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Fetal sex identification in early pregnancy

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ABSTRACT
The need to determine the sex of the fetus in early pregnancy is usually based on medical indications for early implementation of appropriate therapeutic procedures, and is sometimes driven by parental curiosity. Medicine has made tremendous progress in early diagnosis of fetal sex in the last decades. The diagnostic methods described in the literature are characterized by varying degrees of accuracy, cost, and potential risk to the fetus. For these reasons, there is an ongoing debate about when and in which cases a particular method should be used. This paper presents an overview of the current methods of fetal sex determination.
Keywords: chorionic villus biopsy; fetal sex; molecular diagnostics; ultrasound; social science.

INTRODUCTION

The sex of an unborn child has always been of particular curiosity to parents and families. However, until the development of ultrasound diagnostics and, in recent decades, molecular DNA diagnostics, the answer to the question of the child's sex could only be obtained after birth. Nowadays, the possibility of determining the sex of the fetus on the basis of imaging of its external genitalia during ultrasound examination makes the question of the sex of the child the most frequently asked by pregnant women during a visit to the gynecologist. In situations where the desire to find out the sex of the baby early is dictated purely by the parents' curiosity, a standard ultrasound examination at the appropriate gestational age is sufficient. However, there are medical situations in which the physician overseeing the pregnancy should determine the sex of the fetus as early as possible. This is particularly the case when there is a suspicion of the possibility of serious sex-linked genetic diseases.

Advances in medical diagnostics make it possible to determine the sex of the fetus in the early stages of pregnancy. However, some of the diagnostic methods used during this period are associated with the risk of complications or high costs. Amniocentesis, which is performed to collect amniotic fluid and extract fetal cells for cytogenetic analysis, carries the risk of miscarriage, intrauterine infection, and fetal damage. The technique of trophoblast biopsy used by some prenatal pathology centers carries similar risks. The method developed in recent years to determine the sex of the fetus on the basis of cytogenetic analysis of fetal cells isolated from the mother's blood and free fetal DNA detectable in the blood of pregnant women requires a specialized laboratory and highly qualified personnel, and is associated with high costs of testing and frequent failures in obtaining suitable material for testing.

In view of the above, there is a need to look for alternative methods to assess the sex of the fetus as early as possible.

The aim of this work is to review and discuss the effectiveness of fetal sex determination methods in early pregnancy.

IMPORTANCE OF EARLY FETAL SEX IDENTIFICATION

Early identification of fetal sex during the first trimester of pregnancy is of great importance to both physicians and prospective parents in cases of risk for recessive genetic diseases linked to the X chromosome. These include hemophilia A and B, Duchenne muscular dystrophy, Becker muscular dystrophy, X-linked fish scales, agammaglobulinemia (XLA), Hunter syndrome, and fragile X syndrome mental retardation, as well as many rare genetic diseases. In most X-chromosome-related disorders, female fetuses are healthy, while male fetuses have a 50% risk of inheriting the disease. Therefore, the main goal of early fetal sex determination in familial sex-linked disorders is to reduce unnecessary invasive procedures and associated complications in non-risk female fetuses [1, 2].

Misclassification of a female fetus does not change the diagnostic pathway. In contrast, misclassification of a male fetus results in delayed invasive testing and reduced quality of care. In England, early diagnosis of fetal sex in pregnant carriers of X-linked diseases resulted in a 45% reduction in invasive procedures, as the majority of pregnant women carrying female fetuses chose not to undergo chorionic villus biopsy (CVS) [3]. Appropriate early non-invasive fetal sex determination in recessive X-chromosome-associated disorders will halve the number of invasive procedures by limiting them to fetuses of known male sex; early fetal sex determination will also provide other clinically relevant information, such as early assessment of zygosity in twin pregnancies of uncertain chorionic villus, and will satisfy parental curiosity. A more common indication for

first-trimester fetal sex diagnosis is familial congenital adrenal hyperplasia (CAH). In fetuses at risk for this condition associated with 21-hydroxylase deficiency, corticosteroid treatment initiated before the eighth week of gestation can prevent virilization of female fetuses, while male fetuses are not at risk. However, this treatment may be controversial. Because the risks and benefits of this treatment cannot be precisely determined, the American Society of Endocrinology considers it experimental [4]. On the other hand, the European Society for Pediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society have stated that "Early initiation of treatment alleviates genital virilization in all affected female fetuses and eliminates it completely in more than 85%" [5]. Early non-invasive fetal sex determination allows early initiation of treatment for affected female fetuses, and pregnant women with a male fetus can avoid the side effects of glucocorticosteroids and unnecessary treatment.

The indication for fetal sex determination, especially by invasive methods, in Fragile X syndrome (Fra-X) may be questionable. This is due to the fact that this condition also occurs in females. However, due to the phenomenon of lyonization, the female sex is less affected; 50% of girls with fragile X syndrome have no mental retardation and 25% of them have only mild retardation [6, 7, 8]. In view of this, Wald and Morris [9] recommend a non-invasive diagnosis of fetal sex between 8–13.5 weeks of gestation to avoid further unnecessary invasive procedures in female fetuses.

ULTRASONOGRAPHY IN THE SECOND AND THIRD TRIMESTERS

Ultrasonography is a non-invasive diagnostic technique that utilizes the phenomenon of propagation, scattering, and reflection of ultrasound waves. Over the past half-century, ultrasound has become an essential tool for the obstetrician, and ultrasound is now the standard of care for diagnosis in pregnancy.

Pioneering research into the use of ultrasound in diagnosis was conducted during and immediately after World War II. Ultrasound machines were used in diagnostics in the late 1960s and early 1970s. The first application was in fetal diagnostics. In the following years, ultrasound technology developed rapidly. Image resolution was greatly improved, and high-quality probes and digital cameras were produced, allowing for harmonic, three-dimensional, and four-dimensional imaging. Technological advances, and expanded ability to assess organ morphology and function led to a dynamic increase in knowledge and experience in fetal anatomy, physiology, and pathology.

In 1977, Stocker and Evens [10] first presented the diagnosis of fetal sex in the third trimester of pregnancy based on ultrasound visualization of the external genitalia of the fetus. This non-invasive technique for ultrasound assessment of fetal sex, initially in the third and later in the second trimester of pregnancy, was based on the criterion of detecting the presence of the penis and scrotum in a male fetus or the folds of the labia majora and labia minora in a female fetus. In the absence of external genital anomalies, the accuracy of this method reaches 100% after the 20th week of pregnancy.

Initially, obstetricians had doubts about the harmfulness of ultrasound examinations for the fetus. However, the studies conducted have unequivocally confirmed the safety of this diagnostic method. The possibility of ultrasound energy causing fetal malformations has also been ruled out. Ultrasound examination causes only a slight increase in the temperature of the examined tissues of up to 1°C [11, 12]. Only when the Doppler technique is used in the first trimester of pregnancy should the examination time not exceed 30 s. This is due to the proven risk that after this time, the energy transmitted by the ultrasound beam has a detrimental effect on the tissues and the conduction in the fetal neurons [13]. In light of these findings, the European Federation of Medical and Biological Associations recommended that "until further scientific studies are published, pulsed and color Doppler studies should be used with great caution" [14]. This is in line with the generally accepted principle of ALARA (as low as reasonably achievable), i.e. routine measures to minimize the risk of obtaining diagnostic information [15].

Prenatal determination of fetal sex by ultrasound is usually performed in the second and third trimesters [16]. Fetal sex determination in the first trimester by transabdominal or transvaginal ultrasound is a method of considerable reliability only at the end of the first trimester and only in the absence of developmental abnormalities of the external genitalia.

ULTRASONOGRAPHY IN THE FIRST TRIMESTER

The possibility of determining the sex of the fetus by ultrasound in the first trimester of pregnancy is strictly determined by the age-related stages of morphogenesis of the external genital organs of the fetus. The limitations of the applied diagnostic methods are due to the inability to visualize the genital ligaments in the early stages of development, the lack of morphological differences in the early stages of development of the male and female genital organs, and the still too low resolution and precision of the sonographic instruments.

The external genitalia of the fetus develop in the third week of fetal development from a region of primitive steak, from which cells then migrate around the cloacal membrane to form a pair of slightly raised folds. These folds fuse to form the genital node. During the next stage of development, the genital nodule divides into the urethral folds at the front and the anal folds at the back. At about the sixth week of development, two additional prominences are formed on either side of the urethral folds. These protuberances form the scrotum in male fetuses and the labia majora in female fetuses. This difference in the development of male fetuses is due to the influence of androgens secreted by the fetal testes and is characterized by the rapid elongation of the genital tubercle into a penile form. Around the twelfth week of gestation, the urethral folds close over the urethral plate to form the penile urethra. The external genitalia of the female fetus develop under the influence of the estrogens produced by the placenta, the genital bump elongates only slightly to form the clitoris, the urethral folds develop into the labia minora, and the genital protuberances enlarge significantly to form the labia majora [17].

From the above developmental mechanism, it is clear that the structural precursors of the external genitalia are present up to 10 weeks of gestation, but are not sufficiently differentiated to allow a clear distinction between the sexes of the fetus; however, by 12 weeks of gestation, clear changes in the structure of the urogenital sinus are evident. In male fetuses, the urogenital sinus is replaced by the suture of the scrotum and urethra; closure of the urogenital sinus begins at the caudal end of the embryo. This process, combined with the elongation of the genital bulb, results in the gradual displacement of the phallus in a cephalic direction. In females, the genitourinary sinus remains open and eventually becomes the vaginal vestibule [18]. Significant differences in the rate of penile and clitoral growth become apparent in the second trimester. An almost linear acceleration of prenatal penile growth occurs after 14 weeks of gestation [19].

Although it is difficult to determine fetal sex in the first trimester of pregnancy, advances in ultrasound technology, improvements in ultrasound machine resolution, and improved sonographer skills have made it possible to determine fetal sex with satisfactory accuracy as early as 11 weeks of gestation and with very high accuracy by the beginning of the second trimester [20].

At the end of the 1990s, a method for diagnosing fetal sex in the first trimester was developed. It involves using ultrasound in the first trimester of pregnancy to measure the angle between the nuchal fold and a horizontal line drawn through the skin surface of the lumbosacral region, which allows the sex of the fetus to be determined with a high degree of accuracy [21, 22, 23]. This method gives almost 100% sensitivity in determining the sex of the fetus at 13 weeks of gestation but has lower sensitivity at 11 and 12 weeks [21, 24, 25]. The basis for its implementation was the difference in the appearance of the developing genitalia observed in fetal ultrasound screening during the first trimester of pregnancy. At 11–13.6 weeks of gestation, ultrasound images of the male genitalia in the transverse plane can identify a scrotal dome at the base of the prominent penile structure of the fetus (Fig. 1), and in the mid-shaft plane, a prominent anteriorly directed nodule with an echogenic line can be seen at the base of the scrotum under the tip of the developing penis (Fig. 2).

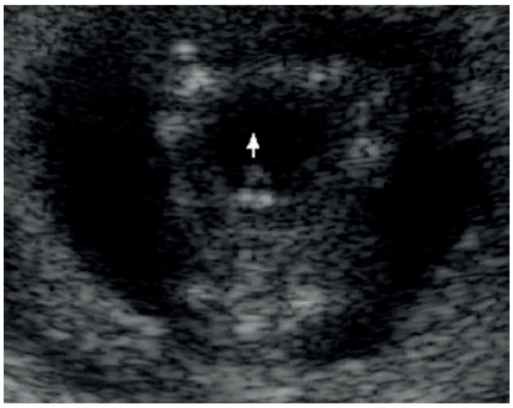

FIGURE 1. Transverse plane image of male genitalia [26]

Female fetal genitalia assessed in the mid-shaft plane are characterized by the absence of any genital nodules (Fig. 3), while a cross-section allows visualization of three or four parallel lines of the greater and lesser labia (Fig. 4). The significant increase in the angle of the genital nodules with increasing crown-rump length (CRL) occurs only in male fetuses. This is explained by the mechanism of embryologic development of the external genitalia described above.

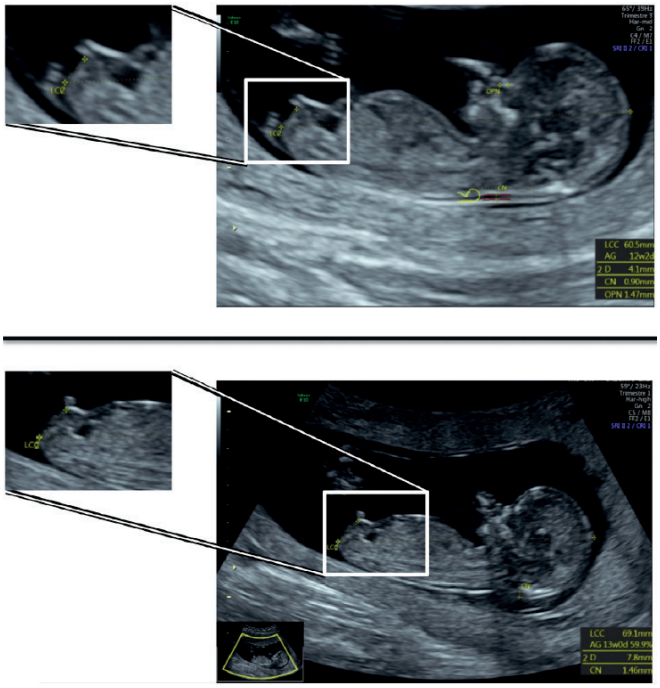

FIGURE 2. Imaging of male genitalia in the first trimester – measurement of anogenital distance [27]

In 1999, Efrat et al. [28] and Whitlow et al. [1] published their findings on fetal sex determination in the first trimester of pregnancy. Whitlow used a combination of cross-sectional and sagittal sections, while Efrat used the mid-arrow plane to measure the angle between the genital prominence and a horizontal line drawn through the skin surface of the lumbosacral region. The results of this study showed an accuracy of identifying the sex of the fetus at 11 weeks' gestation of 78% and 70.3%, respectively, in cases where the sex could be determined. However, when including cases in which the sex of the fetus could not be determined, the accuracy of identification was only 46% and 65%, respectively. In contrast, at 12 weeks' gestation, when the CRL exceeded 57 mm and sex determination was possible, the percentage of correct fetal sex identification was 86% and 92.8%, respectively. According to the evaluation of Lubusky et al. [29], this method cannot reliably predict fetal sex at CRL<50 mm (gestational age <11 + 4). Numerous factors can influence the correct identification of fetal sex in the first trimester of pregnancy. The ability of the operator to obtain a true median plane and correctly assess the direction of the genital cusp in relation to the position of the fetus, as well as the resolution and type of ultrasound transducer, are key [26]. Imaging with a transvaginal transducer is more accurate than with a transabdominal transducer, but most patients prefer a transabdominal examination. Limitations to the feasibility

and accuracy of the examination on the patient side include intestinal gas causing fetal shadowing and maternal obesity [30]. Difficulties on the fetal side may include unfavorable positioning, crossing of the legs, presence of the umbilical cord between the legs, and excessive mobility [1, 31]. It is important to note that only the phenotypic sex of the fetus can be assessed by ultrasound, which may differ from the genotypic sex in the case of developmental disorders such as testicular feminization or severe precocious development [29].

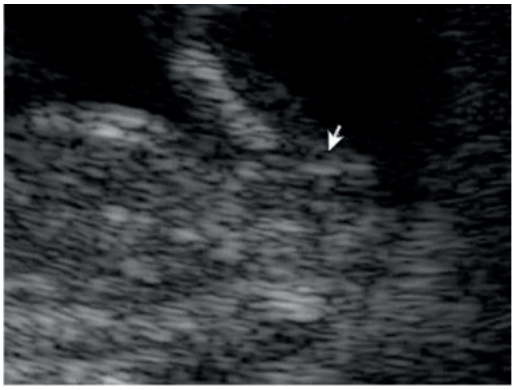

FIGURE 3. Image of female genitalia in the sagittal plane [26]

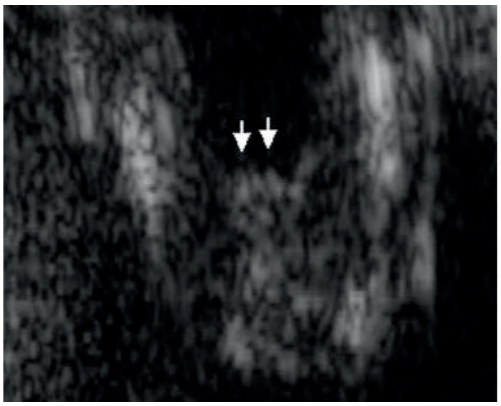

FIGURE 4. Image of female genitalia in the transverse plane [26]

Further studies on fetal sex diagnosis in the first trimester of pregnancy by subsequent authors [1, 26, 28] aimed to evaluate the accuracy of sex assignment and to determine the parietal-leg length (CRL) at which the accuracy is high enough to make a final decision on invasive procedures in a fetus identified as female.

In a study by Chelli et al. [32], ultrasound determination of fetal sex at 11–14 weeks of gestation achieved an accuracy of 87.9%. Efrat et al. [28] achieved an accuracy of 99.6% for male fetuses and 97.4% for female fetuses. The authors of the vast majority of studies confirm a higher accuracy for sex estimation in male fetuses than in female fetuses. Hsiao et al. [26] successfully assigned sex in 91.8% of fetuses. Despite improvements in ultrasound equipment, first-trimester screening does not correctly identify sex in 3–14% of cases [26, 31]. Efrat et al. [28] incorrectly assigned sex in 3% and 0% of fetuses examined in 12- and 13-week pregnancies, respectively. In a study by Whitlow et al. [1] at 12 and 14 weeks of gestation, fetal sex misattribution occurred in 14% and 8%, respectively. Manzanares et al. [31] reported that fetal sex determination at 11–14 weeks of gestation achieved an accuracy rate of 87.5%, with a significant increase in accuracy with increasing fetal head circumference (CRL) and gestational age (GA). In contrast, Benoit [22] successfully assigned sex to 98.5% of fetuses. Many researchers report difficulties in determining fetal sex in some pregnant women. Usually, it cannot be determined in a few to several percent of cases. However, in studies by Benoit [22], Whitlow et al. [1], and Hsiao et al. [26], the percentage exceeds 40%. This may be due to the fact that the angle between the genital nodules and the vertebral column changes on ultrasound, especially early in the first trimester [33]. Lubusky et al. [29] found that 10% of cases of fetal sex nonassignment were due to maternal factors. Numerous studies suggest that correct fetal sex assignment increases significantly with CRL greater than 55–57 mm and GA greater than 12 weeks [25, 27, 34]. Efrat et al. [28] found an overall accuracy of 100% for CRL greater than 68 mm. Lubusky et al. [29] also found the same accuracy for CRL greater than 60 mm.

Advances in ultrasound technology have allowed the use of three dimensional (3D) ultrasound for highly accurate fetal sex determination in the first trimester. The use of 3D ultrasound allows offline analysis with virtual manipulation of the images to obtain the desired images [35, 36].

Another method of fetal sex determination was used in the study by Arfi et al. [27]. This method is based on the measurement of the anogenital distance (AGD). In human fetuses, the anogenital distance (i.e. the distance between the caudal end of the fetus and the base of the genital prominence) is dimorphic and dependent on the action of testosterone and thus on sex. It becomes apparent from the 11th week of pregnancy. The anogenital distance is approximately twice as large in male fetuses [35, 36]. The difference persists until 24–30 months of age and then decreases until adulthood [35, 36, 37]. In the study by Arfi et al. [27], AGD was assessed in the mid-sagittal plane with the fetus in its natural position (neither flexed nor upright), as for CRL measurement. The caudal marker was placed as for the CRL measurement and the genital marker was placed at the base of the genital cusp. A cut-off size of 4.8 mm was used to predict male (>4.8 mm) or female (<4.8 mm) sex of the fetus. Sex was correctly determined in 87% of male and 89% of female fetuses. The authors found the method to be accurate, but further validation in a larger series is needed. In addition to the theoretical basis of the presented method, it should be mentioned that AGD is a marker of fetal exposure to androgens in an animal model [38, 39]. Administration of the androgen receptor antagonist flutamide or dibutylphthalate [40, 41] resulted in shortened AGD in male offspring and increased incidence of cryptorchidism and hypospadias [40]. A shortened AGD was observed in the newborns of male phthalate-exposed mothers [42, 43], while female fetuses of phthalate-exposed mothers had a longer AGD [42]. These observations suggest the need for systematic second-trimester ultrasound screening of male fetuses with low AGD for early detection of genital dysmorphia in the form of hypospadias.

A novel and different concept of fetal sex determination in the first trimester of pregnancy was presented by Borowski et al. [44]

Using precise CRL measurements and advanced statistical methods, they demonstrated significant differences in the CRL of male and female fetuses as early as 8 weeks of gestation. The larger CRL in male fetuses is mainly due to the effects of fetal testosterone. The table created by the authors, showing the relationship between CRL and GA parameters, allows us to determine the sex of the fetus in early pregnancy with a high probability.

DNA MOLECULAR DIAGNOSTICS

The identification in 1997 by Lo et al. [45] of cell-free fetal DNA (cffDNA) in the plasma and serum of pregnant women as a source of fetal genetic material makes it possible to identify Y-chromosome sequences and thus determine the sex of the fetus early, reliably, and minimally invasively, avoiding conventional invasive prenatal diagnostic methods. The most commonly used technique to detect and identify cffDNA sequences is the polymerase chain reaction (PCR). Among the many known types of PCR, quantitative real-time PCR is the most popular [46], as it combines high sensitivity with a closed detection system, thus minimizing the risk of sample contamination. There are a number of clinical applications of cffDNA analysis in prenatal diagnosis, such as fetal sex determination, familial occurrence of conditions associated with ambiguous external genital development, fetal Rh D factor determination, detection of single gene disorders, aneuploidy and some fetal ultrasound findings [47].

Most of the studies that assessed fetal sex based on cffDNA in maternal blood relied on the detection of the SRY gene [48] or the DYS14 marker on the TSPY gene, which was the first gene used to determine fetal sex. Although only a single copy of the SRY gene exists in the genetic material, Lo et al. in 1998 demonstrated its usefulness in confirming the presence of cffDNA in maternal blood [49]. In contrast, the DYS14 marker sequence is present in multiple copies and is more easily detected in plasma at concentrations up to 10 times lower than SRY in the male fetus [50]. In a fetal DNA detection assay, Zhong et al. found that PCR amplification of the DYS14 sequence was more sensitive than PCR amplification of the SRY sequence [51]. Because the X chromosome-associated amelogenin gene (AMELX) differs from the Y chromosome-associated gene (AMELY) by 6 base pairs in intron 3, PCR diagnosis of these genes can also be used to determine fetal sex with almost 100% accuracy [52]. The fetus is presumed to be female if no Y-chromosome DNA is detected.

The lowest sensitivity, 65.9%, for detection of the SRY gene between 11 and 12 weeks of gestation was described by Picchiassi et al. [53]. The authors attributed the low detection rate to the fact that the SRY gene has only one copy and cffDNA is low in the first trimester of pregnancy. However, a similar study from the fifth to the ninth week of pregnancy presented by Ren et al. in 2007 had a sensitivity of 85.4% [54]. Finally, the arguments presented by Picchiassi et al. were refuted by the studies of Costa et al. [55] and Kolialexi et al. [47] in 2012, which found a sensitivity of 100% between 8–13 and 6–11 weeks of gestation, respectively. Similarly, results from numerous studies published in the international literature have reported sensitivities close to 100% from 8 weeks of gestation [3, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67,]. Systematic reviews and meta-analyses by Devaney et al. [68] and Wright et al. [69] reported method sensitivities of 95.4% and 96.6% and specificities of 98.6% and 98.9%, respectively.

The importance of gestational age for the reliability of sex determination by cell-free fetal DNA identification makes it essential to have as accurate a date of conception as possible. Because the date of the last menstrual period may not be accurate enough to determine gestational age [70], ultrasound should be performed as early as possible after pregnancy in at-risk patients to plan the optimal time for cffDNA testing.

In their search for the optimal gestational age to perform the test, Rijnders et al. [71] compared the sensitivity of SRY detection at 5, 7, and 9 weeks of gestation. The earliest detection of SRY was at 5.2 weeks' gestation. The detection rate of SRY in male fetuses was 50% at 5 weeks, 80% at 7 weeks, and 100% at 9 weeks. Using multiplex real-time PCR, Liu et al. found a sensitivity of 100% at 8 weeks of gestation [61].

Martinhago et al. investigated the possibility of determining the sex of the fetus before the 7th week of pregnancy. At 5 weeks' gestation, the concentration of cffDNA in maternal blood was 1% of total DNA, the sensitivity of DYS14 detection was 87% and the specificity was 100%. At 6 weeks' gestation, the cffDNA concentration was 6.8% of total DNA, the sensitivity of detection was 92% and the specificity was 100% [62].

Stanghellini et al. [64] show that cffDNA can be identified as early as 5 weeks of gestation, but that a sensitivity of 100% is not achieved until 8 weeks. Therefore, there is a risk of false negative results if the test is performed before 8 weeks of gestation [48]. Similarly, Devaney et al. [68] suggest in their meta-analysis that tests performed before 7 weeks' gestation are unreliable. Another, less commonly used method of identifying the sex of the fetus is the detection of fetal cells in the mother's blood and analysis of the DNA they contain [55, 72, 73]. However, this method is limited by the continued presence of fetal cells circulating in the mother's blood long after delivery and even after miscarriage, especially if the doctor has not been informed about previous pregnancies [74, 75].

BIOPSY OF THE TROPHOBLAST VILLI (CVS)

Chorionic villus sampling (CVS) is performed on medical indication and at the request of the pregnant woman to determine the karyotype and sex of the fetus as early as possible. Fetal tissue for diagnosis of fetal sex or genetic disease can be obtained by invasive tests such as amniocentesis or chorionic villus sampling (CVS). Chorionic villus sampling under ultrasound guidance was the first approved technique for fetal genetic diagnosis. A pregnant woman may choose to have these tests done for a variety of reasons, such as to determine the sex of the fetus in hereditary X-chromosomal disorders or congenital adrenal hyperplasia.

Chorionic villus biopsy provides reliable fetal karyotype results as early as the seventh week of pregnancy, allowing early termination of pregnancy when medically indicated. The main reason for false results, which are rare with this method, is contamination of the examined trophoblast tissue material with maternal cells during chorionic villus sampling [76]. Therefore,

ultrasound fetal sex determination can be a valuable adjunct to CVS, significantly reducing the risk of false-positive results. Prenatal counseling must take into account the risks associated with invasive procedures. CVS can be difficult to perform. In addition, this test should not be performed before the 11th week of pregnancy because of the risk of pregnancy loss and the risk of limb reduction [2, 77]. As a result, most women have concerns and are reluctant to undergo chorionic villus sampling.

In 2007, Mujezinovic et al. performed a systematic review of all publications on complications after transabdominal CVS performed between 10 and 14 weeks of gestation [78]. The fetal loss rate at 14 days after the procedure was 0.7%and reached an additional 1.3% by 24 weeks' gestation. The overall fetal loss rate was 2%.

In 2010, a review of the literature by Tabor and Alfirevic found that the miscarriage rate associated with the CVS procedure was 0.5–1% [79]. In developing countries with low levels of medical care, this rate may be as high as 5% [80]. Because of these complications, invasive biopsy testing should only be used for definitive prenatal diagnosis.

SUMMARY

The above observations confirm that fetal sex determination by ultrasound in the first trimester of pregnancy is feasible and remains a simple, valuable and cost-effective diagnostic method. Furthermore, in the last week of this trimester, the accuracy of the results obtained is similar to that of invasive examinations. In all studies presented here, ultrasound fetal sex determination was performed by measuring and assessing the anatomy of the genital tubercle area in the last weeks of the first trimester at 11(12)–14 weeks. The cut-off for fetal sex determination according to Lubusky et al. [29] is 50 mm for males (gestational age over 12 weeks) and according to Manzanares et al. [31] 55.7 mm for both sexes (gestational age over 12 + 2 weeks). Gestational age and increasing CRL correlate positively with the feasibility and reliability of fetal sex determination. Further research is needed to validate the tool, including the determination of a feasibility AND reliability threshold adjusted for CRL. A combination of transverse and mid-sagittal projections may further improve the performance of current ultrasound algorithms for sex determination between 11–14 weeks [1].

Regarding the social implications of prenatal sex determination, practice shows that most expectant parents want to know the sex of the fetus as early as possible, despite the risk of misidentification early in pregnancy. Therefore, despite high rates of correct fetal sex identification, any diagnosis of fetal sex at less than 12 weeks of gestation should be accompanied by a warning about the potential for error and the risk of misidentification. The possibility of sex ambiguity, the presence of feminizing testes, and severe presumptogenesis should also be considered.

Genetic testing is highly accurate in determining the sex of the fetus. Enrichment of cell-free fetal DNA from maternal blood allows sex determination in the first trimester of pregnancy with an accuracy of up to 99.5% [71]. However, the use of a non-invasive technique for maternal blood testing in low-income countries may be severely limited by high cost, making it less accessible than ultrasound [9, 81].

The detection of external genitalia abnormalities and the inability to distinguish a male from a female fetus on ultrasound is also an important indication for determining the sex of the fetus by testing for free fetal DNA in the mother's blood. Some of these anomalies are due to chromosomal abnormalities that require karyotyping, but anomalies such as cloaca, bladder protrusion, hypospadias, or virilization of the genital organs of female fetuses caused by congenital adrenal hyperplasia are not associated with karyotype abnormalities [82]. In these cases, the determination of fetal DNA in maternal blood provides important information that influences prognosis and allows planning of postnatal surgical management [3].

Colmant et al. [83] estimate that non-invasive prenatal fetal sex determination using cffDNA in maternal blood is more accurate in terms of sensitivity and specificity and is performed earlier than ultrasound. The sensitivity of maternal blood testing reaches almost 100% at 8 weeks of gestation, which is 3 weeks earlier than CVS. Ultrasound is also an alternative non-invasive method, but an analysis of the data in the literature indicates that the diagnostic efficacy of the method is not fully reliable until the 13th week of pregnancy. The cffDNA test can be performed earlier than ultrasound, significantly reducing the number of invasive tests. In addition, cffDNA analysis is a more reliable technique for fetuses with external genital abnormalities. Ultrasound and cffDNA testing are not an alternative to CVS, but a method of identifying populations of fetuses at risk for whom a CVS result may alter management. CVS is performed later in pregnancy (after 11 weeks of gestation) and, unlike cffDNA, is associated with a risk of complications.

A study of a small number of cases compared the feasibility and accuracy of fetal sex determination by ultrasound and PCR from maternal blood during the first trimester of pregnancy [56, 84]. A series of 28 pregnancies at risk of X-chromosome-associated disease or with fetuses affected by genital anomalies was reported by Hyett et al. [60]. Real-time PCR testing for the SRY gene was performed at 10 weeks' gestation. The possibility and concordance of sex determination was 100%. Ultrasound assessment of fetal sex was performed in 23 patients at a mean gestational age of 12 weeks. The sensitivity was 87% and the concordance was 100%.

Identical results in PCR for the DYS14 gene and ultrasound by Efrat et al. [21, 28] after 12 weeks of gestation in 10 female hemophilia gene carriers were obtained by Chi et al. [84].

In contrast, Mazza et al. [56] show the advantage of diagnostic ultrasound over PCR testing. Using nested PCR for the amelogenin gene, they found 50% sensitivity and 78% concordance. USG visualization showed 100% sensitivity and concordance. The outlier result can be explained by the use of an outdated and inaccurate PCR method.

Ethical and legal issues cannot be ignored in relation to the discussed issues of fetal sex determination in the first trimester of pregnancy.

Non-invasive prenatal diagnosis is currently having a tremendous impact on fetal medicine, but there is a significant risk that its potential will be exploited for non-medical purposes,

such as sex selection of the future child, selective abortion, or unauthorized paternity testing. In many countries, abortion based on the sex of the fetus is not permitted but may be performed for medical reasons in cases of severe sex-related conditions. However, there is a risk that once the sex of the fetus has been determined at the patient's request, she will ask another doctor to remove the fetus of the unwanted sex. Such situations create moral and ethical problems for obstetricians and therefore require additional consideration and legal solutions related to early sex determination [85]. Ethicists believe that the obstetrician should ensure that the patient understands and accepts the moral and legal constraints associated with fetal sex determination performed at her request. Orthodox obstetricians believe that even non-invasive fetal sex determination is unethical because the fetus is being tested without its consent. The counterargument to this view is that non-invasive testing is no different from a screening ultrasound and that a woman always has the right to full information about her pregnancy.

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