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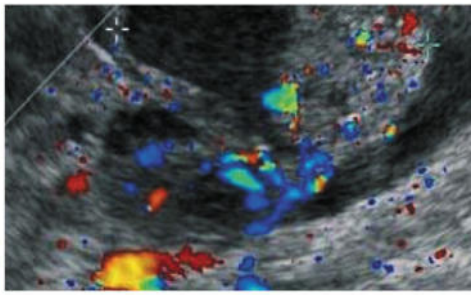

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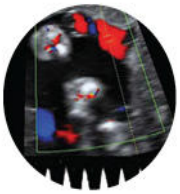

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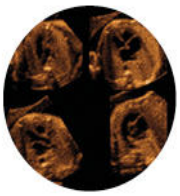

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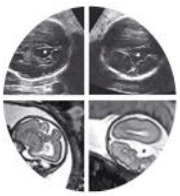

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How to perform an amniocentesis

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BACKGROUND

Amniocentesis is a technique for withdrawing amniotic fluid from the uterine cavity using a needle, via a transabdominal approach and under continuous ultrasound guidance, in order to obtain a sample of fetal exfoliated cells, transudates, urine or secretions. It can be performed from 16 weeks of pregnancy onwards, with various chromosomal, biochemical, molecular and microbial studies being performed on the amniotic fluid sample. The most common reasons for the procedure are to enable prenatal diagnosis of chromosomal abnormalities, single gene disorders, fetal infection and intra-amniotic inflammation, as well as to assess fetal lung maturity and blood or platelet type. The procedure has a risk of fetal loss of approximately 0.5% (range, 0.06–1%)[1] when performed in the second trimester, after the amniotic membrane has fused with the chorion; there is also a risk of amniotic fluid leakage (approximately 0.3% of cases) and other rare complications, such as placental hemorrhage, intra-amniotic infection, abdominal wall hematoma and fetal lesion. There is an important lack of good-quality evidence to support most recommendations for the procedure, and a recent review suggested that operators should use those methods and technique modifications with which they are most familiar when performing an amniocentesis[2].

The aim of this summary article and the full version, included as supplementary material online, is to describe the amniocentesis technique, presenting a practical guideline for its performance. We also describe the use of a Vacutainer® (BD Vacutainer Systems, Plymouth, UK) aspiration system in order to produce a continuous vacuum for amniotic fluid aspiration as an alternative to using manually operated syringes.

PROCEDURE

Ultrasound evaluation

The woman should be positioned as horizontally as possible, to allow better access to the amniotic cavity. Fetal viability should be confirmed by ultrasound, as should gestational age beyond 15 weeks and absence of amniochorionic separation, which may be seen as late as 16–17 weeks of gestation[3].

Ultrasound evaluation before the procedure is based on a sweep of the uterine cavity in serial transverse (horizontal) views of the maternal abdomen, to define placental location, maximum vertical pocket (MVP) or amniotic fluid pool, fetal position and fetal movements (Figure 1). Extreme care must be taken not to have the probe oblique to the maternal abdomen (it should be perpendicular to the maternal abdominal surface), creating a false image of the area beneath the probe. In obese patients, it is important to take into account the distance that the needle must travel towards the amniotic cavity, which may be estimated by ultrasound measurement previous to the puncture. An appropriate needle length (20–22 G) should be chosen based on this distance; 12-, 15- and 20-cm needles are available commercially, although operators must be aware that longer needles are prone to bending.

The MVP should be located in a transverse view of the abdomen, avoiding peripheral pools near the uterine fundus, and ones near the lateral uterine walls, i.e. that are accessible only by a totally lateral needle entry. Fetal movement pattern, which is usually recurrent, should be observed carefully. The image on the ultrasound screen should include the maternal abdominal skin and

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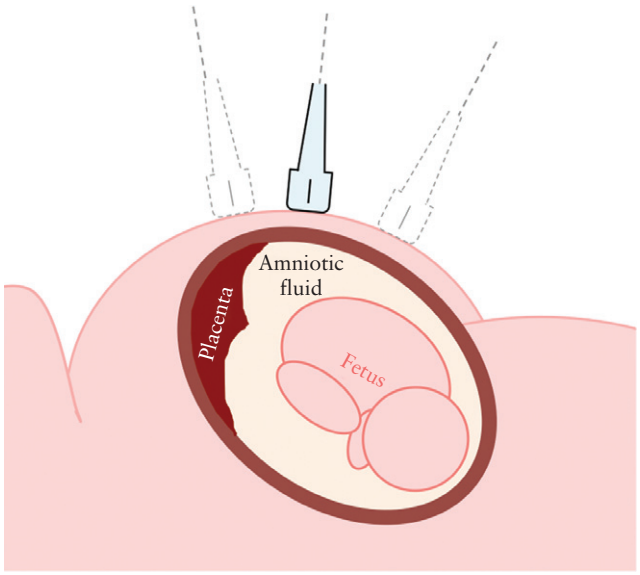

Figure 1 Transverse sweep of the maternal abdomen shown in a sagittal view, with the ultrasound probe perpendicular to the abdominal surface.

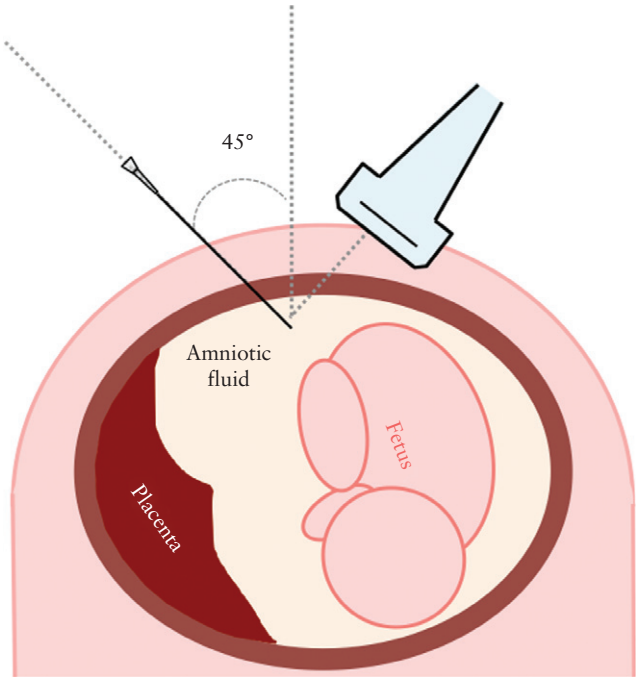

Figure 2 Puncture of the maternal abdominal wall into the uterine cavity, shown in a transverse view; note the needle and the ultrasound probe each at a 45° angle with regard to the mid-sagittal plane of the maternal abdomen.

for this reason image enlargement should be achieved by decreasing depth, rather than by using zoom. Thus, the area of needle insertion can be planned, as can the needle trajectory from the maternal skin to the amniotic fluid pool. Whenever possible, a transplacental insertion should be avoided; this should be used only when the other options would risk a procedure failure, due to the fetal position or an extreme lateral needle entry into the uterine cavity. Transplacental puncture is contraindicated in cases of alloimmunization or viral maternal infection by human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV)[4,5].

Preparation

The operator (and assistant) must scrub with antiseptic and use sterile gloves. The exposed maternal abdominal area must be cleaned and made aseptic with sterile gauze and antiseptics, for example, chlorhexidine or alcoholic povidone[3]. The ultrasound probe must be covered with a sterile plastic cover, with gel in the interior in order to improve sonic transmission. Ideally, sterile gel with individual packaging should be used, to diminish the risk of contamination from multiple-use gel bottles.

Needle insertion

Currently, the complete procedure *must* be performed under ultrasound guidance with *continuous visualization of the needle*[3]. Local protocols will determine whether a single person guides the procedure and inserts the needle (one-operator technique) or an assistant guides the procedure (two-operator technique)[6]. The ultrasound probe is placed perpendicular to the maternal skin, to obtain a transverse plane of the maternal abdomen, taking care that it does not inadvertently slide, as described above (Figure 1). The probe may be tilted up to 45°, with respect to the mid-sagittal plane of the maternal abdomen, away from the intended side of needle entry (Figure 2), whilst remaining in the same transverse plane.

Needle insertion has four stages: abdominal skin puncture, uterine puncture, entry into amniotic cavity and advancement of the needle.

1. Abdominal skin puncture

The needle must be introduced at an angle of 45° with respect to the maternal mid-sagittal plane, contralateral to the probe, so that the probe and the needle are at a 90° angle with respect to each other (Figure 2)[7]. The needle should be inserted lateral to the probe, directly under the middle of the ultrasound beam. The needle is advanced about 3 cm pointing towards the middle of the MVP. The initial skin puncture requires limited pressure while advancing the needle through the patient's subdermal tissue (Figure 3a).

During the trajectory and advancement of the needle between the skin and the uterine wall, it is important to avoid puncture of intestinal loops. This may occur because of incomplete visualization of the needle trajectory due to the lack of insonation lateral to the ultrasound beam (Figure 3c). Inadvertent puncture of intestinal loops may lead to chorioamnionitis, maternal sepsis and, extremely rarely, maternal death[3].

2. Uterine puncture

The moment of uterine puncture can be painful, because of the visceral peritoneum covering the uterus. The

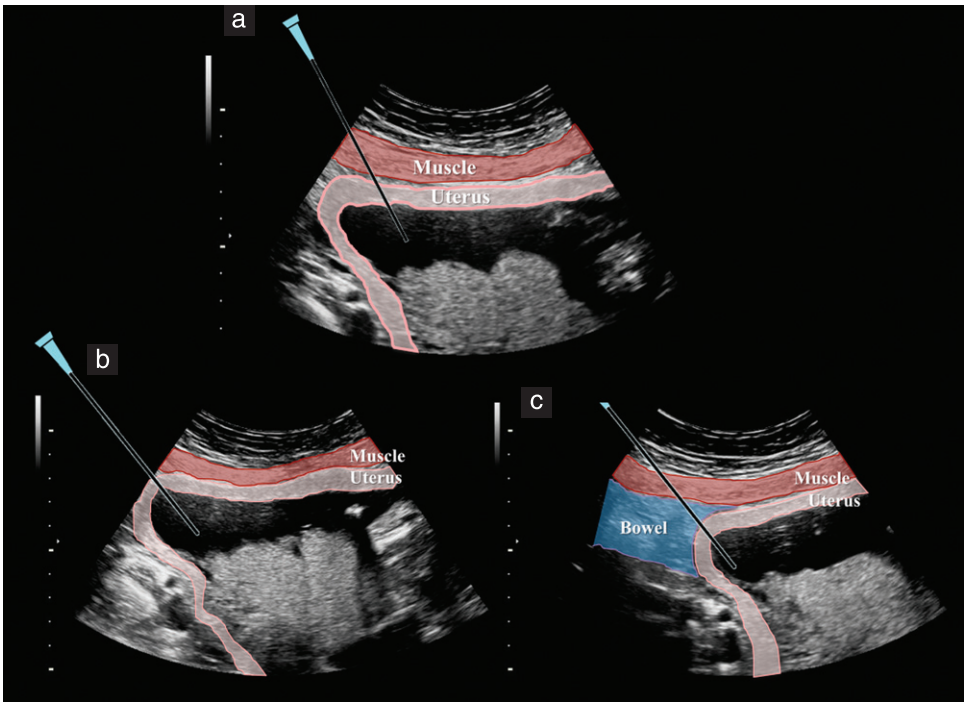

Figure 3 Maternal abdominal puncture into the uterine cavity. The correct technique is illustrated in (a): there is complete visualization of the trajectory of the needle, unlike in (b). Image (c) shows how an error in angle can lead to puncture of intestinal loops in the abdominal cavity.

orientation of the needle must be confirmed immediately before uterine puncture because it may have been altered by maternal movements or contraction of the abdominal musculature.

3. Entry into amniotic cavity

Before entering the amniotic cavity, the entire needle must be located and visualized with precision. The probe must be slightly distant from the needle, because when the angle between them is perpendicular, visualization improves. Partial visualization of the needle can cause puncture of a posterior placenta or of the posterior uterine wall, when the needle exits the uterine cavity. Entry into the amniotic cavity must be achieved with a decisive, fast movement, to avoid 'tenting' of the membranes, which would result in a false entry and no amniotic fluid would be obtained (Figure 4)[3]. Typically, two echogenic signals instead of one are observed in this case, at the needle tip. If tenting occurs, a small thrusting movement or twisting of the needle can assist in puncturing the protruding membrane, allowing entry into the amniotic cavity.

4. Advancement of the needle

Once inside the amniotic cavity, needle advancement must stop approximately 2 cm before reaching the posterior uterine wall, to avoid any anterior wall contracture from dislodging or displacing the needle, and to avoid the fetus being pinched with any sudden or unexpected movement.

In the case of a failed puncture, redirection of the needle can be attempted for no longer than around 1 minute,

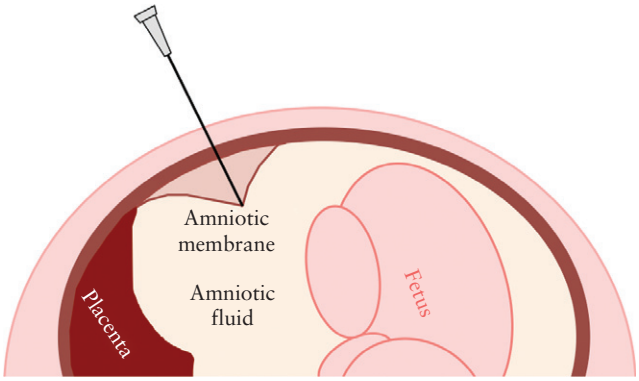

Figure 4 Introduction of the needle into the uterine cavity and 'tenting' of the membrane.

after which it should be extracted if the attempt remains unsuccessful. A new puncture site should be chosen and the needle changed to avoid contamination.

Amniotic fluid aspiration

Once the needle is located correctly in the uterine cavity, the stylet is removed and the syringe, or a Luer adapter attached to a Vacutainer® holder, is connected (Figure 5a). In the case of the latter, a vacuum tube is pushed into the holder so the adapter connects with and perforates the rubber cap on the tube (Figure 5b). The vacuum created aspirates the fluid automatically, without additional manipulation (Figure 5c). The full tube is then removed and a new one is connected and filled in the same way (Figure 5d). Approximately 20 mL amniotic fluid must be obtained from the procedure, ideally without

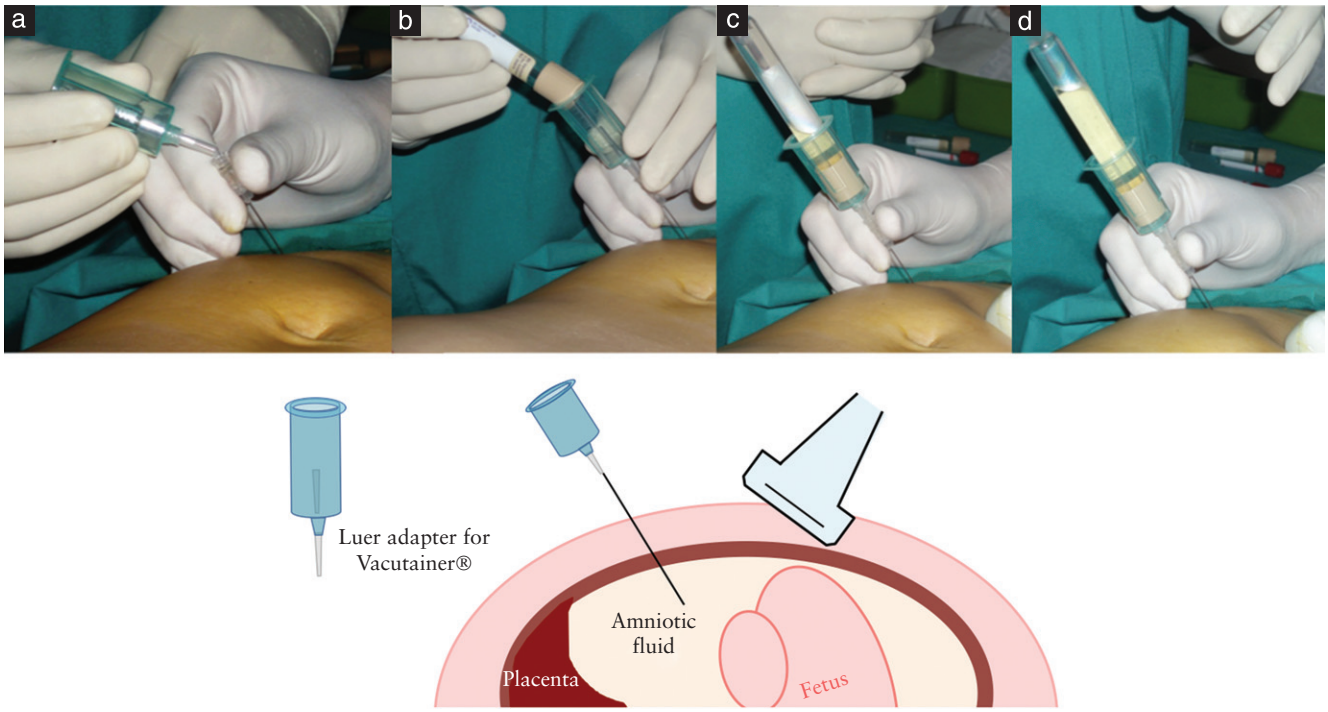

Figure 5 Amniotic fluid aspiration with the Vacutainer® system.

contamination by maternal blood cells. Traditionally, to avoid this, the first 2 mL of fluid were discarded or sent for alpha-fetoprotein level measurements; however, this practice has been discontinued and nowadays quantitative fluorescent polymerase chain reaction (QF-PCR) may be performed on this sample.

If, after two attempts to perform the uterine puncture, the quantity of fluid obtained is minimal or the quality unsatisfactory for analysis, a new procedure may be attempted 1 week later. If only 1 mL is obtained, a QF-PCR/fluorescence *in-situ* hybridization (FISH) result may be obtained[8].

Post-procedure recommendations

Fetal viability must be assessed continuously and any bleeding at the point of insertion must be evaluated by ultrasound. Maternal blood group must be confirmed and entered in the woman's records and all maternal history and clinical data required by the laboratory for sample processing must be verified, as must the woman's name and patient number. The woman must be informed regarding the time required for sample processing and who will deliver to her the results of the rapid test (QF-PCR or FISH), karyotype, array comparative genomic hybridization (array-CGH) or infection studies.

Recommendations for the woman after the procedure are as follows:

- Anti-D immunoglobulin (300 µg) in RhD-negative women.
- Specific anti-HBV immunoglobulin (600 IU intramuscularly in the first 24 h post-procedure) may be administered in women with positive HBsAg (hepatitis B surface antigen) in the case of HBeAg (hepatitis B 'e' antigen) or positive viral load (HBV-DNA), in cases in which transplacental insertion was unavoidable or when third-trimester amniocentesis was performed, although evidence for its prophylactic use in invasive procedures is lacking.
- In transplacental insertions or bloody taps, QF-PCR of both a maternal mouth swab and the amniotic fluid may be useful in identifying maternal cell contamination.
- House rest for 24 h. Normal activities for personal hygiene and in-house activities are allowed. In spite of great social pressure to increase the recommended house-rest period, there is no scientific evidence to justify this.
- Alarming symptoms that require the woman to contact the emergency room include bleeding or amniotic fluid leakage, intense abdominal pain and fever equal to or above 38 °C.
- Ultrasound follow-up may be performed 1 week after the procedure to confirm fetal viability and evaluate the puncture area.
- Usually, results of QF-PCR/FISH are available within 48 h, PCR for fetal infection in 1 week, array-CGH in 2 weeks and karyotyping in 3 weeks. This may vary from center to center.

FINAL RECOMMENDATIONS

- An amniocentesis must be performed or supervised by an experienced operator, in order to diminish the risk of complications.
- The procedure must be performed from 16 weeks of gestation onwards, as confirmed by ultrasound.

- A 20–22-G needle must be used under *continuous ultrasound guidance*[9].
- Antibiotic prophylaxis is not indicated routinely[3,10].
- 10–20 mL amniotic fluid must be obtained for karyotyping/array-CGH.
- Written informed consent must be given by the woman before the procedure and after discussing the risks and information on each of the genetic studies available. The woman or couple must make their choice with all information available[1,9].
- Post-procedure recommendations include anti-D prophylaxis in RhD-negative women[1,3,9].

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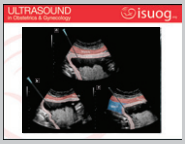

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