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First-trimester serum marker distribution in singleton pregnancies conceived with assisted reproduction

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Abstract

Objective: To evaluate fβ-hCG, PAPP-A and NT thickness in ART pregnancies.

Methods: From a database of 14645 first-trimester combined tests 203 IVF and 192 ICSI cases were selected and matched to 1164 controls for gestational age at sampling, maternal age .

Results: Group characteristics were similar except for maternal weight (higher in the ICSI group). In the IVF group and ICSI group lnPAPP-A was lower (6.74 vs 7.08; P=0.0001; 6.59 vs 7.07; P=0.0001). Lnfβ-hCG and lnNT were lower in the IVF group (3.75 vs 3.90; P=0.005 and 0.236 vs 0.284; P=0.02). Linear regression analysis showed no significant effect of maternal weight on the different parameters in the ICSI group. The computed correction factors for PAPP-A and fβ-hCG were 1,28 and 1,11 for the IVF group and 1,19 and 1,04 for the ICSI group.

The false-positive rate (FPR) in the IVF and ICSI group was higher (10,3% vs 8,6% and 10,9% vs 7.5%). In the overall age-biased group (maternal age significantly lower compared to all ART and control groups) the FPR is 6,8%.

Conclusion: The increase in FPR in the ART groups can be explained by decreased PAPP-A values. Therefore an adjustment in risk analysis for Down syndrome is suggested.

Introduction

Since January 2007 pregnant women in the Netherlands are offered prenatal screening for Down syndrome between the 9th and 14th week of gestation as part of population screening. Combining maternal age, fetal nuchal translucency (NT) thickness and concentrations of maternal serum free β-human chorionic gonadotrophin (fβ-hCG) and pregnancy-associated plasma protein-A (PAPP-A) can identify about 85-90 percent of the affected pregnancies for a screen positive rate of 5 percent (Spencer *et al.,* 1999*;* Wapner R *et al.*, 2003; Nicolaides, 2004; Wald *et al.* 2005; Go *et al.,* 2005; Kagan *et al.* 2008). Conditions as diabetes mellitus, multiple gestation, ethnicity, maternal weight and smoking affect the concentrations of the serum markers (De Graaf *et al.,* 2000; Kagan *et al.* 2008). There is also evidence that in pregnancies conceived with assisted reproduction techniques (ART), like in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), first-trimester parameters differ from spontaneously conceived pregnancies.

Most studies show lower PAPP-A levels in IVF pregnancies with unaltered levels of fβ-HCG (Orlandi *et al*., 2002; Bersinger *et al*., 2004; Maymon and Shulman, 2004; Hui *et al*., 2005; Tul and Novak-Antolič, 2006; Gjerris *et al.*, 2009). Other studies show decreased PAPP-A levels and higher fβ-HCG levels (Liao *et al*., 2001; Ghisoni *et al*., 2003) or lower fβ-HCG levels (Hui *et al*., 2005a) and some show no changes in maternal serum values in IVF pregnancies (Wøjdemann *et al*., 2001; Bellver *et al*., 2005; Lambert-Messerlian *et al*., 2006). In ICSI pregnancies a decrease in PAPP-A levels has been shown with unaltered levels of fβ-HCG (Liao *et a*l., 2001; Hui *et al*., 2005; Tul and Novak-Antolič, 2006; Gjerris *et al.*, 2009). One study showed only increased levels of fβ-HCG (Ghisoni *et al*., 2003) and other studies found no changes in maternal serum values in ICSI pregnancies (Orlandi *et al*., 2002; Bersinger *et al.*, 2004; Bellver *et al*., 2005). NT thickness does not seem to be affected by assisted reproduction technologies. (Maymon *et al*., 1999; Liao *et al*., 2001; Wøjdemann *et a*l., 2001; Ghisoni *et al*., 2003). However, some studies did find an increased NT thickness at IVF or ICSI pregnancies (Lai *et al*., 2003; Maymon and Shulman, 2004; Hui *et al*., 2005b) or a decreased NT thickness (Gjerris *et al*., 2003).

It is suggested that first-trimester screening for Down syndrome in ART pregnancies results in a higher false-positive rate (FPR) than in spontaneously conceived pregnancies. Whether the prevalence of fetal chromosomal abnormalities in ART pregnancies is different from naturally conceived pregnancies is however unclear. Bettio *et al.* reported no higher prevalence for chromosomal abnormalities whereas other studies showed a higher prevalence in ART pregnancies mainly related to a higher number of sex chromosome aneuploïdies (Koulischer *et al.*, 1997; Aboulghar et al., 2001; Bonduelle et al., 2002, Allen *et al*. 2006). A higher FPR is unfavourable because of the increase in invasive diagnostic testing with a iatrogenic fetal loss rate (Evans and Andriole, 2008). To improve the accuracy of the screening applying corrections for serum markers in ART pregnancies might be valuable. In the Netherlands only adjustment criteria for smoking and maternal weight are used in the first trimester risk assessment for Down syndrome.

This study aims at the comparison of NT thickness, fβ-hCG and PAPP-A levels in IVF and ICSI pregnancies versus spontaneously conceived pregnancies in order to evaluate whether adjustments in the risk analysis are necessary.

Material and methods

Down syndrome screening

In this retrospective study data were used collected in the VU University medical center (Amsterdam, the Netherlands) on the first trimester screening program for Down syndrome performed in the period from January 01, 2004 until December 31, 2007 in the province North-Holland in the Netherlands. The screening was performed at 9-14 weeks of gestation using maternal age, fetal nuchal translucency thickness, and maternal serum concentrations of fβ-hCG and PAPP-A (combined test) for risk calculation. In all cases serum was sampled at 9 – 14 weeks of gestation and the serum markers fβ-hCG and PAPP-A were analyzed at the endocrine laboratory of the VUmc, using the Delfia Xpress (Perkin Elmer Wallac Oy Turku Finland) as described before (Linskens *et al.*, 2009). The results were automatically transferred to the software program Elips / Lifecycle 2.2 *(*Perkin Elmer), used for risk assessment. The nuchal translucency (NT) measurements were performed between 11 and 14 weeks of gestation according to the guidelines of the Fetal Medicine Foundation. Gestational age was determined by fetal CRL (Crown Rump Length) at the time of NT measurement. NT measurements were only accepted for risk assessment if the corresponding CRL was between 45 and 79 mm (VUmc NT reference curve) and if the NT thickness was between 0,6 and 3,5 mm. The NT and CRL measurement as well as information on multiple gestation, diabetes, earlier pregnancy with Down syndrome, smoking habits, and maternal weight were taken into account for risk assessment on Down syndrome. Ethnicity was not accurately stated in all cases and therefore not taken into account. All necessary data were transferred to Elips risk software program and Down syndrome risk was calculated.

Patient selection

From the database of 14645 first trimester combined tests (overall study group), all ART (assisted reproduction techniques) pregnancies were selected. Data on the ART protocols were collected from different IVF referral centers (VU University Medical Center Amsterdam, Saint Lucas Andreas Hospital in Amsterdam, Spaarne Hospital in Hoofddorp and the Medical Center in Alkmaar) to make a distinct selection in IVF and ICSI pregnancies. The controls were selected from the same database as the ART cases. For every ART case 3 matched controls were included. They were matched for gestational age at sample date and for maternal age. Matching for gestational age establishes reliable day-specific median serum parameter concentrations in the control group. To assess whether there is a difference in FPR between the ART cases and the controls, maternal age should not be significantly different between both groups. Follow-up was collected on all ART cases and the controls.

Exclusion criteria for the study (ART and controls) were: abnormal karyotype (except for Down syndrome), multiple gestation, diabetes or intrauterine fetal death. Cases with adverse pregnancy outcome, like pregnancy induced hypertension, intrauterine growth restriction and preterm delivery (< 37 weeks), were also excluded because of the association of these conditions with low PAPP-A levels (Ong *et al.* 2000; Smith *et al.* 2002; Spencer *et al.*, 2008; Gagnon *et al.*, 2008). Finally 203 cases were included in the IVF group and 192 cases in the ICSI group.

The control group finally consisted of 1164 spontaneously conceived singleton pregnancies. From the 1185 cases selected 21 cases were excluded because of twin pregnancies (5), XXY karyotype (1), double controls (2) and missing values on NT or maternal serum parameters (13).

Data collection

Of all ART cases and the controls the following data were recorded: age at conception, gestational age at sample date, maternal weight, NT thickness (mm) and the corresponding CRL (mm), the absolute values of fβ-hCG (ng/ml) and PAPP-A (mU/l) and the calculated risk.

From the overall study group data were collected on maternal age and the calculated risk.

Statistical Analysis

Statistical analysis of data was performed with SPSS 15.0 software. Analysis was done on the IVF group versus its controls, on the ICSI group versus its controls and on the IVF and ICSI group together (ART group) versus all controls. All data of fβ-hCG, PAPP-A and NT were log-transformed because of skewed distributions. Student's *t*-tests were used to compare the following parameters: lnfβ-hCG, lnPAPP-A, lnNT, age at conception, gestational age at sample date, maternal weight, CRL and smoking. Secondly linear regression analysis was applied to evaluate whether differences in lnPAPP-A, lnfβ-hCG and lnNT were caused by differences in background characteristics between the ART groups and the controls. Two-sided $P < 0.05$ was considered to reflect statistical significance.

Results

Analysis was done on the IVF group versus its controls, on the ICSI group versus its controls and on the IVF and ICSI group together (combined ART) versus all controls. The group characteristics of the study populations are shown in Table 1. The values of maternal weight (kg), gestational age at sample date (days), age at conception (days) and CRL (mm) were normally distributed in all study populations. In the ICSI group maternal weight was significantly higher than in the controls (70.4 vs 68.0 kg; p=0.026). Comparing all ART cases to all controls no significant differences were found in maternal weight. There were no other significant differences in background characteristics between the different ART groups and their controls (Table 1).

Table 1. Background characteristics of the different study populations.

Group	IVF N = 203	Controls IVF N = 592	*P*- value	ICSI N =192	Controls ICSI N = 572	*P*-value	Combined ART N = 395	All controls N = 1164	*P*-value
Maternal weight (kg)	68.4 (± 12.3)	69.6 (± 12.1)	0.24	70.4 (± 12.1)	68.0 (±12.1)	0.23	68.2 (± 12.3)	68.9 (± 12.1)	0.23
GA at sample date (days)	78.7 (± 8.3)	78.7 (± 8.3)	0.99	77.7 (± 8.4)	77.7 (±8.4)	0.98	78.3 (± 8.3)	78.3 (± 8.3)	0.98
Maternal age (yrs)	36.4 (± .9)	36.3 (± 2.9)	0.96	34.6 (± 3.4)	34.5 (±3.3)	0.98	35.5 (± 3.3)	35.4 (± 3.2)	0.98
CRL (mm)	61.4 (± 6.5)	60.9 (± 7.4)	0.50	61.1 (± 7.3)	60.6 (±7.2)	0.43	61.2 (± 6.9)	60.8 (± 7.3)	0.52
Number of smokers (%)	3 (1.5)	21 (3.6)	0.14	7 (3.6)	23 (4.0)	0.81	10 (2.5)	44 (3.8)	0.25

All values are reported as means (SD), except for smoking.

In all study populations the values of fβ-Hcg, PAPP-A and NT fitted a Gaussian distribution after $\log_2$ transformation. Table 2 shows the comparison of the mean lnPAPP-A, lnfβ-hCG and lnNT values between spontaneously conceived pregnancies and pregnancies achieved by assisted reproduction (ART). Compared with the controls the mean lnPAPP-A is significantly lower in the IVF group (6.73 vs 7.08; P = 0.0001), in the ICSI group (6.59 vs 7.07; P = 0.0001) and in the combined ART group (6.66 vs 7.07; P = 0.0001). In the IVF group the mean lnfβ-hCG is significantly lower compared to the controls (3.75 vs 3.89; P=0.005) in contrary to the ICSI group (3.87 vs 3.93; P=0.272). In the combined ART group lnfβ-hCG is found to be significantly lower (3.81 vs 3.93; P = 0.004).
Correction factors for the PAPP-A and fβ-hCG values necessary for adjustment in the risk analysis were computed in both the IVF and ICSI group. We found 1.28 and 1.11 for PAPP-A and fβ-hCG respectively in the IVF group and 1.19 and 1.04 in the ICSI group.

Linear regression analysis was applied to assess whether the difference in lnPAPP-A and lnfβ-hCG in the ICSI group was caused by the difference in maternal weight between the ICSI group and its controls. A decrease was shown in the difference in lnPAPP-A before and after correction for maternal weight (0.488 versus 0.443), but it was still significantly lower in the ICSI group than in the control group (P=0.0001). For lnfβ-hCG a slight decrease in difference was found (0.071 vs 0.062; P=0.39).

Table 2. Mean lnPAPP-A and lnfβ-hCG values in IVF and ICSI pregnancies and spontaneously conceived pregnancies.

	IVF N = 203	Controls IVF N = 592	*P*- value	ICSI N =192	Controls ICSI N = 572	*P*-value	Combined ART N = 395	All controls N = 1164	*P*-value
lnPAPP-A	6.73 (± 0.93)	7.08 (± 0.83)	0.0001	6.59 (± 0.89)	7.07 (± 0.83)	0.0001	6.66 (± 0.91)	7.07 (± 0.83)	0.0001
Lnfβ-hCG	3.75 (± 0.6)	3.89 (± 0.6)	0.005	3.87 (± 0.6)	3.93 (± 0.7)	0.272	3.81 (± 0.6)	3.91 (± 0.7)	0.004

Values expressed as means (SD).

In all study populations and in the overall study group the false-positive rate (FPR) was determined at a cut-off of 1:200 (Table 3). The FPR in the IVF group compared to the controls is 10.3% versus 8.6%, in the ICSI the FPR is 10.9% versus 7.5% and in the combined ART group 10.6% versus 8.0%.

The FPR of the first-trimester combined test in the overall age-biased group is 6.8%. Maternal age in the overall study group was 33.6 years and this was significantly lower compared to all ART and control groups (P=0.0001).

Table 3. Performance of Down syndrome screening of the first-trimester combined test in ART pregnancies, in spontaneously conceived pregnancies and the overall study group using a cut-off of 1:200.

	IVF N = 203	Controls IVF N = 592	ICSI N = 192	Controls ICSI N = 572	Combined ART N = 395	All controls N = 1164	Overall study group N = 14.645
Number of screen-positive pregnancies	21	51	22	43	43	94	1038
Number of DS cases	0	0	1	0	1	0	42
Number of false-positive pregnancies	21	51	21	43	42	94	996
FPR (%)	10.3	8.6	10.9	7.5	10.6	8.0	6.8

Discussion

The objective of this study was the comparison of fβ-hCG and PAPP-A levels and NT thickness between pregnancies conceived with assisted reproduction technologies (IVF and ICSI) and spontaneously conceived pregnancies in order to evaluate whether adjustments in the risk analysis are necessary.

In this study we found a decrease in PAPP-A concentrations in ART pregnancies in first-trimester screening for Down syndrome. This finding is in agreement with many other studies. (Liao *et al*., 2001; Wøjdemann *et al*., 2001; Maymon and Shulman, 2002; Orlandi *et al*., 2002; Bersinger *et al*., 2004; Hui *et al*., 2005; and Novak-Antolič, 2006; Gjerris *et al.*, 2009). Differences in PAPP-A levels found in this study between the ART groups and their controls are not related to other conditions associated with low PAPP-A levels because all these cases were excluded in our study.

In contrast to the findings of previous studies (Liao *et al*., 2001; Wøjdemann *et al*., 2001; Orlandi *et al.,* 2002*;* Ghisoni *et al*., 2003; Bersinger et al., 2004; Maymon and Shulman, 2004; Hui et al., 2005; Bellver *et al*., 2005; Lambert-Messerlian *et al*., 2006; Tul and Novak-Antolič, 2006; Gjerris *et al.*, 2009) we found a decrease in fβ-HCG concentrations in the IVF group and in the combined ART group.

Several studies found no influence of assisted reproduction technologies on NT thickness (Maymon *et al*., 1999; Liao *et al*., 2001; Wøjdemann *et al*., 2001; Ghisoni *et al*., 2003) and some observed an increase in NT in IVF cases (Lai *et al*., 2003; Maymon and Shulman, 2004; Hui *et al*., 2005b). We found a decrease in NT in IVF pregnancies like Gjerris *et al*., 2003.

The pathogenesis for differences in maternal serum PAPP-A and fβ-HCG concentrations between an ART pregnancy and a spontaneously conceived pregnancy remains unclear.

An explanation may be a delay in placental maturation because an ART pregnancy differs from spontaneously conceived pregnancies also in terms of growth and development of fetus and placenta (Bersinger *et al*, 2004). Also a relationship with multiple corpora lutea, multiple implantation sites or drugs used in the ART treatment leading to an altered metabolism in both the fetus and the placenta has been suggested (Frishman *et al.*, 1997, Wald *et al.*, 1999; Yu Ng EH *et al.*, 2000Lai *et al*., Räty *et al.*, 2002; Maymon and Jauniaux, 2003; Tul *et al*, 2006).

This study suggests that first-trimester screening for Down syndrome in ART pregnancies is associated with a higher FPR than in spontaneously conceived pregnancies. The observed difference in FPR between the combined ART group and their controls was 2,6%. This increase in FPR is caused by a decrease in PAPP-A concentrations in ART pregnancies. The low fβ-hCG values (IVF and combined ART group) and the decrease in NT (IVF group), will both decrease the risk on Down syndrome and lower the FPR. Maternal age was similar in the ART groups and their controls and therefore it cannot explain the difference in FPR. The difference in FPR between the ART control groups and the overall study group was 1,2%. This can be explained by the significantly higher maternal age in the ART control groups.

Consequently a higher FPR in ART pregnancies will increase the invasive diagnostic testing rate leading to a higher fetal loss rate. To perform an accurate risk calculation with the first-trimester combined test in ART pregnancies an adjustment for maternal serum median values is suggested to reduce the overestimate of risk. Before such an adjustment for maternal serum median values can be introduced in the risk assessment for Down syndrome, however, it would be necessary to study a larger number of Down syndrome pregnancies conceived by ART to determine the effectiveness of screening with adjusted medians.

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Table 1. Background characteristics of the different study populations.

Group	Controls IVF n=592	IVF N=203	Controls ICSI N=572	ICSI N=192	All controls N=1164	Combined ART N=395
Maternal weight (kg)	69.6 (12.14)	68.4 (12.35)	68.0 (12.14)	70.4* (12.16)	68.9 (12.16)	68.2 (12.30)
Gestational age at sample date (days)	78.7 (8.30)	78.7 (8.38)	77.7 (8.46)	77.7 (8.40)	78.3 (8.39)	78.3 (8.38)
Age at conception (years)	36.4 (2.95)	36.4 (2.97)	34.6 (3.37)	34.6 (3.40)	35.5 (3.29)	35.5 (3.31)
CRL (mm)	60.9 (7.40)	61.4 (6.52)	60.6 (7.20)	61.1 (7.28)	60.8 (7.31)	61.2 (6.95)
Number of smokers (%)	21 3.6	3 1.5	23 4.0	7 3.6	44 3.8	10 2.5

All values are reported as means (SD), except for smoking.

* = P < 0.05.

Table 2. Mean lnPAPP-A, lnfβ-hCG and lnNT in spontaneously conceived pregnancies and in pregnancies achieved by assisted reproduction.

	IVF N = 203	Controls IVF N = 592	P-value	ICSI N =192	Controls ICSI N = 572	P-value	Combined ART N = 395	All controls N = 1164	P-value
lnPAPP-A	6.73 (0.93)	7.08 (0.83)	0.0001	6.59 (0.89)	7.07 (0.83)	0.0001	6.66 (0.91)	7.07 (0.83)	0.0001
lnfβ-hCG	3.75 (0.60)	3.89 (0.64)	0.005	3.87 (0.60)	3.93 (0.68)	0.272	3.81 (0.60)	3.91 (0.66)	0.004
lnNT	0.236 (0.25)	0.284 (0.26)	0.02	0.275 (0.23)	0.273 (0.26)	0.92	0.255 (0.24)	0.279 (0.26)	0.11

Values expressed as means (SD).

Table 3. Performance of Down syndrome screening of the first trimester combined test in spontaneously conceived pregnancies, ART pregnancies and the overall study group using a cut-off of 1:200.

	IVF N = 203	Controls IVF N = 592	ICSI N = 192	Controls ICSI N =572	Combined ART N = 395	All ART controls N = 1164	Overall study group N = 14645
Number of screen-positive pregnancies (%)	21 10,3	51 8,6	21 10,9	43 7,5	42 10,6	94 8,0	996 6,8