

Male puberty and spermatogenic development



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Disclosures

CONFLICT OF INTEREST

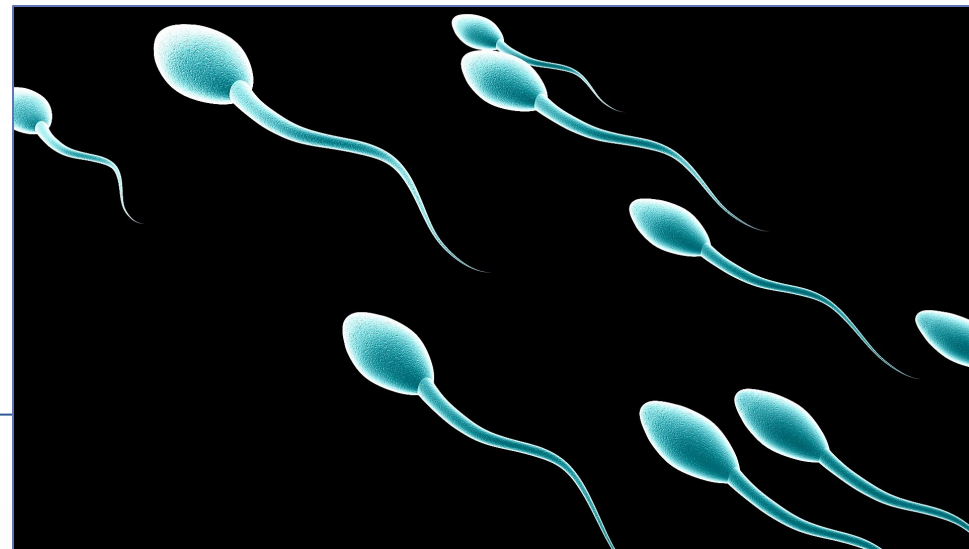
Kate Davies.....

X I have the following potential conflicts of interest to report:

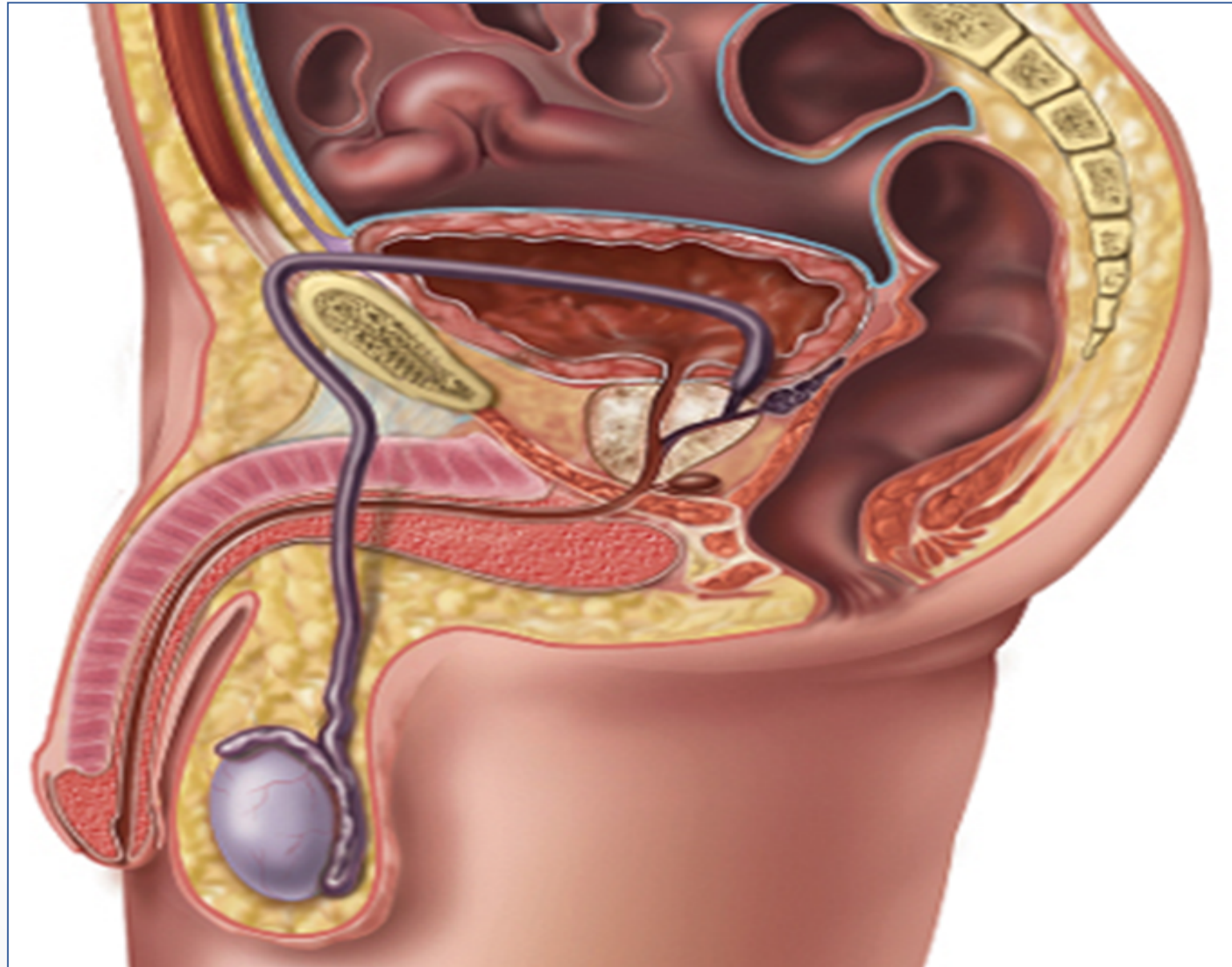
- ☐ Research Contracts
- X Consulting
- ☐ Employment in the Industry
- ☐ Stockholder of a healthcare company
- ☐ Owner of a healthcare company
- ☐ Other(s)

Introduction

- Overview of the male reproductive system
- Spermatogenesis
- Normal puberty in boys
- When things go wrong
- Other clinical indications for assessing puberty

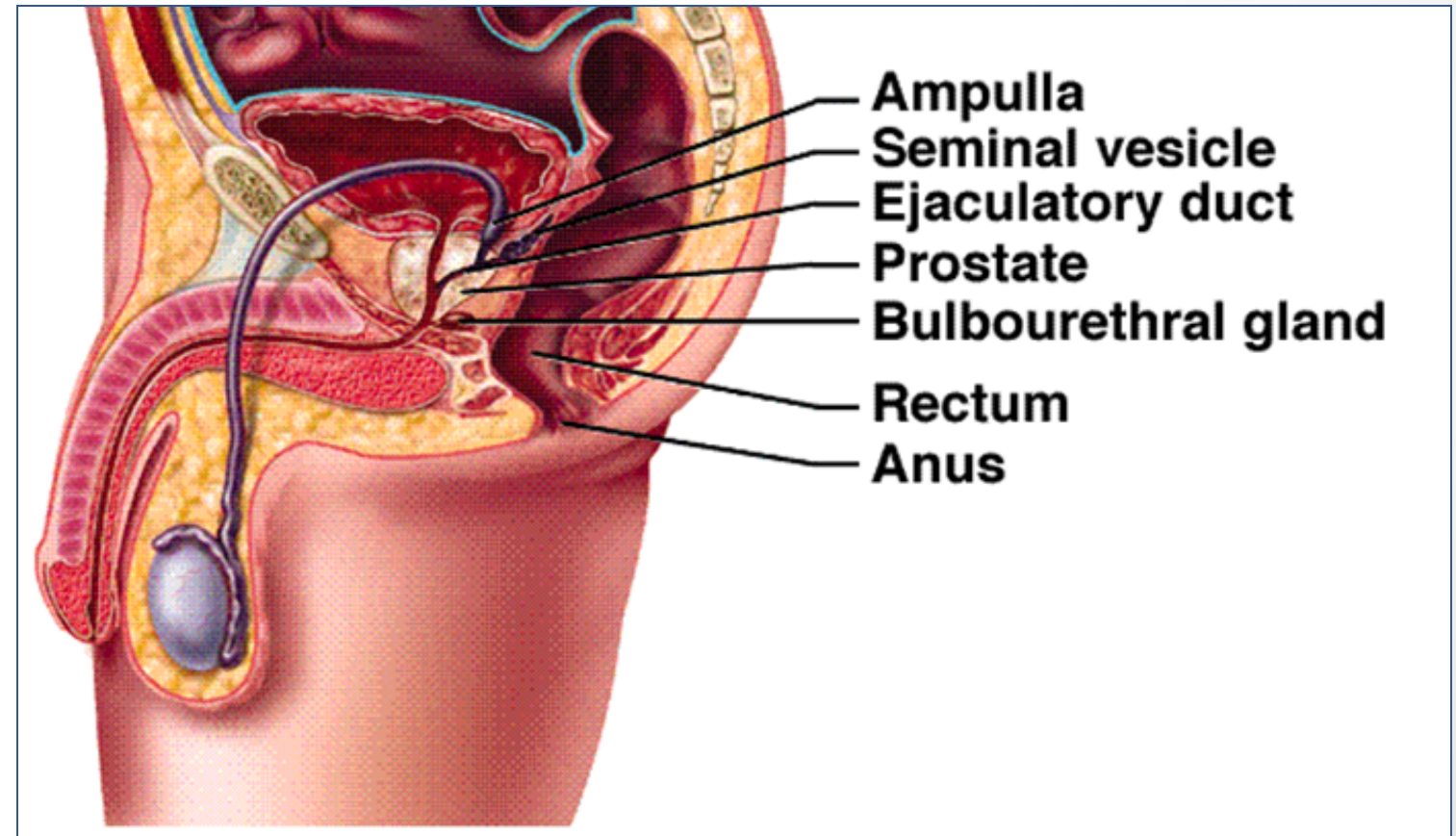
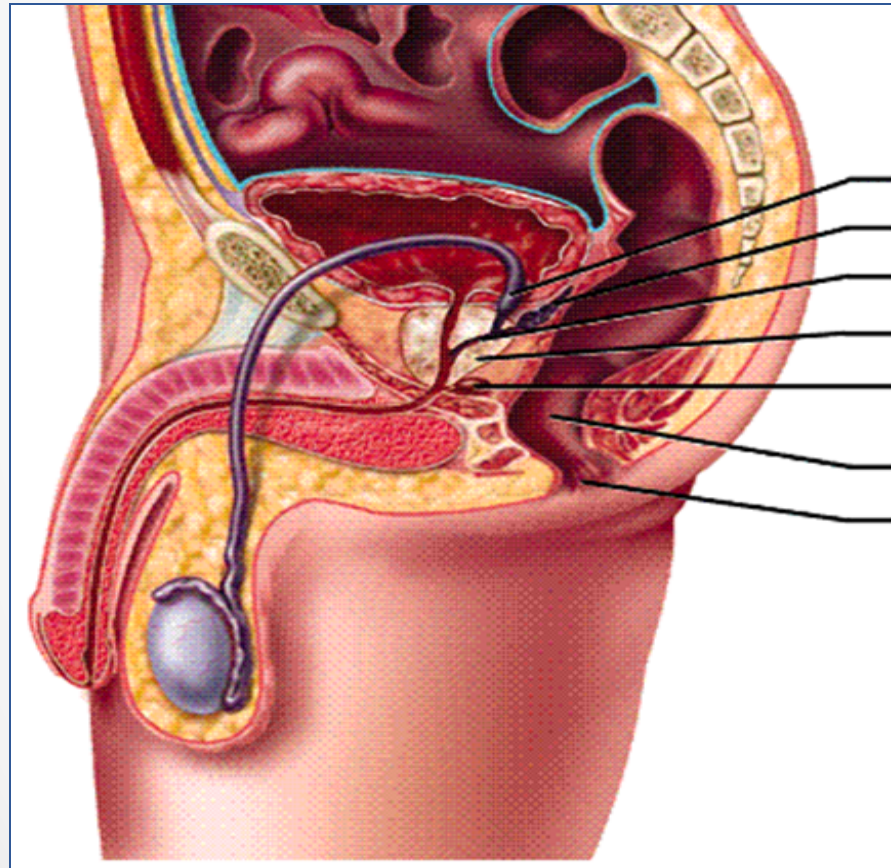


The male reproductive system



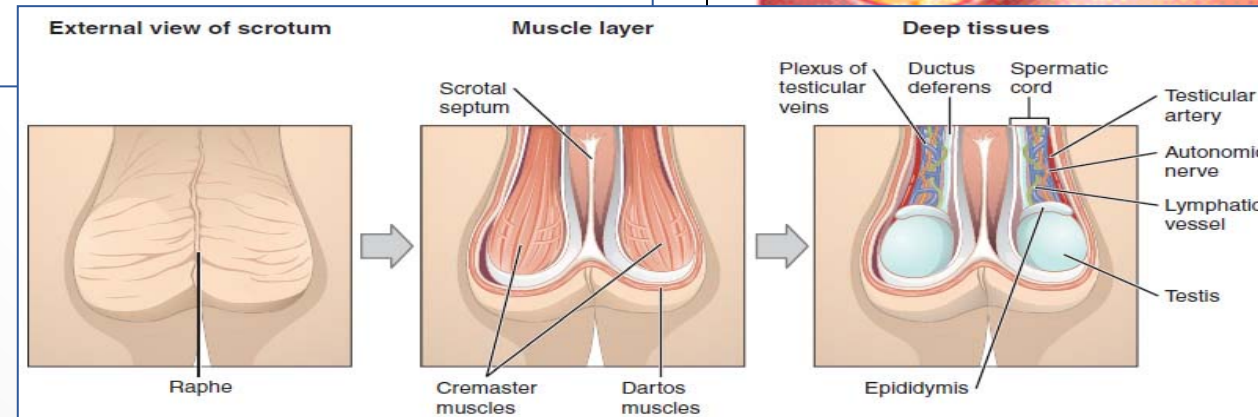
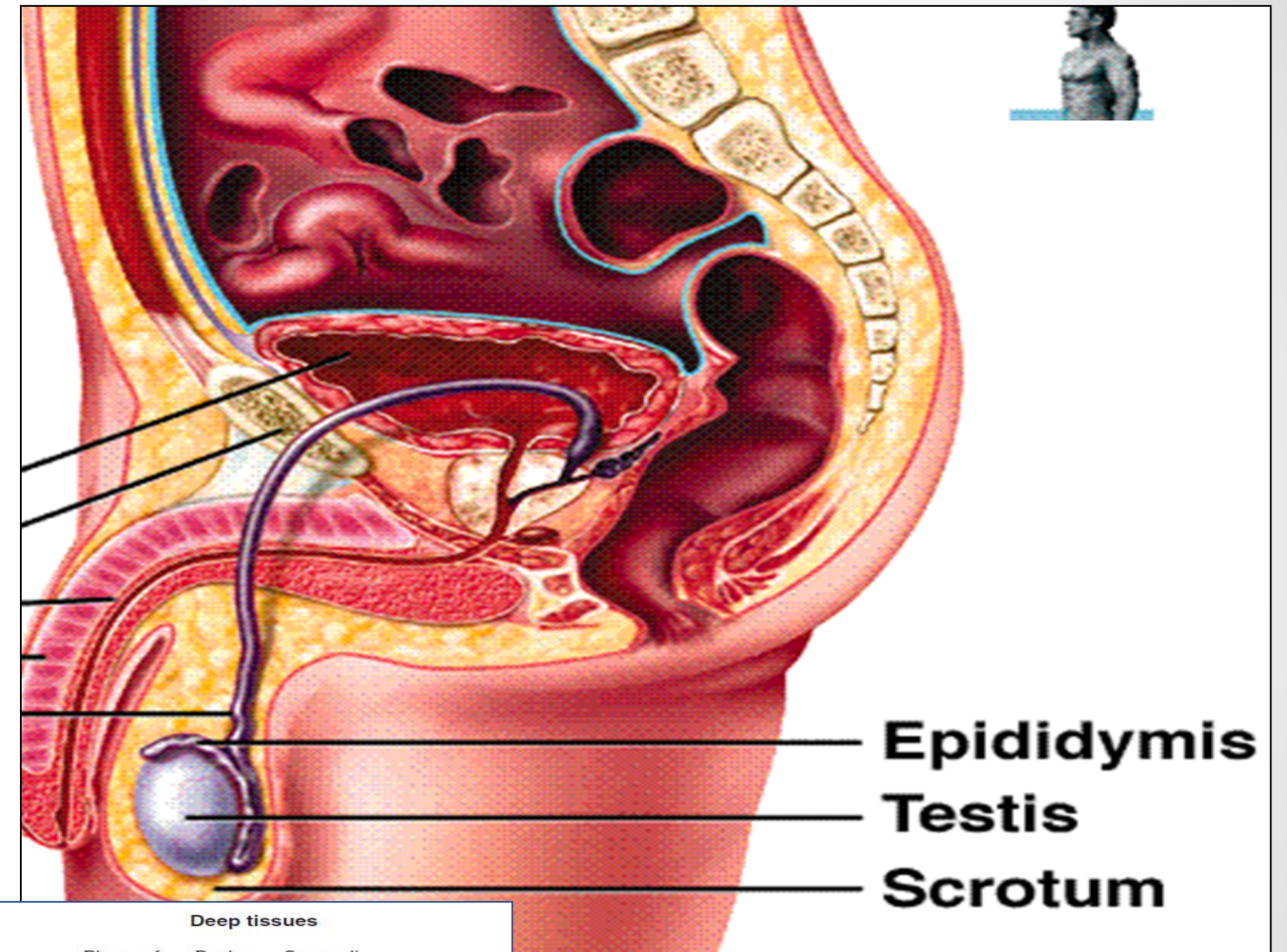
Sex organs

- Primary
 - Testes
- Secondary
 - Ducts, glands, penis

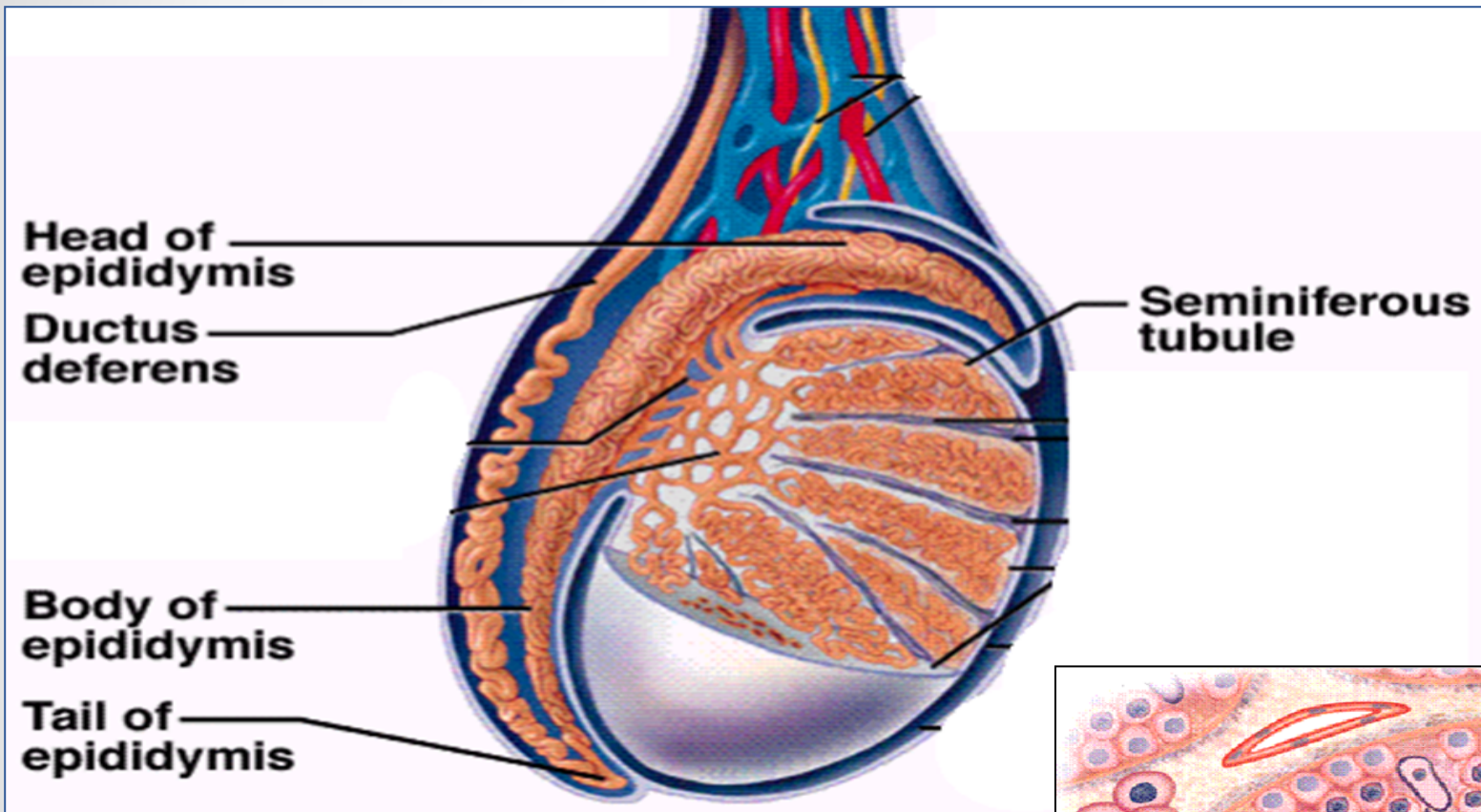


Scrotum

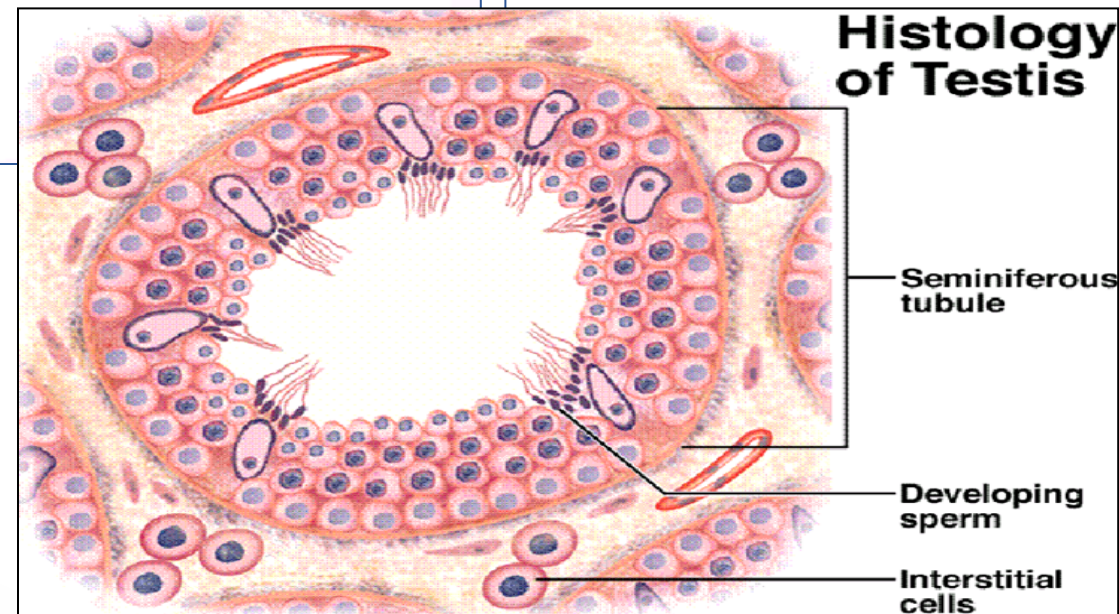
- Protects the testes and maintains the temperature of the testes 2 degrees lower than body temperature
- Dual chambered
- Located under the penis
- One is typically lower than the other



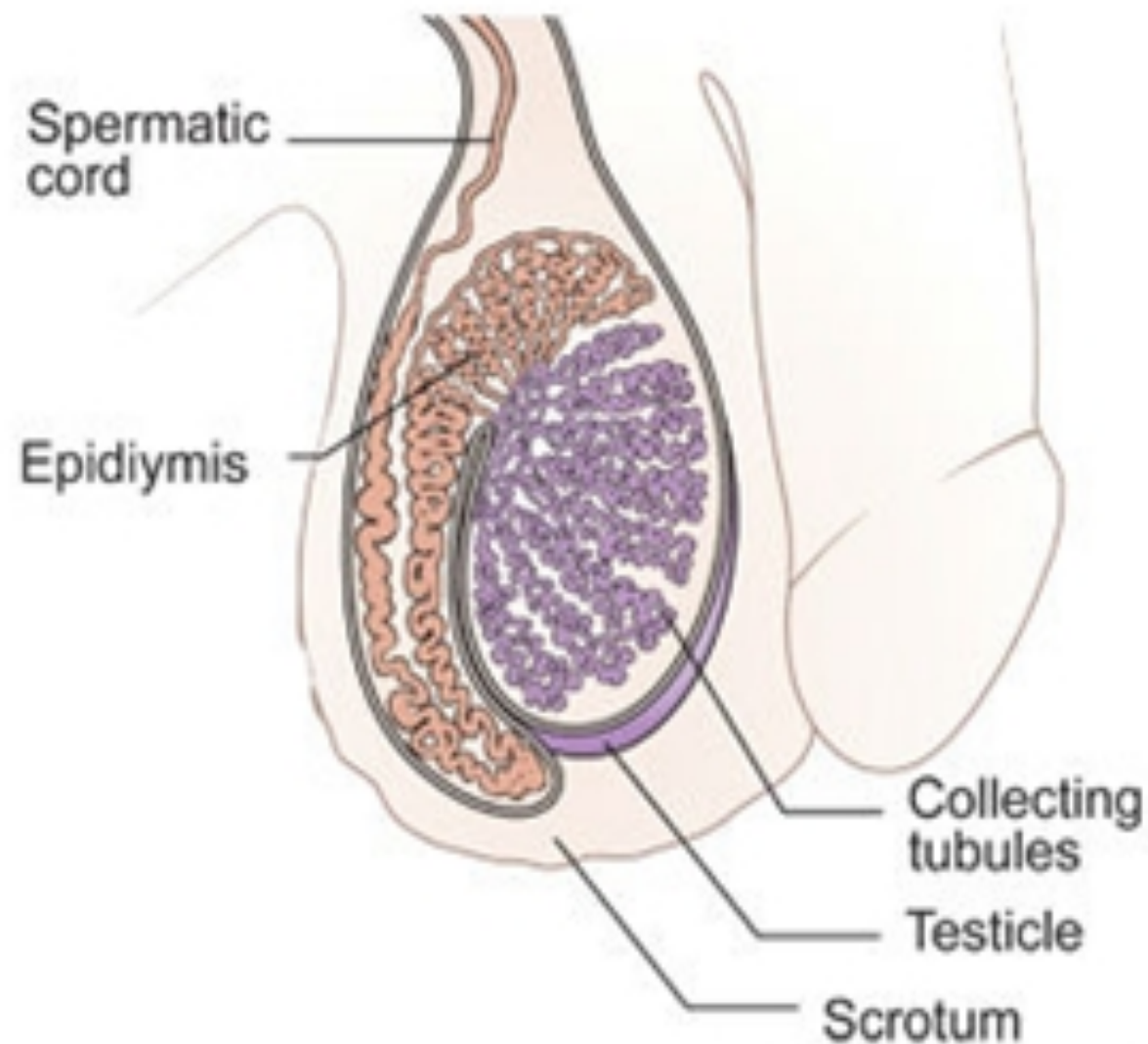
Testes



- Produce sperm cells
- Developed from germ cells in seminiferous tubules
- Secrete testosterone by interstitial / Leydig cells



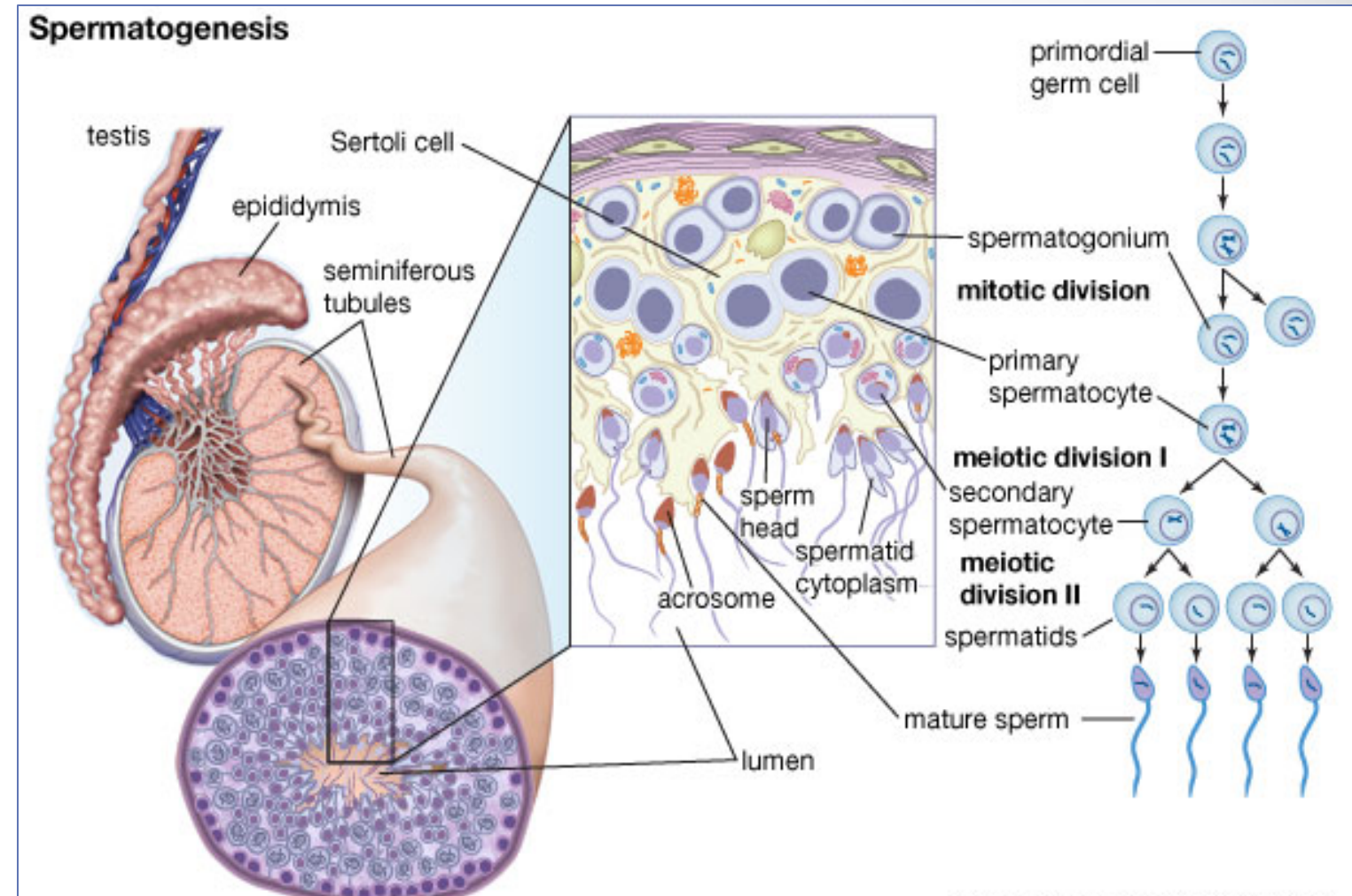
Leydig cells



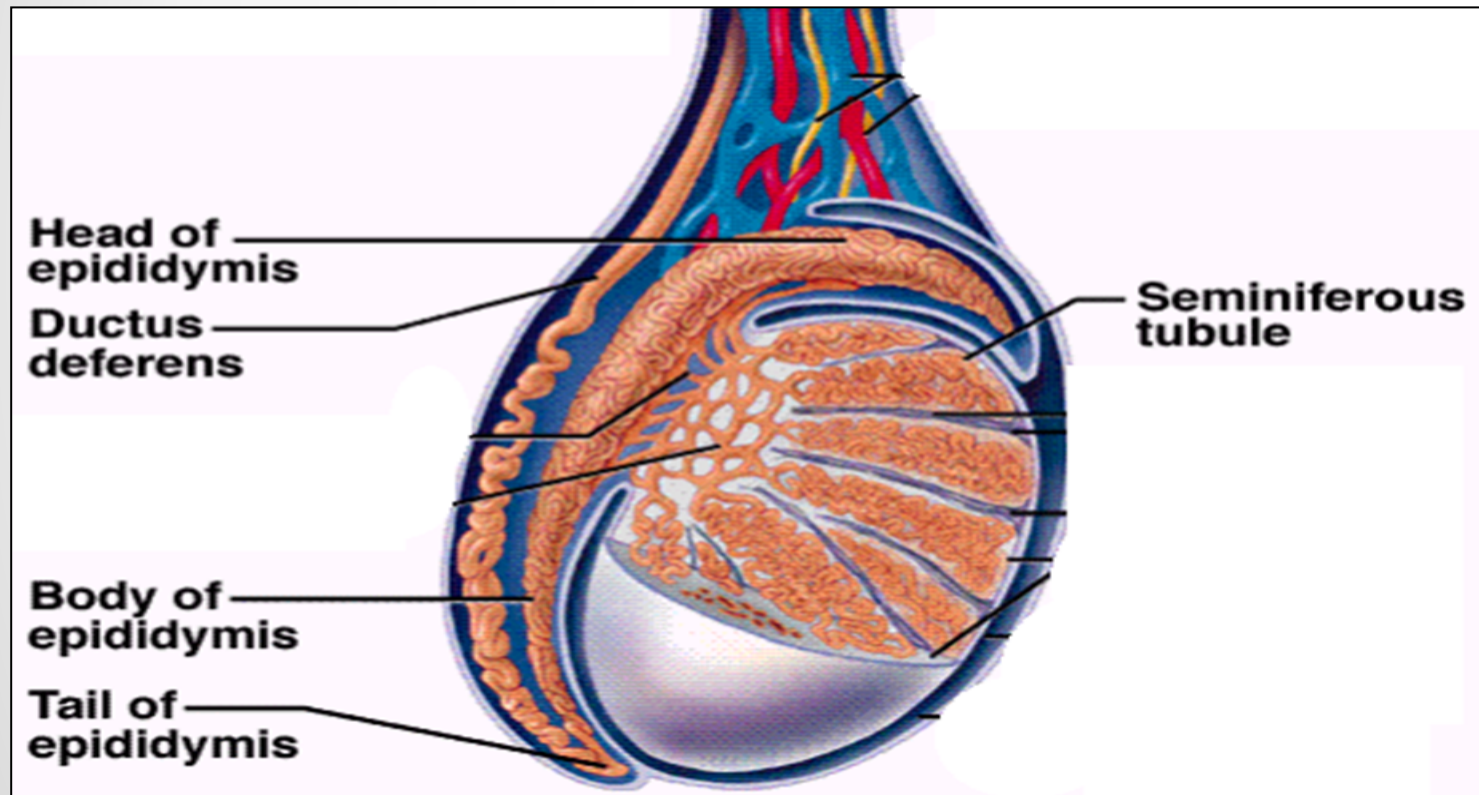
- Found adjacent to the seminiferous tubules in the testicle
- Produce testosterone in the presence of LH
 - LH binds to receptors within the cell

Seminiferous tubules

- Specific location of meiosis
- Epithelium of the tubules contain Sertoli cells
 - Tall type cells that line the tubule
 - Activated by FSH
- Inbetween the Sertoli cells
 - Spermatogenic cells
 - Differentiate through meiosis to sperm cells

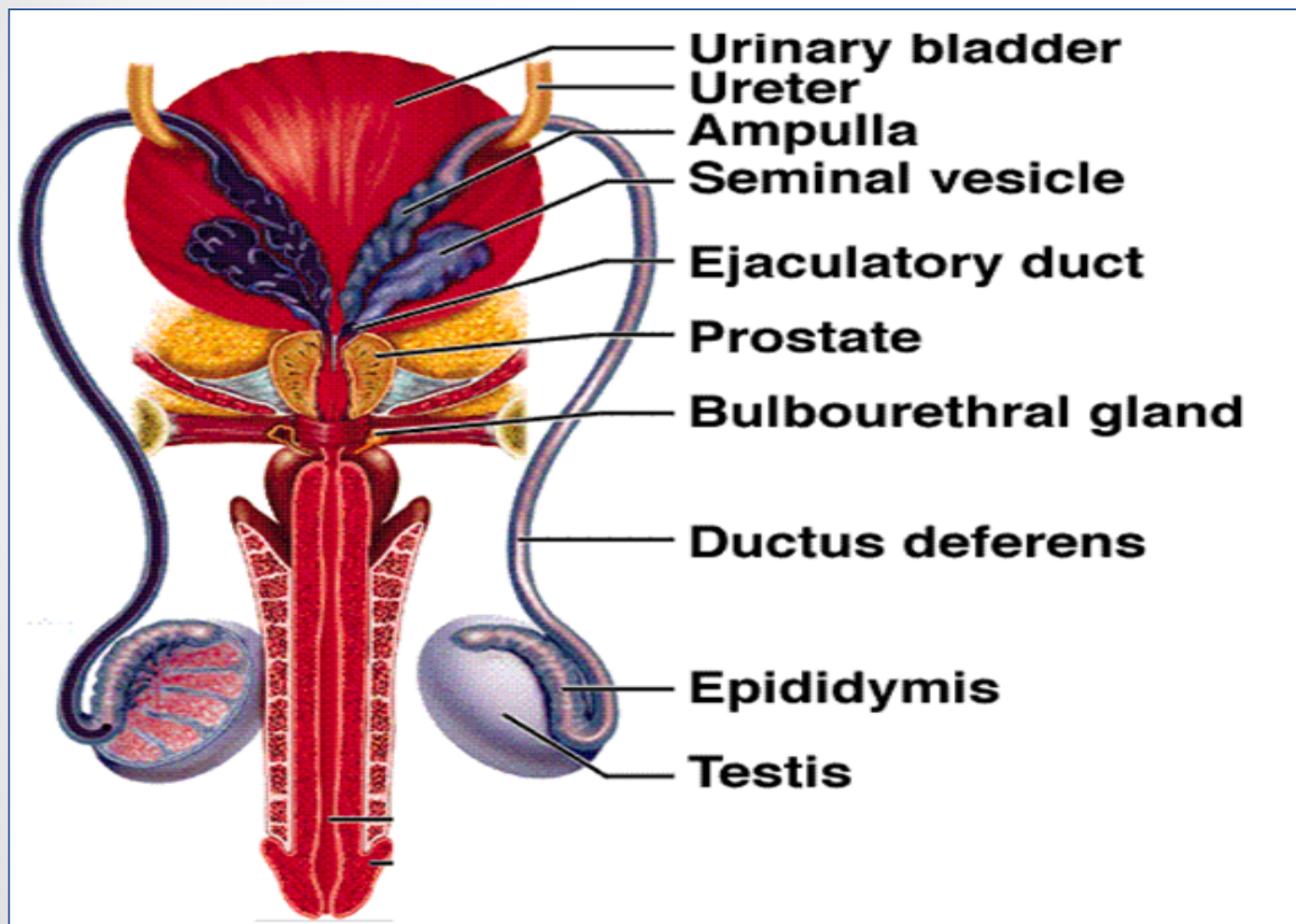


Epididymis



- Storage site of sperm cells
- Absorbs about 90% of the fluid secreted by the testis
- Sperm remain stored here for 40 – 60 days
 - Absorbed if not ejaculated prior to that time

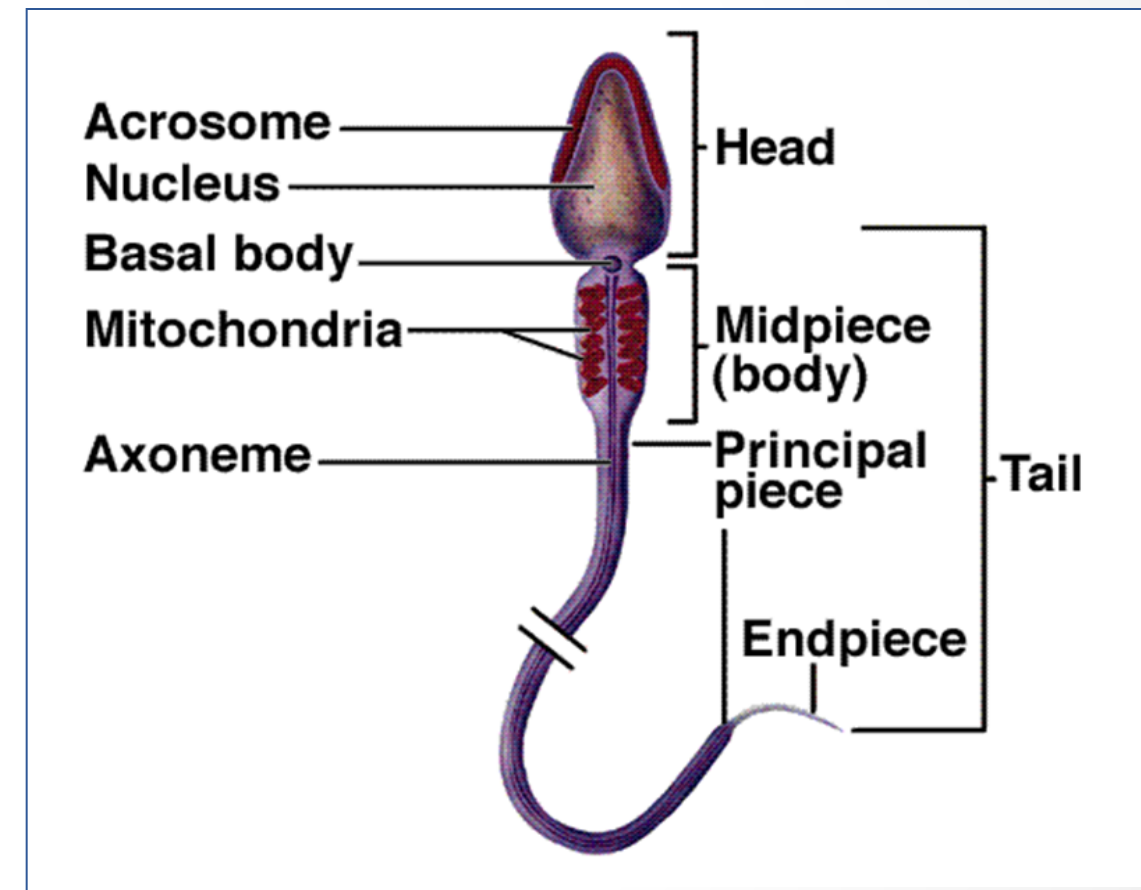
Semen



- Fluid expelled during orgasm
 - Also present in pre-ejaculate
- Mixture of secretions from:
 - Epididymis
 - Seminal vesicle
 - Prostate
 - Bulbourethral gland
- Major constituents
 - Sperm
 - Fructose
 - Clotting and anticoagulant factors
 - Prostaglandins

Sperm

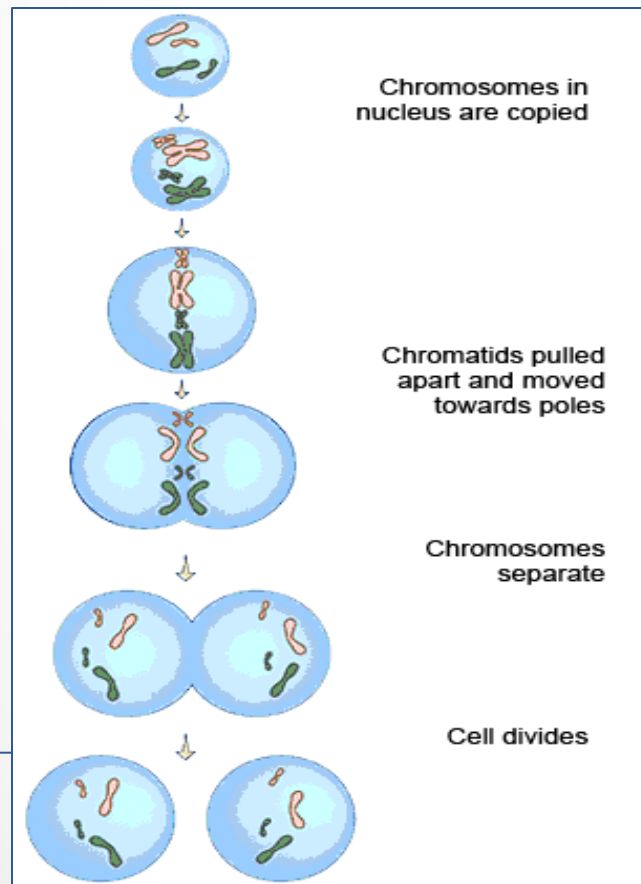
- **Acrosome**
 - Enzymes used to dissolve a path to penetrate the egg
- **Nucleus**
 - Genes
- **Mitochondria**
 - Produce ATP (adenosine triphosphate – intracellular energy transfer) – for sperm motility
- Sperm cells from epididymis are present at a count of 50-120 million sperm/mL
- Sperm count decreased from 113 million sperm/mL in 1940 to 66 million sperm/mL in 1990. Semen volume dropped by 19%.
- < 20-25 million/ml = infertility (sterility)
- **Main function**
 - Transport genomic information from male to female
 - Travels a distance of 15cm
 - Equivalent of a human swimming 40 miles



Cell division

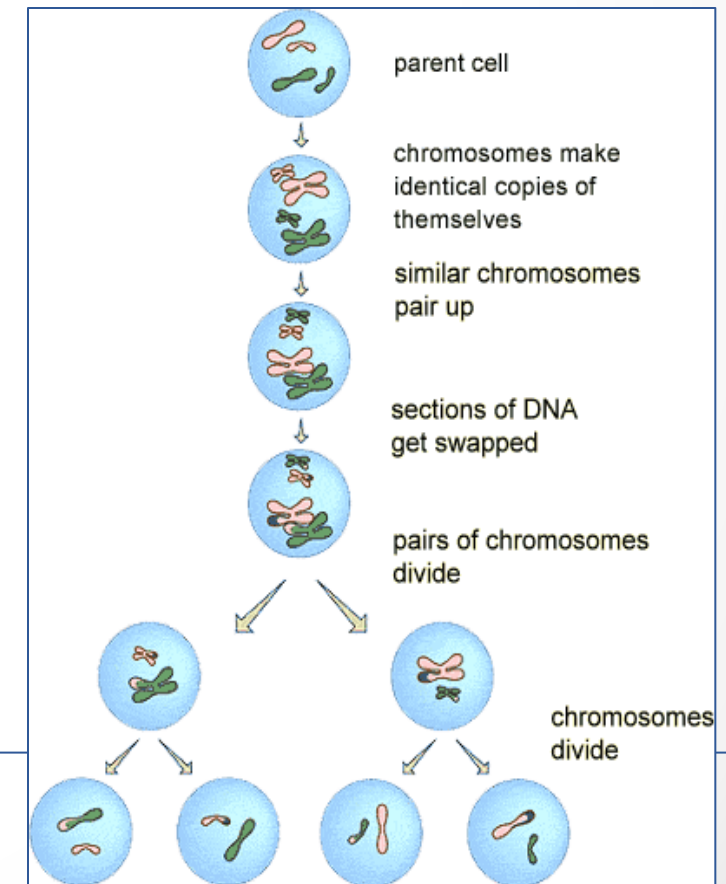
- Mitosis

- Part of the cell cycle where replicated chromosomes are separated into two new nuclei



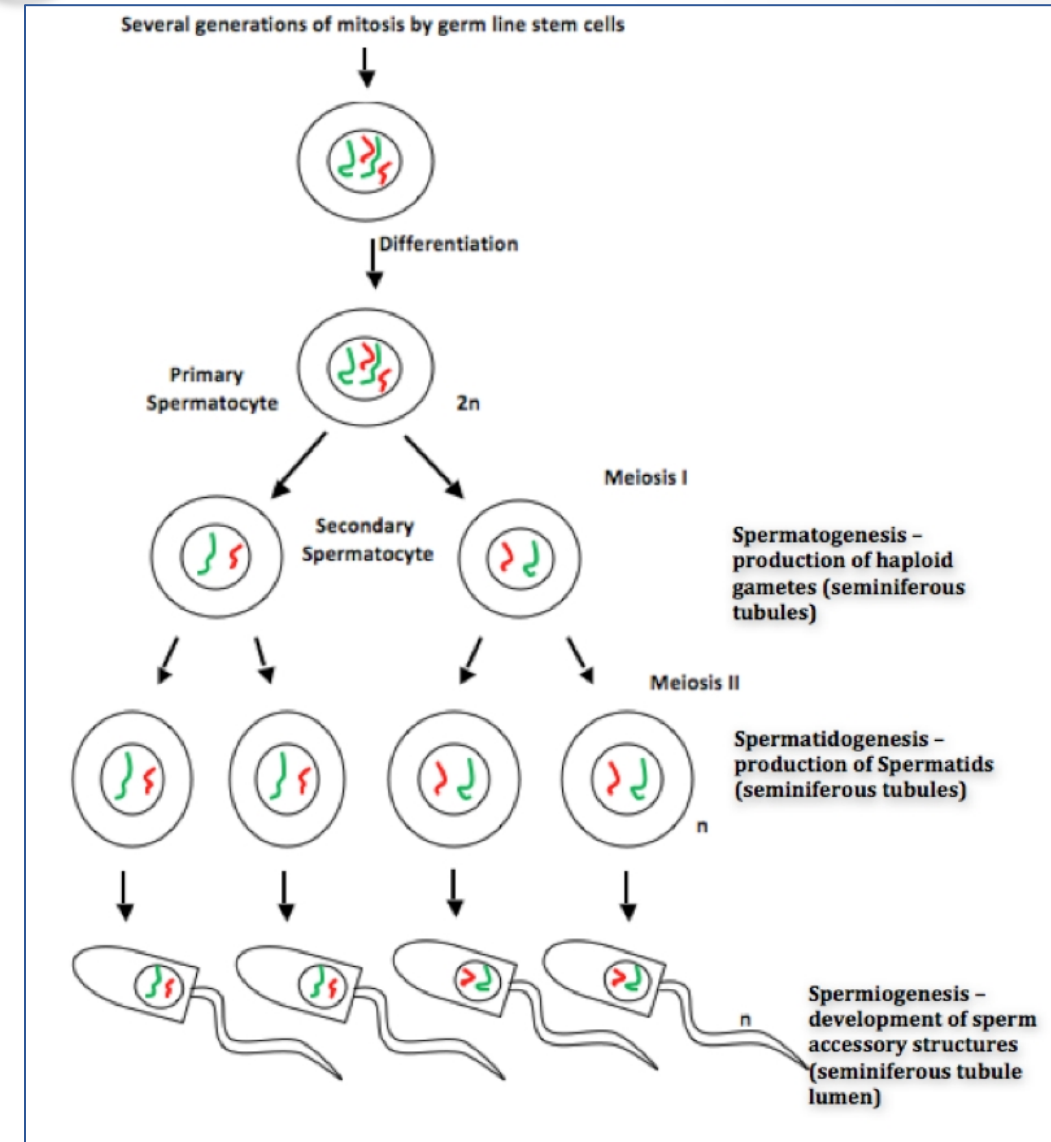
- Meiosis

- Type of cell division that produces gametes



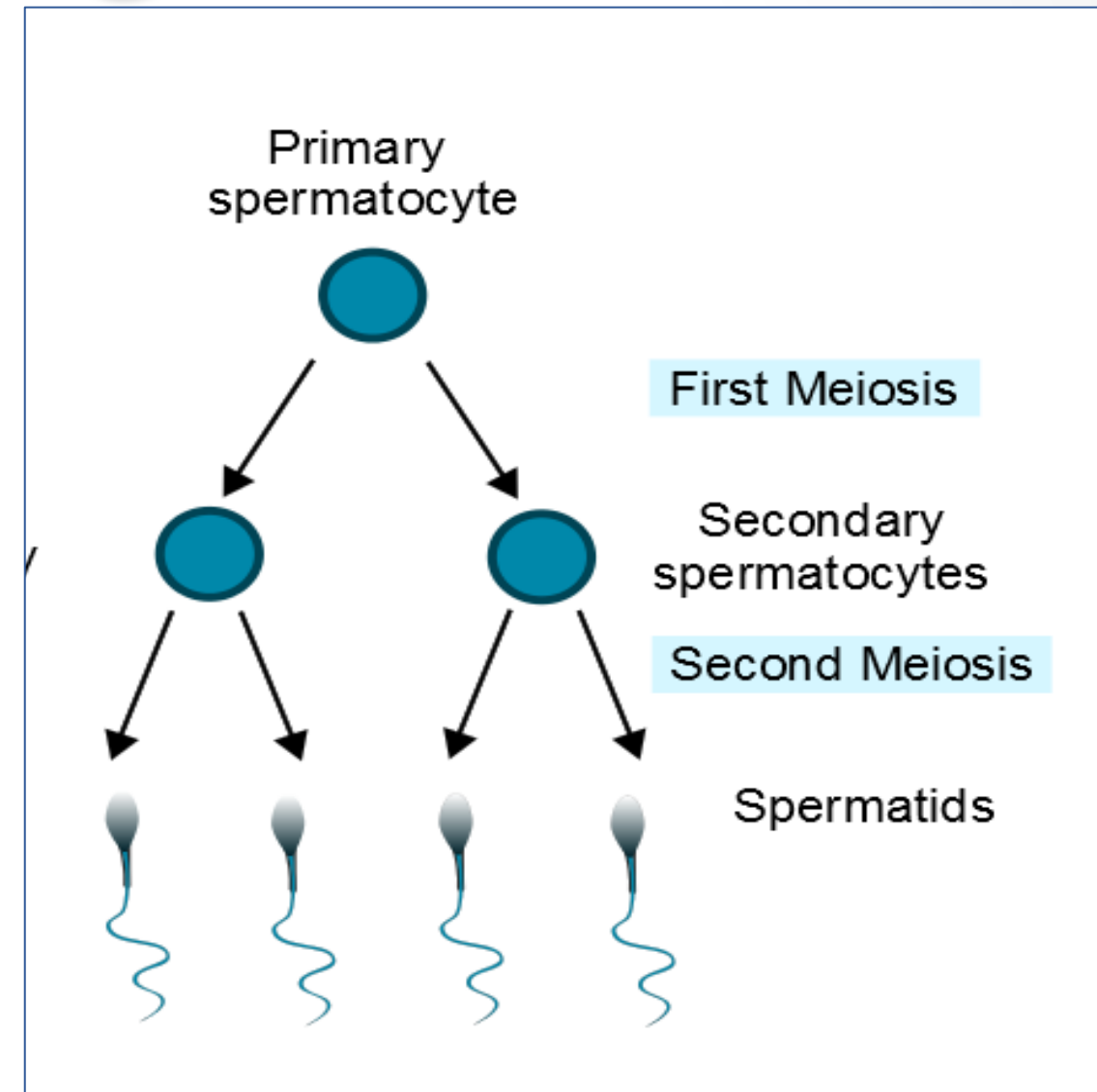
Spermatogenesis

- Spermatogenesis produces haploid male gametes that are able to fertilise a mature oocyte
- Due to the limited life span of sperm cells, production is a continuous process and maintains a daily output of >200 million differentiated sperm
- Sperm production has many steps and from start to finish takes approximately 64 days in humans
- Can be split into 3 stages:
 - Spermatogenesis,
 - Spermatidogenesis
 - Spermiogenesis.



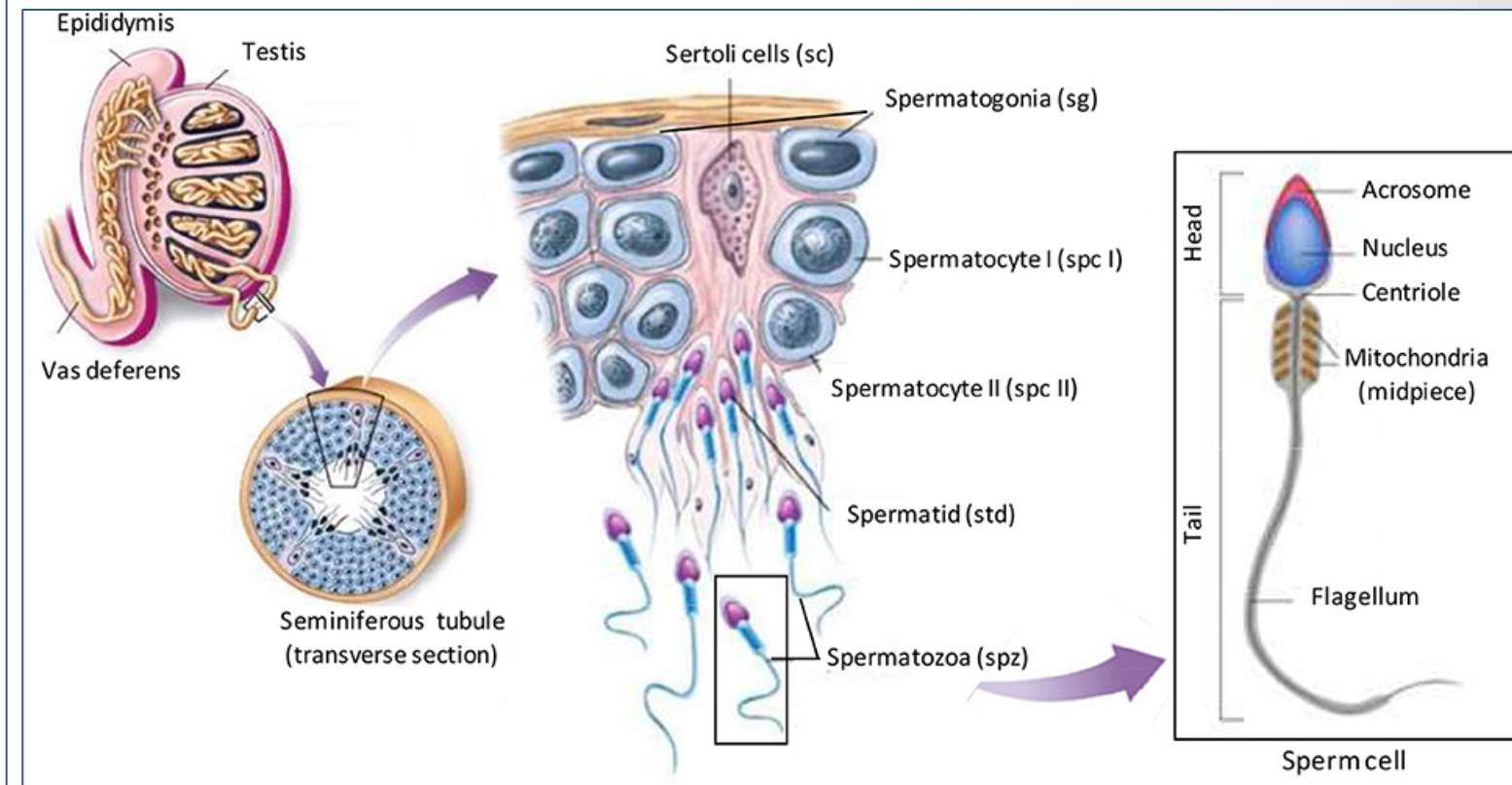
Spermatogenesis

- The production of haploid gametes
- Begins with spermatogonia, the primordial germ cells of sperm production
- Mitotically inactive until the peri pubertal period where an increase in gonadotrophin hormones from leydig cells of the testes induces massive mitotic proliferation
- Spermatogonia then enter meiosis by differentiating into primary spermatocytes
- Primary spermatocytes can either self-renew or divide into two secondary spermatocytes completing meiosis one



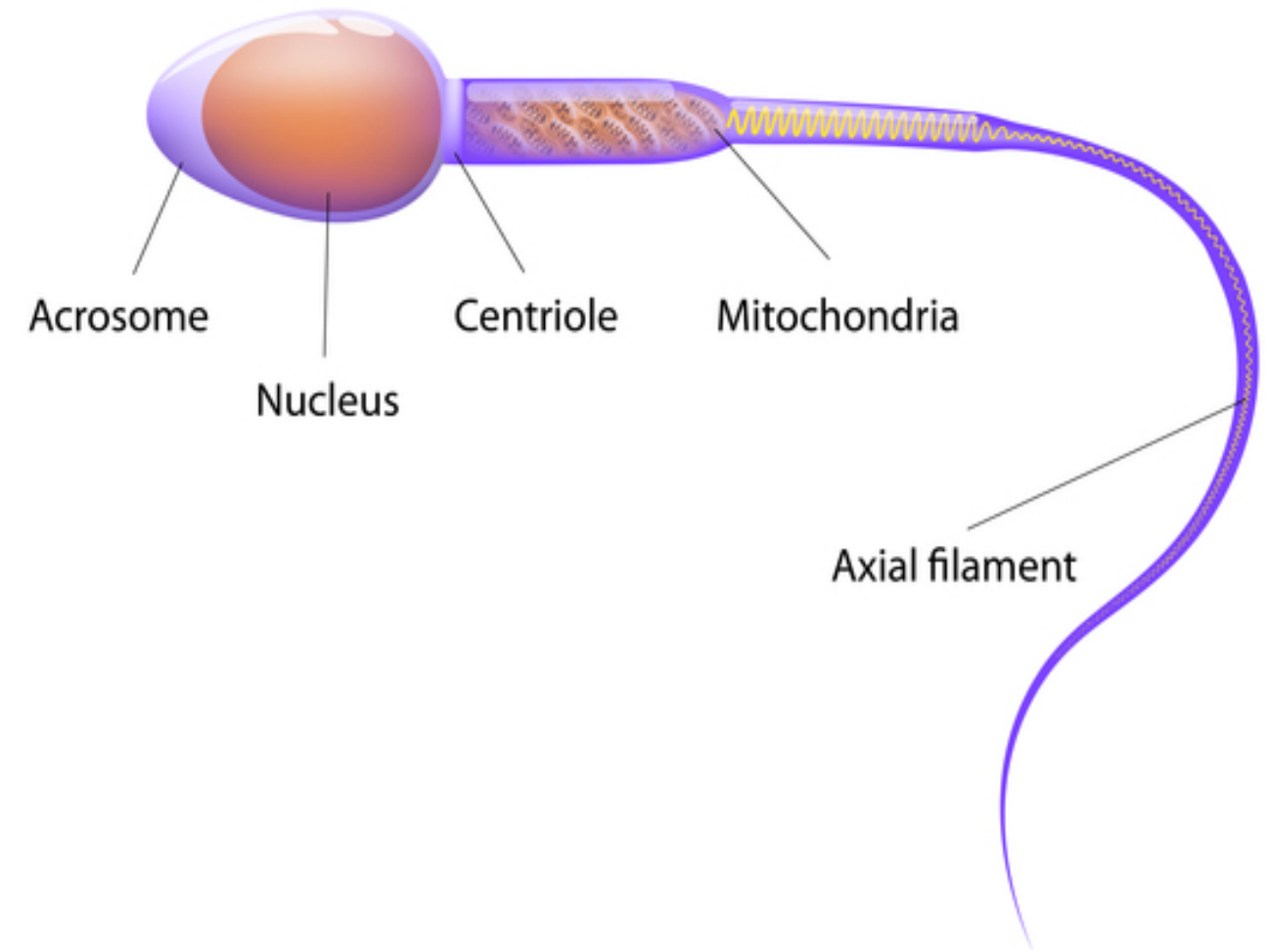
Spermatotidogenesis

- Production of spermatids
- Secondary spermatocytes then divide into four spermatids in order to complete meiosis two
- Spermatids then have many steps of sperm accessory structure to form fully differentiated sperm
 - Spermiogenesis
- Throughout the processes of spermatogenesis, spermatidogenesis and spermiogenesis all spermatogenic cells remain associated with sertoli cells in the seminiferous tubules
- Sertoli cells provide nutrients and signals such as testosterone and FSH required to stimulate and develop each stage of sperm production

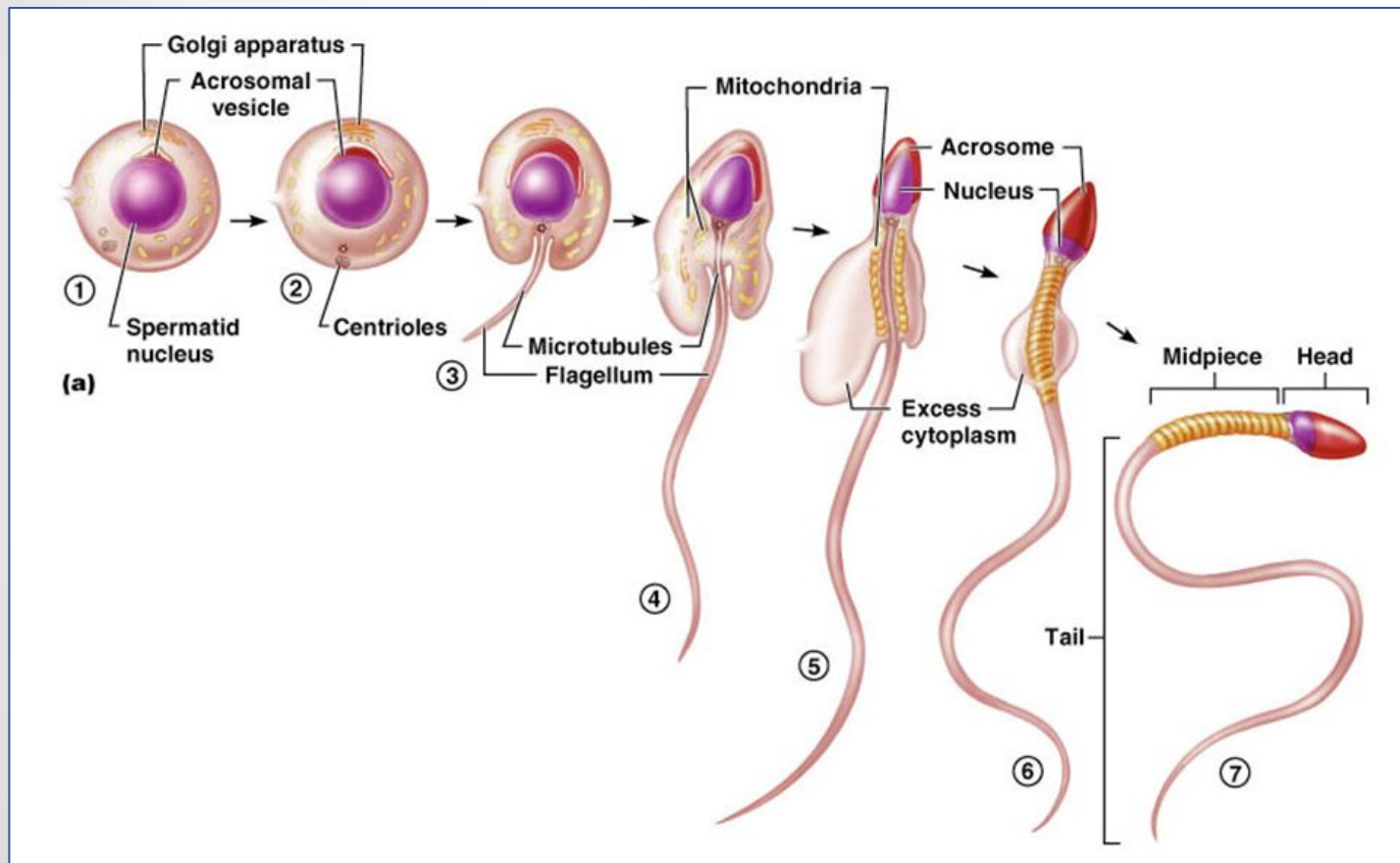


Spermiogenesis

- Elongation to produce tail and all its components
- Morphogenesis of the sperm head to a spatulate shape.
- Removal and degradation of unneeded organelles and proteins in order to achieve motility
- Maturation of the sperm induced by the input of testosterone from sertoli cells
 - Maturation involves the removal of remaining unneeded cytoplasm and organelles, which are then phagocytosed by neighbouring sertoli cells.



Developing spermatids



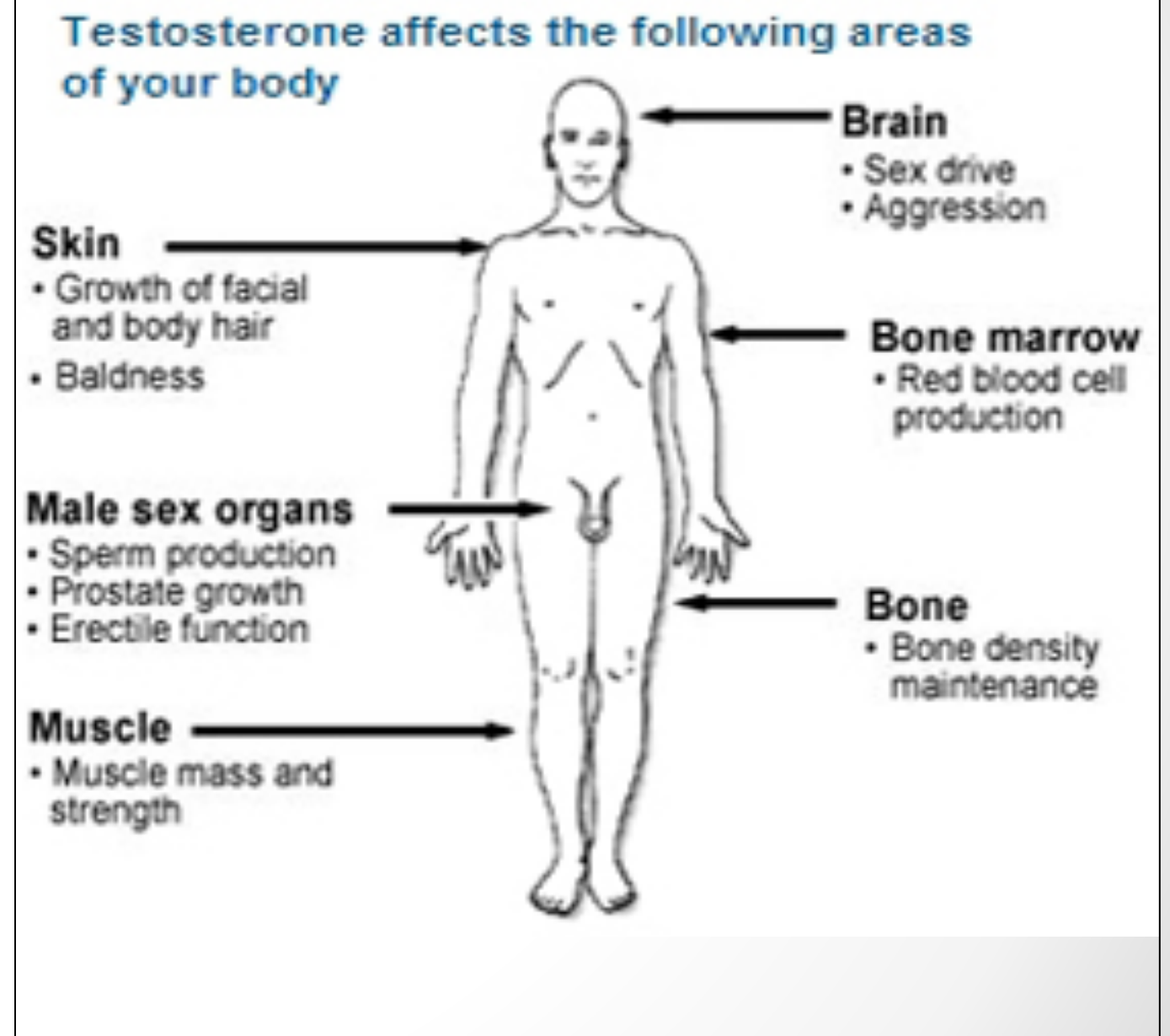
- As spermatids develop, they migrate away from the basement membrane of the seminiferous tubules moving towards the lumen
- When fully differentiated and mature, sperm cells are released from sertoli cells and bud off into the lumen of the seminiferous tubules
- Mature sperm cells are unable to swim so are transported into the epididymis for storage by peristaltic contractions of the seminiferous tubules
- The large mass and number of sperm entering the lumen coupled with active fluid secretion by sertoli cells also produces a pressure that pushes the sperm along the seminiferous tubules
- In the epididymis, maturation of the sperm continues and the sperm gain the ability to swim prior to ejaculation

Hormones in Puberty

- ANTERIOR PITUITARY GLAND
- Luteinizing Hormone (LH)
 - GIRLS
 - Increased levels trigger ovulation
 - BOYS
 - Stimulates Leydig cells to make testosterone
- Follicle stimulating Hormone (FSH)
 - BOYS
 - Induces Sertoli cells → spermatogenesis
 - GIRLS
 - Initiates follicular growth

Testosterone

- Secreted primarily in the testes in males
- Some secretion in the ovaries
- Male levels
 - 7-8 times higher than females
- Development of secondary sexual characteristics
 - Penile / clitoral enlargement
 - Increased libido
 - Hair growth: boys:
 - Facial, chest, periauricular, perianal, pubic, leg
 - Increased muscle mass
 - Broader shoulders
 - Voice deepening



Definition of normal puberty

GIRLS:

Puberty development after the age of 8 years (mean 10 yr)

BOYS:

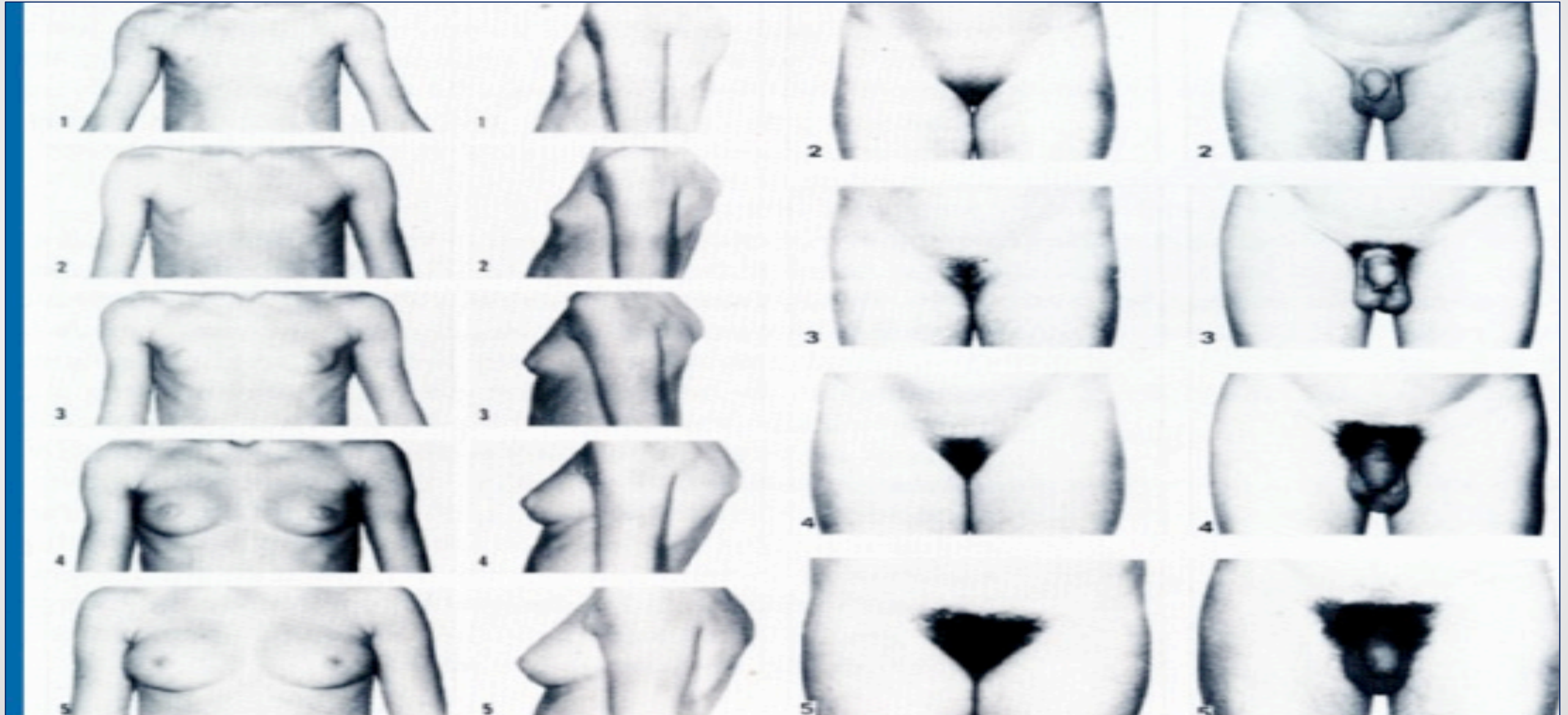
Puberty development after the age of 9 years (mean 12 yr)

Clinical aspects of normal puberty

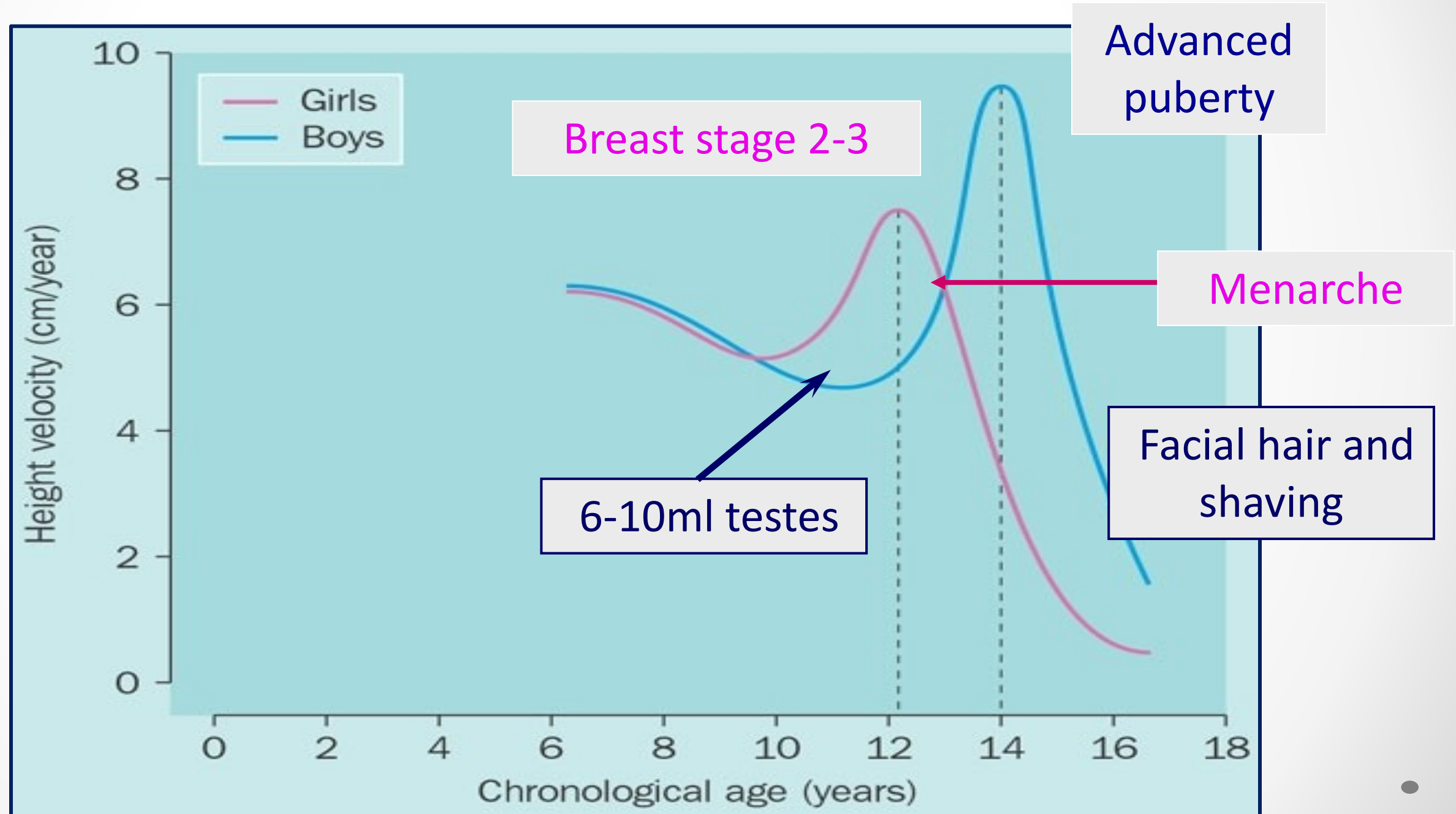
- Females: Breast (B) development
- Males: Genital (G) development
Testicular volume
- Both: Pubic hair (P) development
Axillary (A) hair development

Tanner Stages of Puberty (1 – prepubertal, 5 – mature)

Normal puberty

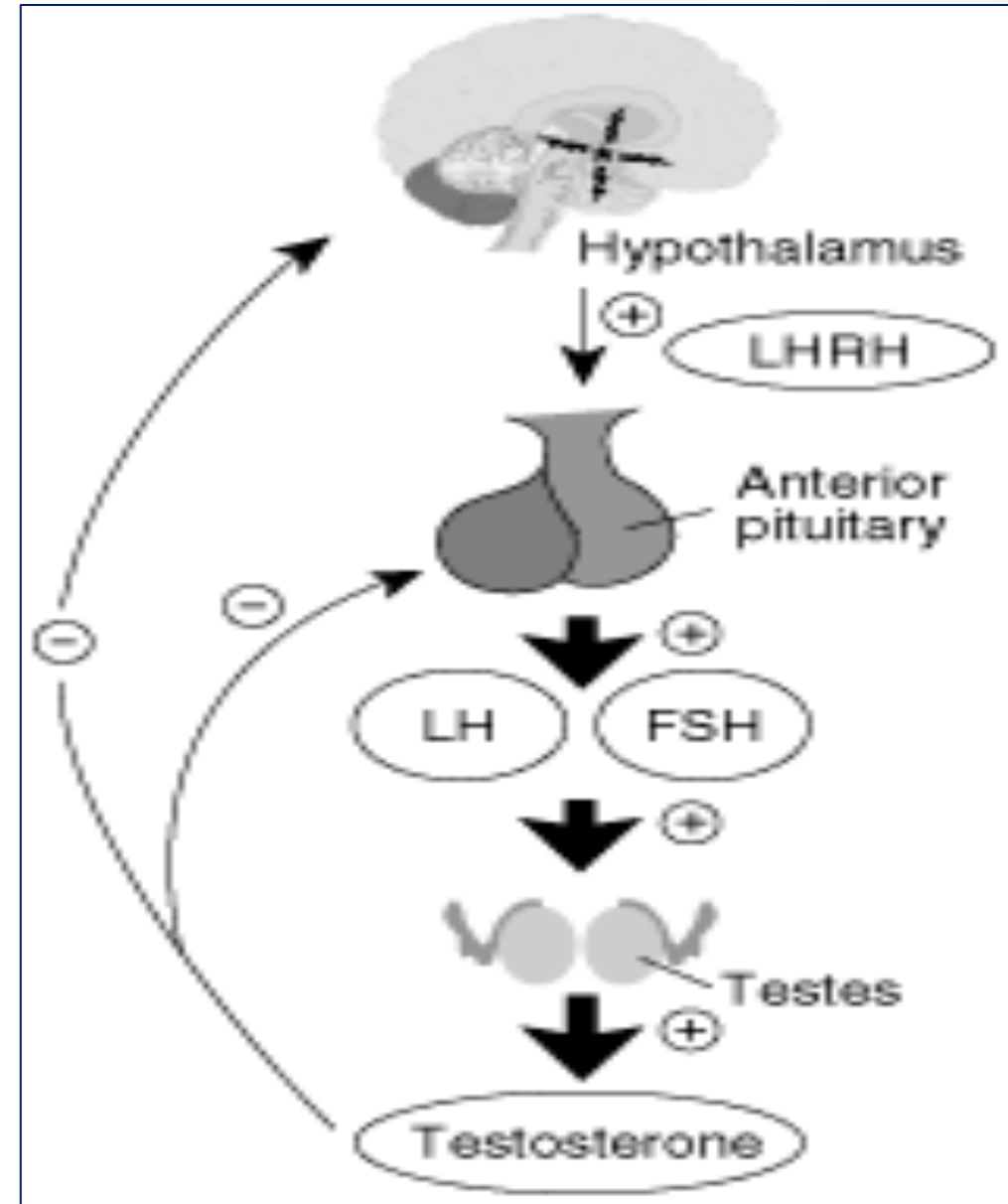


Relationship of secondary sexual features to height velocity



Boys

- LH stimulates Leydig cells to produce Testosterone
- FSH binds to receptors on the Sertoli cells, enhancing spermatogenesis



Boys

Testicular growth



Growth of penis, pubic and axillary hair growth

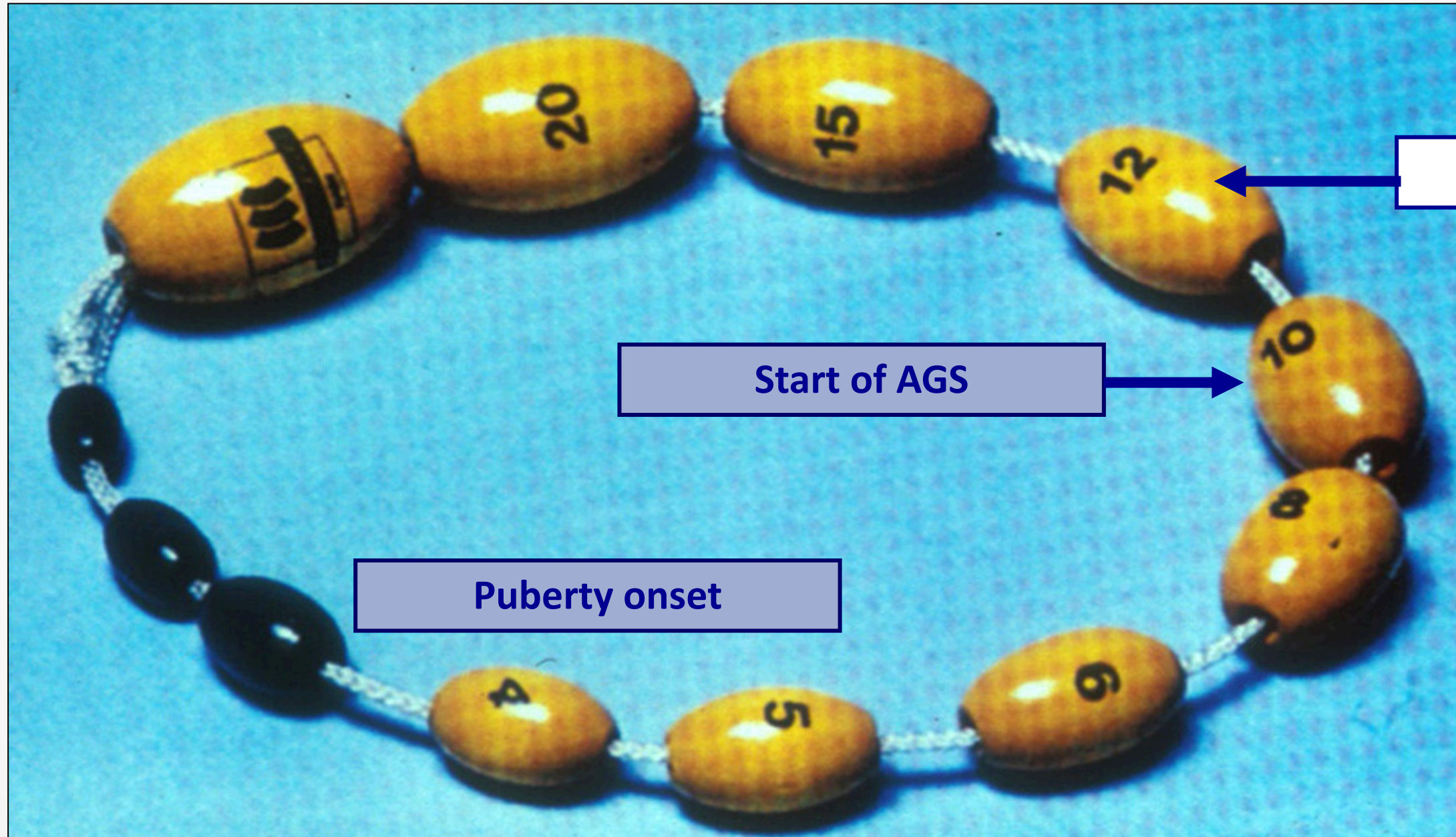


Acceleration in height velocity



Voice deepens, facial hair growth

Prader orchidometer



Testicular staging

- The testis is gently isolated & distinguished from the epididymis
- Scrotal skin is stretched without compressing the testis
- Using a Prader orchidometer, a manual side by side comparison is made between the testis & beads made to identify the bead most similar to the testis

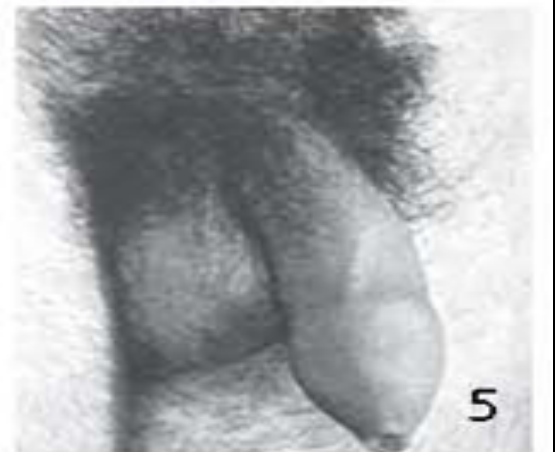
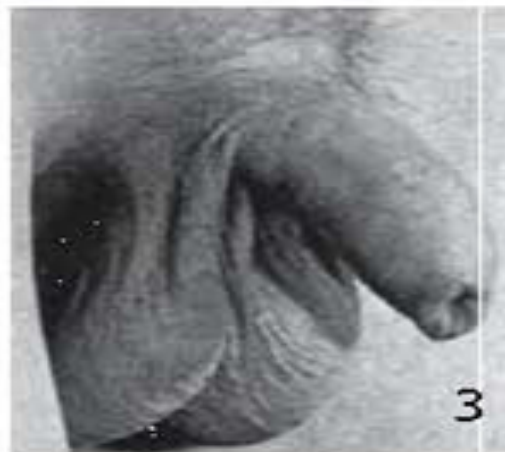
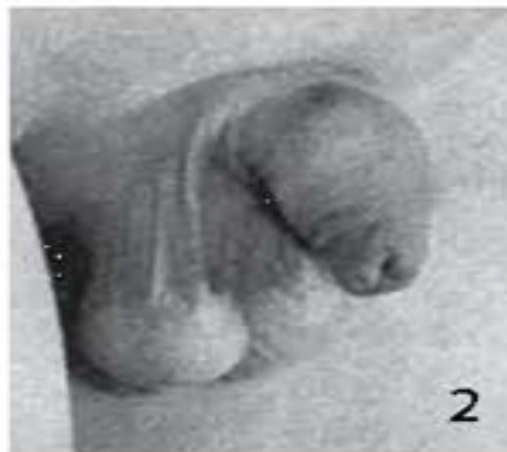
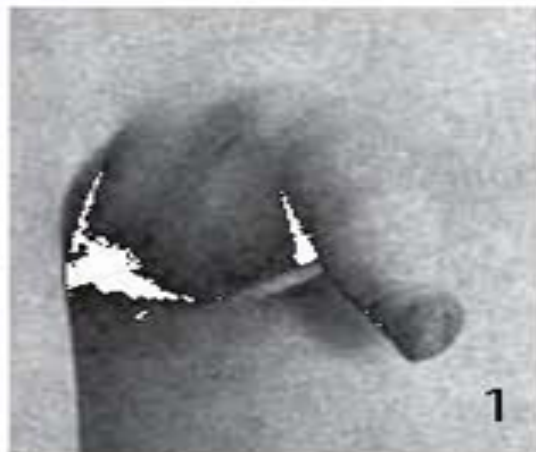


Williams & Dharmaraj (2007)



Genital staging

- **Stage I (Preadolescent)**- The testes, scrotal sac, and penis have a size and proportion similar to those seen in early childhood.
- **Stage II** - There is enlargement of the scrotum and testes and a change in the texture of the scrotal skin. The scrotal skin may also be reddened, a finding not obvious when viewed on a black and white photograph.
- **Stage III** - Further growth of the penis has occurred, initially in length, although with some increase in circumference. There also is increased growth of the testes and scrotum.
- **Stage IV** - The penis is significantly enlarged in length and circumference, with further development of the glans penis. The testes and scrotum continue to enlarge, and there is distinct darkening of the scrotal skin.
- **Stage V** - The genitalia are adult with regard to size and shape



Spermarche

- Beginning of development of sperm in boys' testicles at puberty
- Initiation of spermatozoa production usually occurs during the very early stages of puberty (Nielsen, 1985)
- Median TV was 11.5mls
 - Before peak height spurt
 - Varying from means of 13.3 years – 14.5 years
 - (Schaefer, 1990; Hirsch, 1985; Richardson & Short, 1978; Nielsen, 1985)

When things go wrong

Early puberty

Delayed puberty

Early (precocious) puberty

True precocious puberty:

Pubertal development caused by early activation of the hypothalamic-pituitary-gonadal axis

Pseudo-precocious puberty:

Pubertal development caused by sex steroids secreted without activation of the hypothalamic-pituitary-gonadal axis

Puberty in boys

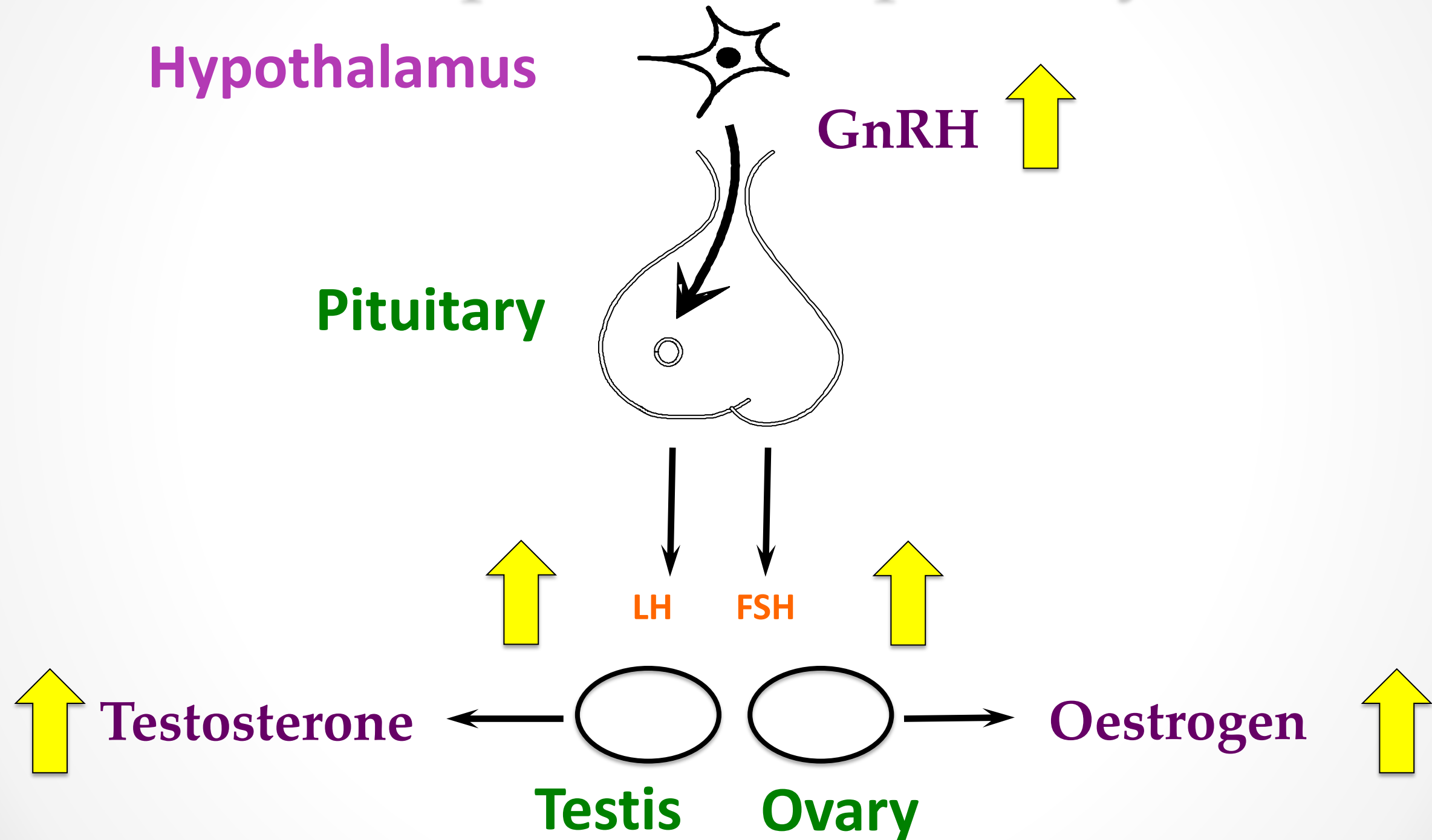
Early Puberty

- Idiopathic central precocious puberty is very rare
- Pseudo precocious puberty is rare
- Exaggerated adrenache
 - More common in girls

Delayed Puberty

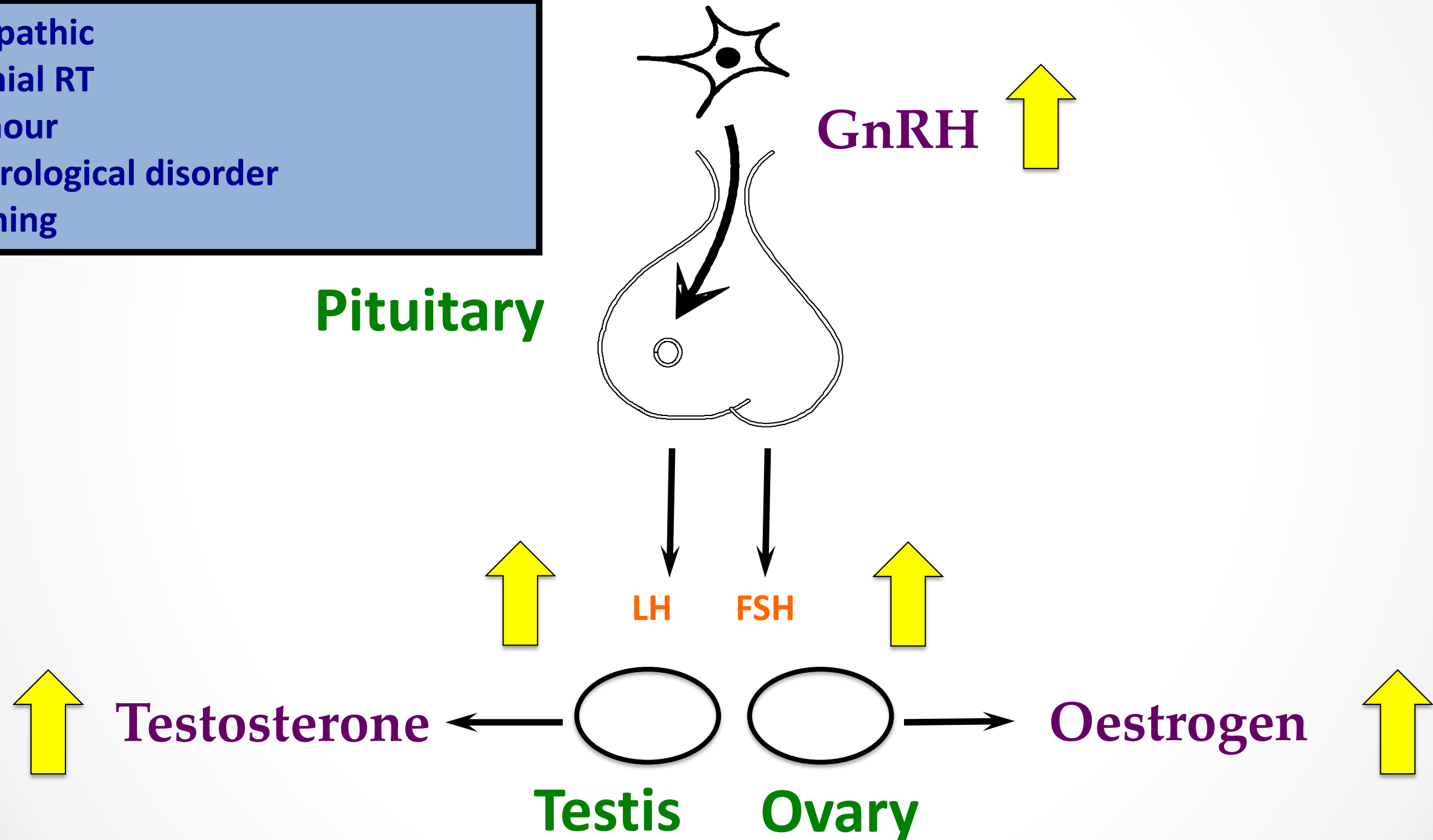
- Constitutional delay of growth and puberty
 - Most common cause of delayed puberty in boys
 - Usually a family history

True precocious puberty



True precocious puberty

- Idiopathic
- Cranial RT
- Tumour
- Neurological disorder
- Priming



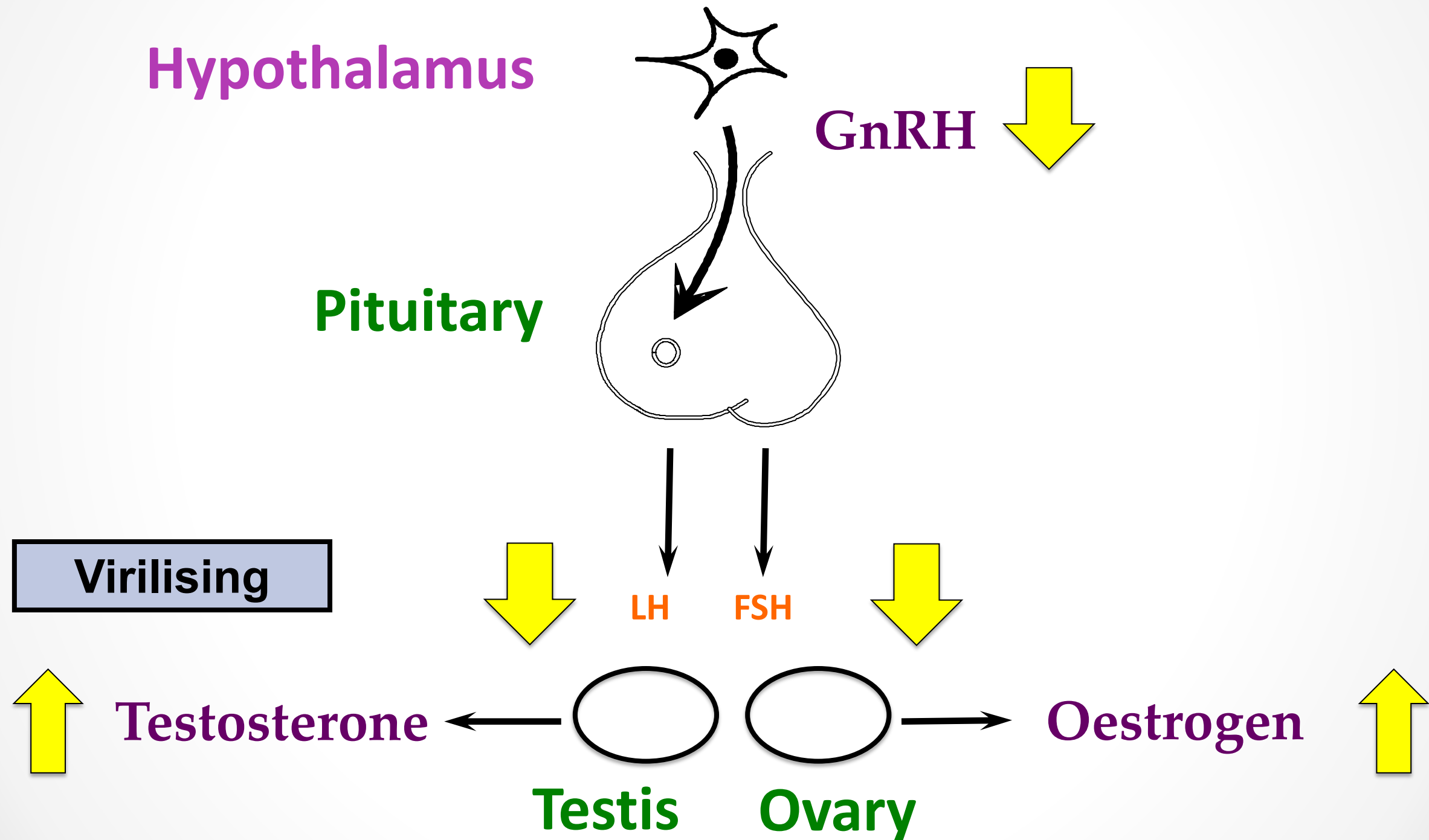
Causes of True Precocious Puberty

Organic CNS disruption:

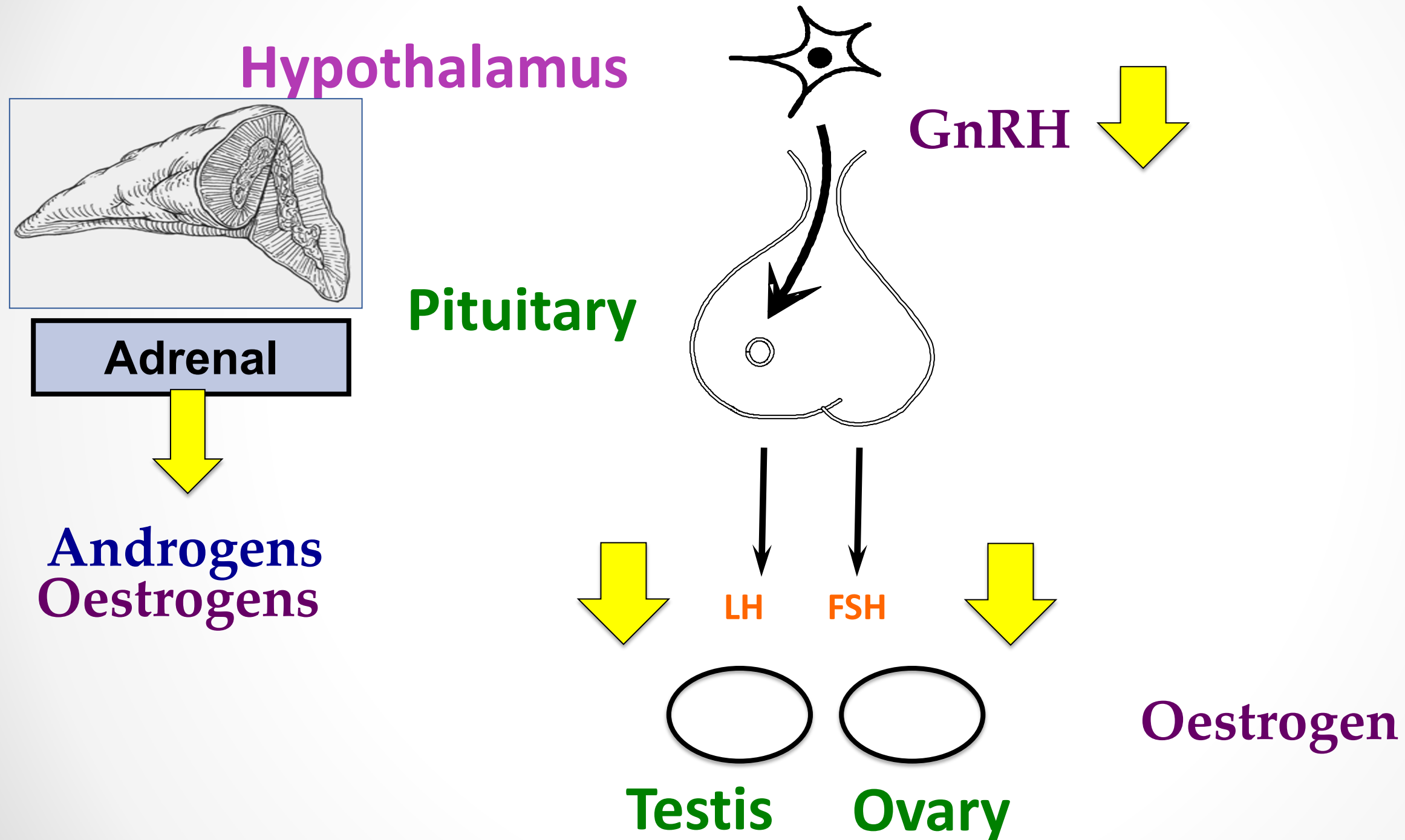
- Tumours of the hypothalamic-pituitary region
- Post head injury / meningitis
- Neurofibromatosis
- Prematurity / Cerebral Palsy
- Hydrocephalus

Post cranial surgery or radiotherapy

Pseudo-precocious puberty



Pseudo-precocious puberty



Causes of Pseudo-Precocious Puberty

Sex steroids from the adrenal:

- Congenital adrenal hyperplasia
- Adrenal tumour
- Premature adrenarche (<6yr)
- Cushing's Syndrome

Sex steroids from the gonad:

- Ovarian tumour, cysts
- McCune-Albright Syndrome
- Testotoxicosis
- HCG – secreting (germ cell) tumours

Exposure to exogenous steroids

3 yr old boy with early puberty

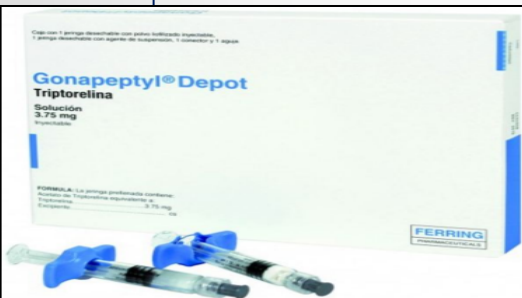
- Penile enlargement
- Pubic hair
- Moodiness
- Body odour
- Growth acceleration
 - HV 17 cm/yr
- Bone age 13 yr
- Height >>90th centile
- Tanner stage
A2 G4 P3 (2/2)



Treatment of early puberty

- GnRH analogues
- Cause a decreased release of FSH and LH
- Act via desensitization and down-regulation of pituitary GnRH receptors and their downstream pathway

Preparation	Route	Dose	Frequency	Brand
Naferelin	Nasal	1 puff	Daily	Synarel
Triptorelin	IM	60 µg/kg	Monthly	Triptorelin 3.75 mg
Leuprolide acetate	IM SC	300 µg/kg	Monthly	Leupride depot 3.75 mg
		900 µg/kg	3-monthly	Leupride depot 11.25
Gosorelin acetate	SC	3.6 mg 10.8 mg	Monthly 3-monthly	Zoladex 3.6 mg Zoladex implant
Histrelin	Implant		Yearly	
IM: Intramuscular, SC: Subcutaneous				



Causes of delayed puberty

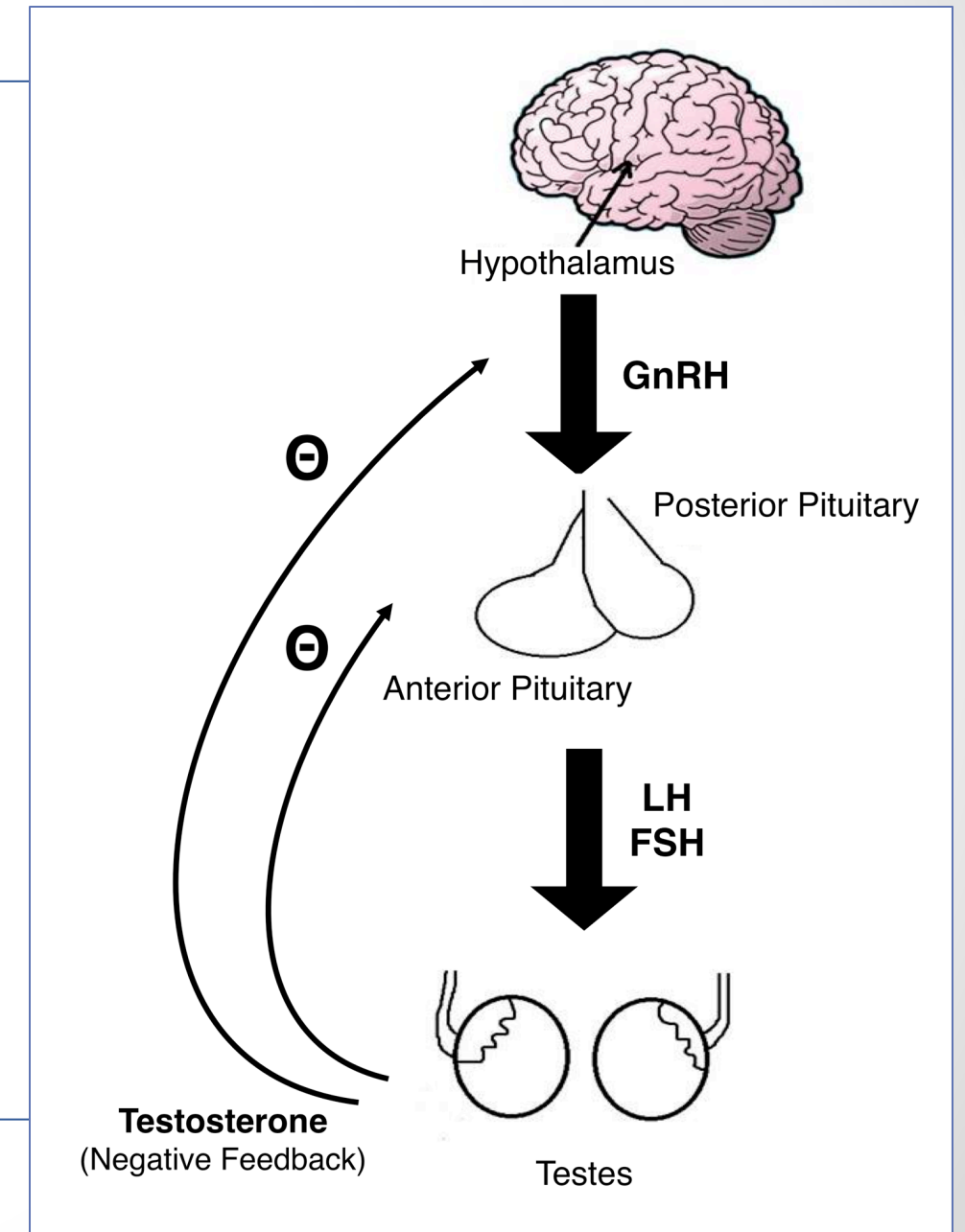
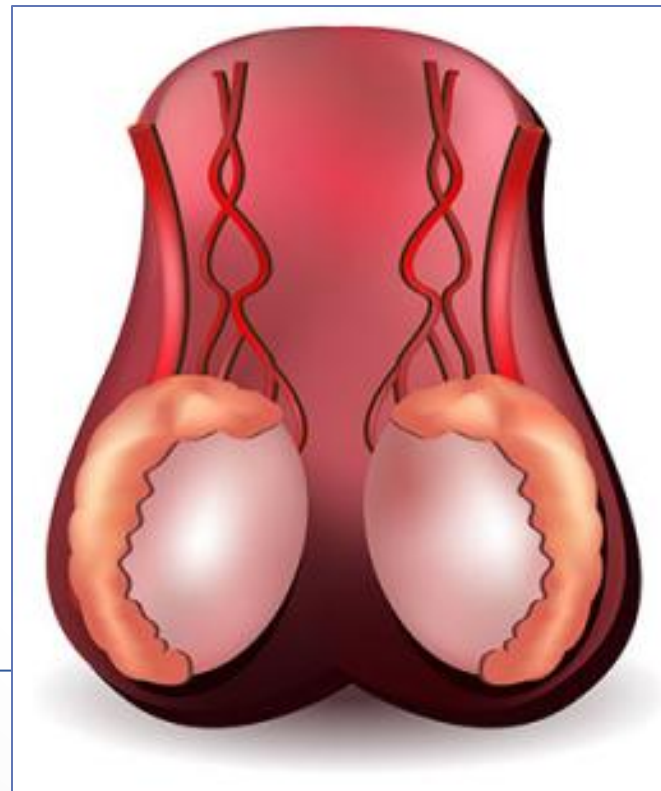
- Constitutional delay in growth and puberty
- Chronic disease
- Central causes:
 - Tumour/irradiation/trauma
 - GnRH or gonadotropin (LH/FSH) deficiency
- Peripheral causes:
 - Testicular damage / cryptorchidism
 - Gonadal dysgenesis (Turner's, Klinefelter's)
 - Irradiation/chemotherapy

Delayed Puberty

- Delayed puberty
 - Absence of secondary sexual development
 - Girl 13 yrs
 - Boy 14 yrs
- Pubertal failure
 - Fails to begin
 - Having begun, fails to complete
- Delayed menarche
 - First period after 15 yrs
- Primary Amenorrhoea
 - Failure to start periods
- Secondary Amenorrhoea
 - When periods stop after having become established

Delayed Puberty

- Central
 - Intact Hypothalamic – Pituitary axis
 - Impaired Hypothalamic – Pituitary axis
- Peripheral



Delayed Puberty

Central

- Intact H-P axis
 - Constitutional Delay of Growth in Adolescence (CDGA)
 - Chronic systemic disease eg asthma
 - Poor nutrition
 - Steroid therapy
 - Hypothyroidism

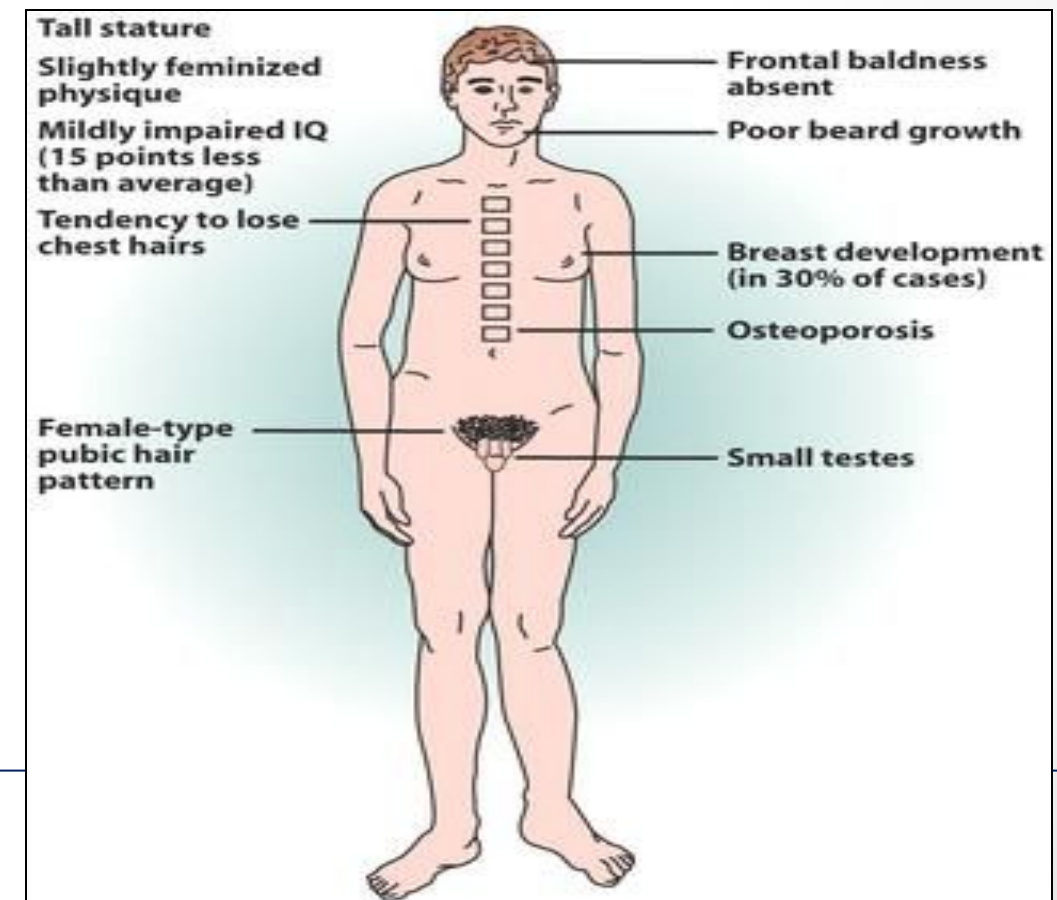
Delayed Puberty

Central

- **Impaired H-P axis**

- Nearby tumours
 - Craniopharyngioma
 - Optic glioma
 - Germinoma
 - Astrocytoma
- Congenital anomalies
 - S.O.D
 - Panhypopituitarism
- Irradiation therapy
- Trauma eg head injury

- GnRH / FSH deficiency
 - Congenital idiopathic
 - Kallmann's syndrome
 - Prader Willi syndrome



Delayed Puberty

Peripheral (Gonadal)

- **Boys**

- Bilateral testicular damage
- Noonans / Prader Willi
- Gonadal dysgenesis
 - Klinefelters
 - XO / XY
- Irradiation / Chemo

- **Girls**

- Gonadal dysgenesis
 - Turners
- Irradiation / Chemo
- DSD disorders
 - Inc. CAIS
- PCOS

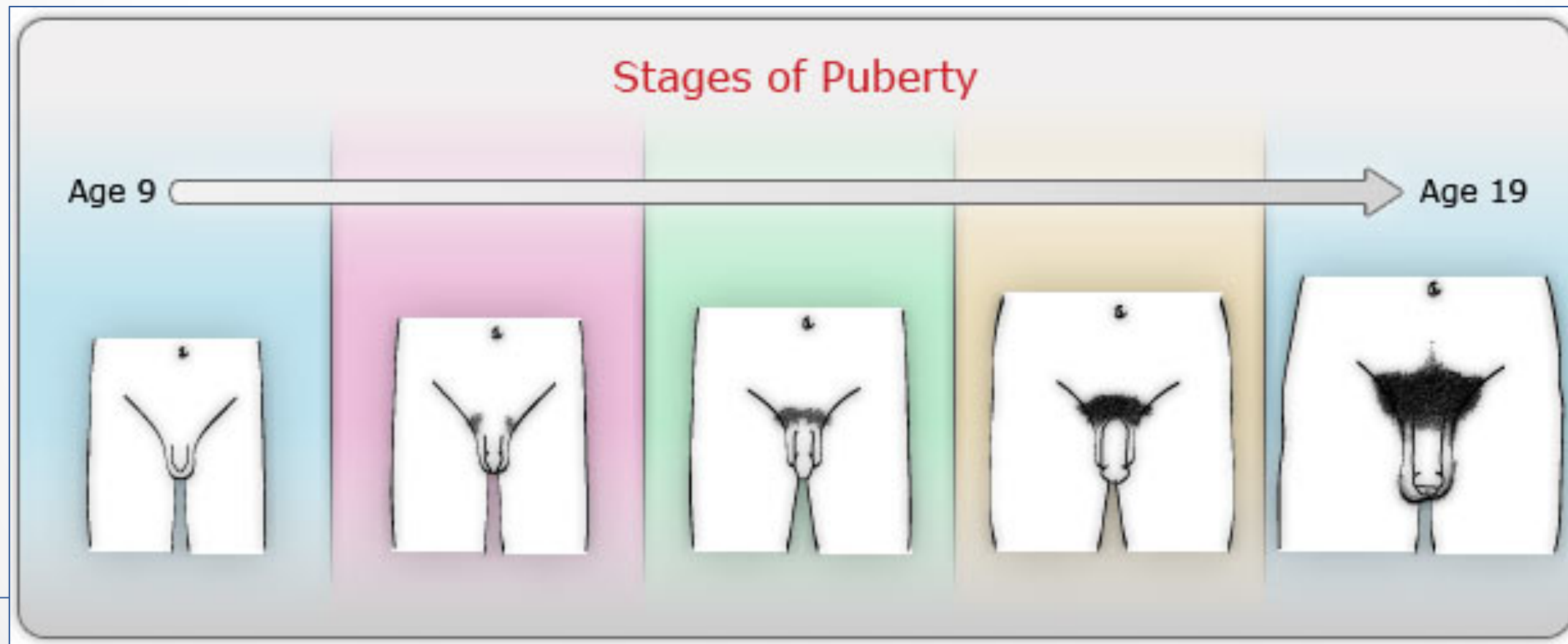
Treatment of delayed puberty



- **Testosterone**: injections, gel
- LH and FSH injections
- **Estradiol**: tablets, patches
- Progesterone
- HRT
- (Oxandrolone)

Other ..

- What other clinical indications in children would it be important to assess puberty in boys?



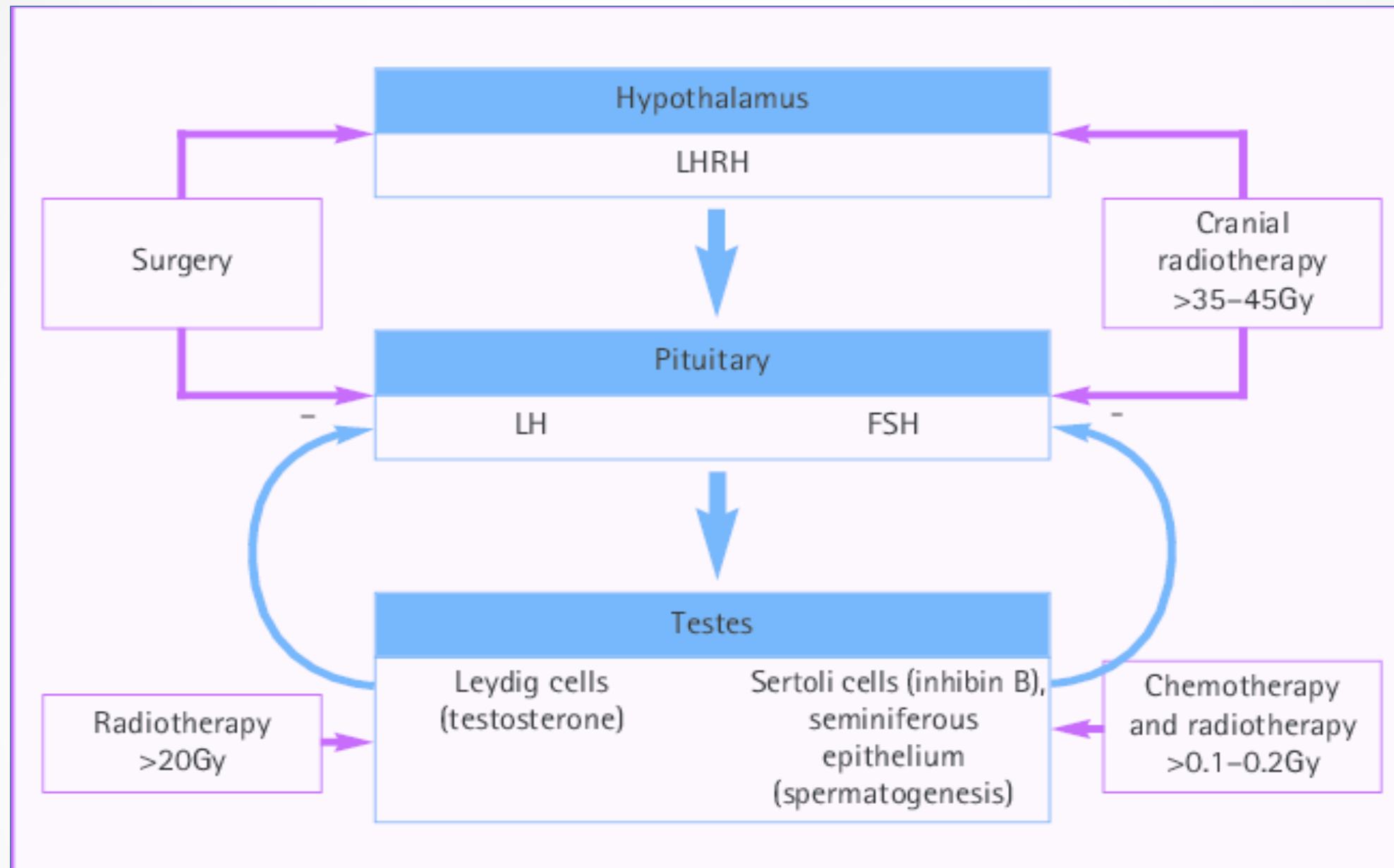
Childhood cancer

- As a result of aggressive yet effective chemo and radiotherapeutic intervention
 - Between 70% and 80% of children with oncological diseases survive their malignancies
 - 1 in 715 people in the UK is a childhood cancer survivor
 - Incidence of cancer is increasing at a rate of 2% per year in adolescents and 1.1% in children
 - Reproductive health is a leading concern in young adult survivors
- (Wyns, 2010)



Cancer treatment

- Treatment not only kills cancer cells but also germ cells
- Radiation induces germinal depletion in a dose dependent manner
 - More immature cells are more sensitive
- Doses as low as 0.1 – 1.2 Gy can damage dividing spermatogonia and result in oligozoospermia
 - Doses more than 4Gy may result in complete sterility
- 85% of adult patients found to be azoospermic after TBI and cyclophosphamide administration



Schematic representation of the hypothalamo-pituitary-testicular axis and potential sites of disruption secondary to various cancer treatment modalities (purple arrows). Thin blue arrows indicate physiological negative feedback mechanisms

(Gan & Spoudeas, 2013)

Fertility preservation

- Cryopreservation of ovarian tissue can preserve unfertilised immature germ cells
 - Still experimental
- Ovarian stimulation and oocyte retrieval
 - Not ethical in pre-pubertal girls
- Testicular cryopreservation
 - Not shown in humans
- Semen cryopreservation
 - Conventional

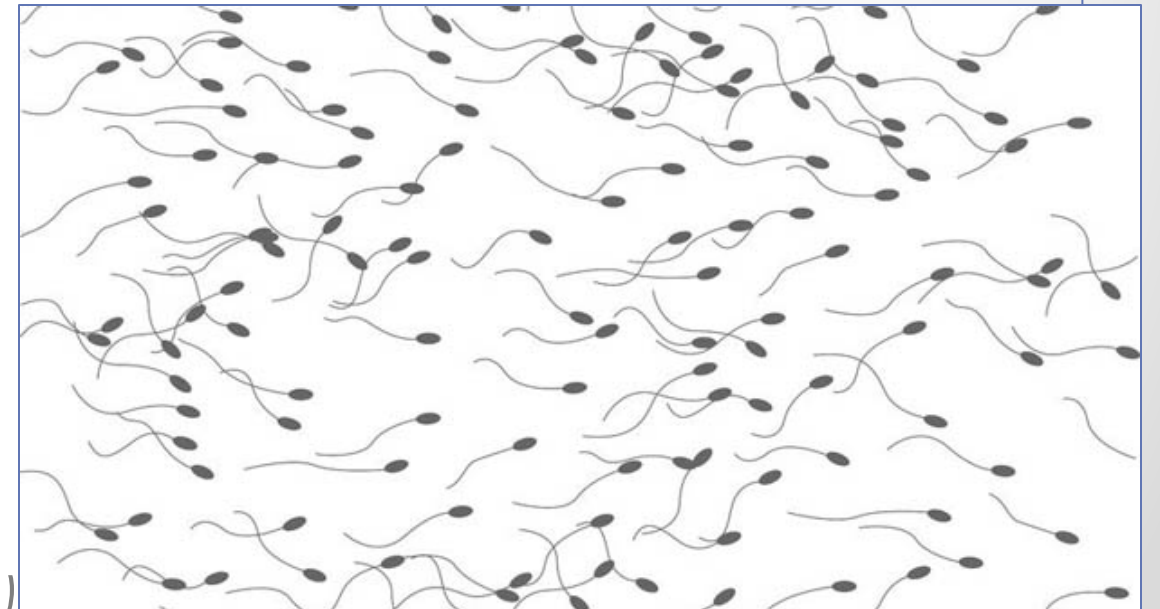
(Oktay, 2005)

Fertility preservation an integral part of paediatric oncology care

Semen cryopreservation in adolescent boys

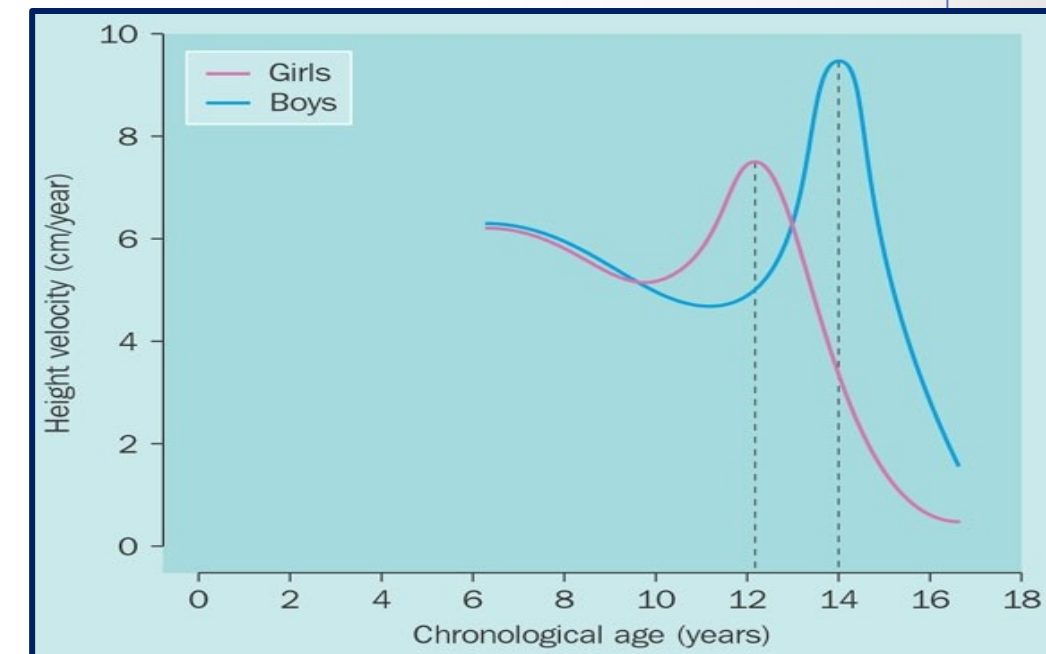
- Poignancy
 - Child / young person and family faced with life threatening diagnosis
 - Guided into decisions about future parenthood
 - ? Welcome expression of medical professional's opinion that their child has a future
- Fertility discussions at diagnosis
 - Professional sensitivity
 - Lack of embarrassment
 - Clarity in information sharing
- High levels of patient satisfaction
 - Being offered the opportunity to bank sperm
 - Needs to be done within days of diagnosis

(Crawshawe, 2009)



Paediatric endocrine intervention

- Pre-treatment plasma endocrine biochemistry
 - LH, FSH, Testosterone
- Reliable measurement
 - Pubertal staging
 - Testicular volumes
- Is the boy old enough to physically be able to produce a sperm sample?
- When is spermarche?
 - Initiation of spermatozoa production usually occurs during the very early stages of puberty (Nielsen, 1985)
 - Median TV was 11.5mls
 - Before peak height spurt
 - Varying from means of 13.3 years – 14.5 years
 - 12.6 years, Tanner stage 3, TV 8mls (Gan & Spoudeas, 2013)



Conclusion

- Importance of understanding male pubertal development
- Clinical incidences in puberty
- Childhood cancer
 - Fertility preservation
 - Advisable to introduce topic of semen cryopreservation to boys from age 12 onwards
 - Issues of concern
 - Modifying adult services to meet adolescent's needs
 - Consent
 - Parental / child
 - HIV, Hepatitis B and C testing
 - Ethical considerations of sperm storage
 - Use of stored samples after possible death / mental incapacitation

