OBSTETRICS

Amniocentesis and chorionic villus sampling in twin gestations: which is the best sampling technique?

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OBJECTIVE: To compare the fetal loss rate <24 weeks and the preterm premature rupture of the membranes <34 weeks' gestation according to type of invasive procedure and to sampling techniques in twins.

STUDY DESIGN: Retrospective cohort study of 204 twin pregnancies, who underwent amniocentesis (100) or chorionic villus sampling (104).

RESULTS: Fetal loss rate <4 weeks was 3.85% in chorionic villus sampling group and 4.00% in amniocentesis group (*P* value not significant). According to sampling technique, fetal loss rate was 4.17% (chorionic villus sampling 1 puncture), 2.70% (amniocentesis 1 puncture), 3.75% (chorionic villus sampling 2 punctures), and 4.76% (amniocentesis 2 punctures), (*P* values not significant). Preterm premature rupture of the membranes rate <34 weeks was 8.2% chorionic villus sampling group and 10% in amniocentesis group (*P* value not significant). According to sampling technique, preterm premature rupture of the membranes rate was 12.5% (chorionic villus sampling 1 puncture), 8.1% (amniocentesis 1 puncture), 6.9% (chorionic villus sampling 2 punctures), and 11.1 % (amniocentesis 2 punctures), (*P* values not significant).

CONCLUSION: Double entry technique does not affect significantly the outcomes evaluated, in both amniocentesis and chorionic villus sampling.

Key words: amniocentesis, chorionic villus sampling, fetal loss, Kaplan-Meier algorithm, sampling technique, twin gestation

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The incidence of twin pregnancies has been increasing over the past 3 decades[1] both due to greater reliance on fertility treatments, such as artificial or nonartificial reproductive technologies, and the rising maternal age secondary to delayed childbearing. Conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) twin rates are 26.9% and 26.02%, respectively, and triplet rates are 2.8% and 2.9%, respectively for an estimated total of approximately 197,000-220,000 infants worldwide.[2]

Patients with multiple gestations are at higher risk for fetal chromosomal anomalies than those with singletons. The overall probability that a multiple gestation contains an aneuploid fetus is directly related to its zygosity. If monozygotic twins most often have the same karyotype and the risk of an affected fetus approximates the maternal age risk of a singleton, each fetus of a dizygotic twin pair has an independent risk of aneuploidy, so that the pregnancy has approximately twice the singleton risk of an affected fetus.[3] Therefore, invasive prenatal diagnosis can be considered an important aspect of the antepartum management of patients with multiple gestation.[4] However, the recommendation to undergo this testing needs careful evaluation, in particular in gestations resulting from fertility treatments, because of the possible increased risk of pregnancy loss because of invasive procedures.

Previous studies[5-15] have reported high success rates in twin gestations for both chorionic villus sampling (CVS) and amniocentesis, whereas the rates of pregnancy loss vary widely. Ghidini et al[8] evaluated the risk of amniocentesis in twins, comparing 101 sampled pregnancies with an unsampled control group scanned at a matching gestational age, and detected no significant difference in total loss rates. The risk of fetal loss in twin pregnancies after CVS varies widely; those studies[13-15] that have compared fetal loss rate in singletons and twins found that CVS was not associated with an increased risk of either total pregnancy losses or single fetal losses.

Only 2 studies[13,15] have compared CVS with amniocentesis in twin pregnancies in contemporaneously sampled groups. They found no difference in the pregnancy loss rate and a potential small benefit for CVS regarding the total fetal loss rate.

No studies have compared second-trimester amniocentesis with first-trimester CVS according to sampling techniques: single vs double entry to the uterus. In some cases, 1 entry to the uterus may be made, sampling before the first placenta and then moving the needle to the other placenta in case of CVS or aspirating amniotic fluid from the sac of the first twin and then advanced through the intertwin membrane into the sac of the second one. The hypothesis is that by introducing a single-entry technique, the fetal loss in twins undergoing

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invasive prenatal diagnosis may be lower and similar to that of singletons.[16,17]

The aim of this study was to assess the invasive prenatal diagnosis-related risk of pregnancy loss in twin gestations and in particular to compare second-trimester amniocentesis with first-trimester CVS according to sampling techniques.

Materials and Methods

Between January 2002 and December 2007, 287 consecutive sets of multiple gestations underwent 153 first-trimester CVS (122 dichorionic, 12 monochorionic, 19 triplet) and 134 second-trimester amniocentesis (101 dichorionic, 27 monochorionic, 5 triplets) at the Unit of Prenatal Medicine (University of Bologna, Italy) and at Tecnobios Prenatale (Bologna, Italy). We excluded triplet pregnancies and 11 twin pregnancies (7 in the CVS group and 4 in the amniocentesis group) reduced to single for chromosomal anomalies in 1 fetus. Moreover, based on cytogenetic analysis, 2 pregnancies in both groups were electively terminated for abnormal results. Karyotyping was successfully obtained in all cases and no diagnostic errors were reported.

Twenty-one patients in the group undergoing CVS and 23 in the amniocentesis group were lost to follow-up.

This is a retrospective cohort study of the remaining 204 women with twin pregnancies of whom 100 underwent amniocentesis and 104 underwent CVS. All the procedures were performed transabdominally; when technically possible, a single entry was performed both in monochorionic and dichorionic pregnancies to reduce the risk of miscarriage. Both twins were sampled in all cases.

The main indication for CVS and amniocentesis was fetal chromosomal evaluation for advanced maternal age (≥35 years) and for parental decision. In a few cases, invasive procedures were performed because of the presence of chromosomal anomalies in previous pregnancies. All the individuals had a genetic counseling session provided by certified physicians. For the vast majority of the cases, the choice of procedure depended on the woman's personal appraisal of the risks and benefits of each technique. In some cases, only amniocentesis was available because of a late admission.

The decision about the sampling technique (single or double entry to the uterus) was made by the operators, based on placental location for CVS (1 entry in fused placentae; 2 entries in separate placentae) and on the technical possibility to perform a single entry for amniocentesis.

CVS was performed at 10-14 weeks' gestation, using a ultrasound-guided technique and a double coaxial needle system (outer needle 18 gauge, inner needle 20 gauge). The guide needle was first introduced into the placenta to be sampled. Thereafter, an aspiration needle was passed through the guide needle and villi obtained by aspiration through the sampling needle whose tip was moved forward and backward. When a single entry to the uterus was made, the first placenta was sampled first and then the needle was moved to the other placenta, taking care to ensure sampling of both placentae by aspirating the remotest possible areas of the 2 placentae.

Amniocentesis was performed at 14-20 weeks' gestation by a transabdominal free hand ultrasound-guided technique. A 22 gauge 0.7 mm needle was generally used and about 15 mL of amniotic fluid were obtained from each sac. When a single-uterine entry was made, after having aspirated amniotic fluid from the sac of the first twin, the syringe was removed, the stylet replaced in the needle, and the needle then advanced through the intertwin membrane under continuous ultrasound guidance into the sac of the second twin.

Ultrasound examination was always performed before the procedure to determine the exact number of fetuses and both placental and fetal location. Fetal heart rate variability was always demonstrated after the procedure.

Neither antibiotics nor tocolytics were given after amniocentesis or CVS. Information on pregnancy outcome was obtained from the patients themselves by telephone call several months after the expected date of birth or from maternal units.

Statistical analysis

Descriptive analysis was performed by routine test. The outcomes of interest of the study were the fetal loss rate before 24 weeks and the premature rupture of the membranes (pPROM) <34 weeks' gestation. The pPROM was diagnosed clinically, in presence of a history of watery vaginal discharge, confirmed on sterile speculum examination.

Data were weighted for the number of fetuses observed (n = 408) because the outcome of the same pregnancy could be different (alive vs lost fetus and/or PPROM of 1 or both the sacs).

Power analysis was performed by using the power analysis sample size (PASS) software at a fixed type I error = 0.05. The fetal loss rate and the pPROM rates were stratified according to type of procedure and to sampling techniques (single vs double entry), and calculated using the Kaplan-Meier algorithm or χ^2 test. Pregnancies that ended before the end of the follow-up constituted the "censor" group. The log rank test was used to explore differences between the generated subcategories (CVS vs amniocentesis and single vs double punctures). Results were considered statistically significant at P value < .05. The occurrence of pPROM was evaluated by χ^2 test.

Results

Mean gestational age (days ± SD) at the time of invasive procedures was 83 ± 5.98 and 112 ± 9.03 ($P < .001$). Table 1 shows the demographic characteristics of the 2 groups. No significant statistical difference was found except for the rate of monochorionic twins. The number of entries according to chorionicity are shown in Table 2.

All the surivival rates have been calculated by Kaplan-Meier algorithm. In our cohort of 204 patients with twin pregnancies, who underwent amniocentesis (100) or CVS (104), the total fetal loss rate <24 weeks was 3.92% (Figure 1). The fetal loss rate <24 weeks was 3.85% in the group of women who underwent CVS and 4.00 % in the group who underwent amniocentesis ($P = .95$, log rank test) (Figure 2).