Midtrimester Maternal Serum Screening After Multifetal Pregnancy Reduction in Pregnancies Conceived by In Vitro Fertilization

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Purpose: Data about the effect of multifetal pregnancy reduction on midtrimester maternal serum levels of α -fetoprotein (AFP), human chorionic gonadotropin (hCG), and unconjugated estriol (uE_3) are scarce and contradictory. Differing gestational ages at fetal reduction, transvaginal versus transabdominal needle insertion, and injection of different fetocidal agents compound the analysis of published data.

Methods: We examined clinical and laboratory data about 27 high-order gestations that were reduced to twins in the first trimester. Fetal reductions were performed transabdominally at 11.41 ± 1.15 weeks' gestation by fetal intrathoracic injection of KCl, and maternal blood sampling was performed at 16.48 ± 1.05 weeks. "Pseudo-risks" for singleton pregnancies were calculated by correcting serum analyte levels for twins.

Results: Twenty-four (88.9%) of 27 patients had maternal serum AFP levels above 2.0 MoM (mean, 4.60 ± 3.48 MoM; range, 1.49–14.85 MoM), however, none of the newborns had structural anomalies. AFP serum levels did not correlate with the number of reduced fetuses or with adverse obstetric outcome. The mean hCG levels were 1.22 ± 0.49 MoM (range, 0.14–2.47), and the mean uE_3 levels were 1.15 ± 0.31 MoM (range, 0.56–1.84). Based on maternal age alone, seven patients (25.9%) would have been offered amniocentesis for a term Down syndrome risk greater than 1:384, whereas combined risk calculations with hCG and uE_3 levels resulted in 1 (3.7%) screen-positive case ($P < 0.01$).

Conclusions: Midtrimester maternal serum AFP levels after multifetal reduction should not be used for screening purposes, whereas incorporation of hCG and uE_3 levels might reduce risk estimates for Down syndrome and the need for invasive testing.

KEY WORDS: MSAFP; hCG; unconjugated estriol; multifetal pregnancy reduction; twins.

INTRODUCTION

The incidence of multifetal pregnancies has increased dramatically since the advent of assisted reproductive technologies (1). These pregnancies are associated with substantially higher perinatal morbidity and mortality, related mainly to preterm birth (2). Multifetal pregnancy reduction from high-order gestations to twins most likely improves perinatal outcome and is, therefore, offered by many centers (3).

Data about the combined screening efficacy of α -fetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (uE_3), and maternal age in twin pregnancies are less robust than in singletons (4). However, estimates based on mathematical modeling indicate that similar false-positive rates, though slightly lower detection rates, can be expected for twin gestations (5). Whether this holds true for twin gestations resulting from multifetal pregnancy reduction is still controversial.

Several studies found midtrimester maternal serum AFP levels to be remarkably elevated following multifetal reduction, suggesting that this analyte could not be used for serum screening (6–8). In contrast, Groutz *et al.* (9) found maternal serum AFP levels to be normal in 24 patients after multifetal reduction and suggested that triple-screen serum analytes could be used without adjustments in this clinical setting. Whether these contradictory findings could be

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explained by differences in study design is unclear. The published studies differed with regard to gestational age at time of multifetal reduction, use of the transvaginal versus the transabdominal route for the procedure, and use of NaCl versus KCl as a fetocidal agent. Another layer of complexity was recently added by findings of Frishman *et al.* (10), who demonstrated that pregnancies conceived after in vitro fertilization (IVF) were twice as likely as naturally conceived gestations to have a positive maternal serum triple-screen test for Down syndrome and neural tube defects. Accordingly, the pooling of pregnancies conceived with different methods of infertility treatments might have contributed to the contradictory findings in the literature.

The purpose of our study was to examine the effect of first-trimester multifetal pregnancy reduction with KCl on midtrimester AFP, hCG, and uE₃ serum levels in twin pregnancies conceived exclusively by IVF.

MATERIALS AND METHODS

Twenty-seven patients who conceived by IVF and embryo transfer were included in this study. Maternal age ranged between 24 and 38 years (mean, 31.26 ± 4.22 years). Prior to multifetal reduction, these pregnancies consisted of 1 sextuplet and 1 quintuplet gestation, 7 quadruplets, and 18 triplets. All pregnancies were reduced to twin gestations by transabdominal ultrasound-guided intrathoracic injection of KCl solution (2 mEq/L) at 11.41 ± 1.15 weeks' gestation, as previously described (11). The needle was kept in place until cardiac standstill was observed for at least 60 sec and then removed.

Maternal blood sampling was performed at 16.48 ± 1.05 weeks. Accordingly, the mean interval between reduction and maternal blood collection was 5.07 ± 1.44 weeks. Prior to blood sampling, a viable twin gestation was confirmed by ultrasound. Maternal serum AFP, hCG, and uE₃ levels were determined by enzyme-linked immunoassay (Delfia, Wallak, Turku, Finland), and results were expressed as multiples of the median (MoM) for gestational age. Measurements from twin pregnancies were interpreted according to the protocol of Wald *et al.* (12). Analyte values were first expressed as multiples of the unaffected singleton population median (MoM). These MoM values were then each divided by two, because at our center normal values for twins are considered twice the normal values for singleton pregnancies.

The corrected MoM values for hCG and uE₃ were then combined with maternal age to calculate a "pseudo-risk" for Down syndrome, as if the values pertained to singleton pregnancies. Patients were considered screen positive when the risk at term exceeded 1:384. Screen-positive rates based on these parameters were compared with "screen-positive" rates based on the maternal age alone, using age 35 as a cutoff for offering amniocentesis. The χ^2 test was used for statistical comparison of screen-positive rates.

We did not collect information about zygosity of the twin gestations. In addition to chart reviews, all patients were contacted by phone after delivery to obtain information about pregnancy complications and newborn outcome.

RESULTS

Individual data about maternal age, number of reduced fetuses, serum analyte levels, and procedure-related and pregnancy complications are listed in Table I. Only 3 (11.1%) of 27 patients had normal maternal serum AFP values (<2.0 MoM) corrected for twin gestations. The mean AFP value was 4.60 ± 3.48 MoM (range, 1.49–14.85 MoM). No structural abnormalities were identified by prenatal targeted ultrasound examinations or neonatal examinations at birth. No correlation could be identified between the number of reduced fetuses and AFP values. Among the 24 pregnancies with elevated maternal serum AFP, 4 (16.7%) were complicated by premature rupture of membranes within 1 week of the fetal reduction, and in 1 case premature rupture of membranes occurred in the third trimester (4.2%), 14 (58.3%) delivered prior to 37 weeks, 8 (33.3%) delivered growth retarded fetuses below the 10th percentile for gestational age (13), and 4 (16.7%) had pregnancy-induced hypertension or preeclampsia develop. One case of intrauterine fetal death occurred in one twin, with an AFP value of 2.52 MoM for this pregnancy.

Maternal serum hCG levels corrected for twin gestations did not exceed 3.0 MoM in any patient. One patient had an hCG level below 0.2 MoM (3.7%). This patient subsequently delivered two karyotypically normal fetuses. The mean hCG levels were 1.22 ± 0.49 MoM (range, 0.14–2.47 MoM).

The mean maternal serum uE₃ levels corrected for twin gestations were 1.15 ± 0.31 MoM (range, 0.56–1.84 MoM).

Five patients underwent amniocentesis based on elevated maternal serum AFP levels and one each for

Table I. Biochemical Profile and Pregnancy Outcome^a

No.	Maternal age	No. of fetuses	AFP (MoM) ^b	HCG (MoM)	uE ₃ (MoM)	Immediate complication	Pregnancy outcome
1	37	3 → 2	1.95	0.91	1.60		PTL
2	33	4 → 2	3.08	0.99	0.56	PROM	IUGR
3	24	3 → 2	3.97	1.95	0.82		
4	31	3 → 2	2.52	1.48	1.09		IUFD, IUGR
5	27	3 → 2	5.24	0.60	1.12		PIH, IUGR
6	28	3 → 2	3.19	1.92	1.43		IUGR
7	38	4 → 2	8.58	0.65	1.00	PROM	
8	27	4 → 2	14.85	1.45	0.90	PROM	
9	30	4 → 2	1.49	1.40	0.66		PTL, IUGR
10	27	3 → 2	2.58	1.37	1.84		
11	36	3 → 2	1.77	1.23	1.04		PIH, IUGR
12	33	3 → 2	2.92	1.96	1.01		PIH
13	37	3 → 2	2.76	1.34	1.01		PTL
14	35	3 → 2	2.07	0.73	1.23		IUGR
15	30	3 → 2	2.90	1.32	1.47		Bleeding 3rd
16	28	4 → 2	4.70	0.84	1.22		PTL, GDM
17	27	3 → 2	3.35	0.14	0.90		IUGR
18	29	3 → 2	4.81	1.21	0.90		
19	34	3 → 2	4.74	1.10	1.40	Bleeding	PIH
20	33	5 → 2	6.72	1.06	0.93	Bleeding	GDM, PTL
21	28	3 → 2	9.4	1.61	1.15		IUGR
22	28	4 → 2	3.35	0.90	1.10		PTL
23	38	6 → 2	3.44	1.31	1.78		PROM, PTL
24	24	3 → 2	11.93	0.87	1.43		IUGR
25	35	3 → 2	5.4	1.00	1.41	PROM	PTL
26	33	4 → 2	2.06	1.16	1.08		PTL
27	34	3 → 2	4.46	2.47	1.08		PTL, IUGR

^a PTL, preterm delivery; PIH, pregnancy-induced hypertension; PROM, premature rupture of membranes; GDM, gestational diabetes mellitus; IUFD, intrauterine fetal demise; Bleeding 3rd, third-trimester bleeding.

^b Data express (MoM) of unaffected pregnancies at the same gestational age.

advanced maternal age and maternal request. No chromosomal abnormalities were found among the 27 patients prenatally or after birth. The mean gestational age at delivery was 35.07 ± 2.77 weeks' gestation (range, 28–39 weeks). The mean birth weight was 2270 ± 585 g (range, 750–3800 g).

Based on maternal age alone, seven patients would have been offered amniocentesis for karyotyping. Based on hCG, uE₃, and maternal age data, one (3.7%) of the patients exceeded a term Down syndrome risk of 1:384. None of the patients were screen-positive for trisomy 18.

DISCUSSION

The main finding of our study is that multifetal pregnancy reduction of high-order gestations to twins causes elevated serum AFP levels at midtrimester maternal blood sampling, whereas hCG and uE₃ levels remain essentially unaffected. Accordingly, even AFP values corrected for twin gestations cannot be used for neural tube defect or Down syndrome screening.

However, hCG and uE₃ levels can be used to reduce substantially the screen-positive rate based on maternal age criteria alone.

With regard to maternal serum AFP levels, our findings are consistent with those in previous reports (6,7,14) but at variance with data presented by Groutz *et al.* (9). Mean AFP levels corrected for twin gestations in our study were 4.6-fold increased, and only 3 of 27 patients had normal AFP levels. Lynch *et al.* (7) similarly reported that midtrimester AFP levels were elevated in all patients following multifetal reduction and correlated positively with the number of reduced fetuses. These findings were subsequently confirmed by Abbas *et al.* (14), who further demonstrated that maternal serum AFP levels correlate negatively with the elapsed time interval from fetal reduction to maternal blood sampling.

In contrast, Groutz *et al.* (9) reported that only 2 of 28 women undergoing first-trimester fetal reduction had elevated maternal serum AFP levels in the second trimester and that both cases were characterized by adverse perinatal outcomes (fetal omphalocele and severe preeclampsia) commonly associated with ele-

vated AFP levels. It has been speculated that the early gestational age (mean, 9.9 ± 0.9 weeks' gestation) at which multifetal reduction was performed in the Groutz study could account for their discrepant findings (15). Indeed, fetal serum AFP levels peak at 12–13 weeks. Furthermore, the total fetal mass of reduced fetuses should be lower at 9–10 weeks in comparison with 11–12 weeks. Both factors would tend to reduce the amount of AFP available for release during the autolytic process and provide for a longer time interval for metabolic clearance of AFP prior to midtrimester maternal blood sampling. However, data from sequential maternal serum testing suggest that even with early fetal reductions, it generally takes 8–12 weeks for AFP levels to return to the normal range (14).

Two technical aspects, however, are distinctly different in the Groutz study in comparison to all other published studies on the subject, including our own: the use of normal saline solution as a fetocidal agent and the transvaginal route for needle insertion. The generally preferred KCl solution for fetal reduction is a hypertonic agent, which could conceivably induce more fetal tissue damage. This, in turn, could increase AFP leakage into the maternal circulation. Furthermore, the transvaginal needle insertion generally leads to reduction of fetuses closest to the cervical os, as opposed to reduction of the fetus closest to the anterior uterine wall with transabdominal procedures. Because the amnion and chorion have not yet fused at the time of the fetal reduction, it is tempting to speculate that some AFP-containing tissue or secretions might leak into the vagina, following transvaginal reduction, as opposed to being systemically absorbed and thereby raising maternal serum levels following the transabdominal procedure.

Because all currently available information about midtrimester maternal serum AFP levels after transabdominally performed multifetal reductions with KCl demonstrates elevated serum AFP levels, it appears that this analyte should not be used for neural tube defect or Down syndrome screening in this setting. Consequently, amniocentesis should probably not be offered routinely for elevated AFP levels after multifetal reduction. Furthermore, use of this serum analyte might falsely lower the risk estimates for Down syndrome. It is important to keep in mind, however, that neural tube defects are more common in twins, even in dizygotic unlike-sexed pairs (16), and a targeted ultrasound examination is therefore indicated.

Elevated AFP levels in twin pregnancies after multifetal reduction in our patient material did not seem to correlate with adverse pregnancy outcome

(Table I), however, sample size limitations need to be considered. Elevated AFP levels in twin pregnancies not resulting from fetal reduction were shown to correlate with risk of fetal and neonatal death, preterm delivery, and twin–twin birth discordance (17). The premature delivery rate in our study population (62.9%) was similar to rates reported from large databases on pregnancies after multifetal reductions (18) but slightly higher than reported for normal twin pregnancies (19).

The aforementioned reservations regarding the use of AFP for screening purposes do not seem to apply to midtrimester maternal serum hCG and uE₃ values, as they pertain to Down syndrome screening. Even though data about the efficacy of triple analyte screening in twins are less robust than in singleton pregnancies, estimates based on mathematical modeling suggest similar false-positive rates but slightly lower detection rates (5). It has been established that standard deviations and correlations between analytes in twin and singleton unaffected pregnancies are not different (12). In our study the median maternal serum hCG and uE₃ values corrected for twin gestations were 1.22 and 1.15 MoM, respectively. Obviously, the limited size of our study group does not allow for the detection of small differences in these median analyte levels in comparison with singleton pregnancies, but these values are reasonably similar to assume that neither hCG nor uE₃ levels are remarkably altered by multifetal reduction. Furthermore, we cannot comment on detection rates estimated for twin pregnancies affected with Down syndrome, because reliable estimates would require at least 30 affected pregnancies. If we were to assume that all twin pregnancies after IVF were dizygotic, and further assumed a prevalence of 1 affected pregnancy per 700 live-born twin pregnancies, we would need to screen 21,000 twin pregnancies following multifetal reduction to establish reliable Down syndrome risk estimates.

Nevertheless, assuming that hCG and uE₃ MoM levels corrected for twin pregnancies are applicable to fetal reduction pregnancies, we were able to reduce the number of amniocenteses offered from 7 (25.9%) of 27 (based on maternal age criteria) to 1 (3.7%) of 27 ($P < 0.01$). Considering that pregnancies conceived by IVF are usually achieved after prolonged infertility, the reduced screen-positive rates are therefore beneficial. Twin pregnancy loss rates after amniocentesis are significantly higher than rates reported from large series of sampled singleton pregnancies (20). In addition, pregnancies after multifetal reduction have already sustained one invasive procedure and have

been reported to carry a 13.1% loss rate between fetal reduction procedure and 24 weeks' gestation (3).

CONCLUSIONS

In conclusion, we found midtrimester maternal serum AFP levels after multifetal pregnancy reduction to be substantially elevated and, therefore, not suitable for neural tube defect or Down syndrome screening. However, hCG and uE₃ levels appear to be unaffected and their incorporation in risk calculations for Down syndrome should be considered. Risk estimates based on maternal age and hCG and uE₃ levels result in significantly lower screen-positive rates, which might reduce the number of amniocenteses performed on these women, who have an already increased risk of miscarriage.

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