

First-trimester screening markers are altered in pregnancies conceived after IVF/ICSI

A. C. GJERRIS*, A. LOFT†, A. PINBORG†, M. CHRISTIANSEN‡ and A. TABOR*

*Department of Fetal Medicine and †The Fertility Clinic, Rigshospitalet, Copenhagen University Hospital and ‡Department of Clinical Biochemistry, Statens Serum Institut, Copenhagen, Denmark

KEYWORDS: ART; FER; first-trimester screening; free β -hCG; ICSI; IVF; nuchal translucency; PAPP-A

ABSTRACT

Objectives To determine the levels of first-trimester screening markers and to assess the false-positive rate for first-trimester combined screening for Down syndrome in a large national population of women pregnant after assisted reproductive technology (ART), in order to decide whether or not to correct risk calculation for mode of conception.

Methods A national prospective cohort study of 1000 pregnancies achieved after ART was compared with a control group of 2543 pregnancies conceived spontaneously. All women completed a first-trimester combined screening program. Risk calculation was performed retrospectively based on the screening parameters to avoid bias due to the use of different algorithms of risk calculation.

Results In chromosomally normal pregnancies conceived after in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), the pregnancy-associated plasma protein-A multiples of the median value was significantly decreased when compared with that of pregnancies conceived spontaneously (0.78 and 0.79 vs. 0.98), while there was no difference in the group treated by frozen embryo replacement. There was no difference in the level of free β -human chorionic gonadotropin between groups. The median nuchal translucency thickness was smaller in the overall ART group compared with controls. The false-positive rate of first-trimester combined screening in the overall ART group, adjusted for maternal age, was significantly higher when compared with controls (9.0% vs. 6.0%).

Conclusions It seems advisable to use a population of IVF/ICSI pregnancies to establish median curves for the first-trimester serum screening parameters and perhaps also for nuchal translucency thickness. However, care

must be taken, as different ART treatment methods and aspects of medical history seem to alter the screening parameters in different ways. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Over the last three decades, prenatal screening has become an integrated part of antenatal care in most developed countries. From the 1970s, second-trimester biochemical screening (the triple test) was used, by which up to 60% of Down syndrome (trisomy 21) pregnancies could be detected with a false-positive rate (FPR) of 5%¹. Lately, prenatal screening has tended to move from the second into the first trimester². A 90% detection rate with a 5% FPR may be achieved by combining maternal age, two first-trimester serum markers (pregnancy associated plasma protein-A (PAPP-A) and free β -human chorionic gonadotropin (β -hCG) – the double test) and nuchal translucency thickness (NT) measurement^{3–5}. In 2004, the Danish National Board of Health made it possible to introduce first-trimester combined screening with double test and NT measurement as a routine offer to all pregnant women in Denmark.

The aim of the current prenatal screening program is to identify women at high risk of carrying a fetus with a chromosomal abnormality. Invasive tests such as amniocentesis or chorionic villus sampling are used to diagnose fetal chromosomal aberrations; however, these procedures carry the risk of miscarriage. Correct risk assessment is essentially dependent on reliable determination of the applied screening markers. Any changes in these risk parameters in the course of various conditions accompanying pregnancy could result in a considerable over- or underestimation of risk.

Correspondence to: Dr A. C. Gjerris, Department of Fetal Medicine 4002, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark (e-mail: ac@gjerris.dk)

Accepted: 16 July 2008

The use of assisted reproductive technology (ART) prior to achieved pregnancy has been shown to be associated with changes in biochemical serum screening markers^{6,7}, although it is unknown whether it is the underlying fertility or the fertility treatment that causes these changes. Several studies from the beginning of the 1990s showed that triple test markers among women who had conceived after *in-vitro* fertilization (IVF) were significantly altered, with higher values of hCG and lower values of alpha-fetoprotein and unconjugated estriol^{6,7}.

Whether first-trimester screening is influenced by mode of conception is a controversial issue. Several studies have found that serum marker levels, especially PAPP-A, seem to be altered in IVF pregnancies^{8–11}, whereas other studies have been unable to confirm this^{12,13}; moreover, the total number of cases examined is limited. Likewise, the NT multiples of the median (MoM) measurement has been reported to be influenced by mode of conception^{14,15}, although the majority of studies report that this is not the case^{9,10,13}. As these altered serum marker levels resemble those of women carrying a fetus with Down syndrome, women pregnant after ART are more likely to be considered at high risk for trisomy 21 compared with women who have conceived spontaneously.

It is believed that women who have conceived after ART are reluctant to undergo invasive testing due to the risk of miscarriage^{16,17}. Yet, women pregnant after ART are generally older than are women with spontaneously conceived pregnancies^{18,19}, and are therefore more likely to be carrying a child affected by a chromosomal disorder (primarily trisomy 21). Furthermore, fetuses conceived after intracytoplasmic sperm injection (ICSI) are known to have an increased risk of chromosomal aberrations^{17,20–22}.

In Denmark, up to 7% of births are the result of some kind of fertility treatment. Three to four percent of pregnancies are conceived with IVF with or without ICSI and there is no reason to believe that this number will decline in the future. Thus, whether or not the performance of first-trimester combined screening is influenced by mode of conception is of considerable importance. The aim of this study, therefore, was to determine the levels of first-trimester screening markers and to assess the FPR for first-trimester combined screening for Down syndrome in a large national population of women pregnant after ART, in order to decide whether or not to correct risk calculation for mode of conception.

PATIENTS AND METHODS

Subjects

In this nationwide prospective cohort study carried out in the period 1 April 2004–31 January 2006 in Denmark, 1666 pregnant women were included and met the inclusion criteria: singleton or twin pregnancy, conceived after IVF, ICSI or frozen embryo replacement (FER) and residing in Denmark to allow follow-up.

Pregnancies conceived after oocyte donation and higher order pregnancies were excluded.

An NT scan was performed in 1449 of the women and 1516 underwent blood sampling for the double test. In 1555 cases, the woman returned a questionnaire or detailed information was retrieved from hospital records. In 109 cases, outcome data could only be retrieved from a register with limited information (birth or miscarriage). Two cases (0.1%) were lost to follow-up due to emigration.

As controls, we used 2618 consecutive women who had conceived spontaneously a singleton pregnancy, who had NT measurement performed at one of two university departments of fetal medicine from 1 January 2004 to 31 December 2006 and whose double test was analyzed at Statens Serum Institut (SSI).

All women gave informed consent in accordance with the Helsinki declaration and the study was approved by the local scientific ethics committee (Jr. nr. KF 01-218/03) and by the Danish Data Protection Agency.

Study design

All Danish fertility clinics were invited to take part in this study, and, with the exception of two, all participated (nine public and nine private clinics). We were able to offer first-trimester combined screening to all included women before it became part of routine antenatal care in Denmark. Regardless of the result of the risk assessment, the women could choose invasive diagnostic testing according to the prevailing guidelines (generally for maternal age ≥ 35 years, ICSI treatment and history of chromosomal abnormality). All women were invited to the study at the fertility clinic at the time of early ultrasound (to assess viability). At the fertility clinic, data on fertility treatment and demographics were recorded and entered into a database. Data on pregnancy outcome were obtained by a self-administered questionnaire given to the women immediately after inclusion and returned after the end of pregnancy. When the questionnaire was not returned, information was retrieved from the fertility clinic or from hospital records.

All chromosomal analyses performed in Denmark both pre- and postnatally are registered centrally in the Danish Cytogenetic Central Registry (DCCR), except for those carried out at Odense University Hospital (OUH), which represents a small proportion of the total number, approximately 5%. The data are reported using the personal identification number (CPR number), allocated by the Centralised Civil Registry. By record linkage, the mothers' and infants' CPR numbers were linked to the DCCR and OUH databanks to obtain information on chromosomal abnormalities.

Because the first-trimester risk calculations were performed at several different hospitals using different risk calculation algorithms, it was impossible to pool these data for analyses. Instead, data on the basic screening parameters were collected: crown–rump length (CRL), NT, PAPP-A (MoM/concentrations) and β -hCG

(MoM/concentrations), including dates of NT measurement and blood sampling. Subsequently, the risks for all women were calculated using the latest Risk Calculation Software of The Fetal Medicine Foundation (FMF) in September 2007 by Chris Harris of Astraia Software, GmbH, Germany, without knowledge of mode of conception.

Screening markers and gestational age

The NT scan was performed by physicians, nurses or midwives certified according to The FMF³. Data from the NT scan were recorded at the departments of fetal medicine. The NT measurements were converted into MoM-NT using the following equation based on this dataset for NT as a function of CRL: Regressed NT = $0.28743 + 0.02161 \times \text{CRL}$; $P < 0.0001$, $R^2 = 0.14$.

Blood samples were taken either at the fertility clinics, by the woman's general practitioner or at the hospital where they had their NT scan performed. Nearly all biochemical analyses were then performed in one laboratory, the SSI in Copenhagen, where measurements of PAPP-A and β -hCG were determined in serum samples as part of the routine first-trimester prenatal screening program. Briefly, the concentrations of the analytes were measured using either the Kryptor platform (Brahms, Henningsdorf, Berlin) or the AutoDelfia platform (Perkin Elmer Life Science, Boston, MA, USA). Concentrations of biochemical parameters were entered into the database of the first-trimester prenatal screening program at SSI and automatically converted into MoMs by the laboratory information management system using underlying reference values that were based on the Danish population and continuously monitored. The MoMs were weight-corrected. All laboratory methods were continually assessed by internal and external quality assurance programs. In 46 cases, the blood sample was analyzed at the local laboratory in the hospital, where the NT scan was performed. The median formulae were similar to those used at the SSI.

In the Danish first-trimester prenatal screening set-up, the blood samples are usually taken prior to the NT scan and gestational age (GA) is provisionally calculated by last menstrual period. We defined 'GA at blood sampling' as the GA used in the analyses of serum markers. Gestational age (GA) was also calculated from CRL, measured at the NT scan, by the formula described by Robinson and Fleming²³: $8.052 \times \text{CRL}^{1/2} + 23.75$, which is used in Astraia's algorithm. At NT measurement, the GA found according to CRL was compared with the provisional GA and if the difference was ≥ 2 days, the GA was changed and serum marker MoMs recalculated.

Statistical analyses

For serum markers, the MoM values were calculated as described above. For NT, regression analysis was carried out to derive the relationship with CRL, then

the expected values of NT were calculated for each case and the measurements converted into MoMs. For the trisomy 21 cases in pregnancies conceived after ART, the ART-adjusted MoM values were calculated using the median formula of PAPP-A and β -hCG in ART pregnancies and converting the individual measurements into MoMs.

The distributions of markers were tested by the Kolmogorov–Smirnov test of fit. When, even after transformation of data, the assumption of normality was not satisfied, non-parametric analysis was used; Kruskal-Wallis test for comparison between more than two groups and Mann–Whitney *U*-test for comparison between two groups. When normality was satisfied, the one-way ANOVA test and *t*-test were used. Categorical data were compared with the χ^2 test. The odds ratios for the risk of Down syndrome $\geq 1:300$ were calculated using logistic regression as described by Tul and Novak-Antolic¹¹. The relationships between the dependent variables (MoMs of PAPP-A and β -hCG and NT) and several possible explanatory variables (treatment protocol, previous spontaneous miscarriage, number of previous pregnancies and ART-treatment cycles, GA at blood sampling, history of polycystic ovaries (PCO) and pregnancy complicated by ovarian hyperstimulation syndrome (OHSS)) were assessed one-by-one using simple linear regression; only those explanatory variables that were significantly related were considered for further investigation. It is an established fact that maternal age is not correlated with first-trimester screening markers⁴, thus it is not an explanatory variable.

All analyses were carried out using SAS Enterprise Guide v 9.2 (SAS Institute Inc., Cary, NC, USA). Statistical significance was defined as $P < 0.05$.

RESULTS

From the ART cohort we identified 1038 pregnancies in which one fetus only was seen at early ultrasound and who completed the first-trimester combined screening program. The control group consisted of 2618 singleton pregnancies. Among the ART pregnancies, 28 (2.7%) ended after NT measurement, nine as elective terminations of pregnancy due to prenatally detected chromosomal abnormality, and 19 (1.8%) as spontaneous miscarriages. Four of the spontaneous miscarriages were found to have a normal karyotype and 15 were not karyotyped.

Except for one case (achieved by ICSI treatment and diagnosed postnatally as having a *de-novo* autosomal structural chromosomal abnormality), no signs of chromosomal disorder were detected in the infants from the study and control groups at birth or at the 1-year follow up. Altogether, 10 cases of chromosomal abnormalities (two with incomplete data) were found among the ART pregnancies (nine pre- and one postnatally), while 12 (one with incomplete data) were detected prenatally in the control group. These were not included in the analyses of screening markers and performance. Six of the ART pregnancies were excluded from further analysis because

of incomplete data on fertility treatment. Thirty-eight ART-pregnancies and 75 controls had incomplete data for risk calculations. Incomplete data included missing data from NT measurements (CRL or NT), CRL outside the GA window for NT measurement (45–89 mm) or missing dates for either blood sampling or NT scan. Thus, the study population regarding screening markers consisted of 992 ART pregnancies and 2532 controls.

Baseline parameters of the ART pregnancies and controls

The women pregnant after ART were significantly older compared with the women who had conceived spontaneously, with a median maternal age of 33 (range, 20–43) vs. 31 (range, 15–46) years ($P < 0.001$, Table 1). The proportion of women of advanced maternal age (≥ 35 years) was also higher, at 33.8% vs. 20.7% ($P < 0.001$). There was no difference in the median maternal age between the different ART treatment groups, but the ICSI-treated women had a lower rate of advanced maternal age compared with the IVF-treated ones.

Women pregnant after ART had their blood samples for analysis of PAPP-A and β -hCG taken significantly earlier in pregnancy compared with the control group, at a median GA of 66 (range, 56–97) vs. 81 (range, 56–97) days ($P < 0.001$). According to the Mann–Whitney U -test, the GA at NT measurement was significantly lower in the ART pregnancies ($P < 0.001$), although the median values were the same (Table 1).

Screening markers

The median MoM levels of PAPP-A, β -hCG and NT together with their corresponding logMoM values and geometric means are shown in Table 2. Secondary analysis was performed comparing IVF, ICSI and FER. The median PAPP-A MoM was significantly lower in the IVF and ICSI treatment groups when compared with controls (0.78 and 0.79 vs. 0.98, $P < 0.001$); this was also the case when the ART group overall was compared with controls (0.80 vs. 0.98, $P < 0.001$). Among the ART groups,

the FER pregnancies differed by having a significantly higher median PAPP-A MoM ($P < 0.001$), which was not significantly different from the control group. The NT was significantly smaller in the overall ART group compared with the control group (median NT MoM, 0.92 vs. 1.00, $P < 0.0001$). Among the different ART treatment groups, the NT in the IVF group had a significantly lower median value compared with the ICSI group ($P = 0.005$). There were no differences in β -hCG concentration.

Invasive testing and chromosomal abnormalities among ART pregnancies

There were 10 chromosomal abnormalities detected among the 1038 ART pregnancies, corresponding to a rate of 0.96%. Nine cases (six of trisomy 21 and three of trisomy 18) were detected prenatally (all had a Down syndrome risk $\geq 1:300$), while one case of autosomal structural abnormality (*de novo* translocation) was found postnatally. This case was a pregnancy conceived after ICSI; both parents were karyotyped prior to fertility treatment and found to be normal. The child was liveborn with a dysmorphic appearance.

The rate of chromosomal abnormalities was twice as high in the ICSI-treated group compared with the IVF-treated group, at 1.5% (6/402, three cases of trisomy 21, two cases of trisomy 18 and one case of autosomal structural abnormality) in the ICSI group compared with 0.78% (4/516, three cases of trisomy 21 and one case of trisomy 18) in the IVF group. Data for the Down syndrome cases found among ART pregnancies are presented in Table 3.

Of the 106 women (Table 4) in the ART group who were found retrospectively from combined screening to have a risk $\geq 1:300$, only 44 (41.5%) had an invasive diagnostic test performed. Overall, in the demographic population, 8.4% (84/1000) of women had an invasive test performed.

False-positive rates and odds ratios

In the ART group, the FPR at a 1 in 300 risk cut-off for combined screening at the time of risk assessment was

Table 1 Baseline parameters of the assisted reproductive technology (ART) and control pregnancies

Parameter	Controls (n = 2543)	ART pregnancies			
		All (n = 1000)	IVF (n = 516)	ICSI (n = 400)	FER (n = 84)
Maternal age (years)	31 (15–46)*	33 (20–43)*	33 (20–43)	32 (20–43)	33 (27–42)
Maternal age ≥ 35 years	527 (20.7)†	338 (33.8)†	194 (37.6)‡	114 (28.5)‡	30 (35.7)
CRL at NT scan (mm)	67 (45–84)*	66 (45–84)*	65 (45–82)§	66 (45–84)§	69 (48–83)§
GA at NT scan (based on CRL) (days)	91 (79–99)*	91 (79–99)*	90 (79–98)§	91 (79–99)§	92 (81–99)§
GA at blood sampling (days)#	81 (56–97)*	66 (56–97)*	65 (56–97)	66 (56–91)	66 (58–82)

Values are given as median (range) or n (%). Thirty-eight ART pregnancies and 75 controls were not included due to incomplete data. #Gestational age (GA) at blood sampling was based on last menstrual period or (if difference between that and GA calculated by crown–rump length (CRL) was ≥ 2 days) on CRL. * $P < 0.001$, controls vs. all ART, Mann–Whitney U -test. † $P < 0.001$, controls vs. all ART, χ^2 test. ‡ $P < 0.05$, ICSI vs. IVF, χ^2 test. § $P < 0.01$, FER vs. IVF and ICSI, Mann–Whitney U -test. FER, frozen embryo replacement; ICSI, intracytoplasmic sperm injection; IVF, *in-vitro* fertilization; NT, nuchal translucency.

Table 2 Median multiples of the median (MoM) levels of pregnancy-associated plasma protein-A (PAPP-A), β -human chorionic gonadotropin (β -hCG) and nuchal translucency thickness (NT) together with their corresponding logMoM values and geometric means in assisted reproductive technology (ART) and control pregnancies

Marker	Controls (n = 2532)	ART pregnancies			
		All (n = 992)	IVF (n = 512)	ICSI (n = 396)	FER (n = 84)
PAPP-A					
Median MoM (range)	0.98 (0.05–15.14)*§	0.80 (0.01–5.01)*	0.78 (0.01–3.93)‡§	0.79 (0.06–4.55)‡§	1.03 (0.25–5.01)‡
Geometric mean MoM (95% CI)	0.96 (0.94–0.99)*	0.77 (0.74–0.81)*	0.74 (0.71–0.79)‡	0.77 (0.72–0.82)‡	1.00 (0.89–1.15)‡
Mean log ₁₀ MoM (SD)	–0.02 (0.26)*	–0.11 (0.28)*	–0.13 (0.28)‡	–0.11 (0.29)‡	0.00 (0.26)‡
β-hCG					
Median MoM (range)	0.99 (0.01–8.89)	0.97 (0.09–6.42)	0.96 (0.18–6.42)	0.98 (0.09–4.90)	1.00 (0.29–4.98)
Geometric mean MoM (95% CI)	1.00 (0.98–1.02)	0.97 (0.93–1.00)	0.96 (0.92–1.01)	0.96 (0.91–1.02)	1.02 (0.91–1.14)
Mean log ₁₀ MoM (SD)	0.00 (0.25)	–0.01 (0.25)	–0.02 (0.25)	–0.02 (0.26)	0.01 (0.23)
NT					
Median mm (range)	1.70 (0.17–5.00)*	1.60 (0.30–4.00)*	1.50 (0.40–3.10)†	1.60 (0.50–4.00)†	1.70 (0.30–3.10)
Median MoM (range)	1.00 (0.09–3.54)*	0.92 (0.18–2.46)*	0.90 (0.25–2.06)†	0.95 (0.27–2.46)†	0.94 (0.18–1.74)
Geometric mean MoM (95% CI)	0.99 (0.99–1.00)*	0.91 (0.89–0.92)*	0.89 (0.87–0.91)†	0.93 (0.91–0.96)†	0.91 (0.85–0.97)
Mean log ₁₀ MoM (SD)	–0.00 (0.10)*	–0.04 (0.12)*	–0.05 (0.12)†	–0.03 (0.12)†	–0.04 (0.13)

Thirty-eight ART pregnancies and 75 controls were not included due to incomplete data, and a further eight ART cases and 11 controls due to chromosomal abnormality. FER, frozen embryo replacement; ICSI, intracytoplasmic sperm injection; IVF, *in-vitro* fertilization.

* $P < 0.001$, controls vs. ART, Mann–Whitney *U*-test. † $P < 0.05$, IVF vs. ICSI, Mann–Whitney *U*-test. ‡ $P < 0.001$, FER vs. ICSI and IVF (no significant difference when compared with controls), Mann–Whitney *U*-test. § $P < 0.001$, controls vs. IVF and ICSI, Mann–Whitney *U*-test.

Table 3 Observed and adjusted assisted reproductive technology (ART) first-trimester screening parameters in the group of chromosomally abnormal ART pregnancies

Karyotype	Treatment method	MA (years)	CRL (mm)	NT (mm)	NT (MoM)	β -hCG (MoM)	PAPP-A (MoM)	Risk				ART-adjusted*	
								MA (1:)	NT (1:)	BC (1:)	Comb. (1:)	β -hCG (MoM)	PAPP-A (MoM)
Overall trisomy 21 (mean)	—	37.3	61	4.2	2.73	1.27	0.29	—	—	—	—	1.39	0.51
Trisomy 21	IVF	32	63	2.7	1.64	0.74	0.56	553	141	15	5	1.00	0.64
Trisomy 21	ICSI	39	54	5.0	3.44	2.82	0.24	97	3	3	2	3.01	0.38
Trisomy 21	ICSI	39	57	5.2	3.42	1.39	0.29	107	3	5	2	1.62	0.59
Trisomy 21	IVF	39	59	6.2	3.97	1.16	0.37	94	3	21	2	1.10	0.83
Trisomy 21	IVF	35	66	2.0	1.17	0.61	0.19	274	899	11	31	0.48	0.57
Trisomy 21†	ICSI	40	65	SH	—	0.88	0.09	88	—	3	—	1.14	0.03
Trisomy 18	IVF	43	55	4.5	3.05	0.74	0.57	33	2	224	7	1.08	1.14
Trisomy 18	ICSI	28	56	8.6	5.74	0.81	1.99	749	5	5342	19	1.03	3.42
Trisomy 18‡	ICSI	34	40	5.2	4.51	0.24	0.17	325	—	17	—	0.37	0.17
Autosomal struc.	ICSI	37	67	1.4	0.81	0.29	0.51	183	871	1308	3657	0.27	0.97

*Regression equations used to calculate the ART-adjusted MoMs: Regressed PAPP-A_{conc} = $10^{2.02+0.00937 \times \text{CRL}}$ and regressed β -hCG_{conc} = $10^{1.83511-0.00026803 \times \text{CRL}}$. †‡Excluded from study due to incomplete data from NT measurement: †NT not measured exactly, and described as septate hygroma; ‡CRL too small for NT risk assessment. β -hCG, β -human chorionic gonadotropin; Autosomal struc., autosomal structural chromosomal abnormality; BC, biochemistry; Comb., combined; CRL, crown–rump length; MA, maternal age; MoM, multiples of the median; NT, nuchal translucency thickness; PAPP-A, pregnancy-associated plasma protein-A; SH, septate hygroma.

10.7%, which was significantly higher when compared with 6.0% in the control group ($P < 0.0001$, Table 4). These FPRs were, however, age-biased due to the higher median maternal age in the ART group. When the observed FPRs for the ART group and the age distribution of the control group were used to model the impact of assisted conception on the FPR, an age-adjusted FPR of 9.0% was obtained, which was also significantly different when compared with the control group ($P < 0.0001$).

When risk was calculated using only the maternal age and NT, there were no differences in the FPRs. The FER group differed (though not significantly so) from the IVF and ICSI groups, having a lower FPR (Table 4).

The odds ratios and 95% CIs of having a ‘positive’ risk assessment ($\geq 1 : 300$) according to mode of conception after adjustment for maternal age are shown in Table 5. There was a significantly increased risk of having a positive first-trimester screening assessment when risk

Table 4 Proportion of women with a trisomy 21 risk $\geq 1:300$ calculated on the basis of maternal age and biochemistry (BC), maternal age and nuchal translucency thickness (NT) or maternal age, BC and NT in the overall assisted reproductive technology (ART) group, in the different ART subgroups and in controls

Group	n (%)		
	Age + NT	Age + BC	Age + NT + BC
All ART (<i>n</i> = 992)	69 (7.0)	186 (18.8)*	106 (10.7)*
IVF (<i>n</i> = 512)	36 (7.0)	100 (19.5)†	57 (11.1)†
ICSI (<i>n</i> = 396)	27 (6.8)	77 (19.4)†	45 (11.4)†
FER (<i>n</i> = 84)	6 (7.1)	9 (10.7)	4 (4.8)
Control group (<i>n</i> = 2532)	163 (6.4)	280 (11.1)*†	151 (6.0)*†

Thirty-eight ART pregnancies and 75 controls were not included due to incomplete data, and a further eight ART cases and 11 controls due to chromosomal abnormality. * $P < 0.0001$, all ART vs. control group, χ^2 -test. † $P < 0.01$, IVF and ICSI vs. control group, χ^2 -test. FER, frozen embryo replacement; ICSI, intracytoplasmic sperm injection; IVF, *in-vitro* fertilization.

was based on maternal age and BC or maternal age, BC and NT for the overall ART group, and for IVF and ICSI pregnancies. For the overall ART group and for IVF and ICSI pregnancies, the odds of being screen positive, when the risk assessment was based only on NT, were decreased when compared with the controls. For the pregnancies conceived by FER, the risk of having a positive risk assessment was not increased.

Possible explanatory variables

There were no associations between gravidity or parity and the values of the screening markers.

By log-linear or linear regression, as appropriate, the relation between number of previous spontaneous miscarriages and serum markers was analyzed and no association was found. Additionally, we identified a group of 18 women who had experienced three or more spontaneous miscarriages and compared their serum marker levels with the remaining ART pregnancies. In the group with three or more spontaneous miscarriages, the median PAPP-A MoM was 0.79 and the median β -hCG MoM was 1.01. When compared with the remaining ART pregnancies, there was no difference.

Table 5 Odds ratios of having a 'positive' risk assessment, with risk calculated on the basis of maternal age and biochemistry (BC), maternal age and nuchal translucency thickness (NT) or maternal age, BC and NT in the overall assisted reproductive technology (ART) group and in the different ART subgroups (*n* = 992)

Group	Odds ratio (95% CI)		
	Age + NT	Age + BC	Age + NT + BC
All ART	0.714 (0.525, 0.972)	1.491 (1.209, 1.838)	1.506 (1.153, 1.968)
IVF	0.689 (0.465, 1.021)	1.512 (1.164, 1.962)	1.521 (1.093, 2.118)
ICSI	0.795 (0.513, 1.231)	1.663 (1.251, 2.210)	1.728 (1.209, 2.470)
FER	0.640 (0.265, 1.545)	0.704 (0.344, 1.441)	0.573 (0.205, 1.604)

Thirty-eight ART pregnancies were not included due to incomplete data, and a further eight cases due to chromosomal abnormality. FER, frozen embryo replacement; ICSI, intracytoplasmic sperm injection; IVF, *in-vitro* fertilization.

There were no associations between number of previous ART treatment cycles and the values of the screening markers. In our cohort, the majority of women had followed a long treatment protocol, with gonadotropin-releasing hormone agonist down-regulation prior to ovarian stimulation in 693 (69.9%) women; 162 (16.3%) had followed a short treatment protocol; in 53 (5.3%) cases this information was not available; and 84 women were treated by FER. There was no significant difference between the median PAPP-A MoM or median β -hCG MoM in the long-protocol group compared with the short-protocol group. The median NT MoM was significantly lower in the long-protocol group, at 0.92 compared with 0.97 ($P = 0.02$).

Women pregnant after ART had their blood sample taken at an earlier GA (Figure 1a,c) and we investigated whether this had any influence on the serum markers. The time of blood sampling had no effect on the PAPP-A MoM levels, but there was a significant relationship between the GA and β -hCG. For the controls this was not the case (Figure 1b,d).

None of these possible explanatory variables was found to have any significant influence on the screening markers when tested in a linear regression model, except for GA at blood sampling in relation to β -hCG and the ART treatment protocol in relation to NT. A full model with all variables analyzed by multiple linear regression was therefore not appropriate.

In the returned questionnaire a small group of women had stated that they were diagnosed with PCO (*n* = 8) before fertility treatment or were diagnosed with OHSS during the pregnancy in question (*n* = 6). Their serum screening markers were considerably decreased: median PAPP-A MoM, 0.50; median β -hCG MoM, 0.72 (PCO: PAPP-A, 0.50; β -hCG, 0.74 and OHSS: PAPP-A, 0.50; β -hCG, 0.64). The number of cases was much too small to make the difference significant.

DISCUSSION

To our knowledge, this is the largest prospective cohort study on ART pregnancies regarding first-trimester screening to date. We have demonstrated that the first-trimester serum screening marker PAPP-A is significantly decreased

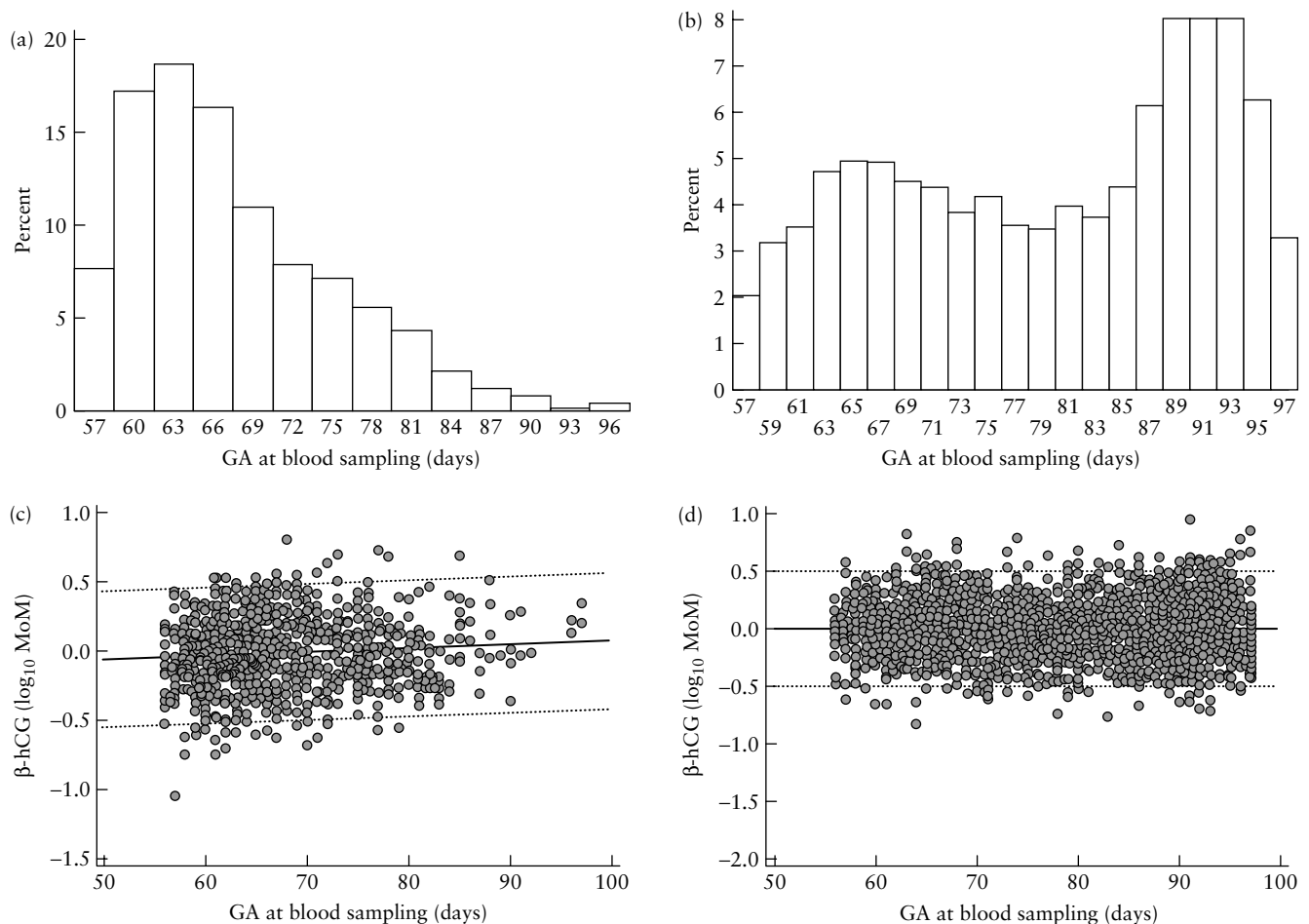


Figure 1 (a,b) Distributions of gestational age (GA) at time of blood sampling in the group of pregnancies achieved by assisted reproduction technology (ART) (a) and in controls (b). (c,d) Scatterplot and regression line (with 95% CI) showing β -human chorionic gonadotropin (β -hCG) (\log_{10} MoM) as a function of gestational age at time of blood sampling (GABS) in cases (c) and controls (d). Regression line for ART pregnancies: β -hCG (MoM) = $10^{-0.20 + 0.00278 \times \text{GABS}}$, $R^2 = 0.0074$, $P = 0.008$ and for controls: β -hCG (MoM) = $10^{-0.04 + 0.00054 \times \text{GABS}}$, $R^2 = 0.0007$, $P = 0.22$.

in IVF and ICSI pregnancies compared with controls who conceived spontaneously. This is in agreement with previous findings^{9–11,15,24}. In pregnancies conceived after FER, this was not the case; however, our cohort contained relatively few FER pregnancies ($n = 85$). A recent study by Anckaert *et al.*²⁵ reported similar findings in a series of 31 FER pregnancies, finding that the median PAPP-A MoM (1.12 MoM) was not significantly different from that in naturally conceived pregnancies (1.10 MoM).

In the overall ART group we found the PAPP-A MoM was 0.8 compared with 0.98 MoM in the control group. We found no differences in the concentrations of free β -hCG, in line with most previous studies^{10–13,15}, although a few papers reported β -hCG to be increased^{8,9,24}. The concentration of free β -hCG in the second trimester has been investigated in a few studies and seems to be slightly increased^{7,26,27}, whereas intact hCG seems to be more significantly increased^{6,28–30}. We found that there was a significant positive correlation between increasing GA and free β -hCG in pregnancies conceived after ART, while this was not the case in the control group. This indicates that distribution curves of serum screening markers might be different in ART pregnancies.

Surprisingly, the NT MoM was significantly smaller in the ART group when compared with the control group. The majority of previous studies found no difference in the size of NT in ART pregnancies compared with spontaneously conceived pregnancies^{9,10,13}, but two studies, although with a limited number of cases, reported a larger NT in ART pregnancies^{14,15}. Interestingly, we found that the thickness of NT was dependent on treatment method; women treated with the long protocol had fetuses with thinner NTs compared with women treated with the short protocol (0.92 MoM vs. 0.97 MoM) and, independently, the same was the case with IVF treatment compared with ICSI treatment (0.90 MoM vs. 0.95 MoM). There does not seem to be any obvious biological explanation for these findings, and indeed, any significant differences might be due to chance as several statistical analyses were performed. In a recent study³¹, we found that GA dating by date of oocyte aspiration and by CRL differed significantly by 1.5 days, the GA dated by CRL being higher. The opposite, GA calculated by date of oocyte aspiration being higher, has been found in other studies^{32–34}; however, these were notably smaller than was ours. The size of NT is believed to be dependent

on GA calculated by the size of the fetus (CRL), although only until week 13/14, after which the NT decreases in size, signifying that not only the size of the fetus but also the biological GA is associated with the size of the NT. Our study suggests that fetuses from ART pregnancies were larger than expected at the NT scan and their real biological GA was lower; one could speculate that this could explain the smaller NT MoM values seen in the ART pregnancies.

As a result of the low PAPP-A, despite the smaller NT, we found that the FPR in pregnancies achieved by ART was significantly increased when compared with pregnancies that were conceived spontaneously (10.7% vs. 6.0%). After adjustment for maternal age, the FPR in the ART group (at 9.0%) was still significantly higher. If we had excluded the FER pregnancies, which did not differ significantly from controls, the FPR for IVF and ICSI pregnancies would have been slightly higher. Consequently, a higher proportion of women pregnant following ART would have been referred for invasive diagnostic testing and been exposed to the associated risk of miscarriage. As the use of ART and ovulation induction is constantly increasing, this tendency might have important implications, certainly for the individual woman. It thus seems necessary to adjust the risk calculation in order to reduce the FPR.

However, contradictory results from previous published work on screening markers and ART might illustrate a very high complexity among women undergoing ART, possible confounding factors including different underlying causes of infertility and different treatment methods, for example. Thus, it seems very important to investigate the reason for the difference in serum marker concentrations in ART pregnancies, which remains unknown. Multiple corpora lutea, multiple implantation sites or drugs used in the fertility treatments have been suggested as possible explanatory factors³⁵. A functional delay in fetal and placental development and/or some unknown underlying pathology leading to various metabolic disturbances as well as the higher risk of obstetric complications (e.g. growth restriction and pre-eclampsia) associated with ART may also cause alterations in serum marker concentrations^{14,36}.

A recent paper by Tul and Novak-Antolic¹¹ reported an inverse association between number of aspirated oocytes and PAPP-A MoM values. The authors hypothesized that the number of oocytes retrieved reflected the number of corpora lutea in pregnancies, supported by their other finding that inhibin A, which is secreted by corpora lutea, was increased with decreasing PAPP-A. They suggested that inhibin A inhibits the secretion of PAPP-A. We did not record information about number of follicles and aspirated oocytes. As an alternative, we identified a small group of women who were diagnosed with PCO before fertility treatment or who were diagnosed with OHSS during the pregnancy in question. Their serum screening markers were indeed altered (median PAPP-A MoM, 0.50 and median β -hCG MoM, 0.72). Since PAPP-A plays an important role in the initial development and later

function of the placenta³⁷⁻⁴⁰, this might be an important finding. As only one corpus luteum is usually present in pregnancies after FER, this might also explain the 'normal' concentration of PAPP-A in these pregnancies.

In the chromosomally normal ART pregnancies, only 41.5% (44/106) of those with a retrospectively calculated Down syndrome risk $\geq 1:300$ had an invasive test performed. Altogether (including the pregnancies with a chromosomally abnormal fetus), 8.4% (84/1000) of cases had an invasive diagnostic test. Women who had an invasive test performed without a positive risk calculation (i.e. with risk $< 1:300$) did so in most cases due to advanced maternal age. Despite the fact that the risk calculation was performed retrospectively and thereby might differ slightly from that actually performed, which is a limitation of this study, it is remarkable that all the prenatally identified chromosomal abnormalities were found in the screen-positive group. As we have follow-up on all infants until the age of at least 1 year these numbers are likely to be valid. The one case that was not found prenatally was an autosomal structural abnormality. The target of routine prenatal screening is to detect the most common chromosomal aberrations (i.e. trisomies 21, 18 and 13). However, evidence shows that ART^{17,21} (perhaps only ICSI) pregnancies have an increased risk of chromosomal aberrations, mainly autosomal structural aberrations inherited from the father or *de novo*. This underlines that genetic assessment of couples, especially fathers with oligospermy, prior to infertility treatment cannot be replaced by prenatal screening.

In our series of six Down syndrome cases, the median MoM values, calculated from the concentration actually measured in ART pregnancies, were 0.51 for PAPP-A and 1.39 for free β -hCG. A meta-analysis of first-trimester cases with trisomy 21 in spontaneously conceived pregnancies found a median PAPP-A MoM of 0.45 ($n = 777$) and a median free β -hCG of 1.98 MoM ($n = 846$)⁴. Comparison of MoM values from Down syndrome pregnancies between studies is difficult due to the different pattern over time in aneuploidy pregnancies ('temporal variation')⁴¹. However, larger cohorts of Down syndrome cases among ART pregnancies are needed to be sure that the FPR is not downsized at the expense of a lower detection rate.

In conclusion, we found that the FPR was significantly increased in pregnancies conceived after IVF and ICSI. Thus, it seems advisable to use a population of ART pregnancies, preferably divided by type of treatment, when establishing median curves for the first-trimester serum screening markers and perhaps also for NT thickness. However, care must be taken as the etiology of the infertility and the response to the ART treatment (e.g. the number of aspirated oocytes and development of OHSS) may cause different changes in the screening markers. Another issue is that low PAPP-A might be used as a marker of obstetric complications later in pregnancy^{37,42} and it is unlikely that first-trimester serum markers in ART pregnancies should be adjusted for this

purpose. Further studies aimed at detecting the cause(s) of the altered screening parameters are needed in order to adjust the first-trimester risk calculation properly.

ACKNOWLEDGMENTS

We thank the doctors and nurses at the fertility clinics and departments of fetal medicine throughout the country, who filled out all the forms with data, and especially we thank all the women who participated in this study. Also thanks to Chris Harris, Astraia Software GmbH, Munich, Germany for making the risk calculations. The study was supported by grants from the Danish Health Foundation (2006B034, 2005B055, 2004B149) and The Danish Medical Research Council.

REFERENCES

- 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.
- Sheppard C, Platt LD. Nuchal translucency and first trimester risk assessment: a systematic review. *Ultrasound Q* 2007; **23**: 107–116.
- Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet* 1998; **352**: 343–346.
- Spencer K. Aneuploidy screening in the first trimester. *Am J Med Genet C Semin Med Genet* 2007; **145**: 18–32.
- Wøjdemann KR, Shalmi AC, Christiansen M, Larsen SO, Sundberg K, Brocks V, Bang J, Nørgaard-Pedersen B, Tabor A. Improved first-trimester Down syndrome screening performance by lowering the false-positive rate: a prospective study of 9941 low-risk women. *Ultrasound Obstet Gynecol* 2005; **25**: 227–233.
- 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.
- 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. *Br J Obstet Gynaecol* 1999; **106**: 1304–1306.
- 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.
- 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.
- 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.
- 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.
- 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.
- Wøjdemann 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.
- Hui PW, Tang MH, Lam YH, Yeung WS, Ng EH, Ho PC. Nuchal translucency in pregnancies conceived after assisted reproduction technology. *Ultrasound Obstet Gynecol* 2005; **25**: 234–238.
- 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.
- Geipel A, Berg C, Katalinic A, Ludwig M, Germer U, Diedrich K, Gembruch U. Different preferences for prenatal diagnosis in pregnancies following assisted reproduction versus spontaneous conception. *Reprod Biomed Online* 2004; **8**: 119–124.
- Gjerris AC, Loft A, Pinborg A, Christiansen M, Tabor A. Prenatal testing among women pregnant after assisted reproductive techniques in Denmark 1995–2000: a national cohort study. *Hum Reprod* 2008; **23**: 1545–1552.
- Geipel A, Gembruch U, Ludwig M, Germer U, Schwinger E, Dormeier A, Diedrich K. Genetic sonography as the preferred option of prenatal diagnosis in patients with pregnancies following intracytoplasmic sperm injection. *Hum Reprod* 1999; **14**: 2629–2634.
- Pinborg A, Loft A, Rasmussen S, Schmidt L, Langhoff-Roos J, Greisen G, Andersen AN. Neonatal outcome in a Danish national cohort of 3438 IVF/ICSI and 10,362 non-IVF/ICSI twins born between 1995 and 2000. *Hum Reprod* 2004; **19**: 435–441.
- Aboulghar H, Aboulghar M, Mansour R, Serour G, Amin Y, Al-Inany H. A prospective controlled study of karyotyping for 430 consecutive babies conceived through intracytoplasmic sperm injection. *Fertil Steril* 2001; **76**: 249–253.
- Bonduelle M, Van AE, Joris H, Keymolen K, Devroey P, Van Steirteghem A, Liebaers I. Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. *Hum Reprod* 2002; **17**: 2600–2614.
- Jozwiak EA, Ulug U, Mesut A, Erden HF, Bahceci M. Prenatal karyotypes of fetuses conceived by intracytoplasmic sperm injection. *Fertil Steril* 2004; **82**: 628–633.
- Robinson HP, Fleming JE. A critical evaluation of sonar "crown-rump length" measurements. *Br J Obstet Gynaecol* 1975; **82**: 702–710.
- 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* 2005; **25**: 390–393.
- Anckaert E, Schiettecatte J, Sleurs E, Devroey P, Smits 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.
- Raty R, Virtanen A, Koskinen P, Laitinen P, Forsstrom J, Salonen R, Morsky P, Ekblad U. Maternal midtrimester serum AFP and free beta-hCG levels in in vitro fertilization twin pregnancies. *Prenat Diagn* 2000; **20**: 221–223.
- 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.
- 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.
- Heinonen S, Ryyanen M, Kirkinen P, Hippelainen M, Saarikoski S. Effect of in vitro fertilization on human chorionic gonadotropin serum concentrations and Down's syndrome screening. *Fertil Steril* 1996; **66**: 398–403.

30. Ribbert LS, Kornman LH, De Wolf BT, Simons AH, Jansen CA, Beekhuis JR, Matingh A. Maternal serum screening for fetal Down syndrome in IVF pregnancies. *Prenat Diagn* 1996; **16**: 35–38.
31. 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.
32. Sladkevicius P, Saltvedt S, Almstrom H, Kublickas M, Grunewald C, Valentin L. Ultrasound dating at 12–14 weeks of gestation. A prospective cross-validation of established dating formulae in in-vitro fertilized pregnancies. *Ultrasound Obstet Gynecol* 2005; **26**: 504–511.
33. Tunon K, Eik-Nes SH, Grottum P, Von Düring V, Kahn JA. Gestational age in pregnancies conceived after in vitro fertilization: a comparison between age assessed from oocyte retrieval, crown–rump length and biparietal diameter. *Ultrasound Obstet Gynecol* 2000; **15**: 41–46.
34. Wennerholm UB, Bergh C, Hagberg H, Sultan B, Wennergren M. Gestational age in pregnancies after in vitro fertilization: comparison between ultrasound measurement and actual age. *Ultrasound Obstet Gynecol* 1998; **12**: 170–174.
35. Weisz B, Rodeck CH. An update on antenatal screening for Down's syndrome and specific implications for assisted reproduction pregnancies. *Hum Reprod Update* 2006; **12**: 513–518.
36. Maymon R, Shulman A. Integrated first- and second-trimester Down syndrome screening test among unaffected IVF pregnancies. *Prenat Diagn* 2004; **24**: 125–129.
37. Dugoff L, Hobbins JC, Malone FD, Porter TF, Luthy D, Comstock CH, Hankins G, Berkowitz RL, Merkatz I, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Vidaver J, D'Alton ME. 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.
38. Santolaya-Forgas J, De Leon JA, Cullen HR, Castracane VD, Kauffman RP, Sifuentes GA. Low pregnancy-associated plasma protein-a at 10(+1) to 14(+6) weeks of gestation and a possible mechanism leading to miscarriage. *Fetal Diagn Ther* 2004; **19**: 456–461.
39. Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early pregnancy levels of pregnancy-associated plasma protein a and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. *J Clin Endocrinol Metab* 2002; **87**: 1762–1767.
40. Tong S, Marjono B, Mulvey S, Wallace EM. Low levels of pregnancy-associated plasma protein-A in asymptomatic women destined for miscarriage. *Fertil Steril* 2004; **82**: 1468–1470.
41. Spencer K, Crossley JA, Aitken DA, Nix AB, Dunstan FD, Williams K. The effect of temporal variation in biochemical markers of trisomy 21 across the first and second trimesters of pregnancy on the estimation of individual patient-specific risks and detection rates for Down's syndrome. *Ann Clin Biochem* 2003; **40**: 219–231.
42. Pihl K, Sorensen TL, Norgaard-Pedersen B, Larsen SO, Nguyen TH, Krebs L, Larsen T, Christiansen M. First-trimester combined screening for Down syndrome: prediction of low birth weight, small for gestational age and pre-term delivery in a cohort of non-selected women. *Prenat Diagn* 2008; **28**: 247–253.