REVIEW ARTICLE

Invasive Diagnostic Procedures in Embryonic Period

Giovanni Monni[1], Valentina Corda[2], Francesca Dessolis[3], Alba Piras[4]

Abstract

In the era of prenatal ultrasound and biochemical screening and also due to the increase of noninvasive prenatal testing and screening (NIPT, NIPS), invasive prenatal techniques are the most appropriate procedures for diagnosing chromosomal, metabolic, and genetic fetal anomalies. Chorionic villous sampling (CVS) in the first trimester of pregnancy is currently the technique of choice for women at high genetic risk. Amniocentesis is more frequently employed in the second trimester while fetal blood sampling (FBS) by cordocentesis is rarely used nowadays. Women who choose to avoid the termination of pregnancy (TOP) of an affected fetus can opt for preimplantation genetic diagnosis (PGD) by assisted reproductive techniques (ART) by transferring *in utero* only the microbiopsied non-affected embryos or blastocysts. All invasive prenatal diagnosis procedures are performed under continuous ultrasound monitoring and can be done both free-hand or by insertion of a spinal needle in the ultrasound probe. Chorionic villous sampling can be performed by transcervical or transabdominal route; this last one is preferred mostly because it can be employed in any trimester of pregnancy but also because it is simpler and therefore easier for hands-on training, faster, less invasive; it is also associated with lower risks of infections and fetal loss. In antenatal diagnosis, the first step is non-directive pretest counseling to explain the risks and efficacy and to provide information about the procedures and the disease. The new laboratory analysis techniques are in continuous progress and their efficacy and success are very high for chromosomal anomalies using traditional karyotype by direct analysis of cytotrophoblastic and cultured metaphases of chorionic tissue. Alternatively, quantitative fluorescent polymerase chain reaction (QF-PCR) and array comparative genetic hybridization (aCGH) can be utilized. DNA amplification by PCR and, recently, next-generation sequencing (NGS) have shown high sensitivity and specificity for single-gene diseases. Audit of clinicians and adequate training of fellows are of paramount importance to have the highest quality results. The authors of this manuscript would like to thank Fondazione di Sardegna; Sardinia Regional Department for Programming; Boyana Petrova Tsikalova, MA in English Philology.

Keywords: Amniocentesis, Chorionic villous sampling, Fetal blood sampling, Invasive prenatal procedures, Preimplantation genetic diagnosis.

Donald School Journal of Ultrasound in Obstetrics and Gynecology (2021): 10.5005/jp-journals-10009-1692

Introduction

Invasive diagnostic sampling procedures are all techniques used for analyzing embryo–fetal and placental tissues, amniotic fluid, or fetal blood to avoid birth defects.[1]

The most common procedures performed are chorionic villous sampling (CVS), amniocentesis, and cordocentesis.[2]

Since 1983 in the embryonic period, the most useful diagnostic procedure is CVS performed either transcervically (TC-CVS) or transabdominally (TA-CVS).

All techniques are performed under continuous ultrasound monitoring and following nondirective genetic-obstetric counseling and informed written consent.

More recently, to avoid genetic diseases and voluntary termination of pregnancy (TOP), women can utilize before pregnancy and using *in vitro* fertilization and intracytoplasmatic injection (IVF-ICSI) the preimplantation genetic diagnosis (PGD).[3,4]

Even if in the past, coelocentesis, early amniocentesis between the 12th and 14th week of gestation, vaginal lavage to obtain fetal cells were described as prenatal invasive procedures during the embryonic period; nowadays, these techniques are completely abandoned.

In this chapter, we would like to give the updated practical recommendations and the most appropriate techniques in invasive prenatal procedures such as CVS and PGD for clinicians and patients while deciding on their reproductive choices available in the embryonic period.

Genetic-obstetric Counseling

Genetic-obstetric counseling must be performed before any invasive procedure by doctors' experts in genetics. Possibly, the counseling must be performed in the preconceptional period or before procedures to suggest and offer to the patients the best information about their reproductive choices.[5]

Counseling must always be informative and nondirective[1] and should include:

- The genetic risks of diseases.
- The possibility of screening, diagnosis, prognosis, and therapy.
- Embryo–fetal, maternal, and neonatal risks following invasive prenatal procedures, diagnostic limits, success and failure rates, and time to obtain a diagnosis.
- Methods of all invasive procedures.
- Other possible techniques and laboratory diagnostic clarifications in case of doubts.
- Choice options of fetal–neonatal therapy as well as discussion of the possibility of performing voluntary TOP in pathologic cases.

[1–4]Department of Prenatal and Preimplantation Genetic Diagnosis and Fetal Therapy, Microcitemico Pediatric Hospital "A. Cao", Via E. Jenner Snc, Cagliari, Italy

Corresponding Author: Giovanni Monni, Department of Prenatal and Preimplantation Genetic Diagnosis and Fetal Therapy, Microcitemico Pediatric Hospital "A. Cao", Via E. Jenner Snc, Cagliari, Italy, Phone: +39-070-5296-5546, e-mail: prenatalmonni@tiscali.it

How to cite this article: Monni G, Corda V, Dessolis F, *et al.* Invasive Diagnostic Procedures in Embryonic Period. Donald School J Ultrasound Obstet Gynecol 2021;15(2):169–174.

Source of support: Nil

Conflict of interest: None

It is suggested to obtain a written informed consent form signed by the patient at the end of the counseling session.

Ultrasound and Prenatal Invasive Techniques

All invasive prenatal procedures would not be possible to perform if in the past years there has not been an impressive enhancement of the ultrasonographic equipment.

Each invasive prenatal procedure must be performed under continuous ultrasound monitoring to find the best spot for the instrument insertion and the sampling itself.

Ultrasonographic monitoring must be performed before the invasive prenatal procedure to define the pregnancy, the viability and the number of fetuses, the gestational age, the placenta location, the amniotic fluid pocket, the umbilical cord insertion and to avoid possible concomitant uterine adnexal pathologies.[6]

Embryonic Invasive Diagnostic Procedures (CVS-PGD)

The most common invasive technique used in perinatal centers is CVS, either transcervical (TC-CVS) or, mostly utilized, transabdominal (TA-CVS).[7]

The transabdominal route is preferable because it can be performed at any period of pregnancy[8] (from 10th to 40th weeks of pregnancy), it is generally better accepted by women since it is faster to perform and implies lower fetal loss and infection risks, less risk of vaginal bleeding, better privacy, and easier reproducibility.[9]

To avoid the voluntary TOP, PGD on the embryo or the blastocyst can be employed.[4]

The techniques used depend on the disease, on whether they are performed before or during pregnancy, on the clinician's experience and hands-on skills, on the capacity of the laboratory, and on the patient's choice. Women usually prefer the simplest technique which provides a result as early as possible.

Chorionic Villous Sampling

Indications of CVS

Karyotype Analysis for Chromosomal Risks

Even if in several countries the maternal age solely (≥35 years) is still employed as an indication for fetal karyotype study, the most appropriate indication is an abnormal screening test following first trimester combined testing (fetal nuchal translucency ultrasound measurement and dosages of free-β hCG and PAPP-A) and, more recently, following cell-free DNA (NIPS—noninvasive prenatal screening) from maternal blood sampling.

Other indications for fetal karyotype study are a previous child with a chromosomal anomaly and ultrasound detection of fetal structural abnormalities in the first trimester of pregnancy.[1]

DNA Analysis for

Increased risk for single-gene diseases with a known DNA mutation, the risk for X-chromosomal inheritance, and autosomal recessive diseases such as thalassemia, cystic fibrosis, Duchenne muscular dystrophy, mental retardation, etc.[1]

Errors of Metabolism[1]

Transabdominal CVS Technique (TA-CVS)

The most commonly employed procedure is TA-CVS by free-hand technique. The free-hand insertion can be done tangentially (Fig. 1) or obliquely (Fig. 2) to the ultrasound scanner using a 20- or 18-gauge spinal needle under continuous ultrasound monitoring.[9,10] Several operators report insertion of the needle into the biopsy rigid adaptor as a guide.[6]

We prefer the free-hand approach using a 20-gauge needle connected to a 2-mL syringe to aspirate chorionic villi by an up-and-down movement because it proves to be less painful for patients and it also allows reaching the shortest route between the maternal abdomen and the chorion site as well as easy correction of the needle trajectory.[11–13]

Neither local anesthesia nor antibiotic treatment is usually required and the sampling is performed in an outpatient facility. In a few cases, antispastics can be administered.

Sampling failure is very rare but it can occur in 1–2% of cases. The quantity and quality of the chorionic villi should be visually controlled by the clinician and usually, 10–20 mg of tissue is sufficient for all analysis.

In a few cases, maternal intestinal loops can be placed above the uterus so it may be useful to exert mild pressure with the screening probe to move the uterus a bit aside and then introduce the needle in the placenta.

In the case of the retroverted uterus and completely posterior placenta (2–3% of cases), transvaginal manipulation of the uterus by the assistant may be required to introduce the needle in the placenta and obtain a sufficient amount of chorionic villi.[14]

All procedures are performed in a sterile area and the needle and syringe must be heparinized to avoid clots, the maternal abdomen must be cleansed with an antiseptic solution beforehand. The screen probe must also be in a sterile drape or a glove.

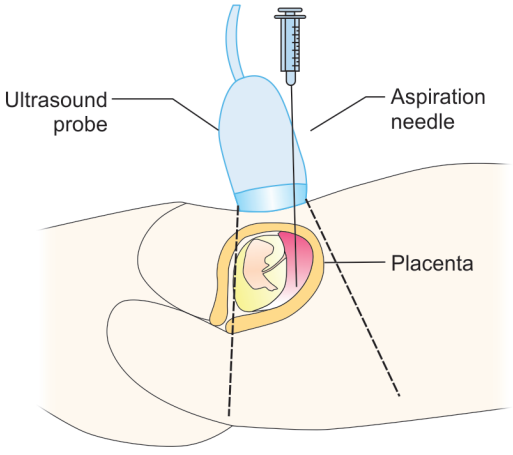

Fig. 1: TA-CVS and tangential free-hand insertion of the needle

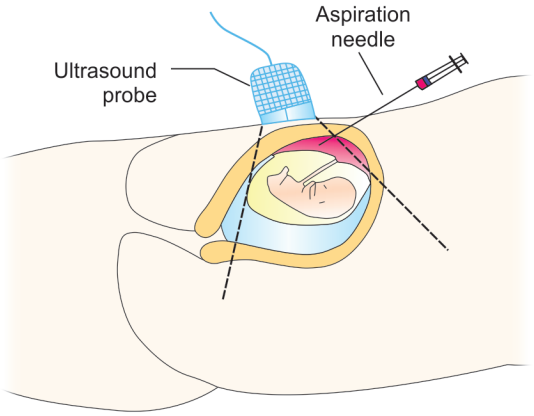

Fig. 2: TA-CVS and oblique free-hand insertion of the needle

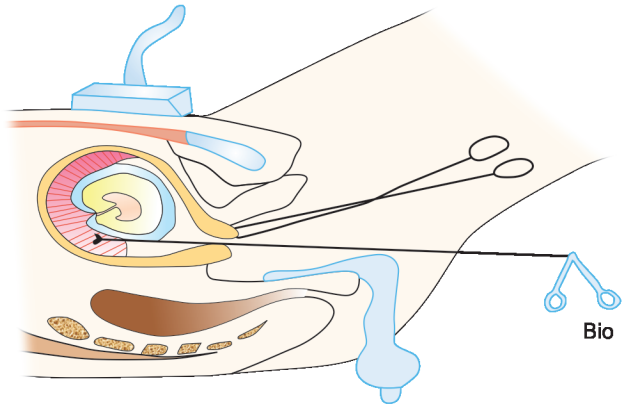

Fig. 4: TC-CVS and rigid biopsy forceps introduction

Transcervical CVS Technique (TC-CVS)

Placenta location by ultrasound and careful disinfection of the vagina must be done before introducing a flexible polyethylene catheter (Fig. 3) with an aluminum mandrel connected to a syringe for aspiration[15] or a rigid biopsy forceps[16] in the cervical canal introduced in chorion frondosum (Fig. 4) between 10 weeks and 14 weeks of gestation under continuous ultrasound monitoring. In several cases, a tenaculum can prove useful for the traction of the cervix and straightening out the uterus to permit a better introduction.

The instruments should not be introduced more than twice and if insufficient chorionic tissue is sampled, it is recommended to use a new sterile device.

Local anesthesia, antibiotics, and hospitalization are not necessary, and only in a few cases, tocolytics can be administered.

Sampling failure is very rare but it is reported to occur in 2–4% of samplings. The sufficient quantity of tissue should be visually controlled by the clinician and may vary from 10 to 20 mL for all genetic or chromosomal analyses.

Timing and Risks of CVS

Even if in the past, several procedures were performed in an earlier period of pregnancy before the 9th week of gestation, CVS must be

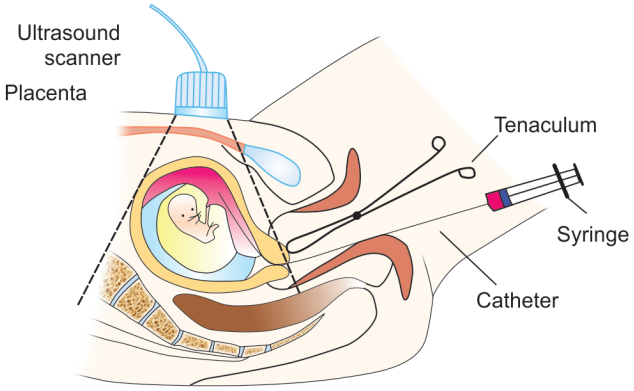

Fig. 3: TC-CVS and catheter introduction

performed following 10 weeks of gestation because several fetal abnormalities such as limb reduction and oromandibular defects were described if performed before the 10th week.[17–19] Therefore, the most appropriate timing is 11–12 weeks.

The additional fetal loss rate following the procedure depends on the clinician's experience and a meta-analysis study reports to be very low, varying between 0.2% and 1%, the same as the one reported for the amniocentesis at 16 weeks.[20–22] The fetal risk is higher in the early period and older women.[23]

It is better not to introduce the instruments more than twice. In Rh-negative patients with a negative Coombs test, RH alloimmunization can be caused by CVS. In such cases, anti-D immunoglobulins prophylaxis is mandatory. In women already immunized, CVS is contraindicated.

It is advisable to perform the procedure after a reasonable period of tutoring under the guiding of a senior clinician.[24] To monitor the capacity of sampling success and to have fewer fetal losses, the clinician must have performed at least 100–200 procedures each year.[25]

Laboratory Analysis of CVS

Prenatal samplings can be analyzed using traditional karyotype, DNA-polymerase chain reaction (DNA-PCR), quantitative fluorescent polymerase chain reaction (QF-PCR), or chromosomal molecular analysis, and for single-gene diseases.

Traditional Karyotype

Metaphase analysis of cultured chorionic tissue and direct analysis of cytotrophoblastic metaphases following CVS is still used in prenatal centers.[26]

In 1–2% several cell lines with different chromosomal kits can be present, this is called mosaicism. Two different types of mosaicism exist the true chromosomal mosaicism in which case the fetus and the placenta have two chromosomal kits and the so-called "confined placental mosaicism" with two cell lines present only in the placenta but not in the fetus. To distinguish the two cases, further analysis by amniocentesis or fetal blood sampling (FBS) is required.[27]

QF-PCR

Recently QF-PCR can be used for the analysis of the most common trisomies and for X and Y chromosomes, but, in few cases, this test

results in more false-positives and false-negatives.[28] Quantitative fluorescent polymerase chain reaction must be confirmed by metaphase long culture analysis and ultrasound examination.

Chromosomal Molecular Analysis

Array comparative genetic hybridization (aCGH) or microarray analysis permits ascertaining submicroscopic chromosomal deletions and duplications. Up till now, targeted, mixed arrays, and genome-wide can be utilized in prenatal diagnosis for the detection of 6% of aberrations in traditional normal karyotype and also in fetal malformations following ultrasound.[29]

Microarray techniques can also increase the detection rate of aberrations in CVS following abnormal screening combined test or following nuchal translucency thickness ≥3.5 mm and congenital cardiac problems.[30,31] Finding a variant of uncertain significance (VoUS) may make genetic counseling difficult.

Sampling for Single Gene Defects

DNA-polymerase chain reaction amplification analysis is the technique of choice for a genetic single gene or Mendelian diseases such as thalassemia, cystic fibrosis, Tay-Sachs disease, etc., with very high analysis accuracy and very low misdiagnosis rate is reported.[32,33]

It is preferable to obtain the DNA by CVS rather than by amniocentesis because of lower contamination probability and better analysis results. Variable number tandem repeat (VNTR) analysis can be added to avoid maternal contamination and misdiagnosis.[34]

Training and Audit of CVS

In the era of declining rates of invasive prenatal procedures due to the decrease of natality, screening tests, and the onset of NIPS by cell-free DNA the importance of training and tutoring of fellows and maintaining the skilled expertise is fundamental.[35–37]

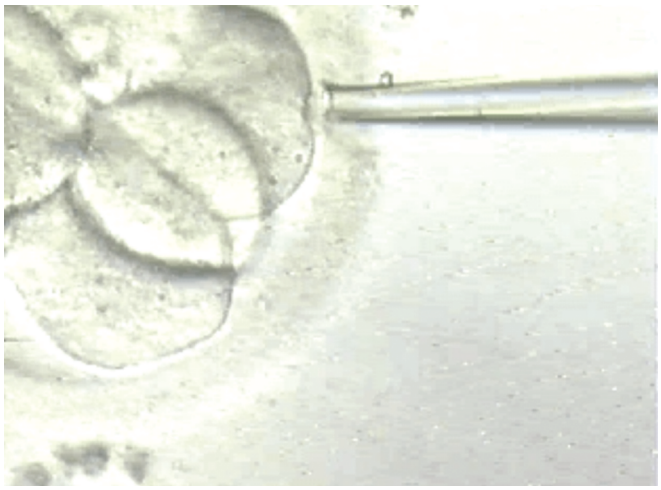

Fig. 5: Single-cell biopsy from an embryo at the 3rd-day cleavage

At least 30–100 amniocenteses and 50–200 CVS are recommended yearly to maintain the manual skills of clinicians. Prenatal centers must create a database for annual monitoring of the sampling procedures and consider all parameters such as failure, success, repetition of procedure, fetal risks, analysis accuracy, as well as several birth defects.[22]

Tutoring fellows in TA-CVS is simpler if they are already expert in ultrasonography and amniocentesis which is generally easier to acquire as a skill, rather than TC-CVS.[35]

A training period of 2–3 weeks with an adequate number of invasive prenatal procedures and in a center with an expert senior tutor is recommendable, with teaching *in vivo* instead of using a model, a mannequin, or simulators.[24]

Performing procedures must be centralized in centers so that they are a considerable number to maintain the skill and thus reduce the fetal loss risk and complications. The expertise of all operators must also be controlled regularly by an annual audit that should be reported during the counseling of the patients.[14]

Preimplantation Genetic Diagnosis

Preimplantation genetic diagnosis (PGD) is a very early form of prenatal diagnosis before the implantation of the embryo in the uterus and involves testing to avoid the transmission of specific genetic or chromosomal birth defects.[38]

It was performed for the first time by Handyside in 1989 in the UK.[39] This technique also aims to bypass the obvious issue of voluntary TOP because it allows to select and transfer in the maternal uterus only the healthy embryos obtained *in vitro* by assisted reproductive techniques (IVF-ICSI).[40]

The technique involves the biopsy of on single or more cells from oocytes or embryos, at the 3rd-day cleavage stage (Fig. 5) or at the 5th day from trophoblastic cells of blastocysts (Fig. 6).[41]

Preimplantation genetic diagnosis is used for genetic diagnosis of autosomal recessive disorders such as thalassemia, cystic fibrosis, Tay-Sachs disease, spinal amiotrophy, etc.,[42] and women at risk for chromosomal disorders, mainly for advanced maternal age, recurrent pregnancy loss and repeated IVF failure, severe sperm factors, carrier of chromosomal rearrangement, and in the last indications is named preimplantation genetic screening (PGS).[43,44]

The biopsied cells are analyzed by DNA-PCR. In the past, the analysis was performed by multiplex polymerase chain reaction for single gene defects and by fluorescent *in situ* hybridization for chromosomal anomalies.

At present time, the most accurate analysis is the whole-genome amplification and genome-wide technologies.[38]

The success rate of analysis is high even if in a few cases a diagnosis may not be available due to DNA amplification problems or contamination. The success of PGD is strongly influenced by

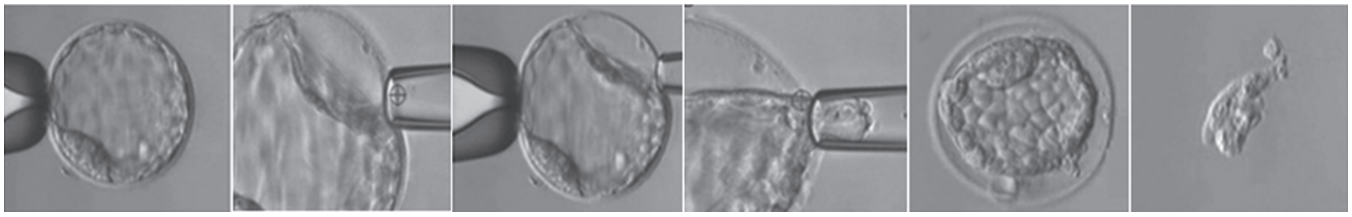

Fig. 6: Blastocyst removal at the 5th-day from trophoblastic cells

maternal age, IVF, quality of embryo culture and biopsy, and also by molecular diagnosis.

Preimplantation genetic diagnosis can also be employed for HLA compatibility by human leukocyte antigen matching in case of bone marrow transplantation after birth even if these techniques raise some ethical problems and controversies.[45]

This paper was previously published in Kurjak A, Chervenak FA. Donald School. Embryo as a Person and as a Patient. New Delhi: Jaypee Brothers Medical Publishers, 2019: 89–95.

References

1. Milunsky A, Milunsky JM, ed. Genetic disorders and the fetus. 6th ed., Chicester, UK: Wiley- Blackwell; 2010.
2. Monni G, Zoppi MA, Axiana C, et al. Changes in the approach for invasive prenatal diagnosis in 35,127 cases at a single center from 1977 to 2004. Fetal Diagn Ther 2006;21(4):348–354. DOI: 10.1159/000092464.
3. Kuliev A, Rechitsky S, Verlinsky O, et al. Preimplantation diagnosis of thalassemias. J Assist Reprod Genet 1998;15(5):219–225. DOI: 10.1023/a:1022571822585.
4. Traeger-Synodinos J. Pre-implantation genetic diagnosis. Best Pract Res Clin Obstet Gynaecol 2017;39:74–88. DOI: 10.1016/j.bpobgyn.2016.10.010.
5. Cao A, Cossu P, Monni G, et al. Chorionic villus sampling and acceptance rate of prenatal diagnosis. Prenat Diagn 1987;7(7):531–533. DOI: 10.1002/pd.1970070710.
6. Ghi T, Sotiriadis A, Calda P, et al. On behalf of the International Society of Ultrasound in Obstetrics and Gynecology, ISUOG practice guidelines: invasive procedures for prenatal diagnosis in obstetrics. Ultrasound Obstet Gynecol 2016;48(2):256–268. DOI: 10.1002/uog.15945.
7. Brambati B, Lanzani A, Tului L. Transabdominal and transcervical chorionic villus sampling: efficiency and risk evaluation of 2,411 cases. Am J Med Genet 1990;35(2):160–164. DOI: 10.1002/ajmg.1320350204.
8. Monni G, Olla G, Rosatelli MC, et al. Second-trimester placental biopsy versus amniocentesis for prenatal diagnosis of beta-thalassemia. N Engl J Med 1990;322(1):60–61. DOI: 10.1056/NEJM199001043220115.
9. Monni G, Olla G, Cao A. Patient's choice between transcervical and transabdominal chorionic villus sampling. Lancet 1988;7(8593):1057. DOI: 10.1016/s0140-6736(88)91880-6.
10. Brambati B, Terzian E, Tognoni G. Randomized clinical trial of transabdominal versus transcervical chorionic villus sampling methods. Prenat Diagn 1991;11(5):285–293. DOI: 10.1002/pd.1970110503.
11. Monni G, Ibba RM, Lai R, et al. Early transabdominal chorionic villus sampling in couples at high genetic risk. Am J Obstet Gynecol 1993;168(1 Pt 1):170–173. DOI: 10.1016/s0002-9378(12)90908-4.
12. Monni G, Ibba RM, Zoppi MA. Prenatal genetic diagnosis through chorionic villus sampling. In: Genetic disorders and the fetus Milunsky A, Milunsky JM, ed. 6th ed., Hoboken, NJ, USA: Wiley-Blackwell; 2010. pp. 160–193.
13. Monni G, Pagani G, Stagnati V, et al. How to perform transabdominal chorionic villus sampling: a practical guideline. J Matern Fetal Neonatal Med 2015(9):1–7. DOI: 10.3109/14767058.2015.1051959.
14. Monni G, Iuculano A. Re: ISUOG practice guidelines: invasive procedures for prenatal diagnosis. Ultrasound Obstet Gynecol 2017;49(3):414–415. DOI: 10.1002/uog.17375.
15. Ward RH, Modell B, Petrou M, et al. Method of sampling chorionic villi in first trimester of pregnancy under guidance of real time ultrasound. Br Med J (Clin Res Ed) 1983;286(6377):1542–1544. DOI: 10.1136/bmj.286.6377.1542.
16. Monni G, Ibba RM, Olla G, et al. Chorionic villus sampling by rigid forceps: experience with 300 cases at risk for thalassemia major. Am J Obstet Gynecol 1987;156(4):912–914. DOI: 10.1016/0002-9378(87)90352-8.
17. Firth HV, Boyd PA, Chamberlain P, et al. Severe limb abnormalities after chorion villus sampling at 56–66 days' gestation. Lancet 1991;337(8744):762–763. DOI: 10.1016/0140-6736(91)91374-4.
18. Monni G, Ibba RM, Lai R, et al. Limb-reduction defects and chorion villus sampling. Lancet 1991;337(8749):1091–1092. DOI: 10.1016/0140-6736(91)91735-D.
19. Froster UG, Jackson L. Limb defects and chorionic villus sampling: results from an international registry, 1992–94. Lancet 1996;347(9000):489–494. DOI: 10.1016/s0140-6736(96)91136-8.
20. Tabor A, Alfirevic Z. Update on procedure-related risks for prenatal diagnosis techniques. Fetal Diagn Ther 2010;27(1):1–7. DOI: 10.1159/000271995.
21. Akolekar R, Beta J, Picciarelli G, et al. 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. DOI: 10.1002/uog.14636.
22. Wulff CB, Gerds TA, Rode L, et al. The risk of fetal loss associated with invasive testing following combined first trimester risk screening for down syndrome – a national cohort of 147 987 singleton pregnancies. Ultrasound Obstet Gynecol 2016;47(1):38–44. DOI: 10.1002/uog.15820.
23. Monni G, Ibba RM, Laj R, et al. Transabdominal chorionic villus sampling: fetal loss rate in relation to maternal and gestational age. Prenat Diagn 1992;12(10):815–820. DOI: 10.1002/pd.1970121007.
24. Monni G, Pagani G, Illescas T, et al. Training for transabdominal villous sampling is feasible and safe. Am J Obstet Gynecol 2015;213(2):248–250. DOI: 10.1016/j.ajog.2015.04.019.
25. Monni G, Zoppi MA. Improved first-trimester aneuploidy risk assessment: An evolving challenge of training in invasive prenatal diagnosis. Ultrasound Obstet Gynecol 2013;41(5):486–488. DOI: 10.1002/uog.12461.
26. Jackson LG, Zachary JM, Fowler SE, et al. A randomized comparison of transcervical and transabdominal chorionic-villus sampling. The U.S. National Institute of Child Health and Human Development chorionic-villus sampling and amniocentesis study group. N Engl J Med 1992;327(9):594–598. DOI: 10.1056/NEJM199208273270903.
27. American College of Obstetricians and Gynecologists. ACOG practice bulletin no.88, December 2007. Invasive prenatal testing for aneuploidy. Obstet Gynecol 2007;110(6):1459–1467. DOI: 10.1097/01.AOG.0000291570.63450.44.
28. Technical and clinical assessment of fluorescence in situ hybridization: an ACMG/ASHG position statement. Technical considerations. American College of Medical Genetics. Genet Med 2000;2(6):356–361. DOI: 10.1097/00125817-200011000-00011.
29. Wapner RJ, Martin CL, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med 2012;367(23):2175–2184. DOI: 10.1056/NEJMoa1203382.
30. Jansen FA, Blumenfeld YJ, Fisher A, et al. Array comparative genomic hybridization and fetal congenital heart defects: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2015;45(1):27–35. DOI: 10.1002/uog.14695.
31. Grande M, Jansen FA, Blumenfeld YJ, et al. Genomic microarray in fetuses with increased nuchal translucency and normal karyotype: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2015;46(6):650–658. DOI: 10.1002/uog.14880.
32. Rosatelli MC, Falchi AM, Tuveri T, et al. Prenatal diagnosis of beta-thalassaemia with the synthetic-oligomer technique. Lancet 1985;1(8423):241–243. DOI: 10.1016/s0140-6736(85)91026-8.
33. Saiki RK, Bugawan TL, Horn GT, et al. Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes. Nature 1986;324(6093):163–166. DOI: 10.1038/324163a0.
34. Batanian JR, Ledbetter DH, Fenwick RG. A simple VNTR-PCR method for detecting maternal cell contamination in prenatal diagnosis. Genet Test 1998;2(4):347–350. DOI: 10.1089/gte.1998.2.347.

35. Lo YMD, Chan KCA, Sun H, et al. Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. Sci Transl Med 2010;2(61):61ra91. DOI: 10.1126/scitranslmed.3001720.
36. Monni G, Zoppi MA, Iuculano A, et al. Invasive or non-invasive prenatal genetic diagnosis? J Perinat Med 2014;42(5):545–548. DOI: 10.1515/jpm-2014-0135.
37. Odibo AO, Dicke JM, Gray DL, et al. Evaluating the rate and risk factors for fetal loss after chorionic villus sampling. Obstet Gynecol 2008;112(4):813–819. DOI: 10.1097/AOG.0b013e3181875b92.
38. Palini S, De Stefani S, Primiterra M, et al. Pre-implantation genetic diagnosis and screening: now and the future. Gynecol Endocrinol 2015;31(10):755–759. DOI: 10.3109/09513590.2015.1068752.
39. Handyside AH, Pattinson JK, Penketh RJ, et al. Biopsy of human preimplantation embryos and sexing by DNA amplification. Lancet 1989;1(8634):347–349. DOI: 10.1016/s0140-6736(89)91723-6.
40. Monni G, Peddes C, Iuculano A, et al. From prenatal to preimplantation genetic diagnosis of β-thalassemia. Prevention model in 8748 cases: 40 years of single center experience. J Clin Med 2018;7(2):35. DOI: 10.3390/jcm7020035.
41. Scott Jr RT, Upham KM, Forman EJ, et al. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril 2013;100(3):624–630. DOI: 10.1016/j.fertnstert.2013.04.039.
42. Monni G, Cau G, Usai V, et al. Preimplantation genetic diagnosis for beta-thalassaemia: the sardinian experience. Prenat Diagn 2004;24(12):949–954. DOI: 10.1002/pd.1051.
43. De Wert G, Dondorp W, Shenfield F, et al. ESHRE task force on ethics and Law22: preimplantation genetic diagnosis. Hum Reprod 2014;29(8):1610–1617. DOI: 10.1093/humrep/deu132.
44. Fiorentino F, Kahraman S, Karadayi H, et al. Short tandem repeats haplotyping of the HLA region in preimplantation HLA matching. Eur J Hum Genet 2005;13(8):953–958. DOI: 10.1038/sj.ejhg.5201435.
45. Tur-Kaspa I, Jeelani R. Clinical guidelines for IVF with PGD for HLA matching. Reprod Biomed Online 2015;30(2):115–119. DOI: 10.1016/j.rbmo.2014.10.007.