Serum levels of soluble vascular cell adhesion molecule-1, tumor necrosis factor-α, and interleukin-6 in in vitro fertilization cycles

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Objective: To assess whether gonadotropin-induced changes in E2 alter serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1) and proinflammatory cytokines.

Design: Prospective collection of serum in patients undergoing IVF.

Setting: University hospital.

Patient(s): Twenty-four infertile women.

Intervention(s): Serum collection at baseline, in the mid and late follicular phases, at oocyte retrieval, and in the mid and late luteal phases.

Main Outcome Measure(s): Samples were assayed for sVCAM-1, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and E2.

Result(s): The VCAM-1 was maximally suppressed in the luteal phase. Luteal sVCAM-1 levels correlated [1] positively with the patient’s age, units of gonadotropins, day 3 FSH levels and [2] negatively with [a] the follicular, retrieval, and luteal E2 levels and [b] the number of preovulatory follicles and oocytes retrieved. Similar correlations were noted in the late luteal phase. Serum TNF-α reached a peak in the mid-follicular phase and a nadir in the luteal phase. The TNF-α levels at retrieval correlated [1] positively with the patient’s age and [2] negatively with E2 and number of preovulatory follicles and retrieved oocytes. The IL-6 levels were suppressed in the follicular phase and correlated negatively with E2 levels.

Conclusion(s): Changes in E2 levels seen during gonadotropin stimulation significantly alter VCAM-1 expression and induce changes in serum IL-6 and TNF-α levels. (Fertil Steril 2009;91:2012–9. ©2009 by American Society for Reproductive Medicine.)

Key Words: Soluble vascular adhesion molecule-1, tumor necrosis factor-α, interleukin-6, E2, in vitro fertilization.

Inflammation has a prominent role in the pathophysiology of atherosclerotic disease promoting the genesis and progression of atherosclerotic lesions and mediating all stages of this disease (1–5). Various circulating inflammatory molecules have garnered significant interest as markers of atherosclerotic risk and few have been shown to predict the development of cardiovascular disease. These markers of systemic inflammation include soluble adhesion molecules (e.g., vascular cell adhesion molecule-1 [VCAM-1]), proinflammatory cytokines, and other acute phase reactants (e.g., C-reactive protein) (6). Vascular cell adhesion molecule-1 is a transmembrane glycoprotein that plays a major role in leukocyte recruitment enhancing adhesion of monocytes to the vascular endothelium (7, 8) and advancing the progression of the atherosclerotic process (9–13). Several studies revealed an association between increased expression of VCAM-1 and increased formation of atherosclerotic plaques (14–16).

The active soluble form of VCAM-1 (sVCAM-1), shed from the cell surface and detected in the peripheral blood and various other biological fluids, retains its biological activity, potentially mediating immune and inflammatory reactions (9, 10, 17–22). Its concentration in the plasma correlates with the level of its cellular expression (21).

In vascular endothelial cells, the expression of VCAM-1 is up-regulated by various cytokines (22) including tumor necrosis factor-alpha (TNF-α) (23–26), a nonglycosylated protein that belongs to the TNF-α superfamily (27). In addition, increasing scientific evidence suggests that interleukin-6 (IL-6)—a cytokine with proinflammatory and proatherogenic activity—promotes endothelial cell adhesiveness by up-regulating VCAM-1 (and other cell adhesion molecules) (6, 28).
The IL-6, TNF-α, and other proinflammatory cytokines are expressed in human atherosclerotic lesions, and several epidemiological studies have shown that basal levels of proinflammatory markers, such as TNF-α and IL-6, are elevated in subjects at increased risk for cardiovascular atherothrombotic events (29–32).

The precise role of E2 in modulating the expression of VCAM-1 in humans and the molecular pathways through which E2 achieves its effect remain largely unknown. However, various studies in pre- and postmenopausal women receiving different formulations of estrogenic compounds have indicated that circulating levels of soluble cell adhesion molecule levels are reduced in users of exogenous estrogens (E) (33–40). These findings are supported by in vitro studies and animal experiments indicating that E may modulate the cytokine-induced cell expression of VCAM-1 (14, 41–46). It is therefore speculated that E2 influences the atherosclerotic progression by regulating the inflammatory component of the atherogenic process and by inhibiting the expression of VCAM-1 protein on the surface of the vascular endothelium, thus inhibiting monocyte adhesion.

The purpose of the present study was to evaluate the effects of acute, short-term, gonadotropin-induced changes in endogenous E2 on the serum levels of sVCAM-1 throughout the various phases of an IVF cycle. We hypothesized that the prolonged cumulative exposure to “supraphysiologic” serum levels of endogenous E2 would have a suppressive effect on the serum levels of sVCAM-1. The secondary objective of our study was to assess the changes in the circulating levels of proinflammatory cytokines, such as TNF-α and IL-6, which are thought to modulate the expression of VCAM-1 in vivo.

MATERIALS AND METHODS

Subjects

The Institutional Review Board (IRB) of the University of California Los Angeles approved the study protocol and all patients gave written informed consent before participation in the study.

Our study group consisted of 24 infertile women (mean age ± SD: 36.0 ± 4.4 years, range 30–43 years; mean body mass index [BMI] ± SD: 24.7 ± 5.2 kg/m²) who were enrolled in the study prospectively and underwent gonadotropin stimulation for IVF at our infertility unit.

At study entry, women were excluded if they had a history of cardiovascular disease, diabetes, cancer, or a venous thromboembolic disorder. Patients who either had a recent acute systemic infection or were using on a regular basis antihypertensive, antithrombotic, or lipid-lowering drugs, were excluded. No subjects had a history of immune system-related disorders, and all were nonsmokers. Participants were encouraged to maintain their usual diet and exercise routine.

Sample Collection

Serum samples were collected at the following time points: [1] early in the follicular phase (EF) (day 2 or 3 of the stimulated cycle) at the time of the baseline ultrasound, before the initiation of gonadotropins; [2] twice in the midfollicular phase: [a] one time after 4–5 days of gonadotropin treatment (day 7 or 8 of the stimulated cycle—phase MF1) and [b] a second time after 6–7 days of gonadotropin treatment (day 9 or 10 of the cycle—phase MF2); [3] in the late follicular phase (LF) at which time the decision was made to administer hCG; [4] at the time of oocyte retrieval (OR); [5] in the midluteal phase of the stimulated cycle (Lut), 8 days after oocyte retrieval; and [6] in the late luteal phase (LLut), 11–12 days after oocyte retrieval and 2–3 days before the scheduled pregnancy test.

All blood samples were collected early in the morning by venipuncture. The samples collected for the determination of E2 were assayed immediately (to monitor the response to gonadotropins). The blood samples collected for the determination of sVCAM-1, TNF-α, and IL-6 levels were allowed to clot in serum tubes coated with retraction accelerator, and then were immediately centrifuged at 3,000 x g for 10 minutes at 4 °C. Serum was aspirated and stored frozen in aliquots at -20 °C, until assayed collectively for sVCAM-1, TNF-α, and IL-6.

Laboratory Measures

Serum concentrations of sVCAM-1, TNF-α, and IL-6 were determined using commercially available kits (Human soluble VCAM-1 Parameter Elisa kit; R and D Systems, Minneapolis, MN; Human Tumor Necrosis Factor-alpha Ultra Sensitive Elisa Kit and Human Interleukin-6 Ultra Sensitive Elisa Kit; Bio Source International, Camarillo, CA). The sVCAM-1 assay uses the quantitative sandwich enzyme immunoassay technique. It reacts with two monoclonal

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Epidemiologic characteristics and IVF parameters of the study population.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>36.0 ± 4.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.9 ± 5.2</td>
</tr>
<tr>
<td>Infertility diagnosis</td>
<td>Decreased ovarian reserve: 28.6%</td>
</tr>
<tr>
<td>Male factor</td>
<td>31.4%</td>
</tr>
<tr>
<td>Anovulation</td>
<td>5.7%</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>14.3%</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>8.6%</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>2.8%</td>
</tr>
<tr>
<td>Other</td>
<td>8.6%</td>
</tr>
<tr>
<td>Day 3 serum</td>
<td>7.9 ± 4.1</td>
</tr>
<tr>
<td>FSH (IU/mL)</td>
<td>2,956 ± 1,238</td>
</tr>
<tr>
<td>Gonadotropins used</td>
<td>1,768 ± 1,053</td>
</tr>
<tr>
<td>Retained oocytes (n)</td>
<td>13.5 ± 15</td>
</tr>
<tr>
<td>Peak E2 (pg/mL) at hCG administration</td>
<td>2,670 ± 2,606</td>
</tr>
<tr>
<td>Pregnancies/cycle initiated</td>
<td>7/24 (29.2%)</td>
</tr>
</tbody>
</table>

antibodies specifically directed against two different epitopes on human sVCAM-1. The TNF-α and IL-6 assays are solid phase sandwich ELISAs. Serum levels of E2 were measured by the Roche Elecsys 2010 immunoassay analyzer (Roche Diagnostics, Indianapolis, IN). The Elecsys-Estradiol II assay is a competitive assay that uses a polyclonal antibody specifically directed against 17-β E2 (detection limit, 18.4 pmol/L; intra-assay coefficient of variation [CV], 3.6%). All procedures were performed using the manufacturer's instructions.

Statistics

Data are expressed as mean ± SD.

Correlations between variables and between times for the same variable were computed using both nonparametric (Spearman rank) and parametric (Pearson) correlation methods. For the parametric analyses E2 was log transformed, as log E2 is more closely approximated by a normal distribution. To assess the effect of cumulative exposure to high serum levels of E2 on the serum levels of sVCAM-1, we also calculated the cumulative log E2, which was defined as the amount of E2 (on the log scale) that the patient had been exposed to, up to a specific phase of the cycle (for example, cum log E2 Lut = log E2 EF + log E2 MF1 + log E2 MF2 + log E2 LF + log E2 OR + log E2 Lut). This was an approximate estimate of the area under the curve, the calculation of which would have required daily E2 values. Mean serum levels of sVCAM-1, TNF-α, and IL-6 were compared across different phases of the cycle using a repeated measures analysis of variance model. For this analysis, IL-6 was log transformed.

Statistical differences of $P < .05$ were considered significant.

RESULTS

Table 1 summarizes the characteristics of the study population. The mean serum concentrations of sVCAM-1, TNF-α, and IL-6 across the entire IVF cycle were within previously reported limits (9, 47–51).

All study patients responded to gonadotropins with multiple follicular development and increased serum E2 levels (mean peak serum E2 levels ± SD: 2,670 ± 2,606 pg/mL in the late follicular phase). All patients underwent a successful oocyte retrieval.
Serum levels of sVCAM-1 were significantly higher at baseline (EF), and in the follicular phases (MF1, MF2, and LF) compared to the Lut and LLut phases, reaching a peak in the MF2 and being maximally suppressed in the luteal phase of the IVF cycle (P = .0006 for MF2 vs. Lut and P = .0005 for MF2 vs. LLut; Fig. 1A). In the luteal phase, serum levels of sVCAM-1 correlated positively with the patient’s age, serum day 3 FSH levels, and number of gonadotropin ampules used for stimulation, whereas it correlated negatively with the number of preovulatory follicles and oocytes retrieved (Table 2). Patients with more than five oocytes retrieved had significantly lower levels of sVCAM-1 in the luteal phase compared with those with a poor oocyte yield (≤5 oocytes retrieved) (371.1 ± 82.9 ng/mL vs. 490.6 ± 103.41 ng/mL and 3,189.7 ± 2,815.1 ng/mL vs. 1,111 ± 649.6 pg/mL for sVCAM-1 and peak E2 levels, respectively, with P < .05 for both comparisons). In the LLut phase, the correlations of sVCAM-1 with these variables were in the same direction but considerably weaker.

As expected, there was a significant inverse correlation between the serum levels of sVCAM-1 in the luteal phase and the mean serum levels of E2 in the various preceding phases (MF1, MF2, LF, OR) and the luteal phase of the IVF cycle (for P and r values, see Table 2). A similar inverse correlation was noted between the serum levels of sVCAM-1 in LLut and those of E2 in the preceding phases (MF1, MF2, LF, OR, Lut) and the Lut phase, reaching statistical significance in the MF1, retrieval, luteal, and late luteal phase (for P and r values, see Table 2).

Serum levels of TNF-α were higher in the follicular (EF, MF1, MF2, and LF) compared with the Lut and LLut phases, reaching a peak in MF1 and a nadir in the luteal phase of the IVF cycle (Fig. 1B). Moreover, the serum levels of TNF-α at retrieval correlated positively with the patient’s age (r = .52, P = .034) and negatively with the number of [1] preovulatory follicles (r = -.67, P = .004) and [2] retrieved oocytes (r = -.59, P = .015; for all correlations, see Fig. 2). Interestingly, there was a significant inverse correlation between the serum TNF-α levels at retrieval and those of E2 in both the same (retrieval phase: r = -.53, P = .034; Fig. 2) and the preceding follicular phase of the cycle (r = -.52, P = .04 for TNF-α at retrieval vs. cumulative E2 at LF).

Mean log IL-6 levels were significantly lower in the follicular (MF1, MF2, LF) compared with the luteal and late luteal phase of the IVF cycle (P < .05 for MF1, MF2, and LF vs. Lut and LLut, see Fig. 1C and Table 3). In the follicular phase (MF2, LF), serum levels of IL-6 correlated negatively with the serum levels of E2 (r = -.52, P = .016 and r = -.45, P = .04 for serum IL-6 vs. E2 and cumulative E2 in the late follicular phase, respectively, and r = -.64, P = .0072, and r = -.48, P = .057 for serum IL-6 vs. E2 and cumulative E2 in the MF2, respectively). Interestingly, the preovulatory IL-6 levels (LF) correlated inversely with the E2 in the

### Table 2

<table>
<thead>
<tr>
<th>Cycle phase</th>
<th>Variable</th>
<th>Spearman rho</th>
<th>P value</th>
<th>Pearson rho</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sVCAM-1 Lut</td>
<td>Age (y)</td>
<td>0.51</td>
<td>.0183*</td>
<td>0.45</td>
<td>.041*</td>
</tr>
<tr>
<td>sVCAM-1 Lut</td>
<td>Day 3 FSH</td>
<td>0.53</td>
<td>.0492*</td>
<td>0.56</td>
<td>.038*</td>
</tr>
<tr>
<td>sVCAM-1 Lut</td>
<td>No. of gonadotropin ampules</td>
<td>0.57</td>
<td>.0071*</td>
<td>0.5</td>
<td>.022*</td>
</tr>
<tr>
<td>sVCAM-1 Lut</td>
<td>No. of follicles</td>
<td>-0.52</td>
<td>.0161*</td>
<td>-0.44</td>
<td>.043*</td>
</tr>
<tr>
<td>sVCAM-1 Lut</td>
<td>No. of oocytes retrieved</td>
<td>-0.52</td>
<td>.0166*</td>
<td>-0.46</td>
<td>.03*</td>
</tr>
<tr>
<td>sVCAM-1 Lut</td>
<td>Cumulative log E2 at MF1</td>
<td>-0.81</td>
<td>.0007*</td>
<td>-0.75</td>
<td>.003*</td>
</tr>
<tr>
<td>sVCAM-1 Lut</td>
<td>Cumulative log E2 at MF2</td>
<td>-0.92</td>
<td>.0005*</td>
<td>-0.76</td>
<td>.018*</td>
</tr>
<tr>
<td>sVCAM-1 Lut</td>
<td>Cumulative log E2 at LF</td>
<td>-0.92</td>
<td>.0005*</td>
<td>-0.76</td>
<td>.017*</td>
</tr>
<tr>
<td>sVCAM-1 Lut</td>
<td>Cumulative log E2 at OR</td>
<td>-0.83</td>
<td>0.0102*</td>
<td>-0.74</td>
<td>0.035*</td>
</tr>
<tr>
<td>sVCAM-1 Lut</td>
<td>Cumulative log E2 Lut</td>
<td>-0.75</td>
<td>0.0522</td>
<td>-0.79</td>
<td>0.036*</td>
</tr>
<tr>
<td>sVCAM-1 LLut</td>
<td>Cumulative log E2 MF1</td>
<td>-0.65</td>
<td>0.0153*</td>
<td>-0.49</td>
<td>0.085</td>
</tr>
<tr>
<td>sVCAM-1 LLut</td>
<td>Cumulative log E2 MF2</td>
<td>-0.63</td>
<td>0.0671</td>
<td>-0.44</td>
<td>0.24</td>
</tr>
<tr>
<td>sVCAM-1 LLut</td>
<td>Cumulative log E2 LF</td>
<td>-0.63</td>
<td>0.0671</td>
<td>-0.43</td>
<td>0.25</td>
</tr>
<tr>
<td>sVCAM-1 LLut</td>
<td>Cumulative log E2 OR</td>
<td>-0.86</td>
<td>0.0137*</td>
<td>-0.67</td>
<td>0.1</td>
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<tr>
<td>sVCAM-1 LLut</td>
<td>Cumulative log E2 Lut</td>
<td>-0.83</td>
<td>0.0416*</td>
<td>-0.79</td>
<td>0.058</td>
</tr>
<tr>
<td>sVCAM-1 LLut</td>
<td>Cumulative log E2 LLut</td>
<td>-0.83</td>
<td>0.0416*</td>
<td>-0.82</td>
<td>0.045*</td>
</tr>
</tbody>
</table>

Note: LF = late follicular phase; MF1 = day 7 or 8 of the stimulated cycle; MF2 = day 9 or 10 of the stimulated cycle; OR = time of oocyte retrieval; sVCAM-1 = soluble vascular cell adhesion molecule-1.

* = Statistically significant.

preceding phase (MF2) as well ($r = -0.59$, $P = .015$ and $r = -0.48$, $P = .032$ for serum IL-6 in the late follicular phase vs. E2 and cumulative E2 in the preceding MF2 phase, respectively). Accordingly, a nearly significant trend toward lower late follicular IL-6 levels was noted in younger patients and those with higher number of preovulatory follicles and retrieved oocytes ($r = -0.4$, $P = .065$ and $r = 0.39$, $P = .078$ for age and number of follicles, respectively).

Patients with male factor infertility as the sole underlying diagnosis did not significantly differ from the ones with female or unexplained factors in age, baseline FSH, peak E2 levels, medication used, and number of oocytes retrieved ($P > .05$ for all comparisons). In addition to this, serum levels of sVCAM-1, TNF-$\alpha$, and IL-6 did not differ significantly between the two groups. Women that received oral contraceptive pills (OCPs) before stimulation did not differ from the ones who did not in the serum levels of sVCAM-1, TNF-$\alpha$, and IL-6 in any cycle phase. Patients who conceived were overall younger than those who had a negative pregnancy test, but serum levels of sVCAM-1, TNF-$\alpha$, and IL-6 did not differ significantly between the two groups.

**DISCUSSION**

Although the exact role of E2 in modulating the expression of VCAM-1 in humans is not completely clarified, the available scientific evidence indicates that E and other estrogenic compounds may modulate the cytokine-induced cell expression of VCAM-1, thus regulating—through complex molecular interactions—the inflammatory component of the atherogenic process. Previous studies evaluating the long-term effect of exogenous Es (various HRT formulations, OCPs) in pre- and postmenopausal women unveiled an association between Es and reduced soluble sVCAM-1 levels (33, 35, 39–40, 47, 51–54). Menstrual cycle changes in endogenous E2 might have a similar effect on sVCAM-1 levels, with the suppression becoming more pronounced in the late luteal phase (47). Gonadotropin stimulation induces rapid and acute changes in endogenous E2 not seen during a normal menstrual cycle and very little is known about the effect of these dramatic, short-term, “supraphysiologic” changes in E2 on the serum levels of sVCAM-1.

Our data support the hypothesis that prolonged exposure to gonadotropin-induced “supra-physiologic” levels of endogenous E2 suppresses the expression of VCAM-1. We found decreased sVCAM-1 levels across the entire luteal phase in women undergoing IVF and noted a significant inverse correlation between [1] sVCAM-1 and E2 in the luteal phase and [2] luteal sVCAM-1 and E2 in all of the preceding phases of the cycle (follicular and retrieval). Similar trends were noted in the late luteal phase. In support of these findings, strong correlations were also noted between the luteal levels of sVCAM-1 and those factors predicting ovarian response (i.e., age, day 3 FSH, gonadotropin units, number of

**FIGURE 2**

Correlation between the levels of tumor necrosis factor-$\alpha$ (TNF-$\alpha$) (in picograms per milliliter) at retrieval and age ($y = 0.18x - 1.07$), number of follicles ($y = -0.23x + 7.6$) and retrieved oocytes ($y = -0.14x + 6.7$), and cumulative serum levels of E2 at retrieval ($y = -3.12x + 14.5$). $P < .05$ for all correlations.

*Souter. VCAM and cytokine levels in IVF cycles. Fertil Steril 2009.*
preovulatory follicles, and retrieved oocytes). Patients with a good response to gonadotropins (younger, requiring less medication, with a larger number of preovulatory follicles and oocytes retrieved) had the highest peak E2 during stimulation and the lowest sVCAM-1 levels in the luteal phase. These findings strongly suggest that the acute, gonadotropin-induced increase in E2 and the prolonged, “cumulative” exposure to “supraphysiologic” levels of endogenous E2 suppress the expression of VCAM-1, with the effect mostly seen across the luteal phase of the cycle. Similarly, in normally menstruating women levels of sVCAM-1 reach a nadir in the late luteal phase (47) and in long-term users of exogenous Es (HRT, OCPs) sVCAM-1 levels are reduced (33, 35, 39–40, 51–54). Our data support the evidence suggesting that the antiatherogenic effects of E2 might be mediated by regulation of the inflammatory component of the atherogenic process.

The exact molecular mechanism by which E2 alters the expression of VCAM-1 is undetermined. Gene transcription of cell adhesion molecules is controlled by NF-κB, a nuclear transcription factor activated by cytokines and reactive oxygen intermediates (55). In the case of VCAM-1, its induction by cytokines is mediated by NF-κB binding sites in the promoter region of its gene. The expression of adhesion molecules is also prevented or attenuated by nitric oxide, which probably acts by preventing the activation of NF-κB by oxidized low density lipoproteins or cytokines. Estrogens can alter the expression of nitric oxide synthase (56). It is therefore possible that the effect of Es on VCAM-1 expression is mediated either by gene regulatory mechanisms or by other cytokine- or nitric oxide-mediated events (46, 56, 57). In the present study we evaluated possible cycle-associated changes in the proinflammatory cytokines (IL-6 and TNF-α) thought to modulate the expression of VCAM-1. It is known that both cytokines [1] exert pleiotropic effects on various sites across the reproductive system (50, 58–62); [2] are present in the follicular fluid (FF); and [3] pituitary hormones and gonadotropins can modulate their production and secretion (IL-6) (50, 63, 64).

With regard to TNF-α, we found that its serum levels fluctuated throughout the various phases of the IVF cycle, being higher during the stimulation phase, reaching the peak in the midfollicular phase, and the nadir in the luteal phase of the cycle. In normally menstruating women (65), TNF-α levels were higher in the follicular phase, and lower in the ovulatory, early and late luteal phase, whereas an increase was noted in the midluteal phase. Other investigators (66, 67) noted a luteal phase TNF-α time curve similar to ours, with no significant midluteal phase increase. No correlation was noted between TNF-α and sVCAM-1 levels; however, the TNF-α changes “mimic” those of sVCAM-1 suggesting that VCAM-1 expression might be regulated by a mechanism involving TNF-α. Concerning the regulation of TNF-α expression by E2, Amato et al. (50) found an inverse correlation between the FF concentration of TNF-α and that of E2. We noted a similar inverse correlation between the retrieval TNF-α levels and those of E2 in the preceding late follicular phase and at retrieval. Accordingly, TNF-α levels at retrieval correlated with ovarian response predictors (i.e., age, number of follicles, and retrieved oocytes).

Similar to TNF-α, IL-6 has a proatherogenic activity. Along with other cytokines, it contributes to an acute phase response and atherogenesis by increasing the production of cell adhesion molecules and C-reactive protein. Regarding its role in reproduction, it facilitates the process of follicular angiogenesis and ovulation, (60, 68) and is detected in the FF (50, 64, 69, 70). Our approach to defining the interactions between IL-6, E2, and VCAM-1 was to examine the relationships among the serum concentration of these indices during an IVF cycle.

Regarding the regulation of IL-6 by E2, published studies yielded conflicting results depending on the conditions used (i.e., cell type, coadministration of other modulatory factors such as hormones and other cytokines) (71–76). Our data show that IL-6 levels were significantly lower during the increase in E2 (follicular phase MF1, MF2, and LF vs. luteal and late luteal phases of the cycle). In the follicular phase (MF2, LF), serum levels of IL-6 correlated negatively with those of E2. In addition, late follicular IL-6 levels correlated negatively with those of E2 in the preceding MF2 phase, findings suggestive of an immediate and a delayed effect of E on IL-6 expression. Similarly, in normally menstruating women (48, 51), serum IL-6 levels [1] declined during the increase in

### Table 3

Comparisons of mean serum levels of sVCAM-1 and IL-6 across the various phases of the cycle.

<table>
<thead>
<tr>
<th>Phase of the IVF cycle</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sVCAM-1 levels</td>
<td>EF vs. Lut: .009*</td>
</tr>
<tr>
<td>sVCAM-1 levels</td>
<td>EF vs. LLut: .05*</td>
</tr>
<tr>
<td>sVCAM-1 levels</td>
<td>MF1 vs. Lut: .0035*</td>
</tr>
<tr>
<td>sVCAM-1 levels</td>
<td>MF1 vs. LLut: .004*</td>
</tr>
<tr>
<td>sVCAM-1 levels</td>
<td>MF2 vs. Lut: .0006*</td>
</tr>
<tr>
<td>sVCAM-1 levels</td>
<td>MF2 vs. LLut: .0005*</td>
</tr>
<tr>
<td>sVCAM-1 levels</td>
<td>LF vs. Lut: .0044*</td>
</tr>
<tr>
<td>sVCAM-1 levels</td>
<td>LF vs. LLut: .007*</td>
</tr>
<tr>
<td>IL-6 levels</td>
<td>EF vs. Lut: .06</td>
</tr>
<tr>
<td>IL-6 levels</td>
<td>EF vs. LLut: .26</td>
</tr>
<tr>
<td>IL-6 levels</td>
<td>MF1 vs. Lut: .005*</td>
</tr>
<tr>
<td>IL-6 levels</td>
<td>MF1 vs. LLut: .038*</td>
</tr>
<tr>
<td>IL-6 levels</td>
<td>MF2 vs. Lut: .0007*</td>
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<tr>
<td>IL-6 levels</td>
<td>MF2 vs. LLut: .012*</td>
</tr>
<tr>
<td>IL-6 levels</td>
<td>LF vs. LLut: .002*</td>
</tr>
<tr>
<td>IL-6 levels</td>
<td>LF vs. LLut: .032*</td>
</tr>
</tbody>
</table>

Note: IL-6 = interleukin-6; LF = late follicular phase; Lut = luteal; LLut = late luteal; MF1 = day 7 or 8 of the stimulated cycle; MF2 = day 9 or 10 of the stimulated cycle; sVCAM-1 = soluble vascular cell adhesion molecule-1; EF = early follicular phase. * = Statistically significant.

Es, and [2] correlated negatively with E2 levels, whereas they were maximally suppressed in long-term users of OCPs (51). Our results and those of other investigators are consistent with the hypothesis that IL-6 expression is modulated by E2. No significant correlation was found between the IL-6 and sVCAM-1 levels.

In conclusion, we found that the short-term, acute, “supraphysiologic” changes in E2 seen during gonadotropin stimulation significantly alter VCAM-1 expression and these changes are associated with changes in the serum IL-6 and TNF-α levels. Further studies are needed to clarify the specific role of these cytokines in the development of atherosclerosis and the exact mechanism by which E2 suppresses the expression of VCAM-1 in vivo. Because the role of inflammation in atherosclerosis is becoming increasingly recognized, elucidating the effect of E2 on inflammatory markers and understanding the relationship between reproductive hormones and the cardiovascular system is becoming more important and may eventually lead to the development of novel therapeutic interventions.

REFERENCES


