# Advancements in ultrasound assessment of fetal genitalia for accurate diagnosis and comprehensive counseling

**Roxana-Elena Bohîlțea[1,2], Bianca-Margareta Salmen[2], Cristiana-Elena Durdu[2]**

*1. Department of Obstetrics and Gynecology, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

*2. Department of Obstetrics, Gynecology and Neonatology, "Filantropia" Clinical Hospital of Obstetrics and Gynecology, Bucharest, Romania*

**Corresponding author:**
Cristiana-Elena Durdu
E-mail: cristianadurdu@gmail.com

## Abstract

*Advancements in ultrasound technology and the integration of cell-free DNA testing into standard prenatal screening services have made it more feasible to identify fetal sex at an early stage. Fetal sex determination through sonography relies on different factors, depending on the gestational stage. In the late second trimester, it is determined by directly visualizing the external genitalia. However, in the late first and early second trimesters, it primarily relies on the direction of the genital tubercle, known as the sagittal sign. Other sonographic landmarks, like the fetal scrotum, midline raphe of the penis, labial lines, uterus, descended testis, and the direction or origin of the fetal micturition jet in males, can aid in the fetal sex determination accuracy. However, malformed external genitalia can lead to inaccurate results. This article focuses on the sonographic determination of fetal sex during the late first trimester, early second trimester and late second trimester, utilizing both transvaginal and transabdominal ultrasound techniques. Additionally, we explore various elements that have contributed to the precision of fetal sex determination. While ultrasound examination of the external genitalia is typically sufficient, there are certain situations where it becomes crucial to identify the internal genitalia for precise prenatal diagnosis and thorough counseling. This paper aims at reviewing these situations in which the evaluation of the external genital organs is necessary, as well as the ways in which ultrasound can be used in the diagnosis of genital malformations.*

***Keywords:*** *ultrasound, fetal genitalia, fetal sex determination, malformations*

## Rezumat

*Progresele în ecografie și integrarea testelor neinvazive prenatale în standardul screeningului prenatal au determinat fezabilitatea identificării sexului fetal în stadiul incipient. Determinarea sexului fetal prin ecografie se bazează pe diferiți factori, în funcție de vârsta de gestație. La sfârșitul trimestrului al doilea, se determină prin vizualizarea directă a organelor genitale externe. Cu toate acestea, la sfârșitul primului trimestru și începutul celui de-al doilea trimestru, se bazează în primul rând pe orientarea tuberculului genital. Alte repere ecografice, cum ar fi scrotul fetal, linia mediană a penisului, liniile labiale, uterul, testicului coborât și direcția sau originea jetului de micțiune fetal la sexul masculin, pot ajuta la acuratețea determinării sexului fetal. Totuși, organele genitale externe malformate pot conduce la rezultate incorecte. Acest articol se concentrează pe determinarea ecografică a sexului fetal la finalul primului trimestru, începutul celui de-al doilea trimestru, precum și la finalul acestuia, utilizând atât tehnici ecografice transvaginale, cât și transabdominale. În plus, explorăm diversele elemente care au contribuit la precizia determinării sexului fetal. Deși examinarea ecografică a organelor genitale externe este de obicei suficientă, există anumite situații în care devine crucială identificarea organelor genitale externe, atât pentru un diagnostic prenatal precis, cât și pentru o consiliere amănunțită. Lucrarea de față își propune să detalieze aceste situații în care este necesară evaluarea organelor genitale externe, precum și modalitățile prin care ecografia poate fi utilizată în diagnosticul malformațiilor genitale.*

***Cuvinte-cheie:*** *ultrasonografie, organe genitale fetale, determinare sex fetal, malformații*

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## Introduction

In the context of several genetic disorders, ultrasound prediction of fetal gender can be of real utility[(1)]. The first indications for ultrasound evaluation of fetal gender were pregnancies at risk of sex and X-linked pathologies. Currently, the fetal gender assessment is commonly done to satisfy the curiosity of the parents[(2)]. Determining fetal sex has several clinical implications. For example, it can dictate the necessity of invasive testing in the case of X-linked conditions such as Duchenne muscular dystrophy and androgen insensitivity syndrome, or adrenoleukodystrophy[(3,4)]. For male fetuses at risk of hemophilia, fetal gender assessment can be very useful in deciding the obstetrical management. Moreover,

fetal sex evaluation can raise suspicion in the case of congenital adrenal hyperplasia which can facilitate early administration of steroids to the mother in order to improve outcomes in female babies[3].

Although fetal sex evaluation is usually conducted in the second or third trimester, it is now possible due to technological advancements to assess fetal sex in the first trimester[2]. Several methods have been proposed and studied to predict fetal sex in the first trimester: the sagittal sign, the anogenital distance, three-dimensional (3D) ultrasound, measuring the angle between genital tubercle and lumbosacral skin surface etc.[5-7] In the first trimester, the efficacy of these methods is lower than in the second or third trimester ultrasound evaluation, with a high probability of false-negative results[8]. Nowadays, the early identification of anomalies allows a lower rate of complications related to pregnancy termination[9].

## Ultrasound assessment of fetal gender in the first trimester

In the second and third trimesters, the visualization of the penis and scrotum suggests a male fetus, while the visualization of the labia minora and majora suggests a female fetus. Up until 14 gestational weeks, the difference in size between the penis and the clitoris is insignificant[10]. Up until the 10$^{th}$ week of pregnancy, there is no difference between male and female external genitalia (Figure 1). After 12 weeks, changes in the urogenital sinus leads to the differentiation of external genital[5]. One of the first ultrasound markers in predicting fetal gender in the first trimester was the direction in which the genital tubercle points: cranially for boys and caudally for girls[11] (Figure 2). Another ultrasound method is represented by the "dome sign", where the fetal scrotum appears as a non-septated dome-shaped structure at the base of the fetal penis. An echogenic line at the base of the penis is suggestive of the penile raphe and can help in diagnosing a male fetus. Visualization of two or four parallel lines is suggestive for both labia minora and majora, and it can help in diagnosing a female fetus[11].

The sagittal sign represents the visualization of a cranial notch in male fetuses or a caudal notch in female fetuses[12] (Figure 3). This sign was evaluated by Mazza et al. in 2002. For a biparietal diameter (BPD) between 21 and 23 mm, the diagnosis of fetal sex was always correct[13]. In another study, starting from a BPD of 22 mm, the fetal gender was correctly assigned in 99% to 100% of cases, the authors concluding that fetal sex prediction should not be done below this threshold[7].

Efrat et al. evaluated another method of predicting fetal gender in the first trimester. Using a midsagittal plane, the angle between a horizontal line through the lumbosacral skin surface and the genital tubercle was determined (Figure 4). The fetus was assigned as male if the angle was greater than 30° and as a female if the angle was less than 30°. The fetal gender evaluation was accurate in 92.3% of cases, the accuracy increasing with gestational age from 70.3% at 11 weeks to 100% at 13 weeks. Therefore, the authors concluded that the decision for invasive testing in X-linked conditions should not be made before 12 weeks[5]. Chelli et al. also used an angle measurement to assess fetal gender: for an angle greater than 30°, male sex was assigned, and for an angle less than 10°, female sex was assigned. The diagnosis was correct in 85.7% of cases[14].

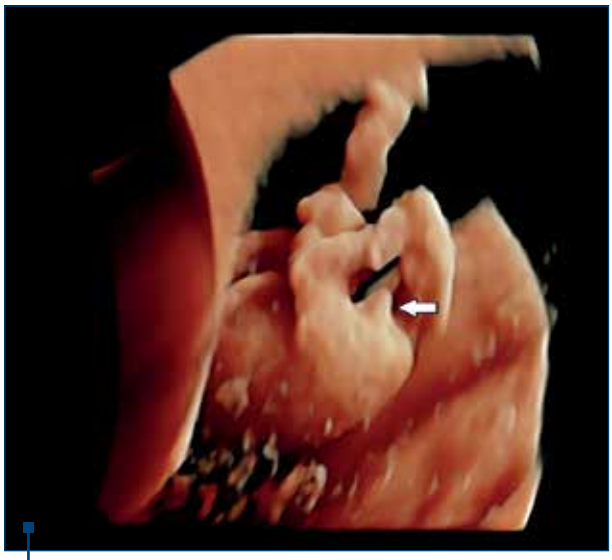

***Figure 1.*** *Three-dimensional ultrasound image of a 10+1 weeks fetus. Note the white arrow pointing to the genital tubercle. Source: R.E. Bohîlțea – personal collection*

Bogers et al. evaluated the efficacy of 3D ultrasound in predicting fetal sex in the first trimester. This technique had a modest efficacy, with an accurate prediction in 54.9% of cases[15]. Better results were provided by Michailidis et al., where two examiners evaluated 3D ultrasound images to predict fetal sex, with a correct prediction in 85.3% of cases[6] (Figure 5).

***Figure 2.*** *Two-dimensional ultrasonographic image exemplifying the sagittal sign in a 10+1 weeks female fetus. Note the asterisk indicating the genital tubercle pointing caudally and the white arrow indicating the caudal notch. Source: R.E. Bohîlțea – personal collection*

***Figure 3.*** *Two-dimensional ultrasound image exemplifying the sagittal sign in a 12-week male fetus. Note the asterisk indicating the genital tubercle pointing cranially and the white arrow indicating the cranial notch. Source: R.E. Bohîlțea – personal collection*

***Figure 4.*** *Two-dimensional ultrasound image – measurement of the angle of the genital tubercle to a horizontal line through the lumbosacral skin surface in a 12+2 weeks male fetus. Source: R.E. Bohîlțea – personal collection*

Anogenital distance as a method of predicting fetal sex was first evaluated by Arfi et al., who, using a cut-off of 4.8 mm, correctly identified 87% of males and 89% of females[16]. In another study, by Sipahi et al., genders were demonstrated accurately for males in 76.7% of cases and for females in 97.1% of cases, using the same technique[17] (Figure 6).

## Ultrasound evaluation of fetal gender in the second and third trimesters

Unlike the first trimester, the determination of the fetal sex in the second and third trimesters is based on the direct visualization of the external genital organs represented by the penis and scrotum in males, respectively the labia minora and labia majora in females. The accuracy of determining the fetal sex after the first trimester varies between 92% and 100% of cases, with a better accuracy in the case of the male sex supported by certain studies and with a better precision in the female sex supported by other studies[8].

***Figure 5.*** *Three-dimensional ultrasound image of a 11+3 weeks fetus. Note the white arrow indicating the genital tubercle. Source: R.E. Bohîlțea – personal collection*

In some cases, the simple evaluation of external genitalia can be insufficient and can require additional evaluation of other structures such as the fetal scrotum, testicular descent, dimensions of the penis, uterus characteristics in girls, and observing micturition in boys[8]. Some of the least studied fetal structures are the fetal scrotum and its contents. The testicular descent does not take place until 24 weeks of pregnancy, which results in not evaluating testicular descent during the second trimester ultrasound. In addition, there are no international recommendations concerning the ultrasound examination of fetal genitalia[18]. Ultrasonography can be useful in the prenatal diagnosis of several conditions such as cryptorchidism, inguinoscrotal hernia, intrauterine testicular torsion and meconium periorchitis[19-23].

Ultrasonographic evaluation of the scrotum and its content is feasible in 100% of cases between 34 and 36 weeks of gestation, the efficacy decreasing to 71.4% at 40 weeks due to reduced amniotic fluid and due to unfavorable fetal decubitus[24]. Ultrasound can evaluate testicle descend, structural anomalies and scrotal masses. Several studies evaluated testicle descend and concluded that the descend is present in 5% of cases between 24 and 25 weeks, and it is present in 97% of cases between 32 and 34 weeks, the incidence of cryptorchidism being 3%[20,24,25]. Structural anomalies involve the number, appearance or position abnormalities of the scrotum or testicles. Some examples of structural anomalies include scrotoschisis, bifid scrotum, or penoscrotal anomalies[26-28]. Scrotal masses include testicular or paratesticular tumors such as rhabdomyosarcomas, lymphomas, teratomas,

hemangiomas and metastases. These masses can be characterized by a solid or cystic aspect, the presence of calcifications, a positive Doppler signal, or the presence of peristalsis[29-31].

Intrauterine testicular torsion (IUTT) is a condition with unknown etiology which is usually diagnosed between 34 and 38 gestational weeks, or it can occur during delivery. Ultrasound evaluation shows a negative Doppler signal and a heterogenous testicle with hypoechoic areas that increased its size[32-34]. Other ultrasound findings include contralateral hydrocele, lateralization of the scrotal septum toward the affected area, and the occurrence of hemorrhagic fluid at the level of the *tunica vaginalis*[35]. Melcer et al. proposed an algorithm for managing IUTT. Bilateral IUTT after 34 gestational weeks requires emergency delivery in order to save at least one of the testicles. In the case of bilateral IUTT under 34 weeks or unilateral IUTT, the therapeutic management is expectant, the birth taking place according to the obstetrical indications. The exception is represented by the situation in which there is a unilateral IUTT with an onset of a maximum of 24 hours diagnosed during successive ultrasound evaluations. This situation requires emergency delivery[19].

Regarding the ultrasound evaluation of the penis, there is no consensus regarding the used methods, as well as regarding the normal upper and lower limits. In addition to the classic measurements of length, width and diameter, structures such as the glans, *corpora cavernosa* and *corpora spongiosa* can now be assessed using ultrasound[18]. The most frequently used penile length measuring method involves measuring the distance between the tip of the penis and the edge of the scrotum, this distance being called outer penile length (OPL)[36]. Besides OPL, other authors used different methods to determine the length of the penis. They used the distance between the tip of the penis and the proximal edge of the cavernous[37], or *spongiosus corpus*[38], or the distance between the tip of the penis and the pubic symphysis[36].

Using the data from clinical studies, the average length of the penis between 19 and 22 weeks should be between 7 and 10 mm, a length below 4 mm suggesting the diagnosis of micropenis. There are no cut-off values for the diagnosis of macropenis[18]. Some authors created reference tables for fetal penis dimensions[39]. When we are dealing with a macropenis, it is most often associated with megalourethra. In this situation, a careful evaluation of the urinary system and amniocentesis are mandatory[40]. In case of an isolated macropenis, it can raise the suspicion of congenital adrenal hyperplasia. In this situation, amniocentesis for karyotype and analysis of steroid metabolites from the amniotic fluid are recommended[41]. The diagnostic approach in case of a micropenis requires a detailed ultrasound scan focused on the genitourinary system that will later guide the choice of a genetic test in order to establish whether the micropenis is in the context of a chromosomal or non-chromosomal abnormality[41].

***Figure 6.*** *Two-dimensional ultrasound image – measurement of the anogenital distance in a 12+2 weeks male fetus. Source: R.E. Bohîlțea – personal collection*

Visualization of fetal micturition can help in the diagnosis of fetal hypospadias[8].

The small size of the ovaries can prevent their visualization by prenatal ultrasonographic evaluation. Therefore, these structures become visible in case of some pathologies that cause their volume to increase. For example, fetal ovarian cysts can be detected prenatally using ultrasound[42]. The fetal uterus has a linear growth and can be visualized sonographically starting at 19 weeks[43]. However, in 20% of cases, the fetal uterus cannot be detected by 2D ultrasound. With regard to 3D ultrasound, it managed to visualize the fetal uterus in less than 50% of cases between 20 and 22 weeks, with the detection efficiency increasing to 80% between 30 and 32 weeks[44]. In situations where the uterus is not visible, an indirect sign can be used, involving the evaluation of the distance between the urinary bladder and the rectum, which is greater in females than in males due to the interposition of the uterus between these organs. Also, the uterus determines a concave indentation at the level of the posterior wall of the urinary bladder. Using this method, Glanc et al. managed to correctly predict the female gender in 98.8% of cases and the male gender in 100% of cases[45].

## Prenatal management of chromosomal sex and phenotypic sex discrepancies, or ambiguous genitalia

The incidence of contradictory results between the ultrasound prediction of the fetal sex and the noninvasive prenatal testing (NIPT) result is below 0.01%[46]. Approximately one-third of these inconsistencies are due to errors such as the wrong interpretation of ultrasound images or the wrong labelling of samples with biological products[46]. Although NIPT has a good accuracy in detecting trisomies 21, 13 and 18, it did not have the same efficacy in detecting sex chromosome aneuploidy, only one-third of these cases being confirmed by karyotyping[47,48]. Repeated contradictory results between the ultrasound prediction of the fetal sex and NIPT require invasive prenatal testing, amniocentesis being the most appropriate method, since chorionic villus sampling has

a high rate of confined placental mosaicism associated with sex chromosome anomalies[48,49].

Despite the fact that ultrasound has good accuracy, rare anomalies are still difficult to diagnose prenatally[50]. When it comes to ambiguous genitalia, the ultrasound findings should be described in detail, with careful attention to female or male genitalia characteristics[42]. Careful ultrasound evaluation of the fetal anatomy can highlight other anomalies or particular signs that can guide the diagnosis. Genetic counseling with anamnesis, including a detailed family history, will guide the type of genetic testing required. Conventional karyotype can bring a lot of information, but karyotype with FISH (fluorescence *in situ* hybridization) for SRY (sex reversal Y) and hormonal analysis of amniotic fluid may be necessary[51]. When a specific syndrome is suspected, specific genetic tests, such as SNP (single nucleotide polymorphism) array and exome or whole genome sequencing, can help the clinician. Prenatal follow-up should include multiple ultrasound assessments in order to detect any fetal impairment, such as intrauterine growth restriction or oligohydramnios[42].

## Conclusions

Beyond satisfying the parents' curiosity, predicting fetal sex can become a necessity in certain situations. This can determine the indication of invasive prenatal testing in the case of X-linked diseases, it can determine the obstetric management of fetuses at high risk of hemophilia, and it can dictate the initiation of treatment in case of congenital adrenal hyperplasia. Although ultrasound evaluation in the first trimester is not as effective as in the second and third trimesters and considering the very good effectiveness of NIPT in detecting fetal sex, ultrasonographic prediction of fetal sex in the first trimester may remain an option in countries with limited financial resources. Fetal genital pathology is vast, with numerous etiologies and, despite its complexity, it is insufficiently studied, with a lack of guidelines to dictate the management of these high-risk pregnancies. Future studies should focus on a better understanding of these pathologies and on the establishment of prenatal diagnosis and management protocols of these complex conditions, thus helping clinicians provide adequate advice to parents and directing complicated cases to centers where postnatal reconstructive surgeries can be performed. ■

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