Serum CA125 level modifications in women undergoing repeated IVF cycles

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Summary

In the present paper, serum CA125 modifications in patients undergoing their first IVF cycle were compared with those of patients in their second attempt. A significant increase of this marker was detected in each group of patients at day 14 after embryo transfer. However, the level of CA125 monitored in the patients in their second attempt was significantly higher than that determined in patients undergoing their first ovarian stimulation. This condition does not influence either ovarian response or oocyte and embryo quality. Moreover similar IVF outcome was obtained. Therefore we propose that patients undergoing repeated assisted reproductive technology (ART) cycles may suffer from ovarian surface epithelial damage and/or altered cellular growth rate.

Key words: Ovarian stimulation; CA125; IVF.

Introduction

Tumor markers are molecules used when malignancy is suspected. They are potentially useful in cancer relapse screening, in predicting response to therapy and in monitoring diagnosed diseases [1, 2]. However, because of lack of sensitivity and specificity, tumor markers can rarely be used for early diagnosis of cancer. Long-term in vitro fertilization (IVF) treatments, indeed, would require new and specific parameters aimed at revealing possible ovarian cell alterations.

CA125 is a surface mucin-like glycoprotein antigen produced by ovarian epithelial, endometrial, tubal and mesothelial cells [3]. High levels of this marker can be found in neoplastic lesions, such as endometrial and ovarian cancer, and in non-neoplastic lesions such as endometriosis, pelvic inflammatory diseases (PID) and ovarian hyperstimulation syndrome (OHSS) [4].

In regular cycling women, serum CA125 is maintained at constant levels during the menstrual cycle, whereas in women undergoing IVF treatments, CA125 concentration is higher in the luteal phase (LP) than in the follicular phase (FP) [3]. This increase appears to be positively correlated with serum concentrations of 17β-estradiol [5] suggesting that corpus luteum function may be involved in CA125 expression [6]. CA125 can also be detected in follicular fluids of periovulatory follicles of IVF patients but intrafollicular CA125 secretion does not correlate with follicular stereodogenesis or IVF outcome [7]. In contrast, a positive correlation has been found between pregnancy rates in assisted reproduction cycles and levels of CA125 in serum [8].

The aim of this work was to investigate whether repeated controlled ovarian hyperstimulation (COH) in IVF patients may be responsible for an increased secretion of CA125. To this end, serum CA125 levels in patients undergoing their first IVF cycle (Group A) were compared with those of patients during their second attempt (Group B). In addition, we investigated whether increased secretion of CA125 is associated with changes in ovarian response and IVF outcome.

Materials and Methods

From January 2001 to November 2003 at the Infertility Unit of the University of L'Aquila, 52 women from those participating in our IVF program were selected for the present study after signing a written consent. They were divided into two groups: patients undergoing their first IVF cycle (Group A) and patients in their second attempt (Group B).

All couples enrolled in the study had solely male factor infertility. Exclusion criteria for this study were endometriosis and serum CA125 > 35 IU/ml. Ovarian stimulation was induced in all patients by using the long down-regulation protocol with gonadotrophin releasing hormone (GnRH) analogue (Enantone 3.75, Takeda) on the 23rd day. Therapy was continued with a daily dose of 225 IU r-FSH (Gonal F, Serono) from day 2 of the next cycle. Follicular monitoring was performed by serum 17β-estradiol measurement and serial transvaginal ultrasound every two or three days. When at least three or more follicles with a diameter ≥ 18 mm were detected, patients received 10,000 IU human chorionic gonadotrophin (HCG; Profasi, Serono). Oocyte retrieval was performed transvaginally under ultrasound control 36 hours after hCG administration. Following a 30-second incubation in culture medium containing hyaluronidase (Medicult), oocytes were individually treated by gentle pipetting to remove the cumulus and corona radiata cells. Oocyte maturation was assessed at x 1200 magnification and the oocytes were classified as metaphase II (MII), metaphase I (MI) and germinal stage (GV). After preparing the semen sample the intracytoplasmic sperm injection (ICSI) procedure was performed following conventional techniques [9]. Oocytes with two pronuclei and two polar bodies 14-18 hours after ICSI were considered as normally fertilized.

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The quality of the embryos was assessed on the day of embryo transfer, ~ 48 hours after egg retrieval. Embryos without fragmentation were categorized as grade 1 and those with 20% of the volume of the embryo fragmented were categorized as grade 2. Embryos with anucleate fragments present in 20-50% of the volume and those with > 50% anucleate fragments were categorized as grade 3 and grade 4, respectively [10]. A urinary pregnancy test was performed 14 days after embryo transfer.

Serum CA125 concentrations were determined by using an enzyme immunometric assay (Can Ag CA125 EIA) on serum samples obtained in a spontaneous cycle (sample 1), 24 hours before oocyte retrieval (sample 2) and 14 days after embryo transfer (sample 3).

Serum estradiol concentrations were determined by using a coated tube radioimmunoassay in samples of blood serum obtained during ovarian stimulation and 24 hours before oocyte retrieval.

Statistical analysis was performed by using a statistics computer program (SigmaStat statistical software version 2.0, Sigma). The Student's *t*-test and rank sum test were used as appropriate and probability values < 0.05 were considered significant. The Spearman correlation test was used to measure the strength of the association between CA125 values with other parameters.

Results

To investigate possible effects of repeated IVF treatments on serum levels of CA125, 26 patients undergoing their first IVF cycle (Group A) and 26 patients in their second attempt (Group B) were selected to participate in the study. To make sure that they did not have any serious ovarian pathologies, especially neoplastic lesions, we verified in advance that their serum levels of CA125 were within normal range (< 35 IU/ml). The first blood sample was collected in the luteal phase of a spontaneous menstrual cycle (I CA125), whereas the second (II CA125) and the third (III CA125) were retrieved 24 hours before oocyte pick up and 14 days after embryo transfer, respectively.

As shown in Table 1, we found that each group of patients showed significant increases in serum CA125. When the two groups were compared CA125 levels in the I and II blood samples did not show any differences. However, the levels of CA125 in the blood samples collected 14 days after embryo transfer (III CA125) of patients in their second attempt were significantly higher than those determined in patients undergoing their first ovarian stimulation ($p < 0.05$).

Table 1. — *Serum CA125 modifications during IVF treatment.*

	CA125* I blood sample	CA125* II blood sample	CA125* III blood sample
Group A	14 ± 10	16 ± 8	31.4 ± 26
Group B	14 ± 8	18.9 ± 11	71.6 ± 40
p value			
(Group A vs Group B)	0.9	0.32	0.02

*CA125 values are reported as IU/ml. Data are reported as means ± SD. Group A: I vs II p = 0.43; II vs III p = 0.006. Group B: I vs II p = 0.067; II vs III p = < 0.001.

Keeping in mind these results we investigated whether the two groups of patients had undergone different ovarian responses and IVF outcome. As shown in Table 2, estradiol levels and the number of mature follicles in Group A and Group B were not significantly different ($p > 0.05$). Oocyte quality, assessed as mean number of retrieved oocytes at metaphase II, was similar in the two groups of patients ($p > 0.05$). Likewise, any difference was detectable when the rates of fertilization, development of good quality embryos and pregnancy were compared ($p > 0.05$).

Table 2. — *Ovarian response and IVF outcome in the two groups of patients.*

Parameter	Group A (n = 26)	Group B (n = 26)	p value
Patient age (years)	35 ± 3	35.5 ± 4.3	0.629
E_2 (pg/ml)	1478 ± 819	1550 ± 750	0.742
No. of follicles	7.4 ± 2.0	6.4 ± 2.3	0.101
No. of metaphase II oocytes	6.16 ± 5.57	5.83 ± 2.71	0.270
No. of immature oocytes	0.04 ± 0.2	0.58 ± 1.55	0.30
Fertilization rate (%)	64.94 ± 26.04	66.84 ± 29.05	0.733
No. of developing embryos	2.4 ± 1.02	3 ± 1.66	0.166
Grade 1-2 embryos (%)	73.83 ± 31	62.31 ± 39.83	0.255
No. of transferred embryos	2.5 ± 0.8	2.3 ± 0.9	0.43
Pregnancy rate *	19	23	1

Data are given as mean ± standard deviation; • % of patients with β hCC serum level > 50 IU/ml

Discussion

Since hormonal and reproduction factors are involved in the etiology of breast or female genital cancer, the risk of assisted reproductive technology (ART) associated treatments requires continuous investigation. Several concerns have been raised about the possible association between ovarian stimulation and ovarian cancer [11]. However, most of the studies conducted so far have not been able to reach solid conclusions due to low statistical power, lack of controls, important confounders and short duration of follow-up [12, 13].

In this work we provide evidence that repeated ovarian stimulation and oocyte retrieval in ART cycles may cause an increase in the serum level of CA125 [14]. Patients undergoing a second IVF treatment (Group B) showed a significant increase in this marker as compared with women in their first attempt (Group A) when the assay was performed 14 days after embryo transfer. A possible interpretation of this finding is that patients from group B may have suffered from repeated epithelial damage during oocyte retrieval. Different kinds of evidence support this hypothesis. First, it is widely reported that ovulation is a predisposing factor in common surface epithelial ovarian cancer. It has been proposed that damage-recognition and repair might be factors involved in the etiology of ovarian metaplasia and carcinogenesis [15]. Second, oxidative distresses to DNA inflicted on ovarian surface epithelial cells could yield a progenitor of tumorigenic potential [16], although oxidative distur-

bances to DNA were reconciled during the consequent luteal phase, before replicative repair of the ovarian rupture wound. Third, discordant cellular growth rates and expression of the cancer antigen CA125 was associated with inhibition of p53 [17]. On the basis of these observations, it is likely that ovarian surface epithelial cell DNA is compromised by reactive oxidants and inflammatory mediators generated during the induction of COH in ART programs.

Increased production of CA125 does not correlate with ovarian response in terms of follicles and oocyte quantity, oocyte maturation, fertilization rate, embryo quality and pregnancy rates. The present data seem to be in accordance with previous works showing that CA125 levels are not predictive of ovarian response [18-20]. The lack of difference in estradiol concentration between the two groups of patients suggests that ovarian steroidogenesis and other granulosa cell functions are not associated with increased production of CA125.

On the other hand, our results showing high CA125 levels in patients undergoing a second IVF cycle lead us to hypothesize that epithelial damage and repair associated with IVF treatment may be responsible for ovarian modifications leading to altered cellular growth rates and expression of CA125.

Since there is no evidence of IVF effects on the incidence of genital or breast cancer, future efforts will be needed to study more in depth the possible correlation between repeated ovarian stimulation and altered epithelial ovarian cell growth.

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