Discordance in Fetal Sex Determination Using cfDNA and Ultrasound: An Overview and Resources for Management

A highly anticipated aspect of pregnancy for many parents is the revealing of fetal sex. Ultrasound, usually in the second trimester, is standardly used to evaluate fetal sex; however, cell-free DNA (cfDNA) testing, which is increasingly being adopted for trisomy screening, can provide fetal sex information as early as 10 weeks gestation. The use of two different screening tools for assessment of fetal sex will inevitably lead to instances of discordant results, the incidence of which is estimated to be 1/1500 to 1/2000 pregnancies.[1] There are many potential explanations for this discordance with the implications ranging from no clinical significance to disorders of sexual development with multisystem involvement (Fig 1). This challenging situation can be distressing for parents and requires careful counseling. The purpose of this document is to provide an overview of the possible explanations and resources to aid in further management.

Limitations of ultrasound and cfDNA testing

Although ultrasound and cfDNA testing have the potential to determine fetal sex with >99% accuracy,[2,3] they are each subject to unique technical limitations. The accuracy of ultrasound is dependent on factors such as gestational age, maternal body habitus, fetal positioning, operator skill, and sonographic equipment. Technical factors influencing the accuracy of cfDNA testing include quality of cfDNA data and insufficient fetal fraction, if measurement of fetal fraction is not employed by a laboratory as a quality metric.

> “Discordance between genotypic sex, as determined by cfDNA testing, and phenotypic sex, based on the sonographic appearance of fetal external genitalia, is fortunately uncommon, occurring in approximately in 1/1845 pregnancies.”
>
> Discordant fetal sex on NIPT and ultrasound. Prenatal Diagnosis. 2020[5]

Additional sources of cfDNA

Testing using cfDNA is sensitive to the presence of DNA in maternal plasma from sources other than the visible fetus. Persistent cfDNA from an anembryonic gestation or demised co-twin is the most common cause for discordance between cfDNA testing and ultrasound in published cases.[4] cfDNA from a nonviable conception has been documented in the maternal bloodstream at least 15 weeks after a demise and if the twins are of a different sex, can produce a result that is not consistent with the appearance of the viable fetus.[5–7] Another known cause for discordance is organ transplant. Donor-derived cfDNA may present in the plasma of transplant recipients and if the organ donor is male, lead to a male cfDNA result in a pregnancy with a female fetus.[8,9] In rare cases, variants in the maternal cfDNA, such as those involving Y chromosome sequences, can confound testing.[1]

Differences of sex development

Differences (or disorders) of sex development (DSD) are associated with atypical development of the external and internal genitalia and may underlie a difference between the genetic (chromosomal) sex as predicted by cfDNA testing and the apparent sex as seen by ultrasound. This is a heterogenous group of conditions with a range of effects from ambiguous genitalia to full sex reversal. Many are syndromic and associated with other abnormalities, some of which may be detectable by ultrasound.

Chromosomal and subchromosomal abnormalities

Sex chromosome abnormalities affect sexual development and, in the mosaic form, can have different effects on both cfDNA testing and the appearance of the genitalia, depending on the level of mosaicism and the tissues (placental and fetal) in which the different cell lines are present. Examples of mosaicism associated with discordant results are 45,X/46,XY; 45,X/46,Xidic(Y).[1,9]

Translocations and submicroscopic deletions involving the sex-determining region Y (SRY), a gene on the Y chromosome that initiates male embryonic development, can cause complete sex reversal.[1] Affected individuals can be XX males (SRY-positive) or XY females (SRY-negative). Submicroscopic changes in other chromosomal regions have also been reported.[1]

Monogenic disorders

In addition to SRY, there are numerous single gene etiologies for DSD.[10] Variants in some of these genes cause complete sex reversal, such as androgen receptor mutations leading to complete androgen insensitivity. In others, they cause abnormal genital development, such as virilization in females with congenital adrenal hyperplasia or ambiguous genitalia in males with Smith-Lemli-Opitz syndrome. Many are syndromic and cause other malformations, some of which may be detectable by ultrasound. For example, potential findings in Smith-Lemli-Opitz syndrome are intrauterine growth retardation (IUGR), cardiac defects, and renal malformations.[11]

Figure 1. Range of clinical implications of discordance in fetal sex between cfDNA and ultrasound

Clinical management

Many of the published review articles about sex discordance (see Resources) contain algorithms for case management. Common aspects of these recommendations are listed below. The extent of the evaluation should be considered in the context of the entire clinical picture and the individual patient's values and reproductive goals.

1. Review of records to ensure accurate documentation of ultrasound and cfDNA results and look for evidence of possible co-twin demise
2. Review of family history for DSD and maternal history for organ transplant, medication exposure, or other potential underlying maternal condition
3. Repeat ultrasound to confirm determination of fetal sex and identify other possible findings suggestive of a syndromic DSD
4. Consideration of amniocentesis with karyotype, microarray, FISH for SRY, or molecular genetic testing for DSD. Molecular testing options are numerous, including targeted testing, gene panels or whole exome/whole genome sequencing depending on the clinical findings
5. Postnatal referral to specialty clinic with DSD for evaluation if prenatal diagnosis is not pursued

Repeat cfDNA testing

Repeat cfDNA testing is appropriate when there is significant concern for a specimen handling error such as mislabeling at the time of phlebotomy. In other circumstances, laboratories performing cfDNA testing will be willing to describe standard measures employed to minimize errors and discuss considerations regarding repeat testing. A second test with a concordant result for fetal sex would not rule out the possibility of a clinically significant etiology for the initial discordance.

Resources

In addition to local specialty consultants in your area, the following two resources may be helpful.

- "Managing fetal sex discordance in cfDNA" is a peer-reviewed article, published in 2020 in the Contemporary OB/GYN Journal; 65(12). It presents a clinical algorithm and four cases to consider. https://www.contemporaryobgyn.net/view/managing-fetal-sex-discordance-in-cfdna
- "Discordant fetal sex on NIPT and ultrasound" is a more in-depth review and contains management guidance. It was published in 2020 in Prenatal Diagnosis; 40:1353-1365. https://doi.org/10.1002/pd.5676

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The Harmony Prenatal Test is a non-invasive prenatal test (NIPT) based on cell-free DNA analysis. Harmony does not screen for potential chromosomal or genetic conditions other than those expressly identified here. The results are intended for prenatal screening and are not intended to be the sole basis for diagnosis. Harmony test results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmatory fetal diagnostic testing, parental evaluation, clinical genetic evaluation, and counseling, as appropriate. This test is offered as a CE-IVD.*This test has not been cleared or approved by the US FDA.

*Note: Because the fetal sex determination test option does not have a medical purpose, it does not meet the definition of an IVD device and therefore is not a CE marked product.

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