63 Amniocentesis

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HISTORY

Amniocentesis is the oldest invasive procedure for prenatal diagnosis. It was first used toward the end of the 19th century for evacuative paracentesis in pregnancies complicated by hydramnion (1). At the beginning of the 1950s, it was used to determine the amniotic fluid composition in cases of Rhesus isoimmunization (2). However, amniocentesis was used for diagnostic reasons in the 1950s, as a method for sex determination by the identification of Barr bodies in the noncultured amniocytes (3). About 10 years later, the karyotype of the embryo was determined through an amniotic fluid cell culture (4). Since then, amniocentesis has been established as a basic invasive method for the prenatal diagnosis of various pregnancy-related conditions, such as fetal karyotyping, diagnosis of metabolic or enzymatic diseases, assessment of the severity of hemolytic disease, establishment of lung maturity, and diagnosis of fetal infections. Additionally, amniocentesis is used for the infusion of various drugs into the amniotic cavity, determination of the composition of the amniotic fluid, and finally for evacuation of hydramnion.

INDICATIONS

Amniocentesis is used for both prenatal diagnosis and fetal therapy (Table 1).

Chromosome and DNA Analysis

Examination of the number and structure of fetal chromosomes (fetal karyotyping) is the main indication for amniocentesis. It is offered to women in cases when the risk for fetal aneuploidies is high such as advanced maternal age (traditionally ≥ 35 years), previous fetal aneuploidy, parental balanced translocation, positive screening test in the first or second trimester, identification of aneuploidy markers in genetic sonogram, and so on. Another indication is an ambiguous result from a previous test such as karyotype mosaicism obtained by the chorionic villus sampling (CVS). Fetal karyotype is examined either in cultured cells or with PCR technology. However, the result of the latter technique should always be confirmed with culture.

Fluorescent-labeled in situ hybridization allows for DNA study, with direct probing, and therefore the diagnosis of fetuses with gene diseases such as hemophilia, β -thalassemia, cystic fibrosis, and so on.

Fetal Infection

Diagnosis of fetal infection from *Toxoplasma gondii*, cytomegalovirus, and so on, in cases of maternal seroconversion is possible by examination of amniotic fluid with PCR-based technologies.

Lung Maturity

Fetal lung maturity can be assessed indirectly using both qualitative and quantitative characteristics of amniotic fluid.

Pulmonary maturity can be assessed by the measurement of lecithin/sphingomyelin ratio or the concentration of phosphatidylglycerol in amniotic fluid. Similarly, the detection of lamellar bodies and the study of other characteristics of the amniotic fluid may give information about lung maturity. However, the improvement in neonatal care and the accurate confirmation of gestational age with ultrasound early in pregnancy have diminished the need for examination of amniotic fluid and thus amniocentesis in order to confirm lung maturity.

Chorioamnionitis

Premature rupture of the membranes (PROM) and preterm labor have been associated with amniotic fluid bacterial colonization (5). Amniocentesis and gram stain, microscopy, and culture of the amniotic fluid have been advocated in cases of premature rupture of membranes or premature labor. Researchers who advocate for amniocentesis state that early diagnosis and management are possible and may result in better perinatal outcome. However, spontaneous labor within 24 hours after membrane rupture is very common and thus there are no data to support amniocentesis as a clinical standard in cases of PROM.

Biochemistry

Amniotic fluid collection and estimation of enzyme level were used in order to diagnose metabolic diseases and other conditions such as cystic fibrosis or congenital adrenal hyperplasia before DNA techniques were applied. This practice has been replaced by the most accurate detection of gene mutation responsible for the disease in fetal cells collected by amniocentesis or CVS.

Another past indication of amniocentesis is the evaluation of fetal hemolytic anemia in cases of Rhesus alloimmunization. Amniocentesis and amniotic fluid spectrophotometry are performed less since middle cerebral artery Doppler velocimetry has been proposed as a routine test for the evaluation of hemolysis and fetal anemia.

Obstetric Cholestasis

Amniocentesis is used in obstetric cholestasis in order to diagnose meconium-stained amniotic fluid, which is a common complication of this pregnancy-related disorder and related to intrauterine fetal demise. However, improvements in understanding both the pathophysiology of the disease and the management of these cases have led to decreased need for amniocentesis in this context.

Fetal Therapy

Amniocentesis is used for uterine decompression in case of hydramnios and twin-to-twin transfusion syndrome in

Table 1 Indications for Amniocentesis

Chromosome analysis
Advanced maternal age
Abnormal biochemical screen
Ultrasound findings
Family or personal history of trisomy
Abnormal parental karyotype
DNA analysis
Genetic testing
Endocrine disorders
Suspected fetal anemia
Rhesus sensitization
Fetal hydrops
Fetal infection
Lung maturity
Chorioamnionitis
Biochemistry
Obstetric cholestasis
Fetal therapy

Figure 1 Amniocentesis in singleton. Note that the whole length and the tip of the needle can be identified when the procedure is done under ultrasound guidance.

monochorionic (MC) twins. Amnioinfusion has been used in cases of severe oligohydramnios, but research is needed in order to define both the indications and the outcome of such an intervention. Finally, thyroxin has been injected into the amniotic sac to treat goitrous hypothyroidism of the fetus.

TECHNIQUE

Amniocentesis is mainly performed during the second trimester but can be performed also during the third trimester when certain indications stand. Amniocentesis for prenatal diagnosis of genetic diseases of the fetus is traditionally carried out between 15 and 18 weeks of gestation when the fundus of the uterus is easily accessible. Additionally in this gestational age, the volume of amniotic fluid is 150 to 200mL, which permits the removal of 15 to 20mL of amniotic fluid, which is adequate for this test without creating problems. Moreover, at this gestational stage, the relationship between the viable and nonviable cells of the amniotic fluid is the optimal in order to have results. For the above reasons, amniocentesis should be carried out at this time (6). Early amniocentesis (between 11 and 14 weeks) has been associated with increased risk of fetal malformations, mainly clubfeet, and has been abandoned.

In any case, before amniocentesis is performed, pregnant women should receive appropriate genetic counseling concerning the risks, the limitations, and the benefits of the procedure and the tests. Informed consent should be obtained before the procedure. A detailed ultrasound examination must be done in order to determine the number of fetuses, gestation age, cardiac function, possible abnormal formation, amniotic fluid quantity, position of the placenta, existence of fibromas in the uterus, existence of adnexal masses, and finally possible cervical inadequacy. When this ultrasound examination has been completed, the entry site of the needle into the amniotic cavity is determined (7,8). Preferred pockets are located away from the fetal face. Sites that must also be determined and avoided as much as possible during amniocentesis is the umbilical cord—depending on its position—and the placenta. However, none of these is absolutely necessary to obtain amniotic fluid safely. The skin around the entry site of the needle is carefully cleaned with an antiseptic solution.

A disposable 22-G spinal needle (Fig. 1) is carefully guided into the amniotic cavity under continuous ultrasound monitoring. When the needle enters the corresponding area, the inner needle is removed and a syringe is attached to the needle hub. If freely flowing fluid is not obtained, the needle is then repositioned. Failure to aspirate amniotic fluid is most commonly due to membrane tenting. When this occurs, the needle appears inside the amniotic cavity, but the needle tip has not actually penetrated the amniotic sac. This usually occurs when an early amniocentesis is carried out, when the amnion and the chorion have not yet fused. In this case, it is recommended that the inner needle is replaced and the needle's entry point to the amniotic cavity is carefully changed so as to avoid injuring the fetus or the umbilical cord. Another reason for failing to take a sample of amniotic fluid is when the fetal membranes plug up the opening of the needle, due to the negative pressure that is exerted here. If this occurs, we then slightly change the needle site without exerting negative pressure. The first 1 to 2 cc of amniotic fluid that is aspirated is discarded because in theory it may contain maternal cells and thus gives us inaccurate results. An amount of approximately 20-cc amniotic fluid is considered to be enough for the results we require when amniocentesis is carried out between the 15th and 20th weeks of gestation. An ultrasound examination is performed after the amniocentesis is completed to document fetal cardiac activity and to look for evidence of bleeding into the amniotic cavity. The pregnant woman is then advised to rest and avoid intercourse for 15 days and informed about possible complications, such as persistent uterine cramping, fever, bleeding, or leakage of the amniotic fluid, which she must report timely.

AMNIOCENTESIS COMPLICATIONS

Amniocentesis is an interventional procedure, and since the feto-maternal unit is injured, a number of complications can ensue. Maternal complications are rare and include perforation of the intra-abdominal viscera with subsequent intra-abdominal infection, sepsis, bleeding, blood group sensitization, and uterine contractions (9). On the other hand, fetal complications are more of a concern and include fetal

Table 2 Fetal Loss Rate in Review by Seeds (15)

Ultrasound use	Preamniocentesis scan	Concurrent ultrasound guidance	p Value
All studies			
Losses (n/N)			
Amniocentesis	714/33,975 (2.1%)	478/34,144 (1.40%)	<0.0001
Control subjects	88/6457 (1.4%)	130/12,097 (1.08%)	NS
Difference (%)	0.74	0.33	
p Value	<0.0001	<0.0007	
95% CI for difference	0.37, 1.1	0.09, 0.56	
Only controlled studies			
Losses (n/N)			
Amniocentesis	170/8607 (2.0%)	191/11,372 (1.68%)	NS
Control subjects	88/6457 (1.4%)	130/12,097 (1.08%)	NS
p Value	0.0042	<0.0001	
Odds ratio	1.46	1.57	
Difference (%)	0.6	0.6	
95% CI for difference	0.19, 1.03	0.31, 0.9	

Source: From Ref. 15.

loss, needle puncture injuries, placenta abruption, preterm labor, injury due to withdrawal of amniotic fluid (e.g., amniotic bands), and rupture of the membranes. Amniocentesis may also cause intra-amniotic infections through the introduction of microorganisms into the amniotic cavity via the needle. This may eventually lead to the abortion or death of the fetus. In order to eliminate the possibility of an infectious complication, rules of aseptic procedures, which apply in all surgical procedures, must be applied here, such as the aseptic cleaning of the skin and using septic gloves and needles. However, endometrial infections are not always related to amniocentesis, but may exist before the prenatal intervention (10,11).

Fetal Loss

Fetal loss is the major risk of second-trimester genetic amniocentesis. When amniocentesis was first introduced in the clinical practice, the risk of miscarriage related to the procedure was not clear mainly because there was lack of ultrasound guidance and lack of determination of fetal viability before the procedure. Since fetal miscarriage does not occur only in association with amniocentesis, it is essential to take into consideration the background loss rate, which is associated with the gestation age, parity, and any other underlying risk factors such as pathologic conditions of the mother (e.g., uncontrolled diabetes mellitus, severe hypertension induced by lupus, etc.) and the fetus (e.g., structural anomalies, chromosomal abnormalities, etc.) as well. Gestational age at the procedure is an important determinant of the observed fetal loss rate, since the earlier the pregnancy, the greater is the pre-procedural risk of miscarriage.

The safety of amniocentesis has been evaluated by several series from single centers and a number of multicentric studies. The difficulties in evaluating the procedure-related loss rate have been shown by the controversial results of these studies.

In 1976, the National Institute of Child Health and Human Development conducted a case-control/cohort study to evaluate the safety and accuracy of second-trimester amniocentesis for prenatal diagnosis and reported that the study group that underwent amniocentesis had a 3.5% total loss rate while the controls had 3.2% (9). The authors also concluded that the loss risk was unrelated to previous miscarriage,

volume of aspirated fluid, or number of attempts. In the same year, the Canadian Collaborative Study reported a loss rate of 3.2%, but there were no controls (12). In this study, an increased risk of loss was reported in cases of failed attempts and large bore needles (>19). The third collaborative case-control study was conducted by the British Working Party on amniocentesis in 1978 (13) and the writers reported a loss rate of 1.5% related to amniocentesis. They reported a 2.4% fetal loss rate <28 weeks in the study group and 1.2% in controls. This study however was strongly criticized mainly on the selection of control individuals who were recruited in the study later in gestation and on the replacement of some of the matched controls who had aborted.

The only randomized trial for the estimation of miscarriage risk related to amniocentesis was done in Denmark in 1986 (14). This study randomized 4606 low-risk women aged 24 to 35 years to have or not to have an amniocentesis, which was carried out using a 20-gauge needle under real-time ultrasound guidance. Most procedures were performed between 16 and 18 weeks of gestation. The amniocentesis group had a loss rate, which exceeded the control group by 1% (1.7% and 0.7% respectively), a figure which has often been quoted in counseling. More than half of the procedures were performed by one operator and the rest by four less experienced operators. The placenta was avoided whenever possible but was perforated in 15% of cases and the study indicated that perforation of the placental was associated with a relative risk of 2.6 for miscarriage.

Since then, a lot of studies reported amniocentesis-related fetal loss rate between 0.2% and 0.9%.

In 2001, Seeds performed a systematic review of 29 controlled and uncontrolled studies with a total of 68,119 amniocenteses (15) and he concluded that amniocentesis with concurrent ultrasound guidance in controlled studies appears to be associated with a procedure-related rate of excess pregnancy loss of 0.6% (95% CI, 0.31, 0.90) (Table 2). The use of concurrent ultrasound guidance appears to reduce the number of punctures and the incidence of blood-stained fluid and eliminates the direct fetal needle trauma. Finally, the review did not substantiate an increased rate of pregnancy loss if placental puncture is required.

In 2006, another trial was published in order to quantify the contemporary procedure-related loss rate after midtrimester amniocentesis using a database generated from patients who were recruited to the First- and Second-Trimester Evaluation of Risk for Aneuploidy trial (16). A total of 35,003 unselected patients from the general population with viable singleton pregnancies were enrolled in this trial and 3096 of them underwent midtrimester amniocentesis. The total spontaneous fetal loss rate less than 24 weeks of gestation in the study group was 1.0% and was not statistically different from the background 0.94% rate seen in the control group ($p=0.74$, 95% CI, 0.26%, 0.49%). The researchers stated that the odds of pregnancy loss were actually *lower* in patients who underwent amniocentesis because of advanced maternal age or screen positive results compared with those who did not. They concluded that this is likely due to the fact that spontaneous pregnancy loss is so strongly associated with aneuploidy, and patients who have an amniocentesis would presumably terminate aneuploid fetuses in most cases before a spontaneous loss could occur. They stated that it is not surprising that patients in these categories who elected not to undergo amniocentesis had a higher rate of spontaneous loss because they were carrying a higher proportion of aneuploid fetuses.

Recently, Mujezinovic and Alfirevic compiled a systematic review of complications related to genetic amniocentesis and CVS to provide benchmark data for counseling and performance assessment of individual operators (17). Twenty-nine articles of amniocentesis were included and pooled pregnancy loss within 14 days was 0.6% (95% CI 0.5–0.7), rising to 0.9% (95% CI 0.6–1.3) for pregnancy loss before 24 weeks and 1.9% (95% CI 1.4–2.5) for total pregnancy loss (Table 3). However, the authors noted that since most of the reviewed studies do not take into account the background risk and therefore do not address the issue of added procedure-related risk, these figures may be useful only for counseling purposes in a broad sense. The authors identified five studies that have included control groups in order to quantify the background risk. Overall, these studies reported higher pregnancy losses compared with uncontrolled case series and the pooled results showed a relative increase of 25% for total pregnancy loss after amniocentesis and 46% for pregnancy losses before 24 or 28 weeks of gestation. However, the results for the latter were

quite heterogeneous, resulting in very wide, statistically no significant confidence intervals. The absolute risk difference between cases and controls was quite similar, showing that second-trimester pregnancy loss and total fetal loss after an amniocentesis increase by approximately 0.6%. The reviewers noted that although the risk of pregnancy loss is relatively low, lack of adequate controls tends to underestimate the true added risk of prenatal invasive procedures.

Midtrimester amniocentesis does not appear to further increase the already increased risk for second-trimester spontaneous abortion in women with uterine myomas (18).

Preterm Delivery

The association of second-trimester amniocentesis and delivery before 37 weeks was evaluated by a case-control study in 2003 by the EUROPOP Group (19). During 1994 to 1997, 3091 preterm births and 5298 controls randomly selected from singleton births born at term were analyzed. An increased risk of preterm delivery was found in women having second-trimester genetic amniocentesis after taking into account other risk factors and confounding variables (OR 1.59, 95% CI 1.31–1.92). The association was statistically significant and similar for spontaneous preterm delivery and induced preterm delivery. Since the data are limited, further research is needed on the causative role of amniocentesis.

Fetal Trauma

The risk of direct fetal needle injury often is presumed to be reduced with the use of simultaneous ultrasound guidance, but several case reports document or attribute cases of fetal injury to the amniocentesis needle, despite the use of simultaneous guidance for the procedure (15). Although several case reports document or attribute cases of fetal injury to the amniocentesis needle (20–22), the attribution has been generally by association only and not on the basis of direct evidence (21,23–31).

Alloimmunization

Feto-maternal hemorrhage occurs in approximately 50% of all women (32) and during amniocentesis on one out of six (33). The attributed risk is 1% greater than the background risk of 1.5% (34). As for preventing the Rhesus sensitization of the mother and when the husband is Rhesus positive, every

Table 3 Pregnancy Loss After Amniocentesis

Type of pregnancy loss	Combined total (n/N)	Combined proportion %	95% CI
Less than 20 weeks of pregnancy	22/2133	1.1	0.7–1.5
More than 20 weeks of pregnancy	17/1775	0.9	0.5–1.4
Less than 24 weeks of pregnancy	122/14,057	0.9	0.6–1.3
More than 24 weeks of pregnancy	57/4195	1.0	0.5–1.8
Less than 28 weeks of pregnancy	283/14,915	1.7	1.3–2.2
More than 28 weeks of pregnancy	134/14,596	0.9	0.7–1.2
Less than 14 days after AC	102/17,047	0.6	0.5–0.7
Less than 30 days after AC	85/10,727	0.8	0.4–1.2
Less than 60 days after AC	66/9406	0.7	0.5–0.9
Total pregnancy loss	627/49,413	1.9	1.4–2.5
Multiple insertions	226/12,142	2.0	0.9–3.6
Bloody or discolored tap	468/10,908	4.1	1.1–8.7

Abbreviations: CI, confidence interval; AC, amniocentesis. Source: From Ref. 17.

Rhesus-negative mother must be given 300- μ g anti-D immunoglobulin after amniocentesis, provided of course the indirect Coombs is negative (35,36).

Other means to minimize the risk is the use of small-gauge needles and to avoid transplacental approach (36).

Respiratory Distress

A significant although small increased risk of neonatal respiratory distress was noted in some studies but not in others (9,37). This complication has been attributed to chronic oligohydramnios, which might follow amniocentesis.

Orthopedic Abnormalities

The British collaboration study (13) implied that amniocentesis is associated with talipes equinovarus, congenital dislocation, and subluxation of the hip. This trend was not confirmed by other studies. This complication has been attributed to chronic oligohydramnios, which might follow amniocentesis.

Transmission of Viral Diseases

Before antiretroviral therapy (ART) use, maternal HIV infection was considered a relative contraindication for amniocentesis and invasive prenatal procedures. Since maternal ART has been used, amniotic fluid viral load is usually undetectable even if the virus was detectable in maternal blood samples. This finding raises the possibility to perform amniocentesis, when it is indicated, to provide the mother with an adequate antiretroviral treatment (38).

Leakage of Amniotic Fluid and Vaginal Bleeding

Uterine contractions and vaginal spotting are quite common (2–3%) after amniocentesis; however, they are almost always transient and self-limited. Leakage of amniotic fluid occurs in 1% of women who underwent second-trimester amniocentesis. It is usually small amount and the outcome is normal in 90% of the patients who experienced fluid leakage (39).

SPECIAL CIRCUMSTANCES

Discolored Amniotic Fluid

The presence of blood in the amniotic fluid occurs in a very small number of cases. It is usually of maternal origin and under no circumstances does it affect the culture or multiplication of the cells. However, the presence of blood in the amniotic fluid is related to the increased possibility that the outcome of the pregnancy will not be good. Thus, the percentage of fetal losses in these cases reaches 33% of all losses (40–42). If at the same time the blood-stained or discolored amniotic fluid is accompanied by increased levels of alpha-fetoprotein (AFP), then in almost all instances the prenatal results will not be good.

Amniocentesis in the Presence of Bleeding

The fetal loss following amniocentesis in women who had bleeding before the procedure is probably increased. Antsaklis et al. found an excess loss rate of 0.6% in women who had experienced bleeding before amniocentesis (43).

History of bleeding is not a contraindication for the procedure but patients should be counseled about the risks.

AMNIOCENTESIS IN MULTIPLE GESTATIONS

Amniocentesis has been recognized as a relatively safe procedure for prenatal diagnosis in singletons. It has been a common practice to extrapolate data derived from singletons to multiples. However, implementation of invasive prenatal diagnostic modalities in twins or higher order pregnancies seems to be more complex. Amniocentesis can be safely performed on twins or higher multiple gestations as long as the sacs have been defined through ultrasound examination. The management and the prenatal diagnosis of a multiple pregnancy are challenging, not only related to the number of fetuses, but also strongly influenced by chorionicity. Chorionicity refers to the placentation whereas zygosity implies the genetic profile of the pregnancy and therefore determines the degree of risk and whether or not the fetuses may be concordant or discordant for chromosomal abnormalities.

The amniocentesis technique in multiple gestations is different from that used in a single gestation and therefore women should be referred to experienced centers. The number of fetuses, their positions, the number of gestation sacs and placentas, the gender, the age and the degree of development of each fetus, and finally the fetal anatomy are all-important and necessary elements of the ultrasound examination that precedes amniocentesis. An important element before amniocentesis is determining the position of each fetus, which is achieved by mapping the site of the placenta that is feeding the specific fetus, because the position of the placenta changes very little over time while the position of the fetus never remains stable. Consequently, in the case where after amniocentesis the problem of selective feticide arises due to an abnormal result, we must know which is the specific fetus in question and its reference point so that we can pinpoint it. This is nothing more than determining the position of the placenta, as determined by the mapping that was carried out before amniocentesis.

The Risk of Aneuploidy in Multiple Gestations

As far as zygosity represents the genetic makeup of the developing entity, accurate determination of this parameter is considered a prerequisite in multiple pregnancy prenatal screening for aneuploidies. In the clinical setting, zygosity is usually inferred from the ultrasound diagnosis of chorionicity (44), the latter best achieved in the first trimester. In diamniotic (DA) pregnancies with fused placentas, measurements of the thickness of the dividing membrane using a cutoff value of 2 mm can differentiate MC from dichorionic (DC) twinning, though a high inter- and intra-observer variability has been reported. Sonographic detection of the “lambda” or “twin peak” sign is reported as a more reliable indicator of DC placentation with an accuracy of 100% in 10 to 14 weeks of gestation (45). Delayed sonographic evaluation in the second trimester is associated with a 10% to 12% chorionicity misinterpretation rate (46,47), while after 20 weeks of gestation, the determination may turn out impossible. Therefore, in the absence of the “lambda” or “twin peak” sign in a DA twin pregnancy, single placentation and monozygosity is concluded, when the rare cases of MC-DZ (dizygotic) gestations following ART reported are not taken into consideration. However, when a single amniotic sac is

Another approach called *double simultaneous amniocentesis* was introduced by Bahado-Singh et al. in 1992 (76). This technique involves two needles inserted separately into the amniotic sacs under ultrasound visualization like in the *technique of double amniocentesis*. The difference is that after aspiration of the amniotic fluid from the first sac, the needle is left in place indicating the sampled cavity and the second insertion is made into the other sac. The main advantage seems to be the documentation of correct sampling from each sac. However, it is not widely used mainly because it is more time consuming and thereby the experience with this approach is limited.

Amniocentesis, like all prenatal diagnostic invasive procedures, must be preceded by a detailed ultrasound evaluation of the multiple pregnancy involving chorionicity and amnionity determination and documentation of the location of the placenta(s). Moreover, relative position, size, and anatomy of each fetus should be specified and “labeling” of the multiples using text and diagram should be performed to ensure correct sampling from each of them. Recently, the role of amniotic fluid alpha-fetoprotein values was evaluated in confirmation of both sacs in a dichorionic pregnancy being sampled (77).

Concern regarding potential post-amniocentesis increase in fetal loss rate in multiples has led to a plethora of studies evaluating this parameter.

The percentage of total fetal loss, as expressed by spontaneous abortions, stillbirths, and neonatal deaths, is approximately 10.8%, a percentage that is almost the same as the statistical miscarriage percentage that is observed in every twin gestation after the 17th week of gestation (42,78).

Early reports suggested a higher fetal loss rate in twin pregnancies than in those with singletons (79–81). However, these studies did not take into account the possibility that the increased fetal wastage might be attributed to the twin pregnancy itself rather than the invasive procedure. Later on,

it was reported that the maternal history of twins per se carries a pregnancy loss rate up to 24 weeks of about 6.3% and severe prematurity (24–28 weeks) rate of about 8% (82). Most series of pregnancy outcome following second-trimester amniocentesis report loss rates before 20 weeks of gestation of between 1% and 2.5% and a much higher loss rate before 28 weeks. In a multicenter European study, the pregnancy loss rate was estimated to be 2.3% and 3.7% before 20 and 28 weeks of gestation respectively (83). In a case-control study, a similar fetal loss rate was reported between sampled twins and unsampled matched twin controls (3.5% vs. 3.2%) (84).

According to other studies, the percentage of fetal losses up to the 28th week of gestation in twin gestations after amniocentesis ranges from 3.57% to 3.78% versus 0.6% for single gestations. This means that the comparatively increased fetal losses observed in twin gestations versus singletons after amniocentesis are not due to amniocentesis, but rather due to the normally increased statistical percentage for abortions seen in twin gestations.

In a recent review by Weisz and Rodeck (85), the pooled data regarding the pregnancy loss rate after amniocentesis are comparable with these background loss rates for twin pregnancies (Table 4).

Finally, amniocentesis post multifetal pregnancy reduction (MFPR) does not seem to significantly increase the rate of fetal loss. However, one group has shown that amniocentesis post MFPR was significantly associated with increased risk of preterm delivery at 25 to 28 weeks (86).

Early Amniocentesis

Early amniocentesis was thought as an alternative option for early prenatal diagnosis in centers in which CVS was not offered. It was suggested to be performed between the 12th and 15th gestational weeks. The technique used for

Table 4 Fetal Loss Rate in Twin Pregnancies Following Amniocentesis

References	No. of twin pregnancies	Fetal loss (%) <20 weeks of gestation	Total fetal loss (%)	Control group (%)	Comment
Anderson et al., 1991 (81)	339	—	3.57	—	Compared with 0.6% miscarriage
Beekhuis et al., 1992 (98)	63	—	4.0	—	A study evaluating maternal Hb as marking dye
Pruggmayer et al., 1991 (83)	529	3.7	8.7	—	
Ghidini et al., 1993 (84)	101	—	3.5	3.2	Control group of twins without amniocentesis
Wapner et al., 1993 (99)	81	—	9.3	—	Compared with 3.9% fetal loss rate after CVS
Tabsh and Theroux, 1995 (100)	53	—	9.4	—	All amniocentesis post-MFPR
Sebire et al., 1996 (101)	176	2.55	4.0	—	Single-needle technique. Total loss attributed to procedure: 2.8%
McLean et al., 1998 (102)	79	5.6	—	—	All amniocentesis post-MFPR
Zoppini et al., 1993 (103)	50	2	—	—	
Horger et al., 2001 (104)	71	—	7.6	—	
Yukobowich et al., 2001 (105)	476	2.73	7.73	0.63	Two control groups. Twins, without amniocentesis and singletons
Antsaklis et al., 2002 (71)	347	—	8.77	—	

Abbreviations: CVS, chorionic villus sampling; MFPR, multifetal pregnancy reduction. Source: From Ref. 85.

performing early amniocentesis is similar to that used in classical amniocentesis and the amount of amniotic fluid removed is equal in milliliters to the weeks of gestation (87). Failure or difficulty in taking out the sample was due to tenting of the membrane caused by the needle (88). Since amniocentesis was performed quite early, it was thought that it could give the opportunity to examine the karyotype of the fetus and determine the AFP and acetylcholinesterase for the early detection of those cases that show increased risk for nervous system abnormalities (89–91) with the same safety as midtrimester amniocentesis. These expectations were confirmed by observational studies although definite conclusions could not be drawn because of the small numbers (92).

The safety of early amniocentesis was not completely clear until the Canadian trial. In two randomized studies (93,94), fetal loss rate was significantly higher for early amniocentesis when compared with transabdominal CVS (TA-CVS). In another randomized trial (95), which compared the first-trimester and midtrimester amniocentesis, fetal losses were more frequently observed after first-trimester than after midtrimester amniocentesis. Additionally, early amniocentesis had a significantly higher rate of amniotic fluid leakage than TA-CVS and midtrimester amniocentesis.

Excessive risk for talipes equinovarus has been uniformly observed in all early amniocentesis studies, with an even greater likelihood when physician confirms amniotic fluid leakage (17.7% vs. 0.5%) (93). Talipes can occur without apparent leakage of amniotic fluid, which likely results from a reduced amniotic fluid volume via transfer of amniotic fluid to the extra-embryonic coelomic space. TA-CVS did not have any effect on malformation rate. The Canadian study therefore indicated that early amniocentesis should be performed only if there is no alternative.

In 2005, Alfirevic et al. conducted a systematic review (96) for the Cochrane collaboration, and the pooled total pregnancy loss after early amniocentesis was 7.6% compared with 5.9% after midtrimester amniocentesis. Similarly, the number of talipes equinovarus was higher in cases of early amniocentesis (1.3% vs. 0.09%). In the same review, combined total loss of pregnancy after early amniocentesis was 6.3% compared with 5% after CVS.

It is therefore justified to say that early amniocentesis should be performed only in extenuating circumstances, especially since TA-CVS has undoubtedly been proved a safe and more effective alternative (97).

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