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INVASIVE DIAGNOSTIC PROCEDURES IN MULTIPLE PREGNANCIES

INVAZIVNI DIJAGNOSTIČKI POSTUPCI U VIŠEPLODNOJ TRUDNOĆI

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Review

Key words: Genetic testing, multiple pregnancy, prenatal diagnosis

SUMMARY. Over the past few years, the rising rate of multiple pregnancies, attributed to both increasing reliance on infertility treatment modalities and delayed childbearing, has expanded the need for prenatal invasive genetic testing. In multiples, first-trimester chorionic villus sampling and second-trimester amniocentesis are relatively safe and efficient alternative procedures, whereas fetal blood sampling is reserved for cases where an indefinite result of fetal karyotyping needs elucidation. The choice of invasive technique should be based on gestational age at referral date, procedure related risks and technical demands, but experience of the center performing the modality should be emphasized in decision making. Technological advances in modern high resolution ultrasound equipment along with increasing operator experience available today result in more accurate and efficacious invasive prenatal diagnosis in twin or higher-order pregnancies, minimizing potential post-procedural fetal loss rate.

Pregled

Ključne riječi: genetsko testiranje, višeplodna trudnoća, prenatalna dijagnostika

SAŽETAK. Monozigotni blizanci čine oko 30% blizanačkih trudnoća, njihova je učestalost stalna, a dvozigotni blizanci čine oko 70% blizanačkih trudnoća, njihova je učestalost u porastu, zbog učestale primjene tehnike pomognute oplodnje i zbog odgađanja prvih trudnoća te posljedične veće životne dobi trudnica. Rizik strukturalnih anomalija u blizanaca je veća nego u jednoplodnih trudnoća, rizik je do tri puta veći u monozigotnih, a u dizigotnih blizanaca je od prilike kao u jednoplodnih trudnoća. Zbog ukupno veće učestalosti višeplodnih trudnoća povećana je potreba za invazivnom prenatalnom dijagnostikom. U višeplodnim trudnoćama su biopsija korionskih resica u prvom i amniocenteza u drugom tromjesečju relativno sigurni i uspješni alternativni postupci, a uzimanje fetalne krvi kordocentezom je rezervirano za slučajeve kada je učinjena kariotipizacija fetusa nesigurna i nejasna. Izbor invazivne tehnike se temelji na dobi trudnoće kad se trudnica javlja, na postojeći rizik postupka i na tehničke zahtjeve, a od velikog je značaja iskustvo prenatalnog centra. Tehnološki napredak suvremene ultrazvučne aparature te rastuće iskustvo prenatalnog operatera doprinose točnoj i učinkovitijoj invazivnoj prenatalnoj dijagnostici u dvojaka i blizanaca višeg stupnja te na najmanju moguću mjeru smanjuju fetalni gubitak nakon invazivnog postupka.

Introduction

The prevalence of multiple pregnancy varies worldwide from 6.7 per 1000 deliveries in Japan to 40 per 1000 deliveries in Nigeria. The respective prevalence in Europe and North America is estimated to be 11 per 1000 deliveries. The incidence of multiple births has increased dramatically over the past three decades since in vitro fertilization (IVF) was first introduced in modern obstetrics and gynaecology. Tremendous advances achieved in assisted reproductive techniques (ART) including IVF and non-ART procedures such as ovulation induction, rendered infertility treatment increasingly popular among infertile and subfertile couples. It has been postulated that women undergoing ovulation induction have an approximately 6% chance of conceiving multiples.[1] Furthermore, implementation of ART is accompanied by a 35% chance or more of accomplishing twin or higher-order pregnancy.[2] Hence, increasing reliance on assisted conception modalities noted nowadays, has been considered the major causative factor of the rising rate of multiple pregnancies. In a lesser degree, delayed childbearing in progressively advanced maternal age currently adopted by many prospective mothers accounts for the rising rate of spontaneously conceived multiplets.

There is no doubt that infertility treatment is associated with an increase in the rate of monozygotic (MZ) twins to greater than 10-fold, the latter being at high risk of functional and structural abnormalities, affecting 10–15% of these twins.[3–5] On the other hand, the incidence of chromosomal abnormalities is strongly related to maternal age. Available data confirms that twin pregnancies *per se* are at increased risk for fetal chromosomal abnormalities than those with singletons.[6,7] Therefore, the increasing incidence of multiple pregnancies illustrates a concomitant increase in the need for invasive genetic testing in these pregnancies.

Zygocity and chorionicity

It is widely accepted that the number of fetuses itself does increase possible maternal and fetal risks and

thereby the potential of an adverse pregnancy outcome. However, the cornerstone in prenatal diagnosis, surveillance and management of a multiple gestation is chorionicity as well as zygocity determination. Chorionicity refers to the placentation whereas zygocity implies the genetic profile of the pregnancy and therefore determines the degree of risk and whether or not the fetuses may be concordant or discordant for chromosomal abnormalities.

It is estimated that more than 30% of twins are identical or MZ and nearly 70% are fraternal or dizygotic (DZ). ***Monozigotic twins*** originate from the division of a single fertilized ovum with an incidence rate of about 3.5/1000 pregnancies.[8] The rate of spontaneous MZ twin pregnancies is constant contrary to the increased frequency reported among pregnancies derived from infertility treatment modalities. MZ twins may be *dichorionic diamniotic* (DC-DA), *monochorionic diamniotic* (MC-DA), *monochorionic monoamniotic* (MC-MA), and even *conjoined,* determined by the period of embryonic development when zygotic splitting takes place. In about 20–30% of cases, splitting occurs within three days of fertilization resulting in separate fetuses with independent placental circulations, therefore being DC-DA, even if placentas may seem to be in continuity or fused. In the majority of cases (about 70%) splitting occurs within the first week but later than the third day, it results in a single monochorionic plate and two distinct amniotic sacs, hence MC-DA twins originate. Delayed zygotic splitting leads to MC-MA twins, accounting for 1% of MZ twins, though later than 13th day is extremely rare, resulting in the formation of the abnormal conjoined (Siamese) twins.

Dizygotic twins arise from the fertilization of two distinct ova, thus may be of the same or different sex. Each twin has its own placenta and amniotic sac (DC-DA). Very infrequent cases of MC-DZ twins originating from the fusion of two separate blastocysts have been recently reported in association with ART,[9] staggering the categorical general rule by far having been accepted in obstetrics that MC twins are exclusively MZ.[10] The incidence rate of DZ twins varies significantly, influenced by race (higher in blacks, lower in Asians), heredity, maternal age (peak between 35–40 years of age), history of previous DZ twin pregnancy, nutrition habitus and anthropometric features (height and weight) of the woman.

ART including in vitro fertilization and non ART procedures such as ovulation induction and subsequent intrauterine insemination using human pituitary gonadotrophic hormones increase the incidence of multiple pregnancy, both MZ and DZ, while clomiphene citrate increases the occurrence rate of DZ pregnancies to about 5–10%.[3–5,11]

It should be emphasized the general aspect that the incidence of multiple pregnancies is correlated with increasing maternal age stands for DZ twins. It is well established that the frequency of MZ twinning remains relatively constant, independent of the age of the woman.

The risk of structural anomalies in multiple gestations

The incidence of structural anomalies in twins is higher compared to singletons. However, the frequency of malformations in DZ twins is thought to be similar to that of singletons (2–3%) contrary to that observed to MZ, which has been reported to be 2–3 times higher.[12] The exact underlying mechanism of the increased prevalence of structural defects in MZ twins remains obscure, although the teratogenic nature of the twinning process itself and vascular events occurring during intrauterine development may account for part of them. Of interest, concordance (both fetuses similarly affected) for a structural anomaly, even in MZ twins, is rare (less than 20%).[13]

Neural tube defects, anencephaly, holoprosencephaly, sirenomelia complex, cloacal exstrophy and abnormalities that fit into the expanded VATER/VACTERL associations are more common in MZ twins. However, the risk of fetal abnormalities in twins may be biased, because multiple pregnancies are intensively scanned, increasing the chances of detecting underlying anomalies. Moreover, twinning is much more common in women of advanced age, in whom prenatal screening is more likely to yield the diagnosis of fetal defects as far as aging is associated with increased risk for fetal abnormalities.

The risk of chromosomal abnormalities in multiple gestations

Inasmuch as zygocity represents the genetic make-up of the developing entity, accurate determination of this parameter is considered a prerequisite in multiple pregnancy prenatal screening for aneuploidies. In the clinical setting, zygocity is usually inferred from the ultrasound diagnosis of chorionicity,[6] the latter best achieved in the first trimester. In DA pregnancies with fused placentas, measurements of the thickness of the dividing membrane using a cut-off value of 2 mm can differentiate MC from DC twinning, though a high inter and intra-observer variability has been reported. Sonographic detection of the »lambda« or »twin peak« sign is reported as a more reliable indicator of DC placentation with an accuracy of 100% at 10–14 weeks' gestation.[14] Delayed in the second trimester sonographic evaluation is associated with a 10–12% chorionicity misinterpretation rate,[15,16] while after 20 weeks' gestation the determination may turn out impossible. Therefore, in the absence of the »lambda« or »twin peak« sign in a DA twin pregnancy, single placentation and monozygocity is concluded, when the rare cases of MC-DZ gestations following ART reported are not taken into consideration. However, when a single amniotic sac is detected, monochorionicity is indisputable. Given that the great proportion (80–90%) of DC twins are DZ,[17,18] chorionicity may roughly correspond to zygocity.[19]

MZ twins are almost always of the same sex and genetically identical and therefore the risk for chromo-

somal abnormalities does not differ from that in singletons. Very infrequently, postzygotic mitotic events (nondisjuction or anaphase lag) or prezygotic meiotic errors can cause genetic discordance between MZ siblings, involving mosaicism, skewed-X-inactivation, differential gene imprinting and small scale mutation.[20] Heterokaryotypic monozygotism is used to define the rare karyotypic discordance, most commonly expressed by one fetus affected by Turner syndrome whereas the other presents either a normal male or normal female karyotype.[21–23] MZ discordance for trisomy 21, Klinefelter syndrome, Patau syndrome, trisomy 1 and 22q11 deletion syndrome has also been described.[23–27] However, these unusual discrepancies are not taken into consideration when calculating aneuploid risk, though should always be assumed when invasive prenatal diagnosis is performed in MC-DA twins dictating sampling from both sacs.

In DZ twins, each embryo has an independent risk for aneuploidy and therefore the risk that at least one fetus being affected will be almost twice the maternal age risk for a singleton (e.g. in respect to trisomy 21, for a 40-year-old woman, 1/100+1/100=1/50). The probability of both fetuses being involved is minimal (1/100x 1/100=1/10,000).[17] In cases with uncertain chorionicity and thus zygocity, aneuploidy risk assessment requires an estimation of the most likely zygocity, which may vary according to maternal age and race. In general, given that one-third of all twin pairs are monozygotic, the risk for one twin being aneuploid in case of unknown zygocity is calculated to five-thirds that of the singleton risk.[15,19] Based on these estimations, a 33-year-old woman bearing twins has a risk for at least one aneuploid offspring, comparable to the risk of a 35-year-old woman bearing a singleton. On this assumption, such women should be offered prenatal testing.[28] However, despite these aspects, reported series show a lower risk for fetal chromosomal abnormalities in live-born twins.

Prenatal screening for fetal anomalies in multiples

Unambiguously, it has been a common practice to extrapolate data derived from singletons to multiples. However, implementation of ultrasound as well as maternal serum analyse screening for fetal abnormalities in twin or higher-order pregnancies, seems to be more complex. Cautious interpretation of screening results is considered mandatory in order to minimize possible erroneous high false positive rate and subsequent high rate of undue invasive procedures.

Given that chorionicity has been definitely determined, in DC multiple pregnancies, first-trimester ultrasound scan offer an invaluable aid in fetal risk assessment for chromosomal abnormalities. In particular, fetal nuchal translucency (NT) screening has yielded comparable results regarding detection rates as well as false positive rates with singleton pregnancies.[29]

In MC twins, a cautious evaluation of increased NT thickness should be reserved in respect to the possible early twin-to-twin transfusion syndrome (TTTS) origin of this finding. A rational approach has been proposed to be the application of the average value of NT measurement of both fetuses in risk assessment in an effort to reduce misinterpretation.[30]

The inability to determine the degree to which each fetus contributes to the overall maternal biochemistry level may reflect a significant shortcoming in first trimester biomarkers' sensitivity and specificity in multiple gestations. Nevertheless, the altered biochemical markers of the aneuploid fetoplacental unit are partly masked by the normal biochemical profile of the euploid co-twin.

Since the application of maternal biochemistry in risk assessment for aneuploides in twin or higher-order multiple gestations remains arguable, ultrasound scan has been proven of great value for early determination of chorionicity and subsequent standardized NT measurement as well as genetic sonogram targeting to illuminate possible sonographic markers of fetal aneuploidy.

Invasive procedures for prenatal diagnosis

It is uniformly accepted that invasive procedures for fetal karyotyping in twins or higher-order multiplets are more challenging than in singletons. First-trimester chorionic villus sampling (CVS) and second-trimester amniocentesis are alternative techniques requiring experienced hands to ensure sampling both fetuses and minimize procedure related risks. Fetal blood sampling for genetic studies is rarely used today.

Amniocentesis

Genetic amniocentesis performed later than 15 weeks of gestation has been proven a safe and accurate procedure for sampling all fetuses of a multiple gestation. Currently, there is little information regarding the risk of amniocentesis between 13 and 15 weeks though this invasive procedure has been associated with increased risk for fetal loss, amniotic fluid leakage and fetal talipes equinovarous and therefore is not recommended.[15,31,32]

Amniocentesis in twins can be reached through a single or double uterine entry. Three methods of tapping multiple sacs have been described so far. One of them uses the single whereas the remaining two use the double uterine entry approach. Each technique can be performed either freehand or with a needle guide.

The first one, initially described by Elias et al in 1980, involves two or more needle insertions, one for each sac, also called the ***double needling technique*** **or** ***technique of double amniocentesis***.[33] In a twin or higher-order multiple pregnancy, two or more 22 gauge 3.5 inch spinal needles are separately and sequentially inserted transabdominally under ultrasound visualization into each sac and about 20 ml of amniotic fluid is readily aspirated and sent for cytogenetic evaluation or fetal karyotyping. A problem not infrequently faced with this

technique is erroneously sampling twice the same amniotic sac. In order to eliminate this possibility, the sampled sac is marked with a blue dye ensuring that the sac is tapped only once. For this purpose indigo carmine has been successfully used without any adverse effects,[34] though a mild vasoconstrictive effect following intravenous injection has been described. However, the instillation of a foreign substance into the amniotic cavity is of concern. A technical disadvantage with the instillation of indigo carmine is that the dye tends to concentrate at the bottom of the sac taking some time before the stained fluid surrounds the fetus. Methylene blue used as a marker dye in the past has been linked to certain toxic manifestations such as fetal hemolysis, fetal small bowel atresias and fetal death.[35–40] Nevertheless, the high resolution ultrasound equipment currently available, in expertise hands, may ensure accurate sampling from each sac,[41,42] reserving the installation of dye for cases of amniotic volume discordance where detection of the septum is uncertain or high-order pregnancies where documentation and »labeling« of sacs turn out insecure.[43]

An alternative approach first described by Jeanty et al. in 1990 is the ***single uterine entry technique or single needle insertion technique***.[44] The needle entry is made into the proximal sac near the insertion of the dividing membrane and 20 ml of amniotic fluid are retrieved. After the stylet is replaced, the needle is advanced through the second sac under direct ultrasound guidance. In order to avoid contamination the first few milliliters of amniotic fluid are discarded and aspiration of 20 ml from the second sac integrates the procedure. Many advantages linked to this technique have been reported: requiring only one needle insertion and being swifter and shorter reduces woman's discomfort as well as the risk of post-procedural complications. Moreover, advancing the needle through the septum between the two sacs under ultrasound guidance provides positive proof of tapping both of them, diminishing the need for dye insertion. However, potential disadvantages render this approach less popular. Possible contamination of the second sample with amniotic fluid and fetal cells from the first one, may lead to an incorrect diagnosis of mosaicism in the second fetus. This complication can be avoided by strictly adhering to the technique, by replacing the stylet prior to intertwin membrane penetration and by discarding the first few milliliters from the second sac. Besides, the possibility of converting DA to pseudo-MA twin pregnancy with the correspondence risks for cord entanglement and the formation of the amniotic band syndrome cannot be precluded.[45] In addition, a technical difficulty in penetrating a »tenting« dividing membrane has been reported.

Two years later, in 1992, the ***double simultaneous visualization technique*** **or** ***double simultaneous amniocentesis*****,** was introduced by Bahado-Singh et al.[46] This technique involves two needles inserted separately into the amniotic sacs under ultrasound visualization like in the ***technique of double amniocentesis.*** The difference is that after aspiration of the amniotic fluid from the first sac, the needle is left in place indicating the sampled cavity and the second insertion is made into the other sac. The main advantage seems to be the documentation of correct sampling from each sac. However, it is not widely used mainly because it is more time consuming and thereby the experience with this approach is limited.

Prenatal diagnostic invasive procedures and thus amniocentesis must be preceded by a detailed ultrasound evaluation of the multiple pregnancy involving chorionicity and amnionicity determination and documentation of the location of the placenta(s). Moreover, relative position, size, anatomy and gender (if possible) consisting distinguishing features of each fetus should be specified, and »labeling« of the multiples using text and diagram should be performed to ensure correct sampling from each of them. Recently, the role of amniotic fluid alpha-fetoprotein (AFAFP) values was evaluated in confirmation of both sacs in a DC pregnancy being sampled.[47]

Concern regarding potential post-amniocentesis increase in fetal loss rate in multiples has led to a plethora of studies evaluating this parameter. Early reports suggested a higher fetal loss rate in twin pregnancies than in those with singletons.[48–50] However, these studies did not take into account the possibility that the increased fetal wastage might be attributed to the twin pregnancy itself rather than the invasive procedure. Later on, it was reported that the maternal history of twins *per se* carries a pregnancy loss rate up to 24 weeks of about 6.3% and severe prematurity (24–28 weeks) rate of about 8%.[51] Most series of single pregnancy outcome following second-trimester amniocentesis report loss rates before 20 weeks' gestation of between 1% and 2.5% and a much higher loss rate before 28 weeks. In a multicenter European study, the pregnancy loss rate was estimated to be 2.3% and 3.7% before 20 and 28 weeks' gestation respectively.[52] In a case control study, a similar fetal loss rate was reported between sampled twins and unsampled matched twin controls (3.5% vs 3.2%).[53]

In conclusion, amniocentesis in twin pregnancies is thought to be a relative safe and accurate diagnostic procedure providing that sampling involves both sacs regardless the zygocity and chorionicity.

Chorionic villus sampling

CVS, also called placental biopsy, is a safe alternative invasive procedure to amniocentesis for prenatal diagnosis in multiples.[54–56] The major advantage of CVS is early diagnosis, obtained in the first trimester of pregnancy. In particular, genetic results are feasible either within hours by direct preparations of the cytotrophoblast layer, or within 3–7 days by tissue culture of chorionic villus mesenchymal core. Early diagnosis provides earlier reassurance of fetal well being and thereby eliminates both maternal anxiety and uncertainty. On the other hand, the diagnosis of one or both abnormal twins allows subsequent selective reduction of the affected fetus or surgical termination of pregnancy rather than

medical induction of labour as early as in the first trimester where complication rates are lower. Moreover, fetal reduction performed earlier in pregnancy may be associated with a higher survival rate of the unaffected twin.[57] In terms of privacy and maternal psychology, the earlier an abnormal pregnancy is terminated the lesser the chance of being widely recognized.

CVS is best performed between 11 and 13 weeks' gestation. Data derived from singleton pregnancies illustrate an association of CVS performed earlier in pregnancy and fetal transverse limb abnormalities, micrognathia and microglosia. In general, first-trimester CVS in multiple gestations is technically more demanding than second-trimester amniocentesis. Transabdominal as well as transcervical approach have been used. Some suggest that the highest success rates are achieved when the clinician is comfortable using either technique. Transcervical CVS is performed either by an aspiration catheter or by a biopsy forceps. Technically, it may be more difficult to perform and the »learning curve« appears to involve many patients. Transabdominal approach uses an aspiration needle and is technically more similar to second-trimester amniocentesis and thus more familiar to the majority of obstetricians. Both techniques can be performed either freehand or with a needle guide.

Continuous ultrasound visualization of the tip of the needle, catheter or biopsy forceps is essential to ensure sampling both chorions. If in doubt, a follow-up procedure should be performed either by an immediate repeat CVS or by second-trimester amniocentesis. A serious drawback of CVS is potential contamination of one sample by villi belonging to the other chorion or less frequent by maternal cells. At that case, a confusing or even misleading diagnosis is unfortunately possible. Although early studies suggested a contamination rate as high as 4%, more recent studies report a much lower rate, almost nullified.[58,59] Still, Weisz and Rodeck suggest that it would be prudent to counsel patients that about 2–3% of twin pregnancies having CVS will need re-sampling because of uncertainty of results.[43]

In rare cases, the combination of transcervical and transabdominal approach along with the increasing clinician experience available today can eliminate such an unfortunate possibility. Furthermore, obtaining samples adjacent to the cord insertion site far away from the dividing membrane is reasonably recommended.

Genetic counseling must include the possibility of a discordant abnormal result necessitating cautious interpretation. Therefore, detailed documentation and »labeling« of the fetuses and the chorions is equally as important with CVS as it is with amniocentesis. Although the position of sacs will remain unchanged during the 2–3 weeks-time following sampling, it is standard practice to re-confirm the original diagnosis in both fetal and chorionic tissues before selective reduction of the affected twin.

The estimated risk of CVS-associated fetal loss in singletons varies widely (1.3–4.3%). Two or more samplings during one procedure have been linked to increased risk of post-procedural miscarriage,[60,61] implying that the risk may be higher in twin sampling. Overall an estimated risk of 2–4% in twin pregnancies has been reported. However, available data demonstrate significant variations. In one study, the risk of CVS-associated fetal loss before 28 weeks' gestation did not seem to differ between twin and singleton pregnancies (4.9 vs 4%).[54] When only chromosomal normal pregnancies are considered, the overall loss rate found in a study of 202 twin pregnancies that underwent CVS became 3.7%, a figure that is considerably less than that of amniocentesis.[54] In another study, the pregnancy loss rate before 20 weeks following CVS was found 3.3% comparable to 2.8% in a control group of twin pregnancies undergone amniocentesis. Hence, it may be claimed that in experienced centers, CVS is as safe as amniocentesis for sampling twins.

The choice of invasive technique for fetal karyotyping should depend on the procedure related risks, on accuracy of obtaining a result from both fetuses, on technical demands and on clinicians' experience. Gestational age at referral date may be crucial in decision making. Eventually, is there a clear benefit of performing CVS than amniocentesis or *vice versa* that would render one procedure by far superior than the other? The answer is absolutely no. Amniocentesis is technically easier and widely adopted, whereas CVS's results are available about one month earlier, thus therapeutic as well as selective terminations are safer. However, it should be emphasized that if the prenatal center is not skilled and experienced in CVS, amniocentesis should be preferred. A rational approach may be as follows: the choice of invasive technique should be based on individual risk calculated from the combination of maternal age and fetal NT thickness measured in the first trimester. When the risk for a chromosomal defect, in at least one of the fetuses, is greater than 1 in 50, it may be preferable to perform CVS. For pregnancies with a lower risk, amniocentesis after 15 weeks may be more appropriate.

Fetal blood-sampling

Fetal cordocentesis for prenatal genetic testing has been previously used to validate abnormal findings in amniocentesis or CVS. It has also been used in case that a rapid chromosomal diagnosis (rapid karyotyping) was pending, since the results are offered in 2–3 days-time. Nowadays, novel molecular techniques allow accurate rapid karyotype determination thereby limiting fetal blood sampling's application.

Likewise in singletons, cordocentesis in multiples is technically challenging requiring skilled operators with extensive experience in other invasive ultrasound-guided needle procedures, such as amniocentesis and CVS. Umbilical cord is usually punctured proximal to its insertion into the placenta. A needle guide or freehand technique may be used.

In a study conducted in 2003, involving 84 twin pregnancies, mainly screened for hemoglobinopathies, the

overall procedure-related fetal loss (up to 2 weeks post-procedurally) was 8.2%, about fourfold higher than the correspondence risk in singletons. However, this technique can be used as an alternative to amniocentesis after 20 weeks' gestation to confirm an abnormal karyotype in a DC pregnancy, when selective feticide is considered a few weeks after the initial procedure.[62]

Conclusions

In conclusion, the rising rate of multiple pregnancies mainly attributed to the widely use of infertility treatment modalities has increased the need for invasive genetic studies in these pregnancies. Diagnosis of fetal aneuploidies and genetic defects can be achieved either by first-trimester CVS or by second-trimester amniocentesis, whereas it is postulated that they are equally safe in experienced hands. The choice of invasive procedure in multiple pregnancies depends on several factors, but the experience of the center performing the modality should be emphasized in decision making. The indications of fetal blood sampling are currently limited and progressively surrogated by novel molecular techniques implemented in CVS or amniocentesis' specimen. High resolution ultrasound equipment available today, along with increasing operator experience gained throughout the years, results in more accurate and efficacious invasive prenatal diagnosis in twin or higher-order pregnancies, minimizing potential post-procedural fetal loss rate.

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