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**Deep nodular endometriosis:
from observational studies to experimental model**

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*A mes proches,
Et plus particulièrement à mes Parents,
Et plus particulièrement à Amandine
Et plus particulièrement à Cassandre et Constantin
Sans oublier Elise*

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« Les mots manquent aux émotions »

Victor Hugo

**« Ce n'est pas tant l'aide de nos amis qui nous aide
Que notre confiance dans cette aide »**

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Summary

Endometriosis is one of the most frequent benign gynecological diseases and is known to occur in 7-10% of women of reproductive age. It is now well established that three different forms of endometriosis must be considered in the pelvis: peritoneal endometriosis, ovarian endometriosis and deep endometriotic nodules of the rectovaginal septum. Most of these lesions originate from the posterior part of the cervix (types II & III) and secondarily infiltrate the anterior wall of the rectum (type III).

This study focuses on the characterization of deep endometriotic nodules, and more specifically type III nodules, which are one the most painful and least characterized forms of endometriosis. Our main objective was to gain further insights into invasion processes leading to infiltration of the rectal wall and surrounding organs by endometriotic lesions (proliferation of glands and stroma). The project was divided into 5 parts:

1. In the first part, we reported the largest clinical series of surgical removal of type III deep endometriotic nodules. Our data confirm that a conservative surgical approach offers good results in terms of quality of life, recurrence and pregnancy. When compared to the existing literature, the shaving technique has a lower complication rate than more radical surgery. The shaving technique should be offered as a first-line surgical approach in case of type III nodules. Bowel resection should be reserved for cases with complete stenosis, whose prevalence is relatively low (less than 2%).
2. In the second part, we described iatrogenic adenomyotic lesions. Radiological, laparoscopic, and histological findings in iatrogenic nodules were similar to those in type III nodular endometriosis. Indeed, iatrogenic lesions were found to resemble adenomyomas, circumscribed nodular aggregates of smooth muscle, endometrial glands and stroma. From analysis of these lesions, the role of the junctional zone (JZ) was highlighted. Indeed, we

proved that a fragment of tissue containing both endometrium and subendometrial myometrium (the so-called JZ) was able to induce adenomyotic tumor development.

3. In the third part, we induced endometriotic nodules in an experimental baboon model, mimicking human deep nodular endometriotic lesions. It was demonstrated that induced nodular endometriotic lesions were significantly larger and showed a stronger invasion process when tissue specimens containing the JZ were grafted. In this experimental model, the JZ was also found to be a key element in the process of proliferation and invasion of induced nodular lesions.

4. In the fourth part, we analyzed nerve densities in type III nodules and induced experimental nodules. As NFD was confirmed to be high in human endometriotic nodules, and most of these nerve fibers were found to be unmyelinated, they could well be implicated in pain.

Moreover, we demonstrated that deep nodular lesions may be neuroattractive through the action of NGF. In the experimental model, nerve fiber density was investigated and the kinetics of neurogenesis was considered. Increased expression of NGF, together with the low NFD observed in experimental lesions, suggest that these lesions actually recruit nerve fibers.

5. Our clinical results (type III nodules and iatrogenic adenomyotic lesions) and data from baboons show morphological similarities, confirming that multicellular coordination between the leading (invasive) edge and the training (cohesive) edge is mandatory. This explains the good results of the surgical technique used in our department, which removes the cohesive part of the nodule.

In conclusion, this study evidences clear similarities between the baboon model and spontaneous disease observed in humans. This model could therefore be used in the future to explore the invasion process of the disease, validate medical or surgical strategies in terms of pain, fertility and disease recurrence, and finally explain the pathophysiology of deep nodular endometriosis.

Abbreviations

ASMA:	Alpha smooth muscle actin
CK22:	Cytokeratin 22
EBM:	Evidence-based medicine
EECs:	Endometrial epithelial cells
EPR:	Electron paramagnetic resonance
ER:	Estrogen receptor
ESCs:	Endometrial stromal cells
FSH:	Follicle-stimulating hormone
H&E:	Hematoxylin and eosin
HOXA:	Homeobox A
IHC:	Immunohistochemistry
IVF:	In vitro fertilization
IVP:	Intravenous pyelography
JZ:	Junctional zone
LASH:	Laparoscopic subtotal hysterectomy
LH:	Luteinizing hormone
MMPs:	Matrix metalloproteinases
MRI:	Magnetic resonance imaging
NF:	Neurofilament
NFD:	Nerve fiber density
NF- κ B:	Nuclear factor kappa-B
NGF:	Nerve growth factor
PCBs:	Polychlorinated biphenyls
PCDDs:	Dioxin-like polychlorinated dibenzo-p-dioxins
PCDFs:	Polychlorodibenzofurans
PGP9.5:	Protein-gene product 9.5
PGs:	Prostaglandins
PR:	Progesterone receptor
RCTs:	Randomized controlled trials
SD:	Standard deviation
SPRM:	Selective progesterone receptor modulator
TEF:	Toxic equivalency factor

TEQ:	Toxi equivalent
TIAR:	Tissue injury and repair
TRUS:	Transrectal ultrasonography
VEGF:	Vascular endothelial growth factor

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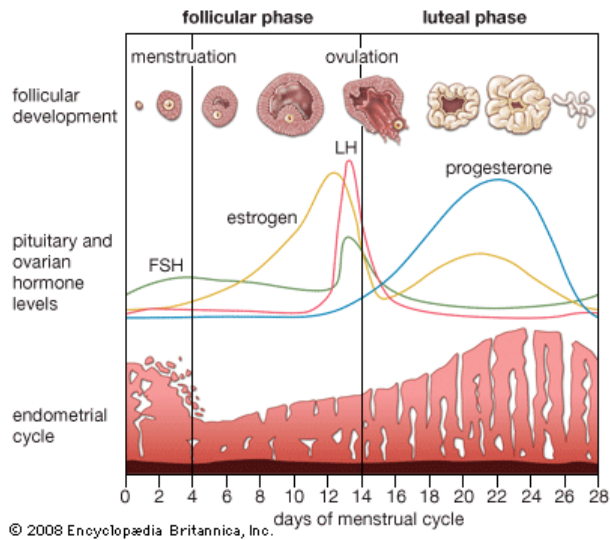
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I. Introduction

I.1. Definitions

I.1.1. Endometrium and the menstrual cycle

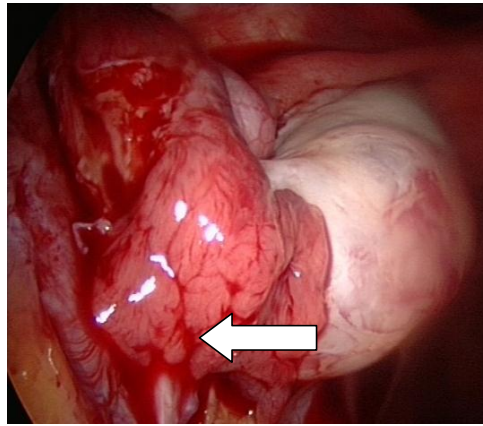
The endometrium is the internal layer of the uterus, composed of endometrial glands and stroma, and undergoes cyclic regeneration (**Figure 1**). It proliferates under the influence of estradiol during the follicular (or proliferative) phase of the menstrual cycle and is prepared for implantation by the action of progesterone during the luteal (or secretory) phase. When fertilization occurs, the blastocyst may implant in the endometrium upon its arrival in the uterus. If pregnancy fails to occur, the corpus luteum regresses and levels of progesterone and estradiol fall suddenly. Progesterone withdrawal is the physiological signal for menstruation. Progesterone receptor (PR)-expressing stromal cells in the superficial zones of the endometrium respond to this decrease and initiate the menstrual cascade. This cascade involves increased production of prostaglandins (PGs) generating vasoconstriction, greater expression of vascular endothelial growth factor (VEGF) and its receptor, elevated levels of stromal matrix metalloproteinases (MMPs), leukocytic invasion, and perivascular expression of cytokines and MMPs. However, hypoxia was not detected by electron paramagnetic resonance (EPR) and minimal biological signs for transient episodes of hypoxia were rarely detected in an experimental model, indicating that this event is not required to trigger menstruation or for tissue repair at least in this model ([Coudyzer et al, 2013](#)). The role of MMPs in the induction of menstrual bleeding was evidenced by Marbaix et al ([1996](#)).

Figure 1: Menstrual cycle

FSH: follicle-stimulating hormone; LH: luteinizing hormone (From Encyclopaedia Britannica, 2008).

During menstruation, the superficial endometrial zones are sloughed off. Menstrual endometrium is eliminated through the uterine cervix and vagina, but retrograde flow of the menstrual slough through the fallopian tubes (**Figure 2**) occurs in most women (Halme et al., 1984).

Figure 2: Retrograde menstruation through the fallopian tube



Laparoscopic view of reflux (white arrow) from the fallopian tube at the time of menstruation.

Endometrial tubular glands consist of simple columnar epithelium (endometrial epithelial cells [EECs]) and are supported by endometrial stroma. Endometrial stroma is composed of a variety of cells (endometrial stromal cells [ESCs]), including spindle-shaped connective tissue cells and bone marrow-derived cells. Both glands and stroma undergo extensive changes during the menstrual cycle.

Two layers have been characterized in the endometrium:

- A superficial layer (or stratum functionalis) that is sloughed off during menstruation and redevelops with each new cycle.
- A deep layer (or stratum basalis) composed of permanent stromal tissue and blind ends of uterine glands. The stratum basalis is maintained permanently during the menstrual cycle and is thought to provide the stratum functionalis with a cell source.

The presence of endometrial stem/progenitor cells has been suggested to be responsible for the highly regenerative capacity of human endometrium ([Chan et al., 2004](#); [Schwab et al., 2005](#)). Stem cells were discovered in the endometrium ([Cervello and Simon, 2009](#)). Recently, it has been demonstrated that human endometrial side population cell lines display similar

phenotypic, molecular signatures, in vitro and in vivo differentiation capabilities as “primary” endometrial side population cells, creating a reliable in vitro model to test relevant targets for endometrial physiology and pathology (Cervello et al, 2011).

Endometrial glands have a relatively flat form and mitotic cells are often found during the proliferative phase. During the secretory phase, epithelial glands are composed of mature secretory cells and their shape is more tortuous.

An important component of the endometrial stroma is its vascular network, which also participates in the menstrual cycle. During menstruation, distal vessels are sloughed off, while spiral arteries retract into the stratum basalis and constrict to limit blood loss. During the proliferative phase, spiral arteries extend again as the stratum functionalis redevelops.

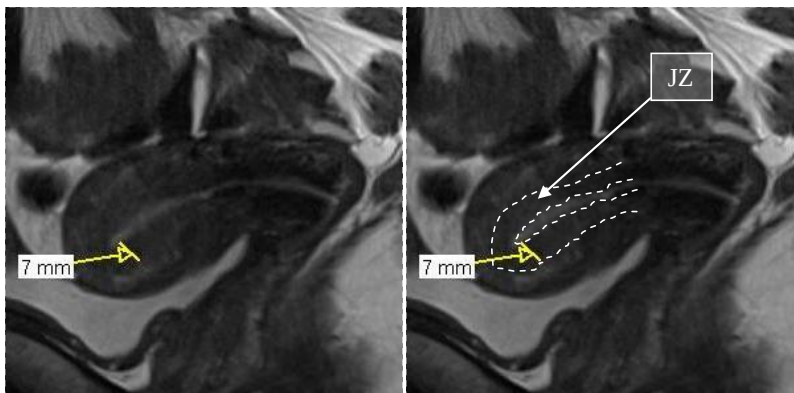
I.1.2. The junctional zone: subendometrial layer

I.1.2.1. Definition, MRI, histology

The uterine wall has been studied for many years using magnetic resonance imaging (MRI) (Hricak et al., 1983; Lee et al., 1985; Bryan et al., 1985) and can be differentiated into three different zones. While the endometrium corresponds to a high-intensity zone, the myometrium consists of a combination of low- and medium-intensity areas. The term junctional zone (JZ) describes this interface observed on MRI: a distinct low signal on T2-weighted sequences separating the endometrium of high signal intensity from the outer myometrium of intermediate signal intensity (Figure 3). It was clearly demonstrated that this zone corresponds to the innermost layer of the myometrium and not to the basal layer of the endometrium (Scoutt et al., 1991). The JZ has been shown to contain myocytes with a greater relative nuclear area, a looser extracellular matrix, and lower water content (McCarthy et al., 1989). The architectural organization of the JZ reveals a concentric arrangement of smooth

muscle fibers in contrast to the longitudinal orientation of the smooth muscle fibers of the outer myometrium (Brown et al., 1990).

Figure 3: Pelvic MRI showing a T2-weighted sagittal view of a normal uterus



The JZ is clearly discernible by its distinct low signal, separating the endometrium of high signal intensity from the outer myometrium of intermediate signal intensity.

I.1.2.2. JZ: physiology and function

The thickness of the JZ is maximal between days 8 and 16 of the menstrual cycle (Wiczek et al., 1988), making it a hormone-dependent structure. After menopause or chemical castration, the JZ becomes indistinct on MRI, although hormone replacement therapy or recovery of ovarian function results in its reappearance (McCarthy et al., 1986).

Contractions of the JZ have been evidenced in the non-gravid uterus by ultrasound (Chalubinski et al., 1993) or MRI with specific dynamic sequences (Ijland et al., 1996). Contractions may be classified according to their direction: from the uterine cervix toward the body, or from the body toward the cervix, or both. In the first part of the menstrual cycle, contractions occur from the cervix toward the body and increase in intensity until ovulation (Kido et al., 2007). It has been suggested that during the luteal phase, any remaining activity

in the form of opposing and random contractions might help the microenvironment of the pre-implantation embryo by facilitating supplies of nutrients and oxygen during the period in which it remains freely floating within the uterine cavity (Ijland et al., 1996). These contractions are probably implicated in many aspects of the physiological reproductive process: endometrial differentiation (Bulletti and De Ziegler, 2006), menstruation (Oki et al., 2002), and implantation (Turnbull et al., 1995). They participate directly in the transport of spermatozooids toward the ovum (Kunz et al., 1996), as they have the ability to carry microspheres placed in the vagina to the peritoneal opening of the tube, mimicking spermatozoa. This hypothesis was confirmed by studying the strong link between contractions issuing from the JZ and fertility (Ijland et al., 1997). Seven days after ovulation, at the time coinciding with embryo implantation, focal disruption of JZ signal intensity is observed (Turnbull et al., 1995). Moreover, in humans, interstitial and intravascular trophoblasts have been found to invade the JZ, but not the outer myometrium (Brosens et al., 2002).

I.1.2.3. Pathological conditions associated with the JZ

The JZ has already been implicated in some pathological conditions, particularly in adenomyosis. Adenomyosis has been defined as "benign invasion of endometrium into the myometrium, producing a diffusely enlarged uterus which microscopically exhibits ectopic non-neoplastic endometrial glands and stroma surrounded by a hypertrophic and hyperplastic myometrium" (Bird et al., 1972). It appears that JZ thickness of up to 8mm is considered normal, while thickness of more than 12mm (Figure 4) is the most widely accepted criterion for establishing the presence of adenomyosis (Reinhold et al., 1999; Bazot et al., 2001; Tamai et al., 2005). Adenomyosis can be present in young women and may be associated with pelvic endometriosis (Kunz et al., 2005; Leyendecker et al., 2006; Zacharia and O'Neill, 2006).

Indeed, Leyendecker et al. suggested that abnormal functioning of the human JZ could represent a common pathogenic factor for endometriosis and adenomyosis development (Leyendecker et al., 1998). The authors postulated that disruption of the basal endometrium may explain structural and functional abnormalities of the JZ (hyperperistalsis, dysperistalsis) and smooth muscle proliferation associated with endometriosis and adenomyosis (Leyendecker et al., 2004). A possible relationship between adenomyosis and deep nodular endometriosis could well be due to this infiltrating growth pattern. However, these common intrinsic abnormalities in eutopic and ectopic endometrium were not confirmed in a recent study by Larsen et al. (2011). The authors evaluated images of the JZ in patients with nodular endometriosis and correlated them with image findings of adenomyosis. They found that women with severe endometriosis showed deeper wall invasion by adenomyosis, but the presence of deep-infiltrating rectovaginal endometriosis (nodular endometriosis) and depth of infiltration did not correlate with the adenomyosis or its depth of infiltration. This was also reflected in one of our recent studies that included 3,298 cases operated on by the shaving technique (Donnez et al., 2013).

Leyendecker suggested that adenomyosis and endometriosis may result from the same physiological mechanism of tissue injury and repair (TIAR), involving local estrogen production in an estrogen-sensitive environment normally controlled by the ovary (Leyendecker et al., 2009). Recently, a correlation was found between adenomyosis and deep endometriosis with a poorer prognosis, particularly endometriosis of the rectosigmoid (Gonzales et al., 2012).

Figure 4: Pelvic MRI showing a T2-weighted sagittal view of an adenomyotic uterus

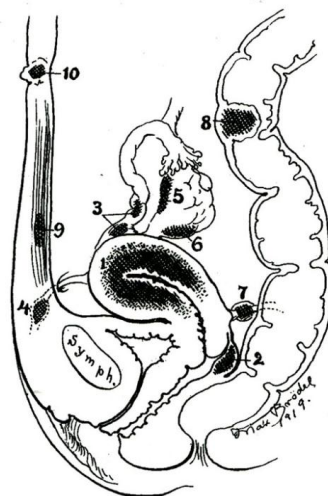


In case of uterine adenomyosis, JZ thickness measures more than 12mm, while less than 8mm is considered normal.

I.1.3. Endometriosis

Endometriosis is defined as the presence of endometrial glands and stroma outside the uterus. The main locations are the pelvic peritoneum and especially the pouch of Douglas and uterosacral ligaments, but endometriosis is also found on the ovaries and in the rectovaginal septum. Endometriosis was documented by Cullen more than one century ago (**Figure 5**). Numerous extrapelvic localizations have been described, including the brain (Thibodeau et al., 1987), lungs (Hilaris et al., 2005), diaphragm (Cooper et al., 1999), liver (Inal et al., 2000), lymph nodes, knee (Patel et al., 1982), vulva, abdominal wall (Markham et al., 1989), and nerves (Anaf et al., 2000).

Figure 5: Localizations of ectopic endometrial tissue (indicated as adenomyomas)



1: uterine wall; 2: rectovaginal septum; 3: fallopian tubes; 4: round ligaments; 5: hilum of ovary; 6: utero-ovarian ligament; 7: uterosacral ligament; 8: colon; 9: musculus rectus; 10: umbilicus (From Cullen, 1919).

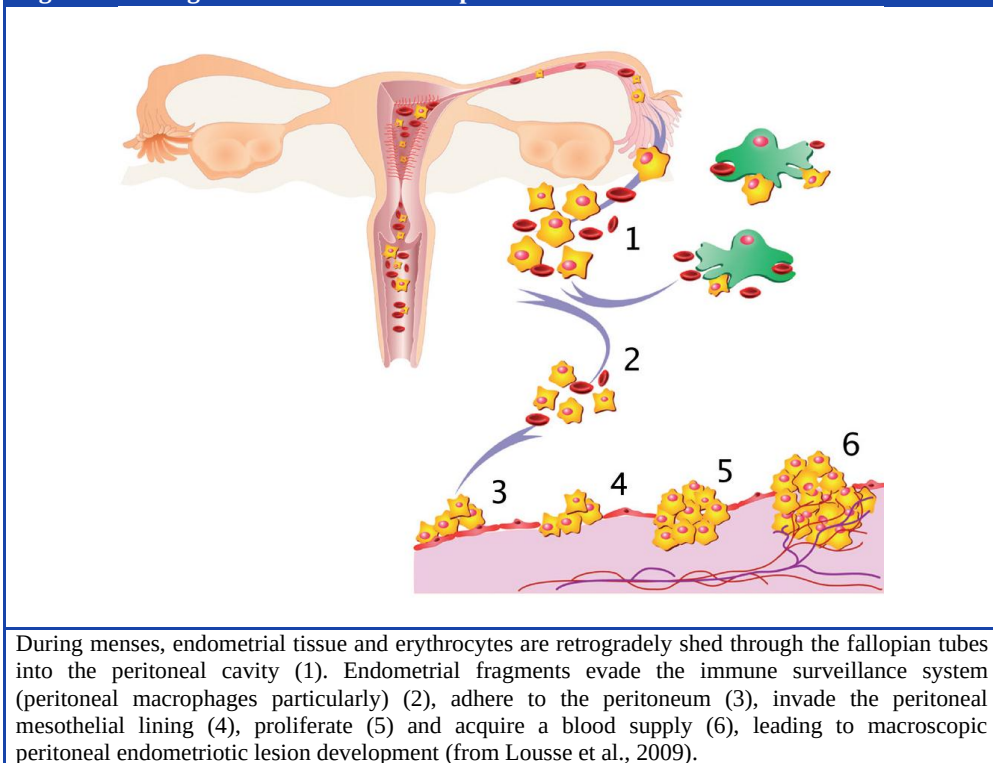
Endometriosis is one of the most frequently encountered benign diseases in gynecology. Its prevalence was found to vary from 6 to 10% in all women and from 35 to 50% in women with pelvic pain and infertility (Nisolle and Donnez, 1997).

1.2. Three different endometriotic entities

In 1996 and 1997, Donnez et al. described three different entities: peritoneal endometriosis, ovarian endometriosis and adenomyotic nodules of the rectovaginal septum, and strongly suggested a different pathogenesis mechanism (Donnez et al., 1996; Nisolle and Donnez, 1997).

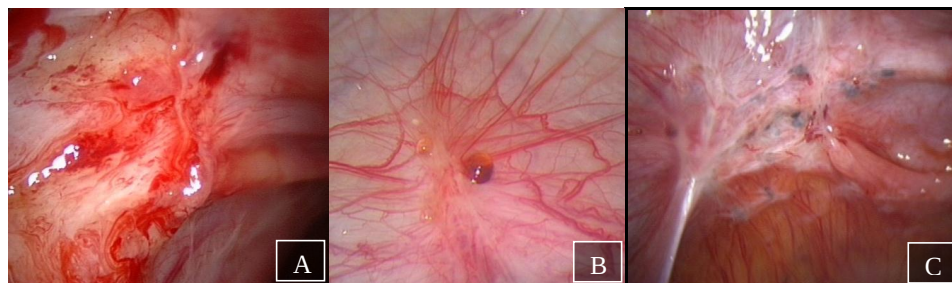
Peritoneal endometriosis (black, red, and white lesions) can be explained by the transplantation theory of Sampson (1922). According to this theory, desquamated endometrial cells are transported into the peritoneal cavity after retrograde menstruation, and still-viable cells subsequently implant and grow, as detailed below (**Figure 6**).

Figure 6: Retrograde menstruation and peritoneal endometriosis



Red lesions are the most active and most highly vascularized lesions and are considered to be the first stage of peritoneal endometriosis (**Figure 7**). Red lesions may regrow after partial shedding. A scarification process is then initiated, and lesions appear as black and, later, white quiescent or latent lesions.

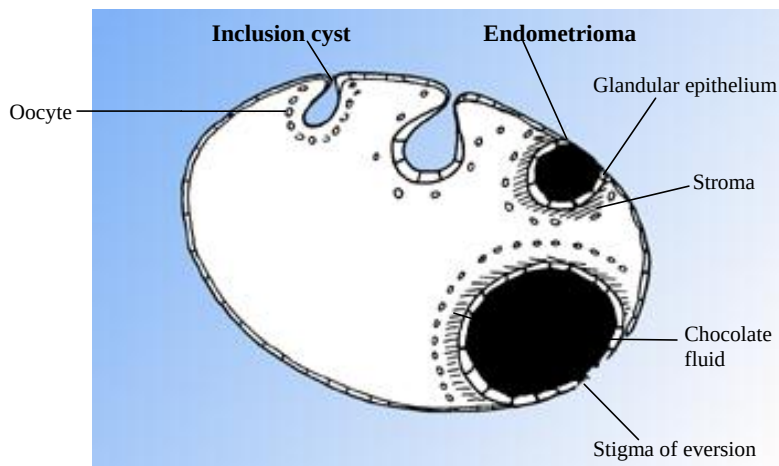
Figure 7: Active red and black endometriotic lesions at laparoscopy



- A. "Fresh" active endometriotic lesion that recently adhered to peritoneum.
 B. Red lesion with hypervascularization.
 C. Typical black endometriotic lesion.

Coelomic metaplasia of invaginated epithelial inclusions could be responsible for the development of ovarian endometriosis. The epithelium covering the ovary, which originally derives from the coelomic epithelium, has great metaplastic potential and provokes epithelial inclusion cysts by colonizing the inside of previous corpus luteum. Under the influence of unknown growth factors, these inclusions could be transformed into intraovarian endometriosis by metaplasia (endometriomas) (**Figure 8**) (Nisolle and Donnez, 1997).

Figure 8: Hypothesis of histogenesis of ovarian endometriomas

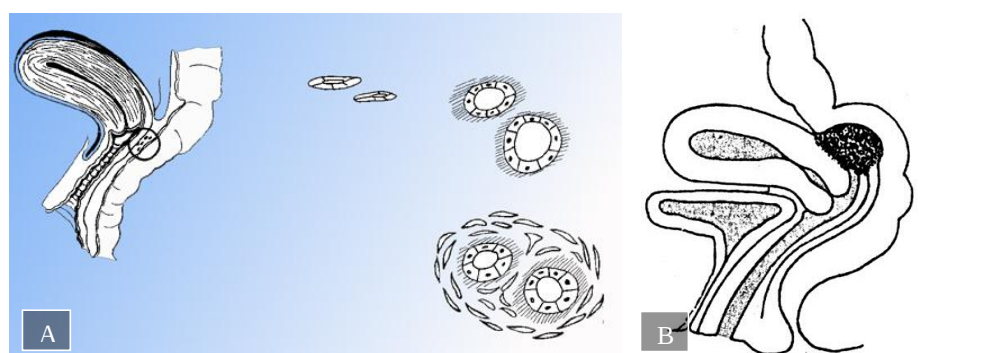


Ovarian endometriosis is caused by metaplasia of invaginated coelomic epithelium (From Nisolle and Donnez, 1997).

The third form of the disease has been defined as deep endometriosis, rectovaginal endometriosis or adenomyosis of the rectovaginal septum. In the literature, it is also called deep-infiltrating endometriosis or posterior deep-infiltrating endometriosis. The term “deep-infiltrating endometriosis” was coined by Koninckx and Martin and first published in 1995 (Koninckx and Martin, 1995). However, Koninckx himself now considers the concept of deep-infiltrating endometriosis no longer valid as an explanation for deep nodular “rectovaginal” lesions (Koninckx and Martin, 1992; Koninckx et al., 2008), recently asking the question: “Deep endometriosis: a consequence of infiltration or retraction or possible adenomyosis externa?” (Koninckx et al., 2012).

In our group, we have always supported the hypothesis that these lesions are retroperitoneal, located in the rectovaginal septum, and may result in some cases (<10%) from metaplasia of Müllerian rests (Figure 9A), and in 90% of cases from an “adenomyotic” process originating from the cervix (Donnez et al., 2011; Donnez and Squifflet, 2010) (Figure 9B).

Figure 9: Hypothesis of histogenesis of rectovaginal endometriosis



A: <10% of rectovaginal endometriosis may result from differentiation of Müllerian rests. Scanty endometrial-type stroma and glandular epithelium are disseminated in smooth muscle hyperplasia (from Nisolle and Donnez, 1997).

B: 90% of rectovaginal nodules result from an adenomyotic process originating from the posterior part of the cervix, where the vagina is attached (picture from Adamyan, 1993).

The origin of these lesions is thus the posterior part of the cervix, where the vagina is attached (Donnez et al, 2010; 2013).

This concept of “retrocervical disease” appears to have already been suggested by Cullen and Sampson (**Figure 10**). As early as 1896, Cullen reported that deep lesions were the consequence of direct extension of lower uterine adenomyosis (Cullen, 1896). As early as 1925, Sampson suggested a link between the cervix and the rectum. He defined cul-de-sac obliteration as “extensive adhesions in the cul-de-sac, obliterating its lower portion and uniting the cervix or the lower portion of the uterus to the rectum, with adenoma of the endometrial type invading the cervical and the uterine tissue and probably also (but to a lesser degree) the anterior wall of the rectum”. It therefore looks increasingly likely that the correct description of deep nodular lesions was actually given over 80 years ago.

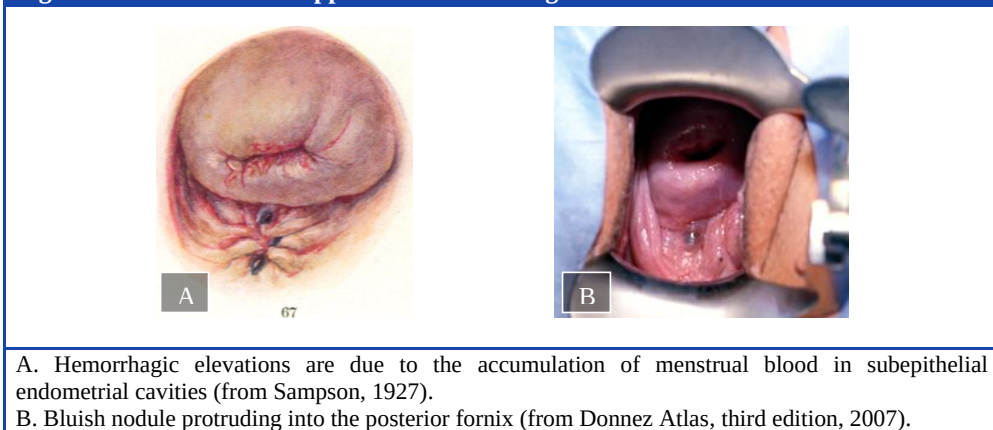
Figure 10: Retrocervical deep nodular endometriosis



Drawing from Sampson's manuscript, clearly demonstrating the cervical origin of the nodular lesion described as “an adenoma of the endometrial type invading the cervical and the uterine tissue and probably also (but to a lesser degree) the anterior wall of the rectum” (from Sampson, 1927).

Deep endometriosis-associated lesions can take the form of nodules, involving the posterior vaginal fornix (Reich et al., 1991; Koninckx and Martin, 1992; Donnez et al., 1995) (**Figure 11**). From a histological point of view, all aspects are similar to those described in uterine adenomyosis.

Figure 11: Characteristic appearance of rectovaginal endometriosis



The aspect of this form of the disease is different from vaginal adenosis, which lacks endometrial stroma and the characteristic inflammatory response of endometriosis (Zaloudek and Norris, 1987). In the uterine corpus, adenomyosis is a common condition characterized pathologically by the presence of endometrial glands and stroma within the myometrium.

Uterine adenomyosis exhibits a varied functional response to ovarian hormones. Proliferative glands and stroma are usually observed in the first half of the menstrual cycle. Adenomyosis may not respond to physiologic levels of progesterone, and secretory changes are frequently absent or incomplete during the second half of the menstrual cycle. Similar histological observations can be made at the level of the endometriotic rectovaginal nodule, which is, like an adenomyoma, a circumscribed nodular aggregate of smooth muscle, endometrial glands, and usually, endometrial stroma (Donnez et al., 1995; Donnez and Nisolle, 1995; Donnez et al., 1996, 2011).

Smooth muscle proliferation and fibrosis, consistently observed, are responsible for the nodular aspect of endometriosis located in the rectovaginal septum. Indeed, 90% of the nodule content consists of smooth muscle hyperplasia.

At least two hypotheses could explain this smooth muscle proliferation: 1) endometriotic foci involving smooth muscle are typically associated with striking proliferation of the smooth muscle, creating an adenomyomatous appearance similar to that of adenomyosis in the uterus (Scully, 1966; Donnez et al., 2012); and 2) endometriotic stroma may exhibit smooth muscle metaplasia, as has occasionally been demonstrated within the wall of ovarian endometriotic cysts (Scully, 1968; van Kaam et al., 2008).

Variations in estrogen receptor (ER) and progesterone receptor (PR) content of nodules throughout the cycle suggest that they are probably not regulated by steroids (Donnez et al., 1997; 2005). The very low glandular epithelial and stromal ER content during the follicular phase may explain the absence of secretory changes in the glandular epithelium of nodules (Nisolle, 1996; Haining et al., 1991; Donnez et al., 1996) and weak response to medical therapy, necessitating surgical excision (Donnez et al. 1995a, 1997, 2010, 2013).

The absence of response to progesterone levels suggests that different regulatory mechanisms of endometriotic steroid receptors expression result in deficient endocrine dependency or that the receptors are present but biologically inactive (Laatikainen et al., 1983; Spirtos et al., 1985). This progesterone resistance could also explain why selective progesterone receptor modulators (SPRMs) (Donnez et al., 2012a and b) are ineffective for deep nodules, in contrast to myoma.

Deep endometriosis should not be considered a progressive disease. Although an endometriotic lesion must clearly have been growing at some point, we challenge the concept of progression; a transition from typical to cystic or deep lesions was never observed (Koninckx et al, 2012). This concept of no or low progression of deep endometriotic disease, is, moreover, consistent with clinical observations that most women experience severe pain for many years, often decades.

I.2.1 DIAGNOSIS

The most frequent and common symptoms of deep endometriotic nodules are dysmenorrhea, dyspareunia, chronic pelvic pain and dyschezia (Donnez et al., 2013). The presence of such symptoms in a woman should lead the clinician to initiate diagnostic tests.

I.2.1.1. Clinical examination

According to Koninckx et al. (2012), only 50% of deep endometriotic nodules >3cm in diameter were diagnosed by clinical examination in the mid-1990s (Koninckx et al., 1996), because the clinician failed to conduct the appropriate examination. Indeed, we have always believed that by good clinical evaluation, the diagnosis could be made in almost 100% of cases (Donnez et al., 2010; 2013).

With experience and awareness, the clinical diagnosis has probably improved. The most important conclusion, however, that the vast majority of deep endometriotic lesions will not be diagnosed by clinical examination, remains valid, essentially because the pelvic examination is not adequately performed by most gynecologists. It also explains why the time

interval between symptom presentation and diagnosis is so long ([Sinaii et al., 2008](#); [Koninckx et al., 2012](#), [Anaf et al., 2002](#)).

Most pathognomonic signs are severe dyschezia, menstrual blood on stools, menstrual diarrhea, severe menstrual mictalgia, and radiation of pain to the perineum ([Donnez and Squifflet, 2010](#); [Koninckx et al., 2012](#); [Donnez et al., 2013](#)). Although robust data, linking these symptoms to size and localization of deep endometriosis, are lacking, clinical symptoms remain key to suspecting deep endometriosis and deciding to perform surgery.

I.2.1.2. Ultrasonography

Clinical suspicion of deep endometriotic nodules can be confirmed by vaginal ultrasonography. The sensitivity and specificity of ultrasonography in the diagnosis of deep endometriosis remains unclear, and although reported to be >85% and even close to 100% ([Hudelst et al., 2009; 2011](#)), operators were never blinded to the clinical symptoms and rarely to the clinical examination. Squifflet reported advantages and limitations of the technique in his thesis published in 2010.

In addition, data linking sensitivity and specificity to the diameter and localization of nodules are lacking. Most important, however, is that the accuracy of ultrasonography varies according to the expertise of the ultrasonographer ([Squifflet, 2010](#)). Ultrasonography is a useful tool when performed by an experienced ultrasonographer in collaboration with the clinician or surgeon.

Roman et al. ([2008](#)) reported that endorectal ultrasonography appears to show poor accuracy for assessment of rectal layer involvement of deep endometriotic nodules, but Keckstein does not agree ([Keckstein and Wiesinger, 2005](#)).

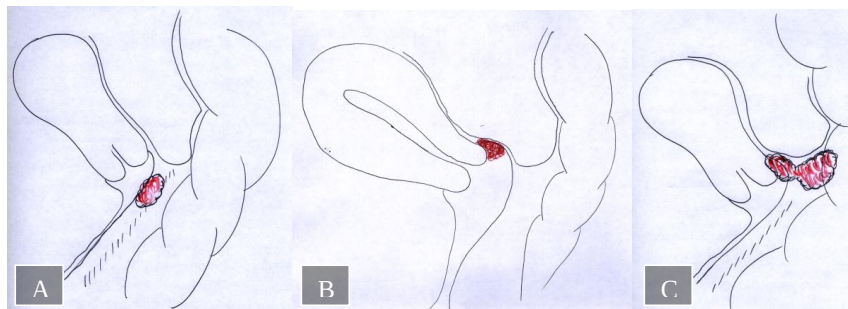
I.2.1.3. Magnetic resonance imaging

MRI might be less operator-dependent and can also provide information about lesions at the level of the sigmoid, but the conclusions reached are similar to those of ultrasonographic examination (Bazot et al., 2009, 2010, 2011; Saba et al., 2012).

Classification of endometriosis of the rectovaginal septum according to MRI

Adamyan et al. (1993), Koninckx et al. (1992), Chapron et al. (2003) and Squifflet et al. (2002) have all tried to classify deep endometriotic lesions, essentially on the basis of clinical findings. In 2002, on the basis of MRI, Squifflet et al. (2002) distinguished three types of deep lesions: a) type I: rectovaginal septum lesions; b) type II: posterior vaginal fornix lesions; c) type III: hourglass-shaped lesions (Figure 12).

Figure 12: Classification of deep retrocervical endometriosis



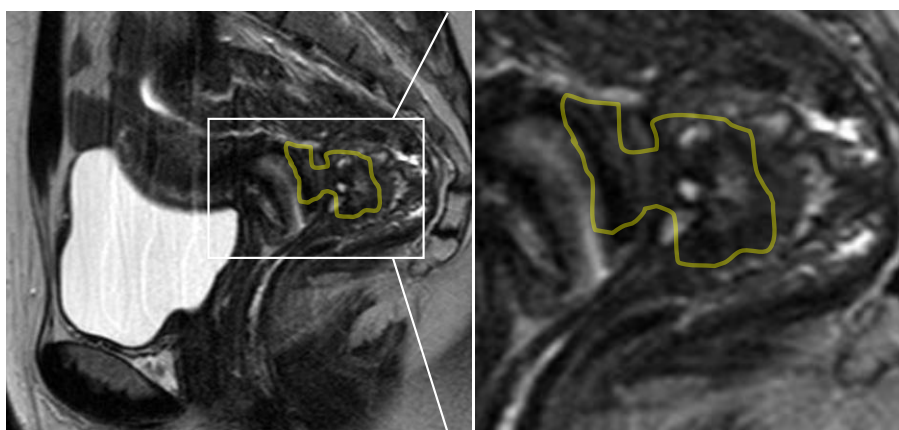
- A. Type I: rectovaginal septum lesion.
 B. Type II: posterior vaginal fornix or retrocervical lesion.
 C. Type III: hourglass-shaped or diaboloid-like lesion.
 (from Squifflet et al., 2002)

Type I lesions are located in the rectovaginal septum and occur in 12% of cases. There is no attachment between the cervix and the nodule.

Type II lesions are the most frequent type of deep lesions (68%). They develop from the posterior fornix towards the rectovaginal septum. The posterior fornix is retrocervical and corresponds, in its attachment to the vaginal wall, to the posterior wall of the posterior lip of the cervix. It is bordered by the joining of the two uterosacral ligaments behind the cervix. Posterior vaginal fornix lesions are often small (around 2-3cm), their average size usually assessed by clinical examination. Extension to the rectal wall is very rare (Squifflet et al., 2002, Donnez and Squifflet, 2010).

Type III lesions, also called hourglass-shaped or diabolo-like lesions, occur when posterior fornix lesions extend cranially to the anterior rectal wall. Their prevalence is 20%. This continuum between the rectal muscularis and the cervix is found to obliterate the rectovaginal septum. As diagnosed by MRI (**Figure 13**), a small but clearly defined continuum exists between these two parts of the lesion. This is why we termed these lesions diabolo-like or hourglass-shaped (Squifflet et al., 2002; Donnez and Squifflet, 2004).

Figure 13: T2-weighted MRI sagittal view of a type III nodule



The nodule extends from the posterior part of the cervix to the anterior rectal wall and an “hourglass” shape can be seen (circled in yellow).

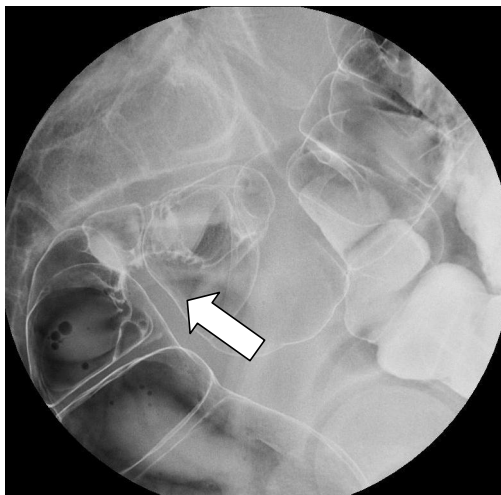
Ultrasonography and MRI can be useful tools to have a preoperative estimation of the size and lateral extension of lesions, larger lesions being more at risk of causing urinary retention after surgery ([Ballester et al., 2011](#)).

I.2.1.4. Contrast barium enema

Typical images of a contrast enema (**Figure 14**) can confirm a sigmoid or high rectosigmoid lesion. Colonoscopy is almost invariably negative. Only in rare cases of very large nodules with a high degree of bowel occlusion will colonoscopy be positive. A contrast enema is important, because it is the only examination that allows evaluation of the degree and length of bowel occlusion at the level of the sigmoid or high rectosigmoid ([Donnez and Squifflet 2010](#), [Donnez et al; 2013](#)).

Is there a place for barium enema?

In our opinion, yes, because, as described in our recent papers ([Donnez and Squifflet 2010](#), [Donnez et al.; 2013](#)), barium enema allows accurate diagnosis of the extent of stenosis, as well as concomitant sigmoid colon or cecal lesions (**Figure 14**).

Figure 14: Barium enema

Infiltration of the muscularis of the anterior rectal wall in a type III lesion (white arrow).

Moreover, the high prevalence of rectal wall involvement (100% for type III and 31.1% for type II lesions, according to Squifflet et al., 2012) demands an accurate diagnosis. Infiltration up to the mucosa and invasion over >50% of the circumference were suggested as an indication for bowel resection ([Abrao et al., 2008](#); [Goncalves et al., 2010](#)), but this remains a subject of debate ([Koninckx et al., 2008](#), [Donnez and Squifflet, 2010](#); [Donnez et al., 2013](#)).

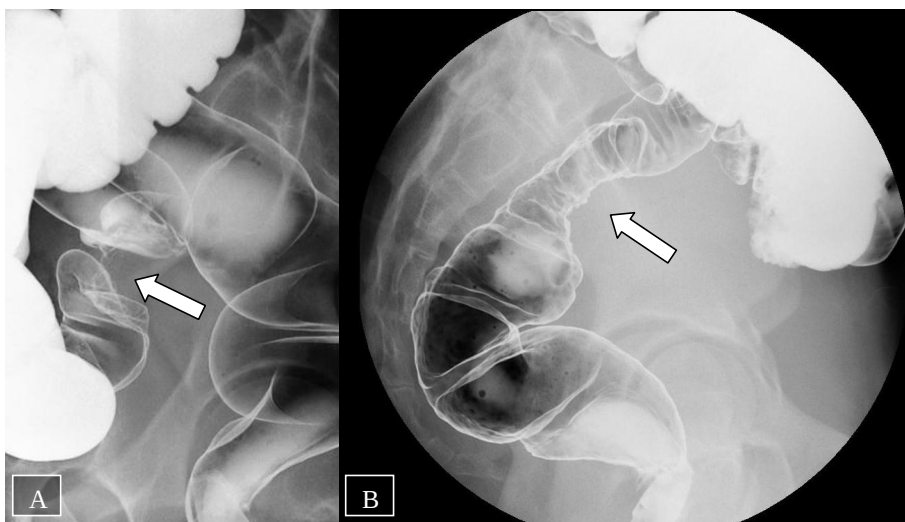
Rectal involvement is considered positive during surgery when:

- a) The nodule is clearly fixed to the rectal wall by dense, fibrotic endometriotic adhesions penetrating the rectum.
- b) There is no free surgical plane of cleavage between the nodule and the rectal muscularis because of infiltration. In this case, resection of the muscularis is performed and infiltration can be histologically analyzed.

Rectal involvement

Perivisceritis, visible on barium enema (**Figure 15**), is due to the inflammatory process and secondary retraction of the rectal muscularis. The absence of evolution of the rectal lesion after removal of the nodule supports our hypothesis concerning its purely retrocervical or rectovaginal septum origin (Donnez et al., 1995a, 1995b, 1997, 2001, 2004, 2007; Nisolle and Donnez, 1997). Indeed, lateral and posterior extension occurs retroperitoneally via the lymphatics or nerves (Anaf et al., 2002; Donnez et al., 2002a and b, 2007). The mode of propagation is very similar to that of cervical cancer. Data on ureteral localization of deep endometriotic nodules (Donnez et al., 2002b) confirm that ureteral endometriosis is, in fact, extension of the disease originating in the retrocervical area in the majority of cases.

Figure 15: Barium enema



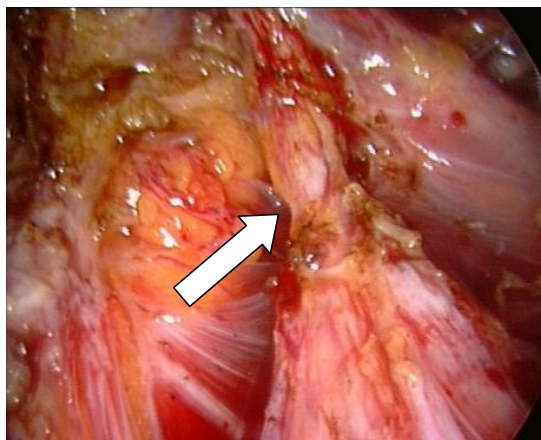
A. Sigmoid stenosis due to a circular endometriotic lesion (white arrow).

B. Mass effect visible on the anterior sigmoid wall with an irregular border (white arrow) due to a large endometrioma.

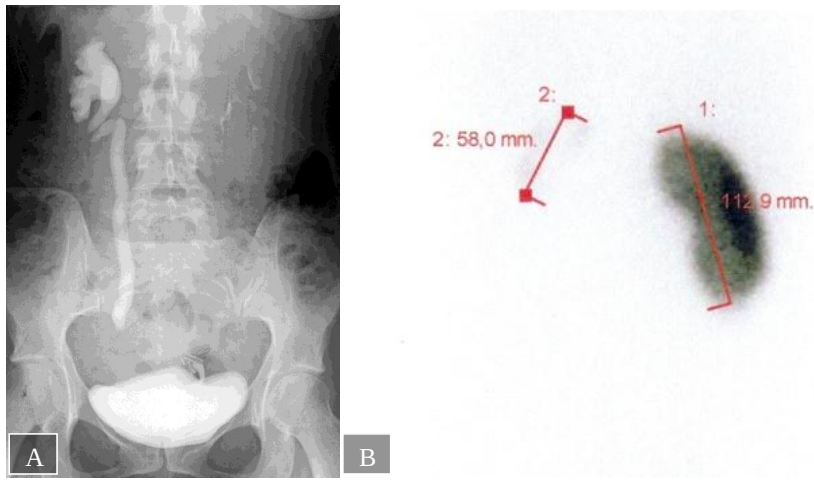
I.2.1.5. Intravenous pyelography and kidney function

Ureterohydronephrosis (Figure 16) should be excluded before surgery because deep nodular endometriosis is associated with 18% of ureteral lesions during surgery and requires a preoperative ureteral stent (De Cicco et al., 2009). As the prevalence of ureterohydronephrosis is more than 10% in case of nodules of more than 3cm in size, MRI should also focus on ureteral dilation. In case of ureteral dilation, intravenous pyelography (IVP) should be carried out and, if severe (Figure 17), insertion of a JJ stent is recommended before surgery to facilitate the procedure and avoid complications.

Figure 16: Ureterohydronephrosis



Laparoscopic view of right ureteral stenosis after ureterolysis (white arrow).

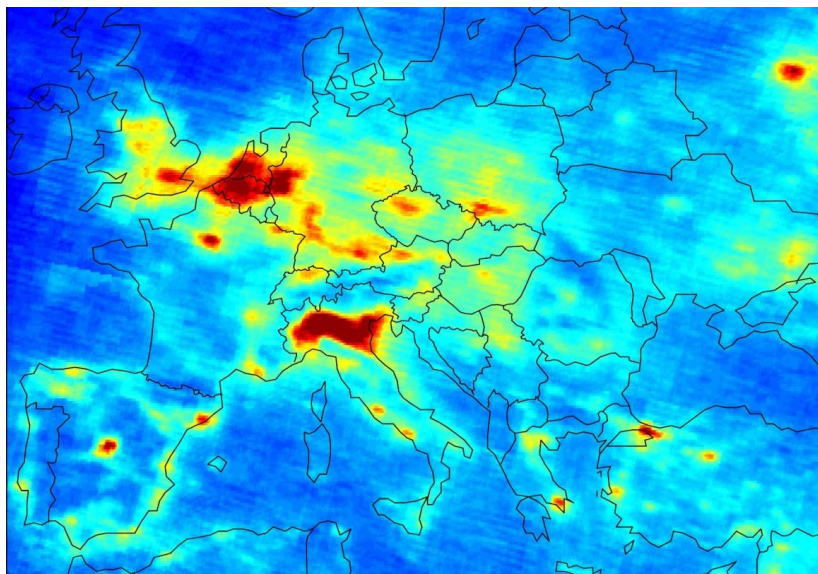
Figure 17: Ureterohydronephrosis

A. Intravenous pyelography: severe right hydronephrosis.

B. Hypofixation of the right kidney identified by renal scintigraphy.

I.2.2. Is the environment responsible for the increasing prevalence of deep endometriotic nodules?

This is the subject of ongoing debate and controversy, but there is no doubt that the prevalence of deep endometriotic nodules in Belgium is one of the highest in the world, where the involvement of environment is still a source of much discussion (**Figure 18**).

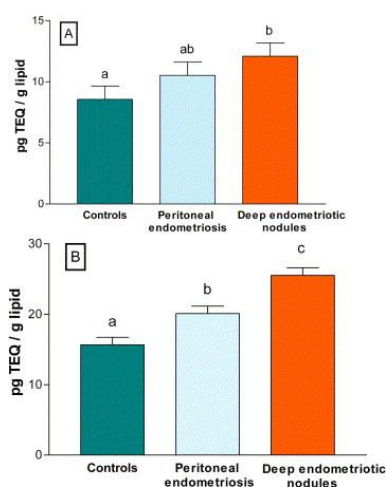
Figure 18: Map of air pollution

The air pollution map produced by European Space Agency shows the most heavily polluted areas (in red and yellow) in Europe. http://www.citymayors.com/environment/urban_pollution.html

It was suggested that dysregulation of the Homeobox A10 (HOXA-10), gene product involved in the development of the Müllerian system, may alter the development of steroid signalling pathways and be one of the factors contributing to the poor response of nodules to hormonal treatment, explaining progesterone resistance (Squifflet, 2010). A mechanism by which HOXA-10 transcription may be altered is through binding of an endocrine disruptor, such as dioxin, to the estrogen receptor (Daftary et al., 2006). The resulting disruptor-receptor complex binds the estrogen-responsive element present in the HOXA-10 promoter, thereby decreasing HOXA-10 gene transcription. Patients with deep nodules present with increased serum levels of dioxin-like compounds (Heilier et al., 2007). (Figure 19)

In 2005, levels of dioxin-like polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated biphenyls (PCBs) were shown to be significantly higher in the serum of women suffering from deep nodular lesions than women with peritoneal endometriosis or controls (Heilier et al., 2005). It has been suggested that these compounds, which act as endocrine disruptors, could be related to the increased prevalence of deep endometriosis observed in recent decades.

Figure 19: PCBs and PCDDs



Dioxin-like polychlorobiphenyls (PCBs) (A) and polychlorodibenzodioxins (PCDDs)/ polychlorodibenzofurans (PCDFs) (B) in serum, standardized for age (30 years) and body mass index (BMI) (22.5kg/m²). Age, parity, breast-feeding, and BMI were all considered in a multiple regression analysis to take into account the lipophilic and cumulative properties of PCDDs/PCDFs and dioxin-like PCBs. For both groups of compounds, age was a significant determinant, contributing to increased serum concentrations, while an increased BMI had a slightly negative influence. Bars represent means and standard deviations (SDs). Concentration of these compounds are expressed as picograms of toxic equivalent (TEQ), which is the sum of the products of the concentration of each compound multiplied by its toxic equivalency factor (TEF). Bars with the same letter are not significantly different (Student-Newman-Keuls test) (from Heilier et al, 2005).

II. Aim of the study

This study focuses on the characterization of deep endometriotic nodules, and more specifically type III nodules, which are one the most painful and least characterized forms of endometriosis.

Our main objective was to gain further insights into invasion processes leading to infiltration of the rectal wall and surrounding organs by endometriotic lesions (proliferation of glands and stroma).

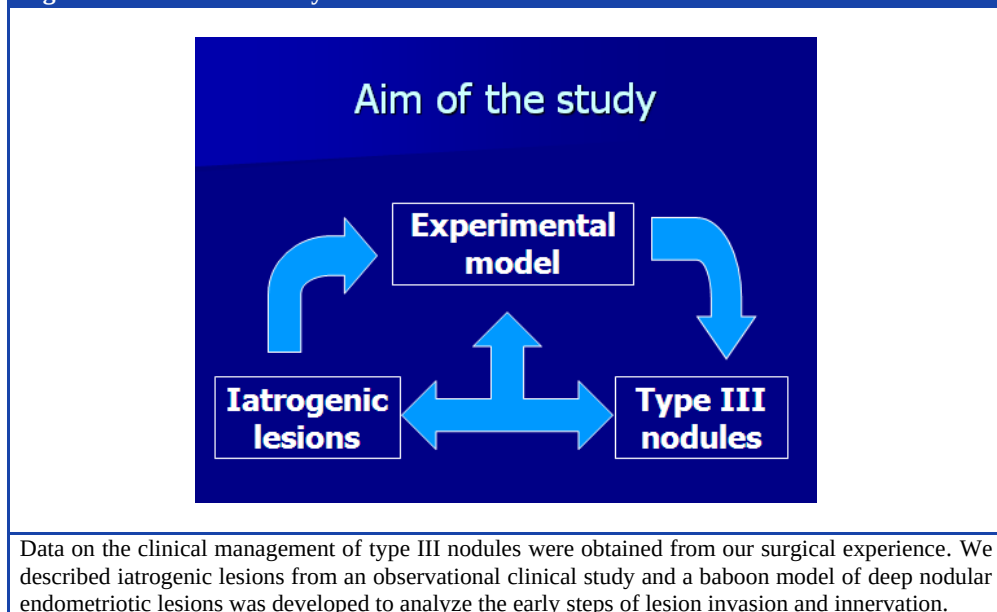
Data on the clinical management of deep-infiltrating nodular lesions were obtained from our surgical experience, while information on the etiology of their invasive phenotype was gathered from an observational clinical study of iatrogenic lesions and an experimental model (Figure 20).

For this purpose, a baboon model of deep nodular endometriotic lesions was developed to analyze the early steps of lesion invasion and innervation.

In the course of this project, we focused on addressing the following questions:

1. Do we have arguments to support the role of the JZ in deep nodular endometriosis?
 - a. Iatrogenic adenomyosis in humans
 - b. Baboon model
2. Do we have arguments to explain the invasive behavior of deep nodular endometriosis?
3. Are nerves implicated in the pathogenesis (pain)?
4. Are nerves implicated in the invasion process?
 1. Do nerves follow endometriotic invasion?
 2. Does endometriotic invasion follow the nerve pathway?
 3. Neurogenesis and angiogenesis: what comes first?

Figure 20: Aim of the study



III. Deep rectovaginal endometriotic nodules: perioperative complications from a series of 3,298 patients operated on by the shaving technique

III.1. Introduction: surgical treatment of type III lesions

The first three large series of surgical management of deep lesions, including 231, 500 and 1,942 women, were published in 1995, 1997 and 2004 respectively ([Donnez et al., 1995a, 1997, 2004](#)) and it was already mentioned in 1995 that it is wise to curtail rather than encourage the widespread use of aggressive and potentially morbid procedures, including bowel resection.

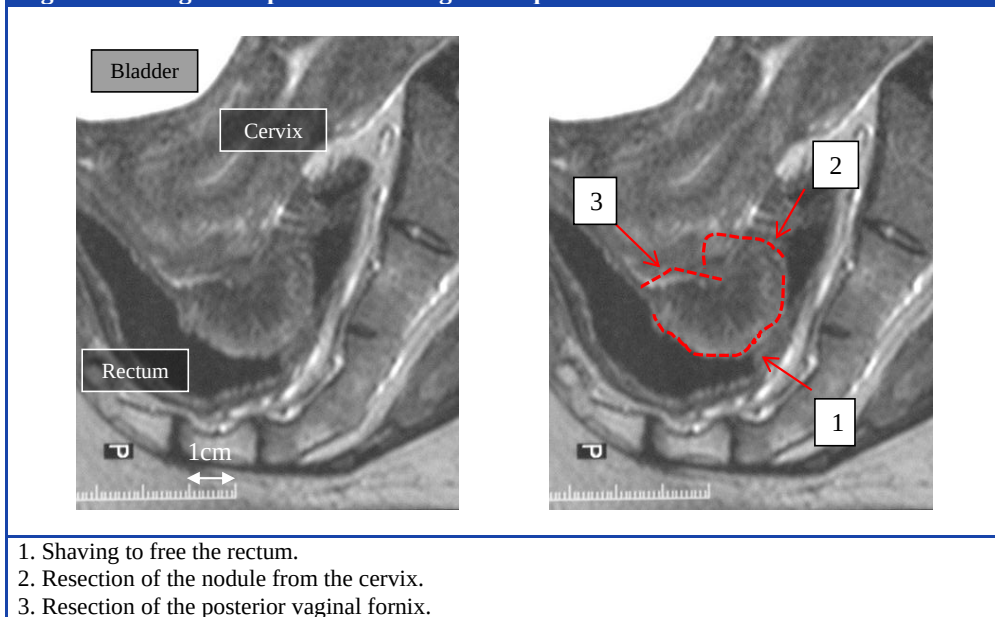
The quality of studies reporting results of surgery is source of debate. Indeed, randomized controlled trials (RCTs) for complex pathologies are practically impossible to conduct

because of the number of cases required ([Batt et al., 2007](#); [Ussia et al., 2008](#)). Moreover, surgical series always evaluate both the technique and the skill of the surgeon, whereas blinding is difficult to implement.

Surgery: the shaving technique

The main steps involve (1) separation of the anterior rectum from the posterior vagina, (2) excision or ablation of deep endometriosis after complete dissection of the nodule from the posterior part of the cervix, and (3) resection of the posterior vaginal fornix and vaginal closure (**Figure 21**).

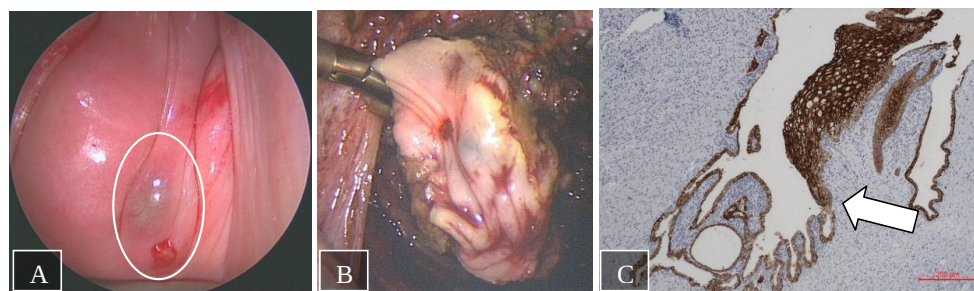
Figure 21: Surgical steps of the shaving technique



The vaginal wall is often penetrated and excision of part of the vagina (posterior vaginal fornix) is essential (**Figure 22**). Indeed, it has been demonstrated that the risk of recurrence of pelvic pain is higher if the posterior vaginal fornix is not removed ([Donnez et al., 2000](#),

2007, 2010, 2013). Dissection is performed accordingly, not only with removal of all visible nodular endometriotic lesions, but also the vaginal mucosa. Lesions extending throughout the vagina are treated with en bloc laparoscopic resection from the cul-de-sac to the posterior vaginal wall.

Figure 22: Vaginal invasion by a type III nodular endometriotic lesion



A. Vaginal view of the lesion with a bluish nodule (circled in white).
 B. Removal of the lesion by laparoscopy: exposure of the vaginal mucosa.
 C. Representative photomicrograph of cytokeratin 22 (CK22) immunostaining in an endometriotic nodule, revealing the continuum between endometriotic glands and vaginal epithelium (white arrow).

Care must always be taken to preserve the pelvic autonomic nerves, as they are the pathway for the neurogenic control of rectal, bladder and sexual arousal function (Maas et al., 1999; Possover et al., 2000; Landi et al., 2006; Roman et al., 2011; Vassiliev et al., 2011). The shaving technique allows preservation of the nerves by avoiding deep lateral rectal dissection (necessary for rectosigmoid resection). Indeed, lateral dissection is mandatory only in case of lateral extension of the disease with ureteral involvement (Donnez et al., 2002b; Donnez and Squifflet, 2010) and, even in this case, rarely involves dissection of the posterolateral compartment of the rectum.

III.2. RESULTS

A series of 3,298 patients was operated for deep rectovaginal endometriotic nodules by laparoscopy at S^t Luc University Hospital between 1989 and 2010 ([Donnez et al., 2013](#)). All patients are presented in [Table 1](#). Operating time ranged from 31 minutes to 238 minutes (median 78 minutes) and median lesion size was 2.8cm (range 1-6cm). In the same period, 37 patients (1.1 %, not included in the series of 3,298) underwent rectosigmoid bowel resection by laparotomy or laparoscopy-mini laparotomy, because of bowel lumen stenosis of more than 80% with circular stenosis (not only anterior involvement), as well as mucosal involvement visible on colonoscopy and confirmed by biopsy and histological evaluation. These three criteria (stenosis of the lumen >80%, circular stenosis, and mucosal involvement) are, in our view, indications for bowel resection, but they are infrequently encountered. Indeed, in this series, bowel resection was only required in 1.1% of cases.

Table 1: A series of 3,298 cases of deep endometriosis treated by the laparoscopic shaving technique without segmental resection

Laparoscopic shaving surgery (n=3,298)	
Lesion size (cm)	2.8 (1-6)
Duration of surgery (min)	70 (31-238)
Hospitalization (days)	2.7 (2-7)
Complications	
• Rectal perforation during shaving	42 (1.3%)
• Fecal peritonitis requiring colostomy	1 (0.03%)
• Rectovaginal fistula	2 (0.06%)
• Delayed haemorrhage (<24h postoperatively)	3 (0.09%)
• Ureteral injury (transection)	4 (0.12%)
• Ureteral fistula (thermal damage)	6 (0.18%)
• Urinary retention (temporary <5 days)	21 (0.64%)
• Long term: recurrence of pain requiring bowel resection	27 (0.81%)
(From Donnez et al, 2013)	

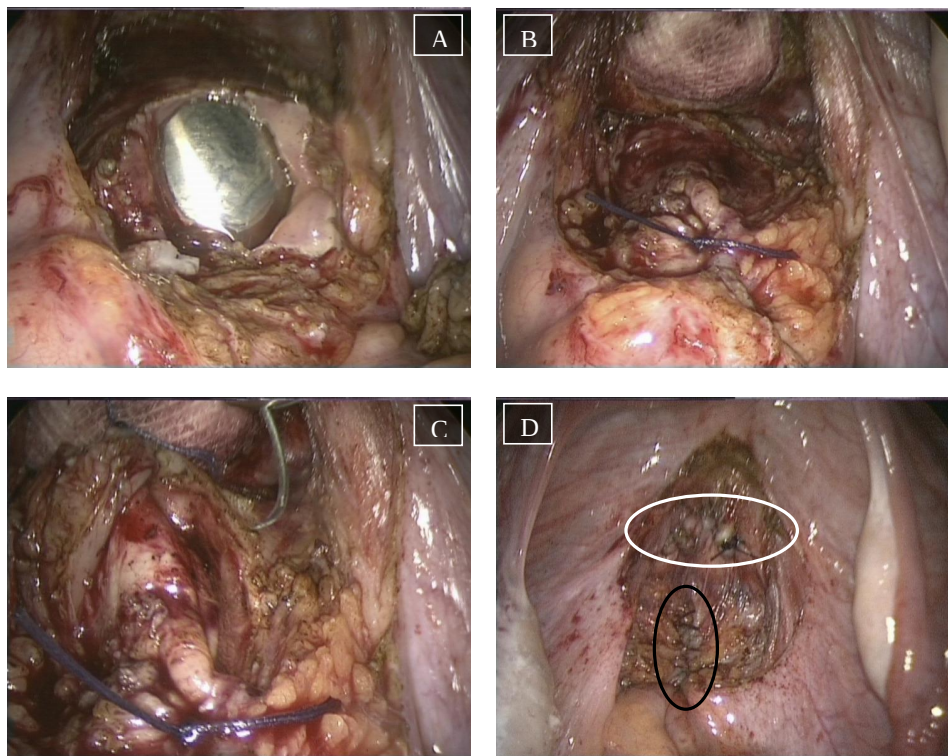
In this series of 3,298 patients, laparoscopic dissection was performed successfully in all cases, even when radiography of the colon showed anterior bowel involvement of up to 5cm or more.

These lesions always occur under the peritoneal fold of the recto-uterine pouch of Douglas. Infiltration of the rectal muscularis is systematically observed in subtype III, as demonstrated by barium enema (profile image) and TRUS, but infiltration is generally limited to the muscularis without mucosal involvement (Donnez et al., 2002; Jadoul et al., 2007; Donnez and Squifflet, 2010; Konninckx et al., 2011).

III.2.1. Peri- and postoperative complications

In the series of 3,298 cases, laparoscopic rectal perforation occurred in 42 cases (1.3%) (**Figure 23**). All perforations were diagnosed at the time of laparoscopy. In the first three cases (in the early 1990s), the rectum was repaired by laparotomy and, in all others, by laparoscopy. Among the 42 cases, neither fistula nor any other complication were observed.

Figure 23: Laparoscopic rectal perforation and repair



A. Rectal perforation showing the intrarectal cannula.

B. Closure of the mucosa (first layer).

C. Closure of the muscularis (second layer).

D. Final view after vaginal closure (white circle); rectal suture is shown in the black circle.

Rectal muscle defects frequently arise during the shaving procedure and are repaired by suturing. If the rectal lumen is not entered, it is not considered a complication, just part of the procedure. It should be noted, however, that in the early years of shaving practice, rectal muscularis defects were not closed and two cases (0.06%) of rectovaginal fistula occurred 6 to 8 days after surgery. Both cases were treated conservatively by antibiotics and diet for 10 days.

One case of fecal peritonitis occurred seven days after surgery. During surgery, bleeding at the site of lateral dissection of the rectum required extensive bipolar coagulation. At the end of surgery, bowel integrity was checked by CO₂ intrarectal insufflation and the blue test. No rectal defect was diagnosed. Seven days later, a hole of 2.5cm in size was detected. Extensive coagulation probably provoked thermal rectal injury, with subsequent necrosis and a fistula. A colostomy was therefore carried out. Three months later, the colostomy was closed.

Ten cases (0.3%) of ureteral injury were noted in the series. Four cases (0.1%) of ureteral transection were diagnosed on the first postoperative day by the presence of abundant fluid in the peritoneal cavity. High levels of urea and creatinine in the “peritoneal” fluid and IVP confirmed the diagnosis. Nephrostomy was carried out. One case resolved spontaneously with complete healing of the ureter two months later. The other three cases required vesico-ureteral reimplantation.

The remaining six cases (0.2%) of ureteral injury were due to thermal damage (bipolar coagulation) and were treated by insertion of a JJ stent, which was removed three months later. Among these six patients, four recovered completely. Two women needed vesico-ureteral reimplantation for hydronephrosis due to stenosis of the lower ureteral segment (fibrosis). In these cases, hydronephrosis was detected approximately one month after

removal of the JJ stent. This is why, in case of large nodules with possible ureteral involvement (suspected by MRI or IVP), a JJ stent is inserted before starting the laparoscopic procedure and ureterolysis is then performed (Donnez et al., 2002b; Donnez and Squifflet 2010). The usefulness of JJ stents in preventing subsequent complications was recently confirmed by Weingartner et al. (2008). The group of Koninckx also published a large series of ureteral injuries (De Cicco et al., 2009). They demonstrated that ureteral repair was often possible by laparoscopy with excellent outcomes.

Urinary retention occurred in 21 cases (0.64%). All were patients with large nodules (>4cm) and bilateral extension, requiring extensive lateral dissection. A Foley catheter was inserted for two days and all but two cases resolved spontaneously. These last two cases required bladder catheterization for five days, then resolved.

Recurrence of severe pain after shaving, necessitating partial bowel resection (n=27, 0.81%), could be considered a complication of the shaving procedure.

We conducted a meta-analysis of complication rates encountered after bowel resection for deep nodular endometriosis (Redwine et al., 2001; Chapron et al., 2004; Daraï et al., 2005a and b; Emmanuel and Davis, 2005; Fleisch et al., 2005; Ford et al., 2005; Keckstein et al., 2006; Mereu et al., 2007; Meuleman et al., 2009, 2011) (Table 2).

Table 2: Complication rate after the shaving technique compared to bowel resection (meta-analysis)

	Shaving technique (personal data, St Luc University Hospital) n=3,335	Rectal resection (meta-analysis by Donnez et al., 2013) (Donnez et al., 2013)	Rectal resection (meta-analysis by Vercellini et al., 2009a) (Vercellini et al., 2009a)
Urinary retention	0.64%	3-10%	4-10%
Ureteral lesions (uoperitoneum)	0.3%	2-4 %	1-2%
Severe anastomotic stenosis	-	3%	0.5-1%
Sepsis (pelvic abscess)	0%	2-4 %	1-2%
Rectal perforation upon shaving (diagnosed and repaired during surgery, no further complications)	1.3%	-	-
Rectovaginal fistula	0.06%	6-9 %	2-10%
Fecal peritonitis, anastomotic leakage	0.03%	3-5 %	1-2%

Our review of recent publications reporting the results and complications of laparoscopy-assisted bowel resection for deep endometriotic nodules reveals a relatively high complication rate compared to the shaving technique (Donnez and Squifflet, 2010; Donnez et al., 2013). Indeed, rates of urinary retention (3-10%), ureteral lesions (2-4%), fecal peritonitis (3-5%), severe anastomotic stenosis (3%), rectovaginal fistulas (6-9%) and pelvic abscesses (2-4%) were found to be significantly higher than with the shaving technique. A possible bias could have been the relatively small number of patients involved in some series, but it should be pointed out that, even in very experienced hands (Daraï et al., 2005; Keckstein and Wiesinger, 2006), the rate of severe complications (rectovaginal fistulas, abscesses, stenosis, fecal peritonitis) can be more than 10%.

In 2009, Meuleman et al. reported an 11% rate of severe complications, some of which were due to the duration of surgery (mean over 7 hours) ([Meuleman et al. 2009](#)). Indeed, lower leg compartment syndrome, which is a serious condition associated with increased intracompartmental pressure and significant morbidity, was observed in three cases (3/56), requiring a fasciotomy. It is important to note that there was no mucosal infiltration of the rectum in this series. This relatively high rate of severe complications was encountered despite a multidisciplinary approach including a urologist and digestive surgeon. In this series, the median operating time was 7h56 (range 180-780 minutes). The duration is also related to costs for the hospital (operating room, nurses, anesthetist).

In our series, the median operating time (including laparoscopy) was just 78 minutes (range 31-248 minutes). Surgery only exceeds three hours when nodules are removed from the bladder, ureter and bowel, sometimes including laparoscopic nephrectomy during the same procedure, as described by Jadoul et al. ([2007](#)).

III.2.2. Recurrence rates

Recurrence rates could only be analyzed in a prospective study. This was done in a prospective series of 500 patients operated on by the shaving technique for deep rectovaginal endometriotic nodules (types II and III). This series was subsequently published ([Donnez and Squifflet, 2010](#)). In types II and III nodules, recurrence of severe pelvic pain was 7% (36/500) ([Donnez and Squifflet, 2010](#)), and was found to be significantly lower ($p<0.05$) in women who became pregnant after surgery (3.6%) than in those who did not (14%).

III.3. DISCUSSION

In our view, removing part of the rectum is simply not justified, since we know that this technique increases the risk of complications. Furthermore, no randomized studies performed to date have been able to prove that it is any more effective than the shaving technique. In 2006, Landi *et al.* reported that full-thickness disc excision using a circular stapler could prevent the potential morbidity associated with low anastomosis. The same group (Mereu *et al.*, 2010) reported a major complication rate that required repeat operations in 20 among 192 cases (10.4%), so they are now probably evaluating a less aggressive surgical approach than bowel resection (Landi *et al.*, 2006). The same trend away from bowel resection toward a less aggressive approach has been described by Slack *et al.* and Roman *et al.* (Slack *et al.*, 2007; Roman *et al.*, 2010). In a series of 75 patients managed by surgery for endometriosis infiltrating the rectum, Roman *et al.* recently observed that reducing the rate of colorectal resection appears to yield better functional outcomes (Roman *et al.*, 2013). This supports the conservative approach over colorectal resection.

We are convinced (Donnez *et al.*, 2013) that the shaving approach is the technique of choice. In most cases, muscularis infiltration observed in all cases of type III lesions may be left in place, at least partially, since the shaving technique already removes what is necessary to alleviate pain. Residual lesions in the muscularis of the rectum do not evolve and remain constant for a long time (Donnez *et al.*, 2001, 2007, 2013; Squifflet and Donnez, 2004). As patients are usually free of symptoms, we consider systematic bowel resection to be unnecessary in case of rectal muscularis involvement. Moreover, such surgery increases morbidity and may be responsible for lower leg compartment syndrome due to the duration of surgery, as observed in a recent series (Meuleman *et al.*, 2009). Indeed, we should bear in mind that radical bowel surgery is associated with long-term morbidity and, as previously

reported in a Cochrane Review ([Pachler and Wille-Jørgensen, 2005](#)), quality of life is significantly impaired following anterior resection.

According to Koninckx et al. ([2012](#)), exactly when discoid excision or bowel resection should be done remains debatable. In Anaf's view ([2002](#)), the most difficult cases are those situated about 3cm from the anal margins, making surgery problematic. Moreover, in two systematic reviews ([Ret Davalos et al., 2007](#); [De Cicco et al, 2011](#)), it was demonstrated that the leakage rate and long-term consequences of bowel resection increase when the resection involves the lower part of the bowel. For sigmoid resection, leaks occur in <1% of cases, almost without long-term complications. For low rectal resections, leaks increase to 15% or more and carry a lifelong risk of bowel, bladder, and sexual problems of 30%, 30%, and 40%, respectively ([Ret Davalos et al., 2007](#)). Of almost 2,000 bowel resections for endometriosis published to date, the large majority were lower resections. It remains unclear, however, what the exact indications were for bowel resection, because it is highly variable between authors. The size of nodules is rarely indicated, the length of resection ranges from 5 to 25cm, and the duration of surgery can be as long as 8 hours, whereas data on sexuality after surgery are lacking ([De Cicco et al., 2011](#), [Koninck et al., 2012](#)).

It should also be pointed out that colorectal segmental resection is a complex procedure, sometimes resulting in pelvic nerve damage and unpleasant urinary and digestive symptoms ([Slack et al., 2007](#); [Roman and Bourdel 2009](#); [Vercellini et al., 2009b](#); [Paya et al., 2011](#); [Roman et al., 2011](#); [Vassilieff et al., 2011](#)). Indeed, urinary retention is quite frequent (3-10%) after bowel resection, as often this type of surgery cannot preserve the pelvic autonomic nerves, which are the pathway for the neurogenic control of bladder function. Interestingly, the shaving technique provokes a very low rate of urinary retention because the dissection usually respects, at least on one side, the sympathetic nerves passing laterally to the rectum, which are frequently cut during extensive rectal or rectosigmoidal resection.

III.3.1. Radical or not radical?

Completeness of surgery with removal of all endometriosis sounds like a precept, as used in cancer surgery. However, evidence that endometriosis surgery needs to be 100% complete is lacking, while circumstantial evidence of the opposite exists ([Koninckx et al., 2012](#)). Absence of evidence that all remaining endometriotic cells should be removed to reduce recurrence rates or improve pain or infertility outcome is very different from carrying out incomplete surgery by leaving a large part of the nodule behind. The lack of evidence of better improvement in case of bowel resection was recently underlined by Vercellini et al. ([2009](#)) and Koninckx et al. ([2012](#)). First of all, it is close to impossible to remove all endometrial/endometriotic cells from all sites, ([Donnez et al., 2013](#)). In >10% of cases of deep endometriosis, lymph nodes contain endometrial/endometriotic cells ([Gong and Tempfer, 2011; Namkung et al., 2011; Tempfer et al., 2011](#)). These lymph nodes do not cause any clinical symptoms and fortunately systematic pelvic lymph node resection was never required ([Koninckx et al., 2012](#)).

Second, deep endometriosis is surrounded by a fibrotic layer ([Donnez et al., 1997, 2010, 2013](#)) which may be left behind. In the absence of evidence of the benefits of more radical surgery, it is worth noting that Koninckx, Wattiez, Nehzat and Roman have become less aggressive surgically than 10 years ago ([Koninckx et al., 2012; Roman et al., 2013 a & b](#)).

Third, recurrence rates of deep endometriosis requiring surgery are so low that it would be virtually impossible to demonstrate the need for complete versus near complete excision. Moreover, when the large series are analyzed, the recurrence rate of severe pelvic pain is low, ranging from 1% to 5%, and thus certainly no higher than that observed after bowel resection, which was recently shown to be 7% at 4 years ([Meuleman et al., 2009, 2011](#)). In the large

series of 3,298 cases treated by the shaving technique, bowel resection was mandatory in 27 cases (<1%) for recurrence of severe pelvic pain after shaving ([Donnez et al., 2013](#)).

III.3.2. Recurrence

According to the literature, the rate of recurrent pain observed in large series of conservative surgery is surely no higher than the rate encountered after more aggressive surgery, including bowel resection. In the majority of series, the recurrence rate of severe pelvic pain is evaluated to be between 5% and 13%. The favorable results observed in our study in terms of pelvic pain recurrence may be explained by the almost complete resection of deep nodular endometriosis, which is innervated abundantly by sensory C chlorogenic and adrenergic nerve fibers, as recently demonstrated by Wang et al. ([2009b](#)). Even if some residual endometriotic foci remain in the bowel muscularis, they are not related to pelvic pain persistence. We should not forget that, in some studies, even if bowel resection is carried out, the margins are not free of disease in up to 10% of cases ([Anaf et al., 2002](#)).

The most difficult surgery is that performed in women who have been insufficiently operated on before, the previous surgeon having left part of the nodule behind because of massive adhesions, and those having undergone several IVF cycles with oocyte pickup and puncture through a deep endometriotic nodule. Koninckx et al. therefore suspect that trauma to endometriotic nodules could reactivate the disease, causing growth and massive adhesions ([Koninck et al., 2012](#)).

In conclusion, in the absence of solid evidence, Koninckx, Wattiez and Donnez suggest that deep endometriosis surgery should be visually complete, but at the level of the bowel, a rim of fibrosis can be left behind ([Koninckx et al., 2012](#)). Because we found most recurrences in the

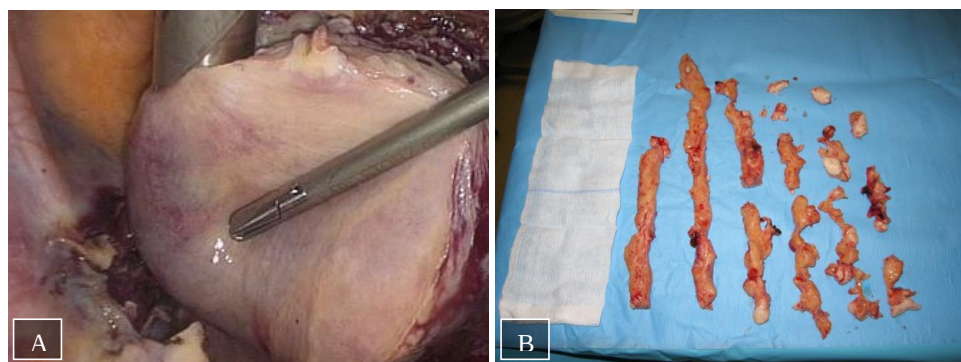
posterior fornix of the vagina, and because the vaginal cuff heals well, we specifically emphasize completeness at this level (Donnez et al., 2000, 2013).

Endometriosis is a benign disease. Women want to be free of symptoms, have normal bladder and digestive function, and enjoy a normal sex life. They do not necessarily want to be completely free of endometriosis. Indeed, as stated by Vercellini *et al.* (1996, 1997), the overall extent of disease correlates neither with the frequency or severity of symptoms, nor with the long-term prognosis in terms of conception or pain recurrence.

IV. IATROGENIC PERITONEAL ADENOMYOMAS

IV.1. Introduction

Very recently, lesions histologically similar to “adenomyotic” nodules (type III) were described and reported after laparoscopic subtotal hysterectomy (LASH) (Figure 24). They were termed iatrogenic peritoneal adenomyomas or pelvic adenomyotic masses (Donnez et al., 2006; 2007). Briefly, the LASH procedure requires coagulation and section of the uterine vascular blood supply. After section of the uterine artery, unipolar scissors are used to cut the cervix below the level of the internal os and separate the cervix from the corpus. The remaining uterus is removed from the abdominal cavity through a 15-mm trocar using electrical laparoscopic morcellator.

Figure 24: Electromechanical morcellation after the LASH procedure

A. Electrical morcellation of the uterus after LASH.
B. Morcellated uterine specimens.

IV.2 Prevalence

In a series of 1,400 LASH procedures, 8 cases (0.57%) of iatrogenic adenomyotic nodules were encountered and subsequently removed 20-192 months later (see Table 3).

IV.2.1 Clinical data

Symptoms appeared within 94.8 ± 59.7 months of the LASH procedure. Major symptoms experienced in all cases were moderate pelvic pain and moderate to severe deep dyspareunia. Clinical examination was painful when the pouch of Douglas and lateral cul-de-sac were explored, and vaginal examination revealed the presence of retrocervical or laterocervical masses no matter the phase of the menstrual cycle. Similar symptoms and clinical examination were observed in patients presenting with deep nodular endometriosis.

Table 3: Characteristics of morcellated specimens and iatrogenic lesions

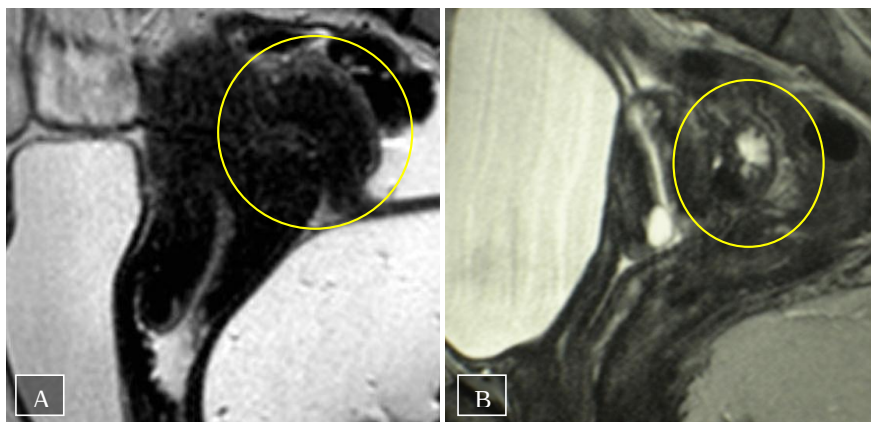
LASH specimens		Iatrogenic lesions				
Histopathology (H&E staining)	Endometrium	Timing between LASH procedure and excision of iatrogenic lesion (months)	Hormonal status	Size (mm)	Cancer Antigen 125 (CA125, serum level, UI/ml)	Histopathology (H&E staining)
Adenomyosis	Secr.	36	Estradiol	85	118	Adenomyosis
Adenomyosis	Prolif.	192	Tibolone	20	64.8	Adenomyosis
Adenomyosis	Secr.	119	Estradiol	80	128	Adenomyosis
Adenomyosis	Prolif.	62	Estradiol	40	39.3	Adenomyosis
Adenomyosis	Prolif.	81	Spont cycles	45	19.5	Adenomyosis
Adenomyosis	Secr.	86	Estradiol	10	19.4	Adenomyosis
Adenomyosis	Secr.	20	Spont cycles	30	21.7	Adenomyosis
Adenomyosis	Secr.	162	Tibolone	25	NA	Adenomyosis
Mean value $\pm$ SD		94.8 $\pm$ 59.7		41.9 $\pm$ 27.		

IV.2.2. Radiological and laparoscopic findings

In case of iatrogenic lesions, pelvic MRI revealed a cervix with normal morphology in all cases. Irregular masses were clearly visible overhanging the cervix, extending up into the pelvis and pressing against the rectum and rectosigmoid. These heterogeneous masses measuring 41.9 ± 27.4 mm (mean $\pm$ SD) were composed of hypo- and hyperintense signals on T2-weighted images. Gadolinium injection evidenced vascularization of the lesions. Hyperintense signals on T1- weighted images with saturation of fatty tissue suggested the presence of old blood. MRI findings revealed the same characteristics in case of type III endometriotic lesions (Squifflet and Donnez, 2002; Donnez and Squifflet, 2004; 2010). In

both type III endometriotic lesions and iatrogenic lesions, rectal attraction was systematically observed. Interestingly, when performed, barium enema showed the same characteristics (Figures 25 and 26) as those observed in type III deep nodules.

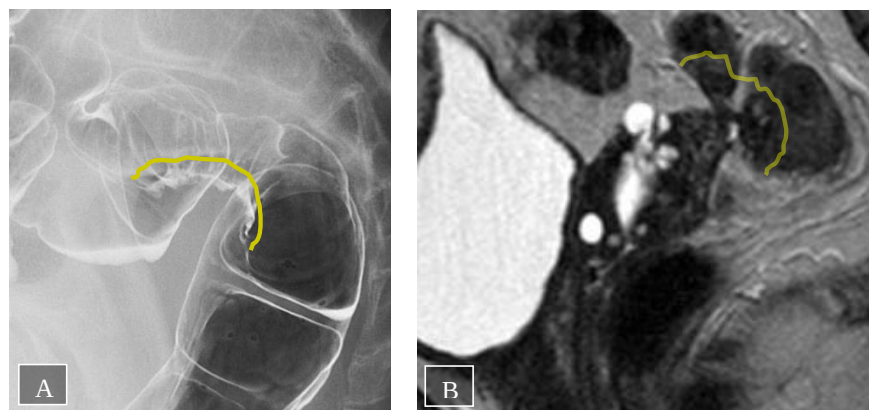
Figure 25: Pelvic MRI showing a T2-weighted sagittal view



A. Type III lesion (circled in yellow) with anterior rectal involvement.

B. Iatrogenic adenomyoma (circled in yellow) situated close to the cervix and the rectum.

Figure 26: Barium enema and pelvic MRI showing a T2-weighted sagittal view



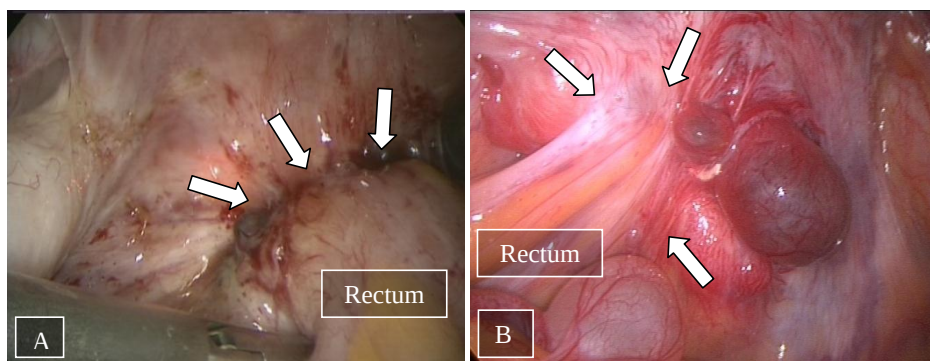
A. Infiltration of the muscularis of the anterior rectal wall in a type III lesion (delimited in yellow).

B. Iatrogenic adenomyoma with involvement of the rectum (delimited in yellow).

Laparoscopic excision was proposed to patients presenting with iatrogenic adenomyosis. Laparoscopy revealed retrocervical masses filling the pouch of Douglas. A macroscopic

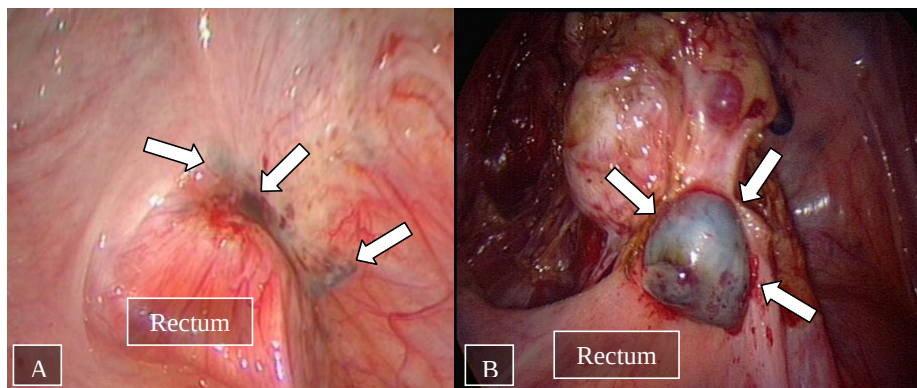
inflammatory reaction could be seen creating retraction of the surrounding organs, such as the cervix and the rectum (**Figure 27**). Adhesions between the pelvic masses and the rectum were found in all cases.

Figure 27: Laparoscopic view of rectal involvement



A. Strong infiltration (white arrows) of the anterior rectal wall by a type III lesion.
 B. Iatrogenic adenomyoma showing **attraction** of the rectum (white arrows).

Extensive dissection of the rectum and pararectal fossa were required to isolate the lesions. During resection of the masses, bluish lesions were identified corresponding to hemorrhagic spots observed on MRI (**Figure 28**). Again, similar surgical observations were made between iatrogenic adenomyosis and type III endometriotic lesions, where a continuum between the rectal muscularis and the cervix was found to obliterate the rectovaginal septum cranially.

Figure 28: Laparoscopic view bluish lesions

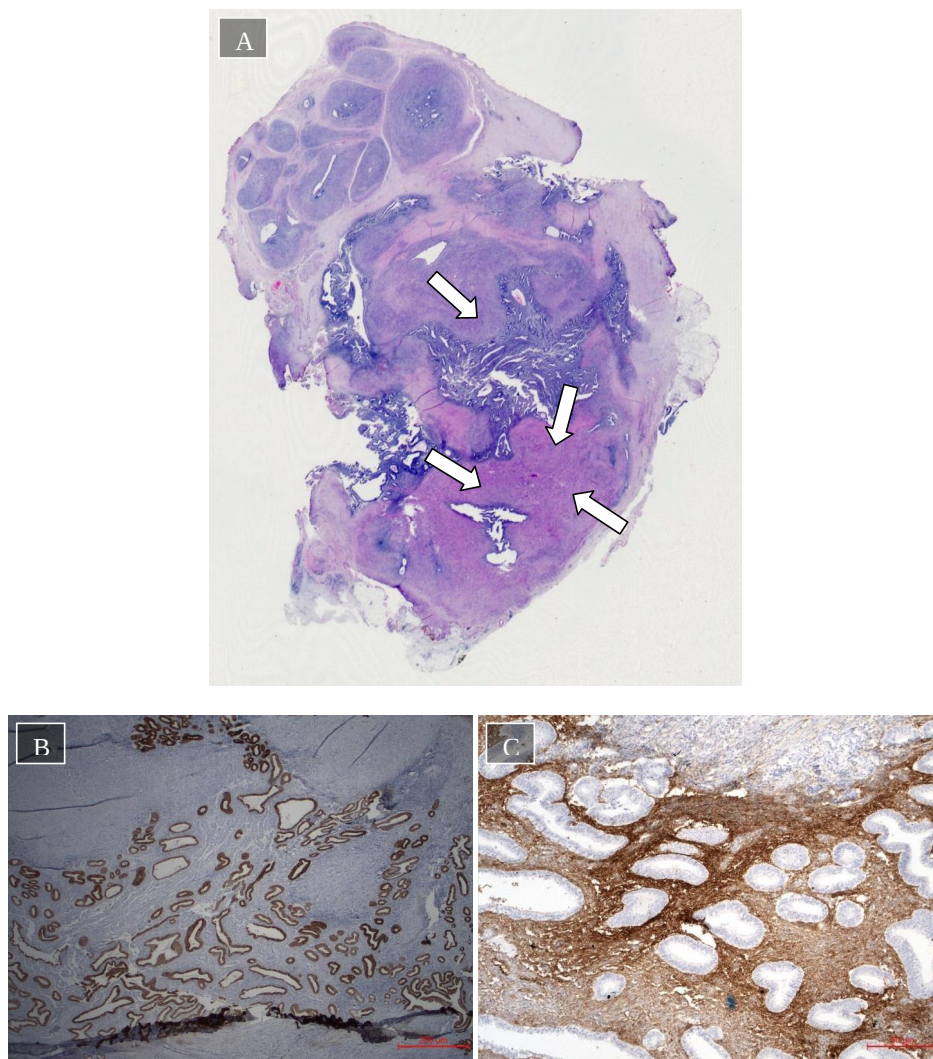
A. Type III lesion showing bluish lesions (white arrows).

B. Iatrogenic adenomyoma showing bluish lesions (white arrows) corresponding to hemorrhagic spots.

IV.2.3. Histological findings

In all 8 cases reported (Donnez et al., 2006 and 2007), microscopic examination of the morcellated uterus showed the presence of benign myomas and revealed proliferation of adenomyotic lesions in the myometrium. The endometrium was in the secretory phase in 5 cases and in the proliferative phase in 3 cases, meaning that the missed fragments were able to survive and grow whatever the phase of the endometrial cycle. Histological examination of the excised iatrogenic lesions confirmed smooth muscle hyperplasia infiltrated by endometrial glands and stroma, as shown by CD10 and CK22 immunohistochemistry (Figure 29 A-C). An inflammatory reaction was also observed around the dilated glands, probably due to old blood retention in the lumen of these glands.

Figure 29: Histological appearance of iatrogenic adenomyosis



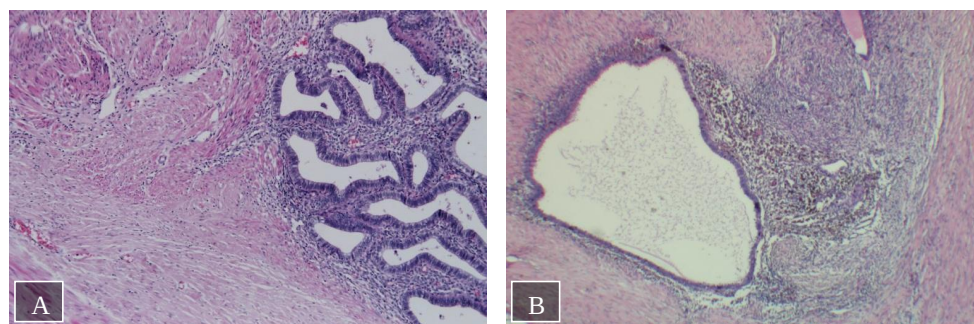
A. Smooth muscle hyperplasia (white arrows) infiltrated by numerous endometrial glands and stroma (from Donnez et al., 2006).

B. CK22 immunostaining of glandular structures (Scale bar 200 μm).

C. CD10 immunostaining of stroma (Scale bar 50 μm).

Similar characteristics were observed in type III nodular endometriosis. Indeed, a circumscribed nodular aggregate of smooth muscle, endometrial glands and, usually, endometrial stroma was typically seen.

Figure 30: Photomicrographs comparing type III lesions (A) and iatrogenic lesions (B)



Both lesion types show numerous glands surrounded by scanty stroma and extensive smooth muscle hyperplasia.

A. Type III lesion (original magnification $\times 80$)

B. Iatrogenic lesion (original magnification $\times 80$, from Donnez et al, 2007)

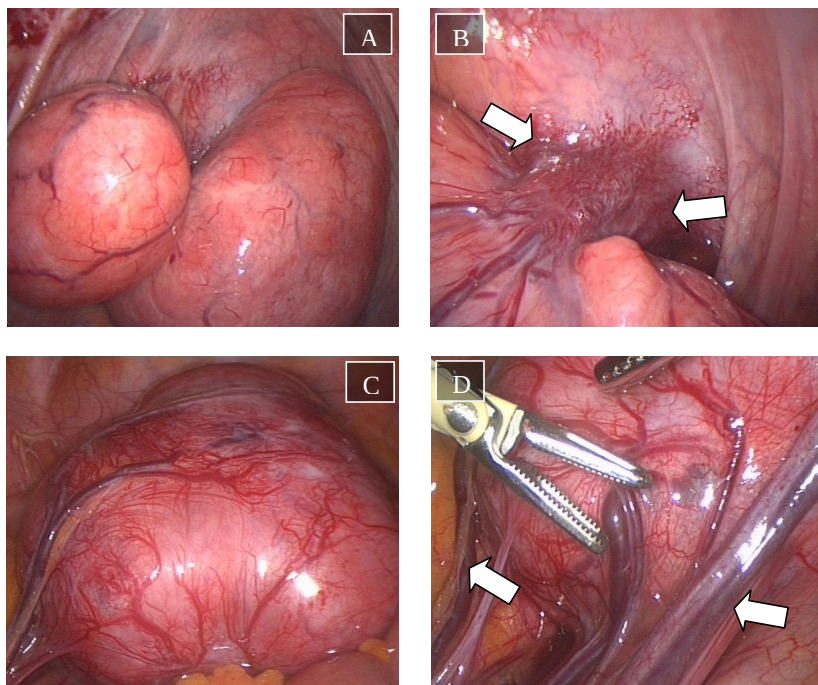
IV.3. What we can learn from observational studies: integration of the JZ

Iatrogenic lesions were identified as heterogeneous masses composed of hypo- and hyperintense signals on T2-weighted images (**Figure 25**). Histology of the removed specimens revealed adenomyosis in all cases, characterized by smooth muscle hyperplasia infiltrated by endometrial glands and stroma (**Figure 30**). We suggest that fragments of uterus containing adenomyosis or fragments of endometrium with myometrium were inadvertently left in place after uterine morcellation following LASH procedure. We thus observed that retained uterine fragments containing both endometrium and myometrium are able to survive in the peritoneal cavity, resulting in adenomyotic lesions closely mimicking deep nodular

endometriosis. Both lesion types present similarities in terms of macroscopic, radiological and histological findings. It is surprising that retained endometrial and myometrial fragments are able to implant and grow in the peritoneal cavity, regardless of the endometrial menstrual cycle phase at the time of hysterectomy.

Such fragments are not the only ones able to attach to peritoneum. Indeed, fragments of resected leiomyomas can also survive in the peritoneal cavity if they are missed during the resection procedure. However, iatrogenic adenomyotic lesions are different from “parasitic myomas” because the latter are never associated with severe adhesions (Paul and Koshy, 2006; Takeda et al., 2007; Moon et al., 2008; Cucinella et al., 2011). Parasitic myomas are generally asymptomatic tumors composed only of smooth muscle and receive an alternative blood supply from the peritoneum or omental vessels (**Figure 31 A-D**).

Figure 31: Laparoscopic view of parasitic myomas



- A. Parasitic myoma in the pouch of Douglas without severe adhesions.
- B. Vascular supply coming from the peritoneum (white arrows).
- C. Abdominal parasitic myoma (recurrence).
- D. Vascular blood supply from the omentum (white arrows).

For us, the most important difference between parasitic myomas and iatrogenic adenomyotic tumors is the presence of endometrium associated with subendometrial myometrium (the so-called JZ). When this association is found in “forgotten” specimens, these iatrogenic lesions are then able to develop tumors characterized by dense tissue composed of smooth muscle hyperplasia with isolated foci of endometrial mucosa and stroma.

V. Induction of endometriotic nodules in an experimental baboon model mimicking deep nodular endometriotic lesions. Can study of the junctional zone help us understand the development of deep nodular endometriotic lesions?

V.1. Introduction: preliminary considerations in the baboon model

Te Linde and Scott (1950) described the first non-human primate (rhesus monkey) model of endometriosis, with autologous transplantation in the pelvis of surgically excised fragments of endometrium. Unfortunately, we do not know if only endometrium or endometrium associated with myometrium was grafted. We know, from several reports, the species of non-human primates presenting with spontaneous endometriosis: 36% of female rhesus monkeys (Hadfield et al., 1997), 27% of baboons (D'Hooghe et al., 1991), and 28.7% of cynomolgus monkeys (Ami et al., 1993). In fact, these authors report a higher incidence of endometriosis in non-human primates than in humans. A number of authors have described non-human primate models to induce endometriosis more rapidly, especially using surgery. D'Hooghe et al. achieved an induced endometriosis rate as high as 69% by increasing the volume of retrograde menstruation in baboons (occlusion of the cervix). In a recent study in the same center (Institute of Primate Research, Nairobi, Kenya), we only observed a 4.8% rate of spontaneous endometriosis among 41 baboons originating from the wild (Dehoux et al., 2011). Endocervical canal resection revealed a 27.6% rate of induced endometriosis, but we never encountered retrograde menstruation after endocervical canal resection in more than 130 laparoscopic procedures (Dehoux et al., 2011). To explain this discrepancy, we could hypothesize that the inoculated tissue was not pure shed endometrium but also contained myometrium because the material of D'Hooghe et al. was obtained by curettage or transabdominal-transmyometrial biopsy when curettage was not possible. Our results suggested that baboons are able to cleanse their peritoneum by maintaining important mechanisms of peritoneal healing lost by women in the course of evolution. We therefore

believe that this species may not be the most appropriate experimental model to study peritoneal endometriosis, although we were surprised to see that uterine fragments left in the abdominal cavity after hysterectomy could induce endometriotic nodules in baboons (see pilot study). For this reason, we decided to establish an experimental protocol with the goal of inducing deep nodular lesions.

Pilot study

Three preliminary pilot studies were carried out:

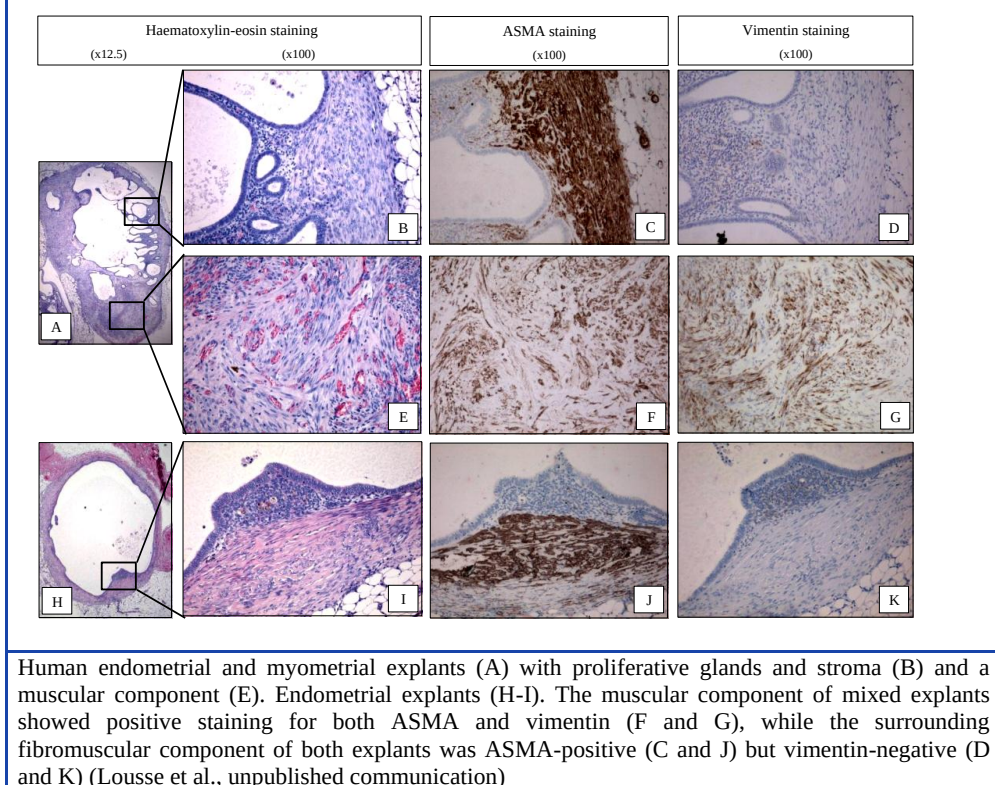
1. Human xenotransplantation
2. Rhesus macaques
3. Baboons

To better understand the pathogenesis of peritoneal adenomyosis, we developed an experimental in vivo model of extrauterine adenomyosis to assess the capacity of combined human endometrial and myometrial explants to implant in the peritoneal cavity of nude mice ([Lousse et al., personal communication](#)). We were able to recreate ectopic adenomyosis by placing human myometrium and endometrium in the peritoneal cavity of nude mice, with an increased rate of successful human endometrial grafts in nude mice when part of the myometrium was combined with endometrium (**Figure 32**).

Histological examination of adherent combined lesions revealed typical proliferative glands and stroma, associated with a recognizable muscular component supported by an extensive vascular network. Interestingly, fibromuscular cells were found surrounding both adenomyosis- and endometriosis-like lesions. In studies performed with J.C. Lousse and J. Squifflet (unpublished data), cells of human origin could be distinguished from mouse cells by positive staining for human-specific vimentin antibody. The muscular component of adenomyosis-like lesions was systematically positive for both markers (vimentin and ASMA),

confirming their human origin, while the surrounding fibromuscular component of both types of lesions systematically expressed ASMA but not vimentin, suggesting a murine origin.

Figure 32: Pilot study, induced adenomyotic lesions in nude mice

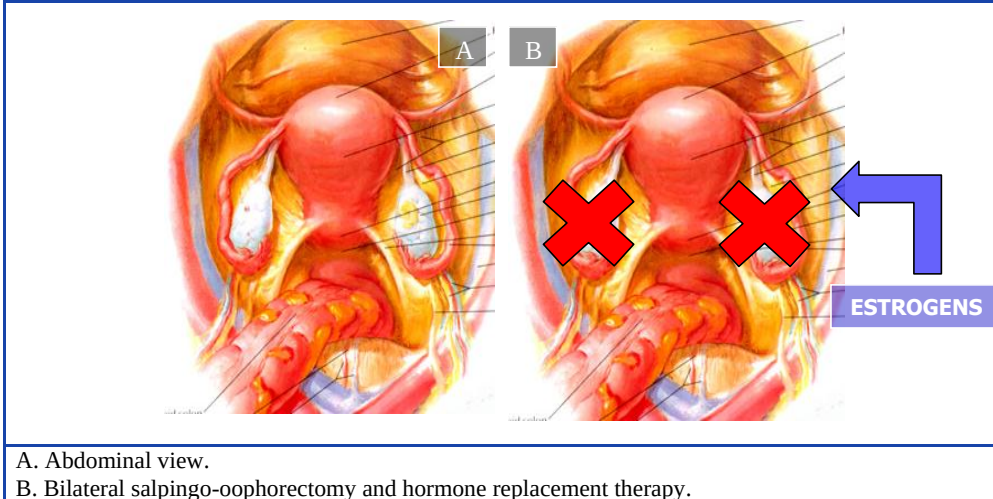


In *Macaca mulatta* and cynomolgus monkeys, we were unable to observe spontaneous or induced peritoneal endometriosis (Defrère et al., 2008; Dehoux et al., 2011). On the contrary, by grafting endometrium and myometrium inside the abdominal cavity, we were able to induce a 3mm lesion composed of endometrial glands and stroma surrounded by fibrotic tissue. In a baboon model (pilot study), however, we were able to induce endometriotic nodules exhibiting the macroscopic characteristics of adenomyotic nodules by grafting combined endometrial and myometrial tissue. In some explants fixed to the rectum,

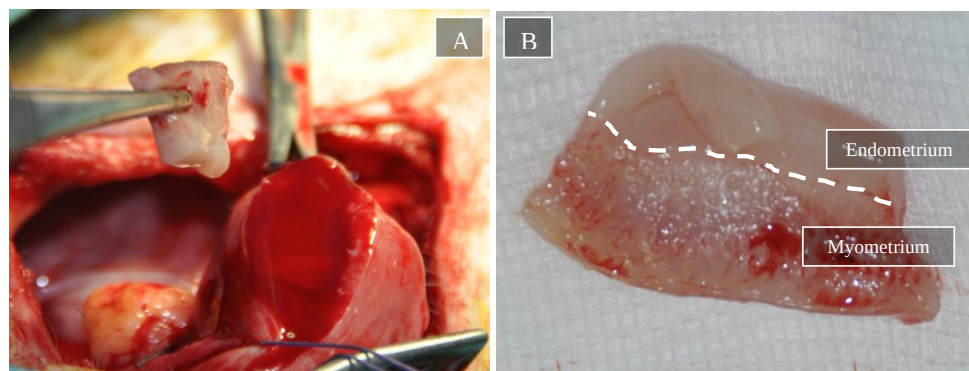
infiltration of the rectal wall by endometriotic glands and stroma could be observed, as seen in type III endometriotic lesions. Our results from observational studies on human iatrogenic adenomyosis, our murine model of peritoneal adenomyosis, and our pilot study on baboons, suggest that myometrium, and more precisely the subendometrial myometrial layer (JZ), plays a role in the attachment and proliferation of endometrial glands in the peritoneal cavity, mimicking adenomyosis. These observations have led us to reconsider the baboon model for induction of endometriotic nodules, and particularly for the study of the JZ.

V.2. Materials and methods

Ten female baboons (*Papio anubis*) were studied at the Institute of Primate Research, Nairobi, Kenya, after more than two years in captivity. The animals underwent median laparotomy. Laparotomy was preferred over laparoscopy because of the subsequent grafting procedure and the reproducibility of the technique that could be more easily achieved by laparotomy than by laparoscopy. Moreover, it was recently demonstrated that repeated laparoscopic procedures without any inoculation of endometrial specimens can induce spontaneous evolution of chronic endometriosis ([Harirchian et al., 2012](#)). After complete pelvic and abdominal exploration, no spontaneous endometriosis was identified in any of the 10 animals. Bilateral salpingo-oophorectomy was performed to avoid individual variations in hormone secretion, and hormone replacement therapy was initiated on the same day (**Figure 33**).

Figure 33: Illustration of median laparotomy

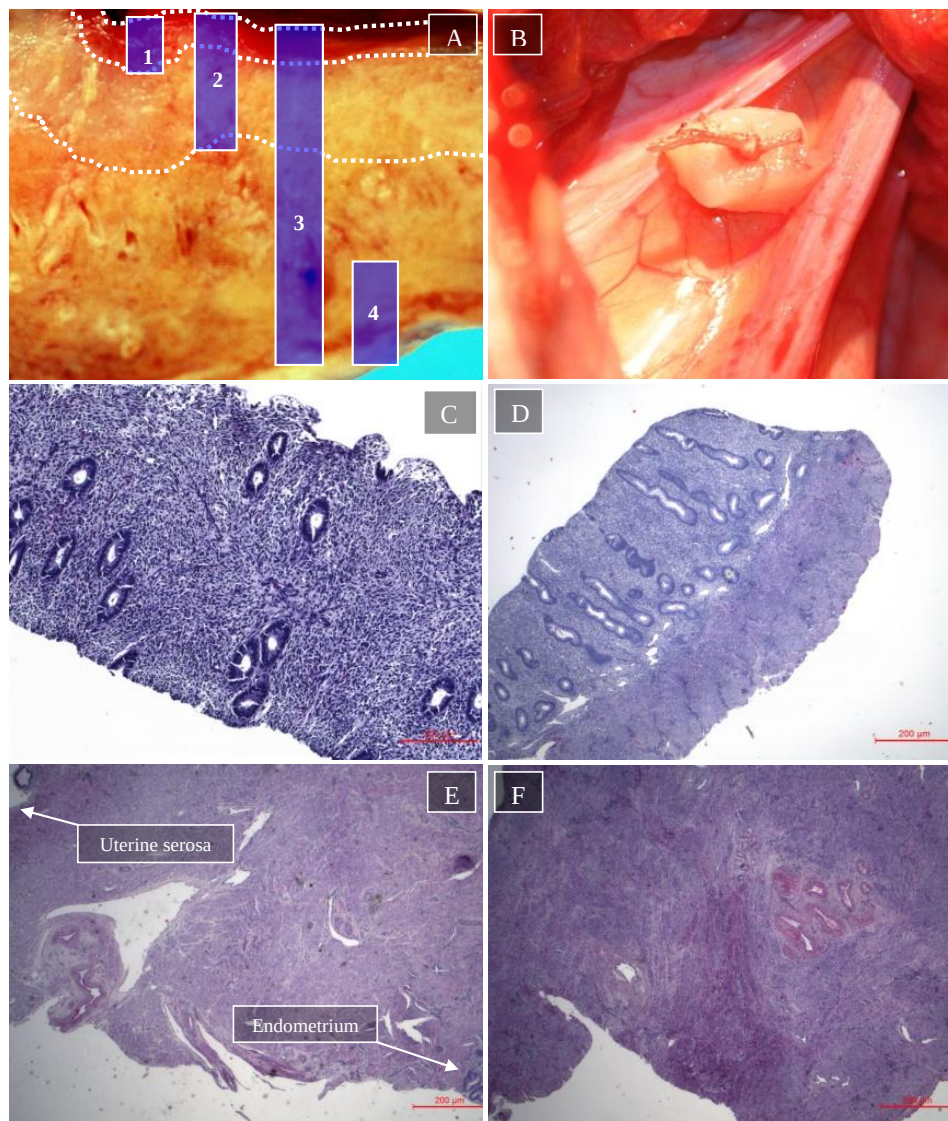
Large uterine biopsies were obtained using a cold knife (**Figure 34 A and B**). Anterior hysterotomy was performed to prevent iatrogenic adhesions in the Douglas pouch.

Figure 34: Uterine biopsy

Fragments (± 10 mm in size) to be grafted were immediately prepared and separated as follows: endometrium alone, endometrium plus the JZ, total uterine thickness, and deep myometrium without endometrium (**Figure 35 A-F**). Endometrial fragments measured

approximately 10×10×5mm, endometrium plus the JZ 10×10×10mm, full-thickness fragments 10×10×15mm, and myometrium alone 10×10×10mm . In future studies, it will be mandatory to measure and weigh the fragments prior to grafting (**Figure 35 B**).

Figure 35: Uterine section and grafted specimens



A. Section of the uterus showing total uterine wall thickness. The endometrium and myometrium are easily distinguishable, as is the JZ (white stippled lines). Four types of specimens were grafted to the peritoneal cavity: endometrium alone ($10 \times 10 \times 5 \text{ mm}$), endometrium plus the JZ ($10 \times 10 \times 10 \text{ mm}$), total uterine thickness ($10 \times 10 \times 15 \text{ mm}$), and myometrium alone ($10 \times 10 \times 10 \text{ mm}$).

B. Specimens of tissue were grafted to different abdominal sites using absorbable sutures.

C. Endometrium alone (Scale bar $200 \mu\text{m}$).

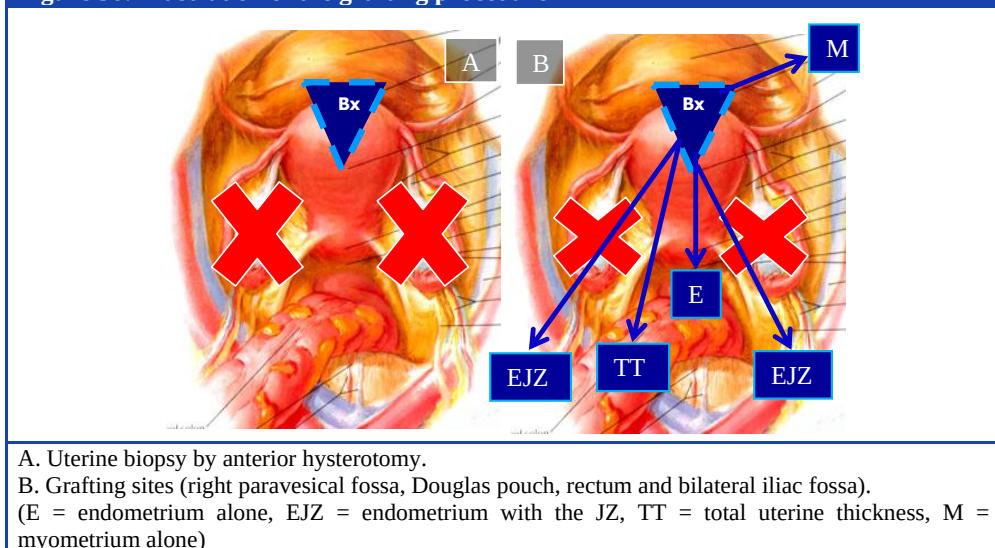
D. Endometrium and JZ (Scale bar $200 \mu\text{m}$).

E. Total uterine thickness (endometrium and uterine serosa) (Scale bar $100 \mu\text{m}$).

F. Myometrium alone (no glands could be visualized) (Scale bar $100 \mu\text{m}$).

Endometrium alone was grafted to the Douglas pouch using one simple suture. Deep myometrium alone was grafted to the peritoneum covering the right paravesical fossa. Total uterine thickness was grafted to the anterior wall of the rectum. Two fragments of endometrium with the JZ were respectively grafted to the left iliac fossa and the right iliac fossa in front of the ureter (**Figure 36**).

Figure 36: Illustration of the grafting procedure



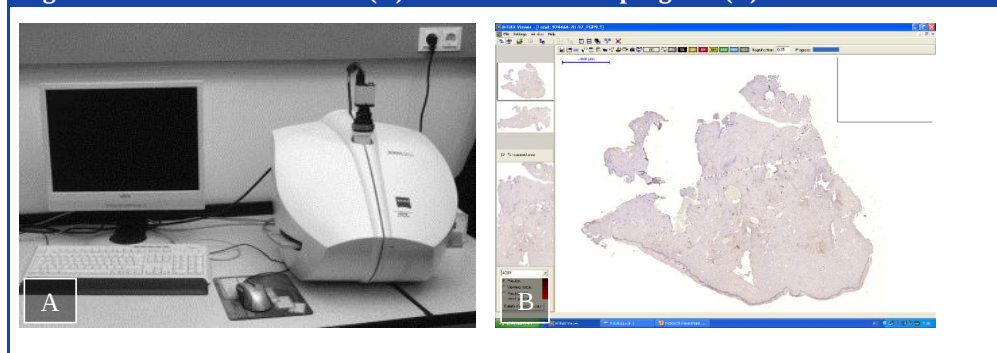
Between 20 and 24 weeks after grafting, laparotomy was performed. All induced lesions were identified and photographed before complete excision. Grafted tissue was fixed in formalin and embedded in paraffin. Histological sections were immunostained with monoclonal anti-CK22 antibody. All sections were analyzed in a blinded manner before correlation with corresponding grafted specimens.

Data were recorded, incorporating both macroscopic and microscopic features. Macroscopic data included the presence/absence of endometriotic lesions and adhesions at the site of grafting. Volume was calculated by measuring the length, width and depth of lesions

macroscopically observed before fixation. Endometriotic adhesions were reported as adhesions with the sigmoid, ureter, cervix, uterus or fatty tissue from the initial grafting site.

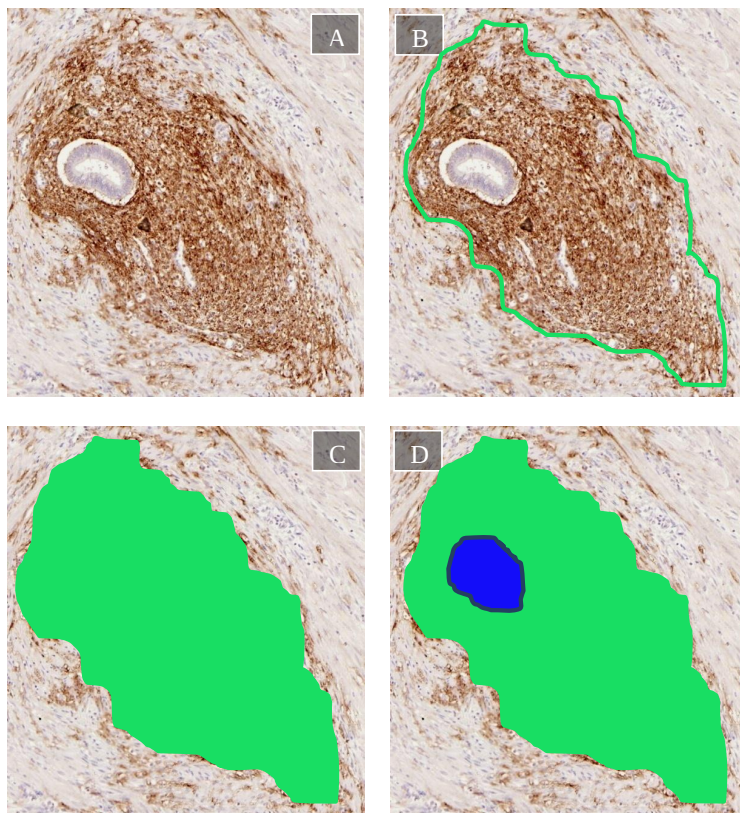
All sections were scanned with the Mirax Midi scanner (**Figure 37**).

Figure 37: Mirax Midi scanner (A) and Mirax Viewer program (B)



This allowed us to measure lesion surface area (glands and stroma) by means of CD10 immunostaining, while volume was calculated by evaluating the length of lesions macroscopically before fixation (**Figure 38**). Microscopic data included the surface area (mm^2) of lesions, glandular density ($\text{glands}/\text{mm}^2$), and surrounding organ invasion, reported as the presence of glands and stroma inside surrounding organs, followed along serial sections.

Figure 38: Illustration of lesion surface area evaluation using the Mirax Midi scanner and Mirax Viewer program



The CD10 immunostained area (A) was delineated with the Mirax Viewer (B), allowing calculation of the surface area (C). Glands (D) were tagged and their density was calculated per mm².

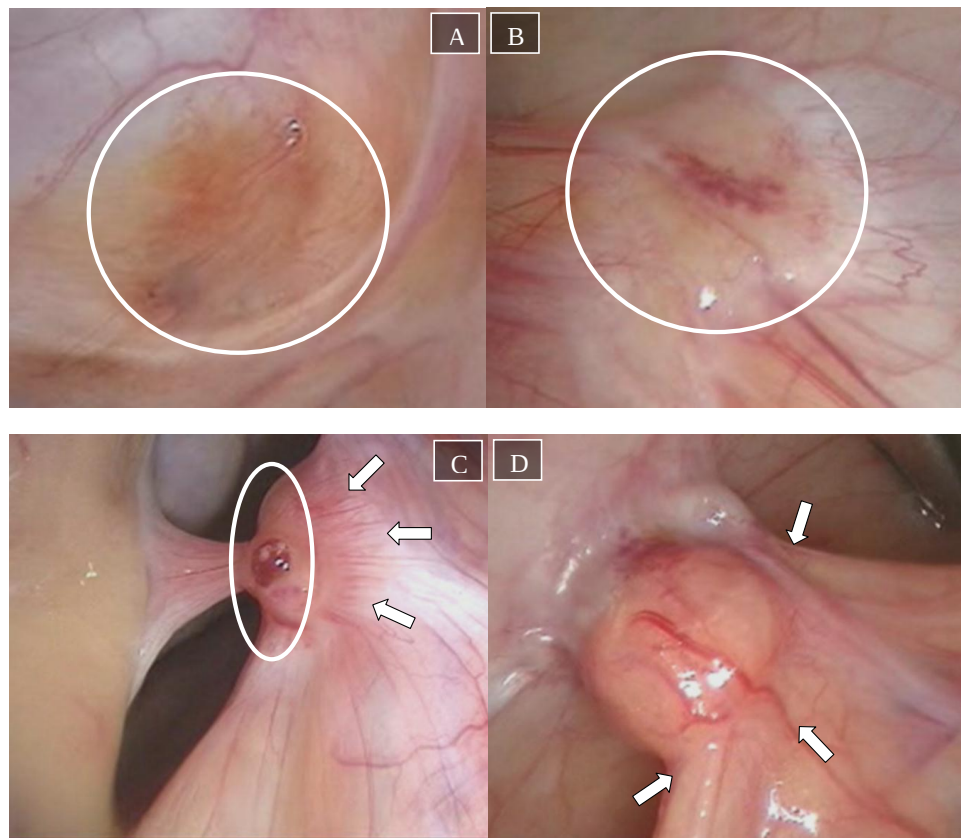
V.3. Results

V.3.1 Laparoscopy four weeks after grafting

A first series of five baboons underwent diagnostic laparoscopy four weeks after grafting in order to carry out a first macroscopic evaluation of the grafts before starting the second series of five baboons. Endometrium alone, grafted to the Douglas pouch, was observed as brownish lesions in all cases (5/5), resembling hemosiderin deposits. Neither red nor black lesions were observed. Grafts of deep myometrium alone (5/5) looked like poorly vascularized fibrotic

tissue. Grafts of endometrium plus the JZ and total thickness of myometrium all appeared as centimetric nodular lesions with neovascularization originating from the surrounding peritoneum. The lesions showed adhesions with the sigmoid and ureter or omentum (**Figure 39**).

Figure 39: Laparoscopic view of induced lesions 4 weeks after grafting



A. Laparoscopic view of a lesion (circled in white) four weeks after grafting endometrium alone. This brownish lesion is not nodular.

B. Laparoscopic view of a lesion (circled in white) four weeks after grafting myometrium alone. This lesion resembles poorly vascularized fibrotic tissue.

C. Laparoscopic view of a nodular lesion (circled in white) four weeks after grafting endometrium and the JZ. Neovascularization from the peritoneum is visible, as well as adhesion with the sigmoid and ureter (white arrows).

D. Laparoscopic view of a nodular lesion four weeks after grafting endometrium and the JZ, revealing adhesions with the omentum (white arrows).

V.3.2. Macroscopic analysis

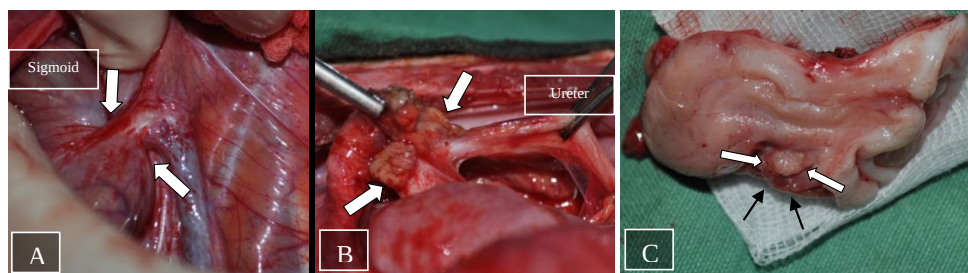
V.3.2.1. Endometriosis and nodular lesions

One baboon was excluded from the study due to protocol violation. Nodular endometriosis was observed in 100% (n=9/9) of the other baboons; 94.6% of all lesions were still attached to the grafted area and 56.7% showed induced adhesions with surrounding organs such as the sigmoid, omentum, ureter, cervix, uterus or pelvic side wall (**Table 4**). No red or black lesions were observed.

V.3.2.2. Adhesions

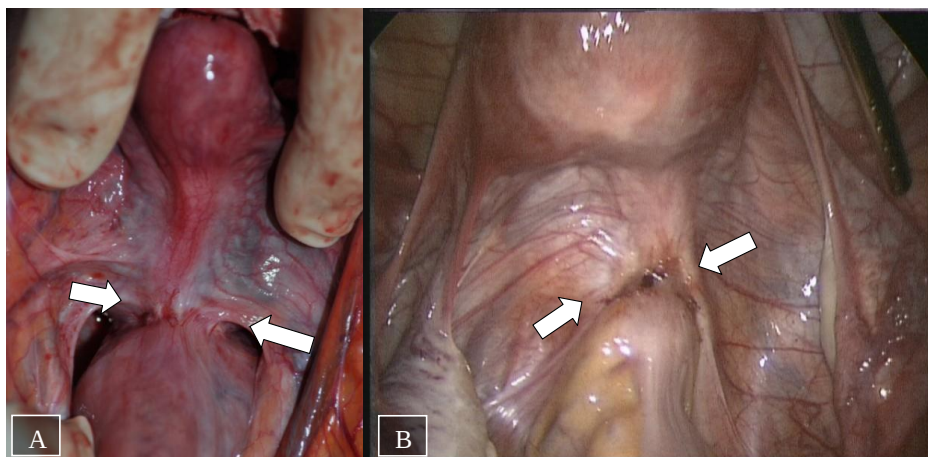
When analyzing adhesions in induced lesions, no endometriotic adhesions were identified in lesions induced after grafting endometrium or myometrium alone. When endometrium was grafted together with the JZ, 66.7% of macroscopic adhesions were present, either with the sigmoid (**Figure 40 A**) or other organs like the ureter (**Figure 40 B**), cervix (**Figure 40 C**) or omentum. When total uterine thickness was grafted, macroscopic adhesion with the cervix was observed in 87.5% of cases (**Table 4**). These lesions (**Figure 41 A**) were macroscopically identical to type III nodules found in women (**Figure 41 B**). Macroscopic adhesions were observed with the sigmoid and uterus.

Figure 40: Laparoscopic view of induced lesions 4 weeks after grafting



A. Nodular lesion observed after grafting endometrium and the JZ. Spontaneous adhesion with the sigmoid is visible (white arrows).
 B. Nodular lesion observed after grafting endometrium and the JZ. Spontaneous adhesion with the ureter is visible (white arrows).
 C. Macroscopic sagittal section of a lesion induced after grafting total uterine thickness reveals a retrocervical nodular lesion (white arrows) and adhesion with the rectum (mucosa shown by black arrows).

Figure 41: Laparoscopic view of induced lesions and type III lesions in humans



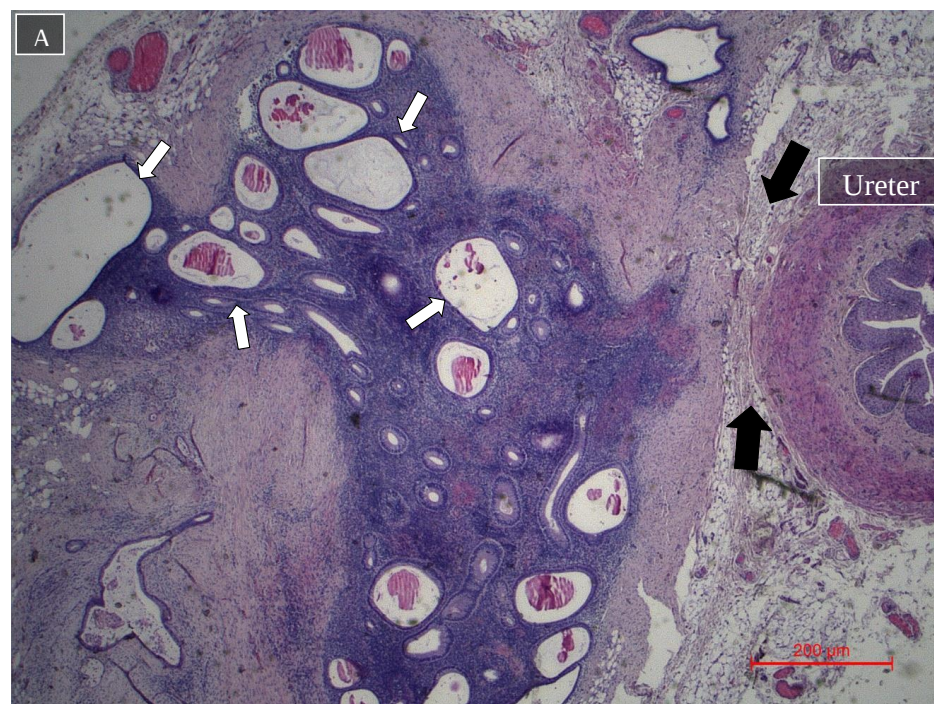
Macroscopic view of nodular lesions from baboon (A) and human (B) subjects, showing striking similarities between lesions induced in baboons and type III nodules in patients (nodular lesion between the white arrows).

V.3.3. Microscopic analysis

V.3.3.1. Endometriosis

Lesions induced after grafting endometrium alone showed endometriosis in 80% of cases, characterized by scattered glands and stroma. Microscopic analysis of the lesions induced after grafting endometrium together with the JZ and total uterine thickness revealed the presence of endometriosis in respectively 77.8% and 85.7% of cases (**Table 4**). These lesions had dilated endometrial glands and stroma associated with smooth muscle hyperplasia (**Figure 42 A**). All endometriotic nodular lesions were characterized by the presence of glands, stroma and smooth muscle hyperplasia. The presence of glands was confirmed using CK22 (**Figure 42 B**) immunostaining and the presence of stroma was confirmed using CD10 immunostaining (**Figure 42 C**). Lesions induced after grafting myometrium alone revealed smooth muscle associated with fibrotic tissue. In one case, a lesion composed of seven isolated glands surrounded by stroma was observed after grafting myometrium alone. After analyzing the uterus of the baboon in question, uterine adenomyosis was diagnosed by the presence of endometrial glands deeply infiltrating the myometrium and extending up to the uterine serosa.

Figure 42: Microscopic view of a lesion induced after grafting endometrium and the JZ



A. Microscopic view of a lesion induced after grafting endometrium and the JZ, showing the presence of dilated glands (white arrows) surrounded by stroma and smooth muscle hyperplasia. Adhesion with the ureter was also observed (black arrows).

B. The presence of glands was confirmed using CK22 immunostaining (Scale bar 200μm).

C. The presence of stroma was confirmed using CD10 immunostaining. CD10 seems to be much fainter in baboon endometrial stroma than in human endometrial stroma (Scale bar 200μm).

V.3.3.2. Size of the lesions

In lesions induced after grafting endometrium alone, mean surface area including stroma and glands was $0.7 \pm 0.23\text{mm}^2$, after grafting endometrium plus the JZ, it was $7.3 \pm 6.7\text{mm}^2$, after grafting total uterine thickness, it was $29.6 \pm 48.6\text{mm}^2$, and after grafting myometrium alone, it was $0.04 \pm 0.32\text{mm}^2$. Statistical analysis of these results revealed that lesions induced after grafting endometrium plus the JZ and total uterine thickness were significantly larger ($p<0.05$) than those induced after grafting endometrium or myometrium alone. In lesions induced after grafting endometrium alone, mean volume was $1.9 \pm 2.4\text{mm}^3$, after grafting endometrium plus the JZ, it was $69.0 \pm 78.1\text{mm}^3$, after grafting total uterine thickness, it was $415.6 \pm 741.6\text{mm}^3$, and after grafting myometrium alone, it was $0.2 \pm 0.6\text{mm}^3$. Statistical analysis of these results revealed that lesions induced after grafting endometrium plus the JZ and total uterine thickness were significantly larger ($p<0.05$) than those induced after grafting endometrium or myometrium alone (**Table 4**).

V.3.3.3. Glandular density

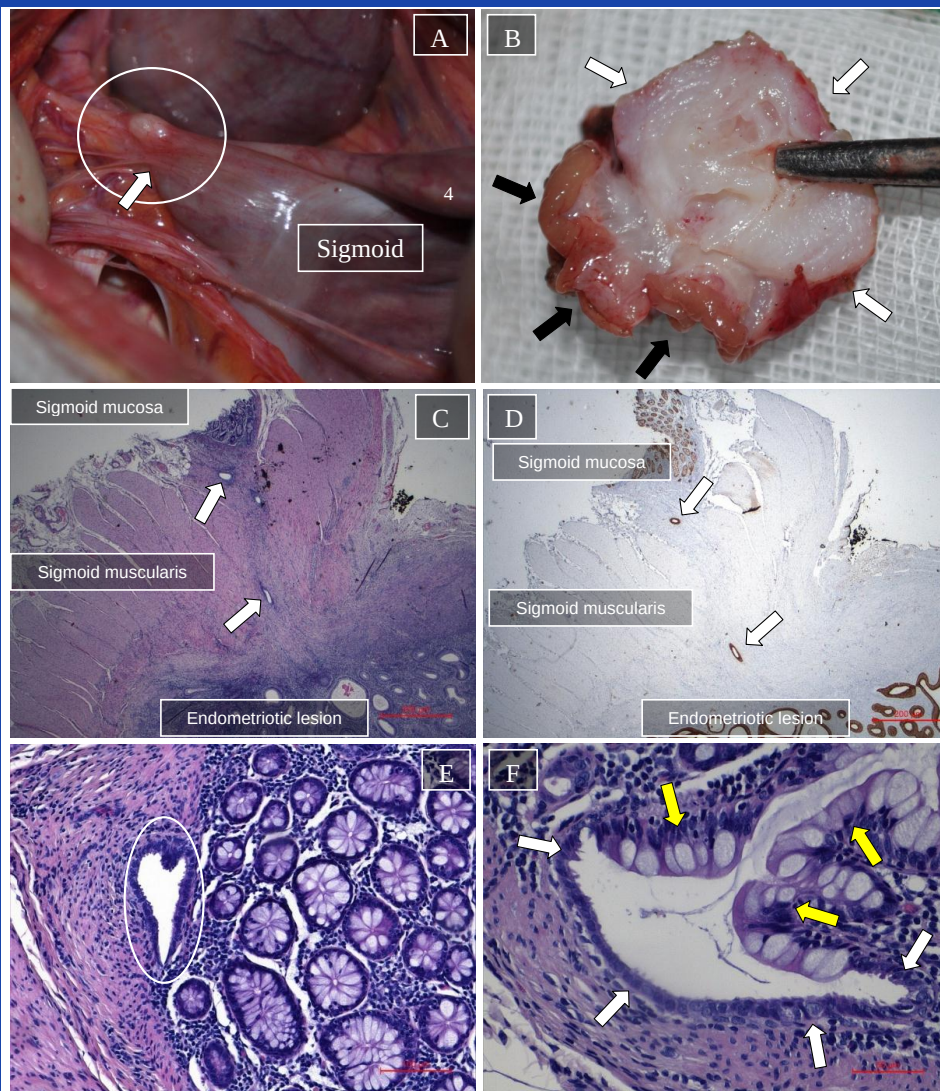
In lesions induced after grafting endometrium alone, mean glandular density was 2.5 ± 2.05 glands/ mm^2 , after grafting endometrium associated with the JZ, it was 4.7 ± 3.25 glands/ mm^2 , and after grafting total uterine thickness, it was 4.9 ± 5.29 glands/ mm^2 . However, mean glandular density was only 0.7 ± 2.21 glands/ mm^2 in lesions obtained from myometrium-only grafts. Statistical analysis revealed that mean glandular density was significantly lower in lesions induced after grafting myometrium alone (**Table 4**).

V.3.3.4. Invasion

No surrounding organ invasion was observed in lesions induced after grafting endometrium or myometrium alone, but it was observed in 41.7% ($n=5/12$) of lesions induced after grafting

endometrium plus the JZ. One case revealed glandular infiltration through the entire thickness of the sigmoid muscularis (**Figure 43 A and B**). On consecutive serial sections, glandular infiltration could be seen from the initial lesion right through the sigmoid wall (**Figure 43 C and D**). In this particular case, endometrial glands and stroma were also present in the submucosal sigmoid layer (**Figure 43 E**), and endometrial glands were able to colonize the sigmoid glandular epithelium (**Figure 43 F**). Surrounding organ invasion was observed in 42.9% of lesions induced after grafting total uterine thickness (**Table 4**). In two cases, glands were identified in fatty tissue surrounding the lesions and through the serosa of the remaining uterus. In these particular cases, the initial lesion secondarily infiltrated the uterus. In one case, glands invaded the sigmoid muscularis, but the submucosal layer was free of endometriosis. The same lesions spontaneously occur in women (**Figure 44 A-C**).

Figure 43: Invasion of the sigmoid by an induced lesion after grafting endometrium and the JZ



A. Nodular lesion (white circle) observed after grafting endometrium and the JZ. Spontaneous adhesion with the sigmoid is visible (white arrow).

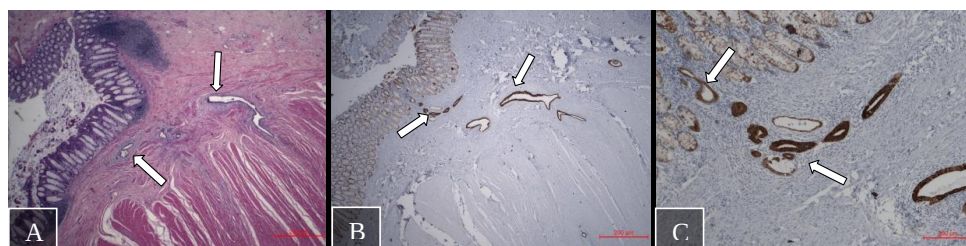
B. Macroscopic section of the previous lesion reveals a solid tumor (white arrows) with a cystic component. Dense adhesion with the sigmoid (mucosa shown by black arrows) is clearly visible (Scale Bar 200µm).

C. Microscopic view of a nodular lesion showing strong adhesion with the sigmoid and infiltration of the muscularis. Glands and stroma (white arrows) are clearly visible through the muscularis (Scale Bar 200µm).

D. CK22 immunostaining confirms the presence of glandular invasion of the sigmoid muscularis (white arrows) (Scale Bar 100µm).

E. White circle showing the presence of a gland in the mucosal layer of the sigmoid.

F. Endometriotic gland (white arrows) infiltrating the sigmoid mucosa and fusing with Lieberkühn crypts (yellow arrows) (Scale Bar 50µm).

Figure 44: Spontaneous endometriotic invasion of the sigmoid in humans

A. Microscopic view (H&E) of spontaneous invasion of the sigmoid submucosal layer in humans by endometriotic glands (white arrows).

B. CK22 immunostaining confirming the presence of glandular invasion of the sigmoid submucosal (B) and mucosal (C) layer (white arrows).

Table 4: Results of microscopic and macroscopic analyses of lesions induced after grafting endometrium alone, endometrium and the JZ, full uterine thickness and myometrium alone

	Endometrium (n=5)	Endometrium and JZ (n=14)	Full uterine thickness (n=9)	Myometrium (n=9)
Adhesions	0%	66.7%*	85.7%*	0%
Endometriosis (H&E)	80%	85.7%	77.8%	11.1%*
Surface (mm ²)	0.7 ± 0.23	7.3 ± 6.7*	29.6 ± 48.6*	0.04 ± 0.32
Volume (mm ³)	1.9 ± 2.4	69.0 ± 78.1*	415.6 ± 741.6*	0.2 ± 0.6
Glandular density (-/mm ²)	2.5 ± 2.05	4.7 ± 3.25	4.9 ± 5.29	0.7 ± 2.21*
Invasion	0%	41.7%*	42.9%*	0%

* $p < 0.05$

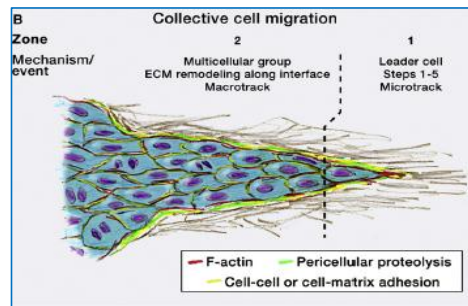
Endometrial fragments measured approximately 10×10×5mm, endometrium plus the JZ 10×10×10mm, full-thickness fragments 10×10×15mm, and myometrium alone 10×10×10mm. Induced nodular endometriotic lesions are significantly larger with greater glandular density after grafting tissue specimens containing the JZ. More than 40% of surrounding organ invasion was observed in lesions induced after grafting specimens containing the JZ.

V.4. Discussion and conclusions on the experimental model

In this new experimental animal model, we observed a 100% rate of induced endometriotic lesions, depending on the tissue grafted. In a recent autologous menstrual endometrium-inoculating baboon model, Harirchian et al. (2012) found significantly more black and blue lesions ($p\text{-value} \leq 0.05$) than powder-burn, blister or multicolored lesions out of a total of 542 peritoneal lesions observed in nine animals 15 months after inoculation. In our series with grafted endometrial specimens, no red or black lesions were observed. This may possibly be explained by the absence of menstrual cycles in our model, but Harirchian et al. did not find any endometriomas or deep-infiltrating endometriosis in their model, suggesting that these lesions may need more time to appear or are caused by different mechanisms, the baboon model primarily reflecting peritoneal disease. We also never observed, neither in this nor a previous study (Dehoux et al., 2011), the development of deep endometriomas nor deep lesions when only endometrial tissue was transplanted. On the contrary, using grafted specimens containing endometrium and superficial/deep layers of myometrium, we were able to induce nodular endometriotic lesions within six months. This was the first time that an experimental animal model has reproduced nodular endometriotic lesions similar to those observed in women presenting with type III nodular endometriotic lesions (Figure 41). Our results already show that induced nodular endometriotic lesions are significantly larger with greater glandular density after grafting tissue specimens containing the JZ. Even when total uterine thickness is grafted, induced lesions are not significantly different from those developing after grafting endometrium together with the JZ. These data indicate that the JZ may be a key element that clearly merits further investigation.

Although the JZ has never been studied in baboons, our results suggest a strong involvement of the JZ in the invasion process. More than 40% of surrounding organ invasion was observed in lesions induced after grafting specimens containing the JZ in our experimental model. Conversely, no invasion was observed in lesions induced after grafting endometrium or myometrium alone. This highlights the aggressive behavior of specimens containing the JZ, seen in the eight cases of iatrogenic adenomyosis we previously described ([Donnez et al., 2006, 2007](#)).

Even if endometriosis is regarded as a benign condition, some authors point out certain characteristics of malignancy, such as distant foci and invasion of other tissue, with subsequent damage ([Stern et al. 2001](#); [Larsen et al., 2011](#)). Friedl and Alexander ([2011](#)) recently described invasion processes in cancer and there are some similarities with endometriosis. Endometriosis and cancer appear to share the same proinflammatory paracrine environment, including common cytokines, growth factors and transduction pathways including NF- κ B ([Defrère et al., 2011](#); [Friedl and Alexander, 2011](#); [Gonzalez-Ramos et al., 2012](#); [Lousse et al., 2012](#)). The theory of collective invasion described by Friedl and Ilina ([Friedl et al., 1995](#); [Ilina and Friedl 2009](#)) requires cell-cell adhesion and multicellular coordination to occur simultaneously with migration (**Figure 45**).

Figure 45: Collective cell migration

From Friedl and Alexander, 2011

According to Gaggioli et al. (2007), in most cases of collective cancer invasion, several leader cells with mesenchymal characteristics generate traction and pericellular proteolysis toward the invaded tissue structure. This is similar to what we observed in our model, especially in case of bowel invasion (Figure 43). Indeed, morphologic similarities can be found with the invasion behavior of deep nodular endometriosis, which is known to invade the anterior rectal muscularis in type III lesions. In lesions induced after grafting, we found sigmoid wall invasion to be morphologically indicative of collective invasion, with native nodular tumors and secondary groups of cells able to invade surrounding organs and even colonize the sigmoid mucosa. This suggests that our model shows high potential for local invasion of surrounding tissue, especially when the JZ is present in grafted specimens.

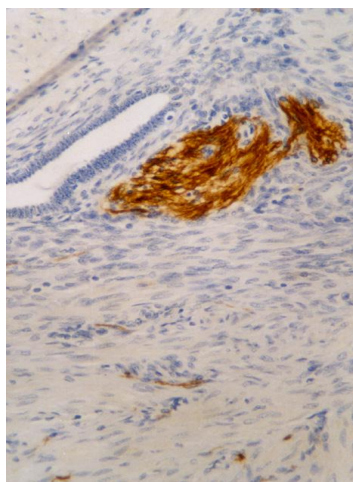
Multicellular coordination appears to be mandatory for extension of the disease. For us, this is an argument in favor of the shaving technique, which could be the treatment of choice for type III lesions, as this surgical technique removes the cohesive part of the nodule and sometimes leaves behind micro-tracks of “leader cells”. This could explain the low recurrence rate of symptoms in our series, and even if some residual disease remains in the bowel muscularis after the shaving technique, it is neither related to pain persistence (Donnez and Squifflet, 2010; Donnez et al., 2013; Koninckx et al., 2013) nor to high recurrence rates.

VI.1. Nerve fibers are absent in both disease-free and eutopic endometrium, but more abundant in deep-infiltrating disease than in other types of endometriosis: do we have arguments to support the involvement of nerve fibers in the invasion process of deep nodular endometriosis?

VI.1. Introduction

In deep nodular endometriosis, Anaf et al. (2000) were the first to pinpoint a close histological relationship between nerves and endometriotic foci, and between nerves and the fibrotic component of nodules. They demonstrated that in lesions from patients with pain scores >7 , the percentage of nerves located within endometriotic lesions was significantly higher than in lesions from patients with pain scores ≤ 7 (Anaf et al., 2000). They postulated that this could partially explain the association between lesions and mediation of pain. In a series of 31 patients undergoing large bowel resection for severe deep nodular endometriosis of the rectum, the same authors (2004) found that $53 \pm 13\%$ of endometriotic lesions were in contact with nerves or invaded neuronal structures (Figure 46).

Figure 46: Invasion of nerves



Typical example of peri- and intraneural invasion by endometriotic stromal cells (S-100 immunostaining) (From Anaf et al, 2002).

Moreover, a rate of 9.7% of resection colorectal margins (>3cm from nodular lesions) presenting endometriotic foci was observed and, in those cases, endometriotic lesions were in contact with nerves. They therefore postulated that nerves could represent a preferential route of invasion, which is why we chose to specifically study nerve fibers in endometriosis.

Nerves in endometriosis

Nerve fibers were also studied and detected in peritoneal and ovarian endometriotic lesions. Their density, calculated after evidencing nerves by protein-gene product 9.5 (PGP9.5, a pan-neuronal marker) immunostaining, was found to be higher in deep-infiltrating endometriosis than in peritoneal lesions (Mechsner et al., 2009; Wang et al., 2009; Arnold et al., 2012; McKinnon et al., 2012). In ovarian lesions, some studies showed high nerve fiber density (NFD) (Tokushige et al., 2010; Zhang et al., 2010), while others reported very poor densities (McKinnon et al., 2012). No difference in NFD was detected during the menstrual cycle in peritoneal lesions (Wang et al., 2011), but hormone therapies were reported to induce a significant decrease in nerve fiber densities in rectovaginal nodules (Tokushige et al., 2008, 2009). Neurofilament (NF) protein, another marker of nerve fibers, is classically used to detect myelinated nerve fibers. Its presence was also evidenced in endometriosis (Fraser, 2010). The presence of nerve fibers in lesions themselves is hypothesized to contribute to the pain encountered by endometriosis patients, as well as inflammation and PGs in the lesion environment (Defrère et al., 2011; Lousse et al., 2012). However, a possible correlation between pain and NFD still remains elusive and controversial (Mechsner et al., 2009; Zhang et al., 2009; McKinnon et al., 2012), and studies on larger cohorts are now clearly warranted to address this question. Indeed, pain is subjective, therefore difficult to investigate, and especially in endometriosis because of the poor correlation between the extent of disease and its morphological characteristics and the intensity and character of pain symptoms (Asante and Taylor, 2010).

Nerve growth factor (NGF), which can potentially play a key role in lesion innervation, has been shown to be present in endometriotic lesions ([Anaf et al., 2002](#); [Tokushige et al., 2006](#); [Mechsner et al., 2007](#)). However, the presence of such a neurotrophic factor in lesions could create an environment favorable to nerve growth. Its role in other important processes encountered in endometriosis (i.e. angiogenesis and inflammation) should not be underestimated ([Nico et al., 2008](#)).

The average time to diagnosis of endometriotic lesions has been shown to be 7-8 years ([Hadfield et al., 1996](#); [Arruda et al., 2003](#); [Husby et al., 2003](#); [Ballard et al., 2006](#); [Sinaii et al., 2008](#)) and was found to be higher in women presenting with pelvic pain than those presenting with infertility ([Dmowski et al., 1997](#); [Arruda et al., 2003](#)). To facilitate diagnosis and hasten the process, there is currently widespread interest in non-invasive diagnostic tools. Notably, a recent and interesting diagnostic approach involves detection of nerve fibers in endometrial biopsies of patients ([Al-Jefout et al., 2007, 2009](#); [Bokor et al., 2009](#)). Indeed, eutopic endometrium recovered from disease-free women in these studies did not appear to contain nerve fibers, but nerve fibers were detected in eutopic endometrium from endometriosis patients. Good diagnostic specificity and sensitivity were obtained in both studies. It was therefore hypothesized that nerve fiber detection in endometrial biopsies could be a valuable non-invasive diagnostic tool for endometriosis, even if a certain degree of caution should be exercised ([Evers and Van Steirteghem, 2009](#)). However, the presence of nerve fibers in the functional layer of endometrium still remains controversial, as some authors demonstrated that weak presence of nerve fibers in the functional endometrial layer of endometriosis patients is pain-related but not endometriosis-dependent ([Zhang et al., 2009](#)). Moreover, a recent study reported the absence of nerve fibers in the functional layer of endometrium in women with endometriosis and adenomyosis and adenomyosis alone

(Barcena de Arellano et al., 2012a). The authors suggested that innervation of this layer in patients with endometriosis should be re-evaluated (Barcena de Arellano et al., 2012a). As stated by Stratton and Berkley (Stratton and Berkley, 2011), finding nerves in the functional layer of the endometrium that is shed monthly is a provocative idea that needs replication by others.

Because of the controversy surrounding the occurrence of nerve fibers in the functional endometrial layer of patients with endometriosis, the objective of the present study was to confirm, by immunohistochemistry (IHC) and morphometry, the presence of nerve fibers and NGF in endometrium from both disease-free women and endometriosis patients, taking into account the different types of endometriosis.

The objective of the present study was to retrospectively investigate nerve fiber presence in different tissues (disease-free endometrium, eutopic endometrium from endometriosis patients, and endometriotic lesions).

VI.2. Materials and methods

VI.2.1. Human endometrial and endometriotic lesion sample collection

Biopsies of endometrium (n=20) were collected from healthy patients (mean age of patients: 39.4 ± 6.9 y). Sixteen were obtained by curette or endometrial resection and 4 were total uterine thickness samples from total hysterectomy. Biopsies of endometrium (n=26) were also collected from endometriosis patients (mean age of patients: 35.9 ± 7.8 y). Twenty were obtained by curette or endometrial resection and 6 were total uterine thickness samples from total hysterectomy. As combined oral contraceptives and progestogens are known to significantly decrease NFD in endometrium and myometrium of women with endometriosis

(Tokushige et al., 2009), endometrial biopsies of patients under hormonal treatment were excluded from the study.

Biopsies of endometriotic lesions (n=54) were obtained by laparoscopy. The lesions were divided into 3 groups: peritoneal lesions (n=11), ovarian lesions (n=16) and deep nodular endometriotic lesions (n=27).

VI.2.2. Immunohistochemistry

PGP9.5, NF (myelinated fibers) and NGF (neurotrophic marker) were used. Human intestinal tissue was used as a positive control for PGP9.5, NF and NGF expression. Negative controls were processed using non-specific IgG, or omitting the specific primary antibodies.

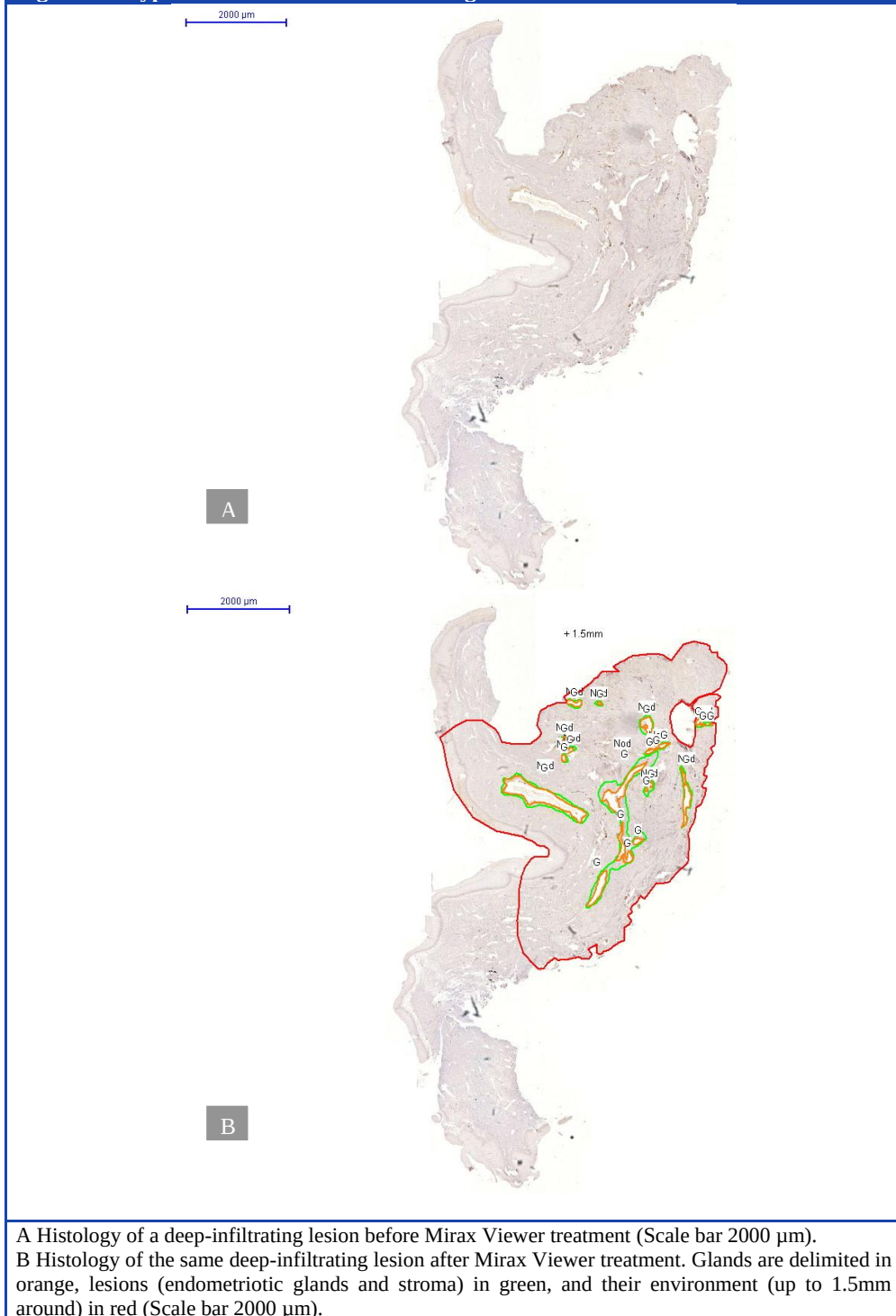
VI.2.3. Digital slide acquisition

Sections were scanned using Mirax, and surface areas of entire endometrial or endometriotic tissues (glands and stroma) and endometrial or endometriotic glands alone were outlined manually and measured with the Mirax Viewer (Figure 37).

VI.2.4. Morphometric analysis

Every fifth slide of each lesion was stained with hematoxylin-eosin (H&E) to identify the section with the largest surface area of endometriotic glands and stroma, and consecutive serial sections were immunostained with PGP9.5, NF and NGF. These sections were scanned as described above, and surface areas of entire endometrial or endometriotic tissues (glands and stroma) and endometrial or endometriotic glands alone were outlined manually and measured with the Mirax Viewer (Figure 47 A). Endometrial or endometriotic stromal surface area was then calculated by subtracting the surface area of glands from the surface area of endometrial or endometriotic tissues. The direct environment of lesions (up to 1.5mm around) was also manually delimited and measured using the Mirax Viewer (Figure 47 B).

Figure 47: Type III lesion and Mirax scanning

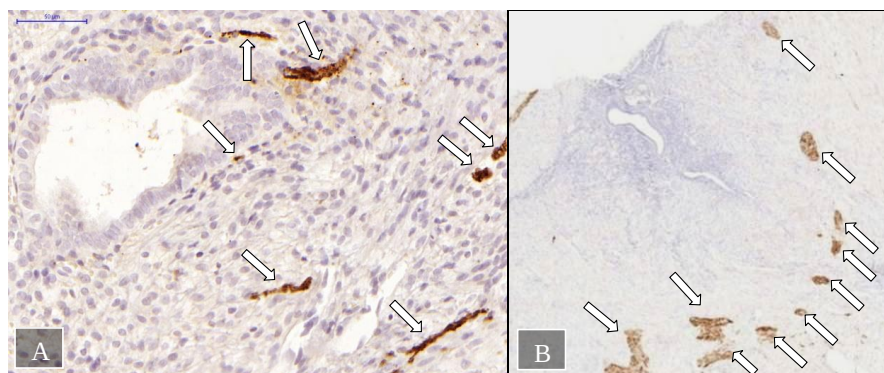


A Histology of a deep-infiltrating lesion before Mirax Viewer treatment (Scale bar 2000 µm).

B Histology of the same deep-infiltrating lesion after Mirax Viewer treatment. Glands are delimited in orange, lesions (endometriotic glands and stroma) in green, and their environment (up to 1.5mm around) in red (Scale bar 2000 µm).

PGP9.5 immunostaining clearly evidenced large and small nerve structures (**Figure 48 A**). NF protein was therefore immunostained in endometrial and rectovaginal biopsies (**Figure 48 B**) to confirm the presence of myelinated nerve fibers (Schlaepfer, 1987; Zhang et al., 2010; Fraser 2010).

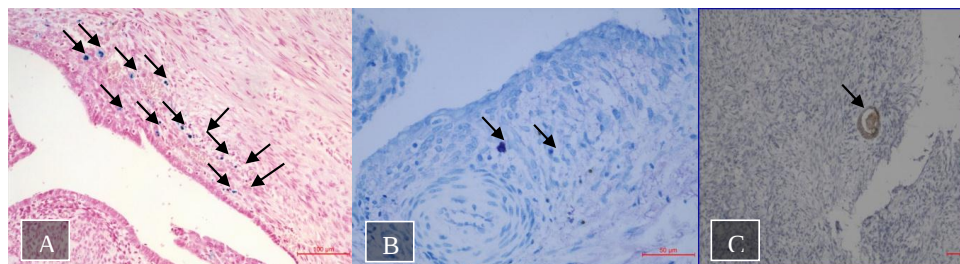
Figure 48: PGP9.5 and NF immunostaining



A. PGP9.5 immunostaining revealing nerve fibers (white arrows).
B. NF immunostaining revealing nerve fibers (white arrows).

Iron deposits, mast cells, and small ovarian follicles were all found to be PGP9.5 immunostained. Iron deposits could be confused with PGP9.5 immunostaining, and mast cells as well as small ovarian follicles were found to show PGP9.5 immunostaining. Perls' Prussian blue (**Figure 49 A**), toluidine blue (**Figure 49 B**) and inhibin α immunostaining (**Figure 49 C**) were respectively performed to differentiate them from nerve fibers.

Figure 49: Stains used to differentiate hemosiderin deposit and PGP9.5 labeling from non nervous structures.



A. Prussian blue (Perls); arrows showing iron deposits in blue.

B. Toluidine blue; arrows showing mast cells in purple.

C. Inhibin α ; arrow showing a small ovarian follicle.

NFD was calculated by manually counting positive nerve fibers on specific surface areas (entire stroma of the lesions and their direct environment) and expressed as median and interquartile range (per mm²).

NGF staining intensity was scored in glands and stroma (0: negative, 1: weak, 2: intermediate, 3: strong).

VI.3. Results

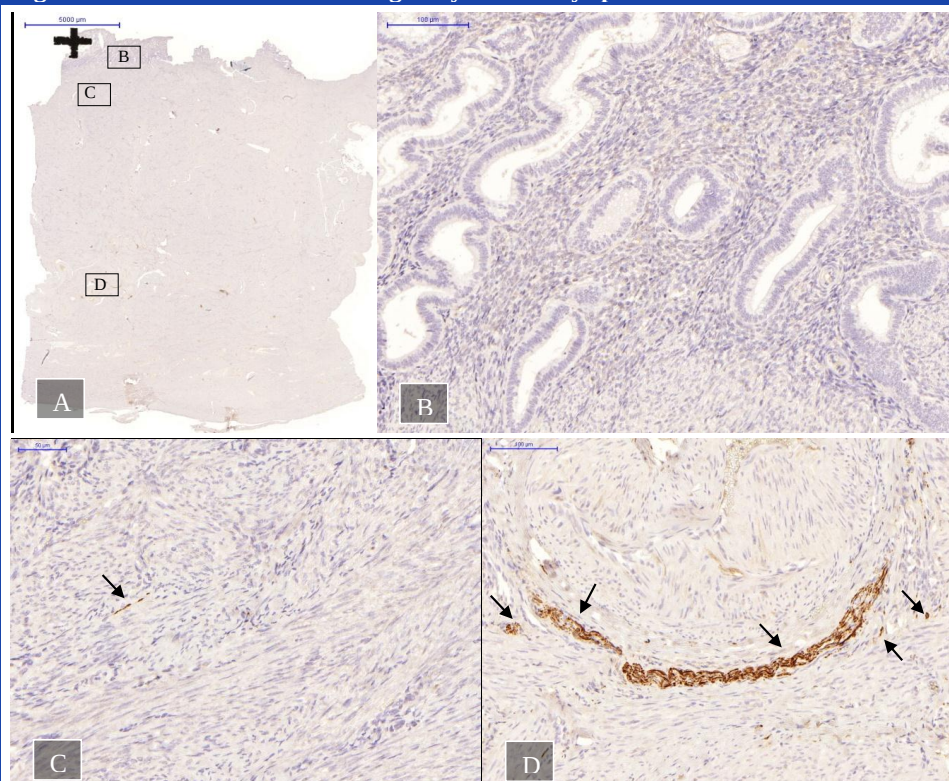
VI.3.1. Nerve fiber density in eutopic endometrium of women with and without endometriosis

Eutopic endometrium (disease-free and endometriotic)

In biopsies recovered by curette from both healthy and endometriosis patients, no nerve fibers were detected by PGP9.5. However, in biopsies recovered after hysterectomy (**Figure 50 A and B**), occasional nerve fibers were detected in the stroma of the basal layer of the endometrium (disease-free: 0.0 [0.0-0.0] PGP9.5-positive fibers/mm²; endometriotic: 0.0 [0.0-0.0] PGP9.5-positive fibers/mm²). In myometrium adjacent to the endometrium (up to 1.5mm), some nerve fibers were detected (disease-free: 1.18 [0.48-2.78] PGP9.5-positive

fibers/mm²; endometriotic: 1.02 [0.45-1.96] PGP9.5-positive fibers/mm²) (**Figure 50 C and D**).

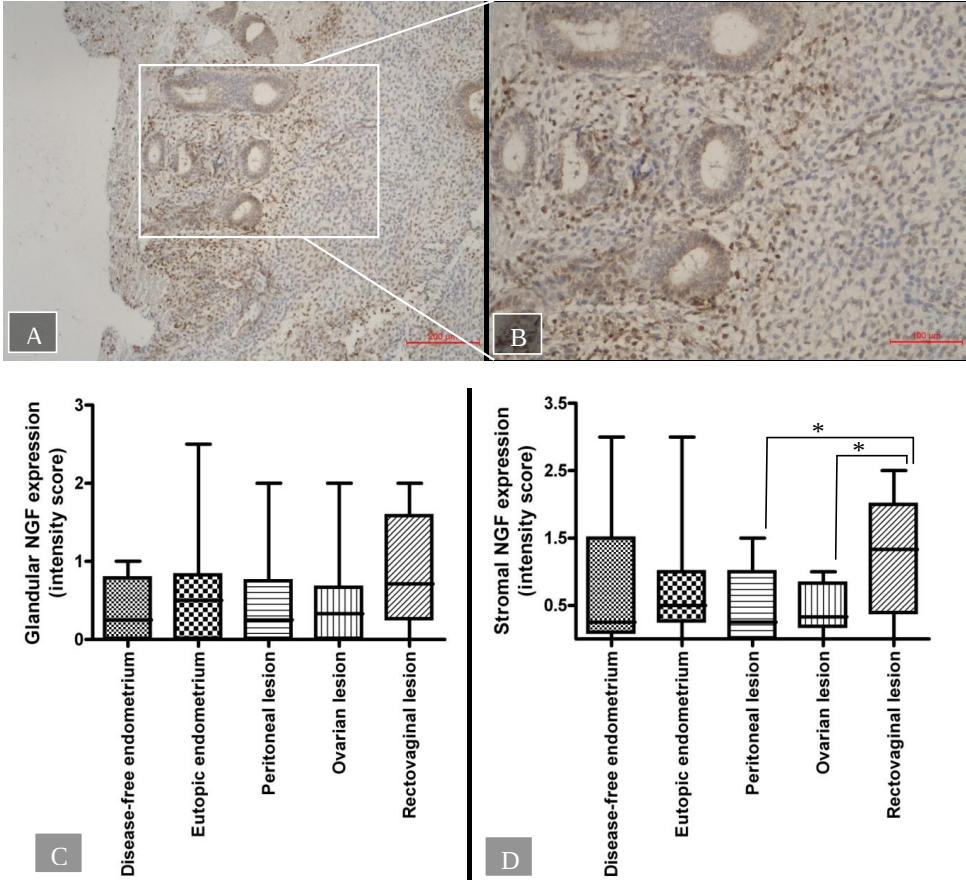
Figure 50: PGP9.5 immunostaining in hysterectomy specimens



Typical pictures of PGP9.5 immunolabeling in hysterectomy specimens recovered from endometriosis patients (A). Enlarged pictures (B to D) show nerve fiber staining (arrows showing PGP9.5-positive nerve fibers).

NGF was equivalently expressed in glandular and stromal compartments of eutopic endometrium from disease-free (glands: 0.25 [0.0-0.79]; stroma: 0.25 [0.08-1.5]) and endometriosis (glands: 0.50 [0.0-0.83]; stroma: 0.58 [0.25-1.0]) patients (**Figure 51 A-D**).

Figure 51: NGF immunolabeling in eutopic endometrium



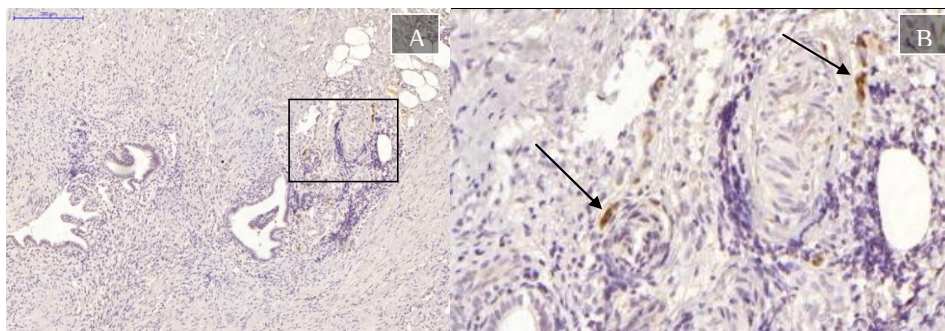
A & B. NGF immunostaining in eutopic endometrium from an endometriosis patient. C & D. NGF expression in endometrial and endometriotic glands (C) and endometrial and endometriotic stroma (D) are shown in graphs. The Kruskal-Wallis test followed by Dunn's post hoc test were used for statistical analysis (median and interquartile range). *: $p < 0.05$

VI.3.2. Nerve fibers in endometriotic lesions

VI.3.2.1. Peritoneal endometriosis

Nerve fibers were detected in 45.5% of peritoneal endometriotic lesions (0.0 [0.0-5.29] PGP9.5 immunostained fibers/mm²), and in the lesion vicinity (<1.5mm) was found to be positive for nerve fiber staining (0.62 [0.39-1.29] PGP9.5 immunostained fibers/mm²) **(Figure 52 A and B).**

Figure 52: PGP9.5 immunostaining in a peritoneal lesion



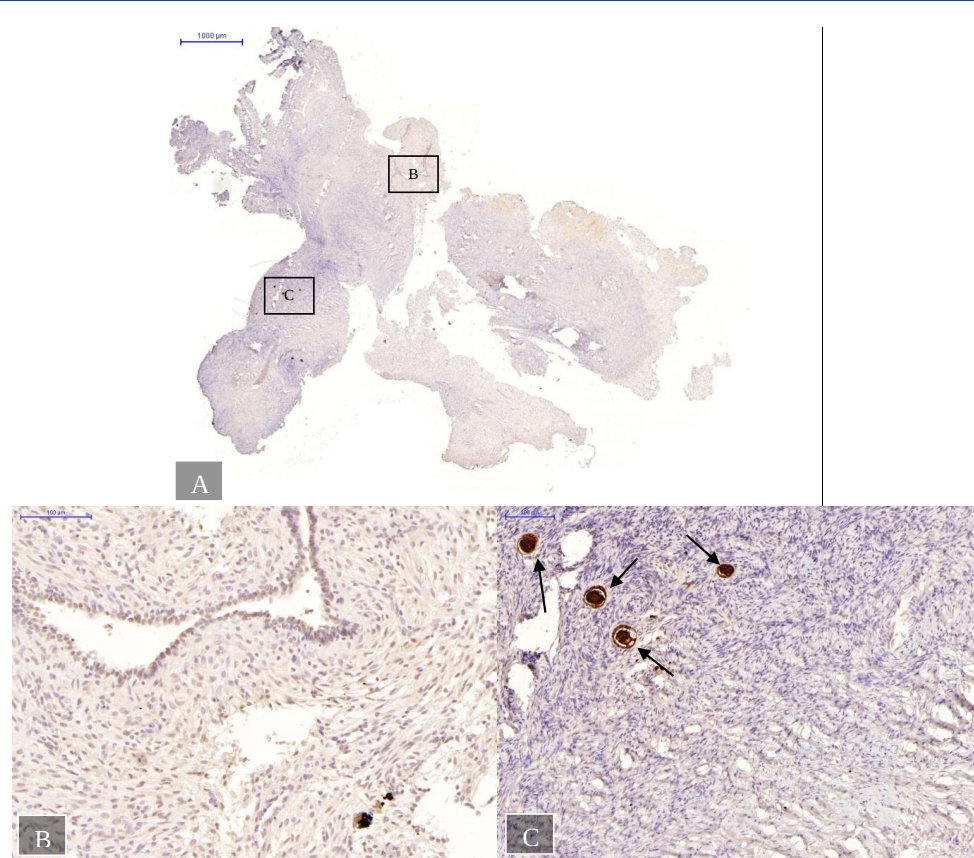
A. Typical picture of PGP9.5 immunolabeling in a peritoneal endometriotic lesion (scale bar 200µm). Enlarged picture (B) shows nerve fiber staining (arrows showing immunolabelling of PGP9.5 nerve fibers, black arrows).

NGF expression was similar to that in matched eutopic endometrium (glands: 0.25 [0.0-0.75]; stroma: 0.25 [0.0-1.0]) **(Figure 51 C and D).**

VI.3.2.2. Ovarian lesions

After discrimination of small follicles thanks to inhibin α staining, nerve fiber densities were determined and found to be very low in ovarian lesions (0.10 [0.0-0.59] PGP9.5 immunostained fibers/mm²), as well as in their direct (up to 1.5mm) vicinity (0.52 [0.14-0.77] PGP9.5 immunostained fibers/mm²) (**Figure 53 A-C**).

Figure 53: PGP9.5 immunostaining in an ovarian lesion



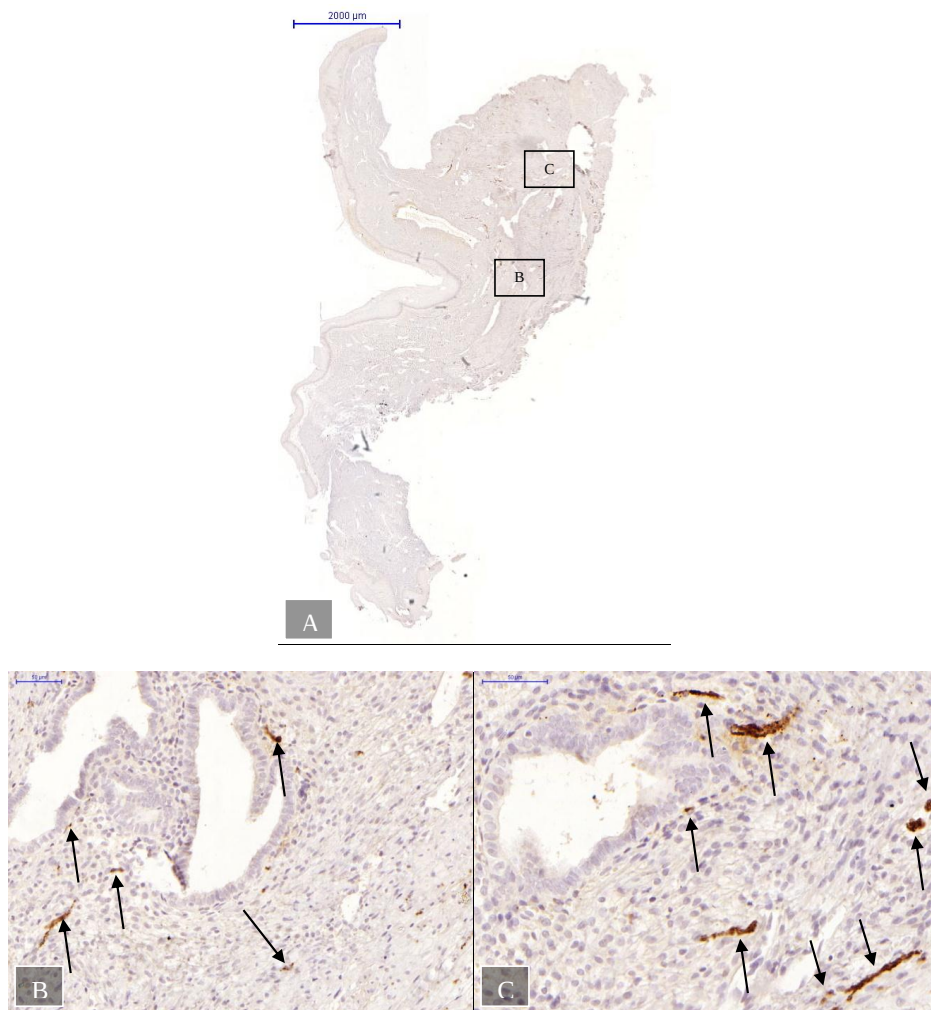
Typical picture of PGP9.5 immunolabeling in an ovarian endometriotic lesion (A). Enlarged pictures (B and C) show nerve fiber staining (arrows showing small follicles stained after PGP9.5 antibody incubation).

NGF expression levels were similar to those detected in eutopic endometrium and peritoneal lesions (glands: 0.33 [0.0-0.67]; stroma: 0.33 [0.17-0.83])) (**Figure 51 C and D**).

VI.3.2.3. Deep nodular endometriosis

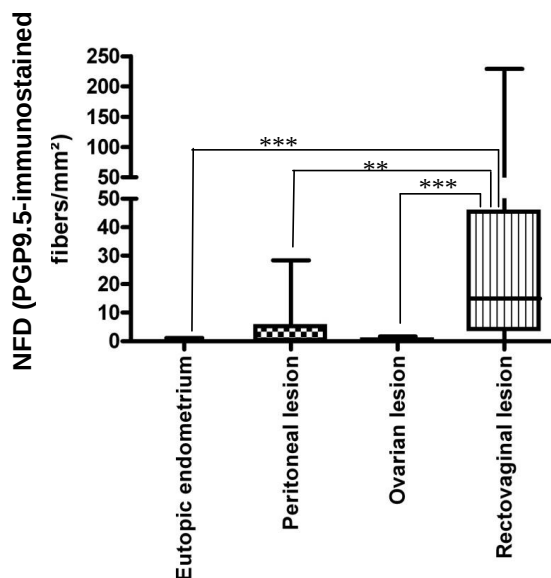
High nerve fiber densities were observed in deep nodular lesions (14.93 [3.76-45.45] PGP9.5 immunostained fibers/mm²), as well as their direct vicinity (up to 1.5mm) (5.16 [3.24-8.07] PGP9.5 immunostained fibers/mm²) (**Figure 54 A and C**).

Figure 54: PGP9.5 immunostaining in a rectovaginal nodule



Typical picture of PGP9.5 immunolabeling in a rectovaginal nodule (A). Enlarged pictures (B and C) show nerve fiber staining (arrows showing PGP9.5-immunostained nerve fibers).

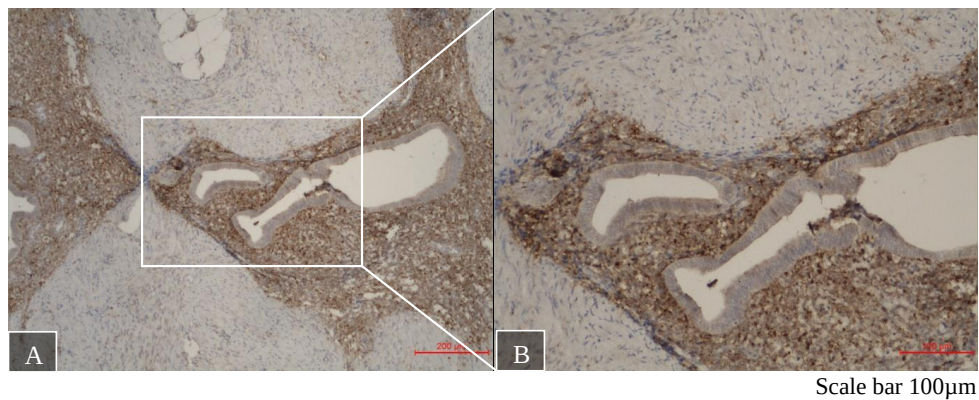
NFD was higher in deep nodular lesions than in peritoneal ($p < .01$) or ovarian ($p < .001$) lesions (Figure 55).

Figure 55: NFD in endometriotic samples

NFD in endometriotic lesions represented in graphs. The Mann-Whitney test was used for statistical analysis in endometrial samples, and the Kruskal-Wallis test followed by Dunn's post hoc test in endometriotic lesions.

** : $p < 0.01$; *** : $p < 0.001$

While glandular NGF expression levels were in the same range as in other lesion types (0.71 [0.25-1.58]), stromal expression levels were found to be higher in case of deep-infiltrating lesions (1.33 [0.37-2.0]) (**Figure 56 A and B**) when compared to peritoneal and ovarian lesions ($p < .05$) (**Figure 51**).

Figure 56: NGF immunostaining in a rectovaginal lesion

VI.3.3. Confirmation by neurofilament immunolabelling

No NF immunolabelling was detected in the functional layer of endometrial biopsies recovered by curette from both healthy and endometriosis patients, in accordance with PGP9.5 results (disease-free: 0.0 [0.0-0.0] NF immunostained fibers/mm²; endometriotic: 0.0 [0.0-0.0] NF immunostained fibers/mm²). High nerve fiber densities observed in deep nodular lesions were confirmed by NF detection (4.75 [1.92-15.83] NF immunostained fibers/mm²); 33.4% of nerves stained for PGP9.5 showed NF immunolabelling.

VI.4. Origin of endometriotic nerve fibers

If we consider Sampson's theory of retrograde menstruation ([Sampson, 1927](#)), the absence of nerve fibers in eutopic endometrium may call into question the origin of nerve fibers in endometriosis, at least in peritoneal lesions. It is evident that they cannot originate from endometrial tissue itself, but could originate from the lesion vicinity, as was recently shown in a heterologous murine model ([Novella-Maestre et al., 2012](#)). Nerve fibers were detected in lesions after 14 days of grafting, while transplanted human endometrial biopsies were free of nerves ([Novella-Maestre et al., 2012](#)). The authors therefore hypothesized that nerve fibers

were of murine origin and grew after establishing implants with appropriate vessel development.

In the present study, nerve fiber densities measured in the direct vicinity (up to 1.5mm) of eutopic endometrium and peritoneal and ovarian lesions were similarly low, while they were significantly higher in case of rectovaginal nodules.

We found that NGF was constitutively expressed in endometrium. As no nerve fibers were detected in this tissue, it could be hypothesized that NGF is involved in processes other than endometrial neurogeneration. Indeed, it was notably shown to play a role in wound healing ([Kawamoto and Matsuda, 2004](#)) and angiogenesis ([Nico et al., 2008](#)), two important events occurring during menstrual cycles. It is possible that endometrial NGF may activate proliferation and growth of nerve fibers when it becomes ectopic after retrograde menstruation ([Sampson, 1927](#)). We showed, like others before us ([Anaf et al., 2002](#); [Mechsner et al., 2007](#); [Tokushige et al., 2008](#); [Wang et al., 2009b](#); [Arnold et al., 2012](#)), that NGF is expressed in endometrium and endometriotic lesions, but at higher levels in lesions. Peritoneal fluid of women with endometriosis was also found to contain high levels of NGF ([Barcena de Arellano et al., 2011a and 2011b](#)), confirming that neurotrophin overexpression is not triggered by eutopic endometrium, but directly by endometriotic lesions and their vicinity. This growth factor could therefore be a potent stimulator of nerve fiber genesis in endometriotic lesions themselves, with higher levels of NGF expression in deep-infiltrating lesions inducing a greater density of nerve fibers. As hypothesized by Anaf ([Anaf et al., 2002](#)), NGF could act as a positive chemotaxin for neurons and may therefore facilitate their contact with target tissues through interaction with its receptors, explaining why deep nodular lesions essentially occur in richly innervated anatomical sites (such as the rectovaginal septum and uterosacral ligaments).

VI.5. What can we learn from the study of nerve fibers?

VI.5.1. Absence of nerve fibers in the functional layer of human endometrium

No or very few nerve fibers were immunostained in the functional layer samples, while the antibody used was the same as that described in the literature. The mean size of samples was $15.0 \pm 25.4 \text{ mm}^2$ and therefore in the range collected in a previous study ([Al-Jefout et al., 2007](#)). In samples recovered from hysterectomies (containing endometrial and myometrial areas), some occasional nerve fibers were detected in the basal layer of endometrium, but never in the functional layer, while myometrial tissue was always infiltrated by nerve fibers. This is at variance with publications by Al-Jefout and Bokor ([Al-Jefout et al., 2009](#); [Bokor et al., 2009](#)), but confirms the findings of another group, who also recently reported that eutopic endometrium from women with adenomyosis and endometriosis ($n=8$) does not show nerve fiber staining nor neurotrophic properties ([Barcena de Arellano et al., 2012 a and b](#)). Even if the number of specimens is small (8 cases) in the above-mentioned study, the results are wholly at odds with NFD as high as $11 \pm 5/\text{mm}^2$ and $18 \pm 8/\text{mm}^2$ (stained with PGP9.5) reported by Tokushige et al ([Tokushige et al., 2007](#)) in the functional and basal layer of endometrium respectively in women with endometriosis. Even though false-positive staining was strictly excluded and morphological measurements were adapted to nerve fiber analysis in the present study, nerve fibers could not be identified in the functional/basal layer of endometrium of 45 women with endometriosis. Zhang et al were able to prove very low NFD (between 0.6 and $1.5 \text{ fibers}/\text{mm}^2$) in the functional layer of endometrium in women with endometriosis with adenomyosis or with endometriosis alone, and showed PGP9.5-immunoreactive NFD to be statistically correlated with the severity of pain symptoms. The authors therefore suggest that the presence of nerve fibers in endometrium of women with endometriosis is dependent on a common symptom of pain, but not on the disease itself.

Our results clearly cast doubt on identification of nerve fibers in endometrium with PGP9.5, as no nerve fibers could be identified in the endometrium of 45 patients with endometriosis. Since false-positive staining was strictly excluded and morphological measurements were adapted to nerve fiber analysis, as previously stated, we believe that nerve fibers are absent or virtually absent in the functional layer of the endometrium.

VI.5.2. Nerve fiber density in endometriotic lesions

The different endometriosis types analyzed in the present study exhibited different PGP9.5 expression patterns. Indeed, patients suffering from ovarian endometriomas presented with the lowest levels of nerve fibers, and these patients are known to be those who experience the least pain ([Vercellini et al., 1997](#); [Fauconnier and Chapron, 2005](#)). On the contrary, patients presenting with deep-infiltrating lesions showed the highest levels of PGP9.5 staining, and are known to suffer most ([Fauconnier et al., 2002](#); [Fauconnier and Chapron, 2005](#)). Even if the objective of the present study was not to find a correlation between pain and the presence of nerve fibers, and knowing that PGP9.5 is not specific for nociceptors, our results nevertheless demonstrate that the more nerve fibers are present, the greater the pain ([McKinnon et al., 2012](#)). However, as pain sensation is a subjective experience, more extensive and specific studies must be conducted to confirm this hypothesis.

In the present study, nerve fiber density in lesions was a little lower than expected from past investigations ([Tokushige et al 2007](#); [Wang et al., 2009b](#)).

Indeed, most of these studies evaluated nerve fiber density in the same way as blood vessels, assessing their number in areas considered as “hot spots”. Since nerve fibers were not found to be homogeneously distributed in our study, as previously observed by Bokor in 2009

([Bokor et al., 2009](#)), the total surface area of endometriotic lesions and their direct vicinity was examined, which may explain the differences observed.

To characterize detected nerve fibers (myelinated or unmyelinated), we investigated the presence of NF protein. We found that in lesions, around 30% of PGP9.5 stained nerve fibers were NF stained, and hence were myelinated. Nerve fibers in lesions were therefore mostly unmyelinated, and possibly implicated in pain, as recently demonstrated by Arnold in peritoneal lesions ([Arnold et al., 2012](#)).

VI.6. Conclusions

The present findings suggest that there is no difference between eutopic endometrium from disease-free women and endometriosis patients in terms of NFD, since almost no nerve fibers were detected in either sample type. As NFD was confirmed to be elevated in endometriotic lesions (especially in deep-infiltrating disease), and most of these nerve fibers were found to be unmyelinated, they could well be implicated in pain, especially in deep nodules where the highest nerve density was observed. Moreover, deep nodular lesions may be more neuroattractive through the action of NGF.

VII. Nerve fiber density in deep nodular endometriotic lesions induced in a baboon experimental model

VII.1. Introduction

A better understanding of neurogenesis occurring during endometriosis development is important for the advancement and improvement of analgesic therapies for the disease. However, investigating the involvement of processes such as neurogenesis in the pathogenesis of endometriosis is impossible in women, since lesions have often already progressed dramatically by the time patients consult their gynecologist. This is why animal endometriosis models are needed, and have been mostly developed in species such as rodents and non-human primates (Colette and Donnez, 2012).

As human deep nodular lesions were found to have the highest NFD, the objective of the present study was to investigate NFD and NGF expression in lesions induced in the model of deep nodular endometriosis we developed in baboons.

VII.2. Results

VII.2.1. PGP9.5 and NGF immunostaining

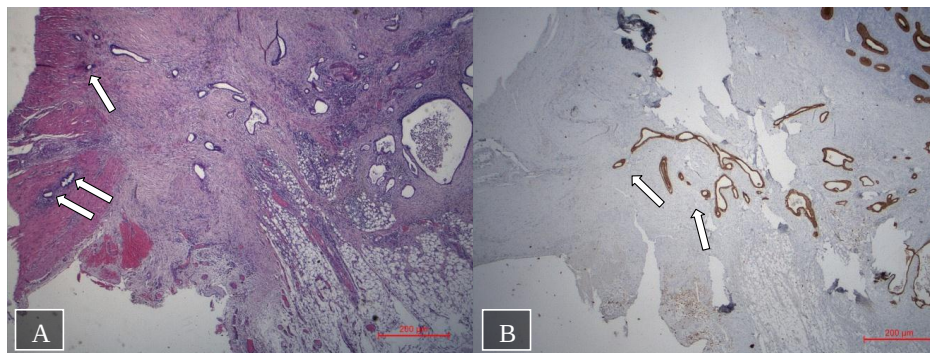
Human and baboon intestinal tissues were used as positive controls for PGP9.5 and NGF immunostaining. Negative controls were processed using non-specific IgG, or omitting the specific primary antibodies. Every fifth slide of each lesion was stained with H&E to identify the section with the largest surface area of endometriotic glands and stroma, and subsequent serial sections, as well as sections of eutopic endometrium, were immunostained with PGP9.5 and NGF. Glandular and total lesion (glands and stroma) and endometrial surface areas, as well as their direct vicinity (up to 1.5mm around), were delimited and measured using the Mirax Viewer (Figures 37 & 38). NFD was then manually determined in stromal areas (total

surface area minus glandular surface area) and in the direct environment of lesions, as well as in myometrium, after careful exclusion of false-positive staining (Perls' Prussian blue and toluidine blue staining for ferric iron deposits and mast cells respectively) (**Figure 49**). NGF staining intensity was scored in glands and in stroma (0: negative, 1: weak, 2: intermediate, 3: strong).

VII.2.2. Lesion invasiveness

As already discussed ([Donnez et al., 2012](#)), 42.1% of induced nodules (n=8/19) were found to invade surrounding organs after grafting pieces of total uterus thickness, as demonstrated by the presence of glands and stroma in organs other than grafting sites (**Figure 57 A and B**). This infiltration was traced along serial sections.

Figure 57: Microscopic appearance of an invasive lesion



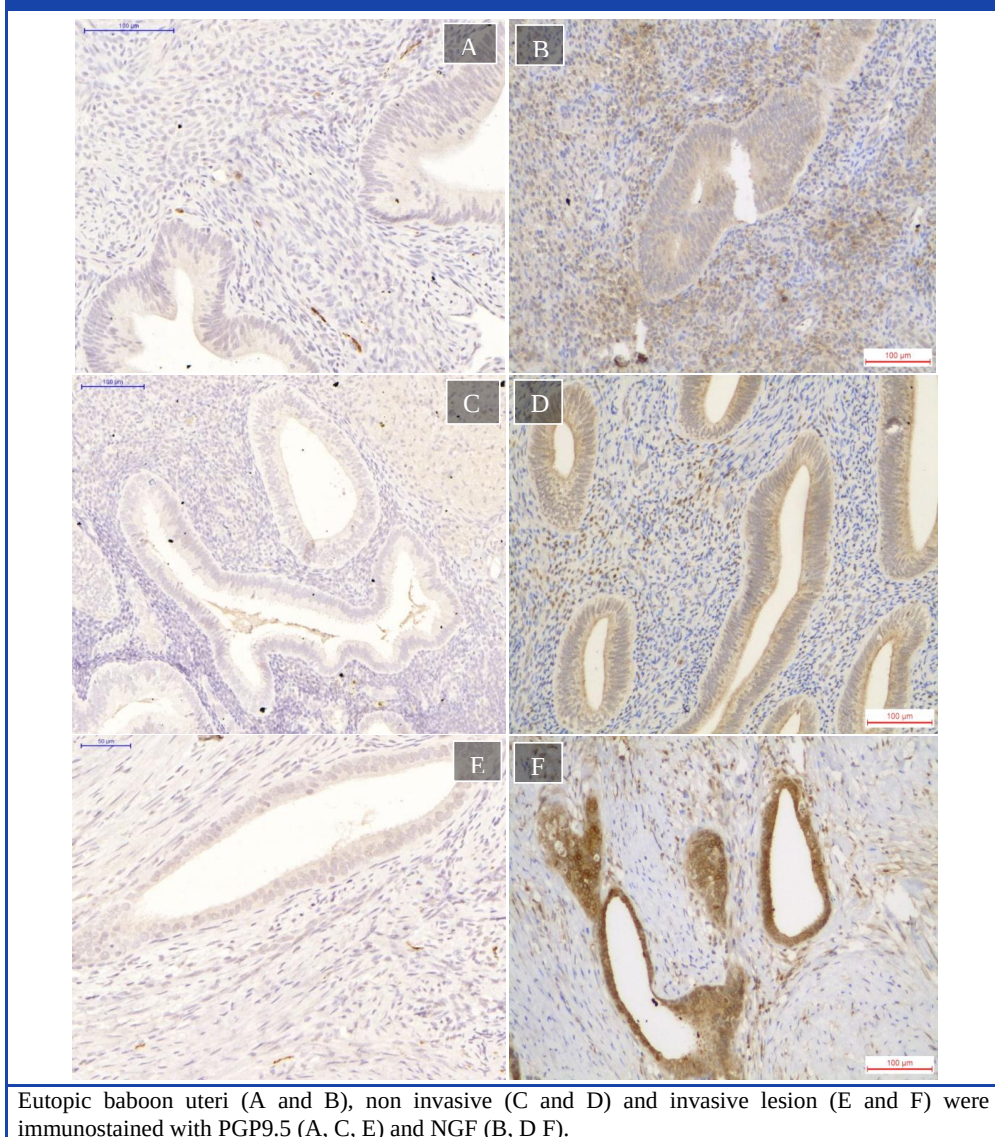
A. Invasive lesions were characterized by the presence of endometriotic glands and stroma inside surrounding organs. Endometriotic glands (white arrows) are visible through the sigmoid muscularis layer.

B. CK22 immunostaining (white arrows) confirming the presence of endometriotic glands.

VII.2.3. Nerve fiber density in deep endometriotic nodules and baboon uteri

PGP9.5 immunostaining revealed the presence of nerve fibers in induced nodules, in eutopic baboon endometrium (mainly in the basal layer), as well as in eutopic myometrium (**Figure 58 A-F**).

Figure 58: PGP9.5 and NGF immunostaining in baboon uteri and endometriotic lesions

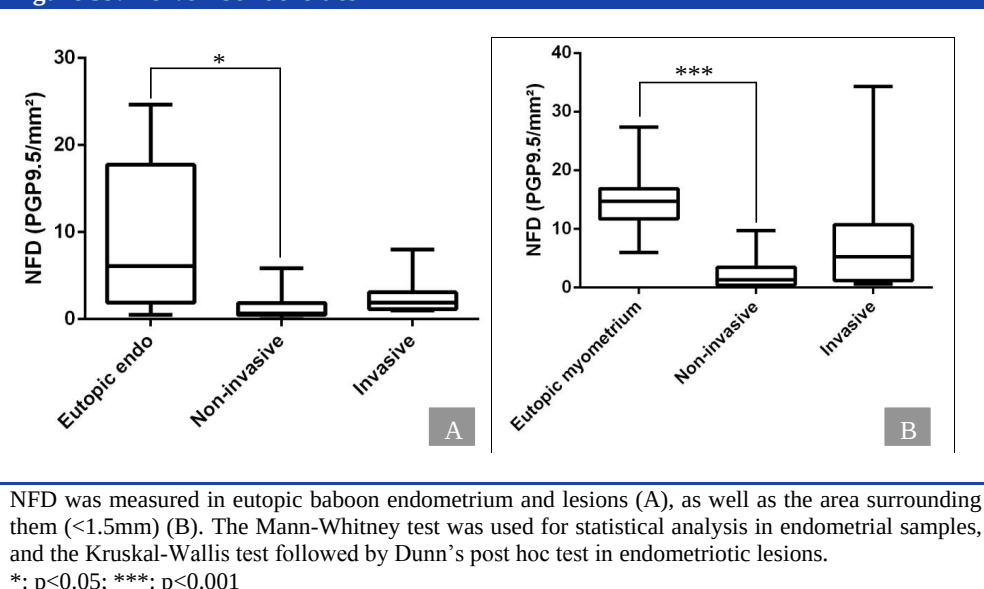


Perls' Prussian blue and toluidine blue staining were performed to distinguish iron deposits and mast cells respectively from aspecific immunostaining.

VII.2.4. Deep endometriotic nodules and eutopic baboon endometrium

Compared to eutopic endometrium (6.1 [1.9-17.7] PGP9.5-immunostained nerve fibers/mm²), deep non-invasive endometriotic lesions showed a significantly lower NFD (0.6 [0.5-1.9] PGP9.5-immunostained nerve fibers/mm², p-value=0.0152), while no significant difference was observed with deep invasive lesions (1.9 [1.2-3.1] PGP9.5-immunostained nerve fibers/mm², p value=0.6331) (**Figure 59 A and B**). However, the difference in NFD between deep invasive and non-invasive lesions was not significant (p-value=0.1074).

Figure 59: Nerve fiber densities



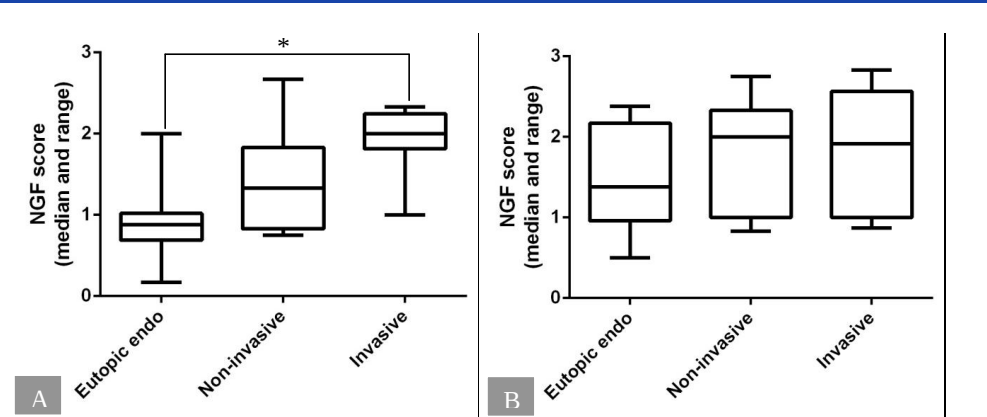
VII.2.5. Lesion vicinity and eutopic baboon myometrium

Similarly, compared to eutopic baboon myometrium (14.7 [11.7-16.8] PGP9.5-immunostained nerve fibers/mm²), a significantly lower NFD was observed in the direct

surroundings of non-invasive lesions (1.3 [0.4-3.5] PGP9.5-immunostained nerve fibers/mm², p-value=0.0004), while no significant difference was noted with the direct surroundings of invasive lesions (5.3 [1.2-10.7] PGP9.5-immunostained nerve fibers/mm², p-value=0.1123) **(Figure 59 B)**. Again, the difference between deep invasive and non-invasive lesions was not significant (p-value=0.1074).

VII.2.6. NGF expression in deep endometriotic nodules and eutopic baboon endometrium

NGF immunolabeling was observed in glands as well as stroma **(Figure 60 A and B)**, with no significant difference between deep invasive and non-invasive lesions (p-value in glands=0.0828 and in stroma>0.9999). However, compared to glands of eutopic baboon endometrium (0.9 [0.7-1.0]), the NGF staining intensity score was found to be higher in glands of deep invasive lesions (2.0 [1.8-2.2], p-value=0.0058), while no significant difference was observed with deep non-invasive lesions (1.3 [0.8-1.8], p-value=0.3305) **(Figure 60 A)**. Similar scores were obtained in stroma (deep invasive lesions: 1.9 [1.0-2.6]; deep non-invasive lesions 2.0 [1.0- 2.3]; eutopic endometrium: 1.4 [1.0-2.2]; p-value>0.9999 for both invasive and non-invasive lesions) **(Figure 60B)**.

Figure 60: NGF expression analysis

NGF expression was scored for baboon glands (A) and stroma (B). The Mann-Whitney test was used for statistical analysis in endometrial samples, and the Kruskal-Wallis test followed by Dunn's post hoc test in endometriotic lesions.

*: $p < 0.05$

VII.3. Discussion

In the present study, we showed that NFD was significantly lower in deep nodular non-invasive lesions 5-6 months after the grafting procedure in baboons (compared to eutopic endometrium), and that NGF expression was higher in glands of invasive nodules. There are only two reports on animal models evidencing the presence and growth of nerve fibers in endometriotic lesions, but both studies focus on peritoneal endometriosis. One was performed in an autologous rat model (Berkley et al., 2004) and the other more recently in a heterologous mouse model (Novella-Maestre et al., 2012). To the best of our knowledge, this is the first study to investigate, in a baboon model, the presence of nerve fibers in deep endometriotic nodules. More than 40% of nodules were found to be invasive after grafting specimens containing the JZ (Donnez O. et al., 2012), the invasion process characterized by the presence of stroma and glandular epithelium inside surrounding organs (Nisolle and Donnez, 1997). Although deep nodular endometriosis was almost always reproduced both macroscopically and microscopically, nerve fiber densities observed were low (<2

fibers/mm²) compared to those observed in nodules recovered from patients (around 15 fibers/mm², [preliminary unpublished data](#)). Several factors could contribute to these results.

First, 6 months of grafting is possibly not long enough to detect the growth of nerve fibers in nodules. This hypothesis is supported by a recent report on peritoneal lesion innervation in non-human primates, in which the authors showed that there were more nerve fibers in long-term grafts (recovered after 15 months), than in short-term grafts (recovered after 3 months) ([Manconi et al., 2011](#)). Nerve regeneration after injury or transplantation appears to be a slow process. After nerve injury, the rate of peripheral nerve regeneration is generally estimated to be 1mm per day ([Pan et al., 2003](#); [Gutmann et al., 1942](#)). Following orthotopic organ transplantation, nervous connections of the organ are disrupted and reinnervation takes at least one year after heart transplantation ([Yap et al., 2006](#)) and up to 9 months after kidney transplantation ([Grisk et al., 2001](#)), although it was completed in 4 months after hepatic transplantation in a rat model ([Takahashi et al., 2001](#)). Similarly, in grafting procedures involving skin tissue (containing numerous sensory nerve fibers), even with the help of tissue-engineered skin ([Siemionow et al., 2010](#)), the reinnervation process after grafting in rodents was found to be only partial 60 days after transplantation ([Biedermann et al., 2013](#); [Gingras et al., 2003](#)), but more developed 120 days after grafting ([Gingras et al., 2003](#); [Caissie et al., 2010](#)). Without tissue-engineered material, it is likely that the innervation process may take longer than the 6-month period investigated in the present study. Our hypothesis of delayed innervation is further supported by the higher NGF expression in glands of invasive lesions than in eutopic endometrium of baboons. This increased expression, together with the low nerve fiber density observed in lesions, could mean that lesions are still recruiting nerve fibers. Second, one cannot exclude the possibility that innervation processes may differ between baboon and human deep endometriotic lesions. This may be explained by the

difference in pathogenesis, i.e. spontaneous occurrence of endometriosis in women and induction of endometriosis in baboons. It will therefore be necessary to evaluate nerve fiber densities in deep nodules at later time points to fully validate the model for neurogenesis studies. Finally, another important factor that may influence reinnervation processes is the site of transplantation, as demonstrated for pancreatic islets (Korsgren et al., 1993). According to Anaf et al. (2002), deep nodular lesions in patients essentially occur in richly innervated anatomical sites and not in sites with scanty underlying nerves. They observed strong expression of NGF-specific receptor in all nerves surrounded and/or invaded by endometriosis or close to endometriotic lesions (Anaf et al., 2002). NGF expressed by nodular lesions (Anaf et al., 2002; Wang et al., 2009b) may thus act as a positive chemotactic agent for neurons lining endometriotic lesions. This could also explain why lower nerve fiber density is observed in lesions induced in the less innervated iliac fossa. It would be interesting to compare different nodular lesion development sites using the current baboon model to further investigate the influence of the grafting bed. In the current study, NGF immunostaining was detected in both stroma and glands of nodular lesions induced in baboons, consistent with the glandular and stromal expression observed by Wang et al. (2009b) in deep nodular lesions in women.

Besides its function as a neurotrophic factor, NGF may also act as a chemoattractant for mast cells in deep nodular lesions and activate their degranulation, as reported by Anaf et al. (Anaf et al., 2006), thereby contributing to the hyperalgesia experienced by patients with deep-infiltrating lesions. NGF expression was found to be higher in glands of invasive lesions than in eutopic endometrium of baboons. This increased expression, together with the low NFD observed in lesions, could mean that lesions are still recruiting nerve fibers.

It is important to note that NGF is also known to potentially play an important role in processes such as wound healing, inflammation and angiogenesis (Kawamoto et al., 2004; Nico et al., 2008), three important events occurring during endometriosis development. Increased NGF expression may therefore also be related to its role in these processes. Moreover, NGF is not the only neurotrophic factor found in endometrium or endometriosis, and it would be of interest to study, in further experiments, molecules such as brain-derived neurotrophic factor, and neurotrophin-3 and -4 (Browne et al., 2012).

VII.4. Conclusion

To conclude, deep nodular endometriotic lesions induced in the baboon were found to closely mimic spontaneous deep-infiltrating endometriotic nodules in terms of macroscopic and microscopic features. However, since we demonstrated lower NFD in deep non-invasive lesions induced after 6 months of grafting, further long-term studies are now warranted to determine whether nerves continue to grow in deep invasive and non-invasive lesions after this time period. It will also be important to differentiate the involvement of NGF in lesion neurogenesis from other processes of early endometriosis development, such as angiogenesis and inflammation, as well as the possible role of the lesion surroundings.

VIII. Conclusions and perspectives

VIII.1. General discussion

VIII.1.1. Lessons learned from clinical experience with the shaving technique

The term deep-infiltrating endometriosis was coined by Koninckx and Martin and first published in 1995 ([Koninckx and Martin, 1995](#)). However, the concept of deep-infiltrating endometriosis is no longer valid as an explanation for deep nodular rectovaginal lesions ([Koninckx et al., 2012](#), [Donnez et al. 2010, 2012, 2013](#)).

In our group, we have always supported the hypothesis that these lesions are retroperitoneal, located in the rectovaginal septum, and may result in some cases (<10%) from metaplasia of Müllerian rests and in 90% of cases from an adenomyotic process originating from the cervix ([Donnez et al., 2011, 2013](#); [Donnez and Squifflet, 2010](#)). The origin of these lesions is thus the posterior part of the cervix, where the vagina is attached ([Donnez et al, 2011; 2012; 2013](#)).

The concept of retrocervical disease appears to have already been suggested by Cullen and Sampson. The aspect of this form of the disease is different from vaginal adenosis, which lacks endometrial stroma and the characteristic inflammatory response of endometriosis ([Zaloudek and Norris, 1987](#)).

This form of the disease is characterized by smooth muscle hyperplasia, which may be explained by endometriotic foci invading smooth muscle, typically associated with striking

proliferation in these areas. This creates an adenomyomatous appearance similar to that of adenomyosis in the endometrium (Scully et al., 1966), and the endometriotic stroma exhibits smooth muscle metaplasia, as has been demonstrated within the wall of ovarian endometriotic cysts (Scully, 1968).

The absence of response to progesterone levels suggests that different regulatory mechanisms of endometriotic steroid receptors expression result in deficient endocrine dependency or that the receptors are present but biologically inactive. This progesterone resistance could also explain why selective progesterone receptor modulators (SPRMs) effective for myomas (Donnez et al., 2012 a and b) are ineffective for deep nodules.

VIII.1.1.1. Concerning the surgical technique

We found that the shaving approach is the technique of choice (Donnez et al., 2013). In most cases, muscularis infiltration observed in all cases of type III lesions may be left in place, at least partially, since the shaving technique already removes what is necessary. Residual lesions in the muscularis of the rectum do not evolve and remain constant for a long time (Donnez et al., 2001, 2007, 2010, 2013).

Radical bowel surgery is associated with long-term morbidity and, as previously reported in a Cochrane Review (Pachler and Wille-Jørgensen, 2005), quality of life is significantly impaired following anterior resection.

Exactly when discoid excision or bowel resection should be carried out remains debatable.

From our clinical study, we learned that conservative surgery yields:

- a. Better functional outcomes in terms of bladder, rectal, and sexual function.
- b. High pregnancy rates.
- c. Low recurrence rates, similar to those observed after rectal resection.

It should also be pointed out that colorectal segmental resection is a complex procedure, sometimes resulting in pelvic nerve damage and unpleasant urinary and digestive symptoms. Indeed, urinary retention is quite frequent (3-10%) after bowel resection, as this type of surgery often cannot preserve the pelvic autonomic nerves, which are the pathway for neurogenic control of bladder function. Interestingly, the shaving technique provokes a very low rate of urinary retention because the dissection usually respects, at least on one side, the sympathetic nerves passing laterally to the rectum, which are frequently cut during extensive rectal or rectosigmoidal resection.

VIII.1.1.2. Radical or not radical?

In conclusion, we found a total lack of evidence of better improvement in case of bowel resection. First of all, it is close to impossible to remove all endometrial/endometriotic cells from all sites ([Donnez et al., 2012, 2013](#)). Second, deep endometriosis is surrounded by a fibrotic layer ([Donnez et al., 1997, 2010, 2012, 2013](#)), which may be left behind. Third, recurrence rates of deep endometriosis requiring surgery are so low that it would be virtually impossible to demonstrate the need for complete versus near complete excision.

In the absence of evidence, it is unsurprising that many surgeons have become less aggressive than 10 years ago.

VIII.1.2. Do we have arguments to support the role of the JZ in deep nodular endometriosis?

VIII.1.2.1. Iatrogenic adenomyosis in humans

The JZ has already been implicated in some pathological conditions, particularly in adenomyosis. Indeed, Leyendecker et al. suggested that abnormal functioning of the human JZ could represent a common pathogenic factor for endometriosis and adenomyosis development ([Leyendecker et al. 1998](#)). A possible relationship between adenomyosis and deep nodular endometriosis could well be due to this infiltrating growth pattern. They found that women with severe endometriosis showed deeper wall invasion by adenomyosis, but the presence of deep-infiltrating rectovaginal endometriosis (nodular endometriosis) and the size of infiltration were not correlated with the adenomyosis or its depth of infiltration ([Larsen et al., 2011](#)). This was also reflected in one of our recent studies that included 3,298 cases operated on by the shaving technique ([Donnez et al., 2013](#)). Leyendecker suggested that adenomyosis and endometriosis may result from the same physiological mechanism of TIAR involving local estrogen production in an estrogen-sensitive environment normally controlled by the ovary ([Leyendecker et al., 2009](#)). Trauma followed by tissue hyper reactive inflammatory response and repair involving specific, albeit physiological cellular, biochemical, and molecular mechanisms may be considered the major events in the development of the disease ([Leyendecker et al., 1998](#)).

As we observed that retained human uterine fragments containing both endometrium and myometrium are able to survive and grow in the peritoneal cavity, we compared such iatrogenic adenomyotic lesions with deep nodular endometriosis. We found close similarities in terms of symptoms, localization, and radiological as well as histological findings.

We identified the subendometrial myometrium (JZ) as potentially involved in the pathogenesis of iatrogenic lesions in humans. Indeed, if the JZ is present, iatrogenic lesions are able to develop tumors characterized by dense tissue composed of smooth muscle hyperplasia with isolated foci of endometrial mucosa resembling “adenomyotic” tumors, for which surgery is almost the same as for endometriotic (adenomyotic) type III nodules. These observational studies led us to develop a pilot study and experimental model for the investigation of deep nodular endometriosis.

VIII.1.2.2. Baboon model

Using grafted specimens containing endometrium and superficial/deep layers of myometrium in a baboon model, we were able to induce nodular endometriotic lesions within six months. Our experimental animal model macroscopically and microscopically reproduced nodular endometriotic lesions similar to those observed in women presenting with type III nodular endometriotic lesions. Induced nodular endometriotic lesions were found to be significantly larger with greater glandular density after grafting tissue specimens containing the JZ. Even when total uterine thickness was grafted, induced lesions were not significantly different from those developing after grafting endometrium together with the JZ. However, when endometrium and myometrium were grafted separately, these tissues were not able to induce nodular endometriotic lesions. These data clearly underline the importance of the endometrium-myometrium interface (JZ).

VIII.1.3. Do we have arguments to explain the invasive behavior of deep nodular endometriosis?

In the baboon model, more than 40% of surrounding organs were invaded if lesions were induced by grafting specimens containing the JZ. Conversely, no invasion was observed in

lesions induced after grafting endometrium or myometrium alone. This highlights the aggressive behavior of tissue containing the JZ, leading to a local invasion phenomenon similar to that observed in type III nodular endometriotic lesions.

Like in cancer, multicellular coordination seems to be mandatory for extension of the disease. When the cohesive part of the nodule (training edge) is separated from the invasion front (leading edge), the remaining endometriotic tissue left in place (after the shaving technique in humans) is not able to develop anymore.

Clinical results (when some tissue is left behind in conservative surgery) support the theory of existence of two different parts of the nodule: the center of the lesion (cohesive part) and the invasion front (invasive part).

VIII.1.4. Are nerves implicated in the pathogenesis (pain)?

We demonstrated that nerve fiber densities measured in the direct vicinity (up to 1.5mm) of lesions were high in case of rectovaginal nodules, but low in peritoneal and ovarian endometriosis.

Patients suffering from ovarian endometriomas presented with the lowest levels of nerve fibers, and these patients are known to be those who experience the least pain. On the contrary, patients presenting with deep-infiltrating lesions showed the highest levels of PGP9.5 staining, and are known to suffer the most pain. Around 30% of PGP9.5-immunostained nerve fibers in lesions were NF-immunostained, and hence myelinated, so the majority were unmyelinated and possibly implicated in pain.

VIII.1.5. Are nerves implicated in the invasion process?

VIII.1.5.1. Do nerves follow endometriotic invasion?

We found that NGF was constitutively expressed in endometrium. As no nerve fibers were detected in this tissue, it could be hypothesized that NGF is involved in processes other than endometrial neurogeneration.

It is possible that endometrial NGF may activate proliferation and growth of nerve fibers in ectopic endometriotic tissue. We showed that NGF is expressed in endometrium and endometriotic lesions, but at higher levels in lesions. This growth factor could therefore be a potent stimulator of nerve fiber genesis in endometriotic lesions themselves, with higher levels of NGF expression in deep-infiltrating lesions inducing a greater density of nerve fibers.

VIII.1.5.2. Does endometriotic invasion follow the nerve pathway?

As hypothesized by Anaf ([Anaf et al., 2002](#)), NGF could act as a positive chemotaxin for neurons and may therefore facilitate their contact with target tissues through interaction with its receptors, explaining why deep nodular lesions essentially occur in richly innervated anatomical sites (such as the rectovaginal septum and uterosacral ligaments).

Nevertheless, it remains to be elucidated whether nerve fibers present in lesions are actually triggered by the lesions, or are simply there because lesions develop in areas already rich in nerves. A combination of the two phenomena (high expression levels of neurotrophic factors like NGF in lesions and presence of numerous nerve fibers in surrounding tissues) should also be considered. According to Anaf ([2002](#)), a strong argument is, in case of bowel resection, the association of endometriotic lesions and nerves in the area of bowel section margins (>3cm from palpated nodular lesions).

In the baboon model, we demonstrated that NFD was low (<2 fibers/mm²) in deep non-invasive nodular lesions 5-6 months after the grafting procedure, and that NGF expression was high in glands of invasive nodules.

Nerve fiber densities observed in the baboon model were different from those observed in type III nodules recovered from patients (around 15 fibers/mm²). Several factors could contribute to these results.

First, a period of 6 months post-grafting is possibly not long enough to detect growth of nerve fibers in nodules. Secondly, one cannot exclude the possibility that innervation processes may differ between baboons and humans in deep endometriotic lesions.

In the experimental model, NGF expression was found to be higher in glands of invasive lesions than in eutopic endometrium. This increased expression, together with low NFD observed in lesions, could mean that lesions are still recruiting nerve fibers, but it could also mean that the grafting site is involved in the development of induced lesions. It may explain the high prevalence of type III nodules in humans, in areas like the retrocervix close to the uterosacral ligaments and the hypogastric plexus.

VIII.1.5.3. Neurogenesis and angiogenesis: what comes first?

In addition, inflammatory and angiogenic ([Mechsner et al., 2007](#); [Asante and Taylor, 2011](#)) processes may be implicated in endometriotic neurogenesis and their role should not be underestimated. As the pathophysiological mechanisms involved in pain in endometriosis remain unclear, it is mandatory to explore new concepts such as neuroangiogenesis. Some authors have proposed that nascent nerve fibers sprouting within ectopic endometriotic

implants may influence the activity of dorsal root neurons within the central nervous system and modify pain perception. As women with endometriosis are known to exhibit monocyte recruitment inside the peritoneal cavity through local production of chemokines ([Khorram et al., 1993](#)), these monocytes could then be prompted to undergo activation by cytokine stimulation. During the activation process, macrophages secrete a cascade of cytokines exacerbating local inflammation ([Lousse et al., 2009](#)). Subsequently activated cells are able to produce growth factors and PGs that may be involved in the pathogenesis of endometriosis. PGs have intrinsic vasoactive and nociceptive properties ([Riley et al., 1999](#)).

Since deep nodular endometriosis is associated with an increase in microvascular density, highly correlated with angiogenic growth factor expression ([Machado et al., 2008](#)), and has been shown to have the highest NFD ([Anaf et al., 2006; 2011; present work](#)), an association with angiogenesis and neurogenesis may be hypothesized. As reported by Weinstein ([2005](#)), profound parallels exist between guidance and patterning in the nervous system and vascular system. He found strong similarities between the two systems in the vertebrate. The interface between these two systems in endometriosis must be investigated using a kinetic animal model for early lesions as well as established lesions. This has also been corroborated by Novella-Maestre ([2012](#)), who demonstrated that administration of an antiangiogenic treatment using a heterologous mouse model of endometriosis was associated with both nerve fiber reduction and a decrease in new blood vessel formation. They observed development of nerve fibers in induced lesions within 14 days of grafting. However, we were not able to identify nerve fibers in a murine endometriosis model used for the study of angiogenesis. No nerve fibers were detected using PGP9.5 in lesions recovered after 8, 14, 21 and 28 days ([personal unpublished data](#)).

This brings to the fore front the question of development of neuroangiogenesis in endometriotic lesions. Neurogenesis and angiogenesis appear to be closely connected and a future challenge will involve investigation of this association.

IV.2. Personal contribution and perspectives

1. In the first part, we reported the largest clinical series of surgical removal of type III deep endometriotic nodules. Our data confirm that a conservative surgical approach offers good results in terms of quality of life, recurrence and pregnancy. When compared to the existing literature, the shaving technique has a lower complication rate than more radical surgery. The shaving technique should be offered as a first-line surgical approach in case of type III nodules. Bowel resection should be reserved for cases with complete stenosis, whose prevalence is relatively low (less than 2%).

[\(Donnez J, Jadoul P, Colette S, Luyckx M, Squifflet J, Donnez O. Deep rectovaginal endometriotic nodules: perioperative complications from a series of 3,298 patients operated on by the shaving technique. Gynecol Surg 2013; 10; 31-40.\)](#)

2. In the second part, we described iatrogenic adenomyotic lesions. Radiological, laparoscopic, and histological findings in iatrogenic nodules were similar to those in type III nodular endometriosis. Indeed, iatrogenic lesions were found to resemble adenomyomas, circumscribed nodular aggregates of smooth muscle, endometrial glands and stroma. From analysis of these lesions, the role of the JZ was highlighted. Indeed, we proved that a fragment of tissue containing both endometrium and subendometrial myometrium (the so-called JZ) was able to induce adenomyotic tumor development.

(Donnez O, Jadoul P, Squifflet J, Donnez J. Iatrogenic peritoneal adenomyoma after laparoscopic subtotal hysterectomy and uterine morcellation. *Fertil Steril* 2006; 86 (5):1511-2.

Donnez O, Jadoul P, Squifflet J, Donnez J. Post hysterectomy pelvic adenomyotic masses observed in 8 cases out of a series of 1405 laparoscopic subtotal hysterectomies. *J Min Inv Gynecol*. 2007; 14:156-60.)

3. In the third part, we induced endometriotic nodules in an experimental baboon model, mimicking human deep nodular endometriotic lesions. It was demonstrated that induced nodular endometriotic lesions were significantly larger and showed a stronger invasion process when tissue specimens containing the JZ were grafted. In this experimental model, the JZ was also found to be a key element in the process of proliferation and invasion of induced nodular lesions.

(Donnez O, Van Langendonck A, Defrère S, Colette c, Van Kerk O, Dehoux JP, Squifflet J, Donnez J. Induction of endometriotic nodules in an experimental baboon model mimicking deep nodular endometriotic lesions *Fertil Steril* 2013; 99: 783-789.)

4. In the fourth part, we analyzed nerve densities in type III nodules and induced experimental nodules. As NFD was confirmed to be high in human endometriotic nodules, and most of these nerve fibers were found to be unmyelinated, they could well be implicated in pain. Moreover, we demonstrated that deep nodular lesions may be neuroattractive through the action of NGF.

In the experimental model, nerve fiber density was investigated and the kinetics of neurogenesis was considered. Increased expression of NGF, together with the low NFD observed in experimental lesions, suggest that these lesions actually recruit nerve fibers.

(Donnez O, Soares M, Defrère S, Van Kerk O, Van Langendonckt A, Donnez J, Dolmans MM, Colette S. Nerve fibers are absent in both disease-free and eutopic endometrium, but more abundant in deep-infiltrating disease than in other types of endometriosis. *J End Pelvic Pain Disorders* 2013 (in press).

Donnez O, Soares M, Defrère S, Dehoux JP, Van Langendonckt A, Donnez J, Dolmans MM, Colette S. Nerve fiber density in deep nodular endometriotic lesions induced in a baboon experimental model. *Fertil Steril* 2013 (in press).)

5. Our clinical results (type III nodules and iatrogenic adenomyotic lesions) and data from baboons show morphological similarities, confirming that multicellular coordination between the leading (invasive) edge and the training (cohesive) edge is mandatory. This explains the good results of the surgical technique used in our department, which removes the cohesive part of the nodule.

In conclusion, this study evidences clear similarities between the baboon model and spontaneous disease observed in humans. This model could therefore be used in the future to explore the invasion process of the disease, validate medical or surgical strategies in terms of pain, fertility and disease recurrence, and finally explain the pathophysiology of deep nodular endometriosis.

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IMAGES IN REPRODUCTIVE MEDICINE

Iatrogenic peritoneal adenomyoma after laparoscopic subtotal hysterectomy and uterine morcellation

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Five years after laparoscopic subtotal hysterectomy and morcellation, pelvic magnetic resonance imaging demonstrated the presence of an irregular and heterogeneous pelvic mass measuring $40 \times 40 \times 30$ mm. Histologic examination confirmed smooth muscle hyperplasia infiltrated by numerous endometrial glands (adenomyosis). (*Fertil Steril*® 2006;86:1511–12. ©2006 by American Society for Reproductive Medicine.)

The first laparoscopic subtotal (supracervical) hysterectomy (LASH) was performed in our department in the early 1990s and the first series was published in 1993 (1). It is now a well established technique for the surgical treatment of uteri with multiple myomas, even in case of enlarged size (2). The advantages of this surgery are widely recognized, with significantly low morbidity rates (2, 3).

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Here we present the magnetic resonance imaging (MRI) picture of a complication not previously described occurring after laparoscopic subtotal hysterectomy and morcellation.

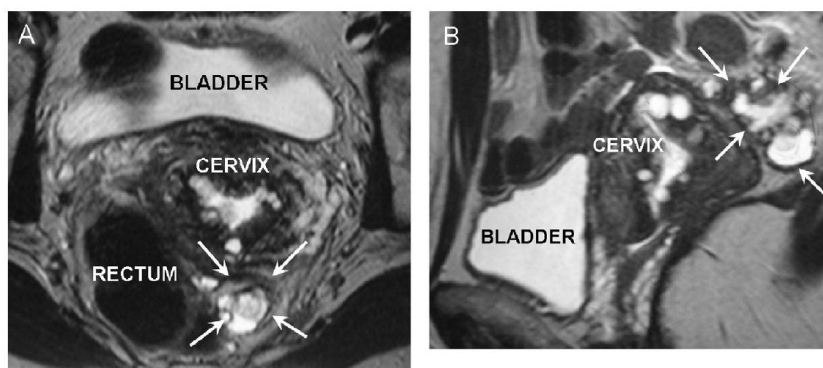
In 2000, we performed laparoscopic subtotal hysterectomy with bilateral oophorectomy in a woman of 48 years of age suffering from menorrhagia and fibroids.

During laparoscopy, no complications occurred, and Steiner's electric morcellator (Storz, Tuttlingen, Germany) (4) was used to morcellate and remove the uterus and myomas.

Histopathologic examination of 240 g of morcellated uterus confirmed the presence of benign myomas associated

FIGURE 1

Pelvic MRI (T2-weighted images) showing a transverse (A) and sagittal (B) view of an iatrogenic adenomyoma (white arrows), situated close to the cervix, but clearly separated from it. This irregular and heterogeneous mass measures $40 \times 40 \times 30$ mm (between arrows). Cystic structures (hyperintense on T2-weighted images) are surrounded by thick walls. Numerous small hyperintense spots, such as those described in adenomyotic tissue, can be seen. The mass is located in the left pararectal fossa, very close to the rectosigmoid.



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Posthysterectomy pelvic adenomyotic masses observed in 8 cases out of a series of 1405 laparoscopic subtotal hysterectomies

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KEYWORDS:

Laparoscopic subtotal hysterectomy;
Adenomyosis;
Electric morcellation;
Retained fragments

Abstract

STUDY OBJECTIVES: To analyze the prevalence of an unexpected complication due to morcellation and to describe the appearance of this complication on magnetic resonance imaging, as well as its therapy.

DESIGN: A well-designed controlled trial without randomization (Canadian Task Force classification II-1).

SETTING: Academic hospital.

PATIENTS: One thousand four-hundred five patients who underwent laparoscopic subtotal hysterectomy (LASH) in our department from 1990 through 2005 by surgeons using the same technique.

INTERVENTION: Morcellation was performed using Steiner's 15-mm electric morcellator.

MEASUREMENTS AND MAIN RESULTS: After 1405 LASH procedures, we encountered 8 cases (0.57%) of deep dyspareunia and pelvic pain caused by heterogeneous masses (median size 45 mm, range 20–80 mm). Symptoms appeared between 2 and 9 years after surgery. Vaginal examination revealed a painful pelvic mass in all 8 patients. The median CA 125 level was 52 IU/mL (range 19.4–128 IU/mL). Magnetic resonance imaging revealed heterogeneous masses containing hyperintense signals on T1-weighted images with saturation of fatty tissue. Injection of gadolinium revealed vascularization of the masses. Laparoscopic excision was performed, and extensive dissection of the rectum and pararectal fossa was required to isolate the masses. Histologic examination showed adenomyosis. Such complications occurred after electric morcellation of myomatous uterine corpora associated with adenomyosis.

CONCLUSION: These lesions probably result from the growth of missed fragments of uterine corpus after previous morcellation, culminating in the development of symptomatic iatrogenic adenomyomas. For this reason, the abdominal cavity must be meticulously inspected after electric morcellation, especially in patients with adenomyotic uteri.

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Laparoscopic subtotal (supracervical) hysterectomy (LASH) is a well-established technique for the surgical treatment of uteri with multiple myomas,^{1–3} even in cases of enlarged size. The first LASH was performed in our department in 1990, and the first series was published in 1993.¹ The advantages of this surgery are now widely recognized, with significantly lower morbidity rates^{2,3} than with lapa-

Induction of endometriotic nodules in an experimental baboon model mimicking human deep nodular lesions

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Objective: To establish an experimental model for the study of deep nodular endometriosis.

Design: Induction of nodular endometriosis in baboons by grafting different uterine specimens to the peritoneal cavity.

Setting: Research and university facilities.

Animal(s): Ten baboons, to develop a model of induced deep nodular endometriosis.

Intervention(s): Biopsies of endometrium, and endometrium plus the junctional zone (JZ), full uterine thickness, and myometrium grafted to the peritoneum.

Main Outcome Measure(s): Macroscopic descriptions recorded for observed induced lesions; staining with hematoxylin and eosin for histological evaluation and specific antibodies (CK22, CD 10) for immunohistochemical studies; and analysis of surface area and volume of lesions, glandular density, and invasion of surrounding organs.

Result(s): The incidence of induced nodular endometriosis was 100%, but the extent depended on the tissue grafted. Lesions induced after grafting specimens containing the JZ were statistically significantly larger than those not containing the JZ. Surrounding organ invasion was reported in more than 40% of lesions after grafting specimens containing the JZ.

Conclusion(s): The first experimental model of nodular endometriosis allows investigation of deeper nodular lesions as well as invasion phenomena associated with nodular lesions. (Fertil Steril 2013;99:783–9. ©2013 by American Society for Reproductive Medicine.)

Key Words: Baboon model, induced endometriosis, junctional zone, nodular endometriosis

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Endometriosis, one of the most frequent benign gynecologic diseases, is known to occur in 7% to 10% of women of reproductive age (1). It is now well established (2) that three different entities of endometriosis must be considered in the pelvis: peri-

toneal endometriosis, ovarian endometriosis, and deep endometriotic nodules of the rectovaginal septum. Classification of deep endometriotic nodules was proposed by our group (3) as well as by others such as Adamyan et al (4) and Koninckx et al (5). We have

found that most of these lesions originate from the posterior part of the cervix (types II and III) and secondarily infiltrate the anterior wall of the rectum (type III) (3).

The pathogenesis of these endometriotic nodules (whose histology reveals typical features of adenomyosis with smooth muscle hyperplasia) remains unclear, and as the time from the onset of the disease to its diagnosis has been evaluated to be 8 to 11 years (6), we clearly need an animal model to study its origin and early development (7). There is ongoing debate about the use of baboons (*Papio anubis*) for the study of peritoneal endometriosis. In a baboon colony at the Institute of

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Deep rectovaginal endometriotic nodules: perioperative complications from a series of 3,298 patients operated on by the shaving technique

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 Mathieu Luyckx · Jean Squifflet · Olivier Donnez

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Abstract The purpose of this study was to analyze complication and recurrence rates after deep endometriotic nodule surgery. A total of 3,298 cases of deep endometriotic nodules were analyzed. The shaving technique was used, avoiding bowel resection. Laparoscopic nodule resection was performed successfully in all cases. Major complications included: (1) rectal perforation in 42 cases (1.3 %), (2) ureteral retention (<5 days) in 21 cases (0.64 %), (3) ureteral injury in 10 cases (0.3 %), and (4) fecal peritonitis in 1 case (0.04 %). This complication rate is much lower than that observed after bowel resection. In the same period, bowel resection was only required in 1.1 % ($n=37$) of cases. Histology revealed circumscribed nodular aggregates of smooth muscle, endometrial glands, and scanty endometrial stroma. Lesions were found to be invaded by nerve fibers. Endometriosis is not cancer and does not require the same treatment approach. In young women, conservative surgery

using the shaving technique means preservation of organs, nerves, and the vascular blood supply. The shaving technique yields low complication and recurrence rates and should be considered the first line in surgical approach in the case of deep endometriotic lesions.

Keywords Deep endometriosis · Nodules · Shaving technique · Bowel resection

Introduction

In 1996, we were the first to publish the concept of three different forms of endometriosis: (1) peritoneal endometriosis, (2) ovarian endometriosis, and (3) deep rectovaginal endometriosis [1, 2]. The third form of the disease has been defined as deep endometriosis, rectovaginal endometriosis, or adenomyosis of the rectovaginal septum. In the literature, it is also called deep-infiltrating endometriosis or posterior deep-infiltrating endometriosis.

Many different surgical approaches have been proposed. The first study reporting the shaving technique and data from a series of 231 cases of laparoscopic management of deep endometriosis was published in *Human Reproduction* in 1995 [3], followed by a second study ($n=500$ patients) published in *BJOG* in 1997 [4] and a third one ($n=1,942$ patients) published in 2004 [5]. But surprisingly, the number of manuscripts advocating bowel resection has dramatically increased over recent years [6–15]. Koninckx et al. (see [16]) and Donnez et al. [17], firm advocates of the shaving technique, are increasingly confounded by this rapidly growing prevalence of bowel resection in the case of deep endometriosis with rectal muscularis involvement. During meetings on this topic, there is often heated debate between advocates of the two respective techniques.

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ORIGINAL ARTICLE

Nerve fibers are absent in disease-free and eutopic endometrium, but present in endometriotic (especially deep) lesions

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Objective: Detection of nerve fibers in endometrial biopsies was recently proposed as a noninvasive diagnostic tool for endometriosis. However, their occurrence in the functional layer of endometrium still remains controversial. Nerve fibers were found to be present in endometriotic lesions themselves, which may account for some of the pain experienced by patients, but their origin is not clear. The objective of the present study was to reevaluate the presence of nerve fibers in endometrium and in different types of endometriotic lesions.

Patients and methods: Nerve fiber density (PGP9.5 immunohistochemical analysis), unmyelinated nerve fiber presence (neurofilament immunohistochemical detection) and nerve growth factor expression were evaluated in endometrial (disease free: $n = 20$; endometriotic: $n = 26$) and endometriotic (peritoneal lesions: $n = 11$; ovarian lesions: $n = 16$; rectovaginal lesions: $n = 27$) samples.

Results: Endometrial biopsies were found to be mostly negative for nerve fibers. Nerve fiber density was higher in deep nodular lesions than in peritoneal ($p < 0.01$) or ovarian ($p < 0.001$) lesions. Around 30% of PGP9.5-positive nerve fibers were confirmed by neurofilament staining. Nerve growth factor expression was detected at higher levels in the stroma of deep-infiltrating lesions ($p < 0.05$).

Conclusions: No nerve fibers were detected in endometrial biopsies (from healthy or endometriosis patients). However, nerve fibers were detected in endometriotic lesions. Most of them were found to be unmyelinated, suggesting they could be implicated in pain. Deep nodular lesions may be more neuroattractive through the action of nerve growth factor.

Keywords: Deep-infiltrating endometriosis, Endometriosis, Endometrium, Nerve fibers

Accepted: April 17, 2013

INTRODUCTION

Endometriosis is defined as the presence of endometrial glands and stroma outside the uterine cavity and is one of the most commonly encountered pathologies in gynecological practice. It can present under different forms, namely peritoneal, ovarian and rectovaginal lesions, each one probably having its own pathogenesis (1, 2).

Dysmenorrhea (painful menstruation), dyspareunia (painful sexual intercourse), chronic pelvic pain and infertility are the most frequent symptoms found in case of endometriosis. Because of the variability in signs and symptoms, and confusion with other disorders, diagnosis of the condition remains problematic. The gold standard for diagnosis of pelvic disease (especially peritoneal endometriosis) is surgical assessment by laparoscopy and biopsy (3). In case of

Nerve fiber density in deep nodular endometriotic lesions induced in a baboon experimental model

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Objective: To study the occurrence of nerve fibers in deep nodular endometriotic lesions after nodules were induced in baboons and nerve fiber densities measured 6 months after the grafting procedure.

Design: Experimental animal study.

Setting: Academic gynecology research unit.

Animals: Ten baboons (*Papio anubis*).

Intervention(s): Recovery of induced endometriotic nodules and eutopic endometrium.

Main Outcome Measure(s): Protein gene product (PGP) 9.5 and nerve growth factor (NGF) immunohistochemistries were performed to evaluate nerve fiber density and NGF expression in induced endometriotic lesions and eutopic endometrium.

Result(s): Eutopic (basalis) endometrium, myometrium, and invasive and noninvasive nodular lesions were analyzed separately. The highest nerve fiber densities were observed in normal myometrium and in the basal layer of eutopic endometrium. No significant differences were observed between the two lesion types. However, the NGF staining intensity score was found to be higher in glands of deep invasive lesions than in glands of eutopic baboon endometrium.

Conclusion(s): This is the first study to show the presence of nerve fibers in eutopic baboon endometrium and induced deep endometriotic nodules. Long-term studies are now warranted to determine if nerves still grow in invasive and noninvasive lesions >6 months after grafting, and to evaluate the role of the lesion environment. (Fertil Steril® 2013;■:■-■. ©2013 by American Society for Reproductive Medicine.)

Key Words: Nerve fibers, endometriosis, baboon, deep nodular lesions

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Endometriosis, one of the most commonly encountered pathologies in gynecologic practice, is a benign disorder defined as the pres-

ence of endometrial tissue (glands and stroma) outside the uterine cavity. Dysmenorrhea, dyspareunia, chronic pelvic pain, and infertility are the

most frequent symptoms found in patients suffering from endometriosis. At present, the fundamental pathologic mechanism underlying endometriosis-associated pain is still unclear. Since the association between nerve fibers and rectovaginal endometriotic nodules was first suggested by Anaf et al. (1), interest in nerve fibers has gained ground, especially over the past decade. Nerve fiber density appears to be higher in deep-infiltrating endometriosis than in peritoneal and ovarian lesions (2–5), and the presence of nerve fibers in lesions is hypothesized to contribute to the pain experienced by endometriosis patients. However, because of the subjectivity of pain, a possible correlation between pain and

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