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First-trimester ultrasound and biochemical markers of aneuploidy and the prediction of impending fetal death

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KEYWORDS: fetal death; free β-hCG; miscarriage; NT; PAPP-A; prenatal screening; trisomy

ABSTRACT

Objectives *To examine the clinical utility of the first-trimester markers of aneuploidy in their ability to predict future fetal loss.*

Methods *We examined 54 722 singleton pregnancies with no chromosomal abnormality and with complete outcome data that had undergone screening for trisomy 21 by a combination of fetal nuchal translucency (NT) thickness, maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 + 0 and 13 + 6 weeks' gestation. The biochemical markers were converted to multiples of the expected normal median for a pregnancy of the same gestation (MoM) and the measurements of fetal NT were expressed as the difference (delta) from the normal median NT for crown–rump length (CRL). The association between free β-hCG, PAPP-A and delta NT and the incidence of fetal loss prior to 24 weeks, at or after 24 weeks or at any time, was assessed by comparing the relative incidence at a number of MoM or delta NT cut-offs and at various centile cut-offs. At various marker levels the likelihood ratio (LR) for fetal loss was also calculated.*

Results *The rate of fetal loss increased with decreasing maternal serum free β-hCG and PAPP-A and increasing delta NT. At the 5th centile of the normal outcome group for free β-hCG (0.41 MoM) the odds ratio for fetal loss before 24 weeks, at or above 24 weeks and at any gestation was 3.1, 1.8 and 2.6, respectively. The respective values for the 5th centile of PAPP-A (0.415 MoM) were 3.3, 1.9 and 2.8 and for the 95th centile of delta NT they were 2.5, 1.9 and 2.2, respectively. There was almost no correlation between reduced levels (≤ 0.50 MoM) of PAPP-A and reduced levels of free β-hCG in either the normal pregnancy group (r = 0.041) or the group with fetal death (r = 0.072), indicating relatively independent prediction by either biochemical marker.*

Conclusions *Low levels of maternal serum PAPP-A and free β-hCG and increased fetal NT are associated, in the absence of an abnormal karyotype, with an increased risk of impending fetal death. The likelihood ratio profiles provided at various levels of PAPP-A or free β-hCG may be of some help in counseling women with such results and raise awareness among health-care professionals for increased surveillance in such cases. Copyright © 2006 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

In the first trimester of pregnancy the placentally derived biochemical markers pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) are increasingly being used in conjunction with the ultrasound measurement of nuchal translucency thickness (NT) as part of screening programs for trisomy 21 and other aneuploidies, of which approximately 90% of such anomalies can be identified[1–7] for a false positive rate of 5%. Preliminary studies[8] have shown that reduced levels of these biochemical markers – particularly PAPP-A – may be of potential value in identifying those pregnancies that may result in adverse outcome. One such adverse outcome is fetal loss, either due to miscarriage (fetal death prior to 24 weeks' gestation) or fetal loss (death at or after 24 weeks' gestation) prior to labor, which affects approximately 1 in 200 pregnancies. Increased risk of fetal loss is associated with advanced gestational and maternal age, smoking, obesity, parity and a poor obstetric history[9].

In this study we examine the clinical utility of the first-trimester markers of aneuploidy in their ability to predict

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future fetal loss in a large cohort of women prospectively screened during the first trimester.

METHODS

All women booked for maternity care at the following UK hospitals were offered screening for trisomy 21 by a combination of fetal NT and maternal serum free β-hCG and PAPP-A at 11 + 0 to 13 + 6 weeks' gestation: Harold Wood Hospital, Romford (between June 1998 and December 2003), King George Hospital, Goodmayes (between July 2001 and December 2003), Kent and Canterbury Hospital, Canterbury (between July 2002 and December 2003), William Harvey Hospital, Ashford (between July 2002 and December 2003), Queen Elizabeth The Queen Mother's Hospital, Margate (between July 2002 and December 2003), King's College Hospital, London (between January 1999 and February 2000) and those attending The Fetal Medicine Centre, London (between July 1999 and December 2003). Women received an information leaflet about the service and gave details about their demographic characteristics and medical history, which were entered into computer databases (PIA-Fetal Database, ViewPoint, Webling, Germany) at the two testing centers, i.e. Harold Wood Hospital and the Fetal Medicine Centre.

Maternal serum free β-hCG and PAPP-A were measured using the Kryptor analyser (Brahms AG, Berlin, Germany) as previously described[1] and an ultrasound examination was carried out to measure fetal NT and crown–rump length (CRL) and to diagnose any major fetal abnormalities. These measurements were used to calculate the difference from the normal median NT for CRL (delta NT). All scans were carried out by sonographers who had obtained The Fetal Medicine Foundation Certificate of Competence in the 11 + 0 to 13 + 6 week scan (www.fetalmedicine.com). Patient-specific risks were calculated by a multivariate approach using biochemical population parameters and likelihood ratios based on delta NT, as outlined in previous studies[1,10], and the gestational age-related risk of trisomy 21 at the time of screening[11]. Data on pregnancy outcome were obtained from the cytogenetics laboratories, the National Chromosomal Anomaly Register, the patients themselves, their general practitioners or the maternity units in which they delivered. In the case of all women screened by the Harold Wood Laboratory pregnancy outcome was additionally obtained from hospital midwifery or patient administration computer records and matched to the prenatal screening records by a locally derived record linkage software.

All biochemical markers were converted to multiples of the median (MoM) of the expected normal median for a pregnancy of the same gestational age using values established in a previous study[8] corrected for maternal weight[12,13], self-recorded smoking status[12,14] and ethnicity[12,15]. To take account of gestational variation in fetal NT, we expressed the measured fetal NT as the difference from the normal median NT[10,16] at the measured CRL (the delta NT). The delta NT approach has been shown to be more appropriate than the conventional MoM approach for handling NT data and calculating patient-specific risks[10].

The two fetal databases were searched for records which had complete outcome information, including gestational age at delivery (completed weeks), fetal birth weight and pregnancy outcome. The normal pregnancy group was defined as those pregnancies in which a live fetus was delivered after 37 completed weeks of gestation and whose birth weight was above the 10th centile of normal for gestational age[17].

The fetal loss group was classified into two subgroups, those with a viable pregnancy at the time of screening but resulting in miscarriage prior to 24 weeks of gestation and those resulting in fetal death at or after 24 weeks of gestation. Cases included in this study were not part of our previous study[8].

The association between free β-hCG, PAPP-A and delta NT and the incidence of fetal loss prior to 24 weeks, at or after 24 weeks or at any time was assessed by comparing the relative incidence at a number of MoM cut-offs and at various centile cut-offs. At various marker levels the likelihood ratio (LR) for fetal loss was also calculated.

Statistical analysis was carried out with the procedures as described using Analyse-It (Analyse-It Software Ltd, Leeds) a statistical add-in for Excel, or SPSS Version 13 (SPSS Ltd, Woking, UK).

RESULTS

The computer search identified 54 722 singleton pregnancies with no chromosomal abnormality and with complete outcome data. Of this group 6952 (12.7%) were either preterm delivery, low birth weight or birth weight less than the 10th centile for gestation or fetal death. The normal birth group therefore comprised 47 770 pregnancies, while the fetal loss group included 531 cases, of which 76 were excluded because they had amniocentesis or chorionic villus sampling (CVS). Those with fetal loss before 24 weeks' gestation included 230 cases (0.48% of pregnancies) and those with fetal loss at or after 24 weeks included 225 cases (0.47% of pregnancies). The characteristics of the normal pregnancy group and the fetal loss groups are summarized in Table 1.

In pregnancies resulting in fetal loss there was a significant reduction in median PAPP-A and free β-hCG, after $\log_{10}$ transformation of MoMs and comparison using *t*-tests of unequal variance (Table 2). At the 5th centile of normal (0.41 MoM free β-hCG and 0.415 MoM PAPP-A) the odds ratio was increased by three-fold in those with fetal loss before 24 weeks and by two-fold in those with fetal loss at or after 24 weeks. Overall the odds ratios for any fetal loss were 2.6 and 2.8 for free β-hCG and PAPP-A, respectively. At a delta NT equivalent to the 95th centile of normal the odds ratios for those with fetal loss before 24 weeks, at or above 24 weeks and all fetal loss were 2.5, 1.9 and 2.2, respectively. The detection rates at these 5% cut-offs are summarized in Table 3.

Table 1 General characteristics of the study groups

Parameter	*Normal*	*Fetal loss (all)*	*Fetal loss < 24 weeks*	*Fetal loss ≥ 24 weeks*
Maternal age, years				
Median	32.00	34.00	35.87	32.00
Mean	31.48	33.28	35.11	31.41
SD	5.52	5.65	5.01	5.65
Maternal weight, kg				
Median	63.50	66.00	66.00	67.25
Mean	63.67	67.90	67.20	69.13
SD	10.89	12.71	12.22	13.49
% Smokers	10.6	12.3		
% Non-smokers	88.4	87.3		
% Not stated	1.0	0.4		
Ethnic group, %				
Afrocaribbean	5.2	8.0		
Asian	9.1	10.0		
Caucasian	81.4	73.0		
Other	4.1	9.0		
Not stated	0.2			
Previous births, %				
None	43.2	41.0		
1	38.1	28.9		
2	14.1	19.9		
3	3.5	6.9		
4	0.7	1.4		
> 4	0.4	1.7		
Crown–rump length, mm				
Median	62.8	62.7		
Mean	63.2	63.1		
SD	8.6	8.4		

Table 2 Median multiples of the median (MoM) of free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), probability of difference from controls and the odds ratios (OR) at the 5th centile of normal (corresponding to 0.41 MoM free β-hCG and 0.415 MoM PAPP-A)

	Median free β-hCG			*Median PAPP-A*		
Fetal loss	*MoM*	P	*OR 5th centile*	*MoM*	P	*OR 5th centile*
< 24 weeks	0.872	< 0.001	3.10	0.893	< 0.001	3.25
≥ 24 weeks	0.909	0.003	1.80	0.818	< 0.001	1.94
All	0.885	< 0.001	2.55	0.848	< 0.001	2.75

Table 3 Percentage detection rates for fetal loss at the 5th centile of normal for pregnancy-associated plasma protein-A (PAPP-A) and β-human chorionic gonadotropin (β-hCG) at the 95th centile for change in nuchal translucency (delta NT)

Group	*PAPP-A*	*Free β-hCG*	*Delta NT*
Fetal loss < 24 weeks	14.8	12.0	11.6
Fetal loss ≥ 24 weeks	8.4	11.7	9.0
All fetal loss	11.7	11.9	10.3

The individual likelihood ratios for fetal loss at each specific MoM level of PAPP-A and free β-hCG are shown in Figures 1 and 2, in which the individual data points and solid lines representing the best fit through the data points, using an S parameter regression data fit, are shown. For the total fetal loss group the regression coefficients (r) were 0.955 for PAPP-A and 0.943 for free β-hCG. PAPP-A was more predictive of fetal loss at lower PAPP-A MoM than free β-hCG, being some 7 times more likely at 0.2 MoM compared with 4 times more likely. The best fit equation for PAPP-A was described by:

$$\text{LR any fetal loss} = \text{Exp}(0.502 \times ((1/\text{PAPP-A MoM}) - 0.813))$$

and that for free β-hCG by:

$$\text{LR any fetal loss} = \text{Exp}(0.363 \times ((1/\text{free β-hCG MoM}) - 0.483))$$

There was almost no correlation between reduced levels (≤ 0.50 MoM) of PAPP-A and reduced levels of free β-hCG in either the normal pregnancy group ($r = 0.041$)

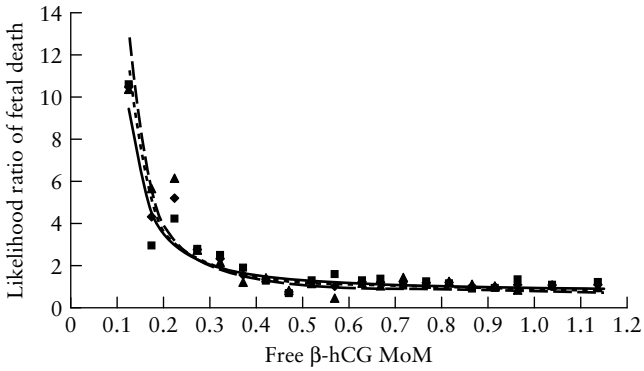

Figure 1 Likelihood ratio for fetal loss at various periods based on the free β-human chorionic gonadotropin multiples of the median (free β-hCG MoM). Symbols represent individual data points, while the smoothed lines represent the best fit to the data. ◆ and ------, all fetal deaths (FD); ■ and — — —, FD < 24 weeks' gestation; ▲ and ———, FD ≥ 24 weeks' gestation.

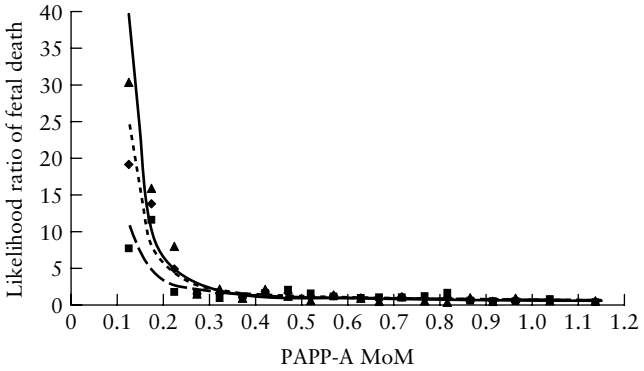

Figure 2 Likelihood ratio for fetal loss at various periods based on the pregnancy-associated plasma protein-A multiples of the median (PAPP-A MoM). Symbols represent individual data points, while the smoothed lines represent the best fit to the data. ◆ and ------, all fetal deaths (FD); ■ and — — —, FD < 24 weeks' gestation; ▲ and ———, FD ≥ 24 weeks' gestation.

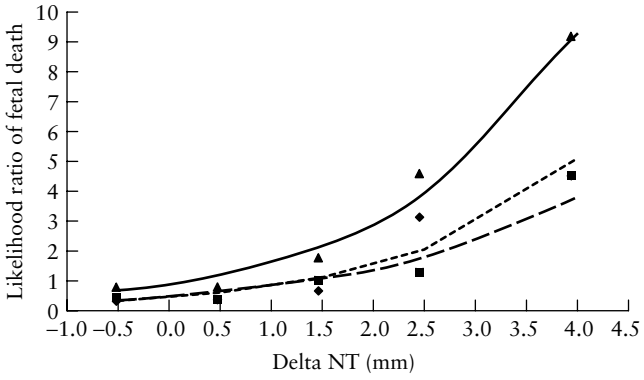

Figure 3 Likelihood ratio for fetal loss at various periods based on the difference from the normal median nuchal translucency for crown–rump length (delta NT). Symbols represent individual data points, while the smoothed lines represent the best fit to the data. ◆ and ------, all fetal deaths (FD); ■ and — — —, FD < 24 weeks' gestation; ▲ and ———, FD ≥ 24 weeks' gestation.

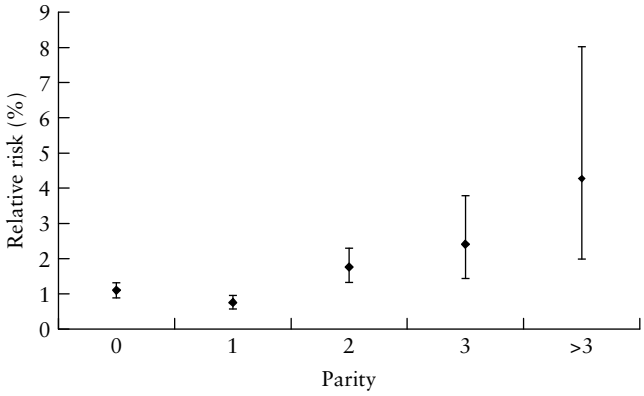

Figure 4 Incidence of fetal loss with increasing parity expressed as percent relative risk; bars represent the 95% CIs.

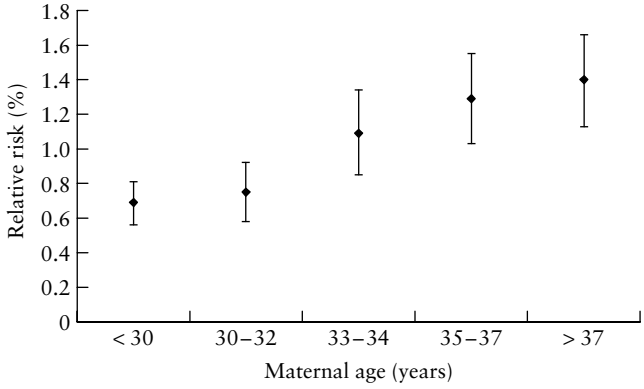

Figure 5 Incidence of fetal loss at any period with increasing maternal age expressed as percent relative risk; bars represent the 95% CIs.

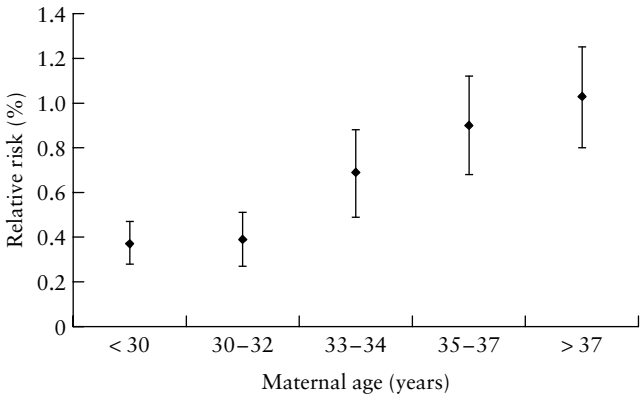

Figure 6 Incidence of fetal loss prior to 24 weeks' gestation with increasing maternal age expressed as percent relative risk; bars represent the 95% CIs.

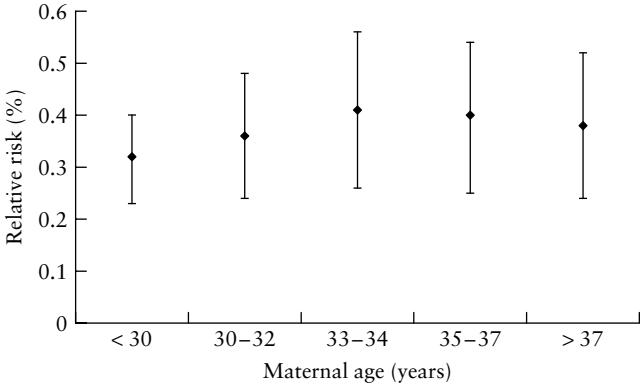

Figure 7 Incidence of fetal loss at or after 24 weeks' gestation with increasing maternal age expressed as percent relative risk; bars represent the 95% CIs.

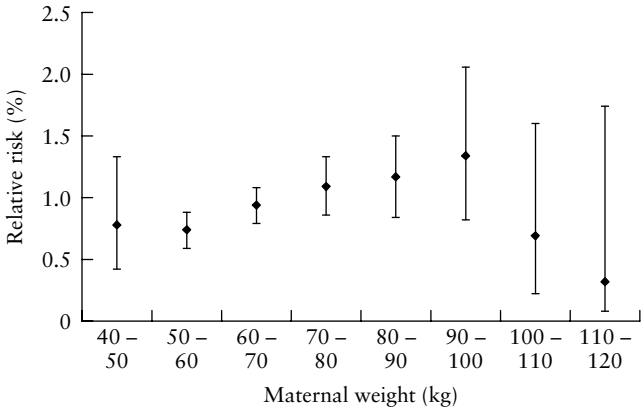

Figure 8 Incidence of fetal loss at any period with increasing maternal weight expressed as percent relative risk; bars represent the 95% CIs.

or the group with fetal death ($r = 0.072$), indicating relatively independent prediction by both biochemical markers. Thus likelihood ratios for PAPP-A and free β-hCG could be combined by multiplication.

When the incidence of fetal loss was examined in relation to delta NT we observed an increasing risk as the delta NT increased in the combined group and also in those with fetal loss before, and at or after, 24 weeks. The data in all three categories appeared to fit an exponential relationship with delta NT, with regression coefficients of 0.974, 0.957 and 0.953 in each case. The individual

grouped data and the fitted curve are shown in Figure 3. The best-fit equation for delta NT was described by:

$$\text{LR any fetal loss} = 0.9132 \times \text{Exp}(0.5804 \times \text{delta NT})$$

At a risk cut-off of the 95th centile or the 99th centile of delta NT the odds ratios for any fetal loss were 2.2 and 7.2, for fetal loss at or after 24 weeks they were 1.9 and 3.8, and for fetal loss prior to 24 weeks they were 2.5 and 10.7.

Examining the impact of parity we observed a step-wise four-fold increase in incidence of fetal loss with increasing parity, as seen in Figure 4. Similarly when we examined the impact of maternal age we observed in the overall group a doubling of the incidence with advancing maternal age, which was primarily as a result of an increased incidence of fetal loss prior to 24 weeks (Figures 5–7). Increasing maternal weight was also shown to double the incidence of fetal loss in the overall group (Figure 8) and this was reflected in both loss before, and at or after, 24 weeks' gestation (data not shown).

DISCUSSION

The data from this study demonstrate that the first-trimester markers of aneuploidy are also predictive of impending fetal loss. These findings are not surprising because low PAPP-A and free β-hCG presumably indicate impaired placentation, and increased NT may be a consequence of a wide range of fetal malformation and genetic syndromes which could result in fetal death.

The data on biochemical markers in relation to fetal death in our current study largely confirm the initial observations we made in our earlier smaller study[8] and confirm the data presented recently from other studies[18–20]. The presentation of our data in terms of likelihood ratios for fetal death at any marker level for either PAPP-A or free β-hCG should enable clinicians to better assess an individual's risk and use this information when counseling the patient on invasive testing or when the results of invasive testing have identified a normal karyotype. Our data also confirm the increased risk of fetal loss associated with advancing maternal age, obesity and parity[9]. The observation of increased NT being associated with increased risk of fetal death is also consistent with our initial findings[21] and those from recent studies[19,20,24].

The potential use of PAPP-A in the prediction of early pregnancy failure was first recognised over 20 years ago by Westergaard *et al.*[22], when they established that at 8 to 14 weeks of pregnancy, levels of PAPP-A were less than the 10th centile of normal in women who showed ultrasonic evidence of fetal life at the time of the investigation. They concluded that the measurement of maternal serum PAPP-A may be of value in the management of threatened abortion, particularly in patients in whom ultrasound examination was normal.

Since the introduction of first-trimester screening for aneuploidy using maternal serum PAPP-A and free β-hCG in conjunction with fetal NT thickness, more data in limited studies have become available – but the clinical usefulness of the data provided is often obscured by the wide range of presentation of the data. Table 4 summarizes the published data with respect to the various biochemical marker levels, odds ratios and detection rates and population sizes in the various studies thus far. In another study, of 170 controls and 97 cases of IUFD in which fetal viability was not confirmed at the time of blood sampling, the median MoMs for PAPP-A and free β-hCG were 0.14 and 0.38, respectively[23]. This study design clearly raises serious questions as to the validity of the data when attempting to predict fetal demise.

For NT it has been shown that the chance of a live birth with no birth defects reduces with increasing NT, such that with an NT of 3.5–4.4 mm the chance was 86%, falling to 31% when the NT was greater than 6.5 mm. For those suffering spontaneous fetal loss the

Table 4 Summary of various studies of pregnancy-associated plasma protein-A (PAPP-A) and β-human chorionic gonadotropin (β-hCG) in intrauterine fetal death

Reference	*Population* (n)	*Cases of fetal loss* (n)	*Loss period (weeks)*	*PAPP-A MoM*	*Free β-hCG MoM*	*OR/RR 5th centile PAPP-A MoM*	*OR/RR 5th centile β-hCG MoM*	*DR 5th centile PAPP-A MoM*	*DR 5th centile β-hCG MoM*	*OR/RR < 0.5 MoM PAPP-A*	*OR/RR < 0.2 β-hCG*
Ong *et al.*[8]	4 351	54	Any	0.755	0.900	1.39	2.07	7.4	9.3		
Smith *et al.*[18,25]	8 817	22	> 24	0.82	0.95	3.6	2.3	18.2	13.6		
Yaron *et al.*[28,29]	1 622	30	< 24							3.78	6.33
Wald *et al.*[24]	1 452	363	Any			4.3	1.9				
Santolaya-Forgas *et al.*[30]	149	10	< 24	0.04	0.90						
De Leon *et al.*[31]		4	> 24	0.80	1.00						
Goetzl *et al.*[20]	7 932	42	< 20			2.8	4.3	12.3	16.7		
		33	> 20			0.6	1.3	3.3	6.6		
Dugoff *et al.*[19]	33 395	294	< 24			2.5		12.9			
		95	> 24			2.15		10.5			
Cuckle *et al.*[32]	229	9	< 12	0.25	0.78						
This study	48 225	230	< 24	0.89	0.87	3.25	3.10	14.8	12.0		
		225	≥ 24	0.82	0.91	1.94	1.80	8.4	11.7		

DR, detection rate; MoM, multiples of the median; OR, odds ratio; RR, relative risk.

incidence increased from 3% with an NT of 3.5–4.4 mm to 16.7% with an NT greater than 6.5 mm[21]. Wald *et al.*[24] also showed that the odds ratio of having a fetal loss was 2.0 at the 95th centile of NT and 6.6 at the 99th centile of NT. Goetzl *et al.*[20] found in fetal death prior to 20 weeks that at the 95th centile of NT the odds ratio was 3.0, rising to 8.3 at the 99th centile, but found odds ratios of only 0.6 and 0 for fetal death after 20 weeks' gestation. Similarly Dugoff *et al.*[19] found that the odds ratio was not significant at the 95th centile of NT for fetal death prior to, or at or after, 24 weeks, but did find an increased odds ratio (3.9) in those with NT above the 99th centile in conjunction with fetal death prior to 24 weeks.

Smith *et al.*[25], based on a small study of 25 stillbirths after 24 weeks' gestation, examined the levels of PAPP-A in relation to the ascribed cause of the stillbirth. They found that a low level of PAPP-A was strongly associated with stillbirth due to placental dysfunction, however they were unable to find any association between free β-hCG levels and risk of stillbirth. Given that both markers are placentally derived proteins and that the findings in nearly all published studies that the relative risk or odds ratio at the 5th centile is of a similar order of magnitude to that for PAPP-A, the observation of Smith *et al.* is probably attributable to the small numbers in their study[25]. Certainly a plausible hypothesis to explain how low PAPP-A can reflect poor placental function and lead to potential fetal death is the role of PAPP-A as an insulin-like growth factor binding protein (IGFBP)-4 and -5 protease[26,27]. Lowered levels of PAPP-A would have less of a protease effect on IGFBPs, leading to higher levels of bound (biologically inactive) insulin-like growth factor-I (IGF-I) and IGF-II, and thus reduced fetal growth.

It is equally possible that lowered PAPP-A and free β-hCG may still just reflect poor placental growth in those fetuses destined to miscarry. Whatever the mechanism, enough information is now available to calculate the risk to the pregnancy based on low PAPP-A, low free β-hCG or high delta NT, or a combination of all three. This information may be useful in counseling women.

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