REPRODUCTIVE ENDOCRINOLOGY

Sandwell & West Birmingham Hospitals

NHS Trust

Alexandra Thurston-Postle

OVERVIEW

- Hypothalamic-Pituitary-Gonadal Axis
  - Females
  - Males
- Overview of reproductive hormones
- Pathophysiology & Investigations
  - Females
  - Males
- Assays and Analytical Considerations
- Cases

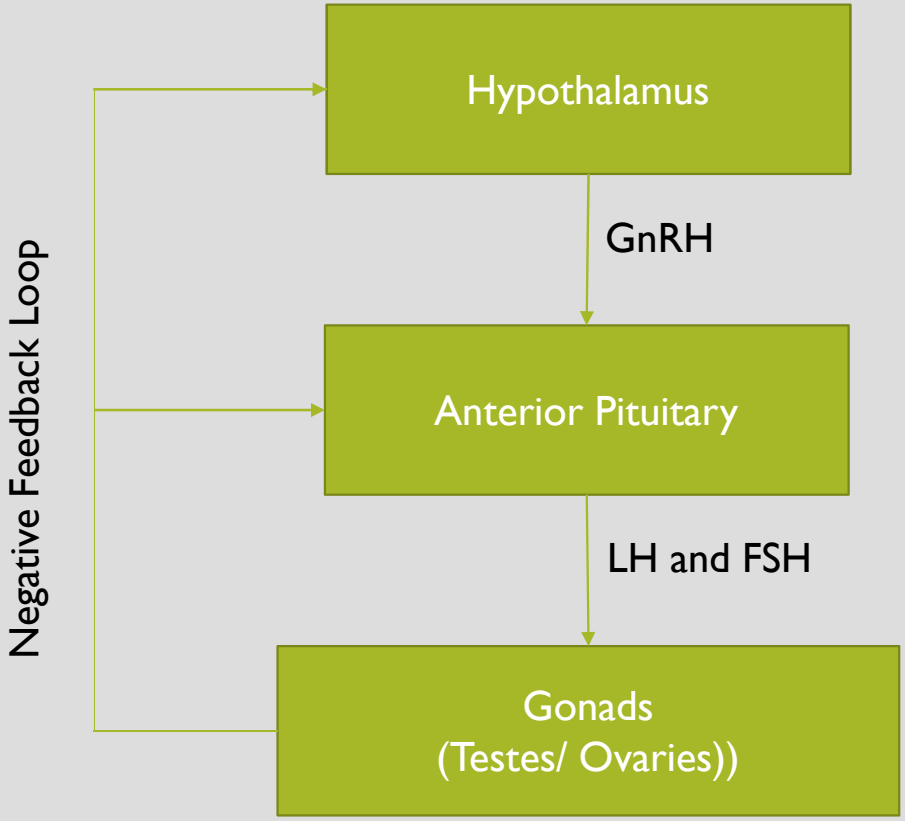
HYPOTHALAMIC-PITUITARY-GONADAL AXIS

- Hypothalamus: episodic secretion of **Gonadotrophin Releasing Hormone (GnRH)**

- GnRH stimulates synthesis and release of the gonadotrophins, **Leutinising Hormone (LH) and Follicular Stimulating Hormone (FSH)**, from the anterior pituitary

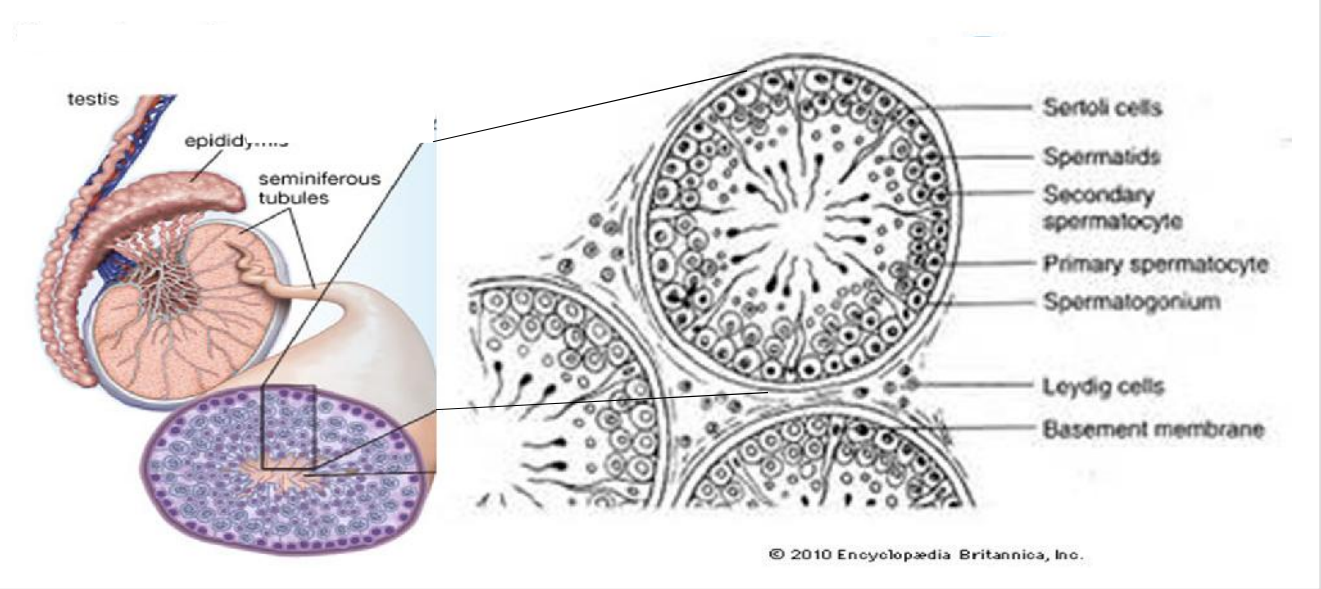
- Both gonadotrophins act co-operatively on the:
  - Ovaries in the woman
  - Testes in the man

to stimulate sex hormone secretion and reproductive processes



MALE GONADAL FUNCTION

- Testes function to
  - Produce hormones: androgens (i.e., testosterone), oestrogen and inhibin
  - Produce spermatozoa

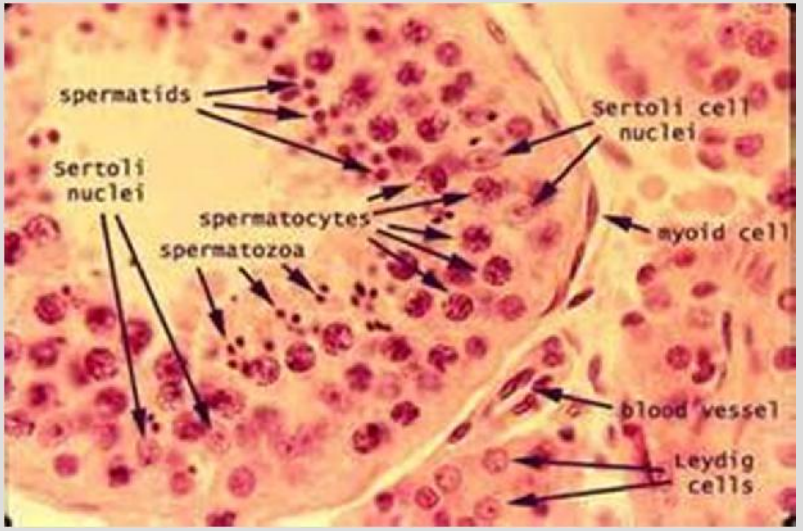


MALE GONADAL FUNCTION

- Testes function to
  - Produce hormones: androgens (i.e., testosterone), oestrogen and inhibin and
  - Produce spermatozoa

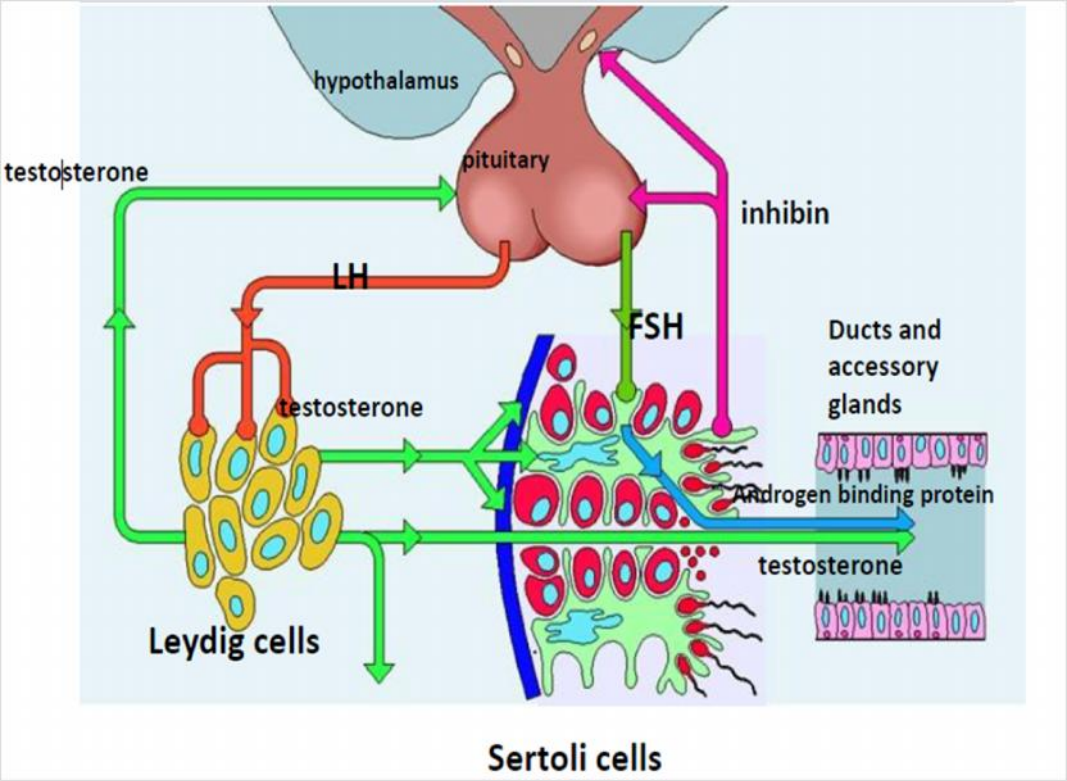
◦ Within the testes: 2 different cell types:
  ◦ Leydig cells: responsible for androgen production (testosterone): influenced by LH

  ◦ Sertoli cells: secrete inhibin and androgen binding protein (ABP): influenced by FSH
    ◦ Within seminiferous tubules where germinal cells (spermatogenic cells) are also interspersed
    ◦ ABP binds testosterone and DHT (dihydrotestosterone: more potent derivative) within the seminiferous tubules which concentrates the hormones to enable spermatogenesis within the seminiferous tubule and maturation within the epididymis



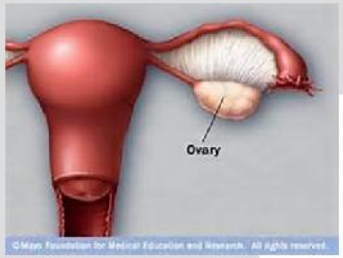
MALE GONADAL FUNCTION

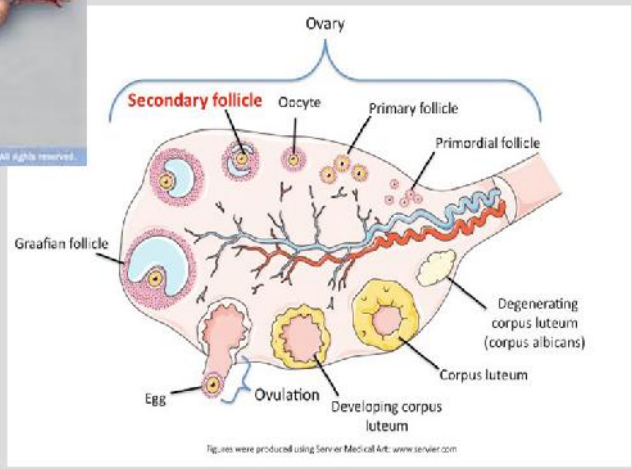
- Spermatogenesis occurs within testes, located within the scrotum
  - Thus keeping the gametes 2-3°C below core body temperature which is important for survival of the spermatozoa
  - Mature spermatozoa carried through the tubules via the vas deferens to the urethra
  - Spermatogenesis commences at puberty, before puberty gonadotrophin and testosterone concentrations in plasma are very low



Inhibin: inhibits production of FSH by the pituitary gland. Inhibins also involved in the control of the production of gametes and embryonic and foetal development.

FEMALE GONADAL FUNCTION



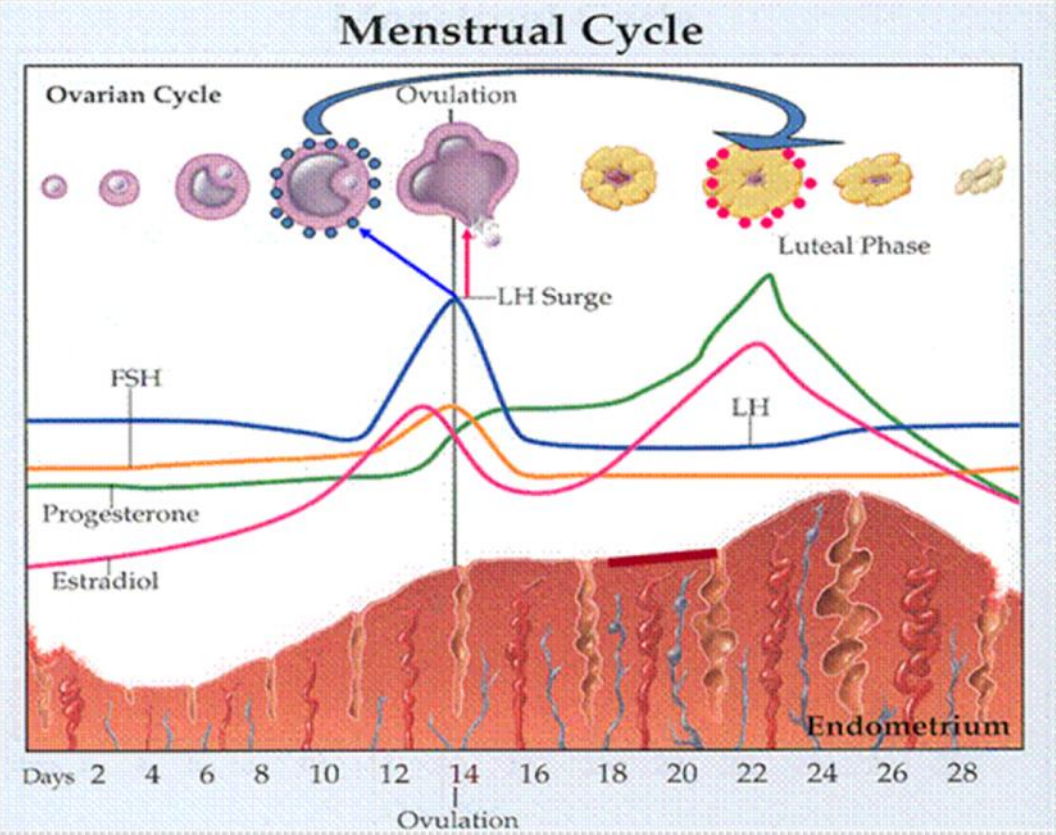


- Hormones secreted by the ovaries involve oestrogens and progesterone
- The ovaries at birth: ~1-2 million immature oocytes within the primordial follicles. Many of these regress within the first decade of life through ovarian follicle atresia (breakdown of the ovarian follicles)
- By puberty, ~ 300,000 to 400,000 left
- Menstrual cycle = reproductive cycle of a female, commences at puberty
- During reproductive life span, 300-400 follicles reach maturity and release an egg
- One mature follicle is produced on approximately the 14th day of each normal menstrual cycle
- The cycle is under hormonal control and involves a co-ordinate interaction of feedback effects between the hypothalamus, anterior pituitary and ovaries

FEMALE GONADAL FUNCTION

- Non-pregnant female, cycle occurs approx. at 4week intervals
- First day of cycle = first day menstrual bleeding
- Cycle consists of a:
  - Follicular phase - day 0 to 14
    - Development of the antral (Graafian) follicle
  - Luteal phase – day 15 to 28

of a 28 day cycle



FEMALE GONADAL FUNCTION

- Follicular Phase:

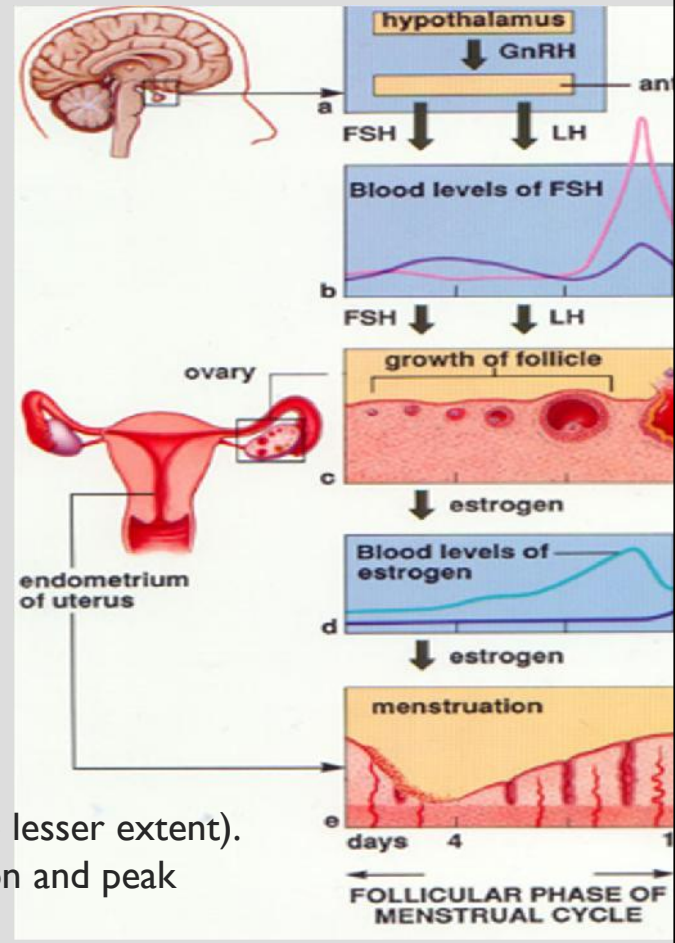
1. Early phase – initially FSH and LH elevated

2. FSH stimulates oestradiol production by granulosa cells of the antral (graafian) follicle: leading to negative feedback

3. LH acts on the thecal cells to produce androgens
   - Androgens can be converted by the enzyme aromatase into oestradiol (within the granulosa cell layer: aromatase under influence of FSH)

4. As graafian follicle enlarges, it produces increasing amounts of oestradiol

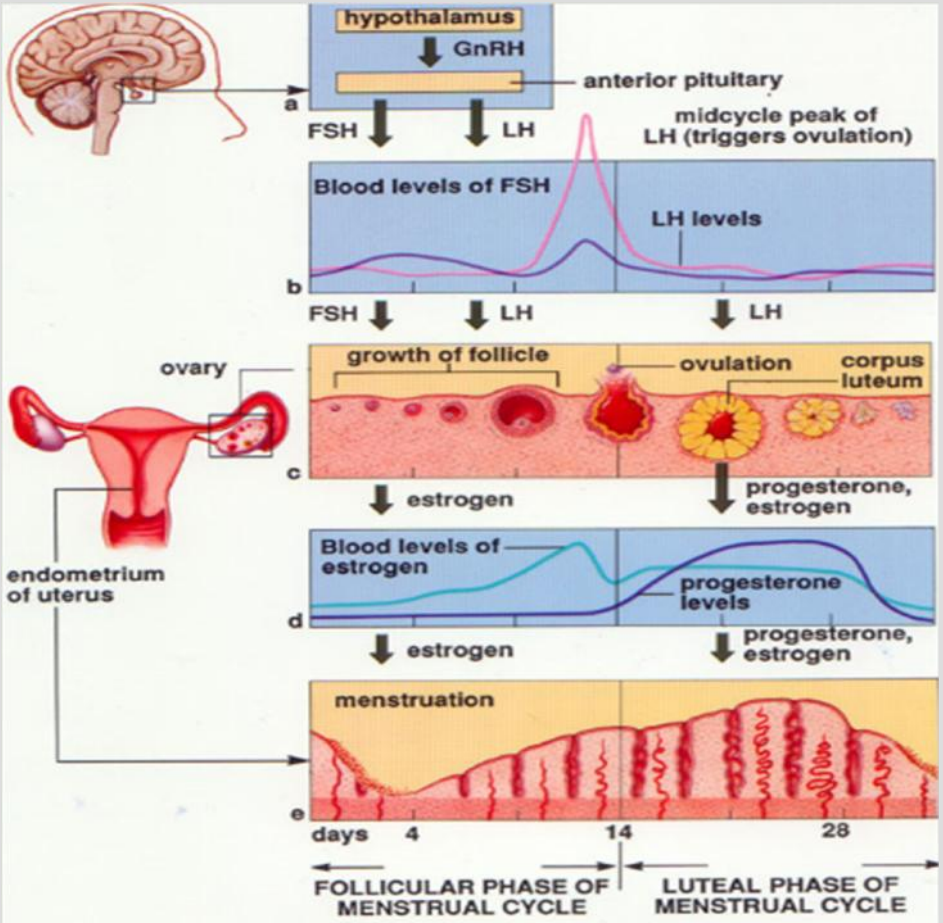
5. Oestrogens themselves stimulate growth of granulosa cells, thus maintaining oestrogen output in spite of falling LH and FSH levels as a consequence of the feedback of oestradiol on the anterior pituitary



- Before ovulation, high oestrogen levels stimulate/ trigger the LH surge (FSH also ↑, but to lesser extent). This surge = reliable predictor of ovulation. Surge occurring 24-36 hours before ovulation and peak occurring 10 – 12 hours before ovulation
- Ovulation – ovum released from follicle

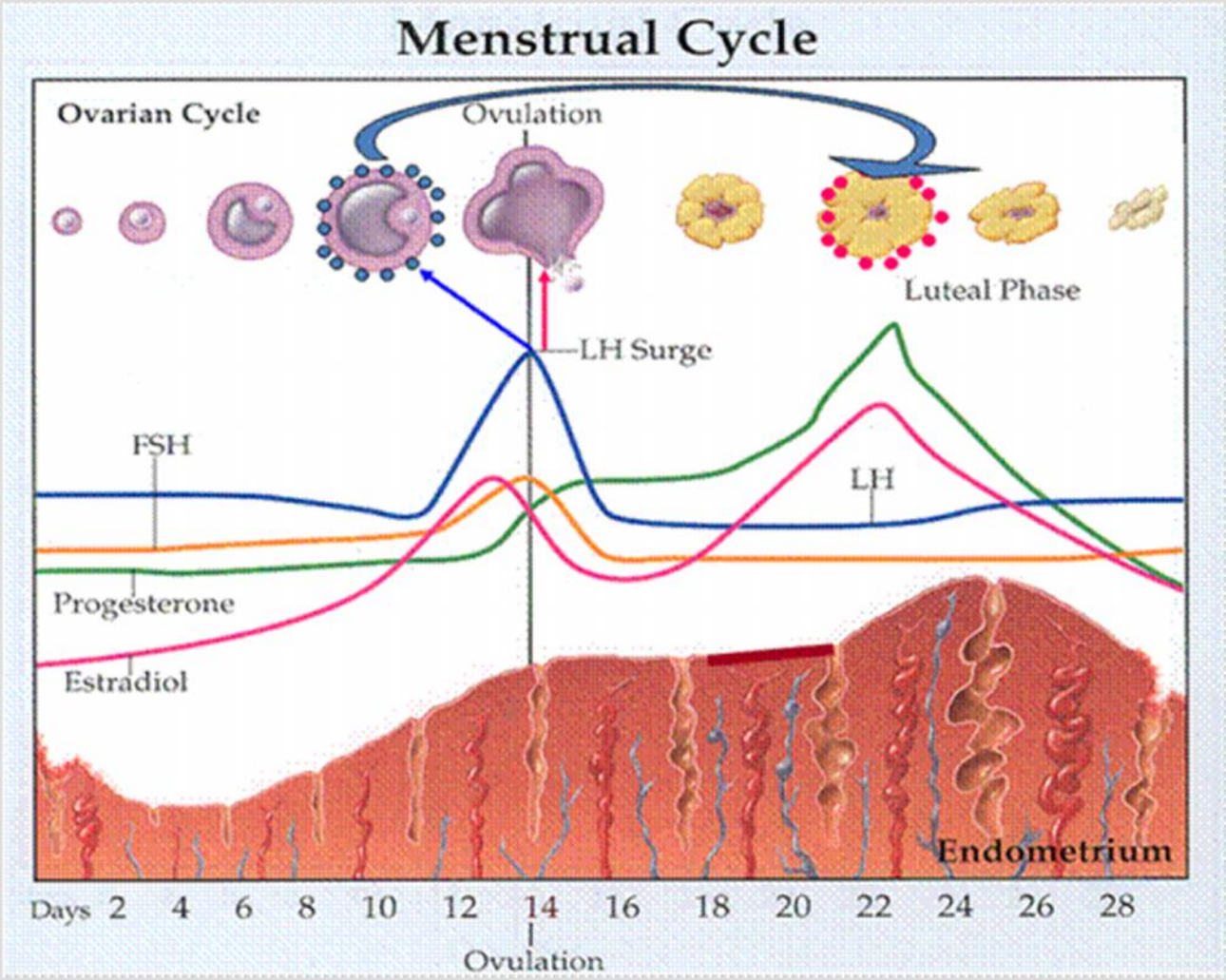
FEMALE GONADAL FUNCTION

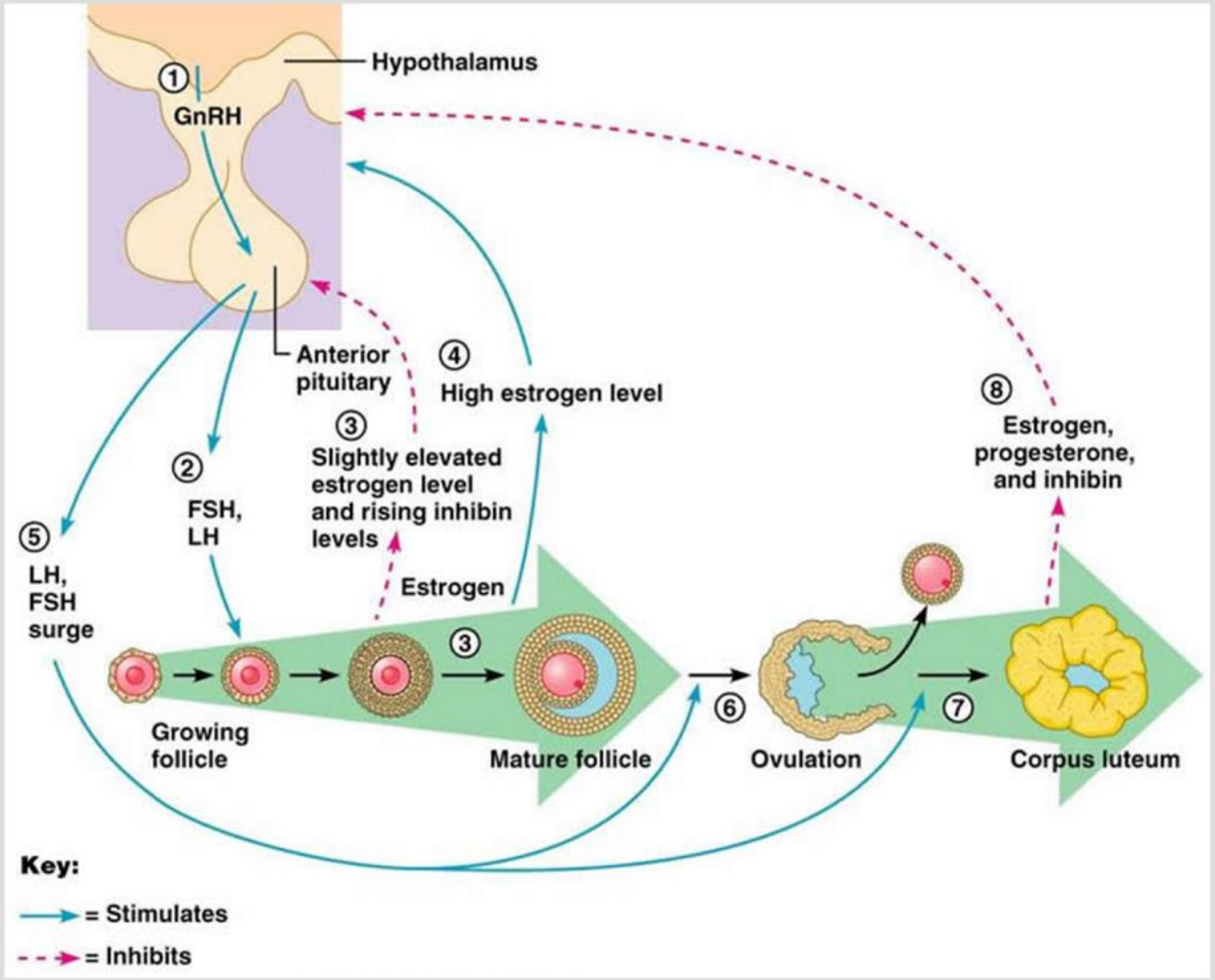
- Luteal Phase: Last half of menstrual cycle
- After ovulation, oestradiol levels ↓ initially, but progesterone secretion ↑ and consequential ↓ in LH and FSH
- Corpus luteum (ruptured follicle) which secretes progesterone
- Note: If ovulation does not occur, the corpus luteum fails to form and the cyclic rise in progesterone is lower than normal
  - Why: If ?Ovulation: progesterone measured at day 21 (of 28d cycle)
- If no conception, corpus luteum degrades & endometrium breaks down, leading to ↓oestrogen and progesterone concentrations. Subsequently FSH and LH start to ↑ as negative feedback to GnRH from progesterone no longer present. An increase in the FSH and LH allows new follicles to mature and triggers the onset of menstruation with shredding of the endometrium
- If female becomes pregnant, human chorionic gonadotrophin (hCG: produced by trophoblastic cells of foetus) maintains corpus luteum, therefore oestrogen and progesterone levels continue to ↑



Changing hormone levels during the menstrual cycle.

FEMALE GONADAL FUNCTION

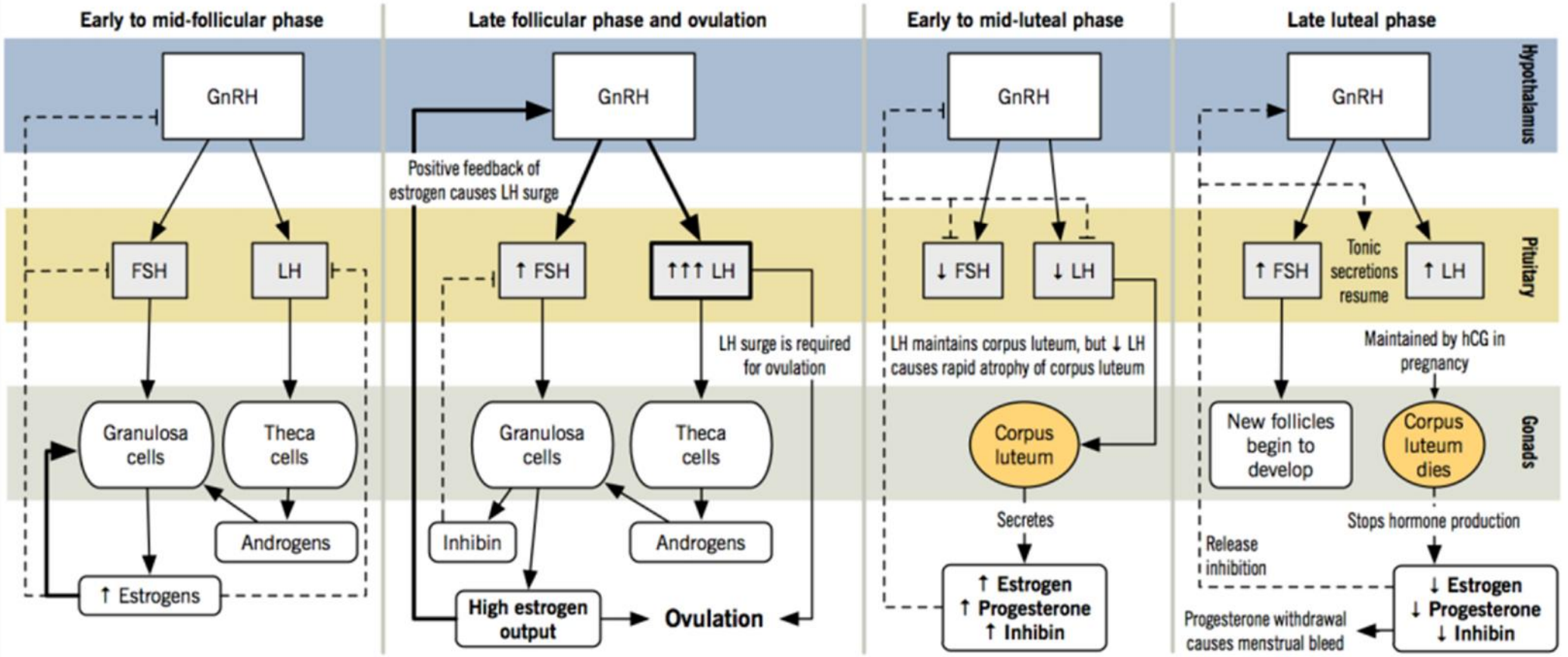



Hypothalamus
1
GnRH
Anterior pituitary
2
FSH, LH
3
Slightly elevated estrogen level and rising inhibin levels
4
High estrogen level
5
LH, FSH surge
Estrogen
3
Growing follicle
Mature follicle
6
Ovulation
7
Corpus luteum
8
Estrogen, progesterone, and inhibin
Key:
= Stimulates
= Inhibits

Hormonal regulation at various parts of the menstrual cycle

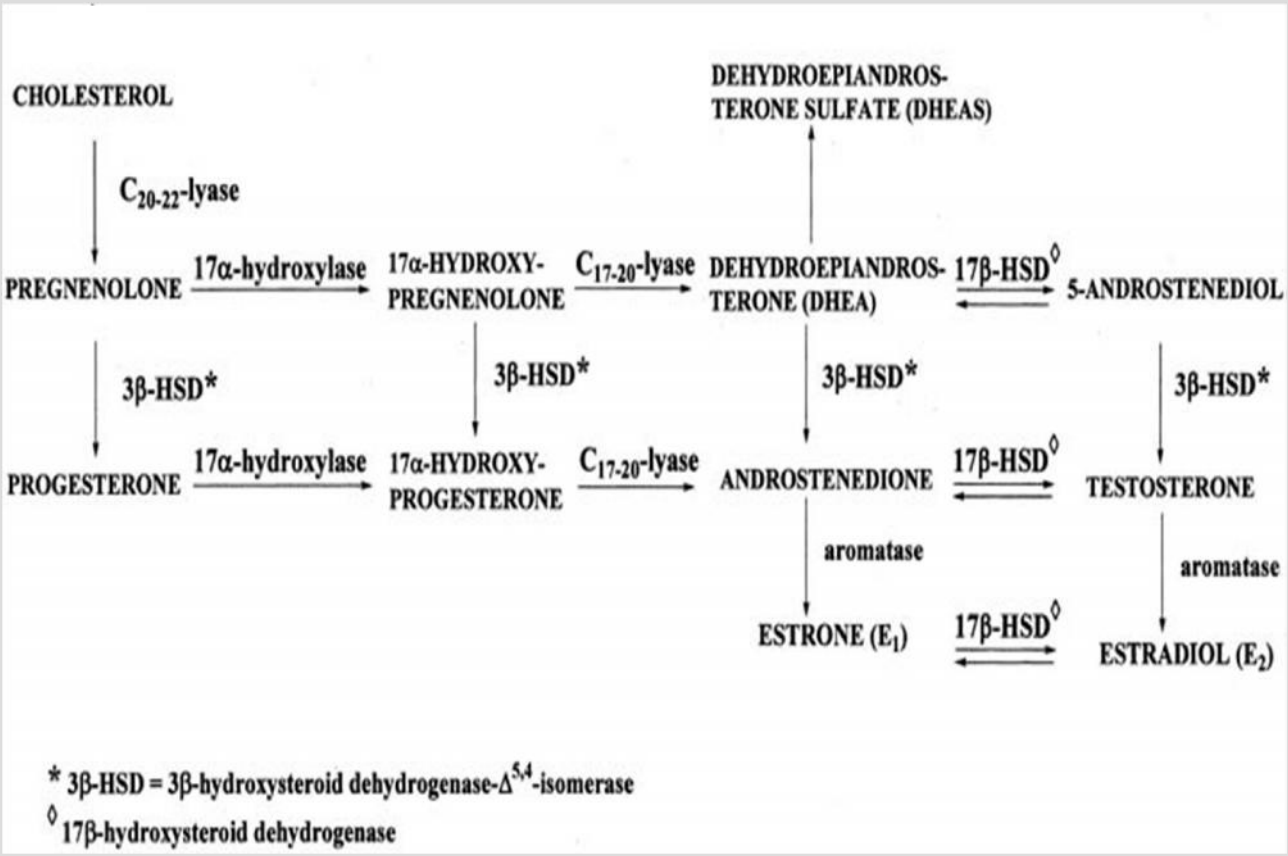
Eric Wong

Adapted from: Silverthorn Human Physiology 4E, figure 26-14



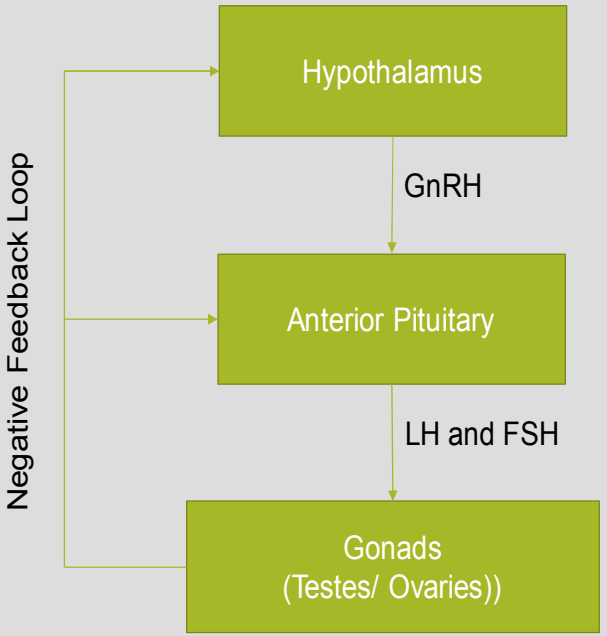
REPRODUCTIVE HORMONES

- FSH and LH
- Oestrogen (oestradiol)
- Progesterone
- Prolactin
- Testosterone
- SHBG



FSH AND LH

- Glycoproteins, both ~30kDa
- Consist of alpha and beta subunit
  - Alpha subunit for both same as for TSH and hCG
  - Beta subunit unique to all hormones
  - Synthesis and release stimulated by hypothalamic hormone, GnRH, such effects being modulated by circulating gonadal steroids feedback



Reference Ranges:
FSH: Fol 3-8IU/L: Luteal 1-5IU/L: Ovulatory 3-17IU/L: Post menopausal 27-133IU/L
LH: Follicular 2-12IU/L: luteal 1-14IU/L: ovulatory 9-89IU/L: post menopausal 5-62IU/L

OESTROGEN & PROGESTERONE

OESTROGENS (I.E., OESTRADIOL)

- Maintains function of reproductive tract
  - Endometrial thickening
  - Stimulate growth of ovarian follicles
  - Mucus secretion at ovulation
- Secondary sexual characteristics
- Systemic effects
  - Bone density
  - Cardioprotective
- In plasma, transported bound to protein: ~60% to albumin and remainder to SHBG.
  - ~2-3% unbound

Reference Ranges
E2: Foll 77-921pmol/L: luteal 77-1145pmol/L: ov peak: 140-2382pmol/L: post menopaus <103pmol/L
Progesterone: Foll <1nmol/L: luteal 1-16nmol/L: post menopausal <1nmol/L

PROGESTERONE

- Concentration ↑ during second half of menstrual cycle, but ↓ if no conception
- Prepares endometrium for implantation
- Also pyrogenic and mediates ↑ in basal body temperature which occurs with ovulation
- Essential for maintenance of early pregnancy:
- Day 21 progesterone – indicates ovulation
  - If menstruation is irregular, samples can be taken several times a week until menstruation occurs

TESTOSTERONE & DIHYDROTESTOSTERONE (DHT)

TESTOSTERONE

- Male reproductive development (foetal & pubertal)
- Male secondary sex characteristics i.e. hair growth, deep voice and characteristic masculinisation
- Libido
- Spermatogenesis
- Bone mass (male & females)
- Also effects on erythropoiesis (low testosterone associated with anaemia) and lipids
- Testosterone has direct & indirect effects
  - In some tissues testosterone is a pro-hormone for DHT

Reference Range:
Female: <2.9nmol/L
Male: Age specific reference levels. Adults: 9.9-27.8nmol/L

DIHYDROTESTOSTERONE (DHT)

- Male reproductive development (foetal & pubertal)
- Male reproductive development requires both T and DHT
- Peripheral conversion from testosterone by 5alpha-reductase (prostate, skin)
- DHT has two to three times greater androgen receptor affinity than testosterone
- DHT measurement indicated in investigating some disorder of sexual development (DSD)

SEX HORMONE BINDING GLOBULIN (SHBG)

- Glycoprotein
- Binds testosterone & oestradiol in plasma: greater affinity for testosterone
- Concentration in males ½ that of females
- Factors affecting SHBG concentration in turn affect ratio of free testosterone to free oestradiol i.e., ↓SHBG = ↑ ratio of free testosterone to free oestradiol
- Therefore, in either gender, the effect of an increase in SHBG is to increase oestrogen-dependent effects, while a decrease in SHBG increases androgen-dependent effects.

Reference Range:
Female: Pre menopausal 20-155nmol/L: post menopausal 14-69nmol/L
Male: 14-71nmol/L

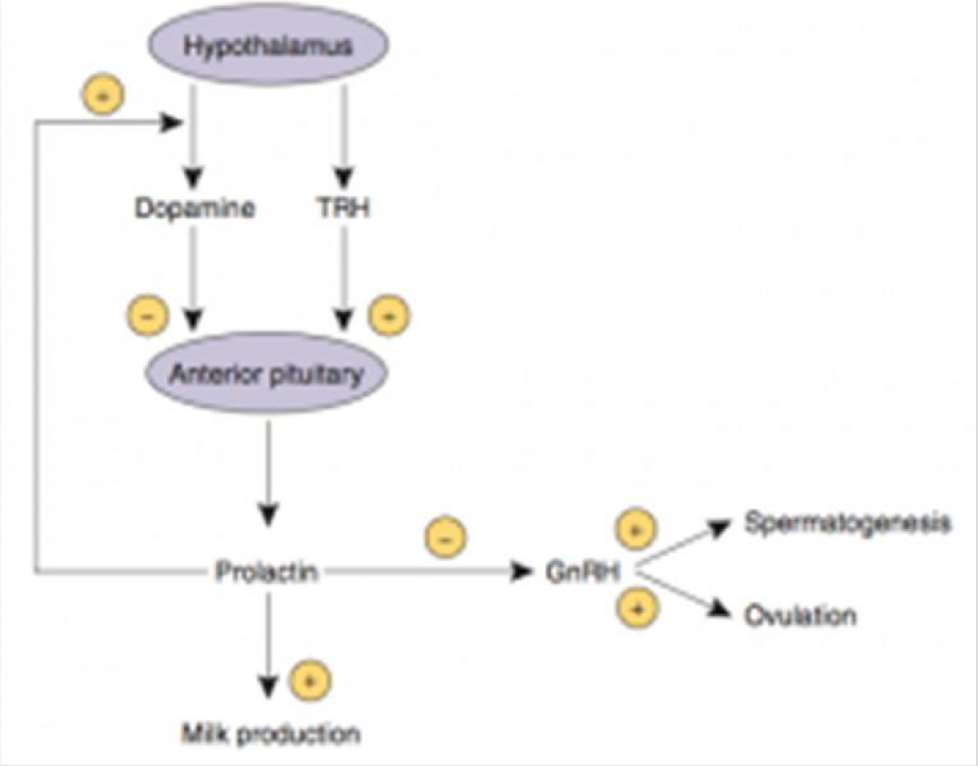
SEX HORMONE BINDING GLOBULIN (SHBG)

FACTORS AFFECTING SHBG CONCENTRATION

- Causes ↑ SHBG
  - Oestrogens (hormone replacement, oral contraceptive, pregnancy)
  - Hyperthyroidism
  - Liver disease i.e., cirrhosis
  - Anorexia
  - Drugs i.e., phenytoin, phenobarbitone, thyroxine
  - Increasing age in men
- Causes ↓ SHBG
  - Androgens (exogenous, endogenous or ↑ sensitivity)
  - Hypothyroidism
  - Cushing's Disease/ Glucocorticoids
  - Malnutrition and Malabsorption
  - Protein-losing states
  - Obesity (particularly in women)
  - Hyperinsulinism/ Insulin resistance
  - Nephrotic syndrome
  - Diabetes
  - Decrease in post-menopausal females

PROLACTIN

- 23-kDa monomeric polypeptide
- Produced by lactotrophs of anterior pituitary
- Principal action: initiate and sustain lactation
- Secretion controlled by hypothalamus through dopamine secretion – inhibitory effects on prolactin
- No known 'prolactin-releasing hormone'. However, TRH and vasoactive intestinal polypeptide (VIP) known to have stimulatory role (why may see ↑ prolactin in primary hypothyroidism)
- Physiological stimuli to prolactin secretion: pregnancy and suckling



- At high concentrations, it inhibits synthesis and release of gonadotrophs from anterior pituitary, inhibiting ovulation in females and spermatogenesis in males

PROLACTIN

- Hyperprolactinaemia Causes:
  - DA Antagonists (therefore loose DA negative feedback)
    - Reserpine, Methyldopa
  - Stress
  - Pregnancy/ Suckling
  - Hypothyroidism (primary: therefore ↑TRH)
  - CKD (reduced clearance)
  - Prolactin secreting tumour - prolactinoma

  - Macroprolactinaemia: Big prolactin (60 kDa) and macroprolactin (150 kDa)
    - IgG complex
    - Minimum Bioactivity
    - Should be screened to avoid unnecessary investigations
    - Prevalence of macroprolactinaemia: 10–25% in patients with hyperprolactinaemia

PATHOPHYSIOLOGY & INVESTIGATIONS

- HYPOGONADISM
- INFERTILITY

HYPOGONADISM

- Diminished functional activity of the gonads

- Clinical syndrome resulting from failure of the testis in males or ovaries in females to produce physiological levels of testosterone & normal number of spermatozoa in males or oestrogen & ovulation in females due to disruption of one or more levels of the hypothalamic-pituitary-gonadal axis.

MALE HYPOGONADISM

- Uncommon for a boy to enter puberty before 9yrs of age
- Boys not entered puberty by 14yrs of age = delayed puberty
  - Usually present <14yrs: concern with short stature (delayed pubertal growth spurt)
  - Causes:
    - Constitutional (slow or delayed) i.e., idiopathic (Family History) – diagnosis of exclusion
    - Chronic illness: CF, Coeliac disease
    - Hypogonadism
      - Implies defective spermatogenesis or testosterone production or both
        - Causes – primary and secondary

PRIMARY MALE HYPOGONADISM

- Primary: i.e., testicular disease
  - Can be due to only defective seminiferous tubule function (spermatogenesis), only defective Leydig cell function (testosterone), or both
    - Former: leads to infertility through decrease production of spermatozoa, but masculinisation is usually normal
    - Latter: leads to failure of testosterone-dependent functions, including spermatogenesis
    - Effects of decreased testosterone secretion depends on age at onset of the disorder. Secondary sexual characteristics are in part preserved if secretion is lost after puberty

PRIMARY MALE HYPOGONADISM

- Hypergonadotrophic hypogonadism: Low testosterone (not always), increased FSH and LH
  - Primary Congenital:
    - Testicular agenesis (absence of one or both testes)
    - Klinefelter's syndrome (47XXY)
    - Inborn errors of testosterone/ DHT biosynthesis
      - Congenital 5α-reductase deficiency: presents with underdeveloped male genitalia and prostate; often raised as girls. Onset of puberty: DHT remains very low and testosterone usually elevated.
    - Androgen Resistance
  - Primary Acquired
    - Acute and chronic systemic disease i.e., mumps (inflammation of the testes)
    - Testicular torsion/ trauma
    - Gonadal toxins (Radiotherapy/ Chemotherapy – cytotoxic drugs)

SECONDARY MALE HYPOGONADISM

- Secondary: i.e., pituitary or hypothalamic disease
  - Hypogonadotrophic hypogonadism: Low testosterone, low FSH and LH
    - Secondary Congenital
      - GnRH Deficiency
        - Kallman's syndrome
          - Characterised by delayed or absent puberty and an impaired sense of smell
      - Isolated LH Deficiency (Fertile Eunuch Syndrome)
      - Prader-Willi Syndrome
    - Secondary Acquired
      - Pituitary disorders (tumours & infarct) i.e.,
        - Tumours esp. if causing hyperprolactinaemia
        - Panhypopituitarism
        - Trauma
      - Haemochromatosis
      - Underweight
      - Aging

FEMALE HYPOGONADISM

- Uncommon for girl to enter puberty before 8yrs of age
- Most girls will have entered puberty at ~13yrs
- Delayed puberty usually presents with absence of breast development (by 13yrs) or amenorrhoea (by 15yrs – primary amenorrhoea)

- Causes:
  - Constitutional (slow or delayed) i.e., idiopathic (Family History) – diagnosis of exclusion
  - Chronic illness - coeliac disease, CAH
  - Hypogonadism
    - Primary: serum oestradiol ↓; FSH and LH ↑ = Hypergonadotrophic hypogonadism
      - Congenital
        - Turners syndrome (45XO) and variants
        - Ovarian (Gonadal) Dysgenesis: progressive loss of germ cells on the developing gonads of an embryo
      - Acquired
        - Chemotherapy/ radiotherapy
    - Secondary: serum oestradiol ↓; FSH and LH ↓ - pituitary or hypothalamic disease = Hypogonadotrophic hypogonadism
      - Pituitary disorders (tumours & infarct) i.e.,
        - Tumours esp. if causing hyperprolactinaemia
        - Panhypopituitarism
        - Trauma
      - Haemochromatosis
      - Underweight

HYPOGONADISM – TREATMENT

Males

- Directed at underlying cause i.e.,
  - Testosterone replacement - testosterone deficiency syndromes
    - Aim to keep within reference range
      - Monitoring pattern: depends on preparation. If implant or injection: baseline values, if transdermal patches or gel, 4-6hrs post application
      - Monitor: LFTs, PSA, haematocrit and plasma lipids yearly
        - Prostate cancer is testosterone dependent
  - Hypogonadotrophic hypogonadism treated with gonadotrophins (infertility):
    - hCG (homology with LH) or human menopausal gonadotrophin (HMG) which contains LH and FSH.
    - Alternatively can prescribe: Pulsatile GnRH
    - If Hyperprolactinaemia: medication &/ or surgery

HYPOGONADISM – TREATMENT

Females

- Directed at underlying cause i.e.,
  - Oral oestrogens – oestrogen deficiency: promote feminisation, sequential oestrogen and progesterone – induce menstruation
  - Hypogonadotrophic hypogonadism treated with gonadotrophins (infertility):
    - Stimulate ovulation: FSH and LH; hCG maybe used to mimic mid-cycle LH peak and stimulate ovulation
      - Monitor oestradiol concentration – detect hyperstimulation: risk of multiple pregnancy and production of ovarian cysts
    - If hypothalamic disease – may prescribe clomifene.
      - Blocks oestradiol receptors in hypothalamus, in turn stimulate GnRH and thus FSH and LH
      - Non-responders prescribed GnRH
    - If Hyperprolactinaemia: medication &/ or surgery

INFERTILITY

- Definition of infertility:
  - A woman of reproductive age who has not conceived after 1 year of unprotected vaginal sexual intercourse, in the absence of any known cause of infertility
  - Such a couple should be offered further clinical assessment and investigation along with her partner.

INFERTILITY

- Prevalence of infertility, quite high: 1 in 7 heterosexual couples (UK)
- Causes:
  - Unexplained infertility (25%)
  - Ovulatory disorders (25%)
    - Hypogonadotrophic hypogonadism
    - Hypothalamic-pituitary-ovarian dysfunction i.e., PCOS, late CAH
    - Ovarian failure
  - Tubal damage (20%)
  - Factors in the male causing infertility (30%)
  - Uterine or peritoneal disorders (10%)
  - Other causes include endometriosis, gamete or embryo defects

  - NOTE: ~40% of cases disorders are found in both the man and woman

INFERTILITY - MALE

- Male infertility causes can be considered as follows:
  - Pituitary/hypothalamic dysfunction (low FSH/ LH, testosterone i.e., secondary hypogonadism)
  - Testicular dysfunction i.e., primary testicular failure (increase FSH, LH, low testosterone +/- small testicular size)
  - Obstruction to ducts (FSH, LH and testosterone are normal)
  - Idiopathic (FSH, LH and testosterone are normal)

INFERTILITY - MALE

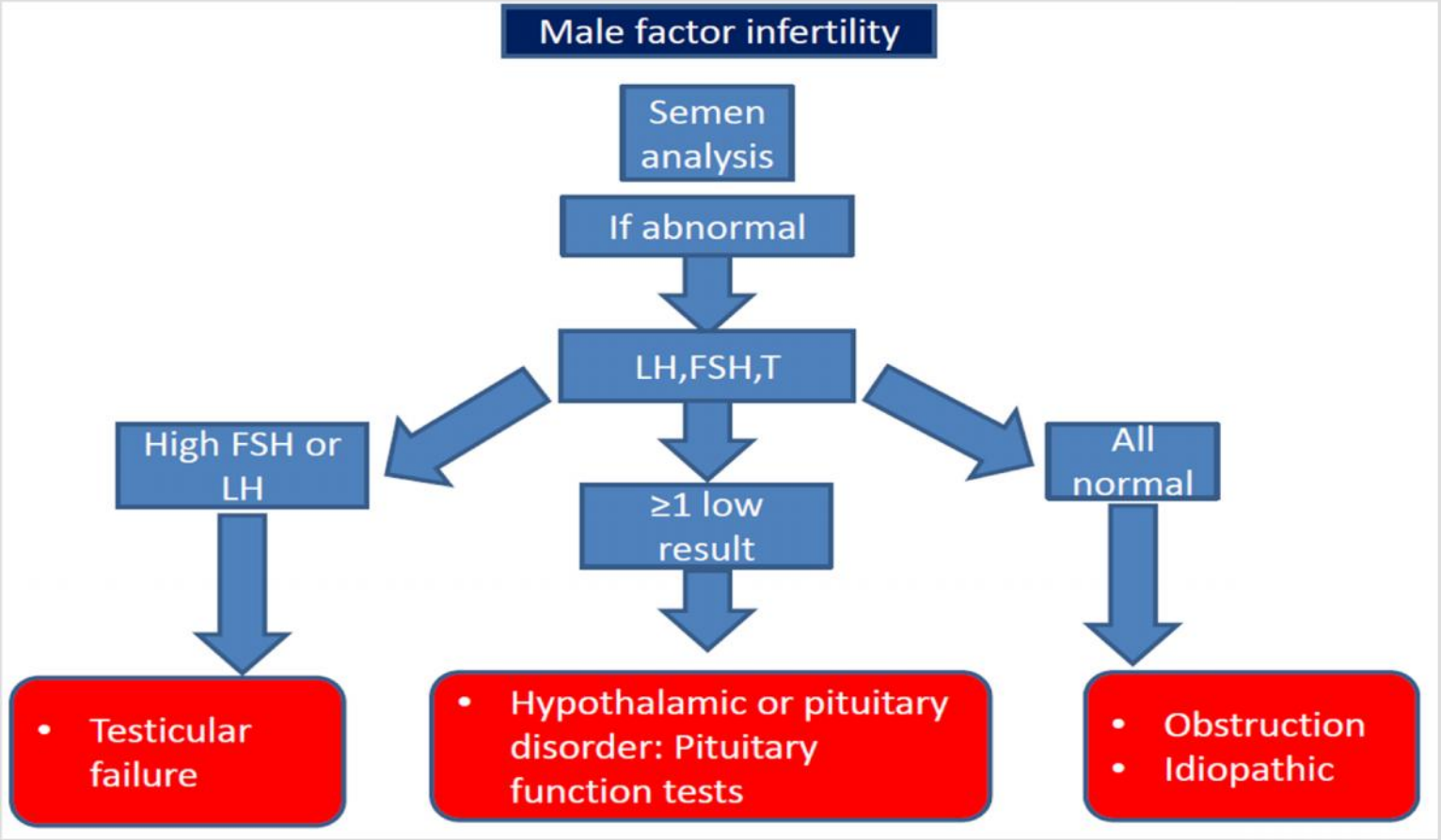
- First line investigation: Semen Analysis (WHO guidelines i.e., volume, pH, concentration, total sperm count, motility, morphology etc)
- Evidence of poor semen quality (i.e., oligo-/azoospermia): further investigations justified – endocrinology

Definitions:
Oligospermia: Deficiency of sperm cells in the semen
Azoospermia: Absence of motile (and hence viable) sperm in the semen

INFERTILITY - MALE

- First line investigation: Semen Analysis (WHO guidelines i.e., volume, pH, concentration, total sperm count, motility, morphology etc)
- Evidence of poor semen quality (i.e., oligo-/azoospermia): further investigations justified – endocrinology
- LH, FSH, testosterone (T): distinguish between primary and secondary hypogonadism
  - Low LH, FSH, T suggest hypogonadotrophic hypogonadism: check pituitary function
  - High FSH/ LH, low T, ± small testicular size suggests primary testicular failure:
    - If ? Primary testicular failure: hCG test
      - Day 0: 9am Baseline testosterone (androstenedione & dihydrotestosterone) , LH and FSH. Then give hCG
      - Day 4: 9am Testosterone (androstenedione & dihydrotestosterone), LH and FSH
        - Normal: ↑ testosterone to > ULN
        - Primary failure: little or no response with exaggerated ↑ in LH and FSH
  - Normal LH, FSH, T suggests ductal obstruction or idiopathic.

INFERTILITY - MALE



INFERTILITY - FEMALE

- Oligomenorrhoea: menstrual cycle length >6 weeks but < 6 months
- Amenorrhoea
  - Primary: failure to establish spontaneous menstruation by the age of 15yrs, regardless of whether secondary sex characteristics have developed
    - ~40% of phenotypic females who have primary amenorrhea (which is nearly always associated with absence of development of secondary sex characteristics) have Turner's syndrome
    - Turners Syndrome: rare genetic disorder characterised by absence of X chromosome: 45XO
  - Secondary: absence of menstruation for >6 months in females who have previously experienced menses or for 12 months in females with a history of oligomenorrhea, or infrequent menstruation (less than 9x per year)
- What is commonest cause of amenorrhoea in women of child-bearing age?

INFERTILITY - FEMALE

- Women presenting with infertility
  - Patient history: description of menstrual patterns, ?galactorrhea (?hyperprolactinaemia), hot flushes, symptoms of hypothyroidism, hirsutism, previous surgery of the abdomen, pelvis or uterus, trauma, medications, nutritional history, exercise patterns, previous contraceptive use, changes in weight, stress, chronic disease

  - Initial investigation: confirm female is ovulating
    - Measure plasma progesterone at mid-luteal phase i.e., day 21 of a 28 day cycle
    - Women with regular cycles of longer or shorter duration essentially 7days before the next menstrual period
    - Women with irregular cycle - difficult to identify, but attempts should be made: samples over a couple days maybe taken
- Other tests include: oestradiol levels, FSH/ LH, testosterone and SHBG
  - ?Ovarian failure
  - ?Androgen excess: PCOS, late-onset CAH, Cushing's syndrome
- Other investigations may also include:

INFERTILITY - FEMALE

- Other investigations may also include:
  - Prolactin
    - If there is evidence of an ovulatory disorder, or galactorrhoea: ?prolactinoma
  - Thyroid function tests
    - Hypothyroidism/ Hyperthyroidism
  - Viral screening
    - Chlamydia (bacteria which can affect the cervix and uterus)
  - Imaging to screen for tubal occlusion/ structural
    - Hysterosalpingography (HSG): radiological procedure to investigate shape of uterine cavity & shape patency of fallopian tubes
    - Tubal ultrasound
    - Laparoscopy

INFERTILITY – FEMALE

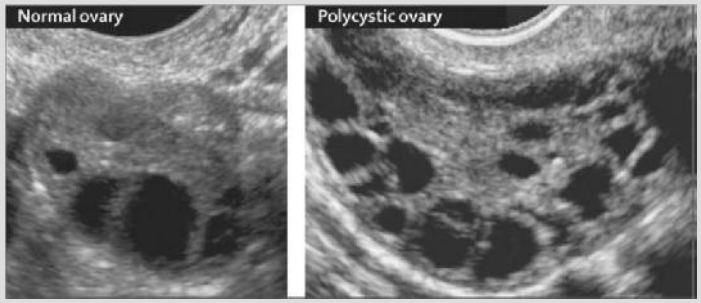
INFERTILITY: 25% DUE TO OVULATORY DISORDERS

- WHO classification of ovulatory disorders: 3 groups:
  - Group 1: Hypogonadotrophic Hypogonadism (i.e., low LH, FSH and oestradiol)
  - Group 2: Hypothalamic-pituitary-ovarian dysfunction (mainly PCOS, also hyperprolactinaemia, late onset CAH)
  - Group 3: Hypergonadotrophic Hypogonadism: (Ovarian Failure)

INFERTILITY – FEMALE
INFERTILITY: 25% DUE TO OVULATORY DISORDERS

- WHO classification of ovulatory disorders: 3 groups:
  - **Group 1: Hypogonadotrophic Hypogonadism (i.e., low LH, FSH and oestradiol)**
    - Causes
      - Severe stress
      - Severe weight loss (e.g. anorexia nervosa)
      - Rigorous athletic training (marathon runners)
      - Pituitary/hypothalamic lesions
  - Treatment: treat according to cause i.e.,
    - Women with a BMI of <19 may respond to weight gain or reduced exercise
    - Pulsatile administration of gonadotrophin-releasing hormone or gonadotrophins with luteinising hormone activity to induce ovulation
      - Essential to monitor follicle development in the ovaries with ultrasound to ensure there is no excessive development of graafian follicles

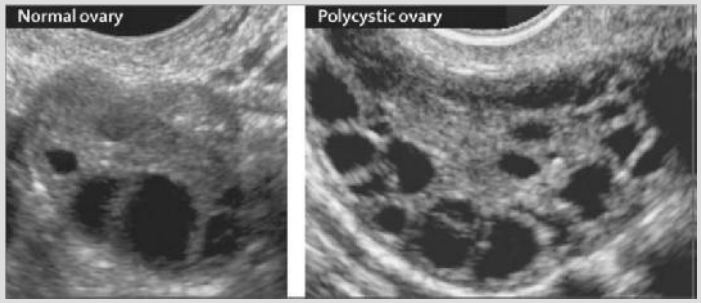
INFERTILITY – FEMALE
INFERTILITY: 25% DUE TO OVULATORY DISORDERS



- WHO classification of ovulatory disorders: 3 groups:
  - **Group 2: Hypothalamic-Pituitary-Ovarian Dysfunction (mainly PCOS)**
    - PCOS: WHO definition of PCOS requires 2 of the following 3 features:
      - Polycystic ovaries (ultrasound)
      - Oligo- or anovulation
      - Hyperandrogenism (biochemical and/ or clinical)
        - Biochemical: ↑ Androgens i.e, testosterone, ↑ high free androgen index
        - Clinical: Hirsutism, male pattern baldness, masculinisation

INFERTILITY – FEMALE

INFERTILITY: 25% DUE TO OVULATORY DISORDERS



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      - Polycystic ovaries (ultrasound)
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      - Hyperandrogenism (biochemical and/ or clinical)
    - Important to exclude other causes of hyperandrogenism e.g.
      - Cushing's syndrome
      - Non-classical (late onset) Congenital Adrenal Hyperplasia (CAH)
        - Can present as infertility: mutations in the 21-hydroxylase gene. Clinical presentation : menstrual irregularities, virilisation (development of male sexual characteristics in female: masculinisation) and infertility.
        - Useful diagnostic test: Synacthen Test - measure 17-hydroxyprogesterone (17-OHP) at 0 and 60mins.
      - Androgen-secreting ovarian or adrenal tumours
      - Pregnancy (total T: ↑ up to 10x pre-pregnancy values: free T should remain constant)
      - Exogenous androgens/ drug induced (androgenic medications i.e., metyrapone, anabolic steroids
      - Increased end-organ sensitivity to androgens

PCOS

- Also known as Stein Levanthal syndrome: Very common cause of menstrual irregularities and subfertility
- Clinical features:
  - Polycystic ovaries on ultrasound (75%)
  - Menstrual irregularities (70-80%)
    - Oligomenorrhoea
      - Interval of >6 weeks between menses
    - Amenorrhoea
      - No menstruation for >6months
  - Infertility (40%): many women with PCOS first present to infertility clinics
  - Obesity
  - Insulin resistance (50-80%)
  - Dyslipidaemia
  - Hyperandrogenism (60-80%) – may include:
    - High free androgen index (T/SHBG ratio: ref range up to 8.5)
    - High androstenedione; DHEA-S
    - Increased LH/FSH ratio
    - Hirsutism (60%); acne (20%); male pattern baldness (5%)
      - Exclude other causes of hyperandrogenism (see previous slide)

FEMALE: EXCESS ANDROGENS

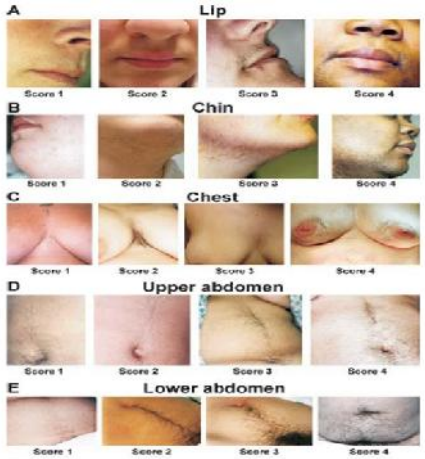
- Hirsutism
  - Excess growth of terminal (coarse) hair in a masculine distribution in women due to excess androgens or increased end-organ sensitivity to androgens.
  - Areas affected may include chin, upper lip, chest, breasts, abdomen, back and anterior thighs.
  - Hirsutism should be distinguished from hypertrichosis:
    - Increased/ abnormal amount of hair growth over the body
    - Not androgen dependent.

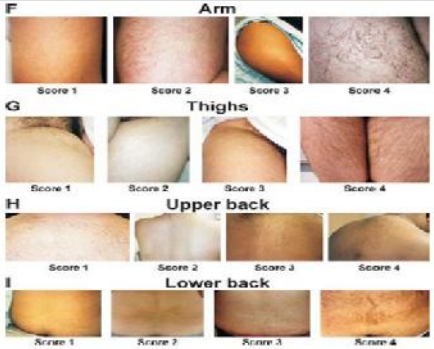
Hypertrichosis

- Virillisation (masculinisation)
  - Usually marked increase in androgens. Manifestations include temporal hair recession, clitoromegaly, increased muscle mass, breast atrophy, deepening of voice, oligo/ amenorrhoea

- Note: grossly elevated testosterone (>5nmol/L) with sudden onset hirsutism/ virilisation more worrying

- Degree or severity of hirsutism - Ferriman–Gallway (FG) scale of measurement
- Reviews nine areas of the body and the degree of hirsutism or hair growth can be compared with pictures and scored from 1-4: maximum score is 36.

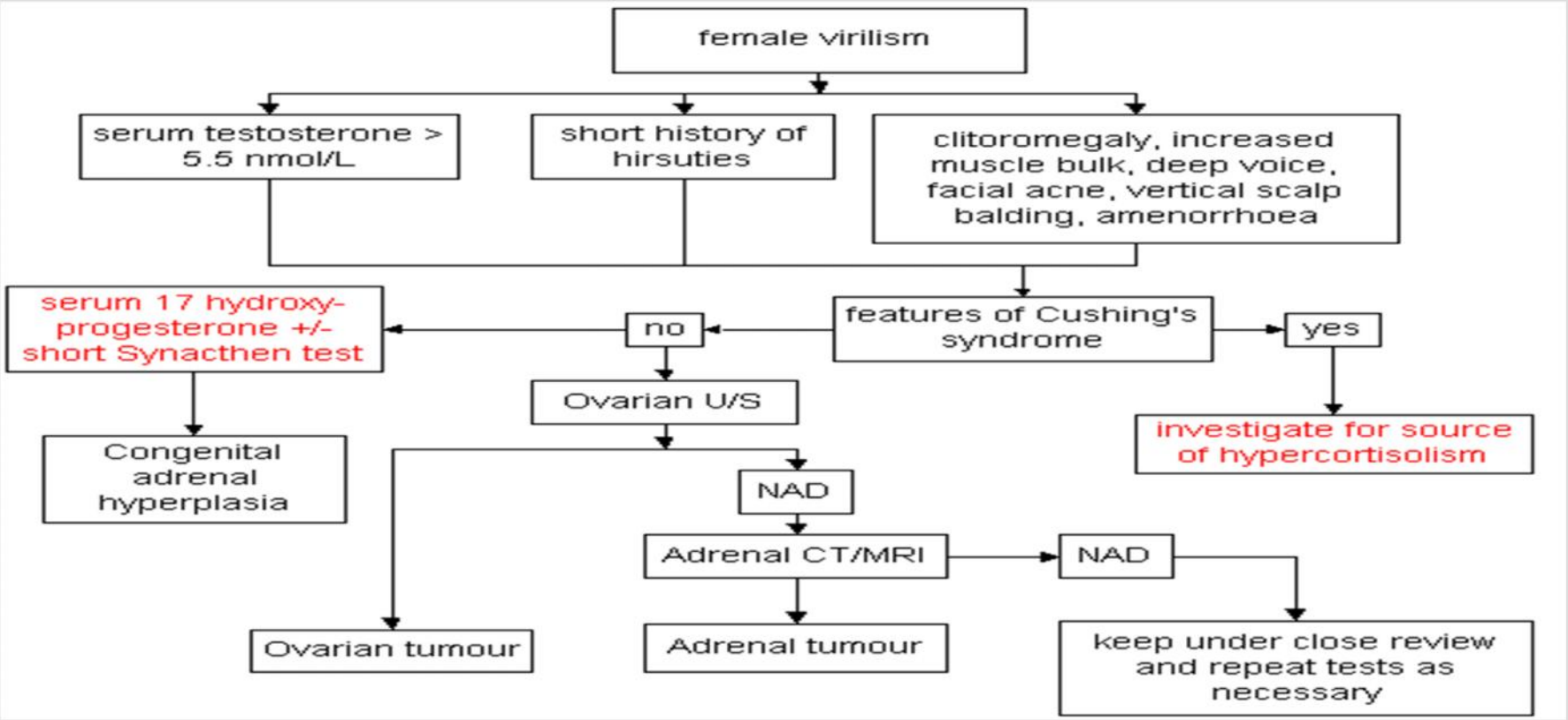




Sum of scores from each of 9 areas (max score 36)
Mild hirsutism: up to a score of 15
Moderate hirsutism: 16 to 25
Severe hirsutism: above 25

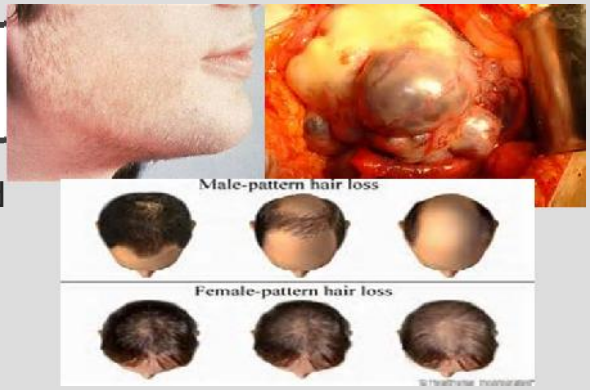
Modified from Yildiz et al. Visually scoring hirsutism. Hum Reprod Update 2010;16:51 –64.

- Biochemical investigations: First line tests include:
  - Testosterone & SHBG and calculate the FAI (free androgen index)
  - LH; FSH; prolactin
  - NB. oral contraceptives lower testosterone levels
- Second line tests:
  - Androstenedione, DHEAS
  - Synacthen stimulation tests (particularly non-classical CAH)
  - Tests for Cushing's syndrome



NAD – no abnormalities detected.

PCOS



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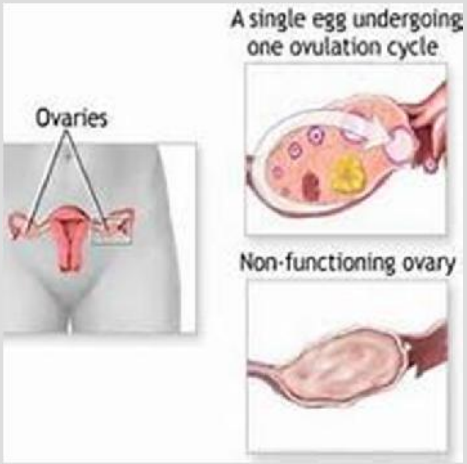
    - High androstenedione; DHEA-S
    - Increased LH/FSH ratio
    - Hirsutism (60%); acne (20%); male pattern baldness (5%)
      - Exclude other causes of hyperandrogenism

- PCOS mechanism unclear:
  - ? Involve peripheral conversion of testosterone to oestrone in adipose tissue, which inhibits FSH and stimulates LH secretion
  - Considering many affected women are obese and have impaired glucose tolerance: hyperinsulinaemia increases ovarian testosterone secretion and inhibits SHBG production, thus proportion of free testosterone (biologically active) is ↑ (leading to a high free androgen index).

- Management/ treatment of PCOS includes life-style advice:
  - Recommend weight loss
  - Oral contraceptives: prescribed as sometimes can break cycle of androgen secretion and disordered anterior pituitary secretion
  - Anti-oestrogens (clomiphene/ clomifene), work by:
    - Occupy oestrogen receptors in hypothalamus
    - Block feedback
    - Induce production of gonadotrophins
    - Stimulate ovarian follicle development
  - Metformin: proved to be useful as it lowers insulin levels therefore by reversing hyperinsulinaemia, may help 'break' the cycle of androgen secretion
  - Laparoscopic ovarian drilling: for reasons which are unclear, causing scaring of the ovaries by laparoscopic ovarian drilling appears to break the cycle of androgen secretion (perhaps due to inflammatory response?)
  - Regular monitoring of glucose tolerance and lipids

INFERTILITY – FEMALE
INFERTILITY: 25% DUE TO OVULATORY DISORDERS



- WHO classification of ovulatory disorders: 3 groups:
  - **Group 3: Ovarian Failure**
  - Ovarian failure, present as premature menopause, usually defined as <45 years of age
    - Main biochemical features of ovarian failure is persistently high FSH
  - Many cases due to autoimmune condition and is commonly associated with other autoimmune disorders
  - May also be due to:
    - Infection
    - Radiation, chemotherapy, surgery
    - Idiopathic

THE CLIMACTERIC

- During climacteric – progressive ovarian failure
  - Declining ovarian oestrogen secretion - - -> menstruation ceases
  - Menopause = last menstrual period: permanent cessation of menstruation
    - The ovaries fail to produce adequate amounts of oestrogen and inhibin, causing gonadotrophin production to increase in a continued attempt to stimulate the ovaries
      - FSH tends to increase first
      - FSH indication of ovarian failure - more reliable versus plasma oestrogen concentration
    - Mean age: 51 years (45-55years)
    - Menopause <45yrs = premature ovarian failure

- Menopause Metabolic Changes:
  - ↑ LDL-C
  - Osteoporosis

- HRT

- **NICE Guidelines NG23 (Nov 2015)**
- **Diagnosis of perimenopause and menopause**
- 1.2.1 *Diagnose the following without laboratory tests* in otherwise healthy women aged *over 45 years* with *menopausal symptoms:*
  - perimenopause based on vasomotor symptoms and irregular periods
  - menopause in women who have not had a period for at least 12 months and are not using hormonal contraception
  - menopause based on symptoms in women without a uterus.

- 1.2.4 Do not use a serum follicle-stimulating hormone (FSH) test to diagnose menopause in women using combined oestrogen and progestogen contraception or high-dose progestogen.

- 1.2.5 Consider using a FSH test to diagnose menopause only:
  - in women aged 40 to 45 years with menopausal symptoms, including a change in their menstrual cycle
  - in women aged under 40 years in whom menopause is suspected

INFERTILITY – FEMALE: TREATMENT

- Role for biochemical investigations:
  - Exclude pregnancy
  - Confirm ovulation – progesterone
  - Investigate irregular menstrual cycles i.e., : FSH, LH, oestradiol, testosterone, SHBG, prolactin with appropriate follow-up tests

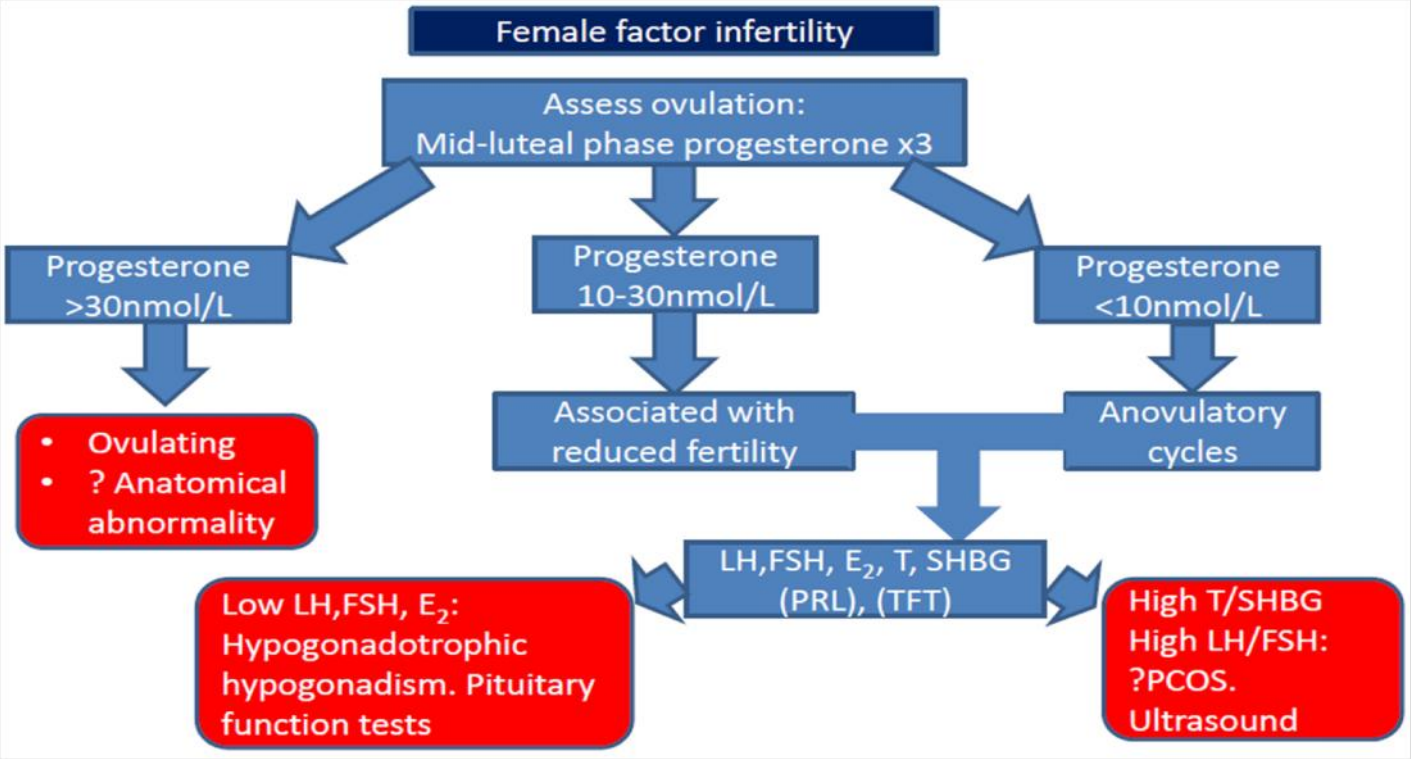
INFERTILITY – FEMALE: TREATMENT

- Treatment affected by cause i.e., PCOS, Pituitary/hypothalamic lesions, anorexia, structural i.e., uterine cavity
  - Prolactinoma: Medical management i.e, cabergoline, surgery
  - Infertility due to hypogonadotrophic hypogonadism treated with gonadotrophins:
    - Stimulate ovulation: FSH and LH; hCG maybe used to mimic mid-cycle LH peak and stimulate ovulation
    - Monitor oestradiol concentration – detect hyperstimulation: risk of multiple pregnancy and production of ovarian cysts
    - If hypothalamic disease – may prescribe clomiphene/ clomifene (selective estrogen receptor modulator)
      - Blocks oestradiol receptors in hypothalamus, in turn stimulate GnRH and thus FSH and LH
    - Non-responders prescribed GnRH

INFERTILITY – FEMALE: TREATMENT

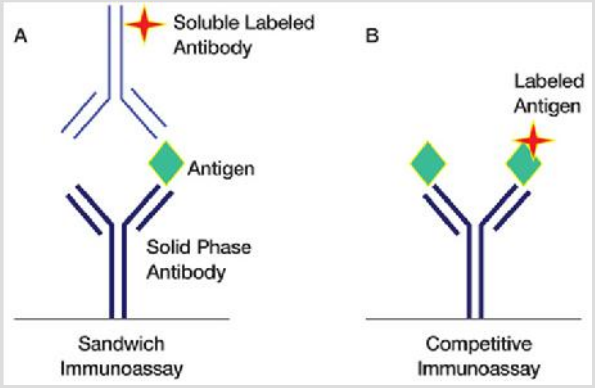
- Other subsequent biochemical investigations (depending on situation):
  - Predicting response to IVF stimulation protocol: Anti-Mullerian hormone (AMH) and FSH
    - Good response prediction:
      - Low FSH levels (< 4 IU/L)
      - High Anti-Mullerian hormone levels (>25pmol/L)
        - Low levels predict declining fertility prior to menopause
  - May exam number of ovarian antral follicles (ultrasound)
    - High number of ovarian antral follicles (>16) = good prognostic sign
    - Low FSH levels (< 4 IU/L)
- Note: AMH alternative test to ultrasound to assess ovarian reserve
  - AMH expressed by granulosa cells of the early pre-antral or small antral follicles. As follicles increase in size during development, the expression of AMH decreases. Therefore plasma levels reflect the number of primordial follicles i.e. ovarian reserve, in the ovaries: these are the follicles which treatment with gonadotrophins aims to stimulate. A low AMH levels predicts declining fertility prior to menopause.

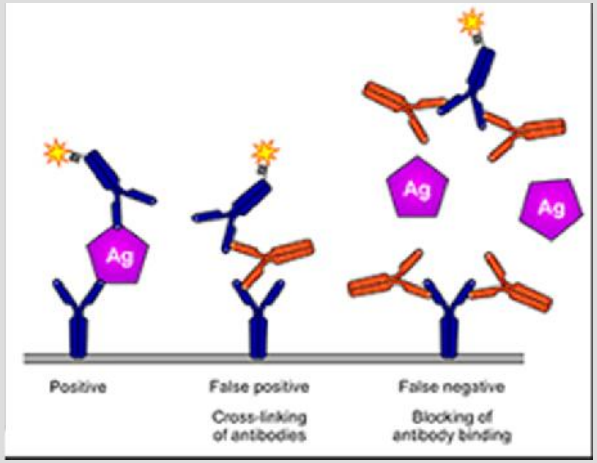
- Summary for the investigation of female infertility is given below:



ANALYTICAL CONSIDERATIONS

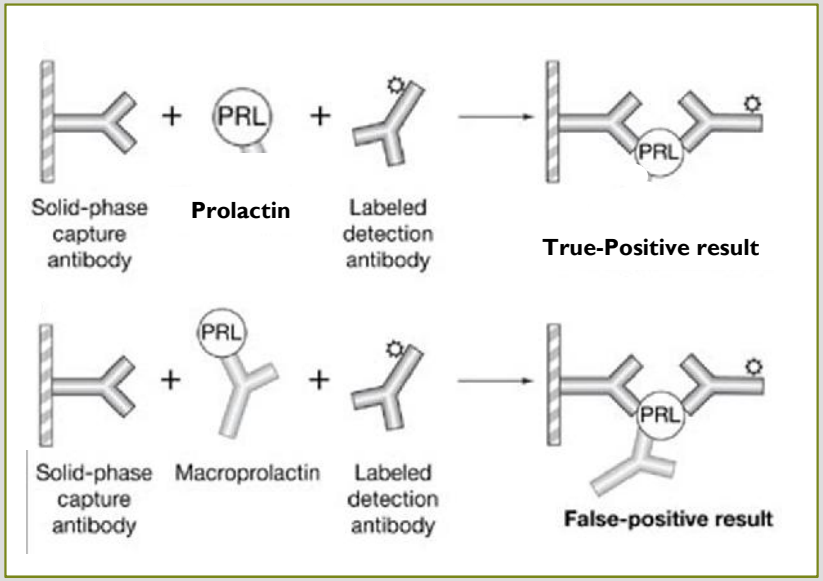
- Immunoassay: 2 site sandwich assay
  - Subunit:
    - FSH, LH, hCG and TSH = share alpha subunit: cross reactivity
    - Most assays today – beta subunit
  - Heterophilic antibodies and HAAAs (human anti-animal antibodies which can develop as a result of pets/ contact with animals i.e., mice etc)
    - Non-specific antibody interactions
      - False positive or false negative results





ANALYTICAL CONSIDERATIONS

- Prolactin
  - Macroprolactin – IgG Complex: cross reactivity
  - Artificially increases prolactin levels
  - Minimum Bioactivity
  - Should be screened to avoid unnecessary investigations
    - PolyEthylene Glycol (PEG) precipitation
      - Precipitate macroprolactin from serum
      - Recovery of prolactin after PEG precipitation shows the presence or absence of macroprolactin



ANALYTICAL CONSIDERATIONS

- Steroids i.e., oestradiol (E2), progesterone, testosterone
  - Immunoassays: heterophilic antibodies, other steroids - interference
  - Total or free hormone (usually total)
  - Detection limits (esp. E2 – pmol/L levels early follicular phase)
    - E2
      - Ref Method: Isotope Dilution GC-MS
      - Current immunoassays: wide measuring range: desired aim of a single assay for clinical application in menopause (low levels) and assisted reproduction (high levels). Also consider measurement of E2 in men and children
        - This analytical trade not been successful, - functional sensitivity of these immunoassays is inadequate for use at low concentrations.
        - Future E2 immunoassay design may require two reagent formulations (?) with different antibody concentrations, providing for precise measurement of E2 at either low- or high-concentration ranges.
        - Alternative: LC-MS/MS

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        - Alternative: LC-MS/MS
    - Progesterone
      - Ref Method: Isotope dilution GC-MS
      - Non-isotopic immunoassays available: has become the predominant method - adapted for use on automated immunoassay analysers

ASSAYS: TESTOSTERONE

Total Testosterone

- Reference method: Isotope dilution-MS
- Immunoassay (IA)
  - Requires displacement of steroid from binding protein
  - Good agreement with GCMS in male range, but poor agreement at low end
  - For pre-pubertal males and females, testosterone direct IA should not be used
    - IA poor accuracy for testosterone and poor analytical sensitivity for clinical use in women and pre-pubertal males
  - Extraction prior to IA may give improved results
  - Recommended: female patients with direct IA total [testosterone] > upper limit of the reference interval should be re-assayed after sample extraction or LC-MS/MS
    - LC-MS/MS (greater specificity and sensitivity: suitable for use in paediatric and female patients)

  - Architect Testo II
    - V high cross-reactivity nandrolone, >300% (19 nortestosterone: synthetic anabolic androgenic steroid (intra-muscular injections: muscle growth, appetite stimulation, increased red blood cell production etc)

ASSAYS: TESTOSTERONE

Free testosterone

- Equilibrium dialysis/ ultrafiltration – gold standard
  - Cumbersome method for use in the routine laboratory.
  - Problems include: sample dilution (ED), adsorption of testosterone to membranes (UF), accuracy of total testosterone assays, and binding of impurities in tracer to SHBG and albumin
- Direct Radio-IA (gives lower values than equilibrium dialysis)
- The clinical use of direct immunoassays for free testosterone is not supported

- Estimated:
  - FAI (female): T/SHBG* 100
  - (Assumes binding capacity of SHBG greatly exceeds testosterone concentration therefore unsuitable for males). Note ref range depends on T an SHBG method
  - Calculated free testosterone (male): Vermuelen equation uses T, SHBG, albumin (http://www.issam.ch/freetesto.htm)

CASE 1

- Female age 28yrs
- Secondary amenorrhoea

- FSH <1IU/L
  - Follicular: 3-8; Luteal 1-5; Ovulatory 3-17; Post-menopausal 27-133
- LH <1IU/L
  - Follicular: 2-12; Luteal 1-14; Ovulatory 9-89; Post-menopausal 5-62
- Oestradiol 9823
  - Follicular: 77-921; Luteal 77-1145; Ov Peak 140-2382; Post-menopausal <103

- What test would you suggest to add?
- If oestradiol low – how would your interpretation change?

CASE 2

- Female age 32yrs
- Oligomenorrhoea, Excessive hair on upper lip, lower abdomen and thighs

- FSH 5IU/L
  - Follicular: 3-8; Luteal 1-5; Ovulatory 3-17; Post-menopausal 27-133
- LH 10IU/L
  - Follicular: 2-12; Luteal 1-14; Ovulatory 9-89; Post-menopausal 5-62
- Testosterone 3.6nmol/L
  - <2.9nmol/L

- What diagnosis do these results suggest?

CASE 3

- Male age 35yrs
- Erectile Dysfunction

- Testosterone: 5nmol/L (9.9 - 30)
- Repeat at 9am: 6.5nmol/L

- Add gonadotrophins
  - LH/ FSH – Inappropriately low
- Add: prolactin, TSH. FT4/ Cortisol – all inappropriately low

- What diagnosis do these results suggest?

CASE 4

- Male age 65yrs
- No clinical details

- Testosterone 49nmol/L (9.9 - 30)

- Add gonadotrophins
  - LH/ FSH – low

- Possible reasons ?