Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for Down syndrome

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BACKGROUND: First trimester screening (FTS) for Down syndrome combines measurement of nuchal translucency, free beta-human chorionic gonadotrophin and pregnancy-associated plasma protein-A (PAPP-A). The aim of this study was to undertake a detailed analysis of FTS results in singleton pregnancies conceived using assisted reproductive technologies (ART) and non-ART pregnancies.

METHODS: A record linkage study compared outcomes in 1739 ART-conceived and 50 253 naturally conceived pregnancies.

RESULTS: Overall, significantly lower PAPP-A levels were detected in ART pregnancies (0.83 multiples of median, MoM) than in controls (1.00 MoM) (t-test P < 0.001). This difference remained after excluding complicated pregnancies. Analysis of factors affecting PAPP-A levels suggested fresh compared with frozen embryo transfers and use of artificial cycles compared with natural cycles for frozen transfers were associated with lower values. The adjusted odds ratio (AdjOR) for receiving a false-positive result was 1.71 (95% CI 1.44–2.04; P < 0.001) for ART pregnancies compared with non-ART pregnancies, and this leads to a higher AdjOR (1.24, 95% CI 1.03–1.49; P = 0.02) for having a chorionic villous sampling (CVS) or amniocentesis.

CONCLUSIONS: ART pregnancies have reduced FTS PAPP-A levels leading to an increased likelihood of receiving a false-positive result and having a CVS/amniocentesis. Lower PAPP-A may reflect impairment of early implantation with some forms of ART.

Key words: ART / PAPP-A / pregnancy screening / pregnancy complications / hormonal stimulation

Introduction

Pregnancy screening for Down syndrome (DS) and other chromosome abnormalities has become part of routine antenatal care over the last 20 years. The measurement of second trimester biochemical markers in the blood of pregnant women to improve screening for DS based on maternal age alone was first described in 1988 (Wald et al., 1988). Over the last 10 years, second trimester serum screening has been progressively replaced by first trimester combined screening. The first trimester combined screen measures maternal serum levels of free beta-human chorionic gonadotrophin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 9–12 weeks
gestation and measures nuchal translucency (NT) by ultrasound at 11–13 weeks gestation. These measurements are combined with maternal age, weight and gestational age to produce a risk estimate of the fetus having DS or trisomy 18 (T18) (Wald et al., 2003). For pregnancies at increased risk, prenatal diagnostic testing, CVS or amniocentesis, can be offered. In the Victorian population, the sensitivity of the first trimester combined screen for DS is 91% (using a risk threshold of 1 in 300 at the time of ultrasound), with the proportion of unaffected pregnancies receiving an increased risk result (false positive rate, FPR) being 3.9% (Jacques et al., 2007).

Pregnancies conceived using assisted reproductive technologies (ART) now account for 3% of all live births in Australia (Wang et al., 2007). Previous studies of second trimester serum screening have shown that serum markers in ART pregnancies differ from natural conceptions, leading to an increased FPR (Barkai et al., 1996; Ribbert et al., 1996; Frishman et al., 1997; Wald et al., 1999; Ray et al., 2002; Lambert-Messerlian et al., 2006). The effect of ART on first trimester combined screening has been examined in 13 studies that have yielded contradictory and inconclusive results, largely due to small sample sizes. Some studies found that hCG (Liao et al., 2001; Niemimaa et al., 2001; Ghisoni et al., 2003; Bersinger et al., 2004) and NT thickness (Maymon and Shulman, 2004; Hui et al., 2005b) were increased in ART pregnancies, yet others found no differences in hCG (Wojdemann et al., 2001; Maymon and Shulman, 2002; Orlandi et al., 2002; Maymon and Shulman, 2004; Bellver et al., 2005; Lambert-Messerlian et al., 2006; Anckaert et al., 2008) or NT (Liao et al., 2001; Niemimaa et al., 2001; Wojdemann et al., 2001; Maymon and Shulman, 2002; Orlandi et al., 2002; Ghisoni et al., 2003; Lambert-Messerlian et al., 2006). PAPP-A levels have been decreased in some studies (Liao et al., 2001; Maymon and Shulman, 2002; Orlandi et al., 2002; Bersinger et al., 2004; Maymon and Shulman, 2004; Hui et al., 2005a; Tul and Novak-Antolic, 2006; Anckaert et al., 2008), but unaltered in others (Niemimaa et al., 2001; Wojdemann et al., 2001; Ghisoni et al., 2003; Bellver et al., 2005; Lambert-Messerlian et al., 2006).

We hypothesized that in pregnancies conceived using ART, factors exist that are absent in natural conceptions, potentially influencing the marker levels, and consequently, risk results of the first trimester combined screen. The aims of this study were to investigate in a large population-based sample, the effect of ART on the individual markers of the first trimester combined screen (hCG, PAPP-A, NT) and on the FPR. We also aimed to investigate the effect of different ART modalities on these markers, and the impact of ART and screening results on the uptake of prenatal diagnostic testing (CVS and amniocentesis) following first trimester combined screening.

### Materials and Methods

The effect of ART (IVF, ICSI, GIFT, embryo cryopreservation and hormone treatment) on the FPR of the first trimester combined screen and its three components (hCG, PAPP-A, NT) was examined in women with singleton pregnancies screened between February 2000 and June 2004 in Victoria, Australia. The pregnancies included did not have a fetus or produce a child with a birth defect, and singleton pregnancies were defined as the presence of one fetus after 20 weeks gestation. Data from three separate databases were linked to obtain the study population (Fig. 1). A fourth prenatal diagnosis database was linked to the study population to investigate the uptake of CVS and amniocentesis. The four databases used are outlined below.

The Victorian Perinatal Data Collection Unit (VPDCU) is responsible for the collection of information on all Victorian births ≥20 weeks gestation or ≥400 g birthweight. Reporting to the VPDCU is mandatory and data collected includes obstetric factors, neonatal outcomes, birth defects and previous pregnancy history. A unique registration number is assigned to each newborn. During the study period there were ∼245 000 singleton births in Victoria.

The Victorian Birth Defects Register collects data on all pregnancies diagnosed with a birth defect, comprising live births, stillbirths, neonatal deaths and terminations. For this study, all pregnancies that resulted in a birth defect were identified and excluded from the study population.

The Victorian Clinical Genetics Service (VCGS) conducts all first trimester combined screening tests in Victoria. The VCGS first trimester

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**Figure 1** Flow diagram showing data linkage between three separate databases to obtain study population.
combined screening programme has been described elsewhere (Jaques et al., 2006, 2007). Briefly, β-hCG and PAPP-A measurements were performed on a KRYPTOR analyser, and NT and crown–rump length (CRL) measurements were made off-site by multiple ultrasonologists in accordance with the technique described by the Fetal Medicine Foundation (Nicolaides, 2004). NT and biochemistry values were calculated from CRL in ART and non-ART pregnancies because this was the only way to directly compare ART and non-ART pregnancies, and because it has been shown that CRL and date of oocyte collection are practically equivalent when calculating gestational age for first trimester screening (FTS) (Gjerris et al., 2008). The biochemistry and risk estimates were calculated using software developed in house and results are monitored by UKNEQAS and audited by ascertaining pregnancy outcomes for calculation of specificity and sensitivity as described elsewhere (Jaques et al., 2006, 2007). The VCGS prenatal screening database contains comprehensive data for all women who had first trimester combined screening in the State.

The data on ART in Victoria were obtained from the three major providers: Melbourne IVF, Monash IVF and Melbourne Assisted Conception Centre, accounting for 98% of ART pregnancies in Victoria. The dataset included detailed information on the ART cycles, including the type of ART treatment, indication for ART, whether embryos were fresh or frozen-thawed, the use of donor gametes or embryos and the number of fetal hearts present at ultrasound at 6 weeks gestation.

Data for all CVS and amniocentesis tests performed in Victoria and their results are provided to the VPDCU by all four cytogenetic laboratories in the State. Information regarding the uptake of CVS and amniocentesis was available for the 41 756 pregnancies screened in 2002–2004. Excluded from this subset of analyses were 10 236 pregnancies screened in 2000–2002 because these pregnancies were not linked to the prenatal diagnostic data.

### Data analysis

Data coding and statistical analyses were conducted using SPSS (version 15; SPSS Inc., Chicago, IL, USA). Mann–Whitney U-tests were done to test the difference between the characteristics (age, weight, CRL and gestational age at testing) of non-ART and ART groups, and chi-square tests were performed to assess the difference in the proportion of women from the different groups who were primigravid. Independent samples t-tests were performed on the means of the log-transformed multiples of median (MoM) values for β-hCG, PAPP-A and NT. Results were then back-transformed and are presented as geometric means. The statistical significance of differences in proportions between groups and results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Univariable logistic regression analysis was done to explore associations between results and a number of covariables, e.g. maternal age, maternal country of birth, gravidity, parity, birthweight and gestational age. Multivariable analysis was adjusted for maternal age and gravidity as these were the only significant confounders.

The ART study population was divided into subgroups to allow more in-depth analyses of the different exposures on outcome: (i) aetiology of infertility (female-only aetiology, male-only aetiology, a combination of male and female aetiology or unknown aetiology); (ii) ART procedure (IVF, ICSI or GIFT); (iii) embryo transfer (fresh or frozen-thawed); (iv) presence or absence of exogenous hormone treatment and (v) presence of single or multiple fetal hearts at 6 weeks gestation. Although treatment protocols varied, hormone treatment accompanying fresh embryo transfers always was defined as follicle stimulating hormone (FSH) or clomiphene, with or without other drugs. All GIFT cycles were done with FSH. A small proportion (n = 13) of fresh embryo transfers were done without any hormone treatment to stimulate multifollicular development before oocyte collection. Hormone treatment accompanying frozen–thawed embryo transfers was defined as any combination of estradiol and progesterone for artificial cycles for oligomenorrhea/amenorrhea, and clomiphene with or without HCG for ovulation induction to time to transfer.

### Ethics approval

Ethics approval was obtained from the Human Research Ethics Committees of the Victorian Department of Human Services, Mercy Health and Aged Care, Royal Women’s Hospital, Freemasons Hospital, Epworth Hospital, Monash University and Monash Surgical Private Hospital.

### Results

Women in the non-ART and ART groups were similar in terms of maternal weight and gestational age at ultrasound (calculated from CRL of the fetus) at the time of screening (Table I). Maternal country of birth was Australia or Europe/UK for 91.7% of ART mothers and 91.8% of non-ART mothers (P = 0.90). The ART group were older and more likely to be primigravid than the non-ART group. The measured CRL was less for fresh embryos than for frozen–thawed embryos.

### Effect of ART on marker levels

PAPP-A levels were significantly lower (P < 0.001) in ART pregnancies (0.83 MoM) compared with non-ART pregnancies (1.0 MoM) (Table II). A small increase in the NT was detected in the ART group (0.91 MoM) compared with the non-ART group (0.90 MoM) (P = 0.004). Analyses of β-hCG levels showed no significant difference between ART (0.99 MoM) and non-ART (0.98 MoM) pregnancies.

Adverse pregnancy complications, comprising pregnancies with adverse perinatal outcomes (stillbirth, neonatal death, prematurity, birthweight <2500 g) and/or obstetric complications (pre-eclampsia, pregnancy-induced hypertension, gestational diabetes) were more common in the ART pregnancies (21.0%) compared with the non-ART pregnancies (13.9%). The PAPP-A levels were lower in the complicated pregnancies overall but there was still a significant difference between the ART and non-ART groups (Table II). Among uncomplicated pregnancies, PAPP-A levels remained significantly reduced in ART pregnancies (0.85 MoM) compared with non-ART pregnancies (1.02 MoM) (P < 0.001) (Table II).

Analyses according to the aetiology of the infertility showed that PAPP-A levels were similarly reduced when the infertility was reported to be of female-only aetiology (0.82 MoM), male-only aetiology (0.85 MoM) and when a combination of male and female aetologies was present in the couple (0.82 MoM). The differences between these categories were not statistically significant.

Further analysis of the effect of ART on marker levels was undertaken using the subset of pregnancies that did not have adverse perinatal and obstetric complications (Table III). Comparison of PAPP-A levels between non-ART pregnancies (1.02 MoM) and each subtype of ART showed that the reduction in PAPP-A applied to all three ART subtypes (IVF, 0.87 MoM; ICSI, 0.84 MoM; GIFT, 0.71 MoM).

Compared with non-ART pregnancies (1.02 MoM), PAPP-A levels were reduced for both fresh embryos transfers (0.79 MoM, t-test P < 0.001) and frozen–thawed embryo transfers (0.95 MoM, t-test
Low levels of PAPP-A in ART pregnancies

Table I Characteristics of the study population

<table>
<thead>
<tr>
<th>ART pregnancies</th>
<th>Non-ART</th>
<th>All</th>
<th>IVF</th>
<th>ICSI</th>
<th>GIFT</th>
<th>IVF/ICSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>33 (15–48)</td>
<td>35 (21–45)*</td>
<td>35 (21–45)*</td>
<td>35 (23–44)*</td>
<td>37 (30–43)*</td>
<td>35 (23–45)*</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>65 (31–155)</td>
<td>65 (41–140)</td>
<td>65 (41–140)*</td>
<td>65 (41–140)*</td>
<td>67 (44–140)</td>
<td>65 (44–135)</td>
</tr>
<tr>
<td>CRL at NT scan (mm)</td>
<td>61 (40–86)</td>
<td>61 (45–84)</td>
<td>61 (45–84)</td>
<td>61 (45–84)</td>
<td>62 (51–80)</td>
<td>60 (45–83)*</td>
</tr>
<tr>
<td>Gestation at NT scan (days)</td>
<td>87 (75–98)</td>
<td>87 (78–97)</td>
<td>87 (78–97)</td>
<td>87 (78–97)</td>
<td>88 (82–96)</td>
<td>87 (78–97)*</td>
</tr>
<tr>
<td>Gestation at blood sampling (days)</td>
<td>77 (44–97)</td>
<td>77 (60–96)</td>
<td>77 (60–96)</td>
<td>77 (60–96)</td>
<td>78 (62–91)</td>
<td>76 (61–96)*</td>
</tr>
<tr>
<td>Primigravid (%)</td>
<td>34.4</td>
<td>46.6*</td>
<td>41.7*</td>
<td>50.0*#</td>
<td>36.4</td>
<td>53.0*</td>
</tr>
</tbody>
</table>

Values are given as median (range) and Mann–Whitney tests were performed for P-value based on independent samples t-test, whereas chi-square tests were performed for values.*Denotes P-value of <0.05 for the different ART groups (All, IVF, ICSI, GIFT, fresh and frozen) versus non-ART comparisions. # denotes P-value of <0.05 for IVF versus ICSI comparison, ^ denotes P-value of <0.05 for fresh versus frozen–thawed comparison.

Table II Effect of ART on marker levels

<table>
<thead>
<tr>
<th>All pregnancies</th>
<th>fβ-hCG</th>
<th>PAPP-A</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ART (n = 1739)</td>
<td>0.99</td>
<td>0.91</td>
<td>0.90</td>
</tr>
<tr>
<td>Complicated pregnancies*</td>
<td>0.94</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>All ART (n = 366)</td>
<td>0.97</td>
<td>0.90</td>
<td>0.99</td>
</tr>
</tbody>
</table>

| All ART (n = 1373) | 1.00 | 0.91 | 0.91 |

<table>
<thead>
<tr>
<th>All ART (n = 1739)</th>
<th>0.99</th>
<th>0.90</th>
<th>0.90</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Non-ART (n = 50 253)</th>
<th>0.98</th>
<th>0.90</th>
<th>0.90</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Non-ART (n = 7001)</th>
<th>0.94</th>
<th>0.90</th>
<th>0.90</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Non-ART (n = 43 252)</th>
<th>0.99</th>
<th>0.90</th>
<th>0.90</th>
</tr>
</thead>
</table>

| All ART (n = 1373) | 1.00 | 0.91 | 0.91 |

Association between ART and false-positive results

Table IV shows the comparison between the non-ART and ART groups for the proportion of women receiving a false-positive result from first trimester combined screening. Women conceiving using ART had a significantly increased likelihood of receiving a false-positive result (OR 2.71, 95% CI 2.19–3.35; P < 0.001) compared with non-ART women. After adjusting for maternal age and gravidity, ART women were still more likely to receive a false-positive result when compared with non-ART women. After adjusting for maternal age and gravidity, ART women were still more likely to receive a false-positive result when compared with non-ART women. After adjusting for maternal age and gravidity, ART women were still more likely to receive a false-positive result when compared with non-ART women.

Number of fetal hearts detected by ultrasound in ART pregnancies

Of the 1739 singleton ART pregnancies, 1604 had one fetal heart, 123 had two or more fetal hearts and results for 12 were not available. PAPP-A levels were 0.83 MoM for pregnancies with one fetal heart and 0.97 MoM for pregnancies with multiple fetal hearts. Results for fβ-hCG were 0.99 MoM for one fetal heart and 1.03 MoM for multiple fetal hearts, and results for NT were 0.91 MoM for one fetal heart and 0.91 MoM for multiple fetal hearts.

False positive results and uptake of CVS and amniocentesis

A higher proportion of women who conceived using ART (10.6%) had a CVS or amniocentesis compared with their non-ART counterparts.
After adjusting for maternal age, the ART group were still more likely to have a CVS or amniocentesis compared with the non-ART group (OR 1.24, 95% CI 1.03–1.49; \( P = 0.023 \)). When the increased risk result was also accounted for, the OR of having a CVS or amniocentesis was reduced to 0.78 (95% CI 0.60–1.00; \( P = 0.054 \)) for the ART group compared with the non-ART group, suggesting that the higher false-positive rate was responsible for the higher uptake of invasive prenatal diagnosis in

### Table III  Effect of ART on marker levels—excluding complicated pregnancies

<table>
<thead>
<tr>
<th>Comparison group</th>
<th>( f\beta-hCG ) Geometric mean MoM</th>
<th>( P)-value*</th>
<th>( P)-value*</th>
<th>( P)-value*</th>
<th>( P)-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-ART (n = 43 252)</td>
<td>0.99</td>
<td>—</td>
<td>1.02</td>
<td>—</td>
<td>0.90</td>
</tr>
<tr>
<td>Subtype of ART</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVF (n = 513)</td>
<td>1.01</td>
<td>0.601</td>
<td>0.87</td>
<td>&lt;0.001</td>
<td>0.92</td>
</tr>
<tr>
<td>ICSI (n = 833)</td>
<td>1.00</td>
<td>0.563</td>
<td>0.84</td>
<td>&lt;0.001</td>
<td>0.91</td>
</tr>
<tr>
<td>GIFT (n = 27)</td>
<td>0.84</td>
<td>0.136</td>
<td>0.71</td>
<td>&lt;0.001</td>
<td>0.94</td>
</tr>
<tr>
<td>Fresh and frozen–thawed embryo transfer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh (n = 773)</td>
<td>0.98</td>
<td>0.554</td>
<td>0.79</td>
<td>&lt;0.001</td>
<td>0.91</td>
</tr>
<tr>
<td>Frozen–thawed (n = 573)</td>
<td>1.03</td>
<td>0.060</td>
<td>0.95</td>
<td>0.001</td>
<td>0.92</td>
</tr>
<tr>
<td>GIFT (n = 27)</td>
<td>0.84</td>
<td>0.136</td>
<td>0.71</td>
<td>&lt;0.001</td>
<td>0.94</td>
</tr>
<tr>
<td>Fresh and frozen–thawed embryo transfer by hormone treatmentb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh with hormone treatment (n = 762)</td>
<td>0.98</td>
<td>0.538</td>
<td>0.78</td>
<td>&lt;0.001</td>
<td>0.91</td>
</tr>
<tr>
<td>Fresh without hormone treatment (n = 11)</td>
<td>1.23</td>
<td>0.871</td>
<td>0.99</td>
<td>0.837</td>
<td>0.82</td>
</tr>
<tr>
<td>Frozen–thawed with hormone treatment (n = 118)</td>
<td>0.98</td>
<td>0.788</td>
<td>0.78</td>
<td>&lt;0.001</td>
<td>0.93</td>
</tr>
<tr>
<td>Frozen–thawed without hormone treatment (n = 455)</td>
<td>1.06</td>
<td>0.024</td>
<td>0.99</td>
<td>0.285</td>
<td>0.92</td>
</tr>
<tr>
<td>GIFT (n = 27)</td>
<td>0.84</td>
<td>0.136</td>
<td>0.71</td>
<td>&lt;0.001</td>
<td>0.94</td>
</tr>
<tr>
<td>All embryo transfers by hormone treatmentb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With hormone treatment (n = 907)</td>
<td>0.98</td>
<td>0.361</td>
<td>0.78</td>
<td>&lt;0.001</td>
<td>0.91</td>
</tr>
<tr>
<td>Without hormone treatment (n = 466)</td>
<td>1.05</td>
<td>0.025</td>
<td>0.99</td>
<td>0.277</td>
<td>0.92</td>
</tr>
</tbody>
</table>

*\( P\)-value based on the independent samples \( t\)-test, values are compared with the non-ART group.

bHormone treatment accompanying fresh embryo transfers always included FSH or clomiphene, often in combination with other drugs. All GIFT cycles were done with FSH. Hormone treatment accompanying frozen–thawed embryo transfers comprised various combinations of estradiol, progesterone, clomiphene and HCG.

### Table IV  Comparison of false-positive results

<table>
<thead>
<tr>
<th>First trimester combined screen result</th>
<th>Total number</th>
<th>Increased risk</th>
<th>Univariate analysis</th>
<th>Multivariate analysis adjusting for maternal age and gravidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Non-ART</td>
<td>50 253</td>
<td>1996</td>
<td>4.0</td>
<td>Reference</td>
</tr>
<tr>
<td>ART</td>
<td>1739</td>
<td>175</td>
<td>10.1</td>
<td>2.71</td>
</tr>
<tr>
<td>IVF</td>
<td>654</td>
<td>65</td>
<td>9.9</td>
<td>2.67</td>
</tr>
<tr>
<td>ICSI</td>
<td>1052</td>
<td>103</td>
<td>9.8</td>
<td>2.62</td>
</tr>
<tr>
<td>GIFT</td>
<td>33</td>
<td>7</td>
<td>21.2</td>
<td>6.51</td>
</tr>
<tr>
<td>ART</td>
<td>1187</td>
<td>129</td>
<td>10.9</td>
<td>2.95</td>
</tr>
<tr>
<td>With hormone treatment</td>
<td>1187</td>
<td>129</td>
<td>10.9</td>
<td>2.95</td>
</tr>
<tr>
<td>Without hormone treatment</td>
<td>553</td>
<td>46</td>
<td>8.3</td>
<td>2.19</td>
</tr>
</tbody>
</table>

(5.3%) (OR 2.10, 95% CI 1.76–2.50; \( P < 0.001 \)) (Table V). After adjusting for maternal age, the ART group were still more likely to have a CVS or amniocentesis compared with the non-ART group (OR 1.24, 95% CI 1.03–1.49; \( P = 0.023 \)). When the increased risk result was also accounted for, the OR of having a CVS or amniocentesis was reduced to 0.78 (95% CI 0.60–1.00; \( P = 0.054 \)) for the ART group compared with the non-ART group, suggesting that the higher false-positive rate was responsible for the higher uptake of invasive prenatal diagnosis in
the ART group. When examining the effect of hormone versus no hormone treatment, we found that after controlling for maternal age, only women whose transfer cycles included hormone treatment were more likely than non-ART women to have a CVS or amniocentesis (AdjOR 1.38, 95% CI 1.11–1.71; \( P = 0.003 \)).

**Discussion**

This is the largest and most comprehensive study to date on the influence of ART conception on first trimester combined screening, and is important because of the increasing use of screening in all pregnancies. This study comprises more than 1700 ART singleton pregnancies and more than 50 000 non-ART singleton pregnancies, representing almost as many ART pregnancies as all previous studies combined.

The primary effect of ART treatment was a significant reduction in the serum PAPP-A level compared with the non-ART conceptions, whereas \( \beta \)-hCG was not significantly altered. A marginal increase in NT was observed in ART pregnancies, however, this very small difference is likely due to operator effects, with ART pregnancies more likely to be scanned by a small subset of operators linked to ART clinics compared with non-ART pregnancies.

The pattern of markers observed in this study is similar to that observed in several previous studies (Liao et al., 2001; Maymon and Shulman, 2002, 2004; Orlandi et al., 2002; Hui et al., 2005b; Anckaert et al., 2008), but differs from others that detected no effect of ART on PAPP-A levels (Niemimaa et al., 2001; Wojdemann et al., 2001; Ghisoni et al., 2003; Belsinger et al., 2004). A likely explanation for the contradictory findings of previous studies is their small sample sizes (sample size range 47–301 ART pregnancies).

We have also shown that as a result of the decreased PAPP-A in ART pregnancies, women conceiving using ART are more likely to receive a false-positive result from the first trimester combined screen and are therefore more likely to have CVS or amniocentesis. This finding is important because an increase in the uptake of CVS and amniocentesis in healthy pregnancies will lead to an increase in parental anxiety and in procedure-related morbidity, including miscarriage. The increase in false-positive results and uptake of prenatal diagnosis is seen for fresh and frozen–thawed embryo transfers, but only in the subset of embryo transfers where the mother was given hormone treatment around the time of embryo transfer. This study only examined the FPR and not other indicators of screening test performance such as sensitivity and specificity; therefore, no conclusion can be drawn in relation to whether adjustments for calculation of risk parameters need to be made. Following further investigation of the effectiveness of the first trimester combined screen in ART pregnancies, it may be possible to modify screening protocols for ART pregnancies in order to reduce the FPR without reducing the sensitivity of the test.

The reduction in PAPP-A levels in ART pregnancies provides further evidence that ART pregnancies are different from non-ART pregnancies, an observation that may have implications beyond the combined screen. PAPP-A is a growth factor that promotes growth by cleaving insulin-like growth factor binding proteins (Lawrence et al., 1999) thereby increasing the bioavailability of insulin-like growth factors.
PAPP-A is present at low concentrations in the blood of women and non-pregnant women, but is detected at high concentrations in the blood of pregnant women (Lin et al., 1974), with blood levels rising soon after implantation and increasing with gestation, peaking in the third trimester (Guibourdenche et al., 2003). In pregnant women, circulating PAPP-A originates at the interface between the placenta and the endometrium, where it is produced by placental trophoblasts and decidualized endometrial stromal cells, and is hypothesized to regulate IGF-II bioavailability in the placenta and to facilitate implantation (Giudice et al., 2002).

Our data provide new insights into the possible mechanisms underlying reduced PAPP-A in ART pregnancies. One consideration is whether the presence of a ‘vanished twin’ in some ART pregnancies might explain the difference in results between ART and non-ART pregnancies. However, in our ART population vanished twins appear to increase PAPP-A levels rather than decrease them; therefore, vanished twins are not responsible for the observed reduced in PAPP-A levels in ART pregnancies. In fact, vanished twins may have the opposite effect, suggesting that the number of fetal hearts at early ultrasound should be considered in any modification of screening protocols in ART pregnancies.

It has previously been suggested that a reduction in PAPP-A in ART pregnancies might be an artefact of testing being undertaken at an earlier gestation in this group (Maymon and Shulman, 2002). In our population, there was no difference between the ART and non-ART pregnancies in the timing of blood sampling or ultrasound. We did detect a slightly greater CRL for frozen–thawed embryos compared with fresh embryos, equivalent to ~1 day of gestational age. This difference may reflect a longer in vitro culture time for frozen–thawed embryos compared with fresh embryos, a difference that would not affect the PAPP-A MoMs because these are adjusted for gestational age.

PAPP-A levels are also known to be associated with adverse pregnancy complications (hypertension, pre-eclampsia and gestational diabetes) and adverse perinatal outcomes (prematurity, low birthweight and neonatal death) (Ong et al., 2000; Dugoff et al., 2004; Smith et al., 2006). ART is associated with an overlapping spectrum of pregnancy and perinatal complications (Maman et al., 1998; Schieve et al., 2002; Helmerhorst et al., 2004; Shevell et al., 2005); therefore, lower PAPP-A in ART pregnancies might simply be a predictor of these complications that are known to be more common in the ART population (Maymon and Shulman, 2002; Bersinger et al., 2004). We were able to analyse our data according to the presence or absence of these complications, and found that PAPP-A levels were reduced in ART pregnancies with or without these complications.

It has also been suggested that lower PAPP-A levels in ART pregnancies might be the result of metabolic impairments related to infertility in the mother (Maymon and Shulman, 2002). This hypothesis is partly refuted by our data that show that PAPP-A is similarly reduced in male-factor infertility compared with female-factor infertility. However, the difference in PAPP-A results between frozen–thawed embryos transferred with and without hormone treatment may be related to underlying ovulatory disorders and defective endometrial development in the mother. This requires further study.

Our results support and extend the hypothesis that exogenous hormone treatment is the principal cause of reduced PAPP-A in ART pregnancies (Bersinger et al., 2004; Hui et al., 2005a; Tul and Novak-Antolic, 2006). We found significantly lower PAPP-A levels for embryos transferred fresh compared with frozen–thawed embryos. This finding is consistent with one previous study that separately examined fresh and frozen–thawed embryo transfers and found a reduction in PAPP-A for fresh embryo transfers (Hui et al., 2005a). A second study analysed PAPP-A levels in relation to the number of oocytes retrieved and found a greater reduction in PAPP-A following fresh embryo transfers in stimulation cycles in which a greater number of oocytes were retrieved (Tul and Novak-Antolic, 2006), further suggesting that hormone stimulation and the woman’s response to hormone treatment is linked to the reduced PAPP-A.

In contrast to previous studies, we also detected significantly lower PAPP-A levels for frozen–thawed embryos compared with non-ART pregnancies, indicating that the reduction in PAPP-A is not restricted to fresh embryo transfers and prompting further analysis. The classification of fresh versus frozen–thawed embryos has potential to mask an effect of hormone treatment because in our study population a significant proportion of frozen–thawed embryo transfers (20.6%) were accompanied by hormone treatment. When our data were stratified according to the presence or absence of any hormone treatment, we found that reduced PAPP-A levels were seen only in the group of pregnancies in which embryo transfer has been accompanied by hormone treatment. In uncomplicated pregnancies where no hormone treatment was used, PAPP-A levels were normal.

The endocrine changes that occur during early pregnancy, including the production of PAPP-A by the endometrium and placenta, are the result of a complex and poorly understood set of interactions between the corpus luteum, endometrium, placenta and embryo. Our data suggest that the administration of exogenous hormones, occurring in fresh and artificial frozen–thawed ART cycles, interferes with the normal endocrine changes of early pregnancy, resulting in reduced PAPP-A levels.

The contribution of different types of hormone treatment to the reduced PAPP-A in ART pregnancies is more difficult to assess because treatment protocols are highly variable and more comprehensive treatment data were not available. It is, however, notable that similarly reduced PAPP-A levels were seen for fresh embryo transfers that were accompanied by FSH and/or clomiphene treatment, and for frozen–thawed embryo transfers that were accompanied by treatment with various combinations of estradiol, progesterone, HCG and clomiphene.

We propose a model whereby hormone treatment accompanying embryo transfers results in abnormal levels of ovarian steroid hormones and other factors yet to be identified, which in turn cause a reduction in PAPP-A production. Although PAPP-A is produced by the placenta, the reduction in PAPP-A is likely to be mediated via an effect of hormones on the endometrium because the effect is seen for hormone treatment administered prior to implantation and establishment of a placenta, possibly reflecting impairment of early implantation with some forms of ART. Lower PAPP-A secretion should lower the availability of IGFs (Giudice et al., 2002) and through this mechanism may directly contribute to low birthweight (Smith et al., 2002). It is well known that singleton babies born after ART conception are at increased risk of being low birthweight or small for gestational age (Schieve et al., 2002; Halliday, 2007), and those with low PAPP-A from FTS are also at increased risk of low birthweight (Dugoff et al., 2004; Barrett et al., 2008). Moreover, it has been observed that babies born from fresh ART cycles are at
increased risk of low birthweight and have a lower mean birthweight compared with babies born from frozen–thawed cycles (Wada et al., 1994; Schieve et al., 2002; Wang et al., 2005; Belva et al., 2008; Shih et al., 2008).

In conclusion, this study has provided conclusive evidence that first trimester maternal serum levels of PAPP-A are decreased in ART pregnancies, resulting in a much higher FPR on first trimester combined screening. Our results highlight the importance of pre- and post-test counselling for women carrying ART pregnancies. Further work should be undertaken to determine the viability of altering the risk calculation for pregnancies conceived via ART, particularly those that underwent hormone treatment. A recent trend towards ‘mild’ IVF with reduced hormone stimulation (Heijnen et al., 2007) might have an additional benefit of reducing the number of false-positive results at first trimester combined screening. Hormone treatment that accompanies many ART cycles appears to be strongly associated with the reduction in PAPP-A and the increased FPR, and may also contribute to the increased risk of low birthweight.

Author’s Role
A.M.J. and D.J.A. formulated the research question, performed the data linkage/merging and analysis, interpreted the results and wrote the paper. J.X.X. performed the statistical analyses, results interpretation and wrote the paper. I.F. provided the data from the maternal screening lab, assisted with interpretation of the results and edited the paper. J.L.H., D.L.H., H.W.G.B. and S.B. provided the ART data, oversaw the project, assisted with the interpretation of results and edited the paper.

Supplementary data
Supplementary data are available at http://humrep.oxfordjournals.org/.

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