Journal of Perinatology (2013) 33, 836–840

www.nature.com/jp

ORIGINAL ARTICLE

Perinatal outcomes in euploid pregnancies with 'double-positive' first trimester prenatal screening for trisomy 18 and 21

LM Yee[1], SG Valderramos[2], S Pena[2], YW Cheng[2] and K Bianco[2]

OBJECTIVE: The objective of this study was to investigate whether women who screened positive for both trisomy 18 (T18) and trisomy 21 (T21) yet had euploid karyotypes were at increased risk for adverse pregnancy outcomes.
STUDY DESIGN: This was a retrospective cohort study of women who had first trimester aneuploidy screening. Double-positive subjects had risks greater than screening cutoffs for T21 and T18 and confirmed euploid karyotypes. Singleton subjects were matched 1:2 by maternal age to controls with normal screening. Perinatal outcomes were investigated using *t*-tests and χ^2-tests; statistical significance was set at $P<0.05$.
RESULT: Of 9733 women who had first trimester screening, 33 euploid pregnancies screened positive for both T21 and T18. Compared with controls, these study subjects were more likely to have abnormalities identified by prenatal ultrasounds, including renal, fetal membrane and fluid, as well as multiple anomalies ($P=0.01$). In addition, double-positive subjects had a lower mean gestational age at birth ($P=0.02$) and lower mean birth weight ($P=0.03$) than controls. Maternal outcomes were not significantly different.
CONCLUSION: Pregnancies with double false-positive first trimester aneuploidy screening were associated with pregnancy/fetal abnormalities.

Journal of Perinatology (2013) **33,** 836–840; doi:10.1038/jp.2013.88; published online 25 July 2013

Keywords: aneuploidy screening; first trimester screening; false-positive screening; ultrasound abnormalities; nuchal translucency

INTRODUCTION

First trimester genetic screening programs utilizing nuchal translucency (NT) measurements and the biochemical serum markers pregnancy-associated plasma protein-A (PAPP-A) and beta-human chorionic gonadotropin have become widespread since their introduction in the 1990s.[1] The combination of maternal age, first trimester serum screening, plus the NT allows for an efficient and sensitive early prediction of aneuploidy risk in the fetus and offers a high detection rate for both Trisomy 21 (79–89% with a 5% false-positive rate) and trisomy 18 (93% at a 0.2% false-positive rate).[2–7]

Beyond the primary role of screening in predicting aneuploidy, several studies have shown that abnormal screening results are associated with an increased risk of adverse perinatal outcomes.[8–9] Earlier studies of false-positive screening for trisomy 21 demonstrated an increased risk of preterm delivery, hypertensive disorders of pregnancy, small for gestational age newborns and intrauterine fetal demise.[10–12] Abnormal levels of PAPP-A and/or beta-human chorionic gonadotropin are associated with increased risk of preterm birth and pre-eclampsia, suggesting the abnormal analytes are markers of possible abnormal placentation.[13,14] A thickened NT is also independently associated with adverse perinatal outcomes, including preterm birth, miscarriage, cardiac defects and inborn errors of metabolism, even in the setting of normal euploid karyotype.[8,15–18]

Yet, scant evidence exists for implications of a combined positive first trimester screen for both trisomy 21 and trisomy 18. One group described perinatal outcomes of 32 pregnancies with 'double-positive' second trimester screen for both trisomies 21 and 18 and observed high proportions with chromosomal abnormalities (37%), spontaneous fetal loss (25%), and structural anomalies (6%).[19] Ten pregnancies (31%) ended in live birth although five (16%) had associated perinatal complications.[19] This group found that 84% of women with a double-positive screen experienced an unfavorable outcome; however, this cohort included aneuploidy fetuses, indicating 'true positive' cases.[19] Thus, while this study sheds light on the prognosis of pregnancies with double-positive screen identified during the second trimester screen, these results cannot be inferred to those pregnancies that were euploid (false-positive screens) and those utilizing first trimester screen.

As there exists little information regarding double-positive first trimester screen in confirmed euploid gestations, we sought to investigate whether such pregnancies were at increased risk for adverse pregnancy outcomes compared with women who screened negative for both trisomies.

METHODS

We conducted a retrospective cohort study of all women undergoing first trimester prenatal aneuploidy screening at the University of California,

[1]Department of Obstetrics and Gynecology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA and [2]Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA. Correspondence: Dr K Bianco, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, 505 Parnassus Avenue, Box 0132, San Francisco, CA 94143, USA.
E-mail: biancok@obgyn.ucsf.edu
The results of this study were presented at 32nd Annual meeting of the Society of Maternal Fetal Medicine in Dallas, Texas on 11 February 2012.
Received 16 January 2013; revised 9 June 2013; accepted 13 June 2013; published online 25 July 2013

San Francisco (UCSF) Prenatal Diagnosis Center between 2004 and 2009. The UCSF Prenatal Diagnosis Center cares for women from a wide geographic area in Northern California. Before 2009, the UCSF Prenatal Diagnosis Center performed aneuploidy screening through a single outside reference laboratory. Since March 2009, the California Department of Public Health Genetic Diseases Screening Program centralized all aneuploidy screening through the California Prenatal Screening Program. The study period was chosen before this change in order to maintain reference lab consistency. Microarray data was not collected during the study period. The Committee on Human Research at UCSF approved this study.

Patients underwent first trimester screening with serum measurements of beta-human chorionic gonadotropin and PAPP-A between 10–13 weeks gestation. NT assessment was performed by trained Fetal Medicine Foundation Program-certified ultrasonographers at the UCSF Prenatal Diagnostic Center according to standard protocols. First trimester risk was calculated from measurements of NT, the two serum markers, maternal age, ethnicity/race, and weight. Karyotype analysis was performed by chorionic villous sampling (CVS) between 10–13 weeks gestation or by amniocentesis beyond 15 weeks gestation.

Double-positive subjects were women with a singleton pregnancy undergoing first trimester aneuploidy screening with a result positive for both trisomy 21 (Down Syndrome) and trisomy 18 (Edwards Syndrome) and with a confirmed euploid fetus. All women classified as double-positive subjects had confirmatory diagnostic procedures (amniocentesis or CVS) at UCSF. Thresholds for a 'positive' screen with the reference lab in use during this period were results $>1{:}300$ for trisomy 21 and $>1{:}150$ for trisomy 18. Women who did not have karyotype analysis by CVS or amniocentesis and those who had an abnormal karyotype were excluded. All subjects and controls were singleton gestations. Deliveries of continuing pregnancies all occurred at UCSF; however, some pregnancy terminations took place at outside institutions. When this occurred, pregnancy outcomes were reported to the UCSF Prenatal Diagnosis Center as a part of the Center's standard protocol.

Controls in this study were matched 1:2 by maternal age. Control women similarly underwent first trimester aneuploidy screening at UCSF during 2004–09 and received negative aneuploidy screening results for both trisomies. Controls had screens $<1{:}300$ for trisomy 21 and $<1{:}150$ for trisomy 18, based on the same first trimester screening strategy as cases. These women did not necessarily undergo amniocentesis or CVS for karyotype confirmation as they all screened negative. Deliveries of all controls took place at UCSF.

All double-positive subjects and controls were identified by review of the UCSF Prenatal Diagnosis Center patient database. Identification of control patients was conducted as a random selection identified by a blinded investigator. Database review identified patient demographic characteristics, NT results, calculated trisomy 21 risk, calculated trisomy 18 risks, type of diagnostic procedure and confirmatory karyotype results. The information from the UCSF Prenatal Diagnosis Center database was linked with the inpatient and outpatient electronic medical records at UCSF to identify perinatal outcomes. Perinatal outcomes examined included: abnormal ultrasound findings on second trimester fetal anatomy survey, pregnancy outcomes, and maternal/neonatal outcomes. Pregnancy outcomes included: live birth, spontaneous abortion and therapeutic abortion. No intrauterine fetal demises beyond 20 weeks gestation occurred. In continuing pregnancies, neonatal outcomes of interest included: birth weight, 5-min Apgar scores, gestational age at delivery and hospital length of stay. Maternal outcomes of interest included: mode of delivery, diagnosis of pre-eclampsia and gestational diabetes mellitus. Perinatal outcomes were then compared between double-positive subjects and controls using two-sample *t*-tests (for continuous outcomes) and Pearson χ^2-statistic (for categorical outcomes). A *P*-value of <0.05 was used to indicate statistical significance. Abnormal ultrasound findings are also described.

A total of 9773 women underwent first trimester aneuploidy screening during the study period. Fifty-six women screened positive for trisomies 21 and 18. Of these, 23 women were excluded for following reasons: 2 for lack of confirmed karyotypes, 1 for karyotype of 45,X and 20 for trisomy karyotypes (13 had trisomy 21, 5 had trisomy 18, 1 had trisomy 13 and 1 had trisomy 9). One excluded woman with an abnormal karyotype (trisomy 21) had a twin gestation. All others were singletons. Among the 20 women with confirmed trisomy, three pregnancies ended in spontaneous abortion and 17 ended in therapeutic termination of pregnancy. Of the three pregnancies ending spontaneously, two were before 14 weeks and one, the twin gestation, ended at 17 weeks after she developed chorioamnionitis following selective reduction of the affected trisomy 21 twin. The 45,X gestation ended with an intrauterine fetal demise at 22 weeks, and the two excluded women without karyotype confirmation had normal term births.

RESULTS

Of the 9733 women who underwent first trimester aneuploidy screening during the study period, 33 identified double-positive subjects and 66 maternal-age matched controls were included for analysis. Mean age was 36. Ethnicity distribution is shown in Table 1.

Among the 33 subjects, 28 experienced a live birth, 2 had spontaneous abortions and 3 underwent therapeutic abortions (Table 2). Among the controls, one experienced spontaneous abortion at 12 weeks; all others experienced a live birth. Double-positive subjects experienced a statistically significant decreased number of live births ($P=0.02$). Of the two spontaneous losses, one occurred at 18 weeks due to spontaneous septic abortion; the other was at 17 weeks due to ruptured membranes and chorioamnionitis 2 weeks after placement of a history-indicated cerclage. Two cases chose termination due to abnormal ultrasound findings of cystic hygroma in one patient and multiple anomalies in the other. The third termination in the case group was an elective procedure for reasons not documented in the medical record. Of the live births, mean birth weight was lower for double-positive subjects compared with controls (3402 vs 3125 g, $P=0.03$) and double-positive subjects delivered approximately 1 week earlier ($P=0.02$). However, the rates of preterm birth (<37 weeks) and low birth weight neonates were not different between groups ($P=0.20$ and 0.28, respectively). In addition, neonatal

Table 1. Population demographics

	Controls N = *66*	*Double-positive subjects* N = *33*	P-*value*
Age (mean)	36	36	—
Ethnicity (%)			008
White	43 (65.1%)	11 (33.3%)	
Black	3 (4.5%)	1 (3.0%)	
Latina	6 (9.1%)	3 (9.1%)	
Asian	14 (21.2%)	18 (54.5%)	

Table 2. Outcomes of double positive with euploid karyotype subjects versus controls with low-risk screening

	Controls N = 66	*Double-positive subjects* N = *33*	P-*value*
Pregnancy outcome (%)			0.02
Live birth	98.5%	84.8%	
Spontaneous abortion	1.5%	6.1%	
Therapeutic abortion	0%	9.1%	
Abnormal US findings (%)	13.9%	36.7%	0.01
Gestational age (mean weeks)	39.5	38.5	0.02
Birth weight (mean g)	3402	3125	0.03
Preterm delivery <37 weeks (%)	6.2%	14.3%	0.20
Low birth weight (%)	4.6%	10.7%	0.28
5 Min Apgar <7 (%)	3.1%	3.6%	0.90
Pre-eclampsia (%)	7.7%	10.7%	0.63
Gestational diabetes (%)	10.8%	10.7%	0.99
Cesarean delivery (%)	33.9%	21.4%	0.23
Neonatal length of stay (mean days)	3.32	5.08	0.19

length of stay was greater for the double-positive subjects (mean 5.08 days, median 2 days, s.d. 9.32 days, range 1–47) compared with the controls (mean 3.32 days, median 2 days, s.d. 3.04 days, range 1–19), although this was not a statistically significant difference ($P = 0.188$). There were no significant differences between Apgar scores or maternal outcomes.

The likelihood of abnormal ultrasound findings was increased among double-positive subjects (36.7% vs 13.9%, $P = 0.01$) compared with controls. The findings noted in the control group were more likely to be soft markers for aneuploidy. In the control group, there were five cases of isolated echogenic intracardiac focus, one isolated choroid plexus cyst, one finding of intragastric debris, one two-vessel umbilical cord and one with both an echogenic intracardiac focus and choroid plexus cyst. As these findings were in the setting of normal first trimester screening, some clinicians may not even consider the majority to be true abnormalities. The abnormalities identified among double-positive subjects included additional findings beyond aneuploidy soft markers. Of the 33 double-positive subjects, 3 did not undergo fetal anatomic survey because of termination or fetal loss before 18 weeks. Eleven of the remaining 30 double-positive subjects had abnormal ultrasound findings. These included: one echogenic intracardiac focus and pelviectasis; three isolated pelviectasis; one pelviectasis with bilateral ureteropelvic junction obstruction, polyhydramnios and hydronephrosis; three cystic hygromas, including one with ventriculomegaly; one polyhydramnios and chorion–amnion separation; one pericardial effusion; and one with oligohydramnios, dysplastic kidney, abdominal calcifications, dilated umbilical vein and abnormal feet. In following these neonates' short-term outcomes, the control group had seven neonates with respiratory distress or decreased respiratory effort, one with thrombocytopenia/anemia in the setting of infection, and two with hyperbilirubinemia. The double-positive group of neonates included three with normal prenatal ultrasounds who had growth restriction, one of whom developed cerebral palsy. One double-positive neonate with a normal ultrasound had apnea, hyperbilirubinemia and a subdural hematoma following an emergent cesarean delivery. A neonate with bilateral pelviectasis on ultrasound had respiratory distress and hyperbilirubinemia from prematurity, as did the neonate with the chorion–amnion separation and polyhydramnios. The neonate with the prenatal finding of bilateral ureteropelvic junction obstruction was additionally noted to have a sacral dimple.

Lastly, NT results differed between the groups. In the control group, mean NT was 1.51 mm (mean 1.4 mm, s.d. 0.37, range 1–2.5 mm), whereas in the double-positive group, mean NT was 3.35 mm (mean 3.25 mm, s.d. 1.3, range 1–6.8), $P < 0.001$. Within the double-positive group, 91% of the double-positive subjects with abnormal ultrasound findings had an abnormal NT (mean 4.16 mm, median 3.8 mm, s.d. 1.47 mm, range 1.7–6.8 mm). Double-positive subjects without ultrasound abnormalities had a lower mean NT measurement of 2.92 mm (median 3.1, s.d. 0.98 mm, range 1–5.4 mm, $P = 0.008$). However, not all adverse outcomes were in the setting of an abnormal NT; both double-positive subjects with second trimester spontaneous losses actually had normal NT measurements.

DISCUSSION

This study investigated perinatal outcomes among women with euploid pregnancies who screened positive for both trisomy 18 and 21. Large trials such as the First- and Second-Trimester Evaluation of Risk trial have reported on the likelihood of false-positive screening, although outcomes for these pregnancies are not reported. The First- and Second-Trimester Evaluation of Risk trial identified that 98% (50 of the 51) of the women who screened false-positive for trisomy 18 in the first trimester also screened false-positive for trisomy 21.[20] Further, although prior studies have found that abnormal serum analytes are predictive of adverse perinatal outcomes, scant literature exists regarding implications for double false-positive first trimester screens. We identified increased risk of ultrasound findings, as well as potential increased perinatal risks in the double-positive population. The mechanism behind this effect likely is multi-fold: first, elevated NT has clearly been shown to be related to both fetal anomalies and adverse pregnancy outcomes, as previously discussed.[8,15–18] Second, the biochemical components of the first trimester screen include PAPP-A and beta-human chorionic gonadotropin, which are analytes that are secreted to maternal circulation from the placental trophoblast. A depressed PAPP-A is associated with abnormal placentation, which increases the risk of such adverse pregnancy outcomes as pregnancy loss, growth restriction, pre-eclampsia and preterm birth.[8] The false-positive screen for both trisomy 18 and 21 can be a result of a low PAPP-A, enlarged NT or combination of factors; regardless of the driving factor, it is likely that the unifying mechanism behind this double-positive effect is the presence of abnormal placental functioning.

We observed that this group was at risk for abnormal ultrasound findings compared with controls with low-risk screening. Our findings may even underestimate the differential rate of ultrasound abnormalities between groups, as the abnormalities noted in controls may not be universally considered clinically significant. This finding suggests the markers used to screen for aneuploidy may be 'falsely' positive due to the presence of non-trisomy-associated fetal anomalies. We additionally noted that the double-positive subjects with ultrasound abnormalities had a greater mean NT than those without such findings. The elevated NT in the double-positive patients likely acts as a marker for abnormal placental functioning and/or other fetal anomalies. The NT may be a key differentiating first trimester marker in identifying pregnancies at risk of abnormalities.

The risk of ultrasound abnormalities in double-positive women is of particular clinical interest in this rapidly evolving era of prenatal screening and diagnostic technologies. Although not performed during this time period, microarray analysis may be revealing in the subgroup with anomalies. Recent data suggest microarray analysis may identify clinically significant cytogenetic information beyond that provided by karyotyping.[21] Further, cell-free fetal DNA analysis has been found to be a highly effective tool for aneuploidy screening and is being adopted widely.[22–24] In 2012 Nicolaides *et al.*[24] reported on the use of cell-free fetal DNA analysis in a low-risk population; although they validated the use of such screening tests for the most common aneuploidies, they additionally emphasized the pivotal role of NT screening in the first trimester. Our data suggest that there may still be a role for first trimester aneuploidy screening even in the era of non-invasive chromosomal evaluation, as components of this screen, such as the NT, may identify women with additional risk. This information may otherwise not be detected at all or until a second trimester ultrasound anatomical survey, should a patient solely undergo cell-free fetal DNA screening. As the field of prenatal diagnosis advances, it will be important to understand how traditional prenatal screening is best integrated with cell-free fetal DNA screening tools.[23,25]

Additionally, double-positive subjects experienced fewer live births than controls. Two had spontaneous losses in the second trimester and two terminated subsequent to abnormal ultrasound findings. Given the very small population, it is difficult to know if differences in live birth rate are clinically significant, and we would encourage further investigation to explore this finding. Among double-positive subjects with continuing pregnancies, we identified an earlier mean gestational age at birth and a lower mean birth weight. Although not statistically significant, the trends in rates of preterm birth and low birth weight also raise the possibility of increased risk of adverse perinatal outcomes for the double-positive cases. However, we lacked the necessary

statistical power to detect significant differences in these findings; for the difference in preterm birth rate identified in this study, our population had a calculated power of only 46% to detect a true difference. A substantially larger number of double-positive subjects would have been required to have sufficient power to detect a difference in preterm birth rate, which is likely impossible in most databases given the rarity of the double-positive outcome. Further data are required to investigate associations between aneuploidy screen abnormalities and risk of adverse perinatal outcomes.

There are limitations to this small study. First, subjects with ultrasound abnormalities may be explained by abnormalities on microarray; however, microarray was not performed during the study period. Second, this double-positive result is uncommon. The small nature of our study allows initial exploration of associations, but a much larger cohort is required to further clarify findings. In addition, although the differences in ultrasound findings or other pregnancy-related information are helpful to clinicians, we acknowledge that the information most useful to parents would be about the long-term status of the child. One limitation of this study is the unavailability of long-term follow-up information about the well-being of the double-positive children; certainly, further information about these children would be worthy of future study.

Further, due to the restrictions of the available databases, we were unable to match cohorts perfectly and unable to collect all information that would be of interest. For example, it was not possible to collect information on all maternal prepregnancy comorbidities. We were additionally unable to match double-positive subjects and controls on the basis of factors such as maternal comorbidities and ethnicity, due to the nature of the available database. As a result, there were some differences between the groups. We noted, for example, a larger proportion of Asians/Asian Americans in the study subject group. Previous literature suggests Asians have a higher likelihood of ultrasound 'soft markers' compared with non-Asians. Although the difference in ethnicity matching is a limitation, the ultrasound findings in the double-positive group actually were more likely to be clinically significant ultrasound findings, rather than soft markers. The control group, with disproportionately fewer Asians, was more likely to have findings of ultrasound soft markers. Nevertheless, we would encourage future work on this issue to explore matching populations on the basis of both ethnicity and medical comorbidities. Finally, it should be noted that the double-positive subjects underwent invasive prenatal diagnostic testing, whereas controls did not; although undergoing a CVS or amniocentesis is unlikely to account for differences in groups, it is a difference between groups that should be noted. That said, we find these limitations acceptable given the exploratory nature of this study and the paucity of existing data on this population.

In summary, there are potential clinical implications for the study findings. Among the most notable findings was the increased rate of abnormal ultrasound findings for double false-positive women. This finding suggests that first trimester screening may still have a role in risk stratification even as we are expanding the use of cell-free fetal DNA technologies. The NT may be an appropriate tool for early identification of women at increased risk who are not otherwise detected by cell-free fetal DNA analysis. The possibility of a screening sequence combining NT, cell-free fetal DNA and second trimester ultrasound deserves further investigation. We would advise providers to consider closer monitoring for double false-positive patients, including careful screening via detailed second trimester ultrasound. Further evidence is required to establish clear guidelines on surveillance for this population. We encourage further research to explore this population and to investigate the role of traditional first trimester aneuploidy screening in this new era of non-invasive chromosomal evaluation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Dr Katherine Bianco is supported by the Reproductive Scientist Development Program Award, NIH, the Eunice Kennedy Shriver National Institute of Child Health and Human Development (K12HD000849) and Clinical investigator Award, NIH, the Eunice Kennedy Shriver National Institute of Child Health and Human Development (K08HD069518-01). Dr Yvonne Cheng is supported by the UCSF Women's Reproductive Health Research Career Development Award, NIH, the Eunice Kennedy Shriver National Institute of Child Health and Human Development (K12 HD001262).

REFERENCES

1 Nakata N, Wang Y, Bhatt S. Trends in prenatal screening and diagnostic testing among women referred for advanced maternal age. *Prenat Diagn* 2010; **30**(3): 198–206.
2 Reddy UM, Wapner RJ. Comparison of first and second trimester aneuploidy risk assessment. *Clin Obstet Gynecol* 2007; **50**(2): 442–453.
3 Wapner RJ, Thom EA, Simpson JL, Pergament E, Silver R, Filkins K *et al.* for the First Trimester Maternal Serum Biochemistry and Fetal Nuchal Translucency Screening (BUN) Study Group. First-trimester screening for trisomies 21 and 18. *N Engl J Med* 2003; **349**: 1405–1413.
4 Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R *et al.* First-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med* 2005; **353**: 2001–2011.
5 Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine, and Ultrasound Screening Study (SURUSS). *J Med Screen* 2003; **10**: 56–104.
6 Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years prospective experience. *BJOG* 2003; **110**: 281–286.
7 Kagan KO, Wright D, Maiz N, Pandeva I, Nicolaides KH. Screening for trisomy 18 by maternal age, fetal nuchal translucency, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 2008; **32**: 488–492.
8 Goetzl L. Adverse pregnancy outcomes after abnormal first-trimester screening for aneuploidy. *Clin Lab Med* 2010; **30**: 613–628.
9 Gagnon A, Wilson RD, Audibert F, Allen VM, Blight C, Brock JA *et al.* Obstetrical complications associated with abnormal maternal serum markers analytes. *J Obstet Gynaecol Can* 2008; **30**: 918–949.
10 Pergament E, Stein AK, Fiddler M, Cho NH, Kupferminc MJ. Adverse pregnancy outcome after a false-positive screen for Down syndrome using multiple markers. *Obstet Gynecol* 1995; **86**(2): 255–258.
11 Spencer K, Cowans NJ, Avgidou K, Nicolaides KH. First-trimester ultrasound and biochemical markers of aneuploidy and the prediction of impending fetal death. *Ultrasound Obstet Gynecol* 2006; **28**(5): 637–643.
12 Ong CY, Liao AW, Spencer K, Munim S, Nicolaides KH. First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *BJOG* 2000; **107**(10): 1265–1270.
13 Goetzinger KR, Cahill AG, Kemma J, Odibo L, Macones GA, Odibo AO. First-trimester prediction of preterm birth using ADAM12, PAPP-A, uterine artery Doppler, and maternal characteristics. *Prenat Diagn* 2012; **31**: 1–6.
14 DiLorenzo G, Ceccarello M, Cecotti V, Ronfani L, Monasta L, Vecchi Brumatti L *et al.* First trimester maternal serum PlGF, free bHCG, PAPP-A, PP-13, uterine artery Doppler and maternal history for the prediction of preeclampsia. *Placenta* 2012; **33**(6): 495–501.
15 Piazze J, Anceschi MM, Cerekja A, La Torre R, Pala A, Papa A *et al.* Nuchal translucency as a predictor of adverse pregnancy outcome. *Int J Gynaecol Obstet* 2007; **98**(1): 5–9.
16 Souka AP, Krampl E, Bakalis S, Heath V, Nicolaides KH. Outcome of pregnancy in chromosomally normal fetuses with increased nuchal translucency in the first trimester. *Ultrasound Obstet Gynecol* 2001; **18**: 9–17.
17 Pergament E, Alamillo C, Sak K, Fiddler M. Genetic assessment following increased nuchal translucency and normal karyotype. *Prenat Diagn* 2011; **31**(3): 307–310.
18 Alamillo C, Fiddler M, Pergament E. Increased nuchal translucency in the presence of normal chromosomes: what's next? *Curr Opin Obstet Gynecol* 2012; **24**: 102–108.

19 Summers AM, Huang T, Wyatt PR. Pregnancy outcomes of women with positive serum screening results for Down syndrome and trisomy 18. Letter to the Editor. *Prenat Diagn* 2002; **22**: 266–271.
20 Breathnach FM, Malone FD, Lambert-Messerlian G, Cuckle HS, Porter TF, Nyberg DA *et al.* for the First and Second Trimester Evaluation of Risk (FASTER) Research Consortium. First- and second-trimester screening: Detection of aneuploidies other than Down Syndrome. *Obstet Gynecol* 2007; **110**: 651–657.
21 Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM *et al.* Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med* 2012; **367**: 2175–2184.
22 Norton ME, Brar H, Weiss J, Karimi A, Laurent LC, Caughey AB *et al.* Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012; **207**(137): e1–e8.
23 Allyse M, Sayres LC, King JS, Norton ME, Cho MK. Cell-free fetal DNA testing for fetal aneuploidy and beyond: clinical integration challenges in the US context. *Hum Reprod* 2012; **27**(11): 3123–3131.
24 Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. *Am J Obstet Gynecol* 2012; **207**(5374): e1–e6.
25 Vora NL, Johnson KL, Lambert-Messerlian G, Tighiouart H, Peter I, Urato AC *et al.* Relationships between cell-free DNA and serum analytes in first and second trimesters of pregnancy. *Obstet Gynecol* 2010; **116**(3): 673–678.