First-trimester screening in pregnancies conceived by assisted reproductive technology: significance of gestational dating by oocyte retrieval or sonographic measurement of crown–rump length

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KEYWORDS: ART; combined screening; first trimester; free β-hCG; gestational age; nuchal translucency; PAPP-A

ABSTRACT

Objectives To evaluate, in pregnancies conceived by assisted reproductive technology, whether determination of gestational age (GA) by date of oocyte aspiration (DOA) or crown–rump length (CRL) at first-trimester screening influences the distribution of serum and sonographic markers or the performance of first-trimester screening for chromosomal abnormalities.

Methods GA was calculated using either DOA or CRL at blood sampling and nuchal translucency thickness (NT) measurement in 729 singleton pregnancies conceived by in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Weight-corrected log multiples of the median (MoM) marker distributions specific for IVF pregnancy were established using multiple log regression and compared for DOA- and CRL-based GA calculation.

Results GA determined by CRL was significantly larger, albeit slightly, than was GA determined by DOA, with a mean difference of 1.50 (SD, 2.4) days (\( P < 0.001 \)). Log MoM distributions of free beta-human chorionic gonadotropin and NT showed that GA dating by CRL resulted in significantly higher, albeit slightly, mean log MoM values compared with DOA dating. The reverse was the case for mean log MoM pregnancy-associated plasma protein-A. The SDs were similar for CRL and DOA dating. According to Monte Carlo simulation, the use of DOA or CRL for GA dating did not appreciably influence the performance of first-trimester screening.

Conclusions DOA and CRL are practically equivalent when calculating GA for first-trimester screening. The correct method of GA dating for other purposes (e.g. estimated time of delivery) in IVF/ICSI pregnancies is still unresolved. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.
thickness (NT) MoM measurement is influenced by mode of conception\textsuperscript{18,19}, although this has not been reported by the majority of studies\textsuperscript{13,14,17}. Furthermore, the total number of cases examined so far is limited.

The aim of this study was to evaluate, in pregnancies conceived by assisted reproductive technology (ART), whether the determination of GA by either DOA or CRL at the time of the NT scan, i.e. in the first trimester, influences the distribution of serum and sonographic markers when using median curves specific for IVF pregnancies. Furthermore, we wanted to assess the potential significance of choice of gestational dating for the performance of first-trimester screening for chromosomal abnormalities.

**SUBJECTS AND METHODS**

**Subjects**

From an ongoing national Danish prospective cohort study (2004–2006) inviting women who conceived after ART treatment to undergo first-trimester combined screening, we identified 729 women who met the following inclusion criteria: singleton pregnancy with delivery of a healthy neonate, child without malformations, pregnant after IVF or intracytoplasmic sperm injection (ICSI), no known chromosomal abnormality detected either pre- or postnatally and complete first-trimester combined screening, with NT measurement and blood sampling with analysis of PAPP-A and free beta-human chorionic gonadotropin (β-hCG). Exclusion criteria were: pregnancy achieved by frozen embryo replacement (FER), multiple pregnancy, pregnancy in which early ultrasound revealed more than one gestational sac and chromosomal aberration detected.

The women were enrolled into the study at the fertility clinics at the time of early ultrasound around weeks 7–8, when viability of the fetus was assessed sonographically (i.e. confirming clinical pregnancy). Demographic data were recorded and entered into a database. Blood samples were taken at the fertility clinics, by the women’s general practitioner or at the hospital where they had their NT scan. Data on pregnancy outcome were obtained by a self-administered questionnaire given to the woman at inclusion and returned by her after conclusion of the pregnancy. Information on non-responders was retrieved from the fertility clinics or from hospital records. All women gave written informed consent to participate and the local scientific ethics committee as well as the Data Protection Agency approved this study.

**Calculation of gestational age**

GA was calculated at the time of blood sampling and at the time of the NT scan based on the DOA and on the CRL. GA based on DOA was calculated by taking the time of conception, the time of the NT scan based on the DOA and on the CRL. GA based on DOA was calculated by taking the time of conception, the time of blood sampling was also calculated using the CRL-GA calculated at the time of the NT scan and subtracting from this the number of days between NT and biochemical measurements.

**Screening markers**

The NT scans were performed by physicians, nurses and midwives, all of whom were certified by The Fetal Medicine Foundation\textsuperscript{21}. The maternal serum concentrations of PAPP-A and β-hCG were determined as part of the routine first-trimester prenatal screening program at Statens Serum Institut, Copenhagen. Briefly, the concentrations of the analytes were measured using either the Kryptor platform (Brahms, Henningsdorf, Berlin) or the AutoDelfia platform (Perkin Elmer Life Science, Boston, MA, USA).

**Monte Carlo Simulation**

The performance of combined PAPP-A, β-hCG and NT screening was estimated using a previously described Monte Carlo-based method\textsuperscript{22}. Briefly, Monte Carlo simulation is a mathematical method used to approximate the probability of certain outcomes by running multiple trial runs (simulations) generated from probability distributions to simulate the process of sampling from an actual population. The selection of a distribution for the inputs is based on data already known (best estimates) and the data generated from the simulation are represented as probability distributions. We assumed that the log MoM distributions of PAPP-A, β-hCG and NT followed a Gaussian distribution, that PAPP-A and β-hCG were correlated with each other and that NT was uncorrelated with the biochemical markers. For each of these three markers there was a mean for the unaffected population, one for the Down syndrome pregnancies (which depended on GA), two SDs (one for unaffected and one for Down syndrome pregnancies) and two correlations (one for unaffected and one for Down syndrome pregnancies). These parameters were required to specify the mean vectors and covariance matrices for the unaffected and Down syndrome distributions. Estimates of the screening parameters from week 10 were obtained from Cuckle and van Lith’s study\textsuperscript{23} (parameter estimates are given in the Appendix). The age distribution of the pregnant women that we used was obtained from the study of Van der Veen et al.\textsuperscript{24} and the age-related risk of Down’s syndrome was obtained from that of Hecht et al.\textsuperscript{25}.

**Statistics**

Means were compared using \( t \)-tests. Pairwise \( t \)-tests were used when appropriate. Compatibility with a normal distribution was ascertained using probability plots. MoM values were established using medians obtained from log
linear regression of markers on either CRL- or DOA-based GA.

RESULTS

Basic demographics and routine screening parameters

Demographic characteristics of the cohort are shown in Table 1. Less than 2% of this population was known to have an ethnicity other than Caucasian, explaining why adjustment for ethnicity was found not to be relevant. The routine screening parameter values are summarized in Table 2. The biochemical markers were based on medians derived from the background population analyzed at Statens Serum Institut, i.e. a mixture of largely spontaneous and to a much lesser extent (<5%) ART pregnancies. The median PAPP-A MoM of 0.76 in our IVF population of was low, whereas the median β-hCG MoM of 0.96 was nearly identical to that obtained in normally conceived pregnancies (MoM values calculated with GA based on CRL).

Comparison of gestational age determined by date of oocyte aspiration and by crown–rump length

The mean difference between GA determined by DOA and by CRL was 1.50 (SD, 2.4) days ($P < 0.001$), with the GA calculated by CRL being greater. The relationship between GA determined by DOA and by CRL at time of blood sampling is shown in Figure 1.

Multiple log linear regressions of PAPP-A, β-hCG and NT on gestational age determined by date of oocyte aspiration or by crown–rump length and maternal weight

In order to establish the log MoM distributions of each of the markers we calculated the median formulae by performing a multiple log linear regression of PAPP-A, β-hCG and NT on GA in days (determined by either DOA or CRL) and maternal weight in kg. Maternal weight contributed significantly to the variation of the analyte explaining why adjustment for ethnicity was found not to be relevant. The routine screening parameter values to be relevant. The routine screening parameter values known to have an ethnicity other than Caucasian, in Table 1. Less than 2% of this population was

### Table 1 Background data on the study population of 729 women pregnant after assisted reproductive technology (in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI))

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>32.8 ± 3.9 [20–43]</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>68.3 ± 12.3 [39–125]</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>169.1 ± 6.4 [150–186]</td>
</tr>
<tr>
<td>Fertility treatment</td>
<td>IVF: 419 (57.5)</td>
</tr>
<tr>
<td></td>
<td>ICSI: 310 (42.5)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Caucasian: 682 (93.6)</td>
</tr>
<tr>
<td></td>
<td>Asian: 8 (1.1)</td>
</tr>
<tr>
<td></td>
<td>Unknown: 39 (5.4)</td>
</tr>
<tr>
<td>Parity</td>
<td>0: 536 (73.5)</td>
</tr>
<tr>
<td></td>
<td>1: 164 (22.5)</td>
</tr>
<tr>
<td></td>
<td>2: 19 (2.6)</td>
</tr>
<tr>
<td></td>
<td>&gt; 2: 6 (0.8)</td>
</tr>
<tr>
<td></td>
<td>Unknown: 4 (0.6)</td>
</tr>
</tbody>
</table>

Values are mean ± SD, n (%).
Distribution of log MoM values of PAPP-A, β-hCG and NT in pregnancies dated by date of oocyte aspiration or by crown–rump length

The regression lines identified above were used to establish log MoM distributions. Table 3 shows the distribution of log MoM values of PAPP-A, β-hCG and NT using either DOA or CRL for GA dating. All distributions were compatible with a normal distribution. The differences for all screening markers (mean log MoM and geometric mean) were statistically significant, but small (< 5%).

Effect on screening performance

The effect on the receiver–operating characteristics curves of combined biochemical and NT screening of the slightly different median log MoMs given in Table 3 was assessed by Monte Carlo simulation. With the false-positive rate fixed at 3% and at a risk cut-off of 1:250 at term, the detection rate was 82% for CRL-based GA dating and 84% for DOA-based dating.

DISCUSSION

In this large national unselected IVF/ICSI population we have shown that DOA and CRL are practically equivalent for establishing GA in ART pregnancies in the first trimester. This is also the case when GA is used to establish MoM distributions of first-trimester biochemical and ultrasound markers for prenatal screening. Likewise, the use of DOA or CRL for GA dating does not appreciably influence the performance of first-trimester screening; i.e., the possibility of obtaining an accurate fetal age by DOA does not improve screening.

In contrast to other studies,7,8 we found that GA calculated from CRL was longer (mean, 1.5 days (~36 h) when compared with GA calculated by DOA. We converted the day of oocyte retrieval into menstrual age by adding 14 days, which is a commonly used algorithm in the fertility sector. However, in a normal menstrual cycle (of 28 days), ovulation occurs 34–36 h after the onset of the luteinizing hormone (LH) surge26, corresponding to 10–12 h after the LH peak. IVF/ICSI treatment protocols prescribe administration of hCG prior (usually around 36 h before) to oocyte aspiration, to mimic the LH surge and to induce final follicular maturation27,28. Using the time of hCG administration instead of the DOA as cycle day 14, would have given us a GA 36 h longer than the DOA-GA, one that was in accordance with the GA that we calculated from CRL.

The difference between our findings and those of other authors could also be caused by differences in ART populations and GA algorithms. Wennerholm et al.9 compared GA calculated from DOA and from BPD measurements in the second trimester and found a mean difference of 1.4 days, GA calculated by ultrasound being the lower. Their study population had mainly conceived after conventional IVF (61.7%; 156/233), only 17.0% (43/233) having conceived after ICSI and 21.3% (54/233) pregnancies achieved after replacement of frozen-thawed embryos (FER). Our study population was considerably larger, with a smaller IVF/ICSI ratio (57.5% (419/729) IVF pregnancies and 42.5% (310/729) ICSI pregnancies). We excluded pregnancies conceived after FER as the growth pattern of frozen-thawed embryos is likely to be different from that of spontaneous pregnancies and pregnancies conceived by other ART methods. GA calculated from DOA was performed by Wennerholm et al. as described here and frozen-thawed embryos were transferred 2 days after ovulation and 3 days after the LH peak, and GA was calculated by adding 14 days to the date of ovulation.

Tunon et al.8 also included FER pregnancies, which accounted for 8.7% (18/208) of their population, ICSI accounting for only 2.4% (5/208). They assessed GA from the time of IVF, and based on CRL and BPD; the mean difference in GA was 0.9 days between IVF and CRL estimates (first trimester), and 2.1 days between IVF and BPD (second trimester). GA according to IVF was calculated in the same way as in our study, by converting DOA into menstrual age by adding 14 days. Frozen-thawed embryo replacement was performed 3 days after ovulation with GA then calculated by adding 14 days to the date of ovulation. For GA dating by CRL they used an equation described by Wisser et al.29; GA = 35.72 + 1.082 × CRL1.2 + 1.472 × CRL − 0.09749 × CRL1.2 and GA according to BPD was calculated with reference to the laboratory’s own standard30. Wennerholm et al.9 used the formula described by Persson and Weldner31 to calculate GA based on BPD: GA = 2.10 × BPD + 39.1.

An ideal ultrasound dating procedure, with no systematic error and a small random error, has yet to be described7,11. Thus, it could be the different choices of GA formula that account for the small but significant differences found between GA dating by DOA and by ultrasound. It is unlikely that choosing another GA

Table 3 Distribution of log multiple of the median (MoM) values of pregnancy-associated plasma protein-A (PAPP-A), beta-human chorionic gonadotropin (β-hCG) and nuchal translucency thickness (NT) using either date of oocyte aspiration (DOA) or crown–rump length (CRL) for gestational-age dating, together with geometric mean MoM values

<table>
<thead>
<tr>
<th>GA based on CRL</th>
<th>Log MoM</th>
<th>MoM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
</tbody>
</table>

| GA based on CRL | PAPP-A*                       | −0.0155          | 0.3046          | 0.965        |
|                 | β-hCG†                        | 0.0093           | 0.2558          | 1.022        |
|                 | NT‡                          | 0.0032           | 0.1191          | 1.007        |

| GA based on DOA | PAPP-A*                       | −0.0011          | 0.3050          | 0.975        |
|                 | β-hCG†                        | −0.0278          | 0.2555          | 0.938        |
|                 | NT‡                          | −0.0014          | 0.1225          | 0.997        |

*MoM CRL–DOA difference = −0.0144 log MoM (P < 0.001).
†MoM CRL–DOA difference = −0.0185 log MoM (P < 0.001).
‡MoM CRL–DOA difference = 0.0046 log MoM (P < 0.001).
ultrasound equation would alter screening markers in a significant way.

One might have expected a mean MoM PAPP-A closer to 1.00 than the value of 0.76 found in the 729 ART pregnancies examined. However, a decreased PAPP-A concentration in ART pregnancies has been found in several other studies\textsuperscript{13–15}. Some studies have also found an altered concentration of first-trimester $\beta$-hCG\textsuperscript{12,13} in ART pregnancies, with mean $\beta$-hCG MoM values as high as 1.21\textsuperscript{13}. The cause of these altered serum screening markers is unknown, but suggestions include a relationship with multiple corpora lutea, multiple implantation sites or the drugs used in fertility treatments\textsuperscript{33}. Other possible explanations are a functional delay in fetal and placental development and the higher risk of obstetric complications, such as IUGR and pre-eclampsia, associated with ART\textsuperscript{32}.

In conclusion, we have shown that DOA and CRL are practically equivalent for establishing GA for first-trimester serum screening. The correct method of GA dating for other purposes (e.g. estimated time of delivery) in IVF/ICSI pregnancies is still unresolved.

**APPENDIX**

Parameters used in Monte Carlo estimation of screening performance in gestational week 10

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unaffected pregnancies</th>
<th>Down syndrome pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median MoM</td>
<td>Free $\beta$-hCG</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>PAPP-A</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>1.00</td>
</tr>
<tr>
<td>Median log MoM</td>
<td>Free $\beta$-hCG</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PAPP-A</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>0</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>Free $\beta$-hCG</td>
<td>0.287</td>
</tr>
<tr>
<td></td>
<td>PAPP-A</td>
<td>0.285</td>
</tr>
<tr>
<td></td>
<td>NT</td>
<td>0.120</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>Free $\beta$-hCG and PAPP-A</td>
<td>0.111</td>
</tr>
<tr>
<td></td>
<td>NT and $\beta$-hCG</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>NT and PAPP-A</td>
<td>0</td>
</tr>
</tbody>
</table>

$\beta$-hCG, beta-human chorionic gonadotropin; MoM, multiples of the median; NT, nuchal translucency thickness; PAPP-A, pregnancy-associated plasma protein-A.

**ACKNOWLEDGMENT**

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