

Peritoneal fluid environment in endometriosis

Clinicopathological implications

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Endometriosis is a puzzling disorder with obscure pathogenesis. The objective of this review was to evaluate the complex role of peritoneal fluid in the etiopathogenesis of endometriosis. Several studies suggest that peritoneal fluid is a key inflammatory environment associated with endometriosis. Many active substances (cytokines, growth factors, hormones and oxidative stress parameters) have been identified in endometriosis patients at different stages of the disease. Inflammatory mediators may be involved in the endometriosis associated-infertility and possibly pain. Furthermore, these mediators may represent a non surgical method for diagnosing endometriosis. Better understanding of the mechanism of cytokines, growth factor and reactive oxygen species production and detoxification and further investigation of their effects on the peritoneal fluid environment are essential to obtain new insight into this disease and eventually develop novel diagnostic and therapeutic remedies.

Key words: **Endometriosis, etiology - Peritoneal fluid - Cytokines - Reactive oxygen species.**

Endometriosis is characterized by the presence and growth of endometrial tissue outside the uterus. It is a common disease among women of reproductive age. The wide range of symptoms associated with endometriosis jeopardize the quality of life. Despite

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a huge number of clinical and basic science researches, endometriosis remains a puzzling disorder and its exact pathogenesis has not never been established yet.

The current consensus is that endometriosis is a local pelvic inflammatory process with altered function of immune-related cells in the peritoneal environment. Supporting this concept are recent studies suggesting that the peritoneal fluid of women with endometriosis contains an increased number of activated macrophages that secrete various local products, such as growth factors, cytokines and possibly free oxygen radicals.¹⁻¹⁶

Studies have reported elevated levels of several cytokines in the peritoneal fluid of women with endometriosis, thus implicating these cytokines in the development and progression of endometriosis and endometriosis-associated infertility. Reactive oxygen species (ROS) are also produced by peritoneal fluid mononuclear leucocytes in endometriosis patients.¹⁷ Production of ROS is known to increase after activation of immune cells, especially polymorphonuclear leukocytes and macrophages. Consequently, ROS appear to be an important mediator in the etiopathogenesis of endometriosis.

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Once endometrium reaches the peritoneal cavity, its growth must be stimulated and maintained to initiate the disease process. Critical to this mechanism is the establishment of a novel blood supply. Angiogenic factors figure prominently in the pathogenesis of endometriosis. Failure of initiation, growth and maintenance may hamper the development of endometriosis. Endometriotic tissue usually behaves like eutopic endometrium in their hormonal responsiveness. Frequently, endometriotic tissues often behave in an aberrant way.

Such data suggest that a combination of factors, including the hormonal milieu and the number and secretory capacity of cells residing in the peritoneal cavity, might be required to sustain the growth of ectopic endometrium and thus induce clinical endometriosis. In this review, the current understanding of the role of the peritoneal fluid environment in the pathogenesis of endometriosis and endometriosis-associated infertility is evaluated.

Peritoneal fluid

Peritoneal fluid is often seen in the vesicouterine cavity or the *cul-de-sac* during gynecologic surgery and bathes the pelvic cavity, uterus, fallopian tubes, and ovaries. It is believed to be a major factor controlling the peritoneal microenvironment that influences the development and progression of endometriosis and endometriosis-associated infertility. Peritoneal fluid is formed in part by the contribution of the follicular activity, corpus luteum vascularity, and hormonal production. The volume of peritoneal fluid is dynamic and phase dependent peaking at the time of ovulation.¹⁸ The peritoneal fluid ingredients are variable in normal menstrual cycles and different pathologic entities.^{19, 20} It has been found that women with endometriosis had a greater peritoneal fluid volume than fertile controls, patients with tubal disease, or those with unexplained infertility. Moreover, an increased volume of peritoneal fluid may be commonly associated not only with endometriosis but also with idiopathic infertility.

Peritoneal fluid is rich with variable cellular components including macrophages, mesothelial cells, lymphocytes, eosinophils, and mast cells. The normal concentration of peritoneal fluid leukocytes is 0.5 to 2.0×10⁶/mL, of which approximately 85% are macrophages.^{19, 20} It has been hypothesized that peritoneal macrophage¹ activation is a pivotal step in the disease initiation and progression. Activated macrophages in the peritoneal cavity of women with endometriosis are potent producers of cytokines. Thus, peritoneal fluid contains a rich mixture of cytokines. Iron overload was also observed in the cellular and peritoneal fluid compartments of the peritoneal cavity of endometriosis patients suggesting a role in its pathogenesis.²¹ Oxidative stress is also a commonly observed process identified in the peritoneal fluid of endometriosis patients.^{22, 23}

Peritoneal fluid immunological factors and endometriosis

Significant role of the immune system in the pathogenesis of endometriosis has been recently documented.²⁴ Based on these recent findings, there is an emerging concept of treating endometriosis as an autoimmune disease.²⁵ Accumulating evidence suggests that systemic T cell activity influences the pathogenesis of endometriosis.^{26, 27} Altered T-helper to T-suppressor ratio and concentration of both cells respectively have been reported in serum, peritoneal fluid (PF)²⁸ and endometriotic tissue²⁹ in endometriosis patients. Moreover, such differences could be detected between eutopic endometrium from women with and without the disease. There is lack of consistency regarding the alterations in T-cells and their role in the pathophysiology of endometriosis.

Natural killer (NK) cells are also altered in endometriosis. Both peripheral and peritoneal fluid NK cells from women with endometriosis showed different characteristics compared with those of the controls.³⁰ Additionally, NK cell cytotoxicity has been shown to be inversely correlated with the stages of

the disease.³¹ Consequently, altered NK cytotoxicity to endometrial tissue may be responsible in part for the initiation, propagation and establishment of pelvic endometriosis. Sera and PF from women with endometriosis have been shown to reduce NK cell activity.³² This is probably caused by monocyte or macrophage activity through their secretions that modulate immune and non-immune cells.

Besides the alterations of T cell functions, many recent findings have shown alterations in B-cell function in endometriosis patients as evidenced by abnormal antigen-antibody reaction and increased B-cell function. Decreased C3 deposition in the endometrium and a corresponding reduction in the serum total complement levels has been shown in endometriosis patients.³³ Antiendometrial antibodies particularly IgG and IgA have been detected in sera, vaginal, and cervical secretions of endometriosis patients.³⁴ The presence of antiphospholipids and antihistones of IgG, IgM, and IgA have been documented by some investigators³⁵ and questioned by others.³⁶ The exact correlation between the stage of endometriosis and autoantibodies ranges from positive³⁷ to negative³⁸ to no relationship at all.³⁹ These observations of immune alterations have lead investigators to believe that markers of immune reactivity, particularly cytokines, may be potentially used as diagnostic aid for endometriosis.

Cytokines: chemistry

Cytokines are polypeptides or glycoproteins secreted into the extracellular compartment mainly by leukocytes. Upon secretion, they exert autocrine, paracrine and sometimes endocrine effects. Moreover, cytokines may exist in cell-membrane-associated forms where they exert juxtacrine activity on adjacent cells. They are essential mediators of cell-cell communication in the immune system. They affect a wide variety of target cells exerting proliferative, cytostatic, chemoattractant, or differentiative effects. Their biological activities are mediated by coupling to intracellular signaling and second-messen-

ger pathways *via* specific high-affinity receptors on target cell membranes. The cytokine nomenclature reflects the historical description of these biological activities.

Cytokines: sources

The main source of cytokines is macrophages, which originate in bone marrow, circulate as monocytes, and migrate to various body cavities. Chemoattractant cytokines particularly RANTES, and IL-8, facilitate macrophages recruitment into the peritoneal cavity. The second major source of cytokines is T lymphocytes. Helper -T cells can be classified into 2 subsets: type 1 (Th1) and type 2 (Th2). Th1 cells produce IL-2, IL-12, and interferon- γ , which are potent inducers of cell-mediated immunity. Th2 cells produce mainly IL-4, IL-5, IL-10, and IL-13, which are involved in suppression of cell-mediated immunity. There is alteration of cytokines secreted by Th1 and Th2 in endometriosis patients particularly in the balance of Th1 and Th2 cells toward the Th2. This may—in part—be responsible for the impaired immunologic defense in endometriosis.³

Tsuda *et al.* hypothesized that cytokines are not only produced by immune competent cells but by endometriotic implants as well.⁴⁰ They demonstrated that endometriotic cells constitutively express IL-6 messenger RNA and produce IL-6 protein and that adding TNF- α stimulated IL-6 gene and protein expression in a dose-dependent manner. On comparing IL-6 production by macrophages and endometriotic stromal cells in-patients with endometriosis, they found that similar levels of IL-6 were produced in stromal cells derived from an endometrioma and by macrophages under basal- and TNF- α -stimulated conditions. This finding supports the hypothesis that endometriotic tissue is another important source of cytokines.⁴⁰

Peritoneal fluid cytokines

Peritoneal fluid is rich with variable cellular components including macrophages, mesothelial cells, lymphocytes, eosinophils, and

mast cells. The normal concentration of PF leukocytes is 0.5 to $2.0 \times 10^6/\text{mL}$, of which approximately 85% are macrophages.^{19, 20} It has been¹ hypothesized that peritoneal macrophage activation is a pivotal step in the disease initiation and progression. Activated macrophages in the peritoneal cavity of women with endometriosis are potent producers of cytokines.²³ Thus, PF contains a rich mixture of cytokines. Iron overload was also observed in the cellular and PF compartments of the peritoneal cavity of endometriosis patients suggesting a role in its pathogenesis.²¹

Individual cytokines

Tumor necrosis factors

The tumor necrosis factors (TNF) are pleiotropic cytokines that exerts an essential role in the inflammatory process. It is believed to be seminal in many physiological and pathological reproductive processes. The spectrum of its effects is very wide with beneficial and hazardous effects. The quantity of TNF produced is the main factor that controls its role in the disease process. The main TNF is TNF- α , which is produced by neutrophils, activated lymphocytes, macrophages, NK cells, and several non-hematopoietic cells. Little is known about TNF- α , which is produced by lymphocytes. The primary function of TNFs is their ability to initiate the cascade of cytokines and other factors associated with inflammatory responses. TNF- α helps to activate helper T cells.

In the human endometrium, TNF- α is a factor in the normal physiology of endometrial proliferation and shedding. TNF- α is expressed mostly in epithelial cells particularly in the secretory phase.⁴¹ Stromal cells stain for TNF- α mostly in the proliferative phase of the cycle. These data suggest a hormonal control of this cytokine.⁴²

Peritoneal fluid TNF- α concentrations are elevated in patients with endometriosis, and some studies show higher concentrations correlate with the stage of the disease.⁴³ Our study did not observe any relationship between levels of TNF- α and stage of the disease.²³ The source of the elevated TNF-

concentration in the PF of endometriosis patients is variable. Some *in vitro* studies suggest that peritoneal macrophages⁴⁴ and peripheral blood monocytes⁴⁵ from these patients have up-regulated TNF- α protein secretion. Activated macrophages play a critical role in the pathogenesis of endometriosis. The secreted TNF- α may play an important role in the local and the systemic manifestations of the disease. Because of its importance in other inflammatory processes, it is likely that this cytokine plays a central role in the pathogenesis of endometriosis.⁴⁶ Moreover, its level in the PF can be used as a foundation for non-surgical diagnosis of endometriosis as well.²³ Recently, the concept of using TNF- α blockers in treating endometriosis is gaining popularity.²⁵

Interleukin-6

IL-6 is a regulator of inflammation and immunity, which may be a physiologic link between the endocrine and the immune systems. It also modulates secretion of other cytokines, promotes T-cell activation and B-cell differentiation, and inhibits growth of various human cell lines.²⁵ Monocytes, macrophages, fibroblasts, endothelial cells, vascular smooth-muscle cells, and endometrial epithelial, stromal cells and several endocrine glands, including the pituitary and the pancreas are all production sites for IL-6.⁴⁷

The role of IL-6 in the pathogenesis of endometriosis was extensively studied. IL-6 response in the peritoneal macrophages,⁴⁸ endometrial stromal cells,⁴⁹ and peripheral macrophages⁴⁵ was dysregulated in patients with endometriosis. The level of IL-6 detected in the PF of patients with endometriosis was inconsistent. Some investigators have demonstrated elevated concentrations,^{4, 5} whereas others have found no elevation.¹⁰ Some studies failed to demonstrate statistically significant differences in IL-6 levels between controls and endometriosis patients.⁵⁰ These inconsistent findings likely are related to antibody specificity of the assay. In our recent study, we found that there is significant elevation of IL-6 in the sera of endometriosis patients but not in the PF as com-

pared to patients with unexplained infertility and tubal ligation/re-anastomosis.²³

Vascular endothelial growth factor

Many studies focused on the proliferation and neovascularization of the endometriotic implants. Vascular endothelial growth factor (VEGF) is one of the most potent and specific angiogenic factors. The main biochemical activity of VEGF when it binds to its targeted receptor is that VEGF-receptor activation leads to a rapid increase in intracellular Ca²⁺ and inositol triphosphate concentrations in endothelial cells.^{51, 52} The basic physiological function of VEGF is that VEGF-induced angiogenesis allows repair of the endometrium following menstruation. It also modulates the characters of the newly formed vessels by controlling the microvascular permeability, permitting the formation of a fibrin matrix for endothelial migration and proliferation.⁵³ This may be responsible for the local endometrial edema which help to prepare the endometrium for embryo implantation.⁵⁴

In endometriosis patients, VEGF was localized in the epithelium of endometriotic implants,⁵⁵ particularly in hemorrhagic red implants.⁵⁶ Moreover, there are increased concentrations of VEGF in PF of endometriosis patients. The exact cellular sources of VEGF in PF have not been precisely defined yet. Although evidence exists to suggest that endometriotic lesions themselves produce this factor,⁵⁵ activated peritoneal macrophages also have the capacity to synthesize and secrete VEGF.¹⁶ Similar to the concept of using TNF- α blockers, antiangiogenic drugs are potential therapeutic agents in endometriosis.

RANTES

RANTES (Regulated on Activation, Normal T-Cell Expressed and Secreted) belongs to the or "C-C" chemokine family. It attracts for monocytes and memory T-cells. RANTES is a secretory product of hematopoietic cells, epithelial and mesenchymal cells and a mediator in both acute and chronic inflammation.⁵⁷

RANTES protein distribution in ectopic endometrium is similar to that found in a eutopic endometrium.⁵⁸ However, *in vitro*

secretion of RANTES by endometrioma-derived stromal-cell cultures is significantly greater than in eutopic endometrium. In this way, PF concentrations of RANTES may be increased in patients with endometriosis.¹¹

Interleukin-1

Interleukin-1 (IL-1) is a key cytokine in the regulation of inflammation and immune responses. IL-1 affects the activation of T-lymphocytes and the differentiation of B-lymphocytes. There are 2 receptors for IL-1, namely IL-1 α and IL-1 β sharing only 18-26% amino acid homology. Both receptors are encoded by different genes but have similar biological activities. It was found that successful implantation in mice was blocked by the administration of exogenous IL-1 receptor antagonist. This illustrates its important role in the implantation of the ectopic endometrium.⁵⁹ IL-1 has been isolated from the PF of patients with endometriosis. Results have been inconsistent, with some investigators demonstrating elevated concentrations in patients with endometriosis⁶⁰ and others finding no elevation.^{2, 23, 44}

Other cytokines

A highly sensitive ELISA kits have made it easy to measure the entire battery of cytokines in the serum and PF of endometriosis patients. Other cytokines have been identified and include IL-4³; IL-5⁴; IL-8^{6, 23}; IL-10⁷; IL-12^{8, 23}; IL-13⁹; interferon- γ ¹⁰; monocyte chemotactic protein-1 (MCP-1)¹²; macrophage colony stimulating factor (MCSF)¹³ and transforming growth factor (TGF)- α ¹⁴. All these cytokines may regulate the actions of leukocytes or may act directly on ectopic endometrium, where they may play various roles in the pathogenesis and pathophysiology of endometriosis. However, their exact role needs further investigation.

Role of peritoneal fluid cytokines and growth factors in endometriosis

The role of peritoneal fluid cytokines and growth factors in the pathophysiology of endometriosis has been investigated exten-

TABLE I.—*The role of peritoneal fluid cytokines and growth factors in the pathophysiology of endometriosis.*

I	Endometrial cells implantation ⁶³
II	Endometrial cell proliferation ^{61, 62}
III	Endometrial tissue remodeling ⁶⁴
IV	Angiogenesis and neovascularization ⁵⁰

sively in the past decade. The hypothesized roles of cytokines in the pathogenesis of endometriosis are summarized in Table I.^{50, 61-64} They are probably responsible for endometrial cell proliferation^{61, 62} and implantation of endometrial cells or tissue.⁶³ Moreover, cytokines increased tissue remodeling through their effects on the matrix metalloproteinases.⁶⁴ Increased angiogenesis of the ectopic endometrial tissue and neovascularization of the affected region is probably the most important effect of cytokines on ectopic endometrial tissue.

Another variable is the role of growth factors. In rodents, epidermal growth factor (EGF), transforming growth factor α , and EGF receptors have been demonstrated in both eutopic and ectopic endometrium.⁶⁵ EGF, insulin-like growth factor I and growth hormone all stimulate the growth of human endometrial stromal cells *in vitro*. Platelet-derived growth factor has been shown to lead to the proliferation of human endometrial stromal cells in a dose-dependent fashion. Additionally, macrophage-derived growth factor (MDGF) enhances endometrial stromal-cell proliferation⁶⁶ with maximal stimulation of growth when MDGF and estrogen are both present in the culture medium. As such, activated macrophages, through the liberation of cytokines and growth factors, could potentially contribute to the early establishment as well as the progression of endometriosis.

Cytokines play a major role in the initiation, propagation, and regulation of immune and inflammatory responses. Immune cell activation results in a burst and cascade of inflammatory cytokines. These cytokines have pleiotropic and redundant activities that culminate in recruitment of numerous cell types to the site of inflammation.

Autoantibodies

A variety of autoantibodies have been detected in endometriosis patients. The most commonly reported types are antiendometrial antibodies^{33, 67} and autoantibodies against oxidative stress parameters.⁶⁸

Antiendometrial antibodies

The antigens used to induce antiendometrial antibodies included sonicated endometrium of women with normal menstrual cycles, endometrial tissue of patients with endometriosis, endometriosis tissue, human endometrial carcinoma cells line, epithelial monolayers or endometrial glands and stromal cells. Moreover, the exact antigen is not known, consequently there is no simple antigen-antibody assay as yet.³⁸

Serum antiendometrial antibodies.—Antiendometrial antibodies have been postulated to be a probable cause of infertility in endometriosis patients as shown by some investigators^{33, 67} but not by others.⁶⁹ Besides the inconsistency of the assay techniques used,⁷⁰ the nature of the antigens used to illicit immune response are inconsistent as well.

The sensitivity and the specificity of serum anti-endometrial antibodies screening were reported by some investigators to be 0.84 and 1.00, respectively.³⁴ On comparing infertile women with endometriosis with unexplained infertility, Wild and Shiver found a sensitivity of 0.71 and a specificity of 1.00.³⁹ Similarly, Meek *et al.*³⁶ found a sensitivity of 0.75 and a specificity of 0.90 while in another study the values were 0.85 and 0.67, respectively.⁷¹ Although serum antiendometrial antibodies matches CA 125 regarding both sensitivity and specificity, it does not satisfy the criteria of an ideal screening test. Despite this limitation, anti-endometrial antibody was proposed not only as a screening marker but as a follow-up marker of treatment results and recurrence as well.⁷²

Peritoneal fluid antiendometrial antibodies.—Although antiendometrial antibodies were found in the PF of endometriosis patients, their sensitivity and specificity are variable. Halme and Mthur found a sensitiv-

ity of 0.23 and a specificity of 0.96 using a passive haemagglutination assay⁷³ while the results were 0.75 and 0.90 using Ouchterlony immune diffusion.³⁶

Autoantibodies to markers of oxidative stress

There is increasing evidence of oxidative stress in the PF of women with endometriosis and showed that oxidatively modified lipid proteins exist in the PF.^{22, 23, 74} In addition, oxidation-specific epitopes and macrophages are present in the endometrium and in endometriosis.⁶⁸ Lipid peroxides interact with proteins, resulting in several types of alterations, and such oxidatively modified proteins are themselves antigenic. Antigenicity is attributed to specific modified epitopes and not to the protein backbone.

In a study to measure autoantibodies to oxidatively modified proteins in the sera of women with surgically proven endometriosis, Murphy *et al.*, included women undergoing surgery for endometriosis or tubal ligation.⁶⁸ They measured serum and PF autoantibody titers to malondialdehyde-modified low-density lipoprotein, oxidized low-density lipoprotein, and lipid peroxide-modified rabbit serum albumin determined by ELISA. They correlated the autoantibody titers with the disease stage, symptoms, and morphologic type of endometriosis.

They found that autoantibodies to markers of oxidative stress were significantly increased in women with endometriosis without any correlation with the stage, symptoms, or morphologic type of the disease. Peritoneal fluid did not contain autoantibodies to any of the 3 antigens. Given the fact that autoantibodies to Ox-LDL have been long considered as a screening tool for atherosclerosis,⁷⁵ a similar role might be claimed in endometriosis.

Peritoneal fluid oxidative stress and endometriosis

Peritoneal fluid reactive oxygen species

The production of ROS by peritoneal fluid mononuclear cells was long reported to be

TABLE II.—*Mechanisms of endometriosis associated infertility.*

I	<i>Ovarian causes</i>
	1. Impaired folliculogenesis
	2. Defective granulosa cell steroidogenesis
	3. Luteinized unruptured follicle syndrome
	4. Reduced oocyte quality
	5. Luteal phase defects
II	<i>Tubal causes</i>
	1. Tubal distortion
	2. Tubal obstruction
	3. Tubal dysfunction
III	<i>Immunological causes</i>
	1. Autoimmunity
	2. Antiendometrial antibody
	3. Antiphospholipid antibody
IV	<i>Hyperprolactinaemia</i>
V	<i>Local peritoneal factors affecting gametes and early embryos</i>
	1. Cytokines
	2. Prostaglandins
	3. Macrophages
VI	<i>Defective implantation</i>

increased in endometriosis. Large amounts of ROS were released after chronic stimulation of peritoneal fluid macrophages in women with endometriosis. Production of ROS is known to increase after activation of immune cells, especially polymorphonuclear leukocytes and macrophages.¹⁷

However, further studies based on direct measurement of reactive oxygen species production failed to show an obvious oxidant or antioxidant imbalance in the peritoneal cavity of patients with endometriosis. Our group²² found similar levels of ROS detected by enhanced chemiluminescence assay using luminol as a probe in the peritoneal fluid of patients with endometriosis and disease-free controls. The same results were confirmed in a later study with large number of cases.²³ Furthermore, the total antioxidant status was not increased in endometriosis,⁷⁶ a finding confirmed recently by Polak *et al.*⁷⁷

Expression of xanthine oxidase, an enzyme which produces ROS, in ectopic and eutopic endometrium remained high throughout the menstrual cycle in women with endometriosis; in contrast, cyclic variations in its expression were seen in controls.⁷⁸ This

TABLE III.—Possible negative effects of cytokine rich peritoneal fluid on gamete function and embryonic development.

I	<i>Spermatozoa</i>
	1. Impairment of acrosome reaction
	2. Impairment of sperm motility
II	<i>Oocyte</i>
	1. Impaired folliculogenesis
	2. Impaired oocyte quality
III	<i>Sperm-oocyte interaction impairment</i>
IV	<i>Impaired embryonic development</i>
	1-2. Cell stage block
	2. Decreased blastulation

apparent discrepancy between results may be due to the fact that only persistent markers of oxidative stress, such as enzymes or stable by-products of oxidative reactions, can still be detected when endometriosis is diagnosed. Another possible explanation is that oxidative stress occurs only locally—for example, at the site of bleeding—and does not result in an increase in total peritoneal fluid concentrations.⁷⁹

Contrary to ROS, no evidence of increased nitric oxide (NO) metabolism was found in the peritoneal fluid of women with and without endometriosis.⁷⁶ However, generation of peroxynitrite by ectopic endometrium was recently demonstrated in patients with adenomyosis.⁸⁰ Expression of endothelial and inducible nitric oxide synthase and peroxynitrite generation were markedly reduced after GnRH agonist therapy, supporting their potential role in the pathophysiology of adenomyosis.⁸⁰

Peritoneal fluid oxidized low-density lipoproteins:

In contrast, Murphy *et al.*⁸¹ found increased oxidation of low-density lipoprotein in patients with pelvic endometriosis and increased concentrations of oxidized low-density lipoproteins in the peritoneal fluid of women in whom the disease was developing.^{81, 82} Oxidative modification of these molecules involves peroxidation of the lipid component, which leads to release of aldehydes, such as malondialdehyde (MDA), and reaction with lysine residues of proteins. Howev-

er, others⁸³ found no relation between levels of malondialdehyde in peritoneal fluid and severity of endometriosis. Higher levels of lysophosphatidyl choline, another indicator of lipoprotein peroxidation, were found in the peritoneal fluid of patients with endometriosis as well.⁷⁴ Murphy *et al.* demonstrated increased modified lipidprotein complexes at the level of the endometrium as well.⁶⁸ Ectopic endometrial cells were also immunostained with antibodies to oxidatively modified proteins.

Peritoneal fluid antioxidants

Several recent studies appear to show that in women with endometriosis, the endometrium shows altered expression of enzymes involved in defense against oxidative stress such as manganese and copper/zinc superoxide dismutase⁸⁴ and glutathione peroxidase.⁸⁵ Expression of manganese superoxide dismutase and glutathione peroxidase, which are induced during increased release of reactive oxygen species, can be considered as an indicator of oxidative stress.⁸⁴ It has been suggested that eutopic endometrium undergoes oxidative stress even in patients who do not develop endometriosis,⁸¹ but probably to a lesser extent.

Vitamin E plays an important role in protecting biological membranes by preventing peroxidation. It may also play a role in preventing activation of redox-sensitive pathways, which have been implicated in abnormal cell proliferation and inflammatory response. Vitamin E levels were found to be significantly lower in the peritoneal fluid of women with endometriosis, perhaps due to their consumption during oxidation reactions.^{74, 81}

A decrease in antioxidant capacity may explain why low-density lipoproteins in the peritoneal fluid of patients with endometriosis are more readily oxidized than are low-density lipoproteins from control patients.⁷⁴ In conclusion, these findings indicate that oxidative stress related mechanisms in the peritoneal cavity of women with endometriosis are contributing to the etiopathogenesis of the disease.

Reproductive implications of peritoneal fluid in endometriosis patients

Endometriosis is frequently associated with infertility, even if affected women are ovulatory or have mechanical patency of the fallopian tubes. An approximate estimate suggests that about 20% to 25% of infertile women have endometriosis, compared with 2% to 5% of women undergoing tubal ligation.⁸⁶ Endometriosis has been associated with infertility even in its early stages, before adhesion or anatomic distortion take place. The exact mechanism of endometriosis-associated infertility is not fully understood, although many possible causes have been suggested (Table II).

Ovulatory dysfunction has been proposed as the main cause of infertility in women with minimal endometriosis. The association between the luteinized unruptured follicle (LUF) syndrome and endometriosis was described in endometriosis patients as well.⁸⁷ In women with the LUF syndrome, steroid hormone concentrations in peritoneal fluid are much lower after the ovulatory cycle.⁸⁸ It was suggested that this lower steroid environment is a contributory factor in the development of endometriosis. Hyperprolactinemia and luteal phase defects have also been also suggested as a possible cause of endometriosis-associated infertility.⁸⁹

Impaired follicular development in endometriosis patients undergoing IVF/ICSI was observed in IVF data published everywhere.⁹⁰ Defective granulosa cell steroidogenesis,⁹² reduced pregnancy rates in IVF cycles in women with minimal and mild endometriosis,⁹⁰ impaired oocyte quality⁹⁰ and defects in implantation⁹⁰ were all proposed as etiological factors for poor IVF outcome in endometriosis patients. In a recent meta-analysis, it was found that patients with endometriosis-associated infertility undergoing IVF respond with significantly decreased levels of all markers of a good reproductive outcome, resulting in a pregnancy rate that is almost one half that of women with other indications for IVF. These data suggest that the effect of endometriosis is not exclusively on the receptivity

of the endometrium but also on the development of the oocyte and embryo, which might be due to local peritoneal fluid effects.⁹³

Gleicher *et al.* noted that a significant proportion of endometriosis patients has elevated autoantibody titers. Autoantibodies have been reported to interfere with various processes during human reproduction, including sperm function, fertilization, and normal progress of pregnancy.⁹⁴

Since the ovaries and fallopian tubes are immersed in the peritoneal fluid, cytokines among other active chemicals present in peritoneal fluid may jeopardize tubal motility, ovum pick-up, or ovulation. Given the fact that oocytes are exposed to the peritoneal environment even after they are captured by the fimbriae, and spermatozoa are present in the peritoneal fluid after intercourse, gametes and early embryos are exposed to cytokine rich peritoneal fluid, which may influence early reproductive process.

The potential mechanisms by which the peritoneal fluid affects fertility in endometriosis

Sperm phagocytosis

Peritoneal fluid contains many phagocytic cells (macrophages). They are responsible for phagocytosis of cellular debris, including sperm, in the pelvis. It has been demonstrated that peritoneal macrophages phagocytosed sperm *in vitro* and that macrophages from women with endometriosis were more active than those from women without the disease. Peritoneal fluid flushing the tubal and endometrial environment may affect sperm and their interaction with the oocyte.⁹⁵ However most indicators of sperm function have been shown to be normal after exposure to peritoneal fluid of endometriosis patients (Table III).⁹⁶

Sperm egg interaction

Studies showed that the peritoneal fluid of patients with endometriosis jeopardizes

sperm function. Sperm motility acrosome reaction, gamete interaction, and ovum pick up by tubal fimbriae have been shown to be affected by peritoneal fluid.⁹⁷⁻⁹⁹ Aeby *et al.*, using a hamster penetration assay, recently showed that peritoneal fluid from patients with endometriosis impaired gamete interaction. In their study, the mean number of eggs penetrated by sperm mixed with peritoneal fluid from patients with endometriosis was significantly less than that observed in controls. These data propose that chemicals in the peritoneal fluid of patients with endometriosis contribute to infertility by impairing sperm egg interaction.¹⁰⁰

Preimplantation embryonic development

Peritoneal fluid effect on preimplantation murine embryo development has also been studied. There are contradictory conclusions regarding the effect of peritoneal fluid obtained from patients with endometriosis on embryonic development *in vitro*. Some studies suggested a negative effect,¹⁰¹ and others have found that peritoneal fluid had no adverse effects at all.¹⁰² However, peritoneal fluid from patients with endometriosis has frequently been shown to be toxic to the preimplantation embryo. Medical treatment of endometriosis was found to reverse the embryotoxicity of the peritoneal fluid.² Moreover, the levels of IL-1 and TNF- α were markedly reduced in the peritoneal fluid of women who received medical treatment for endometriosis.

Conclusions

Randomized clinical trial on the use of surgery for infertility or pain associated with endometriosis have shown a clear benefit.¹⁰³ This clearly shows that the peritoneal environment is a critical part of the pathogenesis and treatment of the disease. Most research on the peritoneal fluid environment has been observational. These studies have reported a variety of inflammatory cytokines and growth factors that are abnormally elevated in patients with endometriosis. It is unclear if

these abnormalities identified are the result or the cause of the disease. However, recent animal data suggests that altering these cytokine levels particularly TNF may have a beneficial effect on endometriotic growth. The mechanism by which the abnormal peritoneal environment causes infertility or chronic pelvic pain is speculative. Future research into the peritoneal fluid environment can lead to more insight into the pathogenesis of endometriosis as well as to potential non-surgical diagnostic and treatment modalities.

Riassunto

Composizione del liquido peritoneale nell'endometriosi: implicazioni clinico-patologiche

L'endometriosi rappresenta una patologia enigmatica, la cui patogenesi è ancora oscura. L'obiettivo di questa review è la valutazione del complesso ruolo svolto dal liquido peritoneale nell'eziopatogenesi dell'endometriosi. Diversi studi suggeriscono che il liquido peritoneale costituisce un bacino infiammatorio di cruciale importanza per lo sviluppo dell'endometriosi. Nei pazienti affetti da endometriosi è stata rinvenuta la presenza, a diversi stadi della malattia, di molteplici sostanze dotate di attività infiammatoria (citochine, fattori di crescita, ormoni e parametri di stress ossidativo). Questi mediatori del processo infiammatorio possono essere coinvolti nell'infertilità associata all'endometriosi e verosimilmente nel dolore. Inoltre, questi mediatori possono rappresentare una metodica non chirurgica per la diagnosi dell'endometriosi.

Una migliore comprensione del meccanismo di produzione delle citochine, dei fattori di crescita e delle specie reattive dell'ossigeno e della loro detossificazione, insieme a indagini più approfondite circa i loro effetti sulla composizione del liquido peritoneale, costituirebbero un passo fondamentale verso la chiarificazione della patogenesi di questa malattia e, in ultima analisi, verso lo sviluppo di nuove strategie diagnostiche e terapeutiche.

Parole chiave: Endometriosi, eziologia – Liquido peritoneale - Citochine - Specie reattive dell'ossigeno.

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