NOVITA' IN ENDOCRINOLOGIA PEDIATRICA

Dalla clinica alla genetica

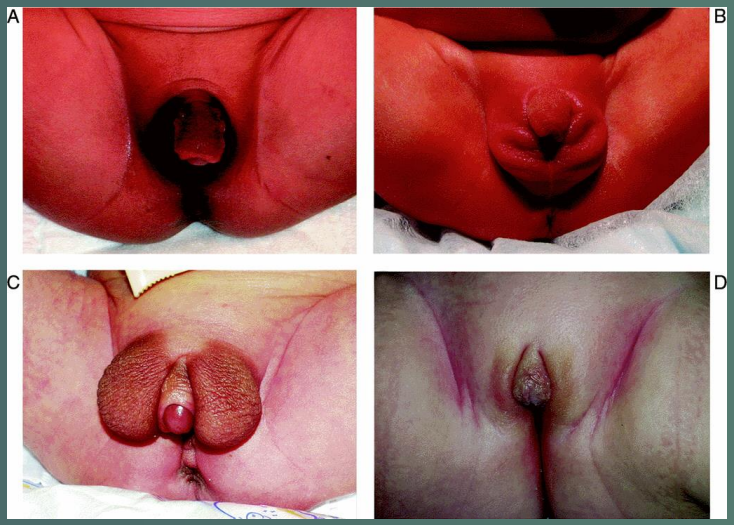
Cagliari, 14 maggio 2016

I disturbi dello sviluppo sessuale (DSD): la clinica

Lucia Ghizzoni
Divisione di Endocrinologia,Diabetologia e Metabolismo
Università degli Studi di Torino
lucia.ghizzoni@unito.it

DISORDINI DELLO SVILUPPO SESSUALE (DSD)

Condizione congenita in cui lo sviluppo del sesso cromosomico, gonadico o anatomico e' atipico



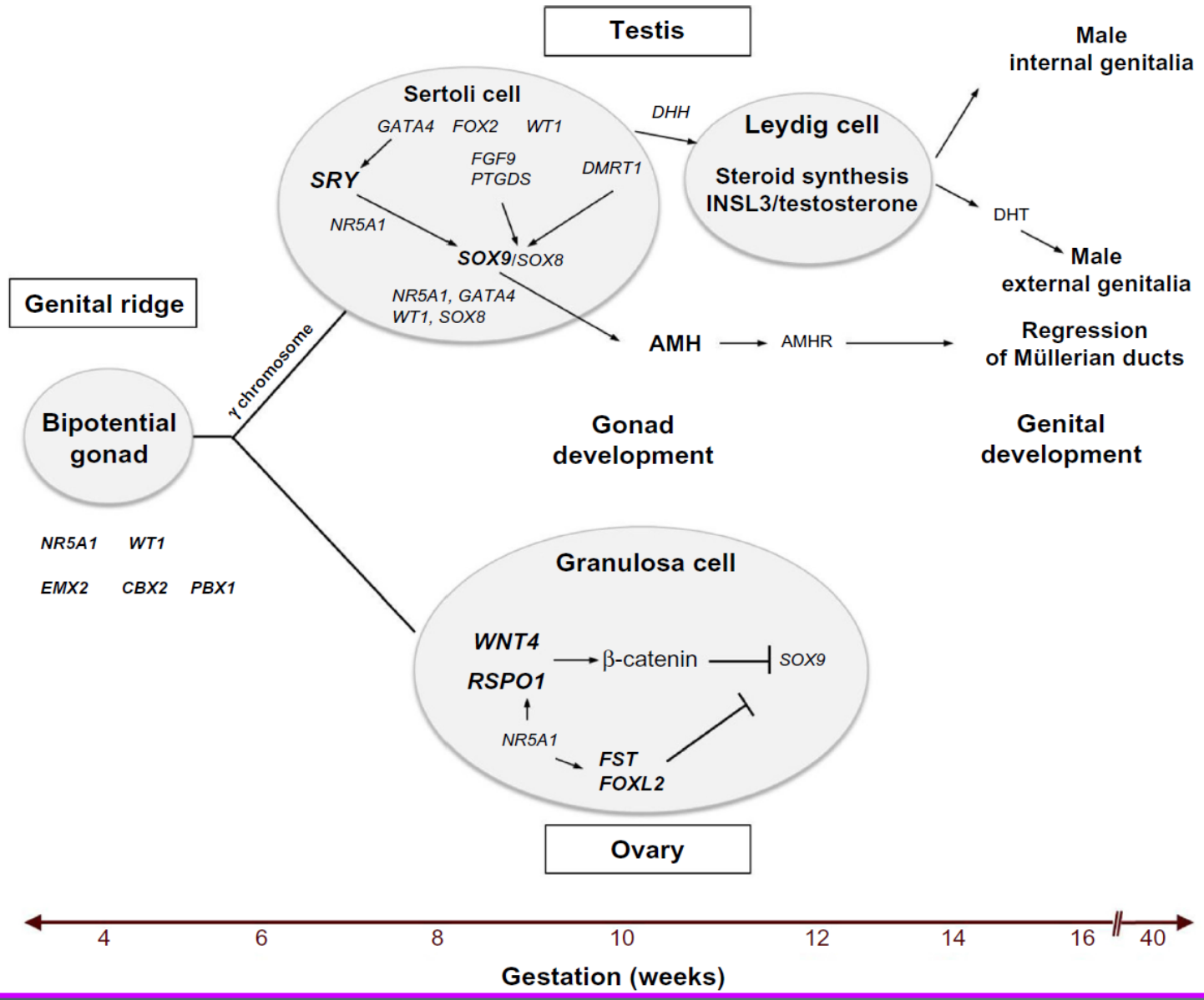
DETERMINAZIONE SESSUALE:

l'insieme dei processi che conducono alla formazione dell'**ovaio** o del **testicolo** a partire dalla gonade indifferenziata embrionale ("cresta genitale")

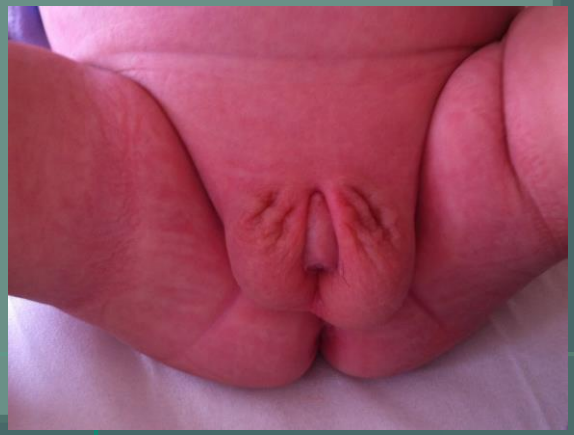
DIFFERENZIAZIONE SESSUALE:

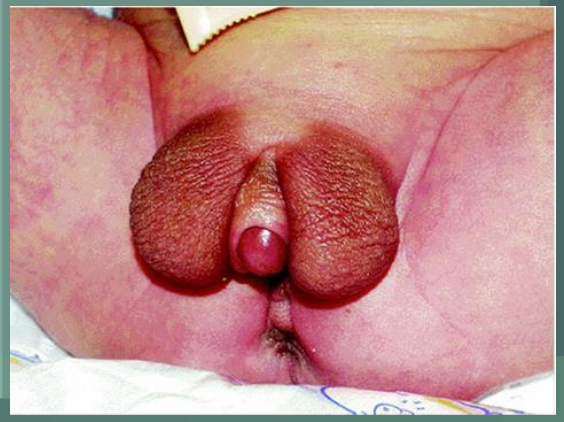
le specifiche azioni ormonali che portano al **sesso fenotipico**, a partire dalla formazione dei genitali interni ed esterni del feto, fino allo sviluppo delle caratteristiche sessuali secondarie durante la pubertà

Patogenesi dei DSD



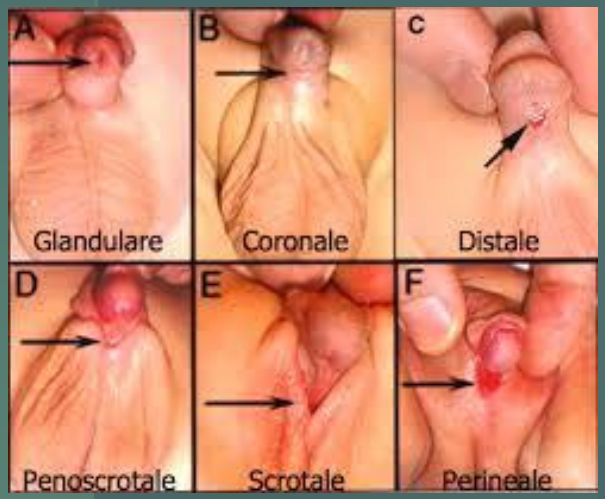
Kyriakou A, Advances in Genomics and Genetics 2015:5 165–177

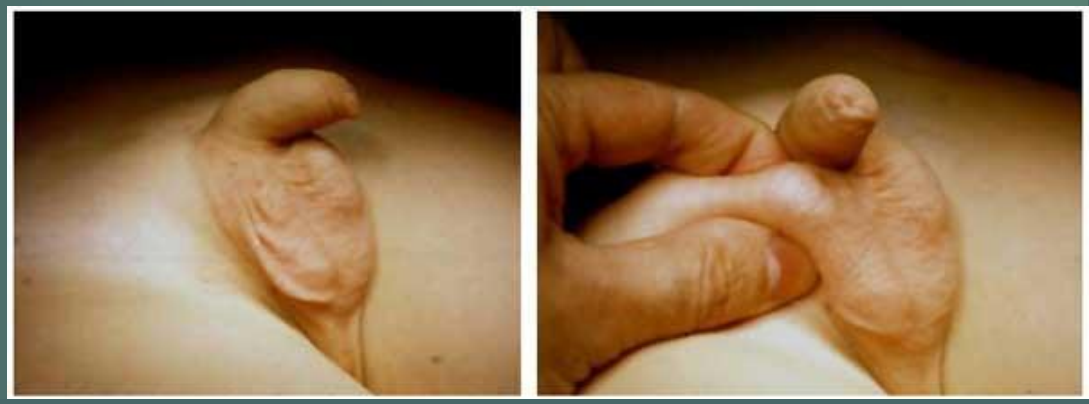




Genitali ambigui **1:4,500 nati vivi**

Anomalie dei genitali **1:300**





A new nomenclature

PREVIOUS	PROPOSED
Intersex	DSD
Male pseudohermaphrodite Undervirilization of an XY male Undermasculinization of an XY male	46, XY DSD
Female pseudohermaphrodite Overvirilization of an XX female Masculinization of an XX female	46, XX DSD
True hermaphrodite	Ovotesticular DSD
XX male or XX sex reversal	46, XX testicular DSD
XY sex reversal	46, XY complete gonadal dysgenesis

(Consensus guidelines, Hughes et al. Arch Dis Child 2006; 91:554-563)

A proposed classification for DSD

Sex Chromosome DSD

A: 47, XXY
(Klinefelter Syndrome & variants)

B: 45, X
(Turner Syndrome & variants)

C: 45, X/46, XY mosaicism
(mixed gonadal dysgenesis)

D: 46, XX/46, XY
(chimerism)

46, XY DSD

A: Disorders of gonadal (testis) development

1. Complete or partial gonadal dysgenesis (e.g. SRY, SOX9, SF1, WT1, DHH, etc)
2. Ovotesticular DSD
3. Testis regression

B: Disorders in androgen synthesis or action

1. Disorder of androgen synthesis
   - LH receptor mutations
   - Smith-Lemli-Opiz syndrome
   - Steroidogenic acute regulatory protein
   - Cholesterol side chain cleavage
   - 3β-hydroxysteroid dehydrogenase II
   - 17α-hydroxylase/17, 20-lyase
   - P450 oxidoreductase
   - 17β-hydroxysteroid dehydrogenase III
   - 5α reductaseII
2. Disorder of androgen action
   - Androgen Insensitivity Syndrome
   - Drugs & enviromental modulators

C: Other

1. Syndromic associations of male genital development (50+) (e.g. cloacal anomalies, Robinow, Aarskog, Hand-Foot-Genital, popliteal, pterygium syndrome, etc)
2. Persistent Müllerian duct syndrome
3. Vanishing testis syndrome
4. Isolated hypospadias
5. Congenital hypogonadotropic hypogonadism
6. Cryptorchidism
7. Environmental influences

46 XX DSD

A: Disorders of gonadal (ovary) development

1. Gonadal dysgenesis
2. Ovotesticular DSD
3. Testicular DSD (SRY+, duoSOX9, RSPO1)

B: Androgen excess

1. Fetal
   - 3β-hydroxysteroid dehydrogenase II
   - 21-hydroxylase
   - P450 oxidoreductase
   - 11β-hydroxylase
   - Glucocorticoid receptor mutations
2. Fetoplacental
   - Aromatase deficiency
   - Oxidoreductase deficiency
3. Maternal
   - Maternal virilizing tumors (e.g. luteomas)
   - Androgenic drugs

C: Other

1. Syndromic associations (e.g. cloacal anomalies)
2. Müllerian agenesis/hypoplasia (e.g. MURCS)
3. Uterine abnormalities (e.g. MODY5)
4. Vaginal atresis (e.g. McKusick-Kaufman)
5. Labial adhesions

(Consensus guidelines, Hughes et al. Arch Dis Child 2006; 91:554-563)

Condition	Gene	Uterus	Adrenal Defect	Associated features	Diagnostic biochemical features
45, X/ 46 XY mosaicism					
Mixed gonadal dysgenesis	-	+/-	-	+/- Turner-like features	-
46, XY DSD- Disorders of testis development (gonadal dysgenesis)					
WAGR, Denys-Drash & Frasier Syndromes	WT1	+/-	-	Wilms tumor, renal abnormalities, gonadal tumors	Proteinuria
Stroidogenic factor-1	NR5A1	+/-	+/-	+/- partial hypog hypog	Adrogen biosyntetic defect too
SRY	SRY	+/-	-	-	-
SOX9	SOX9	+/-	-	Campomelic dysplasia	-
Desert hedgehog	DHH	+	-	+/- Minifascicular neuropathy	-
X-linked lissencephaly	ARX	-	-	Lissencephaly, epilepsy	-
SIDDT syndrome	TSPYL1	-	-	Sudden infant death	-
9p24.3 deletion	DMRT1	+/-	-	Mental retardation	-
Xq13.3 deletion	ATRX	-	-	α-thalassemia, mental retardation	-
Xp21 duplication	DAX1	+/-	-	-	-
1q35 duplication	?WNT4	+/-	-	-	-

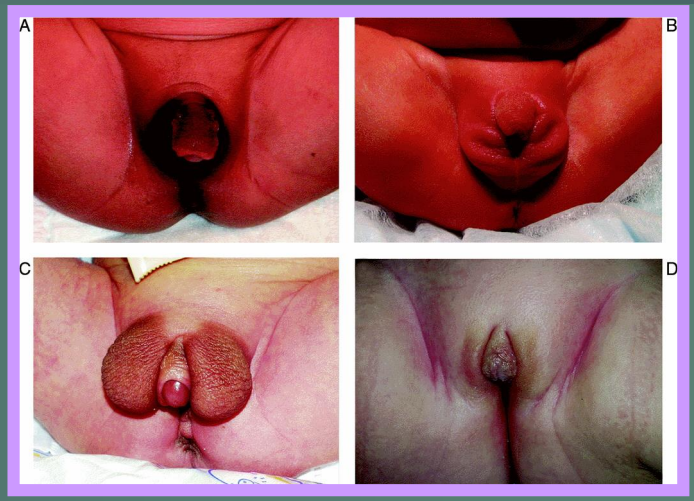
Condition	Gene	Uterus	Adrenal Defect	Associated features	Diagnostic biochemical features
46 XY DSD - Disorders in Androgen Synthesis & Action					
Smith-Lemil-Opitz syndrome	DHCR7	-	+/-	Coarse facies, second-third toe syndactyly, developmental delay, cardiac & visceral abnormalities	↑ 7-dehydrocholesterol
LH resistance	LHGCR	-	-	Leydig cell hypoplasia	↑LH, poor androgen response to hCG
Congenital lipoid adrenal Hyperplasia	STAR	-	+	Lipid-filled adrenals; puberal failure (46,XY), anovulation (46,XX)	Impaired production of all steroids
Cholesterol side-chain cleavage deficiency	CYP11A1	-	+	Puberal failure	Impaired production of all steroids
3β–hydroxysteroid dehydrogenase type II deficiency	HSD3B2	-	+	Puberal failure	↑Δ5: Δ4 ratio +/- mineralcorticoid insufficiency
17α-hydroxylase/17,20-lyase deficiency	CYP17	-	+	Hypertension due to □ ↑ 11-deoxycorticosterone (except in isolated 17,20-lyase deficiency); puberal failure	↑pregnenolone, progesterone, 11-deoxycorticosterone; ↓ 17-hydroxylated dteroids (except in isolated 17,20-lyase deficiency) and adrenal/gonadal androgens; ↑LH
P450 oxidoreductase deficiency	POR	-	+	+/- Antley-Bixler craniosynostosis	Mixed features of 21-hydroxylase deficiency, 17α-hydroxylase/17,20-lyase deficiency; salt loss rare
17 β-hydroxysteroid dehydrogenase deficiency type III	HSD17B3	-	-	Partial androgenization at puberty	↓testosterone: androstenedione ratio (< 0.6)
5α-reductase type II deficiency	SRD5A2	-	-	Partial androgenization at puberty	↑ DHT ratio (often >20, may need hCG stimulation
Androgen receptor	AR	-	-	–	variable↑ testosterone and LH/FSH; ↑AMH

Condition	Gene	Uterus	Adrenal Defect	Associated features	Diagnostic biochemical features
46 XX DSD - Disorders of ovary development					
SRY translocation	SRY	+/-	-	-	hCG responsive ↑ androgens
SOX9 duplication	SOX9	+/-	-	-	hCG responsive ↑ androgens
Palmorplantar hyperkeratosis XX males (testicular DSD)	RSP01	+/-	-	Palmoplantarhyperkeratosis, squamous cell carcinoma	hCG responsive ↑ androgens
46,XX DSD - Androgen excess					
3β-hydroxysteroid dehydrogenase type II deficiency	HSD3B2	+	+	Mild, partial androgenization due to ↑conversion of DHEA	↑ACTH, ↑Δ5: Δ4 ratio +/- mineralcorticoid insufficiency
21-hydroxylase deficiency	CYP21A2	+	+	Premature virilization	↑ACTH, ↑17-hydroxyprogesterone +/- mineralcorticoid insufficiency
11β-hydroxylase deficiency	CYP11B1	+	+	Hypertension due to ↑11-deoxycorticosterone, but often normotensive or even mild salt-loss in early life; premature virilization	↑ACTH, ↑11-deoxycorticosterone, ↑ 11-deoxycortisol
P450 oxidoreductase deficiency	POR	+	+	+/- Antley-Bixler craniosynostosis	Mixed features of 21-hydroxylase deficiency, 17α-hydroxylase/17,20-lyase deficiency;salt loss rare
Aromatase insufficiency	CYP19	+	-	Maternal androgenization during pregnancy; absent breast development at puberty, except in partial cases; polycystic ovaries; delayed bone age	↑A4, testosterone; ↓oestrogens; ↑FSH/LH
Glucocorticoid resistance	GRα	+	-	Hypertension	↑ACTH, 17-OHP, cortisol, mineralocorticoids, and androgens; failure of dexamethasone suppression (NB a patient with ambiguous genitalia was heterozygous for a mutation in CYP21 too)

Criteria suggesting DSD in the neonatal period

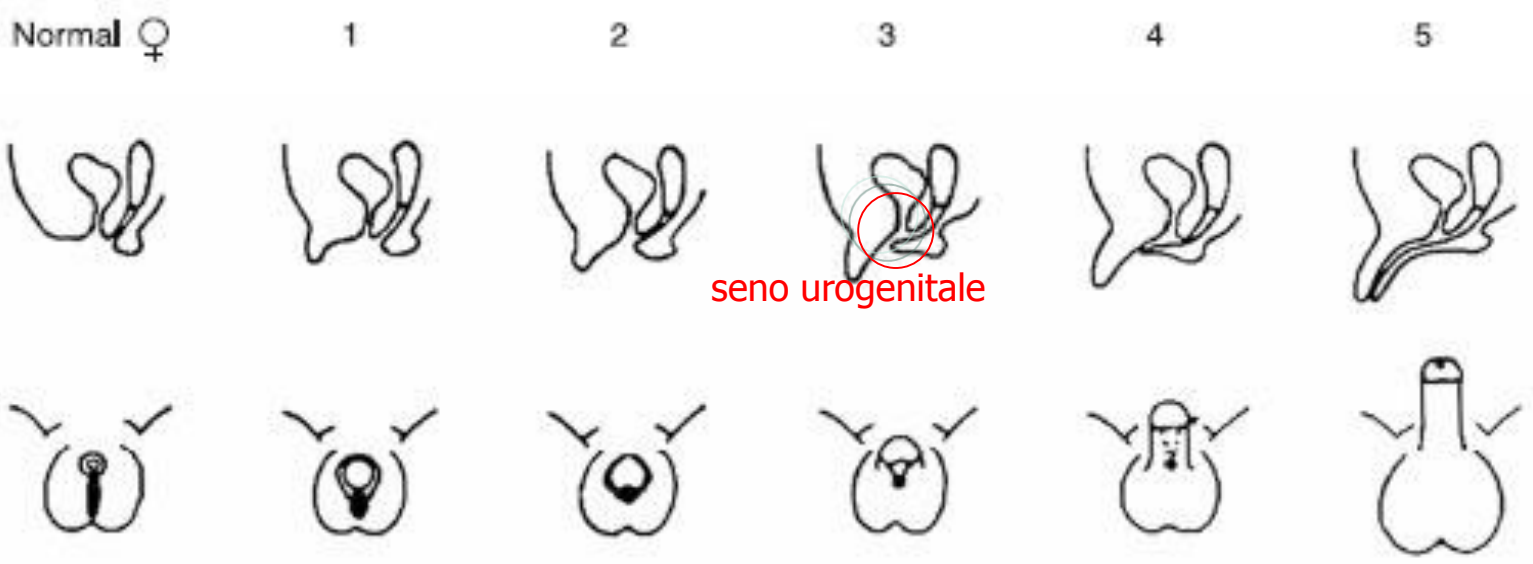
(Consensus guidelines, Hughes et al. Arch Dis Child 2006; 91:554-563)

- Overt genital ambiguity (eg. cloacal exstrophy)
- Apparent female genitalia with an enlarged clitoris, posterior labial fusion, or an inguinal/labial mass
- Apparent male genitalia with bilateral undescended testes, micropenis, isolated perineal hypospadias, or mild hypospadias with undescended testis.
- Family history of DSD such as CAIS
- Discordance between genital appearance and a prenatal karyotype.



Classificazione degli stadi di virilizzazione di Prader

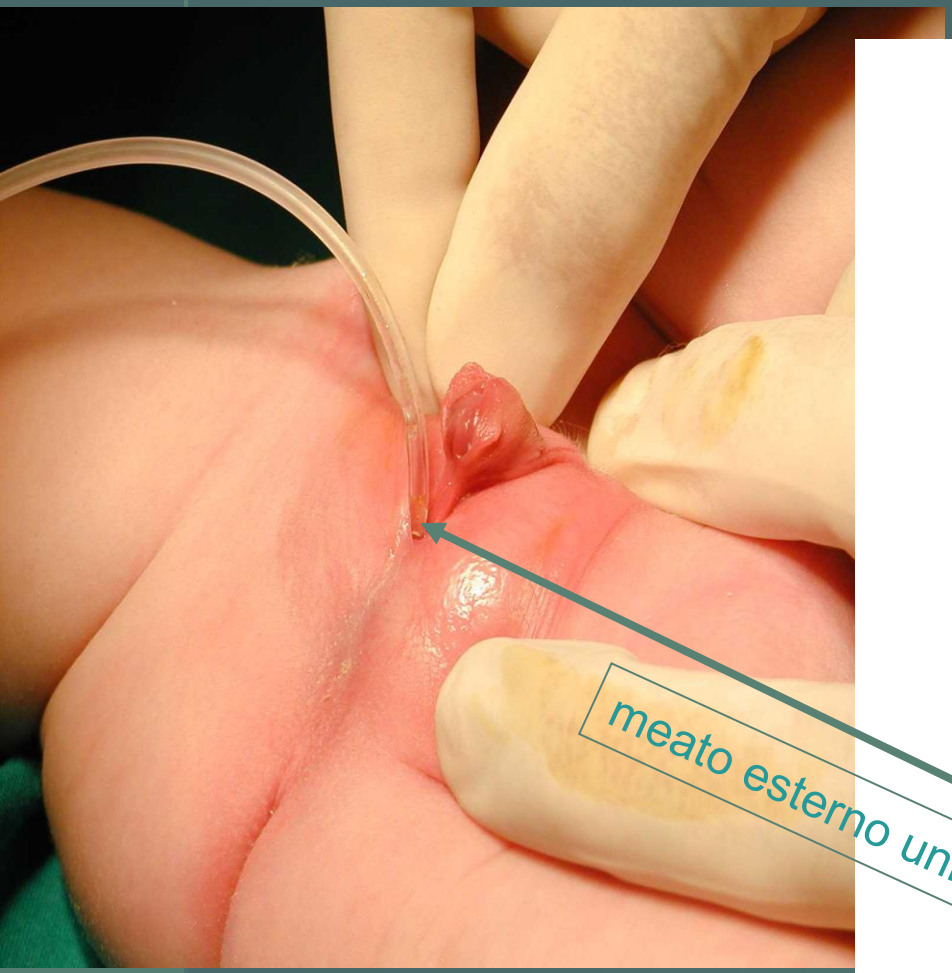
(descrive in modo oggettivo i diversi gradi di virilizzazione del neonato femmina,)



Grade 1 = genitalia of female appearance, with just an enlarged phallus; Grade 2 = phallus further enlarged, associated with posterior fusion of the labioscrotal folds, without a urogenital sinus; Grade 3 = significant increase in phallus size, associated with almost complete fusion of the labioscrotal folds, and the presence of a urogenital sinus with perineal opening; Grade 4 = phallus with penile appearance, associated with complete fusion of labioscrotal folds, and a urogenital sinus with perineal opening at the base or ventral surface of the phallus; Grade 5 = phallus with the appearance of a well-developed penis, associated with complete fusion of the labioscrotal folds, and a urogenital sinus and opening in the body of the phallus or balanic area.

Figure 1 - Prader's classification of grades of genital ambiguity

Il seno urogenitale in una neonata con 21OHD



Genitali esterni

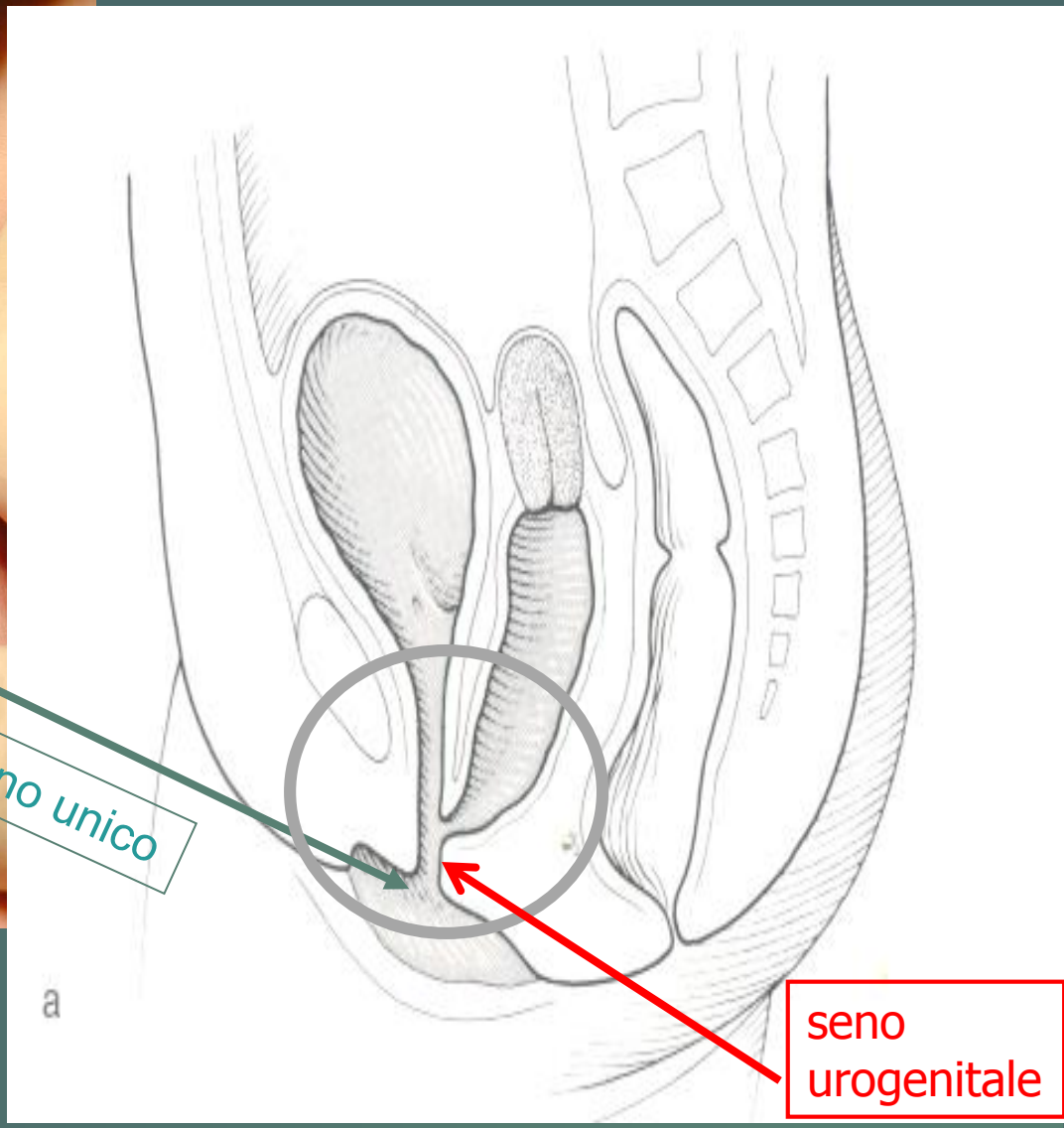


TABLE 3 Anthropometric Measurements of the External Genitalia

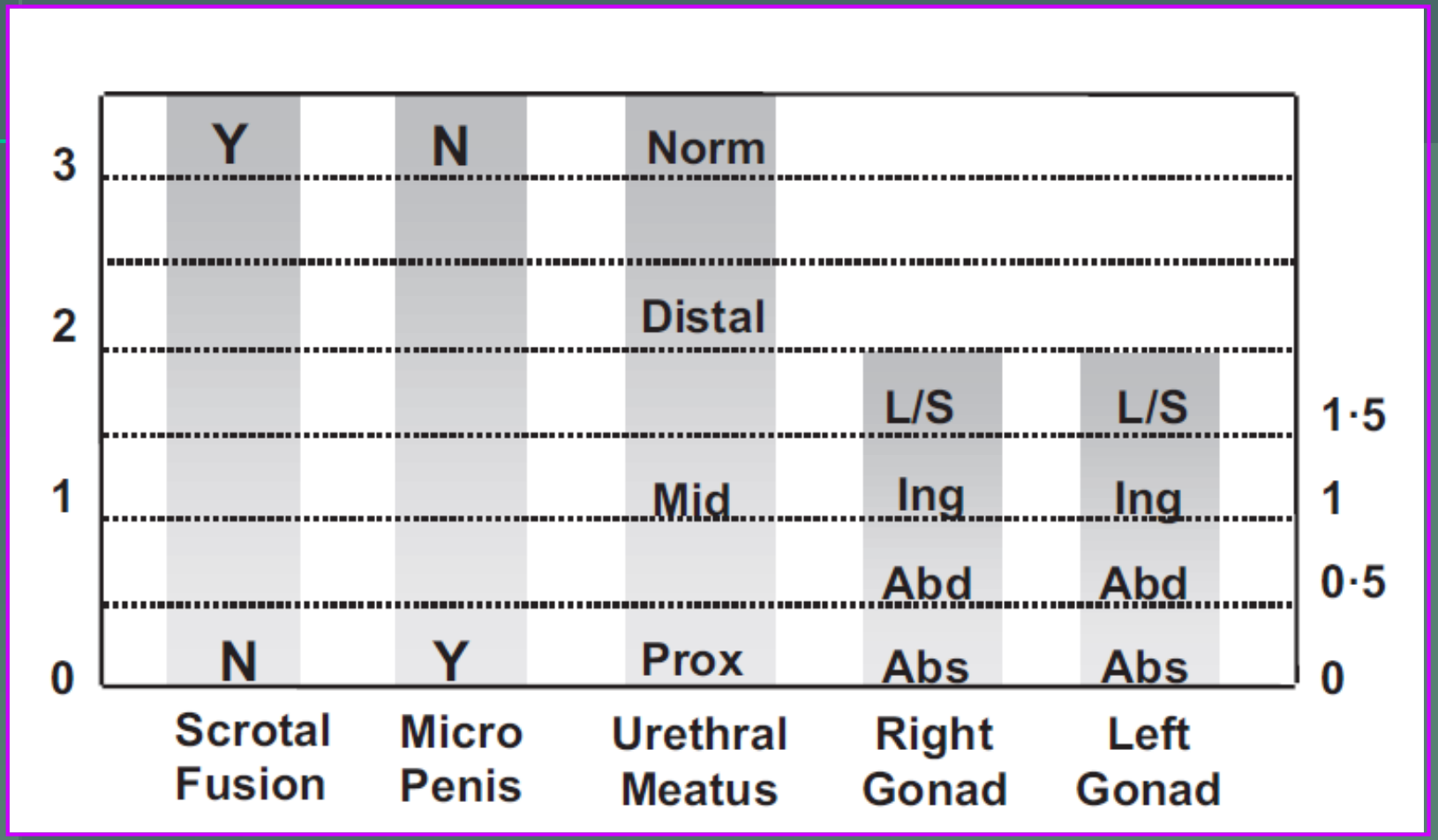
Sex	Population	Age	Stretched Penile Length, Mean ± SD, cm (Males), or Clitoral Length, Mean ± SD, mm (Females)	Penile Width, Mean ± SD, cm (Males), or Clitoral Width, Mean ± SD, mm (Females)	Mean Testicular Volume, mL (Males), or Perineum Length, Mean ± SD, mm (Females)	Ref No.
M	United States	30 wk GA	2.5 ± 0.4			26
M	United States	Term	3.5 ± 0.4	1.1 ± 0.1	0.52 (median)	26 and 27
M	Japan	Term to 14 y	2.9 ± 0.4 – 8.3 ± 0.8			28
M	Australia	24–36 wk GA	2.27 + (0.16 GA)			29
M	China	Term	3.1 ± 0.3	1.07 ± 0.09		30
M	India	Term	3.6 ± 0.4	1.14 ± 0.07		30
M	North America	Term	3.4 ± 0.3	1.13 ± 0.08		30
M	Europe	10 years	6.4 ± 0.4		0.95–1.20	27 and 31
M	Europe	Adult	13.3 ± 1.6		16.5–18.2	27 and 31
F	United States	Term	4.0 ± 1.24	3.32 ± 0.78		32
F	United States	Adult nulliparous	15.4 ± 4.3			33
F	United States	Adult	19.1 ± 8.7	5.5 ± 1.7	31.3 ± 8.5	34

GA indicates gestational age.

(Consensus guidelines, Hughes et al. Arch Dis Child 2006; 91:554-563)

Masculinization Index

Ahmed SF, BJU International 2000, 85:120-24



Soggetti normali: score 12

Vanno indagati i soggetti con score <11

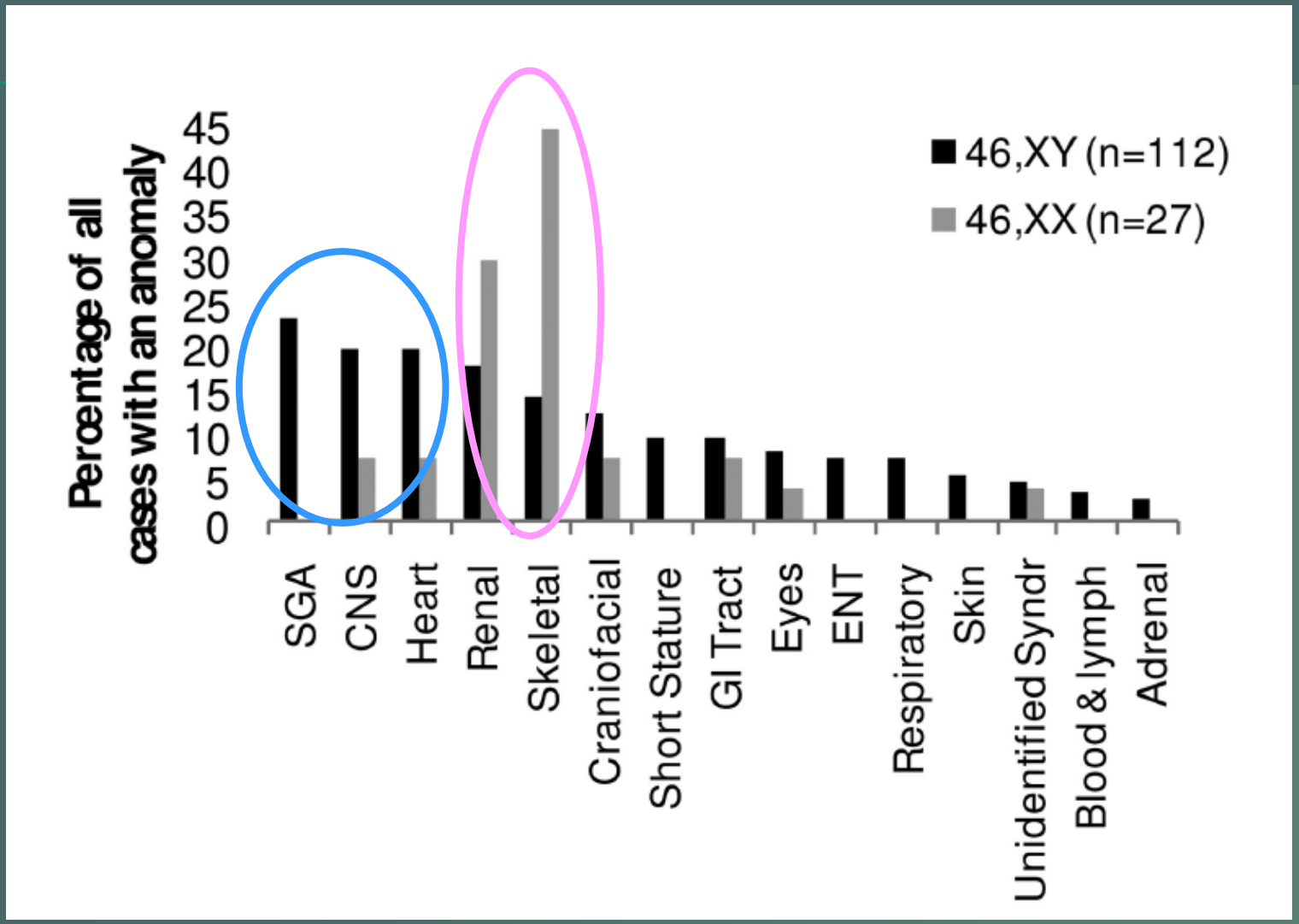
Quali soggetti con sospetto DSD inviare allo specialista

Soggetti con:

- Ipospadia perineale isolata
- Micropene isolato
- Clitoridomegalia isolata
- Tutte le forme di ipospadia su base familiare
- EMS < 11

27% dei DSD presenta un'anomalia associata

Prevalenza di condizioni associate > nei 46XY DSD.

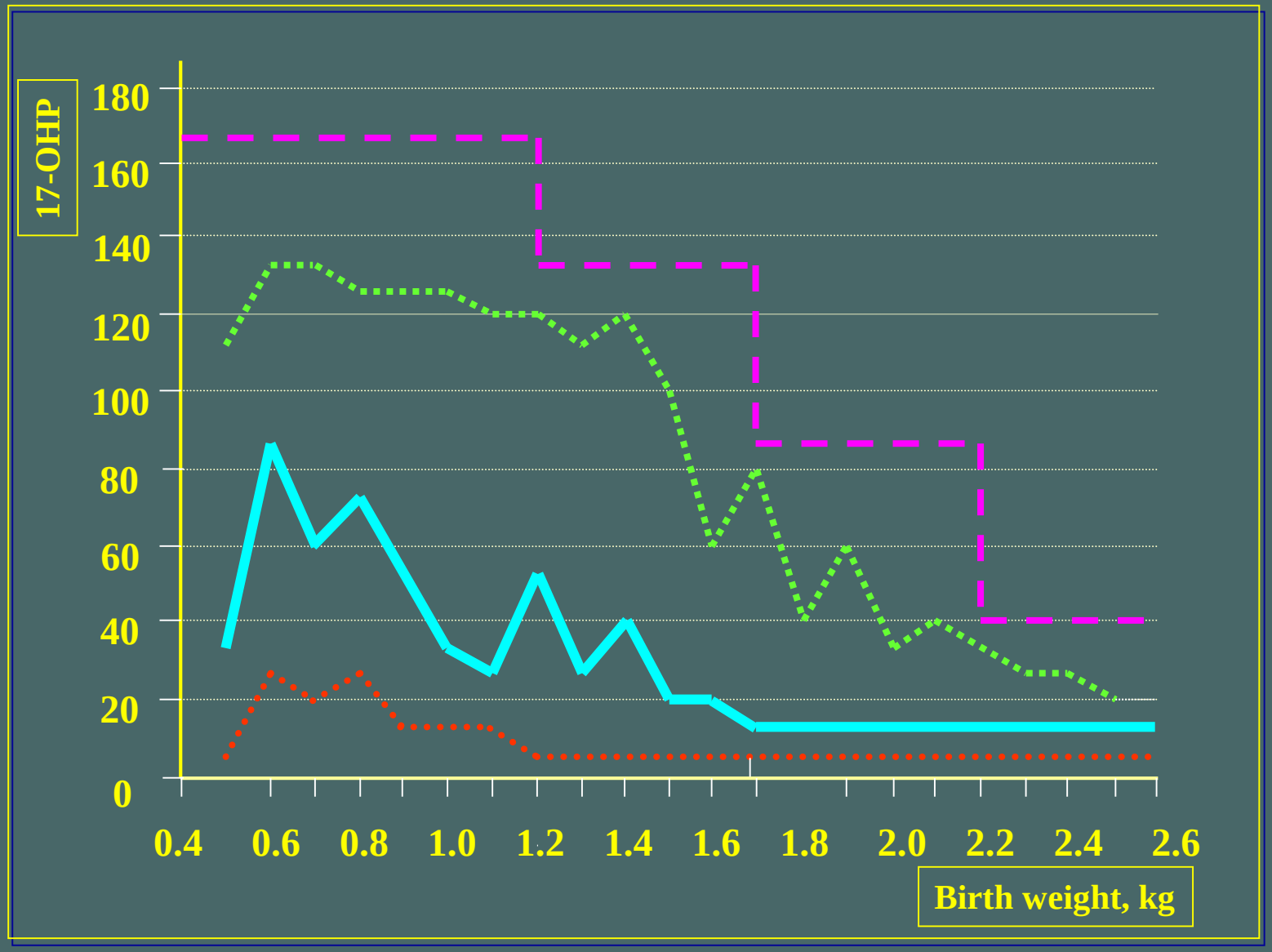


Cox k, et al. JCEM99: E348–E355, 2014

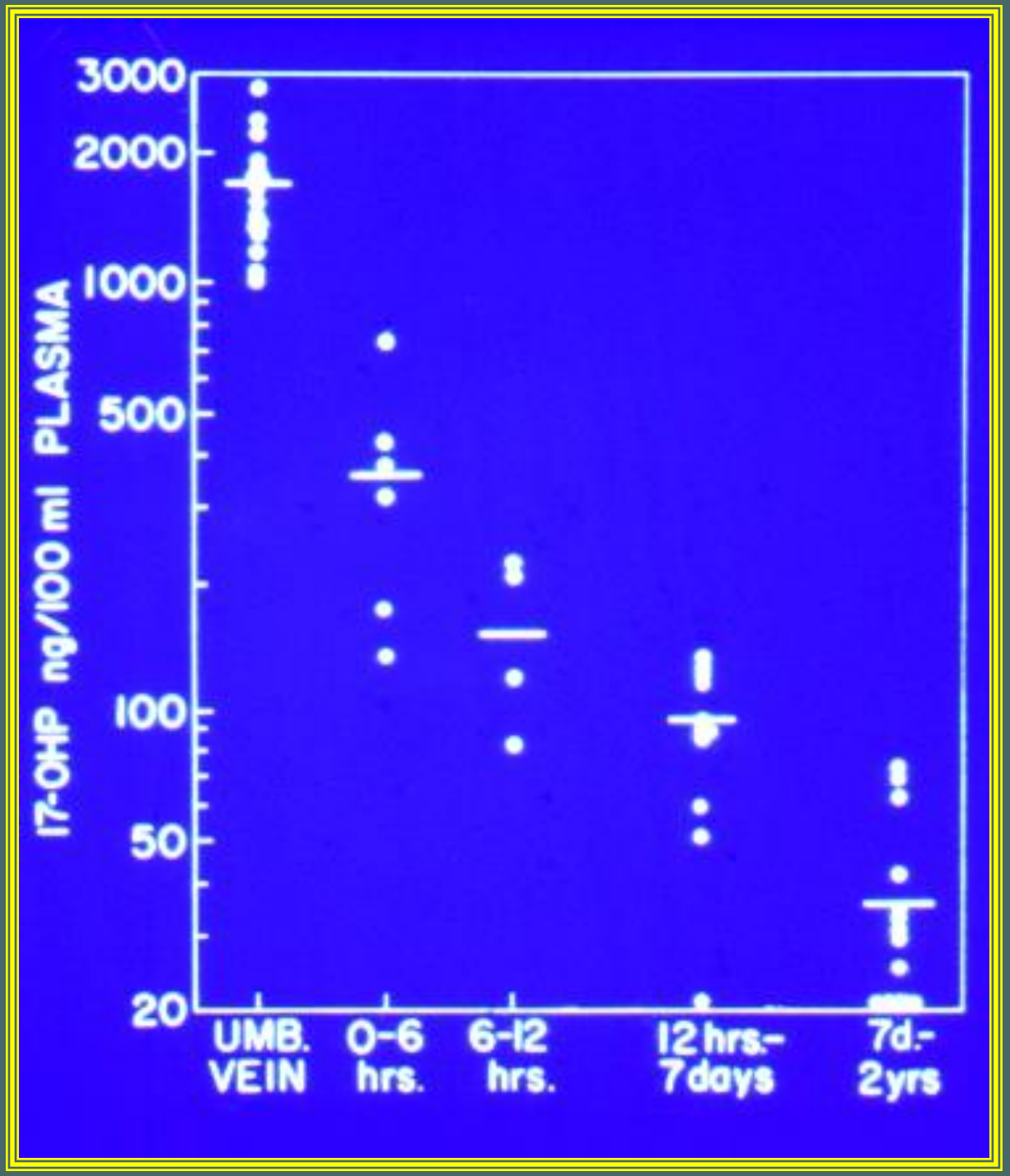
"BASIC"

- **B**onding: parent-infant interactions should be nurtured
- **A**drenal: do not overlook adrenal failure
- **S**ex-assignment: avoid an inexperienced person "best-guessing" sex assignment
- **I**maging: ultrasound is useful, but can be misleading
- **C**ytogenetics: send an appropriate sample early for cytogenetics and FISH analysis for SRY and X-chromosome probes

Levels of 17-OHP in dried blood samples from the Wisconsin neonatal screening



White and Speiser, JCEM 2000


17-OHP ng/100 ml PLASMA
3000
2000
1000
500
100
50
20
UMB. VEIN
0-6 hrs.
6-12 hrs.
12 hrs.-7 days
7d.-2yrs

"BASIC"

- **B**onding: parent-infant interactions should be nurtured
- **A**drenal: do not overlook adrenal failure
- **S**ex-assignment: avoid an inexperienced person "best-guessing" sex assignment
- **I**maging: ultrasound is useful, but can be misleading
- **C**ytogenetics: send an appropriate sample early for cytogenetics and FISH analysis for SRY and X-chromosome probes

Factors influencing gender assignment

(Consensus guidelines, Hughes et al. Arch Dis Child 2006; 91:554-563)

- Diagnosis
- Genital appearance
- Surgical options
- Need for lifelong replacement therapy
- Potential for fertility
- Views of the family
- Cultural practices

Gender assignment in newborns

(Consensus guidelines, Hughes et al. Arch Dis Child 2006; 91:554-563)

- ***CAH:*** >90% of XX infants raised as females identify as females in adulthood
- ***5αRD2:*** 60% of patients assigned female in infancy and virilizing at puberty live as males
- ***CAIS:*** 100% of XY patients identify as females
- ***PAIS, androgen biosynthetic defect, and partial gonadal dysgenesis:*** 25% of cases dissatisfied with their gender assignment

Gender assignment in newborns

(Consensus guidelines, Hughes et al. Arch Dis Child 2006; 91:554-563)

- ***Micropenis:*** raise as male (potential for fertility, no need for surgery, equal satisfaction with assigned gender in those raised male or female)
- ***Ovotesticular DSD:*** consider potential for fertility, and likelihood of having genitalia consistent with the chosen sex
- ***MGD:*** consider prenatal androgen exposure, testicular function at and after puberty, phallic development, gonadal location

Gender assignment in newborns

(Consensus guidelines, Hughes et al. Arch Dis Child 2006; 91:554-563)

- In the presence of testes, a partially differentiated penis with good corpora, and a 46XY karyotype strong consideration should be given to a male sex of rearing

"BASIC"

- **B**onding: parent-infant interactions should be nurtured
- **A**drenal: do not overlook adrenal failure
- **S**ex-assignment: avoid an inexperienced person "best-guessing" sex assignment
- **I**maging: ultrasound is useful, but can be misleading
- **C**ytogenetics: send an appropriate sample early for cytogenetics and FISH analysis for SRY and X-chromosome probes

First line investigations

- FISH (SRY/X Chr)
- Electrolytes
- 17OHP
- Cortisol
- LH/FSH/Testosterone/AMH
- Pelvic US

Second Line Investigations

- ***Adrenal:*** ACTH, pregnenolone, progesterone, 17OH-pregnenolone, 11-deoxycortisol, DHEAS

  Plasma renin activity, aldosterone, DOC

  ACTH stimulation test

- ***Testis:*** Androstenedione, testosterone, DHT, AMH, inhibin B

  hCG stimulation tests

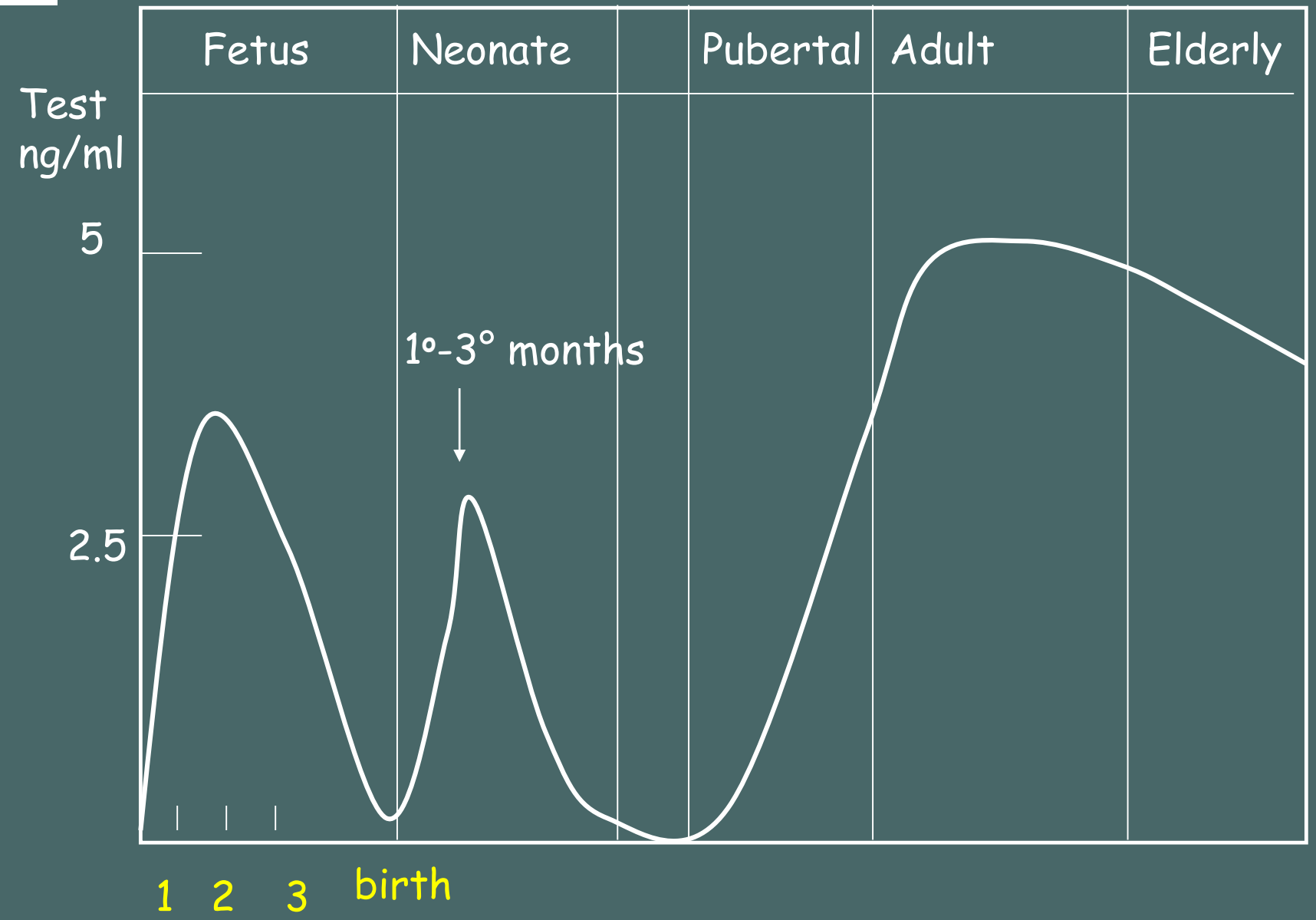
  LHRH stimulation test

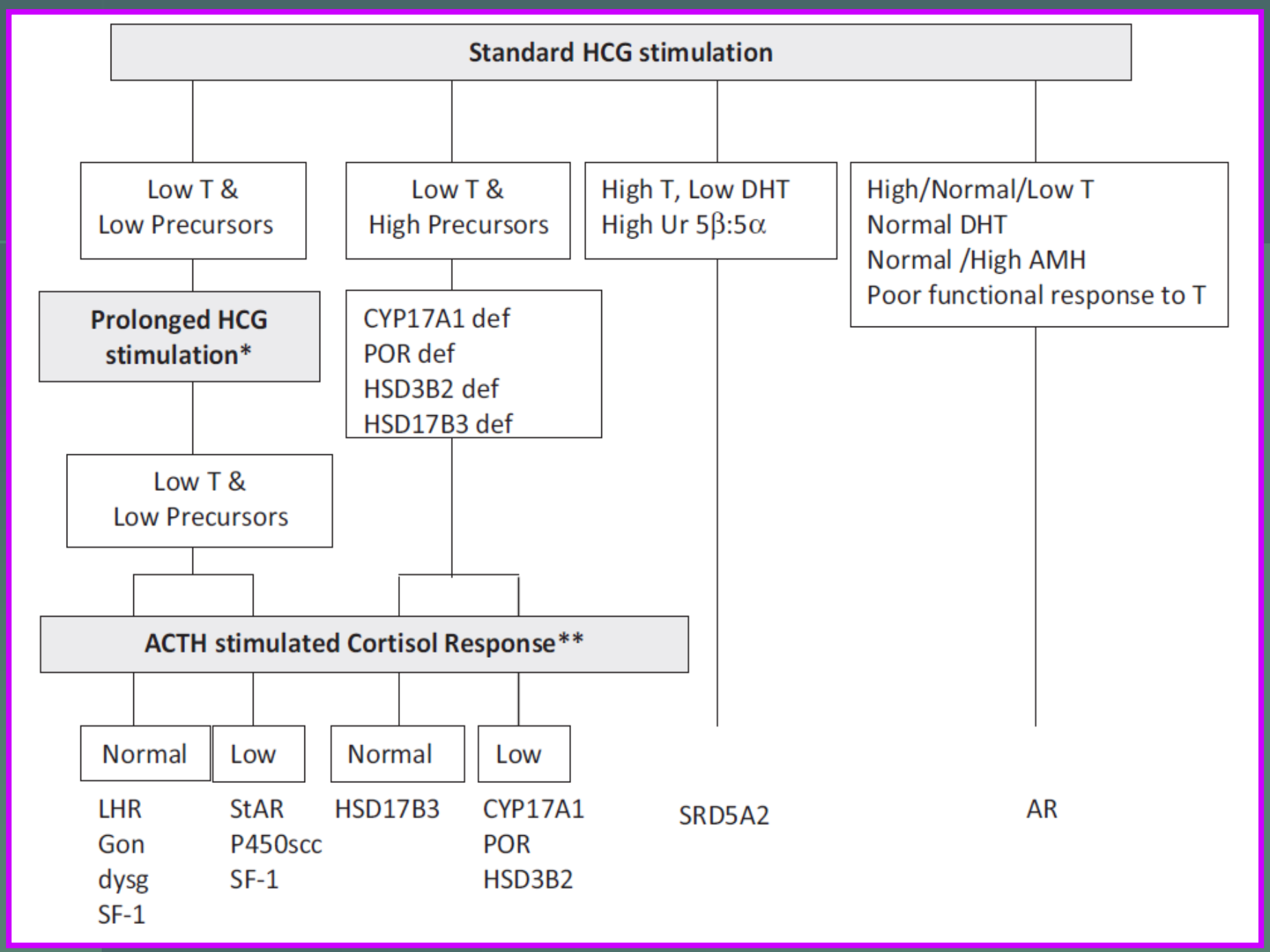
- ***Urine steroid analysis by GC-MS***

- ***Imaging:*** MRI, cystourethroscopy, sinogram/genitogram

- ***Surgical:*** Laparoscopy, gonadal biopsy and histology/cytogenetics/ molecular analysis

Testosterone levels throughout life in males




Standard HCG stimulation
Low T & Low Precursors
Low T & High Precursors
High T, Low DHT
High Ur 5β:5α
High/Normal/Low T
Normal DHT
Normal /High AMH
Poor functional response to T
Prolonged HCG stimulation*
CYP17A1 def
POR def
HSD3B2 def
HSD17B3 def
Low T & Low Precursors
ACTH stimulated Cortisol Response**
Normal
Low
Normal
Low
LHR
Gon
dysg
SF-1
StAR
P450scc
SF-1
HSD17B3
CYP17A1
POR
HSD3B2
SRD5A2
AR

Interpretation of serum AMH concentration in DSD

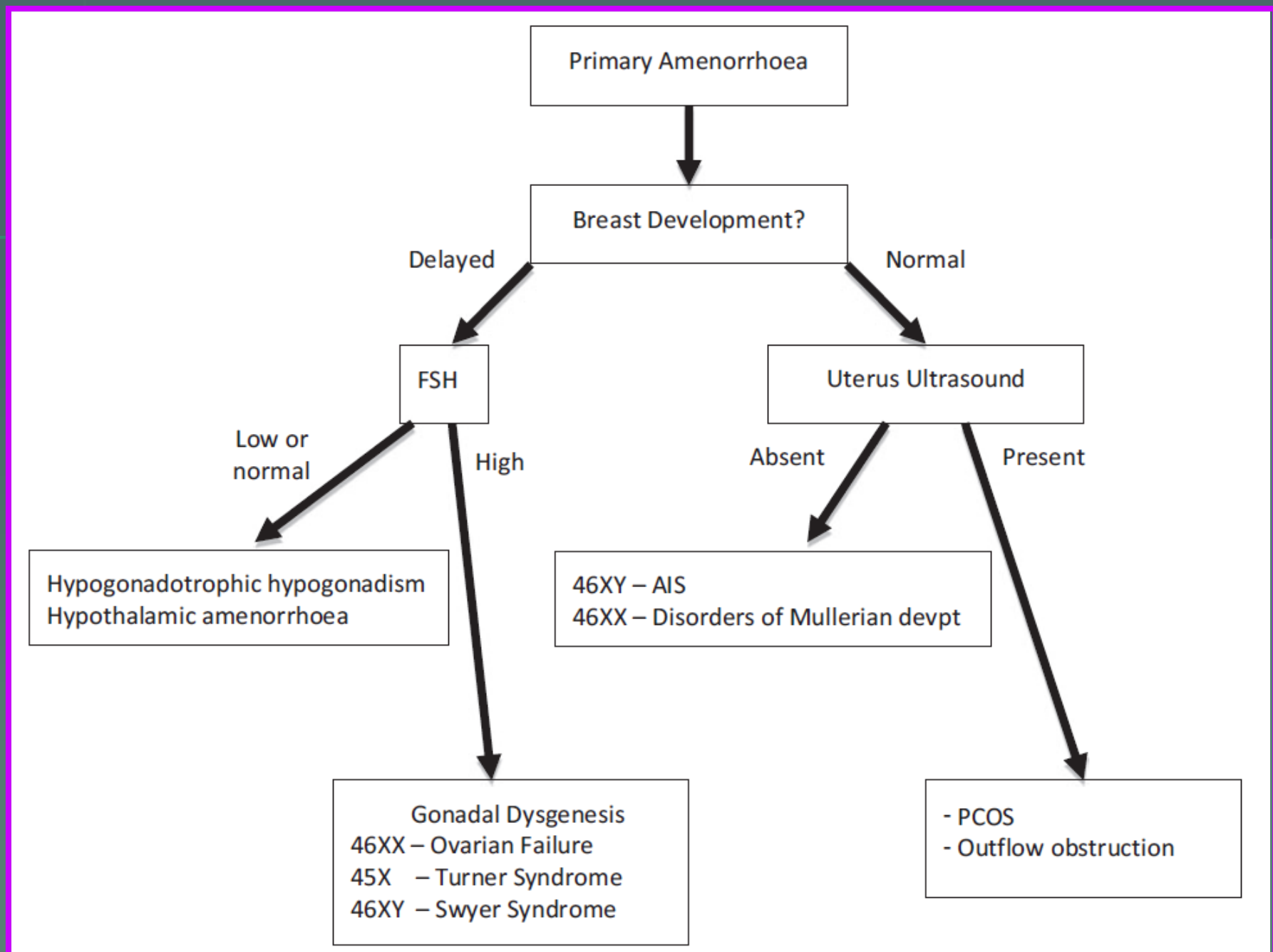
Serum AMH	Testicular tissue	Interpretation
Undetectable or very low	Absent	46,XX CAH Complete gonadal dysgenesis PMDS due to AMH gene defect Congenital anorchia
Within female age-related reference range	Usually absent	46,XX CAH Complete gonadal dysgenesis Dysgenetic testes or ovotestes
Below male or above female age-related reference range	Present	Dysgenetic testes Ovotestes
Within male age-related reference range	Usually normal	Non-specific XY,DSD Hypogonadotrophic hypogonadism PMDS due to AMH-R defect 46,XX testicular DSD Ovotestes
Above male age-related reference range	Present	AIS esp. CAIS 5α-reductase deficiency Testosterone biosynthetic defect Leydig cell hypoplasia

Ahmed SF, et al., Clinical Endocrinology (2016) 84, 771–788

Criteria suggesting DSD in children and young adults

(Consensus guidelines, Hughes et al. Arch Dis Child 2006; 91:554-563)

- Previously unrecognized genital ambiguity
- Inguinal hernia in a female
- Delayed or incomplete puberty
- Virilization in a female
- Primary amenorrhea
- Gross and occasionally cyclic, hematuria in a male


Primary Amenorrhoea
Breast Development?
Delayed
Normal
FSH
Uterus Ultrasound
Low or normal
High
Absent
Present
Hypogonadotrophic hypogonadism
Hypothalamic amenorrhoea
46XY – AIS
46XX – Disorders of Mullerian devpt
Gonadal Dysgenesis
46XX – Ovarian Failure
45X – Turner Syndrome
46XY – Swyer Syndrome
- PCOS
- Outflow obstruction

General Concepts of Care

(Consensus guidelines, Hughes et al. Arch Dis Child 2006; 91:554-563)

- Gender assignment must be avoided before expert evaluation in newborns
- Evaluation and long-term management must be performed at a center with experienced multidisciplinary team
- All individuals should receive a gender assignment
- Open communication with patients and families is essential, and participation in decision-making is encouraged
- Patient and family concerns should be respected and addressed in strict confidence

Gender identity: a person's self-representation as male or female

Gender role: sex-typical behaviors (toy preferences, physical agression)

Sexual orientation: direction of erotic interest (heterosexual, bisexual, homosexual)

Grazie per l'attenzione!

Thank you for your attention!

Table 3. Risk of type-II germinal cell tumors (GCTs) in the various categories of disorders of sex development (DSD) patients, classified into high-, intermediate-, low- and no-risk groups.

Risk group	Disorder	Malignancy risk (%)	Recommended action	Studies (n)	Patients (n)
High	GD[a] (+Y)[b] intra-abdominal	15–35	Gonadectomy[c]	12	>350
	PAIS non-scrotal	50	Gonadectomy[c]	2	24
	Frasier	60	Gonadectomy[c]	1	15
	Denys–Drash (+Y)	40	Gonadectomy[c]	1	5
Intermediate	Turner (+Y)	12	Gonadectomy[c]	11	43
	17β-HSD	28	Monitor	2	7
	GD (+Y)[c]	Unknown	Biopsy[d] and irradiation?	0	0
	PAIS scrotal gonad	Unknown	Biopsy[d] and irradiation?	0	0
Low	CAIS	2	Biopsy[d] and ???	2	55
	Ovotestis DSD	3	Testis tissue removal?	3	426
	Turner (− Y)	1	None	11	557
No (?)	5α-reductase	0	Unresolved	1	3
	Leydig cell hypoplasia	0	Unresolved	2	

CAIS, complete androgen insensitivity syndrome; 17β-HSD, 17β-hydroxysteroid dehydrogenase deficiency; PAIS, partial androgen insensitivity syndrome.

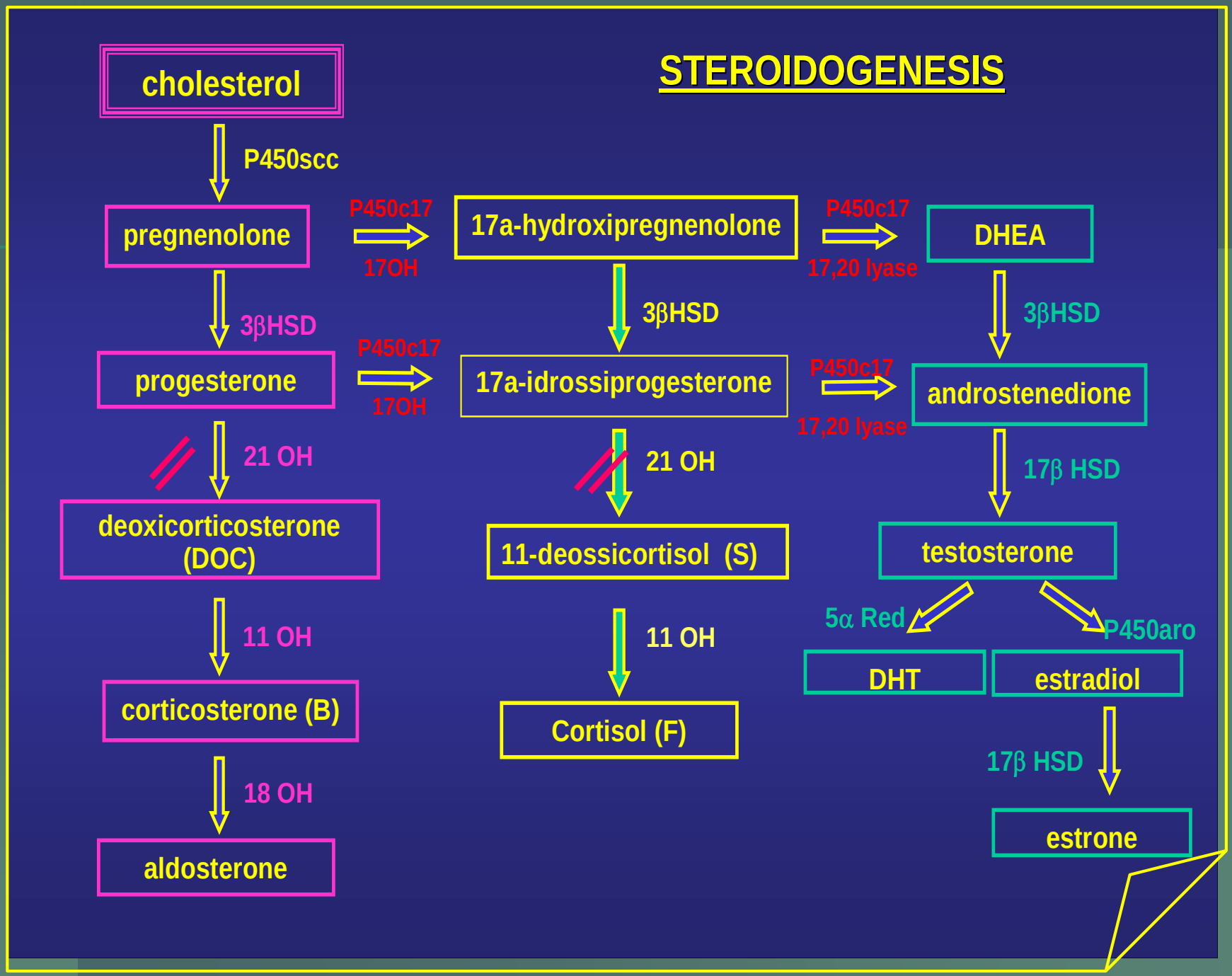
[a] Gonadal dysgenesis (including not further specified, 46XY, 46X/46XY, mixed, partial, complete).

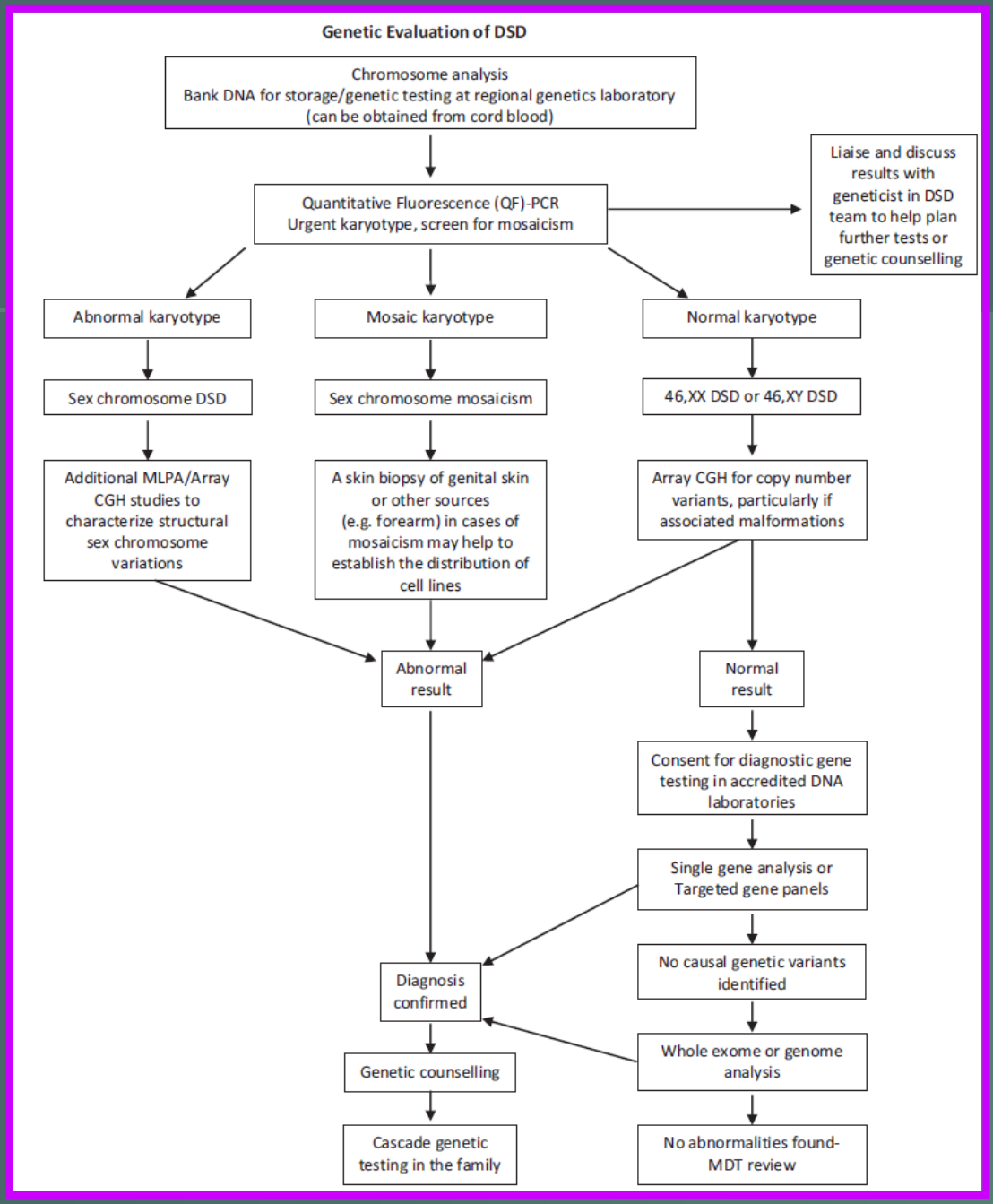
[b] GBY region positive, including the *TSPY* gene.

[c] At time of diagnosis.

[d] At puberty, allowing investigation of at least 30 seminiferous tubules, with diagnosis preferably based on OCT3/4 immunohistochemistry.

(Consensus guidelines, Hughes et al. Arch Dis Child 2006; 91:554-563)


STEROIDOGENESIS
cholesterol
P450scc
pregnenolone
3βHSD
progesterone
21 OH
deoxicorticosterone (DOC)
11 OH
corticosterone (B)
18 OH
aldosterone
P450c17
17OH
17a-hydroxipregnenolone
3βHSD
P450c17
17OH
17a-idrossiprogesterone
21 OH
11-deossicortisol (S)
11 OH
Cortisol (F)
P450c17
17,20 lyase
DHEA
3βHSD
P450c17
17,20 lyase
androstenedione
17β HSD
testosterone
5α Red
P450aro
DHT
estradiol
17β HSD
estrone


Genetic Evaluation of DSD
Chromosome analysis
Bank DNA for storage/genetic testing at regional genetics laboratory
(can be obtained from cord blood)
Quantitative Fluorescence (QF)-PCR
Urgent karyotype, screen for mosaicism
Liaise and discuss results with geneticist in DSD team to help plan further tests or genetic counselling
Abnormal karyotype
Mosaic karyotype
Normal karyotype
Sex chromosome DSD
Sex chromosome mosaicism
46,XX DSD or 46,XY DSD
Additional MLPA/Array CGH studies to characterize structural sex chromosome variations
A skin biopsy of genital skin or other sources (e.g. forearm) in cases of mosaicism may help to establish the distribution of cell lines
Array CGH for copy number variants, particularly if associated malformations
Abnormal result
Normal result
Consent for diagnostic gene testing in accredited DNA laboratories
Single gene analysis or Targeted gene panels
No causal genetic variants identified
Diagnosis confirmed
Whole exome or genome analysis
Genetic counselling
Cascade genetic testing in the family
No abnormalities found- MDT review