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PATHOPHYSIOLOGICAL FACTORS AND GENETIC ASSOCIATION IN ENDOMETRIOSIS

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When it is dark enough, you can see the stars.

/Ralph Waldo Emerson

ABSTRACT

Introduction: Endometriosis is a common benign gynecological disease where endometrial tissue forms lesions outside the uterine cavity. Endometriosis is estimated to affect about 10% of women of reproductive age, rising to 20-40% in patients with infertility, with a significant impact on physical, mental and social well-being of those affected. Today there is no cure for this disease and the most common medical treatments are not suitable for long term treatment due to side-effects.
Although endometriosis is a well-known disease, the pathogenesis remains unclear. The most accepted theory about the pathogenesis is Sampson´s theory about retrograde menstruation. However, different properties, such as adhesion, invasion and proliferation of the shed menstrual cells seem to play an important role in the development of endometriosis. Also, altered immune surveillance, stem cells and genetic predisposition could be involved in the pathogenesis.
Aims: The overall aim of this thesis was to study the aetiology and pathophysiology of endometriosis, specifically the inflammatory profile in the follicular fluid of *in vitro* fertilization (IVF) patients and adhesion, attachment and invasion factors in endometriosis. Furthermore, the aim was to investigate the genetic background of endometriosis and a possible genetic linkage to rheumatoid arthritis (RA) and ovarian cancer.
Results: We found that women with endometriosis undergoing IVF have lower levels of anti-mullerian hormone (AMH) and a lower fertilization rate. These women also have an increased inflammatory profile in the follicular fluid. We observed genetic association of two RA-associated SNPs in CCL21 and HLA-DRB1 in women with moderate/severe disease. However, we could not observe any association of the ovarian cancer associated SNPs in the BNC2 gene. In addition, we found expression of ApoE, ITGB2, ITGB7, LAMC1, CD24 and JAM-1, in endometrium from healthy controls, endometrium from endometriosis patients and in endometriomas.
Conclusions: Our results support a strong inflammatory component in endometriosis, which may affect the ovarian reserve and lead to infertility problems. Furthermore, genetic associations in inflammatory related genes were found in women with moderate/severe disease. An aberrant expression of factors involved in adhesion, attachment and invasion may be important in the establishment of endometriotic lesions and may at least partly be regulated by inflammatory mediators.

LIST OF PUBLICATIONS

I. Henrik Falconer*, **Johanna Sundqvist***, Kristina Gemzell-Danielsson, Bo von Schoultz, Thomas M D'Hooghe, Gabriel Fried (*Equal contribution). **IVF outcome in women with endometriosis in relation to tumour necrosis factor and anti-Müllerian hormone**. *Reprod Biomed Online*. 2009. Apr;18(4). pp.582-8

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III. **Johanna Sundqvist**, Henrik Falconer, Maria Seddighzadeh, Alexandra Vodolazkaia, Amelie Fassbender, Cleophas Kyama, Attila Bokor, Olof Stephansson, Kristina Gemzell-Danielsson*, Thomas M D´Hooghe* (*Equal contribution). **Replication of ovarian cancer associated polymorphisms in the BNC2 gene among women with endometriosis.** *Submitted*

IV. **Johanna Sundqvist**, Karin Louise Andersson, Gianfranco Scarselli, Kristina Gemzell-Danielsson*, P. G. Luther Lalitkumar* (*Equal contribution). **Expression of adhesion, attachment and invasion markers in endometrium and endometriosis – Understanding the aetiology of endometriosis.** *Manuscript*

CONTENTS

LIST OF ABBREVIATIONS

AMH	Anti-mullerian hormone
ApoE	Apolipoprotein E
ASRM	American Society of Reproductive Medicine
BMI	Body mass index
BNC2	Basonuclin 2
CA125	Cancer antigen 125
CCL21	Chemokine CC motif ligand 21
cDNA	Complementary deoxyribonucleic acid
CNV	Copy number variants
CT	Threshold cycles
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide
EAOC	Endometriosis- associated ovarian cancer
ECM	Extracellular matrix
EIA	Enzyme immunoassay
FSH	Follicle stimulating hormone
GM-CSF	Granulocyte-macrophage stimulating factor
GnRH	Gonadotropin-releasing hormone
GWAS	Genome-wide association studies
hCG	Human Chorionic Gonadotrophin
HLA	Human leukocyte antigen
HWE	Hardy-Weinberg equilibrium
ICSI	Intracytoplasmic sperm injection
IFN-γ	Interferon γ
IL	Interleukin
IRF5	Interferon regulatory factor 5
IRMA	Immunoradiometric assay
ITGB2	Integrin β2
ITGB7	Integrin β7
IVF	*In vitro* fertilization
JAM-1	Junctional adhesion molecule-1
K-ras	Kirsten rat sarcoma viral oncogene homologue
L1CAM	L1 cell adhesion molecule
LAMC1	Laminin γ-1
LD	Linkage disequilibrium
LDL	Low density lipoprotein
Lng-IUS	Levonorgestrel-releasing intrauterine system
LOH	Loss of heterozygosity
MAF	Minor allele frequency
MCP-1	Monocyte chemotactic protein-1
MHC	Major histocompatibility complex
MIF	Macrophage migration inhibitory factor
MMPs	Matrix metalloproteinases
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid

NGF	Nerve growth factor
NK	Natural killer
NSAIDs	Non-steroidal anti-inflammatory drugs
PCR	Polymerase chain reaction
PTEN	Phosphatidylinositol-3, 3, 5,- triphosphate 3-phosphate
PTPN22	Protein tyrosine phosphatase non-receptor type 22
RA	Rheumatoid arthritis
RANTES	Regulated on Activation, Normal T-cell Expressed and Secreted
SLE	Systemic lupus erythematosus
SNPs	Single nucleotide polymorphisms
SSRs	Simple sequence repeats
STAT4	Signal transducer and activator of transcription 4
TGF-β	Transforming growth factor-β
TIMPs	Tissue inhibitors of matrix metalloproteinases
TNF-α	Tumor necrosis factor α
TRAF1-C5	TNF receptor-associated factor 1-complement component 5
VEGF	Vascular endothelial growth factor

1 POPULÄRVETENSKAPLIG SAMMANFATTNING

Endometrios är en vanlig sjukdom som drabbar ca 10% av kvinnor i reproduktiv ålder. Sjukdomen är associerad med bäckensmärtor och infertilitet. Endometrios består av små härdar av endometrievävnad (livmodervävnad) som fäster utanför livmodern. Orsaken till sjukdomens uppkomst är fortfarande oklar, men olika teorier finns. Man vet att under menstruationen förekommer en retrograd blödning hos de flesta kvinnor, vilket innebär att blod och endometrievävnad kommer ut i bukhålan. I normala fall tas blodet och vävnaden om hand av immunceller, men hos kvinnor som utvecklar endometrios verkar detta inte ske. Vävnaden fäster sedan till bl.a. bukväggen och äggstockarna, där den regleras hormonellt och blöder såsom det normala endometriet under menscykeln. Man tror att även andra faktorer spelar en roll i utvecklingen av sjukdomen. Vävnaden som fäster på bukväggen/äggstockarna tros ha förändrade egenskaper som gör att dessa celler kan överleva och fästa till/invadera annan vävnad. Dessutom har man sett att kvinnor med endometrios har en ökad inflammatorisk aktivitet i bukvätskan, som skulle kunna påverka utvecklingen av endometrios och fertiliteten. Visst genetiskt anlag skulle också kunna göra att man lättare utvecklar sjukdomen.

Vi har studerat resultat av assisterad befruktning (IVF) hos kvinnor med endometrios i jämförelse med friska kvinnor med infertilitet på grund av skadade äggledare. Dessutom har vi tittat på inflammatoriska markörer i follikelvätskan, dvs. den vätska som omger de mognande äggen i äggstocken. Vi såg att kvinnorna med endometrios hade ökade nivåer av pro-inflammatoriska markörer (TNF-α, IL-15 and GM-CSF) och sänkta nivåer av en anti-inflammatorisk markör (IL-10). Dessa kvinnor hade även lägre nivåer av AMH, en markör för äggreserven i äggstocken, i follikelvätska och serum, vilket tyder på att kvinnor med endometrios har färre ägg som kan mogna och bli befruktade. Vi kunde även se att kvinnor med endometrios svarade bra på IVF behandlingen, men att befruktning av deras ägg var sämre än i kontrollgruppen.

Kvinnor med endometrios har en ökad inflammatorisk aktivitet och man har sett att dessa kvinnor i en högre utsträckning drabbas av autoimmuna sjukdomar, bl.a. reumatism. Samband har hittats mellan olika genetiska variationer och olika autoimmuna sjukdomar. Vi ville därför undersöka om de genetiska variationer som är kopplade till reumatism även är kopplade till endometrios. Vi valde att titta på variationer i gener som är involverade i inflammation, men inget samband kunde hittas mellan endometrios och dessa variationer. Däremot, när vi delade upp kvinnorna med endometrios i två grupper beroende på sjukdomens svårighetsgrad, såg vi en koppling mellan variationer i två olika gener (CCL21 och HLA-DRB1) och moderat/svår endometrios.

Ett samband mellan endometrios och ovarialcancer har också hittats. Det är möjligt att endometrios på äggstockarna skulle kunna omvandlas direkt till cancer. En annan teori är att endometrios och ovarialcancer delar samma mekanismer eller genetiska variationer som gör att drabbade kvinnor lättare utvecklar sjukdomarna. Genetiska variationer i genen BNC2 har tidigare kopplats till ovarialcancer och dessa variationer

skulle även kunna vara kopplade till endometrios. I vår studie kunde vi inte hitta någon koppling till sjukdomen.

Vi har även studerat olika faktorer som påverkar cellernas förmåga att fästa och invadera annan vävnad. Vi såg att alla dessa faktorer (ApoE, ITGB2, ITGB7, LAMC1, CD24 and JAM-1) fanns i endometrium från friska kvinnor, i endometrium från kvinnor med endometrios och i endometriosvävnad. Olika uttryck av dessa faktorer hos friska kvinnor och kvinnor med endometrios skulle kunna göra att den vävnad som hamnar i buken under mensen kan fästa och invadera och därmed leda till att endometrioshärdar bildas.

Endometrios är en komplicerad sjukdom, där många olika faktorer spelar in. Därför är det svårt att veta vad som egentligen orsakar sjukdomen och hur man ska behandla den på bästa sätt. Dessutom påverkas kvinnorna som drabbas av endometrios inte bara fysiskt, utan även psykiskt. Fler studier behövs för att undersöka hur endometrios uppkommer och mina studier är bara ett steg på vägen. Genom att få pusselbitar från olika studier, hoppas jag att pusslet kan bli komplett så att kvinnorna med endometrios kan få ett liv utan smärtor och infertilitet.

2 INTRODUCTION

2.1 ENDOMETRIOSIS

Endometriosis is a benign gynecological disease, characterized by the presence of endometrial stroma and glands outside the uterine cavity. It occurs mostly as peritoneal surface lesions, ovarian lesions and deeply infiltrating lesions of the rectovaginal septum or gut. Endometriosis located within the muscular wall of the uterus is called adenomyosis. Endometriosis is associated with chronic pelvic pain, dysmenorrhoea, dyspareunia, infertility and inflammation, although not all women with endometriosis are symptomatic.

Endometriosis affects about 10% of women of reproductive age (reviewed in (Eskenazi and Warner, 1997)), from all ethnic and social groups. About 20-40% (1994, Strathy, et al., 1982, Verkauf, 1987) of women with infertility have endometriosis. Thus, the disease also has a major impact on general physical, mental and social well-being (Jones, et al., 2004, Lorencatto, et al., 2006). In addition, endometriosis is a large healthcare problem and imposes a substantial economic burden on society. Simoens *et al.* estimated that the annual cost of endometriosis in the USA in 2002 was about 22 billion USD (Simoens, et al., 2007). However, not only hospital visits are costly, but also indirect cost, such as time lost from work due to the disease. Hummelshoj *et al.* reported that 78% of women with endometriosis in UK lose a mean of 5.3 days of work per month, due to their disease (Hummelshoj, et al., 2006).

2.1.1 Diagnosis and staging of endometriosis

Establishing a diagnosis of endometriosis is difficult. Therefore, the time between onset of symptoms and diagnosis can take several years. In USA and UK, the delay of diagnosis is about 7-12 years (Hadfield, et al., 1996). Today, laparoscopy is the gold standard for diagnosis. Noninvasive techniques, such as ultrasound and magnetic resonance imaging (MRI), could also be used to diagnose some types of endometriosis (reviewed in (Brosens, et al., 2004)). Currently, there are no reliable biomarkers for the diagnosis or prognosis of endometriosis. Different markers in serum and peritoneal fluid, including cancer antigen 125 (CA125) (Colacurci, et al., 1996) and cytokines such as Interleukin 6 (IL-6) and tumor necrosis factor (TNF)-α (Bedaiwy, et al., 2002), have been suggested. Although promising results have been shown (Ceyhan, et al., 2010, Seeber, et al., 2010), further studies are needed to evaluate the relevance of these biomarkers for diagnosis. Interestingly, a recent study by Tokushige *et al.* demonstrated that Cytokeratin-19 in urine could be a possible biomarker (Tokushige, et al., 2011).

The severity of endometriosis is assessed by the revised classification system developed by the American Society of Reproductive Medicine (ASRM) (1997), using four stages (I-IV or minimal to severe disease), on the basis of type, location, appearance, depth of invasion and extent of disease. However, there is no correlation between stage and severity of pain symptoms or prediction of infertility treatment.

The extent of endometriosis varies from small lesions to large ovarian endometriotic cysts (endometriomas or chocolate cysts) with extensive fibrosis and adhesions leading to distortion of the pelvic anatomy. Endometrial lesions are mainly divided into three types; 1) peritoneal lesions on the peritoneal surface, 2) deep infiltrating lesions located at least five mm under the peritoneum and 3) ovarian endometriotic cysts. The lesions typically appear as red, black/blue or white, representing different steps in the evolutionary process of endometriosis (reviewed in (Nisolle and Donnez, 1997)). Red lesions are considered to be the first stage, as they are most active and highly vascularized. When these lesions bleed and accumulate blood, they turn into black/blue lesions, a characteristic for advanced endometriosis. White lesions are considered healed and latent, but may remodel into more active lesions (D'Hooghe, et al., 1992).

Infiltration of nerve fibers (Tokushige, et al., 2006, Zhang, et al., 2010) and neuroendocrine cells (Wang, et al., 2010) have been observed in endometriotic lesions. Over-expression of nerve growth factor (NGF) was found in peritoneal fluid from women with endometriosis, which also promoted neurite outgrowth *in vitro* (Barcena de Arellano, et al., 2010). Interestingly, Al-Jefout *et al.* observed that detection of nerve fibers in endometrial biopsies provided a reliable diagnosis of endometriosis (Al-Jefout, et al., 2009).

2.1.2 Treatment of endometriosis

Endometriosis can be treated by surgery and/or pharmacologically, with three aims: to reduce pain, increase fertility/pregnancy and delay recurrence as long as possible. Most pharmacological treatments are based on hormonal suppression, leading to a hypo-estrogenic state and the elimination or reduction in size of the endometriotic lesions. Pharmacological treatment includes progestins, danazol, gonadotropin-releasing hormone (GnRH) analogues, the levonorgestrel-releasing intrauterine system (lng-IUS) and oral contraceptives. Some treatment, like the lng-IUS (Mirena) only alleviates pain, but does not affect the disease (reviewed in (Bahamondes, et al., 2007)). Hormonal suppression with certain drugs, such as GnRH, for a longer time period, leads to undesirable side effects due to a systemic estrogen deficiency.

Other pharmacological drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat chronic pain in endometriosis patients. Regression of lesion size in rats has been observed during treatment with an aromatase inhibitor (letrozole) and reduction of pain symptoms in humans has been observed in the clinic with treatment of either aromatase inhibitors alone or in combination with oral contraceptives (Seal, et al., 2011, Verma and Konje, 2009).

Alternative non-hormonal targets for treatment of endometriosis have been suggested, such as immune-modulators, anti-angiogenic agents and anti-inflammatory drugs. For example, treatment with anti-TNF antibody has been shown to reduce the extent of endometriosis in baboons (Falconer, et al., 2006).

Surgical removal of endometriotic lesions aims at reducing pain by removing the endometriotic tissue and to restore normal pelvic anatomy. Although surgery

effectively alleviates pain (reviewed in (Jacobson, et al., 2009)), endometriotic lesions usually recur within a few years. Also, surgery of ovarian endometriosis is associated with decreased ovarian reserve (Ragni, et al., 2005).

2.1.3 Is endometriosis one disease or several?

Different types of endometriosis can be observed in the pelvis. It is not clear if peritoneal endometriosis is an early stage of the disease, later developing into ovarian and deep infiltrating endometriosis, or if these types of endometriosis could represent three different diseases with different etiologic mechanisms (reviewed in (Nisolle and Donnez, 1997)). It has also been speculated whether mild endometriosis should be regarded as a natural condition occurring in all women (Koninckx, et al., 1994). Gene expression studies have reported differences between ovarian endometriosis and deep infiltrating endometriosis, indicating that the local endocrine control and hormonal microenvironment could differ between these two types of disease (Matsuzaki, et al., 2006). This is strengthened by varied expression of aromatase between different types of endometriosis (Heilier, et al., 2006). A genetic study further supports this theory by reporting genetic association with protein tyrosine phosphatase non-receptor type 22 (PTPN22) and endometriosis, but only in women with moderate/severe disease (Gomes, et al., 2010).

2.2 PATHOGENESIS OF ENDOMETRIOSIS

The pathogenesis of endometriosis is not fully understood. Several different theories have been suggested:

- The *implantation/ transplantation theory* indicates the development of endometriosis by the deposit of viable endometrial cells into the peritoneal cavity by retrograde menstruation through the fallopian tubes, where the endometrial cells are able to attach and grow (Sampson, 1927).

- The *induction theory* suggests that degenerating endometrium in the abdominal cavity releases factors and thus inducing a metaplastic process in mesenchymal cells, leading to endometriosis (Levander and Normann, 1955).

- According to the *in situ development theory* ectopic endometrium develops *in situ* from local tissue, including remnants of the Wolffian and Müllerian ducts (Lauchlan, 1972).

The most widely accepted theory today is the implantation theory proposed by Sampson (Sampson, 1927), which is supported by several observations. Firstly, women with endometriosis have been shown to have shorter cycles (Arumugam and Lim, 1997) and heavier menstrual flow (Vercellini, et al., 1997). Secondly, viable cells in the endometrial effluent have also been observed (Keettel and Stein, 1951). In addition, Ridley and Edwards showed that endometrial tissue, collected from menstrual endometrium, could attach and proliferate in ectopic locations (Ridley and Edwards, 1958). Thirdly, patients with obstructed menstrual outflow have an increased risk of

developing endometriosis (Olive and Henderson, 1987). This has also been confirmed by D´Hooghe *et al.,* who showed that cervical ligation in baboons leads to endometriosis (D'Hooghe, et al., 1994). Furthermore, a correlation between the amount of endometrium used for induction and the extent of endometriosis was observed in baboons (D'Hooghe, et al., 1995). These observations strongly indicate an important role of retrograde menstruation in the development of endometriosis.

It is well known that retrograde menstruation occurs in most women, with an incidence of 76-90% (Halme, et al., 1984, Liu and Hitchcock, 1986), but not all women develop endometriosis. Therefore, the development of endometriosis is likely to not only involve retrograde menstruation, but also other factors such as adhesion and invasion of endometrial cells, proliferation, angiogenesis and immune escape, seen in figure 1. Furthermore, a genetic predisposition seems to be involved.

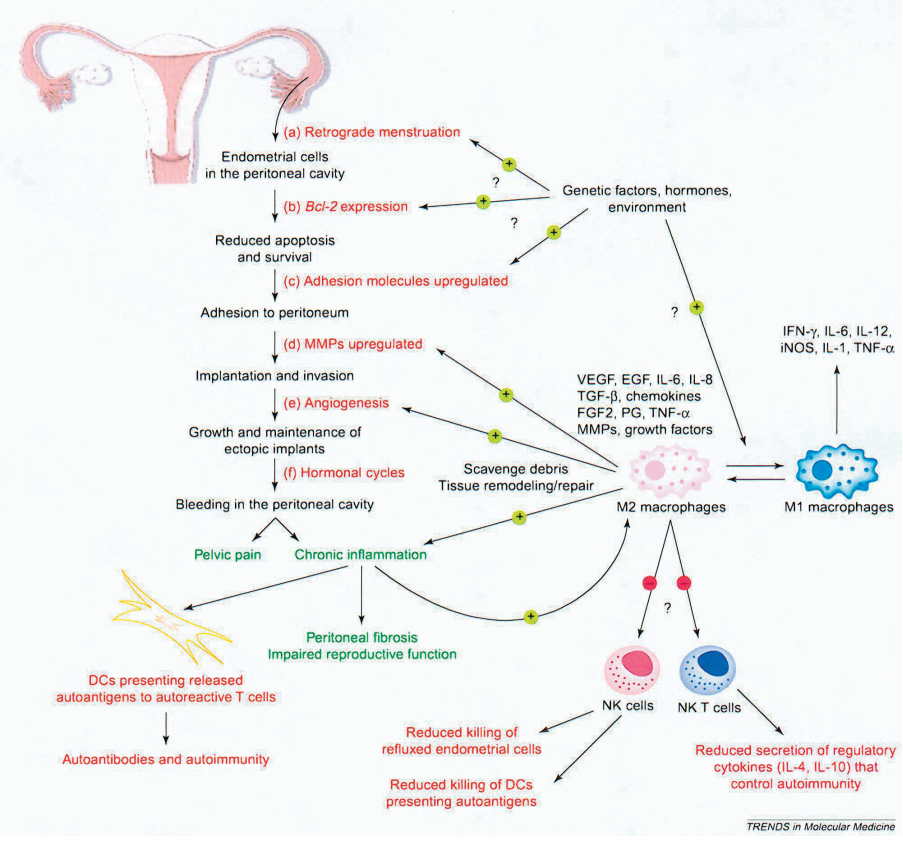

Figure 1. Different factors involved in the pathogenesis of endometriosis. (Reprinted from Matarese *et al.* Trends Mol Med. 2003 May;9(5):223-8 with permission from Elsevier.)

2.2.1 Adhesion, invasion and proliferation of endometrial cells

Apart from only being present in the peritoneal cavity, endometrial cells are able to attach and invade the peritoneum and proliferate to create endometriotic lesions. It appears that both endometrial stromal cells and epithelial cells are able to attach to the peritoneal membrane (Witz, et al., 2001). Evidence of invasion of endometriotic lesions has also been demonstrated by Spuijbroek *et al.* (Spuijbroek, et al., 1992). The invasion of endometrial cells requires local degradation of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs). In normal endometrium MMPs and their inhibitors, tissue inhibitors of matrix metalloproteinases (TIMPs), are needed for the control of tissue breakdown and a higher expression of these factors is seen during the menstrual phase (reviewed in (Pitsos and Kanakas, 2009)). Aberrant expression of MMPs and TIMPs has been seen in endometriosis with increased levels of MMP-9 in endometrium from women with endometriosis (Collette, et al., 2006). Also, the ratio of MMP-9/TIMP-1 protein and mRNA levels were increased. This is strengthened by other studies, which have found decreased levels of TIMP-1 in peritoneal fluid (Szamatowicz, et al., 2002) and higher mRNA levels of MMP-2, -9 and -14 in endometriotic cells (Ueda, et al., 2002). In addition, MMPs are regulated by steroid hormones and cytokines, which are important regulators of endometriosis (reviewed in (Pitsos and Kanakas, 2009)). Interleukin-1 (IL-1) increases the secretion of MMP-3 in endometrial cells from women with endometriosis (Sillem, et al., 2001). Endometrial stromal cells treated with IL-8 showed increased MMP-2 and -9 activities and invasiveness (Mulayim, et al., 2004).

Cells from endometriotic lesions have demonstrated increased capacity to proliferate compared to endometrial cells from women without endometriosis (Li, et al., 1993, Nisolle, et al., 1997). Klemmt *et al.* demonstrated that stromal cells from endometriotic lesions and from the endometrium of women with endometriosis had an increased adhesive and proliferative ability in response to specific ECM components (Klemmt, et al., 2007).

Cell adhesion molecules facilitate cell-cell and cell-ECM adhesion. It has been suggested that cell adhesion molecules, such as integrins and cadherins, are involved in the development of endometriosis. The adhesion of endometrial cells in endometriosis is likely to be modulated in connection with ECM molecules. Integrins, cadherins, laminin and fibronectin have been observed in the endometrium and in endometriosis (Beliard, et al., 1997, van der Linden, et al., 1994, van der Linden, et al., 1994). Beliard *et al.* reported a stronger expression of integrin $\alpha 5$ by stromal cells in endometriosis, compared to stromal cells in healthy endometrium (Beliard, et al., 1997). Furthermore, decreased expression of N-cadherin (Van Patten, et al., 2010), but also E-cadherin and CD44 (Poncelet, et al., 2002), have been observed in peritoneal endometriosis. Other factors, such as L1 cell adhesion molecule (L1CAM) may also be involved in the development of endometriosis (Finas, et al., 2008). Inhibition of L1CAM in an endometriosis epithelial cell line leads to decreased cell proliferation and invasion (Agic, et al., 2010).

2.2.2 Estrogenic effects

It has been known for some time that estrogen is involved in the pathogenesis of endometriosis. Aromatase is the key enzyme in the biosynthesis of estrogen, which is important for the establishment and growth of endometriosis. Expression of aromatase has been found in endometriotic lesions and endometrium from women with endometriosis (Noble, et al., 1996, Velasco, et al., 2006), creating an estrogenic environment. Also, stimulation of endometriotic stromal cells with IL-6 lead to a higher aromatase activity (Velasco, et al., 2006).

The survival and growth of endometriotic lesions requires an adequate blood supply, indicating an important role of angiogenesis in endometriosis. Estrogen has a function in regulating angiogenic growth factors (reviewed in (Hyder and Stancel, 1999)). Increased levels of angiogenic factors, such as vascular endothelial growth factor (VEGF) which is one of the most potent angiogenic factors, are found in the peritoneal fluid of endometriosis patients with advanced disease (Mahnke, et al., 2000). In addition, peritoneal fluid from women with endometriosis increased the expression of VEGF in endometrial cell cultures (Cosin, et al., 2010). McLaren *et al.* observed that VEGF production by macrophages in the peritoneal fluid was increased after stimulation with estrogen and progesterone (McLaren, et al., 1996). Macrophage migration inhibitory factor (MIF), a potential mediator of angiogenesis, is increased in the peritoneal fluid from women with endometriosis and affects the proliferation of endothelial cells (Kats, et al., 2002).

Estrogen also has a role in apoptosis. Removal of estrogen in cell cultures was associated with decreased cell viability and increased number of apoptotic cells (Song, et al., 2002). Estrogen also increases the phosphorylation of Akt, a regulator of apoptosis and cell survival (Guzeloglu Kayisli, et al., 2004). Impaired apoptosis in endometriotic cells of women with endometriosis may contribute to the pathogenesis of the disease. In healthy women, apoptosis is important to maintain the cellular homeostasis during the menstrual cycle (Kokawa, et al., 1996). In women with endometriosis, increased expression of anti-apoptotic factors and decreased expression of pro-apoptotic factors have been reported, supporting an anti-apoptotic phenotype of endometriotic cells (reviewed in (Agic, et al., 2009)). Gebel *et al.* demonstrated decreased apoptosis in eutopic endometrium in women with endometriosis compared to controls, which was further decreased in ectopic endometrium (Gebel, et al., 1998).

2.2.3 Inflammation and immune response

Increased inflammation and altered immune functions have been observed in women with endometriosis. A complex network of locally produced cytokines and immune cells have been proposed to modulate the growth and inflammatory behavior of the endometriotic implants (reviewed in (Osuga, et al., 2010)). An increased number of activated macrophages have been seen in the peritoneal fluid of endometriosis patients (Dunselman, et al., 1988, Keenan, et al., 1995). Oosterlynck *et al.* reported a decreased natural killer (NK) cell activity and cytotoxicity in peritoneal fluid (Oosterlynck, et al., 1991). A reduction of activated T-cells (Ho, et al., 1995) and mature dendritic cells (Schulke, et al., 2009) have been seen in women with endometriosis. In addition,

peritoneal fluid from endometriosis patients contained a significantly higher NK cell suppressive activity than peritoneal fluid from fertile controls (Oosterlynck, et al., 1993).This may partly explain the increased survival of endometriotic cells in the peritoneal cavity.

Inflammatory cytokines play a central role in the regulation of cell proliferation, activation, motility, adhesion, chemotaxis and morphogenesis. Several cytokines, such as IL-1, IL-5, IL-6, IL-8, IL-15, monocyte chemotactic protein-1 (MCP-1), TNF-α, transforming growth factor-β (TGF-β) and Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES) have been implicated in the pathogenesis of endometriosis (reviewed in (Lebovic, et al., 2001)). It has been observed that the level of some cytokines in peritoneal fluid and serum correlates to the severity of the disease. Expression of TNF-α, IL-8 and MCP-1 was higher in early stage of endometriosis and decreased with more advanced disease, while TGF-β expression decreased with the severity of the disease (Pizzo, et al., 2002). RANTES was also increased in the peritoneal fluid in women with more severe disease (Khorram, et al., 1993).

It has been reported that endometrial cells stimulated with IL-8 showed increased survival *in vitro* (Gazvani, et al., 2002). Iwabe *et al.* demonstrated that TNF-α stimulated proliferation of endometriotic stromals cells through the induction of IL-8 (Iwabe, et al., 2000). Moreover, TNF-α increases the adherence of stromal cells to mesothelial cells in culture (Zhang, et al., 1993). Interestingly, TNF-α has a proliferative effect on endometrial cells from endometriosis patients, but not on endometrial cells from healthy women (Braun, et al., 2002). The abnormalities seen in the peritoneal fluid from endometriosis patients may also be linked to endometriosis-associated infertility.

2.2.4 Autoimmune features

It has been proposed that endometriosis is associated with autoimmune disease, due to elevated autoantibody titers in women with the disease (Gleicher, et al., 1987). Sinaii *et al.* have observed higher rates of autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Sjögren´s syndrome, in endometriosis patients (Sinaii, et al., 2002). Several mechanisms, such as familial occurrence, T- and B-cell abnormalities, tissue damage and altered apoptosis, are common in both endometriosis and autoimmune diseases (reviewed in (Matarese, et al., 2003, Nothnick, 2001)).

2.2.5 Endometrial stem cells

Recently, it has been suggested that endometrial stem/progenitor cells are not only involved in the regeneration of endometrium, but also the pathogenesis of endometriosis (reviewed in (Gargett, 2004)). It may be possible that in women developing endometriosis, inappropriately shed stem/progenitor cells reach the peritoneal cavity by retrograde menstruation, and thereby develop endometriotic implants (reviewed in (Sasson and Taylor, 2008)). The first evidence of stem/progenitor cells in the endometrium came from cloning studies, when Chan *et al.*

observed that a small population of human endometrial epithelial and stromal cells possessed clonogenic activity *in vitro* (Chan, et al., 2004). It has also been shown that cells from the endometrium can differentiate into mesodermal lineages *in vitro*, such as chondrogenic (Wolff, et al., 2007), adipose (Dimitrov, et al., 2008), myogenic, and osteogenic (Schwab and Gargett, 2007) cell types, suggesting that these cells are multipotent adult stem cells with an activity similar to mesenchymal stem cells. Another possibility is that bone marrow-derived stem cells could have a role in both the regeneration of the endometrium and in endometriosis (Du and Taylor, 2007).

2.3 INFERTILITY AND ENDOMETRIOSIS

Several studies have found an association between endometriosis and infertility (reviewed in (de Ziegler, et al., 2010)). Endometriosis is present in 20-40% of women with infertility (1994, Strathy, et al., 1982, Verkauf, 1987). A meta-analysis by Barnhart *et al*. reported that women with endometriosis have ≤54% reduction in pregnancy rate after *in vitro* fertilization (IVF), compared to women with tubal factor infertility (Barnhart, et al., 2002). Moreover, decreased pregnancy rate per cycle, pregnancy per transfer and implantation rate have been observed in endometriosis patients (Arici, et al., 1996, Simon, et al., 1994). Women with endometriosis stage III/IV seem to have a worse prognosis for IVF/intracytoplasmic sperm injection (ICSI) treatment (Kuivasaari, et al., 2005).

The underlying reason for endometriosis-associated infertility is debated. Several mechanisms have been proposed, such as distorted pelvic anatomy, endocrine and ovulatory abnormalities, altered peritoneal function, poor oocyte quality and defective implantation capacity (figure 2). *In vitro* studies of fertilized oocytes from endometriosis patients showed a decrease in the number of blastomeres and an increase in the percentage of arrested embryos compared with controls (Pellicer, et al., 1995), indicating a reduced quality of embryos from endometriosis patients. Oocyte donation studies report lower implantation rates in recipients who received oocytes from women with endometriosis (Simon, et al., 1994). Furthermore, cytokines in the peritoneal fluid are believed to be involved in the endometriosis-associated infertility. Since the ovaries and the fallopian tube are exposed to peritoneal fluid, different factors in the peritoneal fluid could have influence on reproductive functions. Thus, early embryos are exposed to the peritoneal fluid, which could be toxic for the preimplantation embryo (Morcos, et al., 1985). This is strengthened by Damewood *et al.,* who reported that serum from endometriosis patients decreases the growth of mouse embryos (Damewood, et al., 1990). High levels of TNF-α and MIF have been found to reduce sperm motility *in vitro* (Carli, et al., 2007, Said, et al., 2005) and high levels of RANTES have a negative effect on the sperm fertilizing ability (Barbonetti, et al., 2008). TNF-α also reduced maturation of porcine oocytes (Ma, et al., 2010).

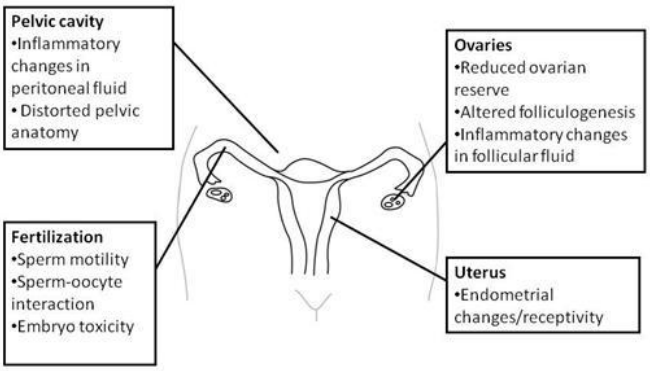

Figure 2. Proposed mechanisms of endometriosis on fertility.

2.3.1 Ovarian reserve

The ovarian reserve has been shown to decrease with increasing age, leading to a lower reproductive capacity (reviewed in (te Velde, et al., 1998)). Several different techniques have been proposed for the assessment of the ovarian reserve. Commonly used tests are the measurement of follicle stimulating hormone (FSH), estradiol and inhibin B in serum on cycle day 3 of the menstrual cycle and the number of antral ovarian follicles (antral follicle count) with ultrasound. Another marker of the ovarian reserve is the serum level of anti-mullerian hormone (AMH) (van Rooij, et al., 2002). AMH is produced by preantral and small antral follicles and may act as a paracrine regulator of early follicular growth (Weenen, et al., 2004). The concentration of AMH is not influenced by the menstrual cycle (Streuli, et al., 2009) or oral contraceptives (Streuli, et al., 2008), making it possible to measure AMH levels at any time of the menstrual cycle during hormonal treatment. Higher levels of AMH in serum have been associated with a higher number of retrieved oocytes (Seifer, et al., 2002) and a higher pregnancy rate (Hazout, et al., 2004). Reduced levels of AMH have been reported in infertile women with endometriosis (Shebl, et al., 2009).

2.4 ENDOMETRIOSIS-ASSOCIATED MALIGNANCY

Although endometriosis is a benign disease, it has been considered to have malignant characteristics. Endometriosis involves attachment and invasion of healthy tissue, loss of control of cell proliferation and is spread both locally and distantly. Furthermore, genetic, angiogenic, endocrine and immunological abnormalities have been observed in endometriosis patients. In malignancy, these properties are also altered. Epidemiological studies show association of different types of malignancy and endometriosis, such as ovarian cancer, breast cancer, colon cancer, endocrine tumours, brain tumours and non-Hodgkin´s lymphoma (Bertelsen, et al., 2007, Brinton, et al., 1997, Brinton, et al., 2005, Gemmill, et al., 2010, Hornstein, et al., 1997, Melin, et al., 2006, Olson, et al., 2002). Interestingly, Melin et al. observed better survival in endometriosis patients with cancer compared to women with the same form of cancer

but without endometriosis, especially those with breast and ovarian cancer (Melin, et al., 2010).

2.4.1 Ovarian cancer

Several studies have found associations between endometriosis and ovarian cancer (Brinton, et al., 1997, Brinton, et al., 2005, Melin, et al., 2006). In a Swedish population study, the risk of ovarian cancer was increased 4.2-fold in subjects with a long-standing history of ovarian endometriosis (Brinton, et al., 1997). Melin et al. reported that women with early diagnosis and long-standing endometriosis had a higher prevalence of ovarian cancer (Gemmill, et al., 2010, Melin, et al., 2006). In addition, cancer was found more often in the ovaries when endometriosis was present in the ovary (Stern, et al., 2001). Clear cell and endometrioid carcinoma were the most commonly seen types of ovarian cancers in endometriosis patients (Fukunaga, et al., 1997, Ogawa, et al., 2000, Stern, et al., 2001). The most frequent location for coexistence of endometriosis and cancer is the ovaries, which has been estimated to occur in 0.7-5.0% of all cases with ovarian cancer (Erzen and Kovacic, 1998, Ogawa, et al., 2000, Stern, et al., 2001).

2.4.2 Transformation of endometriosis into ovarian cancer

The mechanism by which endometriosis is transformed into malignancy is unclear. Two theories have been suggested, seen in figure 3 (reviewed in (Varma, et al., 2004)). It is possible that endometriosis is directly transformed into ovarian cancer via atypical endometriosis. Another theory is that endometriosis and ovarian cancer share common mechanisms and/or predisposing factors, such as an inflammatory environment, genetic susceptibility and hormonal factors.

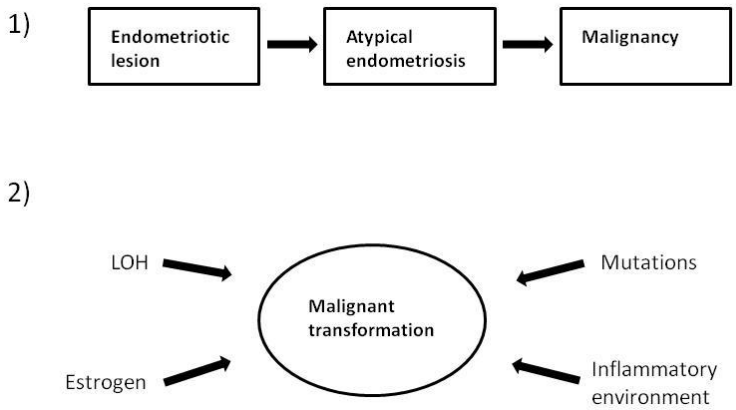

Figure 3. Two possible pathways of the transformation of endometriosis into ovarian cancer.

Ovarian cancer derived from the malignant transformation of endometriosis has been referred to as endometrioisis-associated ovarian cancer (EAOC). Atypia has been observed in EAOC (Fukunaga, et al., 1997, Prefumo, et al., 2002) and Ogawa *et al.* reported evidence of transformation from endometriotic lesions to atypical endometriosis and further into carcinoma (Ogawa, et al., 2000). About 60% of cases of EAOC occur with endometriosis adjacent to the cancer or direct transformation from the endometriotic lesion (Erzen and Kovacic, 1998, Modesitt, et al., 2002). Banz *et al.* observed equally regulated genes in endometriosis and EAOC (Banz, et al., 2010).

A variety of different molecular changes have been suggested to be involved in the malignant transformation of endometriosis. Genetic alterations are a well-known characteristic of malignancy. Especially alterations in oncogenes and tumor suppressor genes, such as p53 alterations, Kirsten rat sarcoma viral oncogene homologue (K-ras) mutations and Phosphatidylinositol-3, 3, 5,- triphosphate 3-phosphate (PTEN) silencing, have been suggested to play a role in malignant transformation of endometriosis. In a mouse model of endometrioid ovarian cancer, PTEN deletion and K-ras activation, gave rise to endometriosis-like lesions, which further developed into endometrioid ovarian carcinoma (Dinulescu, et al., 2005). Mutations in PTEN have also been observed in endometriomas, as well as in ovarian cancer (Sato, et al., 2000) and K-ras mutations have been seen in endometrioid (Amemiya, et al., 2004) and clear cell carcinoma (Okuda, et al., 2003). Sáinz de la Cuesta *et al.* demonstrated an increase in expression of p53 in the transformation from atypical endometriosis to ovarian cancer (Sainz de la Cuesta, et al., 2004). Furthermore, several studies have reported loss of heterozygosity (LOH) as an event in the malignant transformation of endometriosis (Jiang, et al., 1998, Prowse, et al., 2006, Sato, et al., 2000).

Malignant transformation is also influenced by the microenvironment. The inflammatory environment in endometriosis, with increased levels of cytokines, growth factors and hormones, has been proposed to play a role in the malignant transformation of endometriosis (reviewed in (Nezhat, et al., 2008, Varma, et al., 2004)). Ness *et al.* reported similar alterations in inflammatory and immune response in women with endometriosis and ovarian cancer (Ness, 2003). In addition, resistance to apoptosis, angiogenesis and the ability to invade is shared between endometriosis and malignancy.

2.5 GENETIC STUDIES OF ENDOMETRIOSIS

Several studies have indicated a genetic contribution in endometriosis. Familial studies have shown a six to nine fold increase in risk of endometriosis among relatives (reviewed in (Kennedy, et al., 2001)). In addition, Stefansson *et al.* reported a relative risk of 2.20 for sisters and 1.56 for cousins to develop endometriosis (Stefansson, et al., 2002). Twin studies (Moen, 1994) and animal studies (Zondervan, et al., 2004) also support the role of genetics in the pathogenesis of endometriosis. However, not only genes seem to contribute to endometriosis, but also environmental factors, indicating that endometriosis is a complex disease (Kennedy, 1999). Treloar *et al.* reported that 50% of the variance of susceptibility to endometriosis in Australian twins was attributed to genetic factors (Treloar, et al., 1999).

2.5.1 Genetics of complex diseases

Diseases which do not exhibit classical Mendelian inheritance are described as complex diseases. Complex diseases are relatively common in the general population and seem to cluster within families. In addition, complex diseases are believed to be influenced by several genetic and environmental factors, as well as interactions among them. The common variant hypothesis suggests that common genetic variants with relatively high frequency, but low penetrance, are the major contributors to common complex diseases (reviewed in (Altshuler, et al., 2008)). However, rare variants, with a low frequency and high penetrance also contribute to complex diseases (Stratton and Rahman, 2008). Most likely, the genetic etiology is based on a combination of both rare and common variants.

The two most common methods used to investigate complex diseases are linkage and association studies. These two methods basically rely on the same principle; i.e. co-inheritance of adjacent DNA variants. Linkage studies identify haplotypes that are inherited for several generations within families and association studies investigate the retention of adjacent DNA versions for many generations in cases and controls (reviewed in (Cardon and Bell, 2001)).

2.5.2 Sequence variations in the genome

Even though humans are said to be 99.9% identical in respect to DNA, a lot of different variations in the human genome have been observed. Genetic variation can explain the different phenotypes among individuals, although the majority of variants are believed to be neutral with no phenotypic effect. Common variations have a minor allele frequency (MAF) of at least 1%, while rare variations (mutations) have a MAF less than 1% (reviewed in (Frazer, et al., 2009)). Genetic variations include single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs, also known as micro- and minisatellites), as well as structural variants, such as insertions and deletions (indels), inversions, block substitution and copy number variants (CNV) (reviewed in (Feuk, et al., 2006)).

SNPs are the most common genetic variation, where a single nucleotide is changed, inserted or deleted, seen in figure 4. The number of SNPs in the human genome is estimated to be around 3.3 million, with an average of 1 SNP in 1000 bases (reviewed in (Altshuler, et al., 2008, Frazer, et al., 2009)). SNPs are found both in non-coding and coding regions. Synonymous SNPs, localized in coding regions, can cause an amino-acid change, frame shift or termination of translation, thereby giving a functional effect on protein level, or the SNP does not affect the amino-acid sequence. SNPs located in non-coding regions, non-synonymous SNPs, could also have a functional effect, due to localization in regulatory elements such as gene promotors, enhancers or silencers.

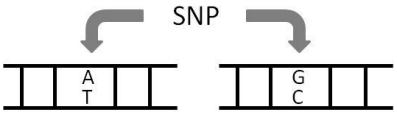

Figure 4. Single nucleotide polymorphism (SNP).

Association studies investigate the correlation between a genetic variation and a trait, thereby comparing the differences in allelic frequency between cases and controls. Often the genetic variant lies within a candidate gene, which is chosen due to its relevance in the pathophysiology of the disease, or from previously found linkage regions. Association between a marker and a phenotype can be due to two reasons; either by marking the causal allele itself or by marking neighboring variants in linkage disequilibrium (LD). LD defines genetic variants positioned close, which tend to be inherited together, and the combination on those linked variants are called haplotypes (Daly, et al., 2001). Associated variants need to be replicated in several independent populations and functional studies are required to determine the effect of the variant.

2.5.3 Linkage and GWAS studies in endometriosis

Genome-wide association studies (GWAS) makes it possible to genotype a million SNPs in one individual at a time (reviewed in (McCarthy and Hirschhorn, 2008)). Until today, only a few linkage studies and GWAS have been performed in the field of endometriosis (reviewed in (Montgomery, et al., 2008)). Treloar *et al.* reported a susceptibility locus for endometriosis on chromosome 10q26 and a suggestive linkage on chromosome 20p13 (Treloar, et al., 2005). Another locus on chromosome 7p13-15 has been found in linkage studies (Zondervan, et al., 2007) and the locus at 7p15.2 was identified in a GWAS performed by Painter *et al.* (Painter, et al., 2011). Furthermore, the CDKN2BAS locus has been associated with endometriosis in Japanese in a GWAS (Uno, et al., 2010) and the region around IL1α on 2q13 has been suggested as a candidate (Adachi, et al., 2010). However, in most linkage and GWAS the candidate genes and their functional relevance remain to be identified.

2.5.4 Candidate genes in endometriosis

Several different candidate genes have been suggested to be involved in endometriosis, including genes involved in inflammation, steroid synthesis, hormone receptors, growth factors, adhesion molecules, cell cycle regulation, apoptosis and oncogenesis (reviewed in (Falconer, et al., 2007)). Association of polymorphisms in inflammatory genes, such as IL-10, IL-16, IL-18, TGF-β, Interferon γ (IFN-γ) and TNF-α, has been observed, mostly in Asian populations (Ayaz, et al., 2011, Gan, et al., 2010, Hsieh, et al., 2005, Juo, et al., 2009, Kitawaki, et al., 2004, Lakshmi, et al., 2010, Teramoto, et al., 2004). Human leukocyte antigen (HLA) genes play a key role in the immune response and are highly polymorphic. Association of different HLA variants have been reported in some Japanese studies (Ishii, et al., 2002, Kitawaki, et al., 2002), while other studies could not find any association (Roszkowski, et al., 2005, Whang, et al., 2006). Studies have also reported conflicting results regarding the association of PTPN22 and

endometriosis, with two studies showing association (Ammendola, et al., 2008, Gomes, et al., 2010) and one showing no association (Ploski, et al., 2009). Many conflicting results like these have been found among the association studies, making it difficult to conclude the genetic effects in endometriosis. Most studies have small populations with poorly-defined control populations. In order to understand more about the genetic contribution to endometriosis, larger and more controlled studies are needed (Colhoun, et al., 2003, Zondervan, et al., 2002). In addition, the results should be replicated in several populations.

3 AIMS

The overall aim of this thesis was to study the aetiology and pathophysiology of endometriosis, in order to obtain a better understanding of the disease. Furthermore, the aim was to investigate the genetic background of endometriosis and genetic linkage to other diseases.

The specific aims were:

- To study the cytokine expression profile in follicular fluid from endometriosis patients undergoing IVF. (Paper I)
- To investigate a possible shared genetic background between endometriosis and RA. (Paper II)
- To evaluate if endometriosis and ovarian cancer shares disease associated genes. (Paper III)
- To identify factors involved in adhesion, attachment and invasion in endometrium and endometriosis. (Paper IV)

4 MATERIAL AND METHODS

4.1 STUDY SUBJECTS

4.1.1 IVF patients (I)

Serum samples and follicular fluid were collected at the fertility unit (RMC) at the Karolinska University Hospital, Sweden. The study included 34 women with laparoscopically-verified endometriosis. In addition, a control group, consisting of 38 women with tubal factor infertility and with no signs of endometriosis upon laparoscopy, was used for comparison. Both groups were similar with respect to age, body mass index (BMI), smoking and duration of infertility. All women underwent IVF and embryo transfer according to standard protocols.

4.1.2 Belgian cases and controls (II, III)

In study II and III, a sample set of 1149 women (798 endometriosis patients and 351 controls) was used. All women were Caucasian and had undergone laparoscopy for subfertility with or without pain at the Leuven University Hospital, Leuven, Belgium during 1998-2007. Peripheral blood samples (n=948; 660 endometriosis patients and 288 healthy controls) were collected and peritoneal biopsies (n= 229; 161 endometriosis patients and 68 controls) were taken during surgery. From some patients and controls both blood sample and tissue were obtained. The absence of endometriosis was confirmed laparoscopically in control patients (age 32 +/- 5 years; BMI 23.4 +/- 4). Women with endometriosis (age 31 +/- 4 years; BMI 23.2 +/- 4) had either minimal (stage I; n=176), mild (stage II; n=116), moderate (stage III; n=88) or severe (stage IV; n = 179) disease, staged according to the revised classification system of the American Society of Reproductive Medicine (1997).

4.1.3 Healthy women and endometriosis patients (IV)

Endometrial biopsies from healthy volunteers were taken at the Karolinska University Hospital, Sweden in both the proliferative and the secretory phase of the menstrual cycle. All women had regular menstrual bleeding and had not used any hormonal medication or an IUS at least 3 months prior to recruitment. Endometrium and endometriomas from endometriosis patients were collected during both the proliferatory and the secretory phase at Danderyds Hospital and Södersjukhuset, Sweden, and the University Hospital of Florence, Italy. The endometriosis patients had not used any IUS or hormonal medication 3 months prior to recruitment. Endometrial biopsies were taken with a Randall curette or a pipelle. The biopsies were divided and placed in RNA later for RNA isolation and formaldehyde/paraformaldehyde for immunohistochemical studies. Endometrial dating was performed histopathologically, to verify the phase of the menstrual cycle, according to Noyes criteria (Noyes, et al., 1975).

4.2 OVARIAN STIMULATION PROTOCOL (I)

All patients followed the long Gonadotrophin releasing hormone agonist (GnRHa) protocol (Fried, et al., 1996). GnRHa was administered (6x200 µg/day) (Suprefact®, Hoechst AB, Stockholm, Sweden), starting on day 21 of the menstrual cycle. It was reduced to half of the dose after the start of FSH injections. Down regulation was verified by vaginal ultrasound scanning, followed by administration of recombinant FSH (75-300 IU/day) (Gonal-F®, Serono Nordic AB, Sollentuna, Sweden). Starting dose of FSH was generally 150 IU up to age 35 and 225 IU above age 35, unless previous FHS stimulation during IVF indicated otherwise. Endometrial growth and follicular development were followed by vaginal ultrasonography, together with serum estradiol levels. After adequate stimulation, i.e. a controlled rise of serum estradiol and a leading follicle diameter of at least 17 mm, human Chorionic Gonadotrophin (hCG) (Profasi®, Serono Nordic AB, Sollentuna, Sweden) was administered. Oocyte retrieval was performed approximately 35 hours later by transvaginal ultrasound-guided follicle aspiration. Progesterone (3x400 mg/day) (Progesteron MIC APL, Sweden) was given until pregnancy test, and with a positive test, continued for 8 weeks after embryo transfer.

4.3 EXPRESSION ANALYSIS

4.3.1 Hormonal assays (I)

The concentration of AMH in serum and follicular fluid was measured by enzyme immunoassay (EIA) (A16507, Immunotech, Marseille, France). Estradiol and FSH in follicular fluid were determined by chemiluminescent enzyme immunometric assay (LKE21 resp. LKFS1, Diagnostic Products Corporation, California, USA). The estradiol samples were diluted 1:1 000 before analysis. All assays were done according to manufacturer's protocol.

4.3.2 Cytokine assays (I)

The level of TNF in follicular fluid was determined by immunoradiometric assay (IRMA), according to manufacturer's protocol (KIC1751, Biosource, California, USA).

4.3.3 Real Time RT-PCR (IV)

RNA extraction was performed using a dismembranation apparatus (Retsch KG, Haan, Germany) together with TRIZOL® reagent (Invitrogen, Carlsbad, CA, USA). RNA was treated with RQ1 RNase- free DNase (Promega Biotech AB, Stockholm, Sweden) before cDNA preparation with Superscript^{TM} II RNase H^{-} Reverse Transcriptase Kit (Invitrogen, Carlsbad, CA, USA). RNA levels were quantified with RealTime RT-PCR, using the Applied Biosystems 7300 RealTime PCR System (Applied Biosystems, Foster City, CA, USA). Primers and probes were purchased from available Taqman gene expression assays (Applied Biosystems).

The threshold cycles (CT), where an increase in reporter fluorescence above the baseline signal could first be detected, were determined. 18S was used as endogenous control. The mean CT value of 18S was used for normalization and was subtracted from the mean CT value of the respective gene, to obtain ΔCT values.

4.3.4 Immunohistochemistry (IV)

For immunohistochemical analysis a panel of antibodies has been used (for detailed information, see paper IV). Biopsies were fixed, paraffin-embedded and sectioned. All staining was performed with the MACH 3^{TM} Mouse-Probe HRP-polymer kit or MACH 3^{TM} Rabbit-Probe HRP-polymer kit (Biocare Medical, CA, USA). Stainings were developed using DAB and counterstained with Mayer´s hematoxylin. Negative controls were performed by omitting the primary antibody or by using primary isotype-matched immunoglobulins; Dako Universal Negative Control Mouse (N1698, Dako, Carpinteria, CA, USA) and ChromPure Rabbit IgG (011-000-003, Jackson Immunoresearch, West Grove, PA, USA). Sections were analysed in a Zeiss Axiovert 200M microscope (Zeiss, Göttingen, Germany) and images were captured with Qcapture, version 3.1.1. (QImagin, Surrey, BC, Canada).

For all immunohistochemical stainings, the intensity of staining and the number of positive cells were observed. Scoring was done blindly by 2 independent observers. The intensity of staining was graded as 0= no staining, 1= weak staining, 2= moderate staining and 3= strong staining. The percentage of stained cells was graded as following; 0= no staining, 1= <10%, 2=11-50%, 3= 51-80% and 4= >81%. The final score was calculated by multiplying the two scores.

4.4 GENETIC ANALYSIS

4.4.1 Genotyping (II, III)

Taqman allelic discrimination assays were performed according to manufacturer´s protocol (Applied Biosystems, Carlsbad, USA) with fluorescent-labeled allele-specific probes. After PCR the plates were read in a 7900 HT Fast-Real Time PCR system (Applied Biosystems) and analyzed with the SDS 2.2 software (Applied Biosystems).

4.4.2 Association analysis (II, III)

Case-control based associations were performed using three different models; the co-dominant model, the dominant-recessive model and allelic model. In paper II and III, the allelic model is presented.

4.4.3 Haploview (II, III)

The Haploview software 4.2 (Cambridge, MA, USA) was used to investigate linkage disequilibrium (LD) between SNPs. Permutation test (10 000 permutations) were used to correct for multiple testing.

4.5 STATISTICAL ANALYSIS

All statistical analyses were performed with the statistical package Graphpad Prism (Graphpad Software Inc., San Diego, USA). In paper I, the Mann-Whitney U-test and Fisher´s exact test was used for comparisons between women with and without endometriosis. Outliers were identified with Grubb´s outlier detection test and correlations were assessed by Spearman´s rank correlation test. In paper II and III, Bonferroni correction was used for multiple testing of SNPs. The Kruskal-Wallis test was applied in paper IV, to compare healthy endometrium, endometrium from endometriosis patients and endometriomas during the menstrual cycle. P-values less than 0.05 were considered significant.

4.6 ETHICAL PERMISSION

Permission from local ethics committees in Sweden, Italy and Belgium was obtained for all studies.

5 RESULTS AND DISCUSSION

5.1 PAPER I- IVF OUTCOME IN WOMEN WITH ENDOMETRIOSIS IN RELATION TO AMH AND INFLAMMATORY MARKERS

Many women with endometriosis suffer from infertility. It has been suggested that the increased inflammatory activity seen in endometriosis contributes to the reduced reproductive potential (reviewed in (Halis and Arici, 2004)). The IVF outcome was investigated in correlation to levels of AMH and inflammatory markers in IVF patients with endometriosis compared to a control group with tubal factor infertility. It was observed that women with endometriosis had significantly lower levels of AMH in both serum and follicular fluid. In addition, levels of the pro-inflammatory markers TNF-α, IL-15 and granulocyte-macrophage stimulating factor (GM-CSF) were increased in follicular fluid, while levels of the anti-inflammatory IL-10 were decreased. Women with endometriosis responded well to the IVF-treatment, but had lower fertilization rates.

AMH is considered to be an indicator of the ovarian reserve, and a poor response in IVF has been associated with low concentrations of serum AMH (Al-Qahtani and Groome, 2006, van Rooij, et al., 2002). The lower levels of serum AMH seen in our study, corroborates earlier finding of low levels of serum AMH at cycle day 3 in women with minimal/mild endometriosis (Lemos, et al., 2008). A positive correlation of AMH levels in follicular fluid with the total number of follicles was also observed. It seems possible that the decreased recruitment of follicles in women with endometriosis could be due to an altered hormone-cytokine balance.

In this study, increased levels of TNF-α in follicular fluid from endometriosis patients was found. These findings support a previous report of increased production of TNF-α in cultured granulosa cells from women with endometriosis (Carlberg, et al., 2000). It has been shown that TNF-α in follicular fluid correlates to oocyte quality with higher levels of TNF-α in poor quality oocytes (Lee, et al., 2000), indicating a role of TNF-α in endometriosis-associated infertility. In addition, it has been demonstrated that TNF-α regulates AMH in mouse testis (Hong, et al., 2003). It is possible that this regulation could also take place in the ovary. An earlier study by Kilic *et al*, could not demonstrate any differences in TNF-α levels in the follicular fluid from women with endometriosis compared to women with unexplained infertility (Kilic, et al., 2007). These contradictory results could be due to differences in control groups or heterogeneity among endometriosis patients.

Increased levels of inflammatory cytokines are seen in endometriosis (Matarese, et al., 2003). Our findings regarding increased levels of TNF-α, IL-15 and GM-CSF support previous results. This inflammatory environment is further supported by decreased levels of the anti-inflammatory cytokine IL-10 seen in our study. This altered cytokine balance may not only affect the folliculogenesis and oocyte development, but also the fertilization process. For example, TNF-α has been reported to influence sperm motility

in vitro and to reduce the maturation of porcine oocytes (Ma, et al., 2010, Said, et al., 2005). Thus, cytokine levels may be important in endometriosis-associated infertility.

Our results indicate that women with endometriosis have a poor IVF outcome compared to controls. The diminished ovarian reserve may be due to an increased inflammatory activity in the ovarian follicles. However, the mechanism of this action needs to be evaluated.

5.2 PAPER II- RA SUSCEPTIBILITY GENES AND ENDOMETRIOSIS

The pathogenesis of endometriosis is still unclear, although evidence suggests that inflammation plays an important role in the disease. Several inflammatory factors, such as TNF-α, RANTES and IL-8 are up regulated in peritoneal fluid from women with endometriosis (Khorram, et al., 1993, Pizzo, et al., 2002) and abnormalities in the immune system have been observed (reviewed in (Osuga, et al., 2010)). Several of these alterations, such as B-and T-cell abnormalities and altered apoptosis are also seen in patients with autoimmune diseases, such as RA. In addition, reports indicate that endometriosis patients have an increased incidence of autoimmune diseases (Sinaii, et al., 2002). Lately, it has been indicated that genetics also could contribute to development of endometriosis. Therefore, we wanted to investigate genetic similarities between molecular and cellular pathways of endometriosis and RA.

Our material consisted of Caucasian women undergoing laparoscopy for subfertility at the Leuven University hospital in Leuven, Belgium. Samples were obtained from 798 patients with endometriosis and 351 healthy controls. We chose to investigate six RA associated SNPs, i.e. PTPN22 rs2476601, STAT4 rs10181656, TRAF1-C5 rs3761847, CCL21 rs2812378, CD40 rs4810485 and IRF5 rs3807306. No association was found between the selected SNPs and endometriosis.

Due to the heterogeneity of endometriosis, the genetic differences between stage I-II (minimal-mild) and III-IV (moderate-severe) was explored. Previous studies have found different gene expression between different types of endometriosis (Matsuzaki, et al., 2006) and differences in cytokine expression have been observed in patients with different stages of the disease (Khorram, et al., 1993, Pizzo, et al., 2002). In our study, an association between CCL21 rs2812378 and moderate/severe endometriosis was found. However, in RA patients an increased frequency was observed with the G allele (Orozco, et al., 2010, Raychaudhuri, et al., 2008), while in endometriosis, an increase in the A allele was found. This suggests a different role of this SNP in the two diseases. Unfortunately, we do not have any record of autoimmune diseases in our material, but it would have been interesting to see if endometriosis patients with increased A allele had a lower frequency of autoimmune diseases.

In RA, genetic associations have been found with HLA-DRB1 variants. Therefore, two RA associated HLA-DRB1 SNPs, rs660895 and rs2395175, were also investigated in our material. No association of these SNPs was found in endometriosis as one group; although association of rs660895 was observed in patients with moderate/sever disease. Previous genetic studies of endometriosis have shown conflicting results regarding

HLA-DRB1. Some studies have found associations (Ishii, et al., 2002, Kitawaki, et al., 2002) while others could not observe any association (Roszkowski, et al., 2005, Whang, et al., 2006). These differences may be due to small study populations, poorly defined controls or differences in ethnicity. Most genetic studies of endometriosis include very few individuals, therefore making it difficult to establish associations, especially associations with low genetic effect. Also, most associations have only been observed in one study population and not replicated in other study populations. Our study is one of the largest on endometriosis, although compared to other diseases, it is relatively small. However, we have a better power to detect associations than previous candidate gene studies. One drawback with our study is that we do not have access to another population to replicate our results, but we hope that other research groups with genetic material from Caucasian populations will find our studies interesting and perform replication of our results. While performing genetic studies, collaborations will be needed to obtain larger populations and replication of results.

In this study, association between SNPs in CCL21 and HLA-DRB1 was observed. However, we do not know the functional effect of these alleles. We can only speculate about the function, relying on previous studies of these genes. Since inflammation is an important event in endometriosis, we could assume that CCL21 and HLA-molecules are involved in the disease. CCL21 is a chemokine, responsible for recruitment of lymphocytes and dendritic cells. In endometriosis, aberrant expression of lymphocytes (reviewed in (Osuga, et al., 2010)) and a reduction of mature dendritic cells have been observed (Schulke, et al., 2009). Furthermore, CCL21 co-stimulates the expansion of naïve T-cells and increases the expression of inflammatory cytokines, such as TNF-α and INF-γ (Flanagan, et al., 2004), which are also increased in endometriosis. Increased expression of CCL21 has been seen in endometriosis patients and could be a reason for this increased inflammatory profile (Chand, et al., 2007). Also, HLA-molecules are important in the immune system, by differentiating T-cells and inhibiting the killing of NK cells. The expression of major histocompatibility complex (MHC) class I on cells protects against NK cell lysis. In endometriosis, a reduction of activated T-cells and defective NK cell activity has been observed (Ho, et al., 1995, Oosterlynck, et al., 1991). In addition, increased expression of HLA class I and II molecules have been reported in the endometrium from women with endometriosis compared to controls (Baka, et al., 2010, Vernet-Tomas Mdel, et al., 2006). An increased proportion of HLA-DR positive stromal and glandular epithelial cells have also been reported (Chiang and Hill, 1997, Nisolle and Donnez, 1997). This indicates an increased resistance against NK cell lysis in endometrial cells in women with endometriosis.

Our results indicate a potential role of CCL21 and HLA in endometriosis. Since the HLA region has a high degree of LD, it is difficult to establish the SNP of functional value. It is possible that it is not our associated SNPs that give a functional effect, but it is in LD with another SNP, causing the effect. Therefore, sequencing of the entire region and functional studies are needed to further investigate our findings.

5.3 PAPER III- REPLICATION OF OVARIAN CANCER SUSCEPTIBILITY GENES IN ENDOMETRIOSIS

Endometriosis is a benign disease, although it is believed to have malignant characteristics. Several epidemiological studies have found an association between endometriosis and malignancy, such as ovarian cancer (Brinton, et al., 1997, Melin, et al., 2006). However, it is not clear how endometriosis may be transformed into ovarian cancer. It has been suggested that endometriosis could transform to cancer via atypical endometriosis, or that both diseases could share common mechanisms and/or predisposing factors, such as genetic susceptibility. A recent GWAS has reported association of several SNPs in the BNC2 gene and ovarian cancer (Song, et al., 2009). We therefore wanted to investigate if the same SNPs would be associated to endometriosis in our Belgian cases and control samples, to find out if BNC2 could be a possible link between endometriosis and ovarian cancer.

In our material, association of the selected SNPs in the BNC2 gene with endometriosis could not be observed, either as one group or divided into minimal/mild and moderate/severe disease. These results indicate that BNC2 is not associated with endometriosis. However, it is possible that we do not have enough power to detect an association and replication of our data is needed in another, preferably larger, study population.

The function of BNC2 is not clear, but it is believed to participate in mRNA processing (Vanhoutteghem and Djian, 2006). Expression of BNC2 has been found in the ovary, uterus and testis (Romano, et al., 2004, Vanhoutteghem and Djian, 2004), indicating an possible role in reproduction. Perhaps BNC2 is affecting mRNA levels of proteins involved in reproduction and thus has a role in endometriosis-associated infertility. To explore this possibility, it would be of interest to investigate the expression of BNC2 in endometriosis patients and especially in infertile women with endometriosis. It would also be interesting to follow the development of ovarian cancer in women with endometriosis, to investigate if women developing ovarian cancer have an aberrant genetic profile compared to the women who do not develop ovarian cancer. Then we can be more certain that BNC2 is not involved in endometriosis.

Other candidate genes have been identified in ovarian cancer, such as genes encoding cell cycle proteins, oncogenes and tumor suppressor genes (Goode, et al., 2010, Obata, et al., 1998, Quaye, et al., 2009). Pathways regulating processes such as apoptosis, angiogenesis and invasion have also been suggested. Similar alteration has been observed in the inflammatory and immune response between ovarian cancer and endometriosis (Ness, 2003), indicating a shared inflammatory pathway. There are several pathways to investigate and the difficulty is to know where to start. Finding proteins with aberrant expression in both diseases may give a clue about which pathways to investigate further. It would also be interesting to collect DNA and biopsies from patients with endometriosis or ovarian cancer and from patients with both diseases, and investigate the possible link between the diseases.

5.4 PAPER IV- EXPRESSION OF ADHESION, ATTACHMENT AND INVASION FACTORS IN ENDOMETRIOSIS

To obtain the normal function of the endometrium, different cell properties such as adhesion, attachment and invasion are important. However, the same properties may also be involved in the establishment of endometriotic lesions. According to Sampson´s theory about retrograde menstruation, viable cells are shed into the peritoneal cavity (Sampson, 1927). These cells need to be able to adhere to, attach to and invade at ectopic sites in order to cause endometriosis. It is possible that factors involved in these abilities could be expressed differently in women with endometriosis. Therefore, we investigated the expression and localization of six factors involved in the above functions, i.e. ApoE, ITGB2, ITGB7, LAMC1, CD24 and JAM-1, in endometrium from healthy controls and endometriosis patients as well as in endometriomas. In order to investigate if these factors were expressed in a cyclic pattern, we observed mRNA and protein expression in both the proliferative and secretory phases of the menstrual cycle.

In this study, we found endometrial expression of ApoE, ITGB2, ITGB7 and LAMC1 in stromal cells and ITGB7, LAMC1, CD24 and JAM-1 in epithelial cells. In endometriomas, we could observe expression of ApoE, ITGB2, ITGB7 and LAMC1 in sub epithelial regions and CD24 and JAM-1 in epithelial cells. The mRNA levels of CD24 and JAM-1 were significantly lower in endometriomas compared with healthy endometrium. Also, significantly lower ApoE mRNA levels were seen in the proliferative phase in endometriosis patients compared to controls. At protein level, a cyclic pattern of CD24 expression was found in epithelial cells from healthy endometrium. Protein levels in endometriomas were not quantified due to heterogeneity in the staining pattern and low protein expression for all studied factors. The lower mRNA and protein levels in endometriomas were surprising, since a higher expression of factors with adhesive, attaching and invasive properties was expected. It could be possible that the studied factors have other properties that promote the establishment of endometriosis when they are down regulated.

ApoE associate with cell surface receptors of plasma lipoprotein particles. It has been reported that women with endometriosis have an unfavourable lipid profile with increased levels of low density lipoproteins (LDL) (Melo, et al., 2010). Oxidised LDL induces MCP-1 production by mesothelial and endometrial cells (Rong, et al., 2002). In addition, oxidised LDL triggers inflammatory signalling via toll like receptors (Stewart, et al., 2010). Thus, ApoE may play a role in the inflammatory profile seen in women with endometriosis. Furthermore, ApoE has been reported to be a marker for cell survival and proliferation (Chen, et al., 2005), events that are important in the establishment of endometriosis. It would be interesting to further look into the function of ApoE in endometriosis, related to inflammation, cell survival and proliferation.

Integrin expression has previously been shown in the endometrium. However, there are many different integrins with different functions. Integrins have been observed in menstrual endometrium (Koks, et al., 2000, van der Linden, et al., 1994) and TNF-α has been shown to increase its adherence (Sillem, et al., 1999). Since TNF-α is an important inflammatory mediator in endometriosis, the expression of integins may also

be important in the development of the disease. Stronger expression of integrin α5 has been observed in stromal cells from endometriosis patients compared to controls (Beliard, et al., 1997). Endometriotic stromal cells are able to adhere to laminin in the ECM and an aberrant integrin expression may be involved in this adhesion (Adachi, et al., 2010). Thus, several observations indicate a possible role of integrins in endometriosis.

An increased adhesive capacity has been observed in stromal cells from endometriotic lesions and endometrium from women with endometriosis in response to specific ECM components (Klemmt, et al., 2007). Laminins are located in the basement membrane of the ECM. LAMC1 have been reported to promote adhesion and migration of monocytes (Pedraza, et al., 2000). An altered expression of LAMC1 could contribute to the aberrant profile of immune cells seen in endometriosis patients. In addition, anti-Laminin antibodies have been associated with endometriosis-associated infertility (Inagaki, et al., 2003) and laminin has been suggested to be involved in the regulation of proliferation and differentiation of trophoblast cells during implantation (Klaffky, et al., 2001).

Previous studies have observed expression of CD24 and JAM-1 in the endometrium (Kim, et al., 2009, Koshiba, et al., 2009). Our findings support these reports. CD24 has been suggested to play a role in tumor progression in the ovaries (Choi, et al., 2005). In addition, aberrant expression of CD24 and JAM-1 have been found in reproductive cancers (Koshiba, et al., 2009, Kristiansen, et al., 2002), indicating that these proteins are involved in tumorgenesis. The establishment of endometriosis has similarities to malignant processes and therefore CD24 and JAM-1 may also be involved in the establishment of the disease, by allowing cells that are shed during menstruation to attach to the peritoneal surface and form lesions.

Our study has shown expression of ApoE, ITGB2, ITGB7, LAMC1 CD24 and JAM-1 in endometrium and endometriomas. However, the function of these proteins in the initiation of endometriosis and lesion needs to be investigated further. In addition, it would be interesting to evaluate the hormonal regulation of these proteins. Furthermore, several of these proteins are involved in malignancy and it would be interesting to evaluate their potential role in the transformation of endometriosis to ovarian cancer.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

The following conclusions can be drawn based on the findings of this thesis:

- Women with endometriosis seem to have a diminished ovarian reserve, indicated by low levels of AMH and a poor IVF outcome. Furthermore, an aberrant inflammatory profile was seen with high levels of TNF-α, IL-15 and GM-CSF and low levels of IL-10. Thus, the diminished ovarian reserve could be due to the increased inflammatory activity in the ovarian follicles.

- We found an association of CCL21 rs2812378 and HLA rs660895 with moderate/severe endometriosis, but not with endometriosis as one group, in a Caucasian population. These findings indicate that the genetic background in endometriosis might differ depending on type and severity of disease. Our results do not indicate shared pathways of endometriosis and RA.

- No association of polymorphisms in the BNC2 gene was observed in endometriosis patients, suggesting that SNPs in BNC2 are unlikely to contribute to the reported risk of ovarian cancer in endometriosis.

- Endometrium from healthy women and endometriosis patients, together with endometriomas, express factors involved in adhesion, attachment and invasion, such as ApoE, ITGB2, ITGB7, LAMC1 CD24 and JAM-1. However, their role in the development of endometriosis needs to be studied further.

Altogether, these results indicate that the increased inflammatory profile seen in women with endometriosis may affect the ovarian reserve and thus lead to infertility problems in these women. Changes in the follicular fluid might influence the maturity and quality of the oocyte, fertilization, early embryonic development and pregnancy outcome. Genetic associations in inflammatory related genes were also found in women with moderate/severe disease, further indicating the importance of inflammation in endometriosis. In addition, the expression of factors involved in adhesion, attachment and invasion may be important in the establishment of endometriotic lesions. These factors may also be regulated by inflammatory mediators.

For future studies, it would be interesting to investigate the function of CCL21 in endometriosis and to find out if the CCL21 rs2812378 has a functional role in the disease. To start with, the expression of CCL21 could be compared to the rs2812378 alleles, to see the impact of the endometriosis-associated A allele. It would also be interesting to investigate other SNPs that are in LD with rs2812378, to see if the endometriosis associated SNP(s) could lie elsewhere.

In general, I would like to collect a new sample set of Swedish cases and controls, which is larger than our previous material and with more information about the patients. It would be interesting to collect data of type and stage of endometriosis, other diseases, such as autoimmune diseases and cancer, and environmental factors. From

these patients, biopsies and serum samples could also be collected to do follow-up functional studies and expression analysis. A GWAS could be used to localize candidate genes and our samples from Belgium could be used for replication.

Since we have found expression of different factors involved in adhesion, attachment and invasion (ApoE, ITGB2, ITGB7, LAMC1 CD24 and JAM-1), it would be interesting to evaluate the possible role of these factors in endometriosis. I would like to perform invasion and proliferation assays with cells expressing these markers and compare between cells from endometrium from healthy controls and endometriosis patients, to see if the function is changed in endometriosis patients. I would also like to investigate the hormonal regulation of these factors, especially the effect of estrogen. Furthermore, it would be interesting to see if there is any connection between stem/progenitor cells and the expression of factors involved in adhesion, attachment and invasion. Is it possible that endometrial stem/progenitor cells with increased adhesive and invasive properties are shed during menstruation in endometriosis patients, but not in healthy women?

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8 REFERENCES

(1994) Prevalence and anatomical distribution of endometriosis in women with selected gynaecological conditions: results from a multicentric Italian study. Gruppo italiano per lo studio dell'endometriosi. *Hum Reprod*, **9**, 1158-1162.

(1997) Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil Steril*, **67**, 817-821.

Adachi, M., Nasu, K., Tsuno, A., Yuge, A., Kawano, Y. and Narahara, H. (2010) Attachment to extracellular matrices is enhanced in human endometriotic stromal cells: a possible mechanism underlying the pathogenesis of endometriosis. *Eur J Obstet Gynecol Reprod Biol*.

Adachi, S., Tajima, A., Quan, J., Haino, K., Yoshihara, K., Masuzaki, H., Katabuchi, H., Ikuma, K., Suginami, H., Nishida, N. et al. (2010) Meta-analysis of genome-wide association scans for genetic susceptibility to endometriosis in Japanese population. *J Hum Genet*.

Agic, A., Djalali, S., Diedrich, K. and Hornung, D. (2009) Apoptosis in endometriosis. *Gynecol Obstet Invest*, **68**, 217-223.

Agic, A., von Wussow, U., Starzinski-Powitz, A., Diedrich, K., Altevogt, P. and Hornung, D. (2010) Inhibition of cell proliferation, adhesion, and invasion with an anti-L1-cell adhesion molecule monoclonal antibody in an in vitro endometriosis model. *Fertil Steril*, **94**, 1102-1104.

Al-Jefout, M., Dezarnaulds, G., Cooper, M., Tokushige, N., Luscombe, G. M., Markham, R. and Fraser, I. S. (2009) Diagnosis of endometriosis by detection of nerve fibres in an endometrial biopsy: a double blind study. *Hum Reprod*, **24**, 3019-3024.

Al-Qahtani, A. and Groome, N. P. (2006) Editorial: Anti-Mullerian hormone: Cinderella finds new admirers. *J Clin Endocrinol Metab*, **91**, 3760-3762.

Altshuler, D., Daly, M. J. and Lander, E. S. (2008) Genetic mapping in human disease. *Science*, **322**, 881-888.

Amemiya, S., Sekizawa, A., Otsuka, J., Tachikawa, T., Saito, H. and Okai, T. (2004) Malignant transformation of endometriosis and genetic alterations of K-ras and microsatellite instability. *Int J Gynaecol Obstet*, **86**, 371-376.

Ammendola, M., Bottini, N., Pietropolli, A., Saccucci, P. and Gloria-Bottini, F. (2008) Association between PTPN22 and endometriosis. *Fertil Steril*, **89**, 993-994.

Arici, A., Oral, E., Bukulmez, O., Duleba, A., Olive, D. L. and Jones, E. E. (1996) The effect of endometriosis on implantation: results from the Yale University in vitro fertilization and embryo transfer program. *Fertil Steril*, **65**, 603-607.

Arumugam, K. and Lim, J. M. (1997) Menstrual characteristics associated with endometriosis. *Br J Obstet Gynaecol*, **104**, 948-950.

Ayaz, L., Celik, S. K., Cayan, F., Aras-Ates, N. and Tamer, L. (2011) Functional association of interleukin-18 gene -607 C/A promoter polymorphisms with endometriosis. *Fertil Steril*.

Bahamondes, L., Petta, C. A., Fernandes, A. and Monteiro, I. (2007) Use of the levonorgestrel-releasing intrauterine system in women with endometriosis, chronic pelvic pain and dysmenorrhea. *Contraception*, **75**, S134-139.

Baka, S., Frangou-Plemenou, M., Panagiotopoulou, E., Makrakis, E., Kaltsakas, G., Hassiakos, D. and Kondi-Pafiti, A. (2010) The expression of human leukocyte antigens class I and II in women with endometriosis or adenomyosis. *Gynecol Endocrinol*.

Banz, C., Ungethuem, U., Kuban, R. J., Diedrich, K., Lengyel, E. and Hornung, D. (2010) The molecular signature of endometriosis-associated endometrioid ovarian cancer differs significantly from endometriosis-independent endometrioid ovarian cancer. *Fertil Steril*, **94**, 1212-1217.

Barbonetti, A., Vassallo, M. R., Antonangelo, C., Nuccetelli, V., D'Angeli, A., Pelliccione, F., Giorgi, M., Francavilla, F. and Francavilla, S. (2008) RANTES and human sperm fertilizing ability: effect on acrosome reaction and sperm/oocyte fusion. *Mol Hum Reprod*, **14**, 387-391.

Barcena de Arellano, M. L., Arnold, J., Vercellino, F., Chiantera, V., Schneider, A. and Mechsner, S. (2010) Overexpression of nerve growth factor in peritoneal fluid from women with endometriosis may promote neurite outgrowth in endometriotic lesions. *Fertil Steril*.

Barnhart, K., Dunsmoor-Su, R. and Coutifaris, C. (2002) Effect of endometriosis on in vitro fertilization. *Fertil Steril*, **77**, 1148-1155.

Bedaiwy, M. A., Falcone, T., Sharma, R. K., Goldberg, J. M., Attaran, M., Nelson, D. R. and Agarwal, A. (2002) Prediction of endometriosis with serum and peritoneal fluid markers: a prospective controlled trial. *Hum Reprod*, **17**, 426-431.

Beliard, A., Donnez, J., Nisolle, M. and Foidart, J. M. (1997) Localization of laminin, fibronectin, E-cadherin, and integrins in endometrium and endometriosis. *Fertil Steril*, **67**, 266-272.

Bertelsen, L., Mellemkjaer, L., Frederiksen, K., Kjaer, S. K., Brinton, L. A., Sakoda, L. C., van Valkengoed, I. and Olsen, J. H. (2007) Risk for breast cancer among women with endometriosis. *Int J Cancer*, **120**, 1372-1375.

Braun, D. P., Ding, J. and Dmowski, W. P. (2002) Peritoneal fluid-mediated enhancement of eutopic and ectopic endometrial cell proliferation is dependent on tumor necrosis factor-alpha in women with endometriosis. *Fertil Steril*, **78**, 727-732.

Brinton, L. A., Gridley, G., Persson, I., Baron, J. and Bergqvist, A. (1997) Cancer risk after a hospital discharge diagnosis of endometriosis. *Am J Obstet Gynecol*, **176**, 572-579.

Brinton, L. A., Westhoff, C. L., Scoccia, B., Lamb, E. J., Althuis, M. D., Mabie, J. E. and Moghissi, K. S. (2005) Causes of infertility as predictors of subsequent cancer risk. *Epidemiology*, **16**, 500-507.

Brosens, I., Puttemans, P., Campo, R., Gordts, S. and Kinkel, K. (2004) Diagnosis of endometriosis: pelvic endoscopy and imaging techniques. *Best Pract Res Clin Obstet Gynaecol*, **18**, 285-303.

Cardon, L. R. and Bell, J. I. (2001) Association study designs for complex diseases. *Nat Rev Genet*, **2**, 91-99.

Carlberg, M., Nejaty, J., Froysa, B., Guan, Y., Soder, O. and Bergqvist, A. (2000) Elevated expression of tumour necrosis factor alpha in cultured granulosa cells from women with endometriosis. *Hum Reprod*, **15**, 1250-1255.

Carli, C., Leclerc, P., Metz, C. N. and Akoum, A. (2007) Direct effect of macrophage migration inhibitory factor on sperm function: possible involvement in endometriosis-associated infertility. *Fertil Steril*, **88**, 1240-1247.

Ceyhan, S. T., Onguru, O., Fidan, U., Ide, T., Yaman, H., Kilic, S. and Baser, I. (2010) Comparison of aromatase inhibitor (letrozole) and immunomodulators (infliximab and etanercept) on the regression of endometriotic implants in a rat model. *Eur J Obstet Gynecol Reprod Biol*.

Chan, R. W., Schwab, K. E. and Gargett, C. E. (2004) Clonogenicity of human endometrial epithelial and stromal cells. *Biol Reprod*, **70**, 1738-1750.

Chand, A. L., Murray, A. S., Jones, R. L., Hannan, N. J., Salamonsen, L. A. and Rombauts, L. (2007) Laser capture microdissection and cDNA array analysis of endometrium identify CCL16 and CCL21 as epithelial-derived inflammatory mediators associated with endometriosis. *Reprod Biol Endocrinol*, **5**, 18.

Chen, Y. C., Pohl, G., Wang, T. L., Morin, P. J., Risberg, B., Kristensen, G. B., Yu, A., Davidson, B. and Shih Ie, M. (2005) Apolipoprotein E is required for cell proliferation and survival in ovarian cancer. *Cancer Res*, **65**, 331-337.

Chiang, C. M. and Hill, J. A. (1997) Localization of T cells, interferon-gamma and HLA-DR in eutopic and ectopic human endometrium. *Gynecol Obstet Invest*, **43**, 245-250.

Choi, Y. L., Kim, S. H., Shin, Y. K., Hong, Y. C., Lee, S. J., Kang, S. Y. and Ahn, G. (2005) Cytoplasmic CD24 expression in advanced ovarian serous borderline tumors. *Gynecol Oncol*, **97**, 379-386.

Colacurci, N., Fortunato, N., De Franciscis, P., Fratta, M., Cioffi, M., Zarcone, R. and Cardone, A. (1996) Serum and peritoneal CA-125 levels as diagnostic test for endometriosis. *Eur J Obstet Gynecol Reprod Biol*, **66**, 41-43.

Colhoun, H. M., McKeigue, P. M. and Davey Smith, G. (2003) Problems of reporting genetic associations with complex outcomes. *Lancet*, **361**, 865-872.

Collette, T., Maheux, R., Mailloux, J. and Akoum, A. (2006) Increased expression of matrix metalloproteinase-9 in the eutopic endometrial tissue of women with endometriosis. *Hum Reprod*, **21**, 3059-3067.
Cosin, R., Gilabert-Estelles, J., Ramon, L. A., Gomez-Lechon, M. J., Gilabert, J., Chirivella, M., Braza-Boils, A., Espana, F. and Estelles, A. (2010) Influence of peritoneal fluid on the expression of angiogenic and proteolytic factors in cultures of endometrial cells from women with endometriosis. *Hum Reprod*, **25**, 398-405.
D'Hooghe, T. M., Bambra, C. S., Isahakia, M. and Koninckx, P. R. (1992) Evolution of spontaneous endometriosis in the baboon (Papio anubis, Papio cynocephalus) over a 12-month period. *Fertil Steril*, **58**, 409-412.
D'Hooghe, T. M., Bambra, C. S., Suleman, M. A., Dunselman, G. A., Evers, H. L. and Koninckx, P. R. (1994) Development of a model of retrograde menstruation in baboons (Papio anubis). *Fertil Steril*, **62**, 635-638.
D'Hooghe, T. M., Bambra, C. S., Raeymaekers, B. M., De Jonge, I., Lauweryns, J. M. and Koninckx, P. R. (1995) Intrapelvic injection of menstrual endometrium causes endometriosis in baboons (Papio cynocephalus and Papio anubis). *Am J Obstet Gynecol*, **173**, 125-134.
Daly, M. J., Rioux, J. D., Schaffner, S. F., Hudson, T. J. and Lander, E. S. (2001) High-resolution haplotype structure in the human genome. *Nat Genet*, **29**, 229-232.
Damewood, M. D., Hesla, J. S., Schlaff, W. D., Hubbard, M., Gearhart, J. D. and Rock, J. A. (1990) Effect of serum from patients with minimal to mild endometriosis on mouse embryo development in vitro. *Fertil Steril*, **54**, 917-920.
de Ziegler, D., Borghese, B. and Chapron, C. (2010) Endometriosis and infertility: pathophysiology and management. *Lancet*, **376**, 730-738.
Dimitrov, R., Timeva, T., Kyurkchiev, D., Stamenova, M., Shterev, A., Kostova, P., Zlatkov, V., Kehayov, I. and Kyurkchiev, S. (2008) Characterization of clonogenic stromal cells isolated from human endometrium. *Reproduction*, **135**, 551-558.
Dinulescu, D. M., Ince, T. A., Quade, B. J., Shafer, S. A., Crowley, D. and Jacks, T. (2005) Role of K-ras and Pten in the development of mouse models of endometriosis and endometrioid ovarian cancer. *Nat Med*, **11**, 63-70.
Du, H. and Taylor, H. S. (2007) Contribution of bone marrow-derived stem cells to endometrium and endometriosis. *Stem Cells*, **25**, 2082-2086.
Dunselman, G. A., Hendrix, M. G., Bouckaert, P. X. and Evers, J. L. (1988) Functional aspects of peritoneal macrophages in endometriosis of women. *J Reprod Fertil*, **82**, 707-710.
Erzen, M. and Kovacic, J. (1998) Relationship between endometriosis and ovarian cancer. *Eur J Gynaecol Oncol*, **19**, 553-555.
Eskenazi, B. and Warner, M. L. (1997) Epidemiology of endometriosis. *Obstet Gynecol Clin North Am*, **24**, 235-258.
Falconer, H., Mwenda, J. M., Chai, D. C., Wagner, C., Song, X. Y., Mihalyi, A., Simsa, P., Kyama, C., Cornillie, F. J., Bergqvist, A. et al. (2006) Treatment with anti-TNF monoclonal antibody (c5N) reduces the extent of induced endometriosis in the baboon. *Hum Reprod*, **21**, 1856-1862.
Falconer, H., D'Hooghe, T. and Fried, G. (2007) Endometriosis and genetic polymorphisms. *Obstet Gynecol Surv*, **62**, 616-628.
Feuk, L., Carson, A. R. and Scherer, S. W. (2006) Structural variation in the human genome. *Nat Rev Genet*, **7**, 85-97.
Finas, D., Huszar, M., Agic, A., Dogan, S., Kiefel, H., Riedle, S., Gast, D., Marcovich, R., Noack, F., Altevogt, P. et al. (2008) L1 cell adhesion molecule (L1CAM) as a pathogenetic factor in endometriosis. *Hum Reprod*, **23**, 1053-1062.
Flanagan, K., Moroziewicz, D., Kwak, H., Horig, H. and Kaufman, H. L. (2004) The lymphoid chemokine CCL21 costimulates naive T cell expansion and Th1 polarization of non-regulatory CD4+ T cells. *Cell Immunol*, **231**, 75-84.
Frazer, K. A., Murray, S. S., Schork, N. J. and Topol, E. J. (2009) Human genetic variation and its contribution to complex traits. *Nat Rev Genet*, **10**, 241-251.
Fried, G., Harlin, J., Csemiczky, G. and Wramsby, H. (1996) Controlled ovarian stimulation using highly purified FSH results in a lower serum oestradiol profile in the follicular phase as compared with HMG. *Hum Reprod*, **11**, 474-477.

Fukunaga, M., Nomura, K., Ishikawa, E. and Ushigome, S. (1997) Ovarian atypical endometriosis: its close association with malignant epithelial tumours. *Histopathology*, **30**, 249-255.
Gan, X. L., Lin, Y. H., Zhang, Y., Yu, T. H. and Hu, L. N. (2010) Association of an interleukin-16 gene polymorphism with the risk and pain phenotype of endometriosis. *DNA Cell Biol*, **29**, 663-667.
Gargett, C. E. (2004) Stem cells in gynaecology. *Aust N Z J Obstet Gynaecol*, **44**, 380-386.
Gazvani, R., Smith, L. and Fowler, P. A. (2002) Effect of interleukin-8 (IL-8), anti-IL-8, and IL-12 on endometrial cell survival in combined endometrial gland and stromal cell cultures derived from women with and without endometriosis. *Fertil Steril*, **77**, 62-67.
Gebel, H. M., Braun, D. P., Tambur, A., Frame, D., Rana, N. and Dmowski, W. P. (1998) Spontaneous apoptosis of endometrial tissue is impaired in women with endometriosis. *Fertil Steril*, **69**, 1042-1047.
Gemmill, J. A., Stratton, P., Cleary, S. D., Ballweg, M. L. and Sinaii, N. (2010) Cancers, infections, and endocrine diseases in women with endometriosis. *Fertil Steril*, **94**, 1627-1631.
Gleicher, N., el-Roeiy, A., Confino, E. and Friberg, J. (1987) Is endometriosis an autoimmune disease? *Obstet Gynecol*, **70**, 115-122.
Gomes, F. M., Bianco, B., Teles, J. S., Christofolini, D. M., de Souza, A. M., Guedes, A. D. and Barbosa, C. P. (2010) PTPN22 C1858T polymorphism in women with endometriosis. *Am J Reprod Immunol*, **63**, 227-232.
Goode, E. L., Chenevix-Trench, G., Song, H., Ramus, S. J., Notaridou, M., Lawrenson, K., Widschwendter, M., Vierkant, R. A., Larson, M. C., Kjaer, S. K. et al. (2010) A genome-wide association study identifies susceptibility loci for ovarian cancer at 2q31 and 8q24. *Nat Genet*, **42**, 874-879.
Guzeloglu Kayisli, O., Kayisli, U. A., Luleci, G. and Arici, A. (2004) In vivo and in vitro regulation of Akt activation in human endometrial cells is estrogen dependent. *Biol Reprod*, **71**, 714-721.
Hadfield, R., Mardon, H., Barlow, D. and Kennedy, S. (1996) Delay in the diagnosis of endometriosis: a survey of women from the USA and the UK. *Hum Reprod*, **11**, 878-880.
Halis, G. and Arici, A. (2004) Endometriosis and inflammation in infertility. *Ann N Y Acad Sci*, **1034**, 300-315.
Halme, J., Hammond, M. G., Hulka, J. F., Raj, S. G. and Talbert, L. M. (1984) Retrograde menstruation in healthy women and in patients with endometriosis. *Obstet Gynecol*, **64**, 151-154.
Hazout, A., Bouchard, P., Seifer, D. B., Aussage, P., Junca, A. M. and Cohen-Bacrie, P. (2004) Serum antimullerian hormone/mullerian-inhibiting substance appears to be a more discriminatory marker of assisted reproductive technology outcome than follicle-stimulating hormone, inhibin B, or estradiol. *Fertil Steril*, **82**, 1323-1329.
Heilier, J. F., Donnez, O., Van Kerckhove, V., Lison, D. and Donnez, J. (2006) Expression of aromatase (P450 aromatase/CYP19) in peritoneal and ovarian endometriotic tissues and deep endometriotic (adenomyotic) nodules of the rectovaginal septum. *Fertil Steril*, **85**, 1516-1518.
Ho, H. N., Chao, K. H., Chen, H. F., Wu, M. Y., Yang, Y. S. and Lee, T. Y. (1995) Peritoneal natural killer cytotoxicity and CD25+ CD3+ lymphocyte subpopulation are decreased in women with stage III-IV endometriosis. *Hum Reprod*, **10**, 2671-2675.
Hong, C. Y., Park, J. H., Seo, K. H., Kim, J. M., Im, S. Y., Lee, J. W., Choi, H. S. and Lee, K. (2003) Expression of MIS in the testis is downregulated by tumor necrosis factor alpha through the negative regulation of SF-1 transactivation by NF-kappa B. *Mol Cell Biol*, **23**, 6000-6012.
Hornstein, M. D., Thomas, P. P., Sober, A. J., Wyshak, G., Albright, N. L. and Frisch, R. E. (1997) Association between endometriosis, dysplastic naevi and history of melanoma in women of reproductive age. *Hum Reprod*, **12**, 143-145.

Hsieh, Y. Y., Chang, C. C., Tsai, F. J., Peng, C. T., Yeh, L. S. and Lin, C. C. (2005) Polymorphism for transforming growth factor beta 1-509 (TGF-B1-509): association with endometriosis. *Biochem Genet*, **43**, 203-210.
Hummelshoj, L., Prentice, A. and Groothuis, P. (2006) Update on endometriosis. *Womens Health (Lond Engl)*, **2**, 53-56.
Hyder, S. M. and Stancel, G. M. (1999) Regulation of angiogenic growth factors in the female reproductive tract by estrogens and progestins. *Mol Endocrinol*, **13**, 806-811.
Inagaki, J., Sugiura-Ogasawara, M., Nomizu, M., Nakatsuka, M., Ikuta, K., Suzuki, N., Kaihara, K., Kobayashi, K., Yasuda, T., Shoenfeld, Y. et al. (2003) An association of IgG anti-laminin-1 autoantibodies with endometriosis in infertile patients. *Hum Reprod*, **18**, 544-549.
Ishii, K., Takakuwa, K., Mitsui, T. and Tanaka, K. (2002) Studies on the human leukocyte antigen-DR in patients with endometriosis: genotyping of HLA-DRB1 alleles. *Hum Reprod*, **17**, 560-563.
Iwabe, T., Harada, T., Tsudo, T., Nagano, Y., Yoshida, S., Tanikawa, M. and Terakawa, N. (2000) Tumor necrosis factor-alpha promotes proliferation of endometriotic stromal cells by inducing interleukin-8 gene and protein expression. *J Clin Endocrinol Metab*, **85**, 824-829.
Jacobson, T. Z., Duffy, J. M., Barlow, D., Koninckx, P. R. and Garry, R. (2009) Laparoscopic surgery for pelvic pain associated with endometriosis. *Cochrane Database Syst Rev*, CD001300.
Jiang, X., Morland, S. J., Hitchcock, A., Thomas, E. J. and Campbell, I. G. (1998) Allelotyping of endometriosis with adjacent ovarian carcinoma reveals evidence of a common lineage. *Cancer Res*, **58**, 1707-1712.
Jones, G., Jenkinson, C. and Kennedy, S. (2004) The impact of endometriosis upon quality of life: a qualitative analysis. *J Psychosom Obstet Gynaecol*, **25**, 123-133.
Juo, S. H., Wu, R., Lin, C. S., Wu, M. T., Lee, J. N. and Tsai, E. M. (2009) A functional promoter polymorphism in interleukin-10 gene influences susceptibility to endometriosis. *Fertil Steril*, **92**, 1228-1233.
Kats, R., Collette, T., Metz, C. N. and Akoum, A. (2002) Marked elevation of macrophage migration inhibitory factor in the peritoneal fluid of women with endometriosis. *Fertil Steril*, **78**, 69-76.
Keenan, J. A., Chen, T. T., Chadwell, N. L., Torry, D. S. and Caudle, M. R. (1995) IL-1 beta, TNF-alpha, and IL-2 in peritoneal fluid and macrophage-conditioned media of women with endometriosis. *Am J Reprod Immunol*, **34**, 381-385.
Keettel, W. C. and Stein, R. J. (1951) The viability of the cast-off menstrual endometrium. *Am J Obstet Gynecol*, **61**, 440-442.
Kennedy, S. (1999) The genetics of endometriosis. *Eur J Obstet Gynecol Reprod Biol*, **82**, 129-133.
Kennedy, S., Bennett, S. and Weeks, D. E. (2001) Affected sib-pair analysis in endometriosis. *Hum Reprod Update*, **7**, 411-418.
Khorram, O., Taylor, R. N., Ryan, I. P., Schall, T. J. and Landers, D. V. (1993) Peritoneal fluid concentrations of the cytokine RANTES correlate with the severity of endometriosis. *Am J Obstet Gynecol*, **169**, 1545-1549.
Kilic, S., Evsen, M., Tasdemir, N., Yilmaz, N., Yuksel, B., Dogan, M. and Batioglu, S. (2007) Follicular fluid vascular endothelial growth factor and tumour necrosis factor alpha concentrations in patients with endometriosis undergoing ICSI. *Reprod Biomed Online*, **15**, 316-320.
Kim, K. H., Choi, J. S., Kim, J. M., Choi, Y. L., Shin, Y. K., Lee, H. C., Seong, I. O., Kim, B. K., Chae, S. W. and Kim, S. H. (2009) Enhanced CD24 expression in endometrial carcinoma and its expression pattern in normal and hyperplastic endometrium. *Histol Histopathol*, **24**, 309-316.
Kitawaki, J., Obayashi, H., Kado, N., Ishihara, H., Koshiba, H., Maruya, E., Saji, H., Ohta, M., Hasegawa, G., Nakamura, N. et al. (2002) Association of HLA class I and class II alleles with susceptibility to endometriosis. *Hum Immunol*, **63**, 1033-1038.
Kitawaki, J., Koshiba, H., Kitaoka, Y., Teramoto, M., Hasegawa, G., Nakamura, N., Yoshikawa, T., Ohta, M., Obayashi, H. and Honjo, H. (2004) Interferon-gamma gene dinucleotide (CA) repeat and interleukin-4 promoter region (-590C/T)

polymorphisms in Japanese patients with endometriosis. *Hum Reprod*, **19**, 1765-1769.
Klaffky, E., Williams, R., Yao, C. C., Ziober, B., Kramer, R. and Sutherland, A. (2001) Trophoblast-specific expression and function of the integrin alpha 7 subunit in the peri-implantation mouse embryo. *Dev Biol*, **239**, 161-175.
Klemmt, P. A., Carver, J. G., Koninckx, P., McVeigh, E. J. and Mardon, H. J. (2007) Endometrial cells from women with endometriosis have increased adhesion and proliferative capacity in response to extracellular matrix components: towards a mechanistic model for endometriosis progression. *Hum Reprod*, **22**, 3139-3147.
Kokawa, K., Shikone, T. and Nakano, R. (1996) Apoptosis in the human uterine endometrium during the menstrual cycle. *J Clin Endocrinol Metab*, **81**, 4144-4147.
Koks, C. A., Groothuis, P. G., Dunselman, G. A., de Goeij, A. F. and Evers, J. L. (2000) Adhesion of menstrual endometrium to extracellular matrix: the possible role of integrin alpha(6)beta(1) and laminin interaction. *Mol Hum Reprod*, **6**, 170-177.
Koninckx, P. R., Oosterlynck, D., D'Hooghe, T. and Meuleman, C. (1994) Deeply infiltrating endometriosis is a disease whereas mild endometriosis could be considered a non-disease. *Ann N Y Acad Sci*, **734**, 333-341.
Koshiba, H., Hosokawa, K., Kubo, A., Tokumitsu, N., Watanabe, A. and Honjo, H. (2009) Junctional adhesion molecule A [corrected] expression in human endometrial carcinoma. *Int J Gynecol Cancer*, **19**, 208-213.
Kristiansen, G., Denkert, C., Schluns, K., Dahl, E., Pilarsky, C. and Hauptmann, S. (2002) CD24 is expressed in ovarian cancer and is a new independent prognostic marker of patient survival. *Am J Pathol*, **161**, 1215-1221.
Kuivasaari, P., Hippelainen, M., Anttila, M. and Heinonen, S. (2005) Effect of endometriosis on IVF/ICSI outcome: stage III/IV endometriosis worsens cumulative pregnancy and live-born rates. *Hum Reprod*, **20**, 3130-3135.
Lakshmi, K. V., Shetty, P., Vottam, K., Govindhan, S., Ahmad, S. N. and Hasan, Q. (2010) Tumor necrosis factor alpha -C850T polymorphism is significantly associated with endometriosis in Asian Indian women. *Fertil Steril*, **94**, 453-456.
Lauchlan, S. C. (1972) The secondary Mullerian system. *Obstet Gynecol Surv*, **27**, 133-146.
Lebovic, D. I., Mueller, M. D. and Taylor, R. N. (2001) Immunobiology of endometriosis. *Fertil Steril*, **75**, 1-10.
Lee, K. S., Joo, B. S., Na, Y. J., Yoon, M. S., Choi, O. H. and Kim, W. W. (2000) Relationships between concentrations of tumor necrosis factor-alpha and nitric oxide in follicular fluid and oocyte quality. *J Assist Reprod Genet*, **17**, 222-228.
Lemos, N. A., Arbo, E., Scalco, R., Weiler, E., Rosa, V. and Cunha-Filho, J. S. (2008) Decreased anti-Mullerian hormone and altered ovarian follicular cohort in infertile patients with mild/minimal endometriosis. *Fertil Steril*, **89**, 1064-1068.
Levander, G. and Normann, P. (1955) The pathogenesis of endometriosis; an experimental study. *Acta Obstet Gynecol Scand*, **34**, 366-398.
Li, S. F., Nakayama, K., Masuzawa, H. and Fujii, S. (1993) The number of proliferating cell nuclear antigen positive cells in endometriotic lesions differs from that in the endometrium. Analysis of PCNA positive cells during the menstrual cycle and in post-menopause. *Virchows Arch A Pathol Anat Histopathol*, **423**, 257-263.
Liu, D. T. and Hitchcock, A. (1986) Endometriosis: its association with retrograde menstruation, dysmenorrhoea and tubal pathology. *Br J Obstet Gynaecol*, **93**, 859-862.
Lorencatto, C., Petta, C. A., Navarro, M. J., Bahamondes, L. and Matos, A. (2006) Depression in women with endometriosis with and without chronic pelvic pain. *Acta Obstet Gynecol Scand*, **85**, 88-92.
Ma, C. H., Yan, L. Y., Qiao, J., Sha, W., Li, L., Chen, Y. and Sun, Q. Y. (2010) Effects of tumor necrosis factor-alpha on porcine oocyte meiosis progression, spindle organization, and chromosome alignment. *Fertil Steril*, **93**, 920-926.
Mahnke, J. L., Dawood, M. Y. and Huang, J. C. (2000) Vascular endothelial growth factor and interleukin-6 in peritoneal fluid of women with endometriosis. *Fertil Steril*, **73**, 166-170.

Matarese, G., De Placido, G., Nikas, Y. and Alviggi, C. (2003) Pathogenesis of endometriosis: natural immunity dysfunction or autoimmune disease? *Trends Mol Med*, **9**, 223-228.
Matsuzaki, S., Canis, M., Pouly, J. L., Botchorishvili, R., Dechelotte, P. J. and Mage, G. (2006) Differential expression of genes in eutopic and ectopic endometrium from patients with ovarian endometriosis. *Fertil Steril*, **86**, 548-553.
McCarthy, M. I. and Hirschhorn, J. N. (2008) Genome-wide association studies: potential next steps on a genetic journey. *Hum Mol Genet*, **17**, R156-165.
McLaren, J., Prentice, A., Charnock-Jones, D. S., Millican, S. A., Muller, K. H., Sharkey, A. M. and Smith, S. K. (1996) Vascular endothelial growth factor is produced by peritoneal fluid macrophages in endometriosis and is regulated by ovarian steroids. *J Clin Invest*, **98**, 482-489.
Melin, A., Sparen, P., Persson, I. and Bergqvist, A. (2006) Endometriosis and the risk of cancer with special emphasis on ovarian cancer. *Hum Reprod*, **21**, 1237-1242.
Melin, A., Lundholm, C., Malki, N., Swahn, M. L., Sparen, P. and Bergqvist, A. (2010) Endometriosis as a prognostic factor for cancer survival. *Int J Cancer*.
Melo, A. S., Rosa-e-Silva, J. C., Rosa-e-Silva, A. C., Poli-Neto, O. B., Ferriani, R. A. and Vieira, C. S. (2010) Unfavorable lipid profile in women with endometriosis. *Fertil Steril*, **93**, 2433-2436.
Modesitt, S. C., Tortolero-Luna, G., Robinson, J. B., Gershenson, D. M. and Wolf, J. K. (2002) Ovarian and extraovarian endometriosis-associated cancer. *Obstet Gynecol*, **100**, 788-795.
Moen, M. H. (1994) Endometriosis in monozygotic twins. *Acta Obstet Gynecol Scand*, **73**, 59-62.
Montgomery, G. W., Nyholt, D. R., Zhao, Z. Z., Treloar, S. A., Painter, J. N., Missmer, S. A., Kennedy, S. H. and Zondervan, K. T. (2008) The search for genes contributing to endometriosis risk. *Hum Reprod Update*, **14**, 447-457.
Morcos, R. N., Gibbons, W. E. and Findley, W. E. (1985) Effect of peritoneal fluid on in vitro cleavage of 2-cell mouse embryos: possible role in infertility associated with endometriosis. *Fertil Steril*, **44**, 678-683.
Mulayim, N., Savlu, A., Guzeloglu-Kayisli, O., Kayisli, U. A. and Arici, A. (2004) Regulation of endometrial stromal cell matrix metalloproteinase activity and invasiveness by interleukin-8. *Fertil Steril*, **81 Suppl 1**, 904-911.
Ness, R. B. (2003) Endometriosis and ovarian cancer: thoughts on shared pathophysiology. *Am J Obstet Gynecol*, **189**, 280-294.
Nezhat, F., Datta, M. S., Hanson, V., Pejovic, T. and Nezhat, C. (2008) The relationship of endometriosis and ovarian malignancy: a review. *Fertil Steril*, **90**, 1559-1570.
Nisolle, M., Casanas-Roux, F. and Donnez, J. (1997) Immunohistochemical analysis of proliferative activity and steroid receptor expression in peritoneal and ovarian endometriosis. *Fertil Steril*, **68**, 912-919.
Nisolle, M. and Donnez, J. (1997) Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil Steril*, **68**, 585-596.
Noble, L. S., Simpson, E. R., Johns, A. and Bulun, S. E. (1996) Aromatase expression in endometriosis. *J Clin Endocrinol Metab*, **81**, 174-179.
Nothnick, W. B. (2001) Treating endometriosis as an autoimmune disease. *Fertil Steril*, **76**, 223-231.
Noyes, R. W., Hertig, A. T. and Rock, J. (1975) Dating the endometrial biopsy. *Am J Obstet Gynecol*, **122**, 262-263.
Obata, K., Morland, S. J., Watson, R. H., Hitchcock, A., Chenevix-Trench, G., Thomas, E. J. and Campbell, I. G. (1998) Frequent PTEN/MMAC mutations in endometrioid but not serous or mucinous epithelial ovarian tumors. *Cancer Res*, **58**, 2095-2097.
Ogawa, S., Kaku, T., Amada, S., Kobayashi, H., Hirakawa, T., Ariyoshi, K., Kamura, T. and Nakano, H. (2000) Ovarian endometriosis associated with ovarian carcinoma: a clinicopathological and immunohistochemical study. *Gynecol Oncol*, **77**, 298-304.
Okuda, T., Otsuka, J., Sekizawa, A., Saito, H., Makino, R., Kushima, M., Farina, A., Kuwano, Y. and Okai, T. (2003) p53 mutations and overexpression affect

prognosis of ovarian endometrioid cancer but not clear cell cancer. *Gynecol Oncol*, **88**, 318-325.
Olive, D. L. and Henderson, D. Y. (1987) Endometriosis and mullerian anomalies. *Obstet Gynecol*, **69**, 412-415.
Olson, J. E., Cerhan, J. R., Janney, C. A., Anderson, K. E., Vachon, C. M. and Sellers, T. A. (2002) Postmenopausal cancer risk after self-reported endometriosis diagnosis in the Iowa Women's Health Study. *Cancer*, **94**, 1612-1618.
Oosterlynck, D. J., Cornillie, F. J., Waer, M., Vandeputte, M. and Koninckx, P. R. (1991) Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. *Fertil Steril*, **56**, 45-51.
Oosterlynck, D. J., Meuleman, C., Waer, M., Koninckx, P. R. and Vandeputte, M. (1993) Immunosuppressive activity of peritoneal fluid in women with endometriosis. *Obstet Gynecol*, **82**, 206-212.
Orozco, G., Eyre, S., Hinks, A., Ke, X., Wilson, A. G., Bax, D. E., Morgan, A. W., Emery, P., Steer, S., Hocking, L. et al. (2010) Association of CD40 with rheumatoid arthritis confirmed in a large UK case-control study. *Ann Rheum Dis*, **69**, 813-816.
Osuga, Y., Koga, K., Hirota, Y., Hirata, T., Yoshino, O. and Taketani, Y. (2010) Lymphocytes in endometriosis. *Am J Reprod Immunol*, **65**, 1-10.
Painter, J. N., Anderson, C. A., Nyholt, D. R., Macgregor, S., Lin, J., Lee, S. H., Lambert, A., Zhao, Z. Z., Roseman, F., Guo, Q. et al. (2011) Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis. *Nat Genet*.
Pedraza, C., Geberhiwot, T., Ingerpuu, S., Assefa, D., Wondimu, Z., Kortesmaa, J., Tryggvason, K., Virtanen, I. and Patarroyo, M. (2000) Monocytic cells synthesize, adhere to, and migrate on laminin-8 (alpha 4 beta 1 gamma 1). *J Immunol*, **165**, 5831-5838.
Pellicer, A., Oliveira, N., Ruiz, A., Remohi, J. and Simon, C. (1995) Exploring the mechanism(s) of endometriosis-related infertility: an analysis of embryo development and implantation in assisted reproduction. *Hum Reprod*, **10 Suppl 2**, 91-97.
Pitsos, M. and Kanakas, N. (2009) The role of matrix metalloproteinases in the pathogenesis of endometriosis. *Reprod Sci*, **16**, 717-726.
Pizzo, A., Salmeri, F. M., Ardita, F. V., Sofo, V., Tripepi, M. and Marsico, S. (2002) Behaviour of cytokine levels in serum and peritoneal fluid of women with endometriosis. *Gynecol Obstet Invest*, **54**, 82-87.
Ploski, R., Dziunycz, P., Kostrzewa, G., Roszkowski, P. I., Barcz, E., Zabek, J., Milewski, L., Kaminski, P. and Malejczyk, J. (2009) PTPN22/LYP 1858C>T gene polymorphism and susceptibility to endometriosis in a Polish population. *J Reprod Immunol*, **79**, 196-200.
Poncelet, C., Leblanc, M., Walker-Combrouze, F., Soriano, D., Feldmann, G., Madelenat, P., Scoazec, J. Y. and Darai, E. (2002) Expression of cadherins and CD44 isoforms in human endometrium and peritoneal endometriosis. *Acta Obstet Gynecol Scand*, **81**, 195-203.
Prefumo, F., Todeschini, F., Fulcheri, E. and Venturini, P. L. (2002) Epithelial abnormalities in cystic ovarian endometriosis. *Gynecol Oncol*, **84**, 280-284.
Prowse, A. H., Manek, S., Varma, R., Liu, J., Godwin, A. K., Maher, E. R., Tomlinson, I. P. and Kennedy, S. H. (2006) Molecular genetic evidence that endometriosis is a precursor of ovarian cancer. *Int J Cancer*, **119**, 556-562.
Quaye, L., Song, H., Ramus, S. J., Gentry-Maharaj, A., Hogdall, E., DiCioccio, R. A., McGuire, V., Wu, A. H., Van Den Berg, D. J., Pike, M. C. et al. (2009) Tagging single-nucleotide polymorphisms in candidate oncogenes and susceptibility to ovarian cancer. *Br J Cancer*, **100**, 993-1001.
Ragni, G., Somigliana, E., Benedetti, F., Paffoni, A., Vegetti, W., Restelli, L. and Crosignani, P. G. (2005) Damage to ovarian reserve associated with laparoscopic excision of endometriomas: a quantitative rather than a qualitative injury. *Am J Obstet Gynecol*, **193**, 1908-1914.
Raychaudhuri, S., Remmers, E. F., Lee, A. T., Hackett, R., Guiducci, C., Burtt, N. P., Gianniny, L., Korman, B. D., Padyukov, L., Kurreeman, F. A. et al. (2008)

Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nat Genet*, **40**, 1216-1223.
Ridley, J. H. and Edwards, I. K. (1958) Experimental endometriosis in the human. *Am J Obstet Gynecol*, **76**, 783-789; discussion 789-790.
Romano, R. A., Li, H., Tummala, R., Maul, R. and Sinha, S. (2004) Identification of Basonuclin2, a DNA-binding zinc-finger protein expressed in germ tissues and skin keratinocytes. *Genomics*, **83**, 821-833.
Rong, R., Ramachandran, S., Santanam, N., Murphy, A. A. and Parthasarathy, S. (2002) Induction of monocyte chemotactic protein-1 in peritoneal mesothelial and endometrial cells by oxidized low-density lipoprotein and peritoneal fluid from women with endometriosis. *Fertil Steril*, **78**, 843-848.
Roszkowski, P. I., Sankowska, M., Jalbrzykowska, A., Radomski, D., Dragowska, K., Ploski, R. and Malejczyk, J. (2005) Susceptibility to ovarian endometriosis in Polish population is not associated with HLA-DRB1 alleles. *Hum Reprod*, **20**, 970-973.
Said, T. M., Agarwal, A., Falcone, T., Sharma, R. K., Bedaiwy, M. A. and Li, L. (2005) Infliximab may reverse the toxic effects induced by tumor necrosis factor alpha in human spermatozoa: an in vitro model. *Fertil Steril*, **83**, 1665-1673.
Sainz de la Cuesta, R., Izquierdo, M., Canamero, M., Granizo, J. J. and Manzarbeitia, F. (2004) Increased prevalence of p53 overexpression from typical endometriosis to atypical endometriosis and ovarian cancer associated with endometriosis. *Eur J Obstet Gynecol Reprod Biol*, **113**, 87-93.
Sampson, J. (1927) Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the pelvic cavity. *Am J Obstet Gynecol*, **14**, 422-469.
Sasson, I. E. and Taylor, H. S. (2008) Stem cells and the pathogenesis of endometriosis. *Ann N Y Acad Sci*, **1127**, 106-115.
Sato, N., Tsunoda, H., Nishida, M., Morishita, Y., Takimoto, Y., Kubo, T. and Noguchi, M. (2000) Loss of heterozygosity on 10q23.3 and mutation of the tumor suppressor gene PTEN in benign endometrial cyst of the ovary: possible sequence progression from benign endometrial cyst to endometrioid carcinoma and clear cell carcinoma of the ovary. *Cancer Res*, **60**, 7052-7056.
Schulke, L., Berbic, M., Manconi, F., Tokushige, N., Markham, R. and Fraser, I. S. (2009) Dendritic cell populations in the eutopic and ectopic endometrium of women with endometriosis. *Hum Reprod*, **24**, 1695-1703.
Schwab, K. E. and Gargett, C. E. (2007) Co-expression of two perivascular cell markers isolates mesenchymal stem-like cells from human endometrium. *Hum Reprod*, **22**, 2903-2911.
Seal, S. L., Kamilya, G., Mukherji, J., De, A., Ghosh, D. and Majhi, A. K. (2011) Aromatase inhibitors in recurrent ovarian endometriomas: report of five cases with literature review. *Fertil Steril*, **95**, 291 e215-298.
Seeber, B., Sammel, M. D., Fan, X., Gerton, G. L., Shaunik, A., Chittams, J. and Barnhart, K. T. (2010) Proteomic analysis of serum yields six candidate proteins that are differentially regulated in a subset of women with endometriosis. *Fertil Steril*, **93**, 2137-2144.
Seifer, D. B., MacLaughlin, D. T., Christian, B. P., Feng, B. and Shelden, R. M. (2002) Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. *Fertil Steril*, **77**, 468-471.
Shebl, O., Ebner, T., Sommergruber, M., Sir, A. and Tews, G. (2009) Anti muellerian hormone serum levels in women with endometriosis: a case-control study. *Gynecol Endocrinol*, **25**, 713-716.
Sillem, M., Prifti, S., Monga, B., Arslic, T. and Runnebaum, B. (1999) Integrin-mediated adhesion of uterine endometrial cells from endometriosis patients to extracellular matrix proteins is enhanced by tumor necrosis factor alpha (TNF alpha) and interleukin-1 (IL-1). *Eur J Obstet Gynecol Reprod Biol*, **87**, 123-127.
Sillem, M., Prifti, S., Koch, A., Neher, M., Jauckus, J. and Runnebaum, B. (2001) Regulation of matrix metalloproteinases and their inhibitors in uterine endometrial cells of patients with and without endometriosis. *Eur J Obstet Gynecol Reprod Biol*, **95**, 167-174.

Simoens, S., Hummelshoj, L. and D'Hooghe, T. (2007) Endometriosis: cost estimates and methodological perspective. *Hum Reprod Update*, **13**, 395-404.
Simon, C., Gutierrez, A., Vidal, A., de los Santos, M. J., Tarin, J. J., Remohi, J. and Pellicer, A. (1994) Outcome of patients with endometriosis in assisted reproduction: results from in-vitro fertilization and oocyte donation. *Hum Reprod*, **9**, 725-729.
Sinaii, N., Cleary, S. D., Ballweg, M. L., Nieman, L. K. and Stratton, P. (2002) High rates of autoimmune and endocrine disorders, fibromyalgia, chronic fatigue syndrome and atopic diseases among women with endometriosis: a survey analysis. *Hum Reprod*, **17**, 2715-2724.
Song, H., Ramus, S. J., Tyrer, J., Bolton, K. L., Gentry-Maharaj, A., Wozniak, E., Anton-Culver, H., Chang-Claude, J., Cramer, D. W., DiCioccio, R. et al. (2009) A genome-wide association study identifies a new ovarian cancer susceptibility locus on 9p22.2. *Nat Genet*, **41**, 996-1000.
Song, J., Rutherford, T., Naftolin, F., Brown, S. and Mor, G. (2002) Hormonal regulation of apoptosis and the Fas and Fas ligand system in human endometrial cells. *Mol Hum Reprod*, **8**, 447-455.
Spuijbroek, M. D., Dunselman, G. A., Menheere, P. P. and Evers, J. L. (1992) Early endometriosis invades the extracellular matrix. *Fertil Steril*, **58**, 929-933.
Stefansson, H., Geirsson, R. T., Steinthorsdottir, V., Jonsson, H., Manolescu, A., Kong, A., Ingadottir, G., Gulcher, J. and Stefansson, K. (2002) Genetic factors contribute to the risk of developing endometriosis. *Hum Reprod*, **17**, 555-559.
Stern, R. C., Dash, R., Bentley, R. C., Snyder, M. J., Haney, A. F. and Robboy, S. J. (2001) Malignancy in endometriosis: frequency and comparison of ovarian and extraovarian types. *Int J Gynecol Pathol*, **20**, 133-139.
Stewart, C. R., Stuart, L. M., Wilkinson, K., van Gils, J. M., Deng, J., Halle, A., Rayner, K. J., Boyer, L., Zhong, R., Frazier, W. A. et al. (2010) CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. *Nat Immunol*, **11**, 155-161.
Strathy, J. H., Molgaard, C. A., Coulam, C. B. and Melton, L. J., 3rd (1982) Endometriosis and infertility: a laparoscopic study of endometriosis among fertile and infertile women. *Fertil Steril*, **38**, 667-672.
Stratton, M. R. and Rahman, N. (2008) The emerging landscape of breast cancer susceptibility. *Nat Genet*, **40**, 17-22.
Streuli, I., Fraisse, T., Pillet, C., Ibecheole, V., Bischof, P. and de Ziegler, D. (2008) Serum antimullerian hormone levels remain stable throughout the menstrual cycle and after oral or vaginal administration of synthetic sex steroids. *Fertil Steril*, **90**, 395-400.
Streuli, I., Fraisse, T., Chapron, C., Bijaoui, G., Bischof, P. and de Ziegler, D. (2009) Clinical uses of anti-Mullerian hormone assays: pitfalls and promises. *Fertil Steril*, **91**, 226-230.
Szamatowicz, J., Laudanski, P. and Tomaszewska, I. (2002) Matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1: a possible role in the pathogenesis of endometriosis. *Hum Reprod*, **17**, 284-288.
te Velde, E. R., Scheffer, G. J., Dorland, M., Broekmans, F. J. and Fauser, B. C. (1998) Developmental and endocrine aspects of normal ovarian aging. *Mol Cell Endocrinol*, **145**, 67-73.
Teramoto, M., Kitawaki, J., Koshiba, H., Kitaoka, Y., Obayashi, H., Hasegawa, G., Nakamura, N., Yoshikawa, T., Matsushita, M., Maruya, E. et al. (2004) Genetic contribution of tumor necrosis factor (TNF)-alpha gene promoter (-1031, -863 and -857) and TNF receptor 2 gene polymorphisms in endometriosis susceptibility. *Am J Reprod Immunol*, **51**, 352-357.
Tokushige, N., Markham, R., Russell, P. and Fraser, I. S. (2006) Nerve fibres in peritoneal endometriosis. *Hum Reprod*, **21**, 3001-3007.
Tokushige, N., Markham, R., Crossett, B., Ahn, S. B., Nelaturi, V. L., Khan, A. and Fraser, I. S. (2011) Discovery of a novel biomarker in the urine in women with endometriosis. *Fertil Steril*, **95**, 46-49.
Treloar, S. A., O'Connor, D. T., O'Connor, V. M. and Martin, N. G. (1999) Genetic influences on endometriosis in an Australian twin sample. sueT@qimr.edu.au. *Fertil Steril*, **71**, 701-710.

Treloar, S. A., Wicks, J., Nyholt, D. R., Montgomery, G. W., Bahlo, M., Smith, V., Dawson, G., Mackay, I. J., Weeks, D. E., Bennett, S. T. et al. (2005) Genomewide linkage study in 1,176 affected sister pair families identifies a significant susceptibility locus for endometriosis on chromosome 10q26. *Am J Hum Genet*, **77**, 365-376.
Ueda, M., Yamashita, Y., Takehara, M., Terai, Y., Kumagai, K., Ueki, K., Kanda, K., Hung, Y. C. and Ueki, M. (2002) Gene expression of adhesion molecules and matrix metalloproteinases in endometriosis. *Gynecol Endocrinol*, **16**, 391-402.
Uno, S., Zembutsu, H., Hirasawa, A., Takahashi, A., Kubo, M., Akahane, T., Aoki, D., Kamatani, N., Hirata, K. and Nakamura, Y. (2010) A genome-wide association study identifies genetic variants in the CDKN2BAS locus associated with endometriosis in Japanese. *Nat Genet*, **42**, 707-710.
van der Linden, P. J., de Goeij, A. F., Dunselman, G. A., Arends, J. W. and Evers, J. L. (1994) P-cadherin expression in human endometrium and endometriosis. *Gynecol Obstet Invest*, **38**, 183-185.
van der Linden, P. J., de Goeij, A. F., Dunselman, G. A., van der Linden, E. P., Ramaekers, F. C. and Evers, J. L. (1994) Expression of integrins and E-cadherin in cells from menstrual effluent, endometrium, peritoneal fluid, peritoneum, and endometriosis. *Fertil Steril*, **61**, 85-90.
Van Patten, K., Parkash, V. and Jain, D. (2010) Cadherin expression in gastrointestinal tract endometriosis: possible role in deep tissue invasion and development of malignancy. *Mod Pathol*, **23**, 38-44.
van Rooij, I. A., Broekmans, F. J., te Velde, E. R., Fauser, B. C., Bancsi, L. F., de Jong, F. H. and Themmen, A. P. (2002) Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod*, **17**, 3065-3071.
Wang, G., Tokushige, N., Russell, P., Dubinovsky, S., Markham, R. and Fraser, I. S. (2010) Neuroendocrine cells in eutopic endometrium of women with endometriosis. *Hum Reprod*, **25**, 387-391.
Vanhoutteghem, A. and Djian, P. (2004) Basonuclin 2: an extremely conserved homolog of the zinc finger protein basonuclin. *Proc Natl Acad Sci U S A*, **101**, 3468-3473.
Vanhoutteghem, A. and Djian, P. (2006) Basonuclins 1 and 2, whose genes share a common origin, are proteins with widely different properties and functions. *Proc Natl Acad Sci U S A*, **103**, 12423-12428.
Varma, R., Rollason, T., Gupta, J. K. and Maher, E. R. (2004) Endometriosis and the neoplastic process. *Reproduction*, **127**, 293-304.
Weenen, C., Laven, J. S., Von Bergh, A. R., Cranfield, M., Groome, N. P., Visser, J. A., Kramer, P., Fauser, B. C. and Themmen, A. P. (2004) Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod*, **10**, 77-83.
Velasco, I., Rueda, J. and Acien, P. (2006) Aromatase expression in endometriotic tissues and cell cultures of patients with endometriosis. *Mol Hum Reprod*, **12**, 377-381.
Vercellini, P., De Giorgi, O., Aimi, G., Panazza, S., Uglietti, A. and Crosignani, P. G. (1997) Menstrual characteristics in women with and without endometriosis. *Obstet Gynecol*, **90**, 264-268.
Verkauf, B. S. (1987) Incidence, symptoms, and signs of endometriosis in fertile and infertile women. *J Fla Med Assoc*, **74**, 671-675.
Verma, A. and Konje, J. C. (2009) Successful treatment of refractory endometriosis-related chronic pelvic pain with aromatase inhibitors in premenopausal patients. *Eur J Obstet Gynecol Reprod Biol*, **143**, 112-115.
Vernet-Tomas Mdel, M., Perez-Ares, C. T., Verdu, N., Molinero, J. L., Fernandez-Figueras, M. T. and Carreras, R. (2006) The endometria of patients with endometriosis show higher expression of class I human leukocyte antigen than the endometria of healthy women. *Fertil Steril*, **85**, 78-83.
Whang, D. H., Kim, S. H., Choi, Y. M., Park, M. H., Noh, J. H. and Kim, Y. B. (2006) No association between HLA-DRB1 alleles and susceptibility to advanced stage endometriosis in a Korean population. *Hum Reprod*, **21**, 129-133.
Witz, C. A., Thomas, M. R., Montoya-Rodriguez, I. A., Nair, A. S., Centonze, V. E. and Schenken, R. S. (2001) Short-term culture of peritoneum explants confirms

attachment of endometrium to intact peritoneal mesothelium. *Fertil Steril*, **75**, 385-390.
Wolff, E. F., Wolff, A. B., Hongling, D. and Taylor, H. S. (2007) Demonstration of multipotent stem cells in the adult human endometrium by in vitro chondrogenesis. *Reprod Sci*, **14**, 524-533.
Zhang, R. J., Wild, R. A. and Ojago, J. M. (1993) Effect of tumor necrosis factor-alpha on adhesion of human endometrial stromal cells to peritoneal mesothelial cells: an in vitro system. *Fertil Steril*, **59**, 1196-1201.
Zhang, X., Yao, H., Huang, X., Lu, B., Xu, H. and Zhou, C. (2010) Nerve fibres in ovarian endometriotic lesions in women with ovarian endometriosis. *Hum Reprod*, **25**, 392-397.
Zondervan, K. T., Cardon, L. R. and Kennedy, S. H. (2002) What makes a good case-control study? Design issues for complex traits such as endometriosis. *Hum Reprod*, **17**, 1415-1423.
Zondervan, K. T., Weeks, D. E., Colman, R., Cardon, L. R., Hadfield, R., Schleffler, J., Trainor, A. G., Coe, C. L., Kemnitz, J. W. and Kennedy, S. H. (2004) Familial aggregation of endometriosis in a large pedigree of rhesus macaques. *Hum Reprod*, **19**, 448-455.
Zondervan, K. T., Treloar, S. A., Lin, J., Weeks, D. E., Nyholt, D. R., Mangion, J., MacKay, I. J., Cardon, L. R., Martin, N. G., Kennedy, S. H. et al. (2007) Significant evidence of one or more susceptibility loci for endometriosis with near-Mendelian inheritance on chromosome 7p13-15. *Hum Reprod*, **22**, 717-728.