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Endometriosis and the Expression of Invasion Factors in both the Endometrium and the Peritoneum

Diploma Thesis

to be conferred with the academic degree “Diplom-Ingenieur”
(Dipl.-Ing.)

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Vienna, 06/2015

Declaration

I hereby declare that I have developed and written the enclosed Master Thesis completely by my own, and have not used sources or means without declaration in the text. Any thoughts from others or literal quotations are clearly marked. During RNA-Isolation and cDNA synthesis I was supported by my dear colleagues (K. Pröstling, S. Balendran, N. Nirtl and E. Marton). K.Pröstling and S. Balendran also helped me with statistical analysis. The Master Thesis was not used in the same or in a similar version to obtain an academic grading or is being published elsewhere.

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ABSTRACT

Background: Different invasion factors including matrix related proteins leading to an early defect in the physiological activity of the endometrium, which result in its overgrowth outside the uterine cavity. Hallmarks of the establishment of endometriosis are the local ECM (extracellular matrix) remodelling and the active invasion of endometrial cells into mesothelium of the peritoneum, which are assisted by peritoneal production of several Matrix Metalloproteinases (MMPs) and ADAMs (A Disintegrin and Metalloproteinase). Tissue inhibitors of metalloproteinases (TIMPs) are the functional inhibitors of MMPs and thus MMP-TIMP imbalance may contribute to the development of ectopic lesions. A total of 114 premenopausal women were recruited in the study, who underwent laparoscopic surgery due to the suspicion of endometriosis with or without infertility.

Methods: *MMP-9, MMP-28, TIMP-1, ADAM-10 and ADAM-17* mRNA expressions were analyzed by qRT-PCR from 53 healthy women and 61 patients with endometriosis. 151 fresh endometrial tissue samples, including 62 ectopic lesions were collected during surgical procedure. In addition 54 fresh tissue samples from normal peritoneum were included in this study.

Results: Significant higher *MMP-9* and *MMP-28* expression was observed in the ectopic endometrium compared to the eutopic endometrium of controls (both $p<0.001$) and compared to the eutopic endometrium of women with endometriosis ($p=0.001$ and $p<0.001$). *ADAM-10* and *ADAM-17* mRNA expressions were significantly increased in ectopic lesions compared to the eutopic endometrium of controls (both $p<0.001$). In 10 matched cases *TIMP-1* expression was significantly decreased in ectopic lesions compared to the eutopic endometrium of patients ($p=0.005$).

Conclusion: *MMP-9* and *MMP-28* expressions were significantly higher expressed in ectopic lesions compared to the eutopic endometrium of controls and patients. Thus suggests that *MMP-9* and *MMP-28* contribute to invasive disease of endometriosis. Additional results suggest that uterine endometrium from women with endometriosis may be biologically different from endometrium in normal women. *ADAM-10* and *ADAM-17* were significantly higher expressed in ectopic lesions and in the eutopic endometrium of patients compared to the eutopic endometrium of healthy women. The eutopic endometrium from endometriosis patients appears to be more invasive and prone to peritoneal implantation than that from women without the disease.

Key Words: endometriosis, endometriotic invasion factors, peritoneum, *MMP-9, MMP-28, TIMP-1, ADAM-10, ADAM-17*

KURZFASSUNG

Hintergrund: Verschiedene Invasionsfaktoren einschließlich Matrix Metalloproteinasen (MMPs) und ADAMs (A Disintegrin and Metalloproteinase) führen zu einem frühen Defekt in der physiologischen Aktivität des Endometriums und die darauffolgende Wucherung aus der Gebärmutterhöhle. Kennzeichen einer Endometriose-Erkrankung sind der extrazellulare Matrix(ECM)-Umbau und die aktive Invasion von Endometriosezellen in das Mesothelium des Peritoneums, das von Produktion mehrerer Matrix Metalloproteinasen (MMPs) und ADAMs (A Disintegrin and Metalloproteinase) unterstützt wird. So genannte Gewebeinhibitoren von Metalloproteinasen (TIMPs) sind die funktionellen Inhibitoren von MMPs und somit kann ein MMP-TIMP Ungleichgewicht zur Entwicklung ektoper Läsionen beitragen. In dieser Studie wurden insgesamt 114 prämenopausale Frauen mit oder ohne Unfruchtbarkeit, die sich einer laparoskopischen Chirurgie auf Verdacht einer Endometriose unterzogen haben, einbezogen.

Methoden: *MMP-9, MMP-28, TIMP-1, ADAM-10* und *ADAM-17* mRNA Expressionen von 53 gesunde Frauen und 61 Endometriose-Patienten wurden mittels qRT-PCR analysiert. Insgesamt wurden 151 endometriale Gewebsproben, die 62 ektopische beinhalten und 54 peritoneale Gewebsproben untersucht.

Ergebnisse: Im Vergleich zum eutopen Endometrium wurde im ektopen Endometrium signifikant höhere *MMP-9* und *MMP-28* Expressionen ($p<0.001$) gefunden. *ADAM-10* und *ADAM-17* mRNA Expressionen waren im Vergleich zum eutopen Endometrium der Kontrollfälle in ektopen Läsionen erhöht ($p<0.001$). In gepaarten Fällen war die *TIMP-1* Expression im ektopen Endometrium signifikant niedriger als im eutopen Endometrium ($p=0.005$).

Schlussfolgerung: Aufgrund der erhöhten MMP-9 und MMP-28 Expressionen in ektopen Läsionen im Vergleich zum eutopen Endometrium lässt sich daraus schließen, dass diese MMPs zur invasiven Erkrankung der Endometriose beitragen. Für ADAM-10 und ADAM-17 wurde selbiges gefunden mit der Ergänzung, dass die Expressionswerte im Endometrium der Patienten signifikant höher waren als im Endometrium der Kontrollfälle. Diese Ergebnisse legen nahe, dass jene eutope Endometrium von Endometriose -Patienten biologisch anders ist als das der gesunden Patienten. Das eutope Endometrium scheint stärker invasiv und anfälliger für eine peritoneale Implantation als das der Frauen ohne Endometriose.

Schlüssel Wörter: Endometriose, endometriale Invasionsfaktoren, Peritoneum, *MMP-9, MMP-28, TIMP-1, ADAM-10, ADAM-17*

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ABBREVIATIONS

ACE	Angiotensin-converting enzyme
ACTB	β-actin
AD	Aqua destillata; distilled water
ADAM	A disintegrin and metalloprotease domain
ADAMTSs	A disintegrin-like and metalloproteinase domain with thrombospondin type 1 repeats
APP	Amyloid precursor protein
β-ME	β-Mercaptoethanol
CCD	Charge-coupled device
CD molecules	Cluster of differentiation molecules
cDNA	Copy deoxyribonucleic acid
COOH	Carboxyl group of carboxylic acid
CT	Cycle threshold
CYS-rich	Cysteine-rich region
DEPC water	Diethypyrocarbonate treated water
DIE	Deeply infiltrating endometriosis
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxynucleoside triphosphate
DTT	Dithiothreitol
EBAF	Endometrial-bleeding-associated factor
ECM	Extracellular Matrix
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMT	Epithelial to mesenchymal transition
ErbB	Erythroblastic Leukaemia Viral Oncogene Homolog
Fas	Type II transmembrane protein

FasL	Fas Ligand
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GnRH	Gonadotropin-releasing hormone
HB-EGF	Heparin-binding EGF-like growth factor
HER	Human epidermal growth factor
HGF	Hepatocyte growth factor
HP	Hepatoglobulin
ICAM I	Intracellular adhesion molecule I
IL	Interleukin
MCP I	Monocyte chemotactic protein I
MCSF I	Macrophage colony stimulating factor I
$MgCl_2$	Magnesium chloride
miRNA	Micro ribonucleic acid
MMP	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
na	not available
NH2	Amino group
NK cells	Natural killer cells
oligo dT	Oligo deoxythymine
PCR	Polymerase chain reaction
qRT- PCR	Quantitative real-time PCR
RAFS	Revised American Fertility Society
RNA	Ribonucleic acid
RNase	Ribonuclease
RT	Room temperature
RT	Reverse transcriptase
RT-PCR	Real time PCR
RXN	Reaction
SPSS	Statistical Package for the Social Sciences
TACE	Tumour necrosis factor-α converting enzyme
TGF-α	Transforming growth factor-α

TGF-β	Transforming growth factor-β
TIMP	Tissue inhibitor of matrix metalloproteinase
TM	Transmembrane domain
TNF-α	Tumour necrosis factor- α
TNFR	TNF receptor
TREG cells	Regulatory T-lymphocytes
TSP-1	Thrombospondin type 1 motif
VCAM I	Vascular cell adhesion molecule I
VEGF	Vascular endothelial growth factor
VEGFR	VEGF receptor
Zn^{2+}	Zinc-Ion

1. INTRODUCTION

1.1. Endometriosis

1.1.1. Background of Endometriosis

Human endometrium is a greatly dynamic tissue, which undergoes periodic growth and regression at each menstrual cycle. Endometriosis is a common oestrogen-dependent inflammatory disease which affects up to 10% of women during the reproductive years (Simpson et al., 1980). It severely affects the health and quality of life of women. Endometriosis is a highly variable condition in terms of age at start and mode of presentation, range of symptoms, anatomical sites and probability of recurrence. In the past few decades, endometriosis has been actively investigated, yet it is still a puzzling disease (Liu and Lang, 2011). The chronic disorder is often associated with pelvic pain and infertility. The uterine endometrial tissue is also present outside of the normal location, primarily on the pelvic peritoneum or on the ovaries (Giudice and Kao, 2004). The disease can furthermore be inherited in a polygenic manner (Simpson et al., 1980). The eutopic endometrium of women with endometriosis shows fundamental differences compared to women without the disease (Lang, 2006). These differences may contribute to the survival of endometrial cells in the pelvic cavity, and thus to the occurrence of endometriosis.

1.1.2. Theories about Pathogenesis

Possible theories on the pathogenesis of endometriosis:

- ⇨ Retrograde menstruation/transplantation
- ⇨ Coelomic metaplasia
- ⇨ Altered cellular immunity
- ⇨ Metastasis
- ⇨ Genetic Basis
- ⇨ Environmental basis

The most widely accepted theory on the pathogenesis of endometriosis is the retrograde menstruation, where endometrial tissue peels through fallopian tubes into peritoneal surface (Sampson, 1927). The theory is supported by the finding that women with endometriosis have higher volumes of refluxed menstrual blood and endometrial tissue fragments than women without endometriosis (Halme et al., 1984). Diagram of events occurring in the pelvis with retrograde menstruation is given below (Figure 1).

This hypothesis is a plausible mechanism for endometrial lesions but does not explain why endometriosis develops only in some women.

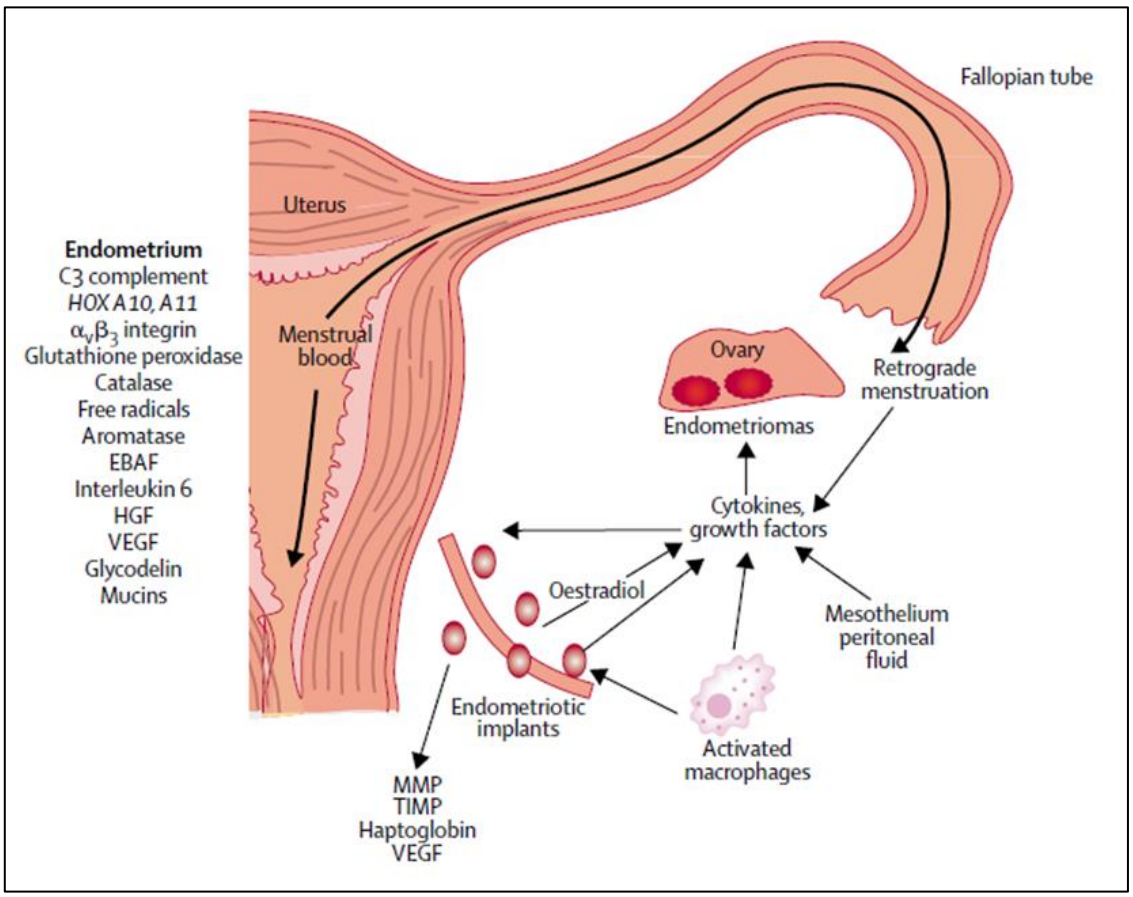

Figure 1 Retrograde menstruation occurring in the pelvis
The above figure shows events occurring in the pelvis with retrograde menstruation, cellular and biochemical principles involved in the pathogenesis of endometriosis. EBAF=endometrial-bleeding-associated factor; HGF=hepatocyte growth factor; VEGF=vascular endothelial growth factor; MMP=matrix metalloproteinase; TIMP=tissue inhibitor of metalloproteinase (Giudice and Kao, 2004).

A further theory may be a failure of the immune system to clear implants from peritoneal cavity (Dmowski et al., 1989; Giudice and Kao, 2004; Osteen and Sierra-Rivera, 1997). The excessive formation of oestrogen and prostaglandin by a stromal cell defect is responsible for increased cell survival, inflammation and incorrect differentiation in endometriosis (Bulun, 2009). It is well established that women with the disease have increased peritoneal fluid cytokines concentrations compared to women without the disease (Oral and Arici, 1997; Zhou and Nothnick, 2005). Further theories on the histogenesis of endometriosis are coelomic metaplasia, embryonic cell rest, induction and lymphatic and vascular propagation (Nezhat et al., 2008).

The heritable features of endometriosis were first recognized more than 20 years ago when the risk for first-degree relatives of women with severe endometriosis was

reported to be six times higher than that for relatives of unaffected women. In the field of environmental factors, there has been no epidemiological study definitively linking one class of chemicals to the risk of endometriosis (Giudice and Kao, 2004).

Bartley et al reviewed that epithelial-mesenchymal transition (EMT) is a prerequisite for the establishment of endometrial lesions (2014). It defines a migratory and invasive process by which stationary epithelial cells convert into highly motile mesenchymal cells (Hay, 1995). That is also why single cells are able to break through the basement membrane, grow invasively and spread metastasis (Boyer et al., 2000). These processes are required for the establishment of an endometrial lesion compliant with Sampson´s implantation theory.

1.1.3. The Mechanism of the Abnormal Eutopic Endometrium

According to the published literature the abnormal eutopic endometrium may be seen as the origin of endometriosis. As indicated in 1.1.2. many events in the endometrium such as defective immune surveillance, retrograde menstruation, endometrial-peritoneal attachment and altered gene expression play a key role in the development of the benign disease. After overcoming a phase of immune tolerance, endometrial cells attach to mesothelium and invade the extracellular matrix. After the last step of angiogenesis, the endometrial cells establish a new blood supply for the survival of implants, continue to proliferative in ectopic sites, and finally result in endometriosis (Figure 2).

1.1.3.1. Immune System

In addition to Sampson´s implantation theory, a second mechanism of the development of endometriosis could be a failure of the immune system to clear implants from peritoneal surface (Bulun, 2009). Guidice et al reviewed that there is much evidence of activation of peritoneal macrophages with increased cytokine production in women with endometriosis (2004). Eyster et al speculated that macrophages stimulated the expression of distinct genes in endometrial stromal cells that lead to the survival of endometrial cells in ectopic sites (2010). Furthermore there is a decreased cytotoxic activity of NK cells in peripheral blood and peritoneal fluid from patients with endometriosis (Wilson et al., 1994). Numerous researchers reviewed that there is defective T-lymphocyte function in endometriosis. Eutopic endometrium may cause apoptosis of surrounding lymphocytes and thereby escape from the attack by lymphocytes, and further develop into ectopic implants (Liu and Lang, 2011). Regulatory T-lymphocytes (Treg cells) suppress activation of the immune system and maintain immunostasis and tolerance to self-antigens (Feuerer et al., 2009). Apoptosis

is a programmed cell death, which is another factor that contributes to the survival of endometrial cells in the peritoneal cavity and the development of endometriosis (Liu and Lang, 2011). Eutopic endometria of women with endometriosis demonstrate reduced apoptosis compared to endometria of controls, especially during late secretory/ menstrual and early proliferative phases (Beliard et al., 2004).

1.1.3.2. Attachment of Endometrial Cells to the Peritoneum

After immune surveillance of endometrial cells in the pelvic cavity the next step is attachment to the peritoneum. Integrins are proteins which mediate adhesion of cells to either neighbouring cells or to extracellular matrix, and they play an important role in this adhesion process (Liu and Lang, 2011). Increased Integrin expression has been discovered in eutopic endometrium of women with endometriosis compared to women without the disease (Kyama et al., 2008). Hyaluronic acid is a major component of the extracellular matrix (ECM) and is expressed on the membrane of peritoneal mesothelial cells. Griffith et al indicated that CD44, a key receptor for hyaluronic acid is highly expressed in endometrial epithelial and stromal cells compared to normal controls without the disease (2010). This hyaluronic acid- CD44 binding complex may be an important participant of the initial attachment event of endometrium to peritoneal mesothelial cells (Hasegawa et al., 2010). Furthermore E-cadherin/ catenin complex is crucial in signal transduction from the outer cell surface to the cytoskeleton and attends in the cell-to-cell attachment between the endometrium and peritoneal mesothelial cells (Liu and Lang, 2011).

1.1.3.3. Invasion of Endometrial Cells into Mesothelium

Tissue invasion requires destruction of the extracellular matrix. Several studies documented that the lysis of the ECM and invasion of mesothelium following attachment could be an important step for the successful invasion of the endometrial cells in endometriosis. Different proteases are involved in ectopic invasion. Among several invasion factors, matrix metalloproteinases (MMPs) mainly favour invasion of endometrial cells into the mesothelium. Many studies which have investigated in eutopic endometria of patients with endometriosis have shown increased expression of MMPs, including *MMP-1, MMP-2, MMP-3, MMP-7, MMP-9 and MMP-11*, and in addition reduced levels of the MMP inhibitors (TIMPs) – *TIMP-1*, TIMP-2, TIMP-3 (Liu and Lang, 2011; Zhou and Nothnick, 2005). Enhanced MMPs expression can promote the invasion of endometrial cells and the establishment of ectopic lesions. Blocking of MMPs activity by expression of TIMPs could result in a significant inhibition of the invasive disease development (Lee et al., 2011). Osteen et al indicated that eutopic

endometrial tissues from women with endometriosis demonstrate a reduced response to progesterone, allowing a continuous expression of MMPs throughout the secretory phase, which reflects the inherent capacity of endometrial tissue to break down the ECM (2005).

1.1.3.4. Blood Supply by Angiogenesis

Liu and Lang reviewed that endometrial fragments located on peritoneum require the establishment of a new blood supply for the survival of implants after local invasion of the basement membrane (2011). Among the identified angiogenic factors, vascular endothelial growth factor (VEGF) is the most potent factor in endometriosis. Eutopic epithelium of women with endometriosis, as compared to endometrium from healthy women, exhibited significantly increased VEGF levels (Donnez et al., 1998). Also VEGF-receptors (VEGFR) have significantly higher expression values in eutopic endometrium from women with endometriosis than in eutopic endometrium from controls without the disease (Wang et al., 2005). Recent research indicated that epidermal growth factor (EGF) and its receptor HER-1 play an important role in angiogenic event (Ejskjaer et al., 2005; Liu and Lang, 2011). The single consecutive steps in the pathogenesis of endometriosis are shown in a simplified view in Figure 2.

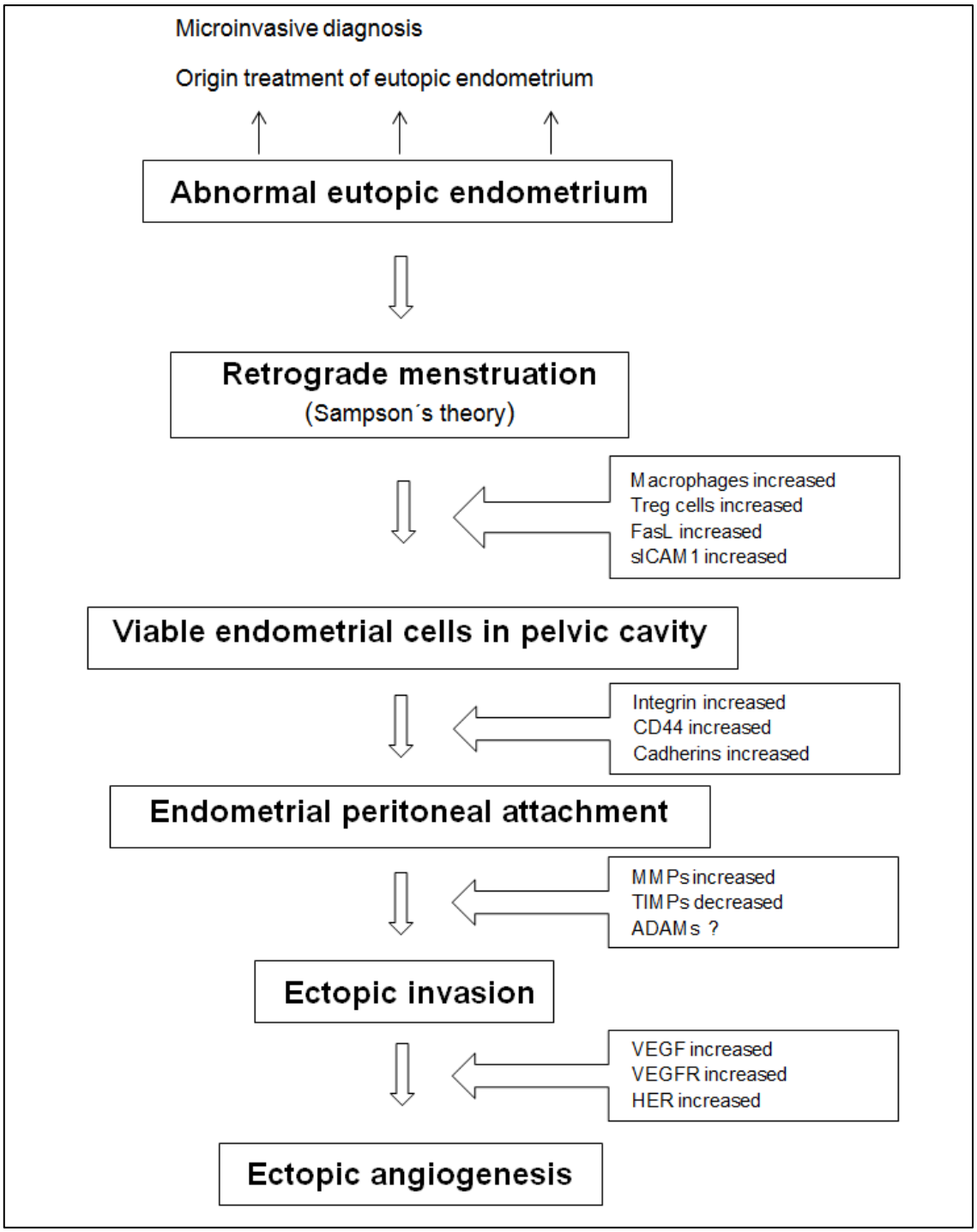

Figure 2 Role of eutopic endometrium in pathogenesis of endometriosis
After overcoming a phase of immune tolerance, endometrial cells attach to mesothelium and invade the extracellular matrix. After the last step of angiogenesis, the endometrial cells establish a new blood supply for the survival of implants, continue to proliferative in ectopic sites, and finally result in endometriosis (down arrow). Up arrows show the clinical implications. SICAM=soluble intercellular adhesion molecule-1; FasL=Fas ligand; treg cells=regulatory T lymphocytes; MMPs=matrix metalloproteinases; TIMPs=tissue inhibitors of matrix metalloproteinases; ADAMs=a disintegrin and metalloproteinases; VEGF=vascular endothelial growth factor; VEGFR=VEGF receptor; HER=human epidermal growth factor (Liu and Lang, 2011).

1.1.4. Role of Peritoneum in Endometriosis

The peritoneum is the largest serous membrane in the body and is composed of three layers: the mesothelium, its basement membrane and the underlying connective tissue (Young et al., 2013). Peritoneal cavity fluid is formed from plasma transudate and ovarian exudate (Oral et al., 1996) and alters in volume throughout the female menstrual cycle. The large surface area of the peritoneal cavity allows passive dialysis of large quantities of substances between peritoneal fluid and blood plasma (Young et al., 2013). The pelvic peritoneum is the most common site for endometrial implants with endometriosis occurring in the Pouch of Douglas in over 80% of patients (Mahmood and Templeton, 1991).

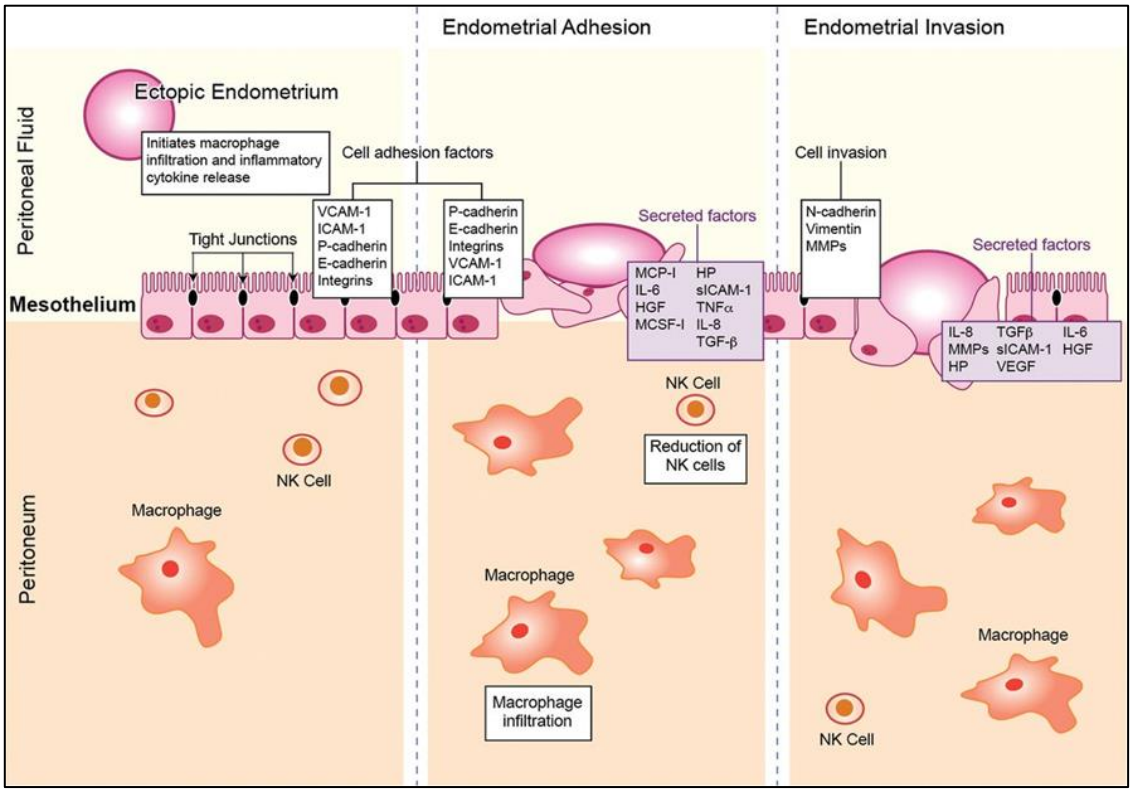

Figure 3 Presentation of the events occurring in the establishment of peritoneal endometriosis
Peritoneum and expressed factors that may contribute to the adhesion and invasion of ectopic endometrial cells during the establishment of peritoneal endometriosis. VCAM-I=vascular cell adhesion molecule I; ICAM-I=intracellular adhesion molecule I; NK cells=natural killer cells; MCP-I=monocyte chemotactic protein; IL=interleukin; HGF=hepatocyte growth factor; MCSF-I= macrophage colony stimulating factor I; HP=hepatoglobulin; TNFα= tumour necrosis factor α; TGFβ= transforming growth factor β; MMP=matrix metalloproteinase; VEGF=vascular endothelial growth factor.

Evidence from a study using a mouse model suggest a close interplay between endometrial and peritoneal cells which can lead to development of ectopic lesions (Hull et al., 2008). After endometrial cell adhesion to pelvic peritoneal mesothelium, the ectopic endometrial cells are able to invade into the peritoneum to establish

themselves as lesions (Young et al., 2013). Peritoneal mesothelial cells are capable of secreting MMPs through regulation by TGF-β. This expression has been linked to ECM remodelling in peritoneal adhesion formation (Figure 3) (Ma et al., 1999).

1.1.5. Endometriosis associated Infertility and Malignant Diseases

1.1.5.1. Infertility

The pathogenesis of endometriosis-related infertility is still unclear. Guidice et al recorded that there is growing evidence supporting abnormal eutopic endometrium and implantation failure in some women with endometriosis as an underlying cause of infertility in this population. Additionally evidence is accumulating of aberrant gene and gene-product expression in eutopic and ectopic endometrium at other periods of the cycle that may be related to infertility or to the development of the disease (2004). Some researchers also found that dysregulation of distinct genes in eutopic endometrium of patients with endometriosis can lead to impaired embryonic attachment, embryo toxicity, immune dysfunction and apoptosis during the event of implantation (Kao et al., 2003; Liu and Lang, 2011).

1.1.5.2. Malignant Disorders

Women with endometriosis face an increased risk of malignant tumours of the pelvis (Van Gorp et al., 2004). Guidice et al reviewed that endometriosis is more frequently found in women undergoing surgery for endometroid, clar-cell and mixed subtypes of ovarian cancers than in women with serous, mucinous, and other subtypes of cancers. Sainz de la Cuesta and colleagues have reported a high frequency of mutations in the tumour suppressor p53 in atypical endometriosis and ovarian cancer associated with endometriosis (2004). A study from Japan found that a mutation in the p53 gene is not associated with endometriosis (Omori et al., 2004), which emphasises the need for more precise assessment of women at risk of endometriosis and ovarian cancers (Giudice and Kao, 2004). Therefore annual physical screening is crucial for early detection of malignant disorders in women with endometriosis.

1.1.6. Clinical Implication in Diagnosis and Treatment of Endometriosis

Severe endometriosis often results in extensive pelvic adhesions and deformation of the pelvic anatomy, which can lead to pain and infertility, as mentioned above (1.1.1.). In women with mild disease and no symptoms the risk that pain will develop is very low (Moen and Stokstad, 2002). The standard for diagnosis of pelvic disease is surgical assessment by laparoscopy. For women with pain, surgery only provides temporary relief as symptoms recur in up to 75% of women within 2 years (Candiani et al., 1991; Olive and Pritts, 2002). Peritoneal implants are cut out or vaporized by usage of electric current or laser. Ovarian lesions and rectovaginal endometrial nodules can be removed with simple dissection (Olive et al., 2004).

Therapies have involved steroids, progestogens and agonists of gonadotropin-releasing hormone (GnRH), as well as androgens and non-steroidal anti-inflammatory agents (Olive et al., 2004; Valle and Sciarra, 2003). These treatments can be used only for a limited time as they are leading to unwanted side-effects, thus changes or additions of medications are definitely needed (Giudice and Kao, 2004).

However endometriosis is defined as the growth of ectopic endometrial tissue. Furthermore altered expression of different invasion factors has been associated with the disease. Therefore research focusing on molecular changes both within the peritoneum and endometrium is essential. Additionally appropriately designed clinical trials are indispensable for determining which therapies are safe and effective.

1.2. Invasion Factors

In the human endometrium and peritoneum, several Matrix Metalloproteinases (MMPs) are expressed and some have already shown to be involved in the pathogenesis of endometriosis and cancers, in particular in invasion and metastasis (Giudice et al., 1998). In endometriosis the endometrial tissue is able to attach itself to the host tissue, then invade it and derive the local vasculature to ensure its own blood supply (Giudice et al., 1998). The degradation of the extracellular matrix (ECM) is a major step for the formation of new vessels in angiogenesis and tissue remodelling (Moses et al., 1996). In addition, the closely related ADAMs (A Disintegrin and Metalloproteinase) also regulate cell invasion and migration by proteolytic ectodomain shedding in cancer progression (Rocks et al., 2008). Tissue inhibitors of matrix metalloproteinases (TIMPs) are secreted in insufficient amounts before menstruation, thus leading to an imbalance between TIMPs and the MMPs which are relevant for the degradation of the endometrium (Marbaix et al., 1996). This study focused on altered expression of invasion factors, such as *MMP-9*, *MMP-28*, *TIMP-1*, *ADAM-10* and *ADAM-17*, in women with and without endometriosis.

1.2.1. Matrix Metalloproteinases

Many factors are essential for the degradation of the ECM needed for the implantation of endometrial tissue in ectopic sites, including matrix metalloproteinases (MMPs) (Chung et al., 2002; Kokorine et al., 1997). MMPs comprise a family of 23 structurally related proteolytic enzymes that require zinc for their activation (Duncan et al., 1998; Luck and Zhao, 1995). Detailed structure is shown in Figure 4. Before their activation they are secreted in a latent form as proenzymes (Van Wart and Birkedal-Hansen, 1990). Among MMPs there is a wide range of different specificities. Together the MMPs degrade most components of the ECM, including the different types of collagens that make up the basement membrane (Freitas et al., 1999). These constituents include also gelatine, fibronectin and laminin. Degradation of the ECM by MMPs occurs in everyday physiological processes such as wound repair, angiogenesis, and different reproductive processes (Curry and Osteen, 2001; Nagase and Woessner, 1999; Sternlicht and Werb, 2001). They regulate biological activity of many proteases, growth factors, cytokines and their specific receptors. A decrease in MMP activity inhibits the invasion of tumour cells both in vivo and in vitro (DeClerck et al., 1992). Within the endometrium, the MMP system is also regulated by steroid hormones, growth factors and cytokines under normal physiological conditions (Curry and Osteen, 2001).

It is evident that an altered expression of MMPs plays an active role in the establishment and progression of endometriosis. Recent studies have shown an increase in the expression of MMPs, especially *MMP-9*, in endometrial tissue (Chung et al., 2001). *MMP-9* is a type IV collagenase which is important for the invasion of trophoblast cells (Behrendtsen et al., 1992) and the progression of tumour (Fishman et al., 1997). In eutopic and ectopic endometria from women with endometriosis, the activity of *MMP-9* is elevated, thus pandering their proteolytic activity and tissue invasion (Chung et al., 2001; Collette et al., 2004; Collette et al., 2006; Liu et al., 2002).

MMP-28 (also called epilysin) is expressed in a number of normal tissues, e.g. testis, lung, intestine and skin (Lohi et al., 2001) as well as in different tumours and tumour cell lines (Marchenko and Strongin, 2001; Nuttall et al., 2003). In epithelial cells, over-expression of *MMP-28* may induce an EMT via activation of latent TGF-β (Illman et al., 2006; Illman et al., 2008). The *MMP-28*-stimulated EMT is associated with the loss of E-cadherin, an important mediator of cell-cell adhesion as well as increased expression of *MMP-9* (Rodgers et al., 2009).

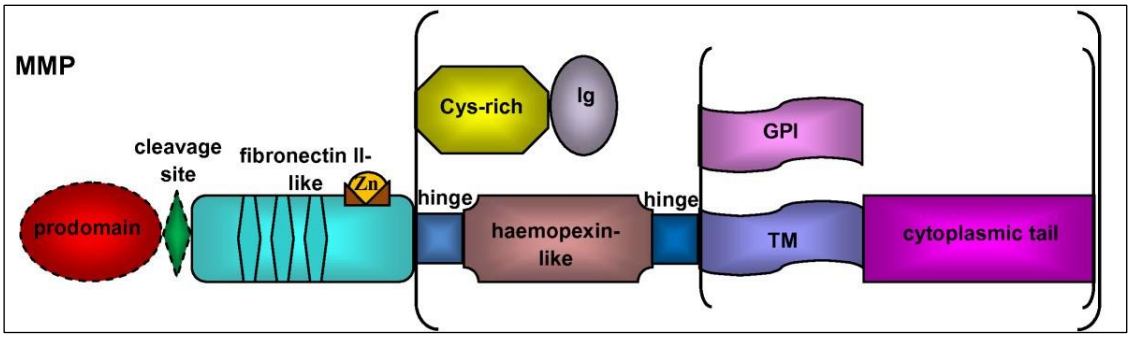

Figure 4 Structural organization of MMPs
The typical structure of MMP is made of prodomain, a furin cleavage site (all Membrane-type MMPs (MT-MMP), MMP-21,-23 and -28), a catalytic metalloproteinase domain with fibronectin type II repeats (MMP-2, *MMP-9*), a linker peptide and a haemopexin domain (except for MMP-7, -26 and -23), a linker peptide, a transmembrane domain and cytoplasmic tail (MMP-14, -15, -16, -24) or glycosylphosphatidylinositol (GPI) anchor (MMP-17,-25). MMP-23 bears C-terminal cysteine-rich (Cys-rich) and Ig-like (Ig) domains and its propeptide lacks a cysteine switch motif.

1.2.2. Tissue Inhibitors of Matrix Metalloproteinases

The matrix metalloproteinase system is composed of the enzymatic component, the MMPs, an enzyme inhibitory component and the tissue inhibitors of metalloproteinases (TIMPs). The production of MMPs and their inhibitors takes place in endometrial stromal and epithelial cells as well as in polymorphic mononuclear leukocytes. Further important sources of the enzymes are macrophages, neutrophils and eosinophils, which are activated during low grade inflammation existent in the peritoneal cavity of women with endometriosis (Jeziorska et al., 1996; Shi et al., 1995; Szamatowicz et al., 2002). TIMPs are secreted in insufficient amounts before menstruation, thus leading to an imbalance between TIMPs and the MMPs which are relevant for the degradation of the endometrium (Marbaix et al., 1996).

TIMP-1 was the first member that was identified. It is a secreted glycoprotein that binds and inhibits active MMPs on a 1:1 stoichiometric basis. Inhibition occurs through the interaction of the N-terminal domain of TIMP with the active site on the catalytic domain and the substrate binding groove (Gomez et al., 1997; Leco et al., 1992). *TIMP-1* may form complexes with pro*MMP-9*, thereby inhibiting the process of *MMP-9* activation (Goldberg et al., 1992). The interaction between *TIMP-1* and *MMP-9* is shown below in Figure 5.

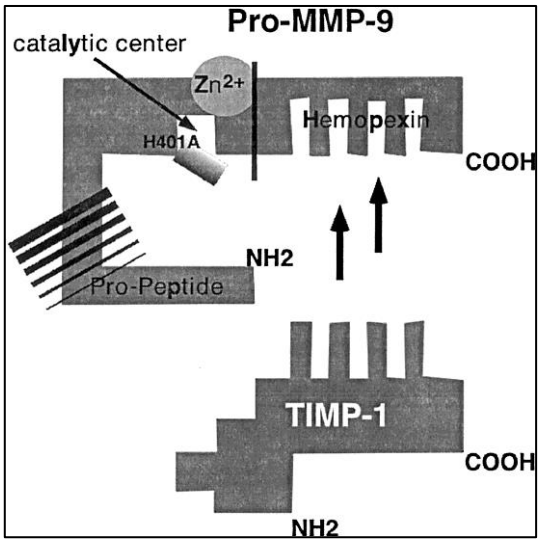

Figure 5 Domain interaction between *TIMP-1* and MMP-9
The rapid tight binding of *MMP-9* to *TIMP-1* is determined by the C-terminal domain of both inhibitor and enzyme. The C-terminal domain of *MMP-9* is not involved in autolytic or cellular activation and does not affect the catalytic activity of the enzyme. However, C-terminal domain interactions between *MMP-9* and *TIMP-1* significantly enhance the rate of complex formation. The N-terminal domain of *TIMP-1* contains the inhibitory site and interactions between *MMP-9* and *TIMP-1* significantly enhance the rate of complex formation. The N-terminal domain of *TIMP-1* contains the inhibitory site and interacts with the N-terminal domain and the catalytic center of the active *MMP-9* (Roeb et al., 2000).

Also *TIMP-1* regulates the active forms of MMP-1 and MMP-3, which inducts to protease inactivation (Hanemaaijer et al., 1993). Laudanski et al. reported, based on their results, that different angiogenesis-related genes, including *TIMP-1* might play an important role in the pathogenesis of endometriosis (2014). In addition Chen et al. referred lower *TIMP-1* and higher *MMP-9* immunostaining in ectopic and eutopic endometrium (2004). Furthermore TIMP-2, TIMP-3 and TIMP-4 have been described (Gomez et al., 1997; Leco et al., 1992). Not all TIMP action is inhibitory of MMP function, thus TIMP-2 has a high affinity for MMP-2 (Curry and Osteen, 2001).

TIMPs also demonstrate selectivity in their inhibition of ADAMs and ADAMTSs which contrasts with their MMP-inhibitory features (Rocks et al., 2008). *ADAM-17* is exclusively inhibited by TIMP-3, *ADAM-10* is sensitive to *TIMP-1* and TIMP-3 (Amour et al., 2000). Moreover TIMPs are able to regulate growth. TIMPs promote embryo growth and development (Satoh et al., 1994), are antiangiogenic agents (Johnson et al., 1994; Murphy et al., 1993) , initiate cell growth in a variety of tissues (Hayakawa et al., 1992), and affect apoptosis (Talhouk et al., 1992).

1.3. The ADAM Metalloproteinases

ADAMs (A Disintegrin and Metalloproteinases) are a family of transmembrane and secreted proteins, which are the principal mediators of proteolytic ectodomain shedding on the cell surface (Figure 7).

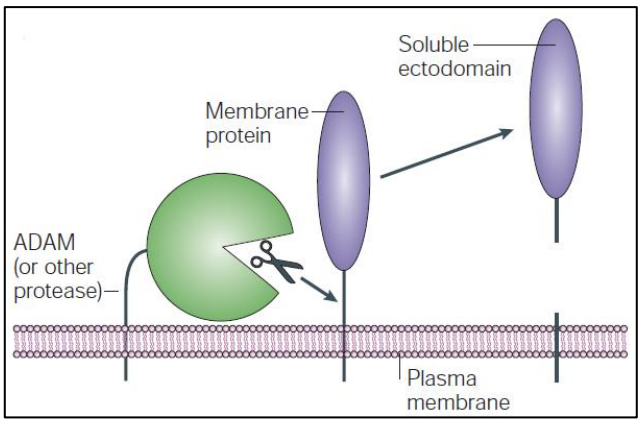

Figure 6 Proteolytic ectodomain shedding
A schematic representation of an ADAM protein that is involved in cleavage of a membrane protein which results in the release of its soluble ectodomain. Structurally and functionally different molecules are engaged in ectodomain shedding. Among these are: cytokines and growth factors - such as tumour-necrosis factor α (TNF-α), transforming growth factor α (TGF-α), heparin-binding epidermal growth factor (HB-EGF); receptors such as TNF receptor-I and -II (TNFRI and TNFRII) and ErbB4); and other molecules (such as Delta, L-selectin, fractalkine, amyloid precursor protein (APP) and angiotensin-converting enzyme (ACE) (Blobel, 2005).

Together with the closely related MMPs and secreted ADAMTSs (a disintegrin-like and metalloproteinase domain with thrombospondin type 1 repeats) they also regulate cellular invasion and migration. ADAMTSs have a proteolytic function an play a predominant role in cancer progression (Rocks et al., 2008). All these enzymes belong to a superfamily of zinc-based proteinases, the metzincins (Edwards et al., 2008; Miller et al., 2013). Detailed structure of ADAMs and ADAMTSs is represented in Figure 6.

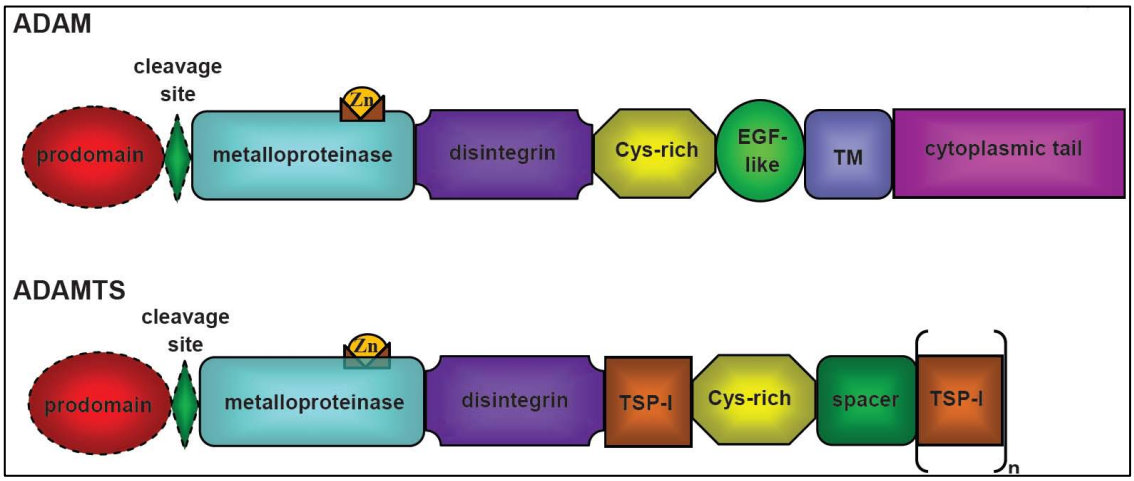

Figure 7 Structural organization of ADAMs and ADAMTSs
Common structure of ADAMs is a prodomain, a cleavage site (by a furin or furin-like proprotein convertase except for ADAM-8 and ADAM-28 which use an autocatalytic process), a metalloproteinase domain, a disintegrin domain, a cysteine-rich region (Cys-rich), an epidermal growth factor repeat (EGF-like), a transmembrane domain (TM) and a cytoplasmic tail. ADAMTSs do not possess a transmembrane domain (TM) but bear a various number of thrombospondin type I motifs (TSP-1) at their C-terminal extremity (Paulissen et al., 2009).

Pollheimer et al. reviewed that about half of the 21 described ADAMs show proteolytic metalloproteinase activity. The function of the ADAMs has been associated with various physiological processes, but has also involved in pathological conditions like inflammation, Alzheimer´s disease and cancer progression (2014).

ADAM-10 and *ADAM-17*, which derive from the same subfamily, are both considered as sheddases and cleave the extracellular domain of membrane-bound proteins, resulting in the release of cytokines, growth factors, adhesion molecules and other enzymes (Brocker et al., 2009).

ADAM-17, also called TACE (tumour-necrosis factor (TNF) α-converting enzyme) and *ADAM-10* (Kuzbanian) are best characterized at present. *ADAM-17* plays an important role in activating EGFR (Blobel, 2005). In addition, *ADAM-17* is upregulated in inflammatory bowel diseases (Brynskov et al., 2002) . Multiple ErbB ligands, including TGFα and HB-EGF are cleaved by *ADAM-17*, whereas EGF and betacellulin are cleaved by *ADAM-10* (Liu et al., 2006; Sahin et al., 2004). *ADAM-10* activates the

Notch signalling by Notch ligand Delta shedding from the cell surface (Hartmann et al., 2002; Qi et al., 1999). However Miller et al found that *ADAM-10* and *ADAM-17* are responsible for cell motility and thus have influence in the invasive disease of endometriosis. They integrate numerous signalling pathways to direct cell motility. Proteomic analysis of samples from endometriosis patients confirmed growth-factor-driven *ADAM-10* activity (2013). Principal, *ADAM-9, -10, -12*, *-15*, *-17* and *-19* are expressed in somatic tissues, it is assumed that these genes show no restricted tissue range (Edwards et al., 2008).

2. GOAL OF THE STUDY

In endometriosis, the success of the ectopic implantation seems to be dependent on changes enabling endometrial cell migration, adhesion and invasive growth, as well as changes in anti-apoptotic signalling, angiogenesis and inflammatory response. MMPs are key enzymes involved in extracellular matrix degradation and therefore allowing cellular movement in foreign tissues. Studies comparing expressions of MMPs in the eutopic endometrium and the ectopic endometrium in women with and without endometriosis exhibit increased expression of MMPs in the ectopic endometrium of women with the disease (Chung et al., 2001; Collette et al., 2004). In ectopic tissue, especially *MMP-9* activity is elevated (Chung et al., 2001), which may be inhibited by *TIMP-1* (Goldberg et al., 1992). Studies have shown that ADAMs play an important role in cell invasion and migration in general (Rocks et al., 2008). *ADAM-10* and *ADAM-17* have also influence on the invasive mechanism of endometriosis via concerted ligand and receptor shedding in numerous signalling pathways (Miller et al., 2013). However, so far no study has yet analyzed the expression of MMPs and their closely related ADAMs in the eutopic and the ectopic endometrium as well in the macroscopically normal peritoneum of women with and without the disease.

The aim of the present study was to investigate mRNA expression levels of *MMP-9*, *MMP-28*, *TIMP-1*, *ADAM-10* and *ADAM-17*. The expression levels were analyzed according to different tissue types, cycle phase, rAFS staging and hormone treatment of women with and without the disease. Possible correlation of gene expressions in the normal peritoneum, the eutopic endometrium and the ectopic endometrium with the development of endometriosis was observed. Differences in gene expressions among the various tissue types might provide theoretical background for progress in diagnosis and treatment of endometriosis. This study should present how tissue remodelling and sheddase activity contributes to cell migration and the development of ectopic lesions.

3. MATERIALS

3.1. Patients and Tissue Samples

3.1.1. Patients

A total of 114 premenopausal women (mean aged 33.3 ± 6.5 years) were recruited in the study, who underwent laparoscopic surgery at the Certified Endometriosis Centre at the university affiliated General Hospital of Vienna due to the suspicion of endometriosis with or without infertility. Among the 114 women, in our study we included 61 patients with endometriosis and 53 control patients without endometriosis. Signed informed consent was obtained from each participant of this study. Staging was performed according to the revised American Fertility Society (rAFS) classification guidelines (I, n=12; II, n=11; III, n=18; IV, n=20) (1997). RAFS is a standardized form for recording pathologic findings which assigns scalar values to the disease status in an effort to predict probability of pregnancy following-treatment. Patients with malignant diseases of the ovaries or the endometrium were excluded. Characteristics of the study population are shown in Table 1.

		total	Cases (n=61)	Controls (n=53)
Age (years)		114	32.11 ± 6.4	34.72 ± 6.3
Cycle Phase	Proliferative	41	24 (58.5%)	26 (63.4%)
	Secretory	57	17 (29.8%)	31 (54.4%)
	na	16	11 (68.8%)	5 (31.3%)
Staging	I or II	23	23 (100%)	-
	III or IV	38	38 (100%)	-
	na	53	-	53 (100%)
Hormone Code three Months	Gestagen Mono	10	7 (70%)	3 (30%)
	Gestagen Combi	12	7 (58.3%)	5 (41.6%)
	others	4	1 (25%)	3 (75%)
	na	88	46 (52.3%)	42 (47.7%)

Table 1 Characteristics of the study population.
Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each column in the proliferative and secretory cycle phases, with low and high stages or taking “Gestagen mono”, “Gestagen Combi” or other treatments. “na”: status not available.

3.1.2. Tissue Samples

Tissue samples were collected between 2010 and 2015 and were analyzed at the Medical University of Vienna. From 114 patients 205 tissue samples in total were obtained, comprising 151 endometrial and 54 peritoneal tissue samples (Figure 8).

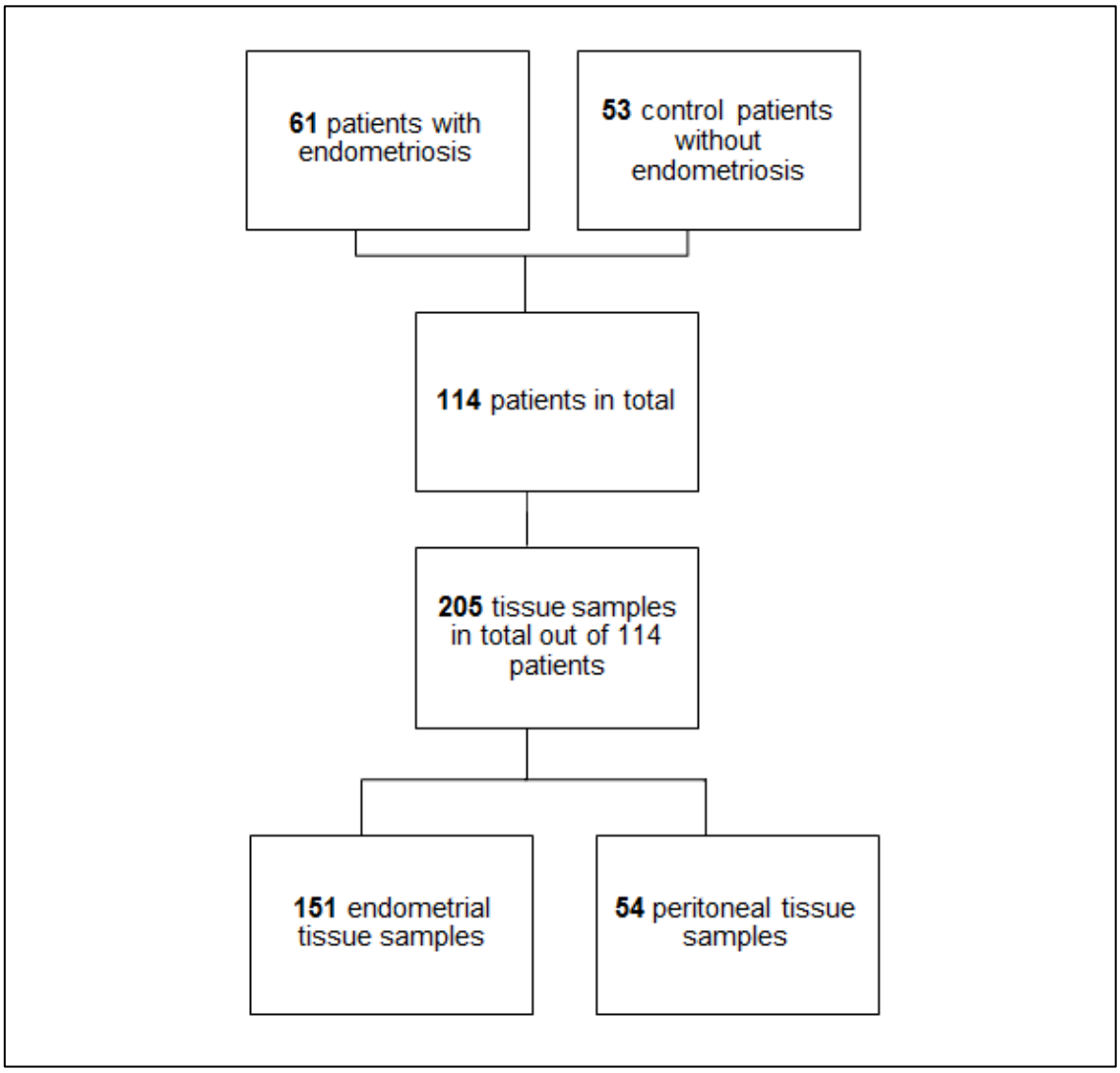

Figure 8 Numbers of patients involved in the study.
Among the 114 women, 61 patients with endometriosis and 53 control patients without endometriosis were included in the study. 205 tissue samples were achieved in total, exclusively 151 samples derived from endometrial tissue and exclusively 54 samples from peritoneal tissue.

3.1.2.1. Endometrial Tissue Samples

151 samples were received from endometrial tissue, which consist of 104 tissue samples from patients with endometriosis and 47 tissue samples from control patients who also underwent hysteroscopy, including dilation and curettage, due to unexplained infertility. Among the 61 cases with endometriosis, we obtained matched samples of

ectopic and eutopic endometrium in 29 cases, exclusively 42 samples of eutopic endometrium and exclusively 62 samples of ectopic endometrium. Ectopic lesions consisted of ovarian lesions (n=40), peritoneal lesions (n=13), and deep infiltrating lesions (n=9).The matched tissue samples were collected during same surgical procedure (Figure 9).

3.1.2.2. Peritoneal Tissue Samples

54 samples from the macroscopically normal peritoneum tissue were collected from lateral wall near the colon, which consisted of 31 tissue samples from cases and 23 tissue samples from controls (Figure 9).

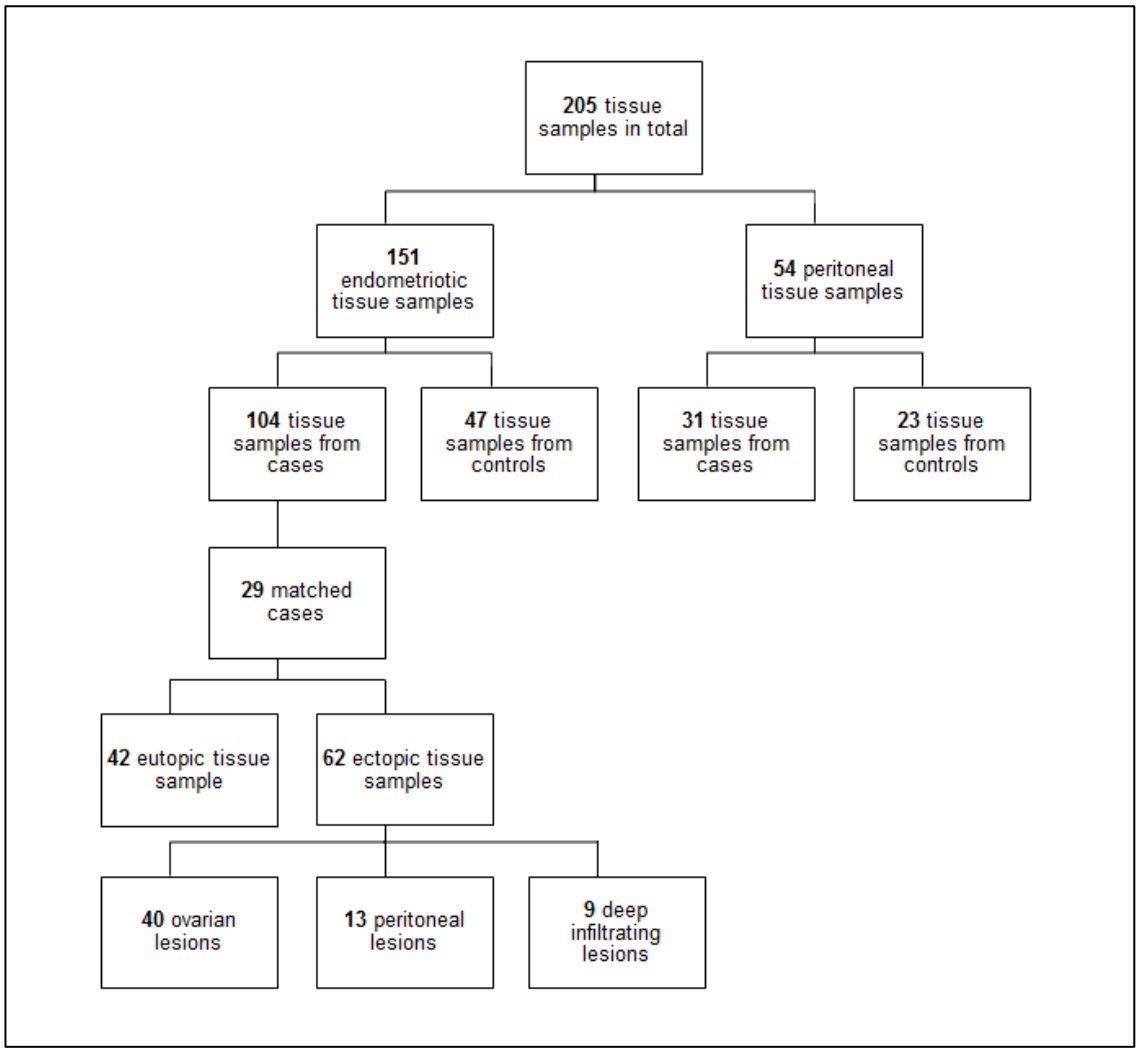

Figure 9 Endometrial and peritoneal tissue samples included in the study
205 tissue samples in total were included in the study. 151 samples were received from endometrial tissue, which consist of 104 tissue samples from patients with endometriosis and 47 tissue samples from control patients. Among the 61 cases with endometriosis, matched samples in 29 cases of ectopic and eutopic endometrium were obtained: exclusively 42 samples of eutopic endometrium and exclusively 31 samples of ectopic endometrium. Ectopic lesions consisted of ovarian lesions (n=40), peritoneal lesions (n=13), and deep infiltrating lesions (n=9). 54 samples were isolated from normal peritoneal tissue, which consisted of 31 tissue samples from cases and 23 tissue samples from controls.

Among the 151 endometrial and the 54 peritoneal tissue samples matched samples of 43 patients were obtained. In 18 cases we achieved matches of eutopic endometrium with peritoneum of control patients without endometriosis. 17 cases included matches of eutopic endometrium with peritoneum of patients with endometriosis, 18 cases included matches of ectopic endometrium with peritoneum of patients with endometriosis (Figure 10).

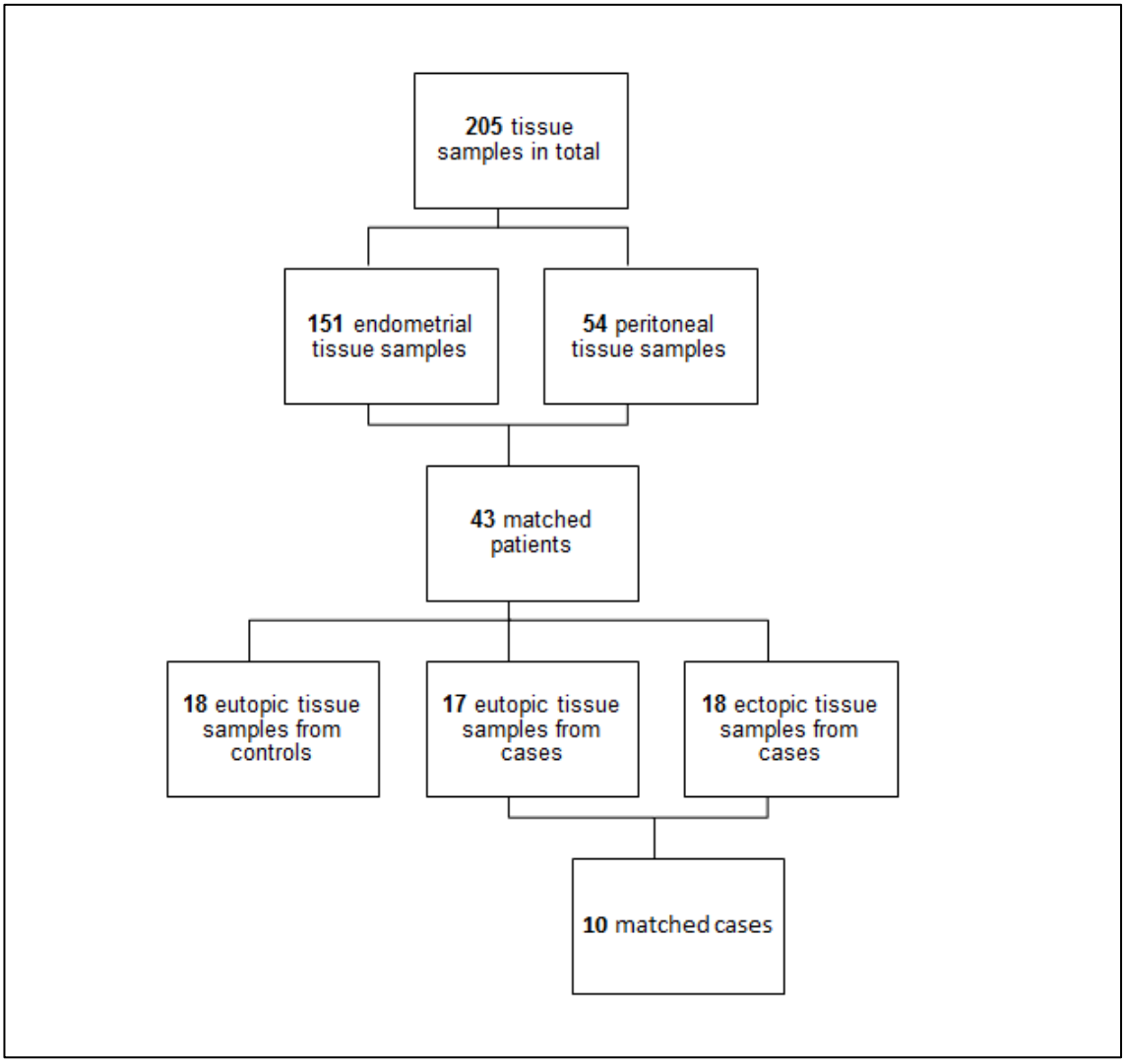

Figure 10 Matched cases between endometrial and peritoneal tissue samples
Among the 151 endometrial and the 54 peritoneal tissue samples, matched samples of 43 patients were obtained. In 18 cases matches between eutopic endometrium with peritoneum of control patients without endometriosis were achieved. 17 cases included matches of eutopic endometrium with peritoneum of patients with endometriosis, 18 cases included matches of ectopic endometrium with peritoneum of patients without endometriosis. 10 matched cases were obtained of eutopic endometrium, ectopic endometrium and peritoneum of patients.

3.2. Working Materials

3.2.1. RNA Isolation

	Company name	Equipment	Catalogue number
RNA Isolation	Peqlab	Precellys 24 Homogenizer	91-PCS24
	Eppendorf	Centrifuge	5415R
	Eppendorf	Thermomixer	5437
	Scientific Industries	Vortex Gene	2
	Gilson	Pipettes	Standard Set
	Swann-Morton	Disposable Scalpels	D501
	Company name	**Kits**	**Catalogue number**
	Agilent Technologies	Absolutely RNA miRNA Kit	400814 Revision B
	Peqlab	Precellys Ceramic Kit	91-PCS-CKM
	Company name	**Reagents**	**Catalogue number**
	Sigma-Aldrich	RNase Zap	R2020
	Sigma-Aldrich	Phenol- Chloroform-Isoamyl alcohol [125:24:1]	77619
	Sigma-Aldrich	Isoamyl alcohol	W203702
	Sigma-Aldrich	TRI Reagent	T9424
	Sigma-Aldrich	Chloroform	288306
	Merck	2-Propanol	1.09634.1011
	VWR Chemicals	absolute Ethanol	20821310
	Ambion	DEPC water	AM 9906
	Company name	**Material**	**Catalogue number**
	-	Tissue samples	**-**

Table 2 working materials for RNA isolation
Total equipment, kits, reagents and material denoted with company name and specific catalogue number used for RNA isolation are shown.

3.2.2. Quantitative and Qualitative RNA Measurement

	Company name	Equipment	Catalogue number
Quantitative and qualitative RNA measurement	Thermo Scientific	NanoDrop Spectrophotometer	ND-1000
	Company name	**Reagents**	**Catalogue number**
	Quiagen	RNase free water	1017979
	Medica Medicare	Aqua ad injectabilia	1-26688
	Ambion	DEPC water	AM 9906

Table 3 Working materials for quantitative and qualitative RNA measurement
Complete equipment and reagents denoted with company name and catalogue number used for quantitative and qualitative RNA measurement are shown.

3.2.3. cDNA Synthesis

	Company name	Equipment	Catalogue number
cDNA Synthesis	Biometra	Thermal cycler	T1
	Sigma-Aldrich	Centrifuge	4K15
	Scientific Industries	Vortex Gene	2
	Eppendorf	Multipette stream	1051647
	Biozym	Multicanalpipette	651130
	Applied Biosystems	96-well reaction plate (0,1 ml)	4346906
	Company name	**Kit**	**Catalogue number**
	Invitrogen by Life Technologies	SuperScript First-Strand Synthesis System	11904-018
	Company name	**Material**	**Catalogue number**
	-	Isolated total RNA	**-**

Table 4 Working materials for cDNA Synthesis
Total equipment, material and specific cDNA synthesis kit denoted with company name and specific catalogue number used for cDNA synthesis are shown.

3.2.4. Quantitative Real Time PCR

Quantitative real time PCR	**Company name**	**Equipment**	**Catalogue number**
	Applied Biosystems	qRT-PCR 7500 fast instrument	4361151 Rev C
	Applied Biosystems	96-well reaction plate (0,1 ml)	4346906
	Applied Biosystems	Micro Amp Optical Adhesive Film	4311971
	Sigma-Aldrich	Centrifuge	4K15
	Eppendorf	Multipette stream	1051647
	Scientific Industries	Vortex Gene	2
	Company name	**Material**	**Catalogue number**
	-	cDNA template	-
	Company name	**Reagents**	**Catalogue number**
	Applied Biosystems	TaqMan Gene Expression Master Mix	4324018
	Company name	**Gene-specific Primer**	**Taq Man Gene Expression ID**
	Applied Biosystems (Taq Man Assay)	ACTB	Hs99999903_m1
		GAPDH	Hs99999905_m1
		MMP-9	Hs01020031_m1
		MMP-28	Hs00234579_m1
		TIMP-1	Hs00171558_m1
		ADAM-10	Hs00153853_m1
		ADAM-17	Hs01041915_m1

Table 5 working materials for quantitative real time PCR
Total equipment, reagents and gene-specific primer denoted with company name, specific catalogue number or Taq Man gene expression ID used for quantitative real time PCR, are shown.

3.2.5. Software

Reference management software	Developer	Software	Version
	Thomson Reuters	EndNote	X4
Data processing	**Company name**	**Software**	**Version**
	Applied Biosystems	Real time PCR System Software	V 2.0.3
	Microsoft Office	Microsoft Excel	2010
Statistical Analyses	**Company name**	**Software**	**Version**
	IBM	SPSS	17.0 and 19.0
	Graph Pad	Graph Pad Prism	5
Graphic design	**Company name**	**Software**	**Version**
	Corel Draw	Corel Draw Graphics	X5

Table 6 working materials for analyses
Total software used for data processing, statistical analyses including graphic design denoted with developer, company name and specific version are shown.

4. METHODS

4.1. mRNA Expression Analysis

4.1.1. RNA Isolation

Before starting with RNA isolation the complete equipment and worktop were cleaned from RNase with RNaseZAP (Table 2). Total RNA from 151 endometrial tissue samples were isolated with Absolutely RNA miRNA Kit (Table 2) according to the manufacturer´s protocol. Total RNA form 54 peritoneal tissue samples were isolated with Tri Reagent (Table 2) according to the manufacturer´s protocol.

4.1.1.1. RNA Isolation from Endometrial Tissue Samples

151 frozen endometrial sections with 5µM in thickness were isolated with using the Absolutely RNA miRNA kit. The reagents such as low-salt wash buffer, high-salt wash buffer, RNase-free DNase I were prepared according to the manufacturer´s protocol. Mixtures of phenol and chloroform [1:1 (v/v)], chloroform and isoamyl alcohol [24:1 (v/v)] for organic extraction were prepared. Flash-frozen tissues were fractured to gain a sample of around 5 to 25mg in weight. The sample kept frozen on dry ice until ready for cell lysis. 7µl of β-Mercaptoethanol (β-ME) was added for each ml of Lysis Buffer required (Table7).

Tissue sample	Lysis Buffer	β-Mercaptoethanol
5-25mg	600µl	4.2µl

Table 7 Amount of Lysis Buffer/β-ME mixture per mg of tissue sample
The amount of Lysis Buffer/β-ME mixture per 5-25 mg of tissue sample is indicated in the table above.

Lysis Buffer- β-ME mixture was prepared fresh for each use. Then the tissue sample was placed in a tube containing the Lysis Buffer-β-ME mixture. The tissue was homogenized using an appropriate RNase-free homogenizer instrument (Table 2). For organic extraction the homogenate was measured before an equal volume of neutral phenol-chloroform [1:1 (v/V)] was added to the sample. The mixture was shaken for 10 seconds and centrifuged at maximum speed for 4 minutes. The aqueous (upper) phase was transferred to a fresh tube and measured. An equal volume of chloroform-isoamyl alcohol [24:1 (v/v)] was added and mixed vigorously for 10 seconds. The mixture was centrifuged at maximum speed for 3 minutes. The aqueous (upper) phase was transferred again in a fresh tube. About 600µl of the extracted lysate were transferred

to a Prefilter Spin Cup that was seated in a 2ml receptacle tube afterwards the spin cup cap was closed. The tube was centrifuged at maximum speed for 3 minutes. Subsequently the spin cup was removed from the receptacle tube and discarded. The filtrate sample was retained in the receptacle tube and then transferred to a fresh tube. 1.25 volumes of 100% ethanol were added to the filtrate and shaken for 15 seconds until the filtrate and ethanol are mixed thoroughly. 600µl of the mixture were transferred to an RNA Binding Spin Cup that was seated in a fresh 2ml receptacle tube, and capped the spin cup. The spin cup was spun in a microcentrifuge at maximum speed for 1 minute. Afterwards the filtrate was removed and the remaining lysate-ethanol mixture was loaded onto the same RNA Binding Spin Cup by repeating the steps as listed above. After the final filtrate was discarded, 600µl of low-salt wash buffer were added and centrifuged at maximum speed for 1 minute. The final filtrate was discarded and the matrix inside the spin cup was dried by spinning the tube in a microcentrifuge at maximum speed for 2 minutes. For the DNase treatment DNase solution was prepared by gently mixing 50µl of DNase Digestion Buffer with 5µl of reconstituted RNase-Free DNase I. Moderate mixing was necessary because DNase I is very sensitive to denaturation. 55µl of DNase solution was added directly onto the matrix inside the spin cup before the sample was incubated at 37°C for 15 minutes in an air incubator (Table 2). Before centrifugation at maximum speed for 1 minute, 600µl of low-salt wash buffer were added to the spin cup. The filtrate was discarded and a second wash was performed using 600µl of low-salt wash buffer repeating steps denoted above. The final filtrate was discarded and the matrix inside the spin cup was dried by spinning the tube in a microcentrifuge at maximum speed for 2 minutes.

For RNA elution the spin cup was transferred to a 1.5ml microcentrifuge tube. Before adding 50µl of elution buffer onto the centre of the matrix inside the spin cup, the elution buffer was warmed to 65°C in an air incubator. The elution buffer was added directly onto the matrix of the spin cup to ensure that the elution buffer permeates the entire matrix. Hereupon the tube was incubated at RT for 1 minute and centrifuged at maximum speed for 1 minute. For maximization of RNA yield the eluate was reloaded onto the matrix and the step of incubation and centrifugation was repeated. In the end the microcentrifuge tube contains the elution buffer including the purified RNA. The sample was stored on ice for quantitative and qualitative RNA measurement. Thereafter it was permanently stored at -80°C.

4.1.1.2. RNA Isolation from Peritoneal Tissue Samples

The RNA isolation method with Tri Reagent was used for peritoneal tissue samples, because of the high fat content of the samples. Tri Reagent is a mixture of guanidine thiocyanate and phenol in a monophase solution, which effectively dissolves DNA, RNA and protein on homogenization or lysis of tissue samples. 54 frozen tissue samples of ~ 5mg in weight were homogenized in 1ml Tri Reagent in a Precellys Homogenizer (Table 2). Because of the high content of fat, an additional step was needed. After homogenization, the homogenate was centrifuged at 12000g for 10 minutes at 4°C to remove insoluble material, such as extracellular membranes, polysaccharides, and high molecular mass DNA. The supernatant consisted of RNA and protein. If the sample had a high fat content, there was a layer of fatty material on the surface of the aqueous phase that was removed. The clear supernatant was transferred to a fresh tube followed by a phase separation. The samples were allowed to stand for 5 minutes at room temperature, to ensure complete dissociation of nucleoprotein complexes. 200µl of chloroform (Table 2) per ml Tri reagent was added. Afterwards the samples were covered tightly, shook vigorously for 15 seconds and stand for further 15 minutes at room temperature. Finally the resulting mixture was centrifuged at 12000g for 15 minutes at 4°C. Centrifugation separated the mixture into 3 phases: a red organic phase containing protein, an interphase containing DNA, and a colourless upper aqueous phase containing RNA (Figure 11).

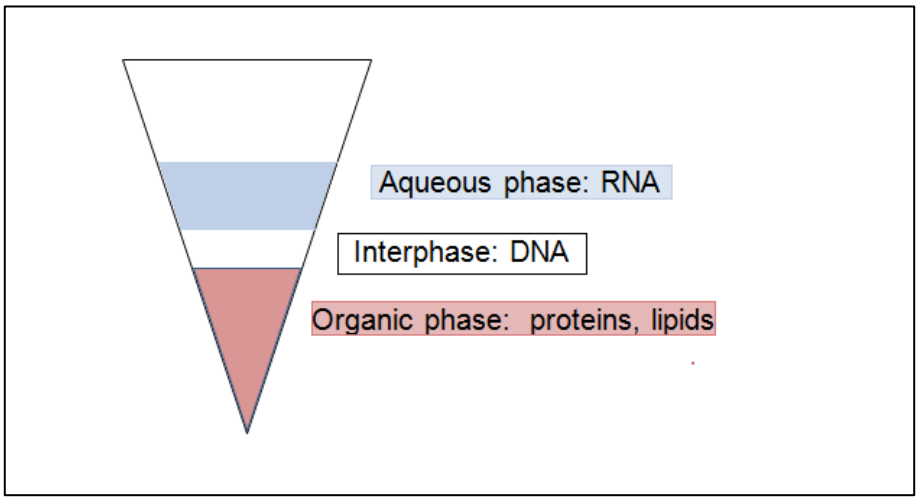

Figure 11 Schematic phase separation using Tri reagent method
After homogenization of tissue samples in 1 ml Tri reagent and adding 0.2 ml Chloroform the mixture separates into three phases: an aqueous phase containing the RNA, the interphase containing DNA and organic phase containing proteins. Each component can then be isolated after separating the phases.

In the next step the upper aqueous phase which contains RNA was transferred to a new tube; 500µl Isopropanol (Table 2) was added, mixed and incubated for 10 minutes at RT before the tubes were centrifuged at 12000g for 10 minutes at 4°C. The RNA precipitate formed a pellet on the side and bottom of the tube. The supernatant was removed and the pellet was washed with 1ml of 75% ethanol (Table 2) and centrifuged at 7500g for 5 minutes at 4°C. Finally, the pellet was air dried at RT for 10 minutes and re-suspended in 30µl DEPC water (Table 2) before the RNA was stored at -80°C.

4.1.2. Quantitative and Qualitative RNA Measurement

Using the NanoDrop Spectrophotometer (Table 3) a 1 or 2µl RNA sample was pipetted onto the end of a fiber optic cable. A second fiber optic cable (the source fiber) was then brought into contact with the liquid sample causing the liquid to bridge the gap between the fiber optic ends. A xenon flash lamp provided the light source and a spectrometer utilizing a linear charge-coupled device (CCD) array was used to analyse the light after passing through the sample. The instrument was controlled by PC based software, and the data is logged in an archive file on the PC. Before starting with quantitative and qualitative RNA measurement, initialization was made with AD (Table 3), blanked with appropriate elution buffer from Agilent kit or DEPC water (Table 3) using tri reagent method. Only non-degraded RNA of high quality with a 260/280 ratio >1.5 and a 260/230 ration >1 was used for cDNA synthesis.

4.1.3. Reverse Transcription of Total-RNA in cDNA

The RNA was adjusted to 50ng/µl with AD for reverse transcription with the SuperScript First-Strand Synthesis System for RT-PCR (Table 4) according to the manufacturers' protocol. The system is optimized to synthesize first-strand cDNA from purified poly (A) + or total RNA. The first-strand cDNA synthesis reaction is catalysed by SuperScript II Reverse Transcriptase (RT). This enzyme reduces the RNase H activity that degrades mRNA during the first-strand reaction, resulting in greater full-length cDNA synthesis and higher yields of cDNA. The enzyme exhibits increased thermal stability and can be used at temperatures up to 50°C. In addition SuperScript II RT is not inhibited significantly by ribosomal and transfer RNA. After cDNA synthesis, the cDNA may be amplified with specific primers for the gene of interest by PCR without intermediate organic extractions or ethanol precipitations. In this work first-strand synthesis was performed using random primers. Random hexamers are the most nonspecific priming method, and are typically used when the mRNA is difficult to copy in its entirety.

Random hexamer primer is a mixture of a single-stranded random hexanucleotides with 5´and 3´-hydroxyl ends. The oligonucleotide sequences of 6 bases which are synthesised entirely randomly give a large range of sequences that have the potential to anneal at many random points on a DNA sequence and act as a primer to initiate first strand cDNA synthesis. Simplified view of the mechanism of random primer is given below in Figure 12.

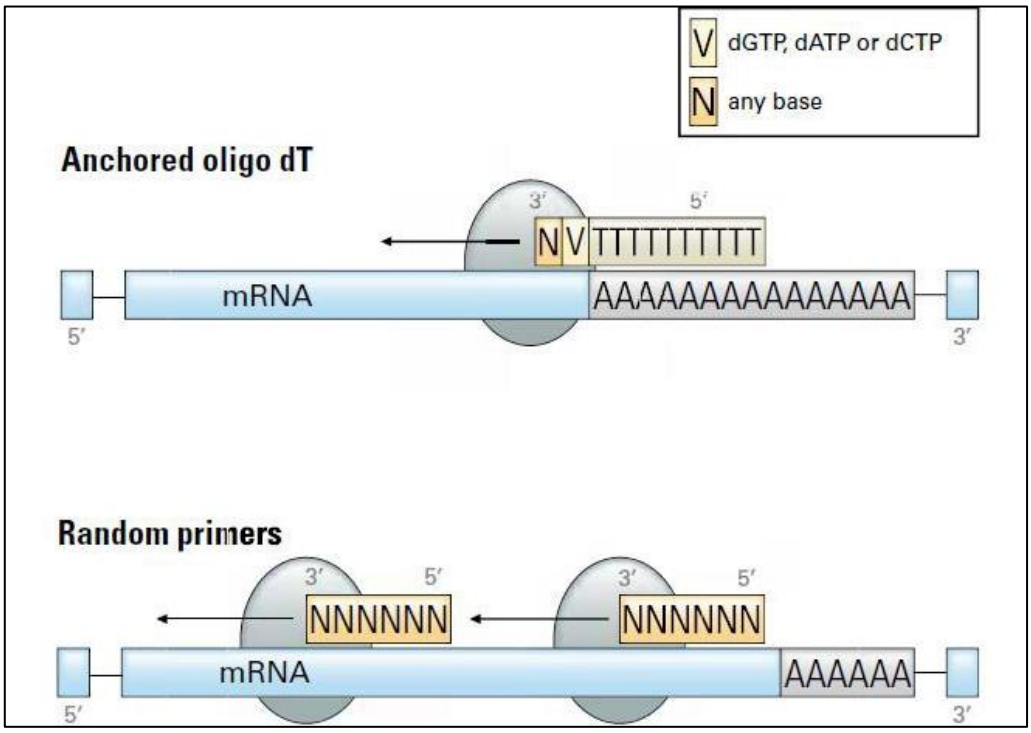

Figure 12 Priming strategies for cDNA Synthesis
Comparison of anchored oligo dT (upper strand) and random primers (lower strand) for RT-qPCR reactions are shown (www.lifetechnologies.com).

Before starting, each component was mixed and briefly centrifuged. For each reaction the following was combined in a sterile 0.5ml tube:

Component	Amount for 1 Rxn
RNA	5µl
10 mM dNTP mix	1µl
Random hexamers (50ng/µl)	4µl

Table 8 Components for RNA/primer mixture
The components for RNA/primer mixture with the appropriate amount of µl for 1 reaction (rxn) are shown.

For cDNA synthesis 10µl RNA/primer mixture (Table 8) was pipetted in each well of a 96-well reaction plate (Table 4) and incubated at 65°C for 5 minutes with a thermal cycler (Table 4) and then placed on ice for at least 1 minute. In a separate tube the 2x Reverse Transcription Master Mix was prepared, adding each component in the indicated order in Table 9.

Component	Amount for 1 Rxn
10x RT buffer	2µl
25mM $MgCl_2$	4µl
0.1M DTT	2µl
RNaseOUT^{TM} (40U/µl)	1µl

Table 9 Components for 2x Reverse Transcription Master Mix
The components for 2x reaction mix with the appropriate amount of µl for 1 reaction (rxn) are indicated.

Next 9µl of the 2x reaction mix was added to each well, mixed gently and collected by brief centrifugation (Table 4) and incubated at RT for 2 minutes. 1µl of SuperScript II RT was added to each well; minus control reaction, in which 1µl DEPC-treated water was added instead of the SuperScript II RT.

Then the mixture was incubated at RT for 10 minutes. With a thermal cycler the mix was incubated at 42°C for 50 minutes and the reaction was terminated at 70°C for 15 minutes and stored on ice. The reaction mixture was collected by brief centrifugation. 1µl of RNase H was added to each well before the reaction mixture was incubated for 20 minutes at 37°C. The single-stranded cDNA was finally diluted 1:2 with AD for real-time PCR.

4.1.4. Quantitative Real-Time-PCR

For detection and quantification of mRNA expression, each sample was analyzed by a quantitative real-time PCR (qRT-PCR) on an Applied Biosystems 7500 fast instrument (Table 5), using gene-specific primers (Table 5) and Universal PCR Master Mix (Table 5) in a total volume of 20µl containing 4µl (6.25ng) cDNA (Table 10)

PCR reaction mix component	Amount for 1 Rxn
20x TaqMan Gene Expression Assay	1µl
2x TaqMan Gene Expression Master Mix	10µl
cDNA template (6.25ng/µl)	4µl
AD	5µl

Table 10 Components of PCR reaction mix
Specific components for the PCR reaction mix are indicated with appropriate amount of µl for 1 reaction (rxn).

For each sample the components indicated in table 10 were added into each well of a 96-well reaction plate (Table 5). Sample volume for each well was 20µl. The plate was sealed with a Micro Amp Optical film (Table 5), centrifuged briefly and loaded into the 7500 fast instrument. The default PCR thermal cycling condition was set for using two-step RT-PCR. As the instrument performs the PCR run, it displays real-time status information and records the fluorescence emissions. A schematic overview of the quantification of gene expression with quantitative RT-PCR is shown in Figure 13.

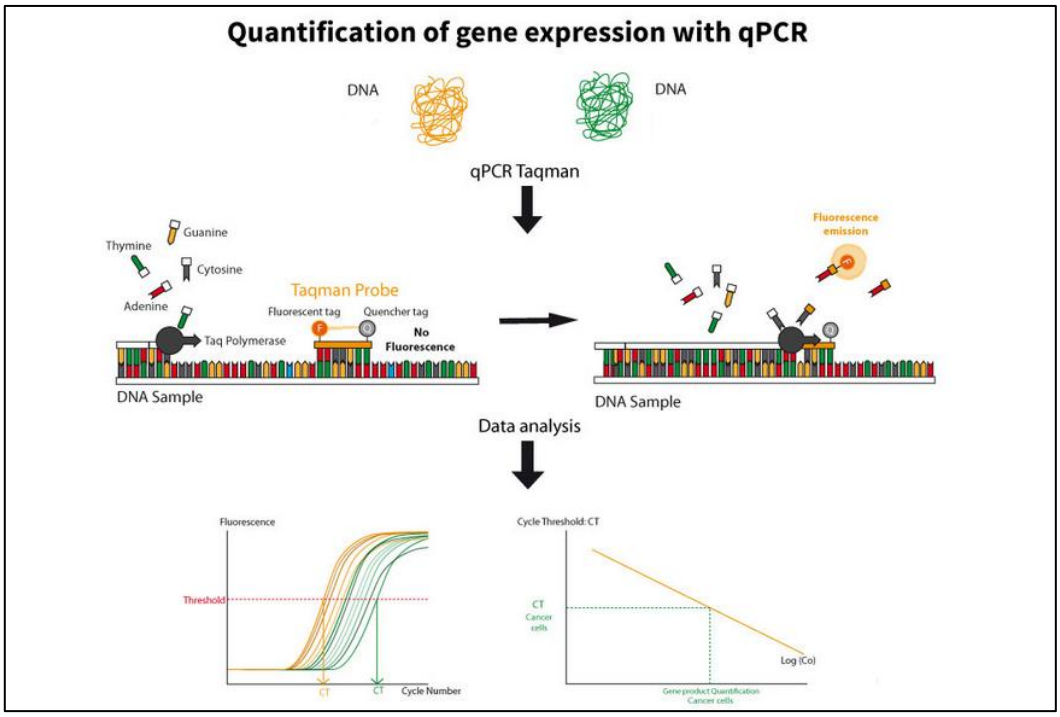

Figure 13 Quantitative RT-qPCR with DNA probes labelled with fluorescent reporter
RT-qPCR is based on the ability to track in "real time" the process of PCR using fluorescence. The intensity of the fluorescent reporter is directly proportional to the amplified target molecule. Data are collected at each PCR cycle and present the amount of product amplified to that point. When the fluorescence exceeds the threshold (exponential phase), the Ct (Threshold Cycle) is defined: this value is used to quantify molecules (Helixio).

4.1.5. Relative Quantification

The mRNAs levels of *MMP-9*, *MMP-28*, *TIMP-1*, *ADAM-10* and *ADAM-17* were normalized to those of ACTB and GAPDH in each sample by subtracting the Ct (cycle threshold) values of the controls from the Ct value of *MMP-9*, *MMP-28*, *TIMP-1*, *ADAM-10* and *ADAM-17*. This subtraction produces ΔCt (delta CT)-values. Relative mRNA expression levels were derived from ΔCt-values as $2^{-\Delta Ct}$. These relative mRNA levels were further normalized to each other, thus deriving $2^{-\Delta\Delta Ct}$ values (Figure 14). In a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The Ct is defined as the number of cycles required for the fluorescent signal to cross the threshold. Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the Ct level the higher the amount of target nucleic acid in the sample).

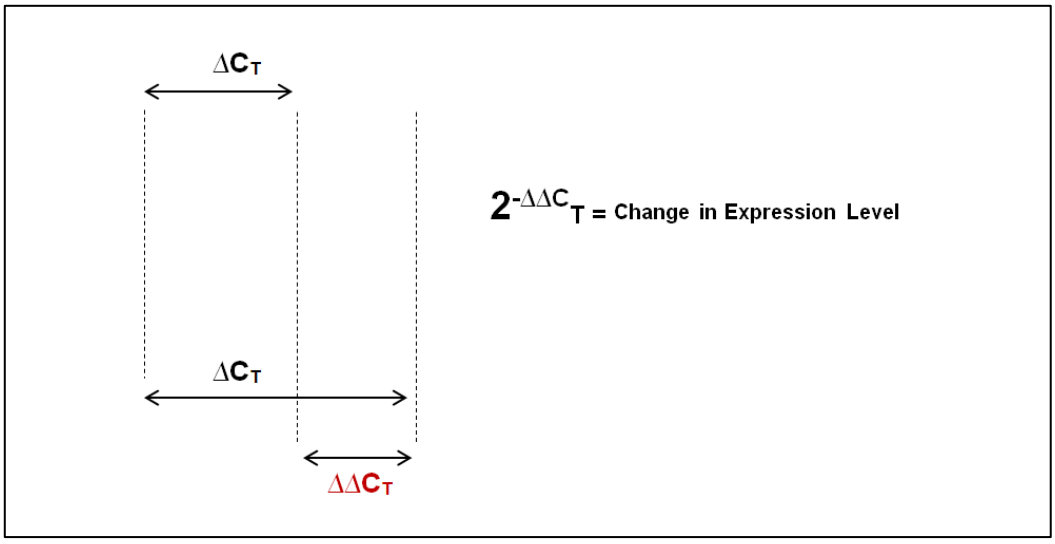

Figure 14 Relative quantification
First the difference in expression of a gene of interest to a housekeeping gene is valuated. This subtraction produces ΔCt (delta CT)-values. Relative mRNA expression levels were derived from ΔCt-values as $2^{-\Delta Ct}$. These relative mRNA levels were further normalized to each other, thus deriving $2^{-\Delta\Delta Ct}$ values. The ΔΔCt value represents an X-fold change in gene expression (www.eppendorf.com)

4.2. Statistics

4.2.1. Statistical Software

Statistical analyses were performed with IBM SPSS software using the version 17.0 and 19.0 (Table 6). SPSS is a widely used program for statistical analysis in social science. SPSS can take data from Microsoft Excel (Table 6) and use it to generate tabulated reports, charts, plots of distributions and trends and descriptive statistics. The base software includes descriptive statistics (cross tabulation, frequencies,

descriptives), bivariate statistics (means, t-tests, anova, correlation), prediction for numerous outcome (linear regression) as well as prediction for identifying groups (factor analysis, cluster analysis, discriminant).

4.2.2. Statistical Analysis

Using SPSS 17.0 and 19.0, mRNA expression levels of *MMP-9*, *MMP-28*, *TIMP-1*, *ADAM-10* and *ADAM-17* were analyzed by the non-parametric Wilcoxon-Mann-Whitney U-Test and the Wilcoxon-Ranked-Sign Test. Correlation analyses were done by non-parametric Spearman´s correlation coefficient. Boxplots were made using SPSS 17.0 and 19.0 as well as using Graph Pad Prism 5, but also redesigned using Corel Draw X5 (Table 6). P-value < 0.05 was considered as significant (Table 11). Correlation coefficient r>0.6 was considered as strong positive correlation, whereas r=1 means perfect positive correlation (Table 12).

P-value Interpretation Table		
< 0.01	(0.001) **	Strong evidence against null hypothesis, very statistically significant
	(0.0002) ***	
0.01 to 0.05	(0.05) *	Some evidence against null hypothesis, statistically significant
> 0.05	-	Insufficient evidence against null hypothesis

Table 11 P-value Interpretation
P-values are indicated with relevance to statistical analyses.

Correlation coefficient r	Interpretation of positive correlation
0	No correlation
0 to 0.2	Much weak correlation
0.2 to 0.4	Weak correlation
0.4 to 0.6	Moderate correlation
0.6 to 0.8	Strong correlation
0.8 to 1	Much strong correlation
1	Perfect correlation

Table 12 Correlation coefficient Interpretation
Correlation coefficient values are indicated with relevance to statistical analyses.

5. RESULTS

5.1. Endometrial Tissue Collective

The following analyzed data include exclusively 151 endometrial tissue samples from eutopic endometrium of control women (n=47), eutopic endometrium of women with endometriosis (n=42) and ectopic lesions (n=62). The different tissue types were used for statistical analyses.

5.1.1. Increase of *MMP-28* and *MMP-9* in Ectopic Lesions

MMP-9 and *MMP-28* mRNA expressions were significantly increased in ectopic lesions compared to the eutopic endometrium of controls (both p<0.001) and compared to the eutopic endometrium of women with endometriosis (p=0.001 and p<0.001; Mann-Whitney U Test, Figure 15A and B).

For *MMP-9* no significant difference was observed between the eutopic endometrium of controls and the eutopic endometrium of patients (Figure 15A). *MMP-28* was significantly higher expressed in eutopic endometrium of patients compared to the eutopic endometrium of controls (p=0.008; Mann-Whitney U Test, Figure 15B)

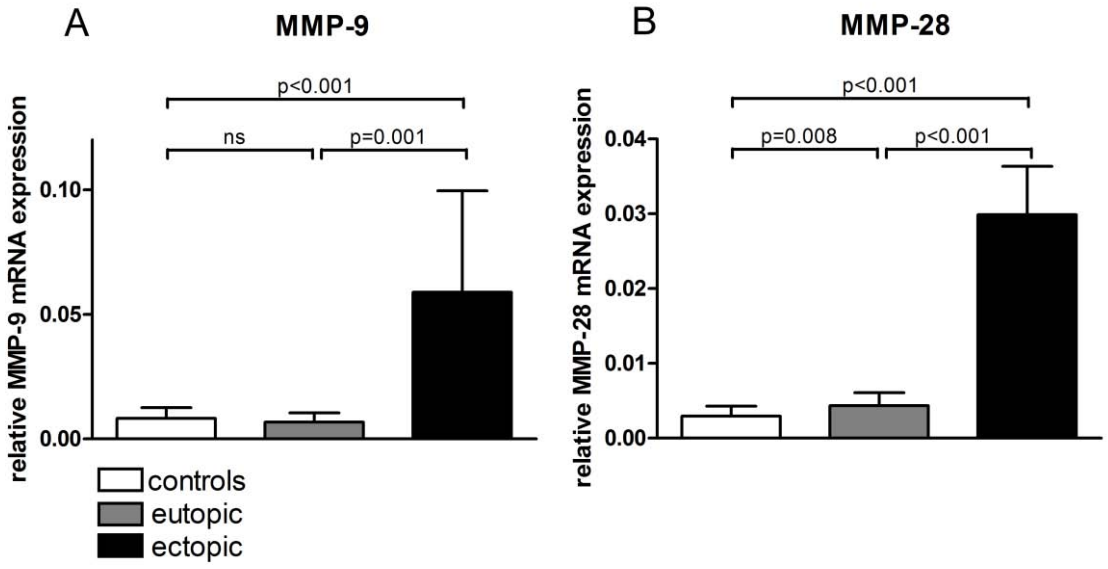

Figure 15 Bar Graph of relative expression levels of *MMP-9* and *MMP-28*
Expression levels of *MMP-9* mRNA (A) and *MMP-28* mRNA (B) are shown for controls (n=47 and 45 for *MMP-9* and *MMP-28* respectively), eutopic (n=42) and ectopic endometrial samples (n=61 and 62 for *MMP-9* and *MMP-28* respectively). Expression levels were normalized to β-actin and GAPDH. All p-values were analyzed by Mann-Whitney U Test.

5.1.2. Increase of *TIMP-1* in Ectopic Lesions

On the contrary to Goldberg et al *TIMP-1* mRNA expression was significantly increased in ectopic lesions compared to the eutopic endometrium of controls and patients (both p<0.001; Mann-Whitney U Test, Figure 16). In addition *TIMP-1* mRNA expression was significantly higher in the eutopic endometrium of patients compared to the eutopic endometrium of controls (p=0.001; Mann-Whitney U Test, Figure 16). The obtained results are different compared to already published findings. *TIMP-1* is actually an inhibitor of *MMP-9* (Goldberg et al., 1992) and thus *TIMP-1* should be decreased in ectopic lesions and increased in the eutopic endometrium.

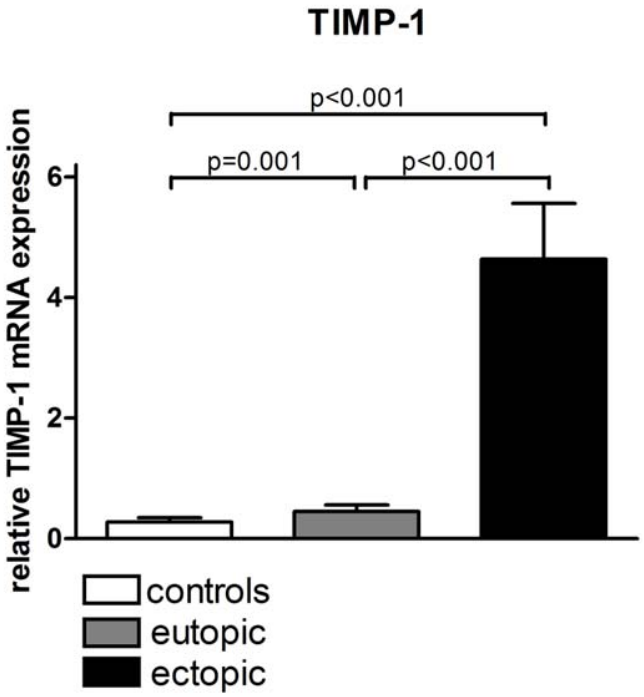

Figure 16 Bar Graph of relative expression level of *TIMP-1*
Expression level of *TIMP-1* mRNA is shown for controls (n=46), eutopic (n=42) and ectopic endometrial samples (n= 62). Expression levels were normalized to β-actin and GAPDH. All p-values were analyzed by Mann-Whitney U Test.

5.1.3. Increase of *ADAM-10* and *ADAM-17* in Ectopic Lesions

ADAM-10 and *ADAM-17* mRNA expressions were significantly increased in ectopic lesions compared to the eutopic endometrium of controls (both p<0.001; Mann-Whitney U Test, Figure 17A and B).

ADAM-10 was significantly higher expressed in the eutopic endometrium of patients compared to the eutopic endometrium of controls (p=0.004; Mann-Whitney U Test, Figure 17A) whereas no difference was observed between the eutopic endometrium of women with endometriosis and ectopic lesions.

ADAM-17 was significantly higher expressed in ectopic lesions compared to the eutopic endometrium of women with endometriosis (p<0.001; Mann-Whitney U Test, Figure 17B), no significant difference was observed between the eutopic endometrium of healthy donors and the eutopic endometrium of patients.

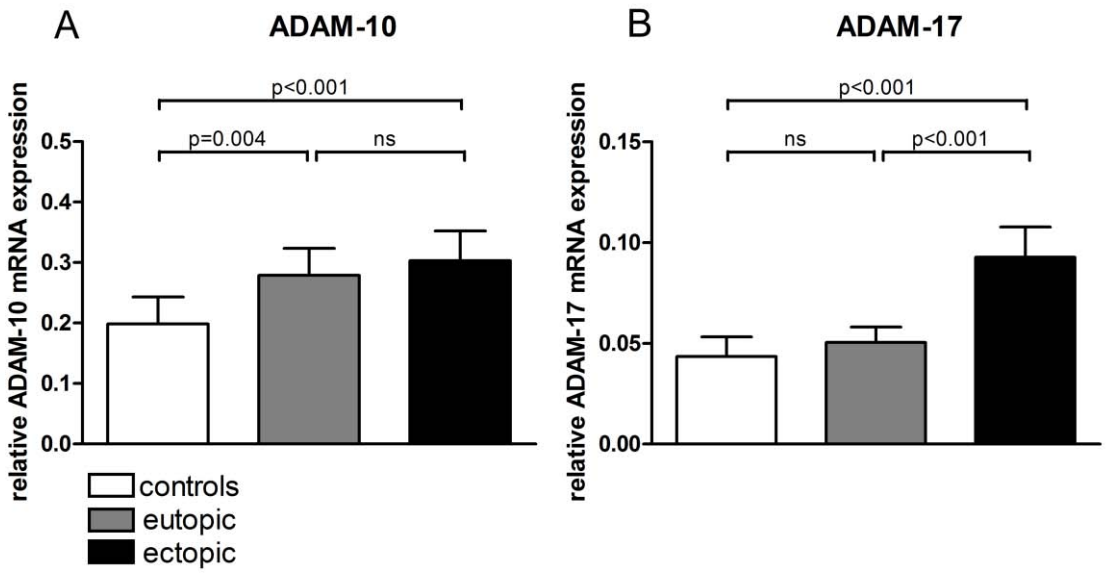

Figure 17 Bar Graph of relative expression levels of *ADAM-10* and *ADAM-17*
Expression levels of *ADAM-10* mRNA (A) and *ADAM-17*mRNA (B) are shown for controls (n=47), eutopic (n=42) and ectopic endometrial samples (n=62). Expression levels were normalized to β-actin and GAPDH. All p-values were analyzed by Mann-Whitney U Test.

5.1.4. Correlation of *MMP-9* and *MMP-28* in Control, Eutopic and Ectopic Samples

In ectopic samples most of the *MMP-28*-positive samples (52.08%) were concordantly negative for MMP-9 expression (p<0.0002; McNemar Test, Table 13). This indicates that MMP-28 expression in ectopic lesions is higher compared to *MMP-9* expression.

No significant differences were observed in control endometrium or in eutopic endometrium of patients.

Controls					
		MMP-28			
		total	neg	pos	p-value
MMP-9	neg	41	40 (73.68%)	1 (36.32%)	0.625
	pos	4	3 (39.29%)	1 (60.71%)	
Eutopic					
		MMP-28			
		total	neg	pos	p-value
MMP-9	neg	40	37 (66.67%)	3 (33.33%)	1.000
	pos	2	2 (57.58%)	0 (42.42%)	
Ectopic					
		MMP-28			
		total	neg	pos	p-value
MMP-9	neg	39	5 (47.92%)	34 (52.08%)	**<0.0002**
	pos	22	2 (14.28%)	20 (85.72%)	

Table 13 Correlation of *MMP-9* and *MMP-28* expressions in control, eutopic and ectopic samples. Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row negative and positive for *MMP-28.* All p-values of subgroup comparisons were analyzed by McNemar Test. (P-value: ectopic: $2.38*10^{-7}$)

5.1.5. Correlation of *TIMP-1* and *MMP-9/MMP-28* in Control, Eutopic and Ectopic Samples

In ectopic samples most of the *TIMP-1*-positive samples (60.98%) were concordantly negative for *MMP-9* expression (p=0.001; McNemar Test, Table 14). In controls and eutopic samples of patients no significant difference was observed. In conclusion, *TIMP-1* was upregulated whereas *MMP-9* expression was significantly lower in ectopic tissues. However this outcome is not as expected as *TIMP-1* should be decreased compared to *MMP-9*.

On the contrary in ectopic samples most of the *MMP-28*-positive samples (75%) were concordantly positive for *TIMP-1* expression (p=0.002; McNemar Test, Table 14). There was no significant difference observed neither in controls endometrium nor in eutopic endometrium of patients. This result fits to the assumption that TIMPs are inhibitors of MMPs. In conclusion *MMP-8* was upregulated whereas *TIMP-1* was downregulated in ectopic tissues.

Controls					
		MMP-9			
		total	neg	pos	p-value
TIMP-1	neg	46	42 (91.30%)	4 (8.70%)	0.125
	pos	0	0 -	0 -	
Eutopic					
		MMP-9			
		total	neg	pos	p-value
TIMP-1	neg	42	40 (95.24%)	2 (4.76%)	0.500
	pos	0	0 -	0 -	
Ectopic					
		MMP-9			
		total	neg	pos	p-value
TIMP-1	neg	20	14 (70%)	6 (30%)	**0.001**
	pos	41	25 (60.98%)	16 (39.02%)	

Table 14 Correlation of *TIMP-1* and *MMP-9* expression in control, eutopic and ectopic samples
Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row negative and positive for *MMP-9*. All p-values of subgroup comparisons were analyzed by McNemar Test.

Controls					
		MMP-28			
		total	neg	pos	p-value
TIMP-1	neg	44	42 (95.45%)	2 (4.55%)	0.500
	pos	0	0 -	0 -	
Eutopic					
		MMP-28			
		total	neg	pos	p-value
TIMP-1	neg	42	39 (92.86%)	3 (7.14%)	0.250
	pos	0	0 -	0 -	
Ectopic					
		MMP-28			
		total	neg	pos	p-value
TIMP-1	neg	20	5 (25%)	25 (75%)	**0.002**
	pos	42	2 (4.76%)	40 (95.24%)	

Table 15 Correlation of *TIMP-1* and *MMP-28* expression in control, eutopic and ectopic samples. Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row negative and positive for *MMP-28*. All p-values of subgroup comparisons were analyzed by McNemar Test.

5.1.6. Correlation of *ADAM-10* and *ADAM-17* in Control, Eutopic and Ectopic Samples

In the eutopic endometrium of patients most of the *ADAM-10*-positive samples (41.67%) were concordantly negative for *ADAM-17* expression (p=0.012; McNemar Test, Table 16). Whereas in ectopic samples, most of the *ADAM-17*-positive samples (40.75%) were concordantly negative for *ADAM-10* expression (p=0.001; McNemar Test, Table 16).

In conclusion, *ADAM-10* was higher expressed in the eutopic endometrium of patients compared to *ADAM-17*, whereas *ADAM-17* was higher expressed in the ectopic lesions compared to *ADAM-10*. No significant difference was observed between *ADAM-10* and *ADAM-17* in control endometrium.

Controls					
		ADAM-17			
		total	neg	pos	p-value
ADAM-10	neg	31	29 (93.55%)	2 (6.45%)	0.687
	pos	16	4 (25%)	12 (75%)	
Eutopic					
		ADAM-17			
		total	neg	pos	p-value
ADAM-10	neg	18	17 (94.44%)	1 (5.56%)	**0.012**
	pos	24	10 (41.67%)	14 (58.33%)	
Ectopic					
		ADAM-17			
		total	neg	pos	p-value
ADAM-10	neg	27	16 (59.25%)	11 (40.75%)	**0.001**
	pos	35	0 (0%)	35 (100%)	

Table 16 Correlation of *ADAM-10* and *ADAM-17* expressions in control, eutopic and ectopic samples.
Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row negative and positive for *ADAM-17*. All p-values of subgroup comparisons were analyzed by McNemar Test.

5.1.7. Correlation of *TIMP-1* and *ADAM-10/ADAM-17* in Control, Eutopic and Ectopic Samples

In the eutopic endometrium of controls and the eutopic endometrium of patients, most *ADAM-10*-positive samples (36.32% and (33.33%) were concordantly negative for *TIMP-1* expression (for both compared groups: p<0.0002; McNemar Test, Table 17). In ectopic lesions no significant difference was observed between *TIMP-1* and *ADAM-10* expressions.

The same was observed for correlation of *TIMP-1* and *ADAM-17*. In the eutopic endometrium of healthy women and the eutopic endometrium of patients, most *ADAM-17*-positive samples (28.26% and 35.71%) were concordantly negative for *TIMP-1* expression (for both compared groups: p<0.0002; McNemar Test, Table 18). In ectopic lesions no significant difference was observed between *TIMP-1* and *ADAM-17* expressions, neither.

In conclusion, in the eutopic endometrium of patients and in eutopic endometrial tissue samples of healthy women *ADAMs* were significant higher expressed than *TIMP-1*.

Controls					
		ADAM-10			
		total	neg	pos	p-value
TIMP-1	neg	46	31 (73.68%)	15 (36.32%)	**<0.0002**
	pos	0	0 -	0 -	
Eutopic					
		ADAM-10			
		total	neg	pos	p-value
TIMP-1	neg	42	18 (66.67%)	24 (33.33%)	**<0.0002**
	pos	0	0 -	0 -	
Ectopic					
		ADAM-10			
		total	neg	pos	p-value
TIMP-1	neg	20	14 (47.92%)	6 (52.08%)	0.167
	pos	42	13 (14.28%)	29 (85.72%)	

Table 17 Correlation of *TIMP-1* and *ADAM-10* expressions in control, eutopic and ectopic samples. Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row negative and positive for *ADAM-10*. All p-values of subgroup comparisons were analyzed by McNemar Test. (P-values: controls: $6.10*10^{-5}$ eutopic: $1.19*10^{-7}$)

Controls					
		ADAM-17			
		total	neg	pos	p-value
TIMP-1	neg	46	33 (71.74%)	13 (28.26%)	**<0.0002**
	pos	0	0 -	0 -	
Eutopic					
		ADAM-17			
		total	neg	pos	p-value
TIMP-1	neg	42	27 (64.29%)	15 (35.71%)	**<0.0002**
	pos	0	0 -	0 -	
Ectopic					
		ADAM-17			
		total	neg	pos	p-value
TIMP-1	neg	20	9 (45%)	11 (55%)	0.481
	pos	42	7 (16.67%)	35 (83.33%)	

Table 18 Correlation of *TIMP-1* and *ADAM-17* expressions in control, eutopic and ectopic samples. Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row negative and positive for *ADAM-17*. All p-values of subgroup comparisons were analyzed by McNemar Test. (P-values: controls: $2.44*10^{-4}$ eutopic: $6.10*10^{-5}$)

5.1.8. Correlation of *MMP-9* and *ADAM-10/ADAM-17* in Control, Eutopic and Ectopic Samples

In the eutopic endometrium of healthy donors and patients, as well in ectopic lesions, most of the *ADAM-10*-positive samples (37.21%, 55%, 48.72%) were concordantly negative for *MMP-9* (p=0.012, p<0.0002, p=0.015; McNemar Test, Table 19).

The same was observed for *MMP-9* expression compared to *ADAM-17*. In all three tissue types, most of the *ADAM-17*-positive samples (32.56%, 35%, 64.10%) were concordantly negative for *MMP-9* (p=0.031, p=0.001, p<0.0002; McNemar Test, Table 20).

In conclusion, in all three analyzed tissue types *ADAM-10* and *ADAM-17* were significantly higher expressed than *MMP-9*.

Controls					
		ADAM-10			
		total	neg	pos	p-value
MMP-9	neg	43	27 (62.79%)	16 (37.21%)	**0.012**
	pos	4	4 (100%)	0 (0%)	
Eutopic					
		ADAM-10			
		total	neg	pos	p-value
MMP-9	neg	40	18 (45%)	22 (55%)	**<0.0002**
	pos	2	0 (0%)	2 (100%)	
Ectopic					
		ADAM-10			
		total	neg	pos	p-value
MMP-9	neg	39	20 (51.28%)	19 (48.72%)	**0.015**
	pos	22	6 (27.27%)	16 (72.73%)	

Table 19 Correlation of *MMP-9* and *ADAM-10* expressions in control, eutopic and ectopic samples. Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row negative and positive for *ADAM-10*. All p-values of subgroup comparisons were analyzed by McNemar Test. (P-values: Eutopic: $4.77*10^{-7}$)

Controls					
		ADAM-17			
		total	neg	pos	p-value
MMP-9	neg	43	29 (67.44%)	14 (32.56%)	**0.031**
	pos	4	4 (100%)	0 (0%)	
Eutopic					
		ADAM-17			
		total	neg	pos	p-value
MMP-9	neg	40	26 (65%)	14 (35%)	**0.001**
	pos	2	1 (50%)	1 (50%)	
Ectopic					
		ADAM-17			
		total	neg	pos	p-value
MMP-9	neg	39	14 (16.56%)	25 (64.10%)	**<0.0002**
	pos	22	1 (5.55%)	21 (95.45%)	

Table 20 Correlation of *MMP-9* and *ADAM-17* expressions in control, eutopic and ectopic samples. Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row negative and positive for *ADAM-17*. All p-values of subgroup comparisons were analyzed by McNemar Test. (P-values: Eutopic: $6.46*10^{-6}$)

5.1.9. Correlation of *MMP-28* and *ADAM10/ADAM-17* in Control, Eutopic and Ectopic Samples

In the eutopic endometrium of controls and patients, most of the *ADAM-10*-positive samples (32.56%, 58.97%) were concordantly negative for *MMP-28* (p=0.001, p<0.0002; McNemar Test, Table 21). In ectopic tissue, most of the *MMP-28*-positive samples (38.18%) were concordantly negative for *ADAM-10* expression (p<0.0002; McNemar Test, Table 20).

The same was observed for *MMP-28* expression compared to *ADAM-17*. In the eutopic endometrium of healthy women and patients, most of the *ADAM-17*-positive samples (30.23%, 33.33%) were concordantly negative for *MMP-28* (p=0.007, p=0.002; McNemar Test, Table 22). In ectopic tissue, most of the *MMP-28*-positive samples (23.63%) were concordantly negative for *ADAM-17* expression (p=0.049; McNemar Test, Table 22).

In conclusion, in eutopic endometrium of healthy women and patients *ADAM-10 and ADAM-17* were higher expressed compared to *MMP-28.* In ectopic lesions *MMP-28* expression was significantly higher compared to *ADAMs* expressions.

Controls					
		ADAM-10			
		total	neg	pos	p-value
MMP-28	neg	43	29 (67.44%)	14 (32.56%)	**0.001**
	pos	2	1 (50%)	1 (50%)	
Eutopic					
		ADAM-10			
		total	neg	pos	p-value
MMP-28	neg	39	16 (41.03%)	23 (58.97%)	**<0.0002**
	pos	3	2 (66.67%)	1 (33.33%)	
Ectopic					
		ADAM-10			
		total	neg	pos	p-value
MMP-28	neg	7	6 (85.71%)	1 (14.29%)	**<0.0002**
	pos	55	21 (38.18%)	34 (61.82%)	

Table 21 Correlation of *MMP-28* and *ADAM-10* expressions in control, eutopic and ectopic samples. Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row negative and positive for *ADAM-10*. All p-values of subgroup comparisons were analyzed by McNemar Test. (P-values: eutopic: $1.94*10^{-5}$ ectopic: $1.10*10^{-5}$)

Controls					
		ADAM-17			
		total	neg	pos	p-value
MMP-28	neg	43	30 (69.77%)	13 (30.23%)	**0.007**
	pos	2	2 (100%)	0 (0%)	
Eutopic					
		ADAM-17			
		total	neg	pos	p-value
MMP-28	neg	39	26 (66.67%)	13 (33.33%)	**0.002**
	pos	3	1 (33.33%)	2 (66.67%)	
Ectopic					
		ADAM-17			
		total	neg	pos	p-value
MMP-28	neg	7	3 (17.29%)	4 (82.71%)	**0.049**
	pos	55	13 (23.63%)	42 (76.37%)	

Table 22 Correlation of *MMP-28* and *ADAM-17* expressions in control eutopic and ectopic samples. Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row negative and positive for *ADAM-17*. All p-values of subgroup comparisons were analyzed by McNemar Test.

5.1.10. Increase of *TIMP-1* in Endometrial Lesions

The following analysis includes only these patients from which multiple ectopic lesions were obtained. Analysis was made in 11 paired samples. The ectopic lesions include peritoneal endometriosis, endometrial endometriosis and deeply infiltrating endometriosis (DIE). The most endometrial lesions were located on the ovaries. DIE lesions include different locations, as rectovaginal septum, retrocerival and paracerival areas.

For *MMPs* and *ADAMs* there were no significant differences observed between the different lesion locations in paired samples.

TIMP-1 expression was significantly increased in endometrial lesions compared to the peritoneal lesions (p=0.015; Mann-Whitney U Test, Figure 18). In DIE lesions no significant differences were observed compared to peritoneal and endometrial sites.

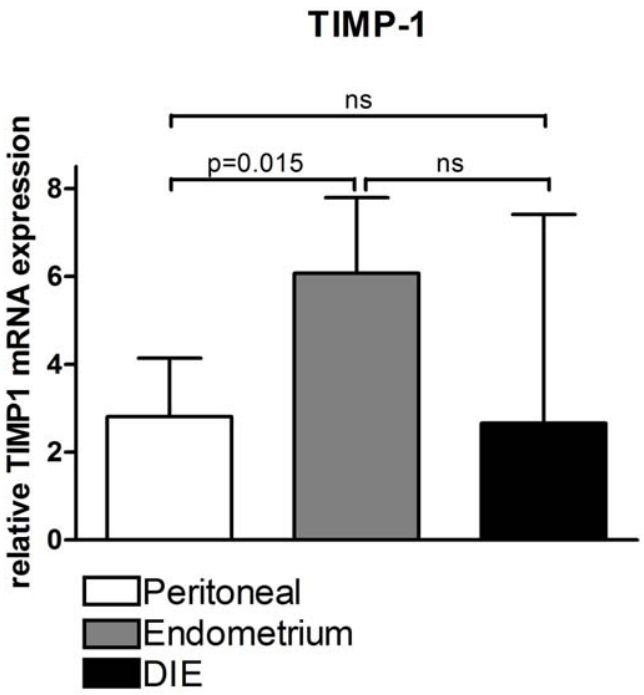

Figure 18 Bar Graph of relative expression level of *TIMP-1* in ectopic lesions
Expression level of *TIMP-1* mRNA is shown for peritoneal lesions (n=7), endometrial lesions (n=19) and DIE lesion samples (n= 4). Expression levels were normalized to β-actin and GAPDH. All p-values were analyzed by Mann-Whitney U Test.

5.1.11. Association between *ADAM-10* and *ADAM-17*

In eutopic samples of controls, eutopic samples of patients, as well ectopic lesions, a significant positive correlation between the *ADAMs* was observed.

In eutopic samples of controls the highly strong positive correlation between *ADAM-10* and *ADAM-17* with the correlation coefficient r=0.914 ($p<0.0002$; Spearman´s rho Test, Figure 19A) indicates a strong association between the two *ADAMs.* In conclusion, *ADAM-10* and *ADAM-17* are similar expressed in eutopic endometrium of controls.

In eutopic samples of patients the highly strong positive correlation between *ADAM-10* and *ADAM-17* with the correlation coefficient r=0.812 ($p<0.0002$; Spearman´s rho Test, Figure 19B) indicates a strong association between the two *ADAMs*. In conclusion, *ADAM-10* and *ADAM-17* are similar expressed in eutopic endometrium of patients.

In ectopic samples the highly strong positive correlation between *ADAM-10* and *ADAM-17* with the correlation coefficient r=0.838 ($p<0.0002$; Spearman´s rho Test, Figure 19C) indicates a strong association between the two *ADAMs*. In conclusion, *ADAM-10* and *ADAM-17* are similar expressed in ectopic lesions.

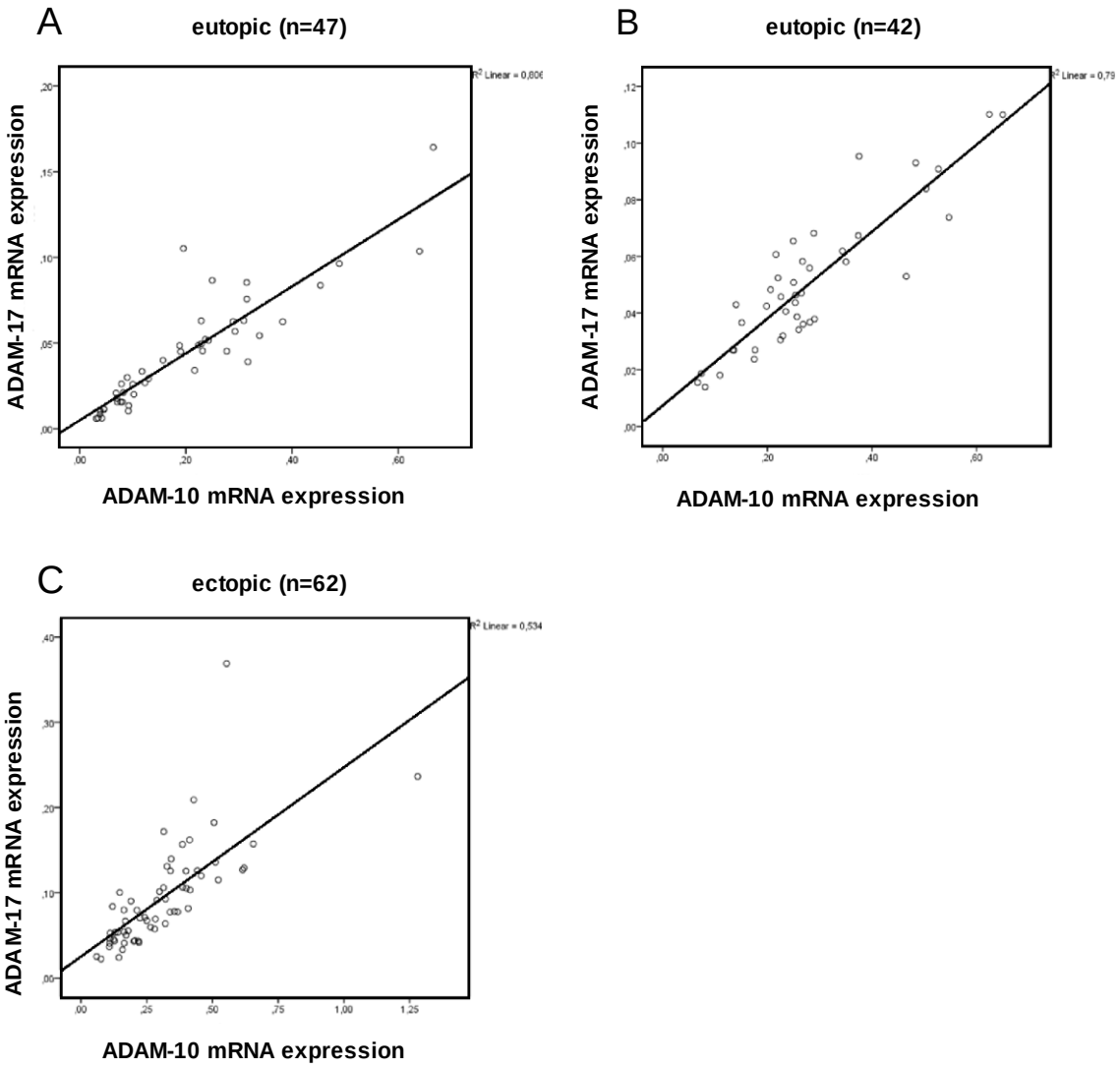

Figure 19 Correlation Graph of relative expression level of *ADAM-10* and *ADAM-17*
Correlation lines of *ADAM-10* and *ADAM-17* are shown for controls (n=47; A), eutopic (n=42; B) and ectopic endometrial samples (n=62; C). Expression levels were normalized to β-actin and GAPDH. All p-values were analyzed by Spearman´s rho Test.

5.1.12. Gene expression in the Eutopic and Ectopic Endometrium of the Same Patient

Among the 104 samples with endometriosis, 29 matched cases of ectopic and eutopic endometrium were obtained.

TIMP-1 expression was significantly increased in ectopic lesions compared to the eutopic endometrium of patients in paired samples ($p<0.0002$; McNemar Test, Table 23). In conclusion, *TIMP-1* was upregulated in ectopic tissues, but didn´t inhibit expression of *MMP-9* and *MMP-28* in ectopic lesions.

MMP-9 and *MMP-28* expressions were significantly increased in ectopic lesions compared to the eutopic endometrium in paired samples ($p=0.003$, $p<0.0002$; McNemar Test, Table 23). In conclusion *MMP-9* and *MMP-28* were upregulated in ectopic tissues, and the high expressions were not influenced by the inhibitor *TIMP-1*.

ADAM-17 expression was significantly increased in ectopic lesions compared to the eutopic endometrium in paired samples ($p<0.0002$; McNemar Test, Table 23). No significant difference was found between the ectopic *ADAM-10* expression and the eutopic *ADAM-10* expression. This led to the suggestion that ADAM-10 expression is in general higher expressed in women with endometriosis than in women without endometriosis. In conclusion *ADAM-17* expression was higher in ectopic lesions compared to the eutopic endometrium, whereas *ADAM-10* was similarly expressed in the ectopic and the eutopic endometrium of the same patient.

			Ectopic			
			TIMP-1			
			total	neg	pos	p-value
Eutopic	***TIMP-1***	neg	29	15 (51.72%)	14 (48.27%)	**<0.0002**
		pos	0	0 -	0 -	
			MMP-9			
			total	neg	pos	p-value
	MMP-9	neg	28	20 (71.43%)	8 (28.57%)	**0.003**
		pos	1	0 (0%)	1 (100%)	
			MMP-28			
			total	neg	pos	p-value
	MMP-28	neg	26	3 (11.54%)	23 (88.46%)	**<0.0002**
		pos	3	0 (0%)	3 (100%)	
			ADAM-10			
			total	neg	pos	p-value
	ADAM-10	neg	15	7 (46.67%)	8 (53.33%)	0.247
		pos	14	9 (64.29%)	5 (35.71%)	
			ADAM-17			
			total	neg	pos	p-value
	ADAM-17	neg	21	9 (42.86%)	12 (57.14%)	**<0.0002**
		pos	8	3 (37.50%)	5 (62.50%)	

Table 23 *TIMP-1*, *MMP-9*, *MMP-28*, *ADAM-10* and *ADAM-17* expressions in the eutopic and ectopic endometrium of the same patient.

Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row in ectopic endometrial lesions negative and positive for *TIMP-1*, *MMP-9*, *MMP-28*, *ADAM-10* or *ADAM-17*. All p-values of subgroup comparisons were analyzed by the McNemar Test.

5.2. Peritoneal Tissue Collective

The following analyzed data include exclusively normal peritoneal tissue samples of women without endometriosis and women with endometriosis. 54 peritoneal tissue samples consisted of 31 tissue samples from cases and 23 tissue samples from healthy women.

5.2.1. Gene Expression in the Peritoneum of Control Women

In peritoneal samples of healthy women most of the *MMP-28*/*ADAM-10*/*ADAM-17*-positive samples (95.83%/100%/91.67%) were concordantly negative for *MMP-9* expression (for all three subgroups: $p<0.0002$; Wilcoxon signed-rank Test, Table 24). This indicates, in comparison to *MMP-9* expression, a higher *MMP-28/ADAM-10/ADAM-17* expression in peritoneum of controls.

All of the *MMP-9*/*MMP-28*/*ADAM-10*/*ADAM-17*-negative samples (100%) were concordantly positive for *TIMP-1* expression (for all four subgroups: $p<0.0002$; Wilcoxon signed-rank Test, Table 24). This indicates, in comparison to *TIMP-1* expression, a lower *MMP-9/MMP-28*/*ADAM-10*/*ADAM-17* expression in peritoneum of controls.

All *ADAM-17*-negative samples (100%) were concordantly positive for *ADAM-10* expression ($p<0.0002$; Wilcoxon signed-rank Test, Table 24). This indicates a significant lower *ADAM-17* expression compared to *ADAM-10* expression in peritoneum of healthy women.

Most of the *ADAM-10*- positive samples (95.83%) were concordantly negative for *MMP-28* expression ($p<0.0002$; Wilcoxon signed-rank Test, Table 24).This indicates, in comparison to *MMP-28* expression, a higher *ADAM-10* expression in peritoneum of control women.

Between *ADAM-17* and *MMP-28* expression no significant difference was found.

In conclusion, *TIMP-1* was upregulated in peritoneal samples of controls and the *ADAMs* were significantly higher expressed than the *MMPs*, but both downregulated.

Peritoneum without Endometriosis					
total				Based on ranks	p-value
24	***MMP-28*** - ***MMP-9***	neg	1 (4.17%)	negative	**<0.0002**
		pos	23 (95.83%)		
	MMP-9 - ***TIMP-1***	neg	24 (100%)	positive	**<0.0002**
		pos	0 (0%)		
	MMP-28 - ***TIMP-1***	neg	24 (100%)	positive	**<0.0002**
		pos	0 (0%)		
	ADAM-17 - ***ADAM-10***	neg	24 (100%)	positive	**<0.0002**
		pos	0 (0%)		
	ADAM-10 - ***TIMP-1***	neg	24 (100%)	positive	**<0.0002**
		pos	0 (0%)		
	ADAM-17 - ***TIMP-1***	neg	24 (100%)	positive	**<0.0002**
		pos	0 (0%)		
	ADAM-10 - ***MMP-9***	neg	0 (0%)	negative	**<0.0002**
		pos	24 (100%)		
	ADAM-17 - ***MMP-9***	neg	2 (8.33%)	negative	**<0.0002**
		pos	22 (91.67%)		
	ADAM-10 - ***MMP-28***	neg	1 (4.17%)	negative	**<0.0002**
		pos	23 (95.83%)		
	ADAM-17 - ***MMP-28***	neg	9 (37.50%)	negative	0.648
		pos	15 (62.50%)		

Table 24 Correlations of two gene expressions in peritoneal samples of controls.
Numbers of patients of the indicated group are shown. Numbers in parentheses indicate the fraction of patients (%) in each row in peritoneum negative ranked and positive ranked for *TIMP-1*, *MMP-9*, *MMP-28*, *ADAM-10* or *ADAM-17*. All p-values of subgroup comparisons were analyzed by Wilcoxon signed-rank Test.

5.2.2. Gene Expressions in the Peritoneum of Patients

In peritoneal samples of women with endometriosis most of the *MMP-28*/*ADAM-10*/*ADAM-17*-positive samples (90%/100%/96.67%) were concordantly negative for *MMP-9* expression ($p<0.0002$; Wilcoxon signed-rank Test, Table 25). This indicates, in comparison to *MMP-9* expression, a higher *MMP-28* expression in peritoneum of controls.

All of the *MMP-9*/*MMP-28*/*ADAM-10*/*ADAM-17*-negative samples (100%) were concordantly positive for *TIMP-1* expression (for all four subgroups: $p<0.0002$; Wilcoxon signed-rank Test, Table 25). This indicates, in comparison to *TIMP-1* expression, a lower *MMP-9* /*MMP-28*/*ADAM-10*/ *ADAM-17* expression in peritoneum of patients.

All *ADAM-17*-negative samples (100%) were concordantly positive for *ADAM-10* expression ($p<0.0002$; Wilcoxon signed-rank Test, Table 25). This indicates a significant lower *ADAM-17* expression compared to *ADAM-10* expression in peritoneum of women with endometriosis.

Most of the *ADAM-10*–positive samples (95.83%) were concordantly negative for *MMP-28* expression ($p<0.0002$; Wilcoxon signed-rank Test, Table 25).This indicates, in comparison to *MMP-28* expression, a higher *ADAM-10* expression in peritoneum of patients.

Between *ADAM-17* and *MMP-18* expression there was no significant difference observed, but a strong association.

In conclusion, *TIMP-1* was upregulated in peritoneal samples of patients and the *ADAMs* were significantly higher expressed than the *MMPs*, but both downregulated

In conclusion*, TIMP-1* was upregulated in peritoneal tissue of women with and without endometriosis, whereas the *ADAMs* and the *MMPs* were downregulated in peritoneal tissue. An interesting view was offered by the additional outcome that the *ADAMs* were significantly higher expressed than the *MMPs*.

Peritoneum with Endometriosis					
total				Based on ranks	p-value
30	***MMP-28* - *MMP-9***	neg	3 (10%)	negative	**<0.0002**
		pos	27 (90%)		
	MMP-9* - *TIMP-1	neg	30 (100%)	positive	**<0.0002**
		pos	0 (0%)		
	MMP-28* - *TIMP-1	neg	30 (100%)	positive	**<0.0002**
		pos	0 (0%)		
	ADAM-17* - *ADAM-10	neg	30 (100%)	positive	**<0.0002**
		pos	0 (0%)		
	ADAM-10* - *TIMP-1	neg	30 (100%)	positive	**<0.0002**
		pos	0 (0%)		
	ADAM-17* - *TIMP-1	neg	30 (100%)	positive	**<0.0002**
		pos	0 (0%)		
	ADAM-10* - *MMP-9	neg	0 (0%)	negative	**<0.0002**
		pos	30 (100%)		
	ADAM-17* - *MMP-9	neg	1 (13.33%)	negative	**<0.0002**
		pos	29 (96.67%)		
	ADAM-10* - *MMP-28	neg	0 (4.17%)	negative	**<0.0002**
		pos	30 (95.83%)		
	ADAM-17* *MMP-28	neg	17 (56.67%)	negative	0.318
		pos	13 (43.33%)		

Table 25 Correlations of two gene expressions in peritoneal samples of patients
Numbers of patients of the indicated group are shown. Numbers in parentheses indicate the fraction of patients (%) in each row in peritoneum negative ranked and positive ranked for *TIMP-1*, *MMP-9*, *MMP-28*, *ADAM-10* or *ADAM-17*. All p-values of subgroup comparisons were analyzed by Wilcoxon signed-rank Test.

5.2.3. Association between *ADAM-10* and *ADAM-17* in the Peritoneum

In peritoneal samples of both women without endometriosis and women with endometriosis a correlation between the ADAM-genes was observed. The observed strong positive correlation between *ADAM-10* and *ADAM-17* with the correlation coefficient r=0.859 for the control group and the correlation coefficient r=0.842 for women with endometriosis were statistically significant (both p<0.0002; Spearman´s rho, Figure 20). This indicates that *ADAM-10* and *ADAM-17* are similarly expressed in peritoneal tissue of women without and with endometriosis. There is a strong association between the two genes, as before analyzed in controls, eutopic endometrium of patients and ectopic lesions.

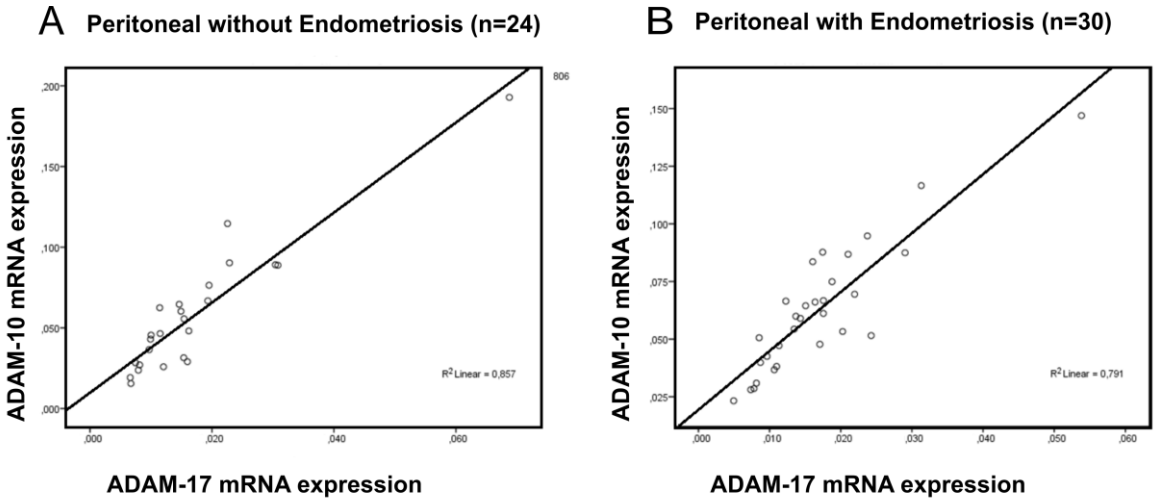

Figure 20 Correlation Graph of relative expression levels of *MMP-10* and *ADAM-17*
Correlation line of *ADAM-10* and *ADAM-17* is shown for peritoneal samples of women without endometriosis (n=24) and women with endometriosis (n=30). Expression levels were normalized to β-actin and GAPDH. All p-values were analyzed by Spearman´s rho Test.

5.2.4. Association between *MMP-28* and *ADAM-17* in the Peritoneum of Patients

In peritoneal samples of women with endometriosis a correlation between the *ADAM-17* and *MMP-28* was observed. The observed moderate positive correlation between *ADAM-17* and *MMP-28* with the correlation coefficient r=0.551 was statistically significant (p=0.002; Spearman´s rho, Figure 20). This indicates that there is a strong association between *ADAM-17* and *MMP-28* in peritoneum of women with endometriosis.

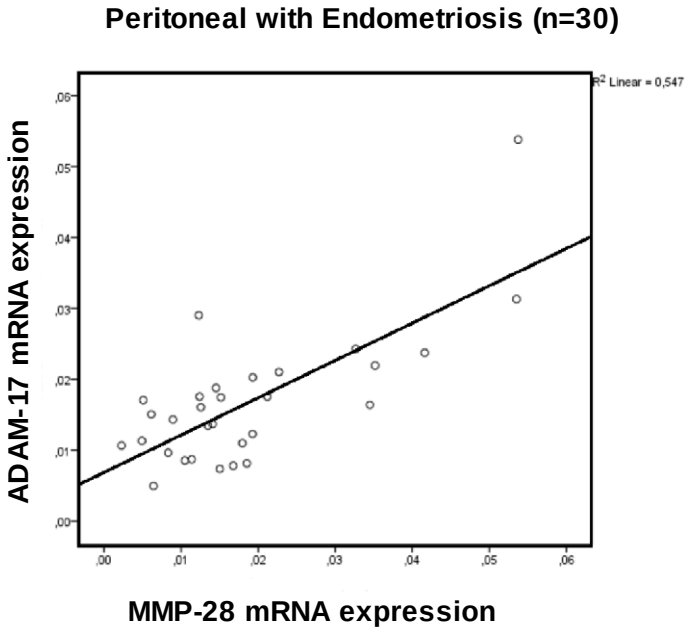

Figure 21 Correlation Graph of relative expression levels of *MMP-28* and *ADAM-17*
Correlation line of *MMP-28* and *ADAM-17* is shown for peritoneal samples of women with endometriosis (n=30). Expression levels were normalized to β-actin and GAPDH. All p-values were analyzed by Spearman´s rho Test.

5.3. Matched cases in Endometrial and Peritoneal Tissues

Among the 151 endometrial and the 54 peritoneal tissue samples, 43 matched samples were obtained. In 18 cases matches were achieved of the eutopic endometrium and normal peritoneum of controls. 17 cases included matches of eutopic endometrium with normal peritoneum of patients, 18 cases included matches of ectopic endometrium with normal peritoneum of patients.

5.3.1. Gene Expressions in the Eutopic Endometrium and the Peritoneum of the Same Control Women

For *TIMP-1* and *MMP-9* expressions no statistical significant difference were observed between the normal peritoneum and the eutopic endometrium of controls.

MMP-28 expression was significantly increased in the normal peritoneum compared to the eutopic endometrium of controls in paired samples (p=0.001; Wilcoxon signed-ranks Test, Table 26).

ADAM-10 and *ADAM-17* expressions were significantly decreased in the normal peritoneum compared to the eutopic endometrium of controls in paired samples (p=0.001 and p=0.006; Wilcoxon signed-ranks Test, Table 26).

In conclusion *MMP-28* was upregulated in the normal peritoneum and the *ADAMs* were downregulated in the peritoneum compared to the eutopic endometrium of controls.

		Peritoneal Controls				
		TIMP-1				
Eutopic Controls	***TIMP-1***	total			Based on ranks	p-value
		18	neg	7 (10%)	negative	0.231
			pos	11 (90%)		
		MMP-9				
	MMP-9	total			Based on ranks	p-value
		18	neg	9 (50%)	positive	0.879
			pos	9 (50%)		
		MMP-28				
	MMP-28	total			Based on ranks	p-value
		17	neg	1 (5.88%)	negative	**0.001**
			pos	16 (94.12%)		
		ADAM-10				
	ADAM-10	total			Based on ranks	p-value
		18	neg	15 (83.33%)	positive	**0.001**
			pos	3 (16.67%)		
		ADAM-17				
	ADAM-17	total			Based on ranks	p-value
		18	neg	15 (83.33%)	positive	**0.006**
			pos	3 (16.67%)		

Table 26 *TIMP-1*, *MMP-9*, *MMP-28*, *ADAM-10* and *ADAM-17* expressions in the eutopic endometrium and peritoneum of the same control

Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row in peritoneal tissue negative ranked and positive ranked for *TIMP-1, MMP-9, MMP-28*, *ADAM-10* or *ADAM-17*. All p-values of subgroup comparisons were analyzed by Wilcoxon signed-ranks Test.

5.3.2. Gene Expressions in the Eutopic Endometrium and Peritoneum of the Same Patient

For *TIMP-1* and *MMP-9* expressions there was no statistical significant difference observed between the normal peritoneum and the eutopic endometrium of patients.

MMP-28 expression was significantly increased in the normal peritoneum compared to the eutopic endometrium of patients in paired samples ($p=0.001$; Wilcoxon signed-ranks Test, Table 27)

ADAM-10 and *ADAM-17* expressions were significantly decreased in the normal peritoneum compared to the eutopic endometrium of patients in paired samples ($p<0.0002$ and $p<0.0002$; Wilcoxon signed-ranks Test, Table 27).

In conclusion *MMP-28* was upregulated in the normal peritoneum of controls and patients and *ADAMs* were downregulated in the peritoneum compared to the eutopic endometrium of controls and patients.

		Peritoneal				
		TIMP-1				
Eutopic	***TIMP-1***	total			Based on ranks	p-value
		17	neg	10 (58.82%)	positive	0.831
			pos	7 (41.18%)		
		MMP-9				
	MMP-9	total			Based on ranks	p-value
		17	neg	12 (70.59%)	positive	0.102
			pos	5 (29.41%)		
		MMP-28				
	MMP-28	total			Based on ranks	p-value
		17	neg	1 (5.88%)	negative	**0.001**
			pos	16 (94.12%)		
		ADAM-10				
	ADAM-10	total			Based on ranks	p-value
		17	neg	17 (100%)	positive	**<0.0002**
			pos	0 (0%)		
		ADAM-17				
	ADAM-17	total			Based on ranks	p-value
		17	neg	17 (100%)	positive	**<0.0002**
			pos	0 (0%)		

Table 27 *TIMP-1*, *MMP-9*, *MMP-28*, *ADAM-10* and *ADAM-17* expressions in the eutopic endometrium and peritoneum of the same patient

Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row in peritoneal tissue negative ranked and positive ranked for *TIMP-1, MMP-9, MMP-28*, *ADAM-10* or *ADAM-17*. All p-values of subgroup comparisons were analyzed by Wilcoxon signed-ranks Test.

5.3.3. Gene Expressions in the Ectopic Endometrium and Peritoneum of the Same Patient

For *MMP-9* expression no statistical significant difference was observed between the peritoneum and the ectopic endometrium of patients.

TIMP-1, *MMP-28*, *ADAM-10* and *ADAM-17* expressions were significantly decreased in the peritoneum compared to the ectopic endometrium of patients in paired samples (for all p<0.001, except for *MMP-28* (p=0.11); Wilcoxon signed-ranks Test, Table 28).

In conclusion, all invasion factors except *MMP-9* were upregulated in ectopic lesions compared to the peritoneum of patients.

		Peritoneal				
		TIMP-1				
Ectopic	***TIMP-1***	total			Based on ranks	p-value
		18	neg	17 (94.44%)	positive	**<0.001**
			pos	1 (5.56%)		
		MMP-9				
	MMP-9	total			Based on ranks	p-value
		18	neg	11 (61.11%)	positive	0.058
			pos	7 (38.89%)		
		MMP-28				
	MMP-28	total			Based on ranks	p-value
		18	neg	14 (77.78%)	positive	**0.011**
			pos	4 (22.22%)		
		ADAM-10				
	ADAM-10	total			Based on ranks	p-value
		18	neg	18 (100%)	positive	**<0.001**
			pos	0 (0%)		
		ADAM-17				
	ADAM-17	total			Based on ranks	p-value
		18	neg	18 (100%)	positive	**<0.001**
			pos	0 (0%)		

Table 28 *TIMP-1, MMP-9, MMP-28, ADAM-10* and *ADAM-17* expressions in the ectopic endometrium and peritoneum of the same patient.
Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row in peritoneal tissue negative ranked and positive ranked for *TIMP-1, MMP-9, MMP-28*, *ADAM-10* or *ADAM-17*. All p-values of subgroup comparisons were analyzed by Wilcoxon signed-ranks Test.

5.3.4. Gene Expressions in the Eutopic and Ectopic Endometrium of the Same Patient

For *MMP-9*, *ADAM-10* and *ADAM-17* expressions no statistical significant differences were observed between the eutopic endometrium and the ectopic endometrium of patients.

TIMP-1 expression was significantly decreased in ectopic lesions compared to the eutopic endometrium of patients (p=0.005; Wilcoxon signed-ranks Test, Table 29).

MMP-28 expression was significantly increased in ectopic lesions compared to the eutopic endometrium of patients (p=0.005; Wilcoxon signed-ranks Test, Table 29).

		Ectopic				
		TIMP-1				
Eutopic	***TIMP-1***	total			Based on ranks	p-value
		10	neg	0 (0%)	positive	**0.005**
			pos	10 (100%)		
		MMP-9				
	MMP-9	total			Based on ranks	p-value
		10	neg	8 (80%)	positive	0.203
			pos	2 (20%)		
		MMP-28				
	MMP-28	total			Based on ranks	p-value
		10	neg	0 (0%)	positive	**0.005**
			pos	10 (100%)		
		ADAM-10				
	ADAM-10	total			Based on ranks	p-value
		10	neg	5 (50%)	positive	0.445
			pos	5 (50%)		
		ADAM-17				
	ADAM-17	total			Based on ranks	p-value
		10	neg	3 (30%)	positive	0.203
			pos	7 (70%)		

Table 29 *TIMP-1, MMP-9, MMP-28, ADAM-10* and *ADAM-17* expressions in the eutopic and ectopic endometrium of the same patient.
Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each row in ectopic lesions negative ranked and positive ranked for *TIMP-1, MMP-9, MMP-28*, *ADAM-10* or *ADAM-17*. All p-values of subgroup comparisons were analyzed by Wilcoxon signed-ranks Test.

5.3.5. Gene Expressions in the Eutopic and the Ectopic Endometrium as well in the Peritoneum of the Same Patient

Among the matched cases of endometrial and peritoneal tissues, 10 matched cases were obtained including eutopic endometrium, ectopic endometrium and normal peritoneum of patients.

5.3.5.1. No significant Differences of *MMP-9* between the Eutopic, the Ectopic Endometrium and the Peritoneum

In the only 10 matched cases, no significant difference was observed of *MMP-9* mRNA expression in the three tissue types, eutopic endometrium, ectopic endometrium and peritoneum (Figure 22). But MMP-9 expression was overall higher expressed in patients compared to controls. Thus suggests, that MMP-9 is in general higher expressed in women with endometriosis compared to endometriosis-free women.

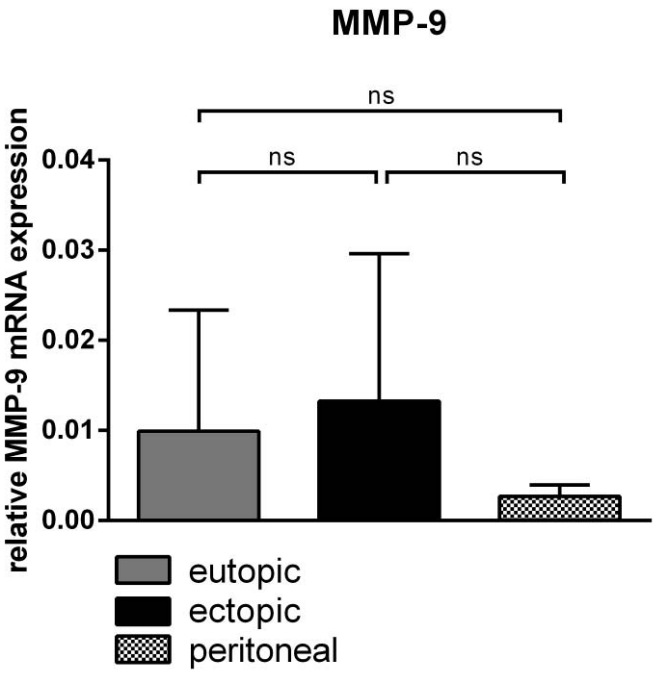

Figure 22 Bar Graph of relative expression level of *MMP-9* in eutopic, ectopic endometrium and peritoneum
Expression level of *MMP-9* mRNA is shown for eutopic (n=10), ectopic (n=10) and peritoneal samples (n= 10). Expression levels were normalized to β-actin and GAPDH. All p-values were analyzed by Mann-Whitney U Test.

5.3.5.2. Significant Differences of *MMP-28* between the Eutopic and the Ectopic endometrium as well the Peritoneum

MMP-28 mRNA expression was significantly increased in ectopic lesions compared to the eutopic endometrium of patients ($p<0.001$; Mann-Whitney U Test, Figure 23). In addition *MMP-28* mRNA expression was significantly increased in the peritoneum compared to the eutopic endometrium of patients ($p<0.001$; Mann-Whitney U Test, Figure 23). No significant difference was observed between the ectopic endometrium and the peritoneum of patients. In conclusion, *MMP-28* was upregulated in ectopic lesions and significantly higher expressed in the peritoneum compared to the eutopic endometrium of the same patients.

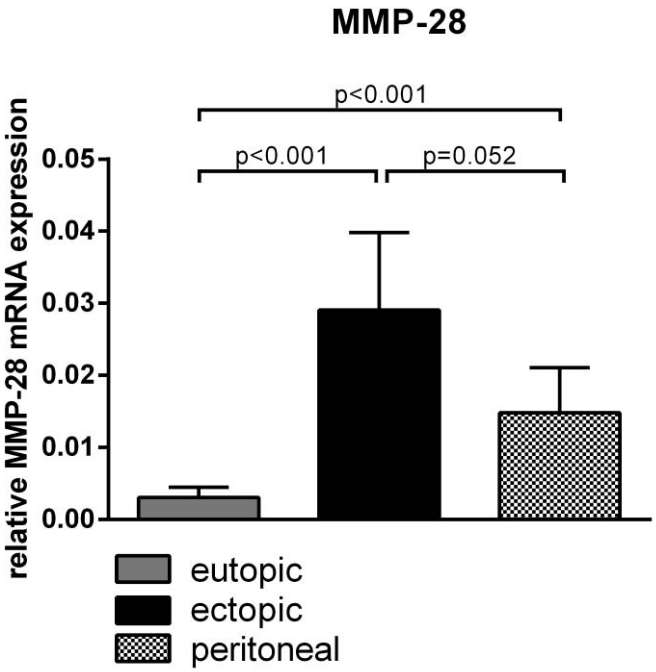

Figure 23 Bar Graph of relative expression level of *MMP-28* in eutopic, ectopic endometrium and peritoneum

Expression level of *MMP-28* mRNA is shown for eutopic (n=10), ectopic (n=10) and peritoneal samples (n= 10). Expression levels were normalized to β-actin and GAPDH. All p-values were analyzed by Mann-Whitney U Test.

5.3.5.3. Significant Differences of *TIMP-1* between the Eutopic and the Ectopic Endometrium as well the Peritoneum

TIMP-1 mRNA expression was significantly increased in ectopic lesions compared to the eutopic endometrium and the peritoneum of patients (both p<0.001; Mann-Whitney U Test, Figure 24). No significant difference was observed between the eutopic endometrium and the peritoneum of patients. In conclusion, *TIMP-1* was upregulated in ectopic lesions and similarly expressed in eutopic endometrium and peritoneum of the same patients.

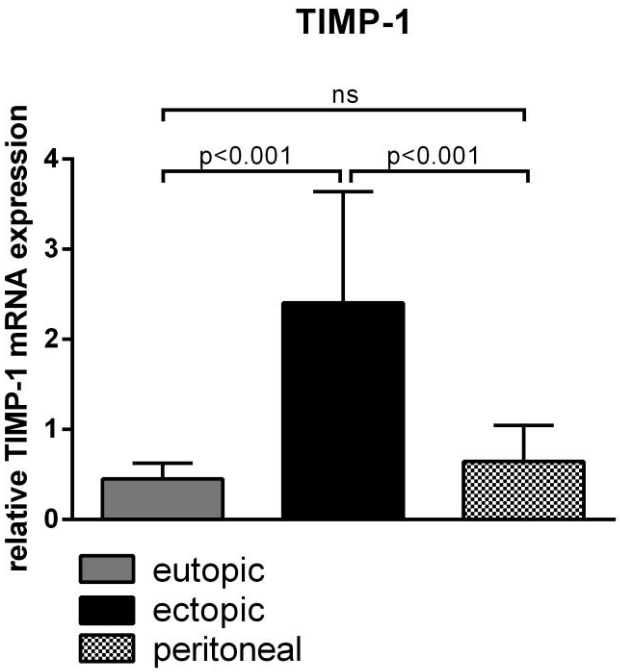

Figure 24 Bar Graph of relative expression level of *TIMP-1* in eutopic, ectopic endometrium and peritoneum
Expression level of *TIMP-1* mRNA is shown for eutopic (n=10), ectopic (n=10) and peritoneal samples (n= 10). Expression levels were normalized to β-actin and GAPDH. All p-values were analyzed by Mann-Whitney U Test.

5.3.5.4. Significant Differences of *ADAM-10* between the Eutopic and the Ectopic Endometrium as well the Peritoneum

ADAM-10 mRNA expression was significantly increased in the peritoneum compared to the eutopic and ectopic endometrium of patients (both p<0.001; Mann-Whitney U Test, Figure 25). No significant difference was observed between the eutopic endometrium and the ectopic endometrium of patients. In conclusion, *ADAM-10* was upregulated in the peritoneum and similarly low expressed in eutopic endometrium and ectopic endometrium of the same patients.

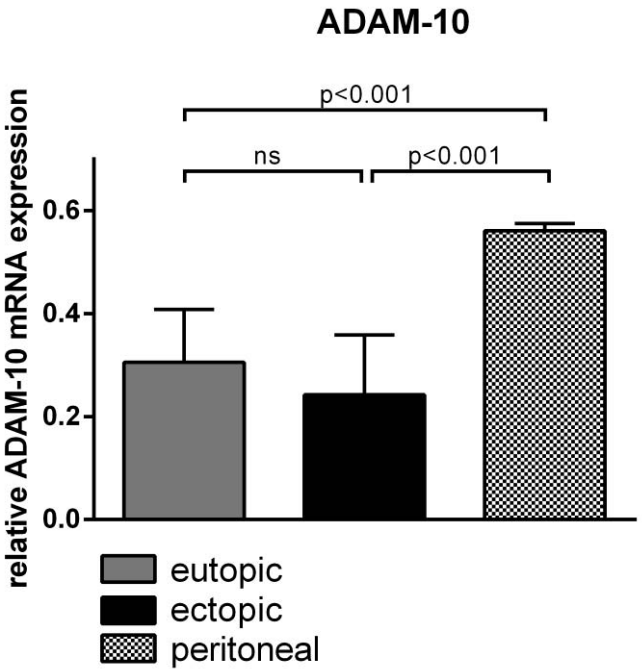

Figure 25 Bar Graph of relative expression level of *ADAM-10* in eutopic, ectopic endometrium and peritoneum
Expression level of *ADAM-10* mRNA is shown for eutopic (n=10), ectopic (n=10) and peritoneal samples (n= 10). Expression levels were normalized to β-actin and GAPDH. All p-values were analyzed by Mann-Whitney U Test.

5.3.5.5. Significant Differences of *ADAM-17* between the Eutopic and the Ectopic endometrium as well the Peritoneum

ADAM-17 mRNA expression was significantly increased in ectopic lesions compared to the peritoneum of patients (p<0.001; Mann-Whitney U Test, Figure 26). In addition *ADAM-17* mRNA expression was significantly increased in the eutopic endometrium compared to the peritoneum of patients (p<0.001; Mann-Whitney U Test, Figure 26). No significant difference was observed between the eutopic endometrium and the peritoneum of patients. In conclusion, *ADAM-17* was upregulated in ectopic lesions and significantly higher expressed in eutopic endometrium compared to the peritoneum of the same patients.

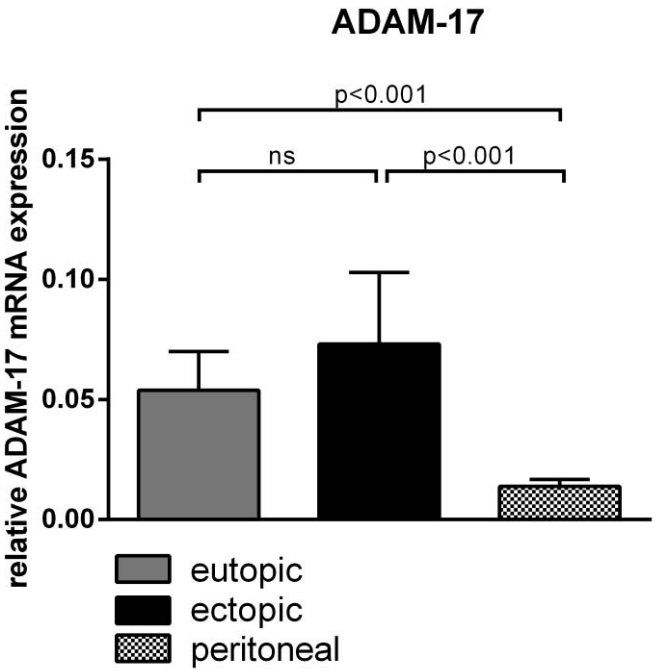

Figure 26 Bar Graph of relative expression level of *ADAM-17* in eutopic, ectopic endometrium and peritoneum

Expression level of *ADAM-17* mRNA is shown for eutopic (n=10), ectopic (n=10) and peritoneal samples (n= 10). Expression levels were normalized to β-actin and GAPDH. All p-values were analysed by Mann-Whitney U Test.

5.4. Analyses according to the Cycle Phase, Hormone Treatment and rAFS Staging

5.4.1. Paired Analysis of Endometrial and Peritoneal Tissue Samples

In endometrial tissue samples as well as in peritoneal tissue samples *MMP-9*, *MMP-28*, *TIMP-1*, *ADAM-10* and *ADAM-17* expressions did not or correlate or little with cycle phase (i.e.: proliferative, secretory), hormone treatment (i.e.: “Gestagen mono”, “Gestagen Combi”, others) and rAFS staging (severity code: I, II, III, IV). Thus the less significant data from unpaired and paired samples were not shown.

Among endometrial tissue samples *MMP-9* mRNA expression was significantly increased in women with “Gestagen Combi” treatment compared to women with other hormone treatments (p=0.049; Mann-Whitney U Test, Bar Graph not shown). Among peritoneal tissue samples *MMP-9* mRNA expression was significantly increased in patients with “Gestagen mono” and “Gestagen Combi” treatment compared to women with other hormone treatments (p=0.036 and p=0.048; Mann-Whitney U Test).

Among endometrial tissue samples *TIMP-1* mRNA expression was significantly increased in endometrial lesions compared to peritoneal lesions and DIE lesions (p=0.009 and p=0.002; Mann-Whitney U Test, Bar Graph not shown). Furthermore *TIMP-1* mRNA expression was significantly increased in patients with disease status 4 (rAFS=4) compared to patients with disease status 1 (rAFS=1) (p=0.047; Mann-Whitney U Test, Bar Graph not shown). Among peritoneal tissue samples *TIMP-1* mRNA expression was significantly increased in patients with disease status 3 (rAFS=3) compared to patients with disease status 1 or 2 (rAFS=1, 2) (p=0.021 or p=0.004; Mann-Whitney U Test).

6. DISCUSSION

Numerous data indicate that the eutopic endometrium may be functioning different compared to the normal endometrium in disease-free women. According to the implantation theory of endometriosis (Sampson, 1927), endometrial cells from retrograde menstruation can attach to and invade the peritoneal membrane to establish ectopic lesions. These cells have specific characteristics to promote survival outside the uterine cavity and to establish well-documented changes at the peritoneum and other ectopic sites (Akoum et al., 2006; Liu and Lang, 2011).

To date the majority of the literature reporting mechanisms responsible for establishment of endometriosis has focused on changes within the endometrial tissue itself and only few has been reported on changes with the peritoneal microenvironment. Establishment of ectopic lesions is assisted by peritoneal production of several Matrix Metalloproteinases (MMPs), allowing for local extracellular membrane (ECM) remodelling (Chung et al., 2002; Kokorine et al., 1997). Together with the closely related ADAMs (A disintegrin and metalloproteinase), MMPs are the mainly responsible factors of cell migration. Many studies showed altered expressions of MMPs and in the eutopic and the ectopic endometrium of women with endometriosis, but no study yet compared the expression levels in the endometrium to the normal peritoneum.

Collette et al found increased expression of *MMP-9* in the uterine endometrial tissue of women with endometriosis and no significant difference between women with and without the disease in the expression of *TIMP-1* (Collette et al., 2006).
Another study from Chung et al showed higher expression level of MMP-9 in ectopic endometrium compared to eutopic endometrium from normal and endometriosis patients.
Miller et al found that ADAM-10 and ADAM-17 integrate in numerous signaling pathways to direct cell motility and further growth-factor-driven ADAM-10 activity in peritoneal fluid from endometriosis patients (2013). However little is known about ADAMs expressions in women with endometriosis. This study analyzed altered expression of invasion factors, such as *MMP-9*, *MMP-28*, *TIMP-1*, *ADAM-10* and *ADAM-17*, in women with and without endometriosis. In addition to deregulated MMPs expressions, deregulated ADAMs expressions were assumed.

In the present study, significant higher *MMP-9*, *MMP-28*, *ADAM-10* and *ADAM-17* expressions were shown in ectopic lesions compared to the eutopic endometrium, suggesting that these genes enhance cellular invasion into the peritoneal cavity. But also a significant higher *TIMP-1* expression was observed in ectopic lesions compared to the eutopic endometrium of women with and without endometriosis. The obtained results are different compared to already published findings. TIMP-1 is actually an inhibitor of MMP-9 (Goldberg et al., 1992) and thus TIMP-1 should be decreased in ectopic lesions. In concordance to Collette et al also a higher *TIMP-1* expression was found in the eutopic endometrium of patients compared to the eutopic endometrium of disease-free women. Singh et al found an extensive involvement of *MMP-9*/*TIMP-1* imbalance in women with endometriosis undergoing in vitro fertilization (2013). Thus it can be hypothesized that *TIMP-1* is generally overexpressed in women with endometriosis, especially in ectopic lesions to attempt combatting the MMPs, primarily *MMP-9*.

In addition, the same as for *TIMP-1* was observed for *ADAM-10* expression level. Higher *ADAM-10* expression was found in the eutopic endometrium of patients compared to the eutopic endometrium of controls.

However, in ectopic lesions of unpaired samples this study was able to demonstrate an overexpression of *MMP-28* compared to *MMP-9* and *TIMP-1* expressions in ectopic lesions. This result led to the suggestion, that *MMP-28* is a strong key contributor to endometriosis and therefore a special attention has to be turned on the regulation of *MMP-28*.

Moreover, *ADAM-17* was significantly higher expressed in ectopic lesions compared to *ADAM-10*, whereas *ADAM-10* was significantly higher expressed in the eutopic endometrium of patients compared to *ADAM-17*. It can be hypothesized that *ADAM-10* was inhibited by *TIMP-1*, and therefore not overexpressed in ectopic lesions. This assumption is confirmed by the finding that *TIMP-1* was significantly higher expressed in ectopic lesions than *ADAM-10*. Rocks et al reviewed *ADAM-10* sensitivity to *TIMP-1* cancer progression (2008).

In this study the contribution of *ADAM-10* and *ADAM-17* to the establishment of endometriosis was observed. In concordance with Miller et al *ADAM-10* and *ADAM-17* regulate endometrial cell migration. It was shown that *ADAM-10* and *ADAM-17* were generally higher expressed than *TIMP-1*, *MMP-9* and *MMP-28* in the eutopic and the ectopic endometrium of women with and without endometriosis. With the exception that *MMP-28* was overexpressed in ectopic lesions compared to *ADAM-10* and *ADAM-17*.

In conclusion, this study presented that *MMP-28* was uppermost expressed in ectopic lesions of unpaired samples, followed by *ADAM-10*, *ADAM-17* and *MMP-9.* Jian et al reported that *MMP-28* was overexpressed in highly invasive gastric cancer sub-cell line (2011), which could be potentially compared with the invasive disease of endometriosis.

Comparing the three analyzed lesion locations of endometrial, peritoneal and DIE sites, a higher expression of *TIMP-1* was found in endometrial lesions compared to peritoneal lesions. This may suggest that TIMP-1 is not that active in deep peritoneal lesions than in endometrial lesions. Among the MMPs and ADAMs, in various lesion locations no significant difference was observed, which may result in too little numbers of involved patients with multiple lesion locations.

In the study of 29 matched endometriosis patients, it was discriminated between eutopic and ectopic mRNA expression for *TIMP-1*, *MMP-9*, *MMP-28*, *ADAM-10* and *ADAM-17*. The results showed that the ectopic expressions of *TIMP-1*, *MMP-9*, *MMP-28* and *ADAM-17* were significantly increased compared to the expression in the eutopic endometrium. *ADAM-10* is not higher expressed in the ectopic endometrium compared to the eutopic endometrium; this is due to *TIMP-1* – sensitivity of *ADAM-10*. Hence, it can be speculated once again that TIMP-1 is a potential inhibitor of ADAM-10. The present study was not able to show inverse expression between *TIMP-1* and *MMP-9* in ectopic tissue, like it was found in the published research of Chen et al (2004). However this outcome is not as expected and the interplay between *TIMP-1* and *MMP-9* has to be adequately explored. However overexpressions of *TIMP-1*, *MMP-9*, *MMP-28* and *ADAM-17* were shown in the ectopic endometrium, which are responsible for tissue remodelling and ectodomain shedding and thus cell migration into foreign tissue.

This study also showed a strong positive correlation of *ADAM-10* and *ADAM-17* in all five analyzed tissue types: the eutopic endometrium and the peritoneum of women without the disease, the eutopic and the ectopic endometrium as well the peritoneum of women with endometriosis. These findings suggest a strong association between *ADAM-10* and *ADAM-17* in women with and without endometriosis or in general. The interaction of *ADAM-10* and *ADAM-17* showed no restricted tissue range, as this was offered by the present results. These findings approved the fact that *ADAM-10* and *ADAM-17* derive from the same subfamily, are distinctly more separated from the rest of ADAM gene family and exhibit similar structures and functions (Brocker et al., 2009). *ADAM-10* was also found to contribute to TNF shedding in cells that lack *ADAM-17*,

indicating a functional overlap between the two genes, despite sharing only 25 per cent amino acid identify (Mezyk-Kopec et al., 2009).

Interestingly, this study was able to show a strong positive correlated expression of *MMP-28* and *ADAM-17* in the peritoneum of women with endometriosis. A more in-depth comprehension of the connection between MMPs and ADAMs and their altered regulation in the endometrium is needed, especially of the interplay between *MMP-28* and *ADAM-17*.

Analyzed normal peritoneum of patients and disease-free women showed an overexpression of *TIMP-1* compared to *MMP-9*, *MMP-28*, *ADAM-10* and *ADAM-17*. In addition a higher expression of *MMP-28*, *ADAM-10* and *ADAM-17* was found compared to *MMP-9* and higher *ADAM-10* expression compared to *ADAM-17* and *MMP-28*. Furthermore the observed higher expression of *MMP-28* and the lower expression of *ADAM-10* and *ADAM-17* in the peritoneum of controls and patients compared to the eutopic endometrium indicate no significant difference between the peritoneum of controls and patients. *MMP-9* and *TIMP-1* were not significantly different expressed in the eutopic endometrium compared to the peritoneum of controls or patients. These findings conclude that *MMP-28*, *ADAM-10* and *ADAM-17* may be different expressed in the normal peritoneum compared to the eutopic endometrium, but it was not possible to differ between women with and without endometriosis. For *MMP-9* and *TIMP-1* the expression levels didn´t differ either between women with endometriosis and disease-free women.

Nevertheless, in the 18 matched cases of ectopic endometrium and peritoneum this study was able to present higher *TIMP-1*, *MMP-28*, *ADAM-10* and *ADAM-17* expression in ectopic lesions compared to the peritoneum of these patients. This indicates higher expression in ovarian, peritoneal or DIE lesions compared to the normal peritoneum and therefore verification that these genes contribute actively to the establishment of endometriosis. Comparing ectopic with normal peritoneal tissue, no significant difference was observed of *MMP-9* expression. This could indicate that *MMP-9* is in general overexpressed in women with endometriosis compared to controls, regardless to the tissue type. Analysis of 10 matched tissue samples of eutopic and ectopic endometrium results in significantly higher *MMP-28* expression compared to *TIMP-1* expression in ectopic lesions. In conclusion, in ectopic tissue *MMP-28* was overexpressed compared to *TIMP-1*. *TIMP-1* is actually an inhibitor of *MMP-9*; nevertheless *MMP-28* was higher expressed and hence is involved in the progression of endometriosis.

Finally 10 paired cases including eutopic, ectopic and peritoneal tissue samples were analyzed. These results presented an overexpression of *MMP-9*, *MMP-28*, *TIMP-1* and *ADAM-17* in the ectopic endometrium and an overexpression of *ADAM-10* in the peritoneum. Furthermore *MMP-9* expression showed no significant difference in eutopic compared to peritoneal tissue and *MMP-28* was less expressed in eutopic compared to peritoneal tissue. The expression levels of *TIMP-1* in the eutopic and peritoneal tissue were not significantly distinct from each other. *ADAM-17* was a bit higher expressed in the eutopic compared to the peritoneal tissue and no significant difference was observed of *ADAM-10* between eutopic and ectopic tissue. These findings suggests that *MMP-9*, *MMP-28*, *TIMP-1*, *ADAM-10*, *ADAM-17* expression levels vary significantly among the different tissue types of endometriosis patients and it is necessary to explore further the functional differences. However all genes except to ADAM-10 were overexpressed in ectopic lesions, which approved hitherto findings of actively contribution to the establishment of endometriosis.

In conclusion, normal peritoneum of women with endometriosis showed no differences in gene expression levels compared to the normal peritoneum of women without the disease. In addition to high *MMP-9*, and *MMP-28* expression in the ectopic endometrium, also a high *TIMP-1* expression was observed. Thus this study was not able to show inverse expression between *MMP-9* and *TIMP-1*, but in paired cases a significant inverse expression between *MMP-28* and *TIMP-1* in ectopic tissue. Overall *ADAM-10* and *ADAM-17* were higher expressed compared to *MMP-9* and *MMP-28*. Furthermore *ADAM-10* and *ADAM-17* are strongly associated in all analyzed tissue types and thus led to the suggestion that they cooperate extensively. *ADAM-17* showed overexpression in ectopic lesions, whereas *ADAM-10* is similarly high expressed in eutopic and ectopic tissue of patients. Regardless of that, the process of cell mobility and facilitating ectopic cell invasion by MMPs and ADAMs may contribute to the development of endometriosis.

These observations might suggest that the matrix related metalloproteinases, such as *MMP-9* and *MMP-28* may be involved in the pathogenesis of endometriosis and should be further investigated. Overexpression of *ADAM-10* and *ADAM-17* may play a critical role in the pathogenesis of the benign disease. Although these proteases are associated with the process of proteolytic shedding and activation of surface proteins including growth factors, cytokines, receptors and their ligands rather than extracellular matrix breakdown.

7. FUTURE PROSPECTS

These findings lead to the implication that the invasive system including inhibitors may be an attractive target for future treatment of endometriosis.

Consequently, an important future issue is to address the functional differences in proteases. Why does ectopic endometrium express so many MMPs and ADAMs? Furthermore it seems rational to conclude that the sum of protease activity promotes cellular motility, and thus development of ectopic lesions. Relatively little is known about the consecutive cooperation between MMPs and ADAMs during the establishment of endometriosis. The produced evidence confirms that the genes *MMP-9*, *MMP-28*, *TIMP-1*, *ADAM-10* and *ADAM-17* may be suitable for diagnostic qRT-PCR of endometriosis. The goal is to obtain a small set of biomarkers that are easily detected on protein level and have a strong prognostic value in endometriosis. Further research may include ELISA or Western Blot to get a potential marker. The future project will be the analysis of expression in different endometriosis cell lines. This study will include SV40 (simian vacuolating virus 40) immortalized cell lines as follows: 11Z, 12Z peritoneal epithelial endometriotic cell line and 22B peritoneal stromal endometriotic cell line. Furthermore a fibroblast immortalized cell line, such as tHESC human stromal cell line will be included in this study. The invasion pathways have been implicated in embryonic development and tumourigenesis and are supposed to be crucial for endometriosis. This coming study will investigate if invasion pathways, like the NOTCH pathway, and which members of MMPs and ADAMs are possibly involved in the pathogenesis of endometriosis. It will furthermore be investigated how MMP-inhibitors, such as TIMP-1, influence the behavior of endometriotic cells in vitro. Different assays, such as proliferation assay, invasion and migration assay, cell survival assay and gene silencing assay will be performed.

However further research is necessary for a better understanding of the proteolytic activity during endometriotic cell invasion. New information about these invasion factors could be crucial for the improvement of diagnosis and treatment of endometriosis.

MMPs in development:

“The rapid and irreversible nature of proteolysis is ideal for pushing forward irreversible decisions in development.”

Basbaum and Werb, 1996

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9. CURRICULUM VITAE

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PROESTLING, K., BIRNER, P., **GAMPERL, S**., NIRTL, N. MARTON, E., YERLIKAYA, G., WENZEL, R., STREUBEL, B., HUSSLEIN, H.; Enhanced epithelial to mesenchymal transition (EMT) and upregulated MYC in ectopic lesions contribute independently to endometriosis

10. ACKNOWLEDGMENT

Special thanks to Ingrid Flick, who enables our research on endometriosis with her generous support (“Ingrid Flick Foundation”).

I would like to express my gratitude to my supervisor Univ.-Prof. Dr. med.univ. Berthold Streubel for the useful comments, remarks and engagement through the learning process of this master thesis. Furthermore I would like to thank my colleague PhD. Mag. Katharina Pröstling for introducing me to the topic as well for her support. Also, I am grateful to my colleague Dr. Sukirthini Balendran for supporting me with statistical analyses and discussion. This work would not have been possible without their mental, experimental and statistical support. In my daily work I have been blessed with further friendly colleagues. BSc. Sandra Liebmann-Reindl has always found the bright site of work. In all the time I have known her: Every day I got a friendly smile and some words of cheer. Dr. med.univ. Theresa Reischer kept us entertained with her huge repertoire of stories. With her you could complain and laugh at once. BSc. Nadine Nirtl often made sure none of us starved. MSc. Erika Marton was one of the best teachers I have ever had. I am glad to have met these lovely persons. Also I would like to thank my second supervisor Assoc. Prof. Dr. Regina Grillari for her supervision and coordination until the last minute.

Beyond my work, Charlotte Brey has been a wonderful follow student and friend. Without her I would not have come this far. I admire her optimism and I appreciate highly what she did for me during our studies. She is a loveable person, and I wish her the best for her professional career and future.

I would like to thank my loved ones, who have supported me throughout all my studies at University. I thank my parents Petra and Josef, especially my mother, for supporting me throughout the entire process. It was not always easy, but you all along believed in me. I will be grateful forever for your love. Also, I want to express my gratitude to my twin sister Stephanie and to my brothers Felix and Thomas. You are so important for me! Special thanks to Günter, without his help this work would never have printed. Further on I would like to thank my grandmother Friederike for her backup during my whole education.

In the end I would like to say thank you to my boyfriend Lukas. He supported me in all my decisions and taught me to be more confident. I am truly grateful.

Finally I want to finish my acknowledgments with a quote, which Lukas told me:

Every accomplishment starts with the decision to try.

[Anonymous]