PREGNANCY

Triple-Test Screening in In Vitro Fertilization Pregnancies

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Purpose: To determine whether the results of triple-test analysis differ between spontaneous and IVF pregnancies.

Methods: The study population consisted of 140 women with singleton pregnancies, 70 by IVF, and 70 by spontaneous conception. The groups were matched for maternal age, gestational week, and laboratory batch. The levels of all triple-test markers—alpha-fetoprotein (AFP), human chorionic gonadotropin (HCG), and unconjugated estriol (u-E₃)—were compared between the groups.

Results: Significantly higher HCG levels were detected in the patients with IVF pregnancies than in the control group (1.31 ± 0.8 vs. 0.95 ± 0.5 multiple of the medians, respectively, ($p < 0.006$), but there were no significant differences in AFP or u-E₃ levels. Overall, 18.5% of the IVF group were found to be screen-positive as compared with 11.4% of the control group (difference not statistically significant). Only 8 IVF pregnancies (11.4%) reached the lowest calculated risk possible (1:9999) compared with 17 (24.2%) in the control group ($p < 0.05$).

Conclusions: Our findings support previous data demonstrating elevated maternal serum HCG in IVF patients in comparison with spontaneous ones.

KEY WORDS: Down syndrome; HCG; IVF; triple-test analysis.

INTRODUCTION

Normal pregnancy is associated with an increase in alpha-fetoprotein (AFP) level and a decrease in human chorionic gonadotropin (HCG) level at 15 to 20 weeks of gestation (1,2). Triple-test screening (AFP, HCG, and unconjugated estriol (u-E₃)), performed during the same weeks, has become a common clinical practice to identify pregnancies at risk for chromosomal anomalies (3). It has been found to successfully detect about 66% of Down syndrome fetuses (4).

In vitro fertilization (IVF) pregnancies are commonly achieved after a prolonged infertility period associated with intensive work-up and devastating psychological, financial, and emotional effects. Under such circumstances, the vast majority of these patients are expected to prefer to avoid invasive procedures (such as amniocentesis and chorionic villus sampling) that may risk their pregnancies and rely on triple-test analysis, even in advanced maternal age. However, the accuracy of triple-test analysis in IVF pregnancies remains unclear (5–7).

The purpose of this study was to determine whether triple-test results differ between spontaneous and IVF pregnancies.

Materials and Methods

A case-control study was conducted at the Assisted Reproduction Technology Unit of the Rabin Medical Center. The study population consisted of 140 women with singleton pregnancies, 70 achieved through IVF (study group) and 70 by spontaneous

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conception (control group). The control group was matched for gestational week, maternal age, and laboratory triple-test batch.

All fetuses/newborns in both groups had a normal karyotype, as determined by either cytogenetic studies or physical examination by a pediatrician. Exclusion criteria for both groups were multiple pregnancy, presence of maternal diabetes, a history of vaginal bleeding during the early weeks of pregnancy, and a history of vanishing twin. For the IVF pregnancies, gestational age was calculated on the basis of the embryo transfer day (two completed gestational weeks) and confirmed by first-trimester ultrasound. In the control group, gestational age was based on the last menstrual period and confirmed by a first trimester ultrasound. Our unit's induction protocols and IVF methodology have been detailed previously (8). As a rule, we do not use repeated HCG injections as a luteal phase support.

The triple-test results of both groups were retrospectively collected from the central laboratory's computerized database. In both groups, blood samples were obtained between 14 and 19 weeks of gestation, and AFP, HCG, and u-E₃ were measured with a clinical diagnostics enzyme immunoassay kit (Johnson & Johnson, Amersham, UK). The sensitivities of the assays are approximately 0.4 mcg/l for AFP, 2 IU/ml for HCG, and 0.1 nmol/l for u-E₃. The intraassay variations are 8%, 7%, and 6%, respectively. To compare individual results, values for AFP, HCG, and u-E₃ were expressed as a multiple of the medians (MOM) for gestational age. For statistical analysis, we used the nonparametric Mann-Whitney test for two independent samples; $p < 0.05$ was considered significant.

RESULTS

There were no differences between the two groups (as expected by the study design) in means maternal age or gestational week (32.8 ± 4.7 vs. 32.8 ± 4.4 years, and 16.9 ± 0.6 vs. 16 ± 0.7 weeks, respectively). Significantly higher HCG levels were detected in the patients with IVF pregnancies than in the control group (1.31 ± 0.8 vs. 0.95 ± 0.5 MOM, $p < 0.006$), but there were no significant between-group differences in AFP or u-E₃ levels (1.13 ± 0.5 vs. 1.02 ± 0.26 MOM, and 0.98 ± 0.3 vs. 1.01 ± 0.3 MOM, respectively; Table I). To verify our results and to minimize the possibility of introducing unnecessary variance, we repeated the analysis, using

Table I. Triple-Test Results for the Study and Control Groups (Mean $\pm$ SD)

	IVF ($n = 70$)	Controls ($n = 70$)	P value (Mann-Whitney test)
AFP	1.13 ± 0.5	1.02 ± 0.26	ns
HCG	1.31 ± 0.8	0.95 ± 0.5	<0.006
μ -E ₃	0.98 ± 0.3	1.01 ± 0.3	ns

Note. Values are given as mean of the multiple of the median. AFP: alpha-fetoprotein; HCG: human chorionic gonadotropin; μ -E₃: unconjugated estriol.

only the data for the 1 week in which most of the examinations were performed (Week 18, 37 examinations in each group). Again, significantly higher HCG levels were detected in the study than the control group (1.35 ± 0.6 vs. 0.93 ± 0.4 MOM, $p < 0.006$). As a result of the test, 13 IVF pregnancies (18.5%) were declared screen-positive, and these patients were offered amniocentesis, compared with 8 (11.4%) in the control group (difference not statistically significant). Only 8 IVF pregnancies (11.4%) reached the lowest calculated risk possible (1:9999) as compared with 17 (24.2%) in the control group ($p < 0.05$).

DISCUSSION

Concerns have been raised regarding the accuracy of triple-test analysis in IVF pregnancies. Both Heinonen *et al.* (9) and Ribbert *et al.* (10) compared the triple-test results in their centers between IVF pregnancies ($n = 41$ and $n = 67$, respectively) and unmatched general spontaneous pregnancies ($n = 4935$ and $n = 4732$, respectively) and found significantly higher HCG levels in the IVF group; Ribbert *et al.* (10) also noted significantly lower AFP levels. Neither study evaluated u-E₃. The present study further assessed differences in the triple-test results using a strict study design with groups matched for gestational age, maternal age, and laboratory triple-test batch. Again, significantly higher levels of HCG were detected in the patients with IVF pregnancies (1.31 ± 0.8 vs. 0.95 ± 0.5 MOM, $p < 0.006$), but there were no differences in AFP or u-E₃ (Table I).

Several mechanisms have been suggested to explain the elevated HCG levels in IVF pregnancies. A miscalculation of gestational age, as suggested by some authors (11), was unlikely in our study because it was verified by a first-trimester ultrasound in both groups. In addition, the exact date of embryo transfer was known in the study group. Marrs *et al.* (12) speculated

that repeated HCG administration as a luteal phase support raises HCG levels. We believe this is doubtful, as HCG has a short half-life and is no longer in the circulation when the triple test is performed. In any case, in our unit, HCG is not administered as a luteal support. A third possibility, suggested by Heinonen *et al.* (9), is that a reduced oxygen supply to the trophoblast may result in increased production of HCG; however, these authors did not determine if indeed there is a decreased oxygen supply in IVF pregnancies. Finally, although the transfer of multiple embryos in IVF pregnancies probably results in an initially high HCG level even when only a singleton clinical pregnancy is identified, this would presumably not affect the triple-test levels which are measured 12 weeks later.

In normal pregnancy, the HCG level rises until 10 weeks' gestation and thereafter progressively drops. This decrease is usually attributed to the differentiation of the trophoblast tissue, resulting in "takeover" of the placenta and resolution of the corpus luteum (1). Since there are multiple corpora lutea in IVF pregnancies, we hypothesize that by some unknown feedback mechanism, the placenta in such circumstances secretes higher levels of HCG. These higher levels may be maintained throughout the first half of pregnancy and therefore may be reflected in the triple-test screening.

Our finding that IVF pregnancies are characterized by higher HCG levels is especially significant because, as demonstrated by Wald *et al.* (4), HCG is the most influential of the triple-test markers. Being that the incidence of Down syndrome is not increased in IVF pregnancies (13), this finding implies that some women with IVF pregnancies are referred for unnecessary amniocentesis owing to an incorrect risk assessment. In the present study, 18.5% of the IVF group was offered amniocentesis as compared with 11.4% of the control group. This difference did not reach statistical significance, but power calculations implied that more than 400 women are needed to reach this level, a number that is currently impossible to achieve in our setting. Although we found a significant difference in the lowest calculated risk group (8 pregnancies reached the lowest calculated risk as compared with 17 controls) it has no clinical implication. Another possible limitation of the study is the different method of determination of gestational age in the two groups: embryo transfer in the IVF pregnancies and last day of menstrual bleeding in the control pregnancies, with verification by first-trimester ultrasound in

both. However, these are the best available ways to calculate gestational age in each of these types of patients. Furthermore, these are also the methods used in daily clinical practice, and our study was carried out to answer a clinical question.

To summarize, our study confirms earlier reports of elevated maternal serum HCG levels in IVF pregnancies. This factor should be considered when the triple-test results in these patients are interpreted.

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