ASSISTED REPRODUCTIVE TECHNOLOGY

Ovarian androgens but not estrogens correlate with the degree of systemic inflammation observed during controlled ovarian hyperstimulation

RAOUL ORVIETO¹,², NAAMA FISCH², VERED YULZARI-ROLL¹,², & ANTONIO LA MARCA³

¹Department of Obstetrics and Gynecology, Rabin Medical Center, Petah Tiqva, Israel, ²Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel, and ³Department of Obstetrics and Gynecology, University of Modena, Italy

Abstract

Aim. To investigate the behavior and association of serum androgen and serum C-reactive protein (CRP) in patients undergoing controlled ovarian hyperstimulation (COH) for in vitro fertilization (IVF).

Design. Prospective, observational study.

Setting. An IVF unit of an academic medical center.

Patients and methods. Blood was drawn three times during the COH cycle from 15 patients undergoing the long gonadotropin-releasing hormone-analog protocol: the day on which adequate suppression was obtained (Day-S); the day of or prior to administration of human chorionic gonadotropin (Day-hCG); and the day of ovum pick-up (Day-OPU). Levels of sex steroids and serum CRP were compared among the three time points.

Results. There was a significant increase in serum ovarian androgen levels during gonadotropin treatment. After hCG administration, there was a significant increase in the levels of both serum CRP and ovarian androgens (testosterone, androstenedione), with no significant change in adrenal androgen (dehydroepiandrosterone). Significant correlations were observed between CRP and ovarian androgen levels but not with dehydroepiandrosterone sulfate or estradiol levels.

Conclusion. In patients undergoing COH for IVF, ovarian androgen levels increase in correlation with the degree of inflammation, as reflected by CRP levels. Further studies are necessary to elucidate whether androgens play a role in or are predictive of the systemic inflammatory response in COH.

Keywords: Androgens, C-reactive protein, controlled ovarian hyperstimulation, in vitro fertilization, ovarian hyperstimulation syndrome

Introduction

Controlled ovarian hyperstimulation (COH) is apparently a key factor in the success of in vitro fertilization-embryo transfer (IVF-ET). One of the major complications of COH is severe ovarian hyperstimulation syndrome (OHSS). OHSS is similar to vascular leak syndrome [1,2], which may be attributable to the massive increase in systemic inflammatory response [3].

C-reactive protein (CRP) serves as a biological marker of systemic inflammation. In a recent study, our group found that serum CRP increased significantly during COH until the peak in estradiol (E₂), and then showed a further significant increase after human chorionic gonadotropin (hCG) administration [4]. There was no correlation between serum CRP and E₂ levels. Furthermore, Sacks and colleagues [5] recently showed that women who developed OHSS had higher CRP levels than those who did not.

Patients with polycystic ovary syndrome (PCOS), which is characterized by hyperandrogenic anovulation, are prone to develop severe OHSS during COH [6]. They also have significantly higher CRP concentrations than normal menstruating women with normal androgen levels [7]. However, their CRP levels do not correlate with basal androgen levels and do not differ from those in controls, matched by age and body mass index (BMI) [8].

Prompted by these findings, together with the observed lack of association between serum E₂ levels and degree of systemic inflammation [9,10], we
sought to prospectively examine the behavior of serum androgens during COH for IVF and the association between stimulated androgen levels and the degree of systemic inflammation, as reflected by serum CRP levels.

**Patients and methods**

The study population consisted of 15 consecutive patients attending the IVF unit of our department for treatment of infertility (male factor, 10 cases; unexplained, five cases). Patients with PCOS or any other endocrinopathy were excluded. The study required no modification of our routine long suppressive gonadotropin-releasing hormone-analog (GnRH-a) COH protocol. Briefly, patients were pretreated with GnRH-a in the mid-luteal phase. Fifteen days later, when adequate suppression was obtained, they underwent ovarian stimulation with gonadotropin. The gonadotropin dosage was adjusted individually according to serum E2 levels and vaginal ultrasound measurements of follicular diameter, obtained every one or two days. When the leading follicles reached a minimum of 18 mm in diameter, 10,000 IU of hCG were administered. Oocytes were aspirated by the transvaginal ultrasonographic route approximately 34 h after hCG injection.

For the purpose of the study, in addition to the routine monitoring during the COH cycle, blood samples were drawn to determine hormonal profile (E2, progesterone) and levels of serum androgens (testosterone, androstenedione and dehydroepiandrosterone sulfate (DHEAS)) and CRP at three time points: (1) the day on which adequate suppression was obtained (Day-S); (2) the day of or prior to hCG administration (Day-hCG); and (3) the day of ovum pick-up (Day-OPU).

Informed consent was obtained from all patients before participation in the study, and the study was approved by the Clinical Research Committee.

**Sex steroid and androgen determinations**

Plasma E2, progesterone and DHEAS levels were determined by a solid-phase, competitive chemiluminescent enzyme immunoassay using the Immulite® 2000 analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). For E2, the sensitivity was 55 pmol/l, and the intra-assay and inter-assay coefficients of variation (CVs) were 6.4% and 7.8%, respectively. For progesterone, the sensitivity was 0.6 nmol/l, and the intra- and inter-assay CVs were 6.7% and 9.5%, respectively. For DHEAS, the sensitivity was 0.08 μmol/l, and intra- and inter-assay CVs were 6.5% and 9.3%, respectively.

Serum androstenedione levels were measured by a radioimmunoassay technique with a sensitivity of 0.07 nmol/l. The intra-assay and inter-assay CVs were 4.3% and 6.3%, respectively.

Serum testosterone levels were measured by an electrochemiluminescence immunoassay using the Roche immunoassay analyzer (Roche Diagnostics Corporation, Indianapolis, IN, USA) with a sensitivity of 0.069 nmol/l. The intra- and inter-assay CVs were 1.8% and 4.3%, respectively.

**Determination of C-reactive protein**

For serum CRP determination, blood samples were centrifuged for 10 min at 1000g, and the plasma was stored in aliquots at −20°C until assayed. Serum concentrations of CRP were determined with a highsensitivity immunoturbidimetric assay (Roche Diagnostics Corporation) using an automated clinical chemistry analyzer. The intra- and inter-assay CVs were 1.0% and 2.9%, respectively. Blanks and controls were included in all experiments.

**Statistical analysis**

The results are expressed as means and standard deviations. Findings were analyzed statistically with Student’s paired t test and correlation analysis. p values of 0.05 or less were considered significant.

**Results**

The mean age of the 15 patients eligible for the study was 28.9 ± 4.2 years. The mean number of gonadotropin ampoules used during the COH cycle was 36.7 ± 13.1, and the mean number of oocytes retrieved was 14.4 ± 5.7.

The mean serum E2, progesterone, testosterone, androstenedione, DHEAS and CRP levels on Day-S, Day-hCG and Day-OPU are presented in Table I. As expected, the levels of serum E2 and progesterone were significantly higher on Day-OPU than on Day-S (p < 0.01 for both). Serum E2 level was significantly higher on Day-hCG than on Day-OPU (p < 0.02), whereas serum progesterone was significantly lower (p < 0.01).

Serum ovarian androgen levels (total testosterone, androstenedione) were significantly higher during gonadotropin treatment (Day-hCG) than on Day-S (p < 0.01 for both). After hCG administration (Day-OPU compared with Day-hCG), serum testosterone and androstenedione again showed a significant increase (p < 0.01 and < 0.03, respectively) and DHEAS, a non-significant decrease.

Aromatase activity, as reflected by the E2/testosterone ratio, increased significantly during COH and up to hCG administration, and subsequently decreased significantly after hCG administration (Table I).

Serum CRP levels were significantly higher on Day-OPU than on Day-hCG and Day-S (p < 0.03 for both), and non-significantly higher on Day-hCG than on Day-S (Table I). Serum CRP significantly correlated with levels of serum testosterone (r = 0.30, p < 0.04) and androstenedione (r = 0.33, p < 0.02) (Figure 1). The correlations with E2, progesterone,
DHEAS levels and aromatase activity were all non-significant.

**Discussion**

The present study shows that serum androgen levels increased during COH until peak $E_2$. After hCG administration, when the ovarian androgens (testosterone and androstenedione) showed a subsequent significant increase, DHEAS decreased. These findings indicate that hCG has no influence on adrenal androgen production during COH. Furthermore, we found that serum CRP levels increased during COH until peak $E_2$, with a further significant increase after hCG administration, in correlation with the changes in the ovarian androgens. There was no association of serum CRP levels with serum $E_2$ levels or IVF treatment variables.

While the increase in androgens during gonadotropin treatment and until the day of hCG administration is well established [11–15], their behavior after hCG administration is hardly reported. In a study of six patients undergoing COH for IVF-ET with GnRH-a, Fanchin and co-workers [16] noted an increase in androgen levels in response to human menopausal gonadotropin treatment, with no further elevation following hCG administration. This observation opposes our finding of an increase in androgen level after hCG administration, and the finding of Slater and colleagues [17] of a significant increase in testosterone and androstenedione levels between Day-$hCG$ and Day-$S$, and between Day-$OPU$ and Day-$hCG$. Assuming that luteinizing hormone drives the ovarian production of androgens, we speculated that the administration of hCG, which is used to trigger ovulation in assisted reproductive techniques, might elevate androgen levels further. This was, indeed, demonstrated by Slater and colleagues [17] and supported by the present study. It is also supported by experimental models showing significantly increased androgen production on exposure of human luteal cells to hCG in vitro [18] and after administration of hCG during the early luteal phase [19].

Our observation of an increase in serum CRP during COH until peak $E_2$, and a further increase after hCG administration, confirms our earlier findings [4]. It is also in accordance with the study of Almagor and associates [20], wherein women treated by IVF had increased blood concentrations of CRP during the first week following oocyte pick-up. Sacks and co-workers [5] also reported higher CRP levels in pregnant women who developed OHSS than in pregnant women who did not. These changes reflect the activation of a systemic inflammatory response during COH, especially following the administration of hCG. They are in line with the suggested role of hCG-induced systemic inflammation in COH, in general, and in the pathophysiology of OHSS, in particular [3].
Furthermore, neither the present study nor our previous one [4] demonstrated a correlation between serum CRP and serum E2 levels, suggesting that E2 plays no role in the prediction or pathophysiology of the inflammatory response in COH or severe OHSS [21].

The present study demonstrated a significant correlation between CRP and ovarian androgen levels. Hyperandrogenic patients with PCOS are prone to develop severe OHSS during COH [6] and have higher serum CRP concentrations than normal menstruating women [7]. Recent studies have described the effects of androgen on inflammation and the cardiovascular system [22] and the vasodilator effects of testosterone in vascular cells [23,24]. Others have noted that CRP transgene expression in mice requires testosterone, and that in females (as opposed to males) CRP transgene induction requires both interleukin-6 and a second message [25]. These findings, together with ours, raise the possibility of androgen involvement in the hCG-induced inflammatory response during COH. Both the increase in CRP expression and the increase in circulatory vasodilatation—which may reflect the androgen involvement in the systemic inflammatory responses—are characteristic of severe OHSS. Therefore, ovarian androgens may be important for the prediction or the pathogenesis of severe OHSS.

Additional studies are required to elucidate the effect of COH and ovarian androgens on the systemic inflammatory response and the pathophysiology of OHSS. These may ultimately lead to new strategies in the prevention and treatment of complications of COH.

References
