First trimester biochemical screening for Down’s syndrome in singleton pregnancies conceived by assisted reproduction

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BACKGROUND: Serum biochemical markers [free βhCG (fβhCG); pregnancy-associated plasma protein-A (PAPP-A)] used in first trimester Down’s syndrome screening have not been fully investigated in pregnancies achieved by assisted reproduction techniques. We present data on pregnancies conceived by all types of assisted reproduction techniques, including pregnancies following ovum donation (OD) and a large sample by ICSI.

METHODS: First trimester Down’s syndrome screening was performed in 1054 normal singleton pregnancies: natural conception (n = 498), ovulation induction (OS, n = 97), IVF (n = 47), ICSI (n = 222) and OD (n = 190).

RESULTS: No differences in maternal levels of fβhCG and PAPP-A, measured by the Kryptor system, appeared between naturally conceived pregnancies (n = 498) and those obtained with assisted reproduction techniques (n = 556). Several differences were apparent when comparing fβhCG levels between different technologies but PAPP-A levels only differed between OS and IVF pregnancies (P < 0.05). In a further small study, no differences were observed using frozen embryos (n = 37), preimplantation genetic diagnosis (n = 53) or sperm from testicular biopsy (n = 21).

CONCLUSIONS: Data accumulated so far suggest that first trimester biochemical markers either do not need any adjustments (e.g. in pregnancies obtained after OS and ICSI), or have very little impact (e.g. IVF pregnancies) or no impact (e.g. OD pregnancies) on the false positive rates.

Key words: assisted reproduction/Down’s syndrome screening/first trimester/free βhCG/pregnancy-associated plasma protein-A

Introduction

Down’s syndrome screening is being shifted from the second to the first trimester of pregnancy because of the higher detection rates and the earlier diagnosis of chromosomal abnormalities. Second trimester screening, based on the combination of maternal age and maternal serum hCG, alpha-fetoprotein (AFP) and unconjugated estradiol (uE3), yields a detection rate ~60–75% for a 5% false positive rate. In contrast, for the same false positive rate, first trimester screening based on the combination of maternal age, nuchal translucency and maternal serum free βhCG (fβhCG) and pregnancy-associated plasma protein-A (PAPP-A) achieves a detection rate ~90% (Nicolaides, 2004) or even higher when other ultrasound parameters (i.e. nasal bone) are added (Cicero et al., 2003). Hence, first trimester screening is currently the method of choice in many settings.

Although ultrasonography is the essential part of the early screening strategy, additional biochemical parameters are also recommended as these are independent of the ultrasound markers and increase the detection rates (Spencer et al., 1999). The application of such biochemical markers to pregnancies achieved by assisted reproduction techniques has not been fully investigated. Accumulated data from the second trimester screening showed that maternal serum levels of hCG, AFP and uE3 seem to be modified in assisted reproduction pregnancies, leading to an increased false positive rate for trisomy 21 (Barkai et al., 1996; Lam et al., 1999; Räty et al., 2001; Maymon and Shulman, 2002; Räty et al., 2002; Hui et al., 2003). The reliability of first trimester biochemical screening in assisted reproduction pregnancies remains controversial. Data on this issue are still relatively scarce and previously published works are usually focused only in a particular assisted reproduction procedure (Liao et al., 2001; Wojdemann et al., 2001; Orlandi et al., 2002; Maymon and Shulman, 2002, 2004; Ghisoni et al., 2003; Lai et al., 2003).

Therefore, the question of whether adjustments of the biochemical levels are, or are not, necessary to maintain a low false positive rate when screening assisted reproduction pregnancies remains unanswered.

In the present study, we wish to report our experience on biochemical first trimester screening in pregnancies obtained by various assisted reproduction procedures compared to those conceived naturally. In this work we present data on pregnancies conceived by all types of assisted reproductive
techniques, including pregnancies following ovum donation and a large sample of pregnancies achieved by ICSI.

Materials and methods
The present study was carried out between January 1, 2001 and December 31, 2003 in the Materno-Fetal Medicine Unit of the Instituto Universitario IVI (Valencia, Spain). First trimester Down’s syndrome screening was performed at 11–13 + 6 weeks of pregnancy (45–84 mm of crown–rump length), after informed consent. The ultrasound examination and the blood extraction for the biochemical screening were performed on the same day. Only singleton pregnancies were included in the study. Exclusion criteria were multiple pregnancies, singleton pregnancies resulting from embryo reduction, pregnancies with structural fetal malformations and those with chromosomal abnormalities at karyotyping. The scans were performed by three experienced examiners (J.B., C.L. and V.S.). First trimester PAPP-A and ßhCG levels were measured by the Kryptor analyser in a reference laboratory (Laboratorios Echevarne, Barcelona, Spain; ISO 9001:2000) without knowledge of the type of conception. Biochemical normal reference parameters were derived from a database of 3586 normal singleton pregnancies conceived naturally. The measured marker levels were expressed as multiples of the gestation-specific normal median value (MoM) after adjusting for maternal weight. In ovum donation pregnancies, the baseline age-derived risk was estimated based on the age of the donor at the time of oocyte retrieval. When using frozen embryos, the baseline age-derived risk was based on the age of the woman at the time of freezing the embryos. The SSD 4.1 software (SBP software, Barcelona, Spain) was used for calculating the final Down’s syndrome risk. A risk threshold of 1 in 250 was used to recommend an invasive procedure.

A total of 498 pregnancies conceived naturally and 556 obtained using assisted reproductive techniques were studied. They all corresponded to singleton pregnancies with normal fetuses. Patients were divided into groups according to the type of conception (Table I): natural, ovulation induction (with or without partner or donor intrauterine insemination), IVF, ICSI, ovum donation with IVF and ovum donation with ICSI. As previous reports had shown some differences in the biochemical markers of Down’s syndrome between IVF or ICSI (Lam et al., 1999; Liao et al., 2001; Orlandi et al., 2002), we assessed separately both procedures. In cases of combination of both methods in a single patient, we assessed whether the transferred embryos came only from IVF or ICSI. When they came from only one procedure, the patient was included in the corresponding group, but when pregnancy was achieved after the transfer of embryos derived from both procedures the patient was excluded from the study.

Finally, we also studied other small series of singleton pregnancies with normal fetuses obtained after transfer of frozen embryos (n = 37), embryos analysed by preimplantation genetic diagnosis (n = 53) and those obtained using sperm from testicular biopsy (n = 21). These cases were not included in the initial series.

The statistical analysis was performed using the Statistical Package for Social Science version 10.0 (SPSS Inc., Chicago, IL, USA). Categorical data were expressed as number and percentage and compared using χ²-analysis. Continuous data were expressed as mean (SD) or median (interquartile range) depending on whether data were or were not normally distributed respectively. Continuous data were analysed using parametric (Student’s t-test and analysis of variance) or non-parametric tests (Mann–Whitney and Kruskal–Wallis tests), where appropriate. P < 0.05 was considered significant.

Results
Table I shows the baseline characteristics of the study population and the rate of false positives after screening for Down’s syndrome according to the type of conception. As expected, the only significant difference between groups was the age of the women, the recipients of donated ova being the eldest and the ova donors the youngest.

In our population of normal fetuses, comparison between naturally conceived pregnancies (n = 498) and those obtained with assisted reproductive techniques (n = 556) showed no significant differences in the maternal serum levels of ßhCG [1.02 (0.88) versus 1.11 (0.92) MoM respectively] and PAPP-A [1.13 (0.88) versus 1.16 (0.84) MoM respectively].

A detailed comparison of ßhCG and PAPP-A maternal serum levels between the different types of conception is shown in Figures 1 and 2 respectively. Slight differences were apparent when comparing separately individual techniques. Hence, with respect to ßhCG, IVF pregnancies showed lower levels (0.83 MoM) compared to ICSI (1.13 MoM) and both ovum donation groups (1.20 MoM) (P < 0.02). Pregnancies obtained after ovum donation with ICSI showed slightly increased ßhCG levels (1.20 MoM) compared to natural conceptions (1.02 MoM) (P < 0.04) or the ovulation induction group (0.98 MoM) (P < 0.05). Pregnancies following ovum donation with IVF showed no

Table I. Characteristics of the study population (n = 1054)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Agea (years)</th>
<th>GA (days)</th>
<th>CRL (mm)</th>
<th>NT (mm)</th>
<th>False positive rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural conception</td>
<td>498</td>
<td>33.1 (3.7)</td>
<td>87.1 (5.5)</td>
<td>63.2 (10.3)</td>
<td>1.46 (0.61)</td>
<td>25 (5.0)</td>
</tr>
<tr>
<td>Ovulation induction</td>
<td>97</td>
<td>34.0 (2.9)</td>
<td>86.2 (4.3)</td>
<td>63.1 (10.1)</td>
<td>1.34 (0.42)</td>
<td>4 (4.1)</td>
</tr>
<tr>
<td>IVF</td>
<td>47</td>
<td>34.4 (2.7)</td>
<td>87.0 (3.7)</td>
<td>62.9 (8.2)</td>
<td>1.39 (0.36)</td>
<td>3 (6.4)</td>
</tr>
<tr>
<td>ICSI</td>
<td>222</td>
<td>34.5 (3.4)</td>
<td>86.4 (4.2)</td>
<td>61.5 (9.8)</td>
<td>1.38 (0.46)</td>
<td>10 (4.5)</td>
</tr>
<tr>
<td>Ovum donation with IVF</td>
<td>71</td>
<td>39.4 (5.1)b</td>
<td>87.1 (5.4)</td>
<td>62.5 (11.3)</td>
<td>1.34 (0.43)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Ovum donation with ICSI</td>
<td>119</td>
<td>39.2 (4.5)c</td>
<td>86.5 (4.2)</td>
<td>62.9 (8.7)</td>
<td>1.39 (0.40)</td>
<td>3 (2.5)</td>
</tr>
</tbody>
</table>

Results are expressed as mean (SD) or n (%); the ovulation induction group includes cycles for planned intercourse or artificial insemination.

*Analysis of variance test: P < 0.001, donor versus recipient.

*Age of recipient.

*Age of donor.

GA = gestational age; CRL = crown–rump length; NT = nuchal translucency.
difference in fβhCG values (1.20 MoM) compared to pregnancies obtained by ovum donation with ICSI (1.20 MoM). When considering both ovum donation pregnancies together (IVF and ICSI), fβhCG levels (1.20 MoM) were higher than those found in naturally conceived pregnancies (1.02 MoM) (P, 0.02). Regarding PAPP-A levels, only pregnancies conceived by ovulation induction showed slightly higher values (1.26 MoM) than IVF pregnancies (1.06 MoM) (P < 0.05). When analysing all the conceptions together (natural, ovulation induction, IVF, ICSI, ovum donation/IVF and ovum donation/ICSI), no differences were found in fβhCG or PAPP-A levels (Kruskal–Wallis test).

Further analyses were also performed to investigate the potential influence on the biochemical markers of other parameters related to assisted reproductive techniques. Although the sample size was small, no differences in fβhCG and PAPP-A MoM values were encountered between pregnancies obtained using frozen embryos and those obtained with fresh embryos after adjusting for the assisted reproductive techniques used (IVF, ICSI or ovum donation). Similarly, no differences in fβhCG and PAPP-A MoM values were observed when comparing ICSI pregnancies with and without preimplantation genetic diagnosis. Lack of differences in fβhCG and PAPP-A MoM values was also evident after comparing ICSI pregnancies obtained using sperm ejaculate with a small group of ICSI pregnancies achieved using sperm retrieved by testicular biopsy.

**Discussion**

A number of changes in first-trimester maternal serum fβhCG and PAPP-A levels have been described associated with chromosomal abnormalities. Hence, serum fβhCG is usually elevated in trisomy 21 and diandric triploidy; decreased in trisomy 18, trisomy 13 and digynic triploidy; and unchanged in sexual chromosome anomalies. PAPP-A is usually decreased in any aneuploidy, but in several degrees depending on the chromosomal aberration (Brizot et al., 1994, 1995; Tul et al., 1999; Spencer et al., 2000a,b,c). In the second trimester, increased serum maternal levels of intact hCG, βhCG or fβhCG, in conjunction with decreased AFP and decreased uE3 levels, have been described in trisomy 21 (Canick et al., 1988; Macri et al., 1990). In pregnancies conceived by assisted reproductive techniques, placental hormonal production during the second trimester might be modified (Räty et al., 2001, 2002; Maymon and Jauniaux, 2002; Hui et al., 2003), leading to changes in Down’s syndrome detection and false positive rates.

Different theories have arisen so far on the impact of assisted reproductive techniques on placental hormonal production. Initial theories suggested that either the multiple corpus lutea generated after a controlled ovarian stimulation, or an early multiple implantation site (not detectable by ultrasound), could be responsible for the elevated second trimester maternal serum hCG levels reported in these pregnancies (Frishman et al., 1997; Wald et al., 1999; Maymon and Jauniaux, 2002; Räty et al., 2002). However, similar changes were later found in pregnancies achieved by ovum donation or embryo freezing–thawing procedures, in which no ovarian stimulation is carried out, demonstrating that different mechanisms might be involved (Maymon and Shulman, 2001; Maymon and Jauniaux, 2002; Perheentupa et al., 2002; Räty et al., 2002; Shulman and Maymon, 2003). Currently, it is believed that hormonal changes could be due to an abnormal
feto-placental unit metabolism caused by the assisted reproductive techniques or by the infertility itself (Räty et al., 2002; Maymon and Jauniaux, 2002; Hui et al., 2003). Hence, a delayed placental or fetal maturation could lead to the release of amounts of placental hormones in the second trimester similar to those of previous stages of pregnancy, resulting in higher serum levels for ßhCG and lower serum levels for AFP than expected in this trimester (Hui et al., 2003).

Interestingly, most hormonal changes reported in pregnancies achieved by assisted reproductive techniques have been described in the second trimester. However, data on first trimester biochemical screening in assisted reproduction pregnancies is still limited (Table II). Previous series have focused mainly on a particular technique and the number of assisted reproduction pregnancies studied has not been very large (Table II).

Despite the above facts, there is general agreement that it is reasonable to offer the combined first trimester screening (ultrasound and biochemistry) to women with singleton pregnancies achieved by assisted reproductive techniques. However, some issues need to be considered. First, maternal age needs to be adjusted when using frozen embryos and in cases of ovum donation. Second, nuchal translucency measurements are known to be unaffected by assisted reproduction (Maymon et al., 1999; Liao et al., 2001; Maymon and Jauniaux, 2002; Nicolaides, 2004). Third, biochemical screening in assisted reproduction pregnancies is more difficult to interpret and merits an in-depth analysis.

As many programmes of first trimester Down’s syndrome screening have been developed in unselected populations that mainly included naturally conceived singleton pregnancies, we will focus on the comparison between assisted reproduction pregnancies and natural conceptions based on our results and those of others (Table II). In our study, measurements of biochemical parameters were performed using the Kryptor system.

For pregnancies achieved by ovulation induction, ßhCG and PAPP-A levels seem to be largely unaffected. For IVF pregnancies, the issue remains controversial with respect to ßhCG as the reported levels have been found either increased or unaffected. Previous studies (Liao et al., 2001) that encountered higher ßhCG levels in IVF pregnancies compared to natural conceptions suggested several options to deal with these changes. One option would simply be to say that there could be a small increase (of ~1%) in the false positive rate when screening IVF pregnancies (Liao et al., 2001). Another option would be to make an adjustment dividing the measured MoM by the median MoM obtained in IVF pregnancies (Wald et al., 1999; Liao et al., 2001). As some pregnancies are obtained after a combined procedure of IVF/ICSI, it would be important to distinguish accurately between both techniques because in our study and that of Liao et al. (2001) ßhCG levels behaved differently in both procedures. In most previous studies, lower PAPP-A values have been observed in IVF pregnancies (Table II). Our own results showed a non-significant trend towards such decreased values.

For ICSI pregnancies, ßhCG and PAPP-A levels seem to be generally unaffected (Table II). To the best of our knowledge, we have provided the largest series of ICSI pregnancies reported so far.

In our study, no differences were observed between the group of assisted reproduction pregnancies as a whole and natural conceptions. Most authors have previously reported similar results, describing a false positive rate for Down’s syndrome in first-trimester not different or only slightly increased (0.9–1.9%) in assisted reproductive techniques compared with natural conceptions (Liao et al., 2001; Wojdemann et al., 2001; Orlandi et al., 2002; Maymon and Shulman, 2002; Ghisoni et al., 2003). However, no previous study included cases of first trimester screening in ovum donation pregnancies. In our study, higher levels of serum

Table II. Comparison of first trimester biochemical markers in singleton pregnancies achieved naturally and by assisted reproduction

<table>
<thead>
<tr>
<th>Study</th>
<th>Natural pregnancies</th>
<th>Assisted reproduction pregnancies</th>
<th>Free ßhCGa</th>
<th>PAPP-Aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liao et al. (2001)</td>
<td>1233</td>
<td>161 (OS)</td>
<td>⇔</td>
<td>⇔</td>
</tr>
<tr>
<td></td>
<td></td>
<td>220 (IVF)</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 (ICSI)</td>
<td>⇔</td>
<td>↓</td>
</tr>
<tr>
<td>Wojdemann et al. (2001)</td>
<td>3026</td>
<td>63 (OS)</td>
<td>⇔</td>
<td>⇔</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47 (IVF)</td>
<td>⇔</td>
<td>⇔</td>
</tr>
<tr>
<td>Orlandi et al. (2002)</td>
<td>370</td>
<td>32 (IVF)</td>
<td>⇔</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42 (ICSI)</td>
<td>⇔</td>
<td>⇔</td>
</tr>
<tr>
<td>Maymon and Shulman (2002)</td>
<td>285</td>
<td>71 (IVF)</td>
<td>⇔</td>
<td>↓</td>
</tr>
<tr>
<td>Lai et al. (2003)</td>
<td>3059</td>
<td>49 (OS)</td>
<td>⇔</td>
<td>↓</td>
</tr>
<tr>
<td>Ghisoni et al. (2003)</td>
<td>426</td>
<td>50 (IVF)</td>
<td>⇔</td>
<td>⇔</td>
</tr>
<tr>
<td>Maymon and Shulman (2004)</td>
<td>1781</td>
<td>92 (ICSI)</td>
<td>⇔</td>
<td>⇔</td>
</tr>
<tr>
<td>Present work</td>
<td>498</td>
<td>99 (IVF)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>97 (OS)</td>
<td>⇔</td>
<td>⇔</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47 (IVF)</td>
<td>⇔</td>
<td>⇔</td>
</tr>
<tr>
<td></td>
<td></td>
<td>222 (ICSI)</td>
<td>⇔</td>
<td>⇔</td>
</tr>
<tr>
<td></td>
<td></td>
<td>71 (OD/IVF)</td>
<td>⇔</td>
<td>⇔</td>
</tr>
<tr>
<td></td>
<td></td>
<td>119 (OD/ICSI)</td>
<td>⇔</td>
<td>⇔</td>
</tr>
</tbody>
</table>

*aComparison with naturally conceived pregnancies (⇔ no difference, ↑ increased, or ↓ decreased serum level).

OS = ovulation induction (with or without artificial insemination); OD = ovum donation; PAPP-A = pregnancy-associated plasma protein-A; N/A = not available.
References


Submitted on March 22, 2005; resubmitted on April 21, 2005; accepted on May 2, 2005.

βhCG were found in ovm donation pregnancies (both in IVF and ICSI procedures). Our results are in agreement with those previously reported in the second trimester (Maymon and Shulman, 2001). These findings could lead to a higher false positive rate in first-trimester Down's syndrome screening in ovum donation pregnancies, but in these cases probably the younger age of the donors largely compensates for this effect, as shown in Table I. In Spain, only women aged 18–35 years are allowed to act as donors, and the mean donor age in our centre was 25 years. This could certainly account for the low false positive rates found in our ovum donation pregnancies.

Our preliminary data on preimplantation genetic diagnosis, frozen embryos and use of sperm retrieved by testicular biopsy showed lack of influence of these factors on the biochemical markers. Larger series are needed to validate or refute these results due to the small sample sizes we present.

In summary, data accumulated so far suggest that first trimester biochemical markers either do not need any adjustments (e.g. in pregnancies obtained after ovulation induction and ICSI), or have very little impact (e.g. IVF pregnancies) or no impact (e.g. ovum donation/IVF pregnancies) on the false positive rates. Therefore, first trimester combined ultrasound and biochemical screening for Down’s syndrome seems feasible in assisted reproduction pregnancies.