CHAPTER 1

INTRODUCTION TO ENDOMETRIOSIS
1.1 Introduction

Study of the female reproductive system (Fig. 1.1) is central to the understanding of the basic biology of conception of life. Particularly, the pivotal role the uterus plays in reproduction is essential to acknowledge, for this is the very site where some of the mysteries of life begin to unfold. The uterus is located inside the pelvis immediately dorsal (and usually somewhat rostral) to the urinary bladder and ventral to the rectum. The uterus is a pear shaped muscular organ which can be divided anatomically into four segments: the fundus, the corpus, the cervix, and the internal os (Fig. 1.1). Outside of pregnancy, its size in humans is several centimeters in diameter.

The uterus is made up of three different layers namely the endometrium, the myometrium, and the perimetrium (Fig. 1.2). These three layers, from the innermost to the outermost regions of the uterus are as follows:

a) Endometrium

Endometrium is the mucosal lining of the uterus, the function of which is to provide a suitable site for implantation and development of a fertilized ovum. The endometrium lines the uterine cavity and consists of the functional endometrium and the basal endometrium from which the former arises. In most mammals, including humans, the endometrium builds a lining, which is periodically shed or reabsorbed if no pregnancy occurs. Shedding of the functional endometrial lining in humans is responsible for menstrual bleeding throughout the fertile years of a female and for some time beyond.

b) Myometrium

Underneath the endometrium is a thick muscular wall, the myometrium, which consists of densely packed, unstriped smooth muscle cells and supporting stromal and vascular tissue. Blood vessels, lymph vessels, and nerves are present in the myometrium.
Myometrium stretches considerably (the smooth muscle cells expand in both size and number) during pregnancy to accommodate, protect, and nourish the fetus, and is responsible for the expulsion of the fetus during birth.

c) Perimetrium

Perimetrium (or serous coat of uterus) is the outer serosa layer of the uterus, equivalent to the peritoneum. It protects the other linings of the uterus. Perimetrium is very thin and covers both the endometrium and the myometrium.

The uterus as a whole is surrounded by the peritoneum.

Figure 1.1 A schematic figure of the human female reproductive system
1.2 Endometrium

1.2.1 Histology of the endometrium

The endometrium consists of a columnar epithelium (ciliated and secretory cells), resting on a layer of connective tissue the stroma, which varies in thickness under hormonal influences (Wynn, 1989; Ferenczy and Bergeron, 1991) (Fig. 1.2). The endometrium is invaginated to form many simple tubular uterine glands (Fig. 1.2 B-E). The glands extend through the entire thickness of the stroma, which also carry a rich blood supply of spiral arteries (Fig. 1.2). The stromal cells of the endometrium are embedded in a network of reticular fibres. Both glands and stroma undergo extensive changes during the menstrual cycle. In a woman of reproductive age, the endometrium comprises of three histologically distinctive layers (Johannisson et al., 1987; Wynn, 1989; Salamonsen, 2007) (Fig. 1.3). These layers are present only in the endometrium lining the cavity of the uterus and not in the lining of the fallopian tubes:

a. The deepest layer adjacent to the myometrium and below the functional layer is the stratum basalis (Fig. 1.3); this is the persisting germinative layer that undergoes minimal changes during the menstrual cycle. It is from this layer, the functional layer develops.

b. The intermediate layer, stratum spongiosum is characterized by the presence of a spongy stroma.

c. The thinner, most superficial layer is the stratum compactum.

The last two layers undergo dramatic and characteristic changes during the menstrual cycle, culminating in menstrual sloughing, and are therefore often jointly referred to as the stratum functionalis (Fig. 1.3). These layers are built up after the end of menstruation during the first part of the menstrual cycle. Proliferation is induced by estrogen (follicular phase of menstrual cycle), and later changes in these layers are engendered by
progesterone which is secreted from the corpus luteum (luteal phase). Stratum functionalis is adapted to provide an optimum environment for the implantation and growth of the embryo. This layer is completely shed during menstruation. In the absence of progesterone, the arteries supplying blood to the functional layer constrict so that cells in that layer become ischaemic and die, leading to menstruation (Ludwig and Spornitz., 1991; Salamonsen et al., 2003; Salamonsen, 2007).

Figure 1.2 Micrograph showing histology of the uterine wall (A) and the endometrium in proliferative (B) and secretory phase at low (C) and high magnifications (D and E)
1.2.2 The menstrual cycle

Endometrium is a highly dynamic tissue that undergoes cyclical variation with every menstrual cycle during the reproductive years. Under the influence of the changing hormonal milieu, cellular proliferation, differentiation, and apoptosis occur in association with changes in extracellular matrix (ECM) composition and leukocyte trafficking. The entire functional layer (functionalis) of the endometrium is shed at menstruation with subsequent regeneration of the tissue from the remaining basal layer (basalis) (Ludwig and Spornitz, 1991). The menstrual cycle is broadly divided into three phases (Fig. 1.4):
a) Menstrual phase: during menses, most of the functionalis layer is shed and re-epithelialization is initiated during this phase in parallel with tissue breakdown in adjacent areas (Ludwig and Spornitz, 1991).

b) Proliferative (follicular) phase: during the proliferative phase, under the influence of estrogen there is rapid cellular proliferation of all cell types and new ECM is laid down.

c) Secretory (luteal) phase: shortly after ovulation, in the secretory phase the endometrium undergoes progesterone dependent functional differentiation which provides a suitable environment for embryo implantation. Characteristically, the glands become increasingly tortuous with considerable secretory activity and the stromal cells begin a differentiation process, termed as decidualization, a prerequisite for successful implantation. The endometrium is receptive to embryonic implantation in the mid-secretory phase, approximately for a period of 5 days and this process coincides with a peak in the levels of progesterone. In the absence of pregnancy, a decline in progesterone levels in the late secretory phase leads to endometrial regression and menstruation (Fig. 1.4) (Salamonsen et al., 2003).

Because of the above mentioned distinct changes, it is possible to identify the phases of the menstrual cycle, namely, the menstrual phase, the proliferative phase, and the secretory phase, based on the histology of the endometrium (Table 1.1 and Fig. 1.4). The characteristic diagnostic features include the thickness and the absence or the presence of the epithelium. This also permits the phasing of the endometrium.
Table 1.1 Histological features that differentiate the various phases of the endometrium

<table>
<thead>
<tr>
<th>Phase</th>
<th>Days</th>
<th>Thickness</th>
<th>Epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstrual</td>
<td>1-4</td>
<td>Thin</td>
<td>Absent</td>
</tr>
<tr>
<td>Proliferative</td>
<td>4-14</td>
<td>Intermediate</td>
<td>Columnar</td>
</tr>
<tr>
<td>Secretory</td>
<td>15-28</td>
<td>Thick</td>
<td>Columnar, also visible are helicine branches of the uterine artery</td>
</tr>
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</table>

Figure 1.4 Changes in the levels of ovarian hormones, estrogen and progesterone (upper panel) in serum; and changes in the endometrial structure (lower panel) during a normalized 28-day menstrual cycle in women
1.2.3 Regulation of endometrial remodeling

Endometrium is a dynamic tissue that undergoes cyclic phases of remarkable periodic growth, remodeling, and breakdown (Ludwig and Spornitz, 1991; Salamonsen et al., 2003; Salamonsen, 2007). The remodeling is regulated by ovarian steroid hormones, estrogen and progesterone (as discussed in section 1.2.2) as well as by various cytokines (Krasnow et al., 1996; von Wolff et al., 2000; Ostanin et al., 2007; Hatta et al., 2009), neuropeptides (Fay and Grudzinskas, 1991; Imai et al., 1996), and growth factors (Nardo, 2005), which are produced locally and secreted in an endocrine, paracrine, as well as autocrine manner. The endometrium initially proliferates under the influence of estrogen (Ferenczy et al., 1979; Jabbour et al., 2006; Groothuis et al., 2007). Prior to ovulation, the role of estrogen is considered to be important in the regeneration and growth of the endometrium and to prepare the tissue to respond to progesterone post-ovulation. However, once ovulation occurs, in addition to estrogen, the ovary also produces progesterone which changes the proliferative pattern of the endometrium to a secretory lining (Jabbour et al., 2006; Young and Lessey, 2010). Eventually, the secretory lining provides a hospitable environment for one or more blastocysts. If no blastocyst is detected by the endometrium, the progesterone level drops and the endometrial lining is shed (menstrual cycle). The process of shedding involves the breaking down of the lining, the tearing of small connective blood vessels, and the loss of tissue and blood (Salamonsen et al., 2003; Salamonsen, 2007).

Estrogen and progesterone elicit their actions primarily by binding to specific high-affinity receptors (Jabbour et al., 2006; Critchley and Saunders, 2009), which then act as transcription factors and modulate the transcription of a large number and variety of genes in the endometrium (Kao et al., 2002; Dey et al., 2004; Jabbour et al., 2006; Groothuis et al., 2007). These genes tightly regulate a series of physiological events occurring during menstrual cycle. Therefore, any aberration in endometrial remodeling or endometrial
physiology would lead to diseases (Ito et al., 2006) like endometriosis (Vinatier et al., 2000; Fowler et al., 2007; Have et al., 2007; Burney et al., 2007; Torres et al., 2009), endometrial polyps, hyperplasia, and endometrial cancer (Yoshizaki et al., 2005; Dubé et al., 2007; Jeong et al., 2009; Liao et al., 2009).

1.3 Pathological conditions related with endometrial physiology

Many of the major issues of women’s health, including abnormal uterine bleeding, endometriosis, and infertility are primarily a result of aberration in endometrial remodeling or endometrial physiology. A few of the diseases are briefly discussed below:

a) Adenomyosis: the growth of the endometrium into the muscle layer of the uterus, the myometrium.

b) Endometriosis: the growth of endometrial tissue outside the uterus.

c) Endometrial cancer: the most common cancer of the human female genital tract.

d) Asherman’s syndrome, also known as intrauterine adhesion, occurs when the basal layer of the endometrium is damaged by instrumentation (eg. dilation and curettage) or infection (eg. endometrial tuberculosis), thus resulting in endometrial sclerosis and adhesions which could either partially or completely obliterate the uterine cavity.

1.4 Endometriosis

Endometriosis is a common, benign, and estrogen-dependent chronic gynecological disorder associated with pelvic pain and infertility. The first description of endometriosis as an entity is attributed to Rokitansky in 1860. It is characterized by the presence of uterine endometrial tissue outside the normal location, mainly on the pelvic peritoneum, but also on the ovaries and in the rectovaginal septum, and more rarely in the pericardium, the pleura, and even the brain. The prevalence of pelvic endometriosis approaches 6–10% in the general female population; in women with pain, infertility, or both, the frequency is 35–50% (Giudice and Kao, 2004). The disorder is most commonly diagnosed in the women of
reproductive age, although at times diagnosis is delayed (mean 11.7 years in USA and 8.0 years in UK) because of variability in symptoms and signs and confusion with other disorders (Hadfield et al., 1996). The gold standard for diagnosis of pelvic disease is surgical assessment, by laparoscopy or laparotomy, and a scoring system has been developed to assess the extent of the disease (American Fertility Society: Classification of endometriosis, 1979). Severe disease results in most cases extensive pelvic adhesions and distortion of pelvic anatomy, which can lead to pain and infertility. Endometriosis can be the result of diverse anatomical or biochemical aberrations of uterine function. For example, endometriosis commonly develops in young women with vaginal obstruction of outflow, possibly because of large quantities of backwashed menstrual tissue that gets implanted on pelvic organs (Rock et al., 1982). In contrast, endometriosis can also involve mechanisms that are independent of anatomical abnormalities; for example, the incidence of endometriosis is increased in women who were exposed in utero to environmental toxins or potent estrogens such as diethylstilbestrol (Missmer et al., 2004).

As cellular and molecular mechanisms involved in endometriosis are being uncovered, the classification of the disease is evolving from a local disorder to a complex, chronic systemic disease. Three clinically distinct forms of endometriosis are endometriotic implants on the surface of the pelvic peritoneum and ovaries (peritoneal endometriosis), ovarian cysts lined by endometrioid mucosa (endometriomas), and a complex solid mass comprising of endometriotic tissue blended with adipose and fibro-muscular tissue, residing between the rectum and the vagina (rectovaginal endometriotic nodule). All three types may be variants of the same pathological process or can be caused by different mechanisms (Brosens, 2004; Garry, 2004). Their common histological features are the presence of endometrial stromal or epithelial cells, chronic bleeding, and signs of inflammation. These lesions can occur singly or in combination and are associated with an increased risk of infertility or chronic pelvic pain (Guzick et al., 1997; Stovall et al., 1997). The inflammation involved in endometriosis can stimulate nerve endings in the pelvis and thereby cause pain,
impair the function of uterine tubes, decrease the receptivity of the endometrium, and hinder the development of the oocyte and the embryo (Barnhart et al., 2002; Berkley et al., 2004). Endometriosis can also cause infertility by physically blocking the fallopian tubes (Guzick et al., 1997; Stovall et al., 1997).

The treatment of infertility caused by endometriosis is surgical removal of endometriotic tissue or assisted reproductive technology whereas the usual treatment of pain is a combination of medical suppression of ovulation and surgery. Peritoneal implants are resected or vaporized by means of an electric current or laser. Ovarian endometriomas and rectovaginal endometriotic nodules, however, can be removed by dissection. For women with pain, surgery generally provides temporary relief as symptoms recur in up to 75% of women within 2 years (Candiani et al., 1991; Kuohung et al., 2002; Olive, 2003) and further surgery is needed in many cases. Medical therapies, historically, have included contraceptive steroids, progestagens, agonists of gonadotropin releasing hormone (GnRH), as well as androgens and non-steroidal anti-inflammatory agents (Lessey, 2000; Valle and Sciarra, 2003; Practice Committee of the American Society for Reproductive Medicine, 2004). Treatments that aim to lower circulating estradiol concentrations (contraceptive steroids, progestagens, and GnRH agonists) can be used only for a limited time owing to unacceptable side-effects, osteoporosis, or both; changes or additions of medications are commonly needed. However, new treatments are being developed through a better understanding of factors synthesized by the endometrial tissue in the disorder and the immune response associated with the presence of endometriosis in the pelvis. For many women, endometriosis imposes a substantial toll in terms of well-being, personal relationships, time of work, and need for surgery and expensive therapies. Furthermore, findings of an increased risk of ovarian cancer and the suggestion of increased risks of autoimmune diseases and breast and skin cancers in women with endometriosis (Swiersz, 2001) support a need for multidisciplinary care for women with this disorder and long-term follow-up for surveillance of associated disorders that may develop in susceptible individuals.
The etiology and pathogenesis of endometriosis remain uncertain. However, important advances have been made during past few years that hold promise for the development of new diagnostic and therapeutic approaches to limit symptoms and to improve fertility. Investigations into the role of genetics, the environment, the immune system, and the estradiol in the pathogenesis of this disorder, as well as postgenomic study of intrinsic abnormalities in eutopic (i.e., within the uterus) and ectopic (i.e., endometriotic lesions outside the uterus) endometrium in women with the disease are providing insights into the pathophysiology of the associated pain and infertility.

1.4.1 Stages of Endometriosis

The American Society for Reproductive Medicine has recognized four stages of endometriosis based on the severity of the disease (Fig. 1.5 and 1.6):

(a) **Stage I** (Minimal): Few or superficial implants are evident in the early stages of endometriosis. A few cells/implants are present, often in the pelvic area; even at this stage some women can suffer significant symptoms. Women are back to stage one, often after surgery to remove the cells/implants (assuming surgery is taken to tackle the latter stages).

(b) **Stage II** (Mild): More implants and deeper involvement. Endometrial cells are found in more areas, often on one or both ovaries. At this stage fertility may start to be affected due to implants.

(c) **Stage III** (Moderate): More implants, with ovaries affected and the presence of adhesions. More cells are found in the pelvic area and can be seen more easily during diagnostic laparoscopy. Pain is often severe and fertility is often impaired as a result of the pain and distress due to the spreading of the cells.

(d) **Stage IV** (Severe): As in Stage III, but with multiple and more dense adhesions. This is the most severe stage; often organs are joined by fibrous strands due to the spread of the endometriosis. Inflammation is often extensive due to the irritation of
the organs as body reacts to the cells. At this stage, fertility is almost certainly affected alongside severe pain.

Figure 1.5 Stages of Endometriosis (according to the revised American Society for Reproductive Medicine)
Stage I

Stage II

Stage III

Stage IV

Figure 1.6 Laparoscopic images of different stages of endometriosis
1.4.2 Theories of pathogenesis

Although endometriosis has been known for over 100 years, its pathogenesis is still poorly understood. Three theories have been proposed to explain this anomaly:

1.4.2.1 Development by metaplasia

As ovary and Mullerian duct are derived from coelomic epithelium, the theory of coelomic metaplasia suggests that the germinal epithelium of the ovary can be transformed by metaplasia into the endometrium (Cullen, 1896). This theory, which explains only ovarian endometriosis, was extended to the peritoneal serosa that is known for its potential of proliferation and differentiation (Meyer, 1903). The metaplasia hypothesis is attractive as it can explain occurrences of endometriosis in the absence of menstruation (Doty et al, 1980; El Mahgoud and Yaseen, 1980) and can also explain the origin of endometriosis, regardless of the sites or the conditions of its occurrence (Suginami, 1991). However several points argue against this theory:

(a) Endometriosis should be possible in the absence of endometrium, for example in patients with a congenital absence of the uterus;
(b) The potential for peritoneal metaplasia should translate into the possibility of endometriosis in males;
(c) Coelomic metaplasia should be manifested wherever one finds the tissue derived from coelomic epithelium;
(d) If coelomic metaplasia resembles the common metaplasia, then the frequency of endometriosis should increase with age.

1.4.2.2 Development from Mullerian remnants (Theory of induction)

Levander and Normann (1955) introduced the induction theory, which is an extension of the coelomic metaplasia theory proposing that one or several endogenous, biochemical, or immunological factors could induce the endometrial differentiation of undifferentiated cells.
This theory is supported by experimentation on female rabbits (Merrill, 1966). In an in vitro experimental model, coelomic metaplasia of mesothelium was observed when ovarian surface epithelium and endometrial stromal cells were co-cultured with 17β estradiol (Matsuura et al., 1999). Morphological observations have confirmed that endometriosis may be manifested as a serial change from the adjacent mesothelial cells (Nakamura et al., 1993). This metaplastic transformation of ovarian epithelium needs 17β estradiol at a concentration 10 times higher than that in the peritoneal fluid of patients with endometriosis. Such a high concentration, found only in the proximity of the ovary, may explain ovarian endometriosis.

1.4.2.3 Implantation and growth of endometrium following menstrual reflux

The implantation theory proposes that during menstruation, there is reflux of endometrial tissue via the fallopian tubes into the abdominal cavity where it can get implanted (Sampson, 1927) as shown in Fig. 1.7. Several points argue in favor of this theory:

a) The reflux of endometrial cells during menstruation is a quasi-universal phenomenon when the fallopian tubes are healthy (Kruitwagen et al., 1991). The endometrium tissue refluxed in the peritoneal cavity is more often and more abundantly found in the follicular phase than in the luteal phase (Koninckx et al., 1980);

b) The observed localization of peritoneal endometrial lesions corresponds to that of a tubal reflux (Hoshiai et al., 1993; Vercellini et al., 1998);

c) Endometrial cells recovered from the abdomen at the end of menstruation are viable and capable of proliferating;

d) The endometrial cells express adhesive molecules (integrins) on their surface permitting them to attach to the peritoneum (Tabibzadeh, 1992);

e) Fragments of endometrium having migrated to the abdomen express metalloproteinase capable of degrading the basal membrane, in particular the extracellular matrix;

f) The endometrium produces angiogenic factors essential for local revascularization;
Microscopic examination by laparoscopy of the healthy peritoneum often identifies (in upto 20% of the cases of unexplained sterility) endometrial implants (Murphy et al., 1986; Nisolle et al., 1990).

Figure 1.7 Schematic diagram of events occurring in the pelvis following retrograde menstruation

1.5 Endometrial anomalies in women with endometriosis

Retrograde menstruation is the permissive factor in endometriosis. The formation of a lesion is therefore, likely to involve the dissemination of uterine endometrium into the peritoneal cavity at the time of menstruation and its survival, attachment, growth, proliferation, neo-angiogenesis, and invasion into ectopic sites within the peritoneal cavity. Since retrograde menstruation is a very common phenomenon among women of
reproductive age, there must be other factors that may contribute to the pathophysiology and/or pathogenesis of endometriosis. Genetic predisposition, environmental factors, and alterations in immune and endocrine functions are believed to play significant roles in the establishment and maintenance of endometriosis. Although the eutopic endometrium of women with and without endometriosis is histologically similar, studies revealed that there are many fundamental differences between these two tissues. These include a variety of anomalies in structure, proliferation, immune components, escape from immune surveillance, adhesion molecules, proteolytic enzymes and inhibitors, steroid and cytokine production and responsiveness, and gene expression and protein production (Sharpe-Timms, 2001; Giudice and Kao, 2004; Bulun, 2009). Invasive properties, decreased apoptosis, alterations in expression of specific genes and proteins, and increased steroid and cytokine production have also been identified in eutopic endometrium of women with endometriosis (Table 1.2). These factors are believed to contribute to the establishment and maintenance of this disease. Furthermore, a significant biochemical difference exists between ectopic endometrium and their putative eutopic precursors even though both the tissues are histologically similar.

However, many of the differences observed between the eutopic and ectopic endometrium of women with endometriosis can be explained by the direct effects of the peritoneal fluid and its contents. The following information provides details of the potential significance of eutopic endometrial anomalies that may be central to the pathogenesis and/or pathophysiology of this disorder.
Table 1.2 Endometrial anomalies in eutopic endometrium of women with endometriosis

<table>
<thead>
<tr>
<th>Anomaly</th>
<th>Description</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td><strong>Structure</strong></td>
<td>* Increased heterogeneity in surface epithelium, reduced glandular and stromal mitosis, basal vacuolated cells</td>
<td>Fedele et al., 1990</td>
</tr>
<tr>
<td></td>
<td>* Reduced endometrial thickness</td>
<td>Shapiro et al., 1995</td>
</tr>
<tr>
<td><strong>Proliferation</strong></td>
<td>* Increased number of proliferating endometrial epithelial, stromal, and endothelial cells</td>
<td>Wingfield et al., 1995; Burlev et al., 2006 &amp; Park et al., 2009</td>
</tr>
<tr>
<td></td>
<td>* No differences in endometrial cell proliferative activity</td>
<td>Kruijswagen et al., 1991 &amp; Jurgensen et al., 1996</td>
</tr>
<tr>
<td><strong>Apoptosis</strong></td>
<td>* Impaired apoptosis</td>
<td>Gebel et al., 1998 &amp; Szymanowski et al., 2006</td>
</tr>
<tr>
<td></td>
<td>* No significant difference in apoptosis or Bcl-2</td>
<td>Jones et al., 1998</td>
</tr>
<tr>
<td><strong>Immune components</strong></td>
<td>* Increased secretion of complement component C3</td>
<td>Isaacson et al., 1990</td>
</tr>
<tr>
<td></td>
<td>* No difference in presence of C3 or C4</td>
<td>Bartosik et al., 1987</td>
</tr>
<tr>
<td></td>
<td>* Presence of endometrial antigens of MW 60 and 66 kDa of the IgG class</td>
<td>Odukoya et al., 1995</td>
</tr>
<tr>
<td></td>
<td>* Decreased mitogenicity for autologous lymphocytes</td>
<td>Helvacioglu et al., 1997</td>
</tr>
<tr>
<td></td>
<td>* Trend for fewer T-suppressor/cytotoxic (CD8+) cells and endometrial granulated lymphocytes but more T-helper/inducer (CD4+) cells, CD68+cells and CD16+ cells</td>
<td>Kientzeris et al., 1995</td>
</tr>
<tr>
<td></td>
<td>* No difference in defined stromal leukocyte subpopulations</td>
<td>Jones et al., 1995</td>
</tr>
<tr>
<td></td>
<td>* Increased numbers of CD45+, CD43+, and CD3+ intraepithelial leukocytes</td>
<td>Bulmer et al., 1998</td>
</tr>
<tr>
<td></td>
<td>* Increased resistance to the cytotoxic effect of heterologous lymphocytes</td>
<td>Oosterlynck et al., 1991</td>
</tr>
<tr>
<td></td>
<td>* Increased expression of HSP 27</td>
<td>Nip et al., 1994 &amp; Ota et al., 1997</td>
</tr>
<tr>
<td>Anomaly</td>
<td>Description</td>
<td>References</td>
</tr>
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</tr>
<tr>
<td>Cell adhesion molecules</td>
<td>• Lack of αv3 expression on endometrial epithelium</td>
<td>Lessey et al., 1992</td>
</tr>
<tr>
<td></td>
<td>• No difference in endometrial epithelial αv3 expression</td>
<td>Hill and Rogers, 1998</td>
</tr>
<tr>
<td></td>
<td>• Increased expression of β-1 integrins and E-cadherin on endometrial glandular epithelium</td>
<td>Ota and Tanaka, 1997</td>
</tr>
<tr>
<td></td>
<td>• Increased expression of L1CAM in epithelial compartment</td>
<td>Finas et al., 2008</td>
</tr>
<tr>
<td>Proteases and their inhibitors</td>
<td>• Aberrant production of matrix metalloproteinases (MMPs) and their inhibitors and the tissue inhibitor of metalloproteinases (TIMPs)</td>
<td>Osteen et al., 1996 &amp; Sharpe-Timms and Cox, 2002</td>
</tr>
<tr>
<td>Steroid production</td>
<td>• Expression of aromatase P450</td>
<td>Noble et al., 1997 &amp; Kitawaki et al., 1997</td>
</tr>
<tr>
<td></td>
<td>• No difference in estrogen or progesterone receptor expression</td>
<td>Nisolle et al., 1994; Jones et al., 1995</td>
</tr>
<tr>
<td></td>
<td>• High estradiol production in endometriotic lesion</td>
<td>Zeitoun and Bulun, 1999</td>
</tr>
<tr>
<td></td>
<td>• Elevated levels of steroid hormones</td>
<td>Zeitoun et al., 1998 &amp; Bulun, 2010</td>
</tr>
<tr>
<td></td>
<td>• Decreased expression of progesterone receptor</td>
<td>Attia et al., 2000 &amp; Sun et al., 2003</td>
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1.5.1 Structure

Structural and ultrastructural defects in preovulatory endometrium of women with minimal or mild endometriosis have been identified by light microscopic, and scanning, and transmission electron microscopic studies (Fedele et al., 1990). These analyses revealed heterogeneity in the endometrial surface epithelium in 77% of the patients with endometriosis and in 16% of the non-endometriosis controls. Glandular and stromal mitosis, basal vacuolated cells, and the ciliated to nonciliated cell ratio were significantly reduced in the endometriosis group as compared to the controls. In women with endometriosis, the peristaltic activity of the uterus is significantly enhanced and may even become dysperistaltic at midcycle (Fedele et al., 1990). Since uterine peristalsis is confined to the endometrium
and the sub-endometrial myometrium with its predominantly circular arrangement of muscular fibres, it was assumed that this dysfunction might be associated with structural abnormalities. Women with endometriosis exhibited an infiltrative expansion of the archimeta; supporting the notion that endometriosis is primarily a uterine disease (Kunz et al., 2000). Although further studies are needed to clarify the role of these observed endometrial anomalies, these data suggest that a primary endometrial factor may be involved in the pathogenesis of infertility in patients with minimal or mild endometriosis. Clinical observations showing inadequate endometrial thickness in women with endometriosis undergoing superovulation and intrauterine insemination as compared to women with other diagnoses undergoing the same protocols support the observations of structural anomalies (Shapiro et al., 1995).

1.5.2 Proliferation

Wingfield et al., (1995) described increased in vivo proliferation of endometrial epithelial, stromal, and endothelial cells in women with endometriosis. Immunohistochemical analyses performed on fixed tissue sections detected proliferation of both endothelial cells and endometrial epithelial and stromal cells. From this study, they concluded that elevated endometrial cell proliferation could lead to enhanced ability of clumps of endometrial cells that reach the peritoneal cavity through retrograde menstruation, to get implanted, induce an angiogenic response, and thus survive outside the uterus. Alternatively, Jurgensen et al. (1996), immunohistochemically studied the frozen endometrial tissues with an antibody against Ki-S3 (an epitope of the Ki-67 antigen) and found no significant difference in endometrial cell proliferation between women with and without endometriosis. The disparities in the findings of these two studies may be attributed to the differences in the patient populations, technical details including tissue processing and antibodies chosen for staining, or methods used for data analyses. Others have failed to show any enhanced proliferation of endometrial cells harvested from the peritoneal fluid of women with and
without endometriosis (Kruitwagen et al., 1991). These studies, however, were performed in vitro and may have lacked paracrine influence from entities in the peritoneal cavity. Burlev et al. (2006), studied proliferative activity of the glandular epithelium and expression of proteins inhibiting or activating apoptosis during the proliferative and secretory phases of the menstrual cycle and showed that the proliferative activity of the glandular epithelium was higher in the proliferative phase than in the secretory phase. They have also shown changes in the expression levels of apoptosis-related proteins in the glandular epithelium of patients with endometriosis during the secretory phase of the menstrual cycle as compared to controls, suggesting that the imbalance between apoptosis-related proteins in the endometrium plays a role in the pathogenesis of peritoneal endometriosis. These results were also supported by Park et al. (2009) who showed increased proliferative activity and increased expression of cell proliferation markers in the endometrium of women with endometriosis than in controls. Ultimately, further studies will have to be performed to resolve the differences between these observations and to determine if aberrant endometrial cell proliferation contributes to the pathogenesis of endometriosis. It is also possible that altered endometrial proliferation may interfere with embryo implantation and thus contribute to the infertility and increased risk of miscarriage associated with endometriosis. This too remains to be proven.

1.5.3 Apoptosis

Apoptosis plays a critical role in maintaining tissue homeostasis and represents a normal function to eliminate excess or dysfunctional cells (Kerr et al., 1972; Hopwood and Levison et al., 1976). Accumulated evidences suggest that apoptosis helps to maintain cellular homeostasis during the menstrual cycle by eliminating senescent cells from the functional layer of the uterine endometrium during the late secretory and menstrual phase of the cycle (Kokawa et al., 1996; Shikone et al., 1996; Harada et al., 2004). Bcl-2 family and Fas/FasL system have been extensively studied in human endometrium and endometriotic
tissues (Otsuki et al., 1994; Garcia-Velasco et al., 1999; Otsuki, 2001; Song et al., 2002; Harada et al., 2004). One of the mechanisms responsible for endometriosis, and that has recently gained a lot of interest, is the finding that apoptosis occurs in eutopic and ectopic endometrium of patients with endometriosis (Harada et al., 2004; Harada et al., 2007).

Gebel et al. (1998) reported that among women with endometriosis, the percentage of sloughed endometrial cells undergoing apoptosis was greatly reduced, implying that the number of surviving cells that enter the peritoneal cavity is greater in women who develop endometriosis. This result was further supported by the studies that demonstrated that apoptosis indices in the eutopic endometrium of women with endometriosis were lower compared to the women without endometriosis (Dmowski et al., 2001; Szymanowski, 2007). One can speculate that if the decrease in apoptosis facilitates ectopic survival and implantation of the endometrial cells, then there may be an inverse correlation between the level of apoptosis and the severity of the disease. To test this hypothesis Dmowski et al. (2001), analyzed the apoptotic index according to the stage of endometriosis and found that there was a trend towards decreased apoptosis with increasing stages of the disease but the difference lacked statistical significance.

Conversely, Jones et al., (1998) reported that neither apoptosis nor expression of Bcl-2 (a cell surface receptor associated with triggering apoptosis) was altered in these tissues. Others have confirmed that Bcl-2 expression is not different in these tissues from women with endometriosis (Watanabe et al., 1997). Further, Goumenou et al. (2004) showed that the apoptotic rate as well as Bcl-2 and Bax expression in ovarian endometriotic cells was not affected by the stage of endometriosis or the phase of the menstrual cycle. An increased expression of Bcl-2 protein was found in proliferative eutopic endometrium of women with endometriosis when compared with normal endometrium from healthy women (Meresman et al., 2000). In the same study, Bax expression was absent in proliferative endometrium whereas there was an increase in its expression in secretory endometrium from women with endometriosis and also in healthy women.
Few studies have been published on the expression of Fas in endometriotic tissues. Harada et al. (1996) found that Fas is expressed randomly in both eutopic and ectopic endometrial tissues. The authors suggested that the expression of Fas antigen, an apoptosis-regulator, may be less involved in apoptosis of eutopic and ectopic endometrial tissues. In accordance with this finding, Watanabe et al. (1997) also observed Fas expression in glandular cells of both ectopic and eutopic endometrium. In contrast with the cyclic expression pattern of Bcl-2, Fas expression was constant in both tissues throughout the menstrual cycle (Song et al., 2002). In contrast with Fas, there are many studies which indicate that higher expression of FasL by endometriotic tissues contributes to their survival and to the development of endometriosis (Garcia-Velasco et al., 2002). These data suggest that apoptosis plays an important role in the development of endometriosis.

1.5.4 Immune components

The role of the immune system in endometriosis has been studied extensively. Yet, whether immune anomalies are the causes or the consequences of endometriosis has not been established. Nonetheless, numerous immune anomalies have been identified in women with this disorder (Dmowski et al., 1989; Osteen and Sierra-Rivera, 1997; Leibovic et al, 2001; Matarrese et al., 2003; Giudice and Kao, 2004). The following information describes anomalous immune components that have been observed in association with the eutopic endometrium from women having endometriosis.

Weed and Arquembour (1980) first described deposition of complement component, C3 in endometrial glandular epithelial cells of women with endometriosis. Subsequently, it was discovered that C3 synthesis and secretion by early proliferative endometrium of patients with minimal endometriosis was significantly greater than that of patients with no endometriosis or patients with severe endometriosis (Bartosik et al., 1987; Isaacson et al., 1990). It is possible that the known biological functions of C3 may have a
role in the pathogenesis or pathophysiology associated with endometriosis. C3 could exert these effects via opsonization for phagocyte (macrophage) activation, stimulation of prostaglandin synthesis from a variety of cell types (Rutherford, 1987), platelet aggregation, and thrombin release (Polley and Nachman, 1978; Devine and Rosse, 1987), or activation of other components of the complement system (Villiers et al., 2008). The presence of endometrial antigens of MW 60 and 66 kDa of the IgG class has been observed in approximately 50% of the patients with endometriosis (Odukoya et al., 1995). It remains unclear whether these patients represent a pathologically distinct subgroup. Autologous lymphocyte and eutopic endometrial cells from women with and without endometriosis were cocultured to observe lymphocyte proliferation (Helvacioglu et al., 1997). A diminished proliferative response of lymphocytes from women with endometriosis was noted. However, when the same lymphocytes were stimulated with phytohemagglutinin (PHA) in a simultaneous assay, it was observed that the proliferative response of lymphocytes from women with endometriosis was probably not the consequence of an intrinsic lymphocyte abnormality. Therefore, it was hypothesized that a fundamental defect in endometriosis may reside within the eutopic endometrial cells. Endometrial leukocyte populations have been studied in women with endometriosis. Although not statistically different, a trend was observed for fewer T-suppressor/cytotoxic (CD8+) cells and endometrial granulated lymphocytes but more T-helper/inducer (CD4+) cells, CD68+ cells, and CD16+ cells in the endometrium of women with endometriosis (Klentzeris et al., 1995). Additional studies of specific endometrial cell types found no difference in defined stromal leukocyte subpopulations (Jones et al., 1995), but increased numbers of CD45+, CD43+, and CD3+ intraepithelial leukocytes were observed when eutopic endometrium of women with endometriosis was compared with that of controls (Bulmer et al., 1998). While providing semi-quantitative information about the leukocyte populations in these tissues, these studies have not provided data about the functional differences of endometrial leukocytes between the two groups. It is possible that although leukocyte numbers were not significantly different, leukocyte subpopulations may differ in their activational status. Activated immune
cells produce cytokines that could be either beneficial or detrimental to the processes of embryo implantation or the disease pathogenesis in the case of endometriosis (Hill, 1992).

The role of natural killer (NK) cells in the decreased cellular immunity of women with endometriosis has also been investigated (Oosterlynck et al., 1991). NK activity (K562-assay) and cytotoxicity against autologous endometrial cells were both decreased in women with endometriosis and this correlated with the severity of the disease. It was concluded that the decreased cytotoxicity to endometrial cells in women with endometriosis was mainly due to a defect in the NK activity, but it was also partially because of a resistance of the eutopic endometrium to NK cytotoxicity.

A number of heat shock proteins are expressed constitutively in mammalian cells. They are also synthesized in response to a variety of physical and chemical stimuli such as heat shock, steroids, and oxidative damage. Studies also suggest that heat shock proteins are involved directly or indirectly with the immune system through antigen processing, antigen presentation, or peptide binding. Certain T cells can recognize heat shock proteins as specific antigens, and heat shock proteins may serve as ligands of T cells. Ota and coworkers (1997) found a significant increase in expression of heat shock protein 27 (hsp 27) in eutopic endometrium from patients with endometriosis and adenomyosis compared with controls regardless of the menstrual cycle stage. The increase in intracellular hsp 27 was implicated in the immune response mediated by macrophages and/or T cells in endometriosis. Furthermore, as Danazol treatment, which inhibits ovarian steroidogenesis resulting in decreased secretion of estradiol, reduces the expression of hsp 27, it was speculated that this protein might participate in the activation process of estrogen. Clinically, eutopic endometrial expression of hsp 27 has been associated with unexplained infertility in association with endometriosis (Nip et al., 1994).
It has also been observed that macrophages residing in the functionalis zone of the endometrium are intensely immunoreactive with anti-human haptoglobin antibody (Sharpe-Timms et al., 2002). They hypothesized that de novo synthesized; extrahepatic, endometrial haptoglobin inhibits macrophage phagocytic function in endometrial functionalis sloughed at menses. The same group has recently (Sharpe-Timms et al., 2010) shown differential production of uniquely glycosylated haptoglobin by eutopic endometrium of women with endometriosis in response to inflammatory cytokines, and suggested that haptoglobin with its immunomodulatory properties (Langlois and Delanghe, 1996; Dobrzychycka, 1997; Sharpe-Timms et al., 2002) may be involved in the pathogenesis and pathophysiology of endometriosis.

1.5.5 Cell adhesion molecules

Integrins are a family of cell adhesion molecules that function in both cell-cell and cell-substratum adhesion (Kim and Yamada, 1997; Morgan et al., 2007; Shattil et al., 2010). They promote cell attachment to proteins within the extracellular matrix (ECM) and potentiate cell migration and invasion. In some cells, integrin attachment to the ECM requires, tyrosine kinase receptor-mediated signaling. It appears that cytokine receptors and adhesion molecules may cooperate functionally to promote cell motility and perhaps invasion (Brooks et al., 1997). Integrins, in particular αvβ3 and αvβ5, have also been shown to form cell surface complexes with matrix metalloproteinases (MMP) to facilitate matrix degradation and motility, thereby facilitating directed cellular invasion (Brooks et al., 1997). Many members of the integrin family are expressed by the endometrium throughout the menstrual cycle. For example, increased expression of β-1 integrins and E-cadherin has been observed on endometrial glandular epithelium of women with endometriosis as compared to controls (Ota and Tanaka, 1997). The expression of αvβ3 by endometrial epithelium is coincident with the period of uterine receptivity (Lessey et al., 1992). Expression of cell adhesion molecules reportedly differs in the eutopic endometrium of women with
endometriosis. The absence of αvβ3 expression on endometrial epithelium of women with endometriosis is notable (Lessey et al., 1994). Others have failed to detect cycle-specific expression of this integrin (Hill and Rogers, 1998); however, these negative findings can be attributed to the technical approaches. Osteen et al. (1996) showed that αvβ3 co-localizes and forms a complex with MMP-2 and that αvβ5 co-localizes and forms complexes with both MMP-2 and MMP-3 on the luminal epithelial surface of eutopic human endometrium. This suggests interactions between endometrial integrins and MMPs that may coordinate cell adhesion, migration, invasion, and targeted proteolytic endometrial remodeling, thereby facilitating embryo implantation. Hence, lack of αvβ3 expression in the eutopic endometrium during the period of uterine receptivity in women with endometriosis may be associated with decreased cycle fecundity due to defects in uterine receptivity. Regidor et al. (1998) investigated the expression pattern of the integrins αv, α2β1, α3β1, α5, α6, β1, β2, and β3 in human ectopic endometrium. They observed that the integrin α6 was expressed in all endometriotic and endometrium samples. Interestingly, they found that integrin α3 was absent in all endometrium tissue samples from endometriosis patients; however, the corresponding endometriotic lesion re-expressed this adhesion molecule. No change was observed in integrin β3 expression pattern in either endometriotic lesion or endometrium samples, regardless of the menstrual cycle phase. They suggested that endometriosis is a dedifferentiated disease as it expressed different integrins in comparison with eutopic endometrium. L1 cell adhesion molecule (L1CAM) expression has also been studied in endometriosis and it was found to be expressed at significantly higher level in the epithelial compartment of patients with endometriosis as compared to the healthy controls (Finas et al., 2008), suggesting that L1CAM could promote endometriosis development by increasing enervation and aggravation.
1.5.6 Proteases and their inhibitors

Aberrant production of matrix metalloproteinases (MMPs) and their inhibitors, the tissue inhibitor of metalloproteinases (TIMPs) have been associated with endometriosis and eutopic endometrium (Osteen et al., 1996; Sharpe-Timms et al., 1995, 2002; Cox et al., 2001; Ruthy Shaco-Levya et al., 2008). The MMPs serve to break down all the components of the extracellular matrix (ECM) and thereby participate in both normal and pathological tissue remodeling. Under normal conditions, MMPs expression is highly regulated by steroid hormones and growth factors (Rodgers et al., 1996). MMPs participate in monthly endometrial remodeling during the menstrual cycle, trophoblast invasion, and decidualization (Murray and Lessey, 1999; Bischof et al., 2000). Expression of several members of the MMP family by endometrium of women with endometriosis is missregulated and may contribute to the establishment of the ectopic endometriotic lesions in the peritoneal cavity (Osteen et al., 1996; Bruner et al., 1997; Cox et al., 2001). As described in section 1.5.5, the MMPs may form complexes with integrin cell adhesion molecules to localize protease activity and facilitate directed cell migration. Tissue inhibitor of metalloproteinase-1 (TIMP-1) serves to control this migration. It has been shown that women with endometriosis have significantly less TIMP-1 in their peritoneal fluid than do women without endometriosis (Sharpe-Timms and Cox, 2002). Since endometriotic lesions and endometrial tissues have been shown to produce TIMP-1 (Sharpe-Timms and Cox, 2002), it remains to be resolved if TIMP-1 production is altered in eutopic endometrium from women with endometriosis.

1.5.7 Steroid production and responsiveness

Various anomalies in steroid production and responsiveness have been reported in eutopic endometrium in women with endometriosis (Bulun, 2009). A variety of molecular and protein techniques have been used to demonstrate that both endometriotic lesions and eutopic endometrium of women with endometriosis contain transcripts for P450 aromatase (Kitawaki et al., 1997; Noble et al., 1997). Aromatase P450 acts in the conversion of C19
steroids to estrogens in various tissues including the ovary and placenta, but not in the normal endometrium from controls. It was concluded that aromatase expression by eutopic endometrium may be related to the capability of these tissues to get implanted on peritoneal surfaces. As the estrogen and progesterone receptor status of eutopic endometrium from women with and without endometriosis does not vary (Nisolle et al., 1994; Jones et al., 1995; Berqvist et al., 1999), the unique ability to produce estrogen in eutopic endometrium of women with endometriosis may serve to selectively promote their growth at ectopic locations. In addition to, or perhaps as a consequence of, immune, environmental, and genetic factors, endometriotic lesions show high estradiol biosynthesis and low estradiol inactivation when compared with endometrium from unaffected women (Zeitoun and Bulun, 1999). Studies by Zeitoun and Bulun (1999) showed abnormally high expression of aromatase in endometriotic lesions and much lower levels in eutopic endometrium of women with endometriosis. This abnormal expression is due to a stimulatory transcription factor that increases aromatase transcription, resulting in conversion of androstenedione to estrone (Zeitoun et al., 1999). The latter is a weak estrogen that is subsequently converted to the more potent estradiol by 17β-hydroxysteroid dehydrogenase 1. Inactivation of estradiol is completed by 17β-hydroxysteroid dehydrogenase 2, normally present in endometrial glandular cells; however, in endometriosis, the glandular cells lack this enzyme, leading to impaired inactivation of estradiol and increased local concentrations of this steroid hormone (Zeitoun et al., 1998; Bulun et al., 2010). In summary, estradiol synthesis is increased while inactivation is decreased resulting in higher local concentrations of the hormone. Estradiol stimulates production of prostaglandins, specifically prostaglandin E2, which further stimulates activity of aromatase (Giudice and Kao, 2004).

In contrast to the clearly unfavorable effect of estrogen on endometriosis, the role of progesterone has remained ambiguous as endometriotic tissue produces large quantities of progesterone and contains much lower levels of progesterone receptors than endometrium (Attia et al., 2000; Sun et al., 2003). Progesterone regulates the levels of estradiol in a
paracrine manner leading to its inactivation. Gene-expression profiles of the endometrium of women with and without endometriosis have shown that a number of genes targeted by progesterone are deregulated during implantation, the time when the endometrium is exposed to the highest levels of progesterone (Kao et al., 2003; Burney et al., 2007; Bulun et al., 2010). These findings suggest that eutopic endometrium of women with endometriosis exhibits progesterone resistance. Progesterone resistance is explicable by the extremely low progesterone-receptor levels in endometriotic tissue (Attia et al., 2000). In endometrium, levels of the progesterone receptor isoforms, PR-A and PR-B progressively increase during the proliferative phase, peak immediately before ovulation, and diminish after ovulation, suggesting that estradiol stimulates progesterone-receptor levels (Attia et al., 2000). In contrast, PR-B is undetectable and PR-A is markedly reduced in endometriotic tissues, leading to progesterone resistance. In combination with high estradiol production due to aberrant aromatase activity, this additional defect contributes to the abnormally high levels of estradiol in endometriotic tissue (Bulun et al., 2009).

1.6 Genetic basis of endometriosis

Endometriosis is commonly regarded as a complex disease, caused by the interplay between genetic and environmental factors. The heritable features of endometriosis were first recognized more than 20 years ago when the risk of getting this disease in first degree relatives of women with severe endometriosis was reported to be six times higher than that in relatives of unaffected women (Simpson et al., 1980). Familial aggregation has been shown in clinical (Simpson et al., 1980; Kennedy et al., 1995; Simpson and Bischoff, 2002) and population-based samples (Stefansson et al., 2002) and in twin studies (Moen, 1994; Hadfield et al., 1997; Treloar et al., 1997; Treloar et al., 2002). The contribution of genetic factors in endometriosis susceptibility is supported by a number of different studies (Kennedy, 1998; Zondervan et al., 2001; Simpson and Bischoff, 2002; Stefansson et al., 2002; Treloar et al., 2002; Vigano` et al., 2003). The International Endogene Study Group is
currently carrying out linkage studies using genome-wide scanning of informative polymorphic microsatellite markers to identify regions of significant sharing in affected siblings (Treloar et al., 2002). By using linkage analysis in affected sibling pairs, various groups have reported candidate genes that have potential biological plausibility in endometriosis (Kennedy et al., 2001; Kennedy, 2003; Bischoff and Simpson, 2004; Falconer et al., 2007; Montgomery et al., 2008). Several of these studies indicate abnormalities in detoxification enzymes, which could lead to susceptibility to environmental stimuli (Babu et al., 2004; Babu et al., 2005; Guo et al., 2006a). Genes associated with malignant transformation like VEGF (Bhanoori et al., 2005; Lamp et al., 2010), eNOS (Bhanoori et al., 2005; Kim et al., 2009), cytokine signalling pathways (Bhanoori et al., 2005; Bhanoori et al., 2007; Riiskjaer et al., 2010), steroid-related genes like estrogen receptor and aromatase (Bianco et al., 2009; Xie et al., 2009), and adhesion molecules and matrix enzymes (Borghese et al., 2008; Han et al., 2009) are also included. Evidences also suggest genetic linkage to chromosomes 7 (Zondervan et al., 2007) and 10 (Treloar et al., 2005a) but the genes (or variants) in these regions contributing to disease risk are yet to be identified.

1.7 Microarray analysis of endometriosis

In pursuit of a global understanding of human embryonic implantation, various groups (Carson et al., 2002; Kao et al., 2002; Borthwick et al., 2003; Kao et al., 2003; Matsuzaki et al., 2004; Burney et al., 2007; Sha et al., 2007; Sherwin et al., 2008) have recently applied array-based experiments to investigate differentially expressed genes and generate an endometrial signature for the window of implantation in normally cycling women without endometriosis. This strategy resulted in the identification of gene families and signaling pathways that are candidates for uterine receptivity, and generation of hypotheses of molecular mechanisms underlying the process of human implantation.
It is well established that ectopic and eutopic endometrium differ in their gene expression profiles (Ryan et al., 2007). For example, ectopic endometrium has high expression of aromatase, which is responsible for E2 production that maintains progression of ectopic lesions by paracrine mechanisms (Noble et al., 1996; Matsuzaki et al., 2004). Various groups have performed global comparative analyses of gene expression of endometrial tissue from women with and without endometriosis. These studies differ mainly in the localization of endometrial tissue analyzed (eutopic or ectopic), microarray platform used, and menstrual cycle phase during which specimens were obtained (Table 1.3).

Kao et al. (2003) analyzed the endometrial transcriptome in women with minimal/mild endometriosis versus without disease during the midsecretory endometrium (MSE) phase, at peak levels of progesterone, and found 91 genes significantly increased and 115 genes significantly decreased (more than two fold) in the endometriosis patients. They identified genes that are likely to have relevance in implantation failure and pathogenesis of the disease including genes for apoptosis, ion transporters, immune functions, secretory proteins, signal transduction, membrane proteins, transcription factors, and others. Furthermore, they found three unique groups of target genes.

One group was up-regulated during the implantation window in women without endometriosis but significantly decreased in those with endometriosis. Group 2 genes were down-regulated during the normal window of implantation but significantly increased in endometrium of women with endometriosis. Group 3 comprised of one gene, neuronal pentraxin II, which was down-regulated during the normal window of implantation and further decreased in endometriosis patients. Dysregulation of several progesterone regulated genes, such as interleukin-15, proline-rich protein, B61, Dickkopf-1, glycodeolin, N-acetylglucosamine-6-O-sulfotransferase, and G0S2 in endometriosis suggests poor response to progesterone (Kao et al., 2003).
Table 1.3 Gene expression studies performed on human endometrial tissue using DNA microarray

<table>
<thead>
<tr>
<th>Endometriosis</th>
<th>Type of Endometrial Tissue</th>
<th>Cycle of Phase</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild/moderate</td>
<td>Eutopic</td>
<td>MSE</td>
<td>Kao et al. (2003)</td>
</tr>
<tr>
<td>I- IV</td>
<td>Eutopic vs ectopic</td>
<td>PE, SE</td>
<td>Matsuzaki et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>(Deep endometriosis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not defined</td>
<td>Eutopic</td>
<td>LPE, ESE</td>
<td>Matsuzaki et al. (2005)</td>
</tr>
<tr>
<td>Epithelial vs Stromal</td>
<td>Eutopic vs ectopic</td>
<td>MSE, LSE</td>
<td>Wu et al. (2006)</td>
</tr>
<tr>
<td>II- IV</td>
<td></td>
<td>PE, SE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eutopic vs ectopic</td>
<td>PE</td>
<td>Mettler et al. (2007)</td>
</tr>
<tr>
<td>I- IV</td>
<td>Eutopic vs ectopic</td>
<td>PE</td>
<td>Eyster et al. (2007)</td>
</tr>
<tr>
<td>Moderate/severe</td>
<td>Eutopic</td>
<td>PE, ESE</td>
<td>Burney et al. (2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MSE</td>
<td></td>
</tr>
<tr>
<td>Minimal/mild moderate/severe</td>
<td>Eutopic</td>
<td>LSE</td>
<td>Sherwin et al. (2008)</td>
</tr>
</tbody>
</table>

PE, proliferative endometrium; SE, secretory endometrium; MSE, midsecretory endometrium; LPE, late proliferative endometrium; ESE, early secretory endometrium; LSE, late secretory endometrium.

Subsequently, global analysis of the endometrial transcriptome across the menstrual cycle revealed that the largest number of statistically significant differentially expressed genes were detected in the early secretory endometrium (ESE) phase in women with moderate/severe endometriosis compared with women without the disease (Burney et al., 2007). A marked downregulation of several progesterone regulated genes (MIG6, FOXO1A,
metallothioneins, glycodelin, IL1R1, stanniocalcin 1) in ESE and midsecretory endometrium (MSE) was observed in women with the disease. Moreover, ESE from women with endometriosis clustered closer to proliferative endometrium (PE) phase, as assessed by principal component analysis, and maintained a fingerprint of cellular mitosis and proliferation commonly observed in PE. The data suggested resistance to progesterone-mediated inhibition of estradiol-induced cellular mitosis. Also, the results demonstrated aberration in expression of progesterone regulated genes, unrelated to the cell cycle and important in cellular differentiation (Burney et al. 2007). Interestingly, late secretory endometrium (LSE) phase is not informative in women with versus without endometriosis (Sherwin et al. 2008). This study demonstrated that the expression of only a relatively small number of genes (eight genes were identified as up-regulated and one gene was down-regulated in all endometriotic samples) differ in late secretory phase endometrium between patients with endometriosis and controls. They have also shown that the median fold changes of these genes are small. No transcripts were identified that could discriminate between minimal/mild and moderate/severe endometriosis. Based on these results, the authors suggested that interrogation of the late secretory endometrial transcriptome is not likely to form the basis of a minimally invasive diagnostic test for endometriosis.

1.8 Proteomic analysis of endometriosis

To date, the quest to have a better understanding of endometriosis and to develop a noninvasive diagnostic test for endometriosis has mostly concentrated on the levels of cytokines and growth factors present in serum, peritoneal fluid, endometrium and endometriotic lesions that are involved in inflammation, angiogenesis, and tissue remodeling (Girling and Rogers, 2005; Khan et al., 2008; Nezhat et al., 2008; Tesone et al., 2008; Omwandho et al., 2010). These approaches were limited with respect to the identification of only a few proteins. However, the advent of proteomics has led to the identification of several proteins that are characteristic of the different phases of the
menstrual cycle in endometriosis (DeSouza et al., 2005; Chen et al., 2009) and some of the identified candidate proteins have been implicated in uterine receptivity and oocyte implantation (Kao et al., 2002; DeSouza et al., 2005; Talbi et al., 2006; Chen et al., 2009). As proteomics allows the comprehensive analysis of complex fluid and tissue samples with good sensitivity and resolution, it is a promising approach in the identification of new players that have potential roles in the initiation and progression of endometriosis and also sets a framework for further investigations on mechanisms underlying the pathogenesis of endometriosis as well as in delivering markers associated with endometriosis. Once identified, the challenge lies in determining the functions of these proteins with respect to endometriosis, as the pathophysiology of this disease is unknown and likely to be complex and multifactorial. Also, with variation between individuals and the influences of steroid hormones during the menstrual cycle, it could be difficult to validate findings relating to a single protein or small groups of proteins differentially expressed in the disease state. Proteomic profiling, using mass spectrometry in combination with sophisticated bioinformatics softwares to identify protein patterns, may provide new insights into endometrial developmental defects that could be responsible for endometriosis.

1.8.1 Proteomic analysis of eutopic endometrium in endometriosis

Earlier studies on differential protein expression in endometriosis using proteomics approach have given us clues about the aberration in the protein expression profiling of eutopic endometrium from women with endometriosis (Zhang et al., 2006; Have et al., 2007; Fowler et al., 2007; Stephens et al., 2010). As on date, only four studies have been carried out to analyze the protein expression in the eutopic endometrium of women with endometriosis using 2D-PAGE and mass spectrometry, either in secretory phase (Zhang et al., 2006; Have et al., 2007; Stephens et al., 2010) or both in secretory and proliferative phases of the menstrual cycle (Fowler et al., 2007).
1.8.1.1 Proteomic analysis using secretory phase eutopic endometrium

Zhang et al. (2006) have compared the protein expression maps of eutopic endometrium and sera of women with stage II, III, and IV endometriosis in secretory phase with controls. They found that 13 protein spots from the serum correlated with 11 known proteins and 11 protein spots from the endometrium correlated with 11 known proteins, to be differentially expressed between women with and without endometriosis. Some of the matched proteins with differential expression were cytoskeletal proteins and others were regulatory proteins of the cell cycle, signal transduction, or were of immunological function. These proteins include the G antigen family B₁ protein, actin-related protein 6, actin-like-7-α, anhydrase I, Dentin matrix acidic phosphoprotein I, CD166 antigen, cyclin A₁, and 14-3-3 protein.

Further, Have et al. (2007) studied the differential protein expression profile of the eutopic endometrium of women without endometriosis (n = 12) and with a confirmed diagnosis of endometriosis (n = 6) in the secretory phase of the cycle. They found a total of 119 proteins to be differentially regulated between endometriotic and control tissue (matched and unmatched spots). Of the 50 highest fold change proteins, 21 proteins were found only in the endometriosis affected sample group. Protein sub-categorisation depending on protein function revealed several proteins including those involved in apoptosis, immune reaction, glycolytic pathway, cell structure, and transcription were either up- or down- regulated.

A recent report by Stephen et al. (2010) suggested considerable post-translational modification of proteins to be a key factor in the pathology of endometriosis. They used 2D-differential in gel electrophoresis (DIGE) and mass spectrometry to identify proteins with altered abundance in the eutopic endometrium of endometriosis patients in the mid-secretory phase of the menstrual cycle. They identified 20 differentially expressed proteins which include vimentin, peroxiredoxin 6 (PRDX6), ribonuclease/angiogenin inhibitor 1 (RNH 1), coronin 1A, and transgelin. In addition, they also reported identification of multiple charge and size isoforms of PRDX6 and vimentin.
1.8.1.2 Proteomic analysis using both secretory and proliferative phase eutopic endometrium

Fowler et al. (2007) reported the effects of endometriosis (stage II) on the proteome of human eutopic endometrium both in the secretory and the proliferative phase of the endometrium. This group reported the identification of 9 of the most abundant and consistently altered spots which include structural proteins like vimentin and β-actin; molecular chaperones including heat shock protein 90 and annexin A2; proteins involved in cellular redox state such as peroxiredoxin 2; proteins involved in protein and DNA formation/breakdown, including ribonucleoside-diphosphate reductase, prohibitin and prollyl 4-hydroxylase; and secretory proteins such as apolipoprotein A1 and glycodelin.

They also analysed endometriosis-related differences in eutopic endometrial protein expression profiles between proliferative and secretory phases. They observed that the number of spots showing altered volumes between the proliferative and secretory phases were similar in women with and without endometriosis. Six protein spots that showed marked endometriosis-related differences in the pattern of changes between the proliferative and secretory phases were positively identified by MALDI MS and include Peroxiredoxin-2, Nucleoside diphosphate kinase A (NDK A), Cystatin E, Ribonucleoside-diphosphate reductase M1 chain (RIR1), Hemoglobin β-chain, and isoforms of actin.

1.8.2 Proteomic analysis of peritoneal fluid from women with endometriosis

Studies have been carried out to analyze proteome profiles of peritoneal fluid (PF) from women with and without endometriosis as evidence suggests that endometriosis modulates the microenvironment of peritoneal cavity. Tabibzadeh et al. (2003) compared 2D-PAGE of PF of women with and without endometriosis. They showed that mild endometriosis was associated with a mild reduction in the amount of several peritoneal protein spots with approximate molecular weights of 35-40 kDa and pI close to 5.7-6.0.
These changes became markedly apparent in the peritoneal fluid of patients who suffered from the severe form of this disease. Severe endometriosis was also associated with appearance of protein spots in the gels that were not detectable in the peritoneal fluids of normal subjects. However, the identity of majority of the protein spots with abnormal expression in endometriosis could not be determined by either immunoblotting or mass spectrometry. However, they were able to show, using enzyme-linked immunosorbent assay, that moderate to severe endometriosis was associated with markedly elevated levels of IL-10 in the peritoneal fluid. This study was followed up by Ferrero et al. (2005) who demonstrated that the PF of women with endometriosis, when compared with that of control subjects, contains higher levels of a particular isoform of Hp-β chain and lower levels of an isoform of vitamin D binding protein. They further carried out studies to search for other specific proteins present in the PF of women with endometriosis using 2D-PAGE combined with liquid chromatography tandem mass spectrometry (Ferrero et al., 2007). They identified eleven protein spots that show significantly higher expression in PF of women with endometriosis when compared with controls. These spots were identified as four isoforms of α2-Heremans Schmidt-glycoprotein, three isoforms of α1-antitrypsin, one isoform of protein S100-A8, one isoform of apolipoprotein A-1, one immunoglobulin (Ig) kappa chain C region, and one serum albumin precursor. Two protein spots had significantly lower expression in PF of women with endometriosis than in PF of controls; they were identified as α-1b-glycoprotein and pantophysin. They also showed that isoforms of α1-antitrypsin and S100-A8 that were upregulated in PF of women with endometriosis had significantly higher levels in subject with severe disease than in those with mild disease. The isoform of α-1b-glycoprotein with decreased expression in PF of women with endometriosis was significantly less expressed in women with severe endometriosis than in those with mild endometriosis. They further compared the expression of protein spots between women with different types of endometriotic lesions: superficial lesions, deep nodules, and endometriotic cysts and observed that S100-A8 spot expression was significantly higher in women with deep endometriosis than in those with superficial lesions. However, protein expression in PF did
not significantly change during the menstrual cycle both in women with endometriosis and in control subjects. Later, the same group compared the expression of PF proteins in women with different severity of endometriosis (Ferrero et al., 2008). Their results demonstrated that isoforms of α1-antitrypsin, S100-A8, transferrin, and α-1b-Glycoprotein have significantly higher expression in women with ASRM stage III–IV endometriosis than in patients with ASRM stage I–II disease whereas the expression of one protein, haptoglobin α chain had significantly higher levels in PF in women with mild–moderate endometriosis than in those with severe and extensive disease. Recently, studies on PF proteome of women with different stages of endometriosis were compared with normal women (Ferrero et al., 2008). This study showed that several protein isoforms have different expression in PF of women with ASRM stage I–II endometriosis than in those with ASRM stage III–IV disease; most of these molecules are involved in inflammation and immune response. One isoform of haptoglobin α chain, α-1b-glycoprotein and one unknown protein had higher expression in PF of women with ASRM stage I–II endometriosis. Four isoforms of α1-antitrypsin, three isoforms of α-1b-glycoprotein, one isoform of S100-A8 and sero-transferrin had higher expression in PF of women with ASRM stage III–IV disease.

1.9 Objectives

Aberration in endometrial physiology would lead to diseases like endometriosis, endometrial polyps, hyperplasia, and endometrial cancer. Therefore, there is a need to understand the molecular basis of function of the endometrium per se. Further, the identification of molecular differences in the endometrium of women with endometriosis is an important step towards understanding the pathogenesis of this condition and for developing novel strategies for the treatment of associated infertility and pain. It also sets a framework for further investigations on mechanisms underlying the pathogenesis of endometriosis.
Thus, this thesis is designed to identify the proteins that are differentially expressed during different stages of endometriosis and to establish the role of one of the differentially expressed proteins in endometriosis.

With this in view, the objectives of the thesis were:

- To establish the proteome of normal human endometrium and to study the differential protein expression in proliferative and secretory phase endometrium.
- To study differential expression of proteins in eutopic endometrium from women with and without endometriosis using proteomic approach.
- To study the functional relevance of the differentially expressed proteins in endometriosis.

2.0 References

All references are listed at the end of the thesis.