

First trimester screening with free β -hCG, PAPP-A and nuchal translucency in pregnancies conceived with assisted reproduction

Francesco Orlandi^{1*}, Cinzia Rossi¹, A. Allegra², David Krantz³, Terrence Hallahan³, Emanuela Orlandi¹ and James Macri³

¹Centro di Diagnosi Prenatale, Palermo, Italy

²Centro Andros, Palermo, Italy

³Research Division, NTD Laboratories, Huntington Station, NY, USA

Objective To evaluate the effect of *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) on free beta-human chorionic gonadotrophin (β -hCG), pregnancy-associated plasma protein A (PAPP-A) and nuchal translucency (NT).

Methods First trimester maternal dried whole blood specimens from 74 singleton pregnancies (32 by IVF and 42 by ICSI) and 30 twin pregnancies (16 by IVF and 14 by ICSI) in which conception was achieved with assisted reproduction techniques were matched with five controls resulting in 370 singleton controls and 150 twin controls. NT was measured using the Fetal Medicine Foundation protocol. Free β -hCG, PAPP-A and NT levels were compared between the IVF and control groups and between the ICSI and control groups using the Mann-Whitney U test.

Results In singleton pregnancies, the only significant difference was a 21% (95% CI: –35%–7%) reduction in PAPP-A in IVF cases. In twin pregnancies, the only significant difference was a 12% (95% CI: –34%–3%) reduction in NT in IVF cases. In singleton pregnancies, the false-positive rate for Down syndrome screening was 1.4% and 1.9% greater for the IVF and ICSI groups, respectively, compared to controls for a general screening population.

Conclusions Patients undergoing assisted reproduction techniques should be counseled about the possibility of increased false-positive rates. Larger studies are needed to confirm this observation and to develop appropriate adjustment factors to reduce false-positive rates. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: Down syndrome screening; free β -hCG; IVF; ICSI; PAPP-A

INTRODUCTION

First trimester Down syndrome screening, which combines the ultrasound measurement of nuchal translucency (NT) (Snijders *et al.*, 1998) with the biochemical measurement of free beta-human chorionic gonadotrophin (β -hCG) and pregnancy-associated plasma protein A (PAPP-A), can identify a subgroup of 5% of the population with the highest risk of carrying a fetus with Down syndrome. There have been several studies (Orlandi *et al.*, 1997; Wald and Hackshaw, 1997; De Biasio *et al.*, 1999; De Graaf *et al.*, 1999; Spencer *et al.*, 1999; Wald *et al.*, 1999a; Krantz *et al.*, 2000) showing that within this subgroup 80–91% of Down syndrome cases could be identified.

Patients undergoing assisted reproduction techniques have usually spent considerable time and money trying to become pregnant so their pregnancies are particularly precious. As a result they have a tendency to prefer to avoid invasive diagnostic procedures and the associated risk of miscarriage (Meschede *et al.*, 1998; Schover *et al.*, 1998; Geipel *et al.*, 1999). Therefore, an effective screening test that would identify only a small proportion of the population as

being at significant risk to require invasive diagnosis would offer distinct advantages to these patients. First trimester screening is an effective test in this regard.

Maternal serum biochemical markers are known to be associated with various maternal factors such as ethnicity, multiple pregnancy, insulin dependency and maternal weight (ACOG, 1996). As part of the screening process it would be important to know whether assisted reproduction techniques have any effect on the biochemical markers or NT that could result in a difference in false-positive and/or detection rates.

MATERIALS AND METHODS

As part of our ongoing prospective study (September 1995 to December 2000) of first trimester Down syndrome screening, 74 singleton pregnancies [32 by *in vitro* fertilization (IVF), 42 by intracytoplasmic sperm injection (ICSI)] and 30 twin pregnancies (16 by IVF, 14 by ICSI), in which assisted reproduction was involved were identified. Each sample was matched with five samples from naturally conceived pregnancies based on gestational age, maternal age, and time of testing. Among these patients, seven controls, three IVF and five ICSI singleton pregnancies did not

*Correspondence to: Dr. F. Orlandi, Centro di Diagnosi Prenatale, 35 Via Villareale, 90141 Palermo, Italy. E-mail: orlandi@tin.it

undergo a NT examination. All patients were Caucasian. Table 1 summarizes the gestational age and maternal age of each group of patients.

Each sample was collected as dried whole blood spots on filter paper (Schleicher and Schuell, Keane, NH, USA). Samples were analyzed for free β -hCG and PAPP-A by in-house enzyme-linked immunosorbent assays (ELISAs). The free β -hCG assay has a detectable range from 0.6 to 68.2 ng/ml. All samples were diluted 1 : 5 prior to being assayed. The inter- and intra-assay coefficient of variation (CV) were 4.1% and 5.9%, respectively. The free β -hCG assay was calibrated against International Reference preparation 75/551. The PAPP-A assay has a detectable range from 0.05 to 10.29 IU/l. Samples were not diluted prior to being assayed. The inter- and intra-assay CV were 6.6% and 1.7%, respectively. The PAPP-A assay was calibrated against International Reference preparation 78/610. Low, medium and high control samples were run on each assay to assess quality control.

NT examinations were conducted according to the protocol of the Fetal Medicine Foundation, London, UK. In the case of twin pregnancies, the larger of the two NT values was used in the analysis.

Multiples of the median values (MoM) were determined based on medians from 10 251 unaffected pregnancies (Krantz *et al.*, 2000). Statistical significance was determined by the Mann-Whitney U test and approximate 95% confidence intervals (CI) were determined using Analyse-It statistical software (Analyse-It Software Ltd, Leeds, UK).

RESULTS

Table 2 shows the free β -hCG, PAPP-A and NT MoM values in singleton and twin pregnancies. For free β -hCG there was no significant difference between the controls and either the IVF or ICSI group in singleton and twin pregnancies. PAPP-A levels were significantly lower for the IVF group but not for the ICSI group in singleton pregnancies. In twin pregnancies there was no significant difference in PAPP-A levels between the control, IVF and ICSI groups. NT measurements were not significantly different between the controls and either the IVF or ICSI group in singleton pregnancies.

In twin pregnancies, NT was significantly lower in the IVF cases. Table 3 shows the correlation of each of the screening markers with gestational age. In singleton pregnancies there were no significant trends in the screening markers with gestational age. In twin pregnancies there was a significant positive correlation for free β -hCG versus gestational age in the IVF group.

The false-positive rate for Down syndrome screening was 22/363 (6.1%), 7/66 (10.6%), 4/29 (13.8%) and 3/37 (8.1%) for controls, the assisted reproduction group, IVF and ICSI, respectively. Using the observed likelihood ratios and modeling for the general age distribution of live births in the US resulted in false-positive rates of 3.3%, 4.7%, 5.2% and 4.2% for controls, the assisted reproduction group, IVF and ICSI, respectively.

For trisomy 18 screening, the false-positive rates were 3/363 (0.8%), 1/66 (1.5%), 1/29 (3.4%) and 0/37 (0.0%), respectively. Modeling the observed likelihood ratios resulted in false-positive rates of 0.9%, 0.9%, 1.9% and 0.1%, respectively.

DISCUSSION

The maximum difference between any two groups was the 21% reduction observed for PAPP-A in IVF pregnancies compared with the controls. These results are similar to those observed in a previous study (Liao *et al.*, 2001) in which the largest observed difference for either free β -hCG, PAPP-A or NT between controls and those undergoing assisted reproduction was also 21%. However, the actual differences varied between the studies. The present study showed no significant difference for free β -hCG among the groups in singleton pregnancy while the previous study showed free β -hCG to be significantly increased in IVF cases. For PAPP-A in singleton pregnancies, the previous study showed significantly reduced levels in both the IVF and ICSI group while the current study showed a significant reduction only in the IVF group. For NT, neither study showed a significant difference in either the IVF or ICSI groups in singleton pregnancies. The previous study did not analyze twin pregnancies.

The present results showed either an insignificant or small significant difference between the levels of the

Table 1—Summary of sample population

| | <i>n</i> | Mean gestational age (in weeks) (SD) | Mean maternal age (in years) (SD) |
|---------------------------|----------|---|--------------------------------------|
| Singleton pregnancies | | | |
| Controls | 370 | 12.13 (0.79) | 31.99 (4.45) |
| All assisted reproduction | 74 | 12.22 (0.71) | 32.47 (3.80) |
| IVF | 32 | 12.23 (0.63) | 32.22 (3.16) |
| ICSI | 42 | 12.20 (0.76) | 32.67 (4.25) |
| Twin pregnancies | | | |
| Controls | 150 | 12.22 (0.61) | 31.34 (3.72) |
| All assisted reproduction | 30 | 12.17 (0.54) | 31.27 (4.07) |
| IVF | 16 | 12.26 (0.54) | 31.31 (4.63) |
| ICSI | 14 | 12.06 (0.54) | 31.21 (3.49) |

ICSI, Intracytoplasmic sperm injection; IVF, *in vitro* fertilisation; SD, standard deviation.

Table 2—Comparison of first trimester free beta-human chorionic gonadotrophin (β -hCG), pregnancy-associated plasma protein-A (PAPP-A) and nuchal translucency (NT) MoM values in pregnancies conceived with assisted reproduction and those conceived spontaneously

| | Median free β -hCG MoM (p) ^a [approximate 95% CI] ^b | Median PAPP-A MoM (p) ^a [approximate 95% CI] ^b | Median NT MoM (p) ^a [approximate 95% CI] ^b |
|---------------------------|---|--|--|
| Singleton pregnancies | | | |
| Controls | 1.00 | 1.00 | 1.00 |
| All assisted reproduction | 0.95 (0.9458) [−0.15–0.13] | 0.89 (0.0143) [−0.22–0.03] | 1.04 (0.6884) [−0.06–0.08] |
| IVF | 0.84 (0.4186) [−0.27–0.12] | 0.79 (0.0029) [−0.35–−0.07] | 1.10 (0.3717) [−0.06–0.15] |
| ICSI | 1.13 (0.4289) [−0.11–0.25] | 0.96 (0.4178) [−0.18–0.07] | 1.02 (0.8193) [−0.08–0.10] |
| Twin pregnancies | | | |
| Controls | 2.01 | 1.52 | 1.00 |
| All assisted reproduction | 1.72 (0.3229) [−0.51–0.20] | 1.61 (0.9464) [−0.30–0.31] | 0.90 (0.0081) [−0.27–−0.04] |
| IVF | 1.84 (0.7139) [−0.57–0.41] | 1.61 (0.9346) [−0.34–0.41] | 0.88 (0.0189) [−0.34–−0.03] |
| ICSI | 1.57 (0.2622) [−0.71–0.25] | 1.68 (0.8483) [−0.41–0.52] | 0.91 (0.1245) [−0.31–0.04] |

^a p Values are based on the Mann-Whitney U test.

^bThe approximate 95% confidence interval (95% CI) is for the difference between the median and the control group median. ICSI, Intracytoplasmic sperm injection; IVF, *in vitro* fertilization; MoM, multiples of the median.

screening markers in control samples and those undergoing assisted reproduction. Indeed, any significant difference might be attributable to chance since several different statistical tests were performed, increasing the likelihood of finding any particular significant difference. This possibility is particularly relevant to the reduced NT levels in twin pregnancy, since neither the present nor the previous study (Liao *et al.*, 2001) found a significant difference in singleton pregnancies. Both studies did agree, however, that PAPP-A levels were lower in pregnancies undergoing assisted reproduction. The cause of such a reduction might be related to a factor associated with pregnancies undergoing assisted reproduction. One such factor is gravidity, since pregnancies undergoing assisted reproduction tend to be first pregnancies. However, three previous studies showed that there was no significant

correlation between gravidity and PAPP-A (Hallahan *et al.*, 1999; De Graaf *et al.*, 2000; Spencer *et al.*, 2000). Another factor might be related to an increased level of progesterone which has been observed in IVF patients (Wald *et al.*, 1999b). However, it seems unlikely that an increase in progesterone would cause a reduction in PAPP-A levels.

Similar to another study of second trimester markers (Lam *et al.*, 1999) the present study showed that the effect of IVF and ICSI might be different. Although it is not clear why these groups have different marker levels, care should be taken to analyze these groups separately. Larger studies are needed to more precisely define the differences in levels observed in the IVF and ICSI groups compared to naturally occurring pregnancies.

There have been several second trimester Down

Table 3—Correlation of first trimester free beta-human chorionic gonadotrophin (β -hCG), pregnancy-associated plasma protein-A (PAPP-A) and nuchal translucency (NT) MoM values versus gestational age in pregnancies conceived with assisted reproduction and those conceived spontaneously

| | Free β -hCG (p) ^a | PAPP-A (p) ^a | NT (p) ^a |
|---------------------------|--|-----------------------------|-------------------------|
| Singleton pregnancies | | | |
| All assisted reproduction | −0.14 (0.2473) | 0.12 (0.2951) | −0.03 (0.8146) |
| IVF | −0.13 (0.4618) | 0.13 (0.4673) | −0.05 (0.8095) |
| ICSI | −0.18 (0.2443) | 0.13 (0.4222) | 0.01 (0.9565) |
| Twin pregnancies | | | |
| All assisted reproduction | 0.38 (0.0359) | 0.35 (0.0614) | −0.20 (0.2842) |
| IVF | 0.71 (0.0022) | 0.19 (0.4815) | −0.24 (0.3684) |
| ICSI | 0.14 (0.6280) | 0.48 (0.0844) | −0.14 (0.6389) |

^aValues for p are based on the Spearman rank correlation test.

ICSI, Intracytoplasmic sperm injection; IVF, *in vitro* fertilization.

syndrome screening studies that indicated that biochemical markers were significantly different in IVF pregnancies compared to spontaneously conceived pregnancies (Barkai *et al.*, 1996; Heinonen *et al.*, 1996; Ribbert *et al.*, 1996; Frishman *et al.*, 1997). However, most of these studies analyzed a combination of alpha-fetoprotein, intact hCG and unconjugated estriol, all of which are not used in first trimester screening. The effect of IVF on free β -hCG, which can be used in either first or second trimester screening, has been analyzed in a few second trimester studies. These studies indicated that there might be a small increase in free β -hCG levels. One study (Wald *et al.*, 1999b) showed that second trimester free β -hCG levels were 9% higher in IVF pregnancies, albeit somewhat less than the 15% increase observed with intact hCG. Another study (Raty *et al.*, 2000) showed free β -hCG levels to be 20% higher in IVF twin pregnancies compared to spontaneously conceived pregnancies, however, this difference was of only marginal statistical significance ($p=0.08$). In the present study, free β -hCG in IVF pregnancies was 16% lower than in controls. A correlation of free β -hCG with gestational age, however, did not show an increasing trend in free β -hCG levels in singleton pregnancies although a trend was observed in the smaller group of twin pregnancies (Table 3). A larger study could ascertain whether the increase in free β -hCG in IVF pregnancies is associated with gestational age across both the first and second trimester.

The present study is in agreement with a previous study (Liao *et al.*, 2001) that the false-positive rate of Down syndrome screening is increased in pregnancies undergoing assisted reproduction. However, based on modeling, this increase is only 1.4% to 1.9% in the present study and 1.2% in the previous study. Patients undergoing assisted reproduction should be counseled about the possible small increase in the false-positive rate but this should not prevent them from undergoing such screening. Larger studies are needed to confirm the present observations. In addition, studies with a significant number of affected cases are needed to develop adjustment factors to screening markers to ensure a lower false-positive rate without reducing detection efficiency.

REFERENCES

- American College of Obstetrics and Gynecology (ACOG). 1996. ACOG Educational Bulletin. Maternal Serum Screening, No. 228, September 1996 (replaces No. 154, April 1991). *Int J Gynaecol Obstet* **55**: 299–308.
- Barkai G, Goldman B, Ries L, *et al.* 1996. Down's syndrome screening marker levels following assisted reproduction. *Prenat Diagn* **16**: 1111–1114.
- De Biasio P, Siccardi M, Volpe G, *et al.* 1999. First trimester screening for Down syndrome using nuchal translucency measurement with free beta-hCG and PAPP-A between 10 and 13 weeks of pregnancy – the combined test. *Prenat Diagn* **19**: 360–363.
- De Graaf IM, Pajkrt E, Bilardo CM, *et al.* 1999. Early pregnancy screening for fetal aneuploidy with serum markers and nuchal translucency. *Prenat Diagn* **19**: 458–462.
- De Graaf IM, Cuckle HS, Pajkrt E, *et al.* 2000. Co-variables in first trimester maternal serum screening. *Prenat Diagn* **20**: 186–189.
- Frishman GN, Canick JA, Hogan JW, *et al.* 1997. Serum triple-marker screening in *in vitro* fertilization and naturally conceived pregnancies. *Obstet Gynecol* **90**: 98–101.
- Geipel A, Gembruch U, Ludwig M, *et al.* 1999. Genetic sonography as the preferred option of prenatal diagnosis in patients with pregnancies following intracytoplasmic sperm injection. *Hum Reprod* **14**: 2629–2634.
- Hallahan TW, Krantz DA, Buchanan PD, *et al.* 1999. The effect of gravidity and maternal age on the first trimester Down syndrome biochemical markers free beta hCG and PAPP-A (Abstract). *Am J Hum Genet* **65**: A176.
- Heinonen S, Ryyanen M, Kirkinen P, *et al.* 1996. Effect of *in vitro* fertilization on human chorionic gonadotropin serum concentrations and Down's syndrome screening. *Fertil Steril* **66**: 398–403.
- Krantz DA, Hallahan TW, Orlandi F, *et al.* 2000. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. *Obstet Gynecol* **96**: 207–213.
- Lam YH, Yeung WS, Tang MH, Ng EH, So WW, Ho PC. 1999. Maternal serum alpha-fetoprotein and human chorionic gonadotropin in pregnancies conceived after intracytoplasmic sperm injection and conventional *in-vitro* fertilization. *Hum Reprod* **14**: 2120–2123.
- Liao AW, Heath V, Kametas N, Spencer K, Nicolaides KH. 2001. First-trimester screening for trisomy 21 in singleton pregnancies achieved by assisted reproduction. *Hum Reprod* **16**: 1501–1504.
- Meschede D, Lemcke B, Stussel J, *et al.* 1998. Strong preference for non-invasive prenatal diagnosis in women pregnant through intracytoplasmic sperm injection (ICSI). *Prenat Diagn* **18**: 700–705.
- Orlandi F, Damiani G, Hallahan TW, *et al.* 1997. First-trimester screening for fetal aneuploidy: biochemistry and nuchal translucency. *Ultrasound Obstet Gynecol* **10**: 381–386.
- Raty R, Virtanen A, Koskinen P, *et al.* 2000. Maternal midtrimester serum AFP and free beta-hCG levels in *in vitro* fertilization twin pregnancies. *Prenat Diagn* **20**: 221–223.
- Ribbert LS, Kornmann LH, De Wolf BT, *et al.* 1996. Maternal serum screening for fetal Down syndrome in IVF pregnancies. *Prenat Diagn* **16**: 35–38.
- Schover LR, Thomas AJ, Falcone T, *et al.* 1998. Attitudes about genetic risk of couples undergoing *in-vitro* fertilization. *Hum Reprod* **13**: 862–866.
- Snijders RJM, Noble P, Sebire N, *et al.* 1998. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. *Lancet* **352**: 343–346.
- Spencer K, Souter V, Tul N, *et al.* 1999. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Ultrasound Obstet Gynecol* **13**: 231–237.
- Spencer K, Ong CYT, Liao AWJ, Nicolaides KH. 2000. The influence of parity and gravidity on first trimester markers for chromosomal abnormality. *Prenat Diagn* **20**: 792–794.
- Wald NJ, Hackshaw AK. 1997. Combining ultrasound and biochemistry in first trimester screening for Down's syndrome. *Prenat Diagn* **17**: 821–829.
- Wald NJ, Watt HC, Hackshaw AK. 1999a. Integrated screening for Down's syndrome based on tests performed during the first and second trimesters. *N Engl J Med* **341**: 461–467.
- Wald NJ, White N, Morris JK, *et al.* 1999b. Serum markers for Down's syndrome in women who have had *in vitro* fertilisation: implications for antenatal screening. *Br J Obstet Gynaecol* **106**: 1304–1306.