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# RECENT ADVANCES IN THE ASSESSMENT OF MULTIFETAL PREGNANCIES BY 3D/4D SONOGRAPHY

## NOVO U PROSUDBI VIŠEPLODNIH TRUDNOĆA 3D/4D ULTRAZVUKOM

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*Review*

*Key words:* multifetal pregnacies, 3D/4D ultrasound, chorionicity, amnionicity, fetal behavior

**SUMMARY.** Introduction of ultrasound has revolutionized the antenatal diagnosis of multiple pregnancies. Indeed, two-dimensional sonography (2D) is still the primary modality for the diagnosis and evaluation of multiple pregnancies. However, 2D sonography may be replaced by three- (3D) and four-dimensional (4D) sonography whenever appropriate. New technique should be accepted as a complementary to 2D sonography, because additional information which assists in the clinical management could sometimes be provided. 3D sonography improves counting of gestational sacs, without any risk of undercounting even for less experienced ultrasonographers. The rate of determinations of appropriate chorionicity by 3D is almost 100% in the second and in the third trimester, as it enables the achievement of »perfectly« oriented picture, which is not always the case with 2D. 3D US improves the visualization of both twins, while the use of volumetric calculations might improve the diagnosis of discordant growth. It also improves the diagnostic capability by offering more diagnostic information in the detection and the evaluation of fetal malformations of the cranium, face, spine, extremities and body surface. 3D and 3D power Doppler permit depiction of curvatures of the umbilical cord and visualization of the number of loops involved in entanglement. The main benefits of 4D sonography include: accurate recognition of an isolated motor activity of a single fetus; distinguishing between spontaneous and stimulated motor activity; and spatial visualization of the inter-twin area. By 4D US, types of movements, isolated movements of each twin or high order multiples, inter-twin contacts and interactions can be easily recognized from early gestation. Studies have shown that twins were at approximately 4-fold increased risk for cerebral palsy compared with singletons, which is important, but not yet solved issue. 4D US is undoubtedly a new powerful imaging tool whose potential in scientific and clinical area should be searched for. In the meantime true clinical value of this new modality should be neither underestimated nor overestimated.

*Pregled*

*Ključne riječi:* višeplodne trudnoće, 3D/4D ultrazvuk, korionicitet, amnionicitet, fetalno ponašanje

**SAŽETAK.** Uvođenje ultrazvuka predstavljalo je veliku pomoć u antenatalnoj dijagnozi višeplodnih trudnoća. Dvodimenzionalni (2D) ultrazvuk je doista još uvijek najvažnija metoda u dijagnostici i praćenju višeplodnih trudnoća, a on se može dopuniti trodimenzionalnim (3D) ili četverodimenzionalnim (4D) ultrazvukom kad god je to neophodno. Novije ultrazvučne tehnike valja prihvatiti kao komplementarne 2D tehnici, jer mogu doprinijeti dobivanju nekih novih spoznaja koje katkada mogu pomoći u kliničkoj procjeni. Primjenom 3D ultrazvuka poboljšava se određivanje broja gestacijskih vrećica, bez bojazni da će se njihov broj podcijeniti čak i u slučaju kad pretragu izvodi neiskusan pretraživač. Određivanje korioniciteta pomoću 3D ultrazvuka će dostići gotovo 100% točnost u drugom i trećem tromjesečju, obzirom da 3D omogućuje dobivanje odlično orijentirane slike, što se međutim ne postiže uvijek pomoću 2D ultrazvuka. 3D poboljšava prikaz obaju dvojaka, dok će primjena volumetrijskih mjerenja poboljšati dijagnozu diskordantnog rasta. Osim toga 3D poboljšava mogućnost otkrivanja prirođenih malformacija, jer se njime mogu prikazati mnoge pojedinosti na različitim dijelovima tijela, te se tako pomoću 3D ultrazvuka češće otkrivaju malformacije kranijuma, lica, kralježnice, ekstremiteta i površine tijela. 3D ultrazvuk i 3D osnaženi dopler omogućuju prikaz petlji pupkovine te broj petlji koje sudjeluju u omatanju pupkovine. Najvažnije prednosti 4D ultrazvuka uključuju: mogućnost prenatalnog otkrivanja pojedinačnih pokreta jednoga od dvojaka, razlikovanje spontane i stimulirane motoričke aktivnosti te zoran prikaz prostora između dvojaka. Osim toga pomoću 4D ultrazvuka se može odrediti vrsta pokreta, pokreti svakog ploda iz višeplodnih trudnoća pojedinačno, a međusobni kontakti i interakcije plodova se mogu vrlo jednostavno prikazati već od najranije gestacijske dobi. Istraživanjima je dokazano četverostruko povećanje rizika pojave cerebralne paralize u djece rođene iz višeplodnih trudnoća u usporedbi s onima rođenim iz jednoplodnih trudnoća, što je važno, no još uvijek neriješeno pitanje. 4D ultrazvuk je nesumnjivo nova metoda prikaza čije mogućnosti u znanstvenom i kliničkom području valja i dalje istraživati. U međuvremenu vrijednost ove nove metode ne bi trebalo ni podcijeniti niti precijeniti.

## Introduction

Introduction of ultrasound has revolutionized the antenatal diagnosis of multiple pregnancies. Thirty years ago, twins were often overlooked, while the triplets were diagnosed only after the delivery of the second infant. After the introduction of ultrasound into routine prenatal care the reliable diagnosis or exclusion of multiple pregnancies became practical reality.[1]

Indeed, two-dimensional sonography (2D) is still the primary modality for the diagnosis and evaluation of multiple pregnancies. However, 2D sonography may be replaced by three- (3D) and four-dimensional (4D) sonography whenever appropriate. New technique should be accepted as a complementary to 2D sonography, because additional information which assists in the clinical management could sometimes be provided. Modern 4D machines are capable of performing spatial imaging in near real-time providing up to 28 frames per second. 4D compared with 2D in multifetal pregnancies can be used for more accurate detection and evaluation of inter-twin contacts, because it allows simultaneous visualization of both fetuses and assessment of their motor activity. The main benefits of 4D sonography include: accurate recognition of an isolated motor activity of a single fetus; distinguishing between spontaneous and stimulated motor activity; and spatial visualization of the inter-twin area.[2] Numerous improvements in data processing capacity during the past years enabled to overcome the previous limitations of 3D/4D ultrasound. In other words, fetal position is not an obstacle to obtain desired section. At present, the only limitation for visualization of desired structure is the amount of adjacent amniotic fluid.[3]

The advantages of 3D imaging in management of multiple pregnancies are described in *Table 1*.[3,4]

*Table 1.* The advantages of 3D imaging[3,4,8]
*Tablica 1.* Prednosti 3D ultrazvuka

| | |
|---|---|
| First trimester<br>Prvo<br>tromjesečje | Less possibility to overlook existing gestational sac (so called »undercounting phenomenon)<br>Manja mogućnost previđanja postojeće gestacijske vrećice (tzv. »undercounting« fenomen) |
| | Improved prediction of spontaneous abortion<br>Poboljšano predviđanje spontanog pobačaja |
| | Improved prenatal classification of uterine anomaly<br>Poboljšana mogućnost klasifikacije malformacija maternice |
| Second trimester<br>Drugo<br>tromjesečje | Improved diagnosis of vanishing phenomenon<br>Poboljšana mogućnost prikaza nestajućeg ploda |
| | Early detection of fetal anomalies<br>Rana dijagnostika fetalnih malformacija |
| | Improved evaluation of fetal malformation<br>Lakša mogućnost evaluacije malformacije |
| | Improved determination of placentation<br>Poboljšana mogućnost određivanja placentacije |

## Diagnosis of multiple pregnancies

The diagnosis of multiple pregnancies (triplets, quadruplets, quintuplets etc.) by 2D sonography usually requires a long examination period and an experienced ultrasonographer. If one uses transvaginal sonography (TVS), multifetal pregnancies can be diagnosed as early as at 5th to 6th gestational week (GW) *(Figure 1)*. An early diagnosis can be associated with numerous pitfalls. One embryo arises usually from a single gestational sac. However, two embryos arise from a single gestational sac in monozygotic twins. The number of gestational sacs always correlates to the numbers of placentas, and very often, but not always, to the number of embryos. Therefore, reliable prediction of the number of embryos on the basis of the number of gestational sacs is not accurate. Even a finding of a gestational sac with a single yolk sac does not exclude the existence of monoamniotic (MA) twins. Only one (large or irregular) yolk sac can be seen in MA twin pregnancies.[4] Therefore, until the 7th week of gestation when the embryo is recognizable sonographically, reliable counting of the embryos is not possible. Only number of embryos is important for final diagnosis of number of multiples *(Figure 2)*.

Three gestational sacs have to be recognized in the case of trichorionic triamniotic triplets *(Figure 3)*. However, even when two gestational sacs are seen, the diagnosis of triplets is not yet excluded, and the possibility of dichorionic triamniotic triplets (complex chorionicity) should be taken in consideration *(Figure 4)*. Counting of the number of embryos is essential for diagnosis of triplets. Determination of cardiac activity is another important prognostic factor. The chance of delivering twins when two chorionic sacs are seen is 57%, but when two cardiac activities (two viable embryos) are present, the chance increases to 87%. Similarly the chance of delivering triplets after visualization of three gestational sacs is 20%, but with three viable embryos the chance increases to 68%.[5] Viability of each embryo can be confirmed using color Doppler imaging of the corresponding fetal circulation.

## Determination of the number of gestational sacs

Ultrasound in early pregnancy confirms that the number of conceived embryos in multiple pregnancies exceeds the multiple birth rate. Spontaneous embryonic/fetal loss in the first trimester of multiple pregnancies is known as the »vanishing twin« phenomenon[6] *(Figure 5.)*. Pregnancy number before the 6th week is determined by counting the number of gestational sacs. Late appearance of twins is recognized on the basis of discrepancy between two sonograms: initial sonogram, usually obtained at 5.0 to 5.7 GW, and a subsequent sonogram at more than 6 GW demonstrates more embryos or fetuses than initially counted number of gestational sacs. According to Manzur et al.[7], the outcome of successful pregnancy after finding 3 chorionic sacs was as follows: for triplets 47.4%, for twins 31.6%, for singletons 18.4%, while miscarriage rate was 2.6%. There are two types of undercounting: polyzygotic and monozygotic. Recent meta-analysis showed that more than 50% of pregnancies with 3 or more gestational sacs have spontaneous reduction before 12th GW.[8] The surviving fetuses weigh less and are born earlier than unreduced pregnancies with the same initial number of fetuses.[8] Polyzygotic undercounting is a result of the limitations of 2D TVS. The anatomy of the female reproductive tract as well as contemporary design of ultrasonic probes limits the number of examination planes to the sagittal and transversal. Because the uterus can be examined only in these two planes, the failure to visualize the total number of gestational sacs on a single screen is quite probable. Stated in other way, one or more gestational sacs may be overlooked. This problem is enhanced in high-order multiple pregnancies in which is impossible to visualize all gestational sacs on a single screen. Therefore, the risk of undercounting is correlated to the number of embryos. Undercounting of the number of gestational sacs is the most common pitfall during the first trimester. This pitfall can be avoided either by conventional or by 3D US.[3,4,8]

Whenever spontaneous reduction is suspected in high-order multiple pregnancies on conventional sonographic exam, the additional use of surface rendering mode is recommended. If one uses surface rendering mode, distinguishing between the spontaneous reduction and normal pregnancy in high-order pregnancy can be easily done. Using 3D US the frontal (coronal) plane enables examination of the uterine cavity in sections which are unobtainable with conventional 2D sonography. Furthermore, 3D sonography enables the appropriate counting of gestational sacs without any risk of undercounting even for less experienced ultrasonographers *(Figure 6)*. Therefore, inter observer variability in detecting the number of gestational sacs is significantly lower. Even quadruplet is recognizable without any difficulties *(Figure 7)*. This advantage strongly suggests that 3D sonography should become the new standard in the early diagnosis of high order multiple pregnancies.[9] Before introduction of 3D ultrasound, 11% of dichorionic twins were initially undercounted as singletons, and 16% of high order multiple gestations were also undercounted.[9]

## Determination of chorionicity and amnionicity

During the first and the second trimester, chorionicity can be determined directly and indirectly *(Figure 8)*. Direct determination of chorionicity is based on the counting of placentas, which is easy to perform when the placentas are separated. However, in multichorionic pregnancies, fusion of different placentas occurs during the second trimester. Distinguishing between single and fused placenta is accomplished by considering of the so-called »twin peak« or lambda sign *(Figure 9)*, which is triangular projection of placental tissue beyond the chorionic surface, extending between two chorionic layers of the inter-twin membrane.[10] It provides reliable evidence that there are two fused placentas (dichorionic, diamniotic) rather than a single shared placenta (monochorionic, diamniotic).[10] However, membranes can be evaluated, counted and measured by 2D US only when they are positioned at 90° to each other. In other words, the orientation of the membranes studied by 2D US should be positioned parallel with the transducer crystal array. 3D US enables to achieve a »perfectly« oriented picture, which is not always the case with 2D. Therefore the rate of appropriate chorionicity determinations by 3D should be ideal (100%) in the second and in the third trimesters.

The junction of three interfetal membranes represents »Y« zone *(Figures 3, 10)*. However, in advanced pregnancy when placentas can not be seen, then examiner should examine junction between the membranes. The absence of the »Y« zone does not exclude trichorionic triplets. In the second trimester, the »Y« zone can be absent in cases in which the inter-fetal membranes do not intersect. Therefore, »Y« zone assessment should be combined with other parameters for determination of chorionicity.[11]

In the case of dichorionic-triamniotic triplets (complex placentation), the placenta is represented by the T-sign and lambda sign with one thin and one thick membrane *(Figure 4)*. Using conventional sonography it is difficult to distinguish single and fused placentas of the twins, which is even more difficult in triplet pregnancy. Such a complex placentation can be more easily examined by 3D US, regardless of gestational age.

## Ultrasound assessment of discordant growth in multiples

Twins discordance which is usually defined as a >20% to 30% difference in birth weight, has been associated with adverse perinatal outcomes.[12] An increasing body of evidence suggests that discordant growth patterns may begin in the first trimester and can be detected sonographically.[13–16] First trimester CRL disparity in dichorionic twin gestations is associated with an increased risk of fetal structural and chromosomal abnormalities.[12] 3D US improves the visualization of both twins, while the use of volumetric calculations might improve the diagnosis of discordant growth *(Figure 11)*. Discordant growth of multiples of > 3 mm or > 5 days during the first trimester is associated with a high loss rate and/or major abnormalities.[17] During the 8 year period, van Gaever et al.[17] observed 6 twin and 2 triplet pregnancies between 8 and 14 GW with discordant growth of over 15%. In two DC/DA (dichorionic/diamniotic) twin pairs one twin had a chromosomal abnormality, in one MC/DA (monochorionic/diamniotic) twin pair increased NT (nuchal translucency) and reduced motility pattern was demonstrated – both twins died before 18 weeks in spite of a normal karyotype. In two DC/DA twins with normal karyotype, normal NT and normal behavioral patterns, growth discrepancy persisted. The outcome of one pair was normal, while in the other pair the smaller fetus presented with VATER association.[17] However, in cases of the first trimester twins with discordant growth, the incidence of congenital malformations does not appear to be increased if the first-trimester scan is normal. Nevertheless, the rates of

IUGR and growth discordance in late pregnancy are increased for this group of patients.[18]

## Detection of fetal malformations in multiples

In a singleton pregnancy, the empiric risk for major fetal malformations is approximately 3%. In trichorionic triplets the empiric risk for major fetal malformations for each fetus is not dependent on the others, so the probability of having at least one malformed fetus is approximately 9%.[19] According to the Eurofetus study, sensitivity of the routine 2D sonography for detecting malformation is 61.4%.[20] The sensitivity of the routine 2D US anomaly scan is lower in multiple pregnancies. 3D US is useful to visualize embryos and early fetuses and to recognize their surface morphology.[21] It also improves the diagnostic capability by offering more diagnostic information in evaluating fetal malformations than fetal growth, particularly in detection of fetal malformations of the cranium, face, spine, extremities and body surface.[22] The ideal visualization rate of desired anatomical structure is the most important improvement of 3D US. This advantage can be used when the finding of 2D US is incomplete in terms of either fetal anatomy or placentation, or due to inappropriate anatomical relations.

There are varieties of anomalies involving multiples. Malformations unique to twinning (MUT) is the term applied to the malformations which occur exclusively in multiple pregnancies, whereas malfomations not unique to twins (MNUT) is the term applied to the malformations which also occur in singletons but are more common in twins *(Table 2)*.[22]

Of the MUT malformations, 3-D sonography is useful for confirmation of MUT like conjoined twins and fetus-in-fetu *(Figure 12)*. The incidence of conjoined twins is somewhere between 1:100 000 births or 1:500–600 twin pregnancies and varies between countries.[23] Maggio et al. [23] reported the first-trimester ultrasonic diagnosis of conjoined twins.[23] Since then several cases of conjoined twins were published using proposed diagnostic criteria.[24–26] Despite the fact that great progress has been made concerning the earlier diagnosis of conjoined twins in the embryonic and early fetal period, delineation of organ sharing could not be done properly before the second trimester.[23] Moreover, examiner should be aware that following criteria proposed by Maggio are sometimes problematic, because two cases of false positive diagnosis of conjoined twins were reported.[27,28] In the case of absence of clear-cut signs of fusion, additional sonographic findings summarized by Koontz et al.[29] include:

1. The lack of a separating membrane,
2. Inability to separate the fetal bodies,
3. Detection of other anomalies in a twin gestation,
4. More than three vessels present in the umbilical cord,
5. Both fetal heads persistently at the same level,
6. Backward flexion of the cervical and upper thoracic spine,
7. No change in the relative position of the fetuses despite attempts of manipulations

It is known that 2D transvaginal sonography limits the number of examination planes to sagittal and transverse. Because the uterus can only be examined in these two planes, there is a possibility of the failure to visualize the coronal section through the fetus and overlook the conjoined twins. This problem can be solved using both modalities of 3D sonography, multiplanar and surface rendering view. Using the multiplanar imaging the visualization rate of coronal section through fetus is about 100%, due to the unlimited number of sections which can be generated by »off-line« data manipulation.

Maymon et al.[30] reported that in a case of conjoined twins at 10$^{th}$ GW, the exact area could be successfully identified by transvaginal 3D US.[30] From Zagreb team Vecek et al.[31] diagnosed this anomaly at 12 GW in a fetus of 27 mm in maximum length, showing two separated heads with twins joined at the level of the thorax *(Figure 12)*. The fetal orientation remained unchanged despite manipulation with transvaginal probe and prolonged scanning by multiple sonographers. An early diagnosis of conjoined twins requires detailed examination of the fetus in the sagittal, transverse, and coronal sections. If this recommendation is not followed, the diagnosis of conjoined twins could be missed, because the appearance of conjoined twins in the sagittal section is almost normal.

Although 2D US is the primary modality for diagnosis and evaluation of conjoined twins, color Doppler and 3D US can sometimes provide additional information which can be helpful in the clinical diagnosis of these twins. 3D US also provides images which are easier for parents to understand and can help them in decision making.[32] Sepulveda et al.[33] reported two cases of con-

*Table 2.* Malformations in multiples[22]
*Tablica 2.* Mane razvoja u blizanaca

| Unique to twinning (MUT)<br>Jedino u blizanaca | Not unique to twinning (MNUT)<br>Ne isključivo u blizanaca |
|---|---|
| Conjoined twins<br>Srasli blizanci | Neural tube defects (NTD)<br>Defekti neuralne cijevi<br>Hydrocephalus – Hidrocefalus |
| Twin reversed arterial perfusion (ACARDIA)<br>Obratni arterijski tok u blizanaca | Congenital heart disease<br>Prirođene srčane greške |
| Fetus in fetu | Anorectal atresias<br>Anorektalna atrezija<br>Intersex – Poremećaji spola |
| Twin to twin transfusion syndrome<br>Blizanački transfuzijski sindrom | Genitourinary anomalies<br>Anomalije spolno bubrežnog sustava<br>Esophageal atresia<br>Atrezija jednjaka |

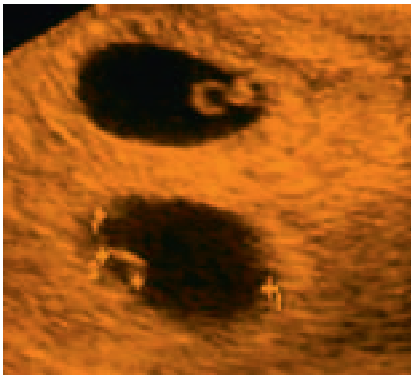

*Figure 1.* The first visible structure of dizygotic and diamniotic twins. Note the number of gestational sacs and the yolk sac.

*Slika 1.* Rani prikaz dizigotnih diamnijskih blizanaca. Prikazan je broj gestacijskih vrećica i žumanjčana vreća.

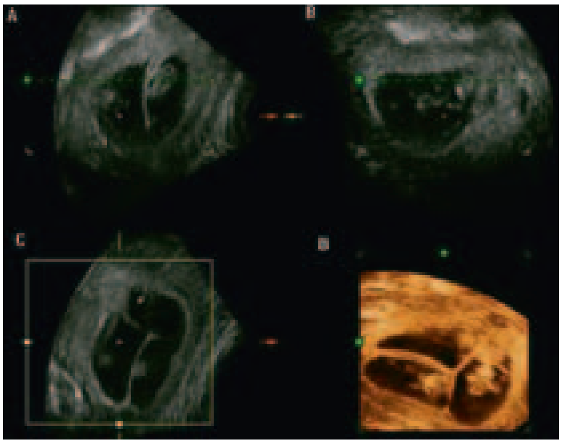

*Figure 2.* 3D multiplanar view in determination of accurate number of gestational sacs of triplet pregnancy at 12th week.

*Slika 2.* Trodimenzionalni (3D) multiplanarni prikaz pravilnog broja gestacijskih vrećica u troplodnoj trudnoći u 12. tjednu trudnoće.

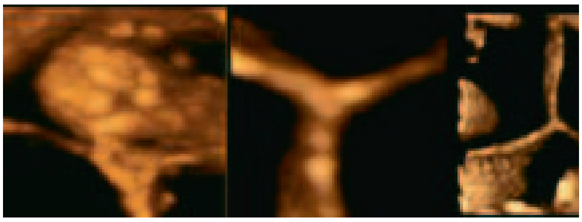

*Figure 3.* 3D ultrasound determination of the chorionicity in the late second trimester. »Mercedes« or Y sign represents the junction of fetal membranes in triplet pregnancy.

*Slika 3.* Određivanje korioniciteta pomoću 3D ultrazvuka potkraj drugog tromjesečja. Veza između plodovih ovoja u troplodnoj trudnoći prikazuje se kao znak »Mercedesa« ili Y znak.

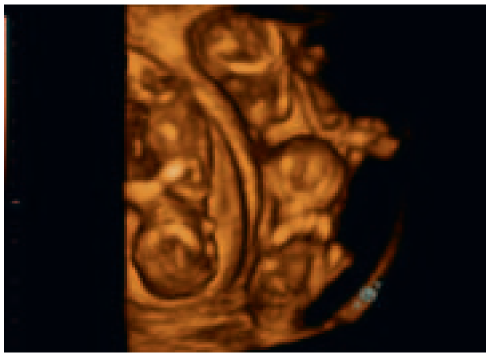

*Figure 4.* 3D US of a bichorionic triamniotic triplet pregnancy at 11 weeks of gestation (demonstrates the advantages of 3D US in the evaluation of multiple pregnancies).

*Slika 4.* Prikaz bikorialnih triamnijalnih trojaka s 11 tjedana gestacije 3D ultrazvukom (3D ultrazvuk olakšava prikaz broja plodova u višeplodnim trudnoćama).

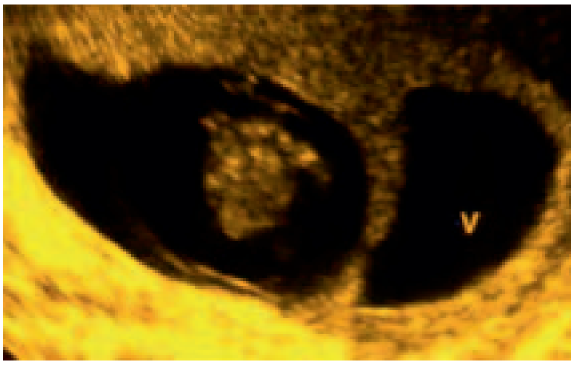


*Figure 5.* Transvaginal scan of dichorionic twin complicated by a vanished twin (right image) at 7 weeks of gestataion.

*Slika 5.* Transvaginalni prikaz bikorijalnih blizanaca sa 7 tjedana trudnoće, od kojih je jedan nestajući blizanac (slika desno).

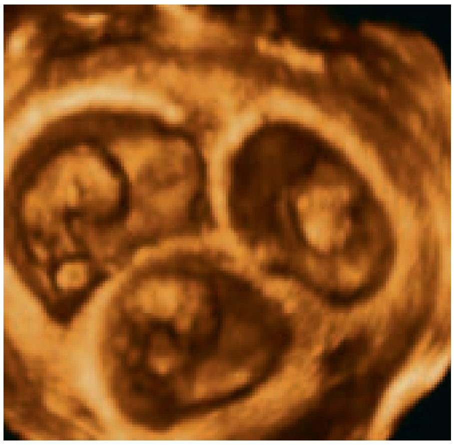

*Figure 6.* 3D ultrasound of triplets at 8th week of gestation: 3 embryos in three gestational sacs (TCTA – trichorionic triamniotic triplets).

*Slika 6.* Prikaz trojaka u 8. gestacijskom tjednu 3D ultrazvukom. Vide se tri zametka u tri gestacijske vrećice (TC/TA trikorijalni triamnijalni trojci).

joined twins complicating a triplet pregnancy diagnosed by 2D US in the first trimester and evaluated further by 3D US. A review of the literature over the last 30 years revealed 11 other cases diagnosed prenatally by ultrasound.[33] On the other hand, literature suggests that 3D US does not improve the diagnosis made by 2D ultrasound, and that very early prenatal diagnosis and first-trimester 3D imaging provide very little additional information compared to the 11–14 GW ultrasound examinations.[34]

Development of a fetus-like mass inside a more mature fetus characterizes the fetus in fetu (FIF) malforma-

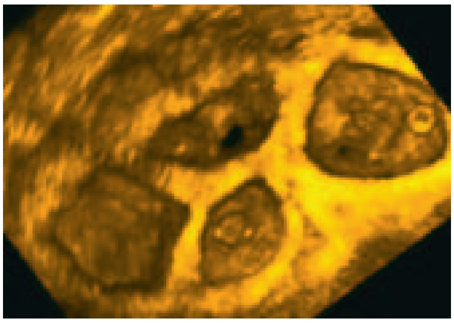

*Figure 7.* 3D scan of an accurate number of gestational sacs. Final diagnosis of quadruplets with 3D surface rendering mode. In contrast to 2D manual slicing, an analysis of 3D volugrams reveals the accurate number of gestational sacs.

*Slika 7.* 3D prikaz broja gestacijskih vrećica. Konačna dijagnoza četvoraka postavljena je površinskim 3D prikazom. U usporedbi s dvodimenzionalnim (2D) manualnim prikazom presjeka, analiza volumena dobivenih 3D ultrazvukom omogućuje određivanje pravilnog broja gestacijskih vrećica.

*Figure 8.* 3D US of a bichorionic twin pregnancy at 9 weeks 3 days gestation. Full length of two umbilical cords with their insertions and the inter-twin membrane are clearly depicted.

*Slika 8.* Prikaz bikorijalnih dvojaka u gestacijskoj dobi od 9 tjedana i 3 dana 3D ultrazvukom. Jasno se prikazuju pupkovine obaju plodova punom duljinom s mjestima njihove insercije kao i membrana između dvojaka.

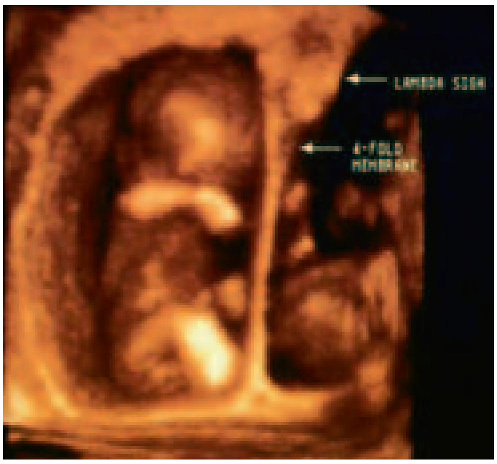


*Figure 9.* Spatial reconstruction of membrane take-off site provides easier differentiation between dichorionic and monochorionic placentation. Furthermore, membrane thickness can be simultaneously evaluated. Therefore 3D US is a better tool for understanding of placentation.

*Slika 9.* Prostorna rekonstrukcija plodovih ovoja omogućuje lakše razlikovanje bikorijalne i monokorijalne placentacije. Osim toga moguće je istovremeno izmjeriti debljinu plodovih ovoja. 3D omogućuje lakše određivanje placentacije.

*Figure 10.* 3D image of triplet pregnancy and external frontal anatomy. »Y« zone is clearly depicted.

*Slika 10.* 3D prikaz troplodne trudnoće u frontalnom prikazu. Vidljiv je »Y« znak.

*Figure 11.* A case of discordant twins at 10 weeks of gestation.

*Slika 11.* Prikaz diskordantnog rasta u dvojaka s 10 tjedana gestacije.

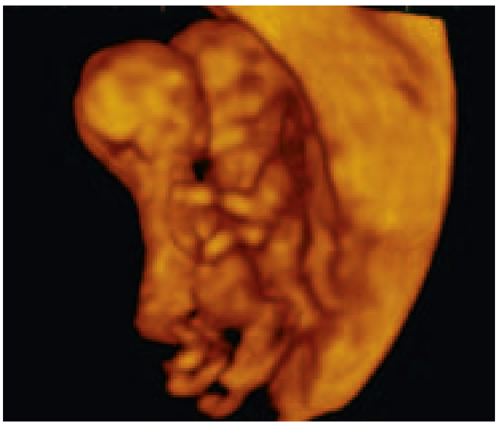

*Figure 12.* 3D US of a twin pregnancy clearly showing a case of thoracopagus at 11 weeks of gestation.

*Slika 12.* 3D prikaz torakopagusa s 11 tjedana gestacije.

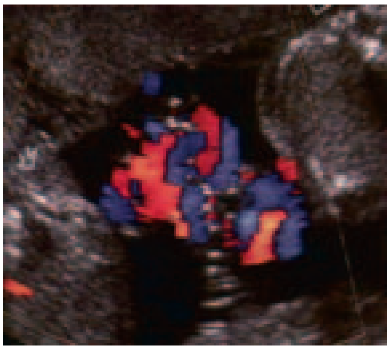

*Figure 13.* Color Doppler imaging improved visualization of cord entanglement in monochorionic-monoamniotic pregnancy.
*Slika 13.* Dopler u boji olakšava prikaz zapletene pupkovine u monokorijalnih monoamnijalnih blizanaca.

*Figure 14.* 3D power Doppler reconstruction of umbilical cord with possibility to differentiate between false and true knots in multiamniotic pregnancies or umbilical cord entanglement and adjacent cords in monoamniotic pregnancies.
*Slika 14.* Prikaz pupkovine 3D osnaženim doplerom s mogućnošću razlikovanja pravih i lažnih čvorova pupkovine u višeplodnim trudnoćama ili zaplitanja pupkovine, te postojanje dodatnih pupkovina u monoamnijalnim trudnoćama.

*Figure 15.* 3D US of a twin pregnancy at 21 weeks gestation showing head to head contact.
*Slika 15.* 3D prikaz dodira glavom blizanaca u 21. tjednu trudnoće.

*Figure 16.* The left twin is touching the nuchal region of the right co-twin.
*Slika 16.* Lijevi dvojak dodiruje nuhalnu regiju desnoga dvojka.

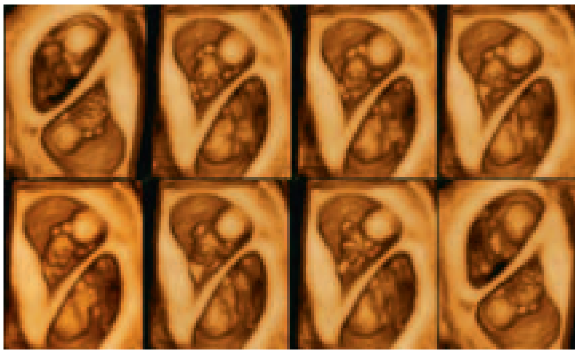

*Figure 17.* 4D US sequence of a BA/BC twin pregnancy at 10 weeks gestation, both twins are active, but there is no inter-twin contact.
*Slika 17.* Prikaz niza slika jedne za drugom (4D) biamnijalnih bikorijalnih (BA/BC) dvojaka s 10 tjedana gestacije. Vidljiva je živa aktivnost obaju dvojaka bez međusobnih kontakata.

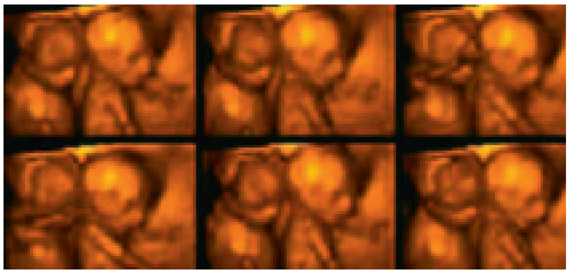

Figure 18. 4D sequence demonstrated intertwin contact. Note active hand movement of the first twin (left image).
Slika 18. Prikaz niza slika jedne za drugom (4D) međusobnih kontakata dvojaka. Zamjetni su pokreti šakom prvoga dvojka (slika lijevo).

tion.[35–37] The true incidence of FIF is unknown, although it has been estimated to be 2 per million births.[36] Jones at al.[38] in 2001 reported 3D sonographic imaging of a highly developed fetus in fetu with spontaneous movement of extremities. In contrast to easy diagnosis by 3D imaging, a FIF with even an extraordinarily high degree of differentiation is very difficult to distinguish by 2D. Nevertheless an increasing number of reports use 2D sonographic examination, which follow minimal diagnostic criteria proposed by Willis.[39] They include the presence of an axial skeleton or fetus with metameric organization, skin coverage, encapsulation and two-vessel cord.[39] Since 3D sonography is capable of generating a 3D surface view of the structure encapsulated within a fluid filled sac, it should be used for this purpose. With

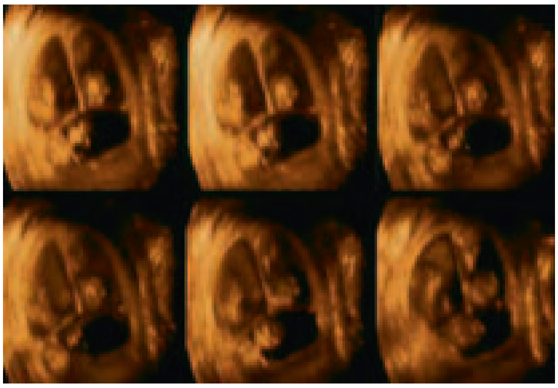

*Figure 19.* 4D sequence illustrated embryonic movements in triplet pregnancy.
*Slika 19.* Prikaz niza slika jedne za drugom (4D) koja ilustrira pokrete zametaka u troplodnoj trudnoći.

*Figure 20.* Missed twin at 13th week of gestation by 4D sonography. On the sequence of images the activity of only one twin can be recognized.
*Slika 20.* Prikaz slika jedne za drugom (4D) odumrlog dvojka u 13. tjednu trudnoće. Vidljiva je aktivnost samo jednoga dvojka.

this technique a highly fetiform shape and axialization could be recognized easily even by less experienced sonographers who could not visualize FIF on the basis of 2D sonography. Distinguishing between FIF and teratoma can be sometimes intriguing. Therefore, whenever a cystic mass is recognized at specific locations within a fetus, an additional 3D exam is recommended. The prenatal sonographic differentiation between FIF and teratoma is related to degree of differentiation of the anomaly. In high degree differentiated FIF, the visualization of fetiform mass is essential. However, in a FIF with a low degree of differentiation the diagnostic criteria are based on the presence of a rudimentary spinal architecture, which confirms the embryonic development beyond the primitive streak stage. This is in the contrast to the classic embryonic concept which postulated, that teratomas do not develop beyond the primitive streak stage of 12 to 15 days.[39]

Among MNUT, 3D sonography can be advantageous for confirmation of suspicion of neural tube defects (NTD). Twins may be concordant and discordant for congenital anomalies. Concordance for congenital malformations is defined as the presence of concordant anomaly in both twins, whereas discordance for congenital malformations is characterized by the presence of an anomaly in only one twin. Monozygotic twins are at higher risk for such anomalies than dizygotic twins.[39] Under these circumstances, discordance for major malformation in a twin pair does not imply dizygotic or dichorionic status.[40]

2D US is the primary modality for detection of congenital anomalies. According to Eurofetus Study, as mentioned before, the sensitivity of routine two-dimensional sonography for detection of malformation in singleton pregnancies was 61.4%, while the sensitivity in multiple pregnancies was between 39% and 87%.[20,41,42] Neural tube defects include: anencephaly, acrania, encephalocoele, and various forms of spina bifida. Screening for these anomalies is recommended during the first trimester because reports document the successful diagnosis in the embryonic period.[43–45] Detection rate of these anomalies in singleton pregnancies is high, except for spina bifida. The morphological anomalies of acrania and anencephaly can be confirmed even before 10 GW. Bonilla-Musoles et al.[45] reported two-dimensional diagnosis of two cases of anencephaly at 10 weeks of gestation. Takeuchi diagnosed acrania by conventional 2D US at 8 weeks and 5 days of amenorrhea in a fetus up to 18 mm of length, showing an irregular shape of the head with neither cranium nor brain vesicle development.[46] 3D surface rendered image facilitated confirmation of this malformation, because it showed characteristics of the anomaly more clearly. It is generally believed that absence of the cranial vault leads to the disintegration of the exposed brain during fetal period, resulting in clinical anencephaly. Bronshtein and Ornoy[43] reported a case with description of the progression from acrania to anencephaly. Spina bifida is associated with anencephaly between 9 to 30% of cases.[47] Bonilla-Musoles[48] reported diagnosis of spina bifida in anencephalic fetus at 9 GW by 3D US. Therefore, whenever anencephaly is found in the embryonic period additional 3D scan of fetal spine is recommended. The multiplanar view is particularly useful in localizing spinal defects accurately in fetuses with spina bifida and in determination of the exact location of extracranial mass and amount of extracranial tissue in fetuses with encephalocele.[44]

## Cord entanglement

The diagnosis of cord entanglement with 2D real-time sonography *(Figure 13)* usually requires long examination period. Due to limitations of sectional imaging, examination is informative only concerning the quality and number of loops and final diagnosis is postponed. The main problem is distinguishing between adjacent and entangled cord. Cords positioned close to each other without torsion around one over the other is defined as adjacent umbilical cords, whereas, cords twisted one around the other is called cord entanglement. Much more information about umbilical cord can be obtained by 3D sonography *(Figure 14)*. 3D power Doppler permits imaging of curvatures of the umbilical cord and the number of involved loops in entanglement can be easily determined. Counting the number of the loops involved in entanglement is useful method for longitudinal evaluation of entanglement. It is currently possible to distinguish prenatally two types of umbilical cord knots by ultrasound: true and false. A focal redundancy of the vessels, which sonographically appear as a vascular protuberance that does not persist in all scanning planes is called false umbilical cord knot.[49] This condition should be differentiated from the true umbilical cord knots, which is a life threatening condition.

## Assessment of fetal behavior in multiple pregnancies by 4D sonography

It seems that application of 3D/4D sonography in the assessment of fetal behavior is most promising. Fetal activity such as kicking has been used as a sign of fetal viability from ancient times. It was found that fetal motor activity is present for some period before the mother feels it. Reinold[5] was one of the first to describe fetal activity using ultrasound, and he stressed the spontaneous character of early prenatal movements. Hooker et al.[50] found out that the fetus responds to a tactile stimulus, which created a hypothesis that the motor activity of a twin could be either spontaneous or induced by the co-twin.[51,52] Endogenous activity is the dominant behavioral pattern in singleton pregnancy.[53] Sometimes, the mother's movements such as walking or running can initiate fetal motor activity. In multiples two types of fetal activities are present: endogenous–spontaneous motor activity and motor activity as a reaction to an exogenous stimulus. Spontaneous motoric activity precedes stimulated motoric activity. Activity evoked by inter-twin contacts is characterized as stimulated activity *(Figure 15)*.

The effect of prenatal reactions evoked by internal stimulus in twinning phenomenon due to inter-twin contacts on the neurologic maturation was in the focus of interest of the systematic research initiated by Arabin et al.[54] They used real-time 2D sonography for detection and evaluation of the inter-twin contacts. Due to sectional imaging, simultaneous visualization of both fetuses and assessment of their motor activity was impossible. Therefore, motor activity of a single fetus was possible to assess and unfortunately only to a limited extent. Similarly the inter-twin area is visualized tomografically, and some of inter-twin contacts are overlooked. Therefore, using this method distinguishing between spontaneous and stimulated motor activity is very difficult and sometimes impossible. From our team Vecek et al.[31] found that movement activity of each fetus in twin or multiple pregnancy can be successfully determined by 4D sonography in the first and in the early second trimester. For the first time it was possible to observe that one twin is active while co-twin or co-triplets are active or not. Simultaneous visualization of the entire anatomy (head, body and extremities) of two or more fetuses along with their movements allows the characterization of the type of movements, isolated movements of each fetus, as well as inter-twin contacts and interactions *(Figure 16)*. By 4D US types of movements, isolated movement of each twin or high order multiples, inter-twin contacts and interactions can be easily recognized from early gestation *(Figures 17–19)*.

Arabin[55] was the first who described inter-twin contacts and determined the gestational age of their onset. She differentiated the following types of inter-twin contacts: first reach and touch (first evidence of touch between twins), first reaction (first movement towards the touch of the co-twin), slow and fast arm, leg, head or body contacts (action and reaction based on the initiating part of the body), mouth contacts and complex interactions.[55] Apart from the descriptive definitions, they also classified the speed of actions and reactions as slow initiations followed by slow or fast reactions, and fast initiations followed by slow or fast reactions.[55] Inter-twin contacts may be explained by the onset of movements whereby incidental touches in utero cannot be avoided and can be defined as early reflexes. Furthermore the initiating and reacting body parts may be randomly involved. However, Piontelli et al.[51] stated that the existence of intrapair stimulation would indicate merely the functioning of fetal tactile and propriceptive sensibility. They stressed out that the existence of intrapair stimulation should not be taken to mean, as suggested by Arabin et al.[55] that fetuses are having complex intra-pair intercourses, but simply that intrapair stimulation exists constantly from the late first trimester and is an active part of its intra-uterine environment. Sadovsky et al.[52] concluded: »The possibility that movements of one twin stimulate the other to move«.

Ferrari et al.[53] came to different conclusion. According to their observations inter-twin contacts have been supposed to cause increased rates of simultaneous twin activities in early pregnancy. The distribution of the periods with or without fetal movements demonstrates high percentage (around 80%) of active periods in twins compared with singletons, which might be the result of inter-twin stimulations.[54] The observation period was 26 to 36 weeks, and a 3-minutes window was used. In contrast, Sadovsky et al.[52] found in twin pregnancies between 33 and 39 weeks that registred fetal movements were independent from the movements of the co-twin. Pitonelli et al.[56] found that each twin, regardless of zygosity, showed individualized behavioral styles. One twin was found to be »dominant« in the sense of more active, bit less reactive, possibly due to the fewer stimuli being generated by its co-twin. Monozygotic twins, as opposed to dizygotic, showed greater similarities in the activity and reactivity levels, but were never behaviorally identical. The behavioral diversity of monozygotic twins is increasing with increasing age. Their data suggest that so-called identical twins are not behaviorally identical from early pregnancy.[55]

It has been demonstrated that the membrane status has a direct impact on the onset and quantity of inter-twin contacts: in MC/MA twins contacts occur earlier than in MC/DA and BC as well as in DC twins, which can be caused by smaller distance between the embryos.[52] Intrapair stimulation before the 11$^{th}$ week of gestation can be considered rather exceptional event and was noted only in monochorionic twins. From 12 weeks onward intra-pair stimulation becomes progressively more frequent both in monochorionic and in dichorionic pregnancies. However these results are from 2D US study, and thus movements occurring outside the plane of scan could have been missed. This is particularly relevant after 13 weeks of gestation, when simultaneous visualization of both fetal bodies is not possible any more.[51] 4D US is useful in evaluation of altered motor development in pathologic and high risk pregancy. The delay in activity pattern is described in twins with tripoidy XXX, and some activities such as yawning and stretching are even not present, which is particularly important in dyzigotic twins.[58]

## Multiple gestations and cerebral palsy

Cerebral palsy (CP) is the most common disability in childhood affecting about 2 per 1000 live-born children. Higher risk of CP in multiple births has been known for many years.[60,61,62] The prevalence of CP is significantly higher in twins compared to singletons, although the type of CP differs as well as the etiology of CP in twins and singletons.[57] Multiple compared with singleton gestations have five- to ten-fold increased risk of CP.[58] Data clearly indicate that the higher the number of fetuses, the higher the prevalence of CP. Moreover the prevalence of CP is increasing exponentially with the number of fetuses. When comparing twin with singleton births, the relative risk of CP was significant only for twins delivered at ≥37 GW. Brain damage of the survivor following single fetal demise is almost exclusively seen in monochorionic twins in which inter-twin transplacentar vascular connections are always found, and on these grounds the »embolic« and the »ischemic« theory were found.[59]

Preterm delivery, intrauterine growth restriction, congenital defects and intrapartal complications occur more frequently in multiple pregnancies. Although CP can be related to all these events, the main reason seems to be associated with preterm birth or the antenatal death of a co-twin or co-triplet.[60–62] *(Figure 5).* The death of one twin can affect the neurological development of the survivor *(Figure 20).* The live-born co-twin of in utero dying fetus has overall risk of 20% for cerebral impairment. The gestational-age-specific prevalence of CP after fetal death of the co-twin is much higher than the reported for the general twin population. Monozygotic twins who have poorer survival rates compared to dizygotic twins are at particular risk. The prevalence of CP in monozygotic twins is 106 per 1000 survivors, while for dizygotic twins it is 29 per 1000 survivors.[59] Other studies showed that twins were at approximately 4-fold increased risk for CP compared with singletons. However, at birth weight of <2500g, twins did better than singletons.[58] Approximately 70% of pregnancies with two sacs/embryos continue as twin gestations, while in contrast only about 50% of pregnancies with three sacs/embryos will continue as triplets, and only 38% of pregnancies with four sacs/embryos will continue as quadruplets.[63] Vanishing rates are more than twice higher in monochorionic compared with dichorionic twins (50% vs. 21%).[64] The increasing use of ultrasound has drawn attention to the vanishing twin phenomenon. The observation that twins of normal birth weight are at increased risk for CP compared with singletons, led to the hypothesis that cerebral palsy of unknown etiology in singleton pregnancies may be attributed to the early loss of previously unrecognized monochorionic twin.[65] The neurological development of the survivor is impaired in pregnancy, with development of spastic CP postnatally.[65] Current data are not supportive for this concept, stating that the vanishing twin syndrome is unlikely to account for a higher proportion of cases of CP.[66]

Against such a concept is a lack of reported cases following multifetal pregnancy reduction, a procedure which is similar to the vanishing twin phenomenon. There was an attempt to determine whether iatrogenic fetal reduction increases the prevalence of CP, and results revealed that the prevalence of CP being 13.8 per 1000 in children from trichorionic triplet pregnancies reduced to twins by selective termination, was similar to that of children from trichorionic triplet pregnancies without loss (18 per 1000).[67] The only difference between groups was that in pregnancies with selective termination, delivery occurred at later gestation.[67] There is an urgent need for clarification of this controversy and it is authors' belief that 4D sonography might help. IVF procedures have often been pointed out as one of the main reason for the increasing incidence of CP in multiple pregnancies. After reports of increased risks of CP after IVF in the Swedish cohort study, Finnström et al.[68] found that there were no difference in type, severity or background characteristics of CP between children born after IVF and non-IVF children. The authors' interpretation is that etiological factors for CP do not differ, but that the increased prevalence of CP observed after IVF is due to higher multiple rate and prematurity.[68]

## Conclusions

Transvaginal sonography is still very important modality in routine prenatal care because it allows accurate diagnosis and clinical management of multiple pregnancies in the first trimester. It was found that early diagnosis of chorionicity and amnionicity has positive influence on mortality and morbidity in multiple pregnancies.

2D US is still the primary modality for diagnosis and evaluation of multiple pregnancies, but due to its limitations, an additional 3D US scan is recommended, because it provides more accurate and more reliable diagnosis with additional information important for management. Moreover, 3D US is beneficial before the onset of fetal movements regarding the determination of accurate number and size of gestational sac which has lead to the elimination of the »undercounting phenomenon« and improved prognosis of spontaneous pregnancy reduction. Furthermore, 3D power Doppler ultrasound enables more accurate differentiation between life threatening cord abnormalities such as cord entanglement and true umbilical cord knots and false positive results achieved with 2D power Doppler ultrasound.

4D US has several advantages over real time 2D sonography in the assessment of twin behavior such as the capability of simultaneous visualization of both fetuses and assessment of their motor activity as well as visualization of the inter-twin area. Therefore, it should be considered as a method of choice for accurate diagnosis of isolated motor activity in twins. 4D US is undoubtedly a new powerful imaging tool whose potential in scientific and clinical area should be searched for. In the meantime true clinical value of this new modality should not be nor underestimated neither overestimated.

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