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# CA-125 is present in significant concentrations in periovulatory follicles of in vitro fertilization patients

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**Objective:** To evaluate the presence of CA-125 in follicular fluid (FF) and its possible correlation to FF estradiol ($E_2$), progesterone (P) and testosterone (T) and in vitro fertilization and embryo transfer (IVF-ET) outcome.

**Design:** Twenty-eight patients undergoing IVF-ET were randomly chosen and 123 FF were sampled.

**Setting:** Clinical IVF-ET program and immunology laboratory for tumor diagnosis in a university tertiary care center.

**Patients:** Pure tubal factor patients treated by midluteal (long) gonadotropin-releasing hormone agonist protocol coupled with follicular phase human menopausal gonadotropin.

**Interventions:** Transvaginal follicular aspiration followed 48 hours later by ET.

**Main Outcome Measures:** The 28 treatment cycles resulted in six gestations including five take-home infants. The mean levels (±SD) were 30.1 ± 66.0 U/ML for CA-125, 28.5 ± 58.1 ng/ML for $E_2$, 2,360.5 ± 2,846.3 ng/ML for P, and 7.22 ± 7.08 ng/ML for T. The FF CA-125 levels were found to be widely divergent in different follicles of the same patient. There was no significant correlation between FF CA-125 and $E_2$, P, T, oocyte fertilization, embryo quality, and pregnancy rates.

**Conclusions:** CA-125 exists in significant amounts in FF of periovulatory follicles of IVF-ET patients. Intrafollicular CA-125 secretion is neither interrelated to follicular steroidogenesis nor is correlated to the outcome of IVF-ET. Fertil Steril 1992;57:377–80

**Key Words:** CA-125, follicular fluid, in vitro fertilization and embryo transfer, assisted reproduction

CA-125 is an antigenic determinant first defined as a murine $IgG_1$ monoclonal antibody that was raised against an epithelial ovarian carcinoma cell line (1–4). Since then, repeated measurements of serum CA-125 serve as one of the most simple and convenient follow-up methods of ovarian cancer patients (5). In addition to ovarian carcinoma, elevated serum concentrations of CA-125 were found in advanced endometriosis (6), pelvic inflammatory disease (7), and pregnancy (8). CA-125 was also demonstrated on the epithelium of the fallopian tube, endometrium, endocervix, peritoneum, pleura, and pericardium (2, 9).

Serum CA-125, in association with or as a result of ovarian follicular secretion, was also investigated (10–13). An increase of CA-125 serum concentrations was reported to be parallel to the growth of the dominant follicle in spontaneous cycles (10). Elevated levels of CA-125 in serum of patients suffer-

Received May 7, 1991; revised and accepted October 22, 1991.

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ing from ovarian hyperstimulation syndrome have been demonstrated (11). Controversial results were observed regarding the correlation between serum CA-125 levels and ovarian output in ovulation induction (12, 13). Zweers et al. (12) reported that after ovarian stimulation for in vitro fertilization (IVF) or intrauterine insemination serum CA-125 concentrations rise considerably in the luteal phase compared with the normally cycling women or those on oral contraceptives. In contrast, Lanzone et al. (13) demonstrated that serum CA-125 levels remained stable at all phases of the cycle regardless of whether ovulation induction was applied or ovulation occurred spontaneously.

It was reported (14) that a possible ovarian tissue-blood barrier may preclude the passage of CA-125 from follicular fluid (FF) to the serum. Therefore the measured serum CA-125 levels may not reflect the true FF CA-125 synthesis. To date, only one study has attempted to investigate CA-125 in FF of IVF patients, and it was concluded that the amount of CA-125 measured in FF was negligible and that at least within the immediate preovulatory phase, CA-125 in FF is poorly expressed (15). These results are somewhat contradictory to the reports showing high serum CA-125 concentrations in women undergoing induction of ovulation (11, 12).

The present study was aimed to define FF CA-125 in a larger group of IVF patients with a much higher number of aspirated periovulatory follicles. An additional goal was to determine the correlation of FF CA-125 to estradiol ($E_2$), progesterone (P), and testosterone (T) secretion in FF, oocyte fertilization, embryo rates, and pregnancy rates (PRs).

## MATERIALS AND METHODS

A total of 28 women underwent IVF-embryo transfer (ET) during January 1989 in our IVF unit. Their ages ranged from 25 to 39 years with a mean of 31.7 ± 4.2. All of them were treated by midluteal (long) gonadotropin-releasing hormone agonist (D-Trp$^6$; Decapeptyl Ferring, Arzneimittel GmbH d-2300 Kiel, Germany) protocol coupled with follicular phase human menopausal gonadotropin (hMG) (75 units of follicle-stimulating hormone + 75 units of luteinizing hormone, Pergonal; Teva, Petach Tikva, Israel). The mean number of hMG ampules given to each woman was 34.4 ± 12.9, and the mean serum $E_2$ at human chorionic gonadotropin administration day was 1,385.0 ± 870.0 pg/ML. The follicular aspiration was performed transvaginally under vaginal sonographic guidance (Pie Medical Scanner 1120, Maastricht, Holland). Each follicle was aspirated separately and collected in a different dish avoiding flushing with medium. The mean aspirated oocyte number was 10.9 ± 5.8. The FFs were stored in a temperature of −70°C. One hundred twenty-three FF were processed. The FF $E_2$, P, and T values were determined using direct radioimmunoassay with solid phase coated tubes (Zer Science Based Industries LTD., Jerusalem, Israel).

The FF CA-125 was evaluated using the ELSA-CA-125 kit (CIS Biointernational, B.P.32, F91192; GIF-SVR-Yvette, Cedex, France). It is a solid phase two-site immunoradiometric assay (IRMA). The CA-125 antigenic determinants that are present in the FF sample are embedded between two differently prepared antibodies, resulting in a coated antibody-antigen-iodinated antibody complex. The redundant unbound tracer is removed by washing. The final amount of measured radioactivity that is bound to IRMA is proportional to the concentration of CA-125 in FF.

The interassay and intra-assay variations for CA-125 were ±0.45 and ±0.12 U/ML, respectively. Internal controls for CA-125 included serum samples of five healthy women, five ovarian carcinoma, and five colon cancer patients with mean values of 17 ± 8.5, 48 ± 23, and 14 ± 12 U/ML in each group, respectively.

The correlations of FF CA-125 to FF $E_2$, P, and T, serum $E_2$, oocyte fertilization, cleavage, quality, and PRs were evaluated. Statistical calculations were performed using SPSS-X (statistical package for social sciences - extended) package. Analysis of variance and multiple regression were employed.

## RESULTS

The 28 treatment cycles generated six gestations including five live surviving infants. The pregnancy and living child per transfer rates were 26% and 22%, respectively.

The FF CA-125 concentrations ranged from 0 to 349.5 U/ML. The mean FF levels (±SD) were 30.1 ± 66.0 U/ML for CA-125, 28.5 ± 58.1 ng/ML for $E_2$, 2,360.5 ± 2,846.3 ng/ML for P, and 7.22 ± 7.08 ng/ML for T.

Figures 1 and 2 demonstrate the FF CA-125 measurable concentrations of each of the 87 follicles. In the other 36, FF zero concentrations of CA-125 were found. There was no significant correlation between

FF CA-125 and FF $E_2$, P, and T, oocyte fertilization, embryo quality, and PRs.

## DISCUSSION

The present work demonstrated that CA-125 is present in significant concentrations in periovulatory follicles of IVF patients. In addition, the FF CA-125 levels were found to be widely divergent in different follicles of the same patient. These variations in FF CA-125 concentrations reach a rate of tens (Fig. 1) and even hundreds of times (Fig. 2). The FF of preovulatory follicle is similar to plasma (16). It is composed of proteoglycans diluted by influx of fluid derived from the plasma enriched by products of synthesis of the theca interna and granulosa cells (16). Therefore it may be hypothesized that, in addition to derivatives of coelomic epithelium, CA-125 is possibly also expressed or secreted by granulosa cells. The present results show that the considerable levels of CA-125 in FF did not correlate with the process of aromatization or IVF outcome. Nevertheless, these CA-125 concentrations may represent some different granulosa cell functions, especially after augmentation by menotropins. Another possibility is that the filtration of CA-125-enriched fluid from serum into some of the follicles is enhanced by exogenous gonadotropins.

It was demonstrated that normal ovarian epithelium does not express CA-125, although CA-125 is seen in inclusion cysts or benign papillary excrescences of the ovary (17). High or rising serum CA-125 densities are a well-established warning sign for a relapsing ovarian carcinoma (5) and were also

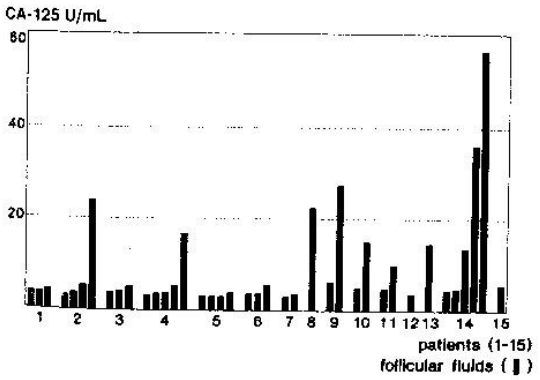


**Figure 1** CA-125 concentrations in FFs of patients 1 to 15. Y axis, CA-125 values from 0 to 60 U/ML. X axis, patient number from 1 to 15; bar lines represent FF of each patient.

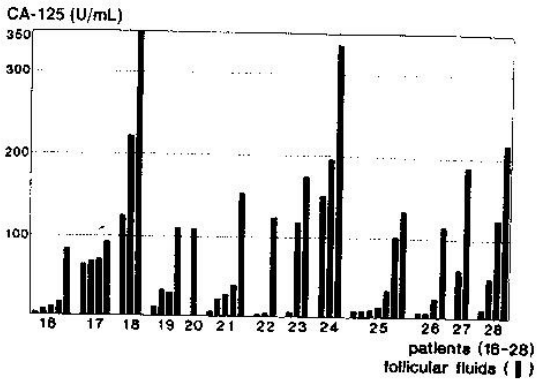


**Figure 2** CA-125 concentrations in FFs of patients 16 to 28. Y axis, CA-125 values from 0 to 350 U/ML. X axis, patient number from 16 to 28; bar lines represent FF of each patient.

suggested for monitoring of advanced endometriosis (7). High FF CA-125 concentrations may have some significance in connection with these aforementioned maladies or possibly mark some other physiological or pathological conditions.

The present study demonstrated that CA-125 exists in significant amounts in FF of periovulatory follicles of IVF-ET patients. It may be concluded that intrafollicular CA-125 secretion is neither interrelated to follicular steroidogenesis nor is correlated to the outcome of IVF-ET.

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