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Case history

• Term neonate from Chuuk
• Predominantly female-appearing ambiguous genitalia
• Gonad palpable within the left labioscrotal fold
• Lab studies:
  – Persistently low sodium
  – High potassium
  – High chloride
  – DHT, FSH, ACTH elevated
  – LH within normal limits
Additional clinical history:

- 39w4d infant born to a 27-year-old G6P2 (SAB 3) mother
- Maternal laboratory values wnl
- Mother lives in shelter, poor PNC
- Maternal history of chewing tobacco and beetle nuts
- C/S for transverse lie
- Apgars 8 and 9, weight 2.8 Kg
- No genital anomalies noted at birth
- On DOL 10, phallus appeared larger and labioscrotal folds darker and more separate
Bilateral testicular biopsies (both with similar histologic appearance):

Low power: Thin, loosely organized tunica albuginea penetrated by branching seminiferous cords
Medium power: Tunica albuginea containing network of seminiferous cords
Medium power: Testicular parenchyma; closely packed seminiferous tubules containing immature Sertoli cells and germ cells.
Medium power; Tubular structures extending into the tunica albuginea
Diagnosis (histologic):

Bilateral gonadal (testicular) dysgenesis
• This patient’s pathology falls into the category of Intersex Disorders. More information is needed in order to provide accurate prognostic, management, and treatment information to the clinicians, surgeons, and parents.....
Intersex disorders

• Reflect a spectrum of phenotypic changes within a single genotype
• Reflect a spectrum of genotypic changes within a single phenotype
• Therefore, the diagnosis relies upon a combination of:
  – Clinical findings
  – Hormonal analysis
  – Gonadal histology
  – Chromosomal analysis
Additional findings for this patient:

- Karyotype: 46,XY
- FISH: Yp11.3 (SRY+)
- Laparoscopic findings:
  - Did not identify Müllerian structures
  - Testes seen in hernia sacs, bilaterally
Previous terminology of intersex disorders:

• 46,XY pure gonadal dysgenesis
  – Bilateral streak gonads
  – Complete sex reversal with unambiguous female genitalia

• Asymmetrical gonadal dysgenesis
  – One gonad displays more complete development and can be identified as ovary or testis (usually testis)
  – Other gonad is streak

• Mixed gonadal dysgenesis (MGD)
  – Individuals with 45,X/46,XY karyotype with testis and streak gonad
  – Patients with varying degrees of asymmetrical gonadal dysgenesis
    • Testicular differentiation with contralateral streak testis or streak gonad
    • Bilateral streak testes
    • Streak testis with streak gonad
    • Bilateral dysgenetic testes
    • Possible karyotypes: 45,X/46,XY, 45,X/47,XYY, 46,XY, others

Previous terminology of intersex disorders:

- **True hermaphroditism (TH)**
  - Rarest disorder of intersexuality
  - Both ovarian and testicular tissues are well developed

- **Streak gonad**
  - Composed almost entirely of ovarian-type stroma without differentiated gonadal structures

- **Streak testis**
  - Streak tissue identified at peripheral portion of differentiated testis

However:

- Problems with the previous terminology:
  - The spectrum of MGD may include patients who may classify as:
    - Dysgenetic male pseudohermaphroditism
    - Pure gonadal dysgenesis
    - Partial gonadal dysgenesis
  - This results in lack of emphasis on significant differences in prognosis and follow-up between 46,XY and 45,X/46,XY patients
  - Confusing terminology
  - Clinician and patient dissatisfaction with terminology
These problems lead to the Consensus Statement on Management of Intersex Disorders, International Consensus Conference on Intersex, 2006, known as the Chicago Consensus
THE CHICAGO CONSENSUS

The European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society jointly organized a meeting of endocrinologists, surgeons, geneticists, psychologists, and patient advocacy group members, all representing a world community involved with the management of intersex disorders. A consensus document was subsequently published. It has become known as the Chicago Consensus by virtue of its generation in the 'windy city'.

The Consensus document is far-ranging and delves into areas of longer-term management and outcome. Already the consequences of proposals contained within the Consensus relating to diagnosis and treatment are being evaluated. A powerful driver to hold a Consensus arose from dissatisfaction with current nomenclature, espoused by both health professionals and patients alike. The generation of a new nomenclature rather serendipitously spawned a radical change in the classification of disorders of sex development; these two components are the subject of this review.

Chicago Consensus Document, 2006
Consensus Statement on Management of Intersex Disorders.
International Consensus Conference on Intersex

<table>
<thead>
<tr>
<th>Previous</th>
<th>Proposed</th>
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<tbody>
<tr>
<td>Intersex</td>
<td>Disorders of sex development (DSDs)</td>
</tr>
<tr>
<td>Male pseudohermaphrodite</td>
<td>46,XY DSD</td>
</tr>
<tr>
<td>Undervirilization of an XY male</td>
<td></td>
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<tr>
<td>Undermasculinization of an XY male</td>
<td></td>
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<tr>
<td>Female pseudohermaphrodite</td>
<td>46,XX DSD</td>
</tr>
<tr>
<td>Overvirilization of an XX female</td>
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<tr>
<td>Masculinization of an XX female</td>
<td></td>
</tr>
<tr>
<td>True hermaphrodite</td>
<td>Ovotesticular DSD</td>
</tr>
<tr>
<td>XX male or XX sex reversal</td>
<td>46,XX testicular DSD</td>
</tr>
<tr>
<td>XY sex reversal</td>
<td>46,XY complete gonadal dysgenesis</td>
</tr>
</tbody>
</table>

Table 2. A proposed classification of causes of disorders of sex development (DSDs).

<table>
<thead>
<tr>
<th>Sex chromosome DSD</th>
<th>46,XY DSD</th>
<th>46,XX DSD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A: Disorders of gonadal (testicular) development</strong></td>
<td></td>
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<tr>
<td>1. Complete or partial gonadal dysgenesis</td>
<td></td>
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<tr>
<td>(e.g. SRY, SOX9, SF1, WT1, DHH etc)</td>
<td></td>
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<tr>
<td>2. Ovotesticular DSD</td>
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<tr>
<td>3. Testicular DSD (e.g. SRY+, dup SOX9, RSP01)</td>
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<tr>
<td><strong>B: Disorders in androgen synthesis or action</strong></td>
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<tr>
<td>1. Disorders of androgen synthesis</td>
<td></td>
<td></td>
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<tr>
<td>LH receptor mutations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith–Lemli–Opitz syndrome</td>
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<td></td>
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<tr>
<td>Steroidogenic acute regulatory protein mutations</td>
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<tr>
<td>Cholesterol side-chain cleavage (CYP11A1)</td>
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<tr>
<td>2β-hydroxysteroid dehydrogenase 2 (HSD3B2)</td>
<td></td>
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<tr>
<td>17α-hydroxylase/17,20-lyase (CYP17)</td>
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<tr>
<td>3β-hydroxysteroid dehydrogenase 2 (HSD3B2)</td>
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<td></td>
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<tr>
<td>2. Ovotesticular DSD</td>
<td></td>
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<tr>
<td>3. Testis regression</td>
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<td></td>
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<tr>
<td><strong>B: Androgen excess</strong></td>
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<tr>
<td>1. Fetal</td>
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</tr>
<tr>
<td>3β-hydroxysteroid dehydrogenase 2 (HSD3B2)</td>
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<tr>
<td>21-hydroxylase (CYP21A2)</td>
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<tr>
<td>11β-hydroxylase (CYP11B1)</td>
<td></td>
<td></td>
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<tr>
<td>Glucocorticoid receptor mutations</td>
<td></td>
<td></td>
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<tr>
<td>2. Fetopelvic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aromatase (CYP19)</td>
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<td></td>
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<tr>
<td>Oxidoreductase (POR)</td>
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<tr>
<td>3. Maternal</td>
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<tr>
<td>Maternal virilizing tumours (e.g. luteomas)</td>
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<tr>
<td>Androgenic drugs</td>
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<td></td>
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<tr>
<td><strong>C: Other</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Syndromic associations of male genital development</td>
<td></td>
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<tr>
<td>(e.g. cloacal anomalies, Robinow, Aarskog, Hand-Foot-Genital, popliteal pterygium)</td>
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<tr>
<td>2. Persistent Mullerian duct syndrome</td>
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<td></td>
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<tr>
<td>3. Vanishing testis syndrome</td>
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<tr>
<td>4. Isolated hypospadias (Coxof6)</td>
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<tr>
<td>5. Congenital hypogonadotropic hypogonadism</td>
<td></td>
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<tr>
<td>6. Cryptorchidism (INS3, GREAT)</td>
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</tr>
<tr>
<td>7. Environmental influences</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From: Chicago Consensus Document, 2006

Further clarification of partial vs. mixed gonadal dysgenesis:

**PGD**
- 46,XY karyotype
- Genital ambiguity due to varying degrees of testicular dysgenesis
- Absence of syndromic picture
- Dysgenetic testes:
  - Bilateral or associated with streak gonads
- Genetics
  - Mutations in SRY rarely seen
  - NR5A1 mutations reported in a few patients

**MGD**
- 45,X/46,XY karyotype
- Similar gonadal and genital features as PGD
- Associated with short stature, dysmorphisms, cardiac and renal anomalies
- Management in these patients include features related to Turner’s syndrome

Dos Santos, AP et al. BMC Medical Genetics 2014;15:115
De Andrade, JGR et al. Arq Bras Endocrinol Metab 2010;54(3):331-4
<table>
<thead>
<tr>
<th>Disorder</th>
<th>Characteristics</th>
<th>External genitalia</th>
<th>Gonads</th>
<th>Mullerian (M)/Wolffian (W) Ducts</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonadal dysgenesis</td>
<td>High LH, high FSH</td>
<td>Female (complete); varying degrees of virilization (partial, mixed)</td>
<td>Streak (complete)</td>
<td>M = present</td>
<td>Subtypes: complete, partial, mild, mixed</td>
</tr>
<tr>
<td></td>
<td>Female phenotype may present with primary amenorrhea</td>
<td></td>
<td></td>
<td>W = absent/hypoplastic</td>
<td>Phenotype related to level of testicular hormones</td>
</tr>
<tr>
<td>Leydig cell dysfunction</td>
<td>Low T, high LH, high FSH</td>
<td>Female or partial virilization</td>
<td>Tests</td>
<td>M = absent</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Persistent Mullerian Duct syndrome</td>
<td>Normal T response to hCG stimulation</td>
<td>Male</td>
<td>Tests</td>
<td>W = hypoplastic</td>
<td>Normal MIS</td>
</tr>
<tr>
<td>Smith–Lemli–Opitz syndrome</td>
<td>Cholesterol synthesis defect = deficiency of 7-dehydrocholesterol reductase Low cholesterol, high 7-dehydrocholesterol, low aldosterone : renin ratio</td>
<td>Female or hypospadia, micropenis, bifid scrotum</td>
<td>Tests</td>
<td>M = may be present</td>
<td>Defect in Sertoli cell function (absent MIS) or MIS receptor function</td>
</tr>
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<td></td>
<td></td>
<td>W = may be present</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>STAR deficiency</td>
<td>Lipid accumulation, enlargement of adrenals, gonads Mineralocorticoid, androgen deficiency Severe CAH, salt loss</td>
<td>Female or mild virilization</td>
<td>Tests</td>
<td>M = absent</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>W = absent/hypoplastic</td>
<td></td>
</tr>
<tr>
<td>P450scc deficiency</td>
<td></td>
<td>Female or mild virilization</td>
<td>Tests</td>
<td>M = absent</td>
<td>Autosomal recessive</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>W = present/hypoplastic</td>
<td></td>
</tr>
<tr>
<td>3ß-hydroxysteroid dehydrogenase II deficiency</td>
<td>Severe CAH</td>
<td>Ambiguous (micropenis, severe hypospadia, bifid scrotum, blind vaginal pouch)</td>
<td>Tests</td>
<td>M = absent</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>W = present</td>
<td></td>
</tr>
<tr>
<td>17ß-hydroxylase deficiency</td>
<td>Low T, high LH, High FSH</td>
<td>Female, blind vaginal pouch</td>
<td>Tests</td>
<td>M = absent</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>W = absent/hypoplastic</td>
<td>May also be associated with 17,20-Lyase deficiency</td>
</tr>
<tr>
<td>17,20-lyase deficiency</td>
<td>Low T, high LH, high FSH</td>
<td>Female or male with mild hypospadia</td>
<td>Tests</td>
<td>M = absent</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>W = absent/hypoplastic</td>
<td>Adrenal function normal</td>
</tr>
<tr>
<td>17ß-hydroxysteroid dehydrogenase III deficiency</td>
<td>hCG stimulation results in increased androstenedione : testosterone ratio</td>
<td>Female or severely ambiguous</td>
<td>Tests</td>
<td>M = absent</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>W = present</td>
<td>Diagnosis rarely made in newborn period</td>
</tr>
<tr>
<td>5α-reductase III deficiency</td>
<td>Increased serum testosterone : DHT ratio</td>
<td>Ambiguous</td>
<td>Tests</td>
<td>M = absent</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>W = present</td>
<td></td>
</tr>
<tr>
<td>Complete androgen insensitivity syndrome</td>
<td>High LH, high T, normal FSH,</td>
<td>Female, blind vaginal pouch</td>
<td>Tests</td>
<td>M = absent</td>
<td>X-linked recessive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>W = present</td>
<td>Absent pubic/axillary hair</td>
</tr>
<tr>
<td>Partial androgen insensitivity syndrome</td>
<td>High LH, high T, normal FSH,</td>
<td>Ambiguous, blind vaginal pouch</td>
<td>Tests</td>
<td>M = absent</td>
<td>X-linked recessive</td>
</tr>
</tbody>
</table>
Causes of 46,XY DSD

• Only 10% of cases of complete gonadal dysgenesis (CGD) are caused by SRY mutation
  – This suggests that other genes may be involved in testis determination
  – Candidate genes include:
    • DAX-1, SF-1, WNT4, SOX3, LHX9, FOG-2
    • Evidence that haplodeficiency of SF-1 resulting from heterozygous mutations are a relatively frequent cause

• Several syndromic causes of gonadal dysgenesis associated with known genes
  – SOX9 – campomelic dysplasia
  – ATRX – alpha-thalassemia/mental retardation
  – WT-1 – Denys-Drash
Gonadal dysgenesis and Y

• Patients with GD and a Y chromosome or Y chromosome material
  – Are at an increased risk for developing germ cell tumors
    • Gonadoblastoma
      – 1/3 of MGD patients
    • Intratubular germ cell neoplasia
      – Potential for transformation to seminoma
Treatment/Management

• MGD, PGD: early bilateral gonadectomy may be indicated for individuals with a Y-chromosome
  – Done to prevent development of malignant germ cell tumors
  – Also important to prevent virilization of patient is to be raised female
  – **Newer thinking:
    • Patients with XY PGD with nonscrotal gonads that cannot be repositioned surgically in to a scrotal position should have bilateral gonadectomy
    • Patients with XY PGD with scrotal gonads being reared as males undergo routine monitoring with self-examination
Treatment/Management

• TH (ovotesticular DSD): removal of opposite gonad from assigned gender and biopsy of remaining tissue may be indicated
  – Normal sexual and reproductive function can be achieved by proper sex assignment at a young age
  – 38% of TH patients with 46,XX karyotype menstruate, ovulate, and have successful pregnancies with surgical removal of testicular portion of ovotestes

• XY CGD (Swyer syndrome)
  – Early gonadectomy indicated
In testicular dysgenesis, the epididymis is often separated from the gonad by thin membranous peritesticular tissue (arrow).
The right gonad represents a streak gonad with an adjacent fallopian tube structure, and the left is a dysgenetic testis. These were removed from a 6-day-old with ambiguous genitalia and a 46,XY karyotype.
Histologic features

- Normal prepubertal testicular tissue
- Solid seminiferous tubules containing immature Sertoli cells and a few primitive germ cells
- Immature Sertoli cell
  - Round to oval nuclei with inconspicuous nucleoli
- Primitive germ cells
  - Adjacent to basement membrane with larger nuclei and abundant cytoplasm
Histologic features

- Normal pre-pubertal ovarian tissue
  - Oocytes
    - 40-60 um in diameter, large central or eccentric nuclei, large nucleolus
    - Surrounded by a single layer of flattened pregranulosa cells
**Histologic features**

- **Ovotestes**
  - Ovarian and testicular tissue often arranged end-to-end
  - Testicular component (left)
    - Solid seminiferous tubules containing immature Sertoli cells and a few primitive germ cells
  - Ovarian component (right)
    - Numerous primordial follicles and a few primary and antral follicles
Histologic features

• Gonadal (testicular) dysgenesis
  • Compact seminiferous tubules in the cortex
  • Thin tunica albuginea penetrated by network of branching seminiferous cords
Histologic features

- **Streak gonad**
  - Contains anastomosing trabeculae or sex cord-like structures
  - Germ cell components are rare or absent in cord-like structures but may be present in fetal or neonatal life
  - May be composed exclusively of ovarian-type stroma with rare atrophic cord-like structures
Immunohistochemistry

- Utilized to help identify cells that may represent intratubular germ cell neoplasia (ITGCN)
Immunohistochemistry

- Placental alkaline phosphatase (PLAP)
  - Expressed by cells of intratubular germ cell neoplasia (ITGCN)
  - Occasionally can be expressed in normal immature germ cells during first year of life
  - Hence not entirely specific for ITGCN
• **POU5F1 (OCT3/4)**
  – Transcriptional regulator
  – Expressed in pluripotent undifferentiated cells, gonadoblastoma, ITGCN, dysgerminoma
  – However, can be normally expressed in testicular tissue in patients <1 year of age
Immunohistochemistry

- CD117 is also used in the diagnosis of ITGCM
ITGCN vs. Atypical intratubular germ cells

- **A** – ITGCN
  - Large cells
  - Hyperchromatic
  - Peripheral location in tubules
- **B** – strongly OCT3/4+
- **C** – testicular dysgenesis
- **D** – Atypical ITGC
  - Large cells
  - Central location in tubules
  - Less hyperchromatic than ITGCN

Note: A, B, and C are from the same patient. D is from a different patient.

Fan R, Ulbright TM. Fetal and Pediatric Pathology 2012;31:21-24
Atypical intratubular germ cells

- May stain with PLAP, OCT3/4, and CD117
- The prognostic significance of these cells in terms of their risk for progression to ITGCN is undetermined
Gonadoblastoma

- Composed of sex cord stromal and germ cell components with cellular nests frequently showing Call-Exner-like bodies
Clinical course of the patient in this case:

- Female gender assignment
- Bilateral gonadectomy performed three days after biopsies
- Feminizing genitoplasty with reduction clitoroplasty and labioplasty performed at 2 years of age
  - Appearance of genitalia prior to surgery at 2 years:
    - Prominent phallus with open introitus and single urogenital opening
    - No labia minora
- Future vaginoplasty planned
Main take home point:

• Differentiation between MGD/PGD, CGD, and TH (ovotesticular DSD)
  – Has important implications for gender assignment and possible early gonadectomy
Additional references:

• Hersmus R, et al. FOXL2 and SOX9 as parameters of female and male gonadal differentiation in patients with various forms of DSD. J Pathol 2008; 215:31-38