Presence of hydrosalpinx correlated to endometrial inflammatory response in vivo

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Objective: To evaluate and compare the inflammatory response and mediators in the endometrium of patients with hydrosalpinges compared with normal controls.
Design: Retrospective case-control study.
Setting: Urban medical center.
Patient(s) and Intervention(s): Hysterectomy samples were identified as being affected by hydrosalpinx or salpingitis (n = 30) and were age-matched with control samples (n = 30).
Interventions: Fallopian tube and endometrial slides were analyzed for leukocytes and immunohistochemical techniques performed for cytokines (interleukin-2 [IL-2]).
Main Outcome Measure(s): Evaluate and compare the endometrial inflammatory response (leukocytes and cytokines) from samples affected and non-affected by hydrosalpinx and salpingitis.
Result(s): Examination of tubal and endometrial slides with hydrosalpinx demonstrated a statistically significant increase in the number of overall inflammatory cells. High-intensity immunohistochemical staining for IL-2 was demonstrated in 7.4% of controls versus 65% of cases.
Conclusion(s): A defined, identifiable, local response to hydrosalpingeal fluid has been demonstrated in the endometrium. This response consists of statistically significant elevations of leukocytes and IL-2. An inflammatory endometrial response may be an independent contributor to the decreased reproductive outcome observed in patients with hydrosalpinges. (Fertil Steril® 2006;86:972–6. ©2006 by American Society for Reproductive Medicine.)

Key Words: Endometrium, hydrosalpinx, inflammatory cells, cytokine, IVF, infertility

Though IVF-ET was originally designed to circumvent tubal factor infertility, it has always been curious that patients with severe tubal disease often do not have the most successful outcome when compared to those with other etiologies of their infertility. Possibly, the reason is that tubal disease is a heterogeneous entity, and that patients with hydrosalpinges represent a subset with the worst prognosis (1).

Specifically, several retrospective studies have demonstrated that patients with hydrosalpinges have lower implantation and pregnancy rates (1, 2). A clear association between the existence of sonographically visible hydrosalpinges and impaired IVF outcome has been demonstrated (3, 4). An increased rate of spontaneous abortion in patients with hydrosalpinges has also been encountered (5, 6).

Several years ago, it was theorized that the hydrosalpinx fluid leaks into the uterine cavity and adversely affects the uterine milieu. Attempts were made, therefore, to interrupt the connection between the fallopian tubes and the uterus, in hopes of restoring an optimal environment for implantation. Patients with hydrosalpinges undergoing salpingectomy before IVF have increased implantation, clinical pregnancy, and delivery rates (7).

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Other retrospective studies have also reported improved outcomes following salpingectomy in patients with sonographically visible hydrosalpinges (3, 4). The precise pathophysiologic mechanism that results in impaired IVF outcome in patients with hydrosalpinges is still unclear.

Many theories have been proposed in explaining the mechanisms for decreased success of IVF in the presence of hydrosalpinx. Our group and others have demonstrated direct embryotoxic properties of the hydrosalpingeal fluid in murine models (8, 9). Others have suggested that refluxed hydrosalpingeal fluid provides a physical barrier or a mechanical hindrance to implantation, resulting in failed implantation and subsequent disintegration of embryos (10, 11).

Significant reduction of motile spermatozoa after 24 hours of incubation with hydrosalpinx fluid, in a concentration-dependent manner, may represent a cause for reduced fertility (12), perhaps by impairing the acrosome reaction (T. Mukherjee, unpublished data). The presence of hydrosalpingeal fluid has even been implicated in promoting diminished ovarian function and follicular development (13).

The predominance of data, however, point to a specific impact of hydrosalpinx on the endometrial environment, primarily because recipients with hydrosalpinges receiving donor oocytes or cryopreserved embryos also experience lower implantation rates and significantly higher abortion rates than do normal control individuals (14, 15). We pro-

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pose that the impaired reproductive outcome in patients with hydrosalpinges is not merely related to the embryotoxicity of the hydrosalpinx fluid but to a resultant inflammatory milieu, which subsequently leads to impaired endometrial receptivity. We therefore designed this study to determine whether patients with evidence of severe tubal disease exhibit an increased presence of endometrial inflammatory cells and inflammatory mediators versus controls.

MATERIALS AND METHODS

A retrospective institutional review board–approved case-control study was designed. We used the CoPath database from Mount Sinai Hospital (New York, NY) to identify hysterectomy samples from 1998–2001. The indications for hysterectomy were those related to general gynecological situations.

A total of 30 cases were identified that demonstrated hydrosalpinx or salpingitis (without history of tubal sterilization). We age-matched these patients to 30 control samples, which were identified as having normal tubal architecture. The samples analyzed in this study were obtained from patients who were not infertile. Archived hematoxylin and eosin (H&E) slides were reviewed to confirm the original pathologic diagnosis of the fallopian tube and the corresponding endometrium. Two observers performed the analysis of the slides for leukocyte counts. Five representative adjacent, nonoverlapping high-power fields (400×) were examined for each fallopian tube slide, and endometrium and leukocyte counts recorded as number of neutrophils, plasma cells, lymphocytes, basophils, eosinophils, and macrophages.

For immunohistochemical studies, blank slides were prepared from archived block tissue. Specimens were deparaffinized using xylene wash. Antigen retrieval was achieved by boiling the slides in a 10-mM citrate buffer (pH = 3) for 15 minutes and rinsing with phosphate-buffered saline (pH = 7.3). A human monoclonal antibody to IL-2 (Chemicon International, Temecula, CA) was applied in a dilution of 1:400 and then incubated for 2 hours at 37°C. The buffer was rinsed and a secondary antibody was applied. Strep-avidin conjugated horseradish peroxidase and diaminobenzidine were applied.

The slides were then counterstained with hematoxylin, and a coverslip was placed. Slides were then evaluated for staining using light microscopy. Positive samples displayed granular membrane or cytoplasmic staining. Samples were evaluated for the extent of staining and the intensity of staining (i.e., none, low, high) according to standard methods. Statistical analysis was performed with the Mann-Whitney *U* test. Significance was reached with a *P* value of $<.05$.

RESULTS

A total of 60 specimens were evaluated for this study. Of the 30 specimens with abnormal tubal architecture, 21 (70%) revealed hydrosalpinges, and 9 (30%) revealed chronic salpingitis. All the control samples (n = 30) demonstrated normal tubal architecture. The phase of cycle distribution as classified by the Noyes, Hertig, and Rock dating system (16) is listed in Table 1.

TABLE 1

Sample distribution by endometrial histology.

Endometrial histology	Cases (n = 30)	Controls (n = 30)
Proliferative	15 (50%)	12 (40%)
Secretory	6 (20%)	8 (27%)
Atrophic	3 (10%)	5 (17%)
Menstrual	1 (3%)	0 (0%)
Hyperplasia	2 (6%)	3 (10%)
Chronic endometritis	3 (10%)	0 (0%)

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In analysis of tubal specimens, cases were found to have a statistically significant increase in inflammatory cells on evaluation of H&E stained slides (106 ± 123.3) versus controls (29.2 ± 22.7) ($P<.001$). Specifically, a statistically significant increase in the number of neutrophils ($P=.04$), lymphocytes ($P=.003$), plasma cells ($P<.004$), and macrophages ($P=.01$), was encountered. No difference was found in the number of eosinophils or basophils (Table 2).

Examination of the H&E stained slides of the endometrium demonstrated a statistically significant increase in the overall number of inflammatory cells in cases (79.9 ± 133.4) versus controls (34.9 ± 40.3) ($P=.08$). There was a statistically significant difference in the number of neutrophils ($P=.04$) and basophils ($P=.04$). No difference was found in the numbers of lymphocytes, plasma cells, eosinophils, or macrophages (Table 3).

Immunohistochemical staining for IL-2 demonstrated high-intensity staining in only 7.4% of controls versus 65% of cases ($P=.0001$), while low-intensity staining was observed in 67% of controls versus 20% of cases ($P=.001$) (see Fig. 1). No staining was observed in 15% of cases versus 26% of controls (nonsignificant).

DISCUSSION

Considerable data suggest that the presence of hydrosalpinges is detrimental to reproductive outcome (17). It is unclear whether this deleterious effect is the result of direct embryotoxicity, an inflammatory response within the endometrial cavity, or a combination of both. An optimal relationship between the embryo and endometrium is essential for optimizing embryo implantation, and is mediated by the secretion and expression of a precise sequence of cytokines and other mediators during the implantation window.

During this period, the endometrium expresses a variety of proteins, many of which have been evaluated as potential

TABLE 2

Inflammatory cells in tubal specimens.

Cell type	Cases (n = 30)	Controls (n = 30)	*P* value
Neutrophils	10.20 ± 26.9	0.1 ± 0.4	.0006[a]
Lymphocytes	82.70 ± 94.2	28.0 ± 21.9	.0025[a]
Plasma cells	11.40 ± 19.9	0.5 ± 1.2	<.0001[a]
Eosinophils	0.70 ± 2.6	0.4 ± 1.2	.84
Basophils	0.70 ± 1.2	0.1 ± 0.4	.06
Macrophages	1.17 ± 2.8	0.1 ± 0.4	.0329[a]
Total cells	106.80 ± 123.3	29.2 ± 22.7	.0005[a]

Note: Values are mean ± SD.
[a] $P<.05$.

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markers of endometrial receptivity (18–20). Optimal implantation appears to occur in the absence of acute and chronic inflammation, thus we chose to also evaluate IL-2 as a common marker for inflammation in this study. We have demonstrated for the first time that there is a defined, identifiable, local response in the endometrium that correlates with the presence of hydrosalpingeal fluid. This response consists of statistically significant elevations of inflammatory cells, specifically, an increase in neutrophils and inflammatory cytokines.

Implantation is a complex, chemically regulated process that requires synchronization between the developing embryo and differentiating endometrium (21). Numerous studies have investigated potential markers of endometrial receptivity as predictors of successful implantation and, in doing so, have helped to define the cellular and molecular mechanisms by which implantation occurs. Cell adhesion molecules (integrin, mucin, and trophinin), pinopodes, cytokines, L-selectin ligands, homeobox (HOX) genes, growth factors, matrix metalloproteinases, and their inhibitors are reportedly involved in appositioning and attachment (22). Daftary and Taylor (23) demonstrated a decrease in HOXA10 expression in the endometrium of patients with hydrosalpinx and described it as one possible mechanism by which hydrosalpinx fluid adversely affects implantation rates.

Recent studies indicate that integrin $\alpha v\beta 3$ binds to and activates matrix metalloproteinases and plasminogen activators in the extracellular matrix. This enables the integrin to act both as a receptor for the embryo at the endometrial surface epithelium and as a stimulator of trophoblastic penetration and invasion (24). Meyer et al. (25) demonstrated decreased endometrial integrin $\alpha v\beta 3$ expression in patients with hydrosalpinx, and found that 70% of the patients had an increase of integrin expression after surgical correction.

Other biomarkers of presumed importance to human implantation are continually being discovered and further studied (18, 26). In 1963, Clyman (27) demonstrated the appearance of mitochondria and nucleolar channel systems in the endometrium before implantation; however, their presence has only been demonstrated to provide an indication of ovulation, and has not been associated with pregnancy out-

TABLE 3

Inflammatory cells in endometrial specimens.

Cell type	Cases, (n = 30)	Controls, (n = 30)	*P* value
Neutrophils	6.8 ± 16.4	0.5 ± 1.5	<.0001[a]
Lymphocytes	50.6 ± 87.9	31.6 ± 37.5	.15
Plasma cells	20.3 ± 92.8	0.2 ± 1.3	.23
Eosinophils	0.4 ± 1.6	0.2 ± 0.8	.5
Basophils	0.4 ± 1.1	0.0 ± 0	.04[a]
Macrophages	1.3 ± 1.8	2.4 ± 3.3	.09
Total cells	79.9 ± 133.4	34.9 ± 40.3	.0315[a]

Note: Values are mean ± SD.
[a] $P<.05$.

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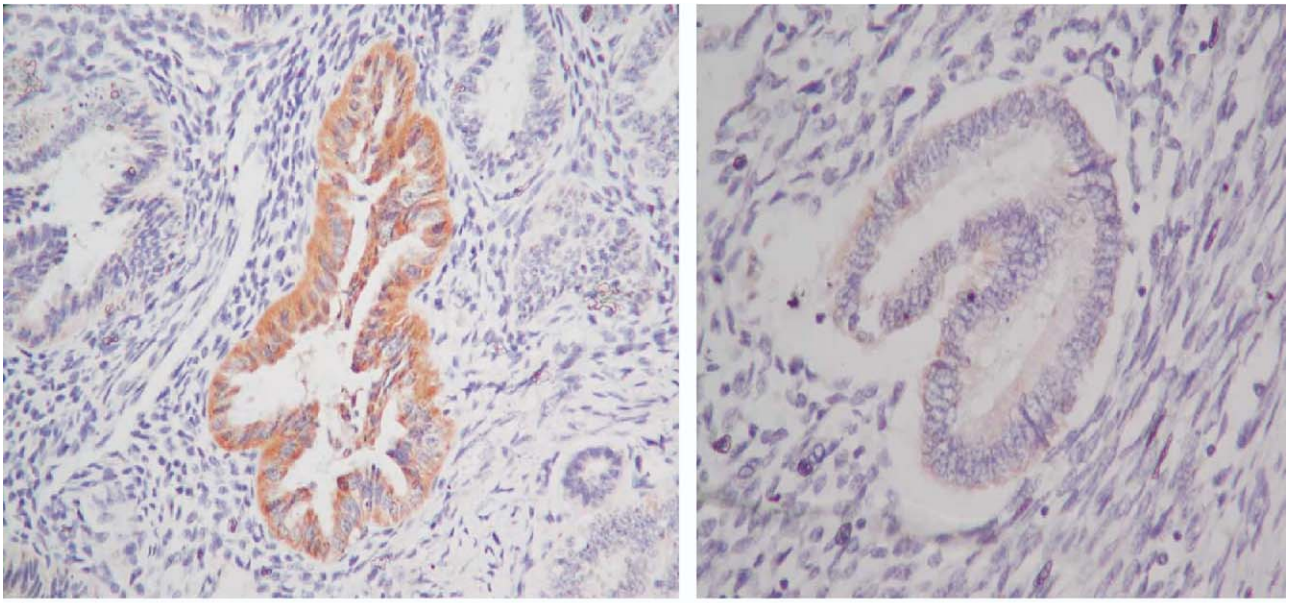
FIGURE 1

High-intensity immunohistocytochemistry staining for IL-2 staining in a patient with bilateral hydrosalpinges (**A**) compared with low-intensity staining in a control (**B**).



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come. Other potential markers of endometrial receptivity studied are pinopodes, which are structures composed of microvilli on the apical surface of the luminal endometrial epithelium (19), which have been positively associated with pregnancy in patients undergoing oocyte donation. Repeated implantation failure was correlated to the absence of pinopodes in the endometrium of these recipients (28).

Various studies report both beneficial and toxic effects of hydrosalpinx fluid on endometrial development. Certain cytokines have a proliferative effect on the endometrium and are known to be produced in the human reproductive tract (leukemia inhibitory factor [LIF], epithelial growth factor [EGF], granulocyte macrophage-colony stimulating factor [GM-CSF]) (29). It has been demonstrated that the presence of these cytokines was beneficial for preimplantation development by increasing the proportion of embryos developing to blastocysts in vitro (29). However, other deleterious primary inflammatory cytokines, such as IL-8, IL-12, IL-α, and tumor necrosis factor-α (TNF-α) have been commonly found in hydrosalpinx fluid (1).

The T helper-1 (Th-1) type cells and T helper-2 (Th-2) type cells are the major subsets of fully differentiated $CD4^+$ T cells, and their specific functions in immune responses correlate with their distinctive cytokine secretion (30). In humans, Th-1 cells synthesize mainly IL-2, TNF-α, and interferon-γ (IFN-γ), whereas Th-2 cells produce IL-4, IL-5, IL-6, and IL-10 (30). The deleterious role of Th-1 cytokines has been proven with IL-2 (31) and with IFN-γ, IL-12, and TNF-β (32). These cytokines have been demonstrated to be detrimental to pregnancy, through direct embryotoxicity, damage to the placental trophoblast, or possibly by activating cells that are deleterious to the conceptus, whereas Th-2 cytokines may directly or indirectly contribute to the success of pregnancy by down-regulating potential Th-1 activity (33–35). Thus, the disruption of any of these pathways may lead to impaired implantation.

Because Th-1 cytokines have been associated to pregnancy rejection, we decided to retrospectively determine the presence of IL-2 in the endometrium of specimens with tubal disease to establish the possible role that hydrosalpingeal fluid plays within the endometrial environment and implantation process. In our study, an increase in IL-2 was observed in patients with active tubal inflammation. The presence of local inflammatory mediators in the endometrium exposed to hydrosalpingeal fluid may lend credence to the notion that increased levels of IL-2 in the endometrial milieu contribute to the inhibition of embryo implantation.

Endometrial receptivity is a temporally and spatially regulated set of circumstances within the endometrium that facilitates successful embryonic implantation. Our findings are consistent with those of previous studies suggesting that hydrosalpinges alter endometrial receptivity. The mechanism of improved outcome following salpingectomy may be attributed to restoration of the normal endometrial endocrine

milieu. However, further studies are required to understand the exact action of these mediators and determine their utility as biochemical markers of endometrial receptivity, as well as establish the possible mechanisms to down-regulate the expression of deleterious cytokines.

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