

Maternal midtrimester serum AFP and free β -hCG levels in *in vitro* fertilization twin pregnancies

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We aimed to compare the levels of alpha-fetoprotein (AFP) and free β -human chorionic gonadotrophin (β -hCG) levels as multiples of the median (MoM) values between spontaneous and *in vitro* fertilized (IVF) twin pregnancies. The control group of spontaneous singleton pregnancies was used for calculating the gestational age specific median levels of the values. Within a cohort of 19 310 pregnancies, 145 twin pregnancies were identified. The data were collected from Down syndrome (DS) screening programmes in four University catchment areas in Finland between 1994–98. Maternal midtrimester serum marker levels were measured across gestational weeks 14–18. There were no fetal chromosome anomalies in either of the twin groups or the singleton group. Serum AFP of 145 and β -hCG values of 39 spontaneous twin pregnancies were compared to the values of 6548 singleton pregnancies. In IVF twins 30 AFP and 29 β -hCG values were compared to the levels of the control group. Both AFP and β -hCG values were twice as high in the spontaneous twin pregnancies (medians 2.18 and 1.83 MoM respectively) as in the singleton group (medians 1.00 and 1.00 MoM respectively). In IVF twin pregnancies β -hCG levels were higher (median 2.20 MoM) than in spontaneous twins ($p = 0.08$), whereas no significant difference was found in AFP levels (2.30 MoM). In conclusion, the higher levels of β -hCG levels in IVF twin pregnancies should be considered in DS screening to avoid high false positive rates. Copyright © 2000 John Wiley & Sons, Ltd.

KEY WORDS: *in vitro* fertilization; twins

INTRODUCTION

Down syndrome screening is performed in many countries by measuring serum alpha-fetoprotein (AFP) and free β -human chorionic gonadotrophin (hCG) at 14–16 weeks gestation. The test detects 66% of affected singleton pregnancies (Wald *et al.*, 1988).

In unaffected twin pregnancies AFP and free β -hCG levels are known to roughly double compared to singleton pregnancies (Spencer *et al.*, 1995). In trisomic twin pregnancies serum AFP and hCG behave in the same manner as in singleton pregnancies and DS screening might be possible also in twin pregnancies with a 51–53% detection rate and 5% false positive rate (FPR) (Neveux *et al.*, 1996; Spencer *et al.*, 1995; Wald *et al.*, 1991).

In singleton pregnancies, maternal serum AFP and/or β -hCG have been reported to differ between spontaneously conceived and assisted reproductive techniques (ART) started pregnancies. Because in pregnancies started with ART serum hCG is usually higher and AFP equal or lower than in spontaneously started pregnancies, this results in higher FPR in DS

screening (Heinonen *et al.*, 1996; Frishman *et al.*, 1997; Ribbert *et al.*, 1996). This has been studied mostly in singleton pregnancies, but no references were found regarding multiple IVF pregnancies. Because the rate of twins in IVF pregnancies is high, we wanted to compare the levels of AFP and β -hCG between spontaneous and IVF multiple pregnancies.

MATERIAL AND METHODS

Our study consisted of 19 310 pregnancies where a DS screening test had been performed. Data were collected from four University Hospital catchment areas in Finland between 1994–96. Maternal serum samples were collected between gestational weeks 14–18. Gestational age was verified by ultrasound dating before sample taking. In *in vitro* fertilized women ultrasound dating was not used, because the exact date of implantation was known. Fourteen days were added to the implantation date in IVF pregnancies to update the gestational age of the pregnancy. AFP and free β -hCG in serum were analysed using the two-site fluoroimmunoassay DELFIA[®] hAFP/free hCG β assay (EG & G Wallac, Turku, Finland).

The data on pregnancy and newborn outcome were collected from four national registers: birth register,

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hospital register, abortion register and malformation register.

In our study there were 145 twin pregnancies, and no chromosome anomalies or neural tube defects were found. Free β -hCG levels were available only in 39 pregnancies, because one of the hospitals changed the sampling method from total hCG to β -hCG only in the last year of data collection. The total number of IVF twin pregnancies was 30. In one of these IVF pregnancies serum β -hCG was analysed as total hCG and could not be used in the final analysis where free β -hCG and AFP levels were compared between IVF twins and spontaneous singleton pregnancies.

The control group consisted of 6548 singleton pregnancies with no chromosome anomalies.

Statistical methods

The MoM values were calculated by dividing each observed AFP and β -hCG value in the same gestational day by the median of every hospital's gestational age specific median. We compared pairwise AFP and free β -hCG MoM levels between IVF twins, spontaneous twins and the control group of normal singleton pregnancies with the Wilcoxon test. A two-sided p -value of 0.05 was considered to indicate statistical significance. The median difference with 95% confidence interval (CI) was also calculated when statistical significance existed (Sachs, 1984). All calculations were done by SAS[®] for Windows 6.11 package (SAS Institute Inc., Cary, NC, USA) and graphic presentations by Microcal Origin[™] for Windows (Microcal Software Inc., Northampton, MA, USA).

RESULTS

The demographic data of spontaneous and IVF twin pregnancies and the control group of singleton pregnancies are shown in Table 1.

Table 1—The demographic data on spontaneously conceived and IVF twin pregnancies and the normal singleton pregnancies. Values are means (SD)

| | Spontaneous twins <i>n</i> = 145 | IVF twins <i>n</i> = 30 | Singleton pregnancies <i>n</i> = 6548 |
|------------------|-------------------------------------|----------------------------|--|
| Age (years) | 32.2 (4.4) | 33.5 (4.5) | 29.7 (4.7) |
| Weight (kg) | 68.7 (13.3) | 65.7 (12.4) | 66.7 (12.3) |
| Gestation (days) | 109.6 (4.2) | 109.7 (3.6) | 109.8 (4.0) |

Table 2—AFP and free β -hCG levels in spontaneously conceived and IVF twins and in singleton pregnancies. Values are medians with 5% and 95% percentiles in parentheses

| | Spontaneous twins <i>n</i> = 145 | IVF twins <i>n</i> = 30 | Singleton pregnancies <i>n</i> = 6548 |
|-----------------------|-------------------------------------|------------------------------------|--|
| AFP MoM | 2.18 (1.24, 3.84) | 2.30 (1.29, 4.68) | 1.00 (0.57, 1.79) |
| Free β -hCG MoM | 1.83 (0.72, 8.40) (<i>n</i> = 39) | 2.20 (1.25, 8.18) (<i>n</i> = 29) | 1.00 (0.37, 3.04) |

AFP and free β -hCG MoM levels were twice as high in spontaneously conceived twin pregnancies as compared to singleton pregnancies (both $p < 0.0001$) (Table 2) (Figures 1 and 2). Median differences with 95% confidence intervals for AFP and β -hCG MoM levels were 1.18 (0.15–2.21) and 0.83 (0.26–1.41) respectively. No significant difference was found in AFP MoM levels between IVF twin pregnancies and spontaneously conceived twins (medians 2.30 and 2.18, respectively). Free β -hCG MoM levels were higher in

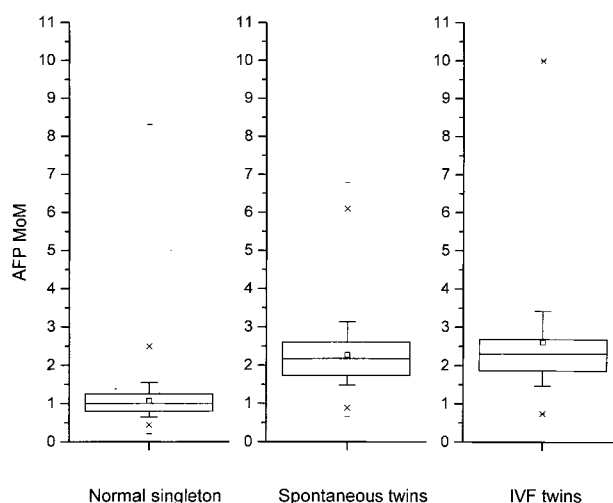


Figure 1—Box plot shows 10th, 25th, 50th and 90th centiles of AFP MoM in singleton pregnancies, spontaneous twins and IVF twins. 1st and 99th centiles (x) and ranges of the values (–) are also shown

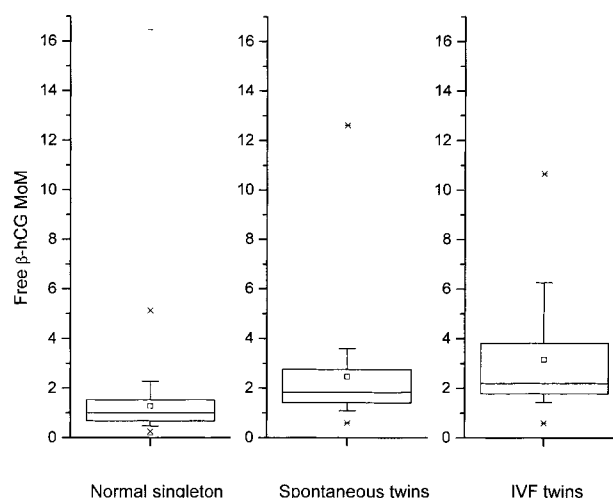


Figure 2—Box plot shows 10th, 25th, 50th and 90th centiles of free β -hCG MoM in singleton pregnancies, spontaneous twins and IVF twins. 1st and 99th centiles (x) and ranges of the values (–) are also shown

IVF twins than in spontaneously conceived twins (2.20 and 1.83 respectively) ($p=0.08$) (Table 2). Median difference was 0.36 between the groups. No difference was observed in AFP MoM levels between the two twin groups (2.30 in IVF and 2.18 in spontaneous twins) ($p=0.39$).

DISCUSSION

There are few papers where mean AFP and hCG values with MoMs in spontaneously conceived twin pregnancies are given (Neveux *et al.*, 1996; Spencer *et al.*, 1995; Wald *et al.*, 1991). To our knowledge there are no other studies where IVF and spontaneous twin pregnancy Down syndrome screening tests had been compared although multiple pregnancies are more common in ART pregnancies than in spontaneous pregnancies. In normal singleton IVF pregnancies AFP values are equal or lower and hCG values higher than in spontaneous pregnancies (Barkai *et al.*, 1996; Frishman *et al.*, 1997; Heinonen *et al.*, 1996; Ribbert *et al.*, 1996) resulting in an increased rate of false-positive results in IVF pregnancies in Down syndrome screening. It has been suggested that the drugs used by IVF, higher maternal age and ovum donation may affect the AFP and hCG levels in IVF pregnancies (Barkai *et al.*, 1996; Frishman *et al.*, 1997). According to our data, the effect of IVF on the levels of AFP and free β -hCG are similar in twin pregnancies, as has been previously published in singleton pregnancies. The background behind these changes may be the factors mentioned above and also the placentation failure in IVF pregnancies, where intrauterine growth retardation has been shown to be more common than in spontaneous pregnancies (Fabris *et al.*, 1990; Tan *et al.*, 1992). The increased false positive rate in IVF pregnancies should be discussed before offering a Down syndrome screening test in twin IVF pregnancies.

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