**Name of Journal: *World Journal of Obstetrics and Gynecology***

**ESPS Manuscript NO: 21500**

**Manuscript Type: Minireviews**

**Amniocentesis: A contemporary review**

Connolly KA *et al*. Amniocentesis: A contemporary review

**Katherine Ann Connolly, Keith Arnold Eddleman**

**Katherine Ann Connolly, Keith Arnold Eddleman,** Division of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology and Reproductive Sciences, Mount Sinai Hospital, New York, NY 10029, United States

**Author contributions:** All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

**Conflict-of-interest statement:** No potential conflicts of interest. No financial support.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Correspondence to: Katherine Ann Connolly, MD,** Division of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology and Reproductive Sciences, Mount Sinai Hospital, 5 East 98th Street, Room 256, New York, NY 10029, United States. katherine.connolly@mssm.edu

**Telephone:** +1-212-2415681

**Fax:** +1-212-3487438

**Received:** August 1, 2015

**Peer-review started:** August 6, 2015

**First decision:** September 16, 2015

**Revised:** October 27, 2015

**Accepted:** December 13, 2015

**Article in press:**

**Published online:**

**Abstract**

Amniocentesis is an essential tool in obstetrics. Invasive testing remains the only modality for diagnostic genetic testing and the only way to provide comprehensive testing for chromosomal abnormalities. Despite increasing use of cell free fetal deoxyribonucleic acid (DNA) testing, amniocentesis should still be offered to all women who desire more complete and accurate genetic testing. Counseling patients on the limitations of screening tests is of the upmost importance and amniocentesis should continue to be recommended to confirm positive results from cell free fetal DNA testing or in the case of failed cell free fetal DNA test. As cell free fetal DNA screening has not adequately been studied in multiple gestations, its use is not recommended in this population and invasive testing should be offered. Amniocentesis is also very useful in providing additional information in settings other than genetic testing the second and third trimester. If intraamniotic infection is suspected, but the clinical findings are not enough to guide management, amniocentesis can provide testing that can both immediately clarify the picture [interleukin-6 (IL-6), gram stain, glucose levels] and finally confirm the presence of infection (culture). It can also be used to detect the presence of intrauterine viral infections. Additionally, amniocentesis may be used to test for markers of fetal lung maturity. The American Congress of Obstetricians and Gynecologists recommends that amniocentesis for this indication not be used in cases where late preterm delivery is indicated. It may be useful in guiding decision-making, however, when late preterm delivery is indicated, but when exact timing is unclear. Regardless of the indication, amniocentesis appears to be a relatively low risk procedure with minimal risk to the patient. Additional randomized controlled trials are not likely, as they are not feasible to due extremely high number of participants that would be needed to detect a difference in loss rates. Based on current literature, however, the risk of pregnancy loss from second trimester amniocentesis is low in both singleton and twin gestations. We counsel patients that technique has changed since the original studies in the 1970s and feel comfortable quoting a loss rate of 1/500-1/1000 based on contemporary data.

**Key words:** Amniocentesis; prenatal diagnosis; Invasive genetic testing; Procedure related loss rates; Cell free fetal DNA testing; Fetal lung maturity; Intraamniotic infection

© **The Author(s) 2015**. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Invasive testing remains the only way to provide comprehensive testing for chromosomal abnormalities. Despite availability of cell free fetal DNA testing, amniocentesis should still be offered to all women who desire complete genetic testing. Amniocentesis is also useful if intraamniotic infection is suspected, but the clinical picture is unclear. Additionally, when late preterm delivery is indicated, amniocentesis need not be used. There are, however, some instances when delivery timing is unclear and amniocentesis for fetal lung maturity may provide information to guide delivery timing. Amniocentesis is a relatively safe procedure. We counsel patients that technique has changed since the original studies in the 1970s and feel comfortable quoting a loss rate of 1/500-1/1000 based on contemporary data.

Connolly KA, Eddleman KA. Amniocentesis: A contemporary review. *World J Obstet Gynecol* 2015; In press

**HISTORICAL CONSIDERATIONS**

Amniocentesis has been widely used since it was first performed in 1956[1]. Originally reported as a method of determining fetal sex *in utero*, Fuchs and Riis then hypothesized that it could be possible to diagnose chromosomal abnormalities in utero *via* this technique. By 1963, it had been confirmed that the karyotype of fetal and amniotic cells are identical[2]. Steele and Brag then showed in 1966 that amniotic cells can be sampled and cultured in sufficient quantity to be karyotyped, showing that in utero chromosome analysis was possible[3]. Shortly thereafter, the first prenatal diagnosis of an abnormal karyotype was reported[4]. Since that time, its utility has only continued to expand in both the second and third trimesters.

In 1979, the National Institutes of Health (NIH) suggested that amniocentesis be routinely offered to women 35 years or older, based on the balance between the risk of procedure-related pregnancy loss and the incidence of aneuploidy based on age[5]. Its use for genetic testing has only continued to expand over the years and is now the most commonly used invasive genetic test in obstetrics[6]. With the recent advent of cell free fetal DNA screening, understanding the continued need for and risks of amniocentesis has become increasingly important.

**TECHNIQUE**

Amniocentesis is generally performed after 15 wk gestation. The amniotic fluid volume at this gestational age is approximately 150cc[5]. Prior to performing procedure, an ultrasound should be performed to evaluate the placental and fetal location and to confirm gestational age and fetal viability. After adequately prepping the maternal abdomen with antiseptic solution, the procedure should be performed under continuous ultrasound guidance to decrease the number of insertions and bloody taps[7]. If possible, transplacental passage of the needle should be avoided. If this is not possible due to an anterior placenta, care should be taken to avoid large vessels and echolucencies seen with ultrasound guidance. In the hands of experienced providers, there does not appear to be an increased loss rate with transplacental amniocentesis compared to procedures during which the needle does not pass through the placenta[8].

The effect of operator experience itself on outcomes of amniocentesis has independently been studied. In a retrospective review, Margioula-Siarkou *et al*[9], examined loss rates of a single operator over a 13 year period (*n* = 5913) and found the loss rates in the first 10% of amniocenteses performed to be significantly higher than the last 10%, suggesting there is benefit to experience. In a review of 6332 amniocentesis specimens that revealed male karyotype (46 XY), maternal cell contamination was seen more frequently in samples obtained by physicians who perform < 50 amniocenteses annually (0.67% *vs* 0.19%, *P* = 0.0021)[10]. There has never been a study defining an exact number of procedures after which a provider becomes “experienced,” though reports of single institutions’ experiences show that fetal loss rates are related to operator experience[11].

Needle size is another technical aspect of the procedure that has been postulated to have effect on loss rates; however studies on this issue are extremely limited. Devlieger *et al*[12], showed that while a larger caliber needle may facilitate a faster collection of fluid, it may also be associated with increased fluid leakage rates. From a review of the literature, it appears that 20 or 22 gauge needles are most commonly used today. When these two sizes were compared in a randomized trial with 200 participants, it was shown that procedure time was statistically significantly lower when a 20 gauge needle was used (9.6 s *vs* 26 s, *P* < 0.0001). There was no difference in intrauterine bleeding at the insertion site, patient discomfort 30 min post-procedure, or complication rates within 2 wk of the procedure between the two groups[12]. This decrease in procedure time is not clinically significant and we use a 22 gauge needle.

**INDICATIONS**

Amniocentesis allows for fetal DNA in the amniotic fluid to be analyzed for chromosomal abnormalities. This can be done in response to abnormal serum genetic screening, an abnormal ultrasound finding, or in order to specifically test for a genetic condition for which a patient or partner is a carrier, including autosomal recessive, autosomal dominant, X-linked conditions or microdeletion/microduplication syndromes. This analysis of the fetal DNA is only possible with diagnostic testing via amniocentesis or chorionic villus sampling (CVS). One advantage of amniocentesis over CVS, however, is the ability to directly analyze fetal DNA. This avoids the potential issue of confined placental mosaicism that may be encountered in CVS samples. In 1%-3% of CVS samples, chromosomal mosaicism is seen[13]. This mosaicism is usually confined to the placenta, however is also present in the fetus in 10% of cases. In all cases of mosaicism on CVS, amniocentesis is recommended in order to determine whether it is confined to the placenta or is seen in the fetus as well.

Another important application for amniocentesis that deserves its own attention is in twin gestations. It has been previously shown that twin gestations are at an increased risk for chromosomal abnormalities. Further, the rate of multiple births is increasing. Between 1980 and 1999, the overall multiple birth ratio increased 59% and by 1999 multiples accounted for 3% of all live births[14]. Women of advanced age have experienced the greatest increase in rates of multiples[14]. As these women who are already at an increased risk for chromosomal abnormalities at baseline become increasingly pregnant with multiples, it is imperative that we have an accurate estimate of the risks of amniocentesis in this setting. As data is limited on cell free fetal DNA screening (see later) in the setting of multiple gestations, amniocentesis remains important for genetic diagnosis in these patients[15].

In addition to its utility in genetic testing, amniocentesis has been used in the third trimester to test the amniotic fluid for biochemical markers suggestive of fetal lung maturity. This indication for amniocentesis has recently come under closer scrutiny. The issue of timing when delivery is indicated in the late-preterm or early-term time period is based mostly on expert opinion. To analyze the obstetric, fetal and maternal conditions that often lead to late-preterm or early-term birth, the National Institute of Child Health and Human Development (NICHD) and the Society for Maternal-Fetal Medicine held a workshop in February 2011. In this meeting, the issue of using amniocentesis for fetal lung maturity to guide decision-making was directly addressed. The consensus was that if there is an indication for delivery, amniocentesis to assess fetal lung maturity should not be used to assist in delivery timing[16]. There are several rationales for this recommendation. The first is that if significant fetal or maternal risk exists, delivery should occur regardless of fetal lung maturity. The second issue is that confirmation of fetal lung maturity with amniocentesis does not translate into maturity of organ systems other than the lungs[17]. A committee opinion from the American Congress of Obstetricians and Gynecologists (ACOG) supports this recommendation, based on the same two issues[18]. In an editorial statement arguing the dissenting opinion, Towers[19] asserts that fetal lung maturity testing provides more information than just lung maturity. He argues that many of the serious morbidities for preterm neonates born after 34 wk (intraventricular hemorrhage, necrotizing enterocolitis) are highest in those infants who are intubated, and that the risk of intubation in the neonatal period after an amniocentesis showing fetal lung maturity is < 1%[20-22]. Further, he argues that there is a role for amniocentesis when the clinical scenario is not clear. For example, in the setting of uncertain dates and a quasi-urgent fetal indication for delivery, such as suspected fetal growth restriction with less-than-optimal interval growth at < 34 wk. We believe that it is reasonable to use amniocentesis to assess fetal lung maturity in this setting, when there is an indication for an early delivery but no imminent danger to mother or fetus would likely occur while awaiting results.

Another use for amniocentesis is in the diagnosis of intraamniotic infection. This diagnosis can usually be made clinically, based on maternal fever often with associated maternal or fetal tachycardia, uterine tenderness, or foul smelling amniotic fluid[23]. There are situations, however, where infection is suspected or likely, but the clinical picture is not this clear. It is extremely important in these situations to clarify the diagnosis and obtain further information to guide management, as undiagnosed infection would put the patient at risk. One example of this is in the case of a candidate for a physical exam indicated cerclage, as subclinical intraamniotic infection is seen in 13%-28% of these women[24]. Amniocentesis can be utilized in these cases, as an amniotic fluid culture remains the most specific test for documentation of intraamniotic infection. There are several other tests that have been used to aid in the diagnosis, as it is not always practical to wait several days for final culture results in the setting of a possible infection. Romero et al. studied the diagnostic value of each of these tests and found that a high IL-6 level was 82% sensitive and a negative gram stain was 99% specific for the detection of amniotic fluid containing bacteria[25]. The correlation between high amniotic fluid IL-6 levels and chorioamniotic infection has been supported by other authors as well[26]. Analysis of amniotic fluid for these parameters remains an invaluable tool in detecting infection when the clinical picture is not straightforward.

Amniocentesis should also be used to diagnose intrauterine viral infections, such as Cytomegalovirus or Parvovirus. Whether there are ultrasonographic signs that a fetus has been affected by one of these viruses or maternal serum indicates infection, amniocentesis can be performed. Polymerase chain reaction studies for these viruses should then be performed on the amniotic fluid obtained[27]. This information is essential to guide further fetal assessment and possible intrauterine treatment, depending on the clinical scenario.

An exciting new application for amniotic fluid and, thus, amniocentesis is its potential use for the ascertainment of stem cells. There has been much attention and research aimed at the potential clinical uses of stem cells from bone marrow, blood, embryonic tissue and umbilical cord blood. Their widespread use has been limited by small cell number, potential tumorigenesis, and some ethical concerns with the use of embryonic tissue. The use of amniotic fluid cells obtained from discarded fluid after second trimester amniocentesis has shown promise as a way to circumvent some of these limitations. The ability to expand these multipotent cells in culture and to cryopreserve them for delayed differentiation and use has already been documented[28]. They have been shown to differentiate along adipogenic, osteogenic, myogenic, endothelial, neurogenic and hepatic pathways without giving rise to tumors[29]. These initial studies make these cells available to be used and studied for potential clinically significant therapeutic purposes.

There is one additional use for amniocentesis that is no longer widely used, though is worthy of discussion: spectral analysis of amniotic fluid (ΔOD450) to quantify the severity of fetal anemia. *In utero*, bilirubin from the fetal pulmonary and tracheal effluents is found in the amniotic fluid. The level of bilirubin in the fluid can be obtained *via* amniocentesis and then be used to estimate fetal hemolysis[30, 31]. This technique was compared to middle cerebral artery (MCA) Doppler assessment in a prospective study by Oepkes *et al*[32]. MCA Doppler assessment was found to be 85% accurate, whereas ΔOD450 measurements were 76% accurate using the Liley curve and 81% accurate using the Queenan curve. Based on the findings of this study, MCA Doppler assessment has been widely accepted as the primary screening tool in the detection of fetal anemia[5]. We agree that amniocentesis should no longer be the first line surveillance tool in this situation, given that the noninvasive option has been shown to be superior.

**COMPLICATIONS**

Amniocentesis is a relatively safe procedure with minimal risk to the patient. With sterile technique, chorioamnionitis is seen in less than 0.1% of cases[11]. Other infrequent complications include transient vaginal spotting or leakage of amniotic fluid. Patients should be counseled that if leakage occurs, it usually occurs within 48 hours and that fetal survival is greater than 90% in these cases[11].

Pregnancy loss is the most serious and feared risk to an amniocentesis. Generally quoted loss rates are primarily based on 3 main studies in the 1970s that were not randomized[33-35]. Based on these studies, the Centers for Disease Control and Prevention (CDC) promulgated a loss rate of 0.5% following amniocentesis. Despite the fact that these studies were not randomized and amniocenteses were not performed with the use of concurrent ultrasound guidance, this CDC estimation of loss after amniocentesis is still often quoted. There is only one randomized trial evaluating loss after amniocentesis published by Tabor *et al*[36] in 1986. This trial reports a 1% increased risk in the amniocentesis group when compared to the control group who did not undergo amniocentesis[36]. This study has been criticized for being carried out on young, low-risk women, which is generally not the group of women most commonly undergoing this procedure. The results from this trial, which was carried out 30 years ago, may not be applicable to our current practice, as their equipment was far inferior to what we have today. Nonetheless, this is the only randomized trial comparing amniocentesis to no amniocentesis, and it is likely to remain so due to societal pressures and ethical concerns.

Absent randomized controlled trials (RCT), researchers have sought to refine the reported risk of amniocentesis by utilizing non-randomized studies that, although not randomized, mitigate some of the criticisms of older studies. For example, Eddleman *et al*[37] used the large database of approximately 35000 patients who were enrolled in the FASTER trial. In this multi-center, prospective clinical trial, there was a 1% loss rate in the amniocentesis group and a 0.94% loss rate in the no amniocentesis group. This difference of 0.06% (1/1600) is the loss rate attributable to the amniocentesis. Another very large, contemporary study that included 51557 patients was done by Odibo *et al*[38], with loss rate of 0.13% (1/769) attributable to amniocentesis. A meta-analysis that included 21 studies performed after 2000 showed a 0.11% procedure-related loss rate[39]. This study only analyzed studies which included greater than 1000 procedures and only those who examined loss rates < 24 wk gestation in order to determine loss rates attributable to amniocentesis. Other recent trials have continued to demonstrate this trend in loss rates lower than previously seen by Tabor *et al*[38,40-43]:Typically in the range of 0-0.5% loss rate attributable to amniocentesis. This more contemporary analysis of loss rates is reassuring and should be included in current patient counseling[38-42].

Overall, reported loss rates for amniocentesis in recent years are consistently low, but have been criticized for various reasons. Due to their nonrandomized nature, many of these studies do not have a control group that would provide a background loss rate. Even in the studies that do have a control group, they are often not appropriately matched in terms of baseline risk factors for the women in each group. Another issue with current literature is that there has not been a standard manner by which to report procedure-related loss rates. Studies to date have used varying definitions of pregnancy loss in terms of cutoffs for gestational age and length of time from procedure to loss. As mentioned earlier, there will likely not be any future RCT to assess contemporary loss rates. An RCT would require > 400000 patients in each arm to have adequate power to detect a difference of 0.05% in loss rates between those who do and do not undergo amniocentesis[37]. Thus, using large scale, multicenter, prospective trials, such as the FASTER Trial, as a surrogate appears to be the best option. Given it was carried out in multiple centers and that there were no specifications as to the technique, the results are generalizable to the larger “national” community. Given that more recent literature suggests loss rates lower than seen in the 1970s, amniocentesis remains a safe option for genetic testing. We believe it is reasonable to counsel patients of an approximate 1/500-1/1000 risk of loss attributable to amniocentesis. All women should be offered genetic testing and we recommend a customized risk assessment for each individual patient, rather than using an arbitrary age cut-off to guide recommendation for amniocentesis. We also believe that patients today, with appropriate counseling, are able to understand the reasons that we cannot give them an exact number for the risk of loss and accept a “range of risk” as our best estimate.

The literature regarding loss rates after amniocentesis in twin gestation is even more limited[11]. There have been several published studies addressing this; however they are limited by small sample size[44-48]. Cahill published a retrospective review of a 16 year time period comparing women who underwent amniocentesis (*n* = 311) to a control group (*n* = 1623) who did not[49]. In this study, the attributable risk of pregnancy loss prior to 24 wk after an amniocentesis was 1.8%. The women who elected to have an amniocentesis were older, more likely to be ≥ 35 years old and more likely to report alcohol exposure. It should be noted, however, that this increased risk of loss after amniocentesis remained after adjustment for maternal age, chorionicity, presence of anomaly on ultrasound, alcohol exposure, or race. Further, amniocentesis remained significantly associated with loss in patients who were younger than 35 years old and had normal ultrasound findings. In agreement with this finding, Yukobowich *et al*[47] found a statistically significant increase in fetal loss rate after amniocentesis (2.7% *vs* 0.6%). Conversely, other authors have found no difference in loss rates[45, 46, 50]. It is clear that additional studies are needed to further elucidate the true impact amniocentesis has on the loss rate in a twin gestation. The literature is extremely scant in regards to loss rates in the setting of higher order multiple gestations and further research is needed to guide counseling.

**OTHER CONSIDERATIONS**

The safety and accuracy of amniocentesis performed prior to 15 wk has been assessed in several randomized clinical trials. One study by the NICHD compared first trimester amniocentesis to first trimester transabdominal CVS[51]. They found an increase in spontaneous loss rate (RR-1.74) and a 4-fold increase in the rate of talipes equinovarus in the amniocentesis group. Those pregnancies which underwent CVS, however, are not an appropriate control group for those undergoing early amniocentesis. CVS is a different procedure that can be done at an earlier gestational age, which involves aspiration of the placental tissue and generally requires a larger gauge needle than used for amniocentesis. Nicolaides *et al*[52], found that the spontaneous loss rate after early amniocentesis (5.8%) was significantly higher than after CVS (1.8%). Other trials have been focused on comparing early amniocentesis to mid-trimester amniocentesis for a more direct comparison. Similarly, in a large study by the Canadian Early and Mid-Trimester Amniocentesis Trial Group[53] that included over 4000 amniocenteses, procedures done at 11-12 wk were compared to those done between 15-20 wk. The only difference between these two groups was the gestational age at the time of amniocentesis. There was a statistically significant increase in post-procedure spontaneous loss rate (2.6% *vs* 0.8%) and talipes equinovarus (1.3% *vs* 0.1%) in the early amniocentesis group. Given the established relative safety of amniocentesis in the mid-trimester, we recommend that these procedures be carried out after 15 wk gestation.

The safety of invasive procedures in the setting of maternal transmittable blood-borne illnesses is an additional concern, due to potential fetal contamination with maternal blood cells. Studies on amniocentesis in the setting of maternal human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are limited. There have been several small studies evaluating the risk of transmission in mothers with HBV. In the limited studies analyzing transmission rates after amniocentesis in women who were HBsAg-positive, there does not appear to be an increased risk of vertical transmission after amniocentesis[54, 55]. In these studies, all infants did receive HBV vaccine and immunoprophylaxis at birth. The data on women with HCV is extremely limited, as there has been one prospective trial including 22 women. The amniotic fluid was positive for hepatitis C RNA in one woman at the time of amniocentesis. None of the infants born to these 22 mothers were positive for hepatitis C on postnatal testing[56]. Due to lack of evidence, there is insufficient data to estimate the risk of transmission in women with high hepatitis B or C viral loads. This preliminary data seems to suggest low risk of vertical transmission, however further studies with larger numbers are needed to adequately assess safety.

There have been more reports on amniocentesis in the setting of maternal HIV infection. There are studies that have shown 2-4 fold increased risk of vertical transmission of HIV after amniocentesis in the second or third trimester[57-59], though these studies were performed prior to the widespread use of combination antiretroviral therapy (ART) and many women had received no treatment at all prior to the procedure. There have been subsequent, promising small series reported after the use of combination ART has been shown to be effective. The previously shown increased risk of vertical transmission was not seen in these women who were effectively treated[11]; in fact, the risk of transmission was not increased when compared to women who did not undergo amniocentesis[60-62]. The United States Department of Health and Human Services guidelines were updated to account for these new studies, stating that the risk of transmission does not appear to be increased in women treated with effective combination ART[63]. They do caution that although there have been no cases of vertical transmission in the 159 reported cases of amniocentesis in women who are on effective combination ART, a small increased risk of transmission may still exist. Due to this potential risk of vertical transmission, it is recommended that women in whom amniocentesis is indicated should be started on combined ART and ideally should have an undetectable viral load at the time of the procedure. Women should be counseled on the potential risk for transmission as well as the risks and benefits of noninvasive testing alternatives[11].

Recently, some obstetrical care providers have opined that with cell free fetal DNA technology, there will no longer be a need for amniocentesis. This technology in the maternal circulation has received much attention and press recently for screening, however it is limited in the information it can provide. Comprehensive pretest counseling is prudent, as this remains a screening modality. Patients must understand that this is not a diagnostic test, a negative test does not ensure a normal pregnancy, and that all positive results should be confirmed by invasive testing[64]. It most commonly only tests for trisomies 21, 18, and 13 and sex chromosome abnormalities, with varying degrees of accuracy[15]. In a study by Bianchi *et al*[65] of patients with abnormal cell free fetal DNA screening, aneuploidy was confirmed by invasive testing in only 93% with trisomy 21, 64% with trisomy 18, 44% with trisomy 13, and 38% with sex chromosomal abnormalities. Similarly, patients must be counseled on the genetic disorders that are not tested for by these blood tests. Women must be counseled that major trisomies screened for with cell free fetal DNA make up only about 50% of the cytogenetic abnormalities that would be found by karyotype following CVS or amniocentesis[5]. Further, there is a chance of test failure using this technique, due to a low fetal fraction of cell free fetal DNA recovered from maternal blood. In this circumstance, we believe that diagnostic testing should be offered due to an increased risk of aneuploidy in this setting (OR 9.2)[66]. Testing with amniocentesis or CVS remain the only ways to definitively obtain genetic information from karyotype and microarray. Thus, ACOG still recommends offering invasive testing to all women[15].

**CONCLUSION**

Amniocentesis is an essential tool in obstetrics. Invasive testing remains the only modality for diagnostic genetic testing and the only way to provide comprehensive testing for aneuploidy and microdeletions. Despite increasing use of cell free fetal DNA testing, this test should still be offered to all women who desire more complete and accurate genetic testing. Counseling patients on the limitations of screening tests is of the upmost importance and amniocentesis should continue to be recommended to confirm positive results from cell free fetal DNA testing, in the case of failed cell free fetal DNA test, or with a positive first or second trimester screen. As cell free fetal DNA screening has not adequately been studied in multiple gestations, its use is not recommended in this population and invasive testing should be offered.

Amniocentesis is also very useful in providing additional information in settings other than genetic testing the second and third trimester. If intraamniotic infection is suspected, but the clinical findings are not enough to guide management, amniocentesis can provide testing that can both immediately clarify the picture (IL-6, gram stain, glucose levels) and confirm or exclude the presence of infection (culture). Additionally, in cases where late preterm delivery is indicated, but exact timing is unclear, amniocentesis to test for fetal lung maturity provides useful information to guide decision-making.

Regardless of the indication, amniocentesis appears to be a relatively low risk procedure with minimal risk to the patient. Though additional randomized controlled trials are not likely, based on current literature, the risk of pregnancy loss from second trimester amniocentesis is low in both singleton and twin gestations. We counsel patients that technology has changed since the original studies in the 1970s and feel comfortable quoting a loss rate of 1/500-1/1000 based on contemporary data.

**REFERENCES**

1 **Fuchs F**, RIIS P. Antenatal sex determination. *Nature* 1956; **177**: 330 [PMID: 13297032 DOI: 10.1038/177330a0]

2 **Carr DH**. Chromosome studies in abortuses and stillborn infants. *Lancet* 1963; **2**: 603-606 [PMID: 14050879 DOI: 10.1016/S0140-6736(63)90396-9]

3 **Steele MW**, Breg WR. Chromosome analysis of human amniotic-fluid cells. *Lancet* 1966; **1**: 383-385 [PMID: 4159775 DOI: 10.1016/S0140-6736(66)91387-0]

4 **Jacobson CB**, Barter RH. Intrauterine diagnosis and management of genetic defects. *Am J Obstet Gynecol* 1967; **99**: 796-807 [PMID: 4231464]

5 **Creasy RK,**Resnick R, Bralow L, Iams JD. Creasy and Resnik's maternal-fetal medicine: principles and practice.  7th ed. Philadelphia: Elsevier/Saunders. xxiv, 2014: 1294

6 **Martin JA**, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Munson ML. Births: final data for 2003. *Natl Vital Stat Rep* 2005; **54**: 1-116 [PMID: 16176060]

7 **Romero R**, Jeanty P, Reece EA, Grannum P, Bracken M, Berkowitz R, Hobbins JC. Sonographically monitored amniocentesis to decrease intraoperative complications. *Obstet Gynecol* 1985; **65**: 426-430 [PMID: 3883268]

8 **Bombard AT**, Powers JF, Carter S, Schwartz A, Nitowsky HM. Procedure-related fetal losses in transplacental versus nontransplacental genetic amniocentesis. *Am J Obstet Gynecol* 1995; **172**: 868-872 [PMID: 7892877 DOI: 10.1016/0002-9378(95)90013-6]

9 **Margioula-Siarkou C**, Karkanaki A, Kalogiannidis I, Petousis S, Dagklis T, Mavromatidis G, Prapas Y, Prapas N, Rousso D. Operator experience reduces the risk of second trimester amniocentesis-related adverse outcomes. *Eur J Obstet Gynecol Reprod Biol* 2013; **169**: 230-233 [PMID: 23664797 DOI: 10.1016/j.ejogrb.2013.03.027]

10 **Welch RA**, Salem-Elgharib S, Wiktor AE, Van Dyke DL, Blessed WB. Operator experience and sample quality in genetic amniocentesis. *Am J Obstet Gynecol* 2006; **194**: 189-191 [PMID: 16389030 DOI: 10.1016/j.ajog.2005.05.033]

11 [**American College of Obstetricians and Gynecologists**](http://www.ncbi.nlm.nih.gov/pubmed/?term=American%20College%20of%20Obstetricians%20and%20Gynecologists%5BCorporate%20Author%5D)**.** ACOG Practice Bulletin No. 88, December 2007. Invasive prenatal testing for aneuploidy. *Obstet Gynecol* 2007; **110**: 1459-1467 [PMID: 18055749 DOI: 10.1097/01.AOG.0000291570.63450.44]

12 **Athanasiadis AP**, Pantazis K, Goulis DG, Chatzigeorgiou K, Vaitsi V, Assimakopoulos E, Tzevelekis F, Tsalikis T, Bontis JN. Comparison between 20G and 22G needle for second trimester amniocentesis in terms of technical aspects and short-term complications. *Prenat Diagn* 2009; **29**: 761-765 [PMID: 19412914 DOI: 10.1002/pd.2283]

13 **Phillips OP**, Tharapel AT, Lerner JL, Park VM, Wachtel SS, Shulman LP. Risk of fetal mosaicism when placental mosaicism is diagnosed by chorionic villus sampling. *Am J Obstet Gynecol* 1996; **174**: 850-855 [PMID: 8633655 DOI: 10.1016/S00029378(96)70312-5]

14 **Russell RB**, Petrini JR, Damus K, Mattison DR, Schwarz RH. The changing epidemiology of multiple births in the United States. *Obstet Gynecol* 2003; **101**: 129-135 [PMID: 12517657 DOI: 10.1016/S0029-7844(02)02316-5]

15 **Society for Maternal-Fetal Medicine (SMFM) Publications Committee.** #36: Prenatal aneuploidy screening using cell-free DNA. *Am J Obstet Gynecol* 2015; **212**: 711-716 [PMID: 25813012]

16 **Spong CY**, Mercer BM, D'alton M, Kilpatrick S, Blackwell S, Saade G. Timing of indicated late-preterm and early-term birth. *Obstet Gynecol* 2011; **118**: 323-333 [PMID: 21775849 DOI: 10.1097/AOG.0b013e3182255999]

17 **Bates E**, Rouse DJ, Mann ML, Chapman V, Carlo WA, Tita AT. Neonatal outcomes after demonstrated fetal lung maturity before 39 weeks of gestation. *Obstet Gynecol* 2010; **116**: 1288-1295 [PMID: 21099593 DOI: 10.1097/AOG.0b013e3181fb7ece]

18 [**American College of Obstetricians and Gynecologists**](http://www.ncbi.nlm.nih.gov/pubmed/?term=American%20College%20of%20Obstetricians%20and%20Gynecologists%5BCorporate%20Author%5D)**.** ACOG committee opinion no. 560: Medically indicated late-preterm and early-term deliveries. *Obstet Gynecol* 2013; **121**: 908-910 [PMID: 23635709 DOI: 10.1097/01.AOG.0000428648.75548.00]

19 **Towers CV**, Freeman RK, Nageotte MP, Garite TJ, Lewis DF, Quilligan EJ. The case for amniocentesis for fetal lung maturity in late-preterm and early-term gestations. *Am J Obstet Gynecol* 2014; **210**: 95-96 [PMID: 24139938 DOI: 10.1016j.ajog.2013.10.004]

20 **Clark RH**, Gordon P, Walker WM, Laughon M, Smith PB, Spitzer AR. Characteristics of patients who die of necrotizing enterocolitis. *J Perinatol* 2012; **32**: 199-204 [PMID: 21593813 DOI: 10.1038/jp.2011.65]

21 **Thorp JA**, Jones PG, Clark RH, Knox E, Peabody JL. Perinatal factors associated with severe intracranial hemorrhage. *Am J Obstet Gynecol* 2001; **185**: 859-862 [PMID: 11641666 DOI: 10.1067/mob.2001.117355]

22 **Gluck L**, Kulovich MV, Borer RC, Brenner PH, Anderson GG, Spellacy WN. Diagnosis of the respiratory distress syndrome by amniocentesis. *Am J Obstet Gynecol* 1971; **109**: 440-445 [PMID: 5107880 DOI: 10.1097/00006254-197110000-00012]

23 **Cunningham FG,** Williams JW. Williams obstetrics. 23rd ed. New York: McGraw-Hill Medical. xv, 2010: 1385

24 **Berghella V**, Ludmir J, Simonazzi G, Owen J. Transvaginal cervical cerclage: evidence for perioperative management strategies. *Am J Obstet Gynecol* 2013; **209**: 181-192 [PMID: 23416155 DOI: 10.1016/j.ajog.2013.02.020]

25 **Romero R**, Yoon BH, Mazor M, Gomez R, Gonzalez R, Diamond MP, Baumann P, Araneda H, Kenney JS, Cotton DB. A comparative study of the diagnostic performance of amniotic fluid glucose, white blood cell count, interleukin-6, and gram stain in the detection of microbial invasion in patients with preterm premature rupture of membranes. *Am J Obstet Gynecol* 1993; **169**: 839-851 [PMID: 7694463 DOI: 10.1016/0002-9378(93)90014-A]

26 **Yoon BH**, Jun JK, Park KH, Syn HC, Gomez R, Romero R. Serum C-reactive protein, white blood cell count, and amniotic fluid white blood cell count in women with preterm premature rupture of membranes. *Obstet Gynecol* 1996; **88**: 1034-1040 [PMID: 8942849 DOI: 10.1016/S0029-7844(96)00339-0]

27 **Norton ME**, Chauhan SP, Dashe JS. Society for maternal-fetal medicine (SMFM) clinical guideline #7: nonimmune hydrops fetalis. *Am J Obstet Gynecol* 2015; **212**: 127-139 [PMID: 25557883 DOI: 10.1016/j.ajog.2014.12.018]

28 [**Young BK**](http://www.ncbi.nlm.nih.gov/pubmed/?term=Young%20BK%5BAuthor%5D&cauthor=true&cauthor_uid=26115489)**,**[Chan MK](http://www.ncbi.nlm.nih.gov/pubmed/?term=Chan%20MK%5BAuthor%5D&cauthor=true&cauthor_uid=26115489), [Liu L](http://www.ncbi.nlm.nih.gov/pubmed/?term=Liu%20L%5BAuthor%5D&cauthor=true&cauthor_uid=26115489), [Basch RS](http://www.ncbi.nlm.nih.gov/pubmed/?term=Basch%20RS%5BAuthor%5D&cauthor=true&cauthor_uid=26115489). Amniotic fluid as a source of multipotent cells for clinical use. *J Perinat Med* 2015 [Epub ahead of print] [PMID: 26115489 DOI: 10.1515/jpm-2015-0152]

29 **De Coppi P**, Bartsch G, Siddiqui MM, Xu T, Santos CC, Perin L, Mostoslavsky G, Serre AC, Snyder EY, Yoo JJ, Furth ME, Soker S, Atala A. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol* 2007; **25**: 100-106 [PMID: 17206138]

30 **Liley AW**. Liquor amnil analysis in the management of the pregnancy complicated by resus sensitization. *Am J Obstet Gynecol* 1961; **82**: 1359-1370 [PMID: 14465271]

31 **Queenan JT**, Tomai TP, Ural SH, King JC. Deviation in amniotic fluid optical density at a wavelength of 450 nm in Rh-immunized pregnancies from 14 to 40 weeks' gestation: a proposal for clinical management. *Am J Obstet Gynecol* 1993; **168**: 1370-1376 [PMID: 8498414 DOI: 10.1016/S0002-9378(11)90767-4]

32 **Oepkes D**, Seaward PG, Vandenbussche FP, Windrim R, Kingdom J, Beyene J, Kanhai HH, Ohlsson A, Ryan G. Doppler ultrasonography versus amniocentesis to predict fetal anemia. *N Engl J Med* 2006; **355**: 156-164 [PMID: 16837679 DOI: 10.1056/NEJMoa052855]

33 Midtrimester amniocentesis for prenatal diagnosis. Safety and accuracy. *JAMA* 1976; **236**: 1471-1476 [PMID: 989112 DOI: 10.1001/jama.1976.03270140023016]

34 **Simpson NE**, Dallaire L, Miller JR, Siminovich L, Hamerton JL, Miller J, McKeen C. Prenatal diagnosis of genetic disease in Canada: report of a collaborative study. *Can Med Assoc J* 1976; **115**: 739-748 [PMID: 61796]

35 An assessment of the hazards of amniocentesis. Report to the Medical Research Council by their Working Party on Amniocentesis. *Br J Obstet Gynaecol* 1978; **85 Suppl 2**: 1-41 [PMID: 104726]

36 **Tabor A**, Philip J, Madsen M, Bang J, Obel EB, Nørgaard-Pedersen B. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet* 1986; **1**: 1287-1293 [PMID: 2423826 DOI: 10.1016/S0140-6736(86)91218-3]

37 **Eddleman KA**, Malone FD, Sullivan L, Dukes K, Berkowitz RL, Kharbutli Y, Porter TF, Luthy DA, Comstock CH, Saade GR, Klugman S, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, D'Alton ME. Pregnancy loss rates after midtrimester amniocentesis. *Obstet Gynecol* 2006; **108**: 1067-1072 [PMID: 17077226]

38 **Odibo AO**, Gray DL, Dicke JM, Stamilio DM, Macones GA, Crane JP. Revisiting the fetal loss rate after second-trimester genetic amniocentesis: a single center's 16-year experience. *Obstet Gynecol* 2008; **111**: 589-595 [PMID: 18310360 DOI: 10.1097/AOG.0b013e318162eb53]

39 **Akolekar R**, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015; **45**(1): 16-26 [PMID: 25042845 DOI: 10.1002/uog.14636]

40 **Anuwutnavin S**, Chanprapaph P, Ruangvutilert P, Eammatta M, Tontisirin P. Short-term outcomes after second-trimester genetic amniocentesis in Siriraj Hospital. *Int J Gynaecol Obstet* 2014; **124**: 222-225 [PMID: 24380612 DOI: 10.1016/j.ijgo.2013.09.019]

41 **Pitukkijronnakorn S**, Promsonthi P, Panburana P, Udomsubpayakul U, Chittacharoen A. Fetal loss associated with second trimester amniocentesis. *Arch Gynecol Obstet* 2011; **284**: 793-797 [PMID: 21052703 DOI: 10.1007/s00404-010-1727-3]

42 **Kozlowski P**, Knippel A, Stressig R. Individual risk of fetal loss following routine second trimester amniocentesis: a controlled study of 20,460 cases. *Ultraschall Med* 2008; **29**: 165-172 [PMID: 17602371 DOI: 10.1055/s-2007-963217]

43 **Mazza V**, Pati M, Bertucci E, Re C, Ranzi A, Percesepe A, Forabosco A, Volpe A. Age-specific risk of fetal loss post second trimester amniocentesis: analysis of 5043 cases. *Prenat Diagn* 2007; **27**: 180-183 [PMID: 17238217]

44 **Wapner RJ.** Genetic diagnosis in multiple pregnancies. *Semin Perinatol* 1995; **19:** 351-62 [DOI: 10.1016/S0146-0005(05)80013-8]

45 **Ghidini A**, Lynch L, Hicks C, Alvarez M, Lockwood CJ. The risk of second-trimester amniocentesis in twin gestations: a case-control study. *Am J Obstet Gynecol* 1993; **169**: 1013-1016 [PMID: 8238111 DOI: 10.1016/0002-9378(93)90045-K]

46 **Millaire M**, Bujold E, Morency AM, Gauthier RJ. Mid-trimester genetic amniocentesis in twin pregnancy and the risk of fetal loss. *J Obstet Gynaecol Can* 2006; **28**: 512-518 [PMID: 16857119]

47 **Yukobowich E**, Anteby EY, Cohen SM, Lavy Y, Granat M, Yagel S. Risk of fetal loss in twin pregnancies undergoing second trimester amniocentesis(1). *Obstet Gynecol* 2001; **98**: 231-234 [PMID: 11506838 DOI: 10.1016/S0029-7844(01)01416-8]

48 **Enzensberger C**, Pulvermacher C, Degenhardt J, Kawecki A, Germer U, Weichert J, Krapp M, Gembruch U, Axt-Fliedner R. Outcome after second-trimester amniocentesis and first-trimester chorionic villus sampling for prenatal diagnosis in multiple gestations. *Ultraschall Med* 2014; **35**: 166-172 [PMID: 23696061 DOI: 10.1055/s-0032-1330700]

49 **Cahill AG**, Macones GA, Stamilio DM, Dicke JM, Crane JP, Odibo AO. Pregnancy loss rate after mid-trimester amniocentesis in twin pregnancies. *Am J Obstet Gynecol* 2009; **200**: 257.e1-257.e6 [PMID: 19136086 DOI: 10.1016/j.ajog.2008.09.872]

50 **Lenis-Cordoba N**, Sánchez MÁ, Bello-Muñoz JC, Sagalá-Martinez J, Campos N, Carreras-Moratonas E, Cabero-Roura L. Amniocentesis and the risk of second trimester fetal loss in twin pregnancies: results from a prospective observational study. *J Matern Fetal Neonatal Med* 2013; **26**: 1537-1541 [PMID: 23544929 DOI: 10.3109/14767058.2013.791271]

51 **Philip J**, Silver RK, Wilson RD, Thom EA, Zachary JM, Mohide P, Mahoney MJ, Simpson JL, Platt LD, Pergament E, Hershey D, Filkins K, Johnson A, Shulman LP, Bang J, MacGregor S, Smith JR, Shaw D, Wapner RJ, Jackson LG. Late first-trimester invasive prenatal diagnosis: results of an international randomized trial. *Obstet Gynecol* 2004; **103**: 1164-1173 [PMID: 15172848 DOI: 10.1097/01.aog.0000128049.73556.fb]

52 **Nicolaides KH**, Brizot ML, Patel F, Snjders R. Comparison of chorion villus sampling and early amniocentesis for karyotyping in 1,492 singleton pregnancies. *Fetal Diagn Ther* 1996; **11**: 9-15 [PMID: 8719715 DOI: 10.1159/000264272]

53 Randomised trial to assess safety and fetal outcome of early and midtrimester amniocentesis. The Canadian Early and Mid-trimester Amniocentesis Trial (CEMAT) Group. *Lancet* 1998; **351**: 242-247 [PMID: 9457093 DOI: 10.1016/S0140-6736(97)12346-7]

54 **Ko TM**, Tseng LH, Chang MH, Chen DS, Hsieh FJ, Chuang SM, Lee TY. Amniocentesis in mothers who are hepatitis B virus carriers does not expose the infant to an increased risk of hepatitis B virus infection. *Arch Gynecol Obstet* 1994; **255**: 25-30 [PMID: 8042875]

55 **Grosheide PM**, Quartero HW, Schalm SW, Heijtink RA, Christiaens GC. Early invasive prenatal diagnosis in HBsAg-positive women. *Prenat Diagn* 1994; **14**: 553-558 [PMID: 7971756]

56 **Delamare C**, Carbonne B, Heim N, Berkane N, Petit JC, Uzan S, Grangé JD. Detection of hepatitis C virus RNA (HCV RNA) in amniotic fluid: a prospective study. *J Hepatol* 1999; **31**: 416-420 [PMID: 10488698]

57 **Shapiro DE**, Sperling RS, Mandelbrot L, Britto P, Cunningham BE. Risk factors for perinatal human immunodeficiency virus transmission in patients receiving zidovudine prophylaxis. Pediatric AIDS Clinical Trials Group protocol 076 Study Group. *Obstet Gynecol* 1999; **94**: 897-908 [PMID: 10576173]

58 **Mandelbrot L**, Jasseron C, Ekoukou D, Batallan A, Bongain A, Pannier E, Blanche S, Tubiana R, Rouzioux C, Warszawski J. Amniocentesis and mother-to-child human immunodeficiency virus transmission in the Agence Nationale de Recherches sur le SIDA et les Hépatites Virales French Perinatal Cohort. *Am J Obstet Gynecol* 2009; **200**: 160.e1-160.e9 [PMID: 18986640 DOI: 10.1016/j.ajog.2008.08.049]

59 **Tess BH**, Rodrigues LC, Newell ML, Dunn DT, Lago TD. Breastfeeding, genetic, obstetric and other risk factors associated with mother-to-child transmission of HIV-1 in Sao Paulo State, Brazil. Sao Paulo Collaborative Study for Vertical Transmission of HIV-1. *AIDS* 1998; **12**: 513-520 [PMID: 9543450]

60 **Somigliana E**, Bucceri AM, Tibaldi C, Alberico S, Ravizza M, Savasi V, Marini S, Matrone R, Pardi G. Early invasive diagnostic techniques in pregnant women who are infected with the HIV: a multicenter case series. *Am J Obstet Gynecol* 2005; **193**: 437-442 [PMID: 16098867]

61 **Coll O**, Suy A, Hernandez S, Pisa S, Lonca M, Thorne C, Borrell A. Prenatal diagnosis in human immunodeficiency virus-infected women: a new screening program for chromosomal anomalies. *Am J Obstet Gynecol* 2006; **194**: 192-198 [PMID: 16389031]

62 **Ekoukou D**, Khuong-Josses MA, Ghibaudo N, Mechali D, Rotten D. Amniocentesis in pregnant HIV-infected patients. Absence of mother-to-child viral transmission in a series of selected patients. *Eur J Obstet Gynecol Reprod Biol* 2008; **140**: 212-217 [PMID: 18584937 DOI: 10.1016/j.ejogrb.2008.04.004]

63 **Panel on Treatment of HIV-infected Pregnant Women and Prevention of Perinatal Transmission.** Recommendations for the Use of Antiretroviral Drugs in Pregnant HIV-1 Infected Women for Maternal Health and Interventions to Reduce Perinatal HIV Transmission in the United States, 2012

64 [**American College of Obstetricians and Gynecologists Committee on Genetics**](http://www.ncbi.nlm.nih.gov/pubmed/?term=American%20College%20of%20Obstetricians%20and%20Gynecologists%20Committee%20on%20Genetics%5BCorporate%20Author%5D)**.** Committee Opinion No. 545: Noninvasive prenatal testing for fetal aneuploidy. *Obstet Gynecol* 2012; **120**: 1532-1534 [PMID: 23168792 DOI: 10.1097/01.AOG.0000423819.85283.f4]

65 **Bianchi DW**, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, Craig JA, Chudova DI, Devers PL, Jones KW, Oliver K, Rava RP, Sehnert AJ. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med* 2014; **370**: 799-808 [PMID: 24571752 DOI: 10.1056/NEJMoa1311037]

66 **Pergament E**, Cuckle H, Zimmermann B, Banjevic M, Sigurjonsson S, Ryan A, Hall MP, Dodd M, Lacroute P, Stosic M, Chopra N, Hunkapiller N, Prosen DE, McAdoo S, Demko Z, Siddiqui A, Hill M, Rabinowitz M. Single-nucleotide polymorphism-based noninvasive prenatal screening in a high-risk and low-risk cohort. *Obstet Gynecol* 2014; **124**: 210-218 [PMID: 25004354 DOI: 10.1097/AOG.0000000000000363]

**P- Reviewer:** Khajehei M, Schulten HJ, Zafrakas M, Zhang XQ

**S- Editor:** Song XX **L- Editor:** **E- Editor:**