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 (fβ-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% (Jaques 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; Raty 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 fβ-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 fβ-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 (fβ-hCG, PAPP-A and 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 (fβ-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
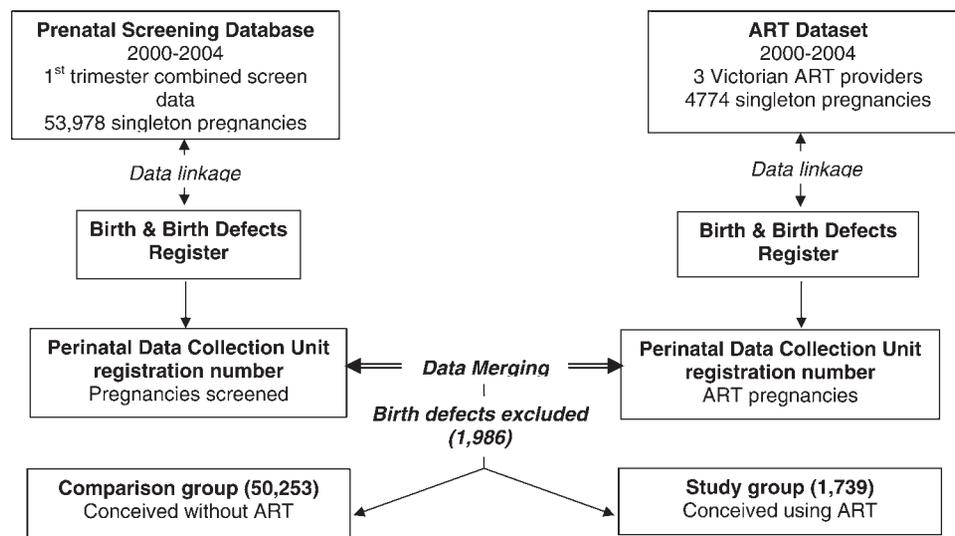
Figure 1 Flow diagram showing data linkage between three separate databases to obtain study population.

combined screening programme has been described elsewhere (Jaques et al., 2006, 2007). Briefly, fβ-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 of 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 fβ-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 aetiologies 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 oligomenorrhoea/amenorrhoea, 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 fβ-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 aetiologies 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

Table I Characteristics of the study population

| | | ART pregnancies | | | | IVF/ICSI | |
| | Non-ART | All | IVF | ICSI | GIFT | Fresh | Frozen−thawed |
	n = 50 253	n = 1739	n = 654	n = 1052	n = 33	n = 1003	n = 703
Maternal age (years)	33 (15−48)	35 (21−45)*	35 (21−45)*	35 (23−44)*	37 (30−43)*	35 (23−45)*	35 (21−44)*
Maternal weight (kg)	65 (31−155)	65 (41−140)	64 (42−136)	65 (41−140)*#	67 (44−140)	65 (44−135)	65 (41−140)
CRL at NT scan (mm)	61 (40−86)	61 (45−84)	61 (45−80)	61 (45−84)	62 (51−80)	60 (45−83)*	62 (45−84)*^
Gestation at NT scan (days)	87 (75−98)	87 (78−97)	87 (78−96)	87 (78−97)	88 (82−96)	87 (78−97)*	88 (78−97)^
Gestation at blood sampling (days)	77 (44−97)	77 (61−96)	77 (62−96)	77 (61−96)	78 (62−91)	76 (61−96)*	78 (62−96)*^
Primigravid (%)	34.4	46.6*	41.7*	50.0*#	36.4	53.0*	38.0^

Values are given as median (range) and Mann−Whitney tests were performed for P-value for maternal age, maternal weight, CRL at NT scan, gestation at NT scan and gestation at blood sampling. Primigravid is given as a percent and chi-square tests were performed for P-values.*Denotes P-value of <0.05 for the different ART groups (All, IVF, ICSI, GIFT, fresh and frozen) versus non-ART comparisons, # 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

| | fβ-hCG | | PAPP-A | | NT | |
	Geometric mean MoM	P-value[a]	Geometric mean MoM	P-value[a]	Geometric mean MoM	P-value[a]
All pregnancies						
Non-ART (n = 50 253)	0.98	—	1.00	—	0.90	—
All ART (n = 1739)	0.99	0.479	0.83	<0.001	0.91	0.004
Complicated pregnancies[b]						
Non-ART (n = 7001)	0.94	—	0.90	—	0.90	—
All ART (n = 366)	0.97	0.281	0.77	<0.001	0.91	0.267
Excluding complicated pregnancies						
Non-ART (n = 43 252)	0.99	—	1.02	—	0.90	—
All ART (n = 1373)	1.00	0.577	0.85	<0.001	0.91	0.005

[a]P-value based on the independent samples t-test, values are compared with the non-ART group.[b]Includes pregnancies with adverse pregnancy outcomes [neonatal death, preterm (<37 weeks) and low birthweight (<2.5 kg)] and pregnancies with obstetric complications (pre-eclampsia, pregnancy-induced hypertension and gestational diabetes).

$P = 0.001$), however, fresh embryo transfers were associated with a significantly lower PAPP-A level when compared directly with frozen−thawed embryos (t-test $P < 0.001$).

When examining the effect of hormone versus no hormone treatment irrespective of fresh or frozen−thawed embryo transfer, we found that transfer cycles that included any hormone treatment resulted in lower PAPP-A levels (0.78 MoM) compared with those without hormone treatment (0.99 MoM, t-test $P < 0.001$).

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.

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 [adjusted OR (AdjOR) 1.71, 95% CI 1.44−2.04; $P < 0.001$]. The likelihood of receiving a false-positive result was higher for fresh embryo transfers than for frozen−thawed embryo transfers.

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

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

	fβ-hCG		PAPP-A		NT	
	Geometric mean MoM	P-value[a]	Geometric mean MoM	P-value[a]	Geometric mean MoM	P-value[a]
Comparison group						
Non-ART (n = 43 252)	0.99	—	1.02	—	0.90	—
Subtype of ART						
IVF (n = 513)	1.01	0.601	0.87	<0.001	0.92	0.064
ICSI (n = 833)	1.00	0.563	0.84	<0.001	0.91	0.065
GIFT (n = 27)	0.84	0.136	0.71	<0.001	0.94	0.384
Fresh and frozen–thawed embryo transfer						
Fresh (n = 773)	0.98	0.554	0.79	<0.001	0.91	0.190
Frozen–thawed (n = 573)	1.03	0.060	0.95	0.001	0.92	0.014
GIFT (n = 27)	0.84	0.136	0.71	<0.001	0.94	0.384
Fresh and frozen–thawed embryo transfer by hormone treatment[b]						
Fresh with hormone treatment (n = 762)	0.98	0.538	0.78	<0.001	0.91	0.149
Fresh without hormone treatment (n = 11)	1.23	0.871	0.99	0.837	0.82	0.305
Frozen–thawed with hormone treatment (n = 118)	0.98	0.788	0.78	<0.001	0.93	0.115
Frozen–thawed without hormone treatment (n = 455)	1.06	0.024	0.99	0.285	0.92	0.050
GIFT (n = 27)	0.84	0.136	0.71	<0.001	0.94	0.384
All embryo transfers by hormone treatment[b]						
With hormone treatment (n = 907)	0.98	0.361	0.78	<0.001	0.91	0.042
Without hormone treatment (n = 466)	1.05	0.025	0.99	0.277	0.92	0.075

[a]P-value based on the independent samples t-test, values are compared with the non-ART group.
[b]Hormone 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

	First trimester combined screen result		Univariate analysis			Multivariate analysis adjusting for maternal age and gravidity		
	Total number	Increased risk						
	n	n	%	OR	95% CI	P-value	OR	95% CI	P-value
Non-ART	50 253	1996	4.0	Reference			Reference		
ART	1739	175	10.1	2.71	2.30–3.18	<0.001	1.71	1.44–2.04	<0.001
IVF	654	65	9.9	2.67	2.06–3.46	<0.001	1.68	1.28–2.21	<0.001
ICSI	1052	103	9.8	2.62	2.13–3.23	<0.001	1.71	1.37–2.13	<0.001
GIFT	33	7	21.2	6.51	2.82–15.01	<0.001	2.20	0.89–5.45	0.088
ART									
Fresh	1003	107	10.7	2.89	2.35–3.55	<0.001	1.80	1.45–2.25	<0.001
Frozen–thawed	703	61	8.7	2.30	1.76–3.00	<0.001	1.55	1.17–2.04	0.002
ART									
With hormone treatment	1187	129	10.9	2.95	2.44–3.56	<0.001	1.91	1.56–2.34	<0.001
Without hormone treatment	553	46	8.3	2.19	1.62–3.00	<0.001	1.32	0.96–1.82	0.08

(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

Table V Analysis of uptake of CVS and amniocentesis

	CVS or amniocentesis		Univariate analysis			Multivariate analysis adjusting for maternal age only			Multivariate analysis adjusting for maternal age and increased risk result		
	n	%	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
All births											
Non-ART	40 327	5.3	Reference			Reference			Reference		
All ART	1429	10.6	2.10	1.76–2.50	<0.001	1.24	1.03–1.49	0.023	0.78	0.60–1.00	0.054
Hormone treatment											
Yes	974	11.4	2.28	1.87–2.80	<0.001	1.38	1.11–1.71	0.003	0.83	0.62–1.13	0.237
No	455	8.8	1.71	1.23–2.37	0.001	0.97	0.69–1.36	0.854	0.65	0.41–1.04	0.077

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 fβ-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; Bellver et al., 2005; Lambert-Messerlian et al., 2006). Some studies have also suggested that ART results in an increase in fβ-hCG (Niemimaa et al., 2001; Ghisoni et al., 2003; Bersinger 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

(IGFs) (Conover *et al.*, 2004). PAPP-A is present at low concentrations in the blood of men 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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References

Anckaert E, Schiettecatte J, Sleurs E, Devroey P, Smitz J. First trimester screening for Down's syndrome after assisted reproductive technology: non-male factor infertility is associated with elevated free beta-human chorionic gonadotropin levels at 10-14 weeks of gestation. Fertil Steril 2008;**90**:1206–1210.

Barkai G, Goldman B, Ries L, Chaki R, Dor J, Cuckle H. Down's syndrome screening marker levels following assisted reproduction. Prenat Diagn 1996;**16**:1111–1114.

Barrett SL, Bower C, Hadlow NC. Use of the combined first-trimester screen result and low PAPP-A to predict risk of adverse fetal outcomes. Prenat Diagn 2008;**28**:28–35.

Bellver J, Lara C, Soares SR, Ramirez A, Pellicer A, Remohi J, Serra V. First trimester biochemical screening for Down's syndrome in singleton pregnancies conceived by assisted reproduction. Hum Reprod 2005;**20**:2623–2627.

Belva F, Henriet S, Van den Abbeel E, Camus M, Devroey P, Van der Elst J, Liebaers I, Haentjens P, Bonduelle M. Neonatal outcome of 937 children born after transfer of cryopreserved embryos obtained by ICSI and IVF and comparison with outcome data of fresh ICSI and IVF cycles. Hum Reprod 2008;**23**:2227–2238.

Bersinger NA, Wunder D, Vanderlick F, Chanson A, Pescia G, Janecek P, Boillat E, Birkhauser MH. Maternal serum levels of placental proteins after in vitro fertilisation and their implications for prenatal screening. Prenat Diagn 2004;**24**:471–477.

Conover CA, Bale LK, Overgaard MT, Johnstone EW, Laursen UH, Fuchtbauer EM, Oxvig C, van Deursen J. Metalloproteinase pregnancy-associated plasma protein A is a critical growth regulatory factor during fetal development. Development 2004;**131**:1187–1194.

Dugoff L, Hobbins JC, Malone FD, Porter TF, Luthy D, Comstock CH, Hankins G, Berkowitz RL, Merkatz I, Craigo SD et al. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). Am J Obstet Gynecol 2004;**191**:1446–1451.

Frishman GN, Canick JA, Hogan JW, Hackett RJ, Kellner LH, Saller DN Jr. Serum triple-marker screening in in vitro fertilization and naturally conceived pregnancies. Obstet Gynecol 1997;**90**:98–101.

Ghisoni L, Ferrazzi E, Castagna C, Levi Setti PE, Masini AC, Pigni A. Prenatal diagnosis after ART success: the role of early combined screening tests in counselling pregnant patients. Placenta 2003;**24**(Suppl B):S99–S103.

Giudice LC, Conover CA, Bale L, Faessen GH, Ilg K, Sun I, Imani B, Suen LF, Irwin JC, Christiansen M et al. Identification and regulation of the IGFBP-4 protease and its physiological inhibitor in human trophoblasts and endometrial stroma: evidence for paracrine regulation of IGF-II bioavailability in the placental bed during human implantation. J Clin Endocrinol Metab 2002;**87**:2359–2366.

Gjerris AC, Loft A, Pinborg A, Tabor A, Christiansen M. First-trimester screening in pregnancies conceived by assisted reproductive technology: significance of gestational dating by oocyte retrieval or sonographic measurement of crown-rump length. Ultrasound Obstet Gynecol 2008;**32**:612–617.

Guibourdenche J, Frendo JL, Pidoux G, Bertin G, Luton D, Muller F, Porquet D, Evain-Brion D. Expression of pregnancy-associated plasma protein-A (PAPP-A) during human villous trophoblast differentiation in vitro. Placenta 2003;**24**:532–539.

Halliday J. Outcomes of IVF conceptions: are they different? Best Pract Res Clin Obstet Gynaecol 2007;**21**:67–81.

Heijnen EM, Eijkemans MJ, De Klerk C, Polinder S, Beckers NG, Klinkert ER, Broekmans FJ, Passchier J, Te Velde ER, Macklon NS et al. A mild treatment strategy for in-vitro fertilisation: a randomised non-inferiority trial. Lancet 2007;**369**:743–749.

Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. BMJ 2004;**328**:261.

Hui PW, Lam YH, Tang MH, Ng EH, Yeung WS, Ho PC. Maternal serum pregnancy-associated plasma protein-A and free beta-human chorionic gonadotrophin in pregnancies conceived with fresh and frozen-thawed embryos from in vitro fertilization and intracytoplasmic sperm injection. Prenat Diagn 2005a;**25**:390–393.

Hui PW, Tang MH, Lam YH, Yeung WS, Ng EH, Ho PC. Nuchal translucency in pregnancies conceived after assisted reproduction technology. Ultrasound Obstet Gynecol 2005b;**25**:234–238.

Jaques AM, Collins VR, Haynes K, Sheffield LJ, Francis I, Forbes R, Halliday JL. Using record linkage and manual follow-up to evaluate the Victorian maternal serum screening quadruple test for Down's syndrome, trisomy 18 and neural tube defects. *J Med Screen* 2006;**13**:8–13.

Jaques AM, Halliday JL, Francis I, Bonacquisto L, Forbes R, Cronin A, Sheffield LJ. Follow up and evaluation of the Victorian first-trimester combined screening programme for Down syndrome and trisomy 18. *BJOG* 2007;**114**:812–818.

Lambert-Messerlian G, Dugoff L, Vidaver J, Canick JA, Malone FD, Ball RH, Comstock CH, Nyberg DA, Saade G, Eddleman K *et al.* First- and second-trimester Down syndrome screening markers in pregnancies achieved through assisted reproductive technologies (ART): a FASTER trial study. *Prenat Diagn* 2006;**26**:672–678.

Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, Gleich GJ, Hays LG, Yates JR III, Conover CA. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc Natl Acad Sci U S A* 1999;**96**:3149–3153.

Liao AW, Heath V, Kametas N, Spencer K, Nicolaides KH. First-trimester screening for trisomy 21 in singleton pregnancies achieved by assisted reproduction. *Hum Reprod* 2001;**16**:1501–1504.

Lin TM, Galbert SP, Kiefer D, Spellacy WN, Gall S. Characterization of four human pregnancy-associated plasma proteins. *Am J Obstet Gynecol* 1974;**118**:223–236.

Maman E, Lunenfeld E, Levy A, Vardi H, Potashnik G. Obstetric outcome of singleton pregnancies conceived by in vitro fertilization and ovulation induction compared with those conceived spontaneously. *Fertil Steril* 1998;**70**:240–245.

Maymon R, Shulman A. Serial first- and second-trimester Down's syndrome screening tests among IVF-versus naturally-conceived singletons. *Hum Reprod* 2002;**17**:1081–1085.

Maymon R, Shulman A. Integrated first- and second-trimester Down syndrome screening test among unaffected IVF pregnancies. *Prenat Diagn* 2004;**24**:125–129.

Nicolaides KH. *The 11-13+6 Weeks Scan*. London: Fetal Medicine Foundation, 2004.

Niemimaa M, Heinonen S, Seppala M, Hippelainen M, Martikainen H, Ryynanen M. First-trimester screening for Down's syndrome in in vitro fertilization pregnancies. *Fertil Steril* 2001;**76**:1282–1283.

Ong CY, Liao AW, Spencer K, Munim S, Nicolaides KH. First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *BJOG* 2000;**107**:1265–1270.

Orlandi F, Rossi C, Allegra A, Krantz D, Hallahan T, Orlandi E, Macri J. First trimester screening with free beta-hCG, PAPP-A and nuchal translucency in pregnancies conceived with assisted reproduction. *Prenat Diagn* 2002;**22**:718–721.

Raty R, Virtanen A, Koskinen P, Anttila L, Forsstrom J, Laitinen P, Morsky P, Tiitinen A, Ekblad U. Serum free beta-HCG and alpha-fetoprotein levels in IVF, ICSI and frozen embryo transfer pregnancies in maternal mid-trimester serum screening for Down's syndrome. *Hum Reprod* 2002;**17**:481–484.

Ribbert LS, Kornman LH, De Wolf BT, Simons AH, Jansen CA, Beekhuis JR, Mantingh A. Maternal serum screening for fetal Down syndrome in IVF pregnancies. *Prenat Diagn* 1996;**16**:35–38.

Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med* 2002;**346**:731–737.

Shevell T, Malone FD, Vidaver J, Porter TF, Luthy DA, Comstock CH, Hankins GD, Eddleman K, Dolan S, Dugoff L *et al.* Assisted reproductive technology and pregnancy outcome. *Obstet Gynecol* 2005;**106**:1039–1045.

Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, Baker HW. Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. *Hum Reprod* 2008;**23**:1644–1653.

Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early-pregnancy origins of low birth weight. *Nature* 2002;**417**:916.

Smith GCS, Shah I, Crossley JA, Aitken DA, Pell JP, Nelson SM, Cameron AD, Connor MJ, Dobbie R. Pregnancy-associated plasma protein A and alpha-fetoprotein and prediction of adverse perinatal outcome. *Obstet Gynecol* 2006;**107**:161–166.

Tul N, Novak-Antolic Z. Serum PAPP-A levels at 10–14 weeks of gestation are altered in women after assisted conception. *Prenat Diagn* 2006;**26**:1206–1211.

Wada I, Macnamee MC, Wick K, Bradfield JM, Brinsden PR. Birth characteristics and perinatal outcome of babies conceived from cryopreserved embryos. *Hum Reprod* 1994;**9**:543–546.

Wald NJ, Cuckle HS, Densem JW, Nanchahal K, Royston P, Chard T, Haddow JE, Knight GJ, Palomaki GE, Canick JA. Maternal serum screening for Down's syndrome in early pregnancy. *BMJ* 1988;**297**:883–887.

Wald NJ, White N, Morris JK, Huttly WJ, Canick JA. Serum markers for Down's syndrome in women who have had in vitro fertilisation: implications for antenatal screening. *BJOG* 1999;**106**:1304–1306.

Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *Health Technol Assess* 2003;**7**:1–77.

Wang YA, Sullivan EA, Black D, Dean J, Bryant J, Chapman M. Preterm birth and low birth weight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. *Fertil Steril* 2005;**83**:1650–1658.

Wang YA, Dean J, Sullivan EA. *Assisted Reproduction Technology in Australia and New Zealand 2005*. Sydney: AIHW National Perinatal Statistics Unit, 2007.

Wojdemann KR, Larsen SO, Shalmi A, Sundberg K, Christiansen M, Tabor A. First trimester screening for Down syndrome and assisted reproduction: no basis for concern. *Prenat Diagn* 2001;**21**:563–565.