Serum CA-125 values on the day of oocyte retrieval are not predictive of subsequent pregnancy with in-vitro fertilization

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In the clinical management of in-vitro fertilization (IVF) patients it would be very useful to know, before the embryo transfer, whether or not there is a significant chance of pregnancy in that cycle. If low, it would be better to freeze the embryos and postpone the embryo transfer to a subsequent cycle. For this reason, a retrospective study was carried out to investigate the correlations between the serum CA-125 values before embryo transfer and the clinical outcome of that IVF cycle. Women aged <40 years undergoing a complete infertility evaluation including laparoscopy and receiving gonadotrophin-releasing hormone analogue (GnRHa) suppression followed by purified follicle stimulating hormone (FSH) for IVF–embryo transfer were entered into the study. Ninety-seven cycles qualified for evaluation (26 pregnant and 71 non-pregnant cycles). CA-125 concentrations on the day of oocyte retrieval were significantly lower in the pregnant versus non-pregnant cycles in both non-endometriosis and endometriosis patients. To evaluate the existence of a cut-off value of CA-125 which would allow the prediction of a possible pregnancy with sufficient specificity and sensitivity, a receiver operating characteristic curve analysis was performed. This analysis demonstrated the absence of any predictive value of the subsequent pregnancy for CA-125 concentrations. For this reason, and in contrast with previous findings, CA-125 determinations before the embryo transfer in IVF patients do not appear to be a useful tool for clinicians to use in predicting the outcome of IVF in any given cycle.

Key words: CA-125/endometrium/in-vitro fertilization and embryo transfer/pregnancy outcome/receptivity

Introduction

One of the current goals of clinicians working in the field of reproductive medicine is to find parameters having a prognostic value for in-vitro fertilization (IVF) and embryo transfer outcome. For this reason, in the last decade several parameters have been investigated as reliable ovarian response indicators. Among these, hormonal determinations and ovarian ultrasound examinations before ovarian stimulation have been proposed (Navot et al., 1987; Scott et al., 1989; Winslow et al., 1991; Smotrich et al., 1995; Martin et al., 1996; Farhi et al., 1997; Laas et al., 1997; Seifer et al., 1997; Tomas et al., 1997).

CA-125 is a cell-surface antigenic determinant on a high molecular-weight glycoprotein that is recognized by the monoclonal antibody OC-125. The CA-125 is regarded as a serum marker of malignant ovarian disease (Jacobs and Bast, 1989) but it can also be detected in apparently healthy women, in a variety of benign disorders including endometriosis and in pregnancy (Kabawat et al., 1983). Furthermore, CA-125 and placental protein 14 concentrations in uterine flushing during the luteal phase of cycle have been studied in recurrent miscarriage patients and a role for these determinations has been suggested as a predictive factor for subsequent pregnancy outcome (Dalton et al., 1998). Regarding the source of CA-125 concentrations found in sera from cycling women, no conclusive data are available but it seems very likely that the primary site of production of this glycoprotein is the endometrium itself (Pittaway and Fayez, 1987; Jager et al., 1988; Abaë et al., 1992).

Recently, it has been suggested that CA-125 measurement in the patients’ serum could be usefully employed as pre-retrieval pregnancy predictor in human IVF. In fact, it has been reported that higher serum CA-125 concentration on the day of human chorionic gonadotrophin (HCG) administration (Miller et al., 1996) or on the day of oocyte retrieval (Chryssikopoulos et al., 1996) was associated with a higher pregnancy rate. These data, however, were obtained from a too-small patient population. Furthermore, a first generation assay for CA-125 measurement was employed. Recently a second generation immunoradiometric assay has been developed (O’Brien et al., 1991). This latter method, based on the combination of two monoclonal antibodies with the ability to bind to the antigen at different epitopes, has improved specificity and sensitivity in measurement of CA-125.

In the clinical management of IVF patients, it would be very useful to know, before embryo transfer, whether or not there is a significant chance of pregnancy in that cycle. If low, it would be better to freeze the embryos and postpone the embryo transfer to a subsequent cycle. Due to the clinical importance of a pre-retrieval predictor of pregnancy in IVF cycles, the serum CA-125 concentrations before embryo transfer were measured in a patient group greater than previously reported and employing a second generation immunoradiometric assay. In addition, the correlations between these values and the clinical outcome of the IVF cycle were investigated.
Materials and methods

A retrospective study of all the patients who had completed an IVF cycle at the Reproductive Unit of the Department of Gynecology and Obstetrics, University of Florence, between January 1996 and December 1996 was initiated. Only women aged <40 years and with a complete infertility evaluation including laparoscopy were included in the study. This was so that the results were not biased by age- or endometriosis-induced hypo-fertility. In addition, only cycles suppressed with gonadotropin-releasing hormone analogue (GnRHa) and stimulated with purified follicle stimulating hormone (FSH) were considered, to minimize the effect of a variable ovarian stimulation. The study was approved by our Department’s ethics committee.

Pituitary desensitization began in the luteal phase of the previous cycle with the daily administration of buserelin acetate (Suprefact, Hoechst, L’Aquila, Italy) 200 µg = 2 s.c. During the subsequent menstrual period, exogenous gonadotrophin therapy was initiated if serum oestradiol concentrations were <50 pg/ml and vaginal ultrasound examination revealed no follicles larger than 10 mm in diameter. Highly purified FSH (Metrodin 75 HP, Serono, Rome, Italy) was employed to promote multiple follicular development. The initial daily dose ranged from three to six ampoules and the dose was subsequently adjusted on an individual basis according to serum oestradiol concentrations and transvaginal ovarian ultrasound scans. HCG (10 000 IU; Profasi HP, Serono, Rome, Italy) was administered in the presence of two or more follicles >18 mm in diameter independent of the patients’ serum oestradiol levels. Transvaginal oocyte retrieval was scheduled 36 h after the injection of HCG. Luteal support was provided by administering 50 mg progesterone in oil (Gestone, Amsa, Rome, Italy) from the day of oocyte retrieval until the pregnancy test 14 days after embryo transfer.

CA-125 concentration was determined in the frozen sera collected on the day of oocyte retrieval using an immunoradiometric kit (IRMAMAT CA-125 II, BYK, Italia, Milano, Italy). The characteristics of the assay were: sensitivity 1.0 IU/ml; precision: intra-assay (CV%) = 4.3, inter-assay (CV%) = 7.3; the recovery test was 99%. To evaluate the effect of freezing on CA-125 determination, 10 fresh serum samples were obtained from normal cycling women at different phases of their cycle. Serum samples were aliquotted in three separated fractions, frozen and stored until assayed. CA-125 level was determined in each aliquot at three different times, at least 6 months apart. The between-assay value of CA-125 was calculated.

The patients were divided into two groups according to the pregnancy success in that IVF treatment cycle. Pregnancy was defined as the presence of an intrauterine gestational sac with fetal heartbeat detected with ultrasound. Hence, ectopic pregnancies, biochemical pregnancies and pregnancies with an intrauterine gestational sac without fetal heartbeat were excluded from the present study, to minimize interference related to abnormal pregnancies. Since CA-125 values were not normally distributed, logarithmic transformation of the data was performed before statistical analysis. Differences on duration of infertility, pharmacological treatment and CA-125 levels were determined by t-test for independent samples. CA-125 values are reported as the log of the mean ± SE. Because endometriosis might affect CA-125 blood levels, values in cycles with and without endometriosis were compared. A P value <0.05 was considered significant. Finally, a receiver operating characteristic (ROC) curve analysis was performed in order to evaluate the existence of a cut-off value of CA-125 which would allow the prediction of a possible pregnancy with sufficient specificity and sensitivity.

Results

A total of 152 consecutive women completed an IVF cycle between January 1996 and December 1996; according to the inclusion criteria for the study, only 97 patients were suitable, 26 of whom achieved pregnancy during the IVF cycle. The 26 pregnant cycles included 12 (46%) singleton, five (19%) twin, four (15%) triplet, and five (19%) spontaneously aborted pregnancies.

Table I shows the characteristics of the study population. No significant differences were found between the pregnant and non-pregnant women, either in their basic composition...
Table II. Log₁₀ CA-125 concentrations (IU/ml, mean ± SE) of the study population in the day of oocyte retrieval (antilogarithmic values are given in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>Pregnant</th>
<th>Non-pregnant</th>
<th>P-values</th>
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<tbody>
<tr>
<td></td>
<td>Mean CA-125</td>
<td>SE</td>
<td>n</td>
</tr>
<tr>
<td>Total cycles (n = 97)</td>
<td>1.07 (11.7)</td>
<td>±0.06</td>
<td>26</td>
</tr>
<tr>
<td>Non-endometriosis cycles</td>
<td>1.04* (11.0)</td>
<td>±0.06</td>
<td>22</td>
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<tr>
<td>(n = 86)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Endometriosis cycles</td>
<td>1.2* (15.8)</td>
<td>±0.08</td>
<td>4</td>
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<tr>
<td>(n = 11)</td>
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*P-values = 0.27 (non-endometriosis versus endometriosis cycles).
†P-values: =0.006 (non-endometriosis versus endometriosis cycles).

Figure 2. Receiver operating characteristics (ROC) curve for CA-125 as predictor of subsequent pregnancy in an in-vitro fertilization cycle. The ROC curve was constructed by plotting the sensitivity (true positive) versus the false positive rate (1 – specificity) for different cut-off CA-125 values. CA-125 values have been logarithmic transformed before the analysis. Open circle represents a single CA-125 cut-off value. The 45 degree line through the origin indicates a test of no diagnostic value (that is, random).

Discussion
In two recent studies, patient serum CA-125 values at the time of HCG administration (Miller et al., 1996) or at the time of oocyte recovery (Chryssikopoulos et al., 1996) have been reported higher in conceptional versus non-conceptional IVF cycles, suggesting that CA-125 determination could be a pre-retrieval predictor of pregnancy. However, as far as we know, this parameter is not currently employed as an important parameter to cancel or continue IVF–embryo transfer treatment cycles.

In contrast with these findings, it is reported here that in women who will become pregnant, the CA-125 values are lower, and not higher, than CA-125 values registered in non-pregnant women, in both non-endometriosis and endometriosis patients. Several considerations may explain this discrepancy. First of all, the case population in this study is larger than previously reported. Moreover, in contrast with previous studies, a different methodology was employed to measure CA-125 in this study. In fact, a second generation assay (CA-125 II) was used which, as previously reported, has improved specificity and sensitivity in measurement of CA-125 (O’Brien et al., 1991).

The data presented here demonstrated, moreover, that CA-125 values cannot predict the probability of obtaining pregnancy in any given cycle. In fact, ROC curve analysis was not able to establish a definite CA-125 cut-off value that could predict pregnancy with sufficient specificity and accuracy. This is due to overlapping CA-125 values in conceptional and non-conceptional cycles. These data also disagree with the previous study (Chryssikopoulos et al., 1996; Miller et al., 1996); the
same aforementioned reasons could account for this discrepancy.

The data in this study showing lower CA-125 values in conceptional compared to non-conceptional cycles are in agreement with the data reported in some of the literature on the subject.

First of all, CA-125 serum concentrations fluctuate throughout the menstrual cycle. The different observations reported in literature on the subject all agree only on the fact that CA-125 levels rise during the menstrual period as a consequence of endometrial disruption (Pittaway and Fayez, 1987; Jager et al., 1988; Abaë et al., 1992). Furthermore, an earlier study (Kobayashi et al., 1989) demonstrated that serum concentrations of CA-125 rise after delivery as a consequence of decidual disruption. Azogui et al. studied 25 consecutive patients, all of whom demonstrated fetal heartbeat, with vaginal bleeding during the first trimester of pregnancy (Azogui et al., 1996). They showed CA-125 serum concentrations that were 133 ± 4.9 IU/ml (mean ± SD) in the patients who subsequently aborted and 32.3 ± 4.3 IU/ml in the women with successful pregnancies. The association between high values of CA-125 and viable pregnancy that was subsequently lost were postulated to be the result of decidual disintegration, preceding the actual loss of the fetus. In a recent study, CA-125 concentrations were measured in stored samples from 32 non-viable first trimester pregnancies (Noci et al., 1995). Nineteen of these women experienced vaginal bleeding while the remaining 13 were non-bleeders. CA-125 concentrations were increased only in the presence of decidual disruption associated with vaginal bleeding.

Considered together, these data suggest that the endometrial/decidual shading is directly responsible for the rise of the serum concentrations of CA-125. Because it is reported here that pregnancy is associated with a lower concentration of CA-125 than the non-pregnant state, it is possible that measurement of CA-125 on the day of oocyte retrieval reflects the actual state of the endometrium and its tendency to shading. It seems likely that the endometrium is more compact, without any trend to shading and somehow more ‘receptive’ to embryo implantation when CA-125 values are low, and irrespective of endometrial thickness (note that there are no differences in endometrial thickness between pregnant and non-pregnant patients). However, the achievement of a clinical pregnancy in an IVF cycle depends on too many variables, such as maternal age, embryo quality (cleave and morphology), endometrial receptivity; and perhaps ‘endometrial compactness’. Hence, CA125 values cannot directly predict the achievement of pregnancy in any given IVF cycle.

In conclusion, the data reported here indicate two primary points. First of all, the results suggest that CA-125 levels on the day of oocyte retrieval are lower in the conceptional versus non-conceptional cycles and, secondly, that CA-125 values on the day of oocyte retrieval are not predictive of subsequent pregnancy. For this reason, CA-125 determinations before the embryo transfer in IVF patients do not appear to be a useful tool for clinicians to use in predicting the outcome of IVF in any given cycle.

References


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