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# Midtrimester Amniocentesis : Experience of 2040 cases

Chanane Wanapirak MD,
Theera Tongsong MD,
Pannee Sirivatanapa MD,
Wirawit Piyamongkol MD,
Supatra Sirirchotiyakul MD,
Acharawan Yampochai BSc,
Apiradee Tuggapijit BSc,
Ratanaporn Sekararithi BA.

*Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*

## ABSTRACT

**Objective** To evaluate the validity of midtrimester amniocentesis.

**Design** Descriptive study.

**Setting** Maharaj Nakorn Chiang Mai Hospital, Chiang Mai University.

**Subjects** Singleton pregnancies at risk of fetal chromosomal abnormalities, attending antenatal care clinic during June 1988- May 1997.

**Main outcome measures** Prevalence of abnormal fetal chromosomes and pregnancy outcomes.

**Methods** Amniocentesis and fetal cell culture were done between 15-24 gestational weeks and the subjects were followed until delivery.

**Results** A total of 2,040 high risk pregnant women underwent midtrimester amniocentesis. Only 1.2% was unable to obtain amniotic fluid. Among 2015 cases of successful amniocentesis, success rate of cell-culture was 92.5%. There were overall 1.88% (35/1864) of abnormal chromosomes. However, only 1.88% (35/1864) were proven to be abnormal, confirmed by either repeated amniocentesis, cordocentesis or sonographic features. Of these, 29 were electively terminated and 6 continued the pregnancies. Pregnancy outcomes in those with normal fetuses included spontaneous abortion 1.5%, dead fetus in utero 1.4%, premature deliveries 11.5%, term deliveries 85.7%.

**Conclusion** The incidence of abnormal fetal chromosome was 1.88%. The rate of fetal loss was similar to general population.

Amniocentesis was first used to obtain fetal cells for karyotyping in 1966[1] and since then it is still the most common invasive procedure used for prenatal diagnosis. Initially, amniocentesis were not ultrasound guided. Most of these attempts were successful and thus blind amniocentesis suggested a low fetal risk and understandably many physicians were reluctant to adapt their technique to include ultrasound. More recently, it has become widely accepted that a technique of continuous ultrasound guidance be used, allowing the operator to perform more safely. It is worldwide accepted that midtrimester amniocentesis permits possibilities in antenatal diagnosis of serious genetic diseases. However, there were only few reports in Thai population.[2-4] Therefore, we conducted the study to assess the prevalence of abnormal fetal chromosome by cell culture of the amniotic fluid in high risk pregnant women and pregnancy outcomes following amniocentesis.

## Materials and Methods

The study was carried out at the Department of Obstetrics and Gynaecology, Faculty of Medicine, Maharaj Nakorn Chiang Mai Hospital. Pregnant women at risk of having a child with abnormal chromosomes, who attended antenatal care unit from June 1988 to May 1996 were counseled and recruited to the study and underwent amniocentesis. Transabdominal amniocentesis was performed between 15-24 weeks of gestation. When the date was uncertain, ultrasonography was performed to establish gestational age prior to performing the procedure. Amniocentesis was done under ultrasonic gui dance. We have used real time scan with abdominal probe of 3.5 MHz (Aloka SSD 238, 620, 680). To avoid maternal cell contamination, the first 2 ml of amniotic fluid was aspirated and discarded in a separate syringe. Fetal chromosome analysis was carried out using trypsin-G-banding technique. The women were followed until delivery. The incidence of fetal abnormal chromosome, pregnancy outcomes and rates of complications would be identified.

## Results

There were altogether 2,051 high risk pregnant women accepted for midtrimester amniocentesis, 11 of them were excluded due to fetal dead prior to the procedure. Of 2,040 cases, 25 (1.2%) were failure in obtaining amniotic fluid. Of 2015 cases whose amniotic fluid samples were cultured, cell-culture chromosomal analysis was successful in 1,864 (92.5%) and 151 (7.5%) had cell-culture failure. The most common indication for amniocentesis was advanced maternal age (86%). Ten percent previously gave birth to trisomy children. Of 2,040 women that amniocenteses were attempted to perform, the mean age (± SD) was 37.5 ± 3.3 years (range 19-53 years), mostly confined to 35-39 years. Most of them were multiparous women (81.8%), 40.6% had only one child. Only 19.2% were primiparae. The mean gestational age (± SD) at the first amniocentesis was 17.3 ± 1.8 weeks (range 14-28 weeks). Nearly 80% was confined to 16-18 weeks.

The appearance of amniotic fluid obtained was normal in most cases (92.8%). Bloody tap was found in 4.2% and abnormal colour (brown or green) was 3%. Based on the first attempt of amniocentesis, the prevalence of abnormal fetal chromosomes in the group of cell-culture success was 2.52% (47/1,864). Most of them were further confirmed the diagnosis with repeated amniocentesis, cordocentesis or sonographic characteristics of fetus with trisomy. However, 12 were antenatally proven to have normal chromosomes.

Therefore, the prevalence of chromosomal abnormalities was 1.88% (35/1,864). Of these 35 fetuses, most were confined to autosomal trisomies including trisomy 21, 18 and 13. The most common chromosomal abnormality was trisomy 21 (Down's syndrome), with prevalence of 0.7%. The rest of them had various disorders of chromosomal rearrangement such as mosaicism, deletion, or other unusual abnormalities.

Of 151 cases of culture failure, only 110 (72.4%) accepted the second procedure for prenatal diagnosis, i.e. repeated amniocentesis (86) or cordocentesis (24), where as the remainings refused to be done. The chromosome results, obtained only in 86.4% (95/110), were all normal. The culture failure rate of the second attempt of chromosome study was 13.6% (15/110).

Among 35 cases with abnormal chromosomes, 29 were electively terminated and 6 continued the pregnancies, giving births of live babies.

All pregnancies excluding those with abnormal fetuses and repeated prenatal diagnosis were followed to evaluate the pregnancy outcomes. However, we had adequate data for analysis in only 1,752 pregnancies. Of these women, fetal loss rate was 2.9%, including abortion 1.5% and dead fetus in utero 1.4% where as 11.5% and 85.7% ended up with premature and term deliveries respectively. Abruptio placenta occurred in 2 cases at 34 and 40 weeks of gestation. No other serious complications related to amniocentesis were found.

**Table 1.** Ages of pregnant women at risk undergone amniocentesis

| Maternal ages | Number | Percent |
|---|---|---|
| 20-24 | 31 | 1.5 |
| 25-29 | 60 | 2.9 |
| 30-34 | 98 | 4.8 |
| 35-39 | 1,490 | 73.0 |
| 40-44 | 353 | 17.3 |
| > 44 | 8 | 0.4 |
| **Total** | **2,040** | **100.0** |

**Table 2.** Gestational ages at time of amniocentesis

| Gestational ages (weeks) | Number | Percent |
|---|---|---|
| < 16 | 94 | 4.6 |
| 16-18 | 1,607 | 78.8 |
| 19-21 | 310 | 15.2 |
| 22-24 | 29 | 1.4 |
| **Total** | **2,040** | **100.0** |

**Table 3.** Indications of amniocentesis

| Indications | Number | Percent |
|---|---|---|
| Advanced maternal age | 1,758 | 86.2 |
| Previous trisomic child | 104 | 5.1 |
| Familial history of chromosome disorders | 41 | 2.0 |
| Suspected of previous fetus with chromosomal disorders | 56 | 2.7 |
| Fetal risk of X-linked disorders | 18 | 0.9 |
| Abnormal sonographic findings | 12 | 0.6 |
| Maternal or paternal translocation | 7 | 0.3 |
| Previous child of mental retardation | 16 | 0.8 |
| Miscellaneous | 28 | 1.4 |
| **Total** | **2,040** | **100.0** |

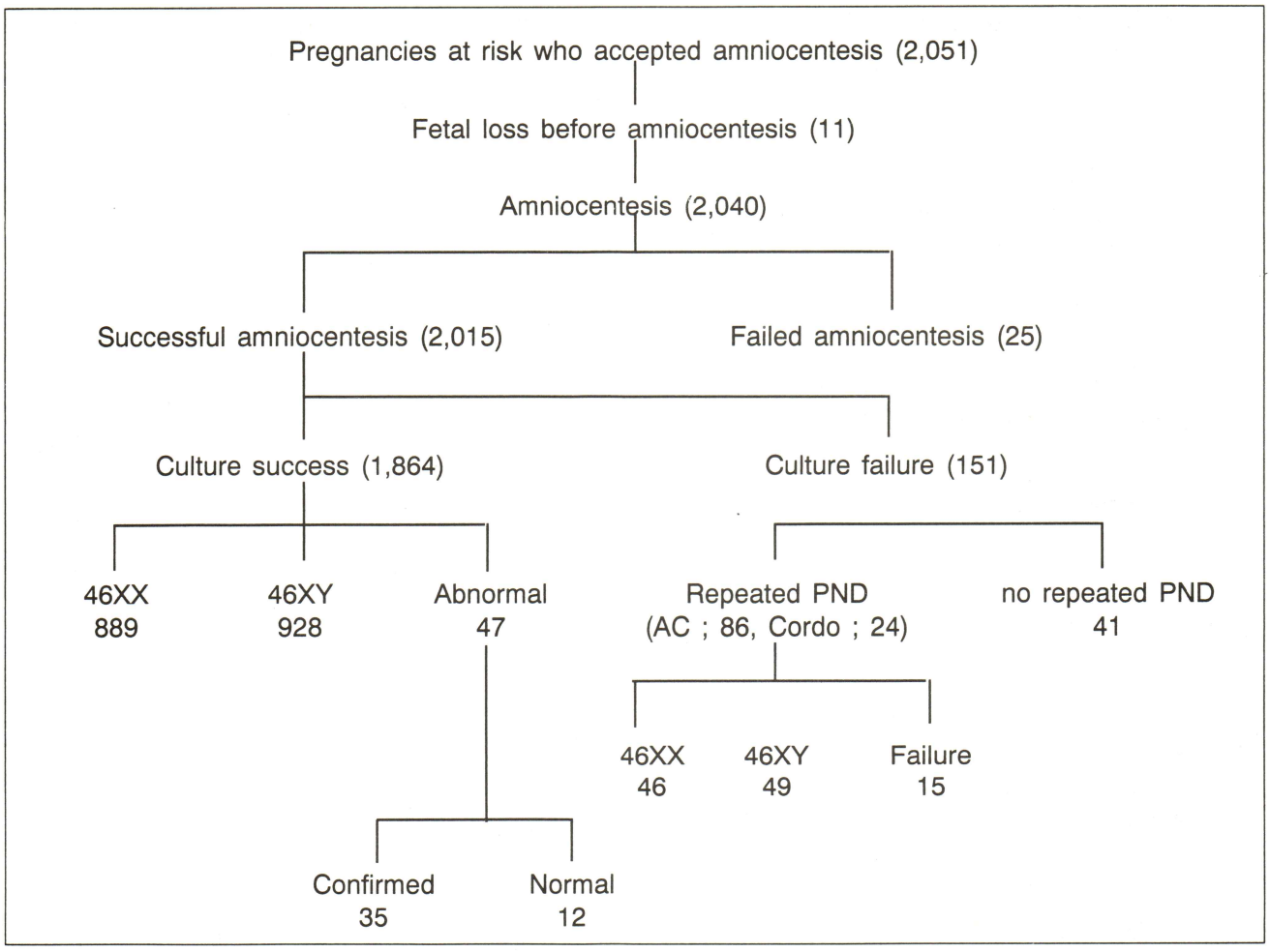
**Table 4.** Final results of the chromosome studies (from 1864 cases)

| Results | Number | Percent |
|---|---|---|
| 46XX | 889 | 47.70 |
| 46XY | 928 | 49.78 |
| Abnormal chromosomes | 47 | 2.52 |
| Trisomy 21 & variants | 13 | |
| Trisomy 18 & variants | 5 | |
| Trisomy 13 & variants | 3 | |
| 47 XXX | 3 | |
| 47 XXY | 2 | |
| Turner syndrome | 2 | |
| Other rearrangement | 7 | |
| Normal | 12 | |

The mean gestational age (± SD) at delivery was 37.8 ± 2.7 weeks (16-42 weeks). The mean birthweight (± SD) was 2,940 ± 615 grams.

## Discussion

Like other reports, we found that chromosomal abnormalities associated with advanced maternal age is the most common indication for amniocentesis and accounts for over 50% of cases. In cases of abnormal chromosomes, we usually repeated amniocentesis or cordocentesis to confirm the initial diagnosis. However, in some




PND = Perinatal diagnosis
AC = Amniocentesis
Cordo = Cordocentesis

**Fig. 1.** Summary of the results of chromosome study.

**Table 5.** Pregnancy outcomes (from 1752 cases)

| **Fetal loss** | **Number** | **Percent** |
|---|---|---|
| Spontaneous abortion (< 28 weeks) | 26 | 1.5 |
| Dead fetus in utero | 24 | 1.4 |
| **Live birth** | | |
| Premature delivery | 201 | 11.5 |
| Term delivery (> 37) | 1,501 | 85.7 |

cases we were so confident in the diagnosis that we could omit the second attempt, especially in cases that we could demonstrate the sonographic stigmata of trisomic fetuses. All of these cases were postnatally confirmed of the diagnosis.

The risk of amniocentesis varies depending on technique and operator's experience. One of the best controlled study describes a risk as high as 1% for spontaneous abortion,[5] although other studies suggest that this may be about 0.5%.[6] The data of Dalhousie University suggests the risk is approximately 0.5% with continuous ultrasound guided amniocentesis. If the placenta is penetrated or bloody amniotic fluid is obtained, the procedure risk is double,[5,7] This emphasizes the importance of ultrasound guidance. Although we did not know the background fetal loss rate because we had no controls, the low total fetal loss rate in this large series suggest the safety of midtrimester amniocentesis. Whichever technique is employed, the risks are minimized by operator experience. In a Dutch study of 3,000 pregnancies, Leschot et al found a fetal loss rate of 1.53% for the first 1,500 cases and 0.47% for the remaining group.[8] Wenstrom et al reported that elevated amniotic fluid interleukin-6 levels, indicating preexisting subclinical infection at time of amniocentesis, is associated with subsequent pregnancy loss.[9]

In this descriptive study, we are unable to calculate the fetal loss rate related to amniocentesis because we had no controls to compare. However, we had 2.9% of overall fetal loss rate (background and amniocentesis-related loss) which is comparable with that of other reports.[5-6]

To minimize the fetal loss rate, we recommend : 1) good counseling before amniocentesis, 2) aseptic technique with ultrasound guidance, 3) avoiding the placenta penetration if at all possible, 4) using a needle no larger than 20 gauge, 5) no more than two insertions, 6) removing the volume of fluid not exceed 20 ml and discarding the first few milliliters of fluid.

Genetic amniocentesis is not only prevent abnormal chromosomal child but it can also reassure, though not absolutely, the couples in cases of normal results. This can usually relieve the anxiety of the couples although this benefit could not be calculated in term of money. This large series indicates that amniocentesis in the couples at risk is worthwhile. The risk from genetic disorder that could be detected is much greater than that of amniocentesis.

The disadvantages of midtrimester amniocentesis are 1) the procedure is performed later (14-17 weeks), when compared to chorionic villi sampling, 2) a 2-3 week laboratory processing time. Consequently, abnormalities are often not identified until late and if pregnancy termination is desired, it is both medically and psychologically more traumatic. It is for these reasons that there has been emphasis to develop a prenatal diagnostic method in the first trimester. To overcome these problems, some authors performed early amniocentesis.[10-13] According to the Centers for Disease Control and Prevention, U.S.A., however, early amniocentesis remains investigational procedure.[12] Godmilow et al[13] reported a cohort study of 1,895 women undergoing early amniocentesis, 2,441 chorionic villi sampling, and 2,880 midtrimester amniocentesis. They found that early amniocentesis (11-14 weeks) and chorionic villi sampling (10-13 weeks) was associated with 2.5-fold increase in pregnancy loss compared with midtrimester amniocentesis (15-20 weeks).

Green or brown discoloured fluid was detected 3% in this study, lower than that of Hankins's report which found 7%.[14] However, they found that either green or brown discoloured fluid was not significantly related to the incidence

of pregnancy loss, neonatal outcomes, or other pregnancy complications when these women were compared with matched controls.[14]

The amniotic fluid was successfully obtained at first attempt (98%) and mostly was clear. When compared with other studies,[2] the culture failure rate was rather high in our studies (7.5 %) despite the fact that it should be lower than 1%. This may be due to a number of different factors including insufficient viable cells in the sample, and heavily blood-stained fluid. Blood-contaminating amniotic fluid may inhibit the replication of fetal cells in culture. However, bloody tap was only 4.2% in this series, similar to other reports.[4] We realized and tried to solve the laboratory problems and the failure rate is currently decreased.

One pitfall of this study was that most cases with normal chromosome were not postnatally studied to confirm the antenatal diagnosis. In addition, we could not follow-up to evaluate pregnancy outcomes in many cases because they attended antenatal clinic but gave birth at other hospitals, despite our attempt to collect all data.

In conclusion, this extensive experience indicates that midtrimester amniocentesis for genetic study is a safe, accurate, and reliable. However it should be performed by a team that provides all the necessary expertise. We recommend that amniocentesis should be performed by obstetricians experienced in this procedure, with all the availability of high quality ultrasonography and, with access to a laboratory with experience in culturing and analyzing amniotic fluid cells.

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