46XY – IS HE ACTUALLY MALE?
AND 46 XX – FEMALE?

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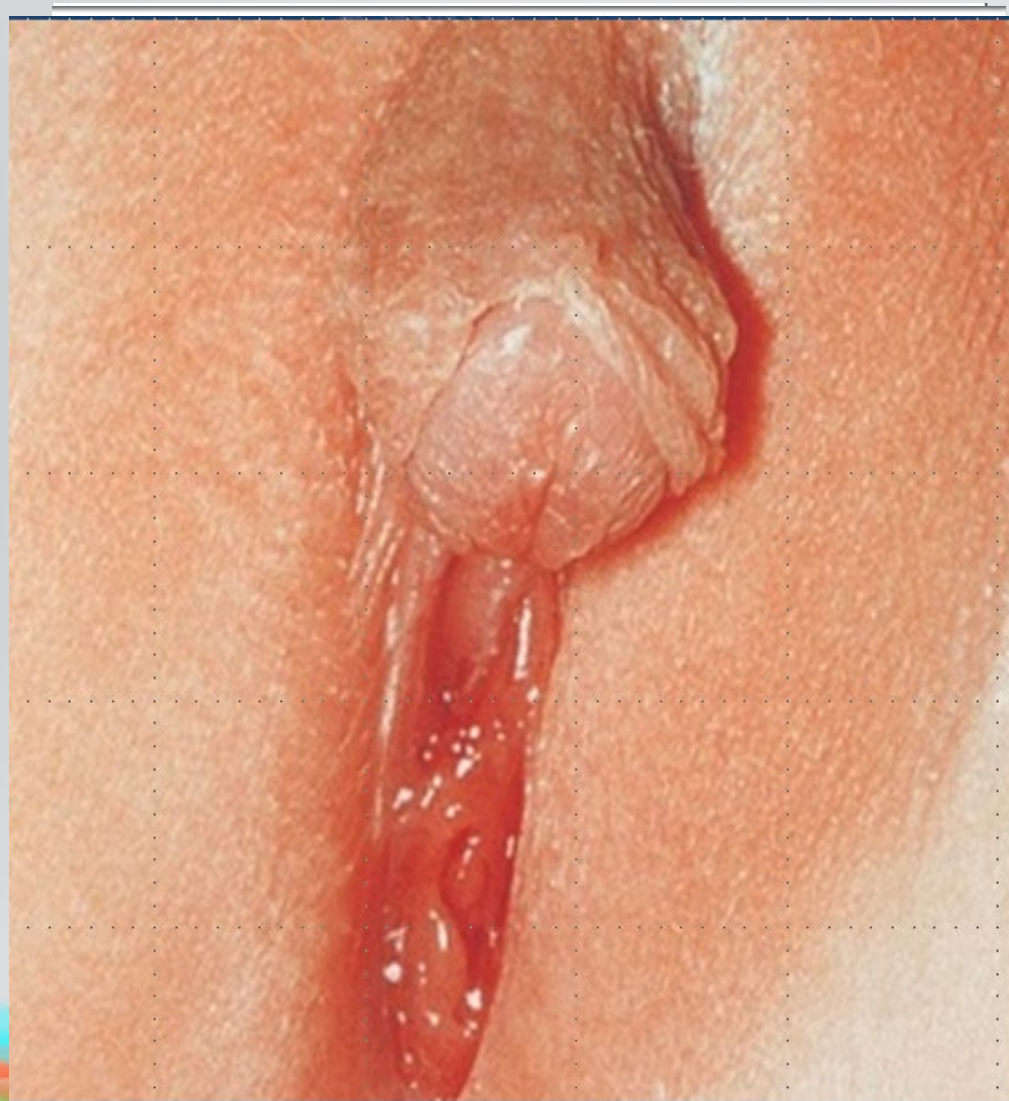
INTRODUCTION

- The first question parents ask after the birth of their child is what the gender is. In recent decades, it has become possible to have the answer to that question much earlier: 3D ultra-sonography, the karyotype in some prenatally examined fetuses can provide an exact answer.
- Not always, though…
- In one out of 4500 newborns, the way external genitalia look makes the answer impossible at the usual time. Differences in Sex Development (DSD) have always posed an enigma, to a greater or lesser extent, to narrow specialists: neonatologists, pediatricians, pediatric surgeons, gynecologists, urologists, geneticists, and of course, to pediatric endocrinologists.
- In recent years /Istanbul Convention/ and months /the Olympic Games and the law prohibiting propaganda for non-traditional sexual orientation in schools/ utterly incompetent people would make decisions and write laws that are at odds with scientific knowledge and professional experience.
- Despite the scientific discoveries of the past decades, each DSD case raises its own singular questions, regarding the determination of the actual gender or the decision on the future gender of the child, due to a number of problems not always sufficiently evident in the newborn period.

DISORDERS OF SEX DEVELOPMENT - DSD

- The normal gender development depends on the precise spatio-temporal sequence and coordination of mutually antagonistic activating and suppressing factors.
- These factors regulate the direction of the unipotential gonad in the binary pathways governing normal sex development
- DSD cover a group of congenital conditions associated with atypical development of internal and/or external genital structures.
- These conditions are the result of genetic disorders, hormonal and anatomical deviations.
- They can be initiated at birth due to unclear phenotypic sex, or at a later age with specific changes - mainly virilization, late or absent puberty



CLASSIFICATION

- The two major DSD groups are:

Abnormal determination the sex - abnormal gonadal development:

XY gonad dysgenesis – SRY gen– Yp11.3 - locus

XX testicular DSD - SOX-9 17q24.3-q25.1

Ovotesticular DSD - SF-1,WT-1,DAX-1, WNT-4,DMRT-1, XH2, DHH.

Disrupted gender differentiation –

in normally developed gonads, the subsequent development of internal and external genitalia is pathological.

CLASSIFICATION

46 XX DSD - ANDROGEN INDUCED

Congenital adrenal hyperplasia (CAH) with virilization

Placental aromatase deficiency

GCS (glucokortikosteroid) receptor mutation

Maternal androgen secreting tumor

Virilizing luteoma of pregnancy

Administration of androgens

(Norethindrone, Ethisterone, Nor-ethynodrel, Medroxyprogesterone, Danazol)

Ovotesticular deseasis

46 XY DSD - Impaired testosterone synthesis

Leydig cell agenesis

LH/HCG receptor mutation (LHCGR)

Inborn lipoid adrenal hyperplasia

Mutation of Cholesterol side chain cleavage (CYP11A1)

Mutation of 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2)

Mutation of 17α-hydroxylase/17,20 lyase (CYP17A1)

Mutation of P450 oxidoreductase (POR)

Smith-Lemli-Opitz (DHCR7) Mutation

Mutation of 17β-hydroxysteroid dehydrogenase type 3 (HSD17B3)

Mutation of 5α-reductase type 2 (SRD5A2)

Cytochrome b5 (CYB5A)

3α- hydroxysteroid dehydrogenase deficit (AKR1C2 and AKR1C3)

Denys-Drash Syndrome (WT1)

CLASSIFICATION

DISORDERS IN ANDROGEN-DEPENDENT ORGANS

Androgen Insensitivity Syndromes (disorders in the androgen receptor or in post-receptor processes):

Complete AIS

Partial AIS

SEX CHROMOSOMES DSD

Turner Syndrome

Klinefelter Syndrome

Mosaicism 45,X/46,XY

Triple XXX Syndrome

XXY Syndrome

XX OR XY DISEASES OF GONADAL DEVELOPMENT

Complete gonadal dysgenesis

Partial gonadal dysgenesis

Regression of the gonads

Ovotesticular DSD

CLASSIFICATION

XY - Persistent Müllerian Duct Syndrome

With low AMH (AMH)

With normal or high AMH (AMHR2)

MALFORMATIVE SYNDROMES

- CHARGE syndrome
- Hand-foot-genital syndrome
- MRKH Syndrome
- MURCS Association
- McKusick-Kaufman Syndrome
- Aphallia
- Cloacal/Bladder Exstrophy
- Isolated Hypospadias
- Penoscrotal Transposition

MICROPENIS

- Kallman - Syndrome
- Growth hormone defician
- GNRH – receptor mutation

CRYPTORCHIDISM

- Kallman - Syndrome
- GNRH – receptor mutation

HYPOSPADIAS

ISOLATED CONGENITAL MALFORMATIONS

MULTIPLE CONGENITAL MALFORMATION SYNDROMES

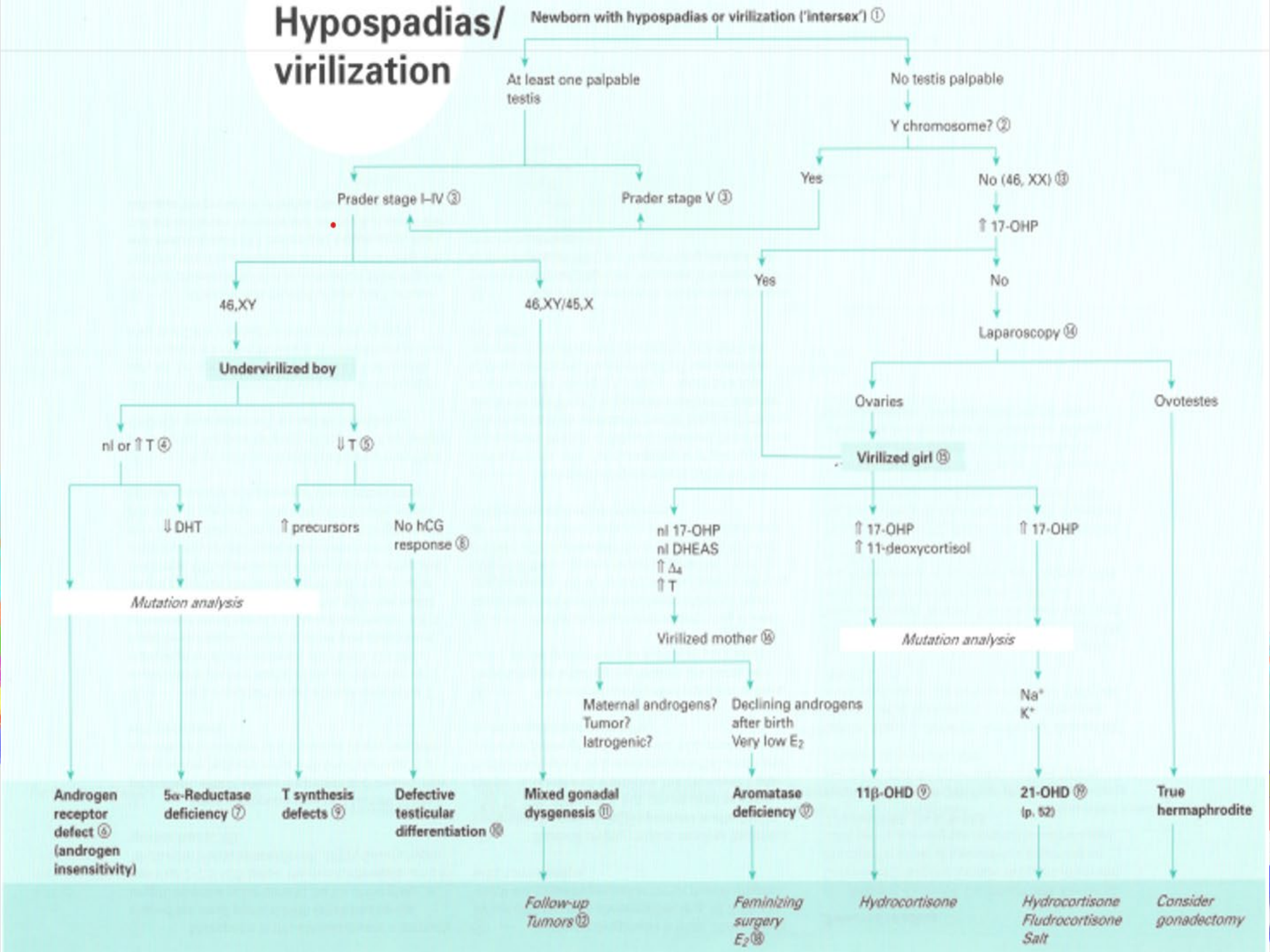
- VACTERL (vertebrae, anus, cardiovascular tree, trachea, esophagus, renal system, limb buds)
- Goldenhar
- Pallister-Hall
- Robinow
- McKusick-Kaufman
- IMAGe (interauterine growth retardation, metaphyseal dysplasia, adrenal

This so a broad classification of DSD, with manifestations of the condition both in the newborn period and in later age - during pubertal maturation, requires a narrow differential-diagnostic approach - without wasting unnecessary time - after birth or at the first worrying doubts about disease - at a later age.

The right decision changes destinies, the mistakes - even more so.

Diagnostic algorithms for the newborn period or for DSD manifesting at puberty can shorten the diagnostic path in terms of the volume of investigations and time.

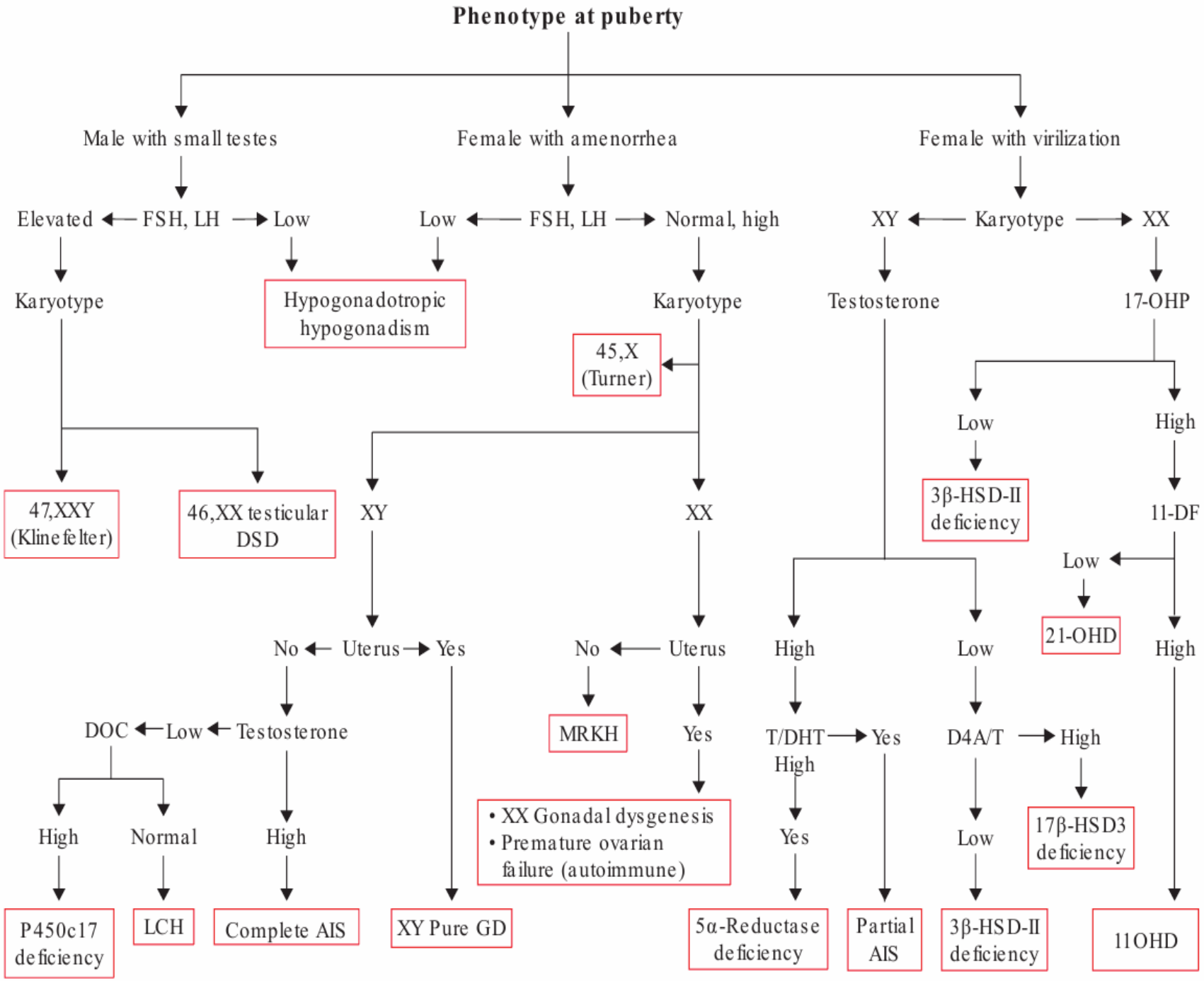
Hypospadias/ virilization



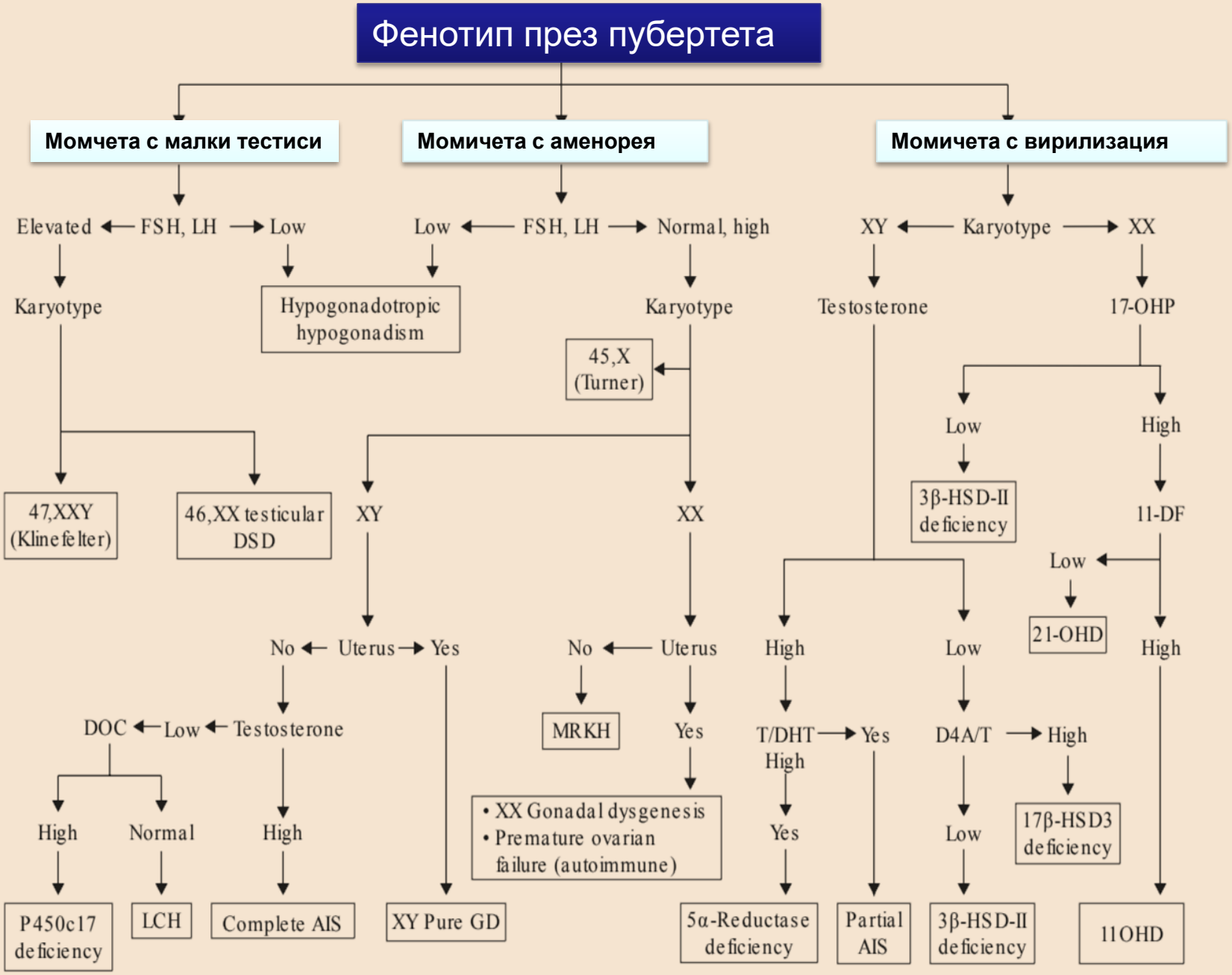
Хипоспадия или вирилизация

DSD IN PUBERTAL AGE

- What happens at the age when the boy is about to become a young man and the girl a young woman?
- In fact, cases with DSD manifesting at the age that should be puberty are much more common than those in the newborn period. In some cases, the cause could be looked into the untimely diagnosis, unsought medical help, incomplete status, undiagnosed arterial hypertension, underestimated short stature, or malformative stigmas.
- Sometimes there is a need for sex reassignment, gonadectomy, hormone replacement therapy, and almost always there is infertility.
- The psychological problems resulting from the diagnoses are often dramatic. Misdiagnosis, incorrect advice, inadequate treatment or surgical correction can be fatal.


Phenotype at puberty
Male with small testes
Female with amenorrhea
Female with virilization
Elevated
FSH, LH
Low
Low
FSH, LH
Normal, high
XY
Karyotype
XX
Karyotype
Hypogonadotropic hypogonadism
Karyotype
Testosterone
17-OHP
45,X (Turner)
Low
High
47,XXY (Klinefelter)
46,XX testicular DSD
XY
XX
3β-HSD-II deficiency
11-DF
Low
21-OHD
No
Uterus
Yes
No
Uterus
High
Low
High
DOC
Low
Testosterone
MRKH
Yes
T/DHT
High
Yes
D4A/T
High
High
Normal
High
• XX Gonadal dysgenesis
• Premature ovarian failure (autoimmune)
Yes
Low
17β-HSD3 deficiency
P450c17 deficiency
LCH
Complete AIS
XY Pure GD
5α-Reductase deficiency
Partial AIS
3β-HSD-II deficiency
11OHD

Фенотип през пубертета



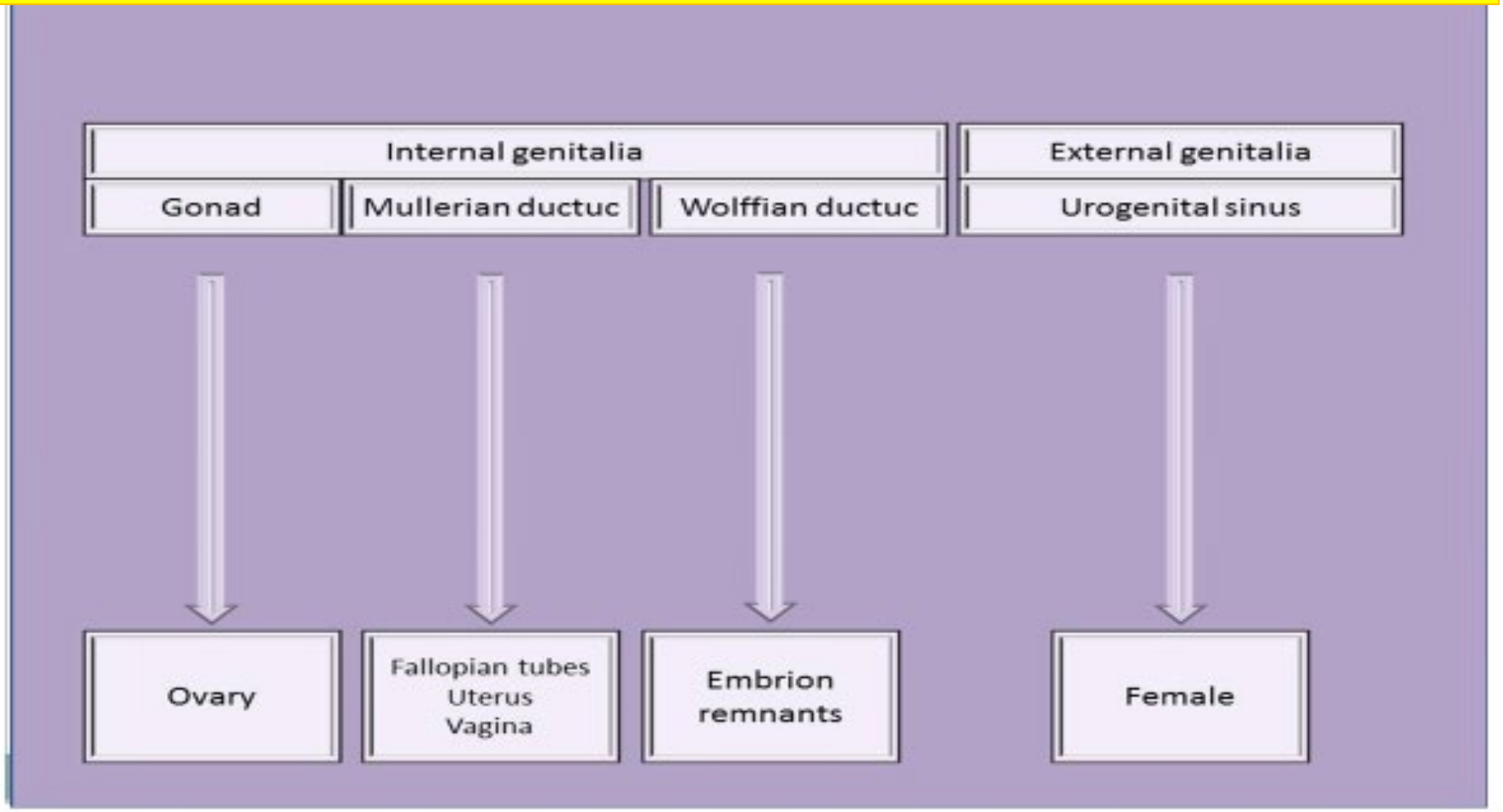
AND AFTER THE LONG INTRODUCTION AND MANDATORY CLASSIFICATION…

- The aim of the presentation is to draw attention to the fact that not all 46 XY individuals are always male, and…
- that in the 46 XX karyotype severe virilization and sexual dysphoria can be observed, as well as to highlight the important role of specialists in each individual case.
- Not less so in the writing laws.

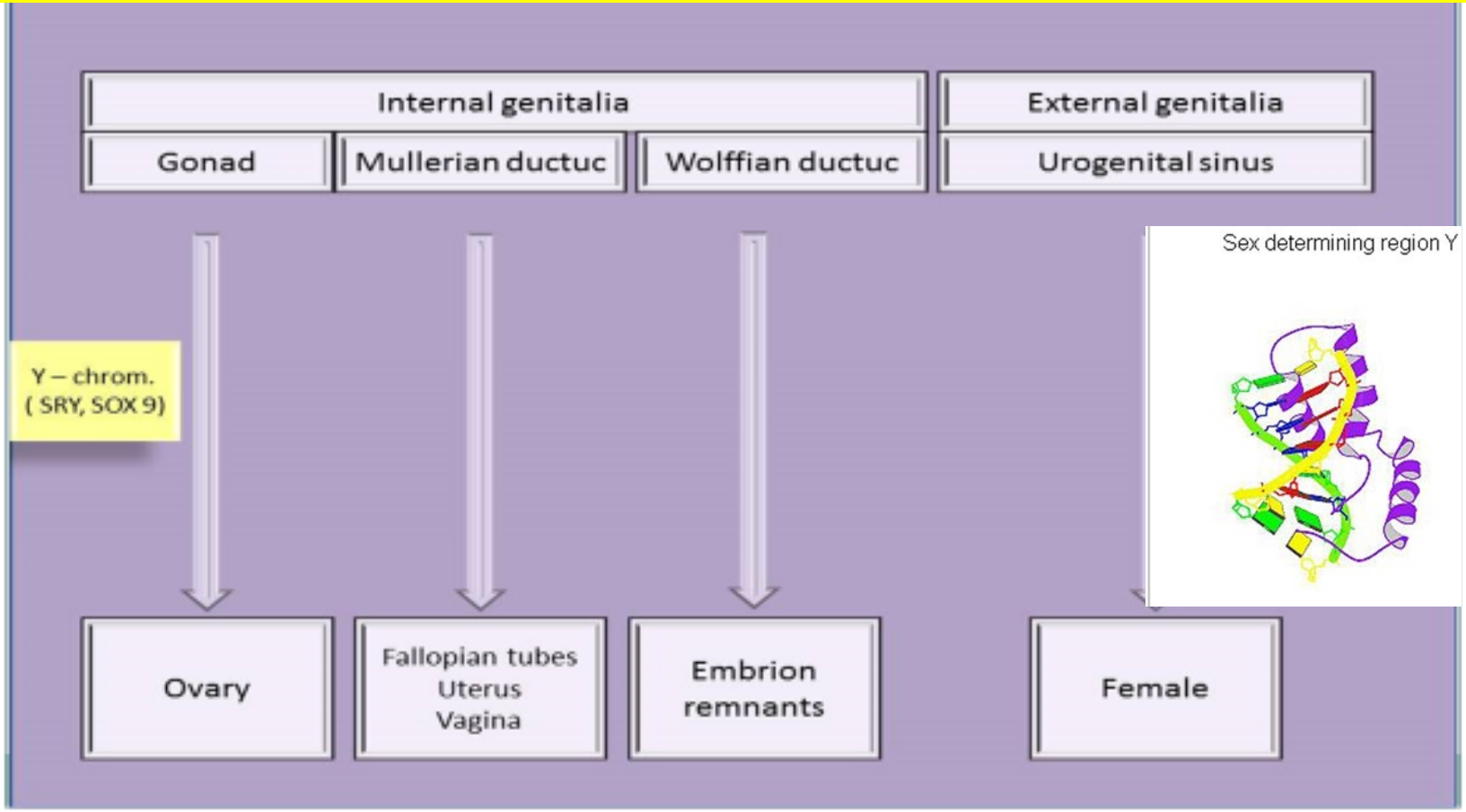
46 XY - IS IT SURELY MALE?

THE ANSWER IS NOT ALWAYS POSITIVE…

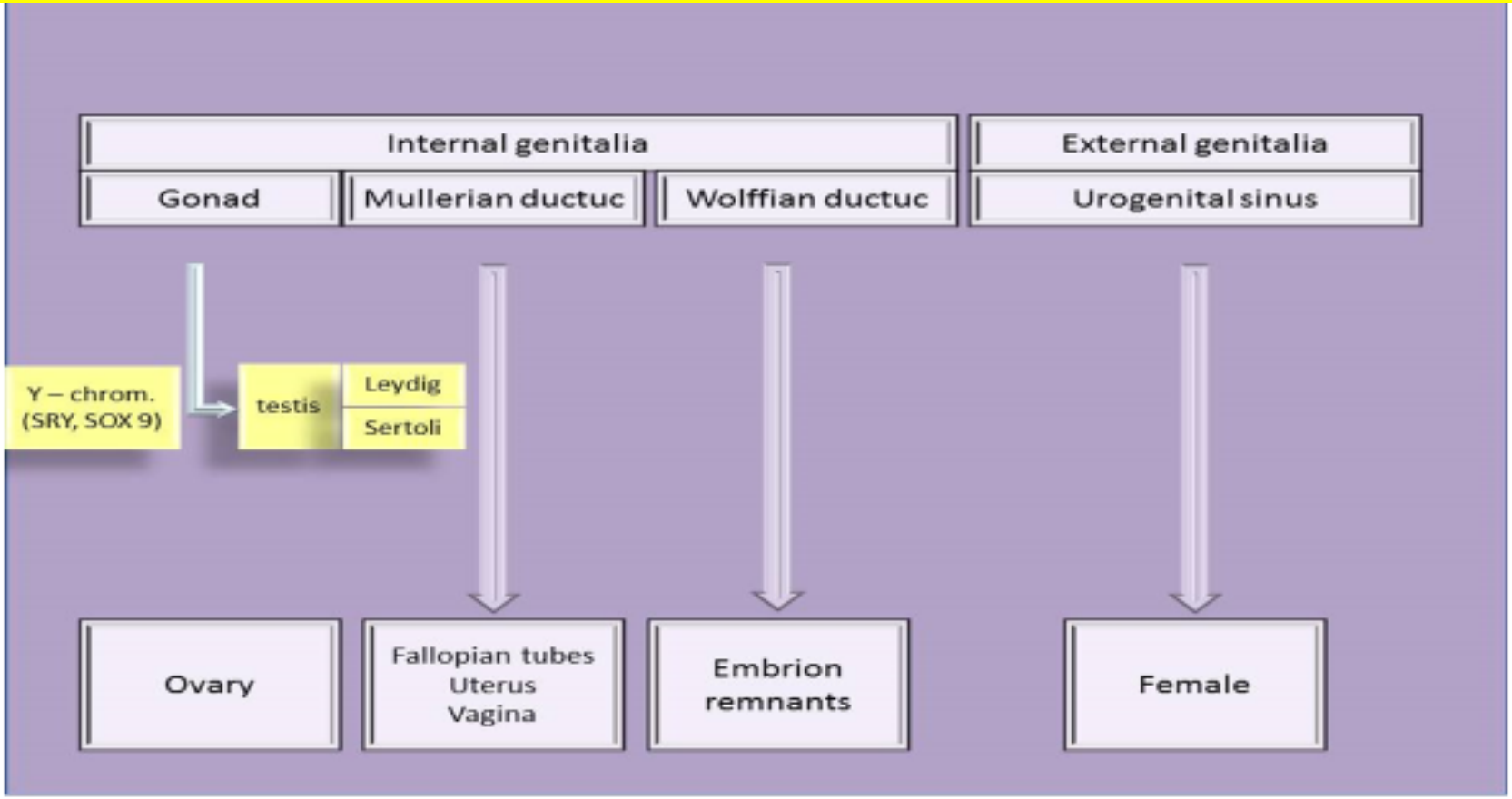
Turning an embryo into a woman is easy



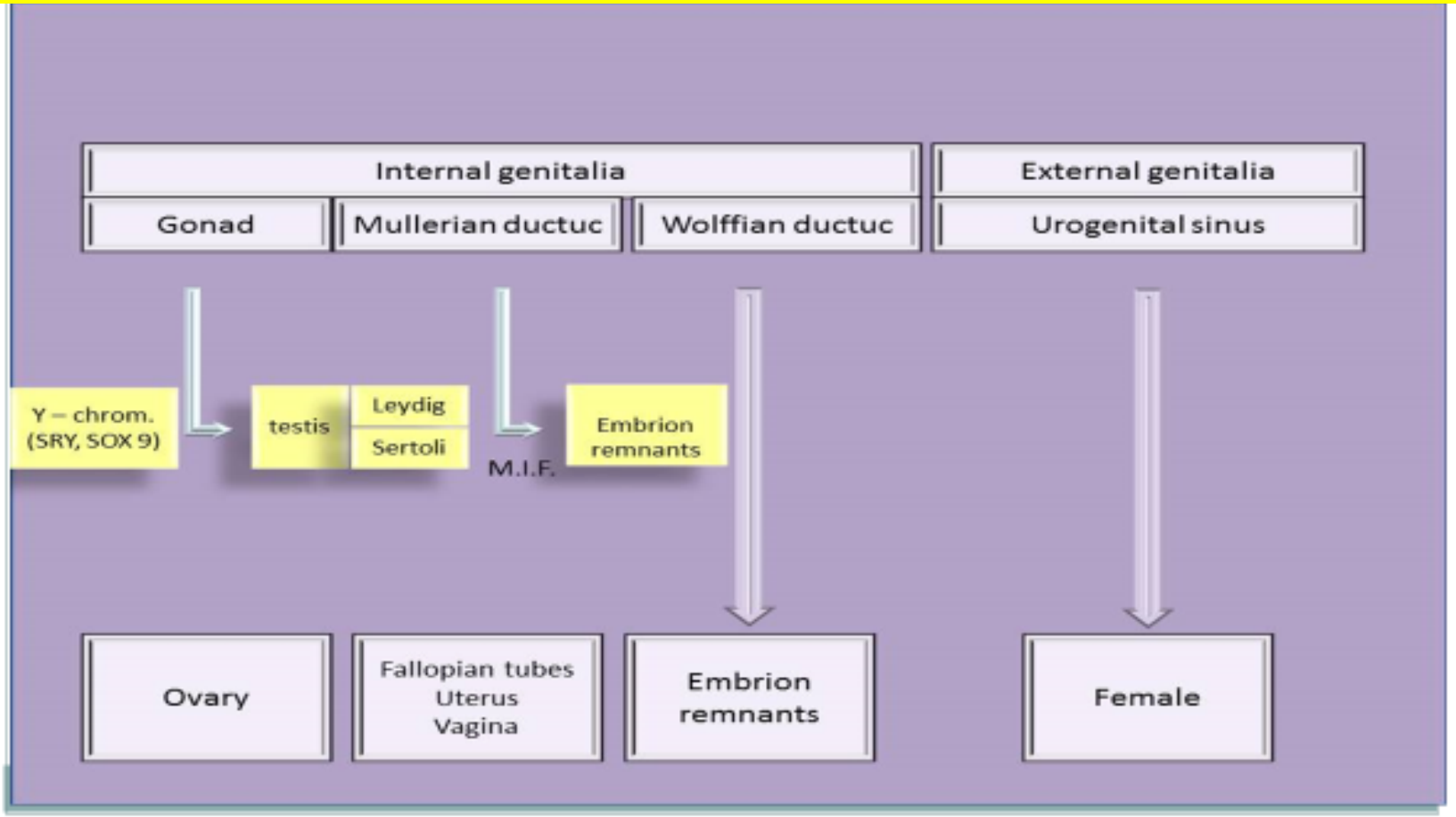
Becoming a man is far more difficult. With the hormones secreted by the testicles, the male fetus overcomes the natural tendency for passive development in a female direction



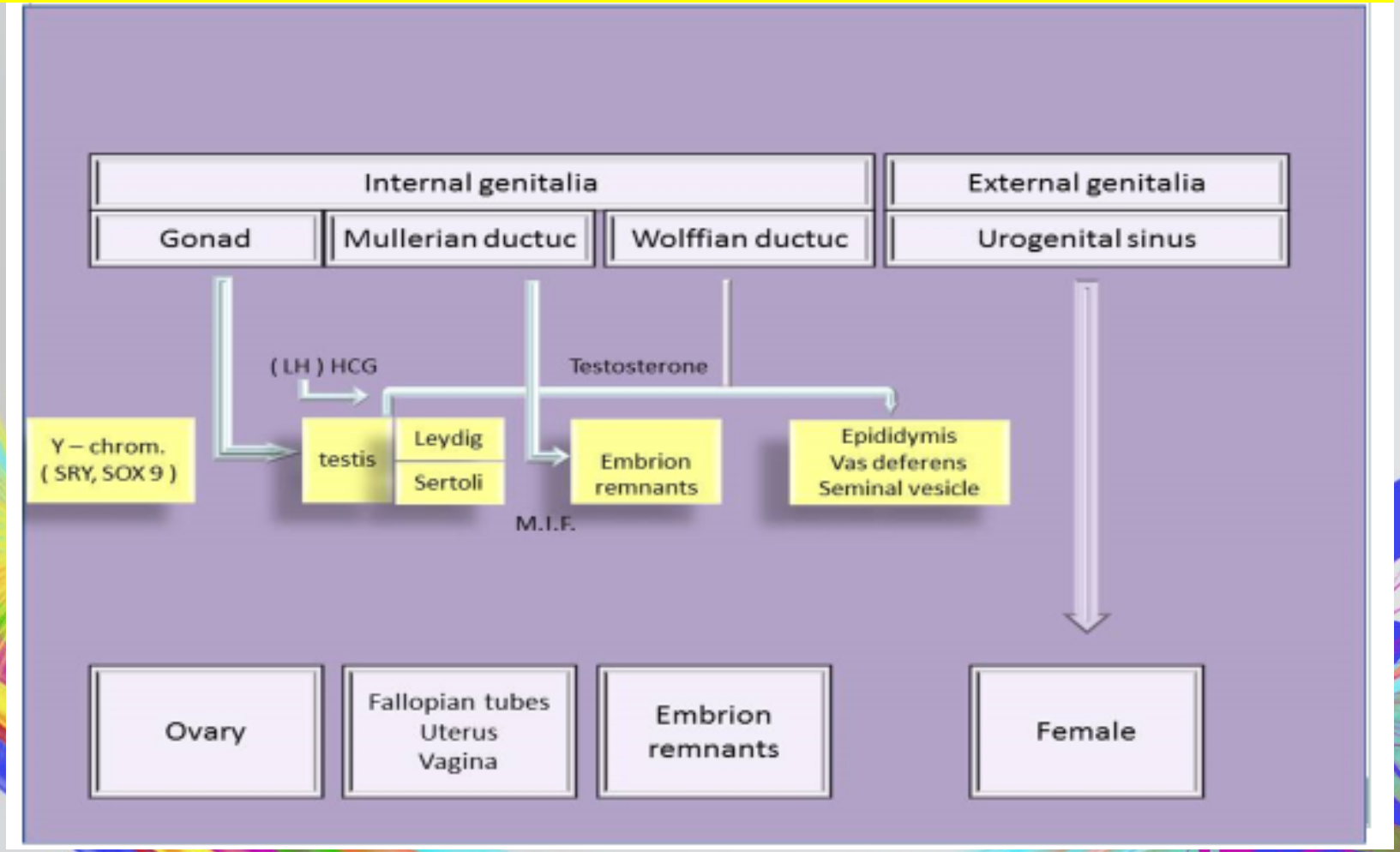
The primary undifferentiated gonad transforms into a testis with Sertoli and Leydig cells



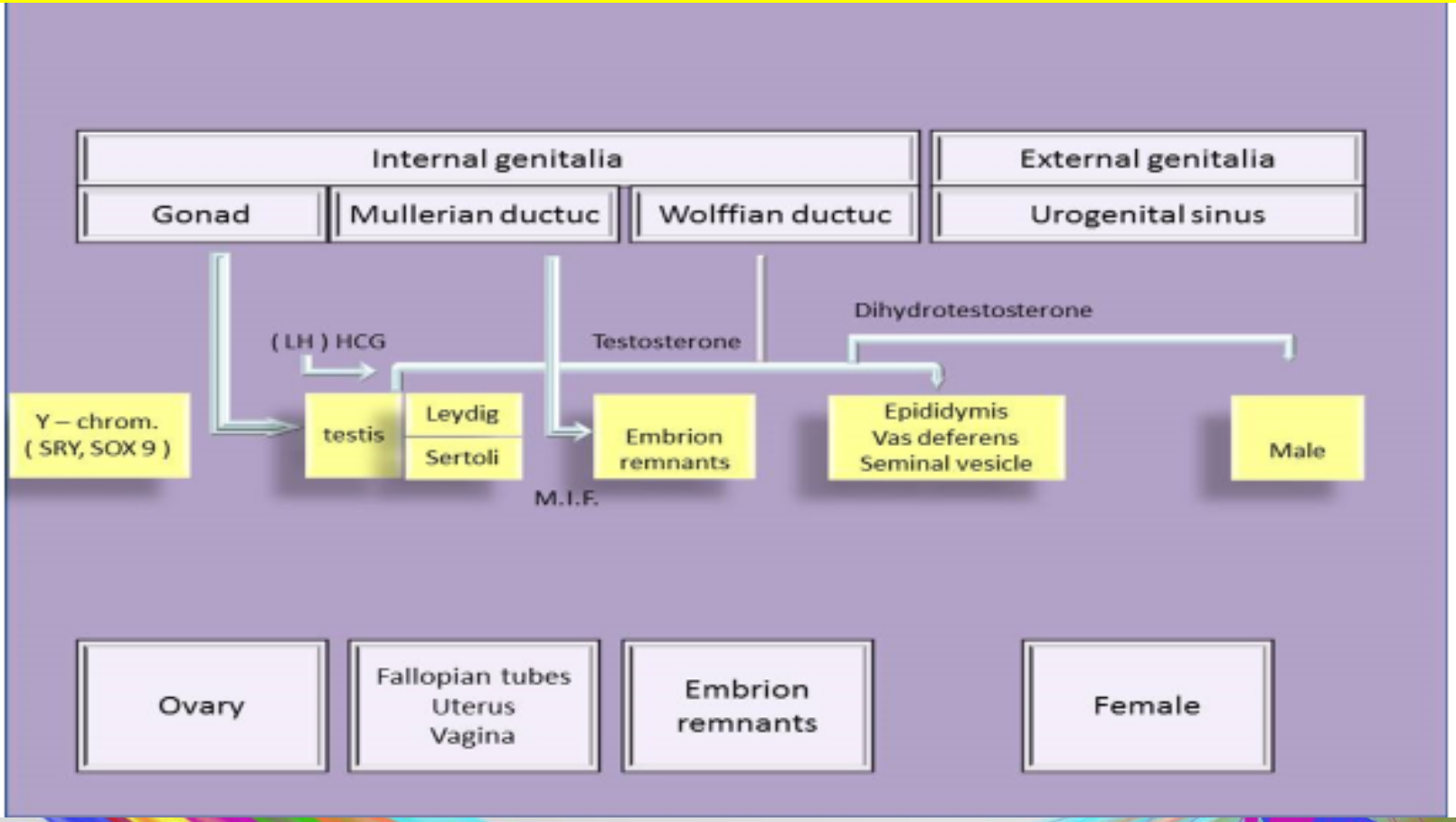
Sertoli cells produce MIF - a Müller inhibitory substance that causes regression of the Müllerian ducts to embryonic remnants.



At the same time, Leydig cells produce testosterone, transforming the Wolffian ducts to the epididymis, vas deferens, and seminal vesicles.



Finally, a man - part of the testosterone, thanks to the enzyme 5-alpha reductase, appearing later in embryonic life, turns into Dihydrotestosterone, important for the final formation of the male phenotype.



HYPERGONADOTROPIC HYPOGONADISM

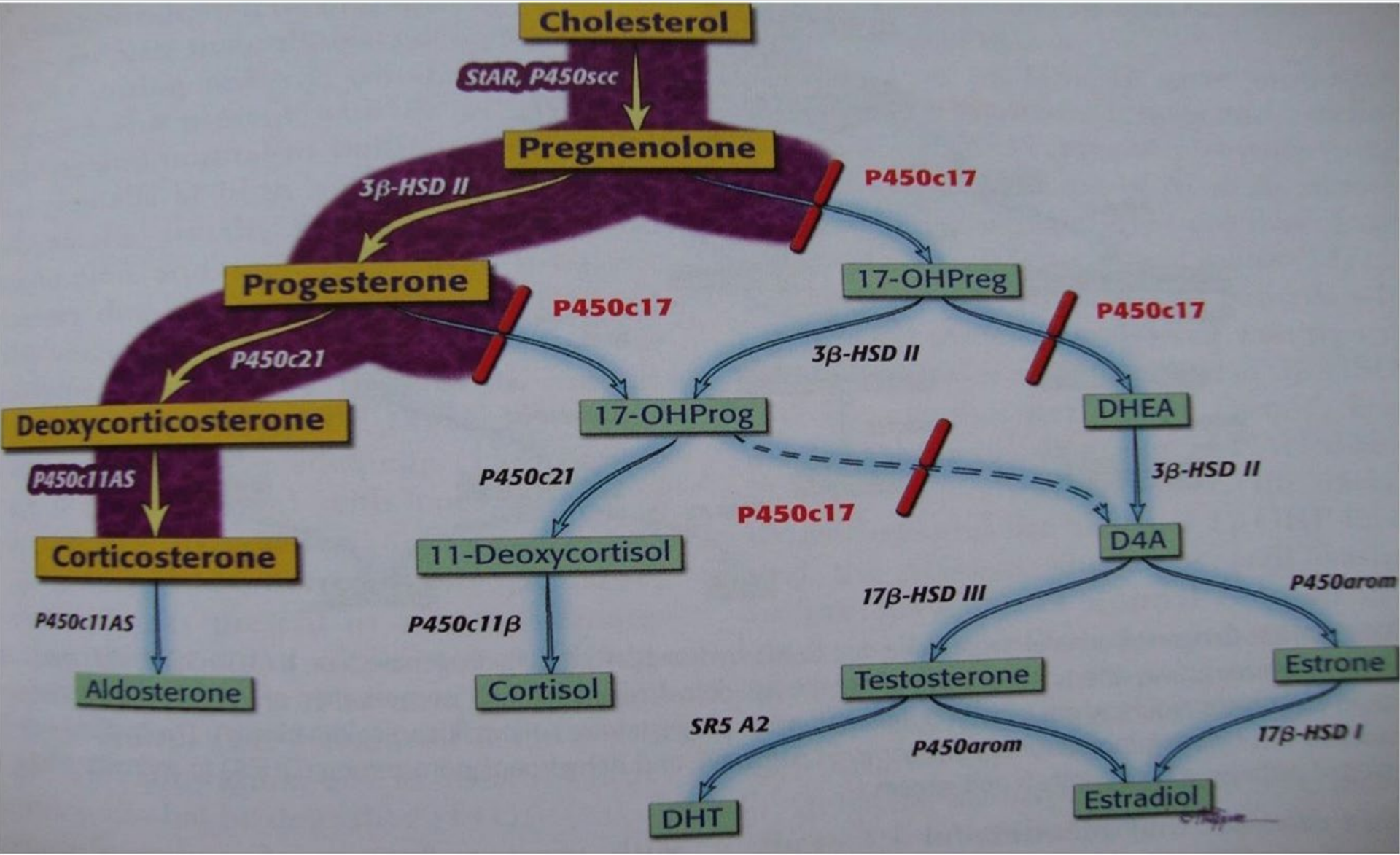
46 XX

- Girl, 14 years, 10 months.
- Normal anthropometric indicators
- Missing pubertal development and arterial hypertension
- Laboratory parameters – **hypokalemia** and uncompensated metabolic **alkalosis**: pH - 7.47, VE +4.6 SB - 28.7
- Hormonal values:Increased **ACTH - 129.1** pg/ml /7.9-66.1 pg/ml/.Gonadotropic hormones - **FSH - 60.47 IU/L, LH - 23.17** IU/LEstradiol - 75 pmol/l - low, pre-pubertal value - Testosterone - 0.19 nl/ml - infantile value for females. **Progesterone - 13.05 nmol/l** - a high value specific only to the luteal phase in menstruating women.17-OH-progesterone - 0.9 ng/ml. DHEA-S – 16 mkg/dl **Renin** - in supine position - 3.47 ng/ml/h /0.2-2.8/Highly elevated **Aldosterone** - 658.68 pg/ml /10-105/
- Significantly **delayed bone age**

46 XY

- Girl, 13 years 6 months.
- Reason for the consultation - missing pubertal development.
- Arterial hypertension registered for a first time
- First degree of obesity.
- Laboratory parameters **– hyperkalemia** and **decompensated metabolic alkalosis**
- Hormonal values: **ACTH – 121 pmol/l** /2-11/**FSH – 46.54 mIU/l /1.7-6.6/, LH – 24.97 IU/l** / 1.7-12.1/Estradiol – 56 pmol/l/44-918/Testosterone – 0 ng/ml **Progesterone – 23.51 nmol/l** /<0.1-4.0/DHEA-s 5.2 mg/dl /8-274/, Cortisol – 19.67 nmol/l /290-770/
- Pediatric gynecologist - presence of a hymenal ring and vestibulum vagina, labia minora and labia majora.
- Ultrasound - **uterus and adnexa are not visualized**.
- **Delayed bone age**
- Diagnosis for both children:**17-HYDROXYLASE DEFICIENCY**
- **The only difference is:**

MUTATION OF 17A-HYDROXYLASE/17,20 LYASE (CYP17A1)



PRIMARY AMENORRHOEA

A girl aged 16 years and 4 months

Past illnesses: operated on for inguinal hernia at 11 months.

Consulted with a gynecologist at the age of 15, due to primary amenorrhoea.

Ultrasound described a hypoplastic uterus and normal ovaries. Prescribed anticoncevtive drugs for one month. No bleeding was observed.

Mother's menarche-15 years old.

Outpatient tests-elevated LH and androgens. During an examination by a pediatric endocrinologist, a blood pressure of 150/90 mmHg was recorded

From the status - **growth higher than the target**, **thelarche - 4th degree**, **without adrenarche.Hypoplastic labia majora.**

BP 150/70 mm.

Laboratory tests:

Normal TBV and gas analysis

Normal biochemical tests

ACTH – 11,9 pg/ml

Cortisol at 8 a.m. – 391.22 nmol/l normal adrenal function

Elevated androgens:

DHEA-S – 844,8 µg/dL (50-286)

Androstendion – 5,45 ng/ml (1,0-2,6)

Testosteron – 6,07ng/mL (0,1-0,5)

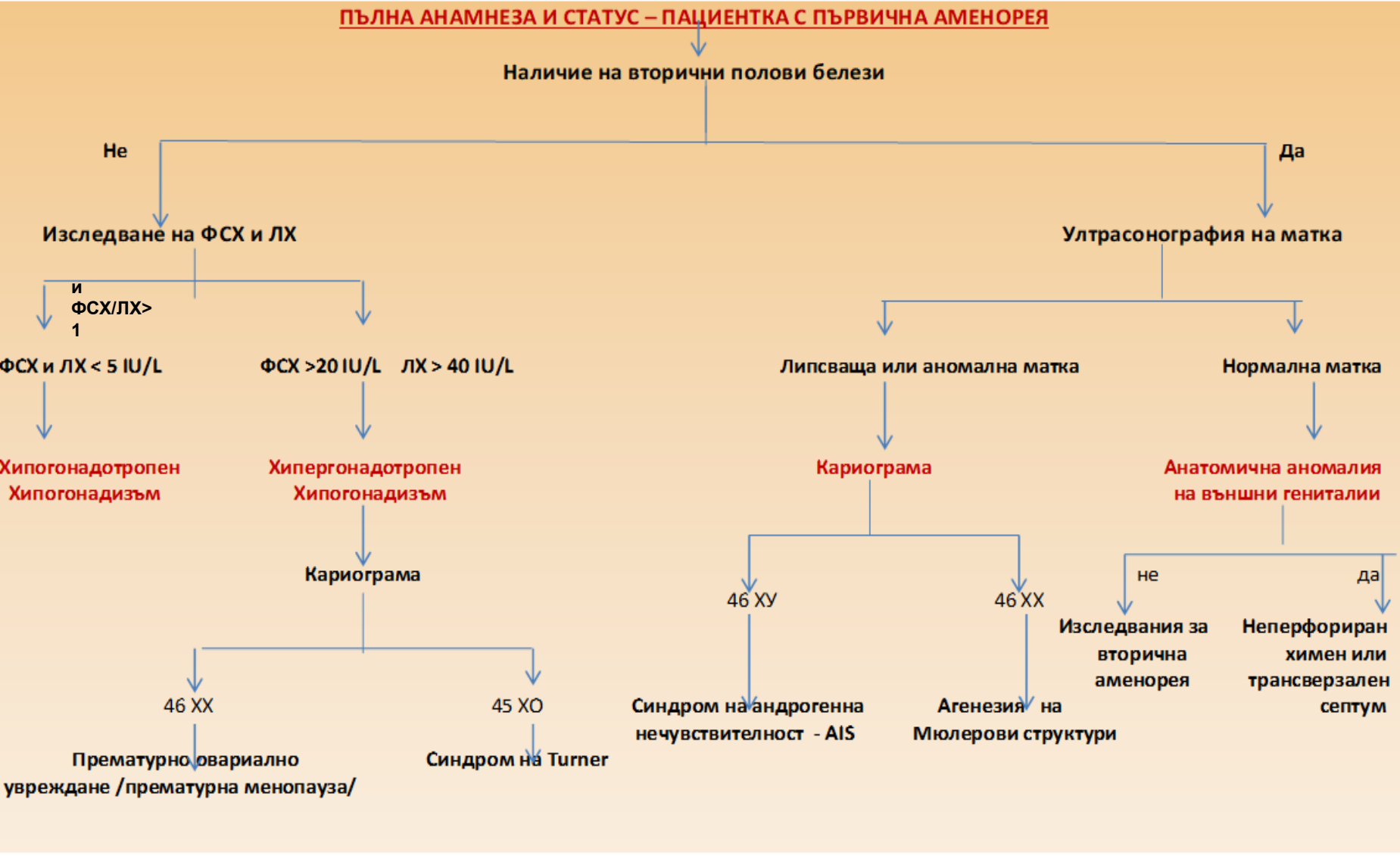
Estradiol – 82,5 pmol/L

LH – 19,51 mIU/mL

FSH – 4,86 mIU/mL.

Genetic test – kariogram: 46 XY

PRIMARY AMENORRHOEA



GENETIC DIAGNOSIS: COMPLEX ANDROGEN INSENSITIVITY SY

- All female external genitalia
- Gonads-normal testes in the abdominal cavity or inguinally.
- A uterus is absent or, in rare cases, a rudimentary embryo is found./normal AMH from Sertoli cells/
- The vagina is shortened and ends blind, and the size varies from 2.5 to 8 cm
- The prostate and other Wolffian structures are absent or rudimentary.
- Hormonal tests: normal/high T, increased LH, FSH normal.

BEHAVIOR:

- They are raised as girls and are perceived as such. There is no risk of gender dysphoria. Gonadectomy is possible at diagnosis and before puberty, but requires hormonal therapy to induce puberty.
- Delaying gonadectomy until after puberty may allow spontaneous puberty\
- After puberty - dilatation of the vagina
- Estrogen-only hormone replacement therapy after gonadectomy.

VIRILISATION OF EXTERNAL GENITALIA - CLITOROMEGALY

- Girl, 9 years, 6 months. She was examined for the first time by a pediatric gynaecologist at the age of 2 - **suspected synechiae** of the labia minora - rejected.
- At the age of 4, she was examined by a pediatric nephrologist because of urethral thickening - no ultrasound pathology was found.
- Newly appeared brownish staining of the underwear at the age of 7.5 years - examined by a pediatric gynaecologist , clitoral enlargement was described and referred for the first time to a pediatric endocrinologist.
- From the status:. External genitalia - clitoromegaly - 2 cm, no vaginal opening visible, well-formed labia majora, fully fused labia minora.
- **Cytogenetic examination - proven male karyotype - 46,XY.**
- MRI of the small pelvis - uterus and ovaries are not visualized. Testes are described bilaterally, located in the anterior abdominal wall, with precise sizes specific for prepubertal age described. A developed spermatic cord is not presented. Conclusion – MRI evidence of bilateral cryptorchidism.
- In the genetic research - DNA analysis - it is proven double heterozygosity for a **5 alpha reductase mutation.**

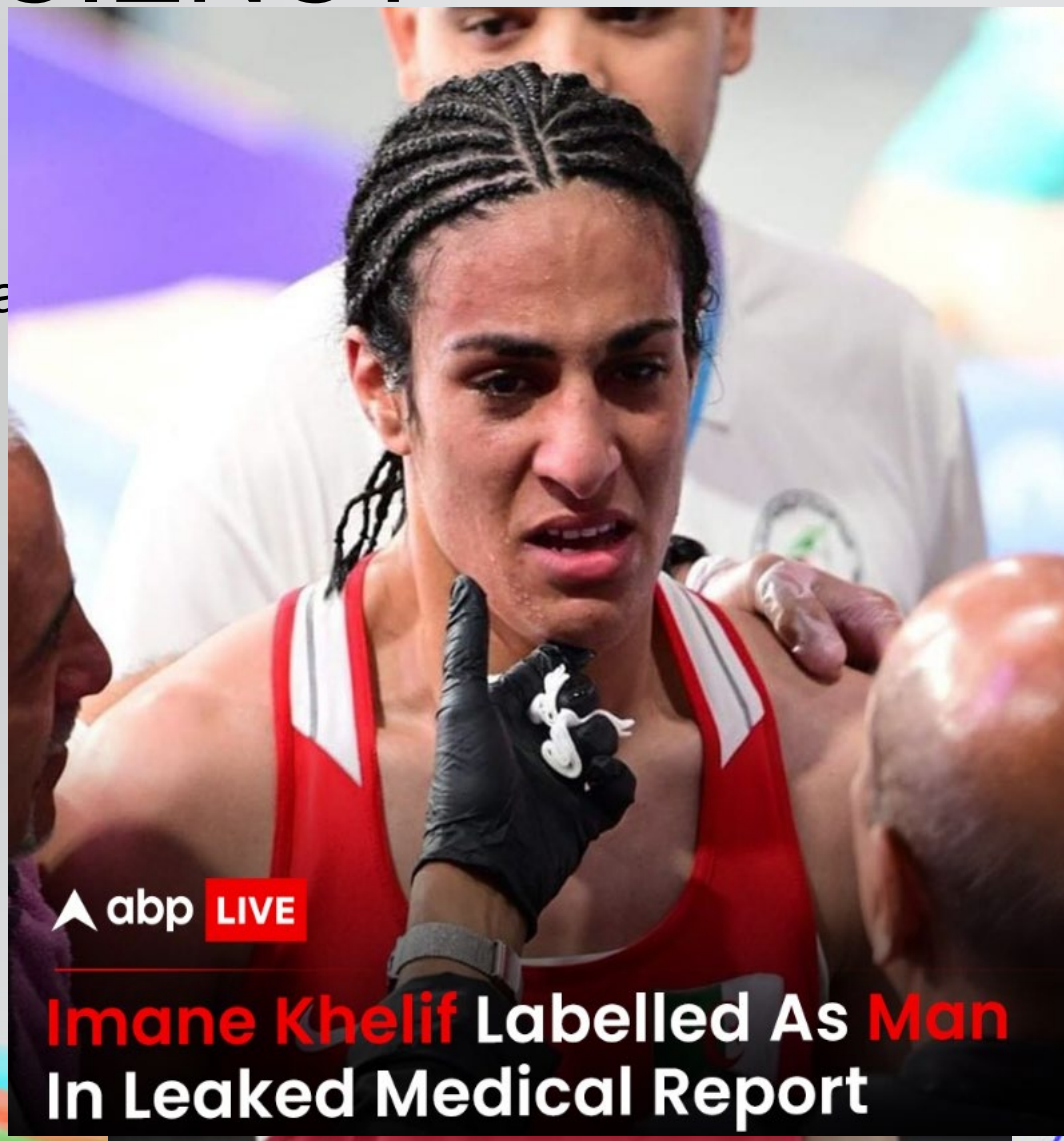
5-ALPHA REDUCTASE DEFICIENCY

- Deficiency of 5-alpha reductase results in a lack of masculinization of dihydrotestosterone-dependent structures—in utero, at birth, and in the years following puberty, despite a male karyotype.
- This condition also leads to a deficiency of the end product of testosterone metabolism - dihydrotestosterone and is one of the forms of DSD / Differences of Sex Development/, in which at birth and in the following years due to a female phenotype of the external genitalia, the sex is usually defined as female .
- DHT plays a key role in the process of sexual differentiation of the external genitalia and prostate during the development of the male fetus. 5-alpha reductase type 2 deficiency /5αR2D/ results from impaired 5αR2 activity, resulting in decreased DHT levels
- This defect results in a spectrum of phenotypes, including lack of masculinization of the external genitalia, hypospadias, and a micropenis, often mistaken for a clitoris—of normal or enlarged size.
- During pubertal development, however, the role of testosterone becomes dominant in terms of general male habitus, gender mental identity, and behavior.

5-ALPHA REDUCTASE DEFICIENCY

Impaired conversion testosterone→DHT.

- Incomplete vitilization and differentiated of external genitalia
- Autosomal recessive.
- Clinical: sexal ambiguity:
- karyotyping:46XY
- range from simple hypospadia→blind vaginal pouch:clittoris-like phallus.
- In puberty
  - Boy undergo to vitilization.
  - Normal girl→fertilization.



PROBLEMS WITH DIAGNOSIS

- Until puberty, children with 5-alpha reductase deficiency - in the absence of a genetic diagnosis - develop and grow up as girls.
- If not diagnosed until puberty, increasing levels of testosterone cause sexual dysphoria - physical and mental masculinization, corresponding to genetic sex.
- Even highly specialized pediatric surgeons refuse to perform an orchidectomy appropriate before puberty when the family decides that the child should remain a girl and receive hormone replacement therapy with estrogens.
- Despite the expected sexual dysphoria and a proven male karyotype, changing sex before the age of 18 is prohibited according to the laws of Bulgaria.

46 XY karyotype with uterus

- A young woman of 18 years and 6 months of age, known at Children's Clinic Plovdiv since the first hours after birth. In connection with intersex external genitalia, which made it difficult to determine the sex, she was hospitalized on the 8th hour after birth. Initial diagnosis - simple virilizing form of CAH /congenital adrenal hyperplasia/ due to elevated values of 17-OH-Progesterone. In support of this diagnosis was the uterus palpated by the pediatric gynecologist.
- **But... Cytogenetic study: 46 XY**
- In the first two years, it was followed up in Sofia - additional genetic and hormonal studies were carried out in the direction of male pseudohermaphroditism.
- At that time, several operations were performed for hypospadias and elongation of a hypoplastic penis. Upon histological examination of the hypoplastic testicles - immature testicular parenchyma, as well as the presence of female internal genital organs - uterus - a feminizing reconstruction of the external genital organs was started - September 2007.
- **Changing the civil gender of the child to female - at the age of 2**

46 XY karyotype with uterus

- At the age of 11, a bilateral gonadectomy was performed for histologically proven hypoplastic testes, but with gonadoblastomas bilaterally.
- Performed an MRI of the small pelvis - with a description of the presence of a uterus.
- Grows and develops normally emotionally and intellectually with the self-awareness of a girl.
- After 12 years of age – started replacement hormone therapy - Climara - with a gradual increase in the dose, and subsequently Trisequens. Currently, the dose of the latter is 2 tablets/day. She has regular menstrual cycles.
- Normal general status. Height 176 cm, weight 63 kg. Glandular epithelium is palpated in slightly spaced mammary glands. When examining external genitalia by a pediatric-adolescent gynecologist - female type. During their additional examination - in the gynecological position - he urinates from a single opening located above the anal ring.
- Hormonal tests – significantly increased gonadotropic hormones.
- Surgical correction of a hypoplastic vagina, as well as additional genetic tests, are pending.

- Probable diagnosis: **XY persistent structures of the Mullerian duct**

ARE 46XX ALWAYS WOMEN?

- It's easy to say „YES“
- There is a complex and rare variant – 46 XX gonadal dysgenesis due to an Xp:Yp translocation containing the SRY segment.
- BUT THERE IS ALSO A MORE COMMON PATHOLOGY, SOMETIMES MISSED
- Girl, 15 years, 7 months. age with a target height above the 50th percentile. Child of chronically ill and meanwhile deceased parents - from non-endocrine pathology. Menarche at 11.5 years of age - irregular cycles.
- Reason for consultation with a pediatric endocrinologist - growth retardation.
- **The girl has sexual dysphoria - she introduces herself with a male name and asks to be addressed in the masculine gender.**
- **From the status -** height 152 cm - at the 3rd percentile, weight 47 kg. Android habitus. Complete pubertal development with poor turgor of the breast epithelium, pronounced hirsutism and acne. No viewing of external genitalia was permitted.
- Laboratory tests – normal Sodium and Potasium.
- **Hormonal tests** - **TSH - 4.75 µIU/ml** /0.53-3.59/ with normal FT4 - 19.20 pmol/L and MAT - 14.5 IU/ml. **Elevated prolactin - 1189 µIU/ml /44.70-391.50/.**FSH – 5.25 mIU/ml, LH – 4.48 mIU/ml.Elevated values of **Testosterone – 2.08 µmol/L**, **DHEA-S – 17.90 µmol/L 17-OH-Progesterone – 3.60 ng/ml**
- **DIAGNOSIS: SIMPLE VIRILIZING FORM OF CONGENITAL ADRENAL HYPERPLASIA – UNDIAGNOSED AND UNTREATED**

CONCLUSION

Disorders of sex development are variations in reproductive tract development. Novel genetic techniques have introduced a new era of the diagnosis of DSDs and elucidation of the molecular factors involved in sex development. Thoughtful respectful care is critical for the management of infants, children, adolescents, and their families to ensure positive and meaningful quality of life.

Goals for individuals with DSDs include psychosocial well being, sexual satisfaction, and fertility options

Correct diagnosis and following - therapeutic behavior represents a multidisciplinary problem in which the role of the pediatric endocrinologist is leading

Knowledge of these problems by a wider range of specialists is imperative - for the correct and timely diagnosis, therapeutic approach and psychological help.

Bulgaria lacks a register of intersex conditions, as well as an expert center for their diagnosis and treatment.

Is it not our fault - the children's endocrinologists' fault, that incompetent politicians are trying to create laws?

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