

INVASIVE PROCEDURES IN TWINS FOR PRENATAL DIAGNOSIS

Aris Antsaklis

Professor in Obstetrics and Gynaecology University of Athens
antsakli@hol.gr

The incidence of twinning and the frequency of fetal aneuploidy increase with advance maternal age. Because of this, requests for prenatal diagnosis have increased recently as many women have delayed childbirth until later in life.

Risk of birth defects in multiple pregnancies

Frequency of monozygotic twins remains constant, while dizygotic twins become more frequent as maternal age increases. In these cases the risk that at least one fetus is aneuploid will be twice the maternal age risk for a singleton while the probability of both fetuses being involved is minimal. Dizygotic twins have a six fold increased risk that one fetus will be chromosomally abnormal. The true incidence of birth defects among twin pregnancies is controversial and it has been suggested that the risk is twice that of singleton. Furthermore prenatal diagnosis of hereditary diseases in dizygotic twins is a complex problem because of the probabilities of transmission of deleterious gene function independently to each fetus.

The risk of an autosomal recessive disorder has a 3 in 8 risk of at least one affected fetus and 1 in 8 chance the both will be affected.

Monozygotic twin are 100% concordant for genetic abnormalities and 2%-10% are concordant for development of defects.

Prenatal diagnosis in multifetal pregnancy may differ in several ways compared to singleton gestations. The obstetric risk following an invasive procedure is likely to be higher.

Before the procedure on should discuss with the couple the likelihood of sampling one or both sacs as well as the possibilities of having discordant results.

The management and the prenatal diagnosis of a multiple pregnancy are strongly influenced by chorionicity.

In a dichorionic pregnancy there are no direct consequences for the co-twin if a fetal demise occurs, but single intrauterine death in a monochorionic pregnancy can have severe repercussions furthermore for the surviving fetus.

In fused placentas the chorionicity must be determined by the ultrasonographic appearance of the dividing membrane. The difference in thickness between the thick dichorionic and the thin monochorionic membranes is much more obvious during the first trimester than it is later in pregnancies.

Furthermore the presence of an echogenic chorionic tissue projection into the base of the inter-twin membrane (Lambda sign) in the first trimester has been shown to be one of the most specific ultrasound landmarks of dichorionic placentation. In the second trimester the lambda sign is progressively more difficult to visualize and its absence after 20 weeks of gestation should be viewed with caution.

Invasive techniques for prenatal diagnosis in twin pregnancies

Amniocentesis must be followed by a detailed ultrasound, evaluation and each fetus must be carefully examined. Amniocentesis is routinely performed between 15 – 17 week's gestation, although procedures performed as early as 10 week's gestation have been reported.

In 1990 the single needle technique has been described in which needle entry was made into the proximal sac near the insertion of the dividing membrane and 20 ml of amniotic fluid was removed. The stylet was then replaced and under ultrasound guidance the needle was advanced through the membranes in the second sac and 20ml of amniotic fluid were removed from the second sac. This technique is particularly easy to perform before 18 weeks of gestation. The first milliliter of amniotic fluid is discarded in order to minimize the risk of contamination. Contamination of the needle with cells from the first sac or the dividing membrane would lead to an incorrect diagnosis of mosaicism for the second fetus. Another drawback is that puncture of the dividing membrane may result in an enlarging hole and pseudomonoamniotic twins with entrapment of fetal parts or the umbilical cord. An alternative approach to assure sampling from each sac utilizes two needles inserted simultaneously.

The possibility of chromosomally discordant results requires that amniotic fluid samples be labeled in such a way as to assure that the specific location of each fetus will remain identifiable. It has been suggested the geographic location of each fetus and placenta both in relationship to each other or to the cervix and a detailed diagram should be drawn at the time of procedure in order to minimize the possibility of confusing the samples. Ghidini et al (1993) reported on a large control study of amniocentesis in 101 twins with 108 control twins. Fetal loss rate was similar in both groups (3,5% and 3,2%) and the technique used involved two needle insertions.

Chorionic villus sampling (CVS) has been demonstrated to be safe and efficacious for sampling twin gestation and has the advantage of an earlier diagnosis than amniocentesis. Is best performed between 10-13 weeks of gestation, under ultrasound guidance. Sampling each sac is performed by either transcervical or transabdominal route. Undetected maternal or twin-twin cell contamination poses a serious potential problem that is the failure to diagnose a chromosomal abnormality in one or both twins.

Twin-twin contamination can occur, if a needle or catheter is dragged through one frondosum, while attempting to sample the other fetus. It can be avoided by using a combination of transcervical or transabdominal techniques. Since there is the possibility of one fetus having an abnormal result, documentation of the location of fetuses is equally as important with CVS as it is with amniocentesis. Although the position of sacs will remain certain even 2-3 weeks after sampling. A standard practice is to confirm the original diagnosis in both fetal and chorionic tissues. Results of chromosomal and genetic analyses can usually be obtain much more rapidly than with amniotic fluid cells.

The risk of fetal loss after CVS did not seem to differ between twin and singleton pregnancies (5% vs. 4%) (Pergament et al 1992)

The postprocedure pregnancy loss rate to 28 weeks has been demonstrated to be 2,4%.

Since CVS and amniocentesis have equal risks of pregnancy loss the question of which procedure is preferable must be addressed. Amniocentesis is technically easier and more widely available and accepted. Therefore, if the center were not skilled and experienced in CVS, then amniocentesis would be preferred. CVS has certain advantages, since the results are available one-month earlier and therapeutic termination as well as, selective termination within the first trimester is safer.

Fetal blood sampling in twins does not present any differences when compared to this procedure in singletons. This technique can be used as an alternative to amniocentesis from 20 weeks' gestation onwards or to confirm an abnormal karyotype in a dichorionic twin pregnancy when selective feticide is considered a few weeks after the initial procedure has been performed.

The Hellenic Experience

The last 15 years 372 patients with multiple pregnancies underwent genetic amniocenteses of which 365 in twin pregnancies. One was lost to follow up, 17 are ongoing pregnancies and we present the data on 347 pregnancies. All patients had an ultrasound examination before the procedure in order to localize the placental site, the fetal position and to determine the chorionicity.

The procedure was performed between the 11th and 23rd weeks' gestation (mean gestational age 17.2 weeks). In seven cases amniocentesis was performed before 14 completed weeks (first trimester amniocentesis) and in the remaining 340, between the 16th and 23rd weeks (second trimester amniocentesis) under ultrasound guidance. Patients were 19-52 years old (mean maternal age 36.18 years).

All procedures were performed with the double entry technique using a 22 Gauge needle and 20ml of amniotic fluid from each sac was withdrawn successfully. In 96 cases both placentas were posterior, in 110 both placentas were anterior and in the remaining 141 there was one anterior and one posterior placenta. The rates of miscarriage were 2.08% (2/96).

The patients were divided into three groups according to indication for amniocentesis. The first group (313 women) includes pregnancies at increased risk for chromosomal abnormalities and consists of women requesting karyotyping because of age more than 35 years, anxiety, abnormal serum biochemistry, increased nuchal translucency or previous pregnancy affected by chromosomal defect. The second group (21 women) includes pregnancies at increased risk of genetic syndromes or structural anomalies (β -thalassaemia, neural tube defects, Potter syndrome and exposure to radiation) and the third group (13 women) are pregnancies with structural anomalies or congenital infections (neural tube defects, cardiac defects, diaphragmatic hernia, omphalocele, multicystic dysplastic kidneys, severe growth restriction, twin-to-twin transfusion syndrome and toxoplasmosis).

In the first group there were 3 cases with chromosomal abnormality (3/313) (2 cases of trisomy 21 and one case with 47XXX, 3/626 fetuses, 0.4%) and selective feticide was performed. Two women with trisomy 21, age 38 and 41, had amniocentesis because of advanced maternal age and the third with the 47XXX because of abnormal triple test. Two pregnancies resulted in a healthy full term neonate and in the third pregnancy the baby died in the neonatal period because of congenital heart defect. Miscarriage of both twins occurred in 12 cases (3.83%). The perinatal loss was 5.91% and 2.03% before and after 28 weeks respectively. In 296 cases (94.56%) the pregnancy resulted in at least one livebirth.

In the second group, selective feticide was performed in 3 cases (3/21), one pregnancy was terminated at 19 weeks because of homozygous β -thalassaemia and one fetus with encephalocele died in the neonatal period, resulting in 6 affected fetuses (6/42, 14.28%). One pregnancy miscarried at 18 weeks (1/21, 4.76%). All 3 women that had selective feticide delivered healthy neonates at term. The perinatal loss was 16.66% and 11.42% before and after 28 weeks respectively. In 19 cases (90.47%) the pregnancy resulted in at least one livebirth.

In the third group, selective feticide was performed in 3 cases (3/13), because of neural tube defects and one pregnancy was terminated at 25 weeks because of severe twin-to-twin transfusion syndrome. One fetus with omphalocele and one with multicystic dysplastic kidneys died in utero and two fetuses with cardiac defects died in the neonatal period, resulting in 9 affected fetuses (9/26, 34.61%). All 3 women that had selective feticide delivered healthy neonates, two at term and the third at 30 weeks. One pregnancy miscarried at 20 weeks (7.69%). The perinatal loss was 26.92% and 21.05% before and after 28 weeks respectively. In 11 cases (84.61%) the pregnancy resulted in at least one livebirth.

In the group of the 7 first trimester amniocenteses there were no miscarriages. Selective feticide was performed in 3 cases because of neural tube defects (2 cases) and homozygous β -thalassaemia (one case) and three healthy babies were delivered two at term and one at 30 weeks. There were no other cases of fetal loss.

In the group of 340 second-trimester amniocenteses, 2 pregnancies were terminated (one because of homozygous β -thalassaemia and one because of severe twin-to-twin transfusion syndrome) and 6 women underwent selective feticide (2 because of trisomy 21, 1 because of 47XXX, 2 because of homozygous β -thalassaemia and 1 because of neural tube defect). One patient delivered a healthy livebirth at 30 weeks and there was one neonatal death of a term baby because of congenital heart defect. In this group 62.5% of pregnancies resulted in one livebirth.

In the 332 remaining second trimester amniocentesis patients there were 14 miscarriages (4.21%) and half of them occurred in the first 3 weeks after the procedure. The rate of preterm delivery before 32 weeks and before 35 weeks was 11.94% and 32.07% respectively. The perinatal loss rate before and after 28 weeks 5.87% and 3.20% respectively. The neonatal mortality rate before and after 28 weeks was 1.26% and 1.78% respectively. Of the 332 pregnancies 94.58% resulted in at least one livebirth.

The same period, 74 chorionic villus sampling procedures (CVS) were performed in multiple pregnancies. Six cases were lost to follow up and we present the data of the remaining 68 cases. The procedure was performed transabdominally in all cases between the 9th and 15th week of gestation (mean maternal age 30.7 years and

the rate of repeat procedure was 11.76% (8/68). There were 5 procedures in pregnancies with more than two fetuses (4 triplet and 1-quadruplet pregnancies) and the indications were thalassaemia-thalassaemia (4) and advanced maternal age (1). There were 6 fetuses affected by homozygous β -thalassaemia and 2 fetuses affected by trisomy 21 and in all cases selective fetocide was carried out at less than 14 weeks of gestation. In this group there were two spontaneous abortions at 20 and 21 weeks of gestation (40%) and one patient delivered prematurely at 34 weeks (33.33%).

CVS were performed in 63 twin pregnancies. The indications were increased risk of chromosomal abnormality in 10 cases (includes advanced maternal age, anxiety, increased nuchal translucency and family history of trisomy 21) and increased risk of genetic syndromes or structural anomalies in 53 women (Duchenne syndrome, phenylketonuria, β -thalassaemia, structural anomalies).

In seven cases the parents decided for termination, in six pregnancies because both fetuses had homozygous β -thalassaemia and in the one case of phenylketonuria the pregnancy was terminated before results became available.

In twelve cases with one fetus affected by homozygous β -thalassaemia selective fetocide was carried out between 11 and 16 weeks of gestation. One pregnancy (1/12, 8.33%) miscarried at 16 weeks and two patients delivered before 32 weeks healthy neonates (2/11, 18.18%). There were no other cases of fetal loss.

In the remaining 44 twin pregnancies there were 2 miscarriages, at 10 and 22 weeks of gestation (2/44, 2.27%). The rate of preterm delivery before 32 weeks and 35 weeks was 16.66% (7/42) and 23.80% (10/42) respectively. The perinatal loss before and after 28 weeks of gestation was 9.09% (8/88) and 1.25% (1/80) respectively. Overall 42 women (95.45%) delivered at least one healthy live baby.

Between 1977 and 2000 we performed 89 fetal blood-sampling procedures in twin pregnancies. Five cases were lost to follow up and we present the data of 84 cases. The patients were 20-44 years old (mean 28.3 years) and the procedures were performed at 18-29 weeks of gestation (mean 20.3 weeks). For the first 28 cases fetoscopy was carried out for fetal blood sampling where as after 1985 we switched to ultrasound guided cordocentesis, except of 7 cases where cardiocentesis was performed.

The indications for fetal blood sampling in 61 cases were the risk for hemoglobinopathies, advanced maternal age (2 cases) increased risk for congenital infection (2 cases) and in seven cases there was a suspicion of fetal defects in the present pregnancy.

Termination of the pregnancy was carried out in 17 cases. In 9 cases both fetuses were affected by homozygous β -thalassaemia and in one case one fetus had severe obstructive uropathy and the other had hydranencephaly. In 6 cases only one fetus was affected 4 cases by homozygous β -thalassaemia, 1 case by trisomy 21, one case by bowel obstruction and the parents opted for termination one patient had termination of her pregnancy because of chorioamnionitis.

Selective fetocide was performed in 12 patients between 20 and 29 weeks of gestation. There were 10 fetuses affected by β -thalassaemia, one fetus with chromosomal abnormality (47XXY), and one with pulmonary hypoplasia. No miscarriages occurred in this group. The rate of preterm delivery before 32 and 35 weeks was 41.66% and 50% respectively.

In the remaining 55 pregnancies there were 2 miscarriages at 20 and 23 weeks of gestation (3.92%). The rate of preterm delivery before 32 and 35 weeks was 16.98% and 28.30% respectively. The perinatal mortality rate before and after 28 weeks was 8.18% and 5.94% respectively. Overall 90.09% of women had at least one live baby.