



Role of Transperineal Ultrasound (TPUS) in Children with Ambiguous Genitalia

Krithika Rangarajan¹ Manisha Jana¹ Nagesh Wadgera¹ Arun Kumar Gupta¹ Minu Bajpai²
Devasenathipathy Kandasamy¹

¹Department of Radiology, All India Institute of Medical Sciences, New Delhi, India

²Department of Paediatric Surgery, All India Institute of Medical Sciences, New Delhi, India

Address for correspondence Manisha Jana, MD, DNB, FRCR, Department of Radiology, All India Institute of Medical Sciences, Room 81C, New Delhi, India (e-mail: manishajana@gmail.com).

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Abstract

Objectives Accurate delineation of anatomy in children with ambiguous genitalia early in life is important. This commonly involves conventional fluoroscopic genitogram (traumatic to the child) and magnetic resonance imaging (MRI) examination (involves sedation). In this study, our objectives were twofold: (1) to describe the findings on transperineal ultrasound (TPUS) in normal children and (2) to describe the findings on TPUS in children with ambiguous genitalia and correlate them with conventional genitogram.

Materials and Methods TPUS was prospectively performed in 10 children without genital ambiguity (5 girls and 5 boys). Subsequently, 15 consecutive children having disorders of sex differentiation (DSDs) with genital ambiguity underwent TPUS. The presence or absence of müllerian structures was documented. Of these patients, 14 also underwent conventional genitogram as a part of routine evaluation. The gold standard was established either by comparison with surgical findings (in patients who underwent surgery) or by comparison with a combination of findings on genitogram and transabdominal ultrasound in patients who did not undergo surgery.

Results In all normal children, lower urogenital tracts could be clearly delineated on TPUS. Out of the 15 children with ambiguous genitalia, TPUS could establish the presence/absence of müllerian structures in 14. This was concordant with findings on conventional genitogram/surgery. In one patient, müllerian structure was missed on TPUS but demonstrated on genitogram. In two children, TPUS showed the müllerian structure, which was not seen on genitogram. When both the controls and the cases were combined, TPUS had an accuracy of 95% and specificity of 100% in the detection of müllerian structures.

Conclusion TPUS is feasible and accurate in demonstration of lower urogenital tract anatomy in children with DSDs having ambiguous genitalia. It can be performed without sedation, and is suitable for use as a screening modality in children with ambiguous genitalia.

Keywords

- ▶ ambiguous genitalia
- ▶ disorders of sex development
- ▶ genitogram
- ▶ müllerian structures
- ▶ perineum
- ▶ transperineal ultrasonography

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Introduction

Ambiguous genitalia in a child are among the most distressing conditions for a parent. It has profound social and psychological consequences. Accurate identification of anatomy is imperative to appropriate gender assignment. The earlier the gender is assigned, the easier the psychological rehabilitation of the child. Management of these children requires a multidisciplinary team including a geneticist, pediatric surgeon, and a pediatric radiologist.

The pediatric radiologist has an important role to play in the accurate identification of anatomy of the internal genital tract of the child. While transabdominal ultrasound is often the first imaging done to identify the gonads (both in male and female children), magnetic resonance imaging (MRI) is often used as a problem-solving tool for evaluation of gonads and internal genital tracts. Nevertheless, conventional genitogram has its own role as the first-line imaging for delineation of the internal genital tracts.

Conventional genitogram is a much widely used technique for evaluation of lower urogenital tract. On the contrary, transperineal ultrasound (TPUS) is not commonly used in evaluation of lower genital tract anatomy in children with disorders of sex differentiation (DSDs) having ambiguous genitalia. TPUS is not a new imaging technique; rather, it has been in practice for the last few decades, both in children and in adults. In children, utility of TPUS is manifold: anorectal malformations, müllerian anomalies, and DSDs; and lower urethral pathologies in male children.¹⁻⁸ The technique is also gaining popularity in pelvic floor studies and incontinence imaging.⁹⁻¹¹ We undertook this study to describe the findings on TPUS in normal children and subsequently to perform TPUS in children with ambiguous genitalia and compare these findings with conventional genitogram.

Materials and Methods

Study Design

This was a prospective study performed with ethical clearance from the institutional review board. Informed consent was obtained from the parents of all the children.

Study period: 6 months (July 2016 to December 2016).

Inclusion Criteria

The inclusion criteria for the study were:

- *Cases:* All children scheduled for genitogram during the study period.
- *Normal/controls:* All children scheduled for a micturating cystourethrogram during the first month of the study period.

Exclusion Criteria

Children were excluded for the following reason: parental refusal to consent.

Method

For the first 1 month, only the controls were recruited. TPUS was performed in all of them. For the next 5 months, only the

cases were recruited. This was done to standardize the TPUS technique and optimize observer training in interpretation of TPUS. All of the cases underwent genitogram and TPUS on the same day.

Genitography Technique

Genitography is routinely performed at our institution in children with ambiguous genitalia.

Under proper aseptic precautions, a careful observation of the number and location of the perineal openings were performed. In cases of male child with hypospadias, female child with clitoromegaly, or common urogenital opening, contrast study was performed from the anterior opening (urethra/common channel). The child was positioned in a lateral/steep oblique position. A thin intravenous cannula or hub of an infant feeding tube of smallest caliber (5 Fr) was placed; iodinated contrast was injected and the anatomy was studied under fluoroscopic guidance. It was emphasized that while injecting contrast, the urethra was properly in profile, without any kink/foreshortening. In all cases, fluoroscopic cine-loop images and videos were saved rather than direct exposure. Images were acquired in true lateral position, to avoid any overlap. Another additional frontal image was acquired whenever a müllerian structure was visualized on lateral profile, in order to assess the number and morphology and to evaluate whether it is in the midline. The presence or absence of müllerian structures, degree of development of the müllerian structure (whether fully formed or a small remnant), and length of the common channel were noted.

In the case of cloaca, the hub of a syringe was used for contrast study to avoid any contrast leakage from the wide external opening. For neonates, a syringe of 10-mL capacity was used, and for older infants/children, 20-mL syringe hub was used.

TPUS Technique

TPUS was performed with a high-frequency (7–12 MHz) linear array transducer. With a generous use of ultrasound jelly, images were acquired in the sagittal and axial plane. On the sagittal plane, the following parameters were noted: the presence or absence of müllerian structure, the presence and length of the common channel, and the distance from the urinary bladder (UB) neck to the junction of the müllerian structure with the urethra (measured in mm). The presence or absence of uterus and its appearance (hypoplastic/well-formed) was also noted.

Image Analysis

Both TPUS and genitogram were analyzed for the following:

- Presence/absence of müllerian structure.
- Presence of a fully formed uterus.
- Visualization and length of the common channel.

The TPUS reader was blinded to results of other investigations and findings of genitogram, and vice versa.

Establishment of Gold Standard

The gold standard was established by comparison with surgical/laparoscopic findings in five patients who

underwent surgery. In the rest, a combination of the findings on genitogram and transabdominal ultrasound was considered the gold standard.

Results

There were 10 control children (5 males and 5 females) without genital ambiguity. The age group of both cases and controls ranged between 2 days and 2 years (mean ages were 5 months in cases and 8 months in control group). The older children had cloacal malformation and congenital adrenal hyperplasia (CAH).

In the control group, on TPUS, three tracts could be seen in all female children corresponding to the urethra, vagina, and the anal canal (►Fig. 1). TPUS of all male children showed only two tracts: the urethra and the anal canal (►Fig. 2). Each tract was seen as a hypoechoic wall with a strip of fluid (anechoic) and echogenic mucosa. Intraluminal foci of air were seen as echogenic areas.

Of the 15 children with ambiguous genitalia, 14 underwent both TPUS and genitogram. One child was only 2 days old and therefore only underwent TPUS, as the parents did not give consent for performing a genitogram.

Out of 15 children with ambiguous genitalia, 6 were being reared up as male and referred for evaluation of hypospadias with or without undescended/absent testis, 4 were being reared up as female and had enlarged clitoris with suspicion of CAH, 3 had a common cloaca, and 2 patients had a common urogenital opening with varying degrees of labial fusion (►Table 1). We divided the patients (according to the clinical findings) into three subsets:

- Group 1: hypospadias.
- Group 2: CAH.
- Group 3: common urogenital sinus (UGS) or common cloaca. This group was further analyzed in two separate sets:
 - Common UGS.
 - Common cloaca.

In group 1 (hypospadias), all six children showed an external male appearance, with undescended impalpable testes. TPUS did not show müllerian structure in any of them (►Fig. 3). Genitogram revealed male-type urethra and no müllerian structure.

In group 2 (CAH), all four children had variable degree of clitoromegaly. On TPUS, three of these four children were

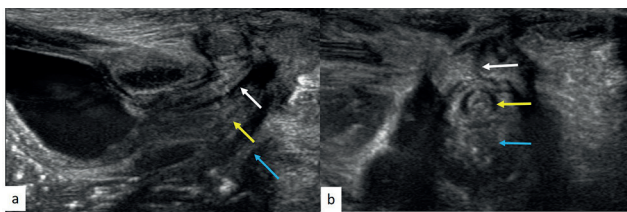


Fig. 1 Normal female anatomy on TPUS. TPUS in a female child without genital ambiguity clearly shows three tracts corresponding to the urethra (white arrow), vagina (yellow arrow), and anal canal (blue arrow) on (a) sagittal and (b) axial images.

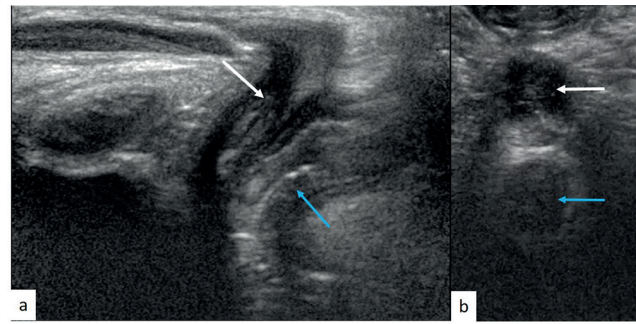


Fig. 2 Normal male anatomy on TPUS. TPUS in a male child without genital ambiguity shows only two tracts corresponding to urethra (white arrow) and anal canal (blue arrow) on (a) sagittal and (b) axial images.

Table 1 Final diagnosis of the cases

Clinical diagnosis	Number of patients
Hypospadias (group 1)	6 (40%)
CAH (group 2)	4 (26.7%)
Common cloaca (group 3)	3 (20%)
Variation of labial fusion (group 3)	2 (13.3%)
Lower vaginal atresia with UGS	1
UGS only	1
Total	15 (100%)

Abbreviations: CAH, congenital adrenal hyperplasia; UGS, urogenital sinus.

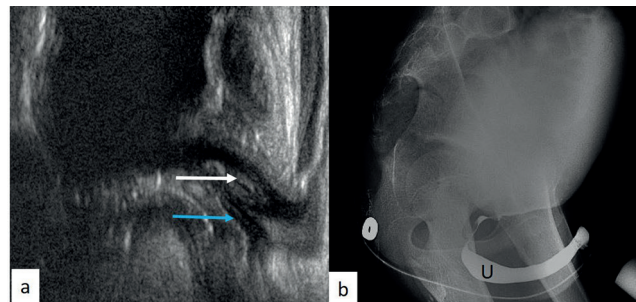


Fig. 3 A patient with hypospadias. (a) TPUS sagittal section (rotated 90 degrees for the sake of similarity in comparison with genitogram) shows only two tracts (urethra: white arrow; rectum: blue arrow) without müllerian structures. (b) Short male-type urethra (U) seen on genitogram without any müllerian structure.

seen to have three separate tracts corresponding to the urethra, vagina, and anal canal. The urethra and vaginal tract merged caudally; the junction was variable in different patients. TPUS was able to demonstrate the presence of uterus in all. Well-developed uterus and ovaries were also seen on transabdominal ultrasound in all.

However, in one patient, diagnostic quality images could not be obtained on TPUS. The images were interpreted as showing only two tracts: the urethra and anal canal. Müllerian structures/remnants were missed on TPUS in this patient.

All four of them showed well-developed müllerian structures with a cervical impression on genitogram. These children were proven to be CAH based on laboratory results.

In group 3 (common UGS or common cloaca), all the five patients presented with varying degrees of labial fusion or truly ambiguous external genital appearance. Of these children, two had common urogenital opening and three had common cloaca.

Of the two children with a common urogenital opening, both showed the presence of three tracts on TPUS. One revealed a blind-ending vagina without any uterus (►Fig. 4), and the other showed presence of uterus. Abdominal ultrasound confirmed the presence of uterus in this second patient. Genitogram was performed in only one child of this group, and it revealed presence of a blind-ending müllerian structure, concordant with the TPUS finding. The findings of TPUS were found concordant on surgery.

All three children with a common cloaca were operated early in life with a diversion colostomy and presented for evaluation of the urogenital tract. Of these, in one child an ovary was seen on transabdominal scan and a testicle was seen in the left scrotum on ultrasound (►Fig. 5). TPUS showed three tracts, and genitogram also revealed a well-developed uterus and vagina. This child was diagnosed as ovotesticular DSD. In two other children, fluid-filled structure was seen on TPUS in between the urethra and anal canal, but no müllerian structure was seen on genitogram. One of them was found to be an obstructed müllerian structure on surgery and another was found to be vaginal atresia (►Fig. 6).

The delineation of lower genital tract anatomy was separately evaluated for TPUS and genitogram. In delineating anatomy, the parameter assessed was presence or absence of müllerian structure. The findings were compared with the gold standard. The findings are summarized in ►Table 2.

The sensitivity of TPUS and genitogram (►Table 2) are 88.88 and 75%, respectively, with the specificity of 100% in both. Genitogram had lower sensitivity as it could not detect müllerian structures in two cases (one in obstructed müllerian structure and another in vaginal atresia).

The spectrum of appearance of müllerian structure is described in ►Table 3. TPUS could demonstrate müllerian structures in eight out of nine patients, whereas genitogram could do so in six out of eight patients (genitogram not performed in one patient of this group).

Length of Common Channel

The analysis of length of the common channel was performed in groups 2 and 3. On genitogram, the length of the common channel was measured from the junction (of the müllerian structure and the urethra) to the tip of the urethra. This measurement was not technically possible on TPUS due to nonvisualization of the entire extent in a single image/plane. On TPUS, the distance from the UB neck to the junction of the müllerian structure with urethra was measured (►Fig. 7).

Discussion

Children whose external genitalia do not fit the anatomical configuration of male or female external genitals are said to have ambiguous genitalia. To counter the use of terms that were considered potentially pejorative (such as intersex and sex reversal), the term “disorders of sex differentiation” (DSDs) has been proposed in the International Consensus Conference on Intersex.^{12,13}

According to the recent terminology, DSDs can be broadly summed up into three categories: 46, XY DSD (earlier known as male pseudohermaphroditism); 46, XX DSD (earlier known as female pseudohermaphroditism); and sex chromosome DSD (such as 45, X or 47, XXY).¹⁴

However, it has to be remembered that not all children with DSD present with an ambiguous genitalia. In some cases, the external genitalia may be that of male/female; yet the chromosomal sex may not be conforming to the external appearance.^{14,15} An example of such situation is complete androgen insensitivity syndrome (AIS), wherein the patient is phenotypically a female (no genital ambiguity) and bears a chromosomal sex of 46, XY. *The presence of an ambiguous genitalia points toward DSD, but not all DSDs have ambiguous genitalia.*

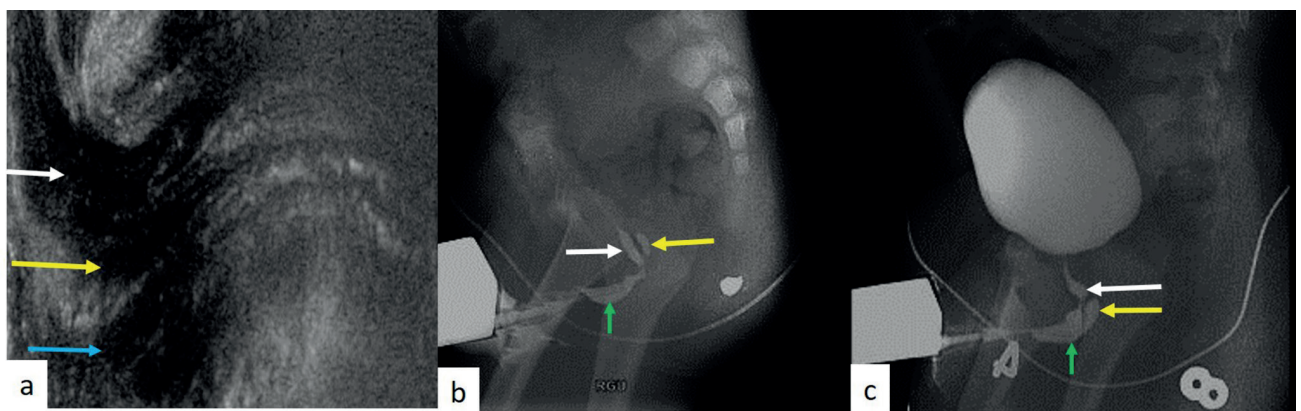


Fig. 4 (a) Common urogenital sinus. TPUS sagittal section (rotated 90 degrees for the sake of similarity in comparison with genitogram) shows the presence of a tract (with upward tapering) representing a blind müllerian structure (yellow arrow). No uterus was seen on TAS of the same patient. (b, c) Genitogram of the same child showed the presence of a müllerian structure without a cervical impression (yellow arrow). Urethra is shown with white arrow, anal canal with blue arrow, and common channel with green arrow.

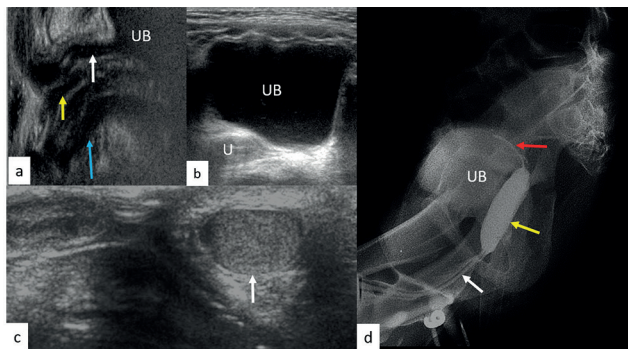


Fig. 5 Ovotesticular DSD. (a) TPUS sagittal section (rotated 90 degrees for the sake of similarity in comparison with genitogram) shows presence of three tracts: urethra (white arrow), vagina (yellow arrow), and anal canal (blue arrow). (b) Uterus (U) is seen on axial transabdominal ultrasound. (c) Testicle (arrow) is also seen in the left scrotal sac. (d) Genitogram shows catheter in the urethra (white arrow), contrast distending the vagina (yellow arrow), and a contrast streak in the uterine cavity (red arrow).

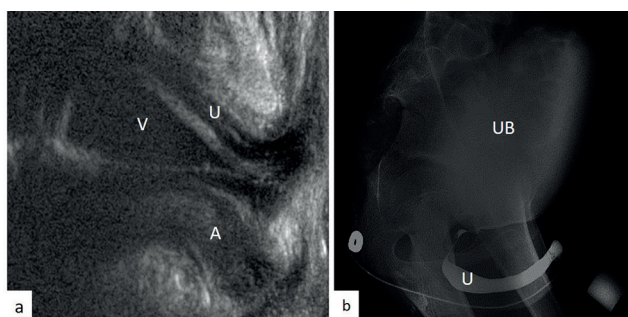


Fig. 6 (A and B) Distal vaginal atresia. (A) TPUS sagittal section (rotated 90 degrees for the sake of similarity in comparison with genitogram) shows the presence of a round-topped fluid filled structure (V) in between the urethra (U) and anal canal (A). (B) Genitogram reveals a male type urethra (U); but does not show the presence of any müllerian structure. (B) On surgery, distal vaginal atresia was seen.

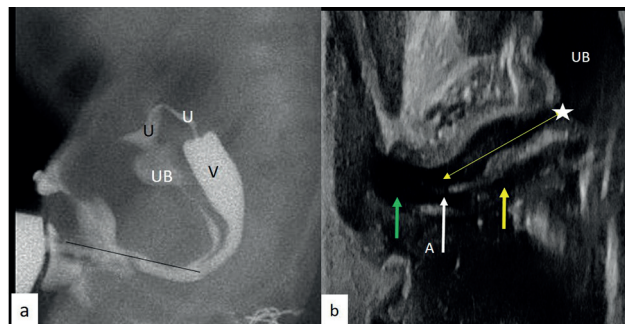


Fig. 7 Ambiguous genitalia in congenital adrenal hyperplasia. Measurement of common channel length/its surrogate. (a) Measurement on genitogram. The length is shown with black line. It is measured from the junction (of the müllerian structure with the urethra) to the tip of the urethra. U, endometrial cavity; V, vagina. (b) Measurement on sagittal TPUS image rotated 90 degrees for the sake of similarity in comparison with genitogram. Distance (double-sided arrow) measured from the bladder neck (asterisk) till the point of junction of both the channels (white arrow). Common channel is shown with a green arrow and vagina with yellow arrow. A, anal canal; UB, urinary bladder.

Common cause of 46, XX DSD who have ambiguous genitalia is CAH (incomplete virilization type). 46, XY DSDs, which commonly have ambiguous genitalia, include incomplete AIS and 5 α -reductase deficiency. Ovotesticular DSD is a rare disorder presenting with ambiguous genitalia and variable combination of abnormal gonadal tissue (ovary/testis/ovotestis).¹⁶ The chromosomal sex in ovotesticular DSD is also variable.

Genital ambiguity is most often recognized in the neonatal period. There may be frank genital ambiguity in the form of a common cloaca; the children present with undermasculinized male genitalia (micropenis, hypospadias, undescended testis) or virilized female genitalia (enlarged clitoris or with varying degrees of labial fusion or a mass in the

Table 2 Visualization of müllerian structures on genitogram and TPUS

	Clinical presentation	Visualization of müllerian structures on TPUS (n = 25, including 10 controls)	Visualization of müllerian structures on genitogram (n = 14)	Gold standard
Group 1	Hypospadias (n=6)	0	0	No müllerian structures
Group 2	CAH (n=4)	3 (75%)	4 (100%)	All had well-developed müllerian structures
Group 3	Common cloaca/ common urogenital opening (n=5)	5 (100%)	2 (out of 4) ^a (50%)	All had some müllerian structures of varying degrees of development
Control (male)		0		
Control (female)		5 (100%)		
Total		8/25 (TP-8, TN-16, FP-0, FN-1)	6/14 (TP-6, TN-6, FP-0, FN-2)	

Abbreviations: CAH, congenital adrenal hyperplasia; TPUS, transperineal ultrasound; FN, false negative; FP, false positive; TN, true negative; TP, true positive; NPV, negative predictive value; PPV, positive predictive value.

Note: Müllerian structures on TPUS: sensitivity = 88.88%, specificity = 100%, PPV = 100%, NPV = 94.11%, accuracy = 95%. Müllerian structures on genitogram: sensitivity = 75%, specificity = 100%, PPV = 100%, NPV = 75%, accuracy = 85.71%.

^aGenitogram was not performed in one.

Table 3 Spectrum of müllerian structures on imaging ($n = 9$, excluding hypospadias group)

Appearance	No.	Detected on TPUS	Detected on genitogram
Well-formed uterus and vagina (4 CAH, 1 cloaca)	5	4	5
Poorly formed vagina, no uterus	3	3	1 ^a
Vaginal atresia, no uterus	1	1	0

Abbreviations: CAH, congenital adrenal hyperplasia; TPUS, transperineal ultrasound.

^aGenitogram was not performed in 1.

inguinal or labial region).¹³ Clinical evaluation should take into account the presence or micropenis versus clitoromegaly, palpable versus impalpable gonads, number of orifices in the perineum, and location of urethral orifices.¹⁶

Radiological investigation comes next, and common investigations advocated in clinical practice are pelvic US for gonads, genitogram for lower urogenital tract anatomy, and MRI as an additional/problem-solving modality for both internal genital organs and gonads.^{14,17-19} MRI has been found to be very useful in demonstration of gonads, differentiation of phallus from the enlarged clitoris, and the presence of some complex anomalies.²⁰⁻²² However, its cost and requirement of sedation/anesthesia remain main points of consideration, especially when used in infants/small children.

Identification of internal genital tract and gonads is an essential component of radiological evaluation. Radiological findings are considered together with karyotyping and hormonal assessment to reach a final diagnosis.²³ Genitogram involves placement of a small catheter in the urogenital opening and injection of an iodinated contrast agent. In combination with pelvic ultrasonography, it is very useful to delineate urogenital anatomy.²⁴ Being a fluoroscopic procedure, it involves irradiation to the gonads of these young children, in addition to being traumatic due to placement of the catheter. Also, a study by Vanderbrink et al on children with CAH reported that genitogram findings were seen to be inaccurate at demonstrating urogenital anatomy in up to 25% of cases.²⁵

In our institute, genitogram is routinely performed for children with ambiguous genitalia to help identify müllerian structures and to accurately delineate lower urogenital tract anatomy. In the case of the presence of a common channel, genitogram also helps measure the length of the common channel.

While both genitogram and MRI have their own imaging advantages, there is also a need for some imaging technique that can be quickly performed on a small child without sedation or without the risk of catheterization and ionizing radiation. TPUS perfectly fits in this situation as a first-line screening imaging tool in ambiguous genitalia. TPUS has been tried in this scenario by some investigators earlier, but no specific imaging guidelines regarding its utility in DSD are widely available.²⁶⁻²⁸

Our study demonstrated the ability of the technique to elucidate the anatomy of pelvic organs. In all the children without genital ambiguity, TPUS clearly demonstrated the tracts of the urethra, anal canal, and vagina in female children. In groups 1 and 2, both TPUS and genitogram were fairly accurate in delineating lower genital tract anatomy.

However, in group 3, TPUS was better than genitogram. This group consisted of complex anomalies such as cloaca, vaginal atresia, and obstructed müllerian structure. Genitogram could not demonstrate the müllerian structures when it did not communicate with the urethra. Across all the three groups, in terms of detection of müllerian structures, TPUS has higher sensitivity than genitogram (88.9 vs. 75%).

When both the controls and the cases were combined, TPUS had an excellent accuracy of 95% and specificity of 100% in the delineation of anatomy and detection of müllerian structures. TPUS also was accurate in assessing the degree of development of the müllerian structures. Moreover, all the children tolerated the noninvasive procedure of TPUS better than the invasive genitogram.

TPUS was inaccurate and missed müllerian structure in one child, where images of diagnostic quality could not be obtained. This was possibly because of the child's body habitus (large amount of labial fat). Also, unlike in genitogram where the length of the common channel (which is of great surgical importance) could be measured directly, this was not possible in TPUS. In an earlier study by Lindert et al, length measurement of the common channel was performed on TPUS in a way similar to usual genitographic measurement, and yielded reasonably good correlation with cystoscopic estimate of common channel length.²⁷ However, in our experience, this method did not result in a very accurate estimate of the common channel length. One explanation to this phenomenon may be the fact that they have children of higher age group compared with ours. Similar to our experience, in the case of vaginal atresia, TPUS was shown to be useful in congenital vaginal anomalies such as atresia in earlier studies as well.^{25,27}

Our study re-emphasized the utility of TPUS as a quick screening tool along with routine transabdominal ultrasonography in cases of DSD. ► **Fig. 8** describes a suggested simplified algorithmic approach to a child with genital ambiguity. It can be noted that to reach an etiological diagnosis, a combination of pelvic US and TPUS with karyotyping may be sufficient. This is because the etiological diagnosis mostly depends on the presence and morphology of müllerian structures and visualization of gonads. Both of the information can be provided by a combination of pelvic US and TPUS. Exceptions include streak gonads or undescended testes nonvisualized by US.

As a surrogate for the length of the common channel, the distance of the junction of both the tracts from the UB neck can be measured.

Moreover, TPUS has some distinct advantages over genitogram in terms of an absence of radiation exposure and being

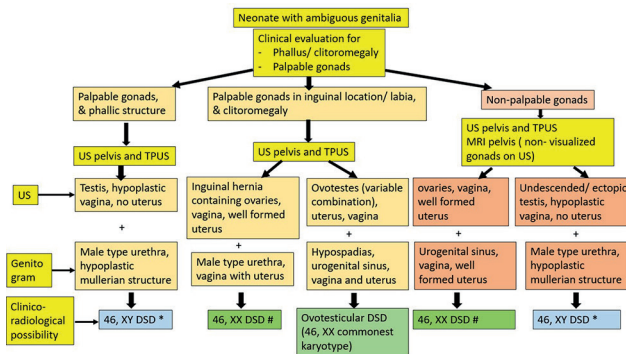


Fig. 8 Suggested clinical and imaging algorithmic approach to a child with genital ambiguity *Common etiologic possibility: partial AIS and 5 α -reductase deficiency. They can present with either palpable (descended) or nonpalpable (undescended/ectopic) gonads. #Common etiological possibility: CAH (partial virilization type). When associated with inguinal hernia containing ovaries, the gonads may be palpable. Degree of virilization is also variable.

less traumatic to the child. In comparison to MRI, TPUS is much cheaper and faster and does not require sedation.

Our study had some limitations in that the size of the study was very small (only 15 patients with genital ambiguity). Besides, we did not compare the TPUS images with MRI, which is widely used now for evaluation of such patients. A larger study that compares findings on TPUS with surgical findings as well as MRI would help further investigation of the modality.

To conclude, while genitogram remains the first and most preferred imaging technique for evaluation of lower genital tract anatomy, and MRI is ideal and is often used as problem-solving tool, TPUS can have its own niche indication as a quick screening tool for lower genital tract in children presenting with ambiguous genitalia.

Declaration of Patient Consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflict of Interest

The authors declare no conflict of interest.

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