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ORIGINAL ARTICLE *Early pregnancy*

Multivariate analysis identifies the estradiol level at ovulation triggering as an independent predictor of the first trimester pregnancy-associated plasma protein-A level in IVF/ICSI pregnancies

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STUDY QUESTION: Can independent predictors of pregnancy-associated plasma protein-A (PAPP-A) levels be identified in a group of women who conceived following IVF/ICSI?

SUMMARY ANSWER: The significantly decreased PAPP-A level in IVF and ICSI pregnancies compared with non-IVF/ICSI pregnancies was correlated strongly with the serum estradiol (E_2) level at ovulation triggering.

WHAT IS KNOWN ALREADY: The first trimester prenatal combined screening test for fetal aneuploidies in pregnancies conceived following assisted reproduction techniques (ART) is complicated by an alteration of the maternal biomarkers free β–hCG and PAPP-A, causing a higher false-positive rate compared with pregnancies which are conceived naturally. The use of controlled ovarian stimulation prior to IVF/ICSI is suggested to be the principle reason for these alterations of biomarkers in ART pregnancies.

STUDY DESIGN, SIZE, DURATION: Between January 2010 and December 2011, 1474 women who conceived naturally and 374 women who conceived following IVF ($n = 89$), ICSI ($n = 204$) or intrauterine insemination (IUI, $n = 81$) were included in this retrospective study. Only singleton pregnancies were eligible for this study. For all women, serum analysis was performed in the same clinical laboratory. Measurement of nuchal translucency (NT) thickness was performed by four physicians belonging to the same infertility centre.

PARTICIPANTS/MATERIALS, SETTING, METHODS: First-trimester combined screening test of aneuploidy parameters (maternal age, PAPP-A and free β–hCG, NT thickness) were compared between non-ART and ART (IVF, ICSI and IUI) singleton pregnancies. Next, a minimal threshold E_2 level at ovulation triggering was suggested for IVF/ICSI pregnancies above which the PAPP-A levels were significantly decreased compared with non-ART pregnancies. Finally, a multivariate analysis was performed to reveal independent predictors of PAPP-A level in IVF/ICSI pregnancies.

MAIN RESULTS AND THE ROLE OF CHANCE: We showed a decrease of the multiple of the median (MoM) PAPP-A level in IVF and ICSI singleton pregnancies compared with non-ART singleton pregnancies ($P < 0.001$), with MoM values of 0.74 (0.16–3.16) and 0.81 (0.12–4.61) versus 0.98 (0.14–5.76), respectively. Analysis of variance of the overall model was highly significant (Fisher test 3.76, $P = 0.01$), indicating that the model explains a significant portion of the variation in the data. No difference in PAPP-A level was found between non-ART and IUI pregnancies. The free β–hCG level and NT thickness did not differ between ART and non-ART pregnancies. PAPP-A levels in IVF and ICSI pregnancies were strongly correlated with the E_2 level at ovulation triggering. We showed by multivariate analysis that an E_2 cut-off level of 1300 pg/ml at the time of ovulation could predict a significantly lower PAPP-A level at first trimester combined screening ($\beta -0.239 \pm 0.088$, $P < 0.005$).

LIMITATION, REASONS FOR CAUTION: The measures of biochemical markers can differ between laboratories and with the used equipment; therefore, extrapolation of the E_2 cut-off level to other infertility centres should be undertaken with caution.

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WIDER IMPLICATIONS OF THE FINDINGS: One should be careful when using correction factors for ART patients undergoing the first trimester combined screening test. The proposed E_2 cut-off level may help to identify a subgroup of women within the population of ART patients for whom use of a correction factor is justified.

STUDY FUNDING/COMPETING INTEREST(S): None.

Key words: pregnancy-associated plasma protein-A / estradiol / first trimester aneuploidy screening / assisted reproduction techniques

Introduction

In most developed countries, the use of a non-invasive combined prenatal screening test for aneuploidies (especially Down syndrome) has become routine practice in the first trimester of pregnancy, including in women who conceived following assisted reproduction techniques (ART). A combination of clinical data (maternal age), biochemical data [maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free β−hCG] and fetal ultrasound nuchal translucency (NT) thickness at 11^{+0}–13^{+6} weeks gestation are used to calculate the risk of fetal aneuploidy. This so-called first trimester combined test identifies 85–95% of trisomy 21 pregnancies and carries a 5% false-positive rate using a risk cut-off of 1/300 (Nicolaides, 2011). Subsequently, amniocentesis or chorionic villous sampling is offered to the women identified as being at high risk of carrying a fetus with chromosomal aberrations. Since June 2009, a French law defined this risk to be 1/250 or higher.

First trimester prenatal screening in pregnancies conceived following ART is complicated by changes in the levels of some biomarkers, causing a higher false-positive rate than in naturally conceived pregnancies, which leads to an increase in the use of invasive diagnosis and unnecessary stress (Amor *et al.*, 2009). For example, a decreased serum level of PAPP-A in pregnancies conceived following IVF and ICSI compared with naturally conceived pregnancies is the most common finding of many reports (Liao *et al.*, 2001; Hui *et al.*, 2005a; Tul and Novak-Antolic, 2006; Anckaert *et al.*, 2008; Kagan *et al.*, 2008; Amor *et al.*, 2009; Gjerris *et al.*, 2009a,b; Bender *et al.*, 2010; Engels *et al.*, 2010; Matilainen *et al.*, 2011). Also, an increase in free β−hCG levels in ART pregnancies has been observed several times (Liao *et al.*, 2001; Ghisoni *et al.*, 2003; Kagan *et al.*, 2008; Bender *et al.*, 2010). Conflicting results have been reported regarding the NT values in ART pregnancies compared with non-ART pregnancies. The majority of the reports found no difference in the mean NT thickness (Liao *et al.*, 2001; Amor *et al.*, 2009; Bender *et al.*, 2010; Matilainen *et al.*, 2011), whereas some studies found thicker NT among IVF and ICSI fetuses (Maymon and Shulman, 2004; Hui *et al.*, 2005b) or even thinner NT in IVF pregnancies (Gjerris *et al.*, 2009a,b; Engels *et al.*, 2010). As a consequence, the false-positive rates for first-trimester combined screening in ART pregnancies have gone up from 5 to 15%, even after adjustment for maternal age or after the use of age-matched controls (Amor *et al.*, 2009; Gjerris *et al.*, 2009a,b; Engels *et al.*, 2010). The principal reason for the alterations of biomarkers in ART pregnancies has been identified as the use of controlled ovarian stimulation (COS) prior to IVF/ICSI. It has been suggested that COS results in marked endocrine changes related to the maturation of multiple follicles and thus the development of multiple corpora lutea (Amor *et al.*, 2009). Moreover, Tul and Novak-Antolic (2006) reported an inverse relationship between the number of aspirated oocytes and the PAPP-A values at first trimester combined screening.

Bearing in mind that ART may account for up to 7% of all births in developed countries (Skakkebaek *et al.*, 2006), many studies have investigated the influence of the ART treatment on the first trimester combined test. This resulted in the development of correction factors related to the mode of conception. Nevertheless, as the etiology of infertility, the treatment protocol and the response to COS may cause different changes in the screening parameters, caution is appropriate when dealing with women who conceived following ART.

The aim of the current study was to compare the first trimester biochemical maternal serum and fetal ultrasound measurements in singleton pregnancies conceived following intrauterine insemination (IUI), IVF and ICSI with naturally conceived singleton pregnancies. Secondly, we determined a cut-off serum estradiol (E_2) level above which the PAPP-A levels were decreased and, finally, independent predictors of the PAPP-A level were explored by multivariate analysis.

Materials and Methods

The Institute of Reproductive Medicine (IMR) of Marseille is a center specialized in the treatment of human subfertility. Additionally, the institute offers first trimester prenatal screening for fetal chromosomal abnormalities to all pregnant women, no matter whether they conceived following ART or naturally. The IMR is connected to the Alphabio lab, which provides the biochemistry analyses for PAPP-A and free β−hCG. From January 2010, the first trimester combined screening was introduced in the center, replacing second trimester sequential screening of aneuploidies. From then until December 2011, all first trimester combined screening test results ($N = 6238$) in singleton pregnancies were collected. All patients with an ART treatment and/or a pregnancy follow-up in a different center were excluded to restrict interobserver variation. In 1848 women, the NT measurement was performed in the IMR facility and only these women were included in the study. Of the 1848 included women, 1474 (79.8%) conceived naturally (non-ART group) and 374 conceived following ART: 81 (4.4%), 89 (4.8%) and 204 (11.0%) following IUI, IVF and ICSI, respectively. Biochemistry analyses were performed on the same day as the NT measurement or 1–2 days later. The combined risk was calculated on the day of biochemistry analysis. Patients with a high risk of aneuploidy were offered an amniocentesis or chorionic villous sampling. Karyotyping following amniocentesis or chorionic villous sampling was performed using conventional RGH banding method.

Fetal NT and crown–rump length (CRL) ultrasound was performed by a group of four physicians and measured according to The Fetal Medicine Foundation guidelines. Briefly, the maximum thickness of the subcutaneous translucency between the skin and the soft tissue overlying the cervical spine was measured in the sagittal section of the fetus lying in the neutral position.

Concentrations of PAPP-A and free β−hCG were measured in serum using an Immulite 2000® analyzer (Siemens Healthcare Diagnostic, Saint Denis, France) at the Alphabio lab. Immulite 2000® PAPP-A is a solid phase, enzyme-labeled chemiluminescent immunometric assay and Immulite 2000® free β−hCG is a solid phase, two site sequential chemiluminescent

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immunometric assay. The concentration of E_2 in serum in ART patients at the time of ovulation triggering was measured at the Alphabio lab using a Centaur XP® analyzer and a direct competitive immunoassay (Siemens Healthcare Diagnostic, Saint Denis, France). Coefficients of variation (CV) were evaluated by testing six different serum levels for PAPP-A and free β–hCG and five levels for E_2. Samples were assayed in duplicate over the course of several days for a total of 80, 80 and 120 replicates for PAPP-A, free β–hCG and E_2, respectively. Ranges of CV were 3.5–12.0, 6.5–11.3 and 6.7–13.6% for PAPP-A, free β–hCG and E_2, respectively. Biochemical parameters (free β–hCG and PAPP-A) as well as the NT thickness were expressed as multiple of median (MoM). MoM values of biochemical parameters were calculated using Prisca V.4.0 (Siemens Healthcare Diagnostics) software following correction for maternal weight, smoking status and ethnic origin as follows: corrected MoM for weight = $1/(a + b/[\text{weight in kg}]) \times$ MoM ($a = -0.19119$ and $b = 74.646$ for PAPP-A and a = 0.67272 and b = 19.654 for free β–hCG), corrected MoM for smoking status = $(1/K) \times$ MoM ($K = 0.85$ for PAPP-A and K = 1.0 for free β–hCG), corrected MoM for ethnic origin = (1/K) x MoM (for PAPP-A, K = 1.00, 1.57, 1.17 and for free β–hCG, K = 1.00, 1.21, 1.04 for Caucasian, Afro-caribbean and Asian descent, respectively) (Spencer, 1998; De Graaf *et al.*, 1999; Spencer *et al.*, 2000). Patient-specific combined risk was calculated using the Prisca V.4.0 software, including maternal age, CRL, NT, PAPP-A and free β–hCG levels. Fertility history, demographic data, NT thickness, biochemistry results and patient-specific risks were entered into a database at the time of assessment.

The practice of first trimester screening has been authorized in France since January 2010. The overall medians used by Prisca V.4.0 are those derived from data from Belgian laboratories. The medians for free β–hCG were established based on 3417, 5394 and 2575 tests performed in the 11th, 12th and 13th week of pregnancy, respectively, whereas the medians for PAPP-A were based on 4369, 6783 and 3181 tests, respectively. The accordance with our own observed values is verified periodically.

Statistical analysis

MoM values from PAPP-A, free β–hCG and NT levels were compared using one-way analysis of variance followed by pairwise comparisons using the non-parametric Mann–Whitney *U*-test. Chi-square test was used to compare between groups the proportion of women aged ≥35 years and the proportion of women having a risk estimate of >1/250. A step-by-step calculation by increasing the E_2 level was performed to set a cut-off level for E_2 at the moment of ovulation triggering, which could predict decreased PAPP-A levels at first trimester screening.

Univariate and multivariate analyses were performed to determine independent predictors of MoM PAPP-A. In the first step, an univariate analysis was performed to select predictors of MoM PAPP-A to be included in the multivariate analysis. The parameters included in the univariate analysis were age of the women, ART indication (male factor, tubal factor, polycystic ovary syndrome, endometriosis, low ovarian reserve or unexplained subfertility), type of down-regulation (GnRH agonist or antagonist), type of gonadotrophin (recombinant FSH (rFSH) or hMG), total dose of gonadotrophin administered, E_2 on the day of ovulation triggering (≥1300 pg/ml or <1300 pg/ml), type of ovulation triggering (hCG 10 000 IU or choriogonadotrophin alfa 250 μg), number of oocytes retrieved, number of metaphase II oocytes, fertilization technique (IVF or ICSI), number and embryo quality score (Giorgetti *et al.*, 1995) of transferred embryos and transfer day (Day 2 or Day 5), MoM free β–hCG. *P*-values below 0.20 following the univariate analysis were considered significant. In the second step, a multivariate analysis was performed. Only significant predictors obtained in the univariate analysis were subsequently included in the multivariate analysis. Fisher *F*-test was used to determine the consistency of the model. Student *T*-test was used to test the relationship between predictors and MoM PAPP-A. *P*-values below 0.05 were considered statistically significant. All calculations were performed using SAS 9.1.3 SP4 Software (SAS® Institute, Cary, NC, USA) or SPSS 20.0 software (SPSS®, IBM corp., NY, USA).

Results

In the ART group, 81 women conceived following IUI, whereas 89 and 204 women conceived following IVF and ICSI respectively. COS in IVF/ICSI patients was achieved following either a GnRH-agonist ($n = 194/293$; 66.2%) or -antagonist ($n = 99/293$; 33.8%) down-regulation combined with either rFSH ($n = 215/293$; 73.4%) or highly purified hMG ($n = 78/293$; 26.6%). Recombinant ($n = 240/293$; 81.9%) or urinary hCG ($n = 53/293$; 18.1%) was administered once three follicles reached a mean diameter ≥18 mm. Oocyte retrieval was performed 35–36 h after ovulation triggering. In the IUI patients, low doses of the same exogenous gonadotrophins were used for mild ovarian stimulation. The non-ART group consisted of 1474 women who conceived naturally. Baseline characteristics of the study population are presented in Table I. Women in the ART groups were older compared with women in the non-ART group, with a significantly higher proportion of women aged ≥35 year in the IVF/ICSI group compared with the non-ART group ($P < 0.001$). The maternal weight in the group of women who conceived following IUI was higher compared with the non-ART group ($P < 0.05$).

Table I Baseline characteristics of women in the study population.

	Non-ART $n = 1474$	IVF/ICSI $n = 293$	IUI $n = 81$
Maternal age (years)	31.5 (18.1–46.1)*#	34.4 (21.7–43.6)*	32.7 (24.3–41.8)#
Maternal age ≥35 years	370 (25.1)$‡	129 (44.0)$	23 (28.4)‡
Maternal weight (kg)	60.0 (39.0–130.0)#	61.0 (36.0–112.0)	63.0 (45.0–130.0)#
CRL at NT scan (mm)	61.0 (45.0–83.4)^	63.8 (45.0–83.0)^	61.2 (47.2–82.0)
GA at NT measurement (days)	87.0 (78–97)^	88.0 (78–97)^	87.0 (79–97)
GA at blood sampling (days)	87.0 (78–94)^	88.0 (78–94)^	87.0 (80–94)

CRL, crown-rump length; NT, nuchal translucency; GA, gestational ages.
Values are given as median (range) or *n* (%).
*$P < 0.001$ and #$P < 0.05$ for the assisted reproduction techniques (ART) groups compared with the non-ART group (Mann–Whitney *U*-test).
$$P < 0.001$ and ‡$P < 0.05$ for the IVF/ICSI and intrauterine insemination (IUI) group compared with the non-ART group (χ^2 test).
^$P < 0.001$ for the IVF/ICSI group compared with the non-ART group (Mann–Whitney *U*-test).

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Table II Effect of ART on first trimester screening parameters and risk estimation.

	Non-ART $n = 1474$	ICSI $n = 204$	IVF $n = 89$	IUI $n = 81$
Median PAPP-A (MoM)	0.98* (0.14–5.76)	0.81* (0.12–4.61)	0.74* (0.16–3.16)	1.00 (0.26–3.74)
Median free β–hCG (MoM)	0.97 (0.18–6.55)	1.09 (0.22–5.90)	0.96 (0.34–5.03)	0.99 (0.29–6.55)
Median NT (MoM)	1.02 (0.45–5.31)	1.06 (0.37–6.29)	1.02 (0.50–6.33)	1.02 (0.57–1.94)
Increased risk >1/250 (%)	6.8#^	16.2#	15.7^	3.7

PAPP-A, pregnancy-associated plasma protein-A; NT, nuchal translucency.
Values are given as multiple of the median (MoM) (min–max).
*$P < 0.001$ for ICSI and IVF groups versus the non-ART group (Mann–Whitney *U*-test).
#$P < 0.001$ and ^$P < 0.01$ for ICSI and IVF groups versus the non-ART group, respectively (χ^2).

Almost all of the included women (98.6%) were of Caucasian descent. The CRL and gestational age (GA) at NT measurement and the GA at blood sampling were significantly higher for the IVF/ICSI than the non-ART pregnancies ($P < 0.001$), whereas this was not the case for pregnancies conceived following IUI.

The PAPP-A levels were significantly lower in the IVF and ICSI pregnancies compared with the non-ART pregnancies ($P < 0.001$) (Table II). The PAPP-A levels in pregnancies conceived following IUI did not differ significantly from the PAPP-A levels in non-ART pregnancies ($P = 0.557$). No difference in the NT was seen comparing non-ART and ART pregnancies ($P = 0.272$). Also the free β–hCG level did not differ between the study groups ($P = 0.157$). Significantly more women in the IVF and ICSI group had a risk estimation >1/250 compared with the women in the non-ART group (15.7 and 16.2% versus 6.8%, respectively). After matching for age (Table III), the PAPP-A levels in the IVF and ICSI group remained significantly lower compared with the non-ART group. Also, the number of women with a risk estimation >1/250 tended to be higher, although this was not significant, in the IVF and ICSI group compared with the non-ART group (Table III). For the IVF/ICSI pregnancies, the outcome was available. A risk estimation of >1/250 was found in 16% (47/293) of the IVF/ICSI pregnancies. Karyotyping results were: euploidy ($n = 35$), trisomy 13 ($n = 3$), trisomy 21 ($n = 2$), microdeletion 13q: (46,XX,t(6;7)(q21;q32)) ($n = 1$) and mosaicism (mos46,X,der(Y)[5]/46,XY[25]) ($n = 1$). Except for the pregnancy in which a mosaic fetus was detected, the other pregnancies with an aneuploid fetus were terminated. Among the 35 euploid fetuses, one pregnancy was interrupted because of a cystic hygroma and severe facial dysmorphy seen on ultrasound. Four women with an estimated risk >1/250 refused to undergo chorionic villous sampling or amniocentesis, and in one other woman invasive testing was not performed because of an increased risk of fetal loss (diagnosis of partial detachment of the placenta). From the 246 pregnancies with a risk estimation <1/250, the outcome could be retrieved for all but two patients (99.3%). Two-hundred forty of these pregnancies led to the birth of an euploid child, and three women miscarried in the second trimester of pregnancy. One pregnancy was interrupted following the diagnosis of severe malformations on ultrasound (euploid fetus born).

As the level of PAPP-A was correlated previously to the number of aspirated oocytes by Tul and Novak-Antolic (2006), we investigated whether the E_2 level at time of ovulation triggering (linked with the number of follicles and oocytes) could be a predictor of the PAPP-A level at first trimester combined screening. At ovulation triggering the median E_2 level of women who conceived following IVF/ICSI was 2000 pg/ml (135–6255 pg/ml), whereas for the women who conceived following IUI this was 370 pg/ml (108–1149 pg/ml). In IVF/ICSI cycles, the median E_2 level at ovulation triggering following agonist down-regulation was significantly higher compared with antagonist down-regulation: 2335 pg/ml (256–6255 pg/ml) versus 1569 pg/ml (135–3999 pg/ml), respectively ($P < 0.0001$). There was no difference in E_2 level following the administration of either rFSH or hMG for ovarian stimulation. The cut-off E_2 level above which a decreased PAPP-A level could be expected was calculated to be 1300 pg/ml. Sixty-four of the women who conceived following IVF/ICSI (21.8%) had an E_2 level below 1300 pg/ml at ovulation triggering and a median MoM PAPP-A level of 0.96 (0.23–3.31), whereas the remaining 229 (78.2%) women who conceived following IVF/ICSI had an E_2 level of ≥1300 pg/ml and a median MoM PAPP-A level of 0.73 (0.12–4.61). The PAPP-A level in the group with high E_2 levels at ovulation triggering (≥1300 pg/ml) was significantly lower than in the group of women with low E_2 levels (<1300 pg/ml) ($P < 0.01$, Fig. 1).

Finally, a multivariate analysis was performed to determine independent predictors of MoM PAPP-A levels in the IVF and ICSI pregnancies ($n = 293$) with the set of parameters that met the significance criterion following univariate analysis: free β–hCG, E_2 and progesterone levels at ovulation triggering, number and score of transferred embryos and transfer day. E_2 level at ovulation triggering and MoM free β–hCG level were independent predictors of the PAPP-A level at first trimester screening (model estimated values ± SD: −0.239 ± 0.088 ($P < 0.01$)

Table III Effect of ART on first trimester screening parameters and risk estimation after adjustment for maternal age.

	Non-ART $n = 293$	IVF/ICSI $n = 293$
PAPP-A (MoM)	0.97*	0.77*
NT (MoM)	1.00	1.04
free β–hCG (MoM)	0.97	1.04
Increased risk >1/250 (%)	11.6	16.0

PAPP-A, pregnancy-associated plasma protein-A; NT, nuchal translucency; MoM, multiple of the medium.
*$P < 0.001$.

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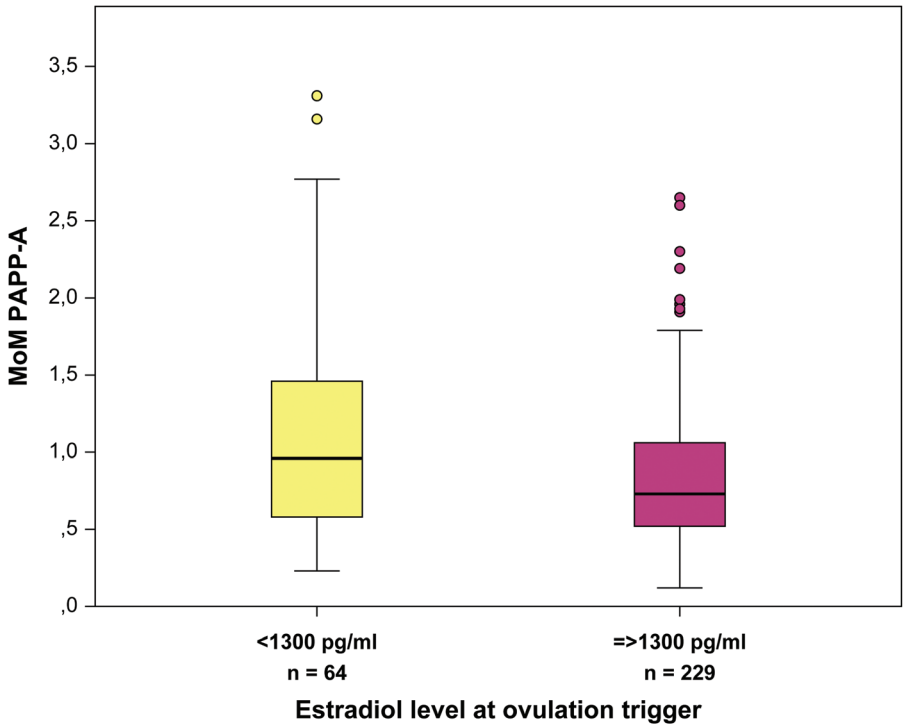

Figure 1 Multiple of the median PAPP-A levels according to the estradiol cut-off. Box-and-Whisker Plot representing MoM PAPP-A levels according to the estradiol cut-off level of 1300 pg/ml. The bottom and the top of the box represent the 25th and 75th percentiles of the MoM levels. The band within the box represents the median. The ends of the whiskers represent the 5th and 95th percentiles of the MoM levels. The dots represent outliers. PAPP-A, pregnancy-associated plasma protein-A; MoM, multiple of the medium.

Table IV Multivariate analysis to determine independent predictors of PAPP-A level in IVF/ICSI pregnancies.

Parameter	Beta	SD	*P*-value
MoM free β–hCG	0.104	0.037	0.005
Estradiol level at ovulation triggering	−0.239	0.088	0.007
Progesterone level at ovulation triggering	−0.022	0.074	0.767
Number of transferred embryos	−0.051	0.058	0.374
Score of transferred embryos	−0.020	0.051	0.691
Transfer day	0.070	0.048	0.149

PAPP-A, pregnancy-associated plasma protein-A; MoM, multiple of the medium.

and 0.104 ± 0.037 ($P < 0.01$), respectively) (Table IV). No other parameter was significantly correlated with the PAPP-A level at first trimester screening.

Discussion

This study confirmed significantly lower first trimester PAPP-A levels in IVF and ICSI singleton pregnancies compared with naturally conceived singleton pregnancies, whereas the PAPP-A levels following IUI did not differ from non-ART pregnancies. On the other hand, free β–hCG levels and NT thickness were similar between ART and non-ART pregnancies. Nevertheless, significantly more women who conceived following IVF/ICSI had an increased first trimester combined screening risk estimation. In this study, a broad range of parameters known to be linked with ART treatment were correlated via multivariate analysis with PAPP-A levels at first trimester combined aneuploidy screening. Our data show that maternal age and eleven other ART parameters do not influence the PAPP-A levels in women who conceived following IVF or ICSI. Few reports have evaluated the influence of different ART parameters on PAPP-A levels, and if they did, solely 1 or 2 parameters were investigated. Anckaert *et al.* (2008) and Amor *et al.* (2009) found no correlation between the PAPP-A levels at first trimester combined screening and the aetiology of infertility, which we have confirmed. It has been shown that the PAPP-A levels decrease with increasing number of retrieved oocytes but this was not confirmed in this study.

The decreased PAPP-A levels found in our cohort of IVF/ICSI patients is in agreement with several other studies (Liao *et al.*, 2001; Hui *et al.*, 2005a; Tul and Novak-Antolic, 2006; Anckaert *et al.*, 2008; Amor *et al.*, 2009; Gjerris *et al.*, 2009a,b; Bender *et al.*, 2010; Engels *et al.*, 2010; Matilainen *et al.*, 2011). Kagan *et al.* (2008) detected a 10% lower PAPP-A level in a large cohort of 2115 women who conceived following IVF. Furthermore, these authors showed that erroneous risks would also be given to women who smoke because the associated decrease in serum PAPP-A could be misinterpreted as an increased risk

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for trisomy 21 and therefore lead to a significant increase in false-positive rate. Hence, the MoM values considered in the current study were also corrected for smoking status, next to maternal weight and ethnic origin. Kagan *et al.* (2008) also detected a 9% rise in free β–hCG in women who conceived by IVF (Kagan *et al.*, 2008), however this was not confirmed in the current cohort.

We found that decreased PAPP-A levels following IVF or ICSI were strongly correlated with the E_2 level at ovulation triggering. Furthermore, we demonstrated that an E_2 level equal to or above 1300 pg/ml at the time of ovulation triggering could predict a decreased PAPP-A level at first trimester combined screening test. This confirms the hypothesis by Tul and Novak-Antolic (2006) and others that exogenous hormone treatment is the principal cause of reduced PAPP-A in IVF or ICSI pregnancies (Bersinger *et al.*, 2004; Hui *et al.*, 2005a; Tul and Novak-Antolic, 2006; Amor *et al.*, 2009). Conversely, in all our included women who conceived following IUI treatment using mild ovarian stimulation, the E_2 levels at ovulation triggering were below the 1300 pg/ml threshold. Moreover, no decrease in PAPP-A levels were found in this study in women who conceived via IUI, which supports the concept of exogenous hormones being the cause of decreased PAPP-A levels in pregnancies following ART (Bersinger *et al.*, 2004; Hui *et al.*, 2005a; Tul and Novak-Antolic, 2006; Amor *et al.*, 2009). In this cohort, an unexpected positive reciprocity was observed between the MoM free β–hCG and the MoM PAPP-A levels. However, the coefficient beta associated with the MoM free β–hCG is low. Therefore, it should be interesting to explore this finding in an independent validation cohort.

During pregnancy, PAPP-A is produced by placental trophoblasts and decidualized endometrial stromal cells at the placenta-endometrium interface (Giudice *et al.*, 2002). The influence of the E_2 level at ovulation triggering on PAPP-A levels can be explained by the fact that E_2 promotes the growth of the endometrium and is a potent vasodilator of uterine arterioles (Chen *et al.*, 2011). It has been shown that high local concentrations of E_2 in the placenta could cause a down-regulation in E_2 receptor expression (Billiar *et al.*, 1997; Bukovky *et al.*, 2003). We hypothesize that an increased E_2 level at ovulation triggering could result in a suboptimal but functional placenta-endometrial interface, resulting in a decreased PAPP-A production.

Despite the fact that inter-observer variation was not investigated, one of the strengths of this study is the fact that NT was measured by four experienced physicians according to The Fetal Medicine Foundation guidelines. In addition, to avoid missing data, we excluded the women who received their ART treatment elsewhere or whose pregnancy follow-up (including NT thickness measurement) was carried out in another center. Another merit of this study is the multivariate analysis, which allowed us to identify the importance of the E_2 level at ovulation triggering.

It is known that the measurement of biochemical markers can differ slightly between clinical laboratories and with the particular equipment used (Reinsberg *et al.*, 2009). Therefore, extrapolation of the E_2 cut-off level to other facilities should be done with caution. Ideally, each facility should define its own E_2 cut-off level in order to refine its first trimester combination screening. However, one should be cautious when using correction factors for all ART patients undergoing first trimester combined screening. The proposed E_2 cut-off level can help to identify a subgroup of women for whom correcting is justified within the population of ART patients. Also to bear in mind is the likely influence of particular ovarian hyperstimulation protocols on the E_2 levels. In our cohort, the E_2 levels at ovulation triggering following GnRH-agonist down-regulation differed significantly from those following GnRH-antagonist down-regulation.

To our knowledge, this is the first study identifying the E_2 level at ovulation triggering in a multivariate analysis as an independent predictor of the MoM PAPP-A levels in IVF and ICSI pregnancies. An E_2 cut-off level defining the need to use a correction factor for the first trimester combination screening test has been suggested. However, more prospective studies are needed to establish the optimal correction algorithm for ART patients.

Authors' roles

C.G. designed the study, collected and analyzed the data and revised the manuscript; C.D. and F.V.M. analyzed the data and wrote the manuscript; G.P. performed the statistical analyses; E.Q. performed NT measurements; V.C.O. revised the manuscript; O.S. performed the laboratory analyses (biochemistry); D.H. collected and analysed karyotype results and revised the manuscript; P.D.S. revised the manuscript and approved the final draft.

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Conflict of interest

None declared.

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