CONVEGNO MACROREGIONALE

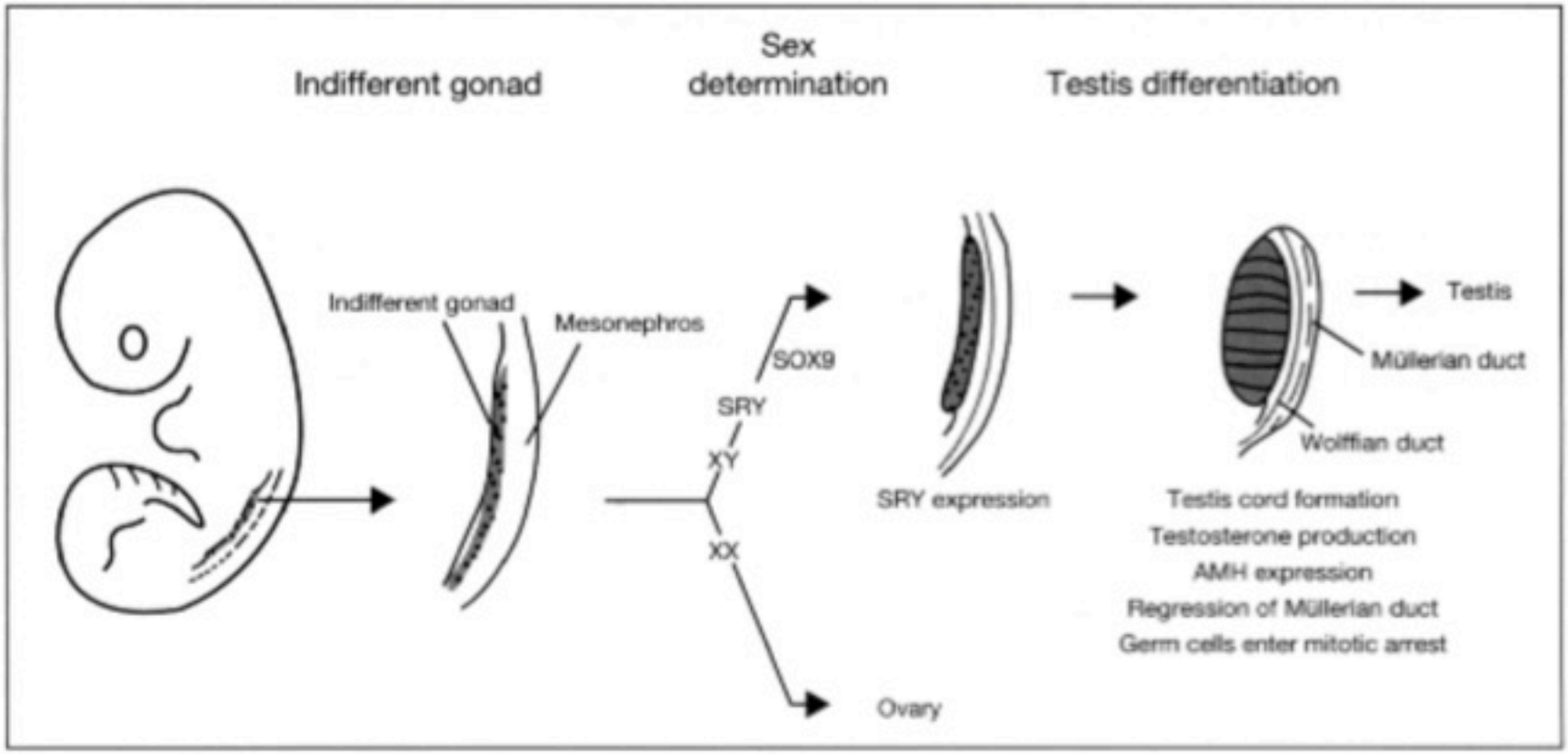
AME DAY

20/21
MAGGIO 2016

"Il testosterone dal grembo materno all' adolescenza"

Piernicola Garofalo

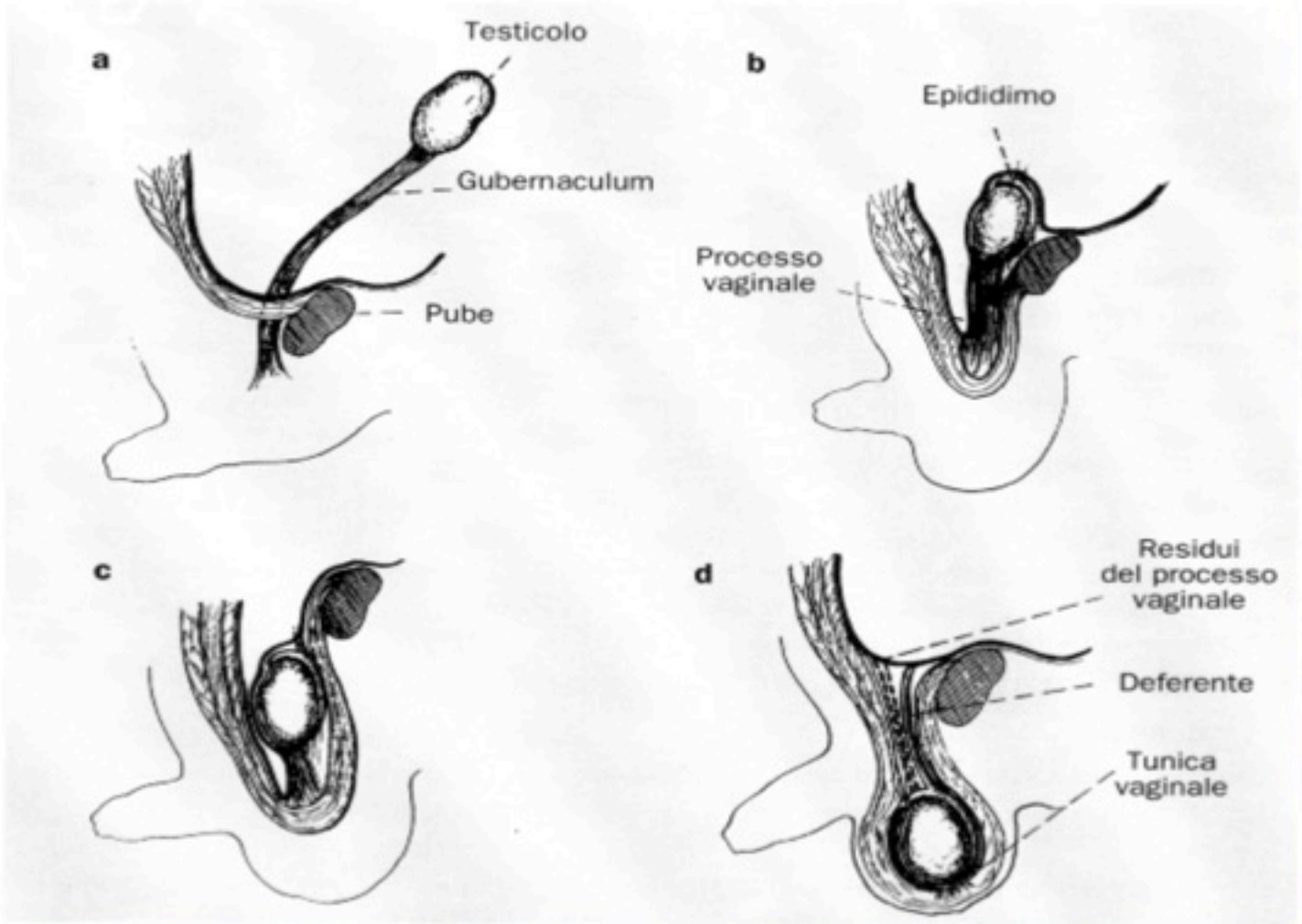
CONVEGNO MACRO REGIONALE AME-SUD | Catania


Indifferent gonad
Sex determination
Testis differentiation
Indifferent gonad
Mesonephros
SOX9
SRY
XY
XX
Ovary
SRY expression
Testis
Müllerian duct
Wolffian duct
Testis cord formation
Testosterone production
AMH expression
Regression of Müllerian duct
Germ cells enter mitotic arrest

Genes and proteins involved in primary sex determination

Gene abbreviated name	Full name	Protein	Function
SRY	Sex determining region of the X chromosome	SRY protein	Male-specific factor; mutations in SRY→XY female
HMGbox	High mobility group box		The DNA-binding domain of a number of trancscription factors including SRY and SOX proteins
SOX	SRY-like HMG box	SOX protein	A family of genes with omology to SRY which are involved in sex determination. Mutation in SOX 9 → XY female; associated with captomelic dysplasia.
DAX-1	Dosage sensitive sex reversal , adrenal hypoplasia congenita on the X chromosome , gene 1	47' aminoacid protein	Involved in gonadothropin, adrenal and gonadal development. mutations → adrenal hypoplasia and gonadothropin deficiency. Duplication →XY rversal.
WT-1	Wilm' stumor 1	WT-1 protein	? Involved early in sex determination. Mutation in 46, XY gonadal dysgenesis and urogenital malformations.
SF-1	Steroidogenic factor 1	461 aminoacid protein	Regulates genes involved in sex determination and differentiation, and steroidogenesis

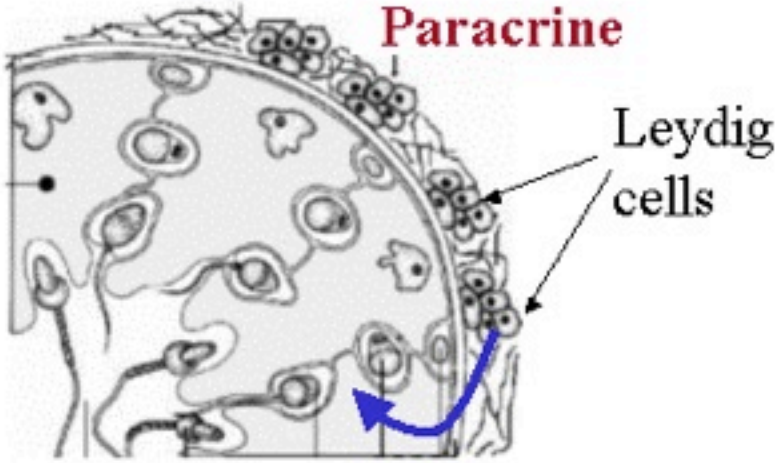
Discesa del testicolo (a. 5ª settimana; b. 3ª-5ª mese; c. 7ª mese; d. 8ª mese).



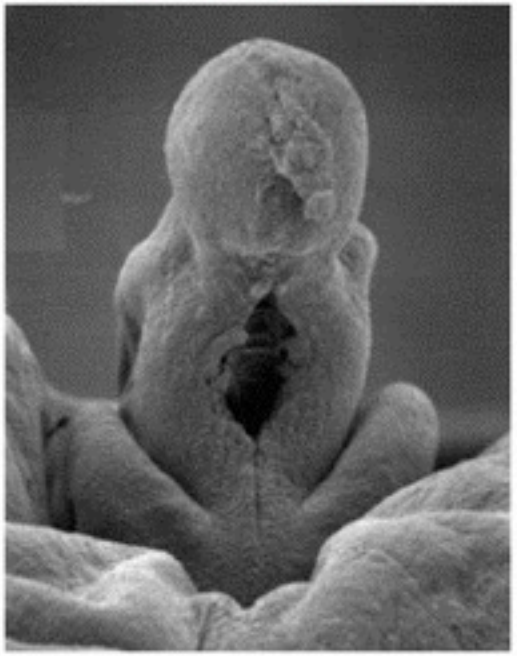
Differential androgen action

Testosteron	⇨ Virilisation of Wolffian ducts ⇨ Regulation of gonadotrophins ⇨ Spermatogenesis ⇨ Masculinisation
DHT	⇨ External Virilisation

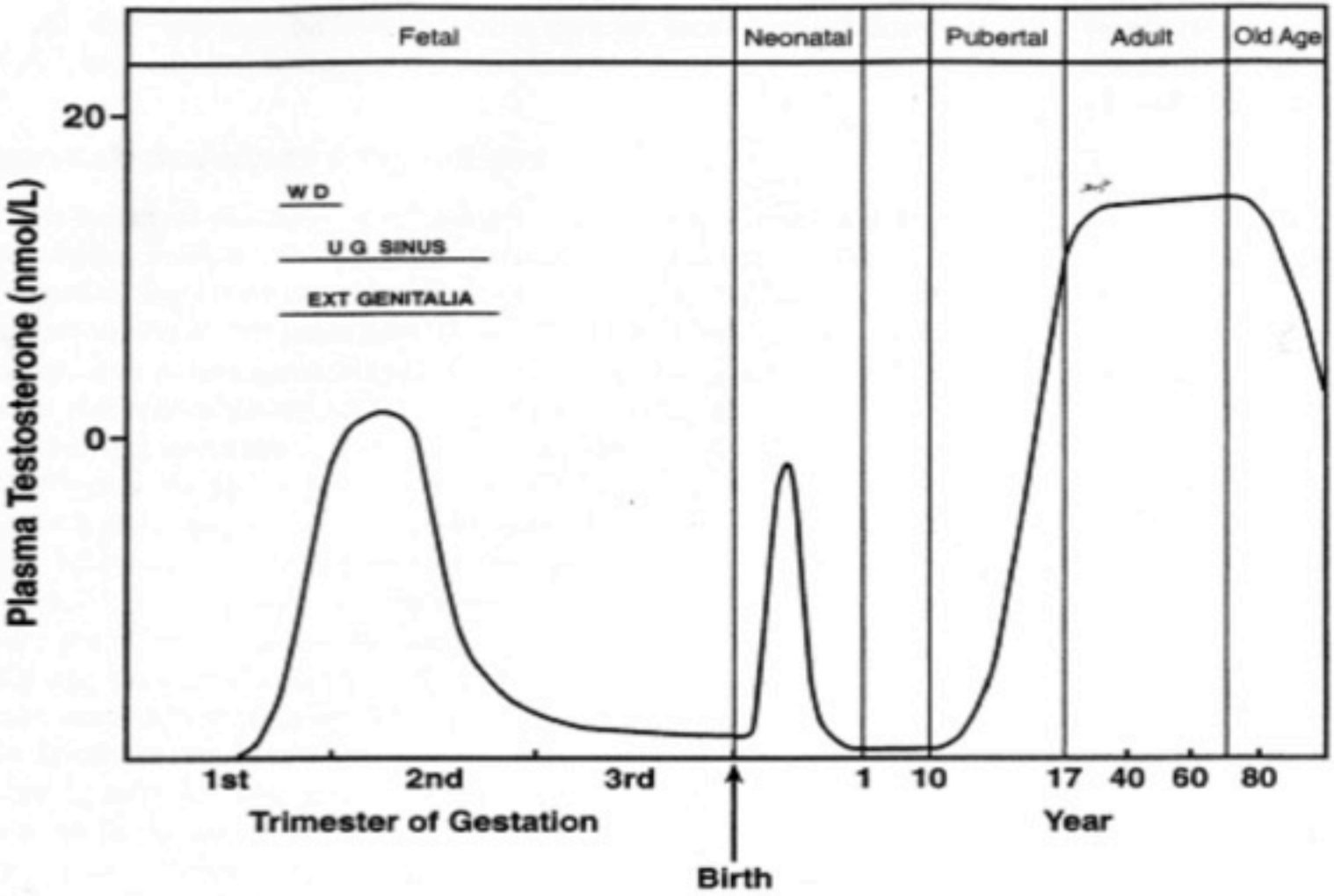
Paracrine



Endocrine

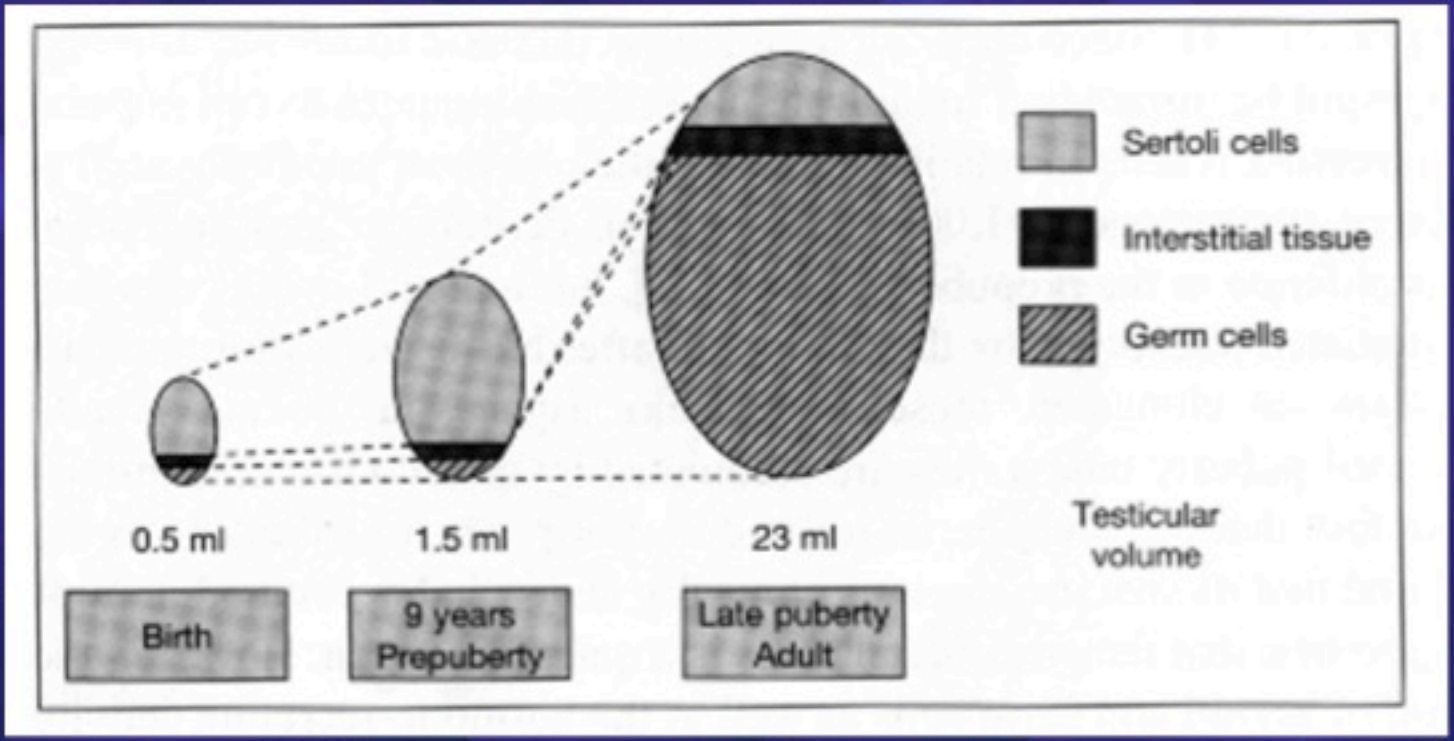


9. Week, male



Phases of testosterone secretion. Kaefer M.

Volume increase of testis in postnatal life



Valori di testosterone nel maschio in relazione allo stadio di Tanner

STADIO 1	< 30 ng/dl
STADIO 2	30- 95 ng/dl
STADIO 3	70 – 210ng/dl
STADIO 4	180 - 550 ng/dl
STADIO 5	> 400 - ng/dl

P. Garofalo

TESTOSTERONEMIA A BASSI DOSAGGI: SOLO SPETTROMETRIA DI MASSA?

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COMMENTO E CONCLUSIONI

I risultati espressi in forma grafica evidenziano come i tre metodi automatizzati, pur in presenza di una sovrastima rispetto al metodo di riferimento, non si discostino in maniera statisticamente significativa dai risultati ottenuti con il metodo di riferimento, mentre il metodo RIA sembra essere quello con peggiori prestazioni sia in termini di accuratezza che di precisione.

Metodo	Range misura (nmol/L)	Coeff. corr.
LC-MS/MS	0.38 – 3.18	/
ICMA	0.35 – 4.65	da 0.829 a 0.943
RIA	0.69 – 5.17	0.705

LOW TESTOSTERONE CONCENTRATIONS: ONLY MASS SPECTROMETRY?

AACC
ANNUAL MEETING
A-161

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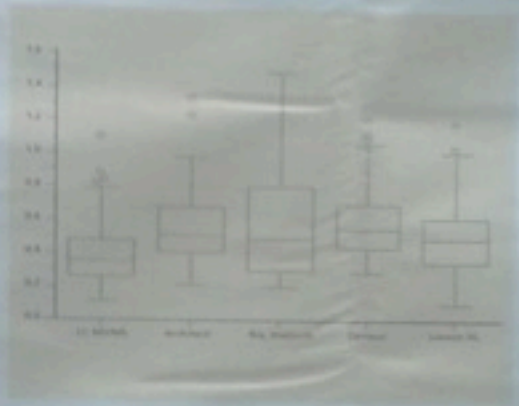
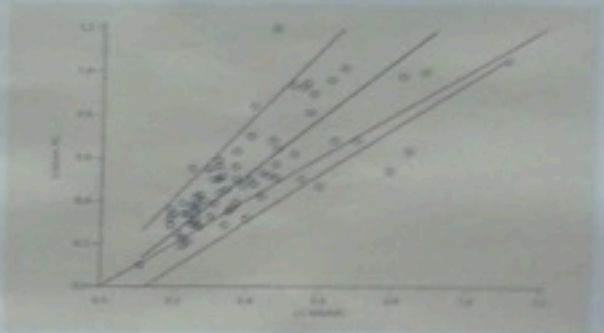
BACKGROUND

Total testosterone level measurement is the most requested one among steroid hormones assays. Unfortunately, the diagnostic accuracy at low concentrations of the most common immunoassays proved to be insufficient. In 2007 the Endocrine Society recommended the determinations of testosterone in children and in women has to be done only with one reference method (extraction, chromatography and determination by mass spectrometry). Due the method related difficulties in most of the laboratories the testosterone determinations are still done by immunoassays.



SAMPLES AND METHODS

We measured testosterone with three different fully automated immunoassays present in most of the clinical labs and repeated the determinations both with a commercial RIA and LC-MS/MS method. The latter one, considered the reference method, has been done in the Perkin Elmer labs (Turku, Finland), with updated equipment, by skilled personnel and determinations carried out in replicated. The serum samples were collected from 70 patients, male and female in pediatric age. The obtained concentrations by LC-MS/MS, considered as reference, ranged from 11 to 110 ng/dL.



RESULTS

The distribution of the concentrations obtained with the methods used should be noted that, although the averages and medians of the concentrations obtained with the LC-MS/MS method are less, the differences are not such as to distort the clinical information can be obtained: the 3 automated methods show values ranging from 10 to 134 ng/dL with correlations coefficients respectively to LC-MS/MS ranging from 0,829 to 0,934, whereas the RIA method shows a higher concentration's dispersal, values ranging from 20 to 149 ng/dL and a worse correlation to the reference method. (r=0,705).

	N	Mean	95% CI	Variance	SD	RSD	SEM	Median	95% CI	Min	Max	2.5 - 97.5 P	Normal Distr
LC-MS/MS	70	0,401	0,36 - 0,45	0,036	0,189	0,423	0,023	0,355	0,31 - 0,41	0,11	1,1	0,190 - 0,870	<0,0001
Architect	70	0,558	0,51 - 0,61	0,046	0,213	0,382	0,026	0,51	0,48 - 0,55	0,21	1,34	0,283 - 1,167	<0,0001
RIA DiaSorin	70	0,583	0,50 - 0,67	0,122	0,349	0,599	0,042	0,49	0,41 - 0,57	0,2	1,49	0,240 - 1,349	0,0001

Influences of androgens on phenotype, gender identity and gender role

Gonosomes
Sex-related genes

Androgens
and
CNS

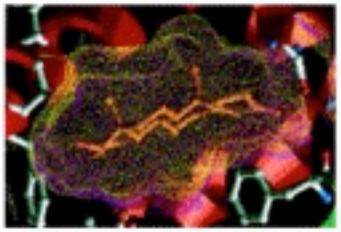
Social influence
Culture

Sex organs

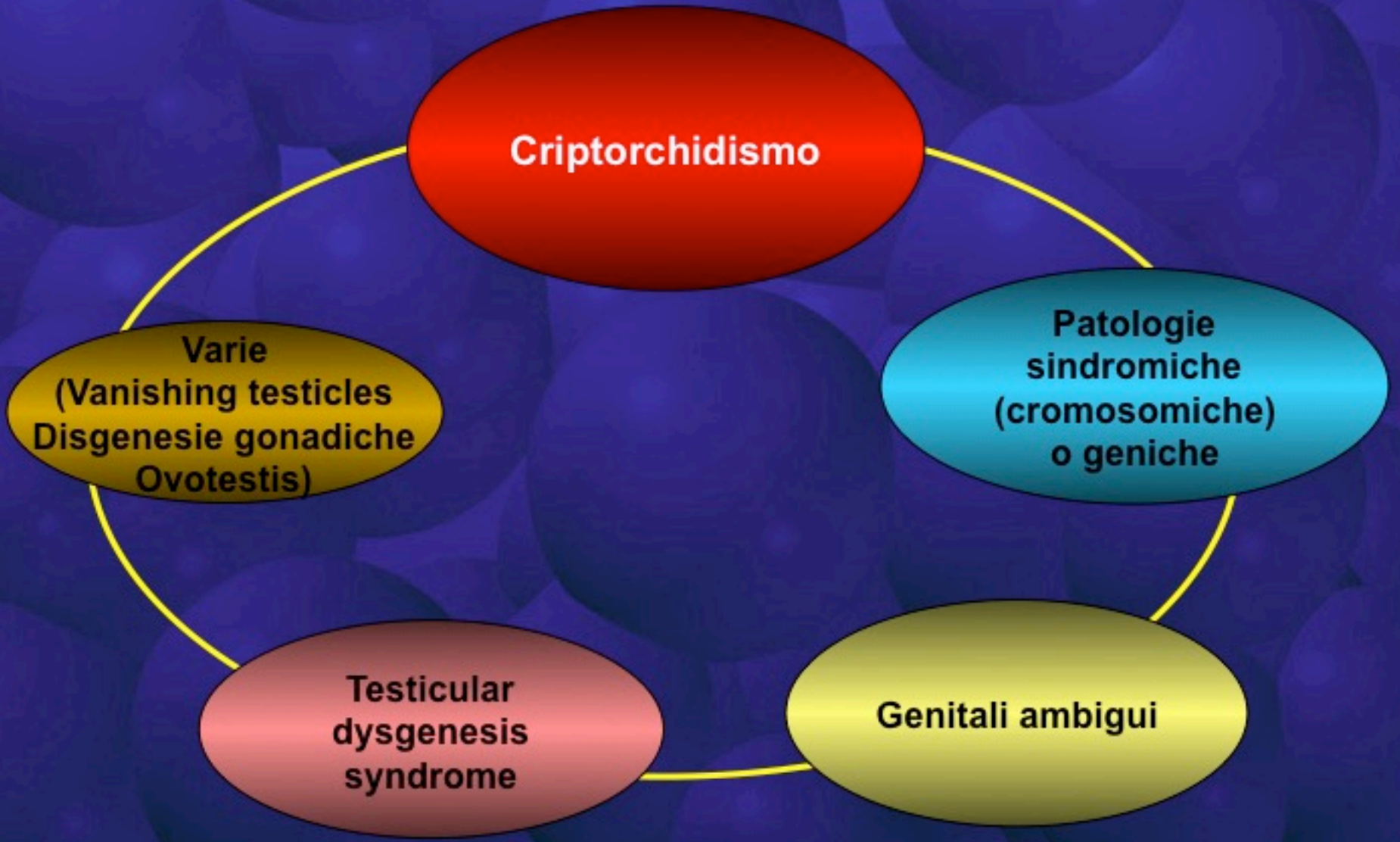
Phenotype

Peripheral
androgen
action

Gender identity
and
Gender role



Androgeni: fenotipi clinici



Prevalenza del criptorchidismo nel primo anno di età

	Peso neonatale (gr)	Scorer C.G: 1964 casi 3612	Berkowits G.S. 1993(casi 6935)
Alla nascita			
	< 2500	21,1	19,8
	2500	2,7	2,2
	Totale	4,3	3,7
A 3 mesi	< 2500	1,7	2,0
	➢2500	0,7	0,9
	Totale	0,8	1,1
A 1 anno			
	< 2500	1,7	1,9
	➢2500	0,9	0,9
	Totale	1,0	1,0

Principali sindromi che presentano criptorchidismo

Aarskog

Carpenter

Dubowitz

Criptoftalmo

Laurence-Moon-Biedl-Bardet

Lowe

Leopard

Noonan

Opitz

Prader-Labhart-Willi

Roberts

Rubinstein-Teybi

Seckel

Smith-Lemli-Opitz

Trisomia 13

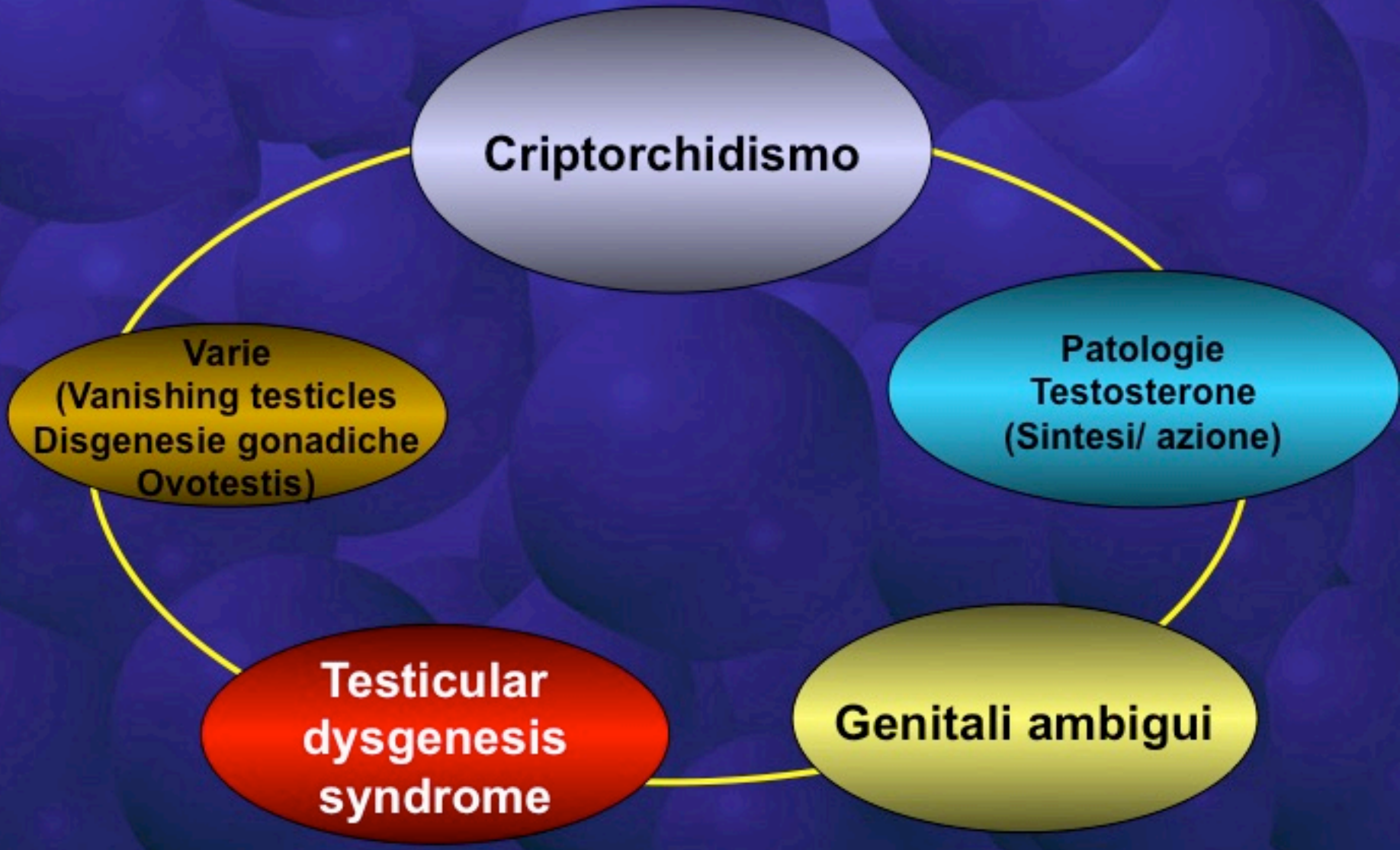
Trisomia 18

XXXXY

Criptorchidismo

- Monolaterale (~ 60% dei casi) quindi non “vere borse scrotali vuote “.
- Cercare ectopie.
- Escludere i casi di testicoli retrattili (c.d. “in ascensore” o migranti).
- Cercare SRY o altri indicatori malformativi con tecniche di genetica molecolare (gonade maschile con c.di Sertoli).
- Attenzione ai pre-termine ed ai LBW: vanno attentamente rivalutati nel tempo.

Androgeni: fenotipi clinici



TESTICULAR DYSGENESIS SYNDROME

Fattori ambientali "endocrine disruptors" che agiscono in utero in una fase embriogeneticamente precoce (PCBs, pesticidi, ftalati, fitoestrogeni, estrogeni di sintesi) legandosi ai recettori per gli estrogeni, androgeni ed ormoni tiroidei.

Anomala funzionalità delle c. del Sertoli

Alterata differenziazione delle c. germinali

OLIGOSPERMIA

TUMORI TESTICOLARI

Androgeni insufficienti

IPOSPADIA

CRIPTOR= CHIDISMO

Ridotta funzionalità delle c. del Leydig

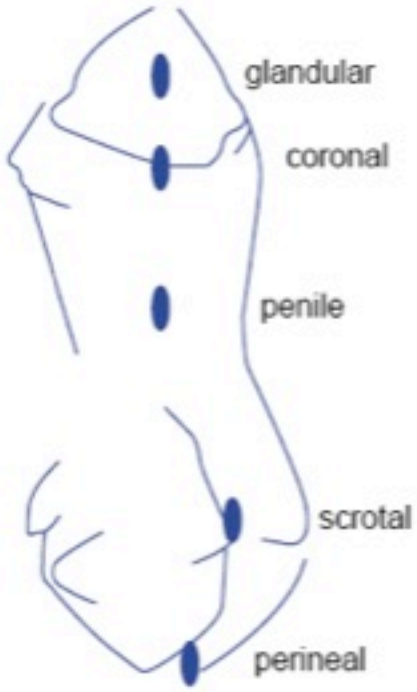


Figure 1. The hypospadias types are classified on the basis of the anatomical location of the urethral meatus (glandular, coronal, penile or scrotal/perineal). The malformation is frequently associated with a ventral curvature of the penis (chordee).

Endocrine disruptors

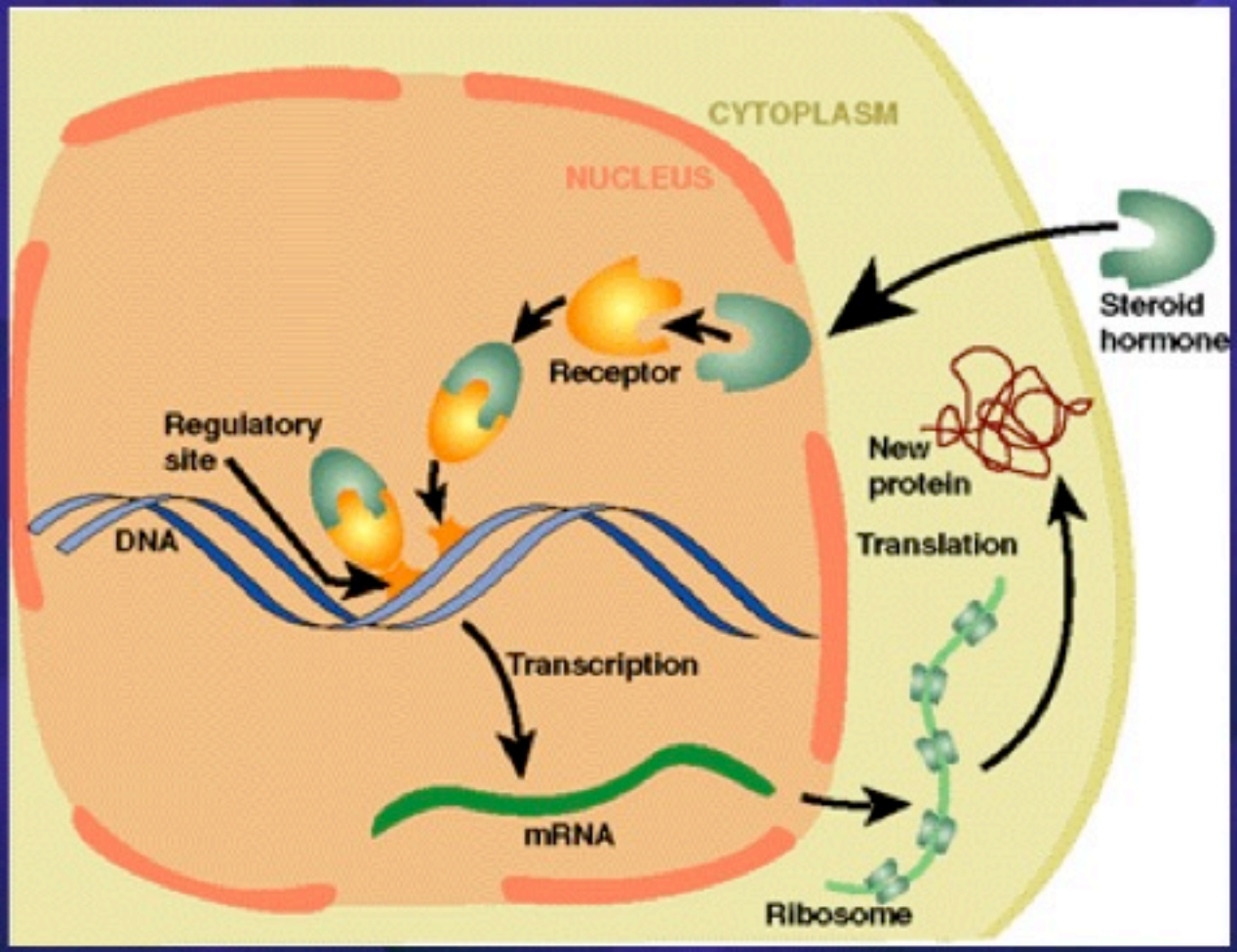
Are natural plant compounds and synthetic chemicals that adversely affect the endocrine system (the communication system of glands, hormones, and cellular receptors that control the body's internal functions) in animals and disrupt normal development.

Many of these substances have been associated with developmental, reproductive, and other health problems in wildlife, laboratory animals, and perhaps humans.

There are four types of endocrine disruptors:

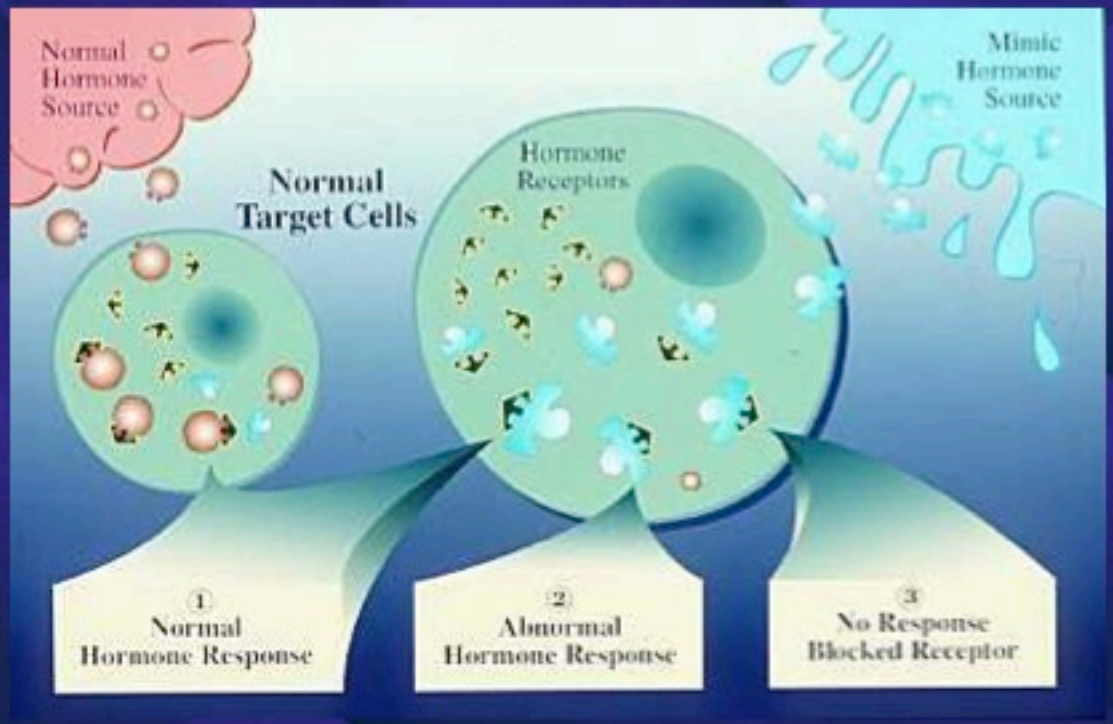
1. environmental estrogens
2. environmental androgens
3. anti-estrogens
4. anti-androgens

"Normal" action mechanism of estrogens

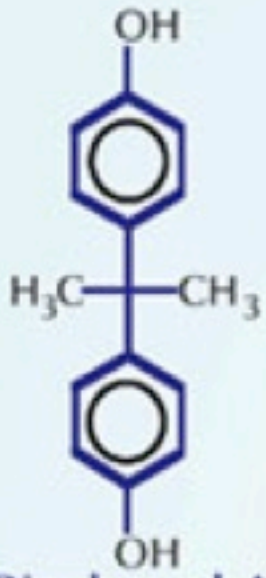


HOW ENDOCRINE DISRUPTORS WORK

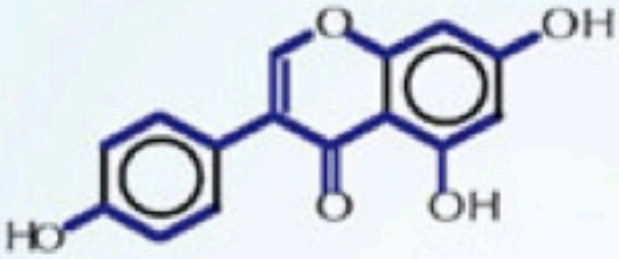
The mechanisms by which endocrine disruptors work is not completely understood. Perhaps the biggest mystery is how substances with different shapes and structures produce similar physiological results. Some compounds simply bind to a hormone receptor and mimic or block normal hormone response, but others appear to produce their effects via elaborate signaling pathways that are independent of binding hormone receptors. Certain substances can mimic hormones by binding to specific hormone receptors inside cells. Receptors are protein molecules that read and respond to hormone signals. DDT, some PCBs, and many phytoestrogens bind to estrogen receptors.



a



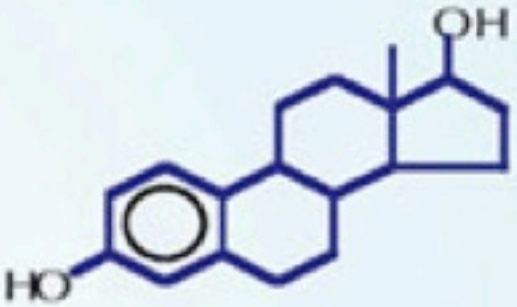
Bisphenol-A
(xenoestrogen)



Genistein
(phytoestrogen)

Cd^{++}

Cadmium
(metalloestrogen)



17β-estradiol
(estrogen)

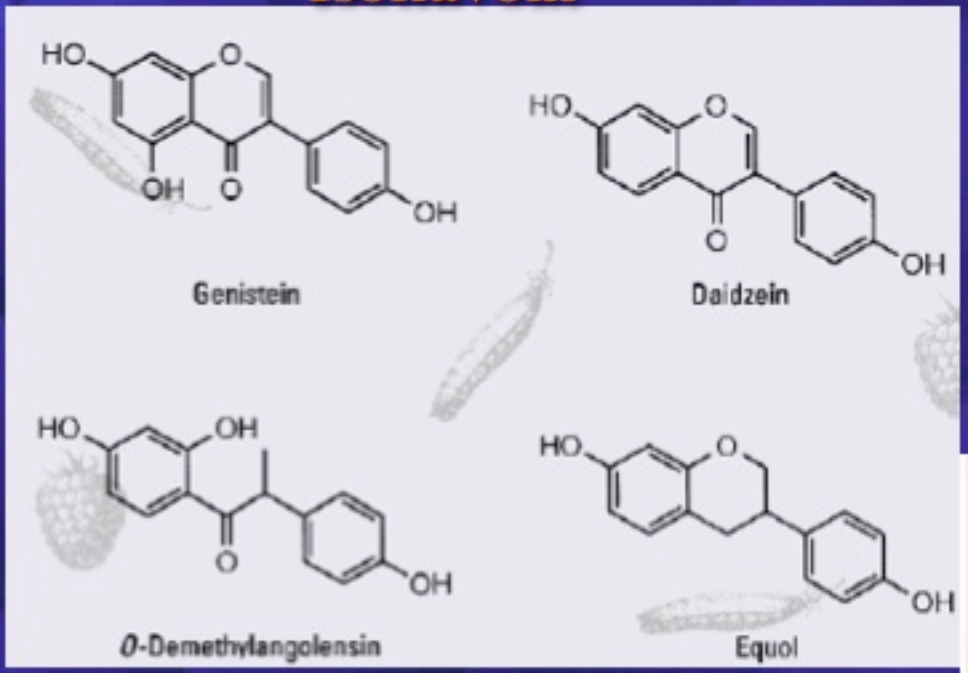
b

Normal physiological functions

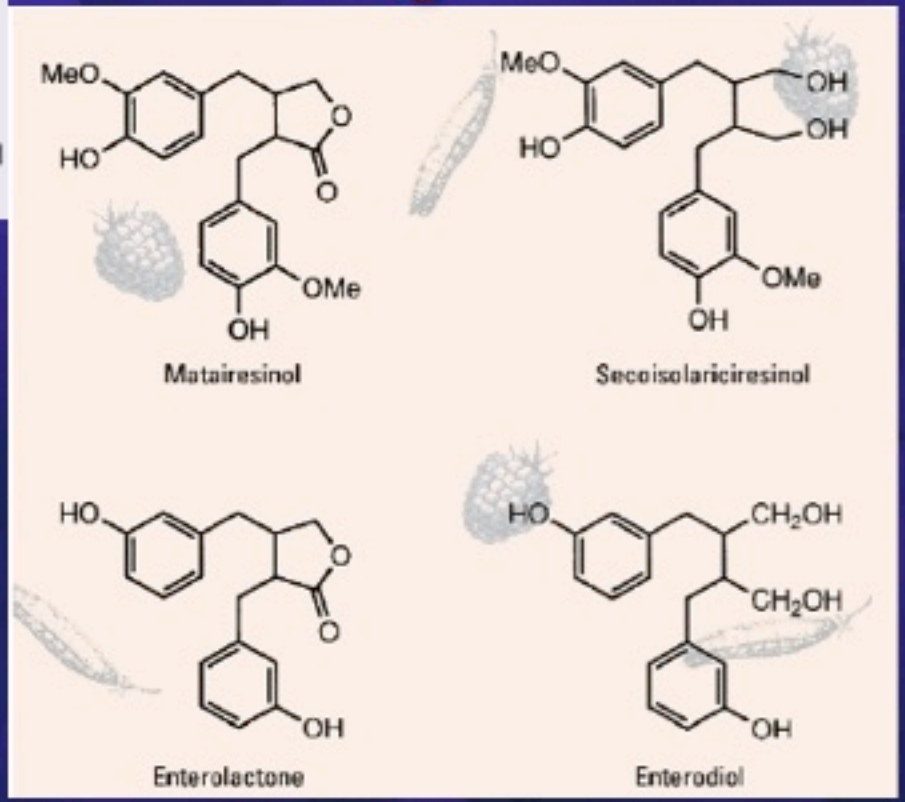
Breast and endometrial cancer

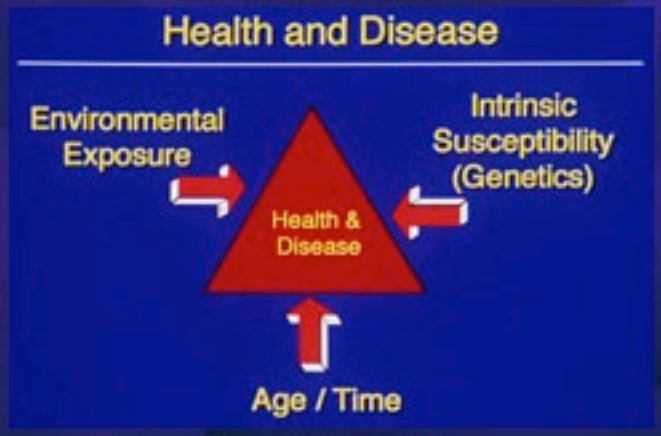
Endocrine disruption

Isoflavoni

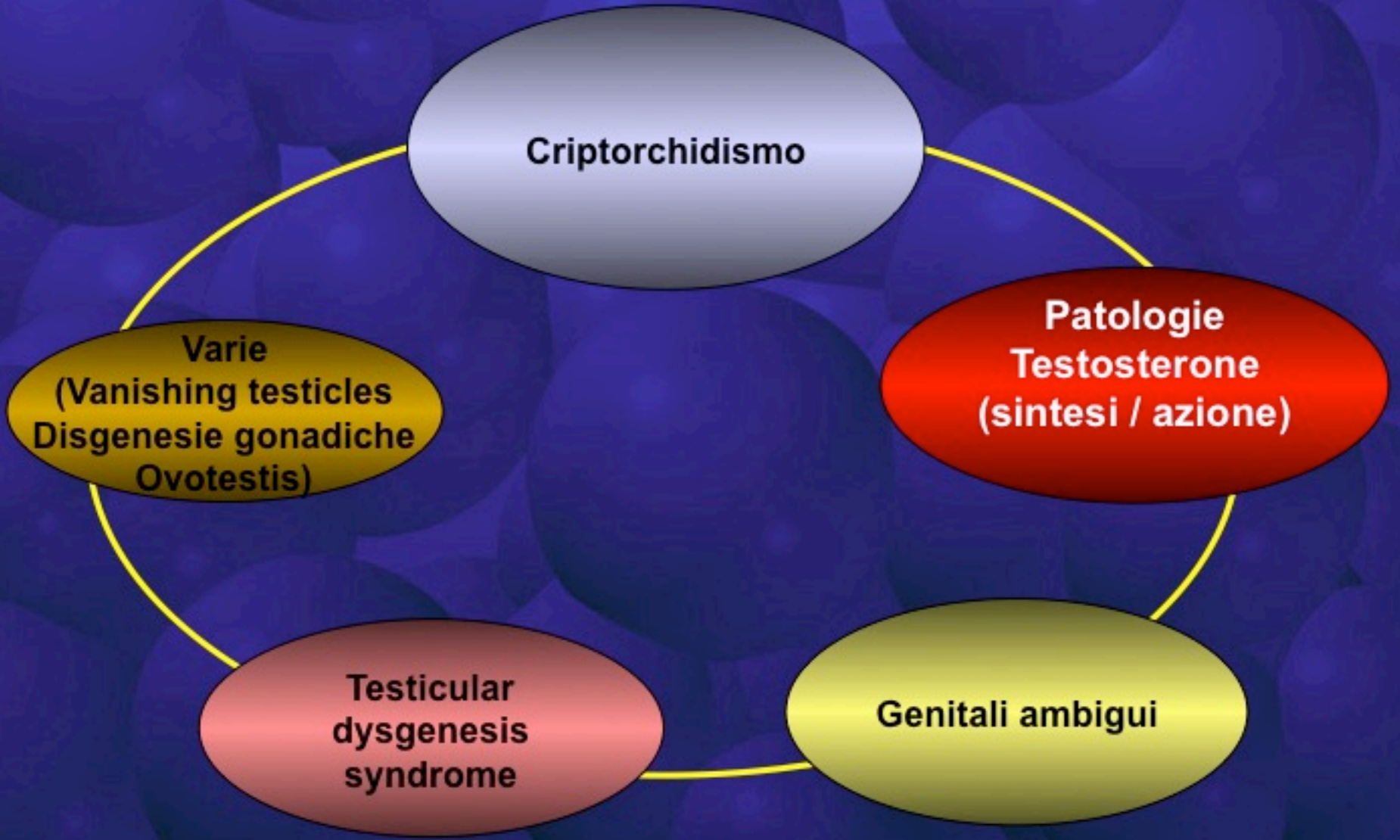


Lignani

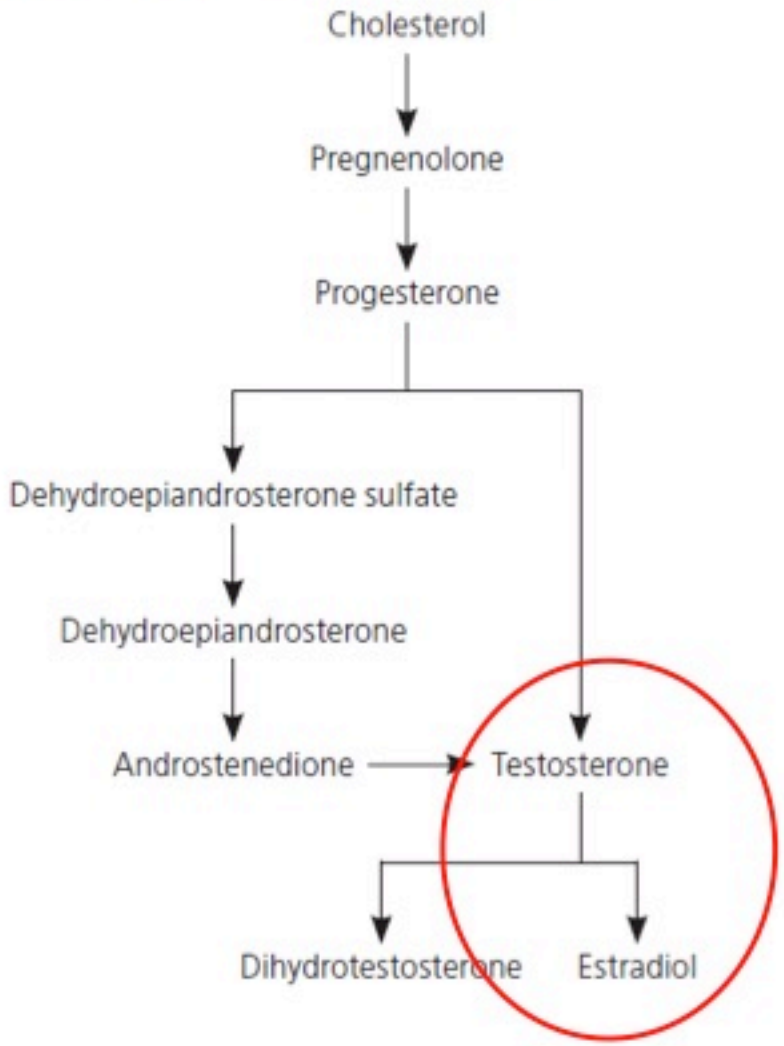


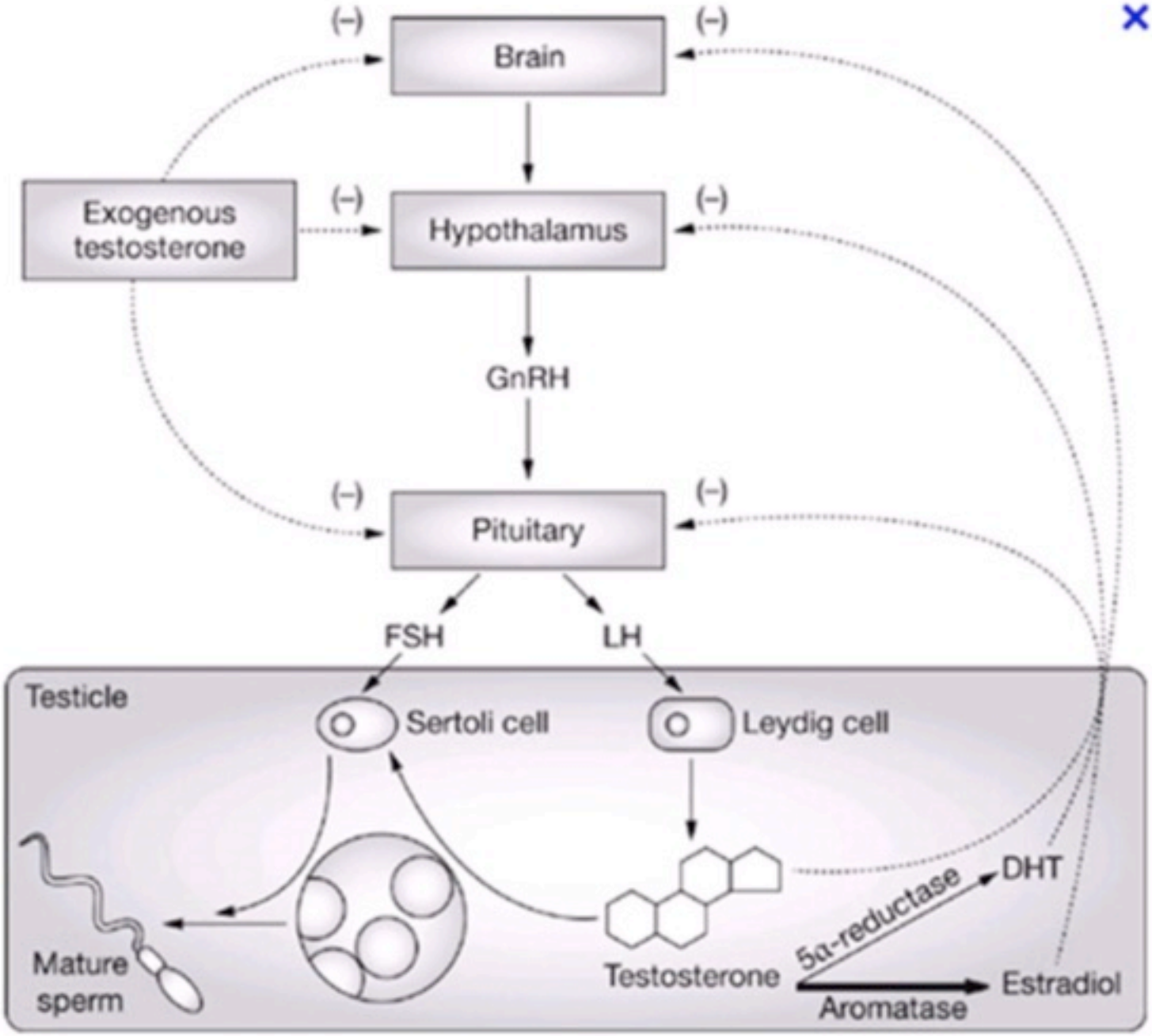


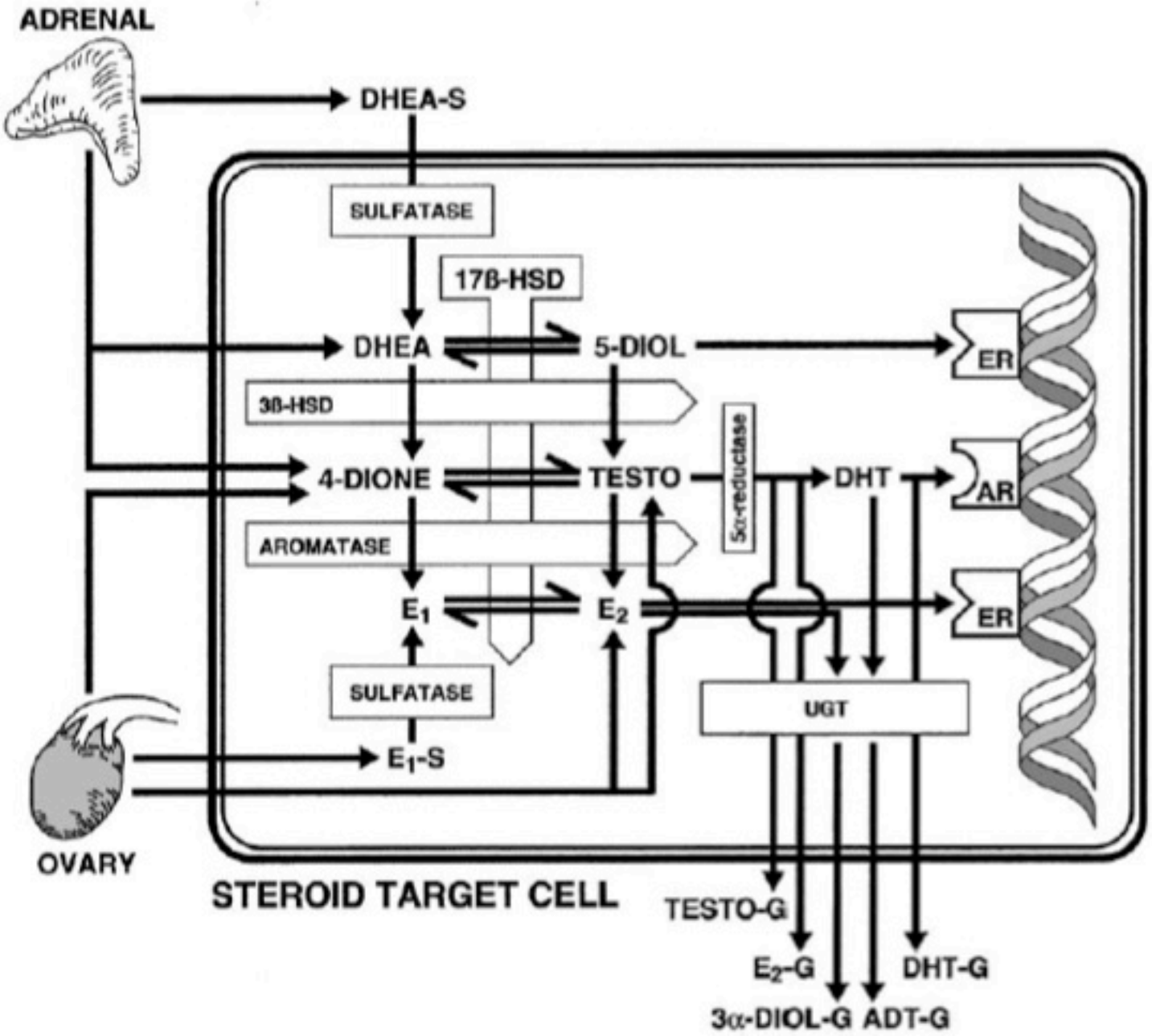
Androgeni: fenotipi clinici



Cholesterol Metabolism




(–)
Brain
(–)
Exogenous testosterone
(–)
Hypothalamus
(–)
GnRH
(–)
Pituitary
(–)
FSH
LH
Testicle
Sertoli cell
Leydig cell
Mature sperm
Testosterone
5α-reductase
DHT
Aromatase
Estradiol


ADRENAL
DHEA-S
SULFATASE
17β-HSD
DHEA
5-DIOL
3β-HSD
4-DIONE
TESTO
5α-reductase
DHT
AROMATASE
E1
E2
SULFATASE
E1-S
UGT
ER
AR
ER
OVARY
STEROID TARGET CELL
TESTO-G
E2-G
DHT-G
3α-DIOL-G
ADT-G

Meccanismo di azione dell'effetto androgenico

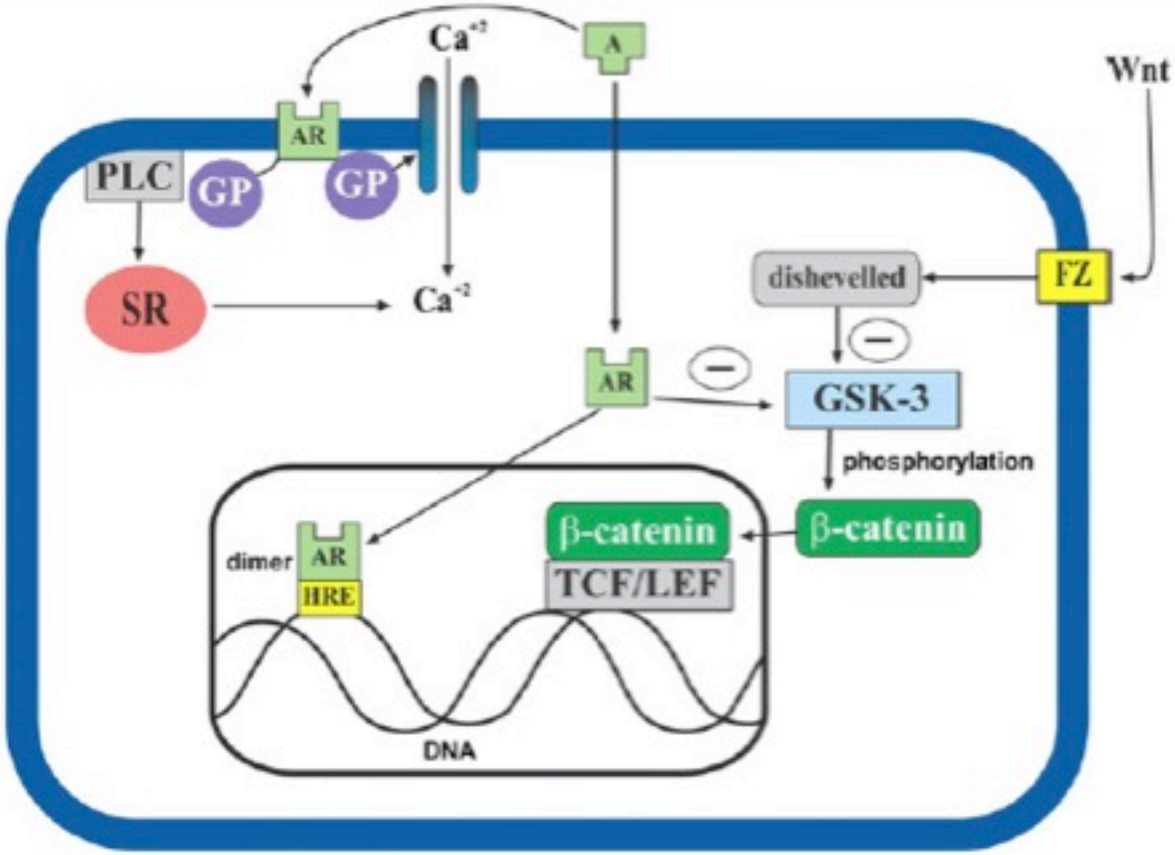


Fig. 1. Androgen receptor signaling pathways. A = androgen, AR = androgen receptor, Ca^{2+} = calcium, FZ = frizzled receptor, GP = G protein, GSK-3 = glycogen synthase kinase 3, HRE = hormone response element, PLC = phospholipase C, SR = sarcoplasmic reticulum, TCF = T cell factor, LEF = lymphoid enhancer factor 1, Wnt = Wingless-Int.

Difetti della sintesi e/o dell'azione del testosterone.

Completa resistenza agli androgeni CAIS (Hutson J.M: 1992)

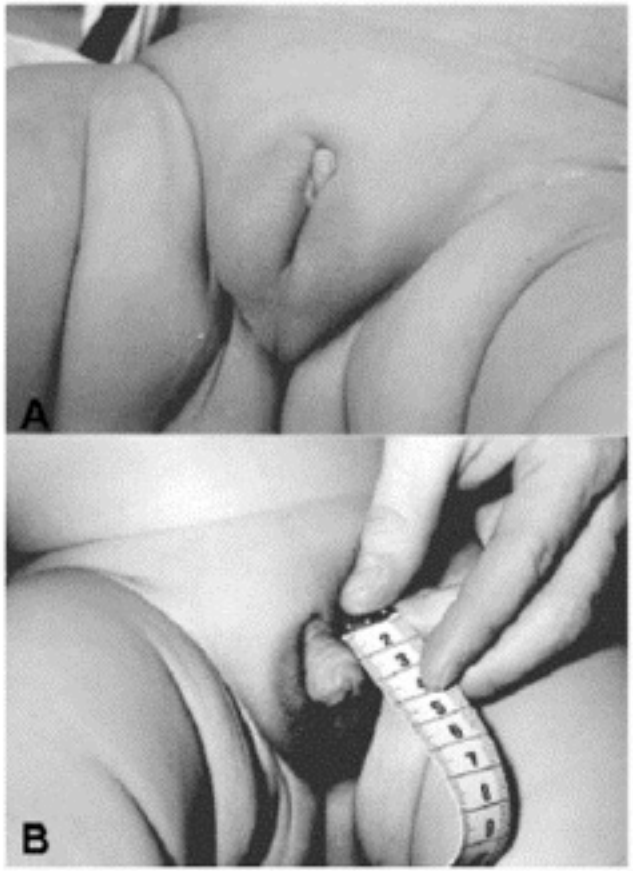
Difetti della sintesi di testosterone (New M. 1984)

Difetti della secrezione di MIF

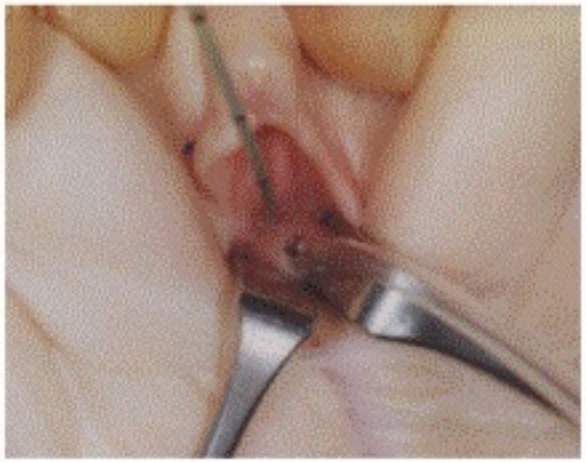
(Hutson J.M: 1992)

Difetti della secrezione di gonadotropine (Santen R.J. 1987)

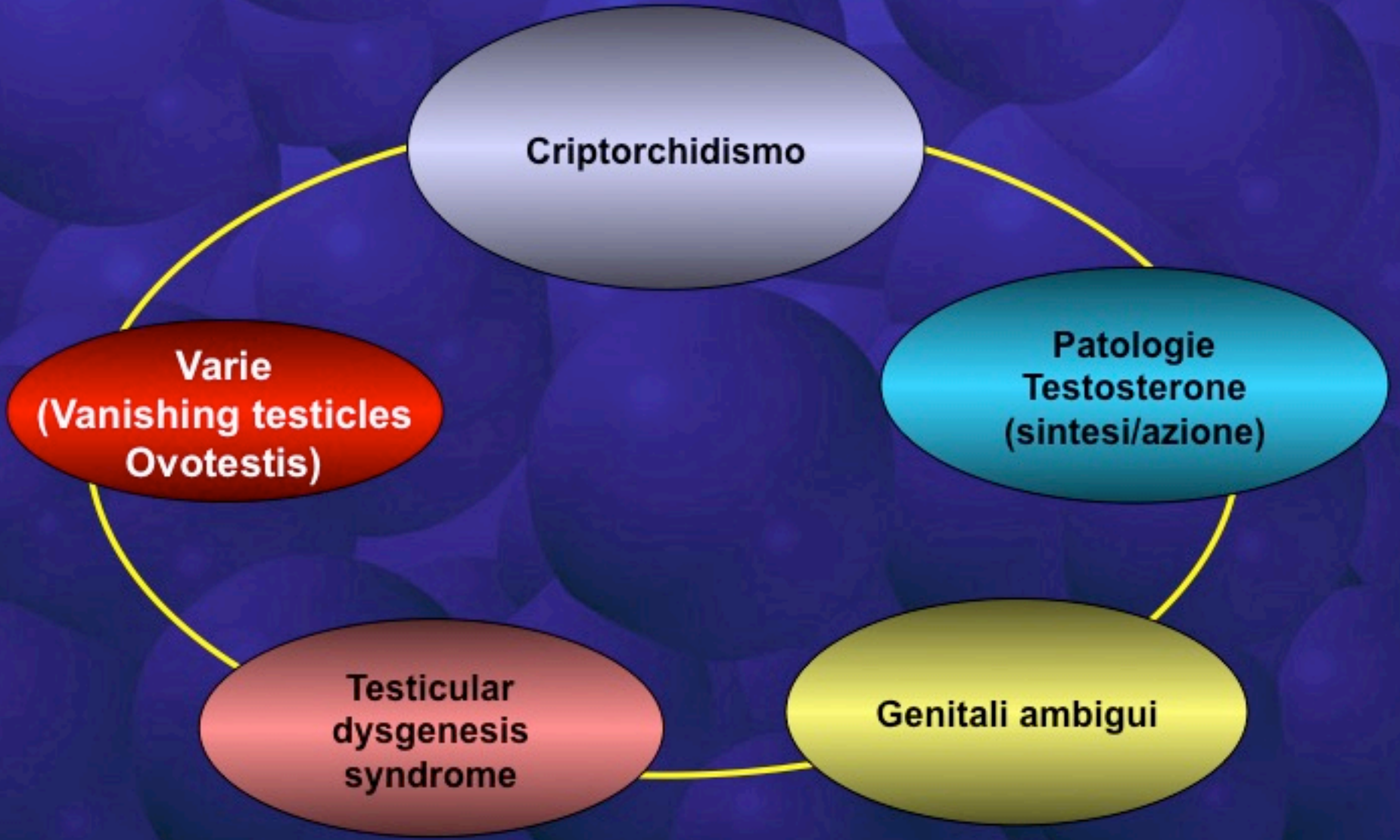
5α-Reductase II deficiency



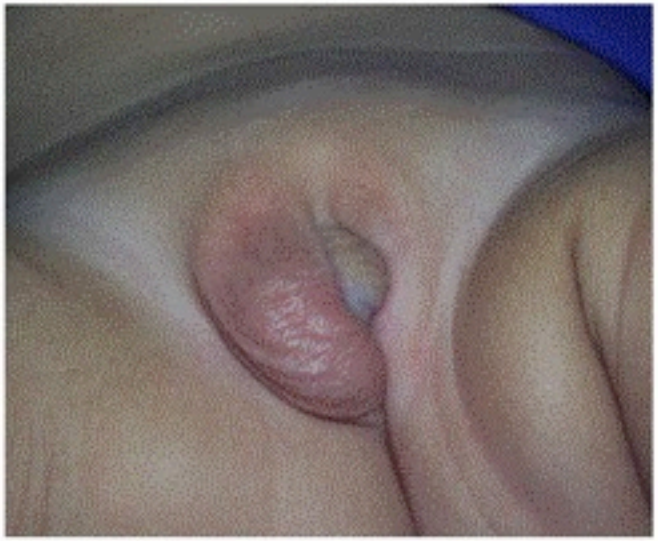
- Defect of peripheral Dihydrotestosterone-Synthesis
- Often female phenotype with partial virilization at birth, however, complete female phenotype possible
- No Uterus /Mullerian structures
- Intact testis, secreting normal testosterone

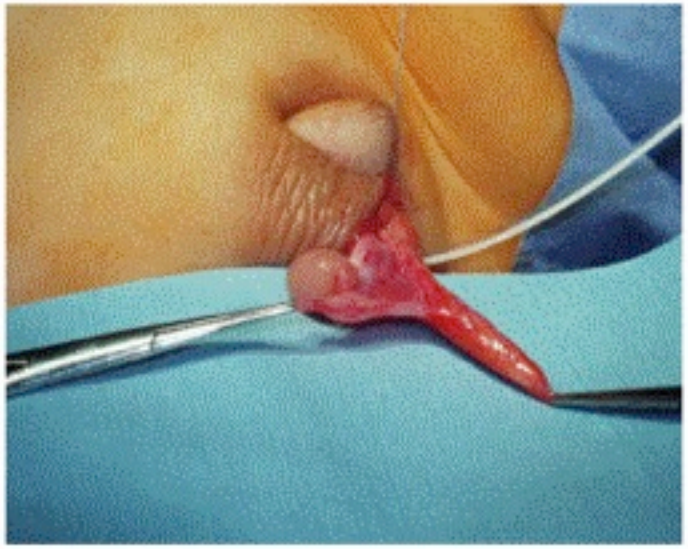


Androgeni: fenotipi clinici



True hermaphroditism





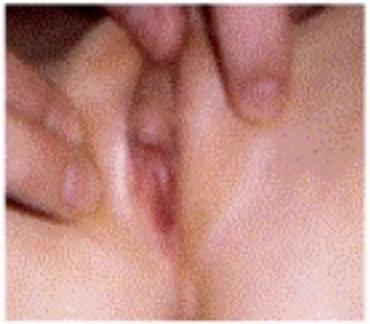
- 46,XX Karyotype, no CAH, Uterus small, on ultrasound testicular tissue on the right side.

- Krob et al. 1994: 283 cases; 96.9 % 46,XX;
- 44.4% Ovotestis, most often on the right side.

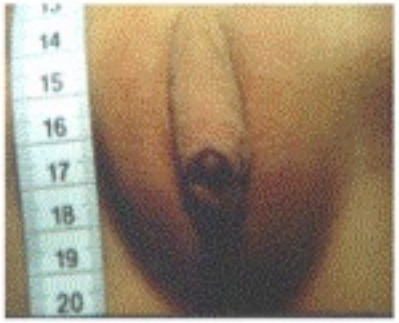
17ß-HSD3 deficiency

- Often detected in infancy or childhood
- Often female phenotype with partial virilization
- Intact testis, secreting high amounts of androstenedione, peripheral conversion to testosterone possible

- At the time of puberty possible high testosterone:
  - Possible clitoromegaly
  - Acne
  - Hirsutism
  - Possible slight breast development (to B3)
  - Deep voice
- If female sex assigment, removal of gonads before puberty !

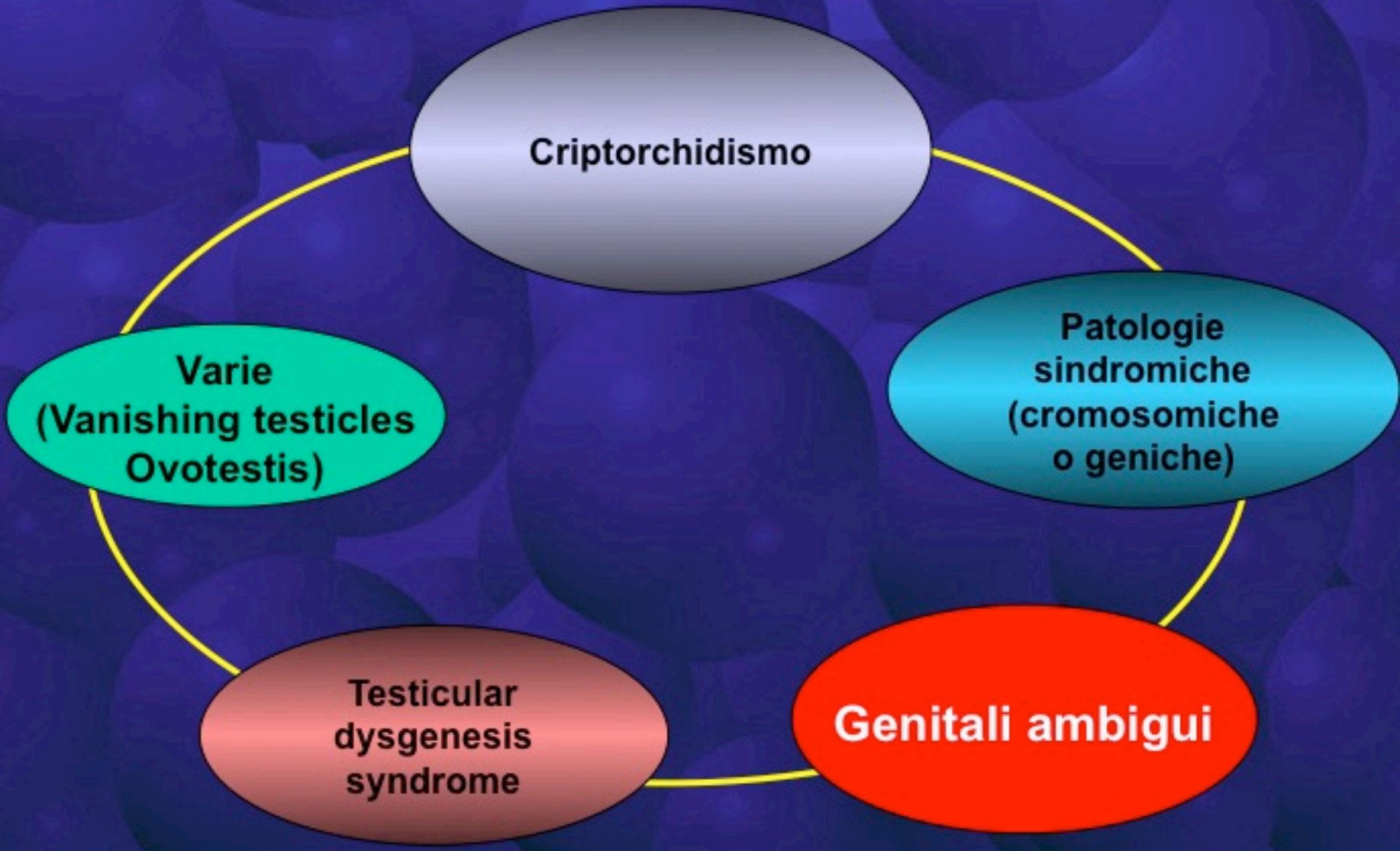


Pre pubertal



Pubertal

Androgeni: fenotipi clinici

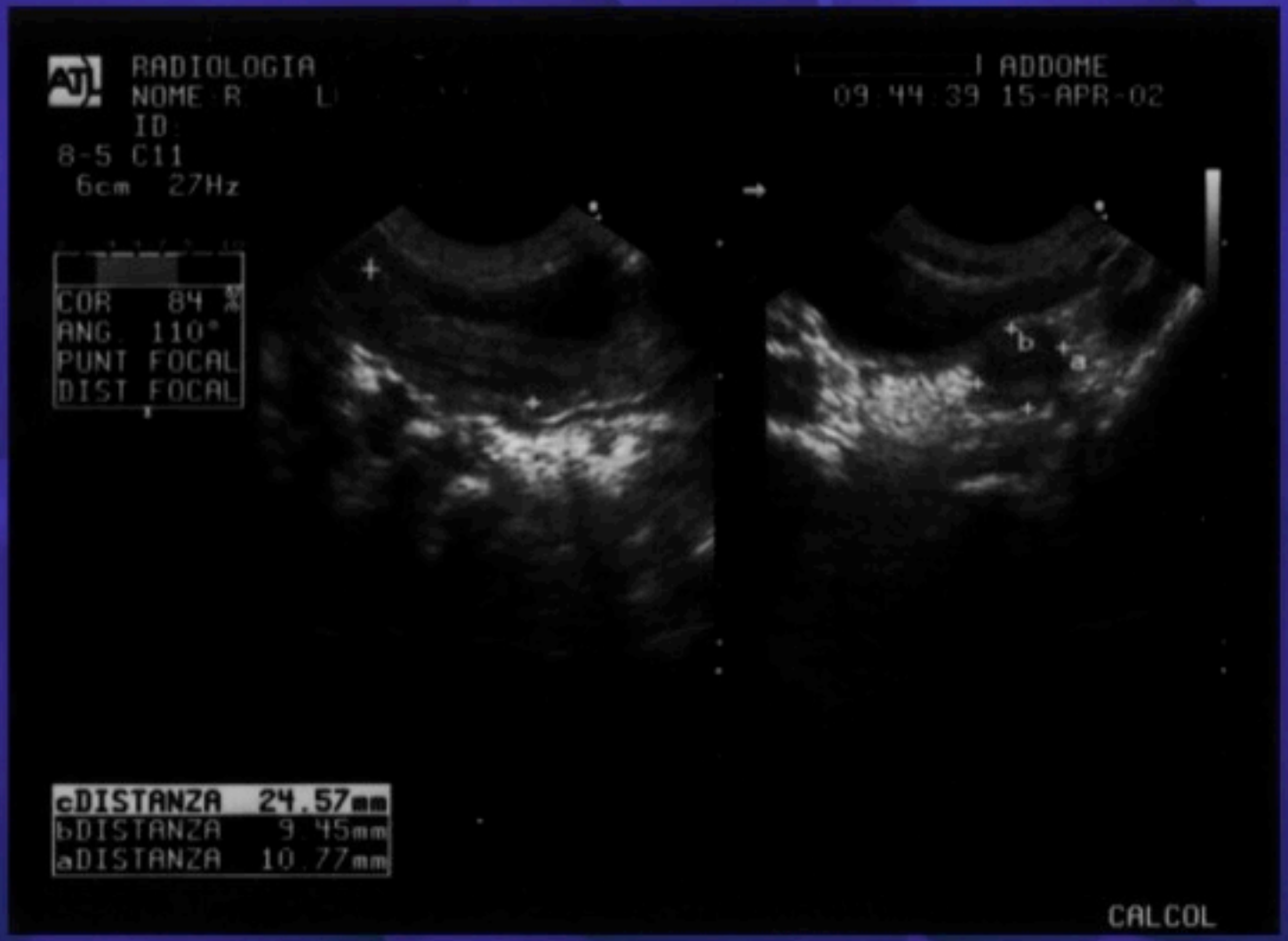


Caso clinico II

Primo scenario

L.R. nasce il 6 aprile 2002

- **Viene evidenziato un criptorchidismo bilaterale con genitali esterni maschili, scroto e pene normoconformati senza note di iperpigmentazione e rafe mediano demarcato.**
- **Viene richiesto un esame ecografico che però non è in grado di reperire i testicoli. Viene richiesto studio citogenetico.**
- **Il neonato viene sottoposto a controllo della pressione e degli elettroliti, risultati nella norma.**
- **Per tal motivo viene dimesso in terza giornata.**
- **Viene consigliato comunque di ripetere l'indagine ecografica a breve (addome completo).**



Referto: non immagini da riferire a testicoli in sede inguinale, bilateralmente.

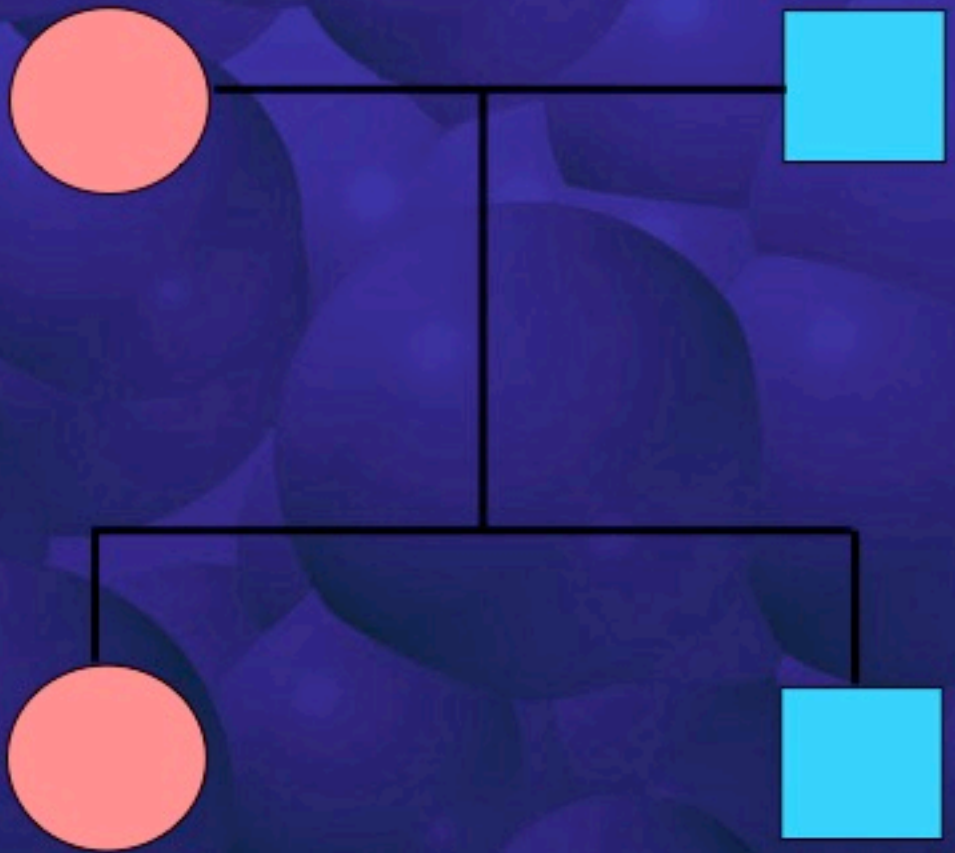
In sede retrovescicale si apprezza formazione solida con linea iperecogena centrale delle dimensioni di 25x11x10 mm da riferire , in prima ipotesi, ad utero meritevole comunque di ulteriore follow-up. Non immagini da riferire ad ovaie in sede pelvica. Nella norma il rimanente reperto ecografico.

Caso clinico II

Secondo scenario

- Il cariotipo è: 46,XX .
- A questo punto il piccolo viene richiamato per un completamento diagnostico (ventesima giornata di vita).
- Si esegue il dosaggio dei seguenti ormoni: 17-OH-P, T, ACTH basale e cortisolo.
- 17-OH-P:> 20, T: 715ng/dl, ACTH basale: 301 pg/ml, cortisolo: 5.6 mcg/dl.
- La diagnosi clinica è: SAG congenita in soggetto di sesso femminile con pseudo ermafroditismo femminile, completa virilizzazione dei genitali esterni e perdita di sali, da verosimile deficit di 21-idrossilasi.
- Viene cominciata la usuale terapia idroelettrolitica e steroidea per ridurre la perdita dei sali.
- Viene eseguita diagnosi di genetica molecolare sulla neonata e sui genitori per individuare le mutazioni del gene per la 21-idrossilasi (CYP21).

Famiglia R.

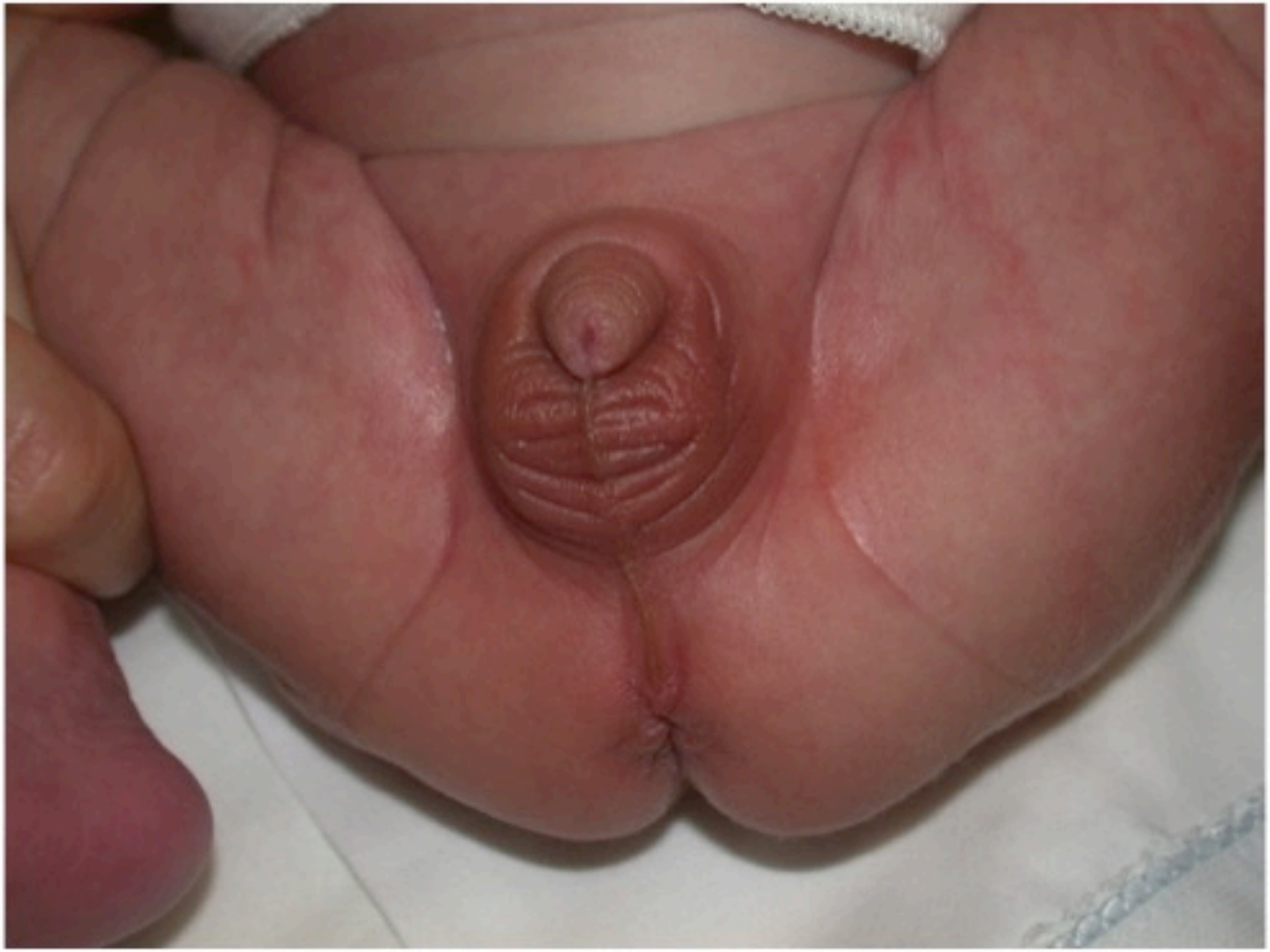


M.L
(madre)
Mut.:655/281
N/N

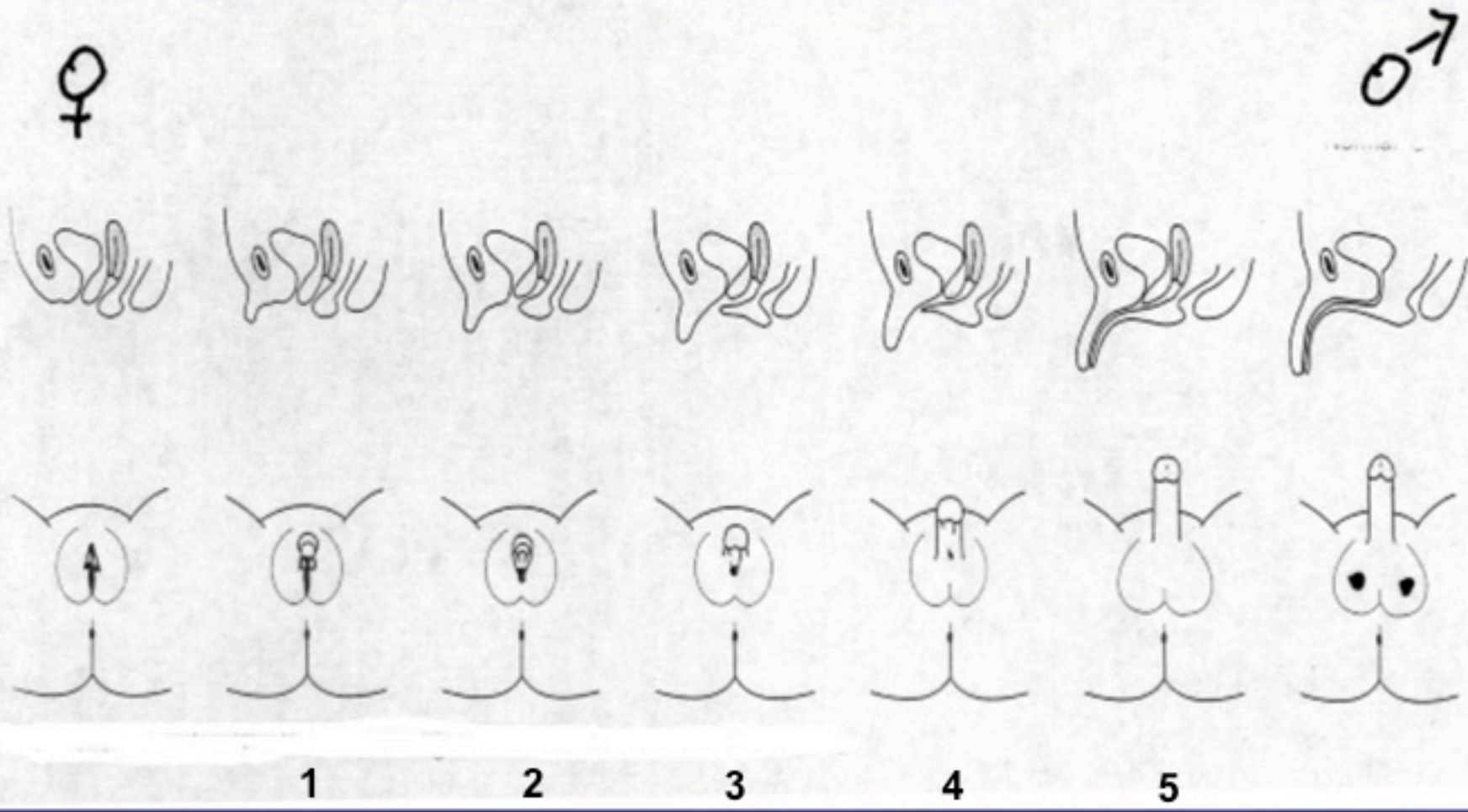
R.S.
(padre)
Mut.:655/N
N/N

R.A
Mut:655/281
655/N

R.A:
Mut:655/281
N/N



Prader classification of five stages of virilization in the female infant



Presentazione clinica della SAG

	Età
SW-CAH (SAG con perdita di sali)	Neonatale
SV-CAH (SAG con virilizzazione semplice)	Neonatale
NCAH (SAG non classica)	< 8 anni
NCAH (SAG non classica)	8-18 anni
NCAH (SAG non classica)	> 18 anni

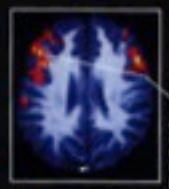
Developmental tasks of adolescence

	Biological	Psychological	Social
Early adolescence	Early puberty. Girls: breast bud and pubic hair development, start of growth spurt. Boys: testicular enlargement , start of genital growth	Concrete thinking but early moral concepts; progression of sexual identity development; possible homosexual peer interest; reassessment of body image	Emotional separations from parents; start of strong peer identification, early exploratory bahaviours (smoking, violence)
Mid-adolescence	Girls: mid-late puberty and end of growth spurt; menarche; development of female body shape with fat deposition. Boys: mid-puberty, spermarche and nocturnal emissions; voice breaks; start of growth spurt.	Abstract thinking, but self still seen as " bullet proof" growing verbal abilities; identification of law with morality; start of fervent ideology (religious, political)	Emotional separation from parents; strong peer identification; increased health risk (smoking, alcohol) ; heterosexual peer interest.
Late adolescence	Boys: end of puberty; continued increase in muscle bulk and body hair	Complex abstract thinking; identification of difference between law and morality; further development of personal identify; further development of or rejection of religious and political ideology	Development of social autonomy; intimate relationships; development of vocational capability and financial independence

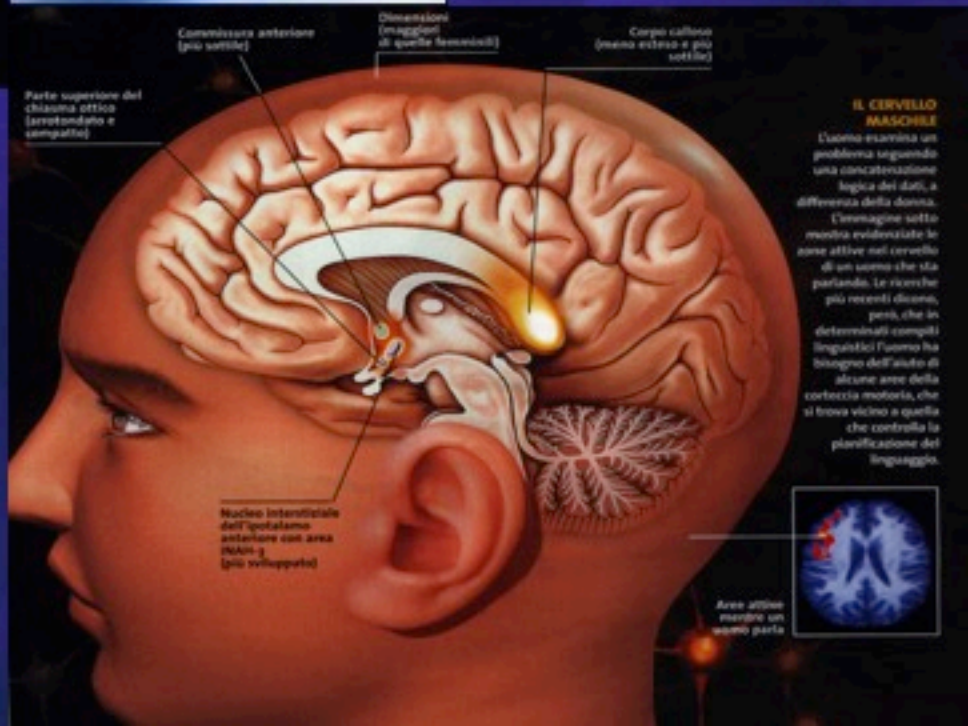
Russel Viner 2009

IL CERVELLO FEMMINILE

Le connessioni tra i due emisferi sono più estese che nell'uomo. Per questo la donna esamina una situazione con una maggiore tendenza a valutare globalmente l'insieme dei dati. Nella foto sotto, sono evidenziate in rosso le aree attive nel cervello di una donna mentre sta parlando. Il nucleo dell'ipotalamo anteriore comprendente la cosiddetta area INAH-3 è meno sviluppato che negli uomini.



Aree attive mentre una donna parla



IL CERVELLO MASCHILE

L'uomo esamina un problema seguendo una concatenazione logica dei dati, a differenza della donna. L'immagine sotto mostra evidenziate le zone attive nel cervello di un uomo che sta parlando. Le ricerche più recenti dicono, però, che in determinati compiti linguistici l'uomo ha bisogno dell'aiuto di alcune aree della corteccia motoria, che si trova vicino a quella che controlla la pianificazione del linguaggio.

Changing your sex changes your brain : influences of testosterone and estrogen on adult human brain structure.

H.E. Hulshoff Pol.

European Journal of Endocrinology (2006)

Aggressività e differenze tra i sessi: effetto degli androgeni (testosterone) sull'aggressività dei maschi

- **Aggressività e riproduzione:** molti comportamenti aggressivi sono legati alla riproduzione e dipendono dagli ormoni sessuali
- **Androgeni (testosterone):** sono implicati nell'aggressività offensiva (quella difensiva è osservabile anche nelle femmine con basso testosterone)
- **Testosterone e aggressività:** l'esposizione post-natale al testosterone (effetto organizzativo) seguito da somministrazione di testosterone in adulti (effetto attivante) aumenta l'aggressività nei maschi

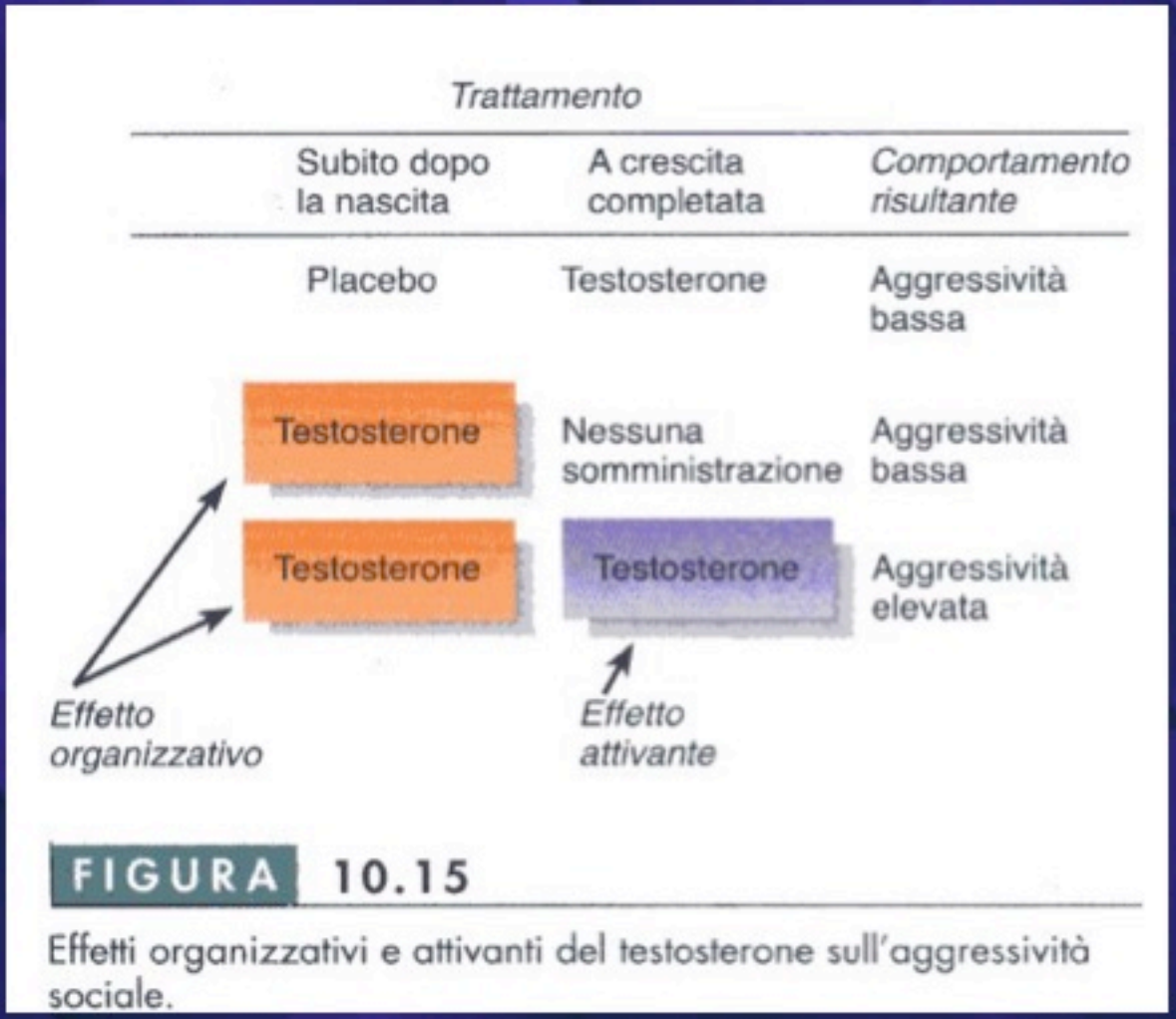


FIGURA 10.15

Effetti organizzativi e attivanti del testosterone sull'aggressività sociale.

TABLE 2. The 2009 WADA list of prohibited androgens and androgen modulators

Exogenous AAS	Endogenous AAS	Gonadotropins	Androgen modulators	Other
Androstendiol	Androstendiol	hCG (men)	Aromatase inhibitors	SARMs
Androstendione	Androstendione	LH (men)	Antiestrogens	
Bolandiol	Dihydrotestosterone		Androstendione	
Bolasterone	Testosterone		DHEA	
Calusterone	Epitestosterone			
Desoxymethyltestosterone				
Fluoxymesterone				
Mestanolone				
Mesterolone				
Methandienone				
Methyltestosterone				
Methylnortestosterone				
Nandrolone				
Oxandrolone				
Oxymesterone				
Oxymetholone				
Stanozolol				
Testosterone				
Tetrahydrogestrinone				
Tibolone				
Trenbolone				

Effetti tossici anabolizzanti

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.2920

World J Gastroenterol 2009 June 21; 15(23): 2920-2922
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Anabolic steroid-induced cardiomyopathy underlying acute liver failure in a young bodybuilder

Testing revealed an **increase in serum transaminases** (aspartate aminotransferase, 7897 IU/L; alanine aminotransferase, 7125 IU/L), **coagulopathy** (international normalized ratio, 3.3; factor V, 15%), **hyperbilirubinemia** (total bilirubin 6.8 mg/dL), **high ammonia levels** (73 umol/L) (normal, 11-32), **acute renal failure** (creatinine, 3.8 mg/dL), **hyponatremia** (126 mmol/L) and **high lactate dehydrogenase** (LDH) level (7140 IU/L).

Journal of Sex & Marital Therapy, 34:287–290, 2008

Letter to the Editor

Is Gender Identity Disorder in Adolescents Coming out of the Closet?

KENNETH J. ZUCKER, PH.D., SUSAN J. BRADLEY, M.D., ALLISON OWEN-ANDERSON, PH.D., SARAH J. KIBBLEWHITE, PH.D., and JAMES M. CANTOR, PH.D.

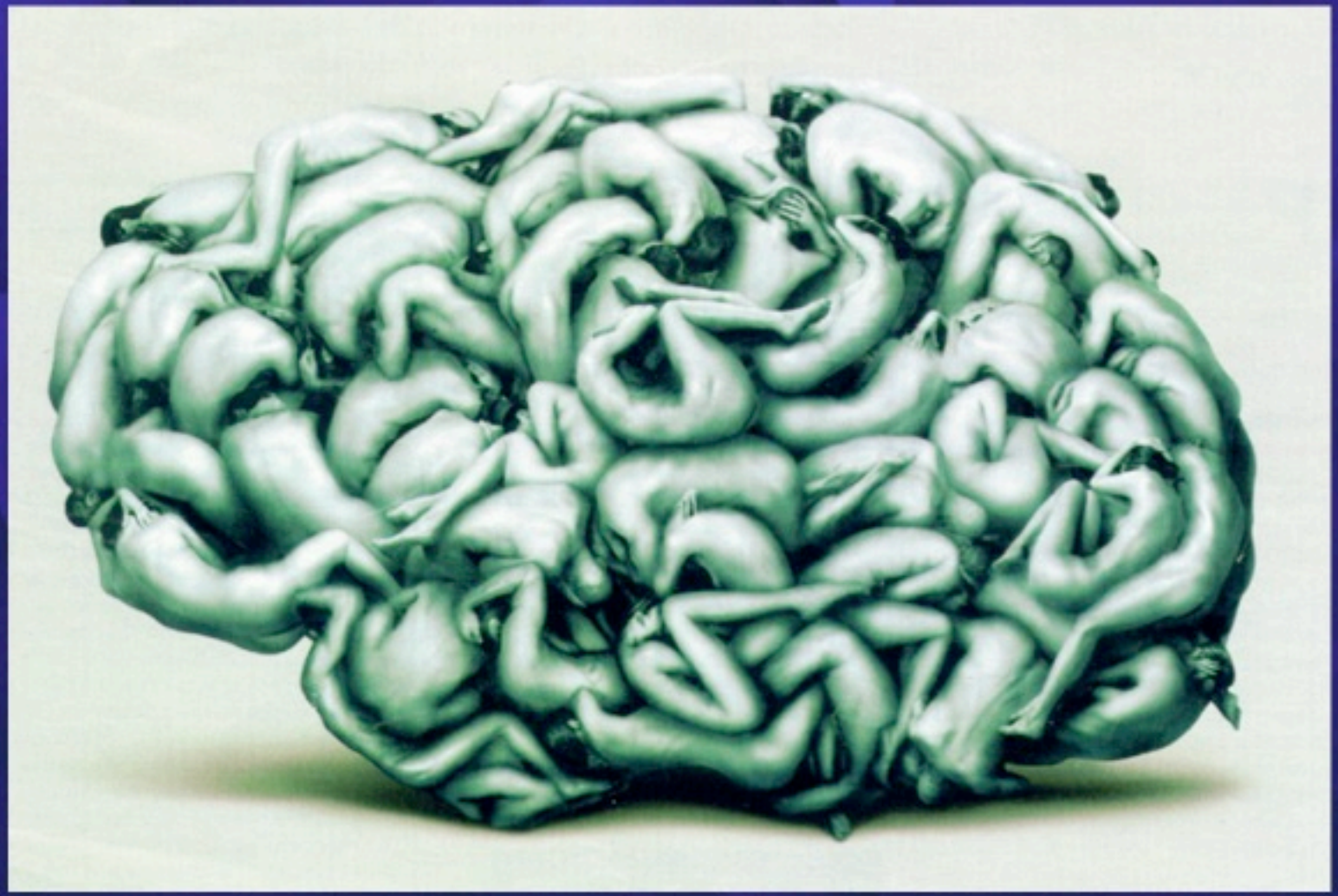
SEX/ GENDER

- SEX = person's biological make-up
- GENDER = person's roles and behaviour ("self image")

GENDER IDENTITY DISORDERS

CAUSES

- Different composition of brain areas ?
- Prenatal exposition to hormones or ECDs ?
- Execessive number of maternal aunts ?
- Birth order ?
- Left handedness ?
- Psycobiological (family, school, society) ?
- Heritabilitu ?



Piernicola Garofalo, SIMA 2007

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CLINICAL MANAGEMENT DEI PAZIENTI CON SAG