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Amniocentesis

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INTRODUCTION

Mid-trimester amniocentesis is the most commonly performed invasive technique in prenatal diagnosis. Valenti et al. reported the first successful diagnosis of Down's syndrome in 1968.[1] Since that time the number of mid trimester amniocentesis has increased dramatically and amniocentesis is nowadays an established standard tool in the assessment of pregnancies that are at risk for a variety of chromosomal disorders, single gene defects, biochemical analysis and fetal infections etc. Chromosome analysis and tests related to risk screening for aneuploidy remain world wide by far the most common laboratory procedures in prenatal diagnosis. Conventional chromosome analysis has maintained its role as a gold standard for the primary exclusion of aneuploidy from amniotic fluid cells. Frequent indications listed in table 1 for offering second trimester amniocentesis are pregnancies considered to be at an increased risk (Table 74.1).

Table 74.1: Common indications for amniocentesis

- Advanced maternal age (= 35 years old)
- Family history for chromosome anomalies, single gene defects etc.
- Abnormal maternal serum screening in the second trimester
- Increased risk for chromosomal anomalies following first trimester screening – if chorionic villus sampling is not available
- Abnormal ultrasound findings
- Maternal infections potentially affecting the fetus

Prior to any prenatal invasive test, patients should be counselled. The risks and benefits of all invasive and non-invasive tests must be discussed as well as the limitations of any procedure. In the cases of specific risk factors or abnormal ultrasound findings the information should be as complete and appropriate as possible. Considering the current developments in prenatal diagnosis by performing advanced methods for individual risk calculation ,e.g. nuchal translucency measurement and biochemical serum marker screening, counselling of pregnant women will become more and more important. It can be assumed that in this context a more selective approach to invasive testing may result in the next years. One of the significant problems in counselling pregnant women is still how to explain women (e.g. social-economical, ethical, cultural variability) the complex implications of risk calculation. Own experiences showed that appropriate individual counselling is more time consuming and requires more appropriate methods.

TECHNIQUE

Mid trimester amniocentesis is commonly performed between 15-16 weeks of gestation (Figs 74.1 and 74.2). Prior to the procedure ultrasound evaluation of the uterus (e.g. exclusion of fibroids), the fetus, amniotic fluid volume, and position of the placenta is recommended, because the failure rate can be

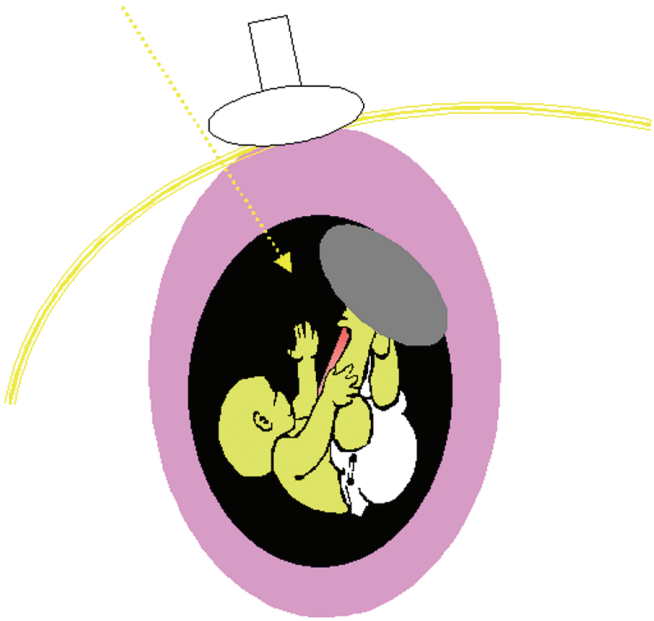

Fig. 74.1: Diagram of amniocentesis. Transplacental perforation should be avoided if possible

reduced by avoiding the removal of bloody fluid or multiple injections.[2-4] Patients anxiety may be reduced if adequate information is given to the patients during the procedure at each step. After disinfection of the maternal abdomen amniocentesis is performed under direct ultrasound guidance generally using a 18-20 gauge needle. The obtained amniotic fluid volume should be not more than 1 ml per gestational week. The first 2 ml of amniotic fluid should be rejected in order to avoid maternal cell contamination. If bloody amniotic fluid is aspirated the analysis of the chromosomes from cultured cells will take longer time and there might be a slightly higher risk for misdiagnosis due to the risk of culturing maternal cells from any other tissue other than blood cells. In women with Rh-negative blood group Rh- immune globuline must be administered, if the father of the child is Rh-positive or his blood group is unknown. Following the procedure it seems helpful to demonstrate the fetus again to the mother by ultrasound evaluation. There is no clear evidence in the literature that ultrasound guidance itself will reduce the fetal loss rate, but these may lower the maternal fears. In contrast it is assumed that the abortion rate increases if the placenta is perforated.[5]

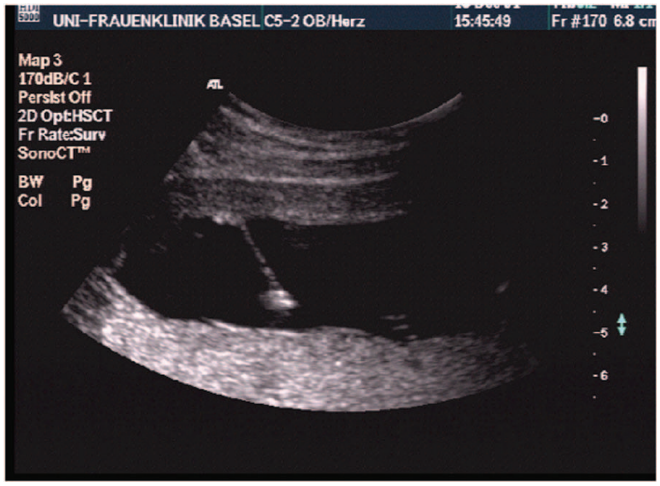

Fig. 74.2: Second - trimester amniocentesis under ultrasound guidance

Operator experience may also prejudice the fetal loss rate but this has not been definitively determined.[6] However there is a general agreement that the number of genetic amniocentesis needed to be trained and maintaining stable ongoing expertise is recommended.[7-9] Exceptionally in multiple pregnancies amniocentesis should be performed by trained investigators. Amniocentesis in twin pregnancies consists in obtaining of amniotic fluid from both cavities. As recommended in the past dye injection in to the first punctured sac was performed routinely.[10] Advanced ultrasound technology allows the evaluation of both fetuses and makes dye injection obsolete in experienced hands. Controversy is ongoing whether both amniotic sacs should be injected in cases of monochorionic diamniotic twins. In dichorionic twins a single needle insertion technique is often favoured, but may result in cytogenetic problems. On the other hand it is unknown whether a single needle technique may induce a rupture of the membrane.

CYTOGENETIC ASPECTS

Compared to other methods for prenatal karyotyping the benefits of an amniocentesis are the simplicity of the implementation of the procedure and the convenience of the analysis for the cytogenetic lab. In clinical practice the level of a-fetoprotein (AFP) in amniotic fluid is determined routinely. Currently it

is debated that these may not longer be needed because high resolution ultrasound is able to detect neural tube defect and abdominal wall defects. However, the detection of spina bifida is depending on the experiences of the sonographer. Therefore evaluation of AFP in the amniotic fluid is still recommended.

Cell culture as a prerequisite for conventional chromosome analysis on amniocytes causes a turn around time of at least one week. In practice the average processing time is probably close to two weeks in most of the labs. Ongoing trends in prenatal diagnosis aim at early and rapid diagnosis as well as at the improvement of risk-screening for aneuploidy. Flourescent in situ hybridization (FISH) on interphase amniocytes and quantitative fluorescence polymerase chain reaction (QF-PCR) are efficient tools for the rapid exclusion of selected aneuploidies.[11] Regarding the overall detection rate of unbalanced chromosome anomalies the same limitations apply for both techniques with current approaches. It is suggested that QF-PCR is advantageous at least in all centers with access to the necessary hardware or those with large sample numbers. We believe, however, that for rapid karyotyping if available, direct preparation from chorionic villi should be the method of first choice when there is an increased risk for unbalanced chromosome anomalies i.e. after the ultrasound diagnosis of fetal malformations. This method carries a false negative rate of below 1% as compared to up to 35% with interphase FISH or QF-PCR.[13] In conclusion it can be stated that FISH and QF-PCR should be used as additional tests.

Particularly in blood - stained probes maternal cell contamination of amniotic fluid cell is are rare but well known cause of diagnostic error in the prenatal diagnosis of fetal disorders (<3/1000).[14] Using flourescent labelled microsatellites permits the differentiation between maternal and fetal cells. Mosaicism is one other remaining problem and is seen in 1/1000 of samples.[15]

COMPLICATIONS

The risks which may be associated with second trimester amniocentesis include leakage of amniotic fluid, vaginal bleeding, contractions, chorio-amnionitis, failure to obtain a sample, pregnancy loss, and possibly fetal injury.[5,16-23]

Actual fetal loss rates related to genetic amniocentesis vary among randomized studies and may be comixed by transplacental needle passage, multiple needle insertion and use of larger needles sizes.[17-19]

The total fetal loss rate related to the procedure is often calculated to be around 0.5%.[24] But in many studies the source for this reported level of risk was nondistinctive, and the background risk for miscarriage was unaccounted. In the three larger multicenter studies the risk for fetal loss following amniocentesis was approximately 1%, but bias due to selection can not be excluded.[3,7,22] These results were comparable with the former findings in the only randomizied controlled trial reported by Tabor.[5] Recently Seeds reviewed 68,119 amniocenteses from both controlled and uncontrolled studies providing straightforward arguments for several conclusions.[25]

Currently midtrimester amniocentesis under ultrasound guidance is associated with a procedure-related rate of excess pregnancy loss of 0.33% (95% CI, 0.09, 0.56). Among only controlled studies, these risk is 0.6% (95% CI, 0.31, 0.90). Adding the natural loss risk of about 1.08% among control groups the total rate of losses can be determined around 1.6%.

Application of ultrasound guidance may reduce the number of injections and may also lower the incidence of blood stained fluid. Analysis of only controlled studies shows that this trend remains, but not statistically significant.

Injury of the fetus is rare can not perfectly prevented by using ultrasound guidance, but may occur more frequently.

Former reported experience of higher risks due to placental perforation does not support an increased rate of miscarriage. As shown in the comprehensive overview other complications (such as vaginal bleeding, infection or leakage of amniotic fluid) could not be analysed due to the limited number of described terms or were not comparable. Following the improvement of the technique and the

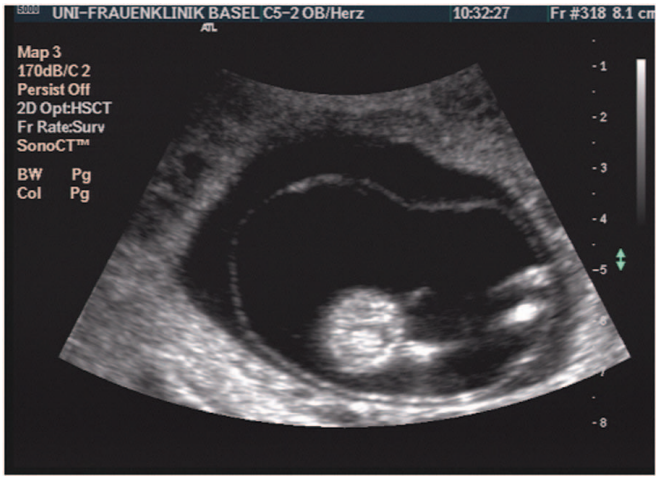

Fig. 74.3: Gestational and amniotic sac at 12 weeks demonstrating absence of fusion between amniotic membranes and the surrounding cavitiy

laboratory methods there has been attempts in bringing forward the time of amniocentesis in the past by performing early amniocentesis which is technically more demanding (Fig. 74.3). The both randomised studies designed to assess the safety and cytogenetic accuracy of early amniocentesis showed increased rate of fetal losses as well as an higher rates of talipes equinovarus and oligohydramnios. Furthermore multiple needle insertions were performed in early pregnancies compared to mid-trimester amniocentesis. The rate of laboratory failures following early investigations was arised. Comparing early vs. late amniocentesis it is suggested that the procedure in the second trimester is more favourable.[26,27]

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