Ambiguous Genitalia

By

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Paediatric Endocrinologist

What is the sex of the baby?

- **Genetic**: XX, XY or other
- **Gonadal**: Testes, ovaries, ovotestes, other
- **Phenotypic**: Male genitalia, female genitalia, ambiguous genitalia
- **Gender identity:** the gender felt by the individual to be most accurate
- **Gender role:** gender typical behaviours

SEX Determination

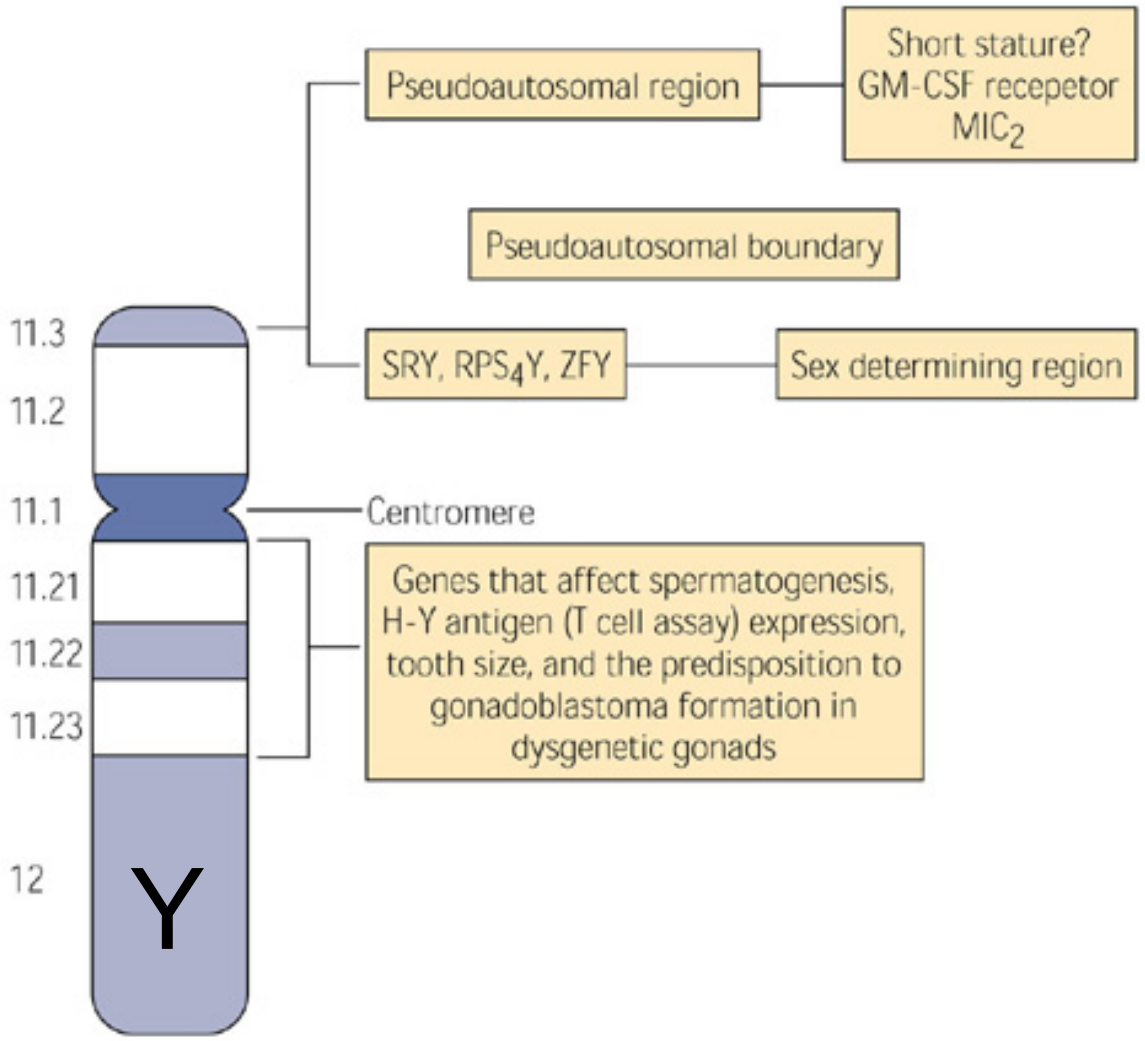
- Gonads
- Internal genital structures
- External genital structures

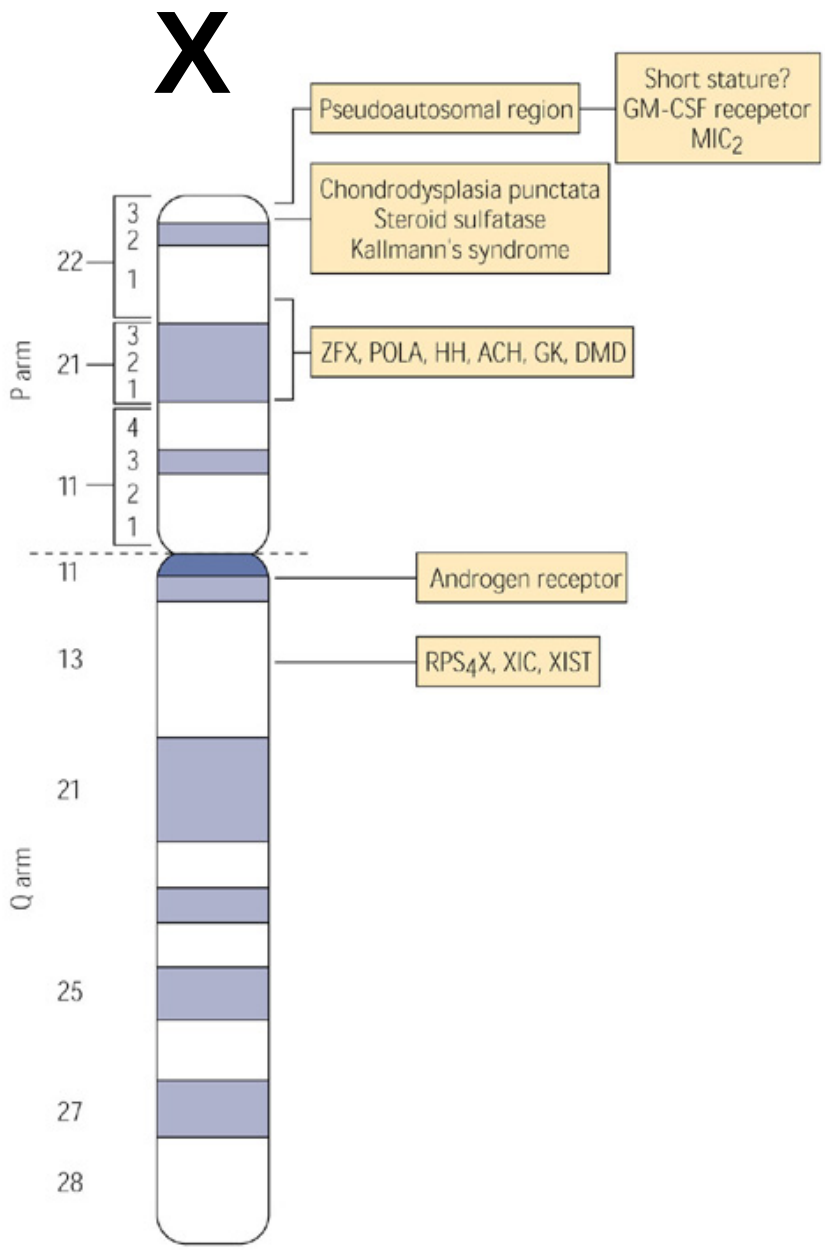
- All develop from bi-potential embryonic tissues

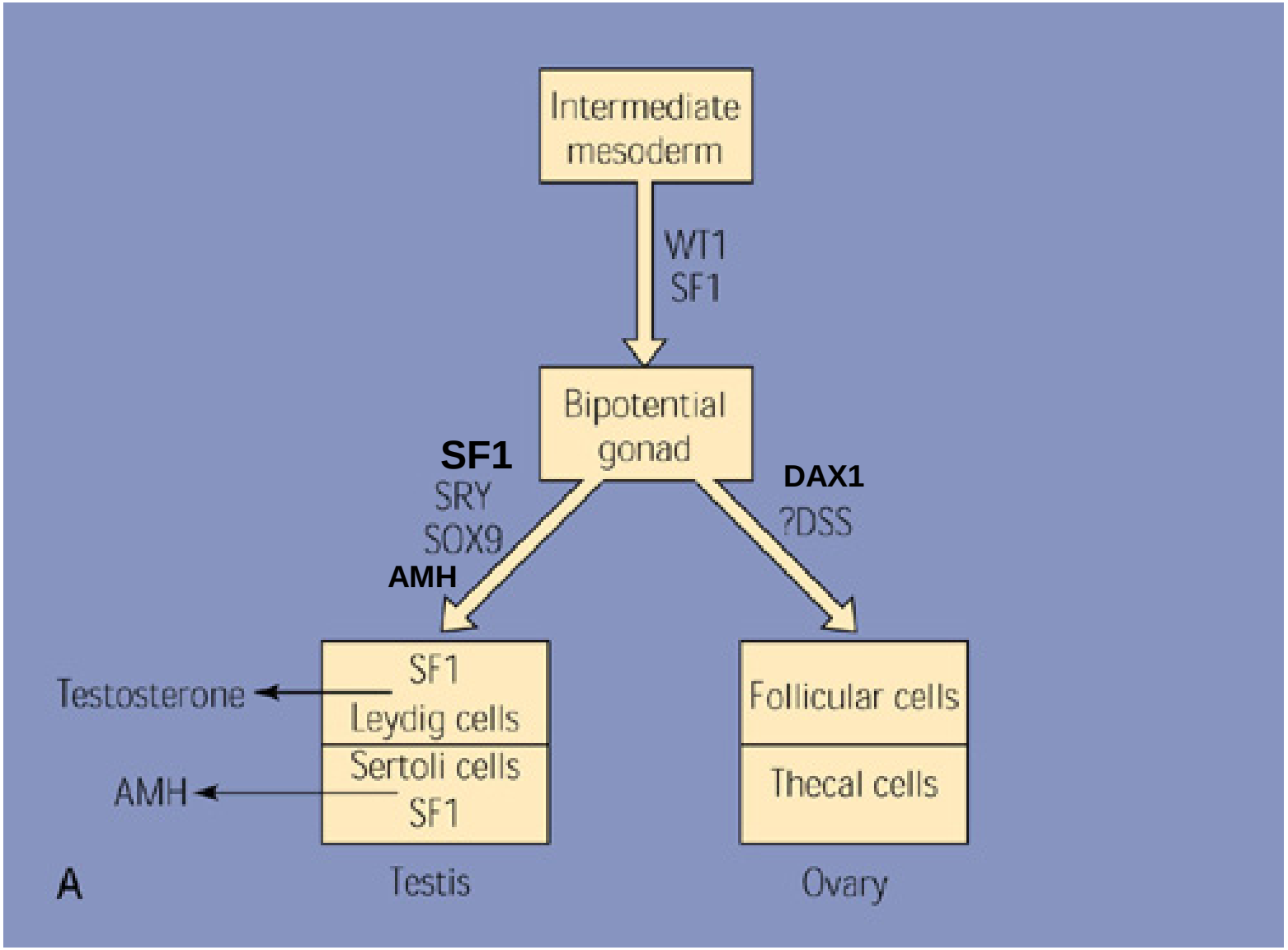
- Determination and differentiation depend on the **regulated expression** and **interaction** of specific **genes** and their **gene products**

- Sex determination:
  - Sex chromosome to gonadal differentiation
  - Sex determination sets the stage for sex differentiation
- Sex differentiation:
  - Sex-specific response of tissues to hormones produced by the gonads

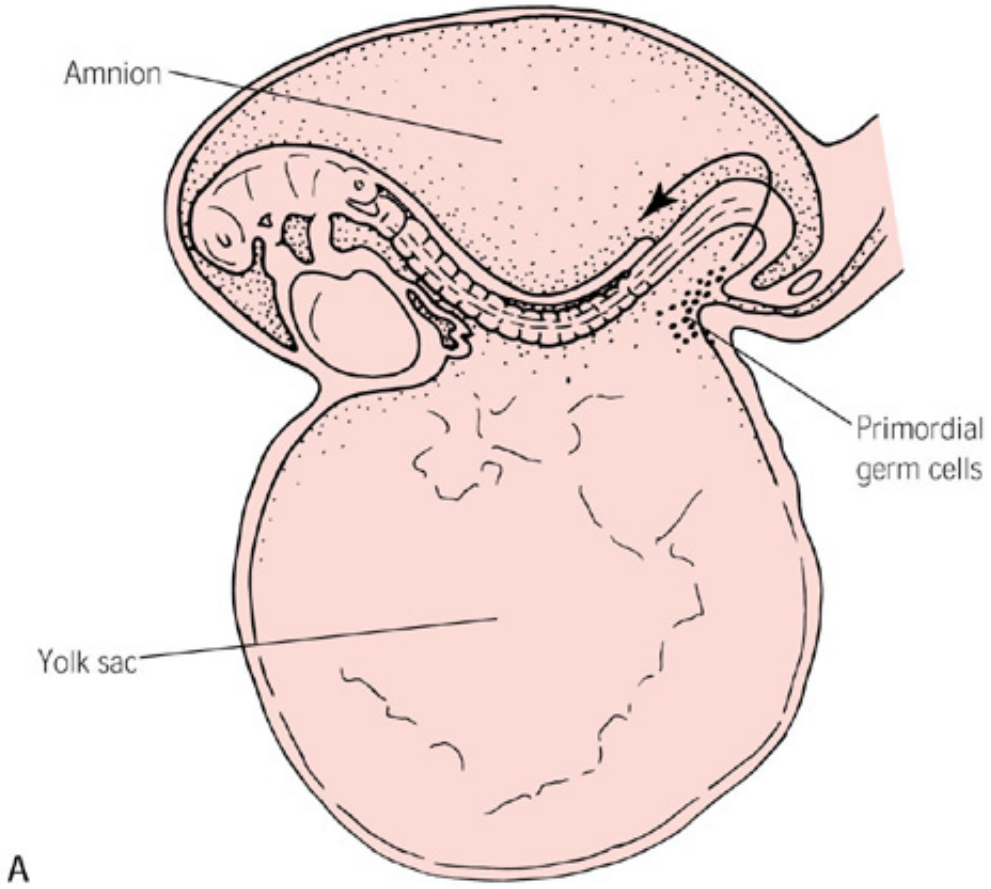
The Players


Pseudoautosomal region
Short stature?
GM-CSF recepetor
MIC2
Pseudoautosomal boundary
11.3
11.2
SRY, RPS4Y, ZFY
Sex determining region
11.1
Centromere
11.21
11.22
11.23
Genes that affect spermatogenesis, H-Y antigen (T cell assay) expression, tooth size, and the predisposition to gonadoblastoma formation in dysgenetic gonads
12
Y

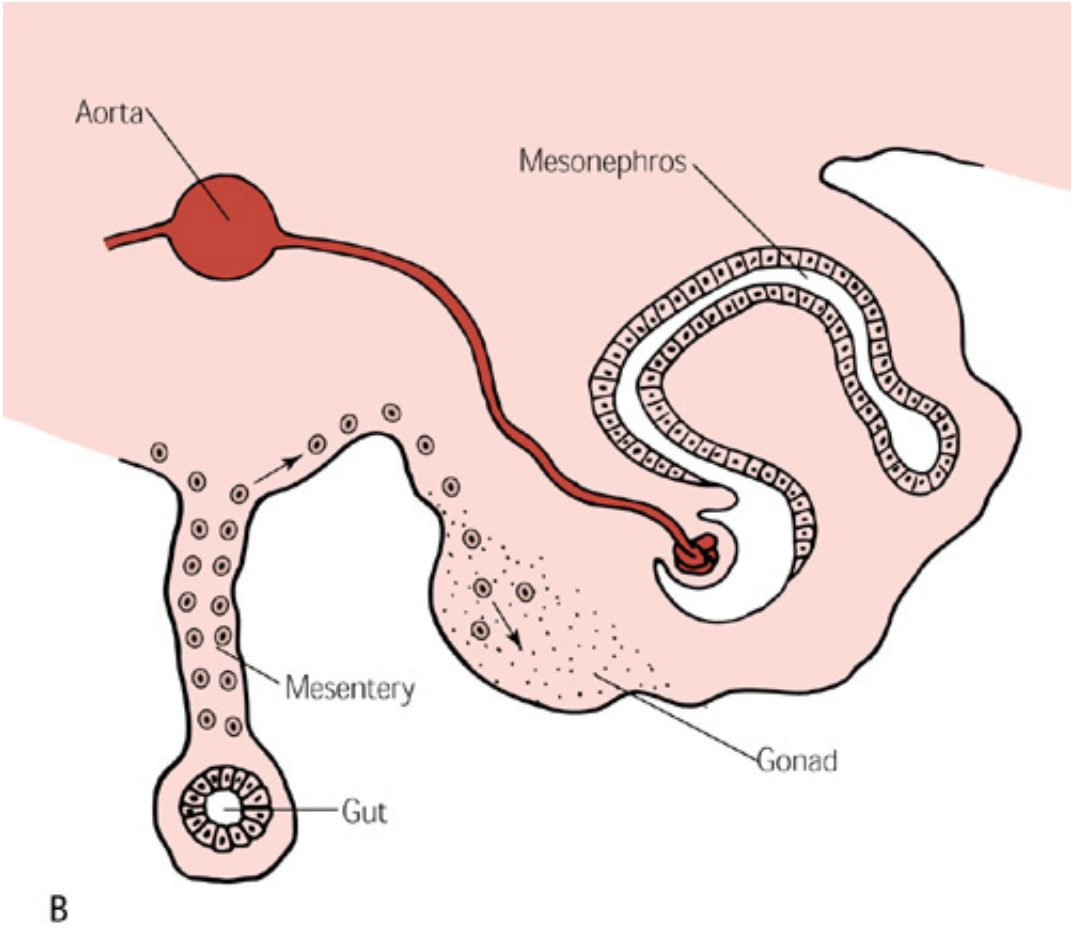

X
Pseudoautosomal region
Short stature?
GM-CSF recepetor
MIC2
Chondrodysplasia punctata
Steroid sulfatase
Kallmann's syndrome
ZFX, POLA, HH, ACH, GK, DMD
Androgen receptor
RPS4X, XIC, XIST
P arm
Q arm
22
21
11
11
13
21
25
27
28


Intermediate mesoderm
WT1
SF1
Bipotential gonad
SF1
SRY
SOX9
AMH
DAX1
?DSS
SF1
Leydig cells
Testosterone
Sertoli cells
SF1
AMH
Follicular cells
Thecal cells
A
Testis
Ovary

Primordial germ cells



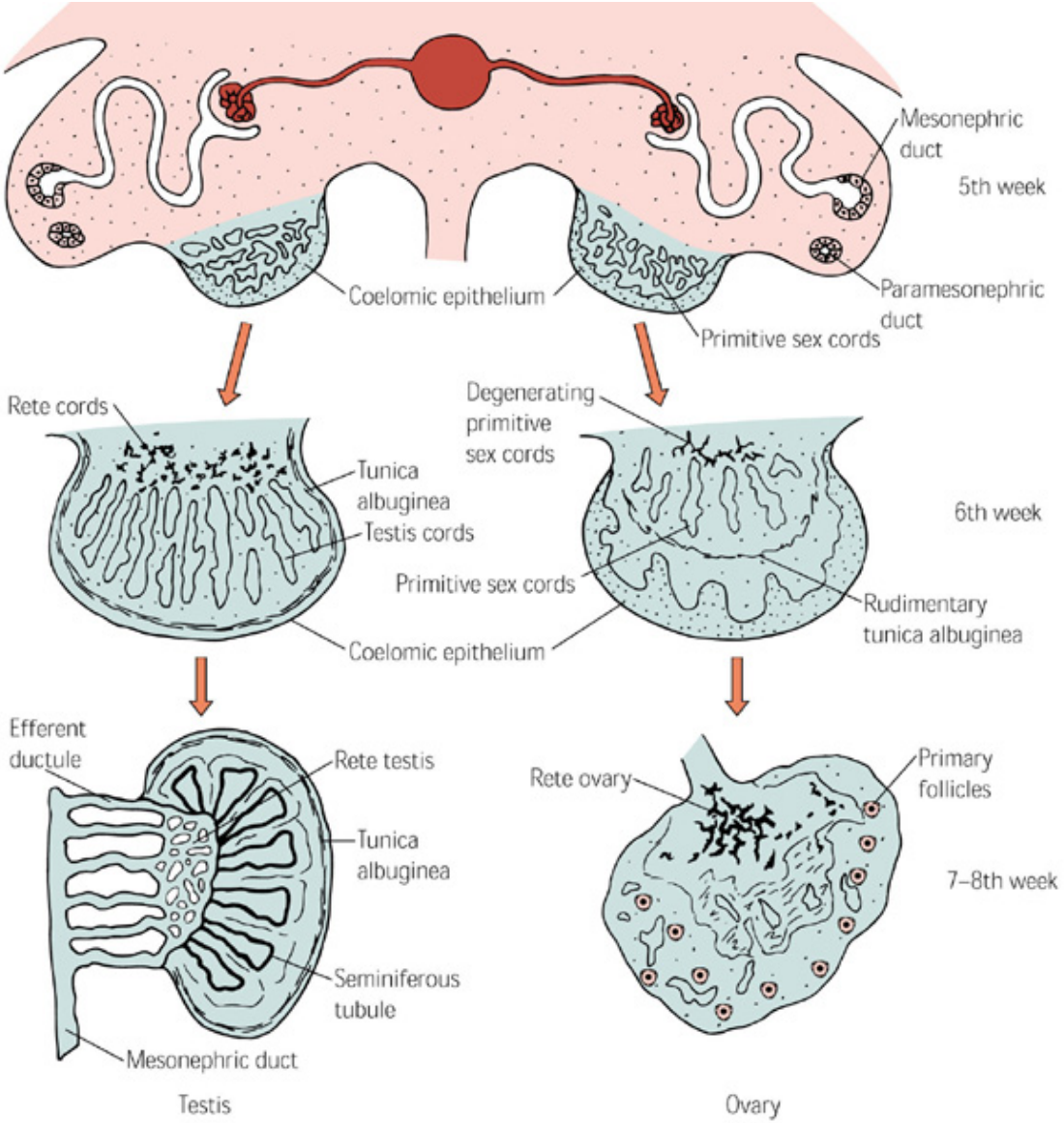
Primordial germ cells



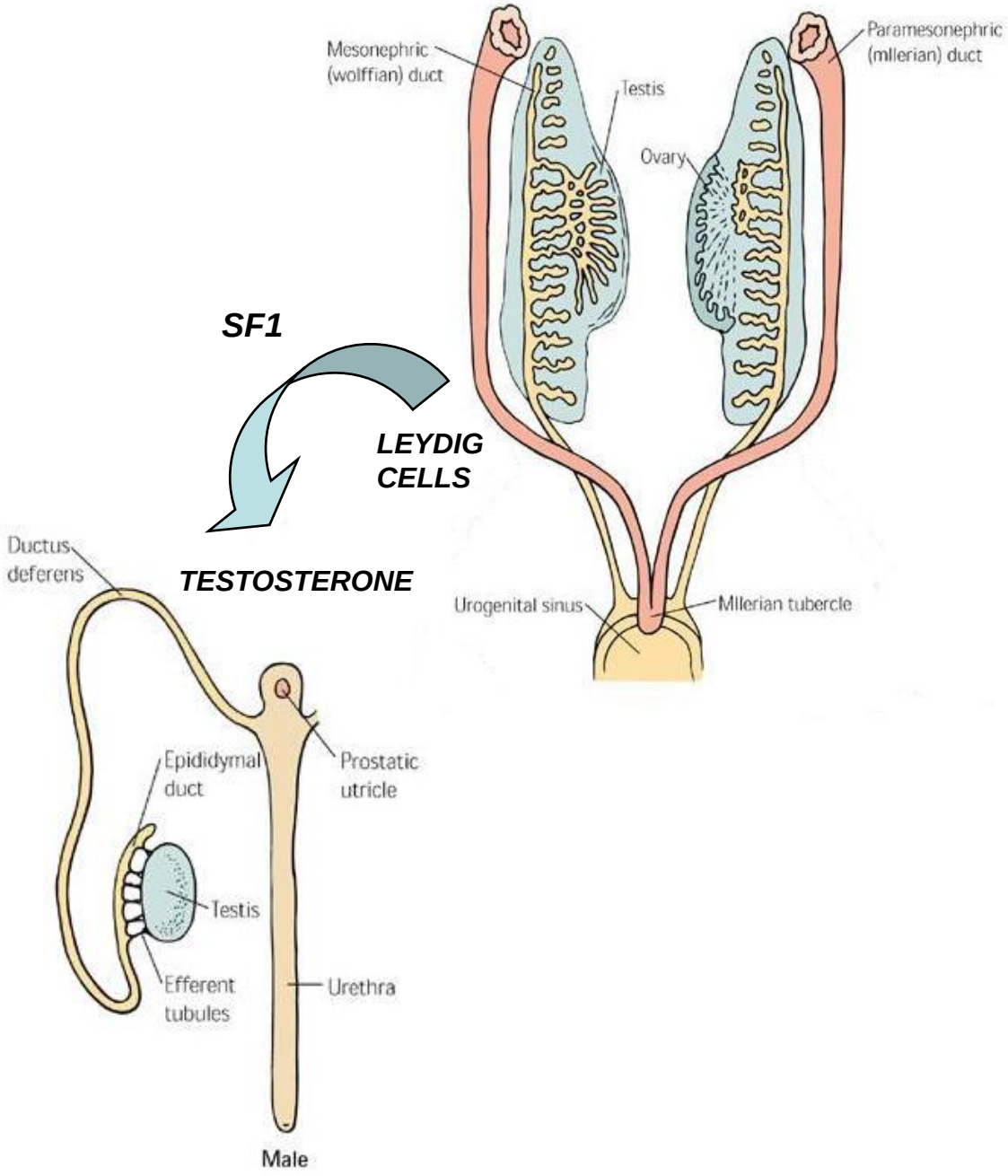
Gonadal differentiation

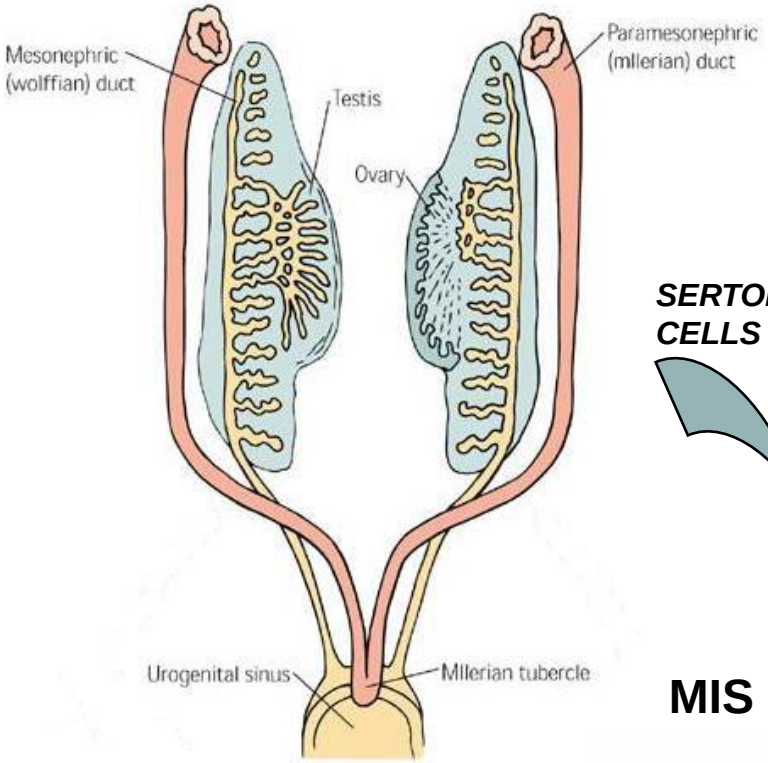
4-6 WEEKS

6-7 WEEKS



<u>3 CELLS TYPES</u>
SUPPORTING
STEROIDOGENIC
GERM

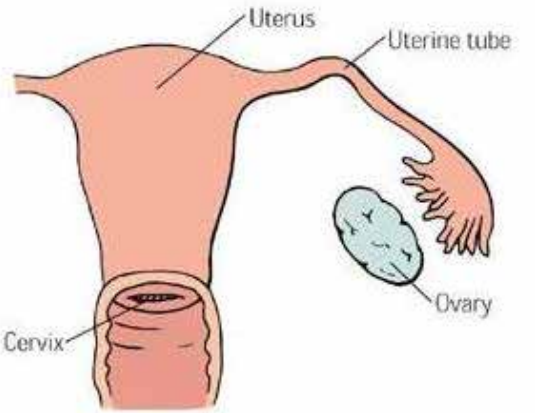

Mesonephric
(wolffian) duct
Testis
Ovary
Paramesonephric
(mllerian) duct
SF1
LEYDIG
CELLS
TESTOSTERONE
Ductus
deferens
Urogenital sinus
Mllerian tubercle
Epididymal
duct
Prostatic
utricle
Testis
Efferent
tubules
Urethra
Male



SERTOLI CELLS

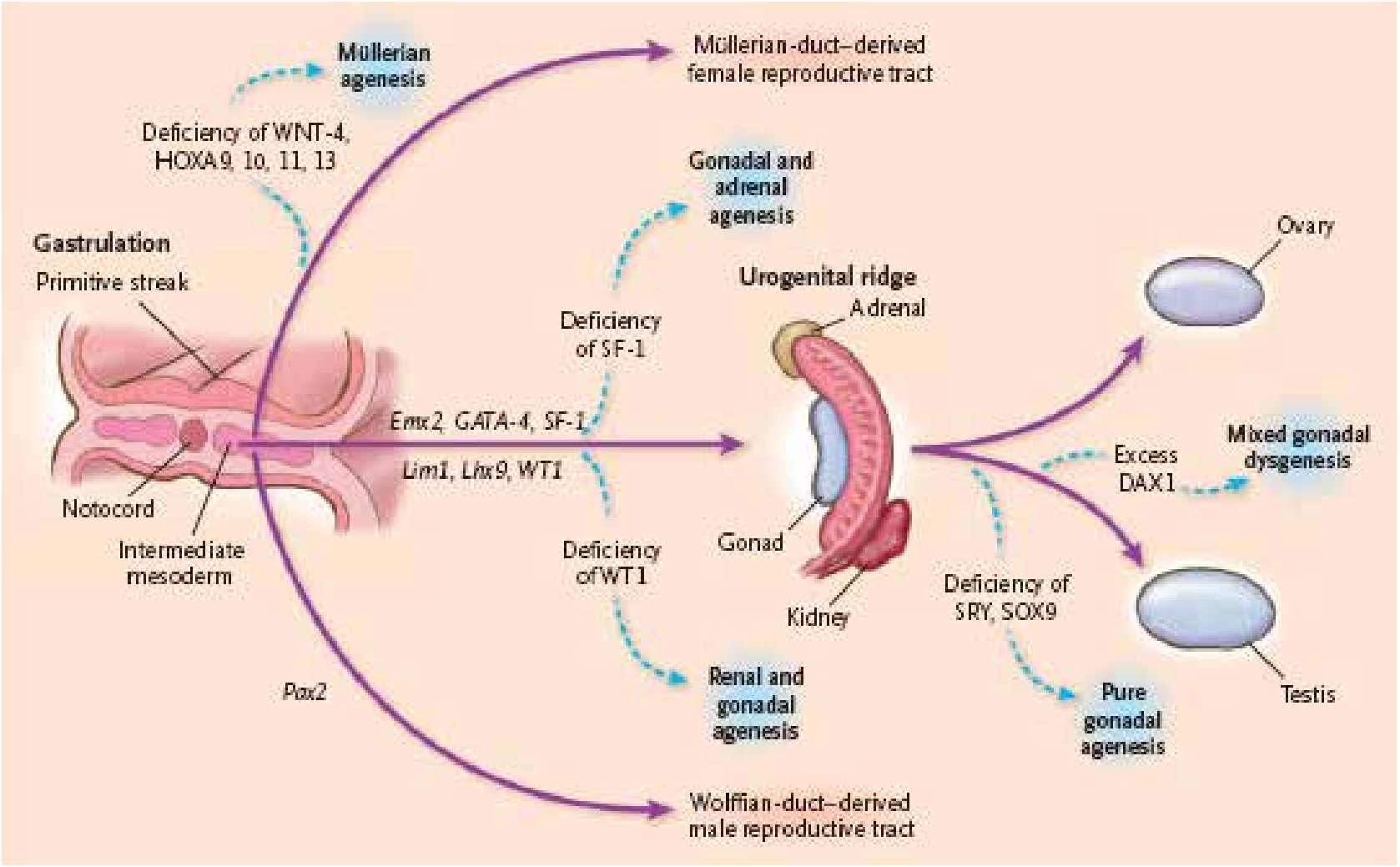
WT1
SF1

MIS



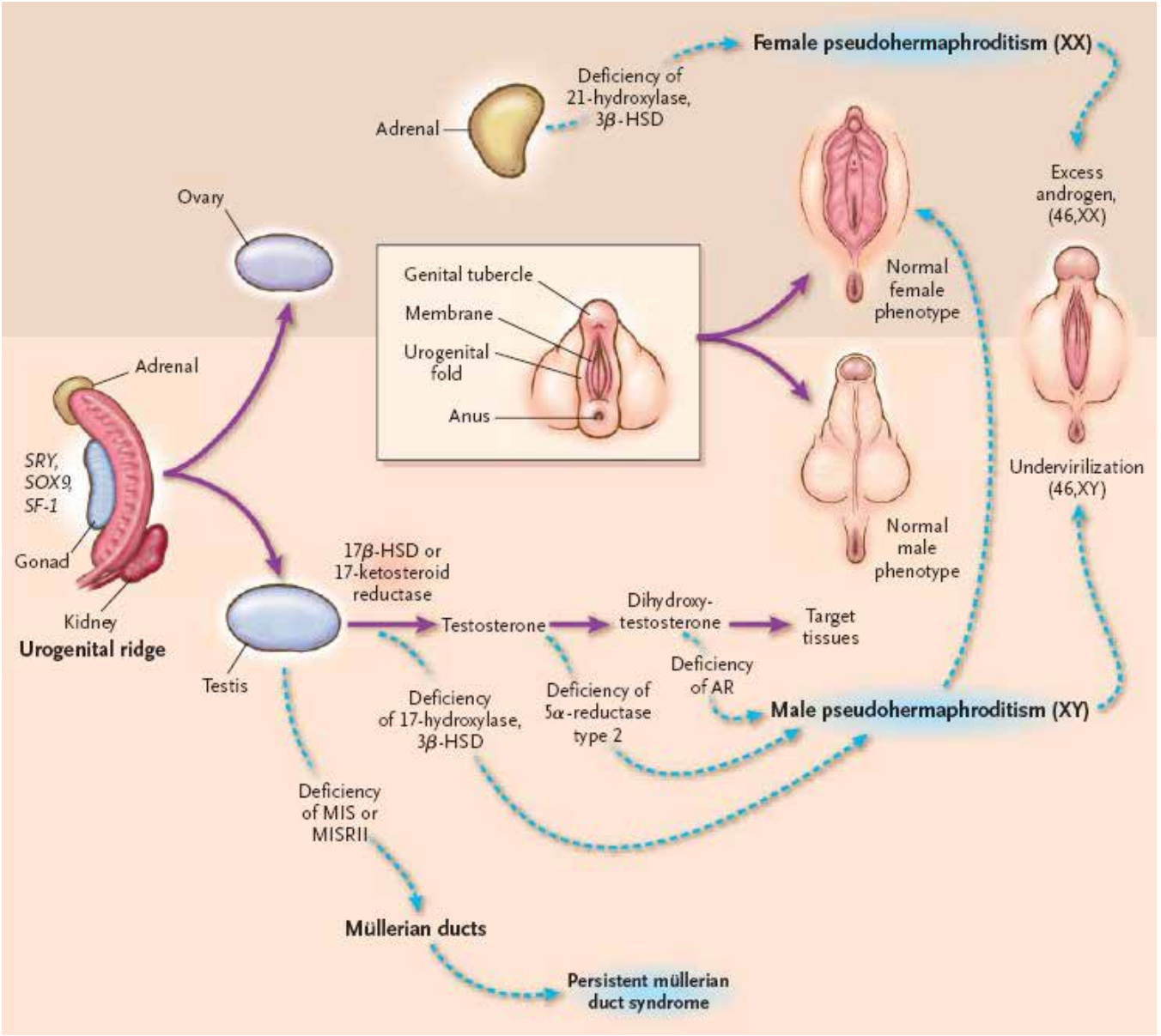
Female

Syndromes of Dysgenesis during the Development of the Urogenital Ridge.



NEJM 350;4 january 22, 2004

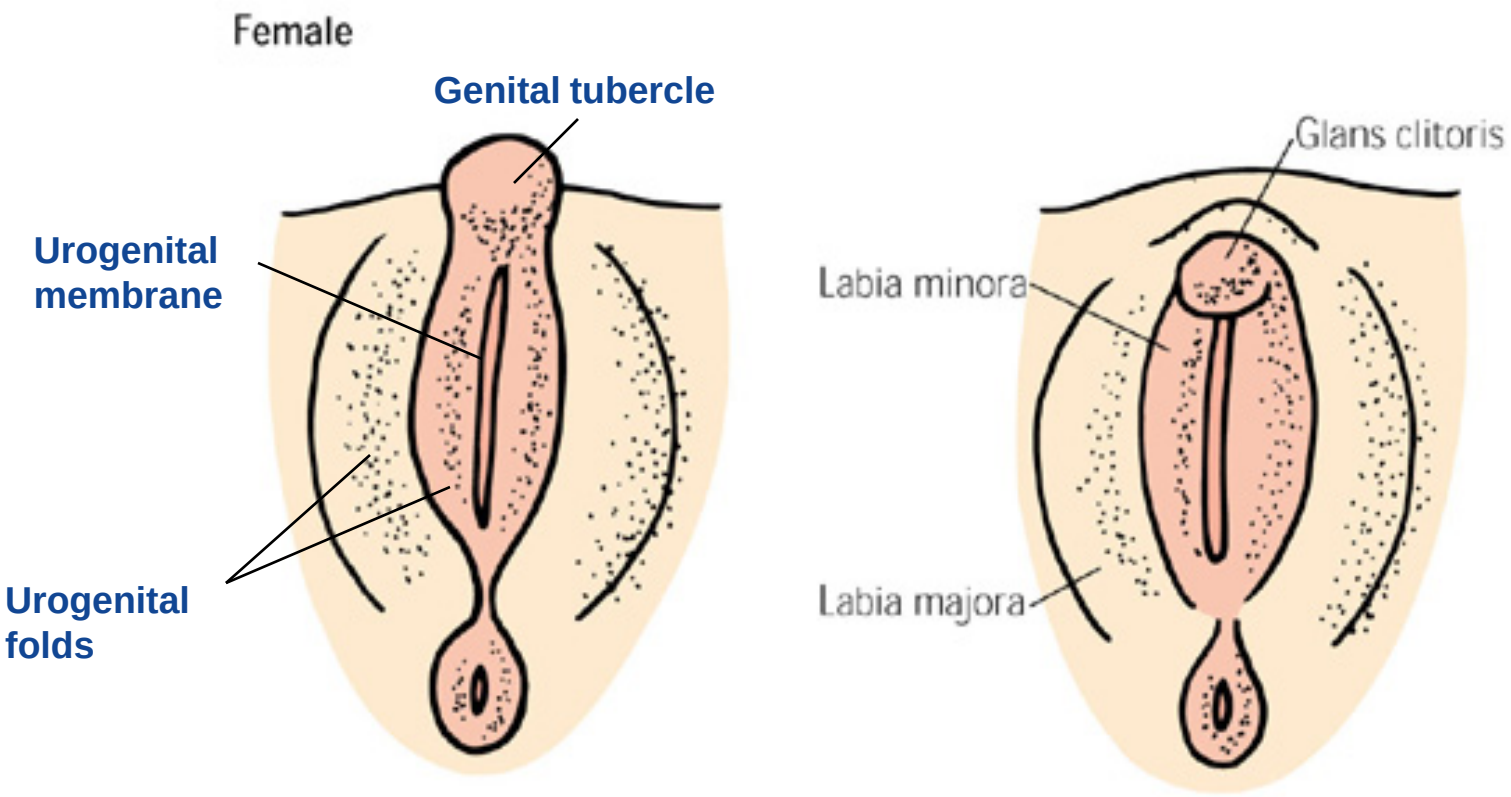
Disorders of Differentiation



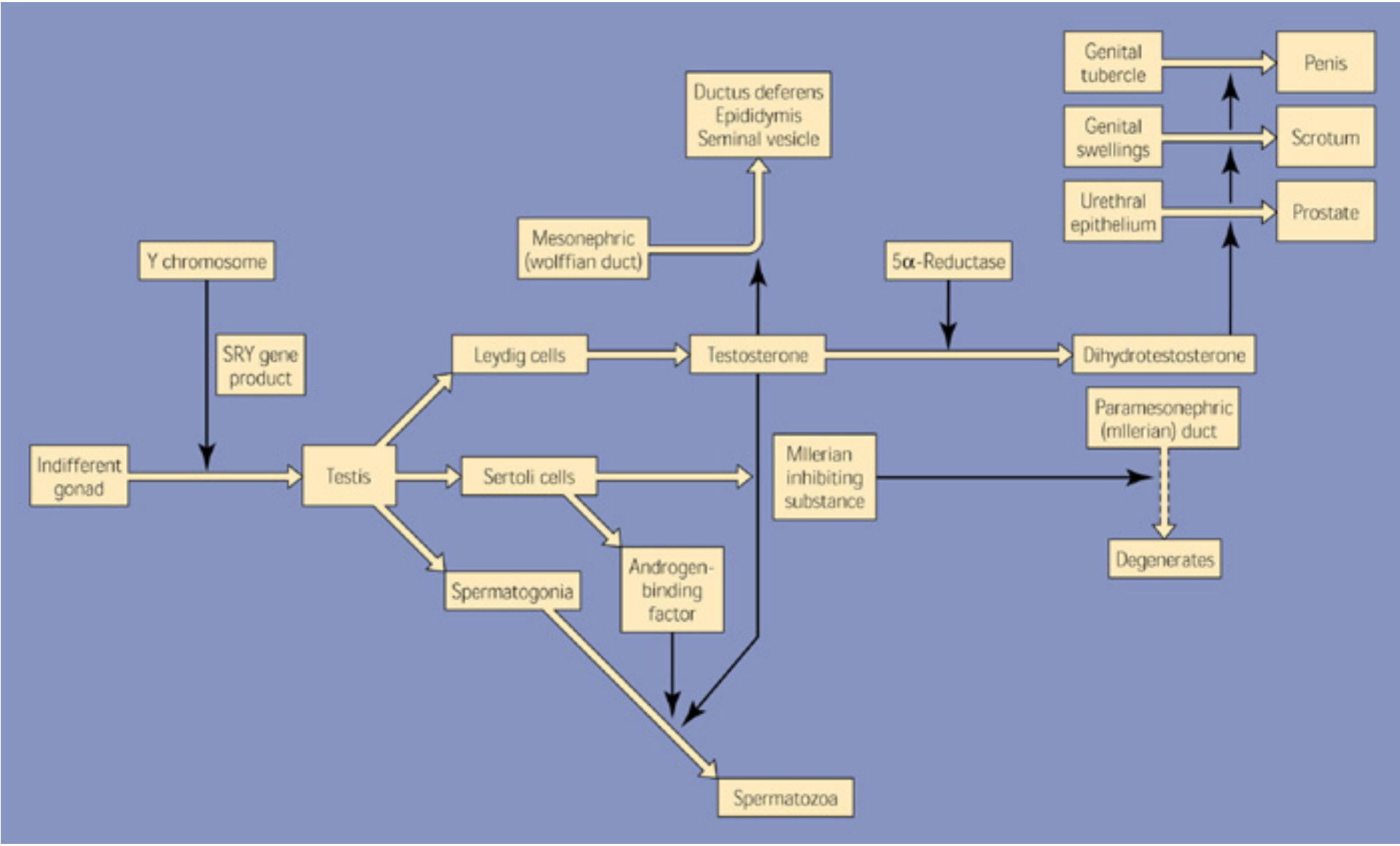
NEJM 350;4 january 22, 2004

Genitalia 7 weeks Indifferent stage

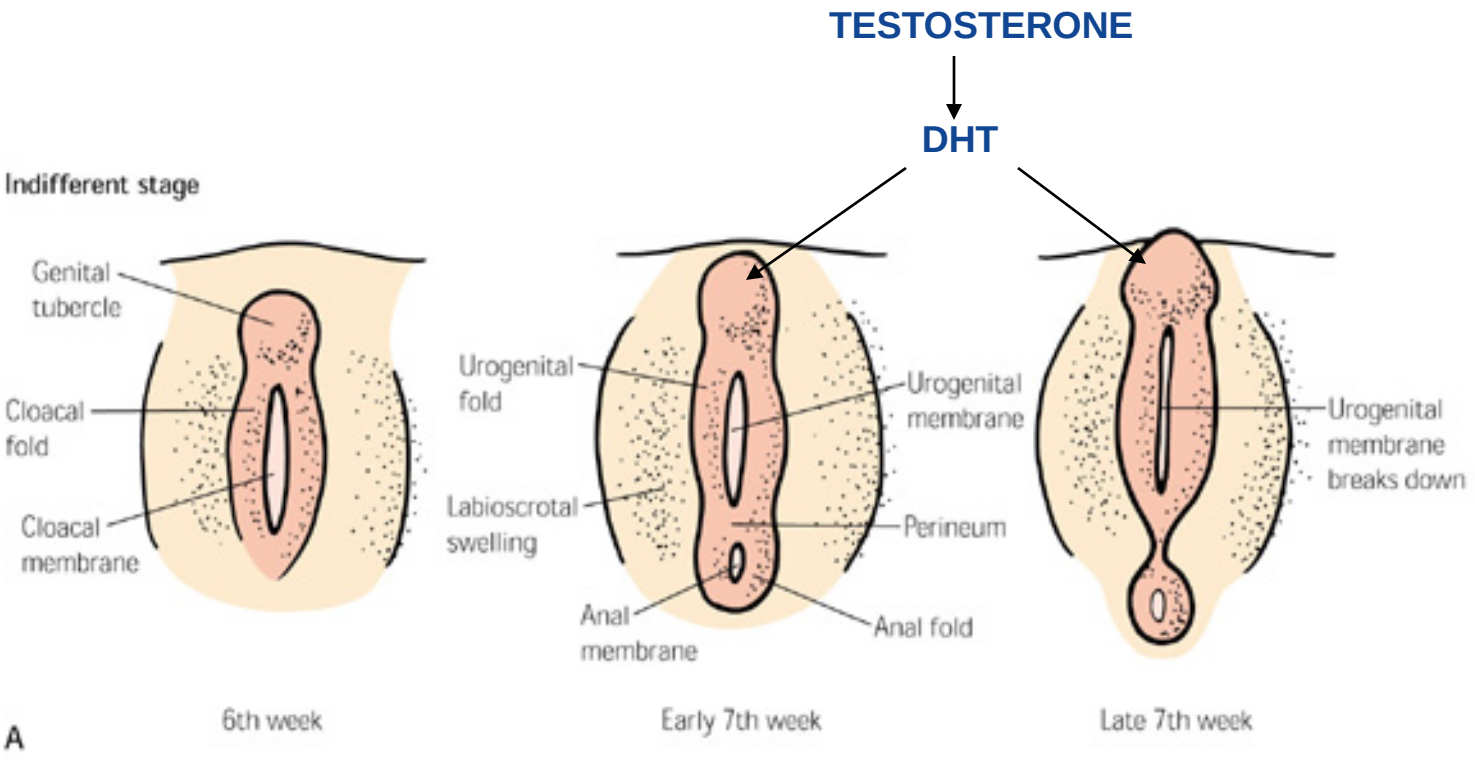
Female external genitalia development



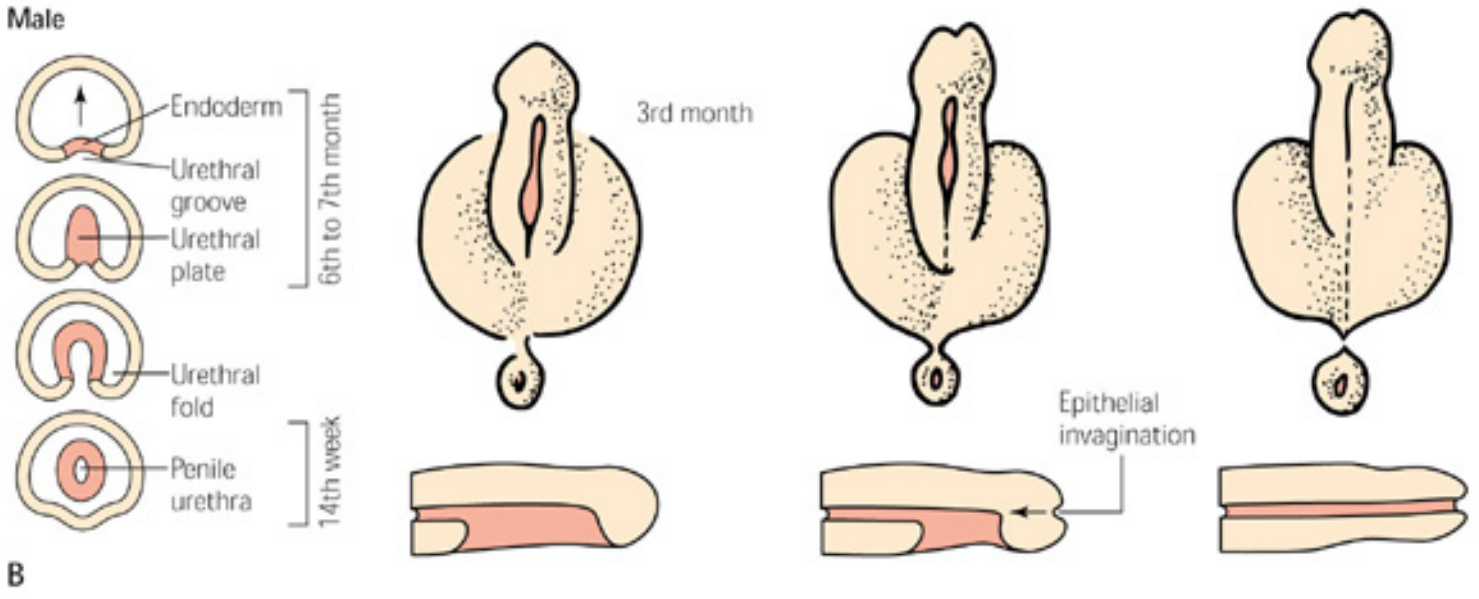
Male sexual differentiation

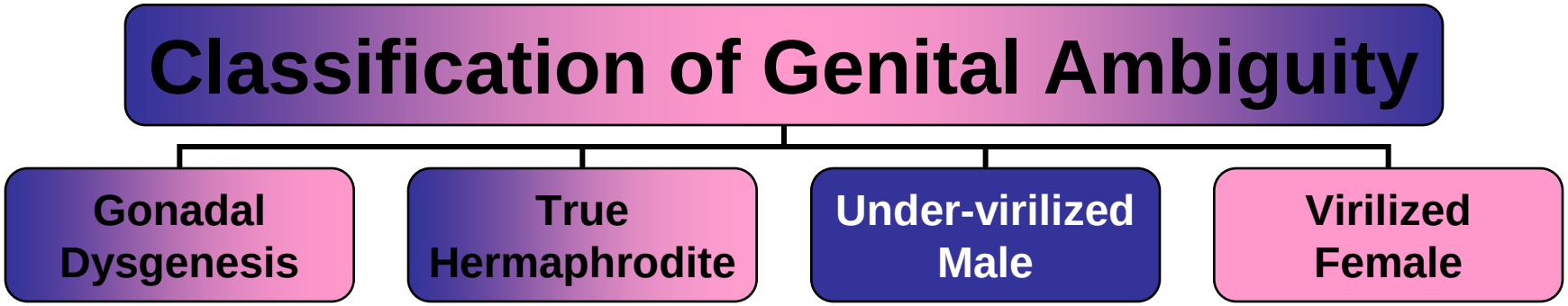


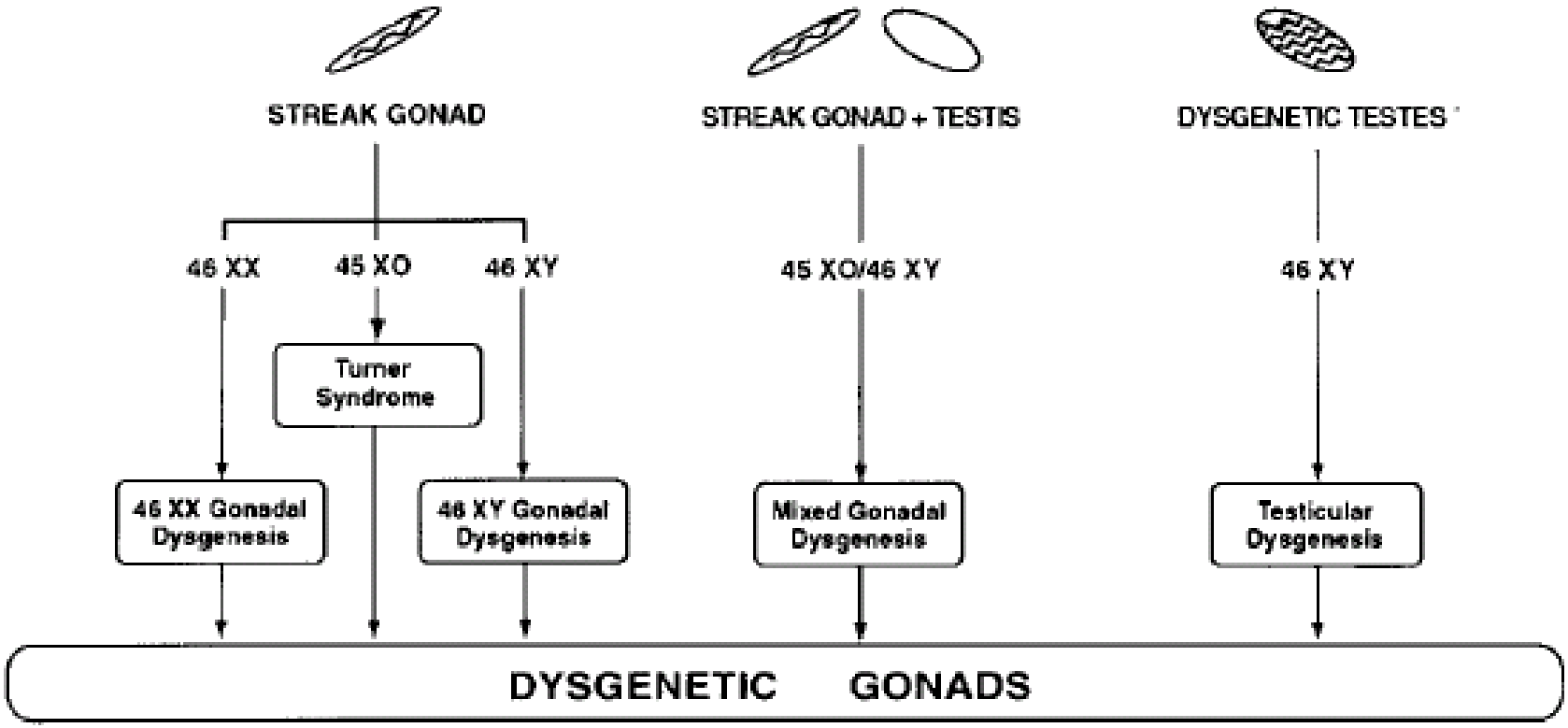
Development of male external genitalia



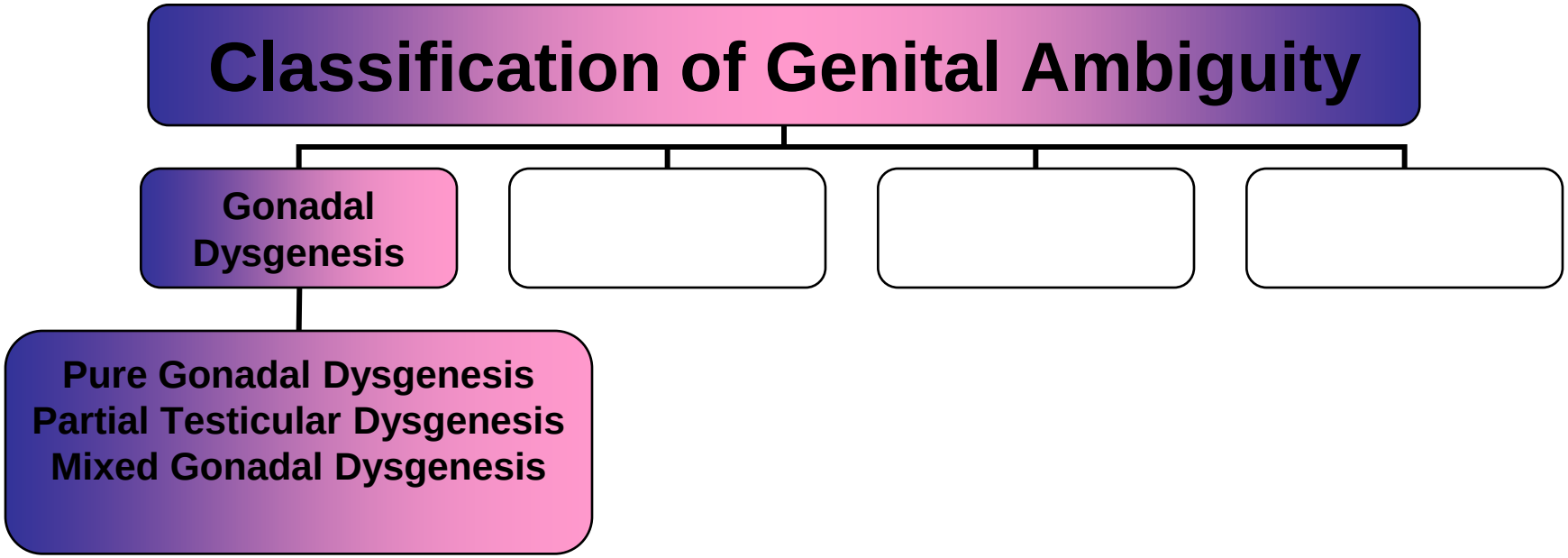
Development of male external genitalia




Classification of Genital Ambiguity
Gonadal Dysgenesis
True Hermaphrodite
Under-virilized Male
Virilized Female


STREAK GONAD
STREAK GONAD + TESTIS
DYSGENETIC TESTES
46 XX
45 XO
46 XY
45 XO/46 XY
46 XY
Turner Syndrome
46 XX Gonadal Dysgenesis
46 XY Gonadal Dysgenesis
Mixed Gonadal Dysgenesis
Testicular Dysgenesis
DYSGENETIC GONADS

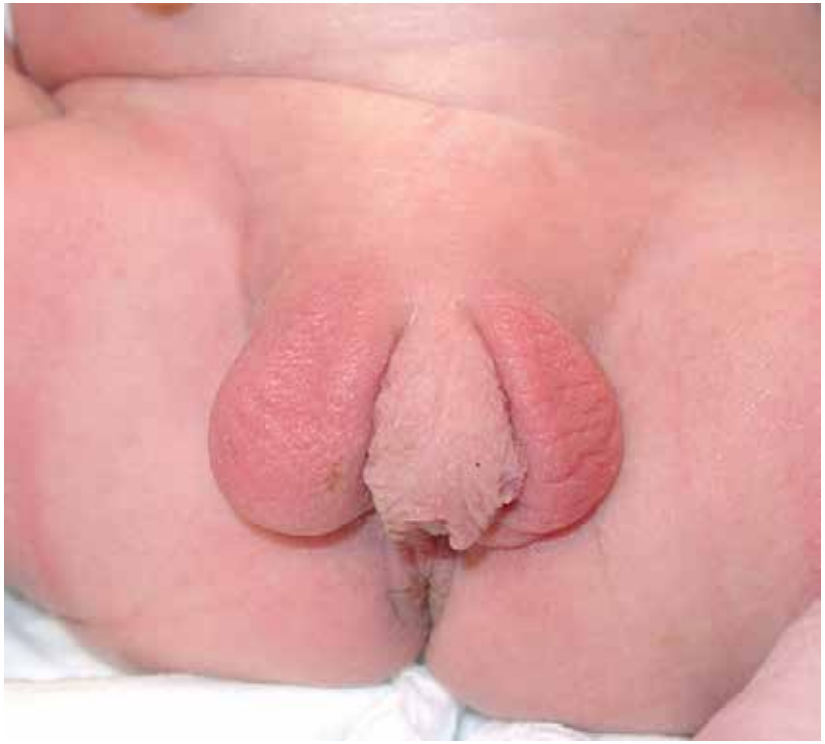
Classification of Genital Ambiguity

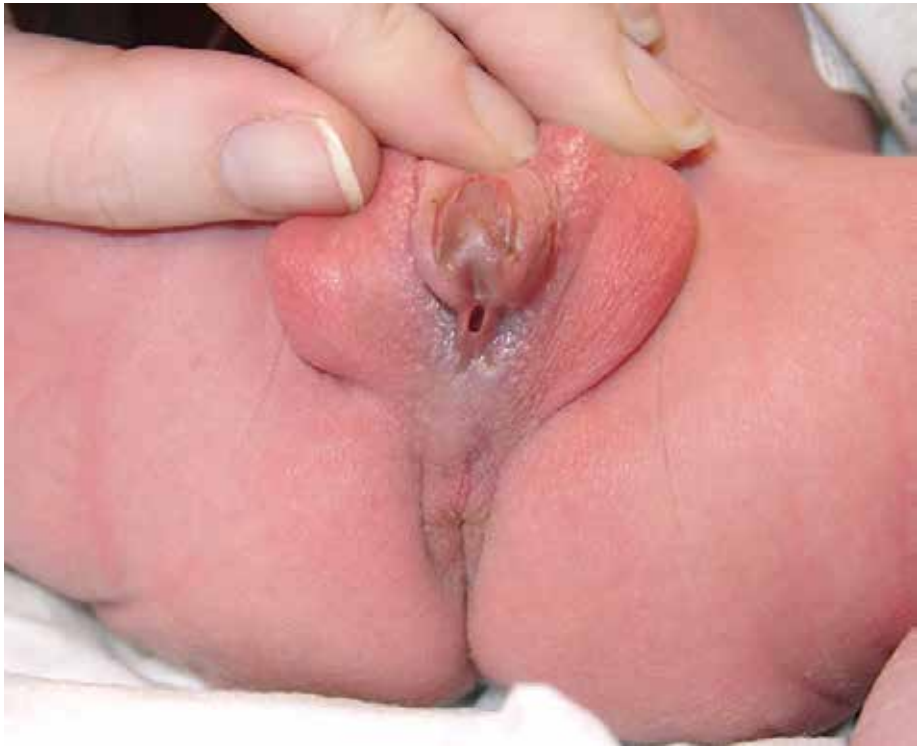


Gonadal dysgenesis

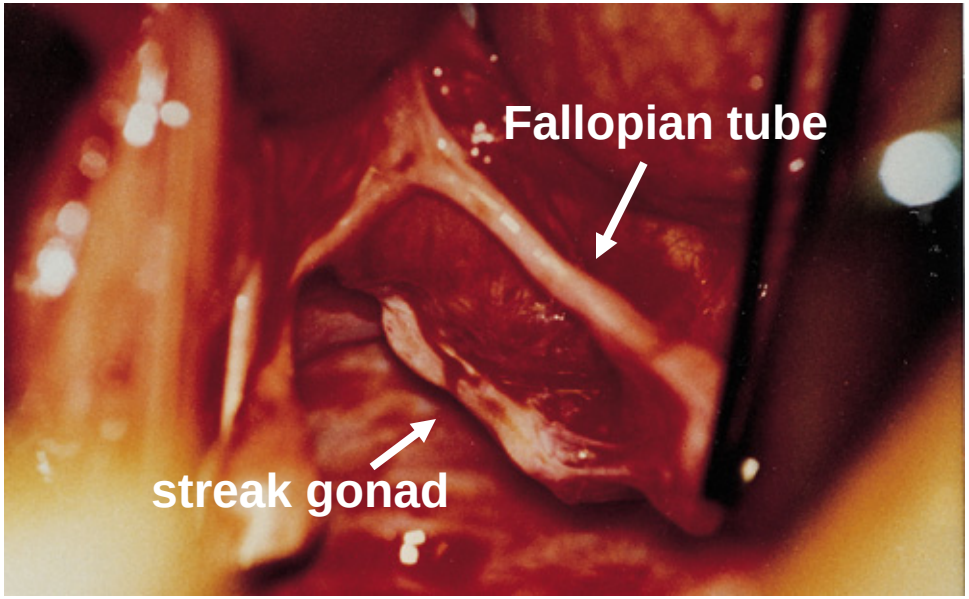
- **Spectrum of disorders leading to maldevelopment of the gonads**
  - **46XY pure gonadal dysgenesis**
  - **Multiple cell lines including a monosomy X mixed gonadal dysgenesis-most often 45,X/46,XY**
  - **True hermaphrodite often 46,XX/46,XY**
  - **46,XX males, 46,XY females**

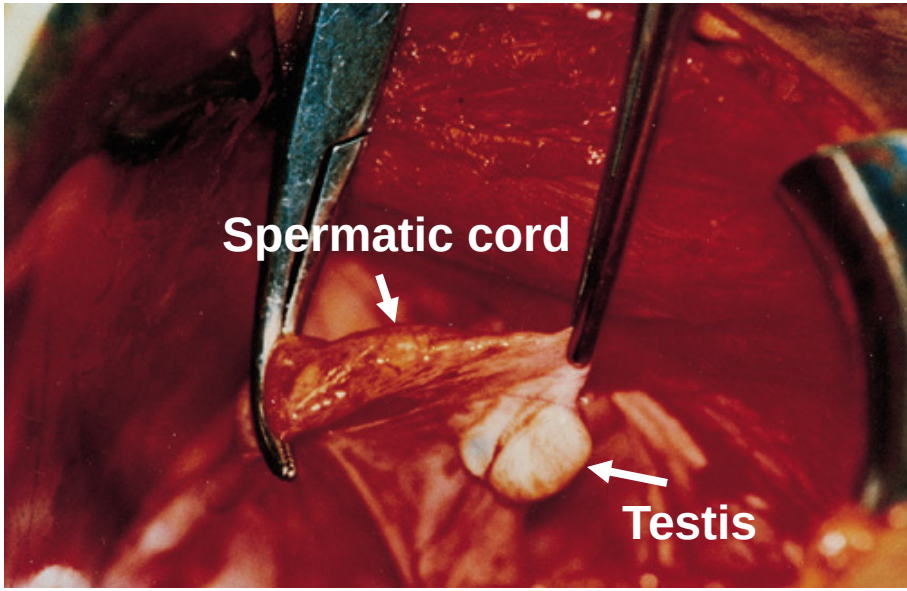
XO/XY/XXY mixed/asymmetric gonadal dysgenesis





16y old female, short stature and sexual infantilism
46,XY asymmetric gonadal dysgenesis





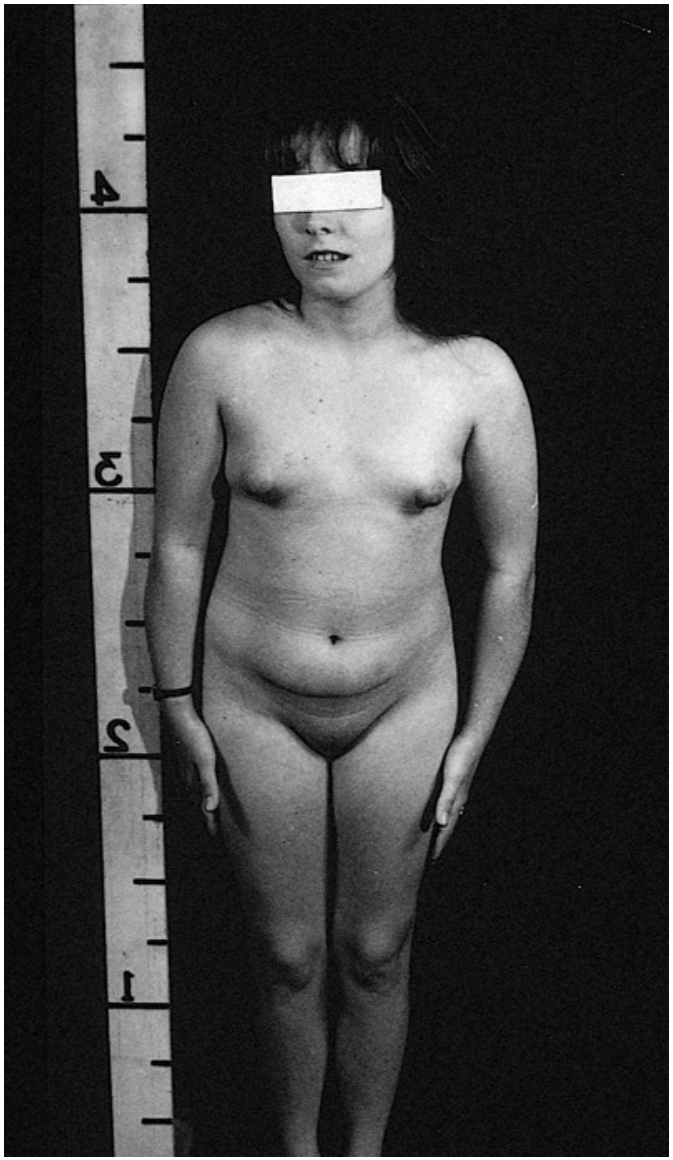
Pure gonadal dysgenesis

14y old female

45,X/46,XY

Short stature

Bilateral streak gonads



Deletion syndrome 45,X/46,XY

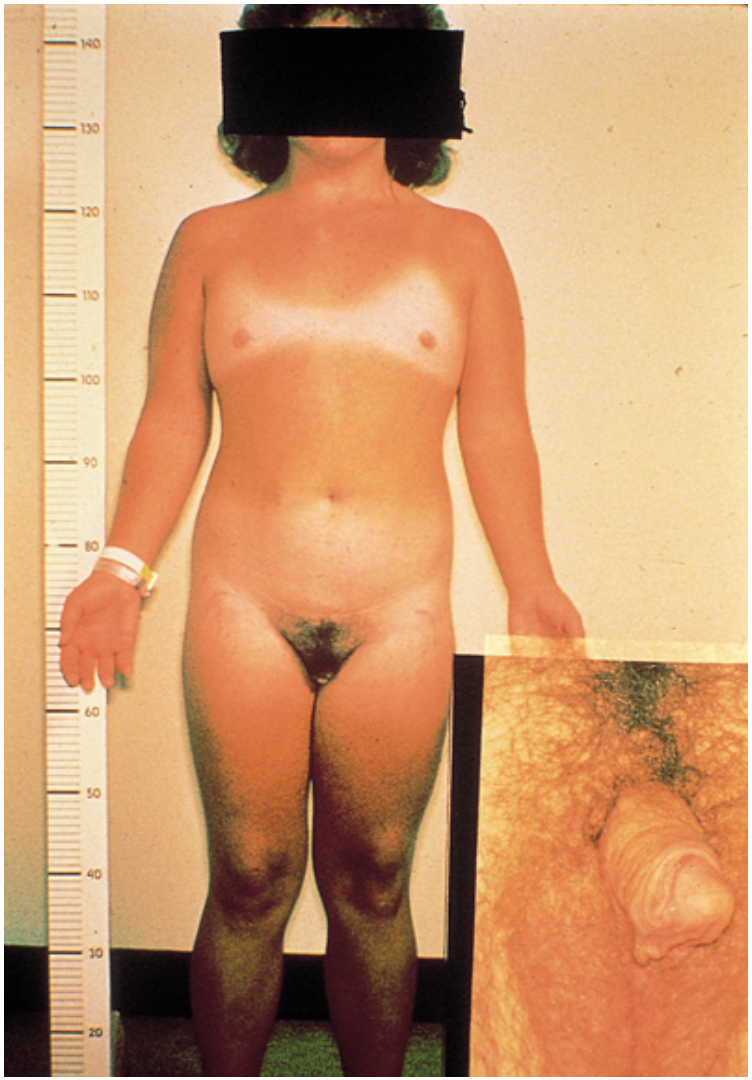
A 15-year-old female

45,X/46,X,idic(Y)/47,XY,+idic(Y)

Short stature

Clitoromegaly

Right streak gonad
Left immature testis.



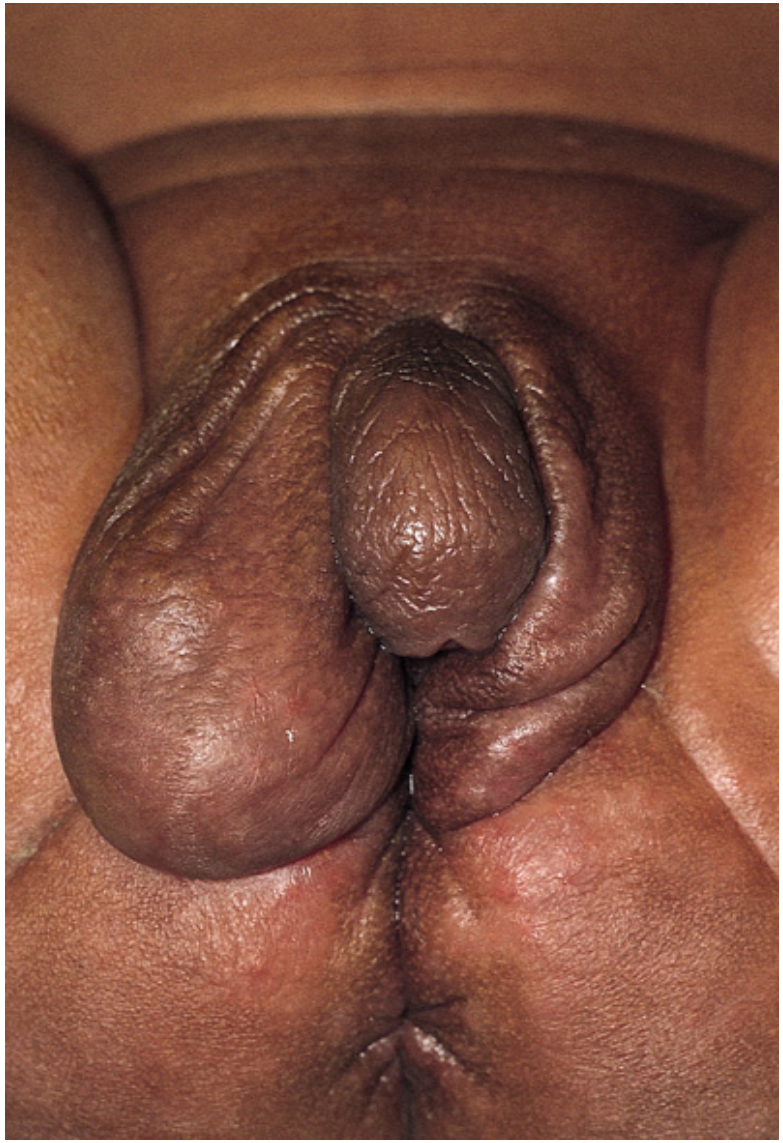
2-mo old female

45,X/46,XY

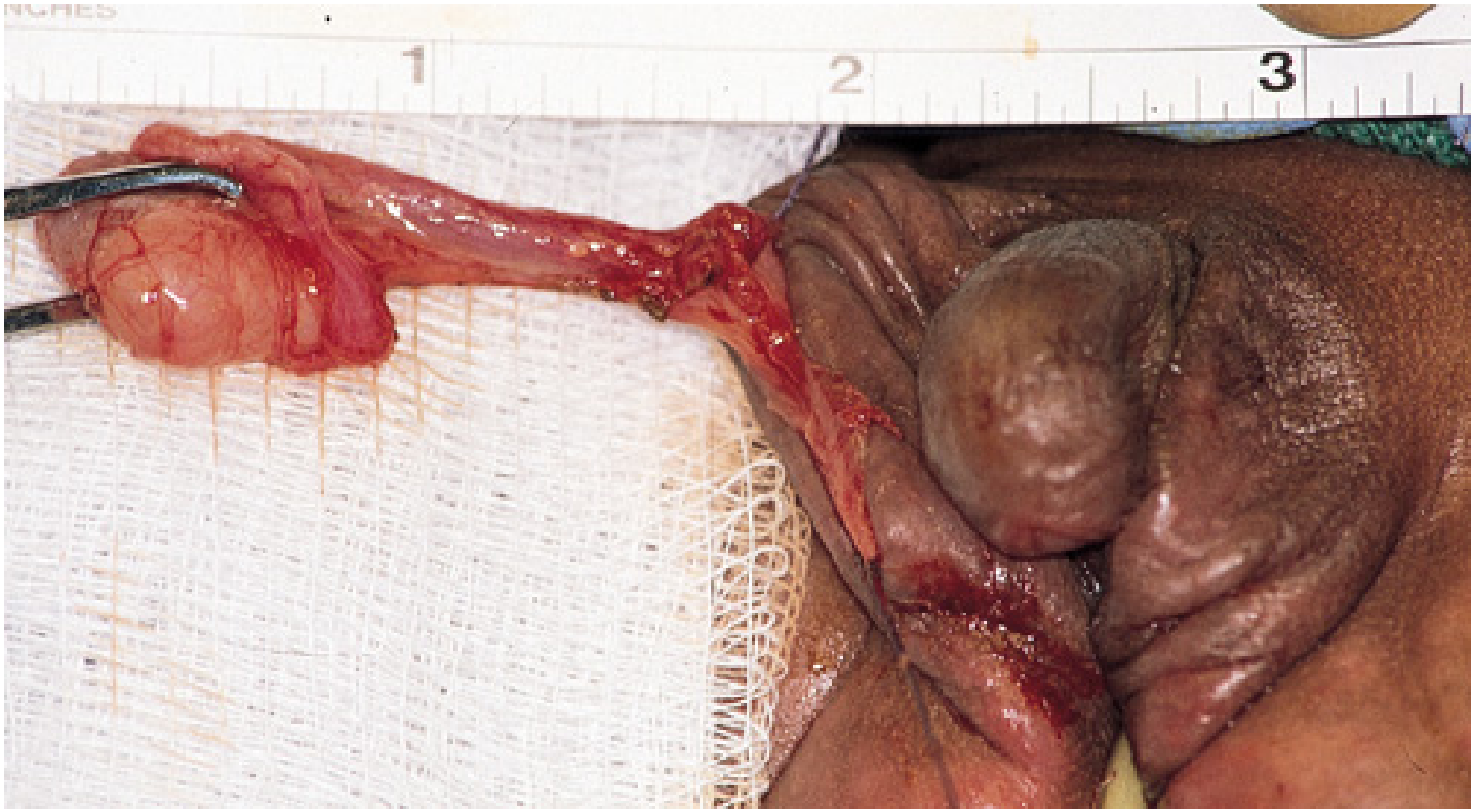
Frank sexual ambiguity

Clitoromegaly

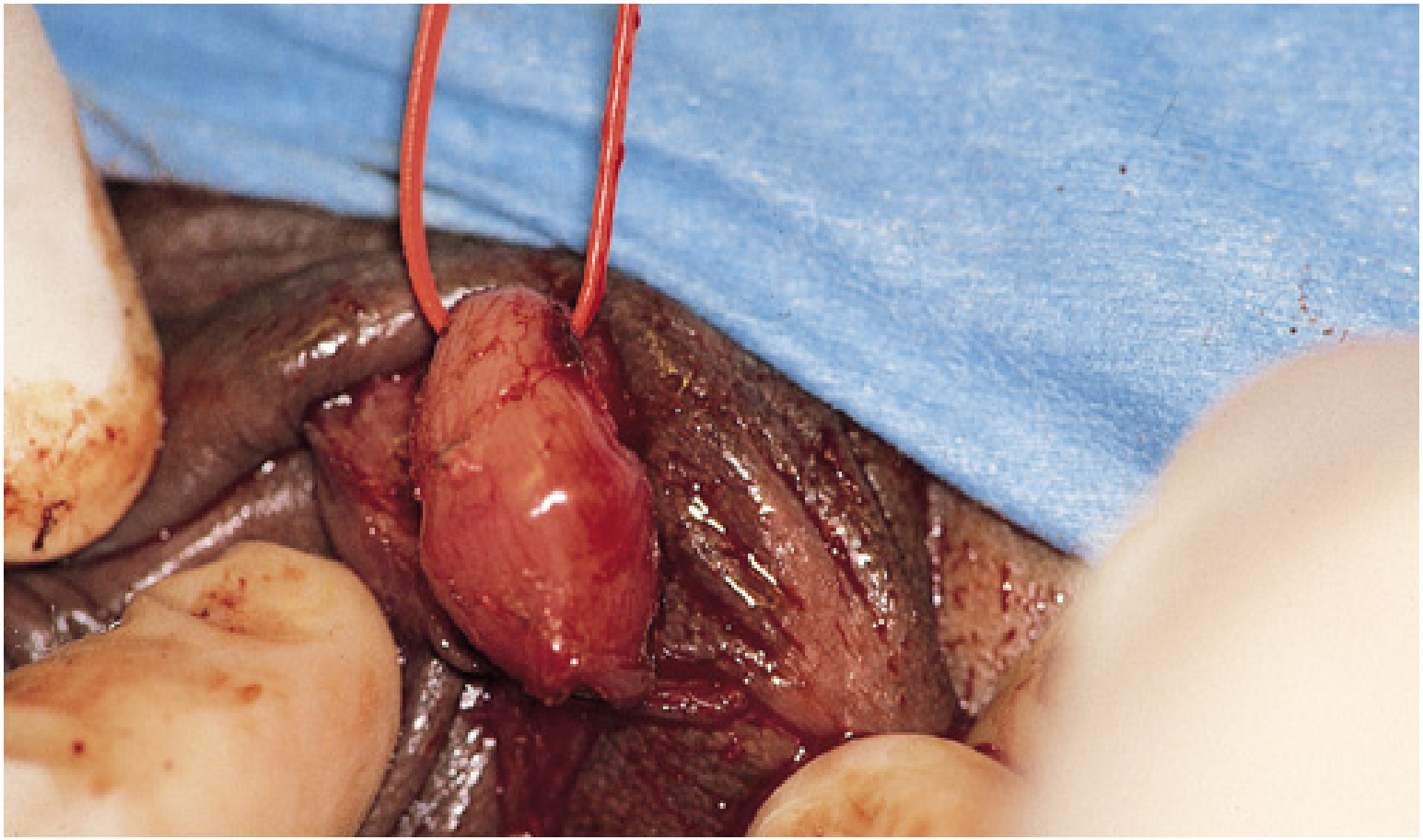
Right scrotal gonad
Empty left labioscrotal pouch



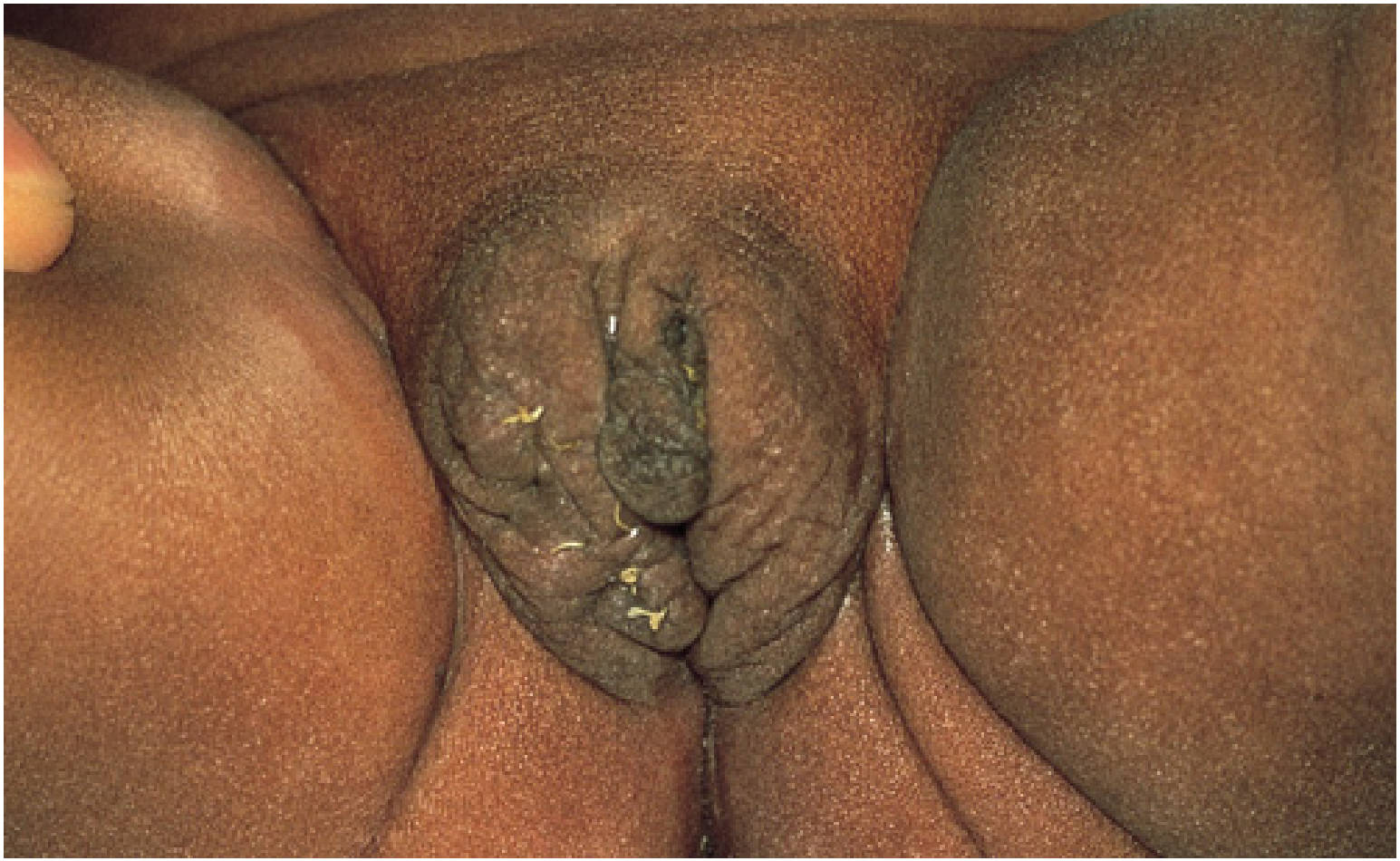
Gonadectomy and reduction clitoroplasty



Gonadectomy and reduction clitoroplasty



Gonadectomy and reduction clitoroplasty



46,XY Gonadal dysgenesis

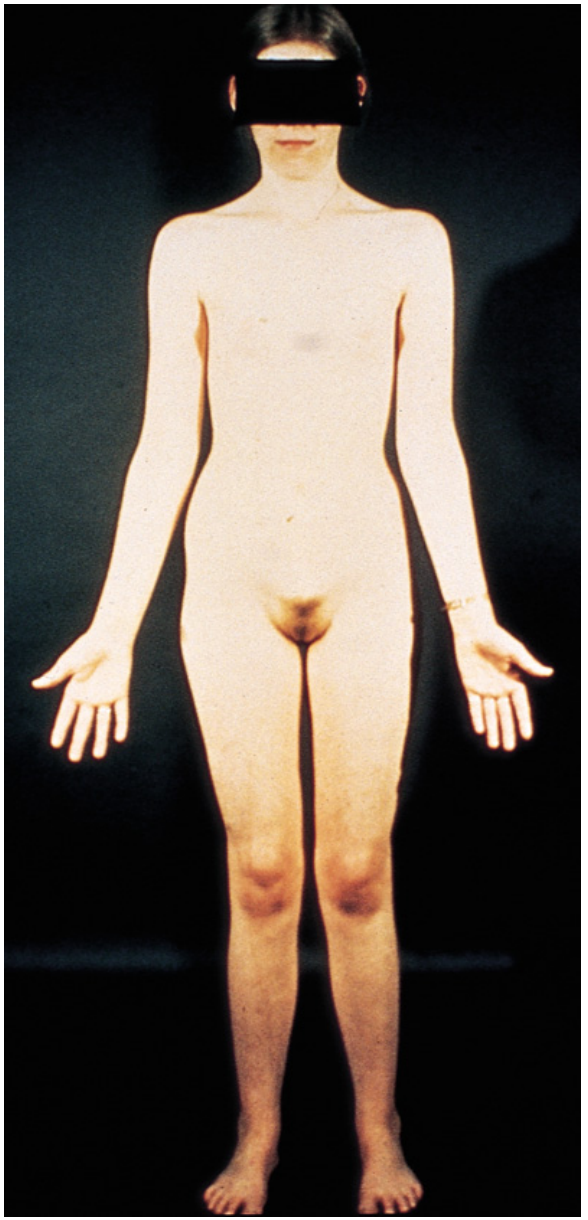
19-y old female

46,XY

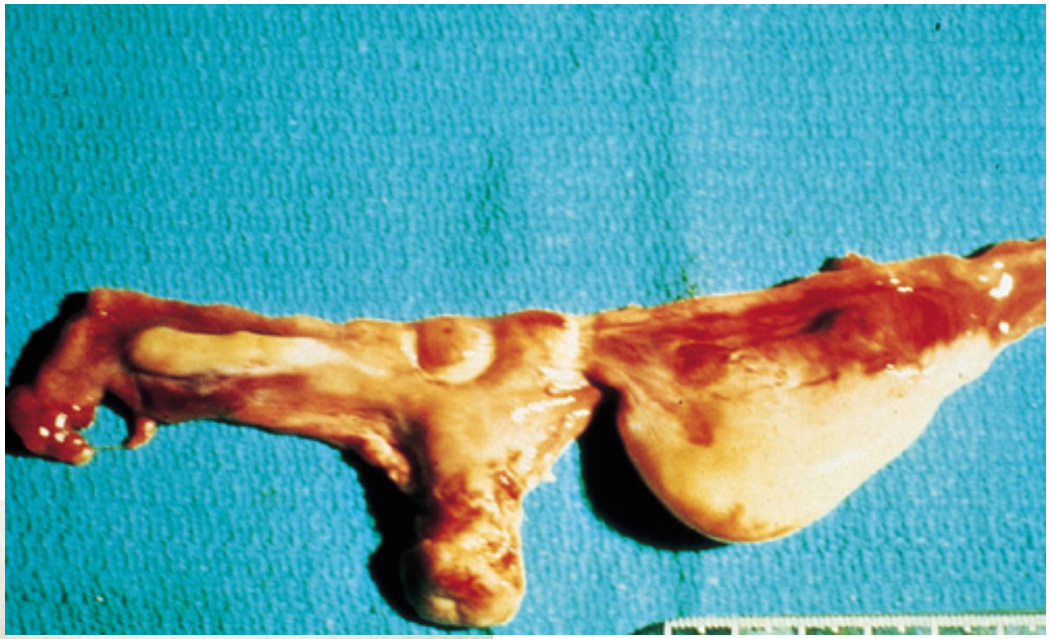
Normal stature

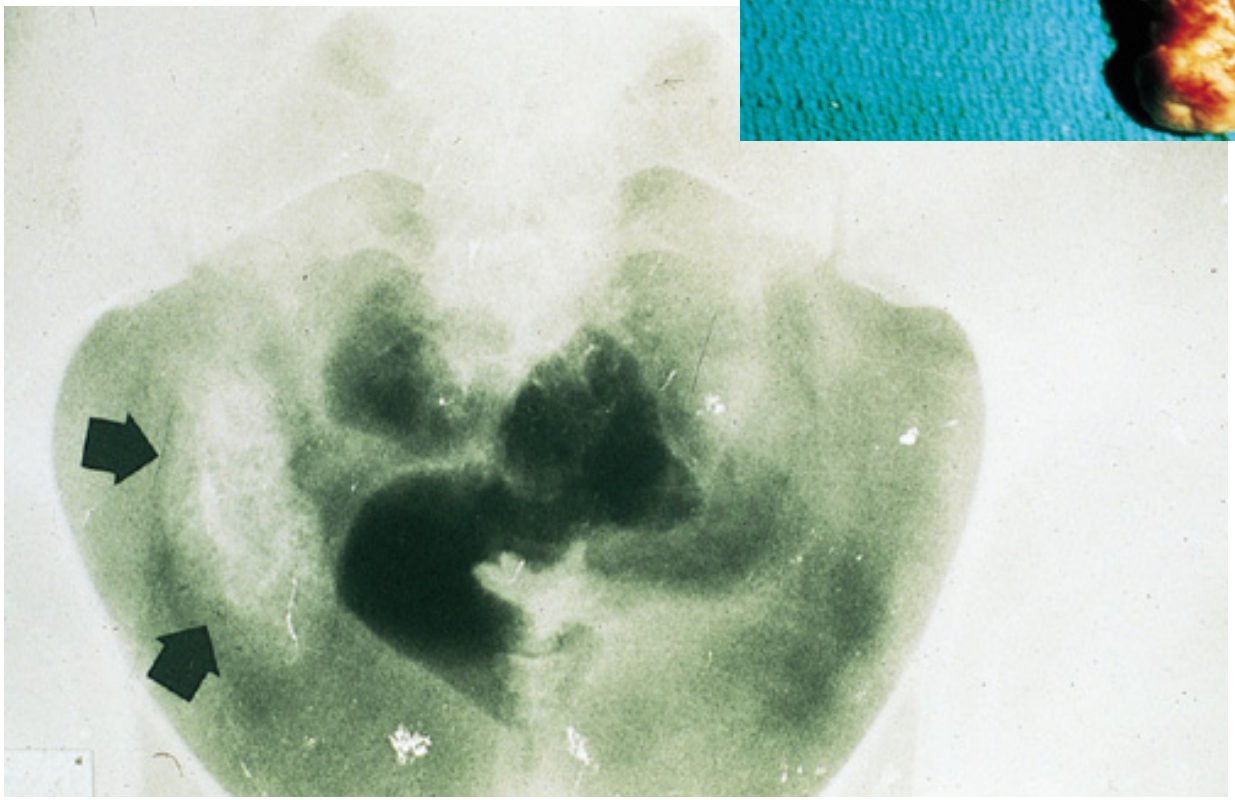
Sexual infantilism

Endocrine profile of primary gonadal failure



46,XY gonadal dysgenesis with calcified gonoblastoma

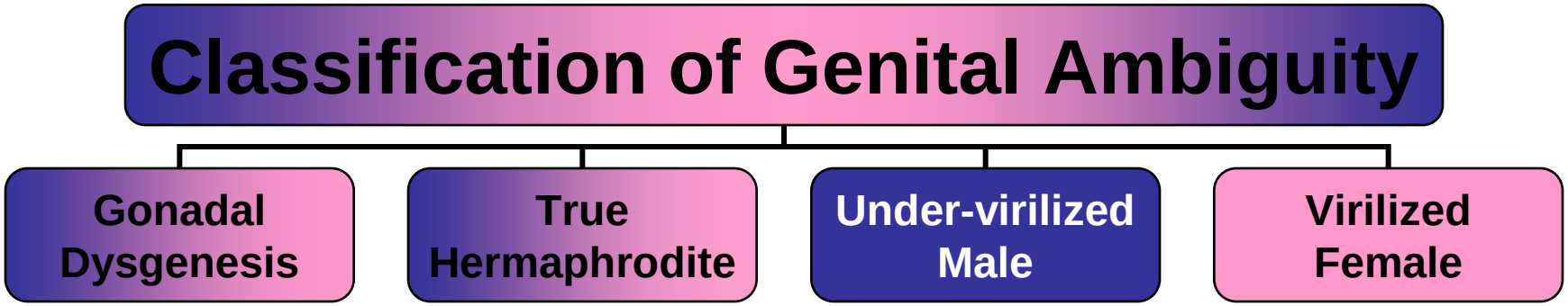




Salient features of the empty pelvis/agonadia/vanishing testes syndrome

C. Salient Features of the Empty Pelvis/Agonadia/Vanishing Testes Syndrome

Karyotype:	46, XY			
Inheritance:	Sporadic; possibly autosomal recessive in familial cases			
Gonads:	Absent (CT, laparoscopy)			
Habitus:	Sexual infantilism at puberty			
Hormone profile:	? plasma FSH and LH, and minimal testosterone			
	Testicular Regression Before 8 Weeks	**Testicular Regression Between 8 and 10 Weeks**	**Testicular Regression After 12 to 14 Weeks**	**Incomplete Regression After 12 to 14 Weeks**
Genitalia	Female	Ambiguous	Male	Micropenis
Wolffian duct derivatives	Absent	Complete absence to partial development	Present	Present
Müllerian duct derivatives	Female	Complete absence to partial development	Absent	Absent
Gonads	Absent	Absent	Absent	Rudimentary testes


Classification of Genital Ambiguity
Gonadal Dysgenesis
True Hermaphrodite
Under-virilized Male
Virilized Female

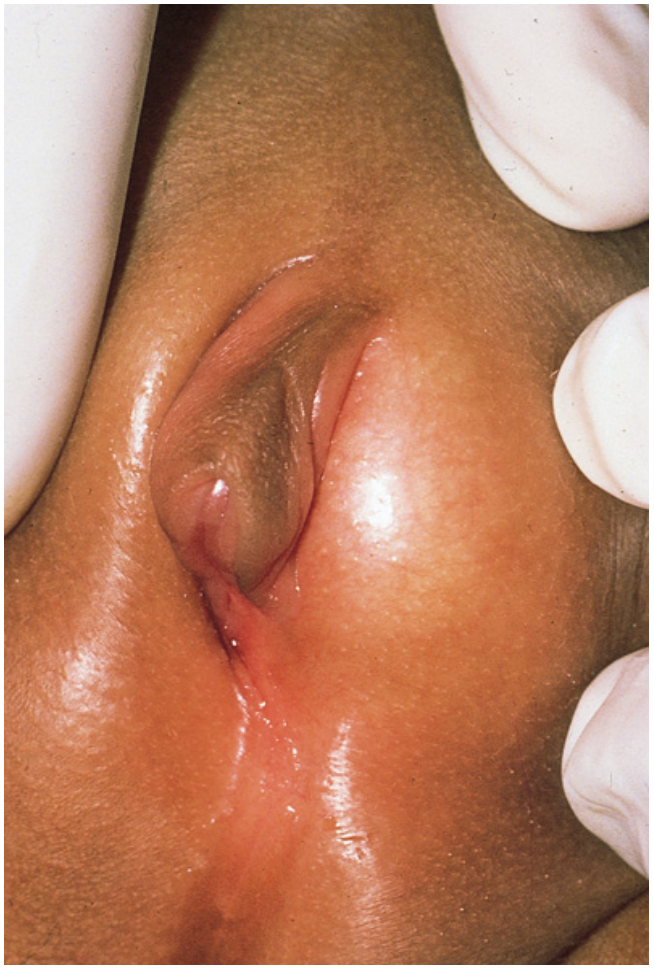
OVARY & TESTIS

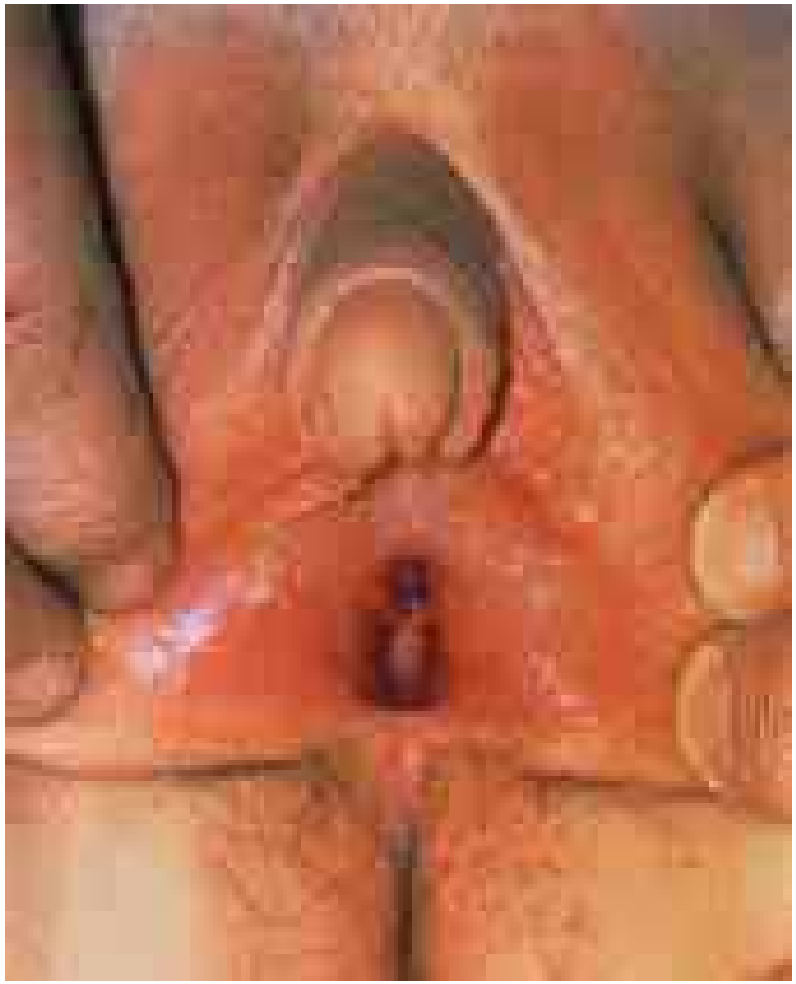
|

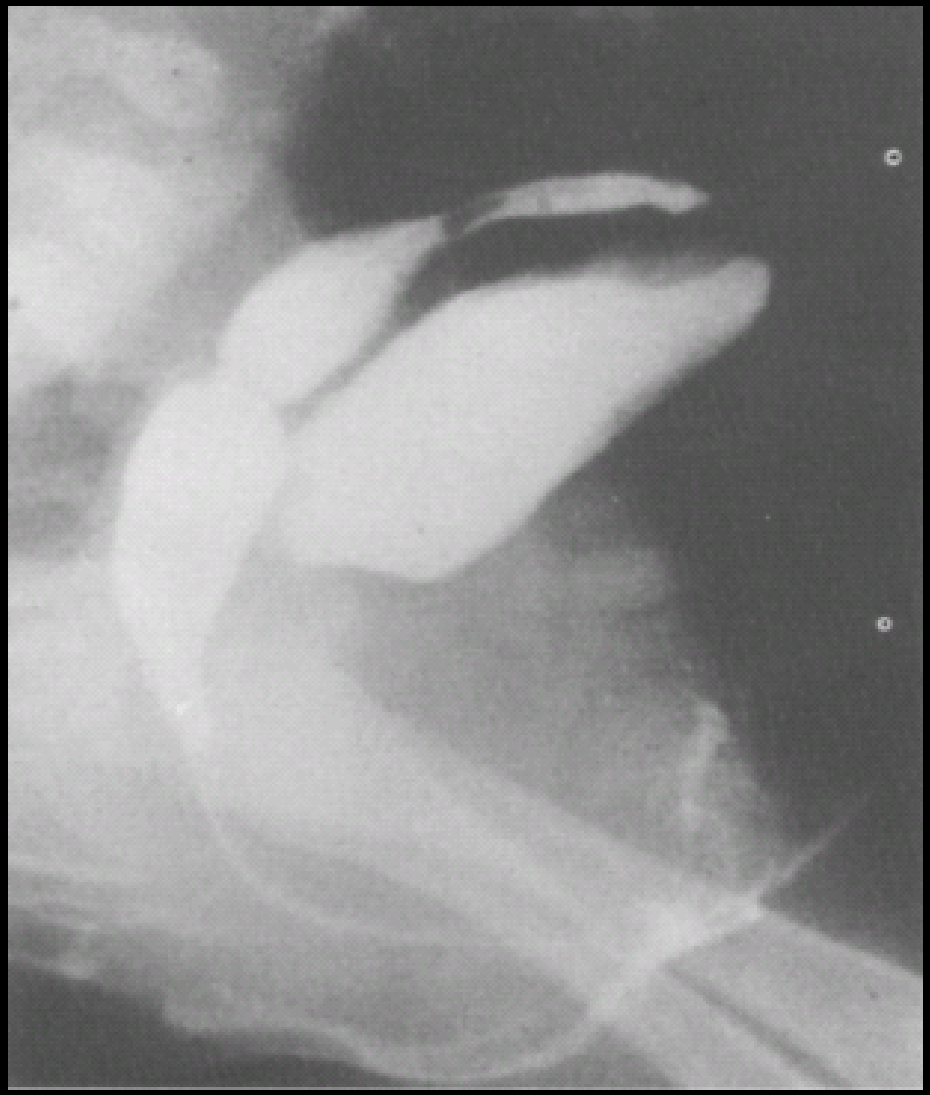
46 XX, 46 XY
46 XX/XY etc.

TRUE HERMAPHRODITISM

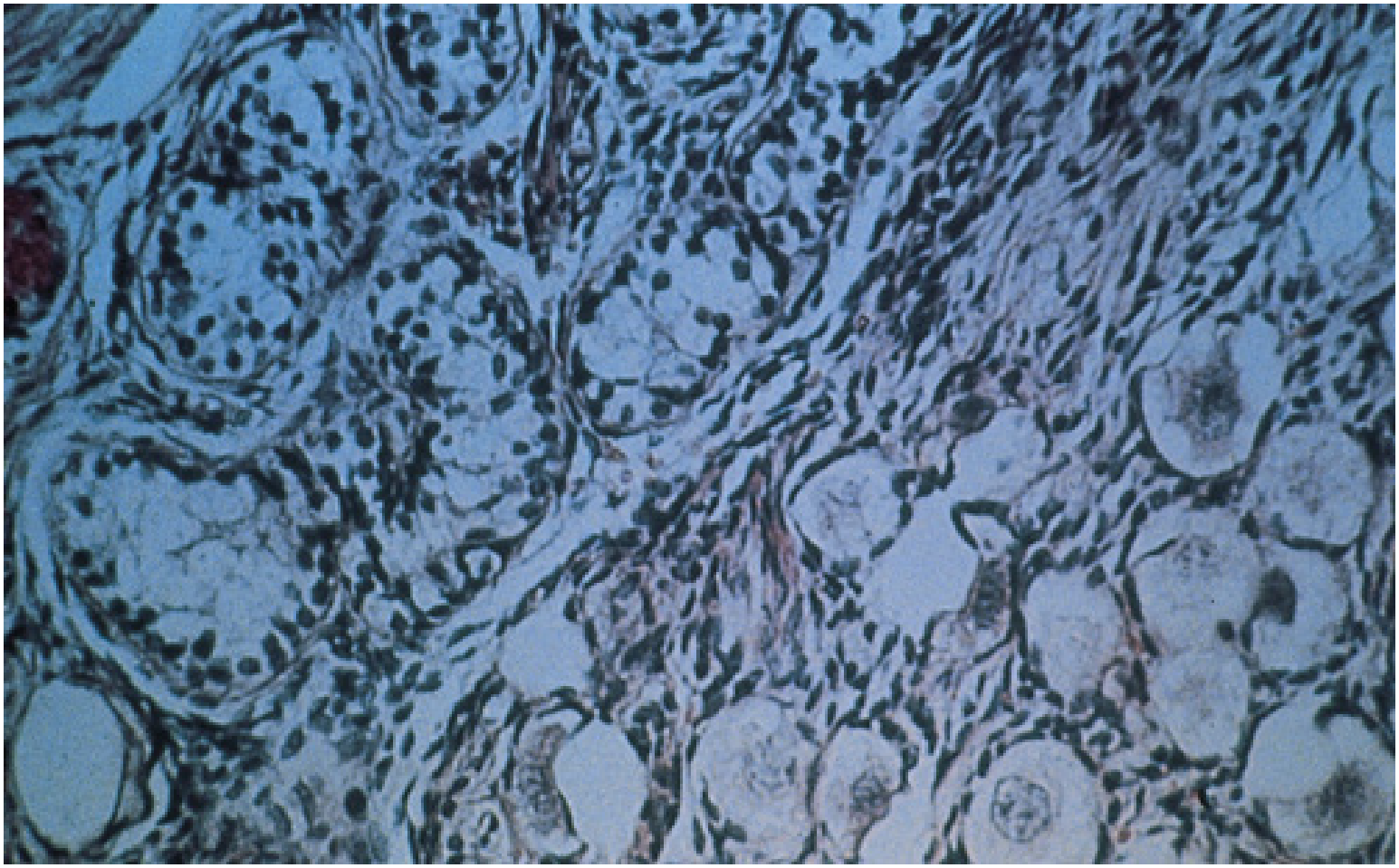
True Hermaphroditism/ Ovotesticular DSD

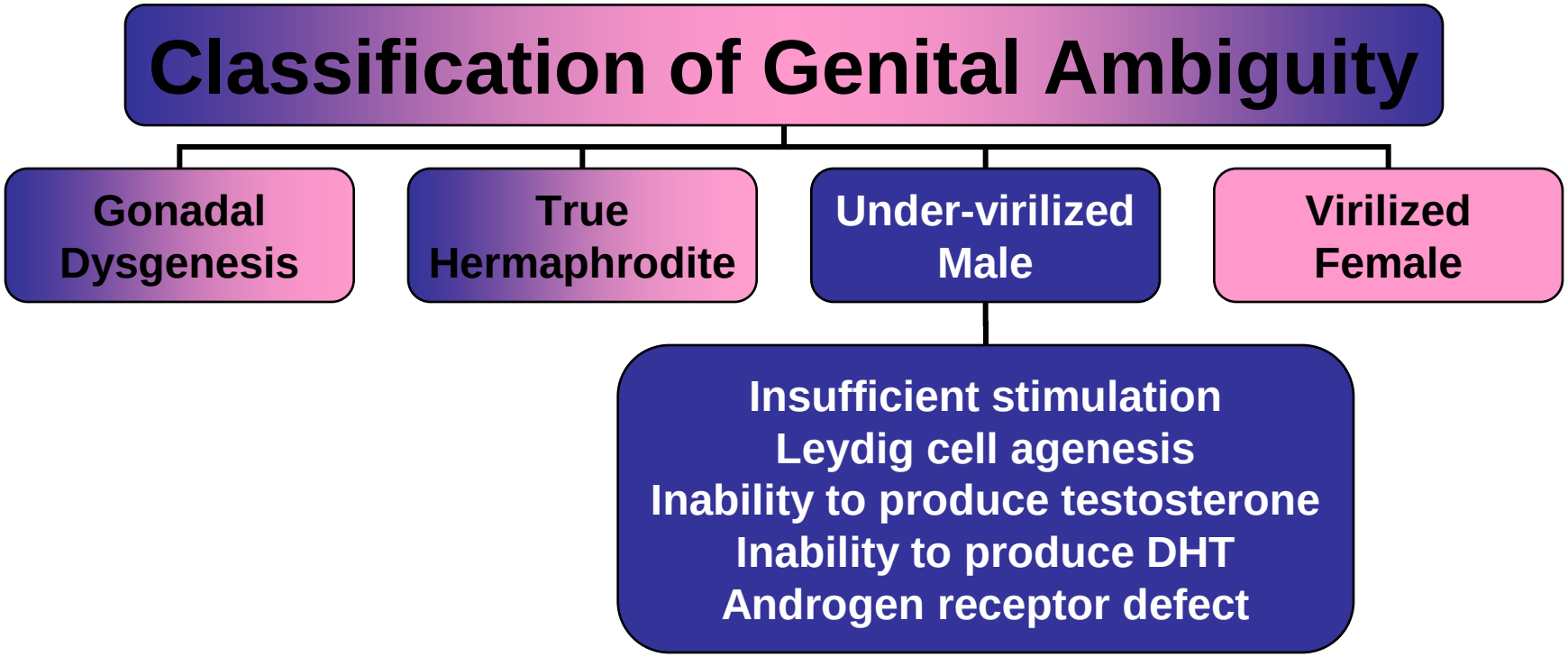


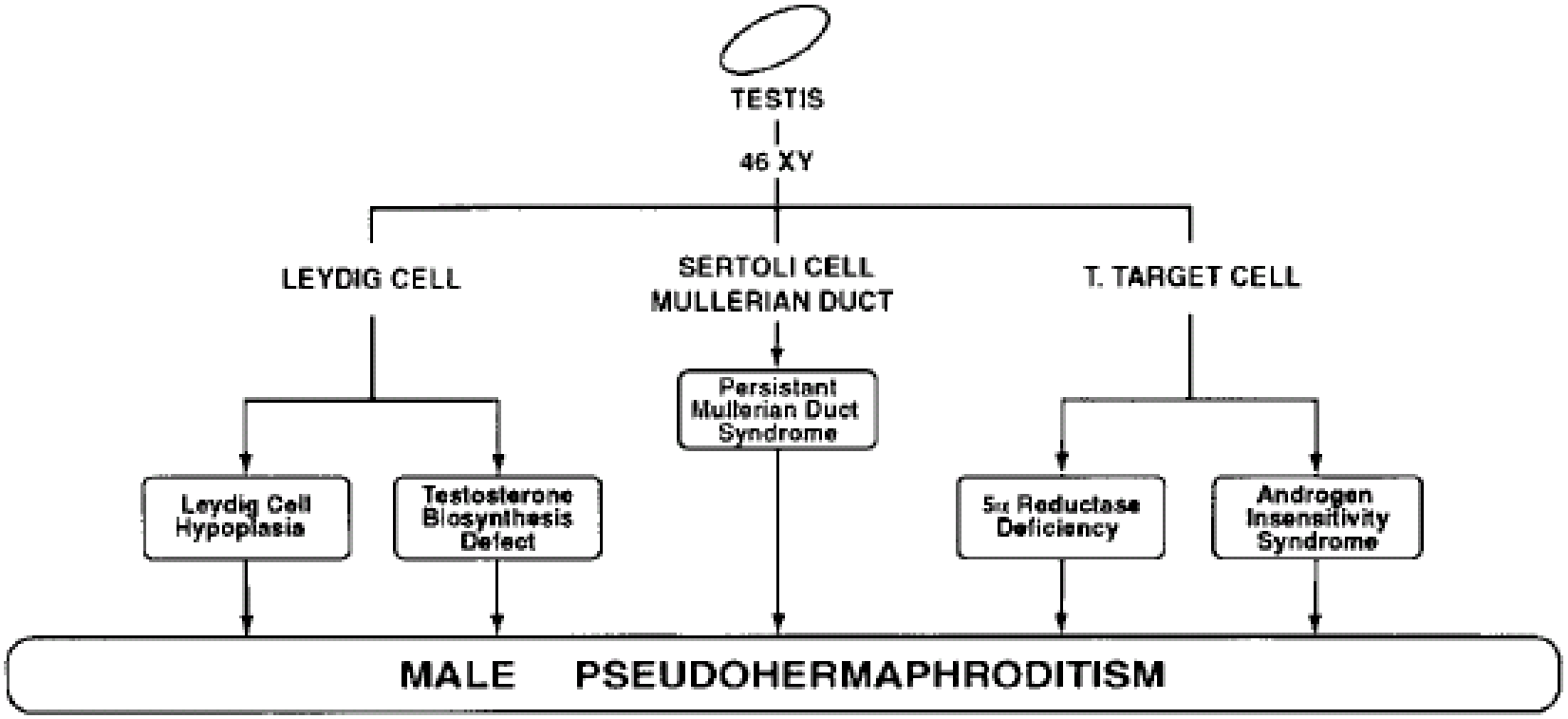


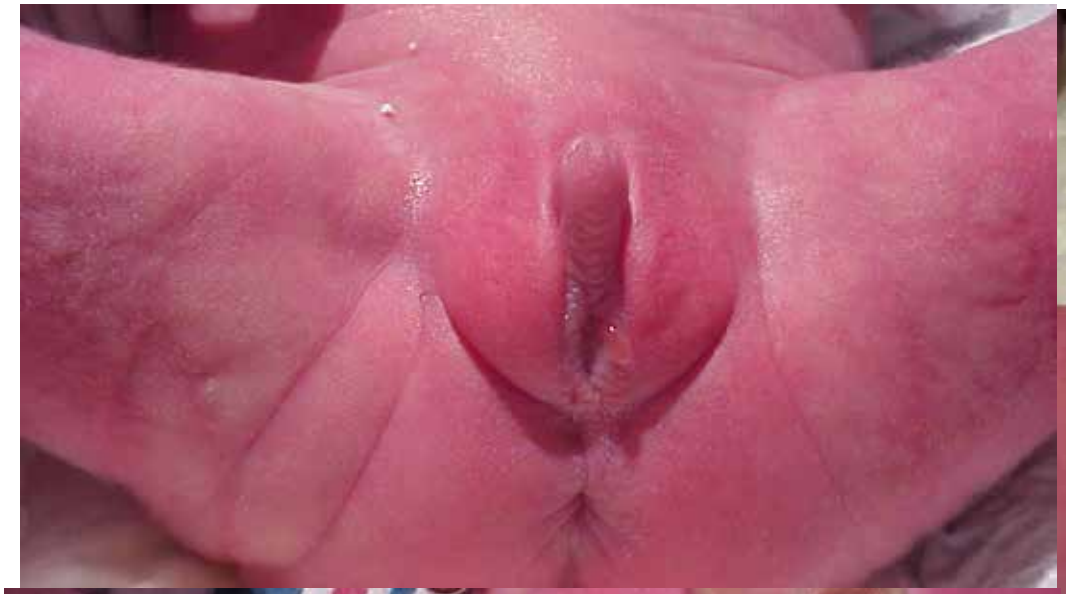


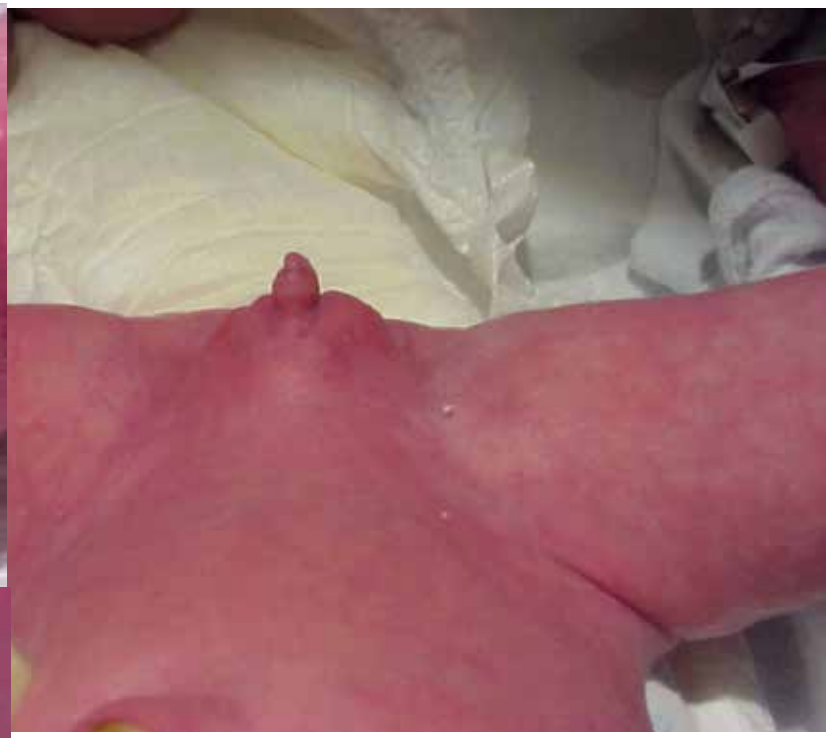
True hermaphroditism

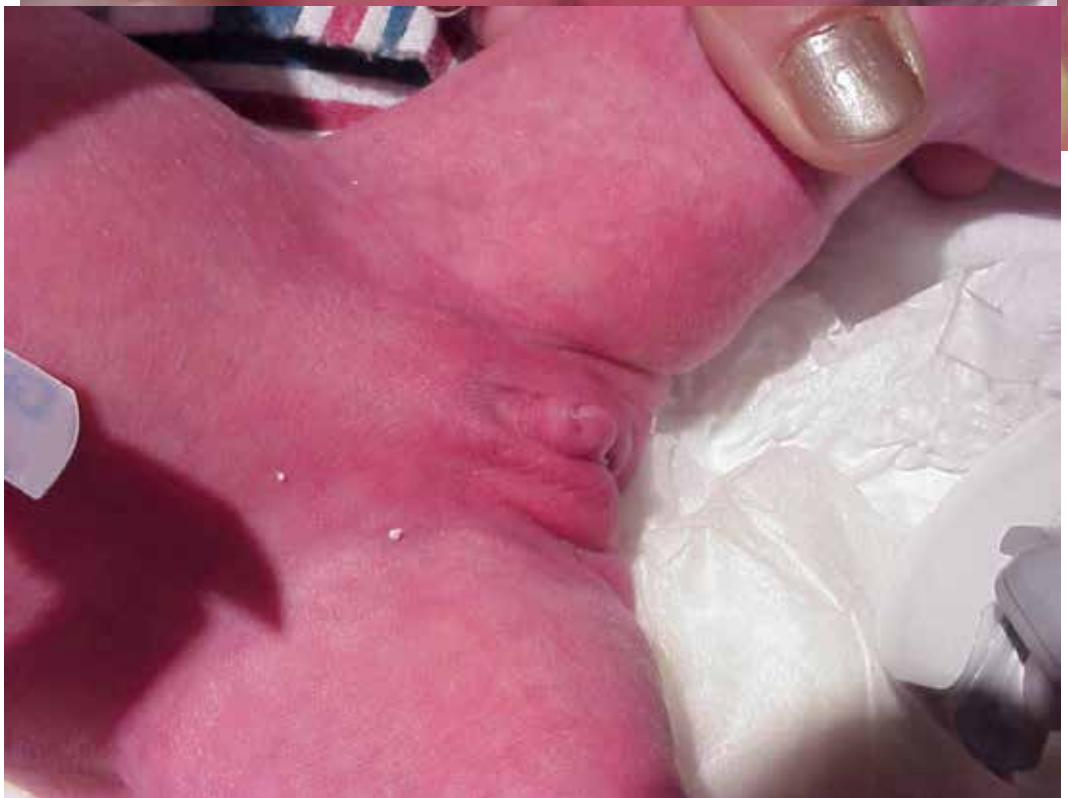



Classification of Genital Ambiguity
Gonadal Dysgenesis
True Hermaphrodite
Under-virilized Male
Virilized Female
Insufficient stimulation
Leydig cell agenesis
Inability to produce testosterone
Inability to produce DHT
Androgen receptor defect


TESTIS
46 XY
LEYDIG CELL
SERTOLI CELL
MULLERIAN DUCT
T. TARGET CELL
Persistant Mullerian Duct Syndrome
Leydig Cell Hypoplasia
Testosterone Biosynthesis Defect
5α Reductase Deficiency
Androgen Insensitivity Syndrome
MALE PSEUDOHERMAPHRODITISM



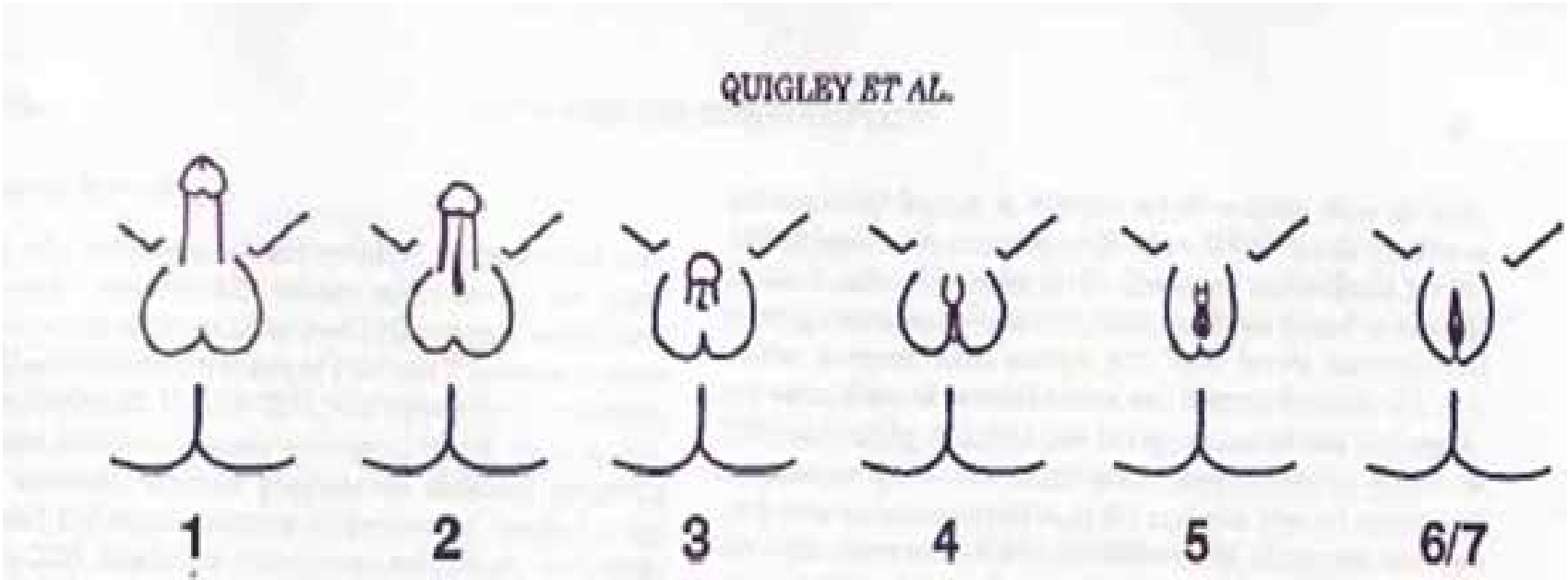




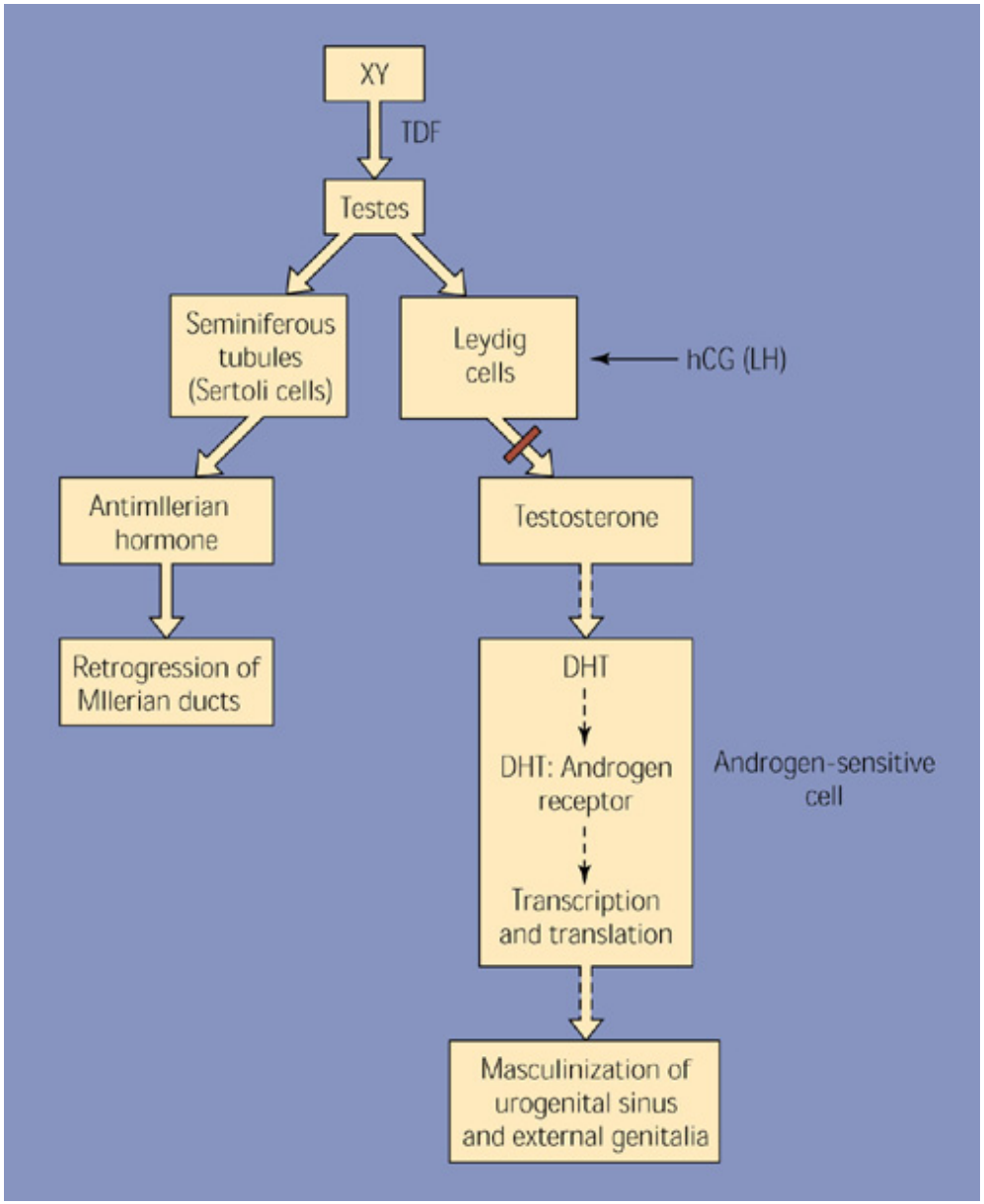
Undervirilized male
Male Pseudohermaphrodite

Absent Uterus
46,XY

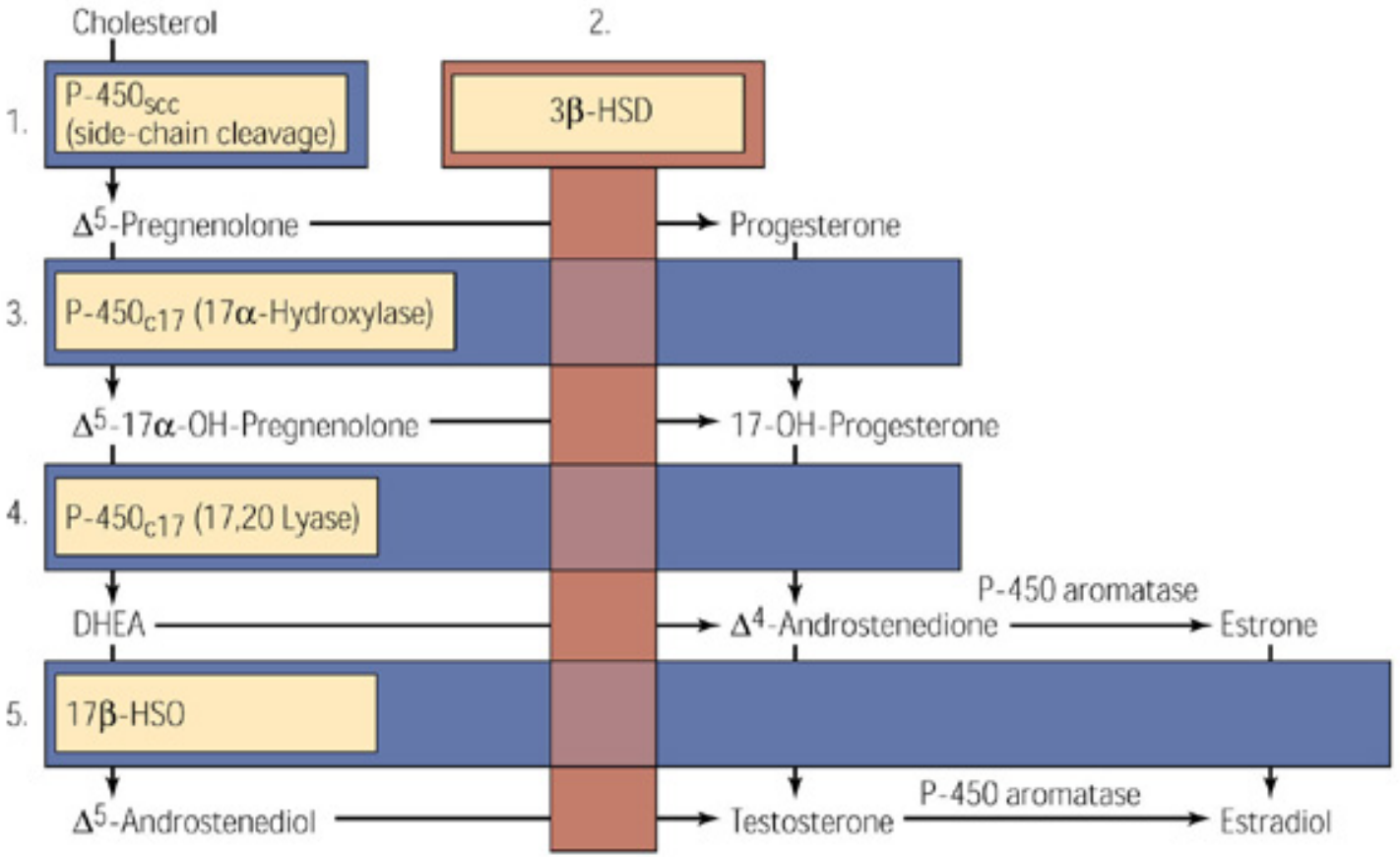
Spectrum of Undervirilization



Testosterone block in male sex determination



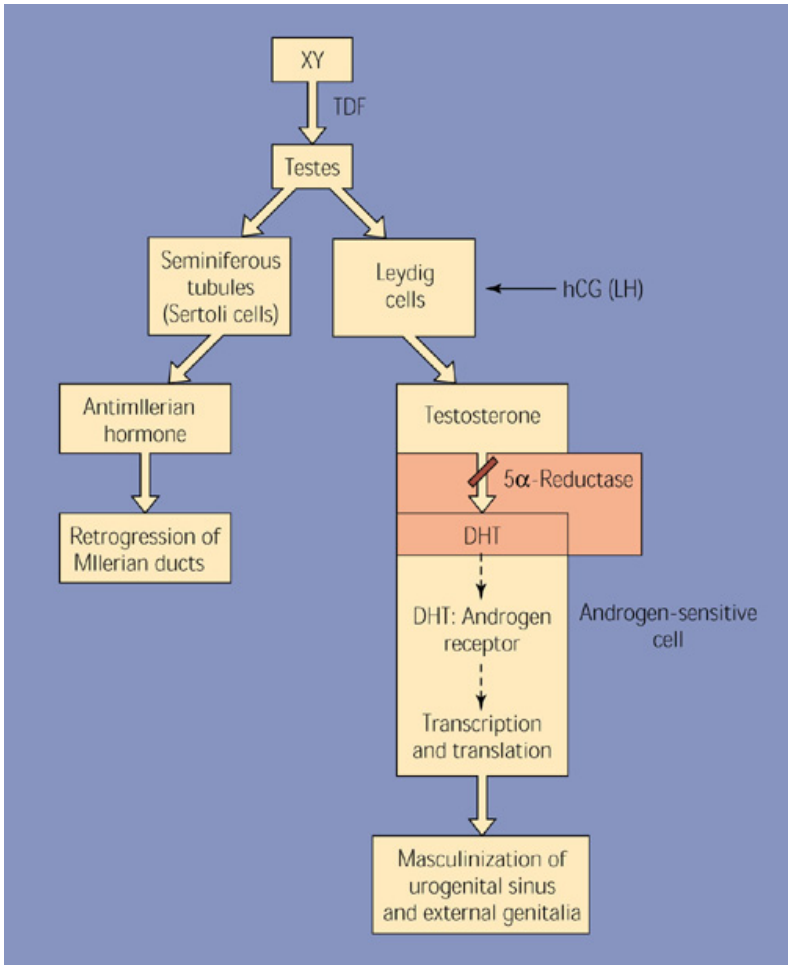
Enzymatic defects in biosynthetic pathway for testosterone



Salient features in Androgenic steroid disorders

	P450scc	**3bHSD**	**17OH**	**17 bHSD**
Karyotype	XY	XY	XY	XY
Inheritance	AR	AR	AR	AR
Gonads	TESTES	TESTES	TESTES	TESTES
Wolfian	–	NL	NL	NL
Mullerian	ABSENT	ABSENT	ABSENT	ABSENT
Genitalia	↗	↗	↗	↗
Habitus	**Adrenal Insufficiency**	**Adrenal Insufficiency**	**Hypertension**	

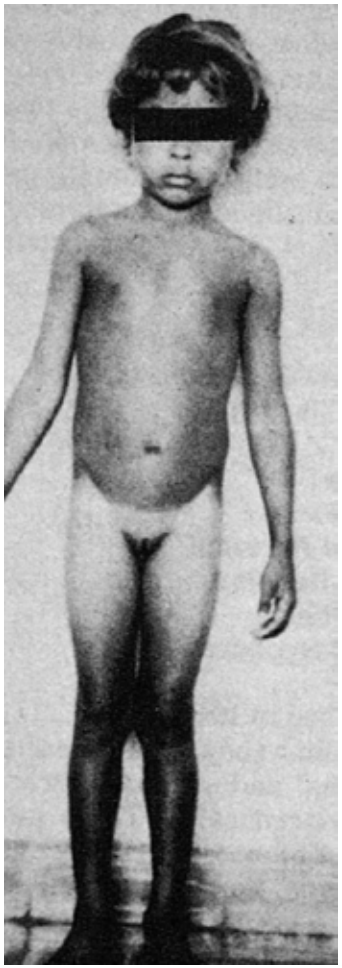
Male pseudohermaphroditism from 5a-reductase deficiency



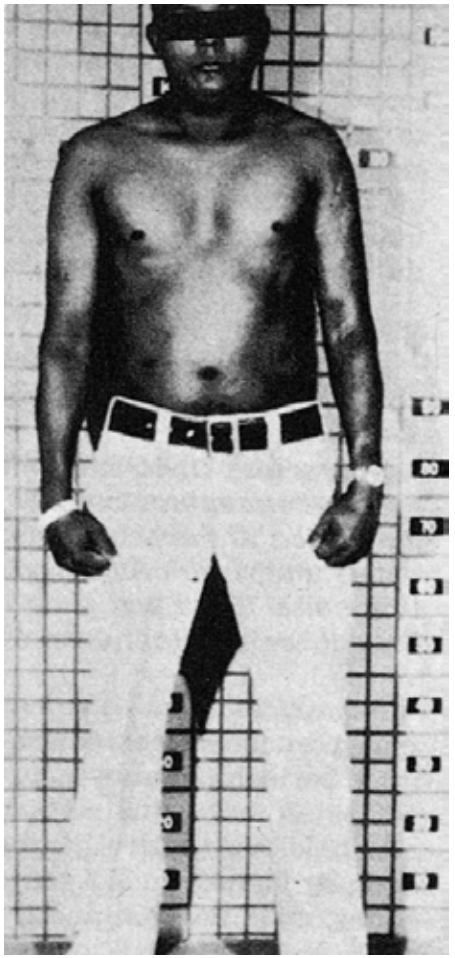
5a-Reductase deficiency

- **AR Mutation in 5aR2 gene**
- **Impaired conversion of T to DHT in target tissues**
- **Impaired development of penis, scrotum, urethra and prostate**
- **At birth, ambiguous genitalia with hypospadic phallus and blind vaginal pouch, normal testes**
- **At puberty virilize without gynaecomastia**

46,XY karyotype with 5a-reductase deficiency



Prepubertal “girl”



Post-pubertal boy

Complete androgen insensitivity

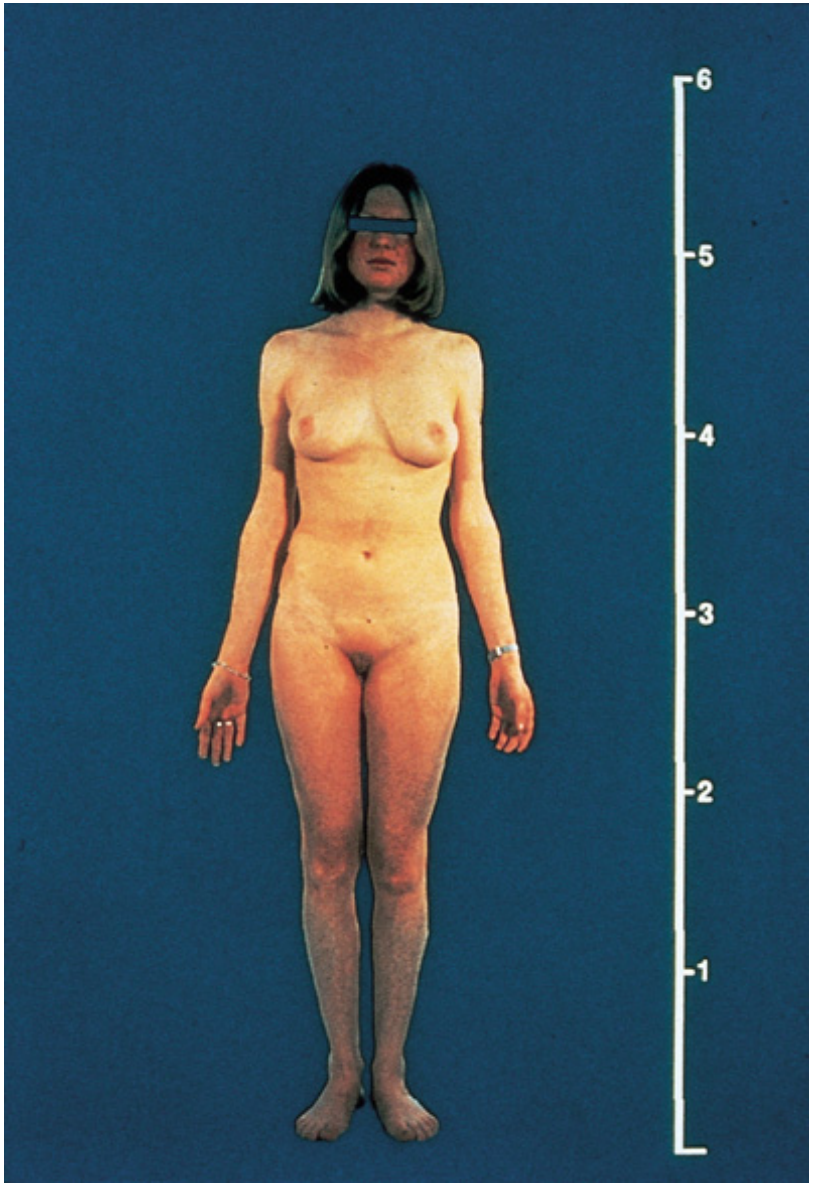
18y old “female”

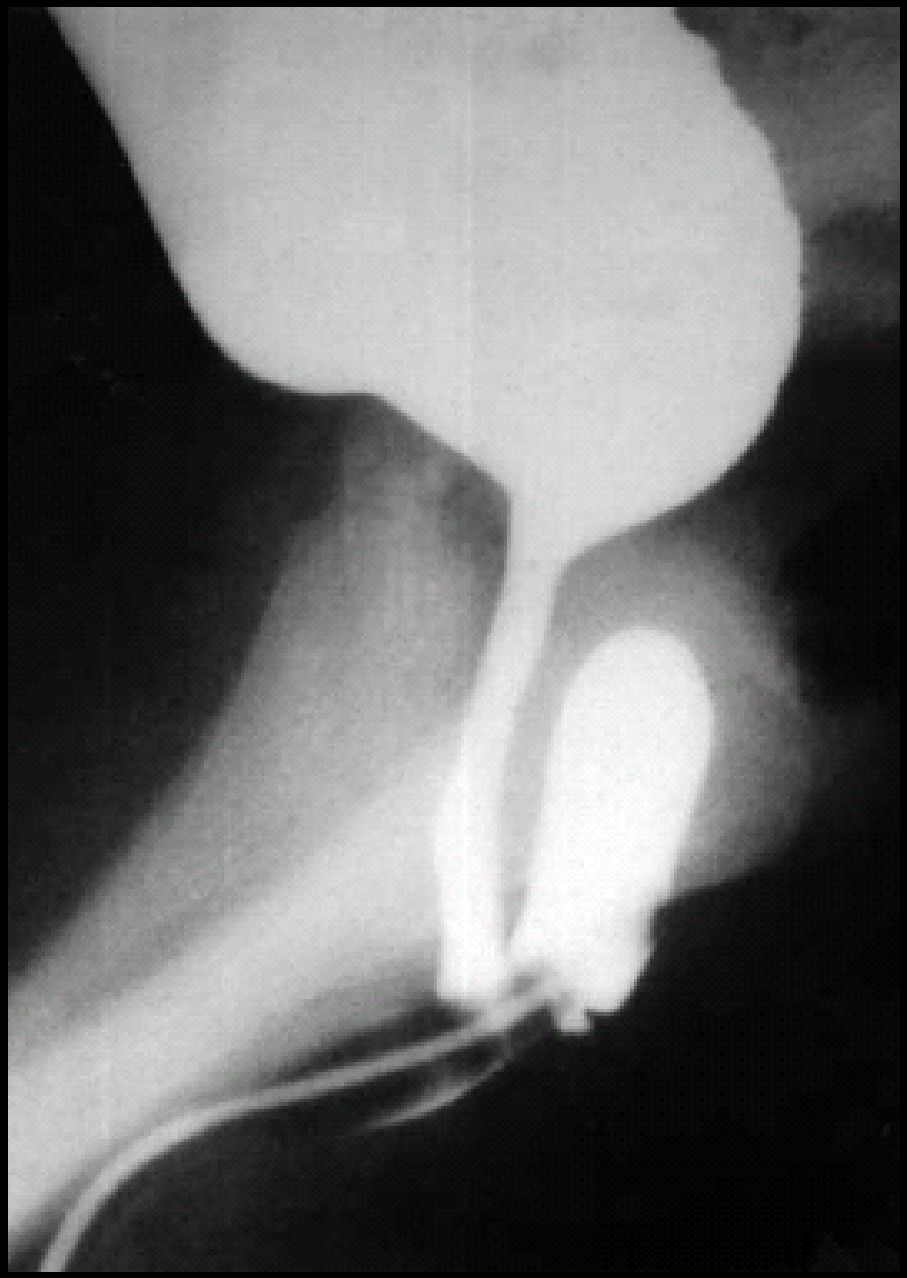
Tanner V breast

Absent Pubic hair

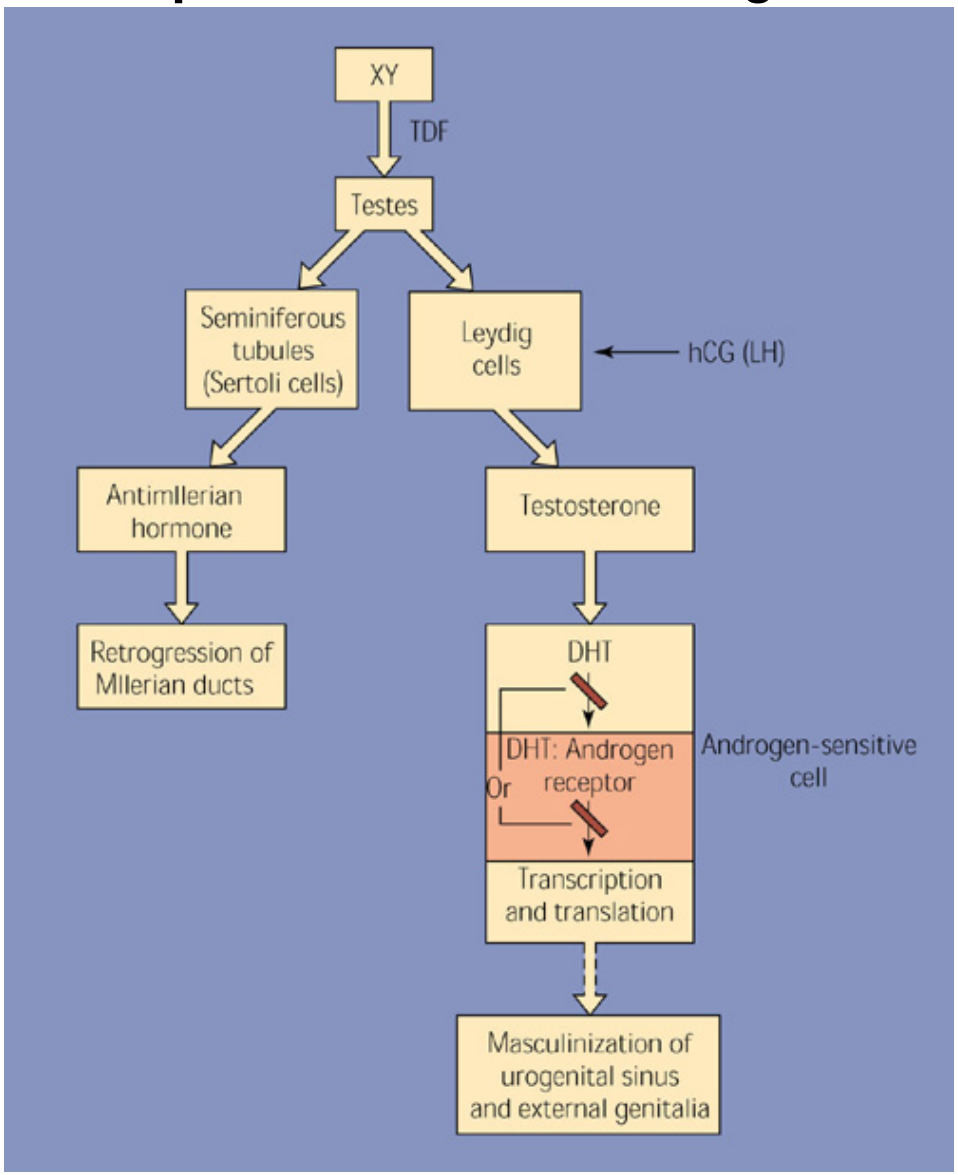
Blind vagina

46,XY

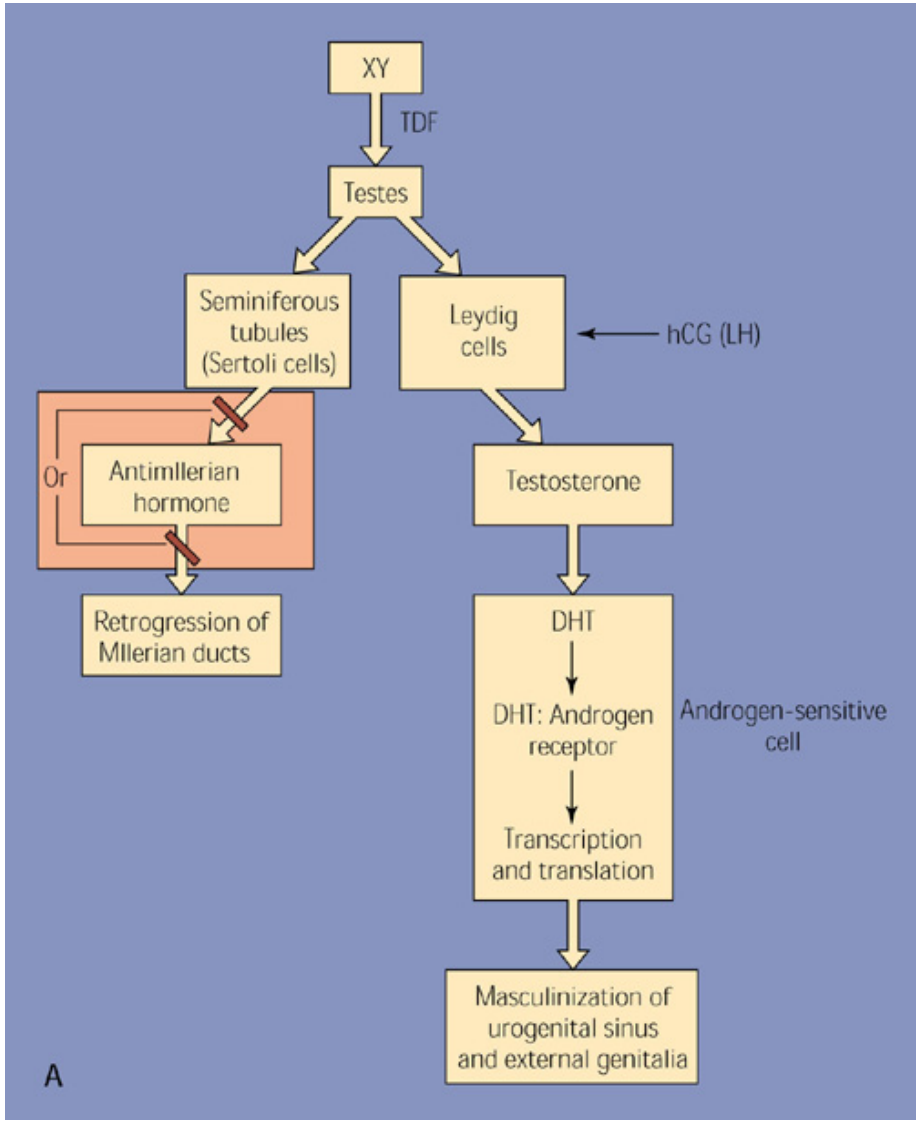




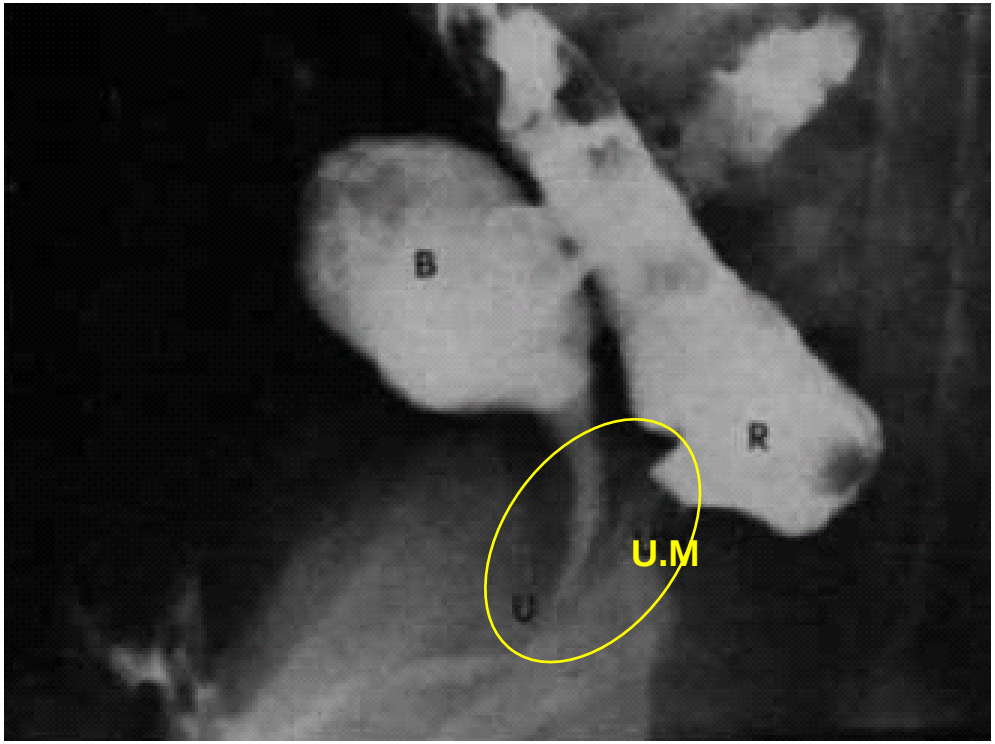
Male pseudohermaphroditism from androgen resistance



Persistent Mullerian duct syndrome

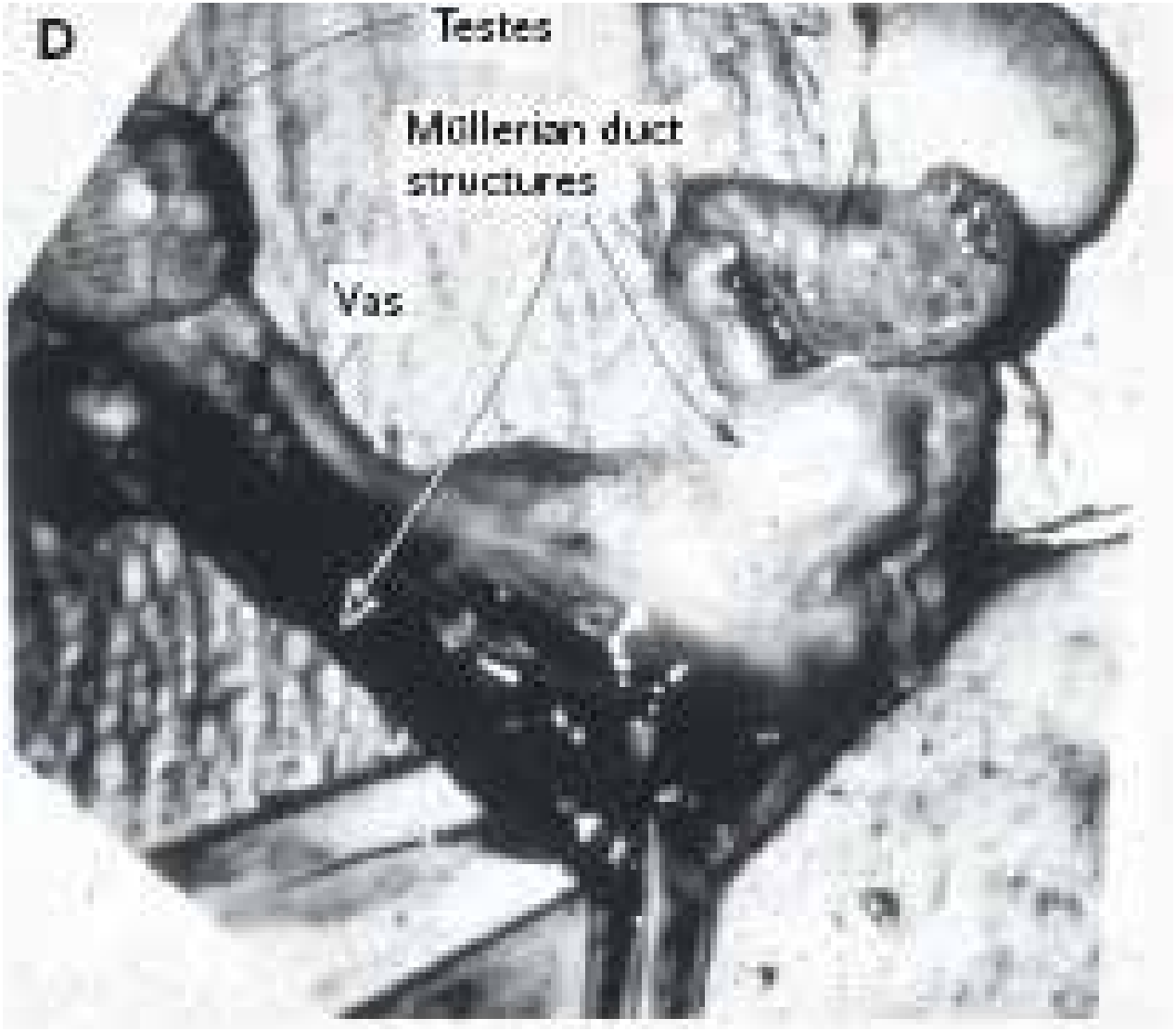


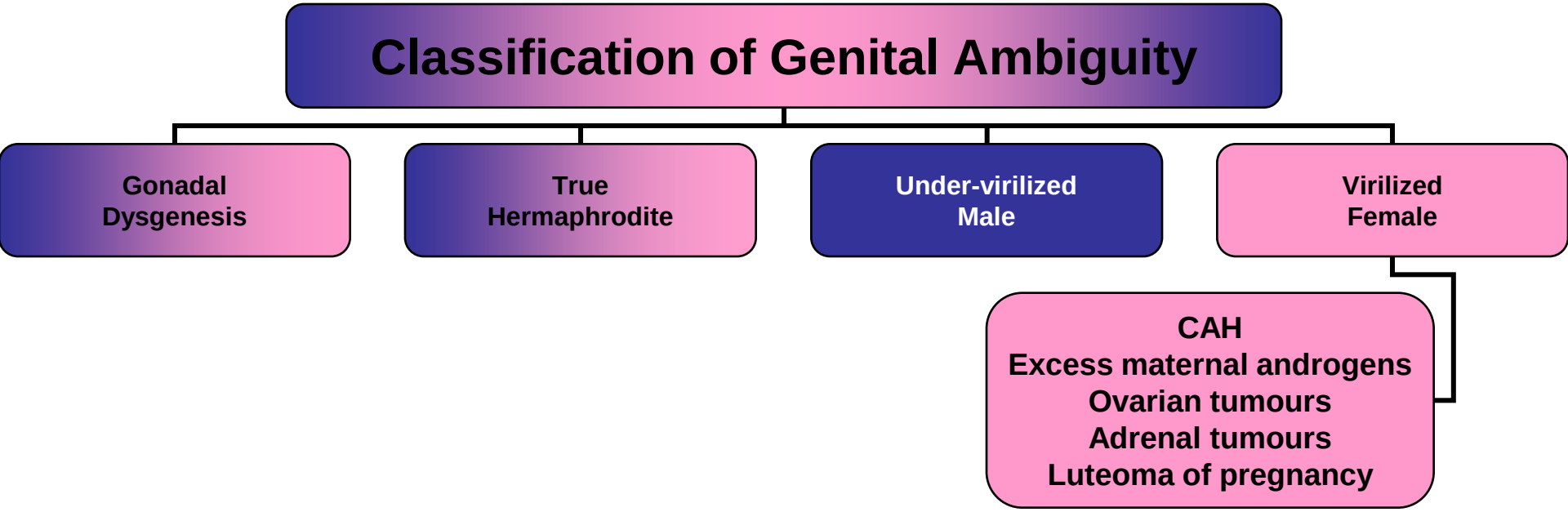
Persistent Mullerian Duct Syndrome (Hernia uteri inguinale)

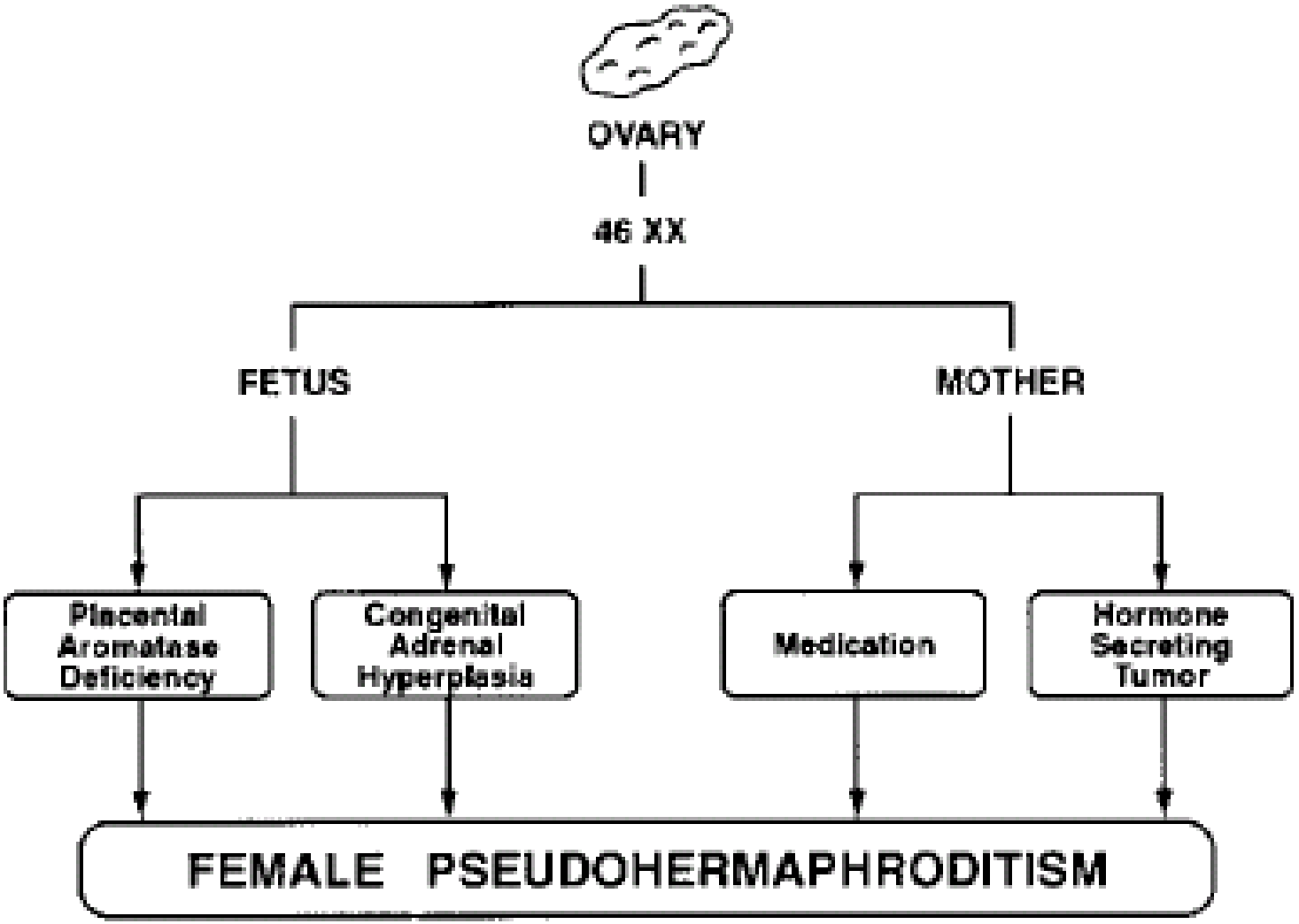


- 46 XY
- Male genitalia
- Undescended testes
- X-linked recessive

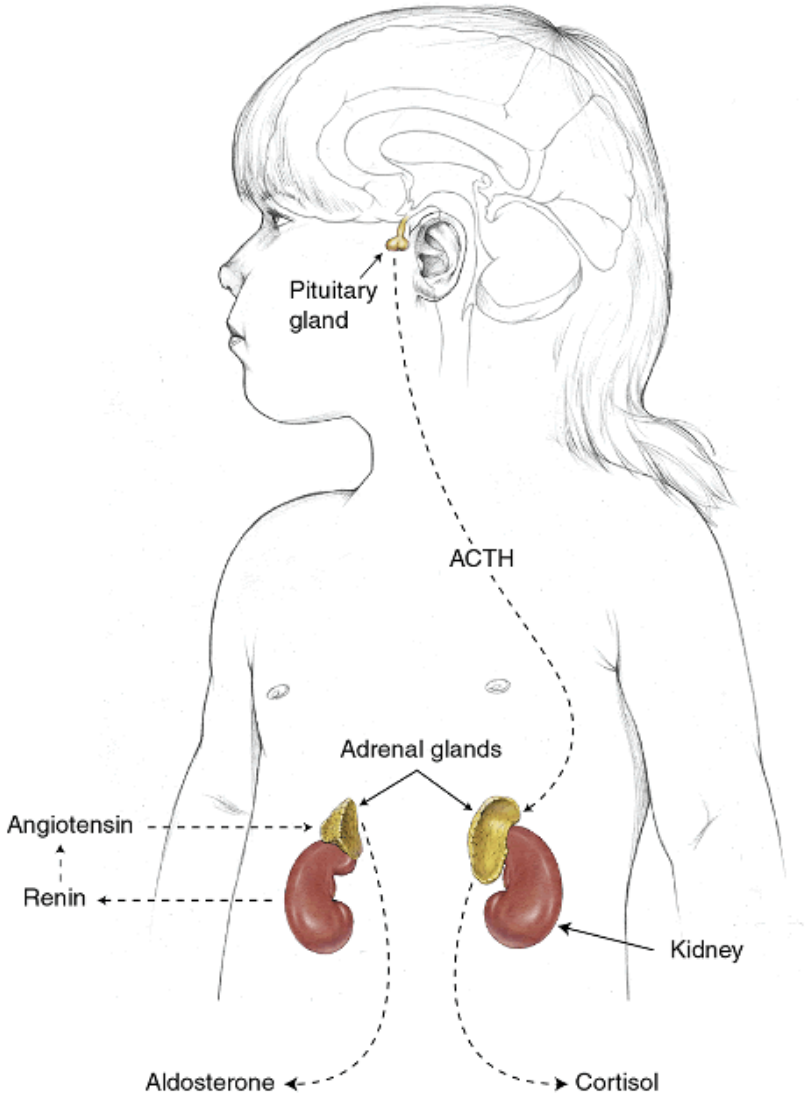
U.M. Utriculus masculinus


D
Testes
Müllerian duct
structures
Vas


Classification of Genital Ambiguity
Gonadal Dysgenesis
True Hermaphrodite
Under-virilized Male
Virilized Female
CAH
Excess maternal androgens
Ovarian tumours
Adrenal tumours
Luteoma of pregnancy


OVARY
46 XX
FETUS
MOTHER
Placental Aromatase Deficiency
Congenital Adrenal Hyperplasia
Medication
Hormone Secreting Tumor
FEMALE PSEUDOHERMAPHRODITISM

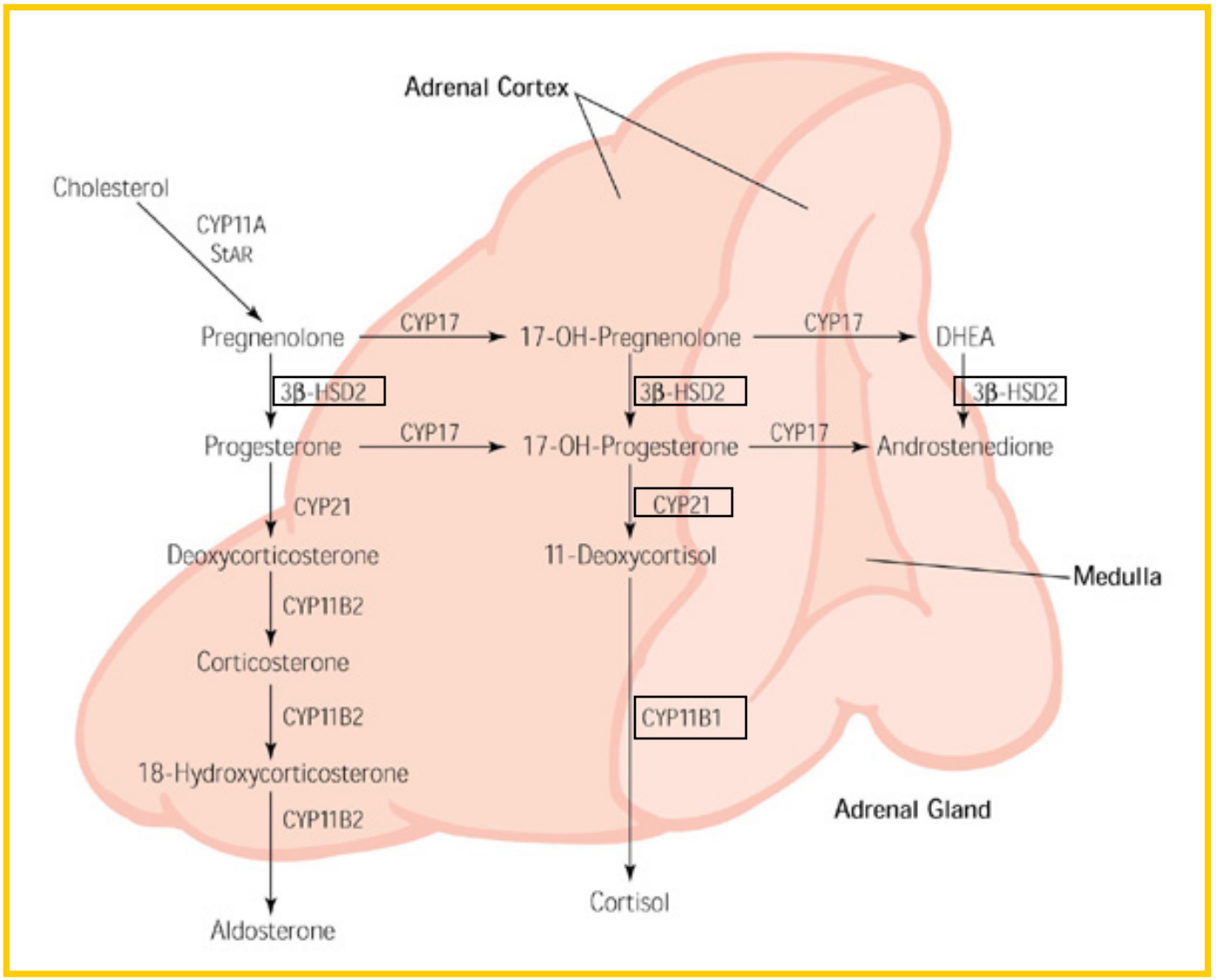
Congenital Adrenal Hyperplasia



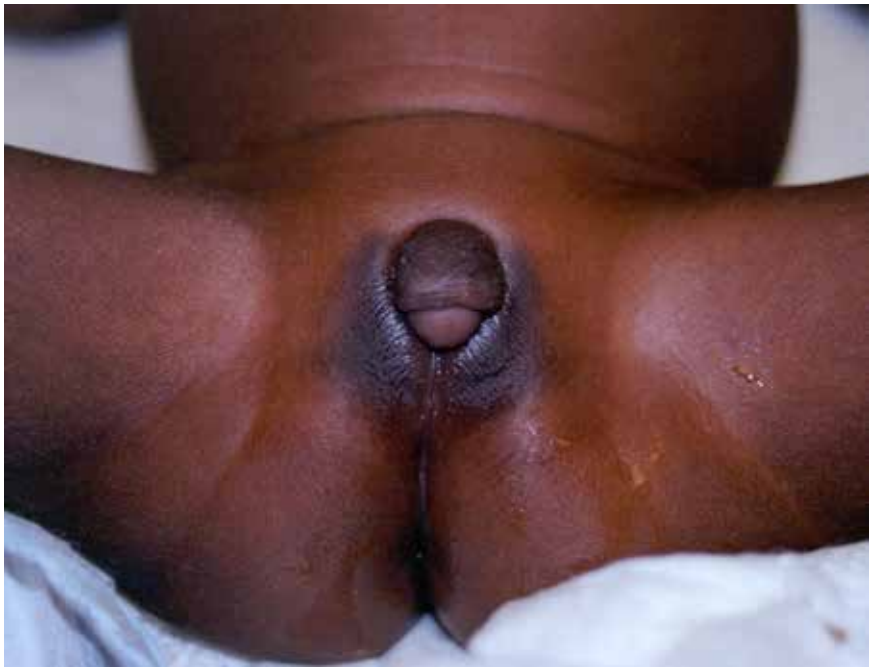
Basics

- 21-OH def (CYP21) 95% of cases
  - Salt-wasting
  - Simple virilizing
  - Non-classic (late onset)
- 11-OH (CYP11)
- 3b-HSD

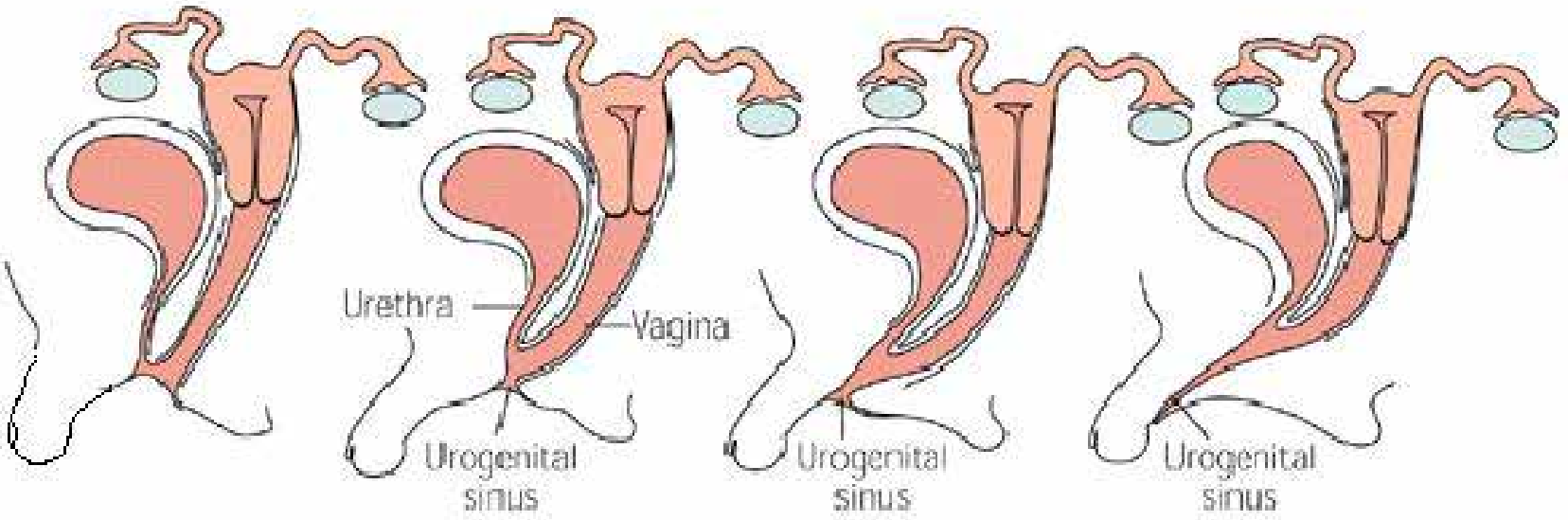
Congenital Adrenal Hyperplasia



CAH- 21-OH deficiency



Spectrum of Masculinization of female external genitalia



Spectrum of virilization CAH

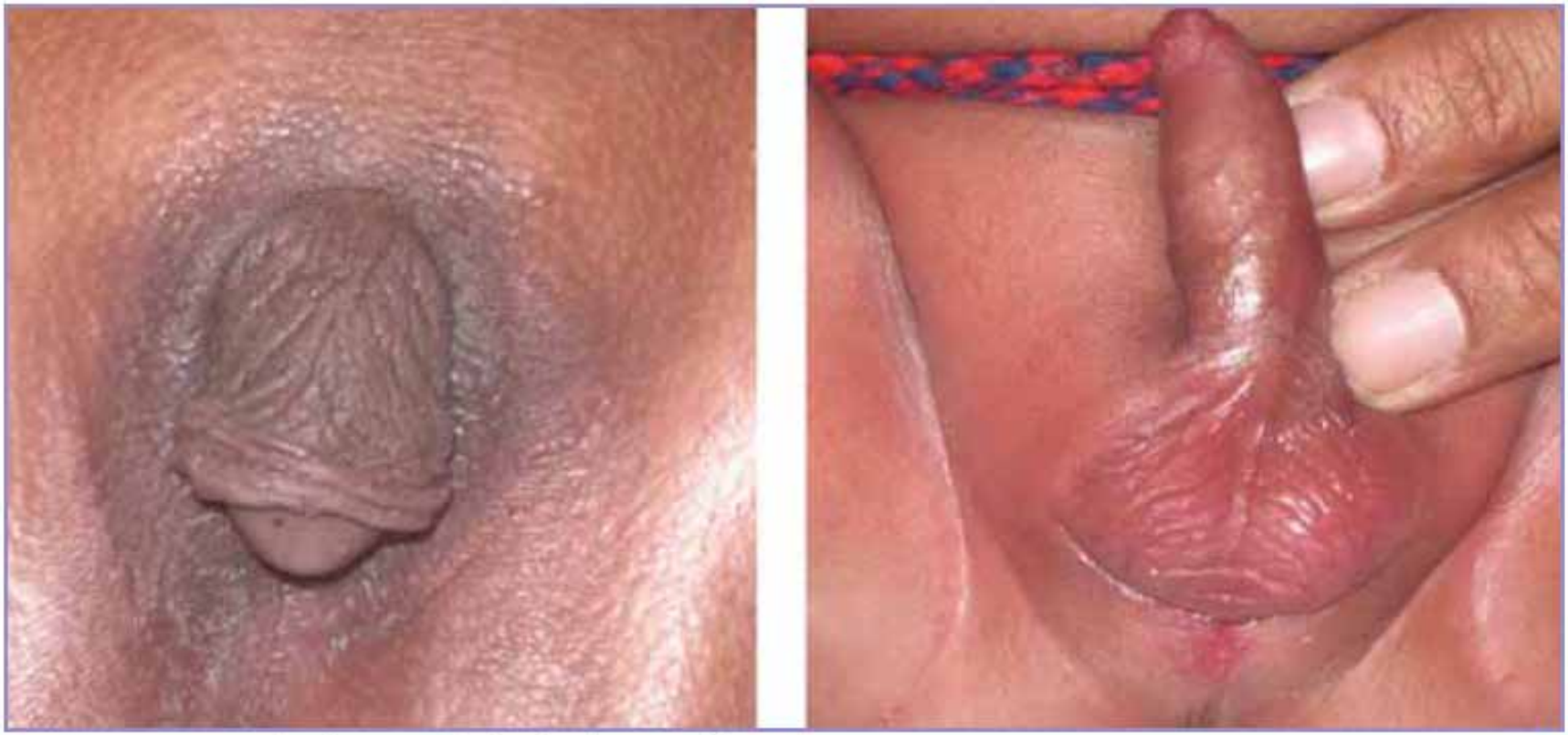
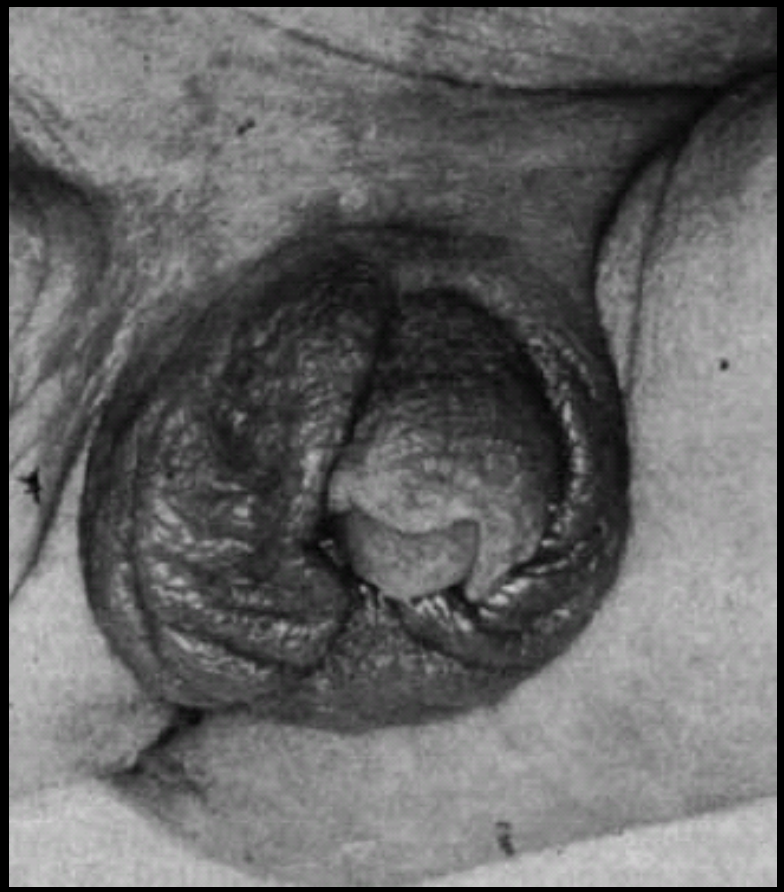
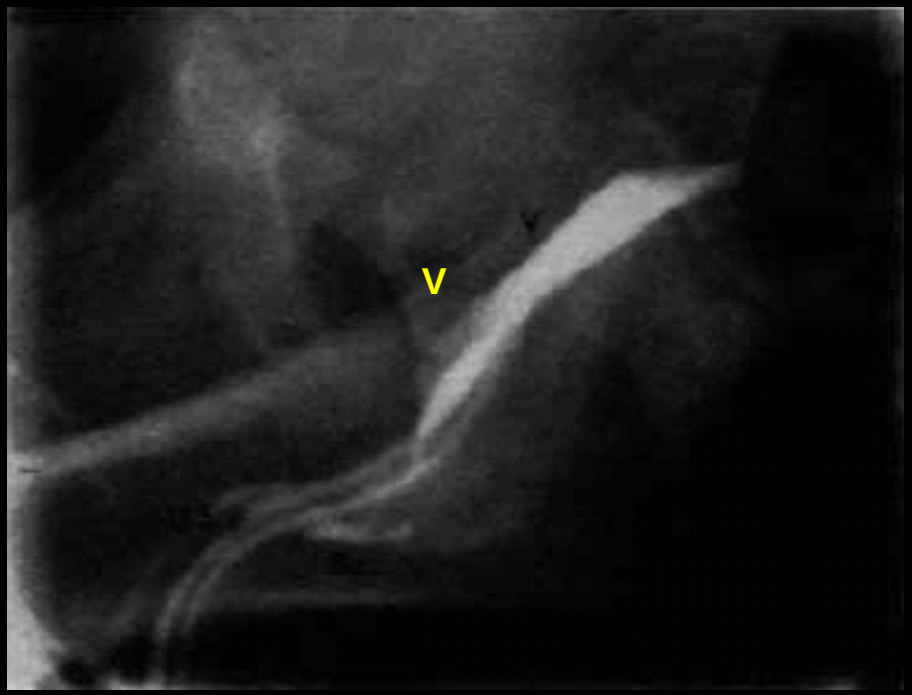
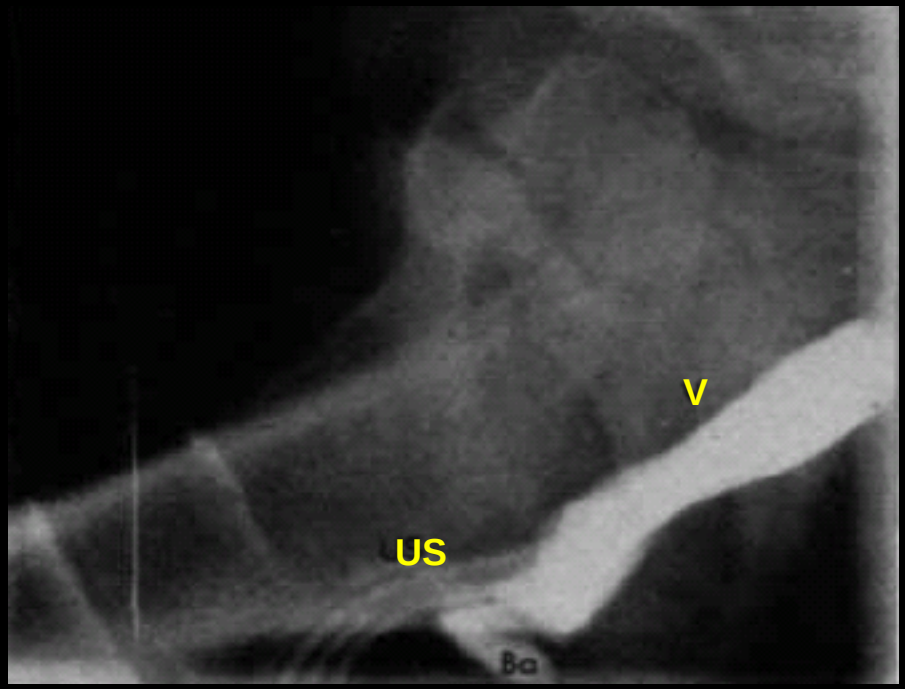
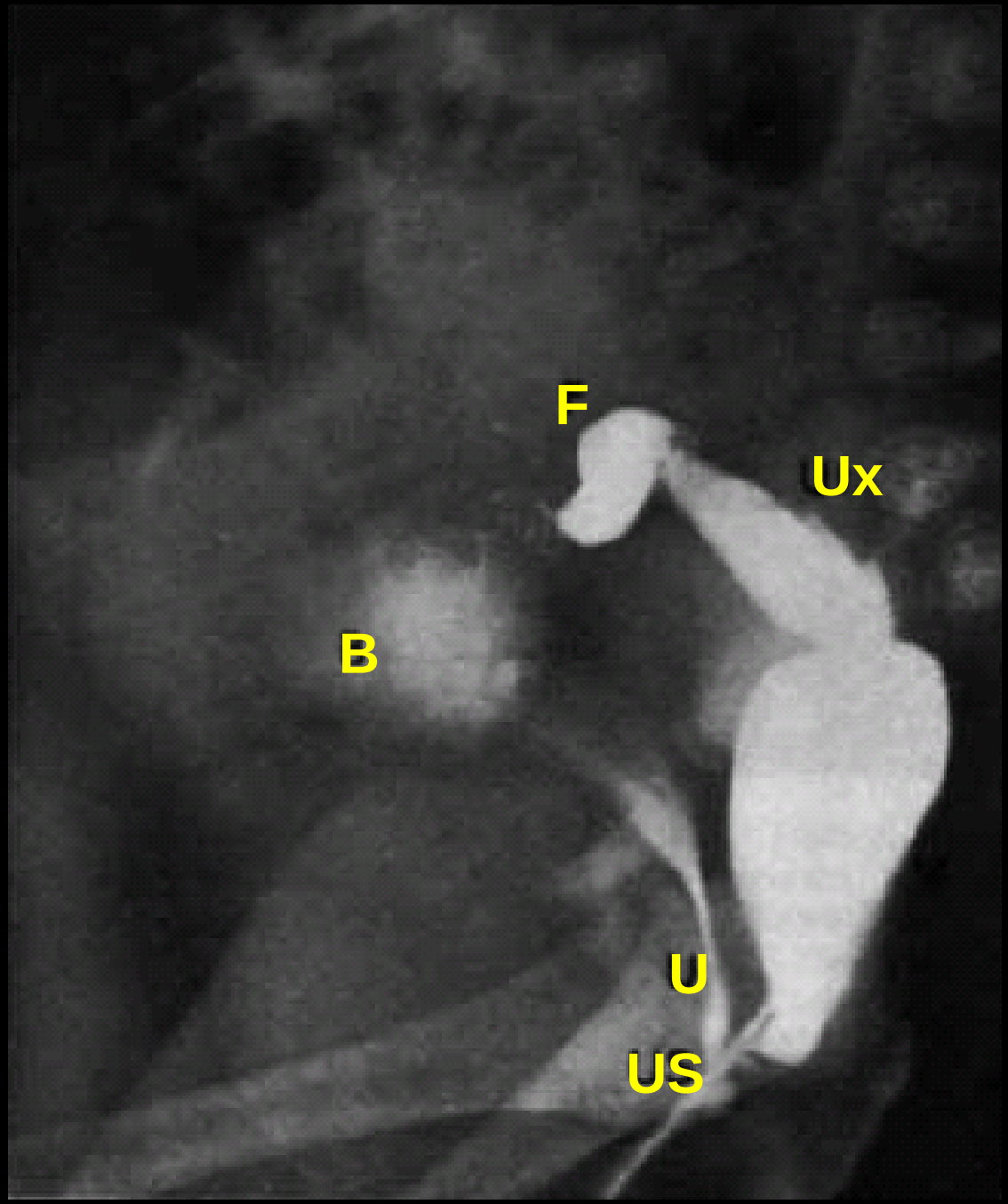


Figure 2: Spectrum of virilisation of a female. (46 XX with CAH)




V


V
US
Ba


F
Ux
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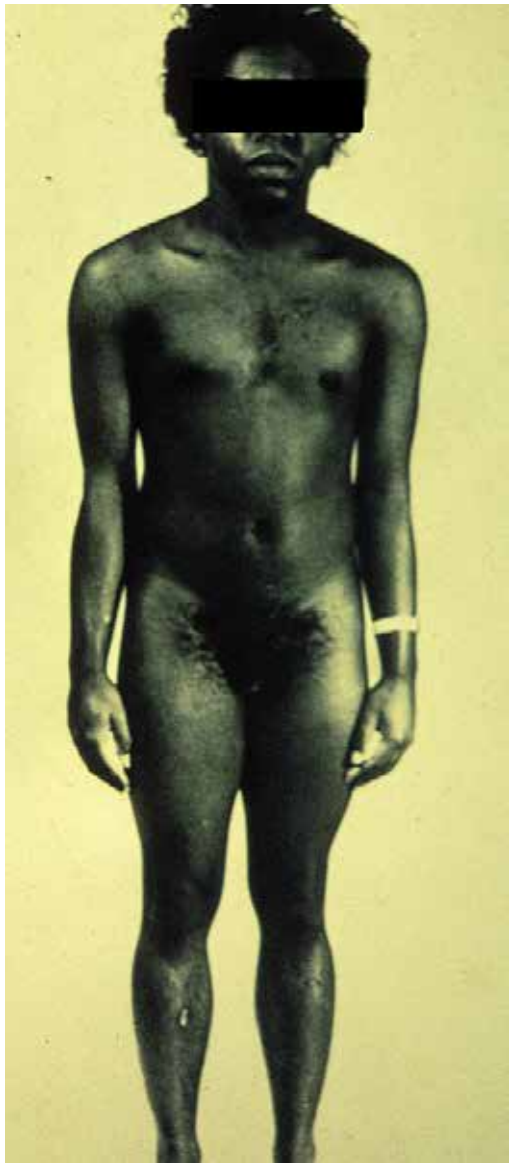
21-Hydroxylase ($P\text{-}450_{c21}$) deficiency



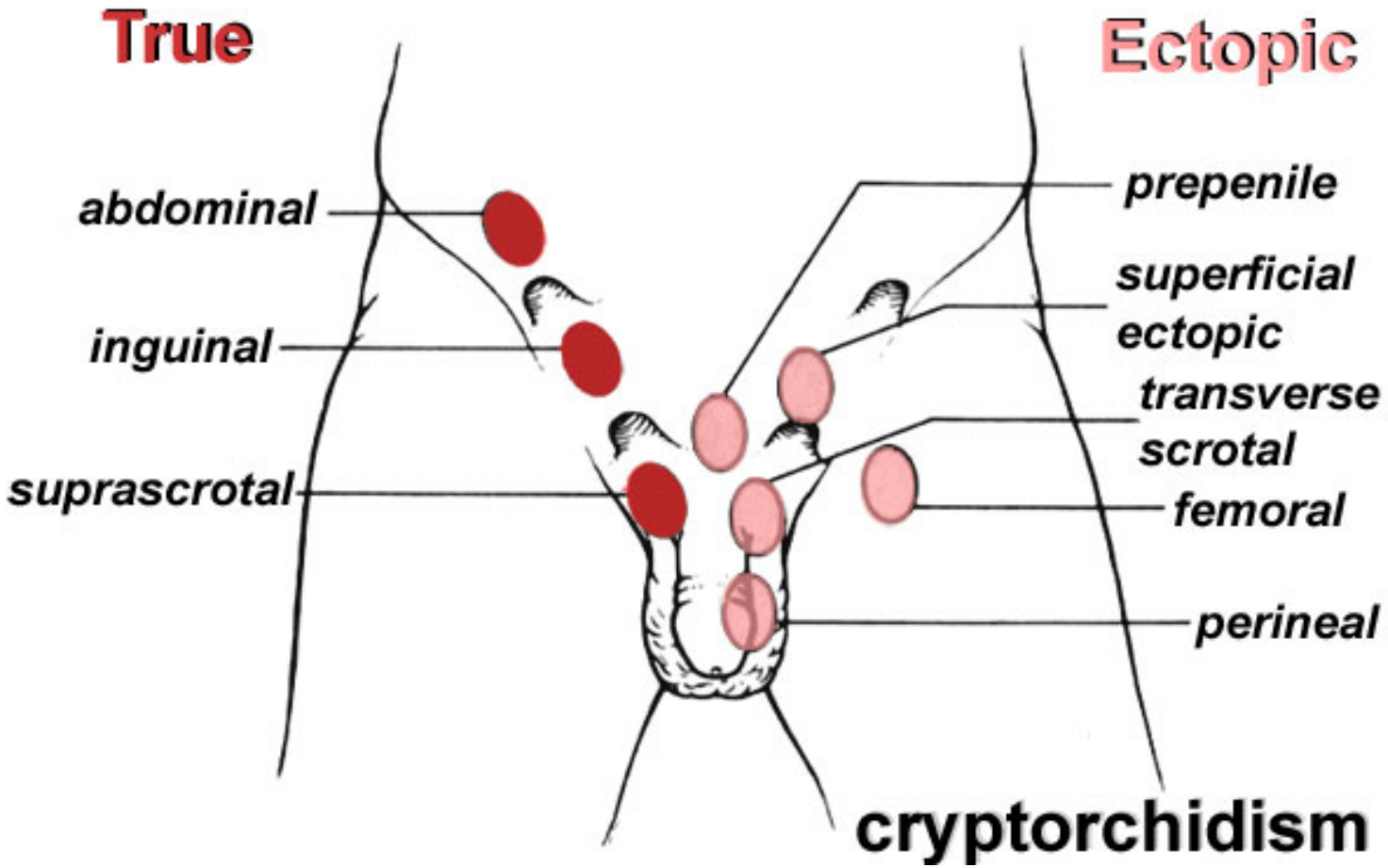
18y old gender identity concern

The Incidence of Intersexuality in children with Cryptorchidism and Hypospadius

Martin Kaefer et al, The Journal of Urology 1999

- Cryptorchidism
  - 1/30 full-term
  - 1/120 1 year olds
- Hypospadius 1/300
- Children with hypospadius *and* cryptorchidism have a 27% incidence of intersexuality
- Both are due to inadequate androgen action

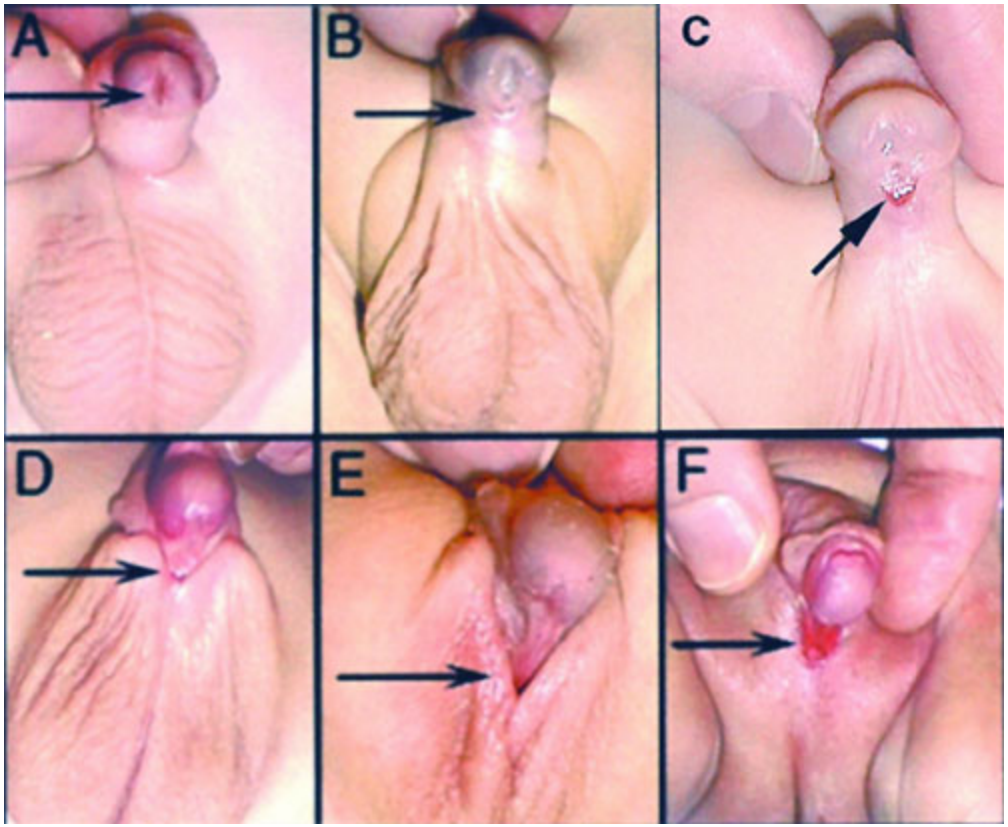
Cryptorchid testes

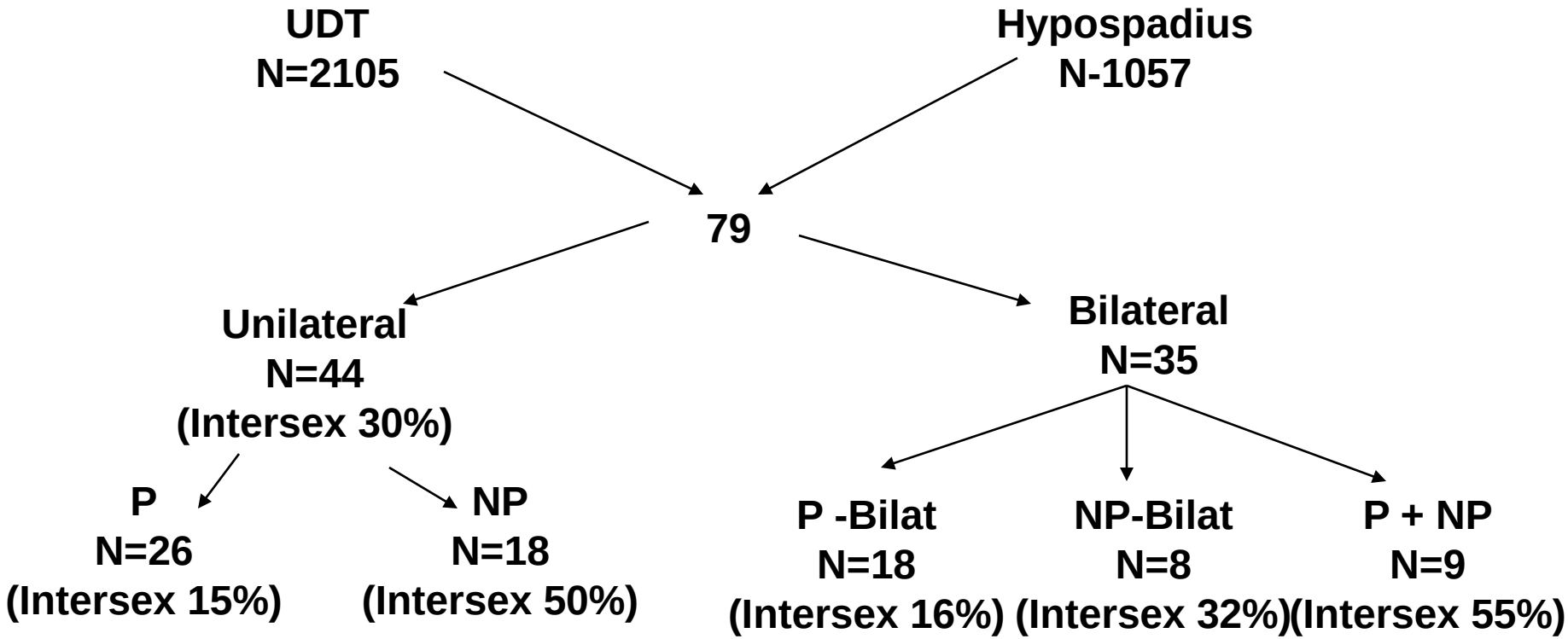


- **Palpable**
- **Non-palpable**

Hypospadius

- **Hypospadius**
  - **Anterior**
    - **Glanular**
    - **Coronal**
  - **Mid**
    - **distal penile**
    - **proximal mid shaft**
  - **Posterior**
    - **Penoscrotal**
    - **Scrotal**
    - **Perineal**





MGD =6
TH = 3
5aR = 2
47XXY = 2

MGD =5
TH = 2
PAIS = 3
Vanish testes = 1

Meatal Position

Meatal position	Intersex
anterior (33%)	8%
mid (26%)	5%
Posterior (41%)	**65%**

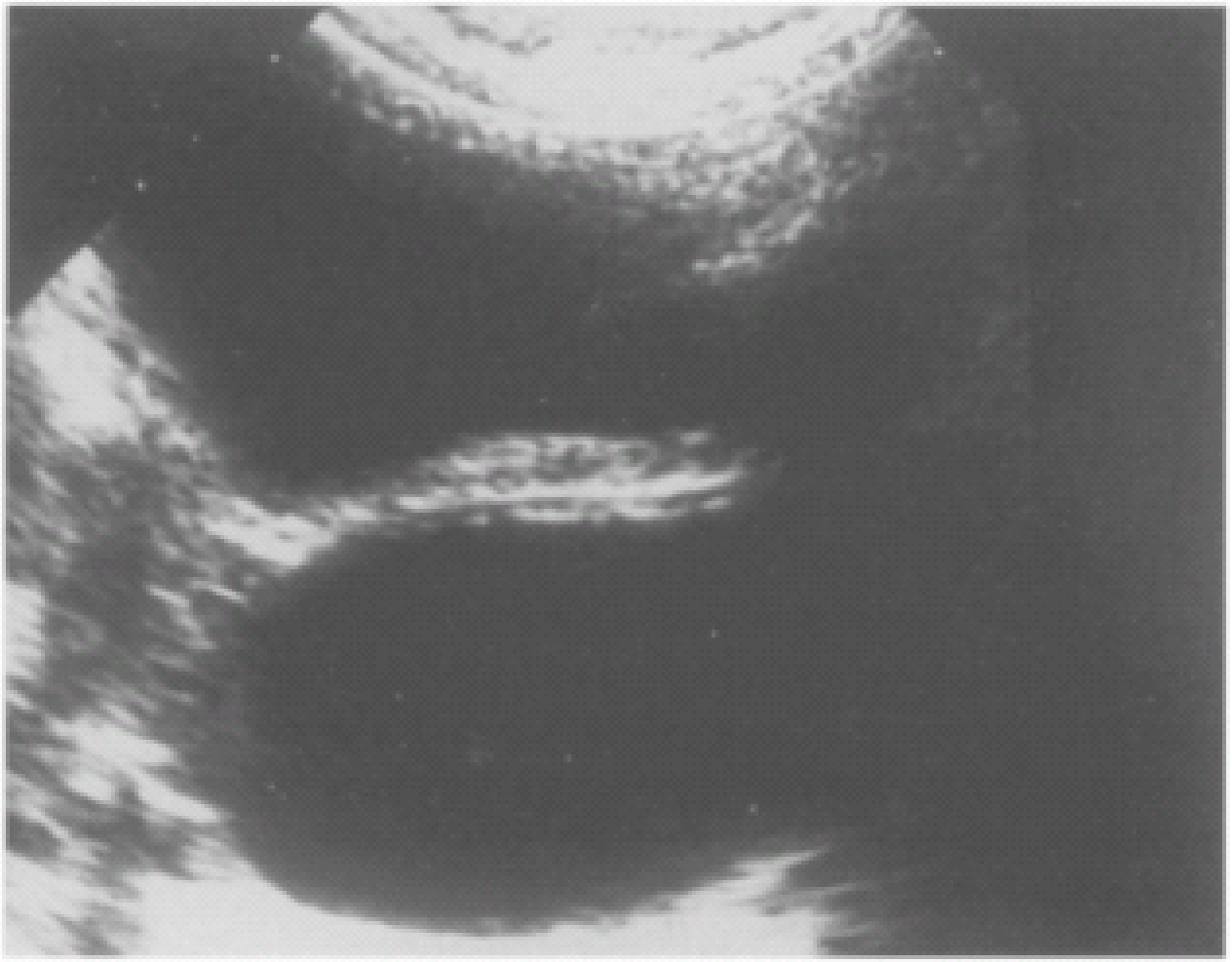


Fig. 1 Pelvic ultrasound in a female pseudohermaphrodite with congenital adrenal hyperplasia showing hydrocolpos consistent with a urogenital sinus malformation.

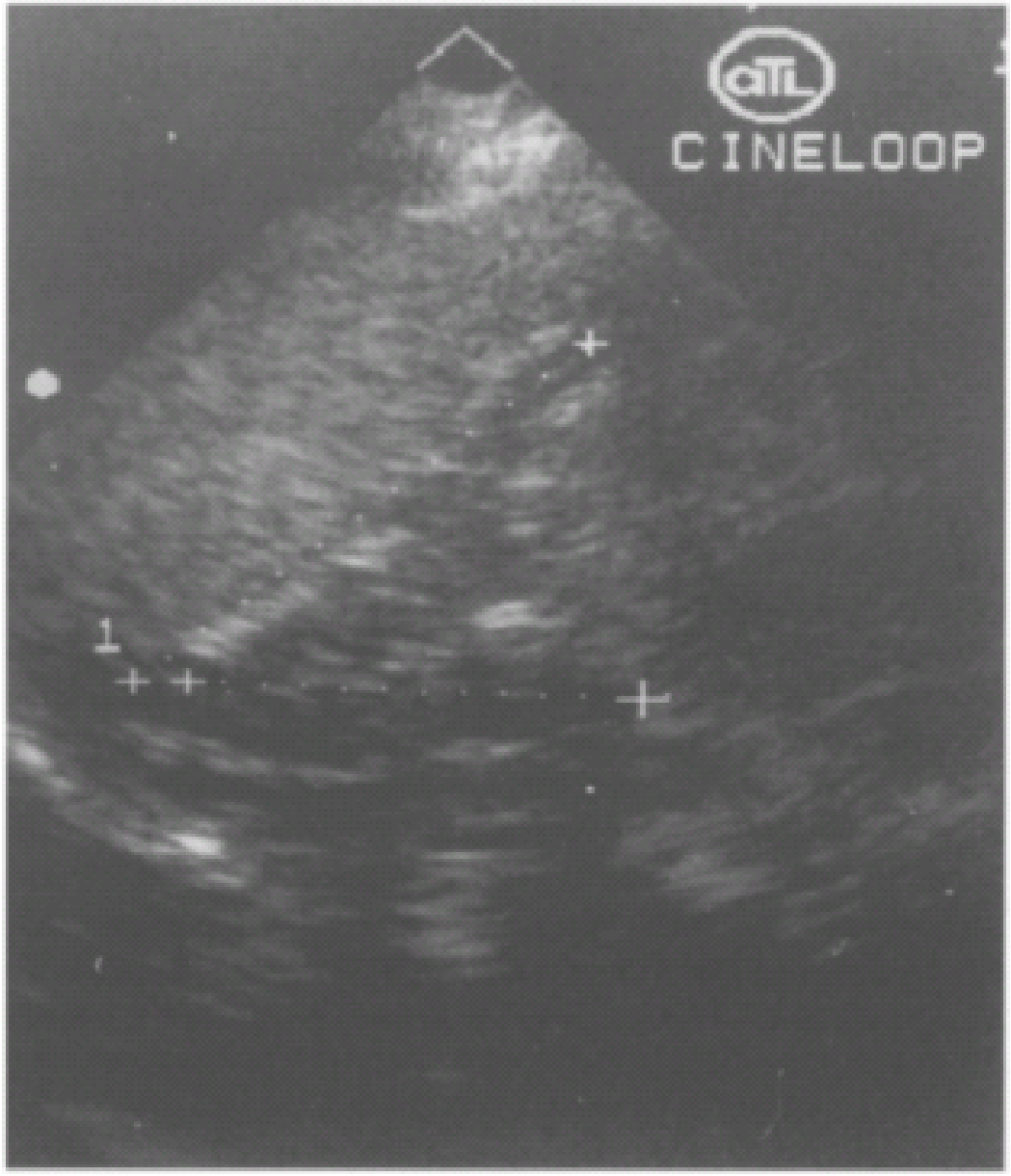
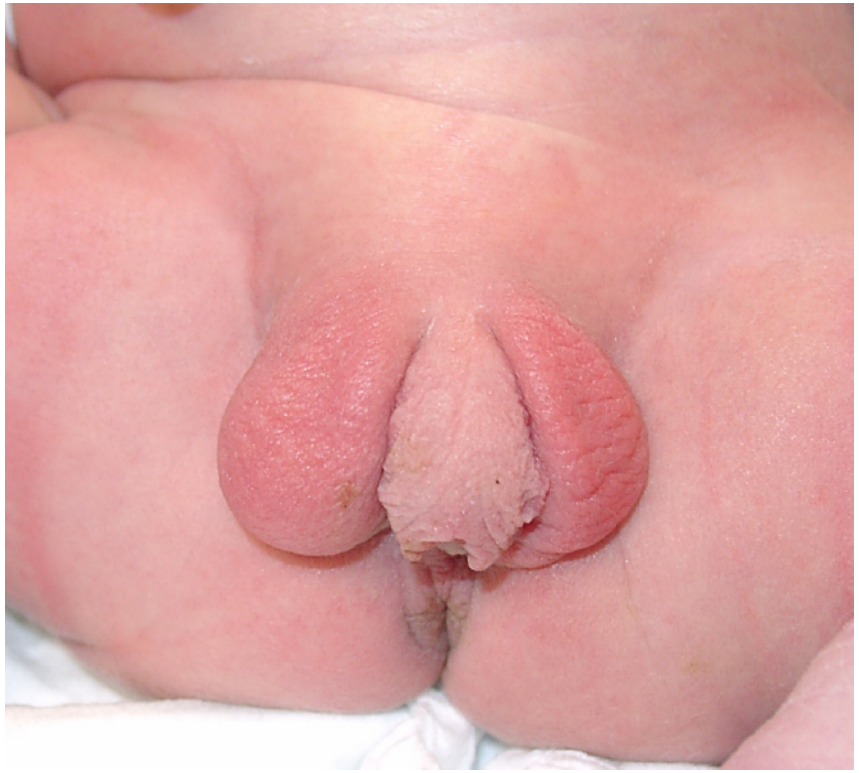


Fig. 3 Ultrasound in a female pseudohermaphrodite with congenital adrenal hyperplasia shows an enlarged adrenal gland.

Ambiguous Genitalia



What to do?

- Call for help
- Karyotype
- Urgent electrolytes
- 17-OHP, DHEAS, androstenedione, testosterone, DHT
- US
- Genitogram
- HCG stimulation
- ACTH stimulation test
- MIS
- Call for help

Management

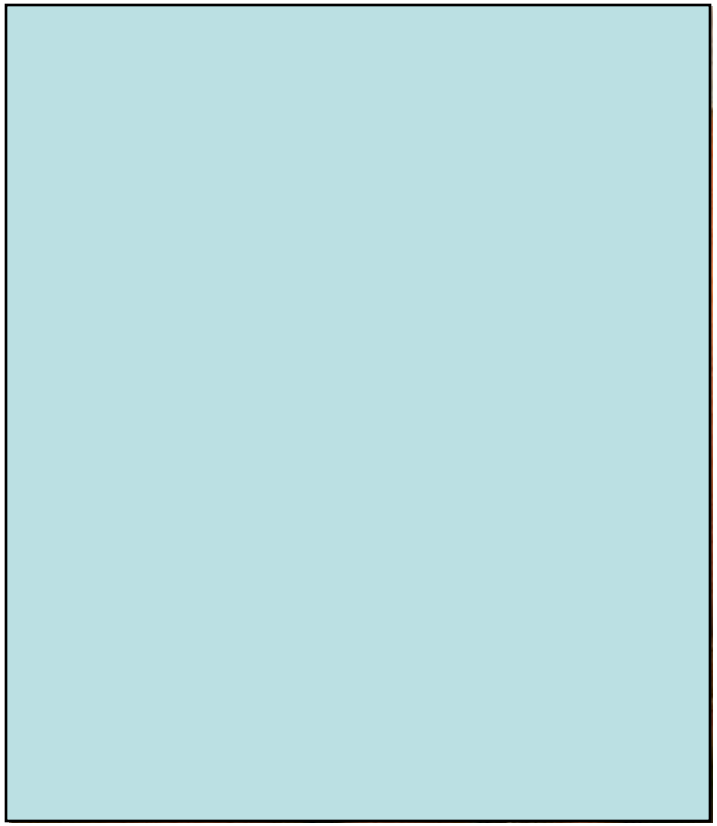
- Specialist team
  - Paediatrician (endocrinologist)
  - Surgeon
  - Genetics
  - Parents

Issues

- Sex assignment
- Surgery
  - Y/N
  - Timing
- Follow-up
- Disclosure

Tying it all together

To prevent this

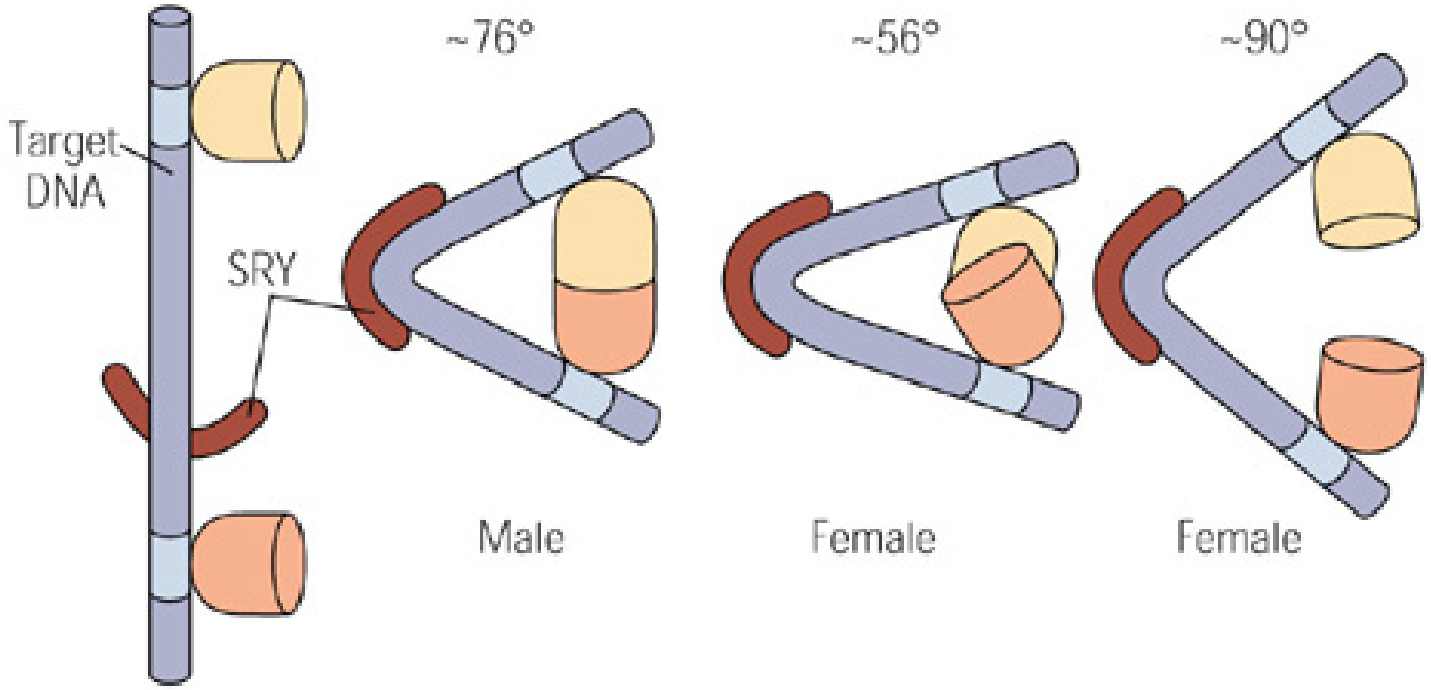


Tying it all together

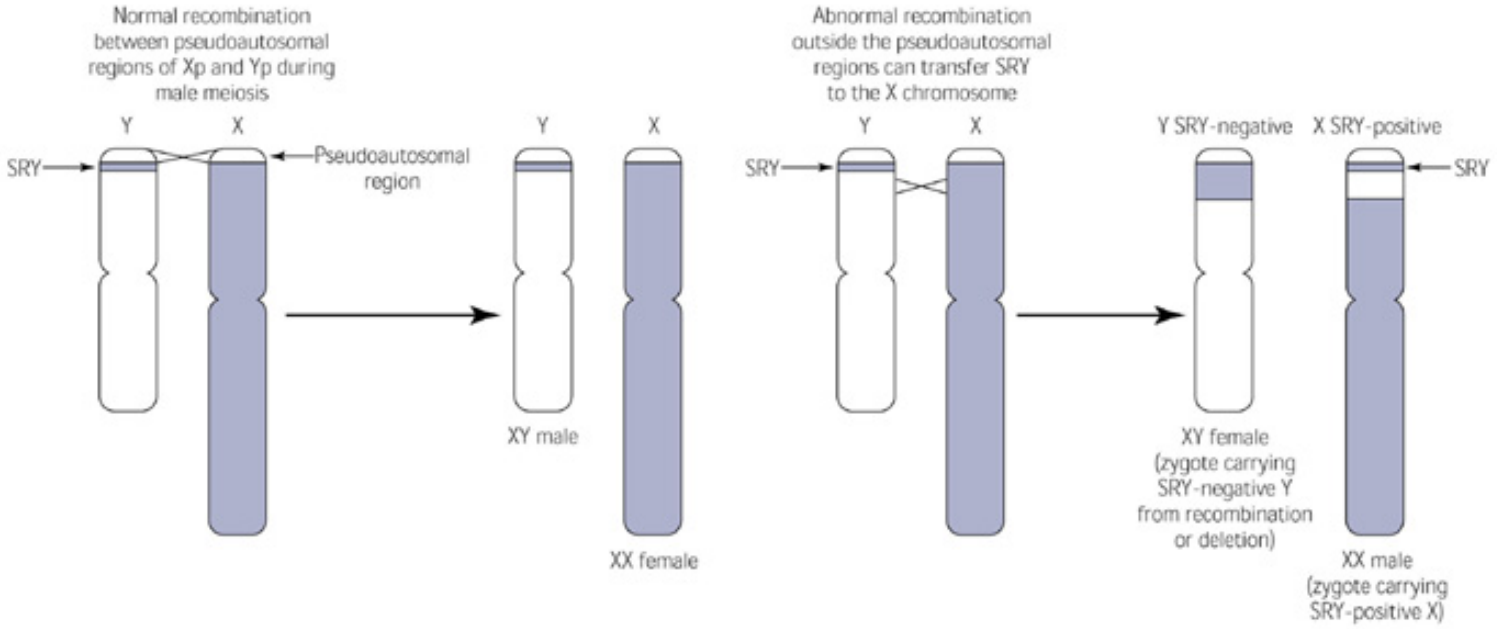
- Team decision
- Based upon all the facts
- Parental wishes
- Adult sexual function
  - The brain is the most important sex organ
- Reproductive capacity
- Gender identity
- Physical characteristics and potential for growth/function

Questions?

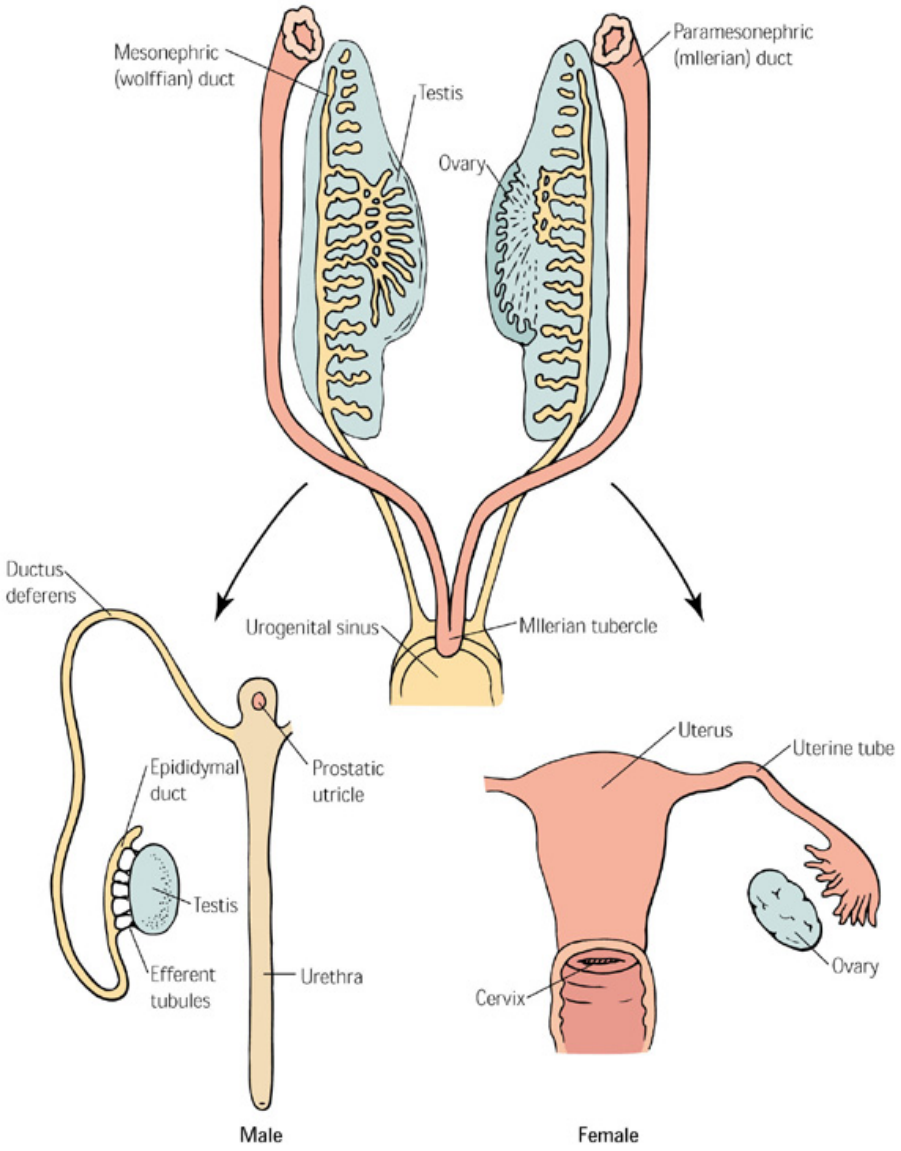
Target DNA bending



X-Y recombination during paternal meiosis



Undifferentiated sexual system

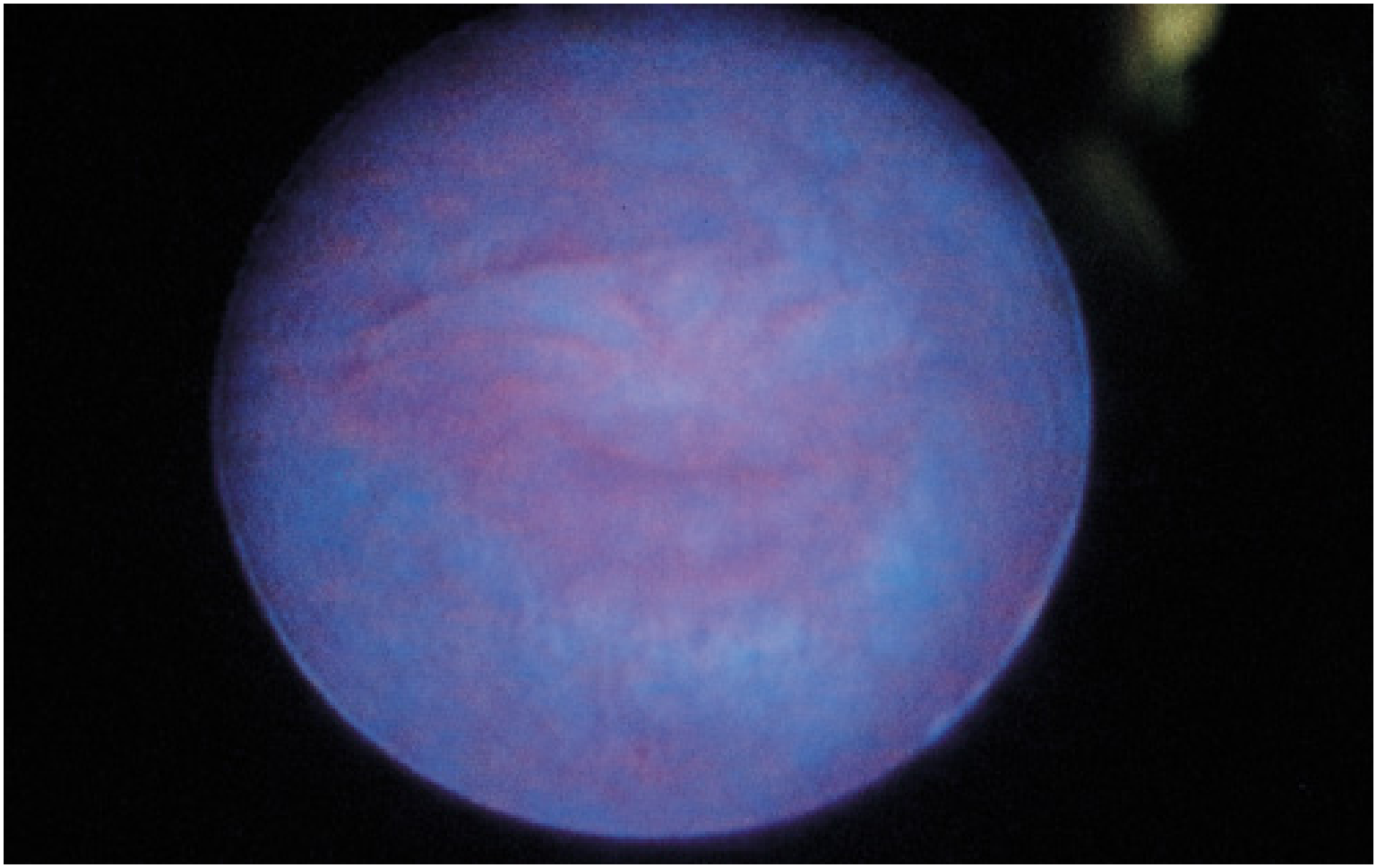


Classifications for patients with abnormalities of sexual differentiation

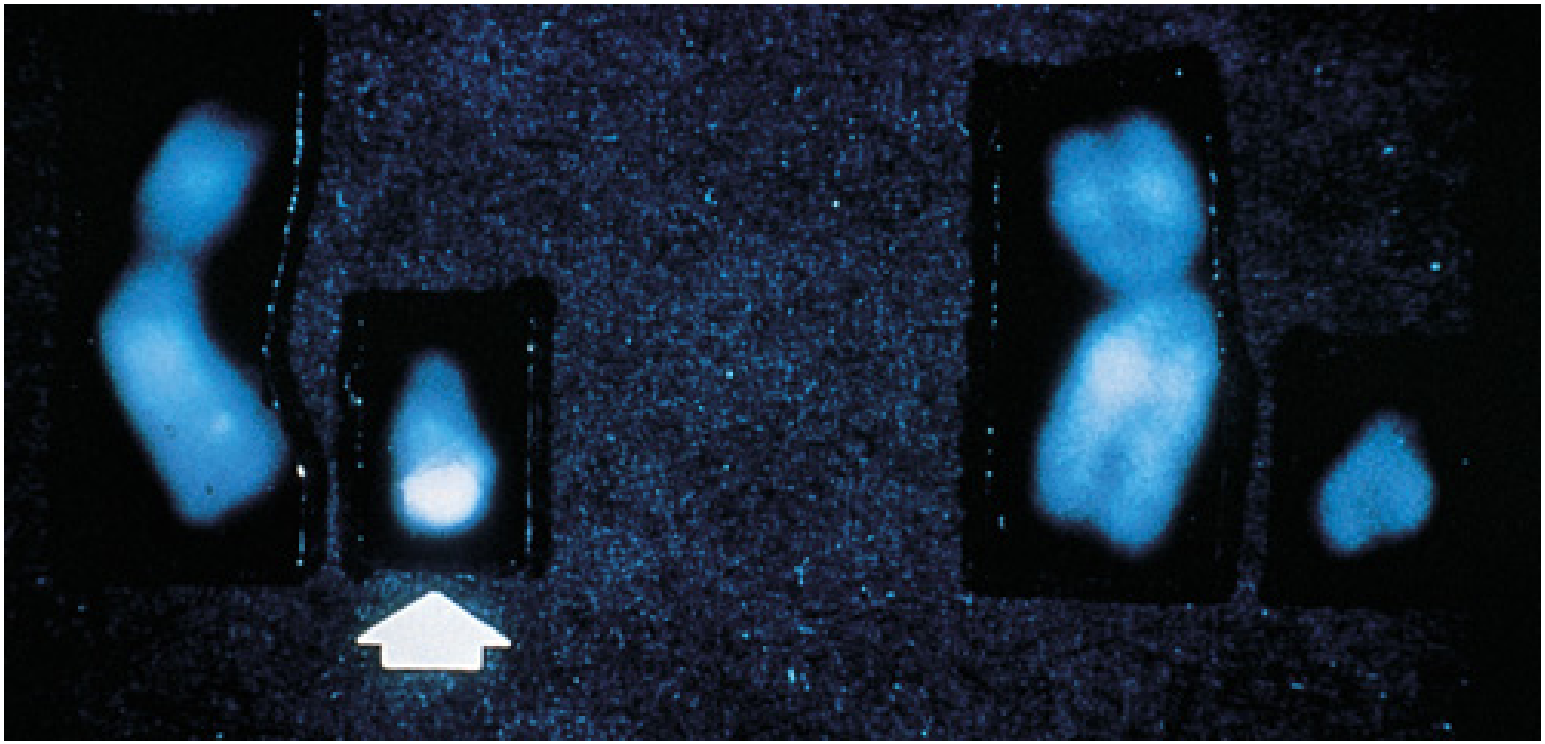
B. Two Classifications for Patients with Abnormalities of Sexual Differentiation

Original Classification	New Classification
Male pseudohermaphroditism	1. Deletion syndromes with Y cell lines (45,X/46,XY)*
	2. 46,XY
	a. Gonadal dysgenesis (Swyer's syndrome)
	b. Empty pelvis: agonadia*
	c. Enzyme deficiencies
	Enzyme deficiencies affecting both adrenals and testes* (variants of CAH)
	—Cholesterol side-chain cleavage (P-450$_{scc}$) deficiency
	—3ß-hydroxysteroid dehydrogenase (3ß-HSD) deficiency
	—17à-hydroxylase (P-450$_{c17}$) deficiency
	Enzyme defects primarily affecting the testes*
	—P-450$_{c17}$ hydroxylase/17,20-lyase deficiency
	—17ß-hydroxysteroid dehydrogenase (17-HSD) or 17ß-kerasteroid reductase deficiency
	5à-reductase deficiency
	d. Androgen insensitivity or resistance
	Complete*
	Incomplete*
	e. Defects in synthesis, secretion, or response to AMH: persistent müllerian duct syndrome
	f. Nonendocrine/nonsex chromosome defects*
	g. 46,XY true hermaphroditism*
	3. 46,XX/46,XY true hermaphroditism*
	4. 46,XX
True hermaphroditism	
Female pseudohermaphroditism	a. 46,XX true hermaphroditism*
	b. 46,XX sex reversed male
	c. Congenital adrenal hyperplasia
	21-hydroxylase deficiency forms*
	11ß-hydroxylase deficiency*
	3ß-ol-dehydrogenase deficiency*
	d. Aromatase (placental) deficiency
	e. Maternal androgen

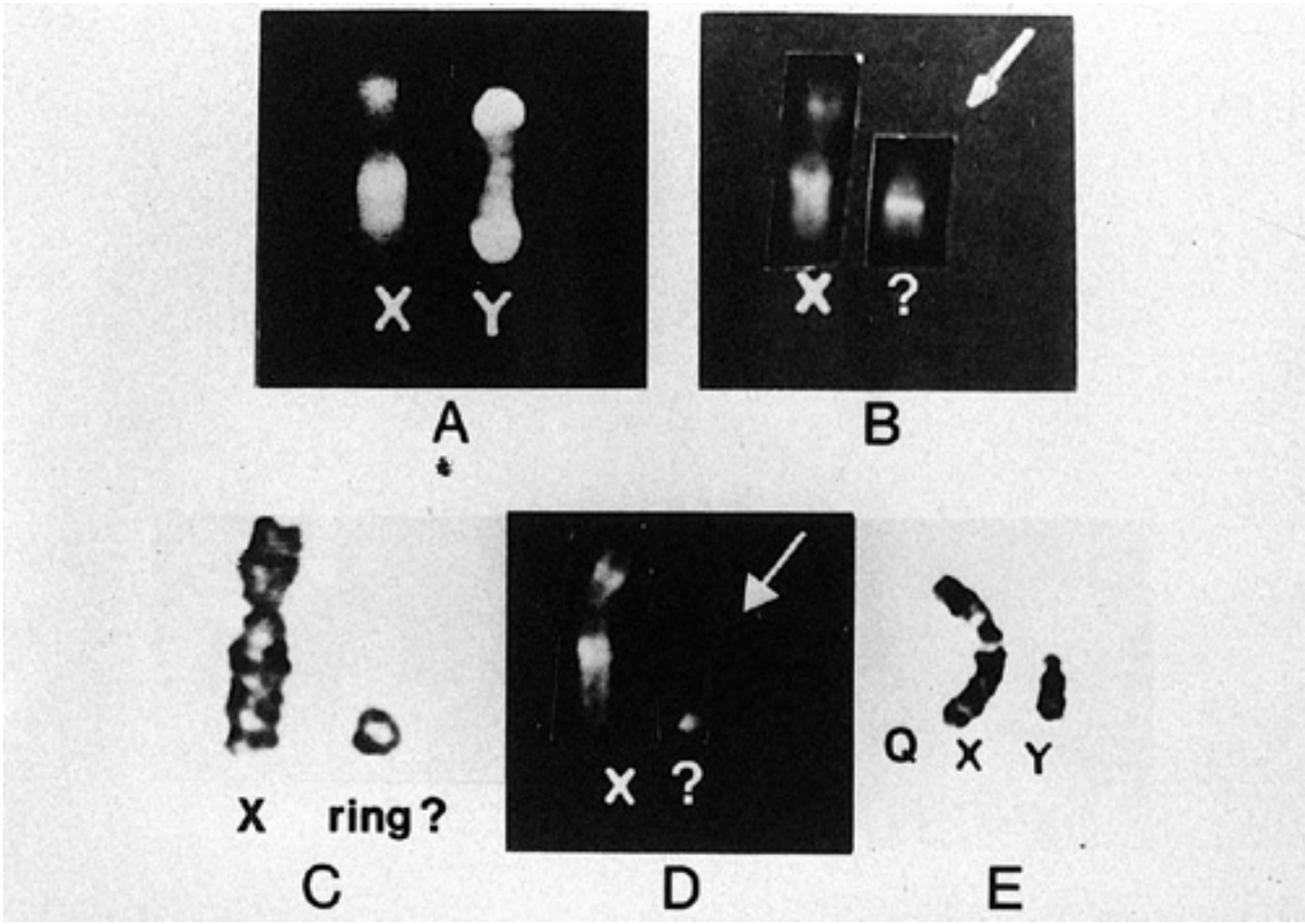
Cervix



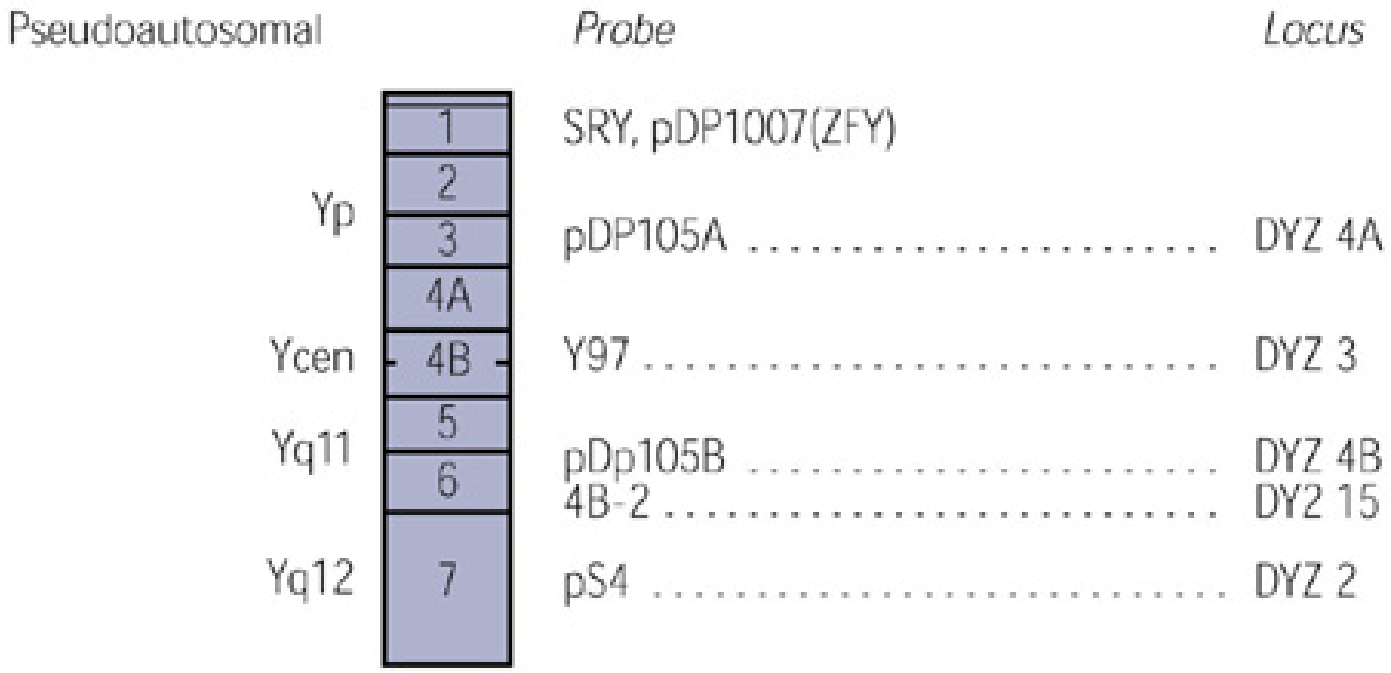
Detection of Y chromosome by Q banding



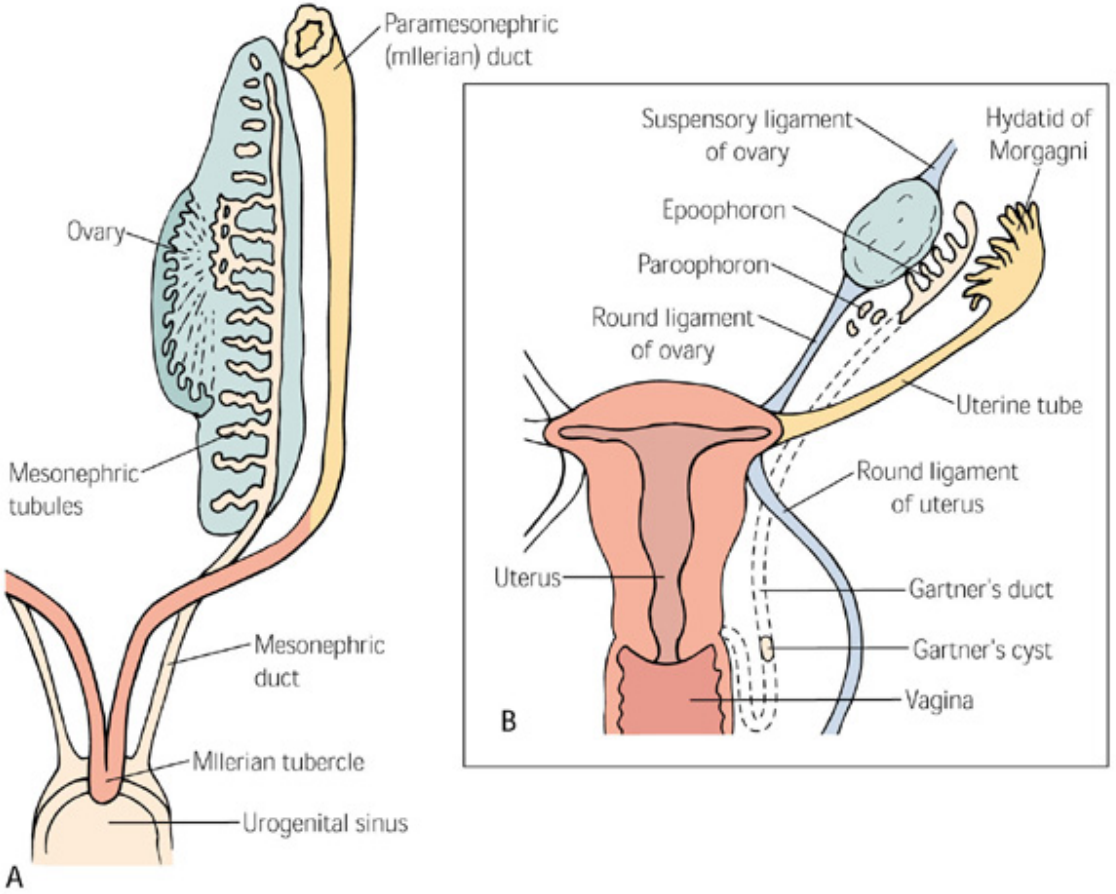
Y variants



Y DNA probes


Pseudoautosomal
Probe
Locus
1
2
3
4A
4B
5
6
7
Yp
Ycen
Yq11
Yq12
SRY, pDP1007(ZFY)
pDP105A ..... DYZ 4A
Y97 ..... DYZ 3
pDp105B ..... DYZ 4B
4B-2 ..... DYZ 15
pS4 ..... DYZ 2

Female genital duct system



Molecular aspects of 21-hydroxylase ($P\text{-}450_{c21}$) deficiency

E. Molecular Aspects of 21-Hydroxylase Deficiency

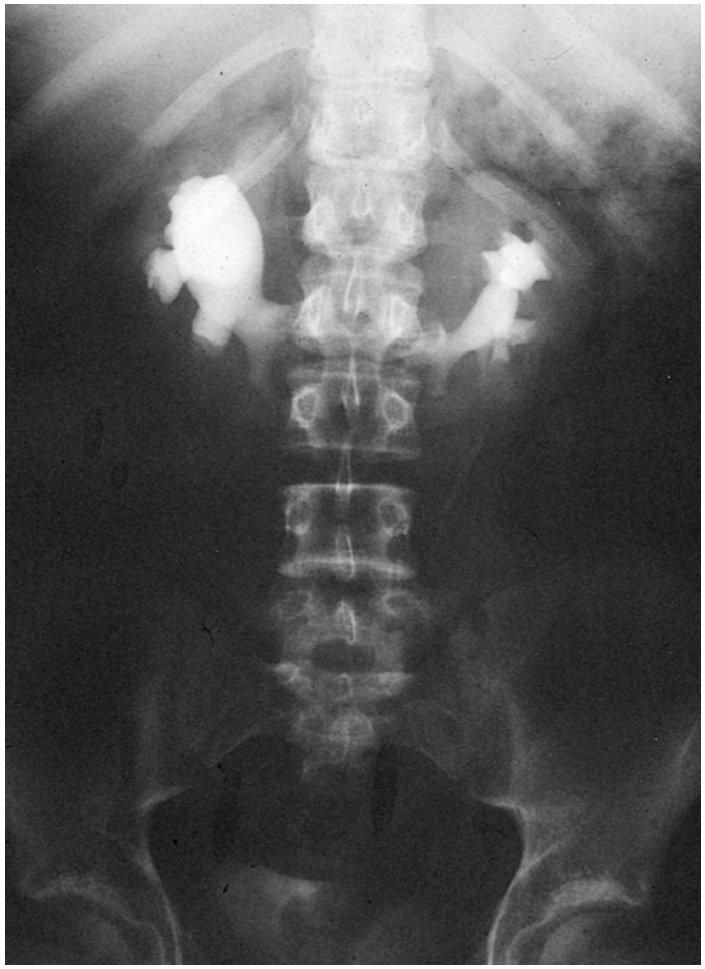
Activate gene CYP21B and pseudogene CYP21A are located in tandem duplication on chromosome 6 and with 98% homology.
Each gene is 3.3 kb long and has 10 exons.
The two genes are flanked in tandem by the C_4A and C_4B genes which encode the fourth component of complement.
Salt wasting form
1. Homozygous large deletion of CYP21B and C_4B loci: 20% to 25% of classic form.
2. Homozygous large gene conversion resulting from transfer to CYP21B of deleterious mutations normally present in CYP21A pseudogene: 10%.
3. Compound heterozygous harboring, for example, a large deletion on one chromosome 6 and a large gene conversion or a nonsense mutation on the other chromosome 6.
4. The most frequent point mutation is the single base pair change from A to G at the end of intron 2 leading to premature truncation of intron 2 and an mRNA splicing defect preventing synthesis of an active protein.
Single virilizing form
1. Mutation involving change from isoleucine to asparagine. This mutation weakens the association of the enzyme with the endoplasmic reticulum and results in reduction of enzyme activity to about 1% of normal.
2. The A to G point mutation in intron 2 is also associated with the simple virilizing form.
Nonclassic form
1. Valine to leucine mutation at position 281 results in the abnormal localization of the mutant enzyme and in the microsome enzyme activity is reduced by 20% to 50% for the substrates progesterone and 17-OHP. Adequate aldosterone production along with excessive androgen lead to variable degrees of hirsutism or virilization.
2. Proline to leucine mutation at position 30 also leads to improper orientation of the enzyme in the membrane of the microsome. This mutation appears to be associated with more severe biochemical changes as well as androgen excess.

Basic features of 3&beta, -hydroxysteroid dehydrogenase deficiency

D. Basic Features of 3ß-HSD Deficiency

3ß-HSD	Two forms of 3ß-HSD Deficiency
Single protein with both dehydrogenase and isomerase activities, localized in the endoplasmic reticulum of the cells of the adrenal cortex, ovary, testis, and placenta.	1. Classic 3ß-HSD deficiency with complete block of 3ß-HSD activity leading to deficient production of glucocorticoids, mineralocorticoids and gonadal steroids.
	Inability to produce cortisol results in:
Responsible for conversion of ß^5-3ß-hydroxysteroids (pregnenolone, 17-hydroxypregnenolone, and DHEA) into ß^4-3ß-ketosteroids (progesterone, 17-hydroxyprogesterone, and androstenedione).	—Increased ACTH secretion and adrenal hyperplasia.
	—Males present with ambiguous genitalia owing to insufficient production of androgens.
Required for synthesis of cortisol, mineralocorticoids, and gonadal steroids.	—Glucocorticoid and mineralocorticoid deficiencies occur, resulting in adrenal insufficiency ? classification into 3ß-HSD/salt wasting and 3ß-HSD/salt conserving.
	2. Nonclassic 3ß-HSD with partial block, late onset, no salt wasting, no genital ambiguity.

Horseshoe kidney



Basic features of P-450$_{SCC}$ (cholesterol side-chain cleavage) deficiency in 46,XY male

B. Basic Features of P-450$_{SCC}$ (Cholesterol Side-Chain Cleavage) Deficiency in 46,XY Male

Cholesterol side-chain cleavage enzyme (P-450$_{SCC}$ or 20, 22-desmolase) is a mitochondrial P-450 enzyme.
Catalyses the initial reaction in the steroidogenic pathway in the gonads and adrenals.
Cleaves a 6-carbon side-chain for cholesterol to form pregnenolone, a C_{21} steroid.
This reaction is rate-limiting
Regulated by ACTH and angiotensin II in the adrenals
Regulated by gonadotropins in the gonads
In P-450$_{SCC}$ deficiency, inability to metabolize cholesterol in the adrenals leads to cholesterol ester deposition resulting in enlarged, yellow, foamy adrenal glands: lipoid adrenal hyperplasia.

Salient features of congenital lipoid adrenal hyperplasia

C. Salient Features of P-450$_{SCC}$ (Cholesterol Side-Chain Cleavage) Deficiency in 46,XY Male: Congenital Lipoid Adrenal Hyperplasia

Karyotype:	46, XY
Inheritance:	Autosomal recessive
Genitalia:	Female ? ambiguous
Wolffian duct derivatives:	Absent ? hypoplastic
Müllerian duct derivatives:	Absent
Gonads:	Testes
Habitus:	Severe adrenal insufficiency in infancy, little or no virilization at puberty
Hormone profile:	? or absent glucocorticoids, mineralocorticoids, and gonadal steroids in plasma and urine
	? plasma LH and FSH
Molecular genetics:	CYP11A$_1$, single gene localized to chromosome 15q23-24, 9 exons, 8 introns
	No mutations found

Salient features of 3&beta, -hydroxysteroid dehydrogenase deficiency in 46,XY males

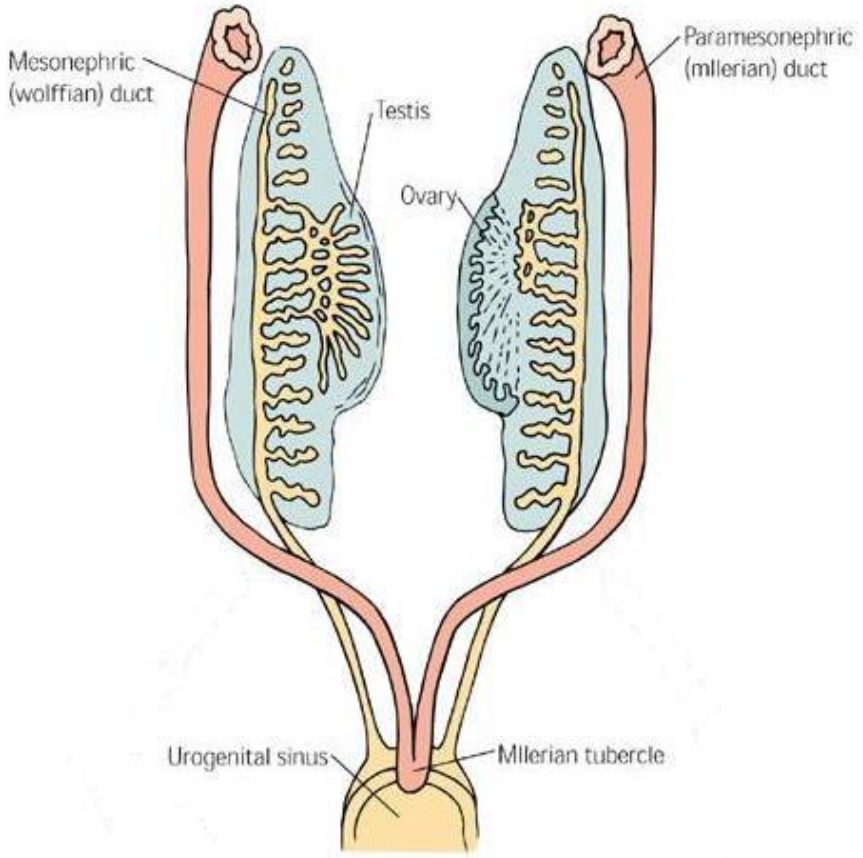
E. Salient Features of 3ß-HSD Deficiency in 46,XY Males

Karyotype:	46, XY
Inheritance:	Autosomal recessive
Genitalia:	Almost normal female to male with degrees of hypospadias
Wolffian duct derivatives:	Normal
Müllerian duct derivatives:	Absent
Gonads:	Testes
Habitus:	Severe adrenal insufficiency in infancy; poor virilization at puberty with gynecomastia. Mild form: no mineralocorticoid deficiency, premature adrenarch ? mild virilization
Hormone profile:	? concentrations of $\delta^5 C_{21}$ and C_{19} steroids (*eg*, 17-hydroxypregnenolone, DHEA, and their sulfates) in urine and plasma
	? ratio of δ^5/δ^4 steroids
	? 17-hydroxypregnenolone and DHEA response to ACTH and/or hCG
Molecular genetics:	17-hydroxypregnenolone and DHEA suppressible by dexamethasone
	Two isoenzymes 3ß-HSD type I and type II
	Type I expressed in placenta and skin
	Type II expressed in adrenal, ovary, and testis
	Genes: localized to chromosome 1q11-13, with 93% homology
	Mutation reported in fourth exon of type II gene, codon 171 converting tryptophan to stop codon leading to formation of a truncated nonfunctional enzyme

Features of androgen resistance or insensitivity

B. Basic Features of Androgen Resistance or Androgen Insensitivity

Testosterone and its reduced metabolite DHT effect their action in androgen-sensitive target cells by binding to androgen receptor.
Androgen receptor is a member of a family of regulatory proteins which include all steroid receptors, and receptor for thyroid hormone, vitamin D, and retinoic acid. These receptors contain (1) NH_2-terminal region involved in transcription activation; (2) cysteine-rich DNA binding with two zinc fingers that bind to specific androgen response elements in target genes and; (3) COOH-terminal ligand binding domain for testosterone and DHT.
Androgen response may be completely or partially blocked in 46,XY males, resulting in absent or partial development of Wolffian ducts and absent or partial development of male external genitalia during fetal development. Because testes produce AMH, müllerian ducts regress leading to absent female internal genital structures.


Mesonephric
(wolffian) duct
Testis
Ovary
Paramesonephric
(mllerian) duct
Urogenital sinus
Mllerian tubercle

Androgen resistance

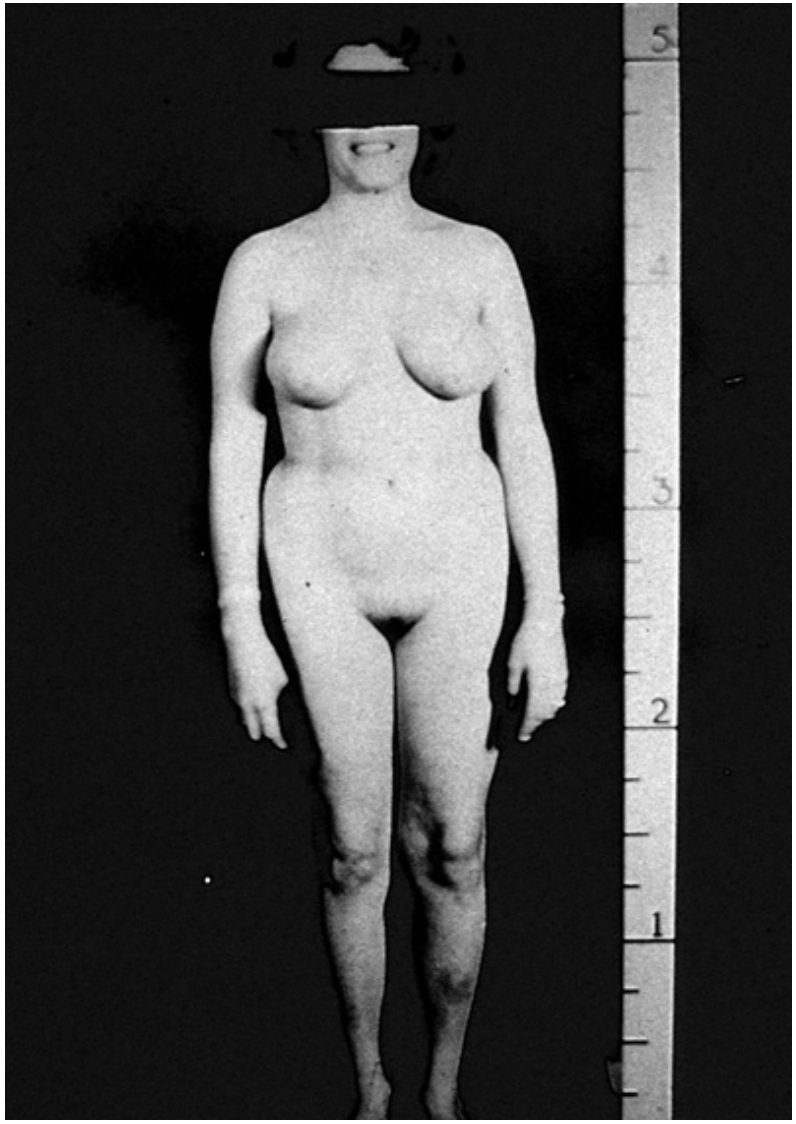
18y old “female”

Tanner IV breast

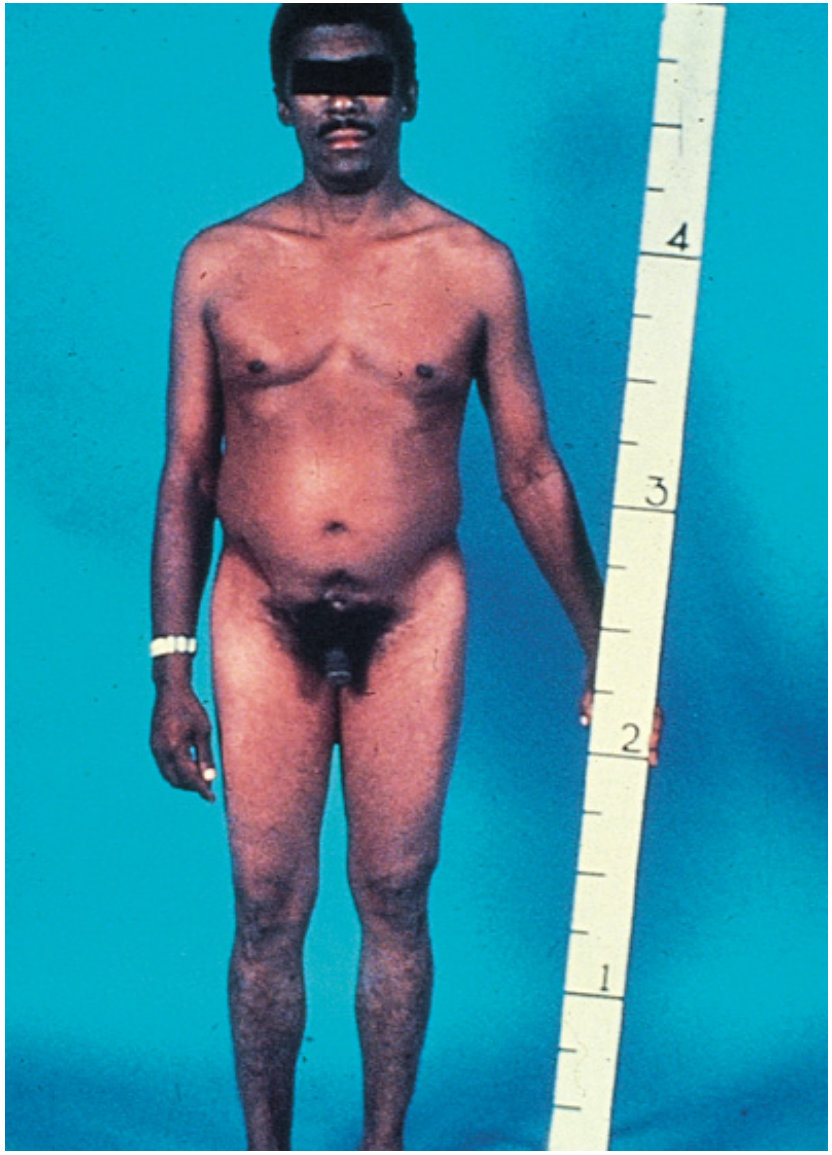
Absent Pubic hair

Blind vagina

46,XY



46,XX “sex-reversed male”



46,XX karyotype reversed male

B. 46,XX Sex Reversed Male

Karyotype:	46, XX
Inheritance:	Sporadic
	Rare kindred including both XX males and XX true hermaphrodites
Genitalia:	Male
	Hypospadias (10%)
Wolffian duct derivatives:	Normal
Müllerian duct derivatives:	Absent
Gonads:	Testes
Habitus:	Normal male, slightly shorter than normal males. Few develop gynecomastia at puberty, owing to hypogonadism; those born with hypospadias tend to develop gynecomastia at puberty. All have azoospermia.
Hormone profile:	Testosterone normal or decreased FSH; LH normal or low
Molecular genetics:	90% result from transfer of SRY from Y to X during paternal meiosis 10% result from mutation of X-linked or autosomal genes involved in gonadal determination