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CA 125 in seminal plasma: correlation with semen parameters

A.Meisser[1], A.Campana and P.Bischof

Clinic of Infertility and Gynaecological Endocrinology, Department of Obstetrics and Gynaecology, University of Geneva, 1211 Geneva 14, Switzerland

[1]To whom correspondence should be addressed

Ovarian cancer marker CA 125 was measured in human seminal plasma, and the concentrations ranged between 22 and 1149 U/ml, and between 39 and 4711 U/ejaculate. This very high patient-to-patient variability was in contrast to a much lower within-patient variability, which was comparable to that of other semen parameters. No significant differences in CA 125 concentration were found in seminal plasma from normospermic patients, patients with male factors, vasectomized men, and in aliquots of samples which led to a pregnancy, via artificial insemination or in-vitro fertilization. The seminal plasma CA 125 concentration was not correlated with sperm count, motility and morphology. In contrast, seminal plasma CA 125 concentrations correlated with the age of the patient ($P < 0.001$) and inversely with the volume of the ejaculate ($P < 0.001$). These correlations were independent of each other. CA 125 did not correlate with the prostatic marker zinc, but did do so with the seminal vesicle marker fructose and the epididymal marker carnitine.

Key words: CA 125/semen/seminal vesicle

Introduction

Cancer antigen 125 (CA 125) was discovered by Bast *et al.* in 1981, by immunizing mice against an ovarian carcinoma cell line. The monoclonal antibody obtained, OC 125, recognized an antigenic determinant present on a high molecular weight glycoprotein, CA 125. The precise biochemical structure of CA 125 is still unknown.

CA 125 is a tumour marker widely used for monitoring ovarian cancer (see Jacobs and Bast, 1989, for review). Beside ovarian cancers, circulating concentrations of CA 125 are elevated in various pathological and physiological conditions such as endometriosis (Giudice *et al.*, 1986; Fedele *et al.*, 1988; Muyldermans *et al.*, 1995), pelvic inflammatory disease (Takahashi *et al.*, 1990), endometrial cancer (Duk *et al.*, 1986), early pregnancy (Haga *et al.*, 1986) and during menses (Pittaway and Fayez, 1987).

Immunohistochemically, CA 125 is localized on covering epithelia, such as peritoneum, pleura, pericardium, endometrium and Fallopian tube. It probably undergoes exocrine secretion into the body's cavities (see Bischof, 1993, for review). CA 125 is also present in uterine fluid (Weintraub *et al.*, 1990), cervical mucus (Crombach *et al.*, 1989), human milk (Hanisch *et al.*, 1985), amniotic fluid (O'Brien *et al.*, 1986), follicular fluid (Mordel *et al.*, 1992) and seminal plasma (Halila, 1985; Dodd *et al.*, 1987; Matorras *et al.*, 1994).

In this study we measured CA 125 in human seminal plasma and studied the possible correlation between CA 125 concentrations and semen parameters.

Materials and methods

Study I

Semen samples (n = 163) were obtained from 126 patients (ages ranged from 26 to 64 years) attending the infertility clinic for in-vitro fertilization (IVF, n = 97), gamete intra-Fallopian transfer (GIFT, n = 1), artificial insemination with the husband's spermatozoa (AIH, n = 50) or sperm preparation tests (n = 15).

The original sperm parameters (volume, sperm concentration, initial motility, morphology) were recorded and then the samples were processed for sperm preparation as follows. After adding 1–3 vol of culture medium (Menezo's B2 from BioMerieux, Geneva, Switzerland, or Whittingham's T6, prepared in our laboratory, with or without 10% patient's own serum), the sample was centrifuged and the supernatant harvested and stored frozen at –20°C. Record was kept of the dilution factor. The pellet was then resuspended and processed as required for sperm preparation.

CA 125 was measured in the supernatant with a commercially available immunoradiometric assay (Centocor CA 125 TM; Medipro AG, Teufen, Switzerland) and the initial concentration was calculated taking into account the dilution ratio. All samples were analysed in duplicates.

Samples were classified as normospermic if the sperm concentration was $\geq 20 \times 10^6$/ml, and there was ≥40% motility and ≥30% normal morphology. Samples not meeting these criteria were considered to have male factor infertility.

For the semen used for AIH, IVF and GIFT, the pregnancies obtained were noted and, for the IVF patients, the fertilization rate was calculated and recorded.

Study II

Semen samples (n = 39) were obtained from patients coming for a semen analysis to the andrology laboratory. After centrifugation, the undiluted seminal plasma was tested for fructose, zinc, carnitine and CA 125.

CA 125 was measured with a commercially available immunoradiometric assay, ELSA-CA 125 II (CIS, Gif-sur-Yvette, France), which we ascertained gave very similar results to the Centocor CA 125 assay (Clément *et al.*, 1995).

Fructose concentrations were measured with the commercial Enzymatic Bioanalysis Kit from Boehringer Mannheim (Rotkreuz, Switzerland), and the results expressed in µmol/ejaculate. Zinc

Table I. Distribution parameters of the variables used in the study

Variable	Skewness	Kurtosis
CA 125 (U/ml)	1.646	2.533
Log CA 125 (U/ml)[a]	0.05	-0.78
CA 125 (U/ejaculate)	3.336	13.536
Log CA 125 (U/ejaculate)[a]	0.008	0.145
Ejaculate volume (ml)	1.522	3.393
Log ejaculate volume (ml)[a]	-0.279	0.688
Age of the male (years)	1.025	1.45
Log age of the male (years)[a]	0.448	0.211
Fructose (µmol/ejaculate)[a]	0.015	-0.716
Log fructose (µmol/ejaculate)	5.075	-2.013
Zinc (µmol/ejaculate)	1.97	3.386
Log zinc (µmol/ejaculate)[a]	0.402	0.152
Carnitine (µmol/ejaculate)	1.243	0.913
Log carnitine (µmol/ejaculate)[a]	0.176	-0.82

[a]Parameters used for statistical evaluation.

Table II. Patient-to-patient and within-patient variation coefficients (%) for seminal plasma CA 125 concentrations and other semen parameters

Variable	Patient-to-patient variation (n = 98)	Within-patient variation (n = 28)
CA 125 (U/ml)	84.3	28.4
CA 125 (U/ejaculate)	107.3	26.5
Ejaculate volume (ml)	50.5	23.4
Sperm count ($\times 10^6$/ml)	103.2	40.9
Motility (%)	44.5	24.6

(Fuentes *et al.*, 1982) and carnitine (Wetterauer and Heite, 1976) were also measured and the results expressed in µmol/ejaculate.

Statistics

Logarithmic transformations were used throughout the study for the parameters which showed a skewed distribution (Table I). Student's t-test and correlation coefficient r were thus calculated on log-transformed data when appropriate.

Results

Study I

To ensure that dilution by culture medium had no effect on CA 125 measurements, six samples were measured diluted and undiluted. After multiplying the diluted CA 125 values by the dilution ratio, a correlation of 0.945 was obtained between the values (results not shown). No differences were observed between the two media used to dilute the samples (B2 and T6), and the presence of 10% serum had no effect either. Furthermore, CA 125 was undetectable in any of the media used.

CA 125 was measurable in the 163 seminal plasma samples tested and the concentrations ranged from 22 to 1149 U/ml and from 39 to 4711 U/ejaculate.

The patient-to-patient variability coefficient of CA 125 was very high (84–107%, Table II). As 28 patients gave more than one semen sample (between two and five samples/patient), a within-patient variability coefficient (mean of the variability coefficients for each patient) could be calculated (Table II). Within-patient variability was much lower (26–28%) and was not different from that of other semen parameters (volume, concentration and motility).

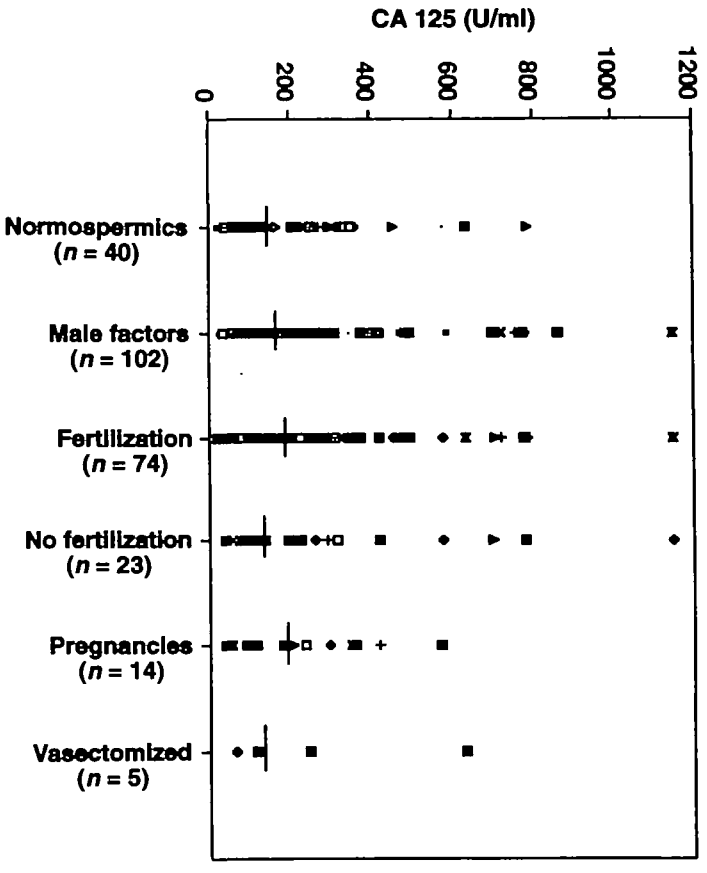

Figure 1. Distribution of CA 125 concentrations in seminal plasma of normospermic men, patients with male factors, patients whose spermatozoa fertilized in IVF and patients whose spermatozoa did not, patients who obtained a pregnancy (by in-vitro fertilization or artificial insemination with husband's spermatozoa) and vasectomized men. Medians are indicated by a horizontal bar.

As shown in Figure 1, CA 125 concentrations in seminal plasma from normospermic men, from patients with male factors, from semen samples which were able or unable to fertilize in IVF, from semen leading to a pregnancy in AIH or IVF, or from vasectomized men were all similar. Furthermore, no correlation could be observed between CA 125 values and sperm concentration, motility, or morphology (results not shown). However, the CA 125 values in seminal plasma were positively correlated with the age of the male patient ($P < 0.001$, Figure 2) but negatively with the volume of the ejaculate ($P < 0.001$, Figure 3). The volume of the ejaculate was weakly negatively correlated with the age of the patient, but not significantly so ($r = -0.14$). Thus partial correlation coefficients were calculated to evaluate the influence of age in the correlation CA 125/volume and inversely the contribution of volume in the correlation CA 125/age. These partial correlation coefficients were slightly lower, but still significant at $P < 0.01$.

Study II

CA 125 was measured in undiluted seminal plasma together with fructose, zinc and carnitine. These parameters ranged from

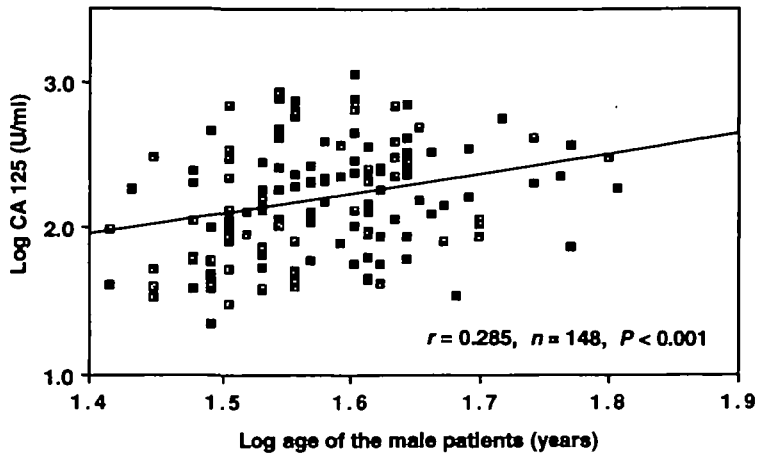

Figure 2. Correlation between seminal plasma CA 125 concentrations and the age of the patient.

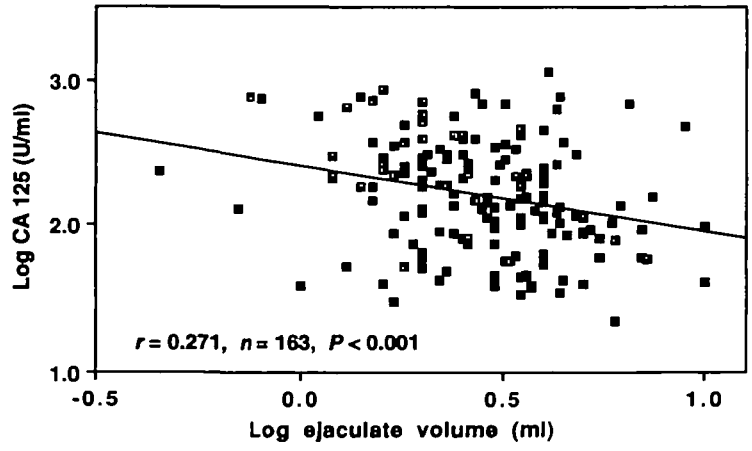

Figure 3. Correlation between seminal plasma CA 125 concentrations and the volume of the ejaculate.

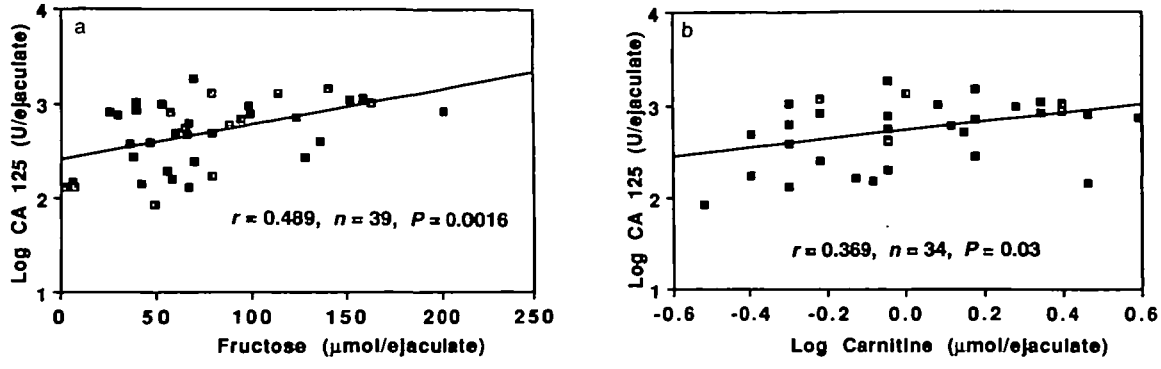

Figure 4. Correlation between seminal plasma CA 125 and fructose (a) or carnitine (b), expressed in units or µmol per ejaculate.

85.8 to 1840.8 U/ejaculate for CA 125, from 1.36 to 201.3 µmol/ejaculate for fructose, from 2.85 to 39.5 µmol/ejaculate for zinc, and from 0.3 to 3.92 µmol/ejaculate for carnitine. There was no significant correlation between concentration of CA 125 per ejaculate and zinc (r = 0.081), but CA 125 concentration (U/ejaculate) correlated significantly with fructose (P = 0.0016, Figure 4a) and carnitine (P = 0.03, Figure 4b).

Discussion

Although the presence of CA 125 in semen is well known, there have been few reports published on the subject. Halila (1985) reported high concentrations of CA 125 in seminal plasma, with a high patient-to-patient variability. He demonstrated that seminal CA 125 reacted similarly to ovarian cancer CA 125 in radioimmunoassay, and had a similar molecular weight (130 kDa) on column chromatography. He could not demonstrate any difference between CA 125 concentrations of normospermic men, patients with suspected infertility and those of vasectomized men, neither could he correlate seminal plasma CA 125 concentrations with the patient's age or any of the semen parameters tested (sperm count, ejaculate volume or motility). Dodd *et al.* (1987) confirmed these results, observing no relationship between CA 125 concentration and

number of spermatozoa, motility, vitality, morphology, or the presence of round cells in the semen. In contrast, Matorras *et al.* (1994) reported a tendency to higher CA 125 values in pathological or borderline semen, and in patients who did not obtain pregnancy.

In our study, the high patient-to-patient variability of the concentrations of seminal plasma CA 125 was confirmed, the values ranging from 22 to >1000 U/ml. On the other hand, the variability of CA 125 values in different samples from the same patient was much lower, and varied to the same extent as other semen parameters (motility, sperm count and ejaculate volume).

We confirmed the results of Halila (1985) that there is no difference in the CA 125 concentrations in samples from normospermic men, patients with male factors or vasectomized men. Furthermore, CA 125 values in seminal plasma were not different between samples failing to fertilize in IVF when compared to samples able to do so. This observation is in contrast to the results reported by Matorras *et al.* (1995), who observed higher CA 125 values in only four samples with no fertilization. This discrepancy is obviously due to the small number of observations in that study. Finally, samples leading to a pregnancy had similar concentrations of CA 125 to those not achieving a pregnancy. This observation confirms previous results (Matorras *et al.*, 1995).

No correlation could be demonstrated between CA 125 values and sperm count, motility or morphology. However, in contrast to the other studies, a correlation (as calculated with the log-transformed values) between CA 125 values and the age of the patient and an inverse correlation between CA 125 values and the volume of the ejaculate were observed. These observations could not be explained by a decrease of the ejaculate volume with age. However, a diluting effect of the fluids secreted by the glands not producing CA 125 could explain this inverse correlation between CA 125 and the volume. There is no clear explanation for the significant correlation between age and CA 125. These correlations may have been revealed because the number of samples tested in the present study was larger than in the studies in which correlations were not observed.

The concentration of CA 125 in seminal plasma is in most cases much higher than the circulating concentrations, suggesting a local production and secretion. With the intention of determining which organ of the male genital tract could produce the CA 125, we measured this antigen in undiluted samples together with fructose, zinc and carnitine. Fructose is a marker of seminal vesicles, carnitine of epididymis and zinc of prostatic function. CA 125 concentration did not correlate with zinc, suggesting that prostate is probably not a source of CA 125. Furthermore, the normal concentrations of CA 125 in seminal plasma of vasectomized men excluded a unique testicular or epididymal source, although the correlation between carnitine and CA 125 would indicate an epididymal origin for that glycoprotein. Finally, the strong correlation between fructose and CA 125 concentrations suggests that this antigen is produced by the seminal vesicles. Immunohistochemically, Kabawat *et al.* (1983) have shown that neither normal testis nor epididymis or prostate reacted with the monoclonal antibody OC 125, the seminal vesicles having apparently not been examined.

Recently, Ohmori *et al.* (1994) reported a patient with an adenocarcinoma of the seminal vesicle, who presented with high circulating concentrations of CA 125 that decreased after surgery was first performed and then rose again as the disease relapsed. Half of the tumour cells were immunohistochemically positive for CA 125. In contrast, in prostatic cancer or in benign prostatic hyperplasia, immunohistochemical studies were unable to detect CA 125 (Alivizatos *et al.*, 1992; Loy *et al.*, 1992). Finally, Kabawat *et al.* (1983) reported no immunoreactivity of OC 125 monoclonal antibody with the following testis tumours: seminoma, embryonic carcinoma and Leydig cell tumour, and Costa *et al.* (1992) showed that Sertoli-stromal cell tumours were not stained for CA 125. Furthermore, Buamah *et al.* (1987) found no elevation of CA 125 in sera from a patient with a non-seminomatous cell tumour of the testis. We would thus conclude that CA 125 seems to be produced mainly by seminal vesicles. Future studies will indicate whether CA 125 could possibly be used as a marker of this gland.

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References

Alivizatos, G., Dimopoulos, A., Mitropoulos, D. *et al.* (1992) Efficacy of eight serially measured markers for diagnosis of prostatic carcinoma. *Arch. Esp. Urol.*, **45**, 269–272.

Bast, R.C., Feeney, M., Lazarus, H. *et al.* (1981) Reactivity of a monoclonal antibody with human ovarian carcinoma. *J. Clin. Invest.*, **68**, 1331–1337.

Bischof, P. (1993) What do we know about the origin of CA 125? *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **49**, 93–98.

Buamah, P.K., Cornell, C., Skillen, A.W. *et al.* (1987) Initial assessment of tumor-associated antigen CA-125 in patients with ovarian, cervical and testicular tumors. *Clin. Chem.*, **33**, 1124–1125.

Clément, M., Bischof, P., Gruffat, C. *et al.* (1995) Clinical validation of the new ELSA-CA 125 II assay. Report of a European multicentre evaluation. *Int. J. Cancer*, **60**, 199–203.

Costa, M.J., Morris, R.J., Wilson, R. and Judd, R. (1992) Utility of immunohistochemistry in distinguishing ovarian Sertoli-stromal cell tumors from carcinosarcomas. *Hum. Pathol.*, **23**, 787–797.

Crombach, G., Scharl, A. and Würz, H. (1989) CA 125 in normal tissues and carcinomas of the uterine cervix, endometrium and Fallopian tube. *Arch. Gynecol. Obstet.*, **244**, 113–122.

Dodd, J., Sculfort, C.J. and Tyler, J.P.P. (1987) Cancer antigen 125 in semen. *Clin. Reprod. Fertil.*, **5**, 85–86.

Duk, J.M., Aalders, J.G., Fleuren, G.J. and de Bruijn, H.W.A. (1986) CA 125: a useful marker in endometrial carcinoma. *Am. J. Obstet. Gynecol.*, **155**, 1097–1102.

Fedele, L., Vercellini, P., Arcaini, L. *et al.* (1988). CA 125 in serum, peritoneal fluid, active lesions and endometrium of patients with endometriosis. *Am. J. Obstet. Gynecol.*, **158**, 166–170.

Fuentes, J., Miro, J., and Riera, J. (1982) Simple colorimetric method for seminal plasma zinc assay. *Andrologia*, **14**, 322–327.

Giudice, L.C., Jacobs, A., Pineda, J., Bell, C.E. and Lippmann, L. (1986) Serum levels of CA 125 in patients with endometriosis: a preliminary report. *Fertil. Steril.*, **45**, 876–878.

Haga, Y., Sakamoto, K., Egami, H., Yoshimura, R. and Agaki, M. (1986) Evaluation of serum CA 125 values in healthy individuals and pregnant women. *Am. J. Med. Sci.*, **292**, 25–29.

Halila, H. (1985) Detection of ovarian cancer marker CA 125 in human seminal plasma. *Tumour Biol.*, **6**, 207–212.

Hanisch, F.G., Uhlenbruck, G., Dienst, C., Stottrop, M., and Hippauf, E. (1985) CA 125 and CA 19–9: two cancer-associated sialylsaccharide antigens on a mucus glycoprotein from human milk. *Eur. J. Biochem.*, **149**, 323–330.

Jacobs, I. and Bast, R.C. (1989) The CA 125 tumour-associated antigen: a review of the literature. *Hum. Reprod.*, **4**, 1–12.

Kabawat, S.E., Bast, R.C., Bhan, A.K. *et al.* (1983) Tissue distribution of a coelomic-epithelium-related antigen recognized by the monoclonal antibody OC 125. *Int. J. Gynecol. Pathol.*, **2**, 275–285.

Loy, T.S., Quesenberry, J.T. and Sharp, S.C. (1992) Distribution of CA125 in adenocarcinomas. An immunohistochemical study of 481 cases. *Am. J. Clin. Pathol.*, **98**, 175–179.

Matorras, R., Genolla, J., Corcostegui, B. *et al.* (1994) Human seminal plasma analysis of five tumor markers: CA 125, alpha-fetoprotein, CA 50, CA 19.9, and CA 195. *Int. J. Fertil.*, **39**, 223–228.

Matorras, R., Genolla, J., Diez, J. *et al.* (1995) Levels of CA-19.9, CA-125, and CA-195 in seminal plasma and in vitro fertilization rates. *Fertil. Steril.*, **63**, 604–607.

Mordel, N., Antaby, S.O., Zajicek, G. *et al.* (1992) CA-125 is present in significant concentrations in periovulatory follicles of in vitro fertilization patients. *Fertil. Steril.*, **57**, 377–380.

Muyldermans, M., Cornillie, F.J. and Koninckx, P.R. (1995) CA 125 and endometriosis. *Hum. Reprod. Update*, **1**, 173–187.

O'Brien, T.J., Hardin, J.W., Bannon, G.A. *et al.* (1986) CA 125 antigen in human amniotic fluid and fetal membranes. *Am. J. Obstet. Gynecol.*, **155**, 50–55.

Ohmori, T., Okada, K., Tabei, R. *et al.* (1994) CA 125-producing adenocarcinoma of the seminal vesicle. *Pathol. Int.*, **44**, 333–337.

Pittaway, D.E. and Fayez, J.A. (1987) Serum CA 125 antigen levels increase during menses. *Am. J. Obstet. Gynecol.*, **156**, 75–76.

Takahashi, K., Nagata, H., Musa, A. *et al.* (1990) Clinical usefulness of CA 125 levels in the menstrual discharge in patients with endometriosis. *Fertil. Steril.*, **54**, 360–362.

Weintraub, J., Bischof, P., Tseng, L., Redard, M. and Vassilakos, P. (1990) CA 125 is an excretory product of human endometrial glands. *Biol. Reprod.*, **42**, 721–726.

Wetterauer, U. and Heite, H.-J. (1976) Der Carnitingehalt im Sperma—ein Parameter für die Nebenhodenfunktion. *Aktuel. Dermatol.*, **2**, 93–103.

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