Outcome of Second Trimester Amniocentesis in Twin Pregnancies at Songklanagarind Hospital

Tharangrut Hanprasertpong MD*, Ounjai Kor-anantakul MD*,
Visit Prasartwanakit MD*, Roengsak Leetanaporn MD*,
Thitima Suntharasaj MD*, Chitkasaem Suwanrath MD*

** Department of Obstetrics and Gynaecology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla*

Objective: *To evaluate the outcome of genetic amniocentesis in twin gestations at Songklanagarind Hospital*
Material and Method: *This was a descriptive study that included all women with twin pregnancies who had a second trimester amniocentesis for chromosome study at the Maternal Fetal Medicine Unit, Department of Obstetrics and Gynecology, Songklanagarind Hospital from January 1998 through June 2006 to assess the outcome including risk of fetal loss in such cases.*
Results: *Advanced maternal age was the most common indication for amniocentesis. The success rate of cell culture was 100%. The fetal loss within 14 days after the procedure was 1.4%.*
Conclusion: *The tendency of fetal loss after amniocentesis in twin pregnancies was higher than in singletons. Pre-procedure counseling personnel should be aware of this potential outcome, and be sure to inform the involved parents prior to the procedure.*

Keywords: *Genetic amniocentesis, Twin pregnancy*

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The incidence of twin pregnancies is increasing in Thailand, partly as a result of the increased use of assisted reproductive techniques, and partly because the pregnancy population is getting older and the rate of twinning increases with maternal age[1,2]. As both twin pregnancies and advanced maternal age are risk factors of adverse outcome. The number of women carrying twins who are advised to undergo invasive prenatal diagnosis testing is significantly increasing. Amniocentesis is the most common invasive prenatal diagnosis procedure at Songklanagarind Hospital. For singletons, the rate of pregnancy loss after genetic amniocentesis was found to be about 0.3% from an earlier report in 1999,[3] however, the fetal loss rate for twin pregnancies after second trimester amniocentesis has never been studied. The authors realized that this information was necessary in order to provide proper pre-procedure counseling, as amniocentesis-related fetal loss seemed to be increased in twin pregnancies.

Correspondence to: Hanprasertpong T, Department of Obstetrics and Gynaecology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand.

Thus, the present study was undertaken to evaluate the risk of fetal loss after second trimester amniocentesis in twin pregnancies at the Maternal Fetal Medicine Unit, Department of Obstetrics and Gynaecology, Songklanagarind Hospital.

Material and Method

The present study included all women with twin pregnancies who had a second trimester amniocentesis for chromosome study at the Maternal Fetal Medicine Unit, Department of Obstetrics and Gynecology, Songklanagarind Hospital from January 1998 through June 2006. This group included the presented patients and referred cases from hospitals in other provinces in southern Thailand, as Songklanagarind Hospital is the only tertiary referral center in southern Thailand. At the time of admission, each patient had an ultrasound scan to confirm the gestational age before the amniocentesis, using real- time ultrasound (Toshiba Sonolayer SSA 250A, 3.75 MHz curvilinear probe and Voluson 730 GE Medical Systems, Kretztechnik, Zipf, Austria device, 2-5 MHz curvilinear probe). The

membrane thickness, twin peak sign, fetal sex and number of placentas were evaluated to determine the chorionicity and amnionicity of the twins. If monochorinonicity or monoamnionicity was diagnosed, single sac amniocentesis was performed. For dichorionic twin pregnancies, amniocentesis was performed from both gestational sacs. Amniocentesis was performed under continuous ultrasound monitoring with a 22-gauge spinal needle. The first 1-2 ml of amniotic fluid was discarded in order to avoid maternal cell contamination and then 15-20 ml of amniotic fluid was aspirated in a separate syringe and sent for cell culture. Fetal karyotyping was performed using the Giemsa-Trypsin-G-banding technique at the Human Genetics Unit, Department of Pathology, Songklanagarind Hospital. Detected complications were immediately recorded in the hospital database.

The authors collected patient demographic data, operative records; late complications and pregnancy outcomes from medical records for patients who delivered at Songklanagarind Hospital. For missing data, the authors interviewed the patient by telephone or mail. Institutional Review Board approval was obtained for the present study.

Results

A total of 74 twin pregnancies underwent genetic amniocentesis during the study period. The mean age of the patients was 37 years (range 21 to 50 years). Approximately 65% of cases were between 35-39 years of age (Table 1). The mean gestational age at the time of amniocentesis was 17.1 weeks (range 14 to 26 weeks), with most procedures performed between 16-18 weeks (89.2%) (Table 2). Advanced maternal age was the most common indication for amniocentesis (93.2%), and the majority of these cases were more than 35 years old at the time of delivery. Other indications for amniocentesis were fetal malformation and previous history of fetal trisomy 21 (Table 3). There was one case of dichorionic diamniotic twins, with a maternal age at the time of delivery of 33. After the couples were counseled about the risk of Down syndrome in one or both fetuses at mid-trimester based on her age assuming that her twin pregnancies were dizygotic, the couple decided to have amniocentesis.

Dichorionic diamnionic twin pregnancies were sonographically diagnosed in 48 cases (64.9%), and monochorionic diamnionic twin pregnancies in 24 cases (32.4%). There was one case of conjoined twins and one acardiac twin. Two cases had indigo carmine injected into the first sac. All operators who performed amniocentesis in the present study were licensed to do as a subspecialty through the Thai board of Maternal Fetal Medicine. The amount of amniotic fluid collected was 18-20 ml per sac. Most of the amniotic fluid was clear (99.2%). A bloody tap was found in 1 monochorionic dichorionic twin pregnancy (0.8%).

The success rate of the cell culture was 100%. There were five abnormal chromosomal results. The first was a dichorionic diamnionic twin pregnancy with trisomy 18 in one sac and 46, XX in the other. The abnormal chromosomal fetus showed an omphalocele in the detailed scan and died at 33 weeks of gestation. The pregnancies continued until 38 weeks of gestation and the infants were delivered by cesarean section. The normal chromosomal baby was a normal female phenotype without anomaly. The trisomy 18 fetus showed maceration. Gross examination confirmed omphalocele. Gross placental examination confirmed the dichorionic diamnionic pregnancy. The second case was a dichorionic diamnionic twin pregnancy with trisomy 21 in one sac and trisomy X in the other. In this case, the parents decided to terminate the pregnancy. The last unusual case was a dichorionic diamnionic twin pregnancy with 46, XX (CSB1q 1q break) in one sac and 46, XX in the other. A scan of the abnormal chromosomal fetus showed cystic hygroma colli. This pregnancy was continued and the babies delivered at another hospital. No fetal outcome was reported.

In two cases, the pregnancies were terminated before the results of the chromosome study were known because of fetal malformation. The fetal malformation in one case was conjoined twins, and in the other case, one fetus was anencephalic and the other had a cardiac malformation. These two cases were excluded from the present study. All of them were normal chromosome.

In all cases, any detected complications were reported within 14 days after the procedure. Amniotic fluid leakage with spontaneous abortion was found in 1 case (1.4%) of a dichorionic diamnionic pregnancy. There was no record of any difficulty during the amniocentesis. The amniotic fluid leakage occurred 7 days after amniocentesis and followed by spontaneous abortion.

No cases of vaginal bleeding were reported, and no cases of chorioamnionitis were diagnosed.

Complications more than 14 days after amniocentesis and pregnancy outcomes of those with fetal chromosome abnormalities were followed in 45 cases. The fetal loss rate was 4.4% including two cases of dead fetus in utero. In the first case of dead fetus in

utero, the mother had systemic lupus erythematosus. She complained that her fetal movement decreased at 20 days after amniocentesis and ultrasound showed that both fetuses had died. In the second case, twin to twin transfusion syndrome (TTTS) was diagnosed at 23 weeks of gestation, and one week later both fetuses died. There were two cases of serious maternal complications. In the first case, the mother died at 38 days after the amniocentesis procedure. She developed hepatic failure without evidence of infection, and a liver biopsy showed cirrhosis of unknown cause. In the second case, the mother was complicated by uncontrolled severe preeclampsia at 23 weeks of gestation and needed termination of pregnancy. All symptoms then eventually resolved. Premature labor occurred in 18 cases (40%) and delivered 16 cases (35%). Most preterm deliveries occurred after 34 weeks of gestation (Table 4).

Table 1. Maternal age group

Age group (years)	Number	Percent
20-24	1	1.4
25-29	2	2.7
30-34	3	4.1
35-39	48	64.9
40-44	17	22.9
> 44	3	4.1
Total	74	100.0

Table 2. Gestational age at time of amniocentesis

Gestational age (weeks)	Number	Percent
< 16	3	4.1
16-18	66	89.2
19-21	5	6.8
Total	74	100.0

Table 3. Indications for amniocentesis

Indications	Number	Percent
Advanced maternal age	69	93.2
Previous abnormal child	1	1.4
Abnormal ultrasonographic finding	4	5.4
Total	74	100.0

Table 4. Pregnancy outcomes of 45 cases

Outcomes	Number	Percent
Fetal loss		
Dead fetus in utero	2	4.4
Maternal complications		
Hepatic failure	1	2.2
Severe pre-eclampsia remote from term	1	2.2
Live birth		
Preterm delivery	16	35.6
Term delivery	25	55.6
Total	74	100.0

Discussion

The risk of genetic abnormalities in twin pregnancies is greater than in singletons[4]. In addition, the rates of twinning are maternal age related, such that women over 35 are three times more likely to conceive twins than are women under the age of 20[5]. Unfortunately, the method for assessing the risk of genetic abnormalities in twin pregnancies is more complicated than the singleton pregnancies and includes genetic screening and invasive procedures. For screening genetic abnormalities, maternal serum and ultrasonographic markers are difficult to evaluate in both the first and second trimesters. For example, a problem with maternal serum screening for genetic abnormalities in a twin pregnancy is that maternal serum marker levels are a reflection of both twins, and the presence of an abnormal fetus may be confounded by the presence of an unaffected co-twin, resulting in a lower detection rate than in singleton pregnancies. An earlier study found that the biochemical for Down syndrome screening tests performed in the second trimester has lower detection rate of approximately 15% than similar tests from singleton pregnancies[6,7]. In the first trimester, the distribution of the serum analyte PAPP-A in twin pregnancies is different from singletons[8], so the real sensitivity of serum screening in the first trimester in twin pregnancies has been questioned. For nuchal translucency thickness, the first trimester ultrasonographic marker has a higher prevalence of increased nuchal translucency thickness in monochorionic twin pregnancies than in singletons and in dichorionic twin pregnancies. The thickness of nuchal translucency is not specific to chromosomal abnormalities. These findings demonstrate that the underlying hemodynamic changes were associated with TTTS as well[9]. Because of these limitations, an invasive diagnostic procedure is indicated for chromosomal detection in twin pregnancies.

The present research was the first study to examine the impact of amniocentesis on the outcome of twin pregnancies in Thailand. The complication rate within 14 days after the procedure in the present study was 1.38%, compared to 0.3% for singletons found in the authors' earlier study[3]. The incidence of complication within 14 days after the procedure was higher than in singleton pregnancies. For twin pregnancies, mid-trimester amniocentesis in previous studies resulted in a pregnancy loss rate between 1.1-3%. Sabire reported a rate of 1.1% pregnancy losses in which amniocentesis could have contributed directly to the losses[10]. Millaire reported a rate of fetal losses in mid-trimester genetic amniocentesis of 3.0%. These loss rates were not significantly different from the rates of twin pregnancies not exposed to amniocentesis[11].

There were some limitations in the present study. The pregnancy outcomes could not be obtained in over 50% of the cases, although the authors tried to collect that data, likely because these cases were referred from other hospitals and were then referred back when the results had been reported. Therefore, only immediate complications, usually within 14 days after the procedures, were reported. The actual fetal loss rate might be higher or lower. In addition, the author did not confirm the present results through postnatal chromosome studies both in normal and abnormal cases. In the present study, the premature delivery rate was 35.6%, which is in line with the normal preterm delivery rate for twin pregnancies[1]. Most preterm deliveries occurred after 34 weeks of gestation.

In the present study, the authors firstly determined the chorionicity and amnionicity revealed by ultrasonographic findings. If the ultrasound finding showed dichorionicity, zygotisity could not be diagnosed, and the authors collected amniotic fluid from both gestational sacs. For monoamnionicity or monochorionicity, the zygotisity could be diagnosed as monozygoticity and the authors collected amniotic fluid from only one sac to reduce risk of fetal loss. The complication rate increases with every gestational sac entry. Sebire reported that the risk of fetal loss after a single uterine entry for genetic amniocentesis in twin pregnancies appeared to be similar to that in singleton pregnancies[10]. Therefore accurate determination of chorionicity assists in risk stratification, genetic counseling and invasive procedure management. A slight problem with this methodology was the less-than-perfect accuracy of an ultrasound for determining chorionicity in twin pregnancies. Scardo reported using a composite of sonographic markers (placental number, fetal sex, membrane thickness and twin peak sign) to predict chorionicity in dichorionic and monochorionic pregnancies, with sensitivity and specificity for the prediction of monochorionicity of 91.7% and 97.3%, respectively. The mean gestational age of the first ultrasonographic examination in the present report, was 22.6 $\pm$ 6.9 weeks. Incorrect counseling or management can be influenced by an incorrect diagnosis of chorionicity[12]. To improve the accuracy of chorionicity testing, the determination of chorionicity prior to 14 weeks should be considered, as several studies have reported that determination of chorionicity is most accurate when performed in this period[13,14]. The authors recommend that ultrasound should be performed in all mothers at high risk to conceive twins since early pregnancy.

In conclusion, the present study found that the tendency of pregnancy loss after amniocentesis in twin pregnancies is slightly higher than in singletons, and counseling of at risk mother/parents before amniocentesis should include this information.

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ผลของการเจาะน้ำคร่ำหญิงตั้งครรภ์แฝดในไตรมาสที่สองที่โรงพยาบาลสงขลานครินทร์

ธารางรัตน์ หาญประเสริฐพงษ์, อุ่นใจ กออนันตกุล, วิศิษฐ์ ประสาธน์วนกิจ, เรืองศักดิ์ ลีธนาภรณ์, ฐิติมา สุนทรสัจ, จิตเกษม สุวรรณรัฐ

วัตถุประสงค์*: เพื่อศึกษาผลของการเจาะน้ำคร่ำหญิงตั้งครรภ์แฝดในไตรมาสที่สองที่โรงพยาบาลสงขลานครินทร์*
วัสดุและวิธีการ*: เป็นการศึกษาเชิงพรรณนา โดยการเก็บข้อมูลหญิงตั้งครรภ์แฝดทุกรายที่เข้ารับการเจาะน้ำคร่ำเพื่อตรวจจำนวนแท่งพันธุกรรมในไตรมาสที่สองของการตั้งครรภ์ ที่หน่วยเวชศาสตร์มารดาและทารกในครรภ์ ภาควิชาสูติศาสตร์นรีเวชวิทยา โรงพยาบาลสงขลานครินทร์ ระหว่างเดือนมกราคม พ.ศ. 2541 ถึง เดือนมิถุนายน พ.ศ. 2549 เพื่อประเมินผลของการเจาะน้ำคร่ำซึ่งรวมถึงการสูญเสียทารก*
ผลการศึกษา*: หญิงตั้งครรภ์แฝดที่เข้ารับการเจาะน้ำคร่ำส่วนใหญ่มีข้อบ่งชี้คือ อายุมารดามาก การเจาะน้ำคร่ำทุกรายสามารถบอกผลโครโมโซมได้ และมีอัตราการสูญเสียทารกภายใน 14 วัน ภายหลังการเจาะน้ำคร่ำประมาณร้อยละ 1.4*
สรุป*: อัตราการสูญเสียทารกภายหลังการเจาะน้ำคร่ำของหญิงตั้งครรภ์แฝดมีแนวโน้มสูงกว่าหญิงตั้งครรภ์เดี่ยว ดังนั้นก่อนการเจาะน้ำคร่ำหญิงตั้งครรภ์แฝดควรให้คำปรึกษาเกี่ยวกับความเสี่ยงที่สูงกว่านี้ด้วย*