Mid-Trimester Genetic Amniocentesis in Twin Pregnancy and the Risk of Fetal Loss

Mélanie Millaire, MD, FRCSC, Emmanuel Bujold, MD, FRCSC, Anne-Maude Morency, MD, Robert J. Gauthier, MD, FRCSC
Department of Obstetrics and Gynaecology, Hôpital Sainte-Justine and Université de Montréal, Montréal (Québec)

Abstract
Objective: To assess the rate of fetal losses in twin pregnancies undergoing genetic mid-trimester amniocentesis.

Methods: In the first part of this investigation, a retrospective cohort study compared a group of women ≥ 32 years old with twin pregnancies who underwent amniocentesis with a similar group unexposed to amniocentesis. Data were compiled from January 1990 to March 2004 for patients from a single institution. Pregnancies complicated by twin-to-twin transfusion syndrome, monoamniotic twins, or lethal fetal anomalies, and those treated by fetal reduction were excluded. The primary outcome was the loss of one or both fetuses prior to 24 weeks’ gestation. In the second part of the investigation, a systematic review of the literature and a meta-analysis were performed.

Results: In the first part of the study, data were collected for 132 women exposed to amniocentesis and 248 women not exposed to amniocentesis. There was no significant difference in the rate of fetal losses between the two groups (3.0% vs. 0.8%, P = 0.10). No losses occurred within four weeks of the procedure. In the second part of the investigation, four studies, including ours, were considered for a meta-analysis of 2026 women with twin pregnancies. Compared with women unexposed to the procedure, amniocentesis in women with twin pregnancies increased the risk of fetal losses prior to 20 to 24 weeks’ gestation (odds ratio 2.42; 95% confidence intervals 1.24–4.74, P = 0.01) with an additional risk of one adverse outcome (1 or 2 fetal losses) for every 64 amniocenteses.

Conclusion: Genetic mid-trimester amniocentesis in twin pregnancies is associated with an increased risk of fetal loss prior to 24 weeks’ gestation.

Key Words: Pregnancy, multiple gestations, twins, amniocentesis, prenatal diagnosis, fetal loss

INTRODUCTION

The incidence of twin pregnancies has increased during the past decade, in part because of childbearing at a more advanced maternal age and the use of assisted reproductive technology. While the risk of chromosomal abnormalities rises with advancing maternal age, it is also well-known that the risk of genetic abnormalities is 1.6 times greater among twins than among singletons.1,2 Therefore, a 33-year-old woman with a twin pregnancy (of unknown zygosity) carries the same risk of chromosomal abnormalities as a 35-year-old woman with singleton pregnancy.3 However, there is a paucity of information in the

Received on January 19, 2006
Accepted on March 20, 2006
literature on the procedure-related risk of amniocentesis in this particular situation.

Although there have been several studies assessing pregnancy outcomes after mid-trimester amniocentesis, only one randomized controlled trial has been performed in singleton pregnancies. In these pregnancies, the rate of fetal losses related to amniocentesis is believed to be between 0.2% and 1%. Since 1990, only four case-control studies have assessed the impact of amniocentesis in twin pregnancies. The results of these previous reports are conflicting, in large part because of the small number of patients included in each of them and the rarity of the outcome (fetal losses) investigated. To give optimal counselling regarding the risks of amniocentesis during the second trimester to women who are carrying twins, we decided (1) to review our experience with twin pregnancies undergoing amniocentesis, and (2) to compile the best available data in the literature in a meta-analysis.

MATERIALS AND METHODS

In the first part of our investigation, we studied women 32 years of age or older with twin pregnancies seen prior to 15 weeks’ gestation at our clinic for pregnancy follow-up. To decrease selection biases, only patients who had their first obstetrical appointment in our prenatal clinic prior to 15 weeks’ gestation were included. Patients who had their first obstetrical appointment or their first obstetrical ultrasound in another centre were excluded. The data were collected from the perinatal database of Saint Justine Hospital for the period January 1990 to March 2004. To ensure that no cases were forgotten, we performed an ICD-9 code survey of the medical records for all twin pregnancies diagnosed at or after 15 weeks by ultrasonographic examination or pathology. Patients were divided into two groups: those who underwent amniocentesis between 15 and 17 weeks’ gestation and those who were not exposed to amniocentesis.

Excluded from the study were (1) women who underwent amniocentesis before 15 weeks’ or after 17 weeks’ gestation, (2) women who underwent chorionic villus sampling, (3) multiple gestations with more than two fetuses, (4) women with multiple gestations treated by fetal reduction, (5) women whose ultrasonographic findings suggested twin-to-twin transfusion syndrome, (6) pregnancies that demonstrated early intra-uterine growth restriction, (7) pregnancies that demonstrated lethal fetal anomalies, (8) pregnancies that demonstrated abnormal karyotype, and (9) women pregnant with monoamniotic twins. The data collected comprised maternal age, parity, gestational age at the time of amniocentesis, medical and obstetrical history, ultrasonographic findings, technical data on the procedure (for patients who had amniocentesis), obstetrical data (gestational age at birth, route of delivery, use of tocolytics, premature rupture of the membranes, clinical chorioamnionitis), and placental pathology (for chorionicity). All second trimester amniocenteses in twin pregnancies were performed under ultrasound guidance by a maternal-fetal medicine subspecialist.

The primary outcome determined prior to data collection was the rate of fetal losses (defined as the loss of 1 or 2 fetuses) between 15 and 24 weeks’ gestation. We used Pearson’s chi-square test and Student t test to analyze discrete and quantitative variables. Statistical analyses were performed using SPSS statistical software, version 11.0 (SPSS Inc., Chicago, IL). We considered P-values of less than 0.05 to be significant. Women 32 years of age or older were selected in order to reduce the potential effect of maternal age on the results and because amniocentesis was routinely offered to this group of patients, as recommended by the Society of Obstetricians and Gynaecologists of Canada. The decision of women to undergo amniocentesis was based on their personal beliefs and evaluation of risks. The risk of fetal loss quoted to women to help them in their decision was 0.5 to 1%.

In the second part of the investigation, we sought studies providing data on the association between amniocentesis in twin pregnancies and the occurrence of fetal loss. These studies were identified by Medline from 1990 to 2005, using the search terms twins, multiple gestations, amniocentesis, and reference lists. We included randomized trials, cohort or case-control studies of women identified as having twin gestations who had an amniocentesis performed between 14 and 20 weeks of gestation with a record of the outcome of pregnancy (at least until 20 to 24 weeks’ gestation). In addition, each study had to contain a comparison group of women who did not have amniocentesis, so that the effect of amniocentesis on pregnancy outcome could be assessed. For inclusion, a study was required to have appeared as a full-text publication, to have been published after 1990, to have contained more than 100 participants, to have had concurrent comparison groups, to have been conducted in a developed country, and to have been published in English or French. We preferred not to include studies published before 1990 because of the inconsistent use of ultrasound guidance during the procedure before 1990. Two investigators (MM and AMM) independently reviewed all titles and abstracts; there was no disagreement on inclusion. Data were entered into the statistical software of the Cochrane Collaboration, RevMan (version 4.2.8) with dichotomous analyses by RevMan 1.0 (The Nordic Cochrane Centre, Rigshospitalet). The dichotomous primary outcome of fetal losses prior to 20 to 24 weeks’ gestation was expressed as a
combined relative risk with 95% confidence intervals. A statistical difference was said to exist if the pooled 95% confidence interval did not include 1.0. The chi-square test for heterogeneity was used to assess the extent to which the results of the studies were in agreement. The significance level was $P < 0.05$.

RESULTS

For the first part of the study, a total of 385 pregnancies met the inclusion criteria. Of these, five were subsequently excluded because they included fetuses with lethal or karyotype abnormalities, leaving 380 pregnancies to be analyzed. Of these pregnant women, 132 (34.7%) underwent amniocentesis, and 248 (65.3%) did not. All patients were routinely evaluated by ultrasound for fetal biometry and anatomy between 18 and 20 weeks of gestation, with reassessment of gestational age, placenta location, and the presence or absence of major abnormalities. All of these patients delivered at our institution. Based on ultrasound findings and postpartum placental pathology, 283 women (74.5%) had dichorionic twins and 97 (25.5%) had monochorionic twins. Placental pathology reports were available to determine chorionicity in 99% (377/380) of cases.

All amniocenteses were conducted under ultrasonographic guidance by a maternal-fetal medicine subspecialist at between 15 and 17 weeks’ gestation. One or two needle insertions (22-gauge) were performed (depending on the position of the fetuses and placenta and on operator preferences), with one or both sacs being punctured (depending on whether the twin pregnancy was monochorionic or dichorionic and on operator preferences). Twelve to 20 mL of amniotic fluid were withdrawn from each sac, and a marker dye, indigo carmine, was injected in some cases, depending on the technique used (1 or 2 needle insertions) and operator preferences. All samples were adequate for amniocyte culture, and there was no need for repetition. The two-puncture technique for sampling both amniotic cavities was performed in 71 cases (54%), and the single-puncture technique for sampling one (22/61) or both (39/61) amniotic cavities was used in 61 cases (46%). In seven cases, an extra puncture was necessary to obtain amniotic fluid from the targeted amniotic cavity. Sampling of both amniotic cavities was more frequent in dichorionic twins (85/87, 98%) than in monochorionic twins (25/45, 56%) ($P < 0.01$).

Table 1 shows a comparison of the characteristics of the two groups. Women who underwent amniocentesis were older ($P < 0.01$) and more likely to have monochorionic twins than those who had no amniocentesis ($P < 0.02$). No significant difference was found between the groups in parity or in the rate of prior first or second trimester spontaneous fetal losses.

Pregnancy outcomes are reported in Table 2. With regard to the primary outcome, there were four spontaneous fetal losses between 15 and 24 weeks’ gestation among women who underwent amniocentesis, and two among those who did not (3.0% vs. 0.8%, respectively; $P = 0.098$). All (total of 6) fetal losses occurred between 21 and 23 weeks’ gestation and in women between 35 and 39 years of age. None of them had used assisted reproductive technologies. None of the fetuses presented lethal anomalies, according to the autopsy reports. Among the four fetal losses in the group of women who underwent amniocentesis, all had dichorionic placentas, all had amniocentesis performed at 16 or 17 weeks’ gestation; one gestational sac was punctured in one patient (25%), and both gestational sacs were punctured in three women (75%), employing the two-puncture technique.
technique in two (50%), and the single-puncture technique for both sacs in one patient (25%). Amniotic indigo carmine dye was not used in these four cases, and none of them required an extra puncture. Maternal age and gestational age at the time of amniocentesis in these cases were not statistically different from maternal age and gestational age in women who did not have fetal losses. On average, fetal losses occurred at six (range 5–7) weeks after the procedure. The initial reason for admission was chorioamnionitis in one, preterm premature rupture of the membranes (PPROM) in one, and preterm labour in two patients. No significant differences were found between the two groups in the other outcomes observed in this study (Table 2).

In the second part of our investigation, we identified three relevant articles, in addition to the current study, that met the inclusion criteria.7–9 The pooled group comprised a total of 893 women with twin gestations who underwent amniocentesis and 1133 controls. The characteristics of each study can be found in Table 3. Although three of the studies found no significant differences in the rate of fetal loss after amniocentesis, Yukobowich et al., in the largest study, reported a significant difference in fetal losses between the two groups (2.73 vs. 0.63%; P = 0.01).8 Although all the studies excluded fetuses with anatomical or karyotype anomalies, the primary outcome was not the same in all Table 3).

The results of the meta-analysis of the primary outcome (rate of fetal loss prior to 20 to 24 weeks’ gestation) are presented in the Figure. Women who underwent amniocentesis had a greater rate of fetal loss before 20 to 24 weeks’ gestation (25/893; 2.8%) than women who did not have amniocentesis (14/1133; 1.2%, P = 0.01). Thus, women with twin gestation who underwent a genetic amniocentesis were more than two times more likely to have a fetal loss than women who did not have a genetic amniocentesis (odds ratio 2.42; 95% confidence interval 1.24, 4.74). Another way of describing this result is by the number needed to treat: 64 women with twin gestations need to undergo amniocentesis for there to be an additional second trimester fetal loss in one of them. The test for heterogeneity was not statistically significant (P = 0.43); therefore, it appears reasonable to use meta-analysis to summarize the primary outcome.

### DISCUSSION

Although our current cohort investigation did not find a significant increase in the rate of fetal loss in women with twin gestation undergoing amniocentesis, systematic review identified three additional cohort studies, and the pooled cohort showed a significant increase in fetal loss prior to 24 weeks’ gestation. This important information needs to be transmitted to women with multiple gestations who are planning to have genetic amniocentesis. Although the risk of fetal loss for singleton pregnancies exposed to amniocentesis is thought to be between 0.5 to 1.0%, the risk for women with multiple gestations is not well-defined.5–13 In 2000, the Society of Obstetricians and Gynaecologists of Canada stated that “All women carrying twin pregnancies should be referred for counselling to a centre for the consideration of invasive testing at age 32 or greater” because “…the chance of a 32-year-old woman who carries twins of

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Amniocentesis performed</th>
<th>No amniocentesis</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal loss &lt; 24 weeks</td>
<td>4 (3.0%)</td>
<td>2 (0.8%)</td>
<td>0.10</td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>70 (53.0%)</td>
<td>138 (55.7%)</td>
<td>0.75</td>
</tr>
<tr>
<td>PPROM</td>
<td>27 (20.6%)</td>
<td>50 (20.3%)</td>
<td>0.95</td>
</tr>
<tr>
<td>Mean gestational age at birth (weeks)</td>
<td>35.3 ± 3.4</td>
<td>35.2 ± 3.3</td>
<td>0.73</td>
</tr>
<tr>
<td>Caesarean delivery</td>
<td>62 (47%)</td>
<td>132 (53%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Apgar 5 min &lt; 7 twin A</td>
<td>10 (7.6%)</td>
<td>14 (5.7%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Apgar 5 min &lt; 7 twin B</td>
<td>17 (12.9%)</td>
<td>22 (8.9%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Clinical chorioamnionitis</td>
<td>3 (2.3%)</td>
<td>8 (3.3%)</td>
<td>0.59</td>
</tr>
</tbody>
</table>

PPROM: Preterm premature rupture of the membranes after 20 weeks’ gestation.
unknown zygosity having at least one child with Down’s syndrome is equivalent to the risks of a 35-year-old with a singleton pregnancy.” The Society based this statement on the findings of Rodis et al., who demonstrated that a patient at 33 years of age with a twin gestation has a risk of Down’s syndrome in at least one of her twins equivalent to that of a 35-year-old with a singleton pregnancy. However, in light of our findings, it becomes an interesting ethical and medical question: should a woman who has an approximate risk of having one fetus affected by Down’s syndrome in 250 twin pregnancies be offered amniocentesis, an invasive procedure that has a 1 in 64 risk of fetal loss? In fact, we believe that women with multiple gestations seeking prenatal diagnosis of Down’s syndrome should have their risk of fetal aneuploidy evaluated on the basis of several factors, including maternal age, nuchal translucency, and other early biochemical serum markers, before making the decision to undergo a procedure (amniocentesis) with such a high risk of fetal loss. Of note, all losses occurred between 20 and 24 weeks’ gestation, contrary to the popular belief that fetal losses occur soon after the procedure, but in agreement with the study of Ghidini et al., who noted that fetal losses do not occur within three weeks of the procedure. According to Töth-Pal et al., fetal losses after amniocentesis performed prior to 20 weeks’ gestation occurred on average four weeks later, and those following amniocentesis performed after 21 weeks’ gestation occurred on average two weeks later. Therefore, we suggest that fetal losses associated with second-trimester amniocentesis may be secondary to a long (up to several weeks) pathological process. Since mid-trimester fetal losses, PPROM, and preterm birth have been associated with early intra-amniotic infection/inflammation, it is possible that amniocentesis accelerates an infectious/inflammatory process that would have potentially led to PPROM, preterm labour, chorioamnionitis, and preterm birth later during the pregnancy. Further studies are needed to resolve this important issue.

On the other hand, we saw no difference in obstetrical outcomes, including preterm birth, PPROM, low Apgar score, chorioamnionitis, and rate of Caesarean section between the two groups. These observations are in agreement with Kidd et al., who performed a cohort study of women with twin gestations from 20 weeks’ gestation to delivery. They found no difference in the stillbirth rate (4.4% vs. 2.6%; $P = 0.16$) and other pregnancy outcomes, such as preterm

### Table 3. Studies reporting the incidence of fetal loss after genetic amniocentesis in twin pregnancies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>Setting</th>
<th>Participants</th>
<th>Mean maternal age (years)</th>
<th>Mean gestational age at amniocentesis</th>
<th>Primary outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghidini et al.</td>
<td>Cohort</td>
<td>New York, USA</td>
<td>All twin gestations between 14 and 20 weeks between 1987 and 1992. 101 women underwent amniocentesis, 108 did not.</td>
<td>Study group: 35.2 Controls: 30.4</td>
<td>17.6 weeks</td>
<td>Fetal loss ≤ 24 weeks</td>
</tr>
<tr>
<td>(1993)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yukobowich et</td>
<td>Cohort</td>
<td>Jerusalem &amp; Tel Aviv,</td>
<td>All women with bichorionic twin pregnancies who underwent ultrasound scanning at 17–18 weeks’ gestation between 1990 and 1997. 476 women underwent amniocentesis and 477 women did not.</td>
<td>Study group: n/a Controls: n/a</td>
<td>17–18 weeks</td>
<td>Fetal loss up to 4 weeks after the ultrasound examination</td>
</tr>
<tr>
<td>al. (2001)</td>
<td></td>
<td>Israel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Töth-Pal et</td>
<td>Cohort</td>
<td>Budapest, Hungary</td>
<td>184 women with twin gestations who underwent amniocentesis were compared with 300 women with twin gestations with no amniocentesis between 1990 and 2000</td>
<td>Study group: 33.7 Controls: 28.9</td>
<td>18.2 weeks</td>
<td>Fetal loss &lt; 24 weeks</td>
</tr>
<tr>
<td>al. (2004)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current study</td>
<td>Cohort</td>
<td>Montreal, Canada</td>
<td>All women ≥ 32 years old with twin gestations seen prior to 15 weeks between 1990 and 2004. 132 women underwent amniocentesis, 248 did not.</td>
<td>Study group: 36.8 Controls: 34.3</td>
<td>16.2 weeks</td>
<td>Fetal loss &lt; 24 weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n/a: not available
birth, between women who had amniocentesis and those who did not.

Our study and others have some limitations. Most data were derived from observational studies with retrospective analysis that could lead to potential selection biases. As an example, mean maternal age was greater in women who underwent amniocentesis than in the control groups in the three studies that reported maternal age in both groups. Although the difference between the two groups was generally small (2 to 5 years), a report has shown an association between maternal age and the risk of fetal loss after amniocentesis. Even if we made efforts to exclude confounding factors for fetal loss, such as abnormal karyotype, it is possible that women who decided to undergo amniocentesis were at higher risk for fetal loss than women who did not. However, this hypothesis is limited by the fact that we did not find differences in other adverse outcomes such as preterm delivery between the groups. Finally, there are insufficient available data to evaluate the role of the amniocentesis technique, such as the number of punctures, in the risk of fetal loss. Prospective studies should be conducted to elucidate this important issue.

**CONCLUSION**

We believe that women with twin gestations seeking genetic amniocentesis should be informed about its potentially higher risk in multiple gestations, and they should also be informed that the risk of subsequent fetal loss prior to 24 weeks’ gestation is probably greater than 1%.

**ACKNOWLEDGEMENTS**

The editorial assistance of Ovid Da Silva is acknowledged.

**REFERENCES**


