

Midtrimester maternal serum inhibin A levels after multifetal pregnancy reduction

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Objective To investigate the relationship between the maternal serum inhibin A concentrations and the number of fetuses. Further, the maternal serum inhibin A levels for twin pregnancies and multiple pregnancies reduced to twins in the second trimester were compared.

Methods Three groups of women with pregnancies following *in vitro* fertilization and embryo transfer were recruited for this study. Groups 1, 2 and 3 included 20 singleton pregnancies, 37 twin pregnancies, and 35 multifetal pregnancies, respectively. In group 3, multifetal reduction was performed during 10–12 weeks of gestation. Blood samples were obtained longitudinally at 10th, 12th, 15th and 18th week of gestation.

Results There was a significant association between the number of fetuses and maternal plasma inhibin A prior to multifetal reduction. The inhibin A levels were not significantly different between twin and multifetal reduced twin pregnancies at 15th and 18th weeks of gestation.

Conclusion In multifetal reduction to twin pregnancies, the maternal serum levels of inhibin A decrease to the level of twin pregnancies during the second trimester. Therefore, inhibin A may be effectively used as a marker for Down syndrome screening in cases of twin pregnancy following multifetal reduction. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: multifetal reduction; inhibin A; second-trimester pregnancy; Down syndrome screening

INTRODUCTION

Maternal serum inhibin A level is raised in Down syndrome pregnancies as compared to normal pregnancies (Aitken *et al.*, 1996; Wallace *et al.*, 1996; Wenstrom *et al.*, 1997) and has been shown to be a useful marker in prenatal screening for Down syndrome during the second trimester of pregnancy (Spencer *et al.*, 1996). Traditionally, triple-test (alpha-fetoprotein (AFP), human chorionic gonadotrophin (HCG), and unconjugated estriol (uE₃)) has been used for Down syndrome screening (Chard and Macintosh, 1995). This test can incorporate inhibin A as a fourth marker for Down syndrome, such that the detection rate for Down syndrome can be improved from 59 to 70%. (Wald *et al.*, 1996).

Data about the combined screening efficacy of AFP, HCG, and uE₃ in twin pregnancies are less robust than in singletons (Barnabei *et al.*, 1995). Down syndrome screening in twin pregnancies can be estimated based on mathematical modeling (Neveux *et al.*, 1996). Inhibin A levels are twice as high in twin pregnancies compared with singleton pregnancies so that its values can be adjusted for Down syndrome screening in twin pregnancies (Watt *et al.*, 1996).

The incidence of multiple pregnancies has increased since the development of assisted reproductive technologies (Schenker *et al.*, 1981). First-trimester multifetal pregnancy reduction from high-order gestations to twins has been often undertaken to decrease the perinatal morbidity and mortality (Evans *et al.*, 1994). Several studies have found midtrimester maternal serum AFP levels to be remarkably elevated following multifetal reduction, suggesting that this analyte cannot be used for serum screening (Grau *et al.*, 1990; Shulman *et al.*, 1996). Goodwin *et al.* reported that a case following the loss of one fetus in a twin pregnancy had an extremely elevated second-trimester serum inhibin A level (Goodwin *et al.*, 2000). Consequently, whether the serum inhibin A used as a marker for Down syndrome screening in twin pregnancy following multifetal reduction remained unknown.

The purposes of this study were to investigate the second-trimester inhibin A levels in twin pregnancies and multiple pregnancies reduced to twins; to examine whether the procedure of multifetal reduction has an effect on maternal serum inhibin A levels; and to evaluate the relationship between the maternal serum inhibin A levels and number of fetuses. To the best of our knowledge, this is the first study to examine the effect of multifetal reduction on maternal serum inhibin A levels in the second-trimester pregnancy.

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MATERIALS AND METHODS

Statistics

Subjects and samples

Three groups of Taiwanese women with pregnancies achieved following superovulation and *in vitro* fertilization and embryo transfer were recruited for studies. Body mass index was between 21 and 28 kg/m². Groups 1 included 20 singleton pregnancies (age range 25–36, median 30) and blood samples were obtained at 10th week of gestation. Group 2 included 37 twin pregnancies (age range 27–39, median 32) and blood sample obtained longitudinally at 10th, 12th, 15th and 18th week of gestation. Group 3 comprised 35 women with multifetal pregnancies (age range 24–39, median 31), undergoing fetal reduction to twin pregnancies (three fetuses, $n = 24$; four fetuses, $n = 6$, five fetuses, $n = 5$). In this group ultrasound-guided embryo reduction to twins was performed by transabdominal injection of potassium chloride into the fetal heart between 10 and 12 week of gestation. Maternal blood samples were obtained before embryo reduction at 10 weeks and at subsequent visits at 12, 15 and 18 weeks. Ovarian stimulation was performed in accordance to a gonadotrophin releasing hormone analogues (Supremon, Hoechst; Frankfurt, Germany) desensitization protocol. All patients gave informed consent to participate in the study. The blood samples were stored at -40°C until assay.

Assays

Dimeric inhibin A concentrations were measured using a commercial available kit which is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) (Serotec, Oxford Bio-Innovarion Ltd, UK). It used the monoclonal antibody specific for the β_A subunit of inhibin. Before the ELISA, samples and standards were mixed with 6% sodium dodecyl-sulphate and heated to 100°C for 3 min, then cooled and incubated at the room temperature with 10% hydrogen peroxide for 30 min. These procedures can enhance the specificity and sensitivity of the ELISA, and even allow analysis of hemolysed samples. The blood samples were assayed in duplicate. The intra-plate and inter-plate coefficients of variation were $<10\%$ and sensitivity of the assay was <20 pg/mL.

Inhibin A levels were expressed as median values (range). Nonparametric test with Kruskal-Wallis was used to determine whether the number of fetuses was significantly associated with inhibin A concentration. Mann-Whitney U test was performed to compare the difference of inhibin A level at 12th, 15th and 18th week of gestation between twin and multifetal reduced twin pregnancies. Statistical analyses were performed with the software SPSS Pack 10.0, differences were considered significant when $P < 0.05$.

RESULTS

Before multifetal reduction, there was a significant association between the number of fetuses and maternal plasma inhibin A ($P < 0.001$); the median (range) inhibin A concentration for fetus numbers are shown in Table 1. Data for inhibin A levels after fetal reduction based on the number of fetuses at conception were shown in Table 2. The circulating levels of inhibin A in women after multifetal pregnancies reduced to twins (Group 3) and nonreduced twin pregnancies (Group 2) pregnancies at 12th, 15th and 18th weeks of gestation were shown in Table 3. The concentrations of inhibin A in Group 2 and Group 3 were converted to multiple of median were presented in Table 4. The inhibin A concentrations were gradually decreased during this period. The inhibin A levels between twin and multifetal reduced twin pregnancies were significant different at only 12th weeks of gestation ($P < 0.05$). Subsequently, inhibin A levels were not significantly different between

Table 1—Maternal serum concentrations of inhibin A (pg/mL) at 10 week of gestation by starting number of fetuses. Values are given as median with the range in parentheses

Number of fetuses	<i>N</i>	Inhibin A concentration
1	20	338.2 (102.8–881.8)
2	37	703.6 (158.2–1723.3)
3	24	1137.7 (551.3–2300.4)
4	6	1640.2 (1335.6–2498.4)
5	5	1801.1 (989.5–2095.3)

Table 2—Maternal serum concentrations of inhibin A (pg/mL) after fetal reduction based on the number of fetuses at conception. Values are given as median with the range in parentheses

Gestation (week)	Number of fetuses			<i>P</i> values
	3 → 2 ($n = 24$)	4 → 2 ($n = 6$)	5 → 2 ($n = 5$)	
12	980.5 (461.2–2010.9)	1021.1 (588.2–1981.4)	755.3 (392.4–1354.7)	0.387
15	467.7 (203.1–1258.6)	708.5 (412.5–939.5)	368.9 (236.3–580.9)	0.109
18	343.6 (208.5–762.4)	395.0 (321.5–594.0)	307.9 (135.0–425.5)	0.130

Kruskal-Wallis test.

Table 3—Maternal plasma levels of inhibin A (pg/mL) in nonreduced twin pregnancies (Group 2, $n = 37$) and multifetal pregnancies reduced to twins (Group 3, $n = 35$). Values are given as median in parentheses. The range and number of cases are shown

Gestation (week)	Group 2	Group 3
12	613.3 (474.2–2010.9) ^a	992.4 (563.3–2217.9) ^a
15	466.3 (189.3–1016.7)	468.8 (203.1–1258.6)
18	352.3 (147.8–723.0)	333.2 (135.0–762.4)

^a $P < 0.05$ (Mann–Whitney U test).

Table 4—Maternal serum levels of inhibin A in nonfetal reduced twin pregnancies (Group 2, $n = 37$) and selective fetal reduced to twin pregnancies (Group 3, $n = 35$). The values are expressed as multiples of the median (range) for gestation of the singleton pregnancy following *in vitro* fertilization and embryo transfer

Gestation (week)	Group 2	Group 3
12	2.05 (1.60–6.78) ^a	3.35 (1.89–7.48) ^a
15	2.07 (0.84–4.51)	2.08 (0.90–5.59)
18	2.04 (2.87–4.25)	1.96 (0.79–4.78)

^a $P < 0.05$ (Mann–Whitney U test).

Table 5—Obstetric complications in nonfetal reduced twin pregnancies (Group 2, $n = 37$) and selective fetal reduced to twin pregnancies (Group 3, $n = 35$)

	Group 2 (n)	Group 3 (n)
Birth weight <10th percentile	8.1% (3)	11.4% (4)
Preterm delivery (<37 weeks)	21.6% (8)	17.1% (6)
Preeclampsia	0	0
Gestational diabetes	2.7% (1)	0

twin and multifetal reduced twin pregnancies at 15th and 18th weeks of gestation.

Obstetric complications in Group 2 and Group 3 were shown in Table 5. There was no significant difference between the two groups in any of complications considered.

DISCUSSION

The finding of this study indicated that in multifetal pregnancies circulating levels of inhibin A increase with number of fetuses, suggesting that feto-placenta unit is the source of inhibin A, confirming previous report (Lockwood *et al.*, 1998). Subsequent to multifetal pregnancy reduction to twins, the maternal serum levels of inhibin A were significantly higher than those of twin pregnancies at 12 weeks of gestation and then fall to the level similar to those of nonreduced twin pregnancies at 15 and 18 weeks of gestation. These data suggest that fetal reduction causes fetoplacental death and therefore some decline in the production of inhibin A by the

surviving placental tissue is similar to the nonreduced twins.

Maternal serum inhibin A levels during pregnancies following multifetal reduction differ from serum AFP levels. In multifetal pregnancies, AFP increase with the number of fetuses. However, following fetal reduction to twins, the AFP concentration remains increased for at least 8 weeks. The most likely explanation for this is that AFP, which is stored in fetal tissues, is released into the amniotic fluid during tissue break down, from where it is absorbed in the maternal circulation (Abbas *et al.*, 1994). Although the levels of inhibin A and AFP are proportionate to the number of fetuses, the differences in maternal circulating levels of inhibin A and AFP following fetal reduction may reflect their main different origins, which are placenta and fetus respectively.

Triple-test screening (AFP, HCG, and unconjugated estriol (uE₃)), performed during the second trimester, has become a common clinical practice to identified pregnancies at risk for chromosomal anomalies (Cuckle *et al.*, 1987). Multifetal reduction of high-order fetal numbers to twins results in elevated serum AFP levels at midtrimester maternal blood sampling, whereas hCG and uE₃ levels remain essentially unaffected (Rotmensch *et al.*, 1999). Maternal serum inhibin A levels for Down syndrome pregnancies are reported to be significantly elevated during the second trimester of pregnancy (Spencer *et al.*, 1996). Further, serum inhibin A levels are around twice as great for twin pregnancies as is the case for singleton pregnancies, such that the value of inhibin A levels can be adjusted for Down syndrome screening in twin pregnancies (Watt *et al.*, 1996). In the present study, serum inhibin A levels did not appear to differ significantly between twin and multifetal reduced twin pregnancies at 15th and 18th weeks of gestation. Although one study has already reported that inhibin A concentrations are not different between reduced and nonreduced twins in the second trimester (Maymon *et al.*, 2006), we provide the novel data concerning the levels of inhibin A at several time points following fetal reduction. This result is an important contribution toward our understanding of the degeneration of inhibin A in human serum. Therefore, inhibin A may be substitute for AFP as a marker for Down syndrome screening in twin pregnancy following multifetal reduction in the second trimester.

This study was restricted to *in vitro* fertilization pregnancies and it is unclear whether maternal serum inhibin A levels may be influenced by method of conception. Wald *et al.* found no significant differences in second-trimester maternal serum levels of inhibin A between singleton IVF and spontaneous conceived pregnancies (Wald *et al.*, 1999). However, other studies showed that inhibin A levels are increased in IVF pregnancy *versus* spontaneous singleton pregnancies (Lambert-Messerlian *et al.*, 2006; Maymon *et al.*, 2006). In twins, the maternal inhibin A level in the second trimester was no difference between IVF and spontaneous pregnancies (Maymon *et al.*, 2006). Therefore, it would appear that inhibin A could be effectively used as a serum maker for Down syndrome in IVF twin pregnancies during the second trimester.

In conclusions, this study demonstrated that maternal serum inhibin A concentrations increase with number of fetuses. For cases of multifetal reduction to twin pregnancies, maternal serum levels of inhibin A fall to those of twin pregnancies in the second trimester. Unlike AFP, multifetal reduction does not cause elevation of inhibin A. Therefore, inhibin A may be effectively used as a marker for Down syndrome screening in twin pregnancies following multifetal reduction.

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