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Maternal Serum Alpha-Fetoprotein in Normal Pregnancy at 11–13 Weeks' Gestation

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Key Words
First-trimester screening · Alpha-Fetoprotein · Free β-hCG · PAPP-A · Pyramid of prenatal care

Abstract
Objective: To establish a reference distribution of maternal serum alpha-fetoprotein (AFP) at 11–13 weeks' gestation and define the contribution of maternal variables that influence the measured concentration of AFP. ***Methods:*** Serum concentration of AFP at 11–13 weeks was measured in 1,500 singleton pregnancies which were not complicated by hypertensive disorders or diabetes mellitus and resulted in the live birth at or after 37 weeks of phenotypically normal neonates with birth weights above the 5th and below the 95th percentile. Multiple regression analysis was used to account for maternal characteristics that influence the measured concentration of AFP and a distribution of log multiples of the median (MoM) values was fitted. ***Results:*** Log_{10} AFP increased with gestational age, decreased with maternal weight and was significantly affected by maternal racial origin, smoking status and method of conception. Compared with values in Caucasian women who were non-smokers and conceived spontaneously, AFP MoM was on average 23% higher in Afro-Caribbeans and 8% lower in East Asians, 11% higher in smokers and 10% higher in those conceiving by in vitro fertilization. ***Conclusion:*** In normal pregnancies at 11–13 weeks, serum AFP increases with gestational age and is affected by maternal race, weight, smoking status and method of conception.

Introduction

Measurement of maternal serum alpha-fetoprotein (AFP) during the second trimester of pregnancy has been shown to be useful in screening both for aneuploidies and neural tube defects [1–3]. Additionally, in pregnancies with normal fetuses, high serum AFP is associated with increased risk for adverse pregnancy outcome, including fetal death, preeclampsia, preterm delivery and fetal growth restriction [4, 5]. In the last few years, the introduction of successful second-trimester sonographic diagnosis of neural tube defects [6, 7] and the improved detection of aneuploidies by a combination of fetal nuchal translucency (NT) thickness and maternal serum free β-hCG and pregancy-associated plasma protein-A (PAPP-A) at 11–13 weeks' gestation, compared to second-trimester biochemical testing, have reduced the

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use of serum AFP [8, 9]. However, recent evidence suggests that in pregnancies resulting in spontaneous early preterm delivery, the maternal serum AFP at 11–13 weeks' gestation is increased and this measurement improves the prediction of preterm delivery provided by maternal characteristics and obstetric history alone [10]. Additionally, there is evidence that in trisomy 21 pregnancies, the maternal serum level of AFP is reduced not only in the second trimester but also in the first trimester and it is possible that measurement of serum AFP may improve the performance of the combined test at 11–13 weeks [11].

The aim of this study is to establish a normal range of maternal serum AFP at 11–13 weeks' gestation and define the contribution of maternal variables that influence the measured concentration of AFP. This will form the basis for subsequent studies examining the potential value of serum AFP in the prediction of adverse pregnancy outcome.

Methods

This study is drawn from a large prospective screening study for adverse obstetric outcomes in women attending for their routine first hospital visit in pregnancy at King's College Hospital, London, UK. In this visit, which is held at 11+0–13+6 weeks of gestation, we recorded maternal characteristics and performed an ultrasound scan to confirm gestational age from the measurement of the fetal crown-rump length, to diagnose any major fetal abnormalities, and to measure fetal nuchal translucency thickness and maternal serum free β-hCG and PAPP-A as part of screening for chromosomal abnormalities [8, 9, 12, 13]. Additionally, blood was collected for research and the separated plasma and serum were stored at –80°C for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by King's College Hospital Ethics Committee.

Maternal characteristics recorded were age, racial origin (Caucasian, Afro-Caribbean, South Asian, East Asian and mixed), smoking status during pregnancy (yes or no) and method of conception (spontaneous or assisted conception requiring the use of ovulation drugs or in vitro fertilization). The maternal weight and height were measured at the time of screening. Data on pregnancy outcome were collected from the hospital maternity records or the general medical practitioners of the women.

Study Population

During the study period (March 2006 to July 2010) we examined 44,982 singleton pregnancies with a live fetus at 11+0–13+6 weeks. We searched the database and selected pregnancies which were not complicated by hypertensive disorders or diabetes mellitus and resulted in the live birth at or after 37 weeks of phenotypically normal neonates with birth weight above the 5th and below the 95th percentile. On the basis of published data from the second trimester, maternal serum AFP is affected by maternal racial origin, smoking status and method of conception. Consequently, we selected pregnancies with the aim of investigating these effects in the first trimester. We selected at random 1,200 pregnancies that were conceived spontaneously and the women did not smoke, including 300 from each of the four racial groups (Caucasian, Afro-Caribbean, South Asian and East Asian). Additionally, we selected 300 pregnancies from Caucasian women, including 100 from each of the following groups: smokers with spontaneous conceptions, non-smokers who conceived by ovulation induction, and non-smokers who conceived by in vitro fertilization.

Maternal serum AFP was measured using the DELFIA XPRESS analyzer (PerkinElmer Life and Analytical Sciences, Waltham, Mass., USA). None of the samples were previously thawed and refrozen.

Literature Search

We searched MEDLINE and EMBASE from January 1970 to May 2011 to identify studies reporting on the association between serum AFP concentration and maternal characteristics.

Statistical Analyses

The distribution of serum AFP was logarithmically transformed to obtain a symmetric distribution of residuals with approximately constant standard deviation. This was assessed by inspecting histograms and probability plots. Multiple regression analysis of log-transformed values was carried out to determine the maternal characteristics that provide a significant contribution to the measured concentration of AFP and to derive the estimates of parameters required to produce log multiple of the median (MoM) AFP [14]. Prior to performing the multiple regression analysis, maternal weight was centered by subtracting the mean from each value and in case of gestational age, 77 was subtracted from each value to allow for assessment of weekly increase in maternal serum AFP compared to values at 11 weeks. We examined whether the association with the log-transformed value was linear or nonlinear and in the case of maternal weight, a quadratic term was used.

The measured concentrations of serum PAPP-A and free β-hCG were adjusted for maternal characteristics and MoM values were derived as previously described [12]. Correlations of $\log_{10}$ AFP MoM with $\log_{10}$ PAPP-A MoM and $\log_{10}$ free β-hCG MoM were estimated.

Results

Maternal characteristics of the study population are summarized in table 1. Maternal serum AFP concentrations increased with gestational age by an estimated 37% per week (95% confidence interval 27–38%) and decreased with maternal weight (fig. 1, 2). Multiple regression analysis demonstrated that $\log_{10}$ AFP was significantly associated with gestational age at sampling, maternal weight, racial origin, smoking status and method of conception. The regression model for the fitted mean $\log_{10}$ AFP concentration is summarized in table 2.

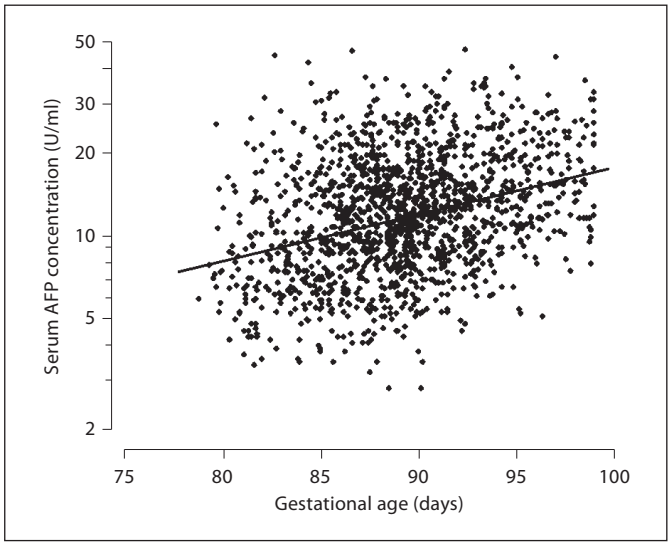

Fig. 1. Relationship of maternal serum AFP concentration with gestational age.

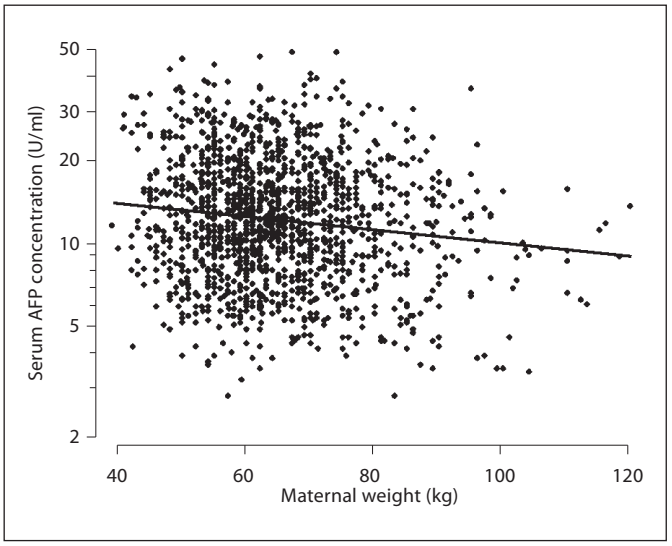

Fig. 2. Relationship of maternal serum AFP concentration with maternal weight.

Table 1. Characteristics of the study population (n = 1,500)

Maternal age, years	31.7 (27.3–35.5)
Maternal weight, kg	62.5 (56.0–71.0)
Gestational age at sampling, days	89 (86–92)
Racial origin	
Caucasian	600 (40.0)
Afro-Caribbean	300 (20.0)
South Asian	300 (20.0)
East Asian	300 (20.0)
Cigarette smokers	100 (6.7)
Method of conception	
Spontaneous	1,300 (86.6)
Ovulation induction	100 (6.7)
In vitro fertilization	100 (6.7)
Gestational age at delivery, weeks	40.2 (39.3–41.0)
Birth weight percentile	44.6 (26.5–63.0)

Values are medians (IQR) or numbers (%).

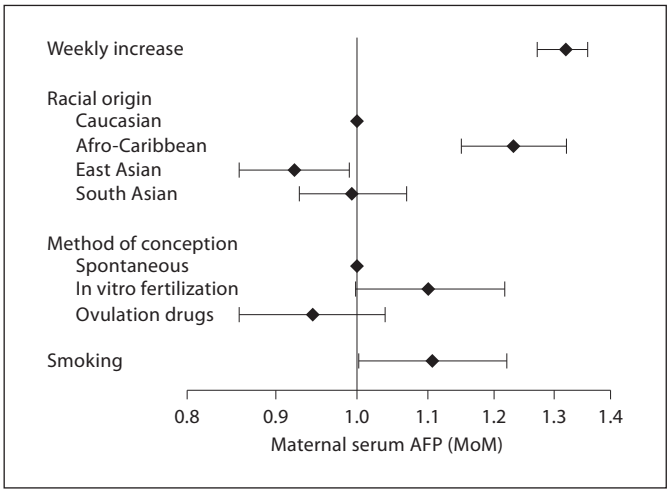

Fig. 3. Estimated effects with 95% confidence interval on serum AFP of different racial origins relative to Caucasians, of smokers relative to non-smokers and of conception by ovulation induction and in vitro fertilization relative to spontaneous conception.

Compared with values in Caucasian women who were non-smokers and conceived spontaneously, AFP MoM was on average 23% higher in women of Afro-Caribbean racial origin and 8% lower in East Asians, 11% higher in smokers and 10% higher in those conceiving by in vitro fertilization (fig. 3).

In the study population the median free β-hCG MoM was 1.02 (IQR 0.69–1.55) and the median PAPP-A MoM was 1.02 (IQR 0.71–1.43). The distributional characteristics of $\log_{10}$ MoM AFP by gestational week and overall are given in table 3.

The results of the literature search on the association between serum AFP concentration and maternal race, smoking status and method of conception are summarized in table 4 [15–39].

Table 2. Fitted regression model for maternal serum AFP in the study population

Coefficient	Log scale		MoM scale	
	estimate	95% confidence interval	estimate	95% confidence interval
Constant	0.834515	0.799686 to 0.869344		6.30501 to 7.401909
Gestational age (days) – 77	0.017114	0.014811 to 0.019416		1.03469 to 1.045722
Maternal weight (kg) – 69	–0.003455	–0.004330 to –0.002580		0.99008 to 0.994077
(Maternal weight (kg) – 69)^2	–0.000015	–0.000056 to 0.000026		0.99987 to 1.000059
Cigarette smoker	0.043371	0.001128 to 0.085615	1.1050	1.0026 to 1.2179
Conception by in vitro fertilization	0.042319	–0.000646 to 0.085285	1.1024	0.9985 to 1.2170
Conception by ovulation drugs	–0.025372	–0.067296 to 0.016552	0.9433	0.8565 to 1.0389
Afro-Caribbean racial origin	0.090205	0.060081 to 0.120329	1.2309	1.1484 to 1.3193
South Asian racial origin	–0.001443	–0.032512 to 0.029626	0.9218	0.8569 to 0.9917
East Asian racial origin	–0.035355	–0.067068 to –0.003643	0.9967	0.9279 to 1.0706

Table 3. Distributional parameters of log10 MoM AFP (estimates and 95% confidence intervals)

Week	n	Standard deviation	Correlation with log MoM PAPP-A	Correlation with log MoM free β-hCG
11	171	0.2103 (0.1902 to 0.2353)	–0.1496 (–0.2931 to 0.0005)	–0.1122 (–0.2580 to 0.0385)
12	858	0.2023 (0.1931 to 0.2123)	–0.0068 (–0.0737 to 0.0601)	–0.0121 (–0.0789 to 0.0549)
13	471	0.1810 (0.1700 to 0.1933)	0.1056 (0.0154 to 0.1941)	0.0599 (–0.0306 to 0.1494)
All	1,500	0.1967 (0.1899 to 0.2040)	0.0084 (–0.0422 to 0.0590)	–0.0020 (–0.0527 to 0.0486)

Table 4. Summary of second-trimester studies on the median serum AFP MoM in women of Afro-Caribbean racial origin compared to Caucasians, smokers compared to non-smokers and pregnancies conceived by in vitro fertilization compared to spontaneous conceptions (in the control groups, the median MoM is 1.0)

Author	Target	Reference	AFP MoM
Afro-Caribbean race compared to Caucasians			
Macri et al., 1976 [15]	46	59	1.07
Crandal et al., 1983 [16]	288	4,822	1.11*
Baumgarten, 1986 [17]	773	14,378	1.20*
Benn et al., 1997 [18]	746	7,560	1.10*
O'Brien et al., 1997 [19]	12,155	56,134	1.13*
Smokers compared to non-smokers			
Thomsen et al., 1983 [20]	120	138	1.22*
Cuckle et al., 1990 [21]	66	319	1.07*
Bartels et al., 1993 [22]	1,018	4,131	1.05*
Spencer, 1998 [23]	5,791	24,936	1.03*
Perona et al., 1998 [24]	3,287	18,835	1.03*
Hafner et al., 1999 [25]	327	1,147	1.00
Crossley et al., 2002 [26]	671	1,514	1.05
Rudnicka et al., 2002 [27]	1,842	6,937	1.05*
In vitro fertilization compared to spontaneous conception			
Barkai et al., 1996 [28]	327	32,623	0.98
Riddert et al., 1996 [29]	67	4,732	0.89*
Frishman et al., 1997 [30]	69	–	0.92
Wald et al., 1999 [31]	151	755	0.99
Lam et al., 1999 [32]	42	2,799	0.94
Bar-Hava et al., 2001 [33]	70	70	1.11
Maymon and Shulman, 2001 [34]	46	–	1.04*
Perheentupa et al., 2002 [35]	96	–	0.98
Maymon and Shulman, 2002 [36]	71	285	1.06*
Räty et al., 2002 [37]	58	6,548	0.95
Muller et al., 2003 [38]	970	21,014	0.94
Rice et al., 2005 [39]	88	596	0.99

* Significant difference from controls.

Discussion

This study has established a reference distribution for maternal serum AFP at 11–13 weeks' gestation. In normal singleton pregnancies, serum AFP increases with fetal gestational age, decreases with maternal weight and is affected by maternal race, smoking status and method of conception. The serum AFP levels were not significantly associated with serum PAPP-A or free β-hCG.

In women of Afro-Caribbean racial origin, serum AFP at 11–13 weeks was on average 23% higher than in Caucasians. This increase is larger than that suggested in studies of second-trimester pregnancies in which the reported level of serum AFP in women of Afro-Caribbean origin was 10–20% higher than in Caucasians [15–19]. Additionally we found that in women of South Asian origin, the levels were similar to those in Caucasians, but in East Asians, serum AFP was reduced by about 8%. There is no obvious explanation for such racial differences in serum AFP, but these have also been reported for other fetoplacental products. In normal pregnancy, serum PAPP-A at 11–13 weeks was 57% higher in women of Afro-Caribbean origin, 3% higher in South Asians and 9% higher in East Asians, compared to Caucasians [12]. Similarly, free β-hCG was 12% higher in women of Afro-Caribbean origin, 9% lower in South Asians and 8% higher in East Asians [12].

In cigarette smokers at 11–13 weeks, serum AFP was on average 11% higher than in non-smokers. In most previous studies of second-trimester pregnancies, the serum AFP in smokers was 2–6% higher than in nonsmokers [20–27]. It therefore appears that the effect of smoking on AFP may decrease with gestational age. Smoking is known to affect serum levels of several biochemical markers used in screening for aneuploidies, including increase in serum inhibin-A and decrease in unconjugated estriol and free β-hCG in the second trimester [27] and decrease in both serum PAPP-A and free β-hCG at 11–13 weeks [12]. The effects of smoking on maternal serum fetoplacental products have been attributed to alterations in placental development and morphology [40–42].

In pregnancies conceived by in vitro fertilization, serum AFP at 11–13 weeks was on overage 10% higher than in spontaneous conceptions, whereas in those conceived by the use of ovulation induction drugs, there was a non-significant decrease in levels. Previous studies in the second trimester reported that in pregnancies conceived by in vitro fertilization, compared to spontaneous conceptions, the levels of serum AFP were either higher, lower or not significantly different [28–39]. Only one previous study examined the effect of ovulation induction in second-trimester serum AFP and although there was no overall significant difference from spontaneous conceptions, there was an increase if treatment was with clomiphene and a decrease when pergonal was used [28]. We are unable to examine further the possible effect of different drugs because in our study the drug regimen for ovulation induction was not recorded.

There is evidence that the standard deviation of $\log_{10}$ MoM AFP decreases with increasing gestational age. At 11 weeks, the estimated standard deviation is 0.21. This decreased to 0.18 at 13 weeks.

In conclusion, this study established a reference distribution for maternal serum AFP at 11–13 weeks' gestation and demonstrates the effect of maternal characteristics on the measured levels. This normal range can be used in further studies investigating the potential value of serum AFP in first-trimester screening for pathological pregnancies.

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References

1 Merkatz IR, Nitowsky HM, Macri JN, Johnson WE: An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. Am J Obstet Gynecol 1984;148:886–894.

2 Cuckle HS: Biochemical screening for Down syndrome. Eur J Obstet Gynecol Rep Biol 2000;92:97–101.

3 Wald NJ, Cuckle H, Brock JH, Peto R, Polani PE, Woodford FP: Maternal serum-alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on alpha-fetoprotein in relation to neural-tube defects. Lancet 1977;1:1323–1332.

4 Dugoff L, Hobbins JC, Malone FD, Vidaver J, Sullivan L, Canick JA, Lambert-Messerlian GM, Porter TF, Luthy DA, Comstock CH, Saade G, Eddleman K, Merkatz IR, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, D'Alton ME; FASTER Trial Research Consortium: Quad screen as a predictor of adverse pregnancy outcome. Obstet Gynecol 2005;106:260–267.

5 Huang T, Hoffman B, Meschino W, Kingdom J, Okun N: Prediction of adverse pregnancy outcomes by combinations of first and second trimester biochemistry markers used in the routine prenatal screening of Down syndrome. Prenat Diagn 2010;30:471–477.

6 Nicolaides KH, Campbell S, Gabbe SG, Guidetti R: Ultrasound screening for spina bifida: cranial and cerebellar signs. Lancet 1986;2:72–74.
7 Ghi T, Pilu G, Falco P, Segata M, Carletti A, Cocchi G, Sanini D, Bonasoni P, Tani G, Rizzo N: Prenatal diagnosis of open and closed spina bifida. Ultrasound Obstet Gynecol 2006;28:899–903.
8 Snijders RMJ, 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. Lancet 1998;351:343–346.
9 Nicolaides KH: Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn 2011;31:7–15.
10 Beta J, Bredaki FE, Calvo JR, Akolekar R, Nicolaides KH: Maternal serum α-fetoprotein at 11–13 weeks' gestation in spontaneous early preterm delivery. Fetal Diagn Ther 2011;30:88–93.
11 Cuckle HS, van Lith JMM: Appropriate biochemical parameters in first-trimester screening for Down syndrome. Prenat Diagn 1999;19:505–512.
12 Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH: First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. Ultrasound Obstet Gynecol 2008;31:493–502.
13 Robinson HP, Fleming JE: A critical evaluation of sonar 'crown-rump length' measurements. Br J Obstet Gynaecol 1975;82:702–710.
14 Draper NR, Smith H: Applied Regression Analysis, ed 2. John Wiley and Sons Ltd., USA, 1981.
15 Macri JN, Weiss RR, Elligers KW, Federgreen WR: Racial differences in maternal serum-alpha-fetoprotein. Lancet 1976;1:207–208.
16 Crandall BF, Lebherz TB, Schroth PC, Matsumoto M: Alpha-fetoprotein concentrations in maternal serum: relation to race and body weight. Clin Chem 1983;29:531–533.
17 Baumgarten A: Racial difference and biological significance of maternal serum alpha-fetoprotein. Lancet 1986;2:573.
18 Benn PA, Clive JM, Collins R: Medians for second-trimester maternal serum alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol; differences between races or ethnic groups. Clin Chem 1997;43:333–337.
19 O'Brien JE, Dvorin E, Drugan A, Johnson MP, Yaron Y, Evans MI. Race-ethnicity-specific variation in multiple-marker biochemical screening: alpha-fetoprotein, hCG, and estriol. Obstet Gynecol 1997;89:355–358.
20 Thomsen SG, Isager-Sally L, Lange AP, Saurbrey N, Schiølier V. Smoking habits and maternal serum alpha-fetoprotein levels during the second trimester of pregnancy. Br J Obstet Gynaecol 1983;90:716–717.
21 Cuckle HS, Wald NJ, Densem JW, Royston P, Knight GJ, Haddow JE, Palomaki GE: The effect of smoking in pregnancy on maternal serum alpha-fetoprotein, unconjugated oestriol, human chorionic gonadotrophin, progesterone and dehydroepiandrosterone sulphate levels. Br J Obstet Gynaecol 1990;97:272–274.
22 Bartels I, Hoppe-Sievert B, Bockel B, Herold S, Caesar J: Adjustment formulae for maternal serum alpha-fetoprotein, human chorionic gonadotropin, and unconjugated oestriol to maternal weight and smoking. Prenat Diagn 1993;13:123–130.
23 Spencer K: The influence of smoking on maternal serum AFP and free beta hCG levels and the impact on screening for Down syndrome. Prenat Diagn 1998;18:225–234.
24 Perona M, Mancini G, Dall'Amico D, Guaraldo V, Carbonara A: Influence of smoking habits on Down's syndrome risk evaluation at mid-trimester through biochemical screening. Int J Clin Lab Res 1998;28:179–182.
25 Hafner E, Stangl G, Rosen A, Schuchter K, Plattner M, Philipp K: Influence of cigarette-smoking on the result of the triple test. Gynecol Obstet Invest 1999;47:188–190.
26 Crossley JA, Aitken DA, Waugh SM, Kelly T, Connor JM: Maternal smoking: age distribution, levels of alpha-fetoprotein and human chorionic gonadotrophin, and effect on detection of Down syndrome pregnancies in second-trimester screening. Prenat Diagn 2002;22:247–255.
27 Rudnicka AR, Wald NJ, Huttly W, Hackshaw AK: Influence of maternal smoking on the birth prevalence of Down syndrome and on second trimester screening performance. Prenat Diagn 2002;22:893–897.
28 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.
29 Ribbert LS, Kornman LH, De Wolf BT, Simons AH, Jansen CA, Beekhuis JR, Mantingh A: Maternal serum screening for fetal Down syndrome in IVF pregnancies. Prenat Diagn 1996;16:35–38.
30 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.
31 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.
32 Lam YH, Yeung WS, Tang MH, Ng EH, So WW, Ho PC: Maternal serum alpha-fetoprotein and human chorionic gonadotrophin in pregnancies conceived after intracytoplasmic sperm injection and conventional in-vitro fertilization. Hum Reprod 1999;14:2120–2123.
33 Bar-Hava I, Yitzhak M, Krissi H, Shohat M, Shalev J, Czitron B, Ben-Rafael Z, Orvieto R: Triple-test screening in in vitro fertilization pregnancies. J Assist Reprod Genet 2001;18:226–229.
34 Maymon R, Shulman A: Comparison of triple serum screening and pregnancy outcome in oocyte donation versus IVF pregnancies. Hum Reprod 2001;16:691–695.
35 Perheentupa A, Ruokonen A, Tuomivaara L, Ryynänen M, Martikainen H: Maternal serum beta-HCG and alpha-fetoprotein concentrations in singleton pregnancies following assisted reproduction. Hum Reprod 2002;17:794–797.
36 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.
37 Räty R, Virtanen A, Koskinen P, Anttila L, Forsström J, Laitinen P, Mörsky 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.
38 Muller F, Dreux S, Lemeur A, Sault C, Desgrès J, Bernard MA, Giorgetti C, Lemay C, Mirallié S, Beauchet A; French Collaborative Group: Medically assisted reproduction and second-trimester maternal serum marker screening for Down syndrome. Prenat Diagn 2003;23:1073–1076.
39 Rice JD, McIntosh SF, Halstead AC: Second-trimester maternal serum screening for Down syndrome in in vitro fertilization pregnancies. Prenat Diagn 2005;25:234–238.
40 Jauniaux, E, Burton, GJ: The effect of smoking in pregnancy on early placental morphology. Obstet Gynecol 1992;79:645–648.
41 Demir R, Demir AY, Yinanc M: Structural changes in placental barrier of smoking mother. Path Res Pract 1994;190:656–667.
42 Genabacev O, Bass KE, Joslin RJ, Fisher SJ: Maternal smoking inhibits early human cytotrophoblast differentiation. Reprod Toxicol 1995;9:245–255.