Low first-trimester PAPP-A in IVF (fresh and frozen-thawed) pregnancies, likely due to a biological cause

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Abstract

Purpose The purpose of this study is to confirm a difference in the first-trimester screen maternal biochemistry and false-positive rates (FPR) between pregnancies conceived spontaneously and those conceived via assisted reproductive technologies (ART).

Methods Retrospective analysis of the complete population of women (17,889 pregnancies) who had undergone first-trimester screening between January 2004 and September 2009 at three private ultrasound clinics in Queensland, Australia was used in the study. The age, gestation, method of conception, ultrasound markers, biochemistry markers (PAPP-A, fβ-hCG), and type of biochemical analyzer platform (Brahms Kryptor, Immulite 2000) data was collated. Univariate analysis of variance (ANOVA), Spearman's rank nonparametric correlation analysis, and Binary Logistic Regression analysis were used to analyze data. Spontaneous pregnancies were used as controls. Results were considered significant when the *p* value was less than 0.05.

Results After exclusions, 16,363 singleton pregnancies, including 1543 conceived via ART, were analyzed.

Results from the two biochemistry platforms, Brahms Kryptor and Immulite 2000 were significantly different ($p < 0.001$); thus, the data was divided for analysis purposes. PAPP-A was universally significantly lower in IVF pregnancies compared to spontaneously conceived pregnancies ($p < 0.001$). Using the Brahms Kryptor platform, ICSI was associated with significantly decreased PAPP-A ($p < 0.046$), and a significantly increased FPR ($p = 0.012$).

Conclusions Consistent with previous studies IVF pregnancies had significantly lower PAPP-A levels supporting the need to appropriately adjust the combined first-trimester screening (cFTS) risk algorithm for IVF conceptions. The Brahms Kryptor and Immulite 2000 platforms are significantly different; however, the universally lower PAPP-A findings support the hypothesis that the lower PAPP-A is due to a biological cause.

Keywords Combined first-trimester screening · First-trimester maternal serum biochemistry · PAPP-A · IVF

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Introduction

Combined first-trimester screening (cFTS) combines the maternal age-related risk with the measurement of maternal serum analytes, free beta-human chorionic gonadotropin (f β -hCG), and pregnancy-associated plasma protein-A (PAPP-A), and the measurement of the fetal nuchal translucency (NT) to produce an estimated risk of Down syndrome (trisomy 21), Edward syndrome (trisomy 18) and Patau syndrome (trisomy 13). The cFTS utilizes the maternal blood levels of f β -hCG and PAPP-A routinely taken between 10 and 12 weeks gestation and the accredited measurement of the NT and crown rump length (CRL) by ultrasonography between 11⁺³ and 13⁺⁶ weeks gestation [1].

The maternal serum levels of PAPP-A and f β -hCG are expressed as multiples of the median (MoM) as compared to population control data. Down syndrome risk is increased when the ratio of the f β -hCG MoM to PAPP-A MoM is increased, whereas low levels of both analytes are associated with an increased risk for Edward syndrome and Patau syndrome. The cFTS has a detection rate of 95% for Down syndrome [1], with a screen-positive rate of 5% [2]. A high risk, or “screen-positive” cFTS result indicates that the adjusted risk from the ultrasonography and biochemistry is higher than 1 in 300. Most screen-positives are unaffected by aneuploidy, and are therefore false-positives [1].

At present, 1–4% of infants born in all developed countries are conceived through assisted reproductive technologies (ART) [3–5]. In 2011, an estimated 3.8% of all births in Australia involved some form of ART [6]. Multiple studies have analyzed the biochemistry and ultrasonography markers used in cFTS, comparing pregnancies conceived via ART with those conceived spontaneously. The studies have varied in sample size and results, but the majority report lower PAPP-A in pregnancies conceived via ART [7]. The objective of this study is to confirm a difference in the first-trimester screen maternal biochemistry and FPR between pregnancies conceived spontaneously and those conceived via ART in this large Queensland cohort.

Methods

Data related to prenatal screening and diagnosis was collected from three private ultrasound clinics in Queensland, Australia. All centers use a commercial program and database, Viewpoint, (General Electric Healthcare, Waukesha, USA) to generate cFTS fetal aneuploidy risks. The clinic databases were searched to identify singleton pregnancies that had cFTS. The data extracted included: PAPP-A and f β -hCG MoM, the biochemical platform utilized (Brahms Kryptor or Immulite 2000), adjusted risk of Down syndrome, method of

conception, and the maternal characteristics in Table 1. The type of embryo transfer (fresh or frozen-thawed) was noted for IVF and ICSI conceptions.

Multiple pregnancies were excluded due to the complications associated with using biochemistry in their cFTS risk assessment. Any fetuses identified with aneuploidy were excluded, as anomalous biochemistry was likely due to the aneuploidy, rather than method of conception. The ART subtype gamete intrafallopian transfer (GIFT) was excluded due to insufficient numbers.

Statistical analysis

Predictive Analytics Software Statistics (PASW) GradPack 17.0, part of Statistical Package for the Social Sciences (SPSS) Incorporated (Chicago, USA) was used to analyze data. When the *p* value was less than 0.05 (*p* < 0.05), the result was considered to be statistically significant.

Univariate analysis of variance (ANOVA) identified significant differences between the two biochemistry platforms used, namely Immulite 2000 and Brahms Kryptor (*p* < 0.001) with the Immulite 2000 platform having significantly lower MoM for the analytes. Therefore, further analyses were conducted as two separate datasets. The spontaneous pregnancy cohort was used as controls. ANOVA and Spearman’s rank nonparametric correlation analyses were used to compare the mean PAPP-A and f β -hCG levels of the ART fresh and frozen-thawed embryo transfer conceptions. Binary Logistic Regression analysis calculated the odds ratio for a screen-positive result from ART subgroups.

Ethics approval

Approval was granted by the Griffith University Human Research Ethics Committee, protocol number BPS/01/09/HREC.

Results

Seventeen thousand eight hundred and eighty-nine women had cFTS at one of the three clinics between January 2004 and September 2009. Multiple pregnancies, fetuses with aneuploidy, and cases where maternal blood biochemistry was not recorded were excluded. Three conceptions were the results of GIFT and thus had an insufficient sample size to be independently analyzed. The final population analyzed contained 16,363 diploid singleton pregnancies, containing 1543 (9.4%) ART conceptions. The process of identifying the population to be analyzed is shown in Fig. 1.

The only significant maternal demographic difference between ART and spontaneous conceptions was an increase in maternal age (*p* < 0.001). See Table 1.

Table 1 Population demographics

	Spontaneous	ART
<i>n</i>	14,820	1543 (9.4%)
Maternal age (years)	32 (15–50)	34 (20–56) ($p < 0.001$)
Mean (range)		
Maternal weight (kilograms)	67 (36–162)	68 (40–130)
Mean (range)		
Ethnicity (% Caucasian)	87%	91%
Current or personal history of smoking	2.7%	1%

The biochemistry results, produced as MoMs were analyzed via two different laboratory systems: Brahms Kryptor (Brahms AG, Berlin, Germany) $n = 9630$ and Immulite 2000 (Diagnostic Products Corporation, Los Angeles, USA) $n = 6733$. The results produced by the two biochemical analyzers were significantly different ($p < 0.001$).

Of the total 16,363 pregnancies, 891 pregnancies received a screen-positive result, giving a false-positive rate (FPR) of 5.4%. Spontaneous pregnancies had a FPR of 6% with Immulite 2000 and 4.9% with Brahms Kryptor. Comparatively, ART pregnancies had a FPR of 10% with Immulite 2000 and 8% with Brahms Kryptor, both $p < 0.001$ prior to adjusting the data for maternal age. Figure 2 compares the FPRs adjusted for maternal age, for the different methods of conception in the Brahms Kryptor and Immulite 2000 populations. The only significant increase in FPR is in the ICSI cohort, when maternal serum was analyzed on the Brahms Kryptor

platform ($p = 0.012$). When biochemistry was analyzed on the Immulite 2000 platform, there was an increased FPR nearing significance ($p = 0.066$) for the IVF cohort. Frozen-thawed embryo transfers had a significantly increased FPR with the Immulite 2000 population ($p = 0.004$) but not with the Brahms Kryptor population. Fresh embryo transfers had an increased FPR but not to significance for either biochemistry platform.

As shown in Tables 2 and 3, compared to spontaneous controls, significantly lower PAPP-A was seen in the IVF subgroup ($p < 0.001$) for both biochemistry platforms. When non-IVF ART conceptions were compared to controls, PAPP-A was not significantly lower. There was significantly lower PAPP-A in ICSI conceptions ($p = 0.046$) measured by the Brahms Kryptor platform. Regardless of whether the embryo transfer was fresh or frozen-thawed, the PAPP-A was significantly lower ($p \leq 0.005$) for both biochemistry platforms. The Immulite 2000 population had no significant difference in β -hCG levels between ART and spontaneous pregnancies. Brahms Kryptor platform data revealed significantly lower β -hCG in pregnancies conceived via IVF ($p = 0.003$).

Discussion

This large study confirmed significantly lower PAPP-A for pregnancies conceived via IVF, consistent with previous studies. The biochemistry platforms Brahms Kryptor and Immulite

Fig. 1 Process of data exclusion to reach final population of 16,363 diploid singleton pregnancies for analysis

Fig. 2 Using binary logistic regression and adjusting for maternal age, odds ratios were calculated to depict the likelihood of receiving a false-positive result when the pregnancy was conceived via a method of ART,

in comparison to pregnancies conceived spontaneously. Spontaneous controls had an odds ratio of 1. Asterisk indicates statistical significance

2000 are known to have significant differences [8]. In this study PAPP-A is significantly lower ($p < 0.001$) for IVF pregnancies regardless of biochemistry platform, thus providing evidence that the lower PAPP-A in IVF pregnancies has a biological cause.

PAPP-A

Lower PAPP-A in IVF pregnancies compared to spontaneous conceptions is consistent with a majority of studies [7, 9–24], though some found no difference [25–28] and one reported a higher PAPP-A [29]. Significantly lower PAPP-A was also observed in pregnancies conceived via ICSI with the

Brahms Kryptor platform ($p = 0.046$). This supports the findings of other studies [7–9, 15, 16, 18–23]. The ICSI technique adds an insult to the zona pellucida of the oocyte by the microinjection procedure, which is postulated to impact the placental development and in turn the first-trimester biochemistry [7]. The ICSI pregnancies with the Immulite 2000 platform did not have significantly lower PAPP-A. This may be due to an insufficient sample size (Immulite 2000 $n = 35/662$, vs. Brahms Kryptor $n = 75/881$), or perhaps there was no PAPP-A reduction, which has been occasionally reported [10, 26, 27], or the difference may be due to the Immulite 2000 platform itself [8].

Table 2 Immulite 2000: PAPP-A, and $\text{f}\beta\text{-hCG}$ levels compared to spontaneous controls, for ART subgroups in the Immulite 2000 population

		PAPP-A		$\text{f}\beta\text{-hCG}$	
		Mean MoM	<i>p</i> value	Mean MoM	<i>p</i> value
Spontaneous	<i>n</i> = 6071	1.00		1.00	
IVF	<i>n</i> = 489	0.86	<0.001	1.02	0.546
ICSI	<i>n</i> = 35	0.85	0.115	1.03	0.784
IUI	<i>n</i> = 81	0.90	0.126	1.05	0.537
Ovulation drugs	<i>n</i> = 57	0.98	0.810	1.11	0.199
IVF/ICSI embryo transfer					
Fresh	<i>n</i> = 370	0.86	<0.001	0.99	0.725
Frozen-thawed	<i>n</i> = 154	0.86	0.005	1.09	0.092

Table 3 Brahms Kryptor: PAPP-A, and fβ-hCG levels compared to spontaneous controls, for ART subgroups in the Brahms Kryptor population

		PAPP-A		fβ-hCG	
		Mean MoM	<i>p</i> value	Mean MoM	<i>p</i> value
Spontaneous	<i>n</i> = 8749	1.00		1.00	
IVF	<i>n</i> = 600	0.87	<0.001	0.92	0.003
ICSI	<i>n</i> = 75	0.88	0.046	1.02	0.866
IUI	<i>n</i> = 143	0.94	0.214	1.03	0.525
Ovulation drugs	<i>n</i> = 63	1.04	0.447	1.01	0.900
IVF/ICSI embryo transfer					
Fresh	<i>n</i> = 513	0.88	<0.001	0.94	0.052
Frozen-thawed	<i>n</i> = 162	0.86	0.001	0.88	0.024

Both fresh and frozen-thawed embryo transfers, regardless of biochemistry platform, had significantly lower PAPP-A ($p \leq 0.005$). Many studies have reported reduced PAPP-A in fresh embryo transfers [7, 9–23, 30], but less have reported a reduction for frozen–thawed embryos transfers [7, 19]. In fact, some studies reported no reduction in PAPP-A with frozen-thawed embryo transfer [13, 16, 18, 22]; however, this may be due to smaller sample sizes than in the studies by Amor et al. [19], Bellver et al. [7] and this study, which identified lower PAPP-A.

Lower PAPP-A levels are known to be associated with pregnancy complications such as pre-eclampsia and gestational diabetes [31–35] and increased risk for adverse perinatal outcomes such as prematurity, fetal growth restriction, and neonatal death [36–40]. Similar pregnancy and perinatal complications are seen at higher frequencies in ART pregnancies [19, 41–44].

Many theories have been proposed to explain the consistently lower PAPP-A observed in IVF pregnancies. One hypothesis is that the cause is due to dating discrepancies between ART procedural dates and ultrasonography dating by CRL. However, this study uses two different biochemical analyzers each with a different method of calculating gestation. Brahms Kryptor generated International Units which were converted to MoMs after CRL was measured by first-trimester ultrasonography, while Immulite 2000 calculated the PAPP-A MoMs on the basis of the oocyte retrieval dates prior to the ultrasonography. Significantly lower PAPP-A levels were observed in IVF pregnancies in both groups thus suggesting a biological, as opposed to a procedural explanation for the lower PAPP-A.

Several studies suggest that exogenous hormone treatment is the principal cause of lower PAPP-A in IVF/ICSI pregnancies [13, 15, 19, 22, 30]. Hormone stimulation protocols can be highly variable, and therefore difficult to assess, but overall hormone treatment has been shown to have the greatest success rate of live births [45]. Giorgetti et al. [23] reported that an estradiol level of 1300 pg/ml or higher at the time of ovulation

could predict a significantly lower PAPP-A level at the cFTS in IVF/ICSI pregnancies. They hypothesize that an increase in estradiol, which promotes the growth of the endometrium and is a potent vasodilator of uterine arterioles [46], could result in a suboptimal, but functional placenta-endometrial interface, resulting in a lower PAPP-A production. Consistent with this hypothesis, IUI pregnancies that resulted from mild ovarian stimulation (estradiol level < 1300 pg/ml) did not have lower PAPP-A [23]. Indeed, Bellver et al. [7] and this present study also support that lower PAPP-A was not observed with IUI pregnancies. If specific dose levels of hormone treatment are confirmed to be associated with lower PAPP-A, this would aid in identifying subgroups within the ART population which require biochemical correction in the cFTS risk algorithm.

Lower PAPP-A has been reported regardless of whether the cause of infertility was due to the male, female, or both [16, 19]. Subfertility has been suggested as a cause for lower PAPP-A; as couples who took greater than 24 months to conceive spontaneously had lower PAPP-A, on-par with IVF conceptions, compared to a control group who conceived spontaneously within 12 months [47].

fβ-hCG

The Brahms Kryptor population had significantly lower fβ-hCG in the IVF cohort ($p = 0.003$). This has been reported in only two other studies [14, 21]. Reduced fβ-hCG is in contrast to many studies which identified increased fβ-hCG [9, 17, 20, 26, 27, 48, 24]. No difference in fβ-hCG levels was reported with the Immulite 2000 ART subgroups, or for the IUI, ICSI, and ovulation drug cohorts with Brahms Kryptor. Most previous studies have reported no change in fβ-hCG levels [9–16, 18, 19, 22, 25, 27–29]. A review by Gjerris et al. [49] suggested the conflicting results for fβ-hCG may be due to samples sizes, no distinction between complicated and non-complicated pregnancies, no distinction between pregnancies with and without hormone treatment [50], and influences from the biochemical platforms [8].

Low first-trimester f β -hCG levels are associated with fetal growth restriction, gestation hypertension, and gestational diabetes [31]. Significant differences in f β -hCG levels have been reported between two culture media, with no difference for PAPP-A [51], and animal models have shown that the in vitro culture influences both fetal and placental development [52, 53].

False-positive rate

ICSI pregnancies with the Brahms Kryptor platform were associated with a significant increase in FPR ($p = 0.012$), compared to the spontaneous controls. Similarly, the IVF cohort with the Immulite 2000 platform had increased FPR, but not to statistical significance. The increased FPR is due to the elevated f β -hCG to PAPP-A ratio, which is positively correlated with Down syndrome. The IVF cohort with Brahms Kryptor had lower PAPP-A and lower f β -hCG, thus it did not increase the FPR.

Correction factors

Current evidence from a large review [7] suggests the need to adjust the first-trimester PAPP-A marker in singleton IVF/ICSI pregnancies, with non-donor oocyte, when calculating a Down syndrome risk estimate, to prevent a higher FPR in this cohort. No correction is required for pregnancies achieved via IUI, oocyte donation, or frozen-thawed embryo transfer because these groups do not consistently have biochemical changes that adjust the FPR [7]. Engels et al. [21] calculated and validated [50] correction factors for the mode of conception for PAPP-A and f β -hCG in non-complicated singleton pregnancies. The degree of correction is dependent on the local median PAPP-A MoM value. Therefore, each screening center needs to calculate their own correction factor based on their non-complicated ART population with hormone treatment [50]. Appropriate application of correction factors may improve the reliability of pre- and post-test counseling for women who have conceived via IVF/ICSI, reduce the FPR, and lower the uptake of invasive testing.

There are many consequences of a higher than expected screen-positive rate; consideration of additional investigations, risk of fetal loss due to an invasive procedure, and psychological stress [54]. Once a patient receives a screen-positive result, they have the option of a diagnostic test or another screening test, such as non-invasive prenatal screening [55]. Conceiving a pregnancy via ART can be a complicated, time consuming, and costly process; and therefore, these patients are often particularly anxious [56, 57] and risk-adverse when it comes to the consideration of an invasive procedure [54]. Hence, it is important that the FPR for ART conceptions is comparable to control groups.

Brahms Kryptor and Immulite 2000 biochemistry platforms

The need to separate the data based on biochemistry platform reduced the potential power of this study. Previous reports have found significant differences in the detection rates between Immulite 2000 and Brahms Kryptor platforms, which potentially impacts on the clinical performance [8]. Interestingly, the Immulite 2000 assay has not been certified by The Fetal Medicine Foundation for use in the first-trimester risk algorithm, whereas Brahms Kryptor has been certified [58].

Other factors that may confound the difference between the biochemical analyzers are the method of gestational dating and the reference data used by each platform. At the time of this study, Immulite 2000 immediately converted the analyte result to MoM, using the maternal age and weight, and gestational age based on oocyte retrieval provided on the request form for IVF/ICSI pregnancies and used the first day of the last menstrual period for spontaneous pregnancies. The laboratory used its own database, which was local in constitution and smaller in size. When the first-trimester ultrasound scan was performed, so long as the CRL placed the fetal gestation within 2 days of the gestation used to calculate the MoM, no biochemistry recalculation was requested by the clinics in this study. (D. Kanowski, personal communication with M. Peterson). The Brahms Kryptor platform generated results in International Units which were entered into Viewpoint, which calculated the MoM based on the CRL, using the larger international Fetal Medicine Foundation (United Kingdom) reference range [58]. Hence Brahms Kryptor relies upon ultrasonography measurements for gestational age, and Immulite 2000 uses oocyte retrieval dates for IVF/ICSI pregnancies. CRL has been reported to be appropriate to determine gestational age for assisted and spontaneous pregnancies, [59–61] although this is debated [62]. Further research is recommended to investigate these differences.

Limitations of study

This study does not capture all ART pregnancies within Queensland. As no IVF services are currently available in the public sector in Queensland, the vast majority of patients who undergo IVF remain in the private sector for their antenatal care once a pregnancy has been achieved. Combining public and private sector data would provide a more complete analysis of the Queensland population.

All data received from the three clinics were de-identified, so any missing data could not be retrieved. Missing data may have been due to information withheld by the patient, data not recorded by the clinic or incorrect data entry.

Conclusions

The Immulite 2000 and Brahms Kryptor platforms produce significantly different results. Further research is required to determine whether there is an optimal platform, reference database, and/or method of determining gestational age which maximizes screening sensitivity without increasing the FPR. However, irrespective of their significant differences, the Brahms Kryptor and Immulite 2000 platforms both reported significantly lower PAPP-A levels for the IVF pregnancies they analyzed, which support the hypothesis that the lower PAPP-A is biological in origin.

Having significantly lower PAPP-A levels at the cFTS compared to spontaneous controls, puts these pregnancies at risk of having an increased FPR, as seen in the ICSI cohort with the Brahms Kryptor platform. Our results confirm the findings of other Australian and international studies for the population of Queensland, Australia, and will contribute to the growing body of evidence for appropriate risk-algorithm adjustments. These results may influence the counseling of IVF/ICSI patients, acknowledging the challenges of biochemistry data and value of detailed ultrasonography, and non-invasive prenatal screening in ascertaining fetal aneuploidy risk.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

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