AMNIOCENTESIS

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1. DEFINITION

Amniocentesis is an invasive procedure that consists of introducing a needle through the maternal abdominal wall to obtain a sample of amniotic fluid.

2. INDICATIONS

- Aneuploidy screening with risk ≥ 1/250 for trisomy 21 or 18 (by combined test or second trimester biochemical screening)
- Chromosomal abnormality in a previous pregnancy
- Parental chromosomal abnormality
- Fetal malformation detected by ultrasound
- Confirmation of a high-risk cell-free fetal DNA
- Confirmation of a preimplantation genetic diagnosis (error < 5% if it was by fluorescence in situ hybridization (FISH) and < 1% if it was by polymerase chain reaction (PCR), microarray or next-generation sequencing (NGS))
- Confirmation of an inconclusive result in chorionic villus sampling
- Early severe fetal growth restriction (FGR) (< 24 weeks)
- Severe FGR <28 weeks with the presence of: ultrasound markers (excluding oligohydramnios), minor malformation or biometrics (femur length or head circumference) < -3 SD (standard deviation).
- Genetic sonogram with resulting risk ≥ 1/250
- Discordant anomaly in monochorionic diamniotic twins
- Risk of monogenic disease with molecular or biochemical diagnosis available
- Risk of fetal infection with available diagnosis by PCR (cytomegalovirus (CMV), toxoplasma, parvovirus-B19, chickenpox, rubella, herpes 1-2, enterovirus)
- Risk of chorioamnionitis or intra-amniotic inflammation

3. BEFORE PROCEDURE

It is useful to do a checklist prior to amniocentesis to prevent mistakes or interruption of the procedure for preventable causes.

3.1. Verification of the indication: it is crucial to be aware of the indication for the procedure and confirm it with the patient.

3.2. Gestational age: as a general criterion it is preferable to perform amniocentesis after 16 weeks.

3.3. Genetic counselling: before scheduling the amniocentesis, genetic counselling by a physician, clinical geneticist or obstetrician about the likelihood of an abnormal result it is mandatory. Information about the genetic studies' scope and limitations is very important. Patients must be informed of the possibility of false-positive, false-negative, and inconclusive results for each genetic test, in order to choose which genetic test should be performed in advance.
The limitations of each technique should be discussed: the "rapid test" (quantitative fluorescent PCR (QF-PCR) or FISH) only determines the number of 5 chromosomes (13, 18, 21, X and Y), the karyotype does not detect sub microscopic changes, the array comparative genomic hybridisation (array-CGH) does not detect the balanced anomalies and none of them detect the single gene defects.

3.4. Informed consent form: the patient should receive information about the procedure, possible complications, sample processing and safety measures after the procedure. All this information must be included in the informed consent form. Ideally, it should be signed at least 24 hours before the procedure.

3.5. Verification of RhD: if the pregnant woman is RhD negative, anti-D gamma globulin should be prescribed within 72 hours post-procedure, except if non-invasive prenatal genotyping has been performed with an RhD negative fetus result. As a general rule, the RhD of the partner should not be taken into consideration.

3.6. Verification of maternal serologic status: human immunodeficiency virus (HIV), hepatitis B (HBV) and, in risk patients, hepatitis C virus (HCV). Positive serologies are not considered an absolute contraindication for the procedure and each case must be assessed individually. In case of infection by HIV, the procedure may be performed under HAART (Highly Active Antiretroviral Therapy) and ideally with an undetectable viral load. In case of HBV "e" antigen (HBeAg) or positive viral load, the administration of specific post-amniocentesis gamma globulin should be recommended (see chapter Chronic viral hepatitis). In all suspected maternal infection (HIV, HBV, HCV, TORCH infection) a transplacental approach should be avoided.

3.7. Relative contraindications:

- Women seropositive for HBV, HCV or HIV with high viral load
- Isoimmunisation
- Fever and/or active maternal infection
- Recent genital bleeding
- Detachment of the chorioamniotic membranes
- Large intracavitary hematoma
- Alteration of maternal coagulation or anticoagulant treatment (*Table 1*).

Table 1. *Adjustment of anticoagulant treatment for amniocentesis (BUTWICK AJ, J Perinatol 2011; 31:73-84).*

ANTICOAGULANT TREATMENT	STOP before treatment	RE-START after treatment
Aspirin (75-300 mg/24h)	Not necessary	Habitual dose
Prophylactic LMWH	10-12 hours	6-8 hours
Therapeutic LMWH	24 hours	24 hours
Dicumarinic drugs	INR ≤1.4	Immediately

LMWH: low molecular weight heparin; INR: international normalised ratio

3.8. Personal and material availability: a principal operator is required to handle the ultrasound transducer and needle, as well as an assistant for the aspiration of amniotic fluid. Regarding instruments, the minimum requirements are (*Figure 1*):

1) Ultrasound
2) Stretcher or gynaecological examination table
3) Sterile gauze pads and an antiseptic solution (chlorohexidine/povidone-iodine)
4) Sterile drapes
5) Sterile cover for the ultrasound transducer
6) Sterile ultrasound transmission gel
7) 22G or 20G needle
8) 10 ml Vacutainer® tubes without any additive
9) 3 ml Vacutainer® tube without any additive
10) 2 Vacutainer® adapters
11) Alternatively, 2 x 10 ml syringes, if Vacutainer® is not used

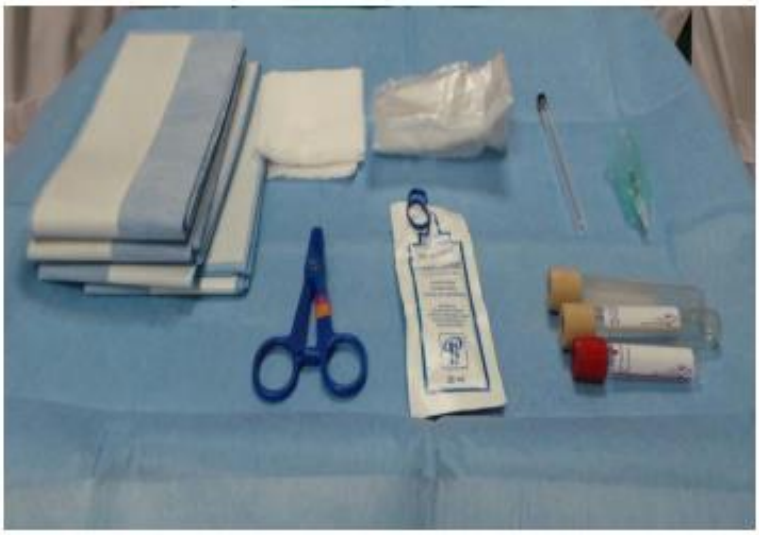

***Figure 1**: Material for the amniocentesis*

4. PLANNING OF PROCEDURE

The pregnant woman should be in a supine position, as horizontal as possible for better access to the amniotic cavity. Fetal viability should be confirmed prior to amniocentesis, as well as a gestational age greater than 15 weeks and a perfect coaptation of chorioamniotic membranes.

The ultrasound evaluation prior to the procedure should be based on a sweep of the entire uterine cavity in transverse view, with the transducer completely horizontal, to define the location of the placenta, the maximum pool of amniotic fluid, and the position and pattern of fetal movements (*Figure 2*). Extreme care must be taken to not deviate from the exact perpendicular plane, otherwise we will be seeing an area that is not located below the transducer.

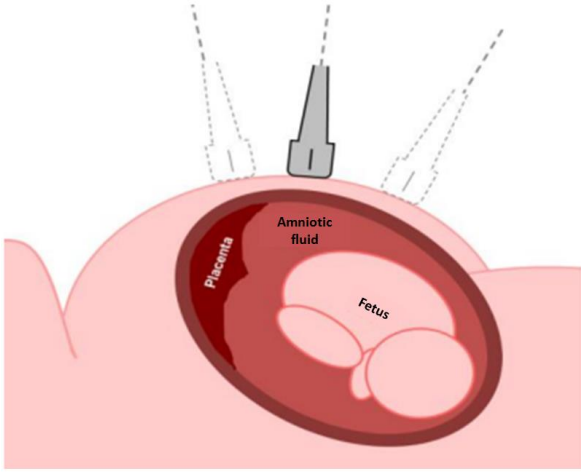

Figure 2. *Different transverse positions of the ultrasound transducer, always perpendicular to the maternal abdomen surface, in a maternal sagittal plane.*

The largest pool of amniotic fluid should be located in a transverse view of the uterus, avoiding peripheral pools (fundic, inferior pole, or very lateral). Maternal skin should be shown on the ultrasound screen so that you can fully see the entrance of the needle from the maternal skin to the pool of amniotic fluid.

Whenever possible, the transplacental approach should be avoided.

5. PERFORMANCE OF THE PROCEDURE

The operator must wash their hands with antiseptic solution (chlorhexidine or Sterillium®) and put on sterile gloves.

The skin of the exposed abdominal area must be cleaned using gauzes and antiseptics (preferably chlorhexidine or alcoholic povidone-iodine).

Ideally, the ultrasound transducer should be decontaminated before and after each use. It is mandatory that the transducer is covered with a sterile sheath, placing an amount of transducer gel on the probe to improve ultrasound transmission.

5.1. Needle introduction

The entire procedure must be performed under ultrasound control with continuous needle vision. Preferably the same operator should handle the needle and the transducer.

The needle introduction has 4 phases: maternal skin puncture, uterine puncture, entry into amniotic fluid cavity and the stopping of the needle's progress into the amniotic fluid cavity.

1. Maternal skin puncture: If the amniotic fluid pool we must access is located in the left hemiabdomen, the transducer should be moved laterally, maintaining the same transverse view, towards the right hemiabdomen, so a 45 degrees angle is formed with the sagittal plane of the mother. The needle should be inserted at 45 degrees to the complementary right lateral plane, forming an angle of 90

degrees with the transducer (*Figure 3*). If it is convenient, you can change the laterality: transducer on the left side and needle on the right side.

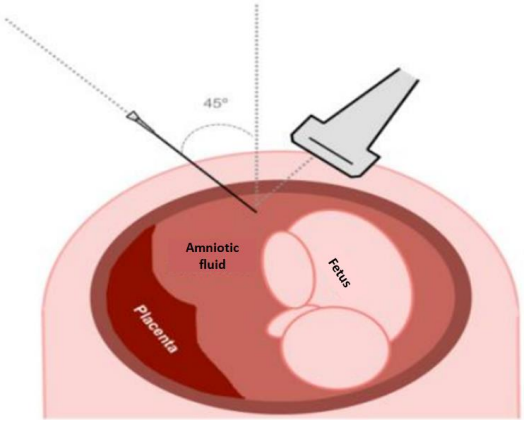

***Figure 3**: Introduction of the needle through the maternal abdominal wall up to the uterus with both needle and transducer at 45 degrees (both in relation to the maternal sagittal plane).*

The needle should enter lateral to the transducer, approximately 3 cm away and matching with the midpoint of the transducer's width. The initial skin puncture requires some self-limited pressure while progressing through the subdermal tissue of the pregnant woman (*Figure 4A)*. It is very important that when we start the puncture we have a whole vision of the needle, including maternal skin, subcutaneous tissue and muscular wall (*Figure 4B*).

During the progress of the needle through the skin and uterus wall, it is very important to avoid an intestinal loop puncture, mostly caused by the lack of vision from the start of the needle path (*Figure 4C*).

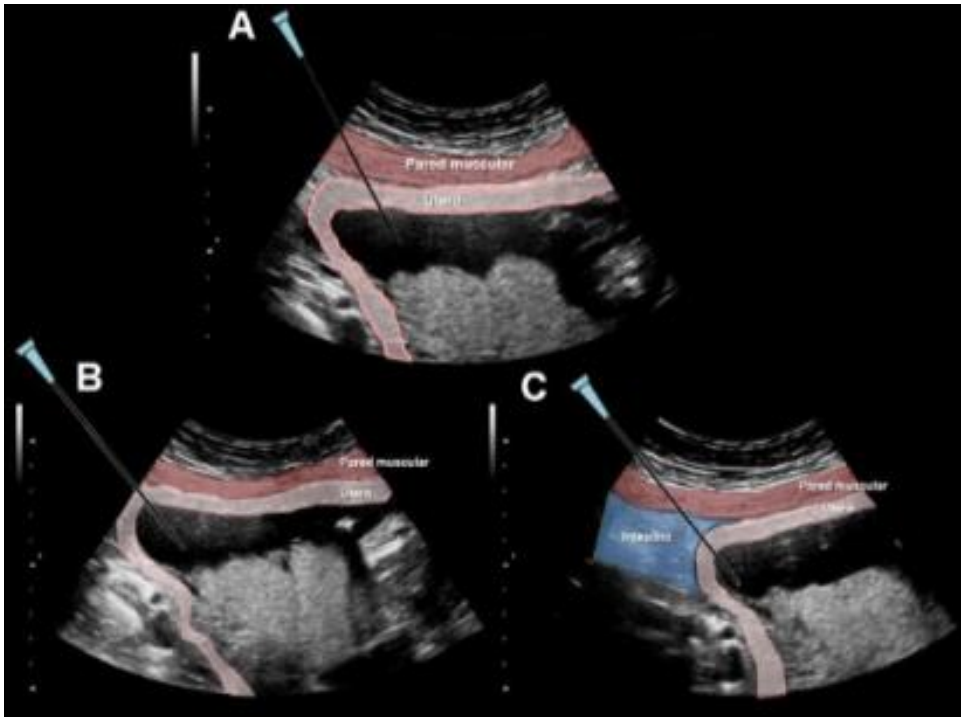

***Figure 4**: Puncture of the maternal abdominal wall up to the uterus. 4A shows the correct way with visualisation of the entire needle. In figure 4B, there is a lack of visualisation of the needle through the skin, subcutaneous tissue and muscular wall. Figure 4C shows an incidental puncture of the intestinal loops.*

2. Uterine puncture: the uterine puncture can be painful because the uterus is covered by peritoneum. The initial needle orientation must be confirmed before puncturing the uterine wall, since it will be more difficult to rectify its orientation, which can be deviated by maternal movements or abdominal muscle contraction.

3. Entry into the amniotic fluid cavity: we must locate the needle before entering the amniotic fluid cavity, seeing the entire needle length within the uterine cavity and not just the needle tip. We must move the transducer to be perpendicular (90 degrees) with the needle and improve its visualisation. A partial vision of the needle can lead to posterior uterus wall puncture. The entrance into the amniotic cavity must be done in a straightforward manner to avoid the tenting of the membranes (*Figure 5*) that happens when the needle is placed outside of the amniotic cavity and results in the needle apparently being seen inside of the cavity without being able to extract amniotic fluid. Sometimes in these cases, two echogenic signals at the needle tip are seen.

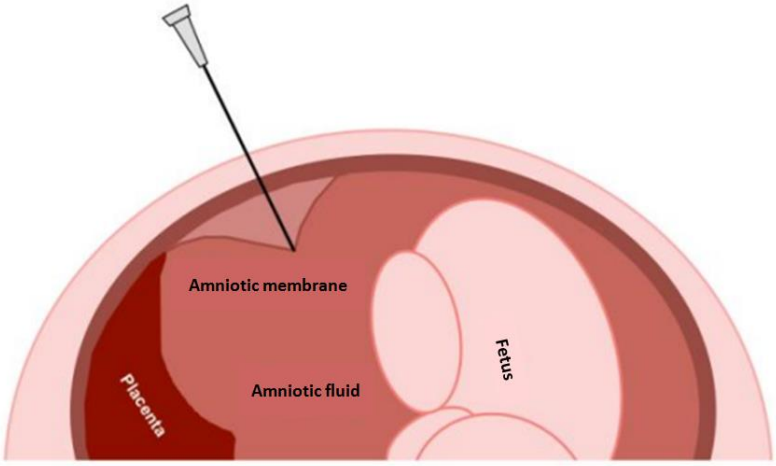

***Figure 5:** Needle introduction into the uterine cavity and "tenting" image.*

4. Stopping of the needle's progress into the amniotic fluid cavity: inside the amniotic cavity, we will not stop the needle progressing up to 2 cm before reaching the posterior wall, to prevent an incontrollable movement of the needle caused by a contracture of the anterior wall. In case of a failed puncture, if we decide to choose a new point of entry, a new needle will be necessary.

5.2. Amniotic fluid aspiration

Once the needle is correctly placed, the assistant removes the stylet and connects the Vacutainer® adapter (*Figure 6A*). The assistant pushes a Vacutainer® tube into the luer adapter so that the adapter's rubber-coated needle pierces the lid of the Vacutainer® tube (*Figure 6B*) and so the tube vacuum suctions liquid and allows self-filling without additional handling (*Figure 6C*). The filled tube is removed and a new one is inserted and filled in the same way (*Figure 6D*).

About 20 mL of amniotic fluid should be obtained, ideally free from maternal blood cell contamination.

If we cannot obtain a minimum amount of amniotic fluid for analysis after two puncture attempts, a new puncture should be delayed until a week later. If you have only obtained 1-2 mL, you can perform a QF-PCR.

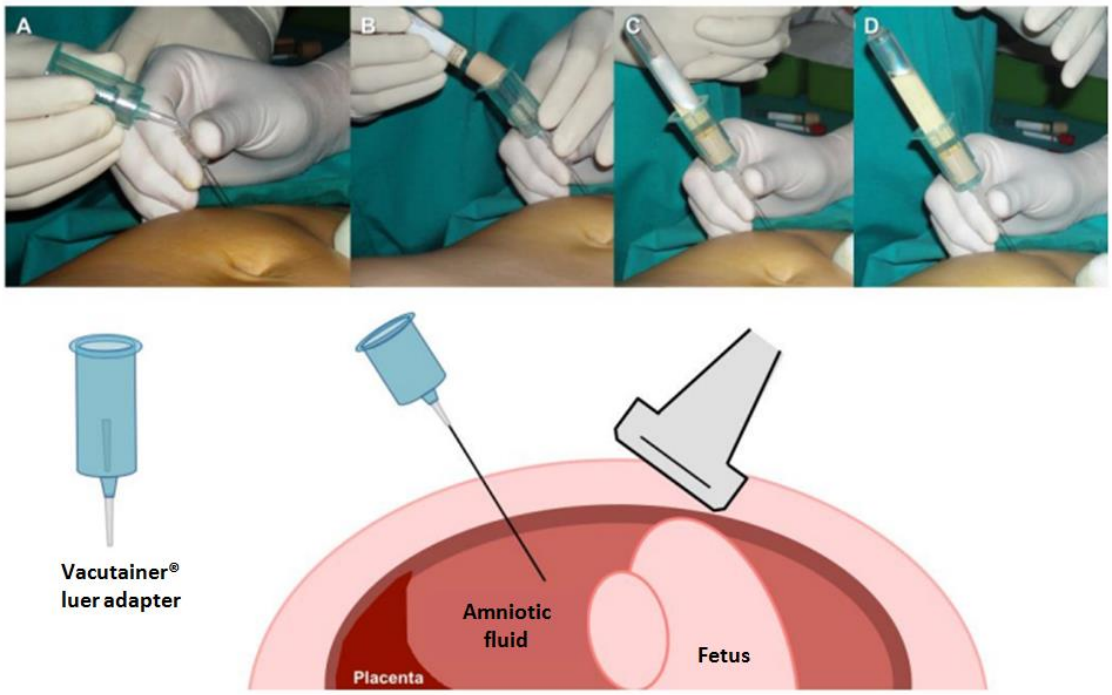

Figure 6: *Process of amniotic fluid aspiration.*

6. AFTER PROCEDURE

- Fetal viability must be confirmed and the needle entry point into the uterine cavity checked for bleeding.
- Administration of anti-gamma globulin-RhD (300 µg) in RhD negative pregnant women (without fetal genotyping or in RhD positive fetus).
- Administration of anti-gamma globulin-HBV in pregnant women with positive HBsAg: in case of positive HBeAg or positive viral load in unavoidable transplacental puncture or third-trimester amniocentesis, specific HBV-immunoglobulin must be administered post procedure (600 IU IM (intramuscular), single dose in the first 24 hours) (See chapter Chronical viral hepatitis).
- In bloody amniotic fluid or transplacental punctures in which a QF-PCR is performed, it is recommended to obtain a maternal sample (mouthwash) to rule out maternal contamination.
- Relative 24 hours home rest is recommended after amniocentesis. Absolute rest is not necessary, since there is no scientific evidence on its beneficial effect. Warning signs for coming to the emergency room are vaginal bleeding, loss of amniotic fluid, severe abdominal pain and fever ≥38ºC.

7. COMPLICATIONS

- Pregnancy loss: there is a risk of 0.1% fetal loss when amniocentesis is performed by an experienced operator, when performed in the second trimester. There are series that report an increase in complications up to 1% in transplacental punctures and 1-2% if there is little training, as well as risks less than 0.01% in centres with great experience.
- Premature rupture of membranes: 0.3% risk.
- Chorioamnionitis: intra-amniotic infection is very rare; however, its incubation time is short and it clinically appears generally 24 hours after the procedure.
- Others: placental haemorrhage, abdominal wall haematoma or fetal trauma, very rare.

8. TWIN PREGNANCY

In the planning of amniocentesis in multiple gestations, it is essential to carry out a detailed mapping of gestational sacs, fetuses, and chorions. To avoid mistakes in sample allocation, it is recommended, as a general rule, to puncture fetus A first and fetus B second.

In the diamniotic twin gestation, amniocentesis is a procedure that allows chromosomal, molecular, biochemical or microbiological diagnosis specific for each fetus. The risk of amniotic bag confusion is very remote, although extreme care must be taken to correctly allocate each sample to the right fetus. The risk of fetal loss could be double compared with unique pregnancies, because we perform two punctures, one for each gestational sac. In exceptional circumstances, when the indication for amniocentesis is a discordant malformation, obtaining a single sample is a possibility in order to reduce the risk of the procedure.

In monochorionic pregnancies, if amniocentesis is performed due to gestational risk of genetic anomaly, a single puncture is enough. It is extremely important to avoid puncturing the dividing membrane due to the high risk of secondary septostomy.

Because the results of amniocentesis are obtained after week 16, chorionic villus sampling should be considered as the best procedure for those couples with a dichorionic gestation that can opt for a selective interruption of a single affected twin. On the contrary, in selected cases of monochorionic-diamniotic pregnancies with discordant ultrasound anomaly, amniocentesis can ensure the obtention of two different samples (risk of heterokaryotic pregnancy).