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

M. Habiba, PhD, PhD, FRCOG (✉)
Department of Obstetrics and Gynaecology,
University Hospitals of Leicester,
Leicester Royal Infirmary, Infirmary Close,
Leicester LE1 5WW, UK

Department of Health Sciences,
University of Leicester, Leicester, UK
e-mail: mah6@le.ac.uk

G. Benagiano, MD, PhD, FRCOG
Department of Gynecology, Obstetrics and Urology,
Sapienza University, Rome 00161, Italy
e-mail: giuseppe.benagiano@uniroma1.it

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'

M. Habiba, G. Benagiano (eds.), *Uterine Adenomyosis*, DOI 10.1007/978-3-319-13012-5_5

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].

References

1. Bird CC, McElin TW, Manalo-Estrella P. The elusive adenomyosis of the uterus – revisited. Am J Obstet Gynecol. 1972;112:583–93.
2. Cullen TS. Adenomyoma of the uterus. Philadelphia/London: W.B. Saunders Company; 1908.
3. Novak ER, Woodruff JD. Adenomyosis (adenomyoma) uteri. In: Novak's gynecologic and obstetric pathology. Philadelphia: W.B. Saunders Company; 1979. p. 280–90.
4. Hendrickson MR, Kempson RL. Non-neoplastic conditions of the myometrium and uterine serosa. In: Surgical pathology of the uterine corpus. Philadelphia: W.B. Saunders; 1980.
5. Langlois PL. The size of the normal uterus. J Reprod Med. 1970;4:220–8.
6. Esmaelzadeh S, Rezaei N, HajiAhmadi M. Normal uterine size in women of reproductive age in northern Islamic Republic of Iran. East Mediterr Health J. 2004;10:437–41.
7. Brosens JJ, de Souza NM, Barker FG, Paraschos T, Winston RM. Endovaginal ultrasonography in the diagnosis of adenomyosis uteri: identifying the predictive characteristics. Br J Obstet Gynaecol. 1995;102:471–4.
8. Ascher SM, Arnold LL, Patt RH, Schruefer JJ, Bagley AS, Semelka RC, et al. Adenomyosis: prospective comparison of MR imaging and transvaginal sonography. Radiology. 1994;190:803–6.
9. Fedele L, Bianchi S, Dorta M, Zanotti F, Brioschi D, Carinelli S. Transvaginal ultrasonography in the differential diagnosis of adenomyoma versus leiomyoma. Am J Obstet Gynecol. 1992;167:603–6.
10. Hirai M, Shibata K, Sagai H, Sekiya S, Goldberg BB. Transvaginal pulsed and color Doppler sonography for the evaluation of adenomyosis. J Ultrasound Med. 1995;14:529–32.
11. Atri M, Reinhold C, Mehio AR, Chapman WB, Bret PM. Adenomyosis: US features with histologic correlation in an in-vitro study. Radiology. 2000;215:783–90.
12. Fedele L, Bianchi S, Raffaelli R, Portuese A, Dorta M. Treatment of adenomyosis-associated menorrhagia with a levonorgestrel-releasing intrauterine device. Fertil Steril. 1997;68:426–9.
13. Reinhold C, Atri M, Mehio A, Zakarian R, Aldis AE, Bret PM. Diffuse uterine adenomyosis: morphologic criteria and diagnostic accuracy of endovaginal sonography. Radiology. 1995;197:609–14.
14. Wiczyk HP, Janus CL, Richards CJ, Graf MJ, Gendal ES, Rabinowitz JG, et al. Comparison of magnetic resonance imaging and ultrasound in evaluating follicular and endometrial development throughout the normal cycle. Fertil Steril. 1988;49:969–72.
15. Demas BE, Hricak H, Jaffe RB. Uterine MR imaging: effects of hormonal stimulation. Radiology. 1986;159:123–6.
16. Mark AS, Hricak H, Heinrichs LW, Hendrickson MR, Winkler ML, Bachica JA, et al. Adenomyosis and leiomyoma: differential diagnosis with MR imaging. Radiology. 1987;163:527–9.
17. Reinhold C, Tafazoli F, Wang L. Imaging features of adenomyosis. Hum Reprod Update. 1998;4:337–49.
18. Fusi L, Cloke B, Brosens JJ. The uterine junctional zone. Best Pract Res Clin Obstet Gynaecol. 2006;20:479–91.
19. Brown HK, Stoll BS, Nicosia SV. Uterine junctional zone: correlation between histologic findings and MR imaging. Radiology. 1991;179:409–13.
20. Lee JK, Gersell DJ, Balfe DM, Worthington JL, Picus D, Gapp G. The uterus: in vitro MR-anatomic correlation of normal and abnormal specimens. Radiology. 1985;157:175–9.
21. Hauth EA, Jaeger HJ, Libera H, Lange S, Forsting M. MR imaging of the uterus and cervix in healthy women: determination of normal values. Eur Radiol. 2007;17:734–42.
22. Hricak H, Alpers C, Crooks LE, Sheldon PE. Magnetic resonance imaging of the female pelvis: initial experience. AJR Am J Roentgenol. 1983;141:1119–28.
23. McCarthy S, Tauber C, Gore J. Female pelvic anatomy: MR assessment of variations during the menstrual cycle and with use of oral contraceptives. Radiology. 1986;160:119–23.
24. McCarthy S, Scott G, Majumdar S, Shapiro B, Thompson S, Lange R, et al. Uterine junctional zone: MR study of water content and relaxation properties. Radiology. 1989;171:241–3.
25. Scoutt LM, Flynn SD, Luthringer DJ, McCauley TR, McCarthy SM. Junctional zone of the uterus: correlation of MR imaging and histologic examination of hysterectomy specimens. Radiology. 1991;179:403–7.
26. Schwalm H, Dubrauszky V. The structure of the musculature of the human uterus – muscles and connective tissue. Am J Obstet Gynecol. 1966;94:391–404.

27. Mehasseb MK, Bell SC, Brown L, Pringle JH, Habiba M. Phenotypic characterisation of the inner and outer myometrium in normal and adenomyotic uteri. Gynecol Obstet Invest. 2011;71:217–24.
28. Ijland M, Evers JL, Dunselman GA, Volovics L, Hoogland HJ. Relation between endometrial wave-like activity and fecundability in spontaneous cycles. Fertil Steril. 1997;67:492–6.
29. Fanchin R, Righini C, Olivennes F, Taylor S, de Ziegler D, Frydman R. Uterine contractions at the time of embryo transfer alter pregnancy rates after in-vitro fertilization. Hum Reprod. 1998;13:1968–74.
30. De Vries K, Lyons EA, Ballard G, Levi CS, Lindsay DJ. Contractions of the inner third of the myometrium. Am J Obstet Gynecol. 1990;679–82.
31. Lyons EA, Taylor PJ, Zheng XH, Ballard G, Levi CS, Kredentser JV. Characterisation of subendometrial myometrial contractions throughout the menstrual cycle in normal fertile women. Fertil Steril. 1991;55:771–4.
32. Brosens JJ, de Souza NM, Barker FG. Uterine junctional zone: function and disease. Lancet. 1995;346:558–60.
33. Fraser IS, McCarron G, Markham R, Resta T, Watts A. Measured menstrual blood loss in women with menorrhagia associated with pelvic disease or coagulation disorder. Obstet Gynecol. 1986;68:630–3.
34. Benson RC, Sneeden VD. Adenomyosis: a reappraisal of symptomatology. Am J Obstet Gynecol. 1958;76:1044–57; discussion 57–61.
35. Meyer R. Ältere und neuere Gesichtspunkte über die Adenomyohyperplasia (adenomyosis) und die extragenitale Fibroadenomatosis. Zentralbl. Gynäk. 1925:49;1170.
36. Kissler S, Hamscho N, Zangos S, Wiegratz I, Schlichter S, Menzel C, et al. Uterotubal transport disorder in adenomyosis and endometriosis--a cause for infertility. BJOG. 2006;113:902–8.
37. Habiba MA. Radionuclide migration through the genital tract in infertile women with endometriosis. Hum Reprod. 1994;9:1193–5.
38. Lundberg S, Wramsby H, Bremmer S, Lundberg HJ, Asard PE. Radionuclide hysterosalpingography does not distinguish between fertile women, before tubal sterilization, and infertile women. Hum Reprod. 1997;12:275–8.
39. Lundberg S, Wramsby H, Bremmer S, Lundberg HJ, Asard PE. Radionuclide hysterosalpingography is not predictive in the diagnosis of infertility. Fertil Steril. 1998;69:216–20.
40. Wanggren K, Wramsby H, Bremmer S, Lundberg S, Jacobsson H. Radionuclide hysterosalpingography is not a reliable tool for investigation of fallopian tube transport--a controlled randomized study using particles of two sizes during three different parts of the menstrual cycle. Gynecol Obstet Invest. 2011;72:20–4.
41. Leyendecker G, Kunz G, Noe M, Herbertz M, Mall G. Endometriosis: a dysfunction and disease of the archimetra. Hum Reprod Update. 1998;4:752–62.
42. Werth R, Grusdew W. Untersuchungen uber die entwicklung und morphologie der menschlichen uterusmuskulatur. Arch Gynakol. 1898;55:325–409.
43. Emge LA. The elusive adenomyosis of the uterus. Its historical past and its present state of recognition. Am J Obstet Gynecol. 1962;83:1541–63.
44. Marcus CC. Relationship of adenomyosis uteri to endometrial hyperplasia and endometrial carcinoma. Am J Obstet Gynecol. 1961;82:408–16.
45. Uduwela AS, Perera MA, Aiqing L, Fraser IS. Endometrial-myometrial interface: relationship to adenomyosis and changes in pregnancy. Obstet Gynecol Surv. 2000;55:390–400.
46. Noe N, Kunz G, Herbertz M, Mall G, Leyendecker G. The cyclic pattern of the immunocytochemical expression of oestrogen and progesterone receptors in human myometrial and endometrial layers: characterization of the endometrial-subendometrial unit. Hum Reprod. 1999;14:190–7.
47. Konishi I, Fujii S, Okamura H, Mori T. Development of smooth muscle in the human fetal uterus: an ultrastructural study. J Anat. 1984;139(Pt 2):239–52.
48. Robboy SJ, Taguchi O, Cunha GR. Normal development of the human female reproductive tract and alterations resulting from experimental exposure to diethylstilbesterol. Hum Pathol. 1982;13:191–8.
49. Wetzstein R. Der Uterusmuskel: Morphologie. Arch Gynecol. 1965;202:1–13.
50. Lebedev NP. Uber den architektouischen Bau des Myometriums. Akush i Ginek. 1952;64.
51. Mehasseb MK, Bell SC, Habiba MA. Neonatal administration of tamoxifen causes disruption of myometrial development but not adenomyosis in the C57/BL6J mouse. Reproduction 2010; In Press.
52. Mehasseb MK, Bell SC, Habiba MA. The effects of tamoxifen and estradiol on myometrial differentiation and organization during early uterine development in the CD1 mouse. Reproduction. 2009;138:341–50.
53. Metaxa-Mariatou V, McGavigan CJ, Robertson K, Stewart C, Cameron IT, Campbell S. Elastin distribution in the myometrial and vascular smooth muscle of the human uterus. Mol Hum Reprod. 2002;8:559–65.
54. Kohnen G, Campbell S, Jeffers MD, Cameron IT. Spatially regulated differentiation of endometrial vascular smooth muscle cells. Hum Reprod. 2000;15:284–92.
55. Leppert PC, Yu SY. Three-dimensional structures of uterine elastic fibers: scanning electron microscopic studies. Connect Tissue Res. 1991;27:15–31.
56. Zheng WQ, Ma R, Zheng JM, Gong ZJ. Elastin distribution in the normal uterus, uterine leiomyomas, adenomyosis and adenomyomas: a comparison. Anal Quant Cytol Histol. 2006;28:115–20.
57. Scharl A, Vierbuchen M, Graupner J, Fischer R, Bolte A. Immunohistochemical study of distribution of estrogen receptors in corpus and cervix uteri. Arch Gynecol Obstet. 1988;241:221–33.
58. Mertens HJMM, Heineman MJ, Theunissen PHMH, de Jong FH, Evers JLH. Androgen, estrogen and pro-

gesterone receptor expression in the human uterus during the menstrual cycle. Eur J Obstet Gynecol Reprod Biol. 2001;98:58–65.
59. Kawaguchi K, Fujii S, Konishi I, Iwai T, Nanbu Y, Nonogaki H, et al. Immunohistochemical analysis of oestrogen receptors, progesterone receptors and Ki-67 in leiomyoma and myometrium during the menstrual cycle and pregnancy. Virchows Arch A Pathol Anat Histopathol. 1991;419: 309–15.
60. Snijders MP, De Goeji AF, Debets-Te Baerts MJ, Rosch MJ, Koudstaal J, Bosman FT. Immunocytochemical analysis of oestrogen receptors and progesterone receptors in the human uterus throughout the menstrual cycle and after the menopause. J Reprod Fertil. 1992;94:363–71.
61. Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL, McCarty Jr KS. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. J Clin Endocrinol Metab. 1988;67:334–40.
62. Amso NN, Crow JC, Shaw RW. Comparative immunohistochemical study of oestrogen and progesterone receptors in the Fallopian tube and uterus at different stages of the menstrual cycle and the menopause. Hum Reprod. 1994;9:1027–37.
63. Richards PA, Tiltman AJ. Anatomical variation of the oestrogen receptor in normal myometrium. Virchows Arch. 1995;427:303–7.
64. Mehasseb MK, Panchal R, Taylor AH, Brown L, Bell SC, Habiba M. Estrogen and progesterone receptor isoform distribution through the menstrual cycle in uteri with and without adenomyosis. Fertil Steril. 2011;95:2228–35, 35 e1.
65. Mylonas I, Jeschke U, Shabani N, Kuhn C, Balle A, Kriegel S, et al. Immunohistochemical analysis of estrogen receptor alpha, estrogen receptor beta and progesterone receptor in normal human endometrium. Acta Histochem. 2004;106:245–52.
66. Mylonas I, Jeschke U, Shabani N, Kuhn C, Kunze S, Dian D, et al. Steroid receptors ERalpha, ERbeta, PR-A and PR-B are differentially expressed in normal and atrophic human endometrium. Histol Histopathol. 2007;22:169–76.
67. Lecce G, Meduri G, Ancelin M, Bergeron C, Perrot-Applanat M. Presence of estrogen receptor b in the human endometrium through the cycle: expression in glandular, stromal, and vascular cells. J Clin Endocrinol Metab. 2001;86:1379–86.
68. Matsuzaki S, Fukaya T, Suzuki T, Murakami T, Sasano H, Yajima A. Oestrogen receptor a and b mRNA expression in human endometrium throughout the menstrual cycle. Mol Hum Reprod. 1999;5:559–64.
69. Critchley HO, Saunders PT. Hormone receptor dynamics in a receptive human endometrium. Reprod Sci. 2009;16:191–9.
70. Speirs V, Green CA, Shaaban AM. Oestrogen receptor b immunohistochemistry: time to get it right? J Clin Pathol. 2008;61:1150–1.
71. Maia Jr H, Casoy J, Correia T, Freitas L, Pimentel K, Athayde C, et al. Effect of the menstrual cycle and oral contraceptives on aromatase and cyclooxygenase-2 expression in adenomyosis. Gynecol Endocrinol. 2006;22:547–51.
72. Takahashi K, Nagata H, Kitao M. Clinical usefulness of determination of estradiol level in the menstrual blood for patients with endometriosis. Nippon Sanka Fujinka Gakkai Zasshi. 1989;41:1849–50.
73. McCormack SA, Glasser SR. Differential response of individual uterine cell types from immature rats treated with estradiol. Endocrinology. 1980;106: 1634–49.
74. King RJ, Townsend PT, Whitehead MI, Young O, Taylor RW. Biochemical analyses of separated epithelium and stroma from endometria of premenopausal and postmenopausal women receiving estrogen and progestins. J Steroid Biochem. 1981;14: 979–87.
75. Shiozawa T, Li S-F, Nakayama K, Fujii S. Relationship between the expression of cyclins/cyclin-dependent kinases and sex-steroid receptors/ Ki67 in normal human endometrial glands and stroma during the menstrual cycle. Mol Hum Reprod. 1996;2: 745–52.
76. Graham JD, Clarke CL. Physiological action of progesterone in target tissues. Endocrinol Rev. 1997;18:502–9.
77. Azziz R. Adenomyosis: current perspectives. Obstet Gynecol Clin North Am. 1989;16:221–35.
78. Moghissi KS. Treatment of endometriosis with estrogen-progestin combination and progestogens alone. Clin Obstet Gynecol. 1988;31:823–8.
79. Wood C. Surgical and medical treatment of adenomyosis. Hum Reprod Update. 1998;4:323–36.
80. Maia Jr H, Maltez A, Coelho G, Athayde C, Coutinho EM. Insertion of mirena after endometrial resection in patients with adenomyosis. J Am Assoc Gynecol Laparosc. 2003;10:512–6.
81. Kunz G, Leyendecker G. Uterine peristaltic activity during the menstrual cycle: characterization, regulation, function and dysfunction. Reprod Biomed Online. 2002;4 Suppl 3:5–9.
82. Kunz G, Bell D, Deininger H, Wildt L, Leyendecker G. The dynamics of rapid sperm transport through the female genital tract. Evidence from vaginal sonography of uterine peristalsis (VSUP) and hysterosalpingoscintigraphy (HSSG). Hum Reprod. 1996;11:627–32.
83. Kunz G, Beil D, Huppert P, Leyendecker G. Structural abnormalities of the uterine wall in women with endometriosis and infertility visualized by vaginal sonography and magnetic resonance imaging. Hum Reprod. 2000;15:76–82.
84. Quinn M. Uterine innervation in adenomyosis. J Obstet Gynaecol. 2007;27:287–91.
85. Laguens R, Lagrutta J. Fine structure of human uterine muscle in pregnancy. Am J Obstet Gynecol. 1964;89:1040–7.

86. Dessouky DA. Ultrastructural observations of the human uterine smooth muscle cells during gestation. Am J Obstet Gynecol. 1976;125:1099–107.
87. Mark JST. An electron microscopy study of uterine smooth muscle. Anat Rec. 1956;125:473–91.
88. Jaeger J. Zur Ultrastruktur der menschliehen Uterus-Muskelzelle unter der Geburt. Archiv fur Gyngkologie. 1963;199:173–81.
89. Jaeger J. Zur Ultrastruktur des bindegewebigen Anteils der Uterusmuskulatur. Arch Gynakol. 1965;202:59–62.
90. Jaeger J. Elektronenmikroskopisehe Untersuehungen an der menschliehen Uterusmuskulatur (znr nervi-isen Innervation der glatten Muskelzellen). Arch Gynakol. 1967;204:63–6.
91. Jaeger J. Der intercelluläre Kontakt der glatten Muskelzellen. Arch Gynakol. 1971;211:351–4.
92. Shoenberg CF. The contractile mechanism and ultrastructure of the myometrium. In: Wynn RM, editor. Biology of the uterus. New York/London: Plenum Press; 1977. p. 497–544.
93. Leoni P, Carli F, Halliday D. Intermediate filaments in smooth muscle from pregnant and non-pregnant human uterus. Biochem J. 1990;269:31–4.
94. Eyden BP, Hale RJ, Richmond I, Buckley CH. Cytoskeletal filaments in the smooth muscle cells of uterine leiomyomata and myometrium: an ultrastructural and immunohistochemical analysis. Virchows Arch A Pathol Anat Histol. 1992;420: 51–8.
95. Huitfeldt HS, Brandtzaeg P. Various keratin antibodies produce immunohistochemical staining of human myocardium and myometrium. Histochemistry. 1985;83:381–9.
96. Brown DC, Theaker JM, Banks PM, Gatter KC, Mason DY. Cytokeratin expression in smooth muscle and smooth muscle tumours. Histopathology. 1987;11:477–86.
97. Norton AJ, Thomas JA, Isaacson PG. Cytokeratin-specific monoclonal antibodies are reactive with tumours of smooth muscle derivation. An immunocytochemical and biochemical study using antibodies to intermediate filament cytoskeletal proteins. Histopathology. 1987;11:487–99.
98. Ramaekers FC, Pruszczynski M, Smedts F. Cytokeratins in smooth muscle cells and smooth muscle tumours. Histopathology. 1988;12: 558–61.
99. Tauchi K, Tsutsumi Y, Yoshimura S, Watanabe K. Immunohistochemical and immunoblotting detection of cytokeratin in smooth muscle tumors. Acta Pathol Jpn. 1990;40:574–80.
100. Turley H, Pulford KA, Gatter KC, Mason DY. Biochemical evidence that cytokeratins are present in smooth muscle. Br J Exp Pathol. 1988;69: 433–40.
101. Evans DJ, Lampert IA, Jacobs M. Intermediate filaments in smooth muscle tumours. J Clin Pathol. 1983;36:57–61.
102. Taggart MJ, Morgan KG. Regulation of the uterine contractile apparatus and cytoskeleton. Semin Cell Dev Biol. 2007;18:296–304.
103. Jaeger J, Pohlmann G. On the ultrastructure of human myometrium cells. Beitr Pathol Anat. 1962;126:113–26.
104. Ross R, Klebanoff SJ. Fine structural changes in uterine smooth muscle and fibroblasts in response to estrogen. J Cell Biol. 1967;32:155–67.
105. Friederici HH, DeCloux RJ. The early response of immature rat myometrium to estrogenic stimulation. J Ultrastruct Res. 1968;22:402–12.
106. Althoff RW, Albert EN. Ultrastructural changes in mouse myometrium during pregnancy. Am J Obstet Gynecol. 1970;108:1224–33.
107. Cole WC, Garfield RE. Ultrastructure of the myometrium. In: Wynn RM, Jollie WP, editors. Biology of the uterus, vol. 455–504. New York: Plenum; 1989.
108. Fujii S, Konishi I, Mori T. Smooth muscle differentiation at endometrio-myometrial junction. An ultrastructural study. Virchows Arch A Pathol Anat Histol. 1989;414:105–12.
109. Gabbiani G, Ryan GB, Majno G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contracture. Experimentia. 1971;27:549.
110. Ghahdially FN. Ultrastructural Pathology of the Cell and Matrix. London: Butterworth; 1982. p. 658–63.
111. Bird CC, Willis RA. The production of smooth muscle by the endometrial stroma of the adult human uterus. J Pathol Bacteriol. 1965;90:75–81.
112. Bo WJ, Odor DL, Rothrock ML. Ultrastructure of uterine smooth muscle following progesterone or progesterone-estrogen treatment. Anat Rec. 1969;163:121–31.
113. Bo WJ, Odor DL, Rothrock M. The fine structure of uterine smooth muscle of the rat uterus at various time intervals following a single injection of estrogen. Am J Anat. 1968;123:369–84.
114. Kawaguchi K, Fujii S, Konishi I, Okamura H, Mori T. Ultrastructural study of cultured smooth muscle cells from uterine leiomyoma and myometrium under the influence of sex steroids. Gynecol Oncol. 1985;21:32–41.
115. Mehasseb MK, Bell SC, Pringle JH, Habiba MA. Uterine adenomyosis is associated with ultrastructural features of altered contractility in the inner myometrium. Fertil Steril. 2010;93: 2130–6.
116. Dito WR, Batsakis JG. Norethynodrel-treated endometriosis: a morphological and histochemical study. Obstet Gynecol. 1961;18:1–12.
117. Cotran RS, Kumar V, Robbins SL. Robbins pathologic basis of disease. WB Saunders, Philadelphia, 1989.
118. Loud AV, Olivetti G, Anversa P. Morphometric measurement of cellular hypertrophy. Lab Invest. 1983;49:230–4.

119. Owens GK, Rabinovitch PS, Schwartz SM. Smooth muscle cell hypertrophy versus hyperplasia in hypertension. Proc Natl Acad Sci U S A. 1981;78:7759–63.
120. Lane BP. Alterations in the cytologic detail of intestinal smooth muscle cells in various stages of contraction. J Cell Biol. 1965;27:199–213.
121. North AJ, Galazkiewicz B, Byers TJ, Glenney JRJ, Small JV. Complementary distributions of vinculin and dystrophin define two distinct sarcolemma domains in smooth muscle. J Cell Biol. 1993;120:1159–67.
122. Fujimoto T. Calcium pump of the plasma membrane is localized in caveolae. J Cell Biol. 1993;5:1147–57.
123. Goto Y, Yoshikane H, Honda M, Morioka S, Yamori Y, Moriyama K. Three-dimensional observation on sarcoplasmic reticulum and caveolae in myocardium of spontaneously hypertensive rats. J Submicrosc Cytol Pathol. 1993;22:535–42.
124. Richards PA, Richards PDG, Tiltman AJ. The ultrastructure of fibromyomatous myometrium and its relationship to infertility. Hum Reprod Update. 1998;4:520–5.
125. Yamamoto T, Noguchi T, Tamura T, Kitawaki J, Okada H. Evidence for estrogen synthesis in adenomyotic tissues. Am J Obstet Gynecol. 1993;169:734–8.
126. Tamaya T, Motoyama T, Ohono Y, Ide N, Tsurusaki T, Okada H. Steroid receptor levels and histology of endometriosis and adenomyosis. Fertil Steril. 1979;31:396–400.