**Session 2:**
**Pathogenesis and Progression of Endometriosis 2**

Gynecologic and Obstetric Investigation

Gynecol Obstet Invest 1999;47(suppl 1):23–33

# Pathogenesis of Endometriosis: The Role of Peritoneal Fluid

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**Key Words**
Endometriosis · Peritoneal fluid · Endocrine · Microenvironment

**Abstract**
Peritoneal fluid is a specific microenvironment. It originates mainly as an ovarian exudation product due to increased vascular permeability, and contains large amounts of macrophages and their secretion products, which include growth factors, cytokines and angiogenic factors. Similarly, within the ovary, there is also a specific microenvironment, best characterised by the follicular milieu with steroid hormone concentrations that are 1,000 times higher than in plasma. Since endometrial cells in peritoneal fluid and superficially implanted cells will be influenced by peritoneal fluid concentrations, any differences found between minimal endometriosis and eutopic endometrium could be the consequence of their different local environments, rather than inherent cellular differences. Superficial endometrial implants may be regulated by factors in peritoneal fluid, while deep endometriosis and cystic ovarian endometriosis may be under the influence of factors in blood and within the ovary, in which case minimal endometriosis may be physiologic only, while deep endometriosis and cystic ovarian endometriosis, both associated with pelvic pain and infertility, may be a pathological state. In conclusion, the local endocrine environment, that is, in blood, peritoneal fluid, and within the ovary, should be taken into account to explain the differences between superficial, deep and cystic ovarian endometriosis, together with inherent differences in the endometrial cells of women with and without endometriosis.

Adapted from the presentation given at the First Japan Conference on Endometriosis and extended from [1].

## Introduction

Endometriosis, defined as the presence of endometrial glands and stroma outside the uterine cavity, is observed in 5–40% of 'normal' women and in up to 60–80% of women with pelvic pain and/or infertility [2]. Its clinical manifestation is heterogeneous, ranging from a few subtle or typical spots to severe forms, such as deeply infiltrating endometriosis, with pelvic pain as the predominant symptom [2, 3] and cystic ovarian endometriosis, which is associated with extensive intrapelvic adhesions, pain, and infertility.

The widely accepted theories to explain the pathophysiology of endometriosis are the Sampson and the metaplasia theory. According to the Sampson theory, viable endometrial cells, shed in the peritoneal cavity by retrograde menstruation, may attach, implant and grow. This hypothesis is attractive since the mechanisms involved have been supported by data [for review, 1, 4]. Retrograde menstruation is a frequent phenomenon in most women

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[5–9] and peritoneal fluid contains viable endometrial cells [10, 11] that can attach to the peritoneum [12, 13]. Also the metaplasia hypothesis has been proven experimentally, since it can be induced with menstrual debris. The metaplasia theory is necessary to explain some occurrences of endometriosis, as endometriosis in the absence of menstruation [14, 15], while it is attractive to attribute a role to the secondary müllerian system [16]. It should be realised, however, that both theories, the implantation and the metaplasia theory, can be viewed to a large extent as complementary or similar, since it is not very important to understand growth and development of endometriosis to know whether it started as metaplasia or as implantation.

Neither theory explains the growth and development of endometriosis. This is assumed, and is based upon indirect observations such as disruption of the basal membrane [17]. These theories do not explain why implantation and endometriosis do not occur in most women, considering that retrograde menstruation occurs in almost all women. They do not explain why severe endometriosis, that is, deep and ovarian endometriosis or endometriotic disease, does not develop in all women with subtle and/or minimal endometriosis. They also do not explain why some women develop cystic ovarian and other deep endometriosis, nor why deep lesions can present as infiltration, or retraction or adenomyosis externa [18]. To explain the growth and development of endometrial cells to severe endometriosis, three concepts were explored: first, cellular differences between endometriosis and endometrium; second, immunologic deficiencies in women with endometriosis; and third, differences in the endocrine environment of the endometrial cells, that is, the bloodstream and the peritoneal fluid. Since the late seventies, over 600 articles have investigated in peritoneal fluid those factors, which might explain the growth of endometrial cells and their development into endometriotic disease [for review, 19]. We want to review critically whether these data improve our understanding of the aetiology, pathophysiology, and natural history of endometriosis and endometriotic disease.

## Peritoneal Fluid: The Intraovarian Milieu as a Specific Microenvironment

*Peritoneal Fluid*

Peritoneal fluid volume is highly variable, ranging between 5 and 200 ml. It can be considered as an ovarian exudate [20]. This exudation is caused by the locally increased vascular permeability around the developing follicle and the corpus luteum, probably a consequence of the high local estrogen concentrations. This mechanism explains that the volume of peritoneal fluid increases progressively during the follicular phase of the menstrual cycle and decreases thereafter, that the volume is increased during ovarian hyperstimulation, and that the volume is low in men and in women without ovarian activity, that is, those with ovariectomy, postmenopausal status, or those taking oral contraceptives [20]. Since endometriosis induces a sterile low-grade inflammation of the peritoneal cavity, a higher volume in peritoneal fluid was anticipated. It remains unclear, however [for review, 21], whether peritoneal fluid volume is similar [22] or slightly higher in women with endometriosis [23]. It is clear that differences in peritoneal fluid volume will not explain the pathophysiology of endometriosis.

Ovarian steroid hormone concentrations in peritoneal fluid are always higher in peritoneal fluid than in plasma, since the ovarian exudate occurs mainly from the developing follicle and the corpus luteum. From the early follicular phase onwards, free 17β-estradiol concentrations in peritoneal fluid are slightly higher than in plasma. Indeed, the total concentration is comparable to the plasma concentration, but the sex hormone binding globulin concentration is only 67% of the plasma concentration. The concentration differences with plasma increase progressively, and when the content of the follicle is released into the peritoneal fluid after ovulation, the 17β-estradiol concentrations increase abruptly, reaching concentrations 100 times higher than in plasma [24, 25–28]. During the luteal phase the 17β-estradiol concentrations decrease progressively, but remain always higher than the plasma concentrations. For progesterone concentrations, a similar picture can be drawn. The concentrations in peritoneal fluid also are much higher, being 5 and 10 ng/ml in the follicular phase, which is comparable to luteal phase concentrations in plasma. The concentrations increase abruptly after ovulation, reaching 2,000 ng/ml in some women, and decrease slowly thereafter.

To emphasise this phenomenon of ovarian exudation, it has been calculated that during the early luteal phase, little progesterone is secreted directly into the bloodstream. Until the corpus luteum is vascularized, a process that takes days to be completed, an exudate containing progesterone leaks from the corpus luteum and is subsequently reabsorbed from the peritoneal cavity. Also, the slightly higher peritoneal fluid protein concentration in the late follicular and early luteal phases in comparison with the follicular phase [24] has been considered as an



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indirect argument for an estrogen-driven vascular permeability as the mechanism of peritoneal fluid formation.

The total surface area of the peritoneal cavity is estimated to be larger than 2 $m^2$ [for review, 29]. This large area permits a quantitatively important passive dialysis of substances between peritoneal fluid and the bloodstream, a phenomenon that is clinically used in peritoneal dialysis. Small molecules, such as urea and electrolytes, diffuse rapidly. Diffusion rates decrease with the molecular weight and become slow for molecules larger than 100,000 as molecular weight [30]. From this it can be anticipated that the concentrations of small molecules in peritoneal fluid and plasma are similar, whereas the concentrations of larger molecules from the blood stream are lower in peritoneal fluid. Prolactin (molecular weight 20,000) and albumin (molecular weight 60,000) concentrations are 67% of those in plasma whereas the concentration of clotting factors (molecular weight larger than 100,000), is less than 30% of the plasma concentrations [24, 31].

Peritoneal fluid also contains cellular elements such as monocytes (mainly macrophages), red blood cells, and endometrial cells, which secrete a range of products as glycodelins (formerly called PP14) [32] and a 60-kD heat shock protein [33]. The peritoneum secretes substances as CA125 [32], and macrophages secrete a range of products as cytokines, growth factors, and angiogenic factors. Since they are secreted locally, these substances are all present in supraphysiologic concentrations, especially those with larger molecular weight.

Peritoneal fluid thus is a specific microenvironment, with concentrations and time courses during the cycle which are different from plasma. Moreover, peritoneal fluid is not circulating rapidly as plasma, but can circulate slowly or not at all. It also can be considered to be compartmentalised, that is, with local concentrations that can be very different. Peritoneal fluid compartmentalisation is well recognised in processes such as postoperative adhesion formation, abscess formation, and peritonitis. Similarly, local concentrations around endometriotic implants, especially when accompanied with adhesions, might be very different. The compartmentalisation concept was used to postulate that ovarian secretion products directly influence the ipsilateral oviduct, since they are drained locally into the peritoneal fluid (fig. 1).

Peritoneal fluid circulates slowly around the abdominal cavity [for review, 29], but this has not been investigated in relation to reproductive biology and endometriosis. Fertilisations have been observed in women with an oviduct on one side, and an ovary on the other side, but we still do not know whether aspiration of the oocyte from the peritoneal fluid is the rule rather than an occasional mechanism. The pregnancy rates after intraperitoneal insemination suggest, however, that this mechanism and peritoneal fluid circulation might be important [34]. This mechanism could, however, explain the effect of intraperitoneal adhesions upon fertility.

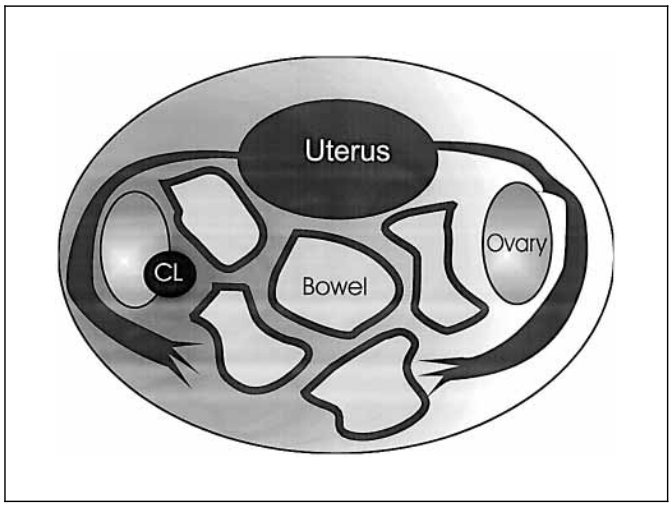


**Fig. 1.** Steroid hormone concentration gradient in peritoneal fluid, which is formed mainly as an ovarian exudate from the growing follicle and/or corpus luteum (reprinted with permission of Human Reproduction).

*The Intra-Ovarian Milieu*

If the concentrations of steroid hormones and other substances in follicular fluid reflect [for review, 35, 36–41] the intra-ovarian concentrations, these are much higher than both the plasma and the peritoneal fluid concentrations. Specific effects of these very high concentrations, such as direct membrane effects of estrogens and immunosuppressive effects of progesterone, could be important to understand why cystic ovarian endometriosis develops almost exclusively in the ovary.

## Physiopathology of Endometriosis

*Peritoneal Fluid in Endometriosis* [for review, 19, 42]

Endometriosis is associated with the luteinized unruptured follicle (LUF) syndrome and with a sterile low-grade inflammatory reaction in the peritoneal cavity as judged by an increased amount of activated macrophages and their secretion products.



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The association between the LUF syndrome and endometriosis was described in women [27, 28, 43–45] and primates [46]. Although this association was challenged in the second half of the eighties, this was recently shown to be due to the changing definition of minimal endometriosis after the description of nonpigmented endometriosis [47], whereas the association between typical endometriosis and LUF syndrome remains valid [48, 49]. In healthy women, the steroid hormone concentrations in peritoneal fluid are much higher after ovulation, but this is not observed in women with LUF syndrome [27, 28, 50]. Since high doses of progesterone were used as a medical treatment of endometriosis, the hypothesis was put forward that an ovulatory cycle is detrimental for endometriosis development, while LUF may facilitate the development of endometriosis. It was therefore suggested that the LUF syndrome was not a consequence, but rather a cause or cofactor, in the development of endometriosis [5].

Peritoneal fluid of women with endometriosis contains more macrophages and activated macrophages, which was initially considered a consequence of low-grade inflammation. More recently, it was recognised that women with endometriosis have higher chemotactic activity for macrophages in their peritoneal fluid, [51] and that medical treatment of endometriosis can reduce this [52]. Candidate molecules are monocyte chemotactic protein-1, secreted by cytokine-stimulated endometriotic cells in vitro [53], the increased concentration of lipid peroxidation products [54] and RANTES, also increased in peritoneal fluid of women with endometriosis [55, 56] which both have potent chemotactic activity for human monocytes and T lymphocytes. These activated macrophages have higher scavenger receptor activity [57] peritoneal fluid of women with endometriosis contains higher concentrations of many macrophage secretion products.

The angiogenic activity in vivo [58] is increased, possibly mediated by increased concentrations of transforming growth factor-beta (TGF-β) [59], or vascular endothelial growth factor (VEGF) [60], secreted by activated macrophages under direct steroid regulation [61]. In peritoneal fluid of women with endometriosis, increased concentrations of cytokines were found for tumor necrosis factor-alpha (TNF-α) [62–64], interleukin (IL-8) [62, 65], IL-10 [62], IL-6 [66–68], the IL-6 soluble receptor [69], IL-8, [67, 68, 70] IL-13 [71], macrophage derived growth factor [72], and macrophage colony stimulating factor (M-CSF) [73]. Interferon-γ [56, 68] was reported not to be elevated, whereas some reports did not confirm the increased concentration of TNF-α [74, 75] and IL-13 [71]. Macrophages of women with endometriosis were reported to secrete higher amounts of fibronectin [76] and mild endometriosis is associated with decreased platelet-activating factor acetylhydrolase activity in peritoneal fluid [77]. Peritoneal fluid also contains the complete insulin-like growth factor (IGF) system, that is, IGFs, their binding proteins (BPs) and an IGFBP protease [78]. Peritoneal fluid of women with endometriosis contains potent mitogens for fibroblasts and endometrium-derived epithelial cells [79], such as the N-terminal truncated form of IGFBP-3 with a preferential mitogenic action on epithelial-derived endometrial cells [80]. Women with endometriosis have higher concentrations of inflammatory prostaglandins in their peritoneal fluid [81–86], and a higher phospholipase-A2 activity [87–89].

Women with endometriosis have a decreased cellular immunity, and more specifically, a decreased natural killer (NK) activity in peripheral blood [for review, 90] and in peritoneal fluid. This decrease in NK cell activity [91] is due to an inhibition of their function [92, 93]. The factor(s) responsible for this were shown to be different from those in follicular fluid [92]. Candidates are intracellular adhesion molecule (ICAM)-1 with an increased shedding by endometrial cells [94] and glycodelins known to be present [32] in concentrations so high that they can inhibit NK activity [95]. Peripheral monocytes from women with endometriosis have a reduced capacity to mediate the destruction of endometrial cells, whereas ectopic endometrial cells have an increased resistance to macrophage-mediated cytolysis [96]. The proportion of Bax-positive peritoneal fluid macrophages is elevated in women without endometriosis, which could have important consequences for the survival and proliferation of the ectopic endometrial tissue by resisting apoptosis [97, 98].

Women with endometriosis have higher concentrations of glycosidases [99] and of a protease inhibitor [100], possibly related to an altered fibrinolytic system [31, 101]. The concentration of the tissue inhibitor of metalloproteinase-1 is decreased [102]. Other proteins in peritoneal fluid with an uncertain role comprise a 32-kD protein [103], glycodelins and CA-125 [32]. Finally, not only macrophages, but also the concentrations of other monocytes [104] were reported to be different in peritoneal fluid of women with endometriosis.

### *The Intra-Ovarian Milieu and Cystic Ovarian Endometriosis*

Isolated reports showed differences in follicular fluid of women with and without endometriosis, such as higher levels of NK cells, B lymphocytes and monocytes [105].



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To the best of our knowledge, there have been no data linking the development of ovarian cystic endometriosis to the intra-ovarian milieu, although the extremely high (steroid) hormones may be a significant factor explaining why large 'chocolate' cysts, only occur in the ovary.

*Are Endometriotic Cells Different from Endometrial Cells or Are Endometrial Cells Different in Women with Endometriosis?* The growth, implantation, and development of endometriosis could be due to differences in the endometriotic cell instead of in the endocrine environment. Therefore, much effort was devoted to find differences between eutopic and ectopic endometrial cells. Numerous reports describe differences in steroid hormone receptors between eutopic and ectopic endometrium. Ectopic endometrium has a higher angiogenesis ans a higher density of mast cells [106, 107] The level of the proteolytic enzyme cathepsin D is higher in endometriotic tissue than in endometrium [108]. The fibronectin receptor expression was different [109], whereas no differences were found in the expression of the adhesion glycoproteins laminin and fibronectin, their receptors alpha 1 beta 1, alpha 2 beta 1, alpha 3 beta 1, alpha 4 beta 1, alpha 5 beta 1, and alpha 6 beta 1, and E-cadherin [110]. In comparison with endometrium, the integrin alpha 3 subunit was up-regulated; the alpha 6 integrin subunit was down-regulated, and the cycle stage-dependent expression of alpha 5 and beta 3 was absent in endometriosis [111]. The expression pattern of IGF-II and IGF-I was different in endometrium and endometriosis, suggesting an alteration in cell proliferation and differentiation in endometriosis [112]. The expression of plasminogen and plasmin was cycle dependent in endometrium, was maintained continuously at a high level in endometriosis [113], suggesting that this could reflect the more invasive nature of the endometriotic implants. These observations should, however, be interpreted with great caution, since it is unclear whether they reflect real differences between eutopic and ectopic endometrium, or whether the differences found are a mere consequence of differences in the endocrine environment between peritoneal fluid and plasma.

Therefore differences in endometrium between women with and without endometriosis could be more important. The observation of a loss in E-cadherin receptors in some foci of endometriosis [114], and the fact that suppressing metalloproteinase secretion in vitro with progesterone or with a natural metalloproteinase inhibitor, inhibits endometriosis formation in an animal model [115] makes it attractive to consider endometriosis as a benign (infiltrating) tumour. Studies on NK cell activity and endometriosis showed that the endometrium of women with severe endometriosis was more resistant to lysis by NK cells than the endometrial cells of normal women. Since the NK cells were derived from a male control person, the defect must be localised in the endometrial cell [116], a conclusion supported by the recent observation that endometrium from women with endometriosis releases more ICAM-1, known to inhibit NK activity [94].

P450arom transcripts, indicating aromatase activity, and IL-6 and IL-11 transcripts were detected in endometriotic implants and in eutopic endometrium from patients with endometriosis, but not in endometrium from women without endometriosis [117]. Increased expression of heat shock protein 27, regardless of the menstrual phase, was found in eutopic endometrium from patients with endometriosis or adenomyosis [118]. The dominant-positive behaviour of the estrogen receptor exon 5 splicing variant might be masked by the functional cascade of estrogen receptor wild type in normal endometria, but not in endometriotic tissue. This might result in an incomplete response to endogenous steroids, and contribute to the growth potential of endometriosis [119]. Finally, angiogenesis is increased in women with endometriosis [120].

## Conclusions and Discussion

The role of peritoneal fluid in the pathophysiology of endometriosis is undoubtedly important. Peritoneal fluid is a self-contained microenvironment with specific products and hormones in concentrations which are very different from those in plasma. This milieu is likely to play a crucial role in the implantation and growth of endometriosis. The studies which investigated cytokines, growth factors, angiogenic factors, and others in peritoneal fluid were performed because of the hypothesis that these substances might stimulate implantation of endometrial cells and growth and development into endometriotic disease. Although stimulatory effects of individual substances are obvious, the concept that peritoneal fluid could explain the development of endometriotic disease has never been proven. Indeed, the overall effect in vitro of peritoneal fluid upon the proliferation of purified endometrial stromal and epithelial cells was stimulatory, but no differences were found between women with and without endometriosis [121].

Possibly more important is the observation that the area of pelvic endometriosis is not directly, but inversely, proportional to the pelvic inflammatory macrophage re-



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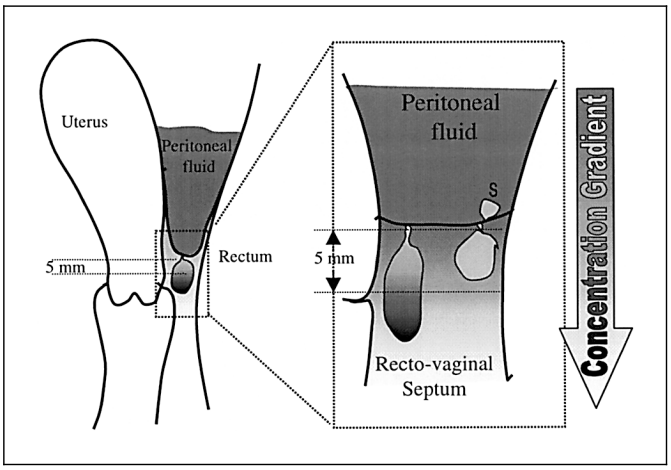


**Fig. 2.** Endocrine gradients from peritoneal fluid and their effect on superficial and deep endometriosis suggest that deep endometriosis has 'escaped' from peritoneal fluid (reprinted with permission of Human Reproduction).

sponse, since it suggests that the overall effect of peritoneal fluid upon growth and development of endometriosis may be inhibitory rather than stimulatory [122]. Considering the variety of secretory products, both concepts that peritoneal fluid may stimulate and inhibit growth of endometriosis, are not necessarily contradictory, since peritoneal fluid could be overall inhibitory for endometrial cells in most women, whereas in some women stimulatory effects could prevail.

Although the precise role of factors such as the LUF syndrome and macrophage secretory products remains unclear, it is obvious that the peritoneal fluid microenvironment regulates endometrial cells floating in peritoneal fluid and superficial endometriosis, whereas the endometrium is regulated by factors in the bloodstream. This concept of different environment and regulation of endometrium and of superficial and deep endometriosis is rarely considered when eutopic endometrium and endometriosis are compared. The observed differences have generally been interpreted as differences in tissue characteristics, rather than a result of the different environment of hormones.

It is well known that estrogens induce progesterone receptors, whereas progesterone decreases the concentration of both receptors in the endometrium. Taking into account the high concentrations of progesterone in peritoneal fluid throughout the menstrual cycle, it is not surprising that the receptor concentrations in superficial endometriosis are different from those in the endometrium. Similarly, the conclusion that deep endometriosis is different from superficial endometriosis because its mitotic activity and vascularization are different, and because the effects of medical therapy are different [123] might be wrong, since the observed differences could result simply from the different local environment.

We believe that this concept of the local endocrine environment is essential for our understanding of the pathophysiology of endometriosis. The Sampson theory emphasises retrograde menstruation and implantation as driving forces, which is not necessarily very different from the metaplasia theory, since degenerating endometrium may release biochemical factor(s) into the peritoneal environment, which induces ectopic endometrium formation [42]. What these theories have in common is that they explain why endometrial cells appear on the peritoneum, assuming that their subsequent growth, albeit at a different speed through modulation by peritoneal fluid, is unavoidable. The newer concept proposed by us, also emphasising the local microenvironment of peritoneal fluid, considers endometrial cells that are implanted superficially on the peritoneum as a normal condition and relatively unimportant. The key question to ask is why these cells infiltrate and behave more aggressively in some women and not in others. Without an answer to this question, endometriosis or rather endometriotic disease cannot be understood. A first element of this answer could be that endometriotic disease has 'escaped' from the influence of peritoneal fluid (fig. 2) [for review, 124, 125]. In addition, other factors such as genetic and immunologic factors, either acquired or hereditary, should be considered.

The role attributed to peritoneal fluid thus varies, whether the implantation theory as opposed to the endometriotic disease theory is considered. In the former theory, peritoneal fluid will stimulate growth and development. If, however, superficial endometriosis is considered a physiologic condition, and if superficial endometriosis is normally removed by the body's defence mechanisms, our concepts of the role of peritoneal fluid need to change. Indeed, instead of considering peritoneal fluid as a stimulus towards endometrial implantation and growth, it may be more appropriate to see it as having a protective role in preventing the development of endometriosis through various mechanisms such as macrophage digestion and the release of inhibitory factors. This is consistent with the observation that peritoneal inflammation is inversely and not directly proportional to the extent of pelvic endometriosis [122]. When the normal peritoneal defense mechanisms fail – for example, because of low



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progesterone concentrations associated with LUF, massive retrograde menstruation, of defective NK cell function [126] – even temporarily, endometriotic cells could infiltrate and escape from the direct influence of peritoneal fluid. These cells may change their behaviour and give rise to deeply infiltrating and cystic ovarian endometriosis, the two forms of severe endometriosis, we suggest, represent the expression of endometriotic disease.

In addition to the concept that endometriotic disease has escaped from peritoneal fluid, compartmentalisation of peritoneal fluid should be considered. Compartmentalisation of peritoneal fluid is well recognised in processes such as local ischemia and adhesion formation. Similarly, in endometriosis associated with adhesions, peritoneal fluid should not be considered an homogenous fluid, and factors secreted locally by endometrial cells, such as glycodelins and ICAM-I, which can inhibit NK function [95], could 'shield' the endometriotic implant from immunologic attack.

Endometrial cells will behave differently in different hormonal environments. We therefore question whether the observed differences between ectopic and eutopic endometrium reflect true differences in tissue characteristics, or whether they are merely the result of the different hormonal environments. Importantly, these observations have been made in superficial endometriotic implants clearly influenced by peritoneal fluid in contrast with the endometrium, which is influenced by the circulation. More important for the endometriotic disease theory are the recent data describing differences between the endometrial cells of women with and without endometriosis, possibly pointing to genetic/hereditary differences between those women. Also the observation that cystic ovarian endometriosis might be monoclonal in origin [127] and the increase in severe endometriosis in rhesus monkeys exposed to dioxin [128] and total body irradiation [129] with a delay of many years, and the metalloproteinase activity, support the concept that endometriotic disease might be considered as a benign tumour.

Besides local hormonal concentrations in peritoneal fluid and cellular differences, the pathophysiology of endometriosis undoubtedly involves genetic and immunologic factors [130, 131]. Affected women have a decreased cellular immunity and decreased natural killer (NK) cell activity [126] both in plasma and in peritoneal fluid. It is still unclear, however, whether this decrease in NK activity is the cause of endometriosis or the mere consequence of inhibition by factors associated with endometriosis. In this context, it is possibly important that decreases in NK activity were not found in baboons with endometriosis [132]. The argument for a genetic factor in the aetiology of endometriosis is strong in both humans and nonhuman primates [133, 134]. The age of onset of pain symptoms is identical in non-twin sisters concordant for endometriosis [135], and there is an increased prevalence among sisters of affected women compared to the general population [136–138]. We therefore believe that ultimately the identification of susceptibility genes could prove to be the best way of identifying those molecular and cellular mechanisms that are aberrant in endometriosis.

In conclusion, we suggest that the endometriotic disease theory could become more important than the implantation/metaplasia theory. We believe that the risk of developing endometriotic disease varies with the type of genetic differences, spontaneous or induced mutations (from environmental agents such as dioxin or total body irradiation). One must acknowledge that the susceptibility may depend on hereditary factors, and take into account tumour mechanisms such as critical mass, extracellular matrix breakdown, and local shielding from immunologic attack. All these factors, in addition to the local hormone concentrations in the ovary and in the peritoneal fluid, will determine whether endometriotic disease is expressed as cystic ovarian endometriosis, as invasive deep endometriosis, or adenomyosis externa, and whether this disease is associated with adhesions.


## Acknowledgements

Mrs. Wolput is thanked for taking care of the manuscript. Mr. Benijts and Dr. Lempereur, Ipsen N.V. Belgium are thanked for their support. This work was partially supported by the NFWO Grant No. 1.5436.97N.





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## Discussion

*Prof. Terakawa:* Could you comment on why peritoneal fluid is stimulatory for endometriosis growth in vitro but inhibitory in vivo?

*Prof. Koninckx:* I know this is provocative and is not mainstream scientific thinking. I tried to take all the data which we have and look at them from a different angle. When you look at each factor in peritoneal fluid separately, we can find many reasons why it would stimulate endometriosis, but if you take the entire fluid in the peritoneal cavity, this seems not to be stimulatory for endometriosis growth. I am only reporting these results and it is too early to interpret them.

*Prof. Taketani:* In your previous talk you postulated that minimal and mild endometriosis is a normal condition and not a disease, whereas rAFS stage III and IV cases should be regarded as a disease state. You have now clearly presented characteristic peritoneal environmental changes associated with endometriosis. Are these characteristic changes in peritoneal fluid a common finding in both the normal minimal and disease states?

*Prof. Koninckx:* Yes, because when you have endometriosis in the pelvic cavity, at that moment, you have an attraction of macrophages and all molecules there are operational. This is a sign that endometriosis is present, but this is not necessarily the cause of the endometriosis. I believe this data supports the tumor concept of endometriosis in which small lesions can be attacked and managed by the immune system. However, once the lesion is large enough, the immune system is no longer able to control the disease.

*Prof. Taketani:* If the LUF syndrome is highly associated with endometriosis in humans and baboons, do you think this syndrome is a causative factor for endometriosis and is it specific to advanced cases?

*Prof. Koninckx:* The LUF syndrome is definitely associated with cystic ovarian and advanced stages of endometriosis as well as typical disease, and there is no evidence that LUF is associated with minimal disease. I would be hesitant about to say that the LUF syndrome causes endometriosis, but perhaps it is more likely that LUF is a factor which can facilitate the development of endometriosis. It is one of a series of predisposing candidates, including genetic predisposition, the environment, and the presence of typical cells. LUF syndrome can be the cause or can be the consequence of endometriosis. There is a series of evidence that women with factors such as atypical psychology, or in a chronic stress situation, have a much higher incidence of LUF, and that these women have a lower pregnancy rate.

*Prof. Taketani:* So do you think there is some mechanism explaining the development of LUF, not a mechanical factor due to the presence of endometrioma?

*Prof. Koninckx:* Yes. Psychology certainly plays a role in the LUF syndrome, although these two factors may be coincident and not directly related. LUF probably helps the endometriotic cells to grow and this may be hormonally related.

*Dr. Harada:* In your remodeling model do you think endometriosis is a progressive disease?

*Prof. Koninckx:* Once it has reached a certain critical point, I believe endometriosis becomes a progressive disease. That is, once there are deep lesions, they increase with age. Also, the size increases with age. Large areas increase with age. Cystic ovarian endometriosis increases with age. When you look at subtle endometriosis, I think there is no evidence that this is progressive in all women.

*Dr. Harada:* If the endometriosis starts from subtle lesions, why not treat all endometriosis at an early stage?

*Prof. Koninckx:* I always treat minimal endometriosis although I think it is not logical and is probably not useful. However, when performing a laparoscopy, it takes only a few minutes using a $CO_2$ laser to treat and I cannot find a reason as a clinician not to do it. Scientifically, I think for most of these women it does not make sense to treat subtle lesions.



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