Introduction

During the reproductive years, the endometrium of the corpus Proper undergoes regular cyclic changes as a response to the release of the ovarian hormones, estrogen and progesterone.

The endometrium consists of simple tubular glands set in a cellular vascular stroma. It is composed of a thin basal layer (basalis), which abuts on the myometrium, and a functional layer on top of the basalis.

The functional layer is highly responsive to hormonal ovarian influence in contrast to the basalis. The functional endometrium consists of a superficial layer with few glands and abundant stroma (the compacta), and a deep layer that has many glands and relatively less stroma (the spongiosa) **(Ferenczy et al., 1991)**.

The structure and activity of a functional endometrium reflect the pattern of ovarian hormone secretion. The histological types of glandular cells are columnar or cuboid. The endometrium undergoes regular growth and maturation and when the cycle ends, in the absence of pregnancy, shedding occurs followed by regeneration. The average duration of the cycle is 28 days. In a normal cycle the postovulatory phase lasts 14 days. Changes in the length of

the cycle are usually due to the duration of the proliferative phase, which can vary from 8 to 21 days **(Mote et al., 2000)**

The endometrial glands are simple tubular glands lined by columnar epithelium or tall, narrow and closely packed cells with elongated and parallel nuclei.

The morphology of the endometrial glands changes during the different phases of the menstrual cycle. *The endometrial stroma* consists of pluripotential mesenchymal cells, which at the beginning of the menstrual cycle are spindle-shaped, poorly- differentiated and joined to one another by cytoplasmic processes. The cells lie firmly anchored within a network of reticulum fibers **(Rogers, 1996)**.

Protein gene product 9.5 **(**PGP9.5**)** is a soluble 27 kDa protein corresponding to an ubiquitin COOH-terminal hydrolase that plays a modulating role in intracellular proteolysis**(Wilkinson et al., 1989).**

Intracellular protein degradation is a tightly regulated process maintaining the normal cellular homeostasis. Multiple system sexist for proteolysis, and the best-described is the highly conserved ubiquitin–proteasome one **(Pickart. 2004, Hershko et al., 2000, Duncan 2002)**.

This complexes a non-lysosomal proteolytic pathway that degrades diverse cellular proteins in the regulation of cell growth, in the modulation of some membrane receptors, in response to heat shock, and in the turnover of cytoskeletal elements. It comprises the enzymes that ubiquitinate/de-ubiquitinate target proteins and the26Sproteasome complex that degrades ubiquitin-conjugated proteins**(Itoh. 2003)**.

Ubiquitination represents the basic cellular process and consists of the covalent attachment of multiple ubiquitin molecules to the protein substrate. This is a reversible step controlled at several levels. One level of regulation involves the large family of de-ubiquitinating enzymes (DUBs) **(Hershko et al., 2000)**.

Standard immunohistochemical techniques have demonstrated the presence of PGP9.5 in neurons and nerve fibers at all levels of the central and peripheral nervous system, in many neuroendocrine cells, in segments of the renal tubules, in spermatogonia and Leydig cells of the testis, in ova and in some cells of both the pregnant and non-pregnant corpus luteum**(Wilson et al., 1988)**.

The concept of using endometrial biopsy in screening for endometriosis was possible after reports of the novel finding of multiple small unmeylinated sensory C-nerve fibers in the functional layer of eutopic endometrium in all

women with endometriosis; although women without endometriosis did not have any nerve fibers in the functional layer **(Tokushige et al., 2006 a, b).** These nerve fibers were also found in the ectopic peritoneal endometriotic lesions and in the deep infiltrating endometriosis **(Wang et al., 2009)** and expressed a wide range of neuronal markers **(Tokushige et al., 2006b).**

The results of the pilot study **(Al- Jefout et al., 2007)** showed that it is possible to make the diagnosis of endometriosis using endometrial biopsy that was verified later on by a double blind study with a large number of patients and the involvement of more surgeons. Results of the new study indicated that a negative endometrial biopsy result would miss endometriosis in only 4 % of women. Performing planned laparoscopy only on a woman with a positive endometrial biopsy would result in endometriosis being confirmed in 80-90% of cases **(Al-Jefout et al., 2009).**

Another study done on patients with endometriosis, adenomyosis and uterine fibroids showed the presence of PGP9.5-immunoactive nerve fibers in the functional layer of endometrium in patients with pain whatever the pathology is. But the study design was retrospective with the sample size needing to be larger **(Zhang et al., 2009)**.

Aim of the work

- **PRIMARY OBJECTIVE:**

To determine the prevalence endometrial nerve fibers in patients with different gynecological pathologies

- **SECONDARY OBJECTIVE:**

To determine the value of endometrial nerve fibers density in relation to the type and degree of pelvic pain if present.

Endometriosis

Introduction

Endometriosis is a benign gynecologic disorder defined as the presence of endometrial glands and stroma outside of the normal location with these ectopic lesions commonly being discovered during the 19th century. **(Burney et al., 2012)**. It is commonly found on the pelvic peritoneum but may also be found on the ovaries, rectovaginal septum and ureter, and rarely in the bladder, pericardium, and pleura.**(Guidice, 2004).**

It is a hormonally dependent disease and as a result is chiefly in reproductive-aged women. Women with endometriosis may be asymptomatic, sub fertile, or suffer varying degrees of pelvic pain **(Comiter et al., 2002)**

The clinical presentation of endometriosis is varied and conclusive diagnosis requires laparoscopy **(Sharpe-Timms K., 2005)**

Epidemiology:

The exact prevalence of endometriosis is difficult to quantify, as women with the disease are often asymptomatic and imaging modalities have low sensitivities for diagnosis.**(Marchino et al., 2005b)**. The primary tool for

diagnosis is laparoscopy with or without biopsy for histologic diagnosis. **(Kennedy et al., 2005).** Using this standard, investigators have estimated the annual incidence of surgically diagnosed endometriosis to be 1.6 cases per 1000 women aged between 15 and 49**(Giudice, 2010).**In asymptomatic women the prevalence of endometriosis ranges form 2-22% depending on the population studied **(Eskenazi, 1997; Mahmood, 1991; Moen, 2004)**. Because of its link with infertility and pelvic pain, endometriosis is notably more prevalent in subpopulation of patients with these complaints being between 20-50% in infertile population and between 40-50% in those with pelvic pain **(Adamson, 2011).**

Risk factors

Familial clustering

A genetic basis for the development of endometriosis is suggested by the reports of familial aggregation, the high risk of endometriosis in those with an affected first-degree relative and the observations of concordance of endometriosis in twins (**Eskenazi et al., 1997).** For example, in a genetic study for women with endometriosis, Simpson and colleagues noted that 6% of Female sibling and 8 % of the mothers of the affected women had endometriosis Compared with 1% of the husband's female first degree relatives **(Simpson et al., 1980)**. Further research has revealed those women with endometriosis and an affected

first degree relative were more likely to have endometriosis (61%) compared to women without affected first degree relative (24%).**(Stefansson et al., 2002)**

Genetic mutations and polymorphisms:

The rate of familial clustering just noted suggests polygenic inheritance, and several candidate genes have been investigated with The largest study to date, examining more than 1000 affected sister- pair families, has identified a region on chromosome 10q26 that demonstrates significant linkage in these sisters affected by endometriosis **(Treloar et al., 2005).** This study also revealed a smaller linkage of chromosome 20p13 with two candidate genes within or near this locus have been identified to be the offenders and at the same time necessary for reproductive tract development, apparently expressed in the endometrium of women with endometriosis **(Daftary et al., 2004)**. The 2nd gene is PTEN a tumor suppressor gene implicated in the malignant transformation of ovarian endometriosis **(Bischoff et al., 2000)**.

Genetic factors may partially explain the susceptibility of certain individuals to develop endometriosis and Genetic aberrations may also give some answers as to the reason of malignant transformation of ovarian endometriosis to

endometriotic adenocarcinoma of the ovary **(Markham et al., 2002).**

Environmental toxins

Numerous studies have suggested that exposure to environmental toxins may play a role in the development of endometriosis with the toxins most commonly implicated are tetrachloro di benzo dioxin (TCDD) and other dioxin like compounds that bind to steroid hormone receptor family leading to gene transcription that is translated to different proteins responsible for tissue remodeling**(Rier et al., 2008)**

Pathogenesis and etiology

Retrograde Menstruation

The earliest and most widely accepted theory relates to retrograde menstruation through the fallopian tubes with subsequent dissemination of endometrial tissue within the peritoneal cavity **(Sampson, 1927)**. Refluxed endometrial fragments adhere to and invade the peritoneal mesothelium and develop a blood supply, which leads to continued implant survival and growth **(Guidice et al., 2004)**.

First proposed in the 1920s, this theory has gained support with the findings of greater volumes of refluxed blood and endometrial tissue in the pelves of women with

endometriosis **(Halme et al.,1984).**Uterine hyper peristalsis and dysperistalsis have been noted in women with endometriosis and resulted in subsequent increased endometrial reflux **(Leyendecker et al., 2004).**Women with amenorrhea due to outflow tract obstruction similarly have a high incidence of endometriosis, which is often relieved by correction of the obstruction **(Sanfilippo et al., 1986).**However, retrograde menstruation occurs in 76%–90% of women with patent fallopian tubes and not all of these women have endometriosis **(Dehoux et al., 2011)**

Lymphatic or Vascular Spread

Evidence also supports the concept of endometriosis originating from aberrant lymphatic or vascular spread of endometrial tissue **(Ueki et al., 1991)**. Findings of endometriosis in unusual locations, such as the perineum or groin, bolster this theory **(Mitchell et al., 1991)**. The retroperitoneal region has abundant lymphatic circulation explaining the cases in which no peritoneal implants are found, but solely isolated retroperitoneal lesions are noted **(Moore et al., 1988)**. Additionally, the tendency of endometrial adenocarcinoma to spread via the lymphatic route indicates the ease at which endometrium can be transported by this route **(McMeekin et al., 2003).**

Coelomic Metaplasia

The theory of Coelomic metaplasia suggests that the parietal peritoneum is a pluripotential tissue that can undergo metaplastic transformation to tissue histologically indistinguishable from normal endometrium **(Figueira et al., 2011)**. Because the ovary and the progenitor of the endometrium, the Müllerian ducts, are both derived from Coelomic epithelium, metaplasia may explain the development of ovarian endometriosis **(Healy et al., 1996).** In addition, the theory has been extended to include the visceral peritoneum because of the proliferative and differentiation potential of the peritoneal mesothelium that explained the pathogenesis of endometriosis in the absence of menstruation, such as in premenarchal and postmenopausal women and in males treated with estrogen and orchiectomy for prostatic carcinoma. However, the absence of endometriosis in other tissues derived from Coelomic epithelium argues against this theory**(Houston et al., 2007).**

Induction Theory

Finally, the induction theory proposes that some hormonal or biologic factor(s) may induce the differentiation of undifferentiated cells into endometrial tissue **(Vinatier et al., 2001)**. These substances maybe exogenous or released directly from the endometrium. In vitro studies have

demonstrated the potential for ovarian surface epithelium, in response to estrogens, to undergo transformation to form endometriotic lesions **(Bontis et al., 1997)**.

Role of the Immune System

Menstrual tissue and endometrium that is refluxed into the peritoneal cavity is usually cleared by immune cells such as macrophages, natural killer (NK) cells, and lymphocytes, for this reason, immune system dysfunction is one likely mechanism for the genesis of endometriosis in the presence of retrograde menstruation **(Sikora et al.,2011)**. Impaired cellular and humoral immunity and altered growth factor and cytokine signaling have each been implicated in the pathogenesis of endometriotic foci implantation with the Macrophages acting as scavenger cells in various tissues and increased numbers have been found in the peritoneal cavity of women with endometriosis **(Osuga et al., 2011)**. Although this increased population might logically act to suppress endometrial proliferation, macrophages in these women have a stimulatory effect on endometriotic tissue **(Christodoulakos et al., 2007)**. In one study, circulating monocytes obtained from women with endometriosis enhanced the in vitro proliferation of cultured endometrial cells, whereas the monocytes from women without endometriosis had the opposite effect **(Braun, 1994).**

Natural killer cells are immune cells that have cytotoxic activity against foreign cells and despite the number of NK cells is unaltered in the peritoneal fluid of women with endometriosis, decreased NK cell cytotoxicity against endometrium has been demonstrated, specifically the peritoneal fluid from women with endometriosis has been found to suppress NK cell activity, suggesting that soluble factors may play a role in NK cell suppression **(Oosterlynck et al., 1993).**

Cellular immunity may also be disordered in women with endometriosis, and T lymphocytes are implicated in such process, such as, in women with endometriosis compared with unaffected women, total lymphocyte numbers or helper/suppressor subpopulation ratios do not differ in peripheral blood, but peritoneal fluid lymphocyte numbers are increased**(Kao et al., 2003)**. Also, the cytotoxic activity of T lymphocytes against autologous endometrium in affected women is impaired **(Lessey et al., 1998)**.

Humoral immunity has also been shown to be altered in affected women and is suggested to play a role in the development of endometriosis via endometrial antibodies of the IgG class that are more frequently detected in the serum of women with endometriosis **(Odukoya et al., 1995)**. One study also identified IgG and IgA auto antibodies against endometrial and ovarian tissues in the sera and in cervical

and vaginal secretions of affected women **(Ulukus et al., 2005)**. These results suggest that endometriosis may be, in part, an autoimmune disease. This may explain some of the factors influencing lower pregnancy and in vitro fertilization (IVF) implantation rates in women with endometriosis **(Dmowski et al., 1995)**.

Cytokines are small, soluble immune factors involved in paracrine and autocrine signaling of other immune cells with interleukins specifically implicated in the pathogenesis of endometriosis **(Marcoux et al., 1997)**. Increased levels of interleukin-1 have been identified in the endometrial fluid of those with endometriosis **(Mori et al., 1991)**.

MoreoverIL-6 has been shown to be increased in endometrial stromal cells of affected women **(Tseng et al., 1996).**

Accordingly, IL-6 serum levels greater than 2 pg./mL and tumor necrosis factor peritoneal fluid levels more than 15 pg./mL may be used to discriminate between those with or without endometriosis **(Bedaiwy et al., 2002).**

Interleukin 8 (IL-8) is a chemoattractant and activating factor for human neutrophils and a potent angiogenic agent with its concentration in peritoneal fluid is significantly higher in patients with endometriosis according to the stage of the disease compared to the control**(Treloar et al., 2005)**.

However potential source of IL-8 in peritoneal fluid are not only the macrophages but also mesothelial cells of the peritoneum and endometrium itself as evidenced by finding that cultured mesothelial cells constitutively express IL-8 mRNA and secrete IL-8 protein, and the expression of IL-8 from mesothelial cells is modulated by other cytokines such as IL-1, TNFa**(Laschke et al ., 2011)** . These latter cytokines appear to play some role in the constitutive secretion of IL-8 as well as being capable of greatly stimulating further production and secretion **(Arici et al: 1996)**

Non classical theories in the pathogenesis of endometriosis

Angiogenesis

Endometrial tissue develops its own vascular supply and becomes an independent, growing mass and mimics the spread of neoplasm with a piece of implanted tissue may subsequently break off from the primary site and travel elsewhere in the peritoneal cavity, setting up a peritoneal location or may enter a blood or lymph vessel and disseminate to distant body sites. As the free floating pieces of endometrial tissue themselves implant, grow, and develop their own blood supply, the process repeats itself (**Healy et al., 1996).**