The levels of C-reactive protein in women treated by IVF

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BACKGROUND: The complex regulation of endometrial receptivity and embryo implantation involves cytokines, several of which are stimulators of the acute-phase reactant C-reactive protein (CRP). The purpose of this study was to evaluate the concentrations of serum CRP in women treated by IVF. METHODS: Seventy-two women who underwent IVF treatment were prospectively studied. The levels of serum CRP were evaluated on the following days: oocyte retrieval, embryo transfer, 5, 6 or 7 days afterwards and 12 days after embryo transfer. RESULTS: CRP levels increased from 6.8 ± 9.5 mg/l on oocyte retrieval day to 14.6 ± 12.5 mg/l on days 5–7 post-transfer (P < 0.0001). The ratios of CRP levels for transfer day/pick-up day were 1.2 in women who conceived versus 2.5 in the non-pregnant group (P = 0.01). CONCLUSION: In women treated by IVF the concentrations of CRP in blood increase significantly during the first week following oocyte retrieval. Successful outcome is associated with a relative small increment in CRP on the day of embryo transfer.

Key words: C-reactive protein/cytokine/IVF/uterine receptivity

Introduction
In humans, successful embryo implantation depends on finely synchronized molecular interactions between the hormonally primed uterus and a mature blastocyst (Norwitz et al., 2001). The synchronization process involves multiple signals of steroid and peptide hormones, growth factors, cytokines, and immunologic factors (Norwitz et al., 2001). Among the various cytokines involved in the process, leukaemia inhibitory factor (LIF) and interleukin (IL)-1 are of special interest. LIF is important for both decidualization and implantation (Cullinan et al., 1996; Lindhard et al., 2002). IL-1 induces the expression of cyclooxygenase-2 genes in endometrial cells and promotes the invasiveness of cytrophoblasts (Huang et al., 1998; Lindhard et al., 2002). Both LIF and IL-1 are known as stimulators of acute-phase proteins (Hilton et al., 1991; Gabay et al., 1999; Pepys et al., 2003). IL-1 has been associated with C-reactive protein (CRP) production (Gabay et al., 1999; Pepys et al., 2003).

The active role of these cytokines in the process of implantation, on the one hand, and their effect on acute phase proteins and CRP generation, on the other, encouraged us to explore the hypothesis that the concentrations of CRP in blood of women undergoing IVF may reflect the outcome of treatment.

CRP is a pentameric protein (mol. wt 1.15 kDa) synthesized by liver hepatocytes in response to acute stimuli (Pepys et al., 2003). Its half-life in the circulation is ~19 h, and it has been reported to reflect ongoing inflammation and/or tissue damage (Pepys et al., 2003).

Since CRP is easily monitored in clinical laboratories and is considerably less expensive to assay in comparison with LIF and IL-1, we undertook a prospective study of women who underwent IVF treatment. The levels of serum CRP were evaluated from the day of oocyte retrieval, on embryo transfer day, during the implantation period and until the 12th day after embryo transfer.

Materials and methods

Patients
A prospective study was conducted between July 2001 and October 2002. All women who had attended our IVF program and underwent intratuterine transfer of either two or three embryos ~48 h after oocyte pick-up were consecutively enrolled in our study. Women who had ICSI, natural cycles or transfer of freeze-thawed embryos were excluded. The main indications for treatment were tubal occlusion and male subfertility (sperm count <10^6 per ejaculate with <20% motility). Analysis of semen from the male spouse involved the measurement of sperm count and motility using a Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) at ×300 magnification. Only clinical pregnancies were considered (gestational sac with fetal heartbeat demonstrated by ultrasound).

Luteal phase support was provided by administration of progesterone (Gestone; Ferring, UK) 50–100 mg/day (i.m.) starting on the day of oocyte retrieval.

Venous blood samples were obtained on admission for oocyte retrieval, on the day of embryo transfer (before procedures were performed), on day 5, 6 or 7 afterwards, as well as on day 12.

Serum was separated by centrifugation (3000 g, 10 min) and stored at −40°C. The serum samples were analyzed for CRP by
immunoturbidimetry using the Integra-700 chemistry analyzer (Roche Diagnostics, Switzerland). The normal values of CRP in our laboratory are <5 mg/l.

The study protocol was reviewed and approved by Bikur Cholim Hospital review board. Informed consent was given by all participants.

**Statistical methods**

CRP concentrations and ratios relative to baseline values at oocyte pick-up were compared between the pregnant and non-pregnant groups using the t-test because the skewness of the distribution of the smaller group was moderate. The use of pooled variances was determined following Levene’s test for equality of variances. A discriminating cut-off value for the transfer/pick-up ratio was determined by the Classification and Regression Trees (CART) methodology (Steinberg et al., 1995). Statistical analysis was performed using SPSS for Windows (version 11) and CART (version 3.6.3). Data were considered statistically significant if \( P \leq 0.05 \).

**Results**

One hundred and sixty eight IVF treatment cycles were initially entered in our study. Ninety-two women had one treatment and 32 had two up to five cycles. Among the 92 women with a single cycle, 20 cases of incomplete sets of CRP results or haemolytic blood specimens were recorded. Therefore, only 72 women who had been treated once during the study period and had a full set of results were enrolled in the final evaluation.

The characteristics of the study participants are presented in Table I. The mean age of the women who achieved pregnancy was not significantly different from that of the non-pregnant group (Table II). The mean concentrations of CRP in the sera of all participants were 6.8 ± 9.5 mg/l on the day of oocyte pick-up, 7.1 ± 7.3 mg/l on the day of embryo transfer and 14.6 ± 12.5 mg/l 5–7 days post-transfer. The 2-fold increase in CRP on days 5–7 was highly significant as compared to the first days of treatment (\( P < 0.0001 \)). Parallel levels of CRP in conception and non-conception groups are summarized in Table III. Although CRP values did not change significantly between the two groups, they seemed higher on oocyte retrieval day in the pregnancy group (Table III). Therefore, we tested the assumption that the ratio of CRP rather than the actual daily concentration may significantly differ between successful and unsuccessful cycles. As shown in Table III, the mean of transfer/pick-up ratios was 1.2 in women who conceived versus 2.5 in the non-pregnant group (\( P = 0.01 \)).

Following the CART method, a transfer/pick-up ratio <1.85 was evaluated as a predictive marker for the outcome of treatment. The sensitivity of this test was 86% (19 of 22 pregnancies with a ratio <1.85) and the specificity was 44% (22 of 50 non-pregnant women with a transfer/pick-up ratio >1.85).

**Discussion**

The present study indicates that in women treated by IVF the concentrations of CRP in blood increase during the first week following oocyte pick-up. These changes were observed in all participants and were not correlated with the outcome of treatment. It is assumed that CRP increases as a result of facilitated signaling of cytokines following IVF. It has been shown that the increase in estradiol during the phase of ovarian stimulation does not affect CRP levels (Ricoux et al., 1994). However, CRP is rapidly induced by IL-1 (Gabay et al., 1999; Pepys et al., 2003) and human preimplantation embryos secrete IL-1, inducing localized changes in the endometrium before adhesion (Sheth et al., 1991; Lindhard et al., 2002).

Although the hypothesis that successful implantation may be associated with a significant change in circulating levels of CRP has been negated, our findings indicate that the CRP ratio (embryo transfer/oocyte pick-up, Table III) was significantly lower in conception cycles as compared to unsuccessful cycles. These results may reflect the low-grade inflammation or proinflammatory effect of CRP in women who achieve pregnancy. It is plausible that the extent of the inflammatory effect of CRP may be a predictive marker for the outcome of treatment.
response of the endometrium may play a role in establishing optimal receptivity. As CRP can interact with both humoral and cellular effector systems of inflammation, it seems that some degree of immunosuppression may be essential for proper maturation of the endometrium and embryo apposition.

Because CRP is rapidly synthesized (Pepys et al., 2003), it may be possible that its increase on transfer day reflects response to the actual procedure of oocyte retrieval. However, the observation that its relative increment was significantly higher in unsuccessful cycles (Table III) implies that intrinsic factors are involved in eliciting the acute reaction.

Previous studies evaluated the timing of implantation in natural conceptions (Wilcox et al., 1999) and in women treated by IVF (Liu et al., 1991, 1993). Implantation was detected 6–12 days after ovulation (Wilcox et al., 1999) and 6–14 days after egg retrieval (Liu et al., 1991, 1993). Since we evaluated CRP only on days 7–9 after egg retrieval, the possibility that CRP levels further elevate on the following 3 days cannot be ruled out.

Brumsted et al. (1990) demonstrated that the tumor marker CA-125 is produced by the endometrium in naturally ovulating women. Because endometrial receptivity is an important factor in IVF pregnancy success, the concentrations of CA-125 as predictors of pregnancy before embryo transfer in IVF (Miller et al., 1996; Chryssikopoulos et al., 1996; Brandenberger et al., 1998; Noci et al., 1999) and ICSI (Tavmergen et al., 2001) were determined. Some reports showed that increased CA-125 levels were associated with pregnancy (Miller et al., 1996; Chryssikopoulos et al., 1996; Tavmergen et al., 2001), whereas others reported no prognostic significance (Brandenberger et al., 1998; Noci et al., 1999). Although the source of high serum CA-125 concentrations was not fully elucidated, Tavmergen et al. (2001) have shown that women with CA-125 >10 IU/ml on the day of oocyte retrieval had very high pregnancy rates. Our attempt to determine a cut-off value for CRP ratio, which may serve as a predictive marker for the early phase of embryo implantation, resulted in acceptable sensitivity, but unsatisfactory specificity. It would be of interest to evaluate simultaneously both CRP and CA-125 in IVF practice. It seems reasonable to assume that in combination, CRP and CA-125 may provide a more sensitive indication of uterine receptivity.

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References


