

Are cytokines and growth factors responsible for the detrimental effects of hydrosalpingeal fluid on pregnancy rates after in vitro fertilization-embryo transfer?

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Objective: To characterize the secretion of cytokines and growth factors in hydrosalpingeal fluid.

Design: Retrospective analysis.

Setting: Hospital-based infertility practice.

Patient(s): Ten infertile women who underwent laparoscopic aspiration of their hydrosalpingeal fluid before salpingectomy or neosalpingostomy.

Intervention(s): Samples were cryopreserved, then thawed and centrifuged to remove cellular debris.

Main Outcome Measure(s): The supernatants were analyzed for the presence of human interferon- γ , epidermal growth factor, transforming growth factor- $\beta 2$, and tumor necrosis factor- α by quantitative enzyme immunoassay kits.

Result(s): Interferon- γ and transforming growth factor- $\beta 2$ were not detected in any of the hydrosalpingeal fluid samples. Epidermal growth factor was present in 5 of 10 hydrosalpingeal fluid samples, with a mean (\pm SE) concentration of 26.7 ± 11.4 pg/mL. Tumor necrosis factor- α was detected in 7 of 10 samples, with a mean (\pm SE) concentration of 6.2 ± 3.6 pg/mL. Three of the 10 samples contained both tumor necrosis factor- α and epidermal growth factor.

Conclusion(s): For the first time, we described the absence of interferon- γ and transforming growth factor- $\beta 2$, and the presence of epidermal growth factor and tumor necrosis factor- α in human hydrosalpingeal fluid. Because the fundamental role of the human fallopian tube is secretory in nature, the alteration in substances secreted from the tubal epithelium that reflux into the uterine cavity may explain the deleterious effects that hydrosalpingeal fluid has on pregnancy rates after IVF-ET. (Fertil Steril® 1999;72:1110-2. ©1999 by American Society for Reproductive Medicine.)

Key Words: Hydrosalpinx, in vitro fertilization, growth factors

Distal tubal obstruction recently has been implicated as a negative prognostic factor for patients undergoing IVF-ET (1). It has been proposed that the deleterious effect of a communicating hydrosalpinx on implantation and pregnancy rates in patients undergoing IVF-ET may be due to an alteration in endometrial receptivity and/or a direct embryotoxic effect. Meyer et al. (2) demonstrated that $\alpha v \beta 3$ integrin expression, a marker of endometrial receptivity, was significantly reduced in patients with hydrosalpinges compared with controls. In addition, a number of in vitro murine embryogenesis studies have demonstrated a re-

duction in rates of blastulation when mouse embryos are exposed to varying concentrations of hydrosalpingeal fluid (3-6).

Although it is postulated that the damaged tubal fluid contains substances that hinder embryonic and/or endometrial development, remarkably little is known about the characterization of these substances. Two recent studies noted an alteration in the content of electrolytes, glucose, and proteins in hydrosalpingeal fluid (3, 6). Because cytokines and growth factors have been associated with inflammatory processes and involved in embryotoxic and embryotrophic effects, we hypothesized that

Received March 25, 1999;
revised and accepted July
7, 1999.

Presented at the 16th
World Congress on Fertility
and Sterility and the 54th
Annual Meeting of the
American Society for
Reproductive Medicine,
San Francisco, California,
October 4-9, 1998.

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0015-0282/99/\$20.00
PII S0015-0282(99)00422-7

their balance in hydrosalpingeal fluid might explain the poor outcome of IVF-ET in women with distally occluded fallopian tubes. Thus, we attempted to characterize the presence of interferon- γ (IFN- γ), epidermal growth factor (EGF), tumor necrosis factor- α (TNF- α), and transforming growth factor- β 2 (TGF- β 2) in hydrosalpingeal fluid.

MATERIALS AND METHODS

Ten infertile women with known hydrosalpinges underwent laparoscopy for either salpingectomy before IVF or neosalpingostomy. All patients had cervical cultures that were negative for *Chlamydia*. At the time of laparoscopy, 1–6 mL of hydrosalpingeal fluid was collected from each patient by needle aspiration before the indicated procedure. In patients with bilateral hydrosalpinges, the fluid was pooled into one sample. The hydrosalpingeal fluid then was centrifuged at $1,000 \times g$ for 5 minutes to remove cellular debris and stored at -70°C until analysis. Institutional review board approval was obtained by the committee at the Crozer-Chester Medical Center for the use of discarded materials.

The hydrosalpingeal fluid supernatants were analyzed for the presence of human IFN- γ , EGF, TGF- β 2, and TNF- α by quantitative enzyme immunoassay kits (Quantikine Immunoassays; R&D Systems, Minneapolis, MN) using ligand-specific monoclonal antibodies. All samples were placed in duplicate in 96-well ELISA plates (Corning, Corning, NY) in parallel with known standards and analyzed in a single assay. The intraassay variability for all cytokines ranged from 1.8%–3.6%. The sensitivities of the assays for IFN- γ , EGF, TGF- β 2, and TNF- α were <3 pg/mL, <0.7 pg/mL, <7 pg/mL, and <0.18 pg/mL, respectively, with ranges of 15.6–1,000 pg/mL, 3.9–250 pg/mL, 31.2–2,000 pg/mL, and 0.5–32.0 pg/mL, respectively. Nonlinear regression analysis (polynomial second and third order) was used to evaluate statistically significant relations between cytokine levels and hydrosalpingeal fluid volumes.

RESULTS

Interferon- γ and TGF- β 2 were not detected in any of the hydrosalpingeal fluid samples. Epidermal growth factor was present in 5 of 10 hydrosalpingeal fluid samples, with a mean (\pm SE) concentration of 26.7 ± 11.4 pg/mL (range, 0–110 pg/mL). Tumor necrosis factor- α was detected in 7 of 10 samples, with a mean (\pm SE) concentration of 6.2 ± 3.6 pg/mL (range, 0–34.9 pg/mL). Our findings are depicted in Table 1, which also includes the laterality and volume of the hydrosalpingeal fluid samples. Three of the 10 samples contained both TNF- α (volume: $R^2 = 0.03$) and EGF (volume: $R^2 = 0.02$). In this small sample, nonlinear regression analysis did not show any statistically significant relation between the cytokine levels and hydrosalpingeal fluid volumes.

TABLE 1

Concentrations of TNF- α , EGF, TGF- β 2, and IFN- γ in hydrosalpingeal fluid as determined by ELISA.

Patient no.	TNF- α (pg/mL)	EGF (pg/mL)	TGF- β 2 (pg/mL)	IFN- γ (pg/mL)	Laterality	Volume (mL)
1	3.2	ND	ND	ND	Unilateral	2
2	4.2	19	ND	ND	Bilateral	6
3	34.9	110	ND	ND	Unilateral	2
4	3.1	ND	ND	ND	Unilateral	3
5	ND	7.5	ND	ND	Unilateral	2
6	ND	ND	ND	ND	Unilateral	2
7	4.9	ND	ND	ND	Unilateral	1
8	2.2	41	ND	ND	Unilateral	3
9	2.6	ND	ND	ND	Bilateral	3
10	ND	49.5	ND	ND	Bilateral	4

Note: ND = not detected.

Barmat. Cytokines growth factors. Fertil Steril 1999.

DISCUSSION

We demonstrated for the first time the presence of the multifunctional proteins EGF and TNF- α , as well as the absence of IFN- γ and TGF- β 2, in aspirated hydrosalpingeal fluid. Because the fundamental role of the human fallopian tube is secretory in nature, the alteration in substances secreted from the tubal epithelium that reflux into the uterine cavity may explain the deleterious effects that hydrosalpingeal fluid has on pregnancy rates after IVF-ET.

Tumor necrosis factor- α was detected in 70% of the hydrosalpingeal fluid samples. This potent cytokine, originally identified as a product of activated macrophages, is now known to be produced by many types of cells. In humans, the TNF transcripts and proteins have been identified in the oviduct and other reproductive tract cells (7). The effects of this cytokine on preimplantation embryo development have not been fully elucidated. In a review article by Haimovici and Anderson, it was noted that some studies have demonstrated the inhibition of both gamete function and the development of murine embryos, whereas other studies have failed to show detrimental effects (8).

We detected the presence of EGF in 50% of our patients' hydrosalpingeal fluid samples. A number of studies have demonstrated menstrual stage-specific expression of EGF ligand and receptors from reproductive tract epithelium, as well as the presence of EGF receptors on embryos (reviewed in (9)). Further, the presence of this mitogenic growth factor in embryo culture medium has been shown to have beneficial effects on preembryo quality, whereas the administration of antibodies to EGF to pregnant mice has increased abortion rates (reviewed in (9)). Therefore, the absence of EGF from half the hydrosalpingeal fluid samples may reflect a lack of production by damaged tubal epithelium resulting in a relative decrease in an embryotrophic factor that could lead to a decrease in pregnancy rates.

Interferon- γ and TGF- β 2 were not detected in the hydrosalpingeal fluid samples. Interferon- γ , a cytokine produced primarily by T lymphocytes and natural killer cells, is predominantly known for its numerous immunologic properties. This cytokine has been detected in human preimplantation culture medium, although the role it plays in early embryo development is currently unknown (10). Although the presence and role of IFN- γ in cases of fetal loss has been investigated, little is known about its presence or absence in the normal oviduct. The synthesis and secretion of TGF- β 2 has been demonstrated in the human fallopian tube and has been suggested to play an important role in gamete maturation and early embryo development (11). The inability of the assay to detect the presence of TGF- β 2 may reflect the damage inflicted on the tubal epithelium as a result of the underlying pathologic process.

Several questions remain to be answered regarding the dynamic interactions that occur at the cellular and molecular levels between the preimplantation embryo and its in vivo environment. The current understanding of growth factor and cytokine expression in the oviduct is based on the analysis of gene expression or protein translation. Because of the dynamic nature of the fallopian tube, lack of static fluid within the fallopian tube, and obvious logistic difficulties in obtaining fluid from nonobstructed oviducts, the literature is unfortunately devoid of normal oviductal secretory concentrations of various growth factors and cytokines. Therefore, although we were able to characterize for the first time the presence or absence of multifunctional proteins in hydrosalpingeal fluid, we could not compare this to a control population of nonobstructed fallopian tubes. This study also demonstrated the heterogeneous nature of hydrosalpingeal fluid,

which may be due to normal interpatient variations as well as to the cause of the hydrosalpinx. Further studies are ongoing at our center to identify the alterations in hydrosalpingeal fluid that may have deleterious effects on human preembryo development, implantation, and ultimate pregnancy rates in patients undergoing IVF.

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