Ultrasound Obstet Gynecol 2010; **35**: 637–646
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.7679

Editorial

Diagnostic approach in prenatally detected genital abnormalities

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INTRODUCTION

Sex differentiation is a complex process that results in the formation of male or female internal and external genitalia. It is the result of interactions between the sex-chromosome complement of the conceptus, gene products which direct the development and migration of germ cells and gonadal hormone production which determines the phenotype. There are three major components involved in sex determination: the chromosomal sex, the gonadal sex and the phenotypic sex.

Typically, prenatal sex determination is performed by ultrasound evaluation of the external genitalia from the second trimester onwards. In the setting of ambiguous external genitalia or a discrepancy between the external genitalia and the chromosomal sex, evaluation of the internal genitalia is of the utmost importance[1]. Although most disorders of sex development (DSD) are isolated, it is important to determine if there are associated congenital abnormalities and to review relevant maternal and family history, which may provide clues to the origin of a DSD. Laboratory investigations can include chromosome analysis, microarray analysis and amniotic fluid hormonal studies[2] as well as mutation analysis when a specific single gene disorder is suspected. The diagnosis of a DSD in a fetus or newborn presents a challenge for both the family and the healthcare team. To optimize the diagnostic and therapeutic plans a multidisciplinary approach involving experts in perinatology, endocrinology, urology and genetics, psychology and social work, is required. The family may need time to adjust to the diagnosis in order to comfortably discuss and decide on issues relating to gender, sexuality and surgical and medical management options.

NORMAL SEXUAL DIFFERENTIATION

The complex process of sex differentiation and determination results in the formation of male or female internal and external genitalia. The process is the result of interaction between the sex-chromosome complement of the conceptus, gene products that direct the development and migration of the germ cells to the urogenital ridge, the formation of the gonads and their differentiation towards testes or ovaries and gonadal hormone production which determines the phenotype – the external genitalia.

The primordial germ cells first appear in the outer ectodermal layer (proximal epiblast) of the embryo, from where they migrate through the primitive streak along the wall of the hindgut to the urogenital ridge, the site of the future gonad. The formation of the bipotential gonad is influenced by many genes, including *SRY, SOX9, SF1, Dax1, WT1, WNT4*α and *LHX* among others.

In the male (XY), the XY germ cells undergo mitosis during their migration to the primordial gonads, but soon after reaching the gonads, the protein coded by the *SRY* gene on the short arm of the Y-chromosome acts on the *SOX9* gene by stimulating its product, which in turn drives the formation of the Sertoli cell and turns the undifferentiated gonad into a testis. The germ cells' growth becomes arrested and they remain in the testis in the G0 phase of the cell cycle until after birth. They then resume the cell cycle and undergo meiotic division, producing the haploid spermatogonia which complete spermatogenesis at puberty, under the influence of gonadotropins (follicle stimulating hormone and luteinizing hormone) secreted by the pituitary gland[3,4].

In the female (XX), the XX germ cells undergo mitosis during their migration to the female genital ridge and gonads. In the absence of the *SRY* gene or when it is mutated, the *SOX9* gene is silenced, follicular cells develop and the gonad develops as an ovary. The cells then progress through the initial stages of the first meiotic division and are arrested at prophase I. At this stage the germ cells become surrounded by a single layer of granulosa cells, forming primordial follicles. At puberty, the resting primordial follicles are stimulated to grow as

primary, secondary, and preovulatory follicles under the influence of follicle-stimulating hormone.

The phenotypic sex is determined by the influence of hormones on the internal and external genitalia. Regarding the internal urogenital tract, in the male, the Wolffian ducts give rise to the epididymis, vas deferens, seminal vesicles and ejaculatory ducts. The Müllerian ducts regress under the influence of Müllerian inhibiting factor (MIF), produced by the testicular Sertoli cells. MIF acts locally through its receptors, located on the Müllerian ducts. In the female, the paramesonephric ducts (Müllerian ducts) develop and give rise to the Fallopian tubes, uterus and the upper part of the vagina. The mesonephric ducts (Wolffian ducts) persist in vestigial form.

Regarding the external genitalia, three organs exist before differentiation: the genital swelling or labioscrotal swelling, the genital folds and the genital tubercle. In the male, the testes produce the androgen testosterone, which is reduced to dihydrotestosterone (DHT) by the action of the 5α reductase enzyme. DHT has a stronger affinity than does testosterone to the androgen receptor and amplifies the androgen action on the external genitalia. This results in their anterior displacement, fusion of the genital swelling to form a scrotum and fusion of the genital folds to form the penile shaft. The genital tubercle forms the glans penis. In the female, in the absence of testes and independent of ovarian hormone secretion, the external genitalia develop into a female phenotype. Thus, the genital swellings form the labia majora, the genital folds form the labia minora and the genital tubercle forms the clitoris.

Thus, the three major components in sex differentiation are:

1. chromosomal sex, determined at fertilization (XY being male and XX female);
2. gonadal sex, including ovary and testis; development begins around 6–7 weeks after fertilization;
3. phenotypic sex, determined by hormonal influence on external and internal genitalia.

For the first 6 weeks, human development in the two sexes is identical, in a bipotential state. Subsequent development of the external genitalia into the characteristic male or female structures is completed by week 12 in the male and somewhat later in the female.

PRENATAL SEX DETERMINATION USING FETAL ULTRASOUND AND MRI

Determination of normal fetal gender by visualization of external genitalia

Prenatal diagnosis of the fetal phenotypic sex can be made non-invasively, typically by prenatal ultrasound or less commonly by magnetic resonance imaging (MRI). Diagnosing the fetal sex using real-time ultrasound was first reported in 1977 by Stocker and Evens[5], who examined the fetal perineum after 30 weeks' gestation in singleton pregnancies. They identified the male external genitalia and by exclusion made the diagnosis of female external genitalia. Using this technique they accomplished fetal sexing in 366 cases with an overall accuracy rate of 95.6% (correct in 99.5% of those diagnosed as males and in 91.5% of diagnosed females). Accurate diagnosis was limited by the fetal position, presentation and amniotic fluid volume. Although technical improvements in ultrasound have permitted earlier and more confident diagnosis, these factors continue to limit evaluation. Emerson *et al.*[6] in 1989 were the first to report the 'sagittal sign' for determination of fetal sex in early gestation. With the fetus being scanned in the midline sagittal plane, following the rump from dorsal to ventral, the focal bulge, representing the penis or clitoris, can be seen ventrally. The clitoris presents as a caudal notch while the penis presents as a cranial notch. Using this sagittal sign, the fetal sex could be determined in five of seven fetuses at 10.0–11.9 weeks of gestation. However, the diagnosis was correct only in three of the five. The detection rate improved with advanced gestational age, and was 75%, 100%, 98% and 100% at 12.0–13.9, 14.0–15.9, 16.0–17.9 and 18.0–20.4 weeks' gestation, respectively. Using the sagittal sign and additional sonographic findings, others have also reported sonographic fetal sex determination in the late first and early second trimesters with varying success. Dunne and Cunat[7] failed to diagnose the fetal sex at 10–14 weeks' gestation, while Natsuyama[8], adding the anogenital and anoperineogenital distance (both being longer in males) at 12–40 weeks' gestation, reported an accuracy of 85.3%. The same author also noted that the direction of the developing fetal phallus differs, with the penis pointing cranially and the clitoris pointing caudally. Using transvaginal sonography between 12 and 23 weeks of gestation, Bronshtein *et al.*[9] were able to detect fetal sex accurately in 76% cases at 13–14 weeks' gestation and in 88% cases at 15–16 weeks' gestation. They also found the detection rate to increase with experience: following 2 years' experience, the detection rate increased to 80% at 13–14 weeks' gestation and 96.7% at 15–16 weeks' gestation. In this study the sonographic determination of male genitalia was based on the identification of a non-septate, dome-shaped structure at the base of the penis ('dome sign'), indicating a scrotum. Female external genitalia were identified based on the visualization of two or four parallel lines, representing the labia majora and minora. Other important signs used for accurate prenatal detection of male genitalia include the longitudinal, midline, echogenic line at the base of the fetal penis in the tangential plane, representing the median penile raphe. Mielke *et al.*[10] reported 100% accuracy in fetal sex determination in both sexes using transabdominal ultrasound at 11–16 weeks' gestation and Benoit[11], using mainly transvaginal ultrasound, was able to accurately determine the fetal sex in 98.5% cases at 12 weeks' gestation and 100% at 13 weeks' gestation. Improvement in using the sagittal sign was

achieved by Efrat *et al.*, initially in 1999[12] and later in 2006[13]. In the later publication they performed transabdominal ultrasound in 656 singleton pregnancies at 12–14 weeks of gestation and determined the fetal sex by measuring the angle of the genital tubercle to a horizontal line through the lumbosacral skin surface in the mid-sagittal plane. Using this method the fetus was assigned male sex if the angle was $>30°$ and female if it was $<10°$; when the angle was 10–30°, the gender could not be determined. Using this method the phenotypic sex was confirmed in 555 newborns with an accuracy for male gender of 99–100% at all gestational ages. In females the detection rate was less accurate and more dependent on gestational age: 91.5% at 12 + 0 to 12 + 3 weeks, 99% at 12 + 4 to 12 + 6 weeks and 100% at 13 + 0 to 13 + 6 weeks. Using the same method, Chelli *et al.*[14] were able to diagnose accurately 87.9% of males and 83.3% of females at 11–14 weeks' gestation.

Better visualization can be achieved in the second compared with the first trimester, and gender determination can rely on visualization of the penis and scrotum themselves in males and on the two or four parallel labial lines in the female. Detecting these genital signs can increase normal gender identification in the second trimester to 100%[11,15] (Figures 1 and 2).

Determination of normal fetal gender by visualization of internal genitalia

Fetal testes

Identification of the fetal gonadal sex can facilitate sex determination. Testes do not descend to the scrotum prior to 25 weeks' gestation. Birnholz[16] found the testes to be descended in 12.5% of male fetuses at 26.0–27.9 weeks, in 60% of cases at 28.0–31.6 weeks, in 85.7% of cases at 32.0–33.6 weeks and in 94.7% of cases thereafter. Using better ultrasound resolution, Achiron *et al.*[17] in 1998 were able to detect at least one descended testis in 30% of male fetuses at 25.0–16.0 weeks' gestation and in 70% of fetuses at 26 weeks. By 32 weeks, bilateral testicular descent was observed in 97% of cases.

Fetal uterus

Soriano *et al.*[18] were able to visualize the intra-abdominal fetal ovaries as early as 19 weeks' gestation and reported the normal values of the fetal female uterus by measuring its transverse diameter and circumference between 19 and 38 weeks. They found that the mean transverse diameter increased from 6 mm at 19 weeks to 20 mm at 38 weeks and the uterine circumference increased from 20.5 mm at 19 weeks to 58.2 mm at 38 weeks. However,

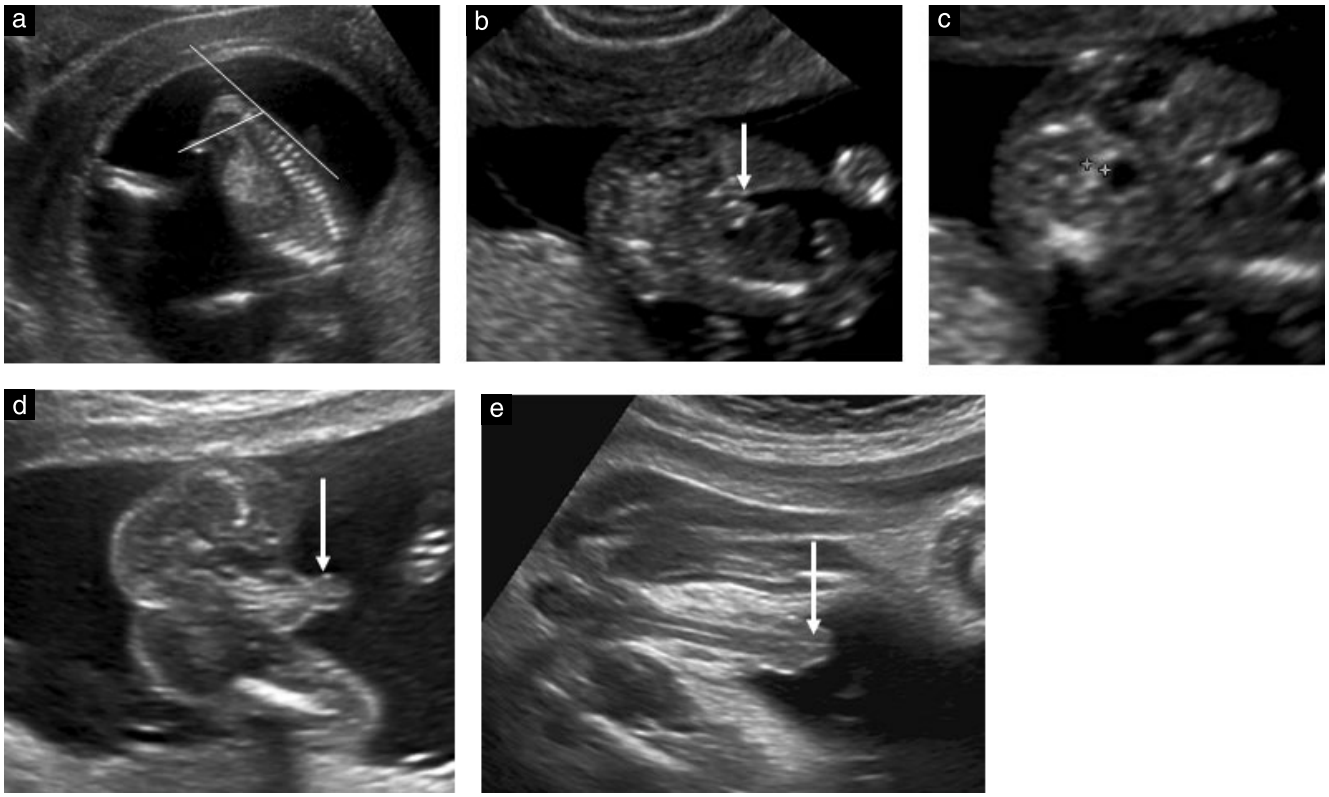

Figure 1 Sagittal (a) and transverse (b–e) ultrasound images showing normal male phenotypic genitalia: (a) at 12.8 weeks, showing anterior-directed genital tubercle, with an angle to a horizontal line through the lumbosacral skin surface of > 30°; (b,c) at 14.4 weeks, showing penile shaft (arrow) (b) and internal genitalia with bladder–rectum distance of 1.4 mm (calipers) and concave interface with bladder consistent with male gender (c); (d) at 19 weeks, showing penile shaft and dome-shaped scrotal sac (arrow); (e) at 32 weeks, showing length of penile shaft, with bulbous appearance of glans penis and urethra visible extending to tip of glans penis as a thin echogenic line (arrow).

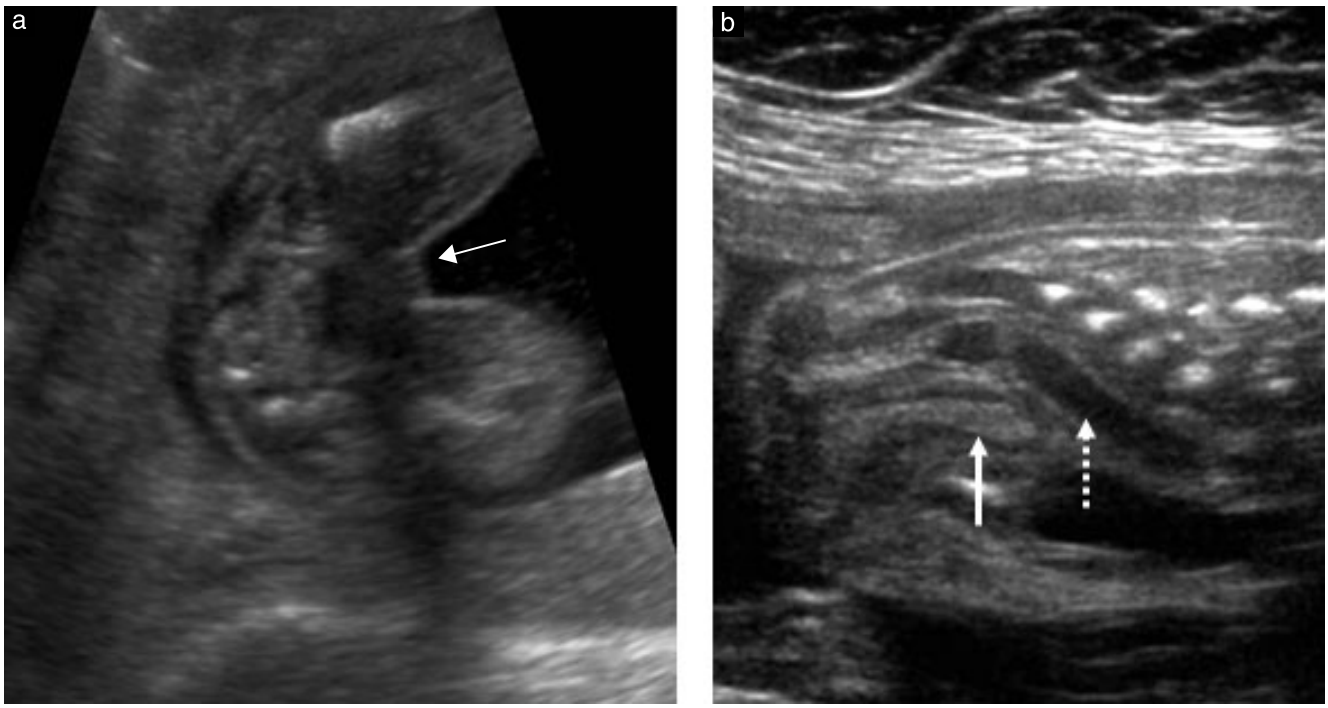

Figure 2 Transverse (a) and sagittal (b) ultrasound images showing normal female phenotypic genitalia: (a) at 19 weeks, showing external genitalia as four parallel lines of labia majora and labia minora (arrow); and (b) at 24 weeks, showing uterus and cervix with echogenic endometrium and cervical mucosa (arrow) surrounded by relatively hypoechoic myometrium, with rectum immediately posterior (dashed arrow).

in 40/180 (22%) female fetuses the uterus could not be visualized. Using three-dimensional (3D) volume contrast imaging (VCI), Jouannic *et al.*[19] were able to visualize the uterus at 20–22 weeks' gestation in 50% of cases using conventional two-dimensional (2D) ultrasound and in 82–87% of cases using VCI. At 32 and 34 weeks' gestation the accuracy increased to 85% and 95–100%, respectively.

Fetal rectovesical interspace

The presence of the uterus in female fetuses results in an increased distance between the bladder and rectum compared to male fetuses. Measuring the distance between the posterior wall of the bladder and the anterior wall of the rectum allowed Glanc *et al.*[1] to determine fetal sex correctly in 98.8% of female fetuses and 100% of male fetuses between 14 and 40 weeks of gestation. Using linear discriminant analysis they found that the best way to separate male from female fetuses was to plot the graph given by: distance (in mm) = (0.26 × gestational age in weeks) − 2.17. Using this equation, 100% of male fetuses were below and 99% of female fetuses were above the line. They found that, of all fetuses studied by them, a total of 98.2% of all concave interfaces were found in female fetuses, and 92.6% of straight or concave interfaces were found in male fetuses. Thus, among female fetuses, concave interfaces were found in 88.5%, and other interfaces were found in 11.5% whereas among male fetuses, convex interfaces were found in 1.1% and other interfaces were found in 98.9%. In the setting of ambiguous external genitalia, evaluation of the rectovesical interspace may aid in gender assignment (Figure 3).

PRENATAL DIAGNOSIS OF DISORDERS OF SEXUAL DEVELOPMENT: A STEPWISE APPROACH

Fetal gender abnormality can be diagnosed by fetal ultrasound and/or MRI examination, or by finding a discrepancy between the fetal phenotypic and chromosomal sex. This is a complex situation described by confusing eponyms such as 'intersex', 'hermaphrodite' and 'pseudohermaphrodite', terms that only add to parental confusion and anxiety.

A consensus statement recommended using the term 'disorder of sex development' (DSD), as 'a generic definition encompassing any problem noted at birth where the genitalia are atypical in relation to the chromosomes or gonads'[20], rather than a descriptive definition. According to this consensus statement the sex chromosome is used as a prefix to define the category of DSD. Thus, what used to be called male pseudohermaphroditism is now called XY DSD, what used to be called female pseudohermaphroditism is now known as XX DSD and true hermaphroditism is now called ovotesticular DSD. The category to which the disorder belongs is thus a combination of the chromosomal sex, phenotypic sex and gonadal sex. Prenatal diagnosis and accurate categorization of the DSD is complicated by the ultrasound resolution and radiographer's expertise in detecting accurately the phenotypic and gonadal sex and

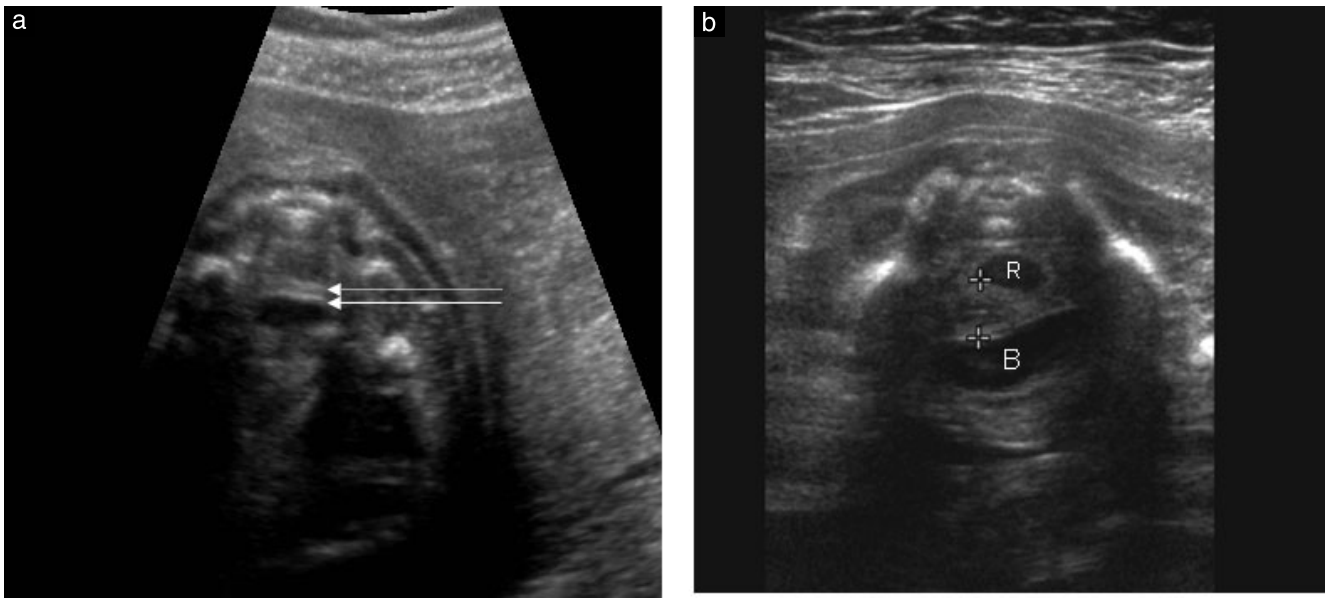

Figure 3 Assessment of internal genitalia and rectovesical interspace. (a) Male fetus at 24 weeks, showing posterior serosal surface of the bladder (lower arrow) and anterior serosal surface of the rectum (upper arrow); the interface is flat and the distance is 1 mm, consistent with a male fetus. (b) Female fetus at 25 weeks, showing uterus (between calipers) with a rectovesical interspace of 6.7 mm consistent with a female fetus. Note the rectovesical interspace produces a convex indentation into the posterior aspect of bladder wall due to the anterior convex contour of uterus. The endometrium is visualized as a thin echogenic line centrally. B, bladder; R, rectum.

the fact that determination of the chromosomal sex can only be done by an invasive procedure (chorionic villus sampling (CVS), amniocentesis or cordocentesis), with each procedure carrying its own risk for miscarriage or premature delivery (depending on gestational age at the time of the procedure). Thus, the chromosomal sex is not always readily available. Furthermore, unlike other fetal abnormalities, the most severe abnormality can look normal, though of the opposite sex, and may therefore go undetected; for example, severe masculinization of the female external genitalia, as in 21 hydroxylase deficiency which results in Prader 4, will be indistinguishable from the normal male external genitalia, while severe incomplete masculinization of the male external genitalia, as seen in androgen insensitivity syndrome, will appear like normal female external genitalia. It is therefore important to assess fetal DSD in a stepwise fashion to obtain as much information as possible and make the most likely diagnosis in order to enable the woman to make an informed decision regarding the outcome of the pregnancy.

Step I: describing genital abnormalities

Genital abnormalities can be diagnosed by fetal ultrasound and/or MRI examination from around 13–14 weeks' gestation, once development of the fetal external genitalia is complete[21]. They may also be noted when there is a discrepancy between the fetal phenotypic (detected by ultrasound examination) and chromosomal (detected by CVS or amniocentesis) sex. Incomplete masculinization of the external genitalia will be suspected prenatally if the fetal ultrasound/MRI examination shows abnormal phallic structure (absent, short or abnormal shape) and/or scrotum (absent or bifid) with absent or undescended testes later in the pregnancy or a discordance between the assessment of external fetal gender and internal fetal gender by assessment of the pelvic organs and the distance between the anterior wall of the rectum and the posterior wall of the bladder[1]. Masculinization of female external genitalia will be suspected when the fetal ultrasound/MRI shows an enlarged phallic structure and abnormal/fused labia instead of a scrotum, with identifiable uterus or a relatively large rectovesical distance.

Step II: non-isolated vs. isolated DSD

Once fetal genital abnormalities have been identified it is important to look for other fetal abnormalities to determine whether the genital abnormality is isolated. When genital abnormalities are associated with other, non-genital detectable abnormalities they are more likely to be the result of chromosomal abnormalities, single gene disorders or teratogen exposure (Table 1). They are less likely to be the result of a multifactorial condition or single gene disorder affecting sexual differentiation (*SF1* gene mutation, mutation in the *MIF* gene or its receptors), abnormal metabolization (5α reductase deficiency) or overproduction of androgens (congenital adrenal hyperplasia (CAH)) or abnormal response to male hormones (androgen insensitivity). However, since fetal ultrasound does not detect all fetal abnormalities, what is thought prenatally to be an isolated genital abnormality may turn out, postnatally, to be associated with other abnormalities.

Table 1 Conditions associated with non-isolated disorders of sex development

Chromosome abnormalities
Trisomy 13
Trisomy 18
Triploidy
del(4)(p16.3)
del(11)(q23.3)
del(13)(q33.2)
Single gene disorders
Fraser syndrome (AR)
Smith–Lemli–Opitz syndrome (AR)
Fryns syndrome (AR)
Carpenter syndrome (AR)
McKusick–Kaufman syndrome (AR)
Robinow syndrome (AR and AD)
Noonan syndrome (AD)
CHARGE syndrome (AD)
Frasier syndrome (AD)
Cornelia de Lange (AD)
ATRX syndrome (X-linked)
Aarskog syndrome (X-linked)
Imprinting disorders
Prader–Willi syndrome
Associations
CHARGE association
EEC association (epispadias, bladder exstrophy, cloacal exstrophy, OEIS)
MURCS association

For single gene disorders, mode of inheritance is indicated in parentheses (AD, autosomal dominant; AR, autosomal recessive).

Step III: pregnancy history

The pregnancy history should include information regarding maternal diseases resulting in virilization (hirsutism, excessive cystic acne and enlarged clitoris due to maternal androgen-producing tumors or placental aromatase deficiency) or maternal exposure to androgens, endocrine disrupters (phenytoin, aminoglutethimide) or teratogens (pesticides, isotretinoin)[22]. The results of the screening for Down syndrome should be reviewed to check whether the maternal serum unconjugated estriol level is low, which is typical in fetal conditions such as Smith–Lemli–Opitz syndrome, CAH and placental aromatase deficiency as well as chromosomal abnormalities such as trisomy 18. Furthermore, high alpha-fetoprotein and human chorionic gonadotropin and low pregnancy-associated plasma protein-A levels may indicate placental insufficiency, known to be associated with a higher incidence of hypospadias in male fetuses[23].

Step IV: family history

A three-generation family history using standardized pedigree symbols[24] should be obtained. Information regarding family members with genital or other abnormalities, recurrent miscarriages, stillbirths, mental retardation and inherited conditions should be obtained. A family history of consanguinity can suggest recessive disorders, such as CAH, disorders of androgen biosynthesis and Fraser syndrome. It also increases the risk for multifactorial conditions such as hypospadias. A family history of childless or amenorrheic females points towards the possibility of androgen insensitivity and unexplained infant death raises the possibility of salt-losing CAH. Similar abnormalities in sibs or other family members should be taken into consideration when the fetal ultrasound examination and other investigations are being done, looking for recurrence.

Step V: laboratory investigation

The initial investigation of fetal genital abnormalities should include chromosome analysis as well as microarray analysis, if indicated. According to the fetal ultrasound findings, the amniotic fluid can be checked for additional hormones, including 17-hydroxy-progesterone, testosterone, androstenedione, 11-dexoycortisol and 7-dehydrocholesterol[2]. Mutation analysis for specific conditions based on the family history and/or the fetal ultrasound findings can be done using fetal DNA for conditions such as 21 hydroxylase deficiency, 5α reductase deficiency or *SRY* mutation. However, mutation analysis is a lengthy procedure when no specific mutation is analyzed based on family history, and the results may only be available late in the pregnancy or after delivery. Since in most cases further investigation will be required after delivery, an attempt should be made to obtain a piece of cord as well as a cord blood sample, stored in EDTA and sodium heparin tubes, to minimize blood drawing from the newborn.

Step VI: compiling information and differential diagnosis

Non-isolated DSD indicates the possibility of maternal exposure to teratogens, fetal chromosome abnormalities and single gene disorders or may indicate sporadic conditions such as cloacal dysgenesis and omphalocele–exstrophy of the bladder–imperforate anus–spine abnormality (OEIS) (Table 1).

Most cases with DSD detected prenatally have isolated genital abnormalities and since the same phenotype can result from various conditions, further investigation in the form of amniocentesis for chromosome analysis and amniotic fluid hormonal studies should be offered to the woman if she wishes to obtain precise information regarding the etiology and thus the prognosis.

XX DSD

The fetal abnormalities in the XX DSD group of patients are, in most cases, the result of intrauterine exposure to high levels of androgens. This can be extrinsic and due to maternal consumption or production of androgen, or intrinsic and due to placental aromatase deficiency or fetal CAH[22,25,26]. The prenatal ultrasound findings include enlarged phallic structure (clitoris) and redundant/incompletely separated or fused labia majora. More than 90% of these cases are the result of

CAH and more than 90% of these are due to 21 hydroxylase deficiency. In cases with CAH the fetal adrenal glands can be visualized in the third trimester as being enlarged in volume and with a discoid rather than triangular shape, with a large hypoechogenic cortex[27]. Other, although much less common, causes of CAH are 11β-hydroxylase deficiency and 3β-hydroxysteroid dehydrogenase deficiency. Of note, the extreme cases (Prader Stage 4) with complete labial fusion and urogenital opening at the base of the phallic shaft can resemble 46,XY DSD or isolated hypospadias in a male. Thus, in the absence of known fetal karyotype, one of the most important ultrasound findings to differentiate XX DSD from XY DSD is the direct visualization of the uterus or appropriate measurement of the distance between bladder and rectum, utilizing the technique established by Glanc *et al.*[1]. Consideration of CAH is crucial as it may represent a life-threatening condition postnatally which requires early neonatal diagnosis and treatment. Furthermore, in affected families, early prenatal diagnosis will allow maternal prenatal treatment by dexamethasone to reduce genital virilization in affected female fetuses[28].

XY DSD

This group of patients is more complicated regarding the etiology and postnatal treatment. It is the result of incomplete masculinization (or undervirilization) of the male external genitalia. The first step in making the diagnosis is determining the chromosomal sex (46,XY) and the existence of a normal *SRY* gene. In the absence of fetal karyotyping, visualization of testes by fetal ultrasound is crucial in making the diagnosis and can distinguish these cases from XX DSD. It can be the result of: (1) abnormalities in testicular formation due to *SRY* gene mutation or deletion (other rare causes are *DAX-1* duplication and *SF-1* mutations), sex chromosome abnormalities (45,X/46,XY mosaicism), testicular regression syndrome and vanishing testes syndrome (of unknown etiology); (2) abnormal production of testosterone due to absence of luteinizing hormone or lack of testicular response to pituitary hormones; (3) abnormal synthesis of testosterone due to testicular enzyme deficiency (steroidogenic acute regulatory protein deficiency, 3β-hydroxysteroid dehydrogenase, 17β hydroxysteroid dehydrogenase or 17 lyase deficiency); (4) abnormal conversion of testosterone to DHT in the external genitalia (5α reductase deficiency); (5) abnormal/lack of response to testosterone (partial or complete androgen insensitivity). The absence of testes, testosterone deficiency, DHT deficiency and complete androgen insensitivity cannot be distinguished prenatally from normal female genitalia and if the fetal karyotype is not known these conditions will not be suspected prenatally.

Other conditions associated with DSD which can be detected prenatally

Hypospadias. The estimated prevalence of hypospadias is 1 : 500 live male births[29]. The condition is classified according to the meatal location, which also reflects the severity. In first-degree (or glandular/coronal) hypospadias, the urethral opening is below the penis tip, in second-degree hypospadias the urethra opens along the penile shaft and in third degree (perineal) hypospadias, the most severe form which occurs in 20% of cases, the urethral opening is at the penoscrotal junction or below the penis. In second- and third-degree forms the penis has a ventral curvature due to chordee, caused by atresia of the corpus spongiosum distal to the urethral meatus, which facilitates the prenatal diagnosis. Some of these cases show a ventral urinary stream which is also characteristic of the condition. Some cases with severe hypospadias are diagnosed initially as female genitalia due to the downward orientation of the genital tubercle at 13–15 weeks, and the observation of penile shaft and scrotum later in the pregnancy. Meizner *et al.*[30] described the 'tulip sign', in cases with severe hypospadias, a sonographic finding caused by the severe curvature of the penis in association with penoscrotal transposition due to a bifid scrotum. 3D ultrasound can help in making the diagnosis of hypospadias by showing the downward direction of the penis. An association was reported recently between hypospadias and intrauterine growth restriction (IUGR), which may indicate a placental role in the formation of the male external genitalia. Thus, hypospadias should be sought in cases with IUGR (Figure 4).

The direction of the fetal phallus results in a different angle of micturition which can help in sex determination. Boopathy Vijayaraghavan[31] reported visualization of fetal micturition in males and females, using both gray-scale and color Doppler sonography. In 19 of 21 male fetuses and in five female fetuses with bilateral pyelectasis, micturition was observed with visualization of urinary bladder contraction, slight fluid distension of the urethra and a urinary stream from the external urethral meatus being ventral in females and caudal in males. In the three male fetuses with hypospadias (with variable closure of the urethra along the ventral phallus), a ventral jet of urine was visualized, rather than the jet originating from the tip of the penis.

Exstrophy-epispadias complex (EEC). EEC consists of a spectrum of genitourinary malformations including epispadias, bladder exstrophy, exstrophy of the cloaca and OEIS. This complex is rare, with a prevalence at birth of 1/30 000 for bladder exstrophy and 1/200 000 for exstrophy of the cloaca and OEIS, and is more common in males than in females. EEC results from mechanical disruption of the cloacal membrane, with the severity of the malformation being determined by the timing of the rupture. The etiology is unknown and most cases seem to be multifactorial. EEC may be detected prenatally by ultrasound, the most common finding being non-visualized fetal bladder with the umbilical arteries running alongside a mass in the abdominal wall, below the insertion of the umbilicus, in the presence of normal amniotic fluid volume and abnormal external genitalia and, in OEIS, in the presence of omphalocele and lower spine segmentation[32] (Figure 5).

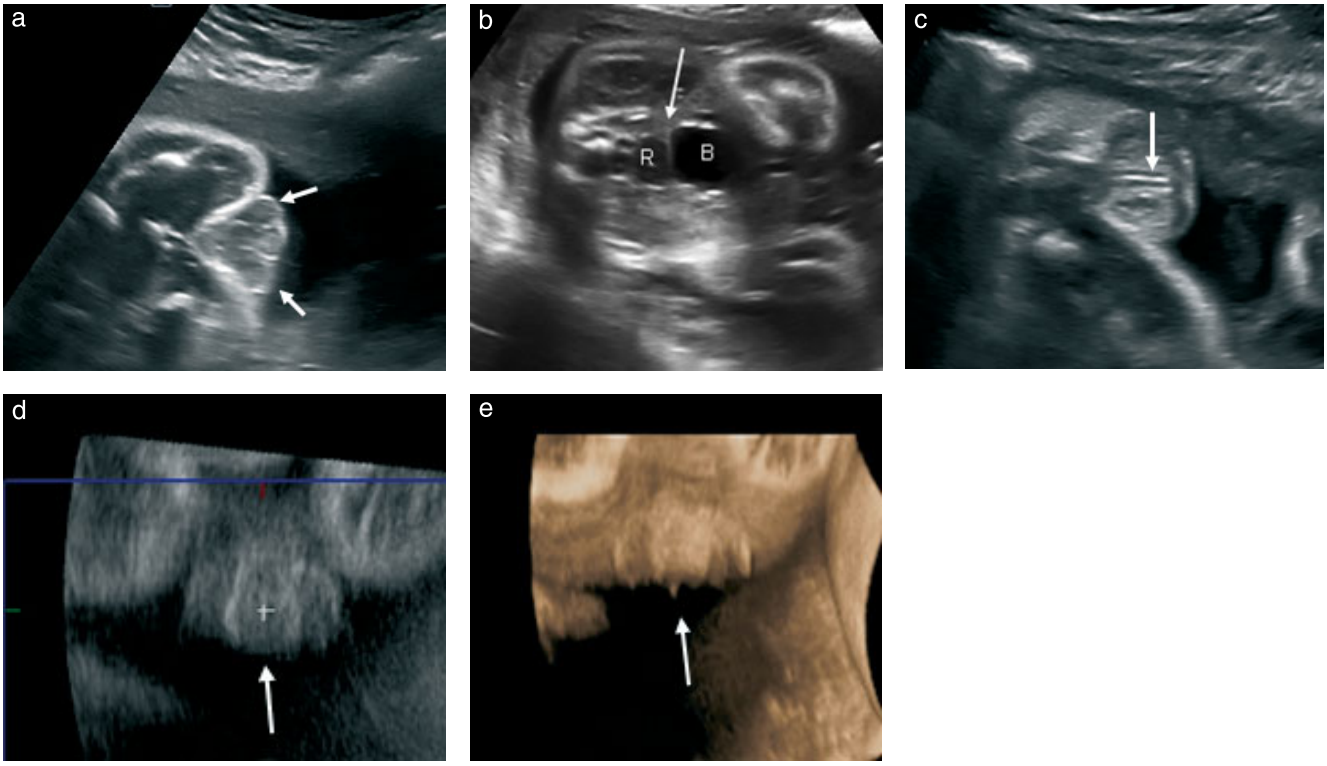

Figure 4 Imaging in a case of hypospadias at 29 weeks' gestation in a male fetus with severe early onset intrauterine growth restriction. Addition of an internal genitalia view in combination with the pregnancy history helped to confirm male gender. At delivery, micturition occurred at the penoscrotal junction, consistent with Grade 3 hypospadias with penoscrotal transposition. (a–c) Transverse ultrasound images demonstrating ambiguous genitalia. (a) Three structures create the 'tulip' sign, with the short penile shaft nestled between the bifid scrotum (arrows). (b) The thin echogenic line (arrow) is the short rectovesical distance creating a flat interface with the bladder (B) consistent with male internal genitalia. R, rectum. (c) The urethra is visualized as an echogenic line (arrow) ending along the base/shaft of the short penis, consistent with hypospadias. (d) Coronal reconstruction image demonstrating the micropenis with bulbous ending and downward direction between the bifid scrotum (arrow). (e) Volume-rendered coronal image demonstrating downward direction of the micropenis between the bifid scrotum (arrow).

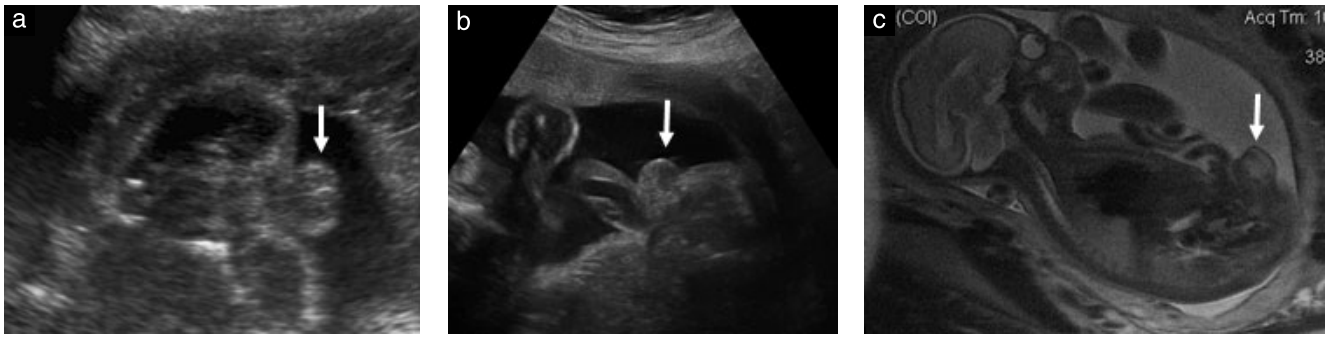

Figure 5 Imaging in a case of a male with bladder exstrophy initially diagnosed at 23 weeks' gestation on ultrasound. (a) Transverse ultrasound image demonstrating ambiguous genitalia (arrow) with no phallus identified. (b) Parasagittal ultrasound image demonstrating a soft tissue mass below the umbilical cord (arrow) with no bladder identified. (c) Sagittal magnetic resonance image at 27 weeks. Inferior to the low umbilical cord is a homogeneous soft tissue mass (arrow) in keeping with bladder exstrophy, and immediately below is the scrotum. No phallic structure identified.

An approach toward the differential diagnosis of fetal DSD based on ultrasound findings and chromosome sex is presented in Figure 6.

PREGNANCY AND DELIVERY

The prenatal finding of fetal genital abnormalities is a medical and psychosocial emergency and should be investigated and presented to the family by a multidisciplinary team that includes perinatologists, endocrinologists, medical geneticists, neonatologists, urologists, psychologists and social workers with expertise in this field. Information regarding the abnormality, the differential diagnosis and postnatal treatment and prognosis should be discussed. It should also be made clear that ultrasound cannot identify all abnormalities and that

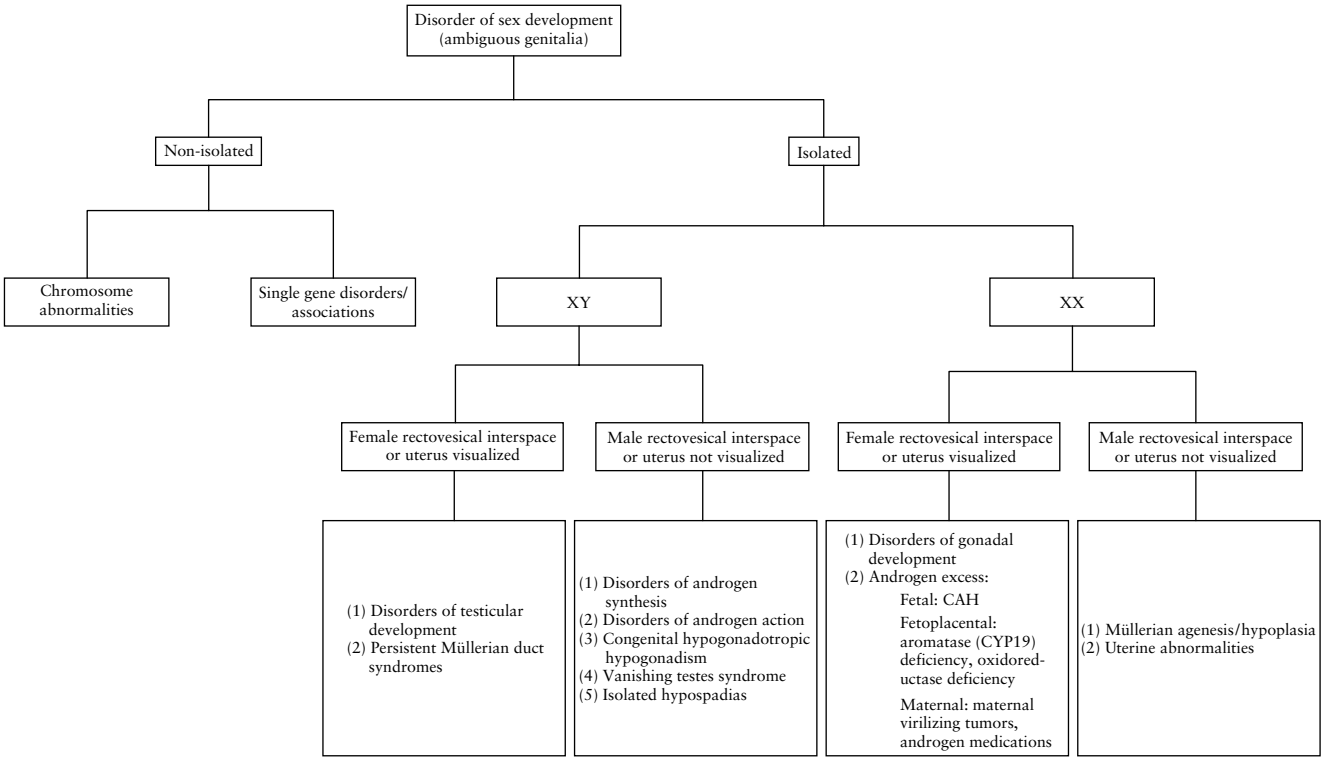

Figure 6 An approach toward the differential diagnosis of fetal disorders of sex development based on ultrasound findings and chromosome sex.

the findings at delivery may differ from the prenatal ultrasound description. The embryology of normal and abnormal external genitalia should be explained and it should be emphasized that there is nothing that the parents did or did not do that caused the abnormality. The discussion should be targeted to meet the parents' intellectual capabilities, taking into consideration cultural and religious background. Parents should be presented with the options of continuing the pregnancy or terminating the pregnancy. They can also decide to have further investigation to define the etiology prior to making a decision or to continue the pregnancy and have maternal prenatal treatment, if applicable. The parents should be given time to adjust before making this decision[33]. The issue of a possible need to defer newborn gender determination and naming should be discussed.

Prenatal treatment options are limited and include resection in the case of a maternal androgen-secreting tumor, taking into consideration the risk for premature delivery, and maternal treatment with dexamethasone in cases of XX DSD due to CAH, to reduce prenatal clitoral growth. If parents decide to undergo further prenatal investigation the first and most important study is determination of the fetal karyotype. A cell-free amniotic fluid sample should be frozen for possible further investigation, amniocytes should be banked and DNA should be extracted from amniocytes to allow further investigation if needed.

The finding of DSD has no impact on the mode of delivery; however, if the decision is made to carry the pregnancy to term, the team involved in prenatal care should continue postnatal care regarding gender determination and, if needed, treatment. A piece of cord should be obtained to establish a cell line for further investigation and the newborn's cord blood should be obtained for hormone studies as well as for DNA extraction (in an EDTA tube) and chromosome analysis (in a sodium heparin tube) to minimize the amount of blood that needs to be drawn from the newborn.

The initial investigation should be guided by the findings on chromosome analysis and physical examination and should usually include laboratory studies (determination of sex chromosomes, if not done prenatally, and determination of levels of 17-hydroxyprogesterone, serum electrolytes and serum glucose) as well as neonatal imaging: ultrasonography of the abdomen and pelvis to determine the presence of gonads, a uterus, and/or a vagina (neonatal adrenal glands may be assessed at this time; abdominal/pelvic MRI may play a role in inconclusive or challenging cases); retrograde urethrogram and/or cystoscopy/vaginoscopy; and possibly laparoscopic visualization to delineate the internal reproductive organs/anatomy including gonadal biopsy for histological/genetic evaluation. Ultrasound is the first line imaging investigation for establishing the presence of normal uterus and ovaries/testes. If the testes are undescended, inspection of the inguinal region and along the iliopsoas muscle should be performed. The adrenal glands should be assessed for hypertrophy/hyperplasia. In more complex forms of DSD, perineal assessment for the presence of fistulous tracts may be required.

CONCLUSION

Abnormal genitalia can be diagnosed prenatally by the ultrasound finding of abnormal external genitalia or discrepancy between fetal chromosome and phenotypic sex. Detection of abnormal genitalia is possible only in the second and third trimesters since external genitalia development is complete only after 12 weeks' gestation. The first step should be to determine if the genital findings are isolated or associated with other abnormalities. Fetal ultrasound measurement of the rectovesical distance and/or identification of the fetal uterus are important in directing the investigation and determination of the fetal karyotype as well as amniotic fluid endocrine studies, if indicated, are of utmost importance if parents decide to undergo amniocentesis. Information regarding the fetal abnormalities detected, possible postnatal management and prognosis as well as the prenatal options should be presented to the parents by a multidisciplinary team that includes perinatologists, endocrinologists, medical geneticists, neonatologists, urologists, radiologists, psychologists and social workers with expertise in this field. Throughout the process of investigation and counseling, parents should feel secure that they will be supported by the team regardless of the decision they make.

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