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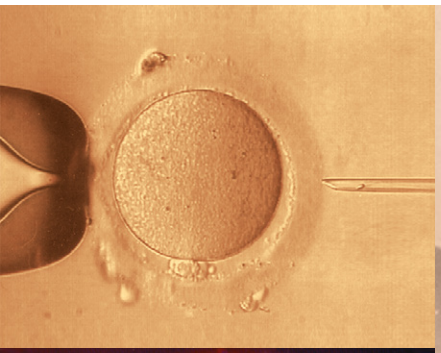

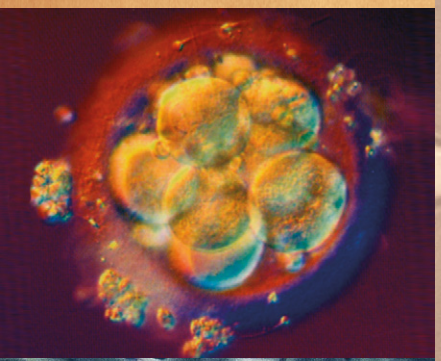

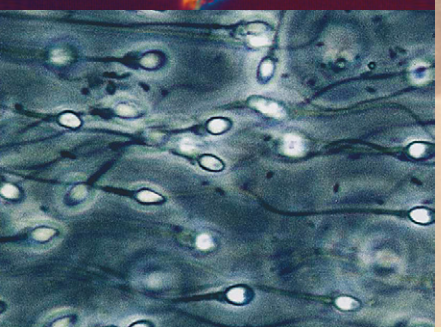

Adenomyosis – Facts and Questions 13th World Congress on Endometriosis, 2017 May 17–20, 2017 Vancouver, CAN Extended Abstracts

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Extended Abstracts*

miRNA Regulation of Cell Function in Adenomyosis and Endometriosis

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Abstract

microRNAs (miRNAs) play an important role in benign and malignant diseases through post-transcriptional regulation of various proteins. Various miRNA-dysregulations have been shown in endometriosis and adenomyosis. Based on their function and technical properties, miRNAs could potentially be promising biomarkers and therapeutical targets in endometriosis and adenomyosis.

In endometriosis miRNA-145, miRNA-200b and miRNA-142-3p downregulations account for enhanced proliferation, invasion and stem cell functions in endometriotic tissues through regulation of multiple pathways as NFKB, cytoskeletal impairment, alteration of cellular adhesions and stem cell factors.

In adenomyosis miR-10b is the most promising target. Dysregulation of various genes and miRNAs have been found, but detailed studies are still needed to decode the pathways.

Micro RNAs in Endometriosis

MicroRNAs (mi-RNAs) non-coding RNA molecules. As a small, singular strand RNA molecule they target mRNA to inhibit protein expression through gene regulation (gene silencing) on a post-transcriptional level. The mRNA is degraded or translation is inhibited [1].

Various studies demonstrate the impact of miRNAs on every aspect of the invasion, viability and proliferation in endometriosis. They affect inflammation, apoptosis, cell cycle, angiogenesis and much more. MiRNAs are also investigated as potential biomarkers in endometriosis and adenomyosis.

miRNAs as Serum Markers of Endometriosis – Current conclusions

Based on their function and technical properties, miRNAs could potentially be promising biomarkers in endometriosis. For large and reliable studies standardized guidelines, sample types and collection methods as well as reference genes are needed. For a solid estimation these studies should be performed on a large and uniform patient collective. All of the following miRNAs could be targets of such studies with the potential to revolutionize endometriosis diagnostics.

Important miRNAs for regulation of Endometriosis

MiR-145 is commonly expressed in mesenchymal cells (e.g. fibroblasts and smooth muscle cells) and inhibits expression of cytoskeletal elements as FASCIN-1, tight-junction and adhesion molecule JAM-A and protease-inhibitor – resulting in decreased proteolysis, cell mobility and adhesion. It also reduces stem cell factors as SOX-2, MSI2 and OCT-4 leading to less proliferation and motility. In endometriosis, lower levels of miRNA-145 have been detected, accounting for increased proliferation and lesion invasiveness [2].

As the cell composition of those cell types is different in the three entities of endometriosis differences between the forms should be expected. Also, the miRNA-145 levels differ and contribute to different invasiveness in the entities. In studies miRNA-145 dysregulation and decrease occur in more invasive and aggressive lesions [2–3].

MiR-142-3p likely inhibits endometriosis by decreasing cell proliferation and enhancing apoptosis through NFKB by repressing IL-6 ST. Through regulation of WASL, Integrins as well as actin and vinculin in endometriosis, the building of spikes and focal adhesion formation is reduced, resulting in diminished invasiveness. miRNA-142-3p is reduced in endometriosis – possibly enhancing invasion and progress of the lesions through cytoskeletal changes, enhanced proliferation, immunological responses, angiogenesis and altered oestrogen levels [4].

MiR-200b increases E-cadherin expression through decreased ZEB-1 and ZEB 2 levels resulting in decreased cell mobility, decreased declamping from the cell layer and diminished invasion. In endometriosis miRNA 200b expression is reduced, inducing lesion growth, enhanced invasion and progression of the disease [5].

*Appraised by the Chairman, Prof. L. Kiesel, Münster

Table 1. Dysregulation of miRNAs in endometriosis. Mod. from [6, 7].

Downregulated miRNAs [6] (sign. Microarray and qRT-PCR)	Upregulated miRNAs [6] (sign. Microarray and qRT-PCR)	Downregulated miRNAs [7]
miR-10b	miR-143	miR-9-1
miR-371b-5p	miR-513a	miR-139
miR-92b-5p	miR-466	miR-149
miR-30c		miR-197
		miR-326
		miR-339

Those miRNAs and many more may be future targets for therapies and biomarker research.

MiRNAs in Adenomyosis

For Adenomyosis few studies exist and data concerning miRNA changes is rare.

In large transcription studies and microarray studies several miRNA expressions were dysregulated in ectopic endometrium in Adenomyosis versus normal endometrium [6–7] (Tab.1).

Expression of miRNA 10b is downregulated most significantly in microarray and RT-PCR in endometrial tissue in adenomyosis compared to normal tissue and even less expressed in ectopic endometrium in adenomyosis. At the same time cell migration and invasion are increased in endometrium of adenomyosis patients and even more increased in ectopic endometrium of adenomyosis patients. In summary, in adenomyosis invasion and cell migration of endometriotic cells are increased, possibly due to severely decreased levels of miR-10b [6]. miR-10b regulates apoptosis and proliferation and is known to be dysregulated in various cancers [8] (Fig. 1).

Also, endometrial epithelial cells are targets of miRNA regulation. As a potential indicator of invasiveness or severity ZEB1 is also known to be regulated by miRNAs and to be dysregulated in adenomyosis. This indicates miRNA-dysregulation. In deep infiltrating endometriosis as well as in adenomyosis ZEB1 was expressed excessively. In normal endometrium ZEB1 was not expressed at all [9].

As in endometriosis, IL6-ST regulated pathways seem to be involved.

As these few data show, research is still insufficient regarding pathophysiology and regulation of adenomyosis in general and concerning miRNA in particular. If results about regulation of endometriosis can be applied for adenomyosis as well should be validated, followed by adenomyosis specific factors and pathways.

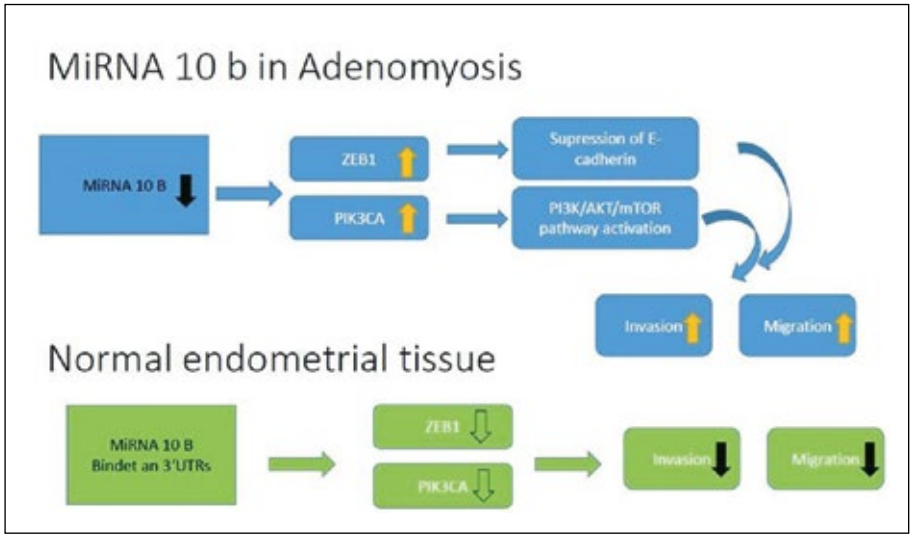

Figure 1. miR-10 b in endometriosis after Guo et al 2015. Mod. from [5], open access (CC BY-NC).

Relevancy to Practice

- miRNAs can be future targets for individualized therapies.
- miRNAs may be valid candidates for biomarkers in diagnosis of endometriosis and adenomyosis.
- Adenomyosis and endometriosis have different pathophysiological characteristics and need to be evaluated separately.
- Standardized research is needed for endometriosis and adenomyosis, providing opportunities for researchers.

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Adenomyosis – Possible Cause for Infertility in Women with Endometriosis?

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Abstract

Adenomyosis is a heterogeneous and poorly understood gynaecologic disease. Most frequently the major symptoms are troublesome, heavy menstrual bleeding, dysmenorrhoea and sometimes a tender uterus. Since more women are tending to delay their first pregnancy till their late thirties or early forties, adenomyosis has become more relevant in the context of subfertility. In order to be able to answer the question referred, a literature research was performed.

As possible causes for infertility in women with adenomyosis can be seen: intrauterine abnormalities, intensified uterine peristalsis and altered endometrial milieu. But, there is a lack of sufficient data to show doubtless evidence that adenomyosis alone is the cause of subfertility. To improve fertility, surgical treatment or/and reproductive medicine ± GnRH analogues pre-treatment are recommended. It is necessary to provide a complete removal of all adenomyosis or to induce a reduction of the inflammatory process by a GnRH agonist (ultra-)long pre-treatment before fertility enhancing procedures are started.

Introduction

Adenomyosis is a heterogeneous gynaecologic disease. It is a poorly understood and so often called enigmatic and/or elusive due to the difficulties in diagnosis and the lack of agreement concerning the definition. Most frequently the major symptoms are troublesome, heavy menstrual bleeding, excruciating dysmenorrhoea and sometimes a tender uterus. But sometimes it can also be asymptomatic.

Adenomyosis is a benign disease of the uterus characterised by an invasion of ectopic endometrial glands and stroma within the myometrium producing a diffusely enlarged uterus which microscopically exhibits ectopic, non-neoplastic, endometrial glands and stroma surrounded by the hypertrophic and hyperplastic myometrium [1]. A first scientific description of adenomyosis was done by C. Rokitansky in his publication: "Über Uterusdrüsen-Neubildung" in 1860 [2].

Adenomyosis is seen mostly in women at their late 4th or 5th life decade and so during long time the problem adenomyosis and fertility seemed not to be existent! Since more women are tending to delay their first pregnancy till their late thirties or early forties, adenomyosis has become more relevant in the context of subfertility. It is a welcome figure that in Germany in 2016 the number of newborns re-approached the level reached in 1996 but on the other side the number of mothers at the age of 40 years and more increased since 2011 of 13.4% up to nearly 40,000 women [3]. When looking at ART treatment we have to realise that in 2016 every 5th woman treated by IVF was at the age of ≥ 40 years and 3.2% even at the age of ≥ 44 years [4]. These figures explain very well that adenomyosis has become an increasing problem in fertility treatment although the incidence peak is about 5–10 years later compared to endometriosis [5]. Nevertheless, endometriosis and adenomyosis often occur together in about 27% to 49% and is significantly associated with deep infiltrating endometriosis, parity, dysmenorrhea intensity and women's age [6].

Methods

In order to be able to answer the question referred, a literature research was performed looking to the following items:

- adenomyosis
- fertility
- endometriosis
- surgery in patients with adenomyosis ± endometriosis
- medical treatment in patients with adenomyosis ± endometriosis.

Adenomyosis in Patients with Fertility Disturbances

In animal studies it is quite easier to examine the association between adenomyosis and fertility. In a population of captive baboons endometriosis was strongly associated with the presence of adenomyosis with an OR of 31.5. Adenomyosis was associated with the presence of lifelong infertility (OR = 20.6), even if cases with coexisting endometriosis were excluded (OR = 20.1) [7].

In human, long time, the only way to be certain of adenomyosis is to examine the uterine tissue under the microscope after surgery. Only with the possibility to diagnose adenomyosis at the "living object" by ultrasound – US – and/or magnetic resonance imaging – MRI – the question of impact on fertility has emerged. But there are conflicting results reported in the literature. One group reported in women with infertility and symptoms of dysmenorrhoea or menorrhagia that in 54% adenomyosis could be detected by MRI. But finally, only 26 women were included in the study [8]. Puente et al. [9] diagnosed adenomyosis in women before starting an ART treatment or after recurrent pregnancy loss by 3D-US. They found signs for adenomyosis in 24.4%, significantly more often in women aged ≥ 40, in 29.7% as compared to women < 40 years, 22%. The prevalence was also significantly higher in those cases of recurrent pregnancy loss, 38.2 %, and previous ART failure, 34.7%. Patients with fibroids showed also adenomyosis in 18%. Among the patients with endometriosis, 35.1% were also diagnosed with adenomyosis. This is in contrast to the group of Bazot et al. which reported the coincidence of pelvic endometriosis and adenomyosis only in 27% (44/163 – diagnostic by MRI) [10]. In a further study the same group demonstrated in women with dysmenorrhea, nonmenstrual pelvic pain, dyspareunia, and pain on defecation treated with laparoscopic

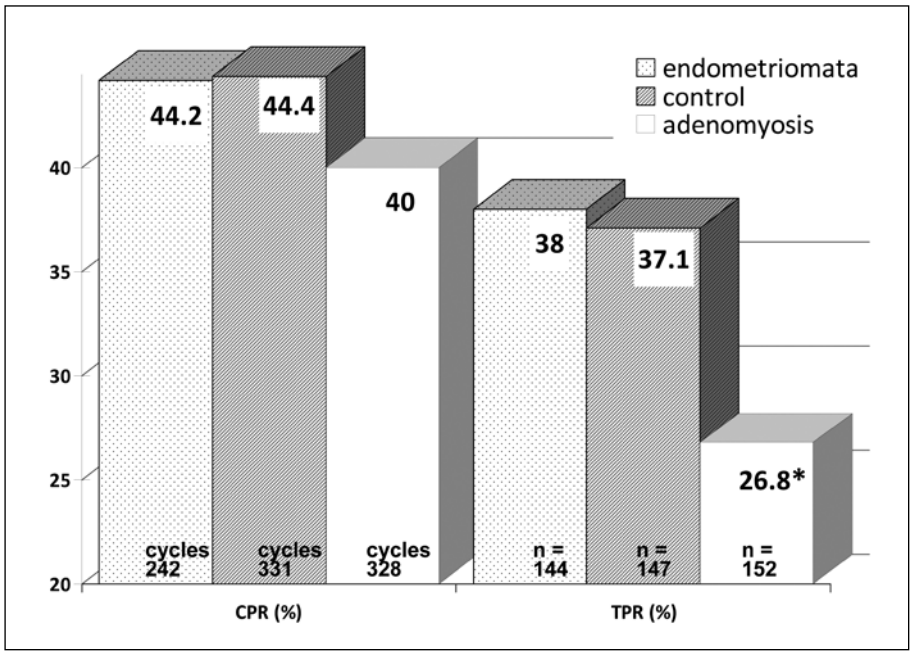

Figure 1. Cumulative pregnancy (CPR) and term pregnancy (TPR) rates after embryo transfer with donated oocytes of women without adenomyosis. Mod. from [14].
*significant difference

colorectal resection only 4 women of 22 – 18.2% – who wanted to conceive showed also adenomyosis. 45.5% of the women got pregnant after the surgery, but none of them with adenomyosis [11].

Kunz et al. looked in 227 infertile women about the prevalence of adenomyosis, diagnosed by MRI, and endometriosis and its impact on fertility. In 28% of the women without endometriosis signs of adenomyosis were found in 28% of the women and when in them there was an andrological reason of the infertility only in 9%. But in 160 women with proven endometriosis 79% showed also adenomyosis and in 30 women of them, younger than 36 years and with fertile partners even in 90% adenomyosis could be detected with MRI [12]. These differences are due to the different definitions applied.

As possible causes for infertility in women with adenomyosis can be seen:

Intrauterine abnormalities:
distortion of the uterine cavity, obstruction of tubal ostium, interference with sperm & embryo

Uterine peristalsis:
dysfunctional uterine hyperperistalsis, increased intrauterine pressure, junctional zone hyperactivity → contractions (→ ectopic pregnancies ↑), disturbed uterotubal sperm transport

Altered endometrial milieu:
higher macrophage density, more proinflammatory cytokines, such as TNF-α or IL-1, reduction of progesterone receptor expression

Very often, a reduced endometrial receptivity is discussed also. So, Huang et al. discussed if an abnormal expression of oxytocin receptors in the uterine junctional zone may result in abnormal uterine contractile activity and reduces so fertility [13].

The group of the *Instituto Valenciano de Infertilidad* evaluated the endometrial receptivity in patients with adenomyosis by two aspects: endometrial gene expression and the model of oocyte donation (OD) [14]. Endometrial samples were collected from six women in whom adenomyosis had been diagnosed by MRI and transvaginal US. There is a similar endometrial gene expression pattern in both the adenomyosis group and controls, and nonparametric tests revealed 34 dysregulated genes in adenomyosis patients but none belonged to the group of window of implantation genes. Pregnancy rates in OD did not differ among the three groups, control, women with endometrioma, women with adenomyosis. However, miscarriage was significantly higher in the adenomyosis group vs. the endometriosis and control groups and so, term pregnancy rate was also significantly lower in the adenomyosis group compared with others (Fig. 1).

Treatment Options in Patients with Adenomyosis and Infertility

Surgical treatment to improve fertility

Limited data are available concerning the efficacy of the different treatment options of adenomyosis on fertility outcome and mostly the data are retrospective evaluations or small case series [15]. As different surgical techniques are described: adenomyomectomy with unilateral salpingectomy, microsurgical adenomyomectomy, adenomyomectomy using double or triple-flap method, or e. g. adenomyomectomy with continuous horizontal mattress technique. Rocha et al. reported in a recent review [16] a pooled clinical pregnancy rate after surgical resection of adenomyosis of 38.8%, mostly achieved by ART, and a miscarriage rate and live birth rate of 17.9% and 30.4% respectively (Fig. 2).

Reproductive medicine to improve fertility

Once again, mostly retrospective studies are available. In the pooled analysis from Rocha et al. [16] the overall pregnancy rate was 36.1%, live birth/ongoing pregnancy rate 29.9% and miscarriage rate 25.9%. There is a significant difference in the outcome of IVF/ICSI treatment cycles depending of the type of GnRH analogue used (Fig. 3). If the long agonist regimen was applied pregnancy and live birth rates were significantly increased and the miscarriage rate significantly lower. This was also showed by a meta-analysis [17]. There was no difference in ART outcome in women with adenomyosis compared to women without adenomyosis when the long downregulation protocol was used but with the short protocol the results were significantly lower. And also looking at miscarriage rates there was no difference with the long downregulation but with the short protocol when adenomyosis was present.

Obviously, the myometrial thickness can serve as a marker for the ART treatment outcome. If in transvaginal US a myometrial thickening of more than 2.50 cm was seen at the day of hCG triggering the implantation, clinical pregnancy, and live

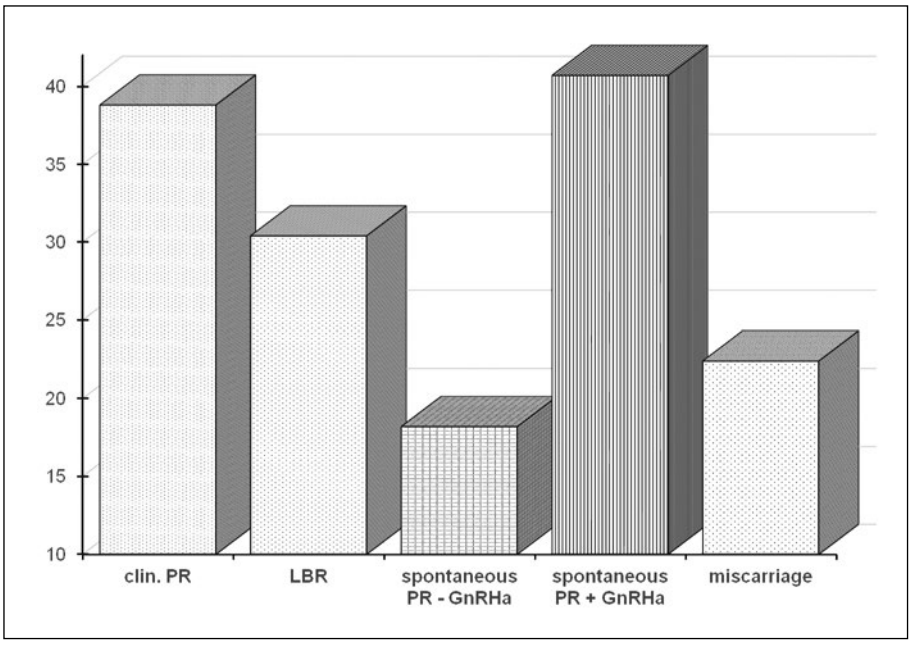

Figure 2. Pregnancy and live birth rates (%) after surgical treatment of adenomyosis. Mod. from [16]. PR = pregnancy rate, LBR = live birth rate, GnRHa = Gonadotropin Releasing Hormone agonist

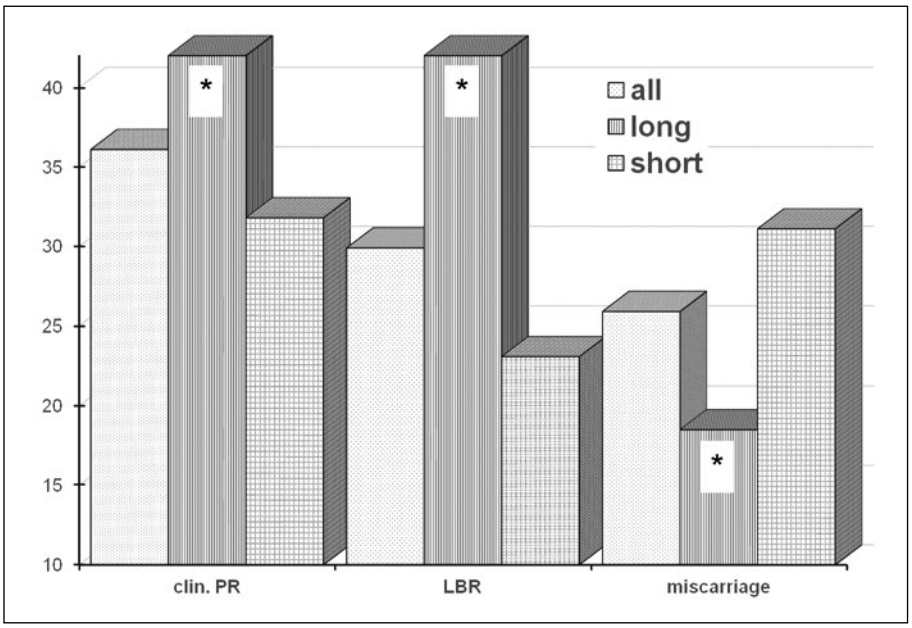

Figure 3. Pregnancy and live birth rates (%) after ART treatment in women with adenomyosis. Mod. from [16]. PR = pregnancy rate, LBR = live birth/ongoing pregnancy rate, all = overall, long = GnRH agonist regimen, short = short GnRH agonist or GnRH antagonist regimen
*significant difference

birth rates were significantly lower in the IVF-ET treatment with the short protocol [18].

Also, if frozen embryo transfer (FET) was performed long downregulation with GnRH agonist showed a beneficial effect: significantly higher ongoing pregnancy/live birth rates [19, 20]. It seems that GnRH-a treatment decreases the size and demarcation of adenomyotic lesions, as demonstrated by MRI [21], and treatment has a positive effect on endometrial implantations markers [22].

Conclusion

Adenomyosis represents a chronically inflammatory process yielding particularly in early pregnancy loss. Concerning a possibly reduced implantation the present evidence is too poor and more studies are needed to reach a definitive conclusion. Only if adenomyosis and pelvic endometriosis occur together a clear fertility reduction is established. So, there is a lack of sufficient data to show doubtless evidence that adenomyosis alone is the cause of subfertility. It seems that adenomyosis is the reason for higher miscarriage rates and reduced fertility is more due to often concomitant endometriosis. But in both pathologies it is necessary in order to ameliorate fertility to provide a complete removal of all adenomyosis and/or endometriotic foci or to induce a reduction of the inflammatory process by a GnRH agonist (ultra-)long pre-treatment before fertility enhancing procedures are started.

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Pathogenesis of Adenomyosis uteri (AM) – New Concepts

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Abstract

Adenomyosis (AM) means the occurrence of endometrial cells within the myometrium. It could be present as a diffuse superficial form or with deeper infiltration. There are also focal forms, which are able to affect the adjacent organs such as the bladder or rectum. Over the years the real prevalence of AM uteri has been underestimated. However, the ongoing better quality of ultrasounds and the possibility of MRIs have demonstrated a high prevalence of AM in patients with endometriosis (EM) especially in patients with infertility and ranked the prevalence between 80–91 % of all endometriosis patients.

This high prevalence may indicate the possible involvement of AM on the pathogenesis of AM and EM-associated infertility. Furthermore, some new data suggest that AM might also have an impact on miscarriages. Patients suffer from severe dysmenorrhea, heavy menstrual bleeding, pelvic pain and dyspareunia.

The pathogenesis of AM still remains unclear. There is the idea of a peristaltic-dependent induction of microscopic tissue injury at the endometrial junctional zone (EMJZ), which in turn leads to the translocation of the basal endometrium into the myometrium with the consecutive development of adenomyotic lesions. This tissue injury might stimulate a process of tissue repair in which estrogen is locally produced, which in turn stimulates angiogenesis, proliferation and a local OT synthesis. The local OT activated oxytocin receptors on the myometrial smooth muscle cells cause more hyperperistalsis. Subsequently, the tissue injury increases and a vicious circle is established. Microscopic findings underlay this theory. Furthermore, ultrastructural analysis of the junctional zone identifies stem cell like cells, which might be involved in the pathogenesis of this disease.

The adenomyotic-associated myometrial cells show an over expression of the oxytocin receptor as well as changes in their architecture. Both might lead to dysperistalsis of the myometrial cells and the reduced uterine blood flow, which causes ischemic pain. The myometrial innervation seems to be disturbed and the deletion of sympathetic nerve fibers might be a further factor in the functional changes of the myometrium.

Background

Adenomyosis (AM) seems to be a fix linked objective to endometriosis. However, over the years the real prevalence of adenomyosis uteri has been underestimated. The ongoing better quality of ultrasounds and the possibility of MRIs have demonstrated a high prevalence of adenomyosis in patients with endometriosis, especially in those with infertility [1]. This prevalence is currently ranked between 80–91% of all endometriosis patients.

The high prevalence may indicate the possible involvement of AM on a range of endometriosis related symptoms like dysmenorrhoea and heavy menstrual bleeding, pelvic pain and dyspareunia [2]. But furthermore, there seems to be a direct link to the pathogenesis of AM and EM-associated infertility and some new data suggests that AM could be an impact on miscarriages. AM is of great underestimated clinical importance.

AM means the occurrence of endometrial cells within the myometrium. It could be present as a diffuse superficial form characterised by a thicker junctional zone or with deeper infiltration. In these cases, the complete myometrial wall would be thicker and both uterine walls are more asymmetric to each other. If the infiltration affects both sides, there will be an overall enlargement of the uterus. There are also focal forms, which are able to affect the adjacent organs such the bladder or lead to retrocervical endometriosis with bowel affection [3] (Fig. 1).

It is very important development for clinicians to focus on AM, especially when descriptions on the ultrasonographic features of uterine changes are becoming more and more popular [4]. However, all these visible changes have already shown a disturbed uterus. It is a shame that we have no possibility to recognize uterine changes before irreversible damage has occurred. AM is a hard topic and the brilliant review of Yen [5] was summarised at the end: *"The mechanisms and pathogenesis of AM and its clinical impacts are still far from being understood. Consequently, it is difficult to diagnose AM at an early stage."*

This is what we have to focus on to analyse in the near future. All these features need time for development and the beginning of these changes has not yet been defined. Maybe it is linked to the primary dysmenorrhoea, which over 90%

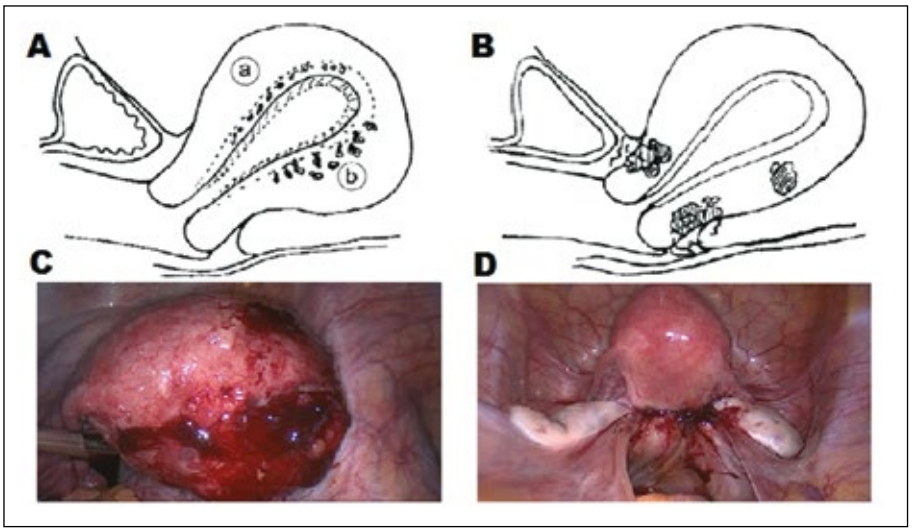

Figure 1. Adenomyosis uteri. Mod. from [3].
A: Diffuse forms of adenomyosis uteri: a) superficial and b) deep; **B:** focal forms with bladder or bowel affection; **C:** clinical presentation of deep infiltration diffuse adenomyosis; **D:** clinical presentation of focal retrocervical adenomyosis with bowel infiltration

of endometriosis patients have reported (Leyendecker). There are also hints of presence of adenomyosis in adolescents with severe dysmenorrhoea [6–9]. We know that dysmenorrhoea is associated with a disturbed pattern of contractility and this might be the beginning of the tissue injury and ongoing changes. Maybe AM is more undetectable in its early stages, but already present. In order to investigate this in more detail, we examined 38 adolescent patients with severe dysmenorrhoea for the early signs of adenomyosis such as the halo phenomenon, inhomogenous myometrium and asymmetry of the uterine walls and found changes in 91% of the cases [unpublished data, Abesadze et al., in prep].

Pathogenesis – Changes of the Junctional Zone (JZ)

However, so far, we have no idea of the pathogenesis of adenomyosis and as long as this remains to be unclear the innovative therapeutic approaches to fight this disease are missing. The current leading idea of the pathogenesis of AM in uteri of reproductive aged women is the tissue injury and repair concept postulated by Leyendecker. What does this mean.: The uteri of patients with adenomyosis exhibit hyperperistalsis [10]. This is linked with a peristaltic-dependent induction of microscopic tissue injury at the endometrial junctional zone (EMJZ), which in turn leads to the translocation of the basal endometrium into the myometrium with consecutive development of adenomyotic lesions. This tissue injury might stimulate a process of tissue repair mechanisms in which oestrogen is locally produced, this in turn stimulates angiogenesis, proliferation and a local oxytocin (OT) synthesis. The local OT activated oxytocin receptors on the myometrial smooth muscle cells cause more hyperperistalsis. Subsequently, more tissue injury occurs and a vicious circle is established [11]. In agreement with this, further ongoing studies have analysed the border between the endometrium and myometrium in patients with and without AM and confirmed the concept of disrupture of the JZ. The endo-myometrial border in patients with fibroids is very smooth, whereas the endo-myometrial border in patients with AM is disruptured [12, 13]. Interestingly, other than these morphological changes, there is also an upregulation of smooth muscle actin present in the basal endometrial layer of patients with AM. This is a strong hint for a fibroblast to myofibroblast transition in the JZ [14]. Myofibroblasts are known to secrete Collagen 1, an important factor for fibrosis, which is also upregulated in the adenomyotic uteri [14–16]. So, fibroblast to myofibroblast transdifferentiation (FMT) seems to be an important part of the process, which might be activated by tissue injury. Ongoing platelet aggregation seems to induce EMT and FMT in a adenomyosis mice model [15].

The junctional zone was also analysed by ultrastructural investigations and showed a range of changes which are also in line with mechanical alternations. For example, normally the nucleus of non-adenomyosis patients is very smooth, whereas the nucleus of patients with adenomyosis is irregular with invaginations. This could be a result of mechanical alteration due to the strong contractions in this area [13]. Transelectric microscopy gives a deep insight into the composition of the epithelial and stromal cells of the junctional zone. In uteri of adenomyosis patients the arrangement of these cells is interrupted by the myometrial cells. Furthermore, in non-adenomyotic uteri the orientation of the myometrial smooth muscle cells of the inner myometrium is parallel to each other, but in adenomyotic uteri the myometrial cells have no clear orientation as they are directed in diverse alignments. Interestingly, there was a so far undescribed cell population present in patients with and without AM. These cells are electro-lucent and mitochondria – and ribosome rich because these are characteristics from stem cell-like cells, which are called pale cells. These cells are mostly localised on the basal of the glands and are sometimes also concentric to the lumen of the gland. Pale cells have no desmosomes and are partially detached from the surrounding cells. A total detachment of these cells was only observable in patients with adenomyosis and interestingly the cell bodies of the neighbouring cells seem to drift apart from each other, with the finding of a thin basal cellular interdigitations. In a rare number of patients, a micro-perforation of the basement membrane was found with the building of pseudo-pods and even a cytoplasmic migration. The migration of these cells was only seen in AM specimens. So pale cells might also be involved in pathogenesis of AM (Fig. 2). There seems to be some so far unknown factors (perhaps the severe peristaltic) which lead in turn to an activation of these cells with the induction of migration. Translocated pale cells might be able to develop into AM or endometriosis [13].

Changes of the Adenomyotic Lesions

The adenomyotic lesions also show a range of morphological changes: In AM lesions there are not only isolated isles of endometrial and stromal cells, but also smooth muscle like cells present. These cells are located within the stromal cells, but are also surrounded by the lesions.

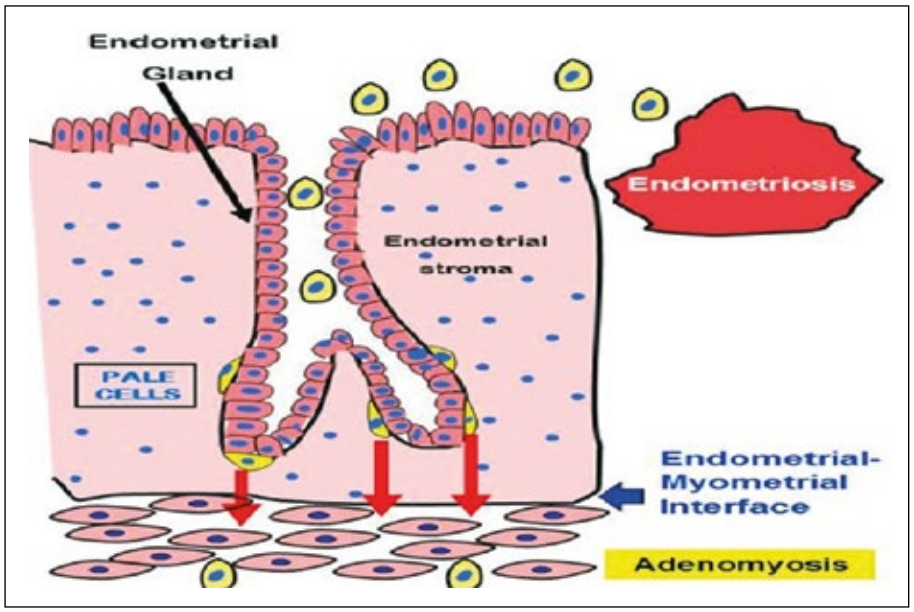

Figure 2. Concept of adenomyosis and endometriosis pathogenesis. Reprint from [13] with permission from Elsevier.

The adjacent myometrium seems to be very compact with a changed architectural arrangement of the myometrium in comparison to the unaffected myometrium. Similar to the junctional zone smooth muscle metaplasia seems to be present in this area, resulting in a compact packed myometrium around the lesions. These myometrial cells show a moderate to strong oxytocin and vasopressin receptor (OTR/VPR) expression. The adenomyotic stromal cells are mostly OTR negative with the exception of some single cells, which fuse with the surrounding smooth muscle cells of the myometrium. Using the antibody against sm-Actin, this spindle like cell population has been characterized as smooth muscle cells, which are in direct contact with the adenomyotic stroma cells. OTR and VPR are known as being very important uterotonic peptides and may play a crucial role in the dispersalist of AM. In comparison to the unaffected myometrium there seems to be an overexpression of the OTR in myometrial cells surrounding the adenomyotic stroma and epithelium, however it might be possible that the OTR-overexpression is due to the compact morphology of the myometrial cells.

OTR expression is present in the normal endometrium of patients with and without AM with cyclical changes especially in the functional layer. In AM lesions there is only a weak expression of OTR in epithelial cells present, interestingly the cells do not show a cyclic variation. OTR-expression in the AM lesion is similar to the expression described for the basal layer of the endometrium [12]. VPR is also expressed in all layers of the myometrium of the non-pregnant uterus. Interestingly, an immunoreactive staining in the smooth muscle cells of the blood vessels is also present. In contrast to the OTR expression there is no VPR expression in endometrial epithelial cells. In adenomyotic epithelial and stromal cells there are no VPR expression detectable. In contrast, the smooth muscle cells of the myometrium and blood vessels do show a moderate to strong VPR expression. So both receptor systems might be involved in hyperperistalsis.

Pathogenesis of Hyperperistalsis and Dysmenorrhoea

Myometrial hyperactivity and reduced uterine blood flow causes ischemic pain. This is a well-established phenomenon in women with primary dysmenorrhoea, meaning women have a painful menstruation starting with their first period [17]. Oxytocin, Vasopressin as well as prostaglandin (PG) F2a are involved in causing myometrial contractility. Peptides like Vasopressin, Endothelin, noradrenaline and endoperoxides might contribute to the vasoconstriction. However, the relationship between adenomyosis and dysmenorrhoea needs to be clarified. There is still little knowledge about the pathophysiology of adenomyosis-associated dysmenorrhoea. Recently it has been demonstrated that the uteri from patients with adenomyosis and endometriosis show hyper- and dysperistalsis [18–20]. The physiological role of OT and VP in non-pregnant uteri has not been fully understood. There is a body of evidence, that these peptides play an essential role in uterine contractility and dysmenorrhoea. The changed architectural myometrial composition might be the cause of the ongoing hyper- and dysperistalisis, sometimes also with insufficient contraction resulting in high menstrual bleeding.

Changes in the Uterine Innervation of AM

One of the main topic of the last few years was the question: Are there changes in the uterine innervation of patients with AM and does this have an impact on the pathogenesis? A systematic investigation regarding the innervation of uteri with AM was performed: full thickness specimens after a hysterectomy were selected and the innervation in all layers were investigated separately. Four different kinds of pan marker for myelinated and unmyelinated nerve fibers (NF) were used. No significant difference was found between patients with and without AM. There were nearly no nerve fibers in the functional layer, only single ones. More NF were detected in the basal and junctional layer and nearly 4 NF per mm² in the myometrium. The adenomyotic lesions were also rarely innervated [21].

In order to understand the pathophysiology and the pain mechanisms in more detail the adenomyosis associated nerve fibers had to be characterised. In general, we were interested in the correlation of sympathetic and sensory nerve fibers, because these two nerve fiber systems seem to have a crucial role in chronic inflammatory diseases. For this, different nerve fiber markers were used to distinguish between sensory and sympathetic nerve fibers. Interestingly in adenomyotic myometria there were a reduced number of sympathetic nerve fibers observed [21]. Changes in the sympathetic uterine innervation are a known phenomenon physiologically during cycle and pregnancy. There seems to be hormone dependent changes present in the myometrium of rodents. In the estrogen rich phase, the sympathetic nerve fibers decrease, and remodel in the estrogen-poor phases [22, 23]. This is also observed in

humans with ongoing pregnancy, with the lowest level at term. Maybe this has an influence on the contractility and make the uterus calm with ongoing pregnancy. But so far there is no data available about the biological reason for this.

But the question is what happens in adenomyosis, what is the reason for the reduced number of sympathetic nerve fibers in AM? If the higher estrogen expression is involved in this process, then maybe the aromatase overexpression with local estrogen synthesis is the trigger for the disturbed remodelling of sympathetic nerve fibers in adenomyotic uteri. Perhaps this also has a functional effect on adenomyosis associated dysmenorrhea and infertility with disorder in the contraction pattern with dysperistalsis.

Conclusion

There seems to be evidence for a microtrauma associated pathway involved in the pathogenesis of AM like fibroblast to myofibroblast transdifferentiation, especially in the area of the JZ. Activation of pale cells might be involved in the pathogenesis of EM/AM. The translocated pale cells could have the potential to develop into fully differentiated lesions including stromal, epithelial as well as smooth muscle cells.

Changes of the architectural morphology of the myometrium might be a reason for the dys-and hyperperistalsis. The Oxytocin as well as the Vasopressin receptor system might be involved here as well. OTR is expressed in epithelial and smooth muscle cells in adenomyosis uteri. Oxytocin mediates the induction of PGF2α synthesis in epithelial cells, which mediates pain and oxytocin induces smooth muscle contraction. The change in the myometrial architecture amplifies the dys- and hyperperistalsis of the myometrial cells. The selective inhibition of this receptors system might be a promising new treatment option for endometriosis-associated dysmenorrhoea and cyclic pelvic pain, especially for women who want to become pregnant. The disturbed remodeling of sympathetic NF might be another functional problem in the coordination of the myometrial acitivity. Altogether adenomyosis seems to be a disorder which causes defects in the coordination of uterine contractions.

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