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Original Article

Quadruple test parameters in art pregnancies

Nilgün Güdücü[1], Uzay Görmüş[2], Ebru İlhan Güner[3], Ömer Güzel[3], Zehra Neşe Kavak[4]

Departments of [1]Obstetrics and Gynecology, [2]Biochemistry, İstanbul Bilim University, İstanbul, Turkey; [3]Centro Laboratories, Gursel Mahallesi, Kagithane Caddesi, 14/3 Kagithane, Istanbul, Turkey; [4]Department of Obstetrics and Gynecology, Marmara University School of Medicine, Marmara University Hospital, Istanbul, Turkey

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Abstract: Aim: Quadruple test is used for Down's syndrome screening in the second trimester of pregnancy. The aim of this study was to investigate differences in quadruple test parameters between pregnancies achieved by assisted reproductive treatments (ART) and spontaneous conception. Materials and methods: We retrospectively compared levels of alfa-fetoprotein (AFP), unconjugated Estriol (uE3), inhibin-A and hCG and also screen positive test results. Results: Levels of all quadruple test parameters were statistically significantly increased in ART pregnancies when compared to spontaneous pregnancies, AFP was 1.4±0.74 and 1.16±0.53, (p=0.001), uE3 was 1.10±0.37 and 1.00±0.28, (p=0.004), hCG was 1.56±1.04 and 1.26±0.76, (p=0.001), inhibin A was 1.38±0.76 and 1.08±0.57, (p=0.001), screen positive tests were nearly doubled (4.8% and 8.4%). Conclusions: Increased screen positive test results and quadruple test parameters in ART pregnancies may lead to unnecessary amniocentesis.

Keywords: AFP, estriol, inhibin-A, hCG, quadruple test, ART

Introduction

Second trimester Down's syndrome screening with triple test has been replaced by first trimester screening due to higher detection rates, earlier diagnosis of chromosomal abnormalities and use of increased NT as a marker of cardiac abnormalities and other structural defects [1]. Previous studies comparing components of first trimester screening tests between pregnancies achieved by assisted reproductive treat-ments (ART) and spontaneous conception demonstrated some variations, increased free beta human chorionic gonadotropin (hCG) and/or decreased pregnancy associated plasma protein-A (PAPP-A) values have been reported [2-7].

Previous studies also demonstrated that women with pregnancies achieved by ART were less likely to accept invasive testing [8, 9]. Therefore these patients may request a second test in case of a positive first trimester screening test result or may want to increase the detection rate with a second test when the maternal age is older than 35 years. There is evidence that medications and/or technologies used in ART or intrinsic metabolic abnormalities unique to this subgroup of patients affect second trimester serum markers, but some controversies exist between the studies [10-15]. To the best of our knowledge only two studies searched the effect of ART on inhibin-A levels [15, 16]. The aim of the present study was to identify whether the levels of quadruple test biomarkers were affected from ART. Furthermore the rate of screen positive test results of the two groups were compared.

Materials and methods

This study was conducted by searching the database of Centro laboratories for quadruple test results between January 2008 and June 2013. We checked the data entered by physicians for quadruple test. Exclusion criteria were the presence of twin pregnancy and incomplete information about the presence of ART (642 patients were excluded because of incomplete data). Data of singleton pregnancies that gave blood for quadruple test for Down's syndrome screening in the second trimester between 16 and 19 weeks of pregnancy were included. All maternal blood samples were collected on the same day

Table 1. MoM levels of quadruple test parameters in spontaneous and ART pregnancies

	ART pregnancies (n=143)	Spontaneous pregnancies (n=3661)	*p*-value
	Mean±SD (Median)	Mean±SD (Median)	
AFP	1.40±0.74 (1.23)	1.16±0.53 (1.06)	0.001**
uE3	1.10±0.37 (1.05)	1.00±0.28 (0.96)	0.004**
hCG	1.56±1.04 (1.34)	1.26±0.76 (1.07)	0.001**
Inhibin A	1.38±0.76 (1.18)	1.08±0.57 (0.95)	0.001**

Mann-Whitney U Test. *p<0.05. **p<0.01.

with transabdominal ultrasound. We did not investigate information on pregnancy outcome and chromosomal abnormalities and we did not further classify patients according to the type of ART used to achieve pregnancy. The study protocol was in confirmation with the Helsinki Declaration and was approved by the Ethical Committe of İstanbul Bilim University.

Values of alfa-fetoprotein (AFP), unconjugated Estriol (uE3), inhibin-A and hCG were determined by CLIA chemiluminescence, Beckman Coulter Dxl 600. The maternal values of serum biomarkers were expressed as multiples of median (MoM) after correction for maternal weight and adjustment according to biparietal diameter measurement obtained by transabdominal ultrasonography. The software used was Benetech PRA Ver. 2.4.1.1.

Statistical analyses were performed using the NCSS (Number Cruncher Statistical System) 2007 & PASS (Power Analysis and Sample Size) 2008 Statistical Software (Utah, USA). Data showing anthropometric parameters were presented as mean standard deviation. Quantitave parameters showing normal distribution were compared with Student T test, other parameters were compared with Mann Whitney U test. Qualitative data were compared with Fisher's Exact test and Chi-square test with Yates Continuity Correction. Within 95% conficence interval, *p*-values <0.01 and <0.05 were considered statistically significant.

Results

We compared the quadruple test results of 3661 spontaneous and 143 ART pregnancies. There was no statistically significant difference between the mean age of ART and spontaneous pregnancies (30.34±5.26 and 30.16±5.01 respectively, p=0.673). Thirteen patients had insulin dependent diabetes mellitus (IDDM) (0.3%) and 305 patients (8%) were smokers. There was no statistically significant difference between the number of IDDM in ART and spontaneous pregnancies, 2 (1.4%) and 11 (0.3%) respectively, p=0.08. There was no statistically significant difference between the number of smokers in ART and spontaneous pregnancies, 8 (5.6%) and 297 (8.1%) respectively, p=0.352.

In **Table 1** we can see the statistically significant difference between AFP, uE3, inhibin-A and hCG levels of ART and spontaneous pregnancies. Median risk of Down's syndrome was higher in ART pregnancies (1/6170) when compared to spontaneous pregnancies (1/8535), p=0.01. Median risk of Trisomy 18 syndrome was higher in ART pregnancies (1/86400) when compared to spontaneous pregnancies (1/112000), p=0.004. When a cut-off level of 1/250 was accepted for detection of Down's syndrome, 12 ART (8.4%) and 176 spontaneous pregnancies (4.8%) had increased risk for Down's syndrome (p=0.081) (**Table 2**).

Discussion

In this study we determined that all parameters of quadruple test increased statistically significantly in ART pregnancies when compared to spontaneous pregnancies. We detected increased hCG levels in ART pregnancies. Previous studies mostly reported increased hCG levels in the second [10, 11, 15-17] and also in the first trimester of pregnancy [2-7]. Others reported no difference in hCG levels between ART and spontaneous pregnancies [13, 18, 19]. HCG levels rise up to 8-9 weeks of pregnancy and then begin to fall. An explanation proposed for increased hCG levels in ART pregnancies was the high dose of gonadotropins used in ovarian stimulation but there was no association between the total dose of gonadotropins and the values of maternal serum biomarkers in the second-trimester of pregnancy [20]. High levels of hCG in ART was suggested to be related to higher progesterone levels due to multiple corpora lutea [15], but the highest hCG levels

Table 2. Down's syndrome risk of spontaneous and ART pregnancies

Down's syndrome Risk	ART pregnancies	Spontaneous pregnancies	*p*-value
	n (%)	n (%)	
<1/250	12 (8.4%)	176 (4.8%)	0.081
>1/250	131 (91.6%)	3484 (95.2%)	

Chi-square Test with Yates Continuity Correction.

were in frozen embryos where no multiple corpora lutea existed [14, 20] and also in women who conceived after oocyte donation without ovulation induction [12]. Increased hCG levels in ART pregnancies was attributed to delayed maturation of the fetoplacental unit [21] or could be explained with an aberrant feto-placental unit metabolism in ART pregnancies [22].

In previous studies, either lower [10, 18] or unchanged [11-13, 15, 19] AFP levels were detected in ART pregnancies when compared to spontaneous pregnancies. AFP was reported to increase in pregnancies achieved via oocyte donation [16, 23], but levels were normal in IVF pregnancies [11, 12]. Although AFP levels were reported to increase with maternal age [15] the increased AFP levels in pregnancies achieved via oocyte donation [12, 23] could not be explained with high maternal age. Because of its previously reported role in angiogenesis of the fetomaternal unit [24] and in poor pregnancy outcome [25, 26], abnormal placentation or an intrinsic metabolic abnormality that also played role in infertility could be a more reasonable mechanism. Tendency of false positive screening results to repeat in subsequent pregnancies [27] also supports the idea of metabolic abnormality. In this study we found increased AFP levels in ART pregnancies, as Perheentupa et al but because that we did not examine subgroups of ART, we could not explain whether the increase was related to oocyte donation (Oocyte and sperm donation are forbidden in Turkey, but patients travel to other countries for the procedure) or frozen embryo transfer.

In this study uE3 levels were statistically significantly increased in the ART group, previous studies reported lower [10, 15], higher [16] or unchanged [13, 19] uE3 levels. Previously only two studies reported about inhibin-A levels in IVF pregnancies and levels were similar to spontaneous pregnancies in one study [15] and in the other inhibin-A levels were increased [16]. In this study we detected increased inhibin-A levels in ART pregnancies. Inhibin-A is a product of fetal membranes, villi and other tissues including the ovaries [28]. IVF pregnancies were reported to be associated with a higher risk of preterm delivery, SGA and preeclampsia [11, 17]. Increased inhibin-A level was reported as the maternal serum biomarker that was most commonly associated with preeclampsia [29]. Premature accelerated differentiation of the villous cytotrophoblasts was proposed as the mechanism that lead to increased levels of inhibin-A and hCG in the second trimester of pregnancy [30].

In this study the screen positive rate for Down's syndrome was doubled in ART pregnancies when compared to spontaneous pregnancies, similar to previous studies [10, 11, 15]. Some other studies reported no difference in serum biomarkers and as a result in Down's syndrome screen positive rate [13, 19]. As we did not investigate pregnancy outcomes, we could not report false positive rates. Increased screen positive rates lead to higher invasive testing and anxiety, which might be more intolerable in ART pregnancies.

The main limitation of this study was incomplete information on pregnancy outcome and chromosomal abnormalities. Another limitation was unknown ART type used to achieve pregnancy. Also various etiologies and broader indication rates for IVF were reported to end up in different results and screen positive rates [12, 20] and ICSI pregnancies had a higher rate of chromosomal abnormalities [31].

In conclusion quadruple test results of ART pregnancies gave higher than expected screen positive results and serum levels of all four biomarkers were increased. When we consider that most of these women achieved their pregnancy after a long-standing infertility and treatment, avoiding unnecessary amniocentesis might be more important for this subgroup of patients. Nowadays screening with cell-free fetal DNA in maternal serum seems to be a better option in this subgroup of pregnants.

Disclosure of conflict of interest

None to declare.

Address correspondence to: Dr. Nilgün Güdücü, Department of Obstetrics and Gynecology, İstanbul Bilim University, Kisikli Cad, No: 106 Altunizade 34696, İstanbul, Turkey. Tel: +90 0533 6404010; Fax: +90 02163250104; E-mail: nilgun.kutay@gmail.com

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