. ***Cystic degeneration of a leiomyoma***: when the epithelial lining is absent.
D. ***Unclassified cyst***: if the cyst wall has not been subjected to histological examination.

We have also proposed that reports should include a description that allows adequate comparison following the acronym MUSCLE (Table 11.1).

Treatment

Medical Treatment

Patients may not respond or show only partial response to initial empirical treatment including the use of oral contraceptives [14, 37]. As the lining of submucous and intramural cysts expresses steroid hormone receptors, cyst size may increase during the menstrual cycle and regress at the time of menstruation [2, 11].

Surgical Treatment

Surgical excision is necessary in cases that do not respond to medical treatment. Nabeshima et al. reported laparoscopic total resection of a cystic adenomyoma using transtrocar ultrasonic guidance after a previous unsuccessful resection by laparotomy [16]. In a series of nine cases Takeuchi et al. observed that laparoscopic excision resulted in a statistically and clinically significant reduction of symptoms [12]. Bedaiwy et al. favoured the laparoscopic approach as it allows sufficient access [24].

Other surgical approaches have been proposed include ultrasound guided radiofrequency needle ablation [21], the use of single-incision with monopolar cautery [17] or the use of robotic surgery [39].

Giana et al. and Kumar described the accidental hysteroscopic drainage of a submucous adenomyotic cyst [27, 45]. This approach was used in one reported case [44].

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Current Treatment for Adenomyosis

12

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Abstract

Prior to the identification of diagnostic criteria for adenomyosis using modern imaging, the condition could only be diagnosed in hysterectomy specimens. The use of Magnetic Resonance Imaging and modern Ultrasound created the conditions for less radical treatment. This may include symptomatic treatment for heavy bleeding or dysmenorrhea, gonadotropin-releasing hormone agonists, levonorgestrel-releasing intrauterine device, continuous administration of the oral contraceptive pill, progestogens, danazol or aromatase inhibitors but the evidence base for these options is limited and the disease recurs following discontinuation. Uterine sparing adenomyomectomy, hysteroscopic endomyometrial ablation or uterine artery embolisation may have a role. But these options remain limited because of the often diffuse nature of adenomyosis and because of uncertainty about the effect on future pregnancy

Keywords

GnRH agonists • Levonorgestrel-releasing intrauterine device • Oral contraceptive pill • Progestogens • Danazol • Aromatase inhibitors • Valproic acid • Bromocriptine • Hysterectomy • Uterus-sparing surgery • Hysteroscopic • Endomyometrial ablation

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Introduction

Recent progress has enabled adenomyosis to be diagnosed in women not undergoing a hysterectomy and it is no longer believed that adenomyosis only affects parous women.

Awareness and knowledge about adenomyosis have grown considerably in recent years. Preoperative diagnosis has become increasingly feasible following the advent of transvaginal ultrasound scanning (TVS) and Magnetic Resonance Imaging (MRI) [1]. As a result, it is now possible to develop individual patient management plans in advance. This has the advantage of being able to take into consideration all available medical and surgical treatment options.

Factors That Influence the Choice of Treatment

There are many important factors to be considered when counseling a patient with adenomyosis.

A critical question is whether to remove or preserve the uterus. Hysterectomy remains the standard treatment for symptomatic adenomyosis if fertility is not an issue. More challenging are cases where women decline hysterectomy or wish to pursue future pregnancy.

Planned intervention should take into consideration the indication for treatment. Symptoms more frequently associated with adenomyosis are dysmenorrhea and menorrhagia, less commonly women present with dyspareunia or chronic pelvic pain, but intervention may not be required in as many as 35 % of women as they are asymptomatic [2]. Adenomyosis identified in the course of infertility investigations creates a challenging dilemma whether or not there are other associated symptoms. These cases are likely to become increasingly common as women delay their first pregnancy into their late 30s or early 40s. Adenomyosis is known to be more common in the fourth and fifth decade of life [3]. A recent review [4] linked adenomyosis to subfertility in women seeking conception after surgery for severe rectovaginal and colorectal endometriosis. In women seeking pregnancy, 7/59 (11.9 %) women with concomitant adenomyosis conceived, compared with 74/172 (43.0 %) in those without adenomyosis. Adenomyosis was associated with a 68 % reduction in the likelihood of pregnancy in women seeking conception after surgery for rectovaginal and colorectal endometriosis [4]. In another meta-analysis of published literature women with adenomyosis had a 28 % reduction in pregnancy rate after IVF/ICSI compared to women without adenomyosis. This reduction is a factor of reduced clinical pregnancy and implantation and increased early pregnancy loss [5].

Adenomyosis may be diffuse involving all or a large part of the myometrium or localized to a limited portion of the myometrium. The extent of adenomyosis is critical to the choice of uterus-sparing excision for adenomyosis.

Also important is the question of coexisting pelvic disease. It is estimated that endometriosis is present in 6–22 % of patients with adenomyosis, and that myomas are present in 35–55 % [6]. Treatment plans may need to be tailored in the presence of coexisting pathology.

Medical Treatment

Traditionally, treatment for heavy periods and dysmenorrhoea has not been tailored to adenomyosis because of the lack of reliable nonsurgical diagnosis. Treatments still include the anti-fibrinolytic agent -tranexamic acid-, non-steroidal anti-inflammatory agents or the combined oral contraceptive pill. Yet, there is no research that specifically addresses the effectiveness of these agents in adenomyosis. Women may have also undergone endometrial destructive procedures (resection, or ablation) only for the condition to be diagnosed in a proportion of those who do not have favourable outcomes.

Medical treatments that specifically target adenomyosis may rely on the observation that the disease is hormone dependent and on the similarities to endometriosis [7]. Although adenomyosis and endometriosis are distinct conditions with different epidemiological features, ectopic endometrium can be a common target for hormonal

therapy. Therapies that affect endometriosis can potentially be useful for adenomyosis.

The main limitation of medical treatments is that they are not cytoreductive and available compounds induce regression but not eradication of ectopic endometrium. Consequently, symptoms may recur after treatment is discontinued. Moreover, medical treatments induce atrophy of eutopic endometrium and frequently inhibit ovulation, which are problematic in women seeking pregnancy.

Medical treatments for adenomyosis include gonadotropin-releasing hormone (GnRH) agonists, levonorgestrel-releasing intrauterine device (LNG-IUD), continuous administration of the oral contraceptive pill, progestogens, danazol or aromatase inhibitors.

Experimental medical treatments include valproic acid, which has an antiproliferative action on endometrial and endometriotic cells and bromocriptine, which can prevent prolactin-induced adenomyosis in mice.

GnRH Agonists

GnRH agonists bind to GnRH receptor in the pituitary gland resulting in a reversible hypo-estrogenic hormonal state. The pronounced lowering of estrogen levels explains the efficacy as well as the primary limitation of GnRH agonists. Adverse effects of GnRH agonists include hot flushes and reduced bone mineral density with prolonged treatment. These side effects may be diminished using add-back oral contraceptive pill or hormone replacement therapy [8]. GnRH agonists can be administered via intramuscular or subcutaneous injections, nasal spray and, more recently, orally.

GnRH agonists were among the first drugs used for adenomyosis. Since 1991, case reports or small case series reported that GnRH agonists reduce uterine volume and resolve symptoms associated with adenomyosis [9–11]. Other studies reported successful pregnancies within 6 months of discontinuing GnRH agonists in infertile patients with adenomyosis [12–14]. GnRH agonists have also been administered prior to surgical excision of adenomyosis in an attempt to improve outcome, reduce menstrual blood loss and improve preoperative hematological indices. The reduction of adenomyosis burden may make surgery technically easier, but may also render smaller foci difficult to identify. Postoperative GnRH agonists may have a role in relation to smaller adenomyotic foci that cannot be removed surgically. In one small retrospective study, the use of GnRH agonist in women with adenomyosis ($n=37$) was compared to a group ($n=28$) who were treated by conservative surgery with or without GnRH agonists. The live birth rate in the group who underwent conservative surgery was higher (32 %) compared to those who received GnRH agonist alone (8 %), the difference was statistically significant ($p=0.022$) [15].

Levonorgestrel-Releasing Intrauterine Device

The use of the levonorgestrel intrauterine device (LNG-IUD) which released 20 μg of levonorgestrel per day induces marked atrophy of endometrial glands and stromal decidualization and significantly reduces menstrual blood loss [16]. LNG-IUD improves dysmenorrhea by reduction of prostaglandin within the endometrium [17]. LNG-IUD acts directly on adenomyotic deposits resulting in down-regulation of the oestrogen receptors at least for the first year of use [18], as a result, adenomyotic deposits and the uterus reduce in size. The LNG-IUD is licensed for idiopathic menorrhagia where it can be used for 5 years. Serum levonorgestrel varies in women using the device but may be in the region of 276 ± 119 pg/ml –177 ± 70 pg/ml. A disadvantage of the device is prolonged duration of bleeding and frequent and variable intermenstrual spotting and bleeding especially during the first months of use.

Fedele et al. [19] evaluated the use of LNG-IUD in 25 women with recurrent menorrhagia (blood loss >80 ml) for at least 6 months. Adenomyosis was diagnosed by transvaginal ultrasound and MRI in 9 cases. At the end of the first year of follow-up, all women had reduced blood loss and a rise in haemoglobin. There was

a moderate but significant reduction in uterine volume during treatment. Only two patients discontinued treatment prematurely; one due to expulsion of the LNG-IUD and the other had persistent irregular bleeding.

Sheng et al. [20] reported 85 % reduction in the visual analogue scale for dysmenorrhea from 77.9 at baseline to 11.8 after 36 months of the LNG-IUD insertion in women with adenomyosis. In another study, a significant decrease in pain score was reported 3–6 months after LNG-IUD insertion [21].

LNG-IUD has also been used to improve the clinical outcome of endometrial resection in women with adenomyosis-associated menorrhagia. In a retrospective study, 53 women who had a LNG-IUD inserted following endometrial resection were compared to 42 women who underwent a resection and no further treatment. After a 1-year follow-up, all women in the LNG-IUD group compared to only 9 % of women in the control group were amenorrhoeic and no women in the LNG-IUD group and 8 women in the control group required a second endometrial resection. Women in the LNG-IUD group reported a significant reduction in pain symptoms [22]. The data remain limited but the combination of endometrial ablation and LNG-IUD may have a place as an alternative to hysterectomy, while LNG-IUD is a possible option for women who wish to preserve fertility [23].

Oral Contraceptives and Progestogens

The combined oral contraceptive pill (OCP), especially when taken continuously, results in symptom control and inhibits disease progression in women with endometriosis [24], but no studies have evaluated the effectiveness in adenomyosis.

Oral progestogens have also been reported to be effective in the treatment of endometriosis [25]. The mechanism of action may involve modulation of mitotic activity, local growth factors and their receptors or modulation of paracrine and anti-inflammatory reactions [26, 27]. Muneyyrci-Delale et al. [26] evaluated the role of norethindrone acetate (norethisterone, NA), a synthetic progestogen, in the management of adenomyosis. In this study, 28 women with adenomyosis-associated moderate or severe pelvic pain and abnormal uterine bleeding received a low dose of 5 mg of norethisterone based on "three weeks on, one week off" treatment protocol advocated to minimize breakthrough bleeding. There was an 82 % reduction in dysmenorrhea and a 71 % reduction in bleeding score at 3-months and no breakthrough bleeding after 2 months of therapy. This suggests that norethisterone can be a useful medical treatment option but the long-term use requires careful consideration.

Danazol

Danazol is a derivative of ethisterone with progestin-like effects. It exerts its uterine action through induction of a hypogonadotropic state and by a direct interaction with endometrial androgens and progesterone receptors [7] causing atrophy. However, there is limited research on the use of danazol in patients with adenomyosis. For the treatment of endometriosis, danazol is now superseded by GnRH analogues – in part because of the androgenic side effects and because of its association with ovarian cancer [28]. Ishihara et al. demonstrated that systemic treatment with danazol improves adenomyosis-related symptoms and reduces uterine size [29]. A recent study compared the use of low-dose danazol with low-dose dienogest for the long-term treatment of adenomyosis [30], the study suggested that both treatments are effective and safe for the long-term management but details of this small size study are not available. Takebayashi et al. [31] injected 10 mg of danazol into the cervix in patients (n = 22) affected by adenomyosis at 2-week intervals for a period of 12 weeks. They observed a significant improvement in pelvic pain and uterine bleeding and a reduction in uterine size, without significant adverse effects. Igarashi et al. [32] described the effects of the insertion of an intrauterine device loaded with 300–400 mg of danazol in 14 women with symptomatic adenomyosis. The results were encouraging with a reduction in pain and bleeding in the majority of the enrolled patients. In addition, three of the four infertile women conceived after

removal of the device. Serum levels of danazol remained below the limit of detection and no systemic side-effects were noted. Despite encouraging preliminary results, it is unlikely that danazol will have a major role in the management of adenomyosis because of the recognised risk profile.

Aromatase Inhibitors

Both eutopic and ectopic endometrium in women affected by endometriosis, adenomyosis, and leiomyomas express aromatase P450, an enzyme implicated in the conversion of C19 androgens to C18 estrogenic steroids [33, 34]. Inhibition of aromatase P450 results in reduction in local estrogens and thus interferes with an important mechanism implicated in the development of the disease [7]. Badawy et al. compared the efficacy of aromatase inhibitor (letrozole) and GnRH agonists in the treatment of adenomyosis. Thirty-three patients were randomly allocated to receive oral letrozole (2.5 mg/day) or a subcutaneous GnRH agonist for 12 weeks. GnRH agonist was more effective than letrozole in relieving chronic pelvic pain, dysmenorrhea, menorrhagia, metrorrhagia and dyspareunia, but the difference was not significant. They claimed that no women in the letrozole group had hot flushes. Worryingly, two women in the letrozole group became pregnant whilst on treatment [35].

Experimental Drugs

Valproic Acid

Valproic acid (VPA) is a specific and potent histone deacetylase inhibitor (HDI), approved by the US Food and Drug Administration for the treatment of epilepsy. In 2010 Liu et al. [36] published a case series on the efficacy of the off-label use of VPA in the treatment of adenomyosis, with encouraging results. They recruited 12 patients with confirmed adenomyosis, dysmenorrhea and an enlarged uterus. All patients received VPA for 3 months and afterwards they were randomly assigned to either the insertion of a LNG- IUD or to no further treatment. There was a significant reduction in uterine size and dysmenorrhea at 6 months in both groups. VPA efficacy could be related to its antiproliferative action on endometrial and endometriotic cells or to its ability to impair myogenesis. In addition HDIs have been reported to suppress growth factors, like vascular endothelial growth factor which play a key role in regulating uterine bleeding and could be involved in adenomyosis related menorrhagia. Studies on the effectiveness of VPA for adenomyosis remain very limited, and no information is available on the medium- and long- term effects.

Bromocriptine

In a mouse, anterior pituitary isografting results in raised prolactin and adenomyosis [37]. Pretreatment with bromocriptine-mesilate (a suppressor of pituitary prolactin) between 4 and 8 weeks of age decreased the incidence of adenomyosis to the level of controls. This led to speculation of a possible role in the human, but no studies have been published that explored the putative effect.

Surgical Treatment

Hysterectomy

Hysterectomy is the definitive cure for women affected by adenomyosis-related dysmenorrhea and menorrhagia who do not desire a future pregnancy and who accept the operation. Hysterectomy should be performed laparoscopically or vaginally whenever technically feasible. Factors to be considered are uterine size, parity and the surgeon's experience. However, vaginal hysterectomy in women with adenomyosis has been associated with an increased rate of bladder injuries when compared to women with uterine fibroids [38]. This may be due to difficult dissection of the vesicovaginal and vesicuterine planes in the anterior adenomyosis or to the presence of deep infiltrating endometriosis of the vesico-uterine pouch [39]. Hysterectomy for uterine adenomyosis may be technically challenging in cases associated with pelvic disease such as recto-vaginal endometriosis and adhesions. In these cases, laparoscopic hysterectomy may be feasible.

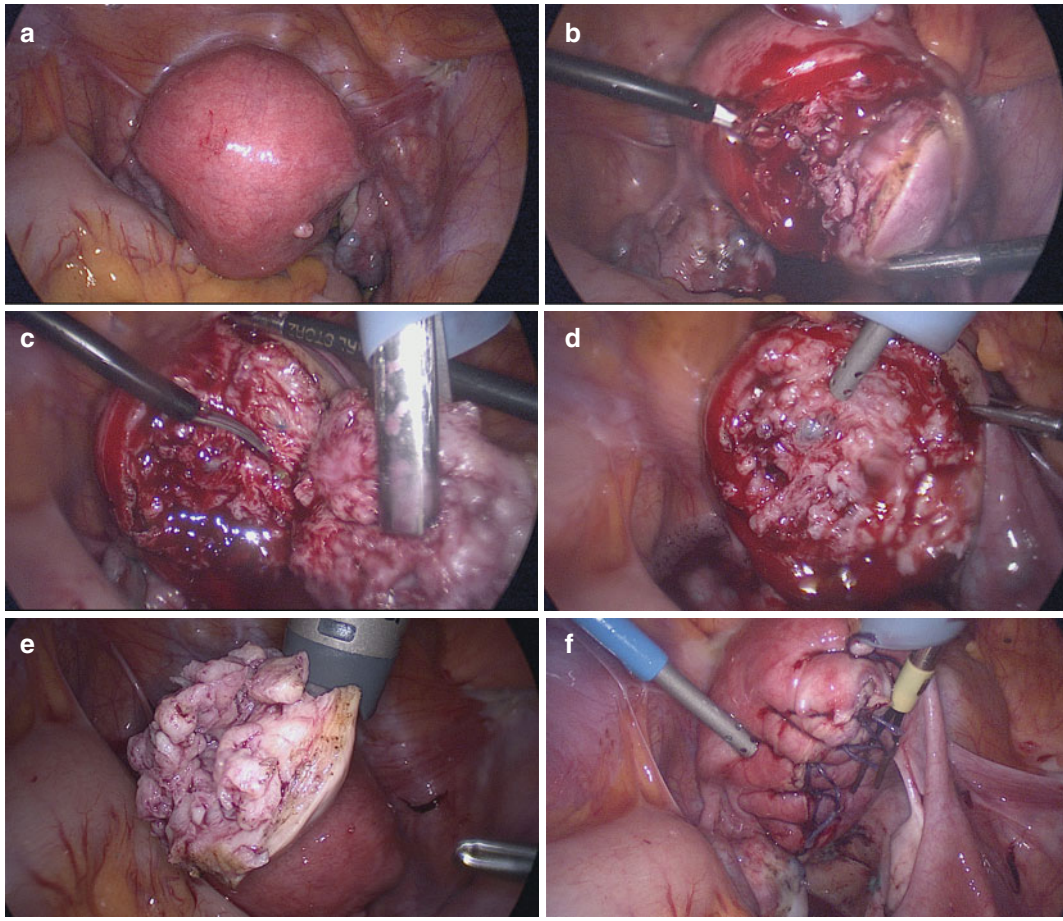

Fig. 12.1 (**a**) Laparoscopic view of the uterus with a posterior adenomyoma of 6 cm. (**b**, **c**) Sharp excision of the adenomyoma. Typically, a cleavage plane from healthy myometrium is absent. (**d**) After the removal of the adenomyoma, residual adenomyotic tissue is removed in order to minimize residual disease. (**e**) Adenomyoma is removed from the abdominal cavity. (**f**) Uterine myometrium and serosa are closed with multiple interrupted suture

However, although laparoscopic hysterectomy seems to be effective in reducing the rate of bladder injuries, it is associated with a higher ureteric injury rate compared to vaginal hysterectomy [40]. No studies have evaluated the possible role of preoperative administration of GnRH agonists in reducing intraoperative complications.

Uterus-Sparing Surgery for Adenomyosis

Uterus-Sparing Surgery for Focal Adenomyosis

Focal adenomyosis is characterized by localized lesions that are distinct from the surrounding normal myometrium [41]. Focal adenomyosis has been referred to as adenomyoma reflecting some similarity to leiomyoma. The surgical technique for adenomyomectomy can be more challenging than myomectomy because of the lack of a demarcation plane in adenomyosis. The first series of adenomyomectomies were performed through laparotomy [42–44] but more recent series demonstrated that most cases can be safely and effectively performed laparoscopically. The surgical steps, as shown in Fig. 12.1, are as follows: (1) an incision is made along the uterine wall overlying the adenomyoma; (2) the adenomyotic lesion is dissected from the surrounding myometrium by gentle sharp dissection. This step requires the use of sharp dissection or mono-

polar cautery to separate the adenomyoma from the healthy myometrium (Fig. 12.1b–d). This can be more readily achieved in cases of focal adenomyosis but in more diffuse disease some affected tissue has to be preserved to allow satisfactory uterine reconstruction; (3) Closure of the uterine cavity if it was opened during dissection; (4) Closure of the uterine incision (Fig. 12.1f). An alternative technique to adenomyomectomy involves wedge resection of the adenomyoma. In published surgical series, wedge resection is less frequently performed compared to adenomyomectomy as it may not be sufficiently effective in removing lesions. One study reported comparable outcomes in terms of symptom control, but wedge resection was associated with a significantly higher rate of sonographic recurrences (69.2 % vs. 15.0 %; $P<0.001$) [45].

Table 12.1 summarizes the outcome of adenomyomectomy in published medium sized and large series, excluding individual case reports and small series of less than five cases. The five studies reviewed here included 497 patients [45–49]. Overall, reduction of dysmenorrhea was achieved in 97 % of cases and the mean pain reduction was 70 %. Reduction of menorrhagia was achieved in 93 % of cases with a mean reduction of bleeding of 65 %. At a mean follow-up of 23 months, relapse of symptoms occurred in 16 % of cases. At a mean follow up of 27 month, there were 75 (34 %) conceptions and the live birth rate was 28 % (Table 12.2) amongst the 221 women wishing to conceive following adenomyomectomy [45–48, 50].

Uterus-Sparing Surgery for Diffuse Adenomyosis

Diffuse adenomyosis typically involves the myometrium with digitations and irregularly shaped lesions with unclear borders. Consequently, complete excision of adenomyotic tissue is not technically possible and in most instances there will be some loss of healthy myometrium. Excision of diffuse adenomyosis is best performed via laparotomy because digital palpation of the uterus is fundamental to the identification of affected areas. This allows selective and piecemeal removal of lesions whilst sparing unaffected myometrium. A few surgical techniques have been described which differ mainly in the type of uterine incision used and in the approach used for uterine reconstruction. Most cases can be done through a transverse laparotomy. A tourniquet placed to compress the uterine arteries reduces blood loss. Adenomyosis lesions can be exposed by bisecting the uterus longitudinally in the midline from the fundus to the endometrial cavity. The index finger is introduced into the endometrial cavity to protect and guide the excision of the lesion [51]. But whilst some authors reported routinely opening the uterine cavity [52, 53], others count breach of the endometrium as a surgical complication [54]. Adenomyotic tissue is resected with the aid of digital palpation. Preservation of at least 1–1.5 cm of myometrial thickness is needed for uterine reconstruction. The endometrial cavity is then closed with interrupted sutures and the myometrium is re-approximated. Reconstruction following extensive ademyomectomy can be particularly challenging. Multiple layers of interrupted sutures are always required for a good repair and particular attention should be paid to avoid intramural dead spaces which can lead to a weak scar with implications on future pregnancies.

Modification of dysmenorrhea and menorrhagia was evaluated in six studies involving 296 women who underwent excision of diffuse adenomyosis [15, 52–56]. Overall, dysmenorrhea and menorrhagia were reduced in 46 % and 69 % of cases respectively with a mean improvement of 60 %. Symptoms relapsed in 5 % of cases after a mean follow-up of 22 months (Table 12.3). Fertility outcomes were reported in seven studies [15, 43, 44, 52–55] involving 219 women wishing to conceive after excision of diffuse adenomyosis. The conception and live birth rates were 36 % and 28 % respectively over a mean follow up of 25 month (Table 12.4).

Comparison of Outcomes of Uterus Sparing Surgery for Focal or Diffuse Adenomyosis

Available evidence suggests that excision of adenomyosis is associated with a 60–70 % reduction in dysmenorrhea and menorrhagia. However, the

Table 12.1 Improvement of symptoms after surgical excision of focal adenomyosis

Author, year	No. of patients	Follow up (months)	Percentage reduction in pain	Reduction of dysmenorrhea n (%)	Percentage reduction in bleeding	Reduction of menorrhagia n (%)	Symptoms relapse n (%)
Wang et al., 2009 [46]							
Surgical group	51	24	70	–	59	–	25 (49)
Surgical-medical	114	24	80	–	75	–	32 (28)
Takeuchi et al., 2006 [47]	14	–	75	–	Improved	–	–
Dai et al., 2011 [48]	86	6	80	–	–	–	6 (7)
Sun et al., 2011 [45]							
Adenomyomectomy	40[a]	28	–	31/34 (91)	–	4/10	2 (5)[c]
Wedge resection	13[b]	21	–	8/9 (89)	–	5/10	3 (23) [d]
Liu et al., 2014 [49]	179	36	45	176/179 (98)	60	176/179 (98)	14 (8)
Total	497	23	70	215/222 (97)	65	185/199 (93)	82 (16)

[a]34 pain/10 bleeding
[b]9 pain/10 bleeding
[c]Ultrasonographic recurrence: 15 %
[d]Ultrasonographic recurrence: 69 %

Table 12.2 Fertility outcomes after surgical excision of focal adenomyosis

Author, year	Patients wishing to conceive	Follow up (months)	Total conceptions n (%)	Miscarriages n (%)	Total deliveries n (%)
Wang et al., 2009 [46]					
Surgical group	27	24–31	20 (74)	3 (15)	17 (63)
Surgical-medical	44	24–31	35 (80)	3 (9)	32 (73)
Takeuchi et al., 2006 [47]	14	–	2 (14)	0	2 (14)
Dai et al., 2011 [48]	86	6–36	2[a]	0	1 (1.2)
Sun et al., 2011 [45]					
Adenomyomectomy	24	28	8 (33)	–	3 (13)
Wedge resection	8	21	0	0	0
Al Jama et al., 2011 [50]	18	36	8 (44)	2 (25)	6 (33)
Total	221	27	75 (34)	8 (11)	61 (28)

[a]1 medical abortion due to early conception (2 months after surgery)

Table 12.3 Improvement of symptoms after surgical excision of diffuse adenomyosis

Author, year	No. of patients	Duration of follow up: months	Percentage reduction in pain	Reduction of dysmenorrhea n (%)	Percentage reduction in bleeding	Reduction of menorrhagia n (%)	Symptoms relapse n (%)
Osada et al., 2011 [52]	104	24	83	–	71	–	4 (4)
Wang PH et al., 2009 [15]	28	36	63	–	–	–	–
Fujishita et al., 2004 [54]							
Modified	6	23–69	55	–	–	–	1 (17)
Classic	5	23–69	18	–	–	–	4 (80)
Nishida et al., 2010 [55]	44	3	91	–	Improved	–	3 (7)
Saremi et al., 2014 [53]	100	24	–	24/59 (41)	–	13/20 (65)	1 (1)
Kim et al., 2014 [56]	9	12	52	7/9 (78)	48	7/9 (78)	3 (33)
Total	296	22	60	31/68 (46)	60	20/29 (69)	16 (5)

proportion of women experiencing such postoperative symptomatic relief at 2 years is higher (90 %) in women with focal adenomyosis, as compared to the lower symptomatic relief of dysmenorrhea (46 %) and of menorrhagia (69 %) in women with diffuse adenomyosis. As for fertility outcome, there was no difference between women who underwent excision of focal or diffuse adenomyosis, with an identical live birth rate of 28 % in both groups. In comparison, Grimbizis et al., reported a delivery rate of 50 % (74 of 147) for total adenomyomectomy and 34 % (11 of 32) for partial adenomyomectomy [41]. Maheshwari et al. reported an overall live birth rate of 36 % (21 of 58) following conservative surgery for adenomyosis [57]. There is a wide variation in fertility outcome reported by studies of conservative surgical treatment, with

Table 12.4 Fertility outcomes after surgical excision of diffuse adenomyosis

Author, year	Patients wishing to conceive n (%)	Follow up (months)	Total conceptions n (%)	Miscarriages n (%)	Total deliveries n (%)
Osada et al., 2011 [52]	26	24	16 (62)	2 (12)	14 (54)
Wang et al., 2009 [15]	28	36	13 (46)	4 (31)	9 (32)
Fujishita et al., 2004 (modified) [54]	4	23–69	2 (50)	0	2 (50)
Fujishita et al., 2004 (classic) [54]	3	23–69	0	0	0
Nishida et al., 2010 [55]	44	na	2[a] (4, 5)	0	1 (2.2)
Tadjerouni et al., 1995 [44]	36	na	21 (58)	6 (29)	15 (42)
Strizhakov and Davydov, 1995 [43]	8	12	4 (50)	0	4 (50)
Saremi et al., 2014 [53]	70	24	21[b] (30)	4 (19)	16 (23)
Total	219	25	79[a,b] (36)	16 (20)	61 (28)

[a]1 interstitial pregnancy
[b]1 stillbirth afterine uterine rupture at 37 weeks
na not applicable

live birth rates ranging from 1 % [48] to 73 % [46]. It is difficult to ascertain if this reflects differences in surgical technique or in patient characteristics.

The potential risks of uterine-sparing surgery for adenomyosis include uterine rupture, Asherman syndrome, uterine deformities and reduced uterine capacity. However, the incidence of these complications is unknown. There thus remains a need for well-designed, prospective clinical trials.

It is possible that decidualization of residual adenomyotic fragments further weakens the scar leading to uterine rupture during pregnancy [58]. Attention to myometrial reconstruction may reduce the risk of rupture. Recently, Saremi et al. reported a series of 103 women who underwent excision of diffuse adenomyosis through laparotomy, 70 of whom wished to conceive. There were 21 pregnancies including 16 that reached term. The residual myometrial thickness was at least 0.5 mm [53]. There were 2 (9.5 %) cases of uterine rupture which occurred at 32 and 37 weeks. Postoperative Asherman syndrome was diagnosed in 4 out of 103 (3.9 %) patients. Osada et al. described a similar surgical technique, but preserved at least 1 cm of myometrial thickness. They reported no uterine rupture among 14 women who subsequently had a term delivery [52]. Until more data becomes available, it is advisable to preserve at least 1 cm of myometrial thickness for uterine reconstruction.

In conclusion, the decision to proceed with surgical excision of extensive adenomyosis in women desiring future pregnancy should only be taken after extensive counseling and consideration of alternatives and there should be a low index of suspicion with regards to uterine rupture in women who conceive after uterine sparing surgery [59].

Hysteroscopic Endomyometrial Ablation/Resection

Hysteroscopic endomyometrial resection has been, perhaps also inadvertently, performed for the treatment of adenomyosis-related menorrhagia

in women not desiring future pregnancy. The rationale for hysteroscopic treatment is based on the assumption that adenomyosis located at the endomyometrial junction is responsible of excessive uterine bleeding. McCausland and McCausland [60] demonstrated that the success rate of roller-ball endometrial resection was inversely correlated with the depth of penetration of endometrium within the myometrium. Patients with superficial adenomyosis with penetration <2 mm experienced postoperative amenorrhea or light menses, whereas patients with deep adenomyosis (penetrating >2 mm) experienced poor outcomes and ultimately required a hysterectomy. Endometrial ablation >3 mm is contraindicated due to the presence of significant myometrial arteries approximately 5 mm deep within the myometrium [61]. Wood (1998) reported that 10 out of 18 (55 %) patients who underwent endometrial ablation for adenomyosis-related menorrhagia were free of symptoms at 24 months follow-up [61]. In another study evaluating hysteroscopic roller-ball ablation in 190 patients with adenomyosis, 98 % of treated women reported improved symptoms and 3 % required a hysterectomy [62]. The cause of failure of hysteroscopic endomyometrial ablation may be that deeper ectopic endometrial glands persist and eventually regrow causing recurrent menorrhagia or new onset dysmenorrhea. Alternative treatment options for women who had failed ablation include repeat ablation [63], insertion of LNG-IUD [22] or hysterectomy.

Uterine Artery Embolization

Fifteen studies published between 1999 and 2010, evaluated the outcomes of uterine artery embolization (UAE) in the treatment of symptomatic adenomyosis. These included 511 affected women [64]. Overall, 387 patients (75.7 %) had symptomatic improvement in bleeding, pain and bulk-related discomfort in long term follow-up (median follow-up of 26.9 months). Recent studies have confirmed these findings. Froeling et al. [65] and Smeets et al. [66] reported similar outcomes: 29 out of 40 (72.5 %) women treated with UAE for symptomatic adenomyosis were free of symptoms after a follow up of 40 and 65 months. Ten patients underwent hysterectomy because of failed UAE in the study by Froeling et al. (2012) and 7 needed a hysterectomy in the study by Smeets et al., (2012) . Similarly, Nijenhuis et al. [67] reported that 22 out of 29 (76 %) patients with severe adenomyosis-related symptoms, were asymptomatic 37 months after UAE.

No data are available regarding fertility among women with adenomyosis following UAE. However, a recent review evaluated existing evidence about fertility after UAE for uterine fibroids. Out of 738 women affected by uterine fibroids who expressly wished to conceive at the time of UAE, fertility, miscarriage and take-home baby rates were 36.3, 29.7 and 19.6 %, respectively [68]. This study by Torre et al., also prospectively evaluated pregnancy rates in a cohort of 66 women who desired future pregnancy and who were treated with UAE for symptomatic fibroids. Fibroid symptoms including menorrhagia, metrorrhagia, pain and bulk syndrome were significantly improved after UAE. However, in spite of seeking pregnancy of 33.4 ± 14.5 months, only 1 out of 31 women became pregnant and she finally miscarried. In this series, 5 patients resorted to in-vitro fertilization and 22 embryos were transferred, including 8 embryos from oocyte donation. No pregnancy was achieved, suggesting deficient implantation. Based on the low pregnancy rates, the authors concluded that UAE might have had a negative impact on fertility and suggested that UAE should not be performed routinely in young women of childbearing age with extensive fibroids.

In conclusion, UAE may improve symptoms in more than 70 % of women with adenomyosis but future fertility rates and obstetric outcomes are unknown and need to be assessed by means of randomized controlled trials.

Conclusions

Medical treatments for uterine adenomyosis remain poorly investigated. Most existing medical treatment options have been evaluated only in short-term studies involving small populations. However, what we know from the little data available and from more extensive experience with hypo-estrogenizing drugs for the treatment of endometriosis, is that

medical therapies are effective for as long as they are administered, and symptoms may promptly recur after treatment is discontinued. Therefore, although adenomyosis manifests at a more advanced age compared to endometriosis, medical treatment of adenomyosis may need to be administered long term, hypothetically from onset of symptoms to the time when affected women seek pregnancy or to the onset of the menopause.

Hypo-estrogenic status can be induced by GnRH analogues, which are reportedly very effective in the treatment of adenomyosis-related menorrhagia and dysmenorrhea. However, the use of these drugs in the long term is limited by significant adverse effects including hot flushes and osteoporosis as well as by high costs. In comparison, inexpensive and well-tolerated drugs such as progestogens, if proven effective and safe in the long term, might represent an alternative. Prospective studies are needed to evaluate the efficacy of progestogens and estro-progestogens in the long term. Finally, a promising option is the LNG-IUD, which reportedly decreases or eliminates symptoms of dysmenorrhea and menorrhagia, with a low adverse effects profile.

Although endometrial ablation have been reported to improve symptoms in a high proportion of women with adenomyosis [62], this technique has the major disadvantages of being indicated only for superficial disease (penetrating < 2 mm) and of precluding the possibility of a future pregnancy.

In light of the availability of effective medical treatment options, uterus sparing surgery in patients not wanting children has a very limited place. In fact, postoperative symptom relief may be comparable to medical treatment alone. Adenomyomectomy or wedge resection may be as burdensome for patients as a hysterectomy, but with a lower short and long term success rates. In women not desiring a pregnancy, when medical and possibly hysteroscopic treatments have failed, hysterectomy should be considered as the definitive treatment.

Uterus-sparing surgery has a role in women affected by adenomyosis who desire future pregnancy. The only study comparing surgery plus GnRH agonists and GnRH agonists alone showed higher pregnancy rates in the surgical group [15]. Nowadays, the demand for conservative, fertility-enhancing surgical treatment of adenomyosis is increasing because more women delay their first pregnancy until 30 or 40 years of age. Results are difficult to compare between surgical series but it seems that live birth rate after excision of adenomyosis may be around 30 %. It has to be kept in mind that surgical excision of adenomyosis is technically demanding, especially in cases of diffuse disease, and that myometrial reconstruction has to be performed meticulously, leaving at least 1 cm of myometrial thickness and no intramural dead spaces. Finally, women have to be extensively counseled about the risk of uterine rupture in a future pregnancy.

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