ZJE-UoE Institute – ERB3

ENDOCRINOLOGY AND REPRODUCTIVE BIOLOGY

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Notes for ERB3 revision

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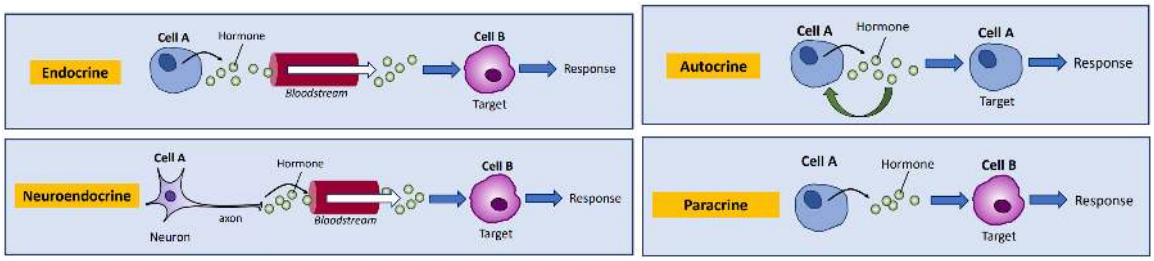
1 OVERVIEW

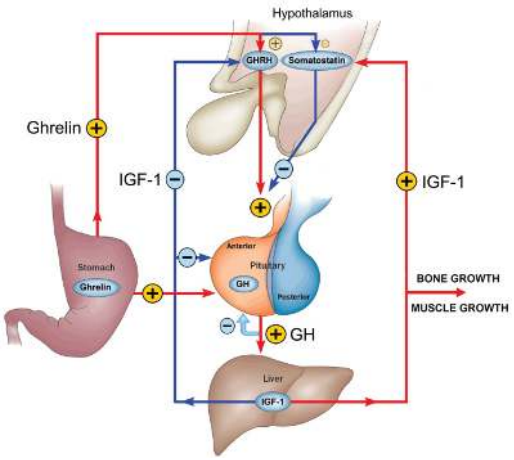
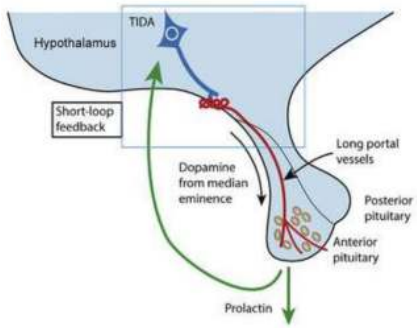
Hormones can be defined as chemical messengers or signalling molecules

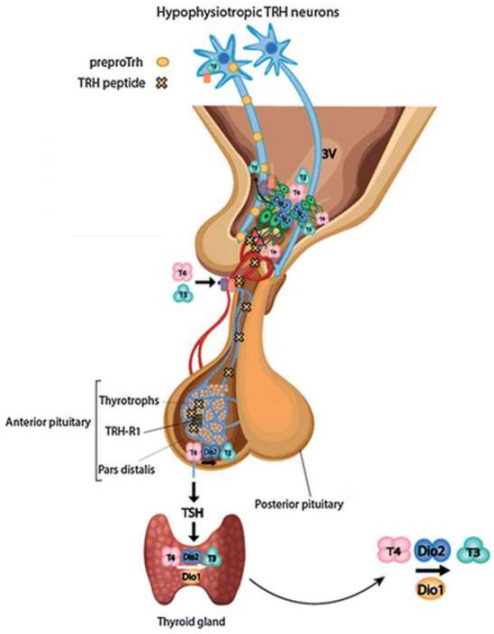
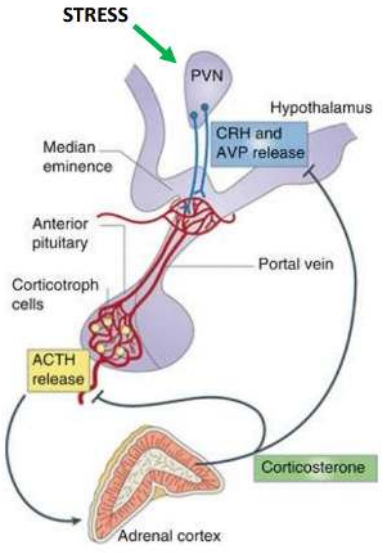
Hormones can be produced in dedicated endocrine cells, i.e., the endocrine glands, or in cells in organs that have additional roles, e.g., adipose, stomach…

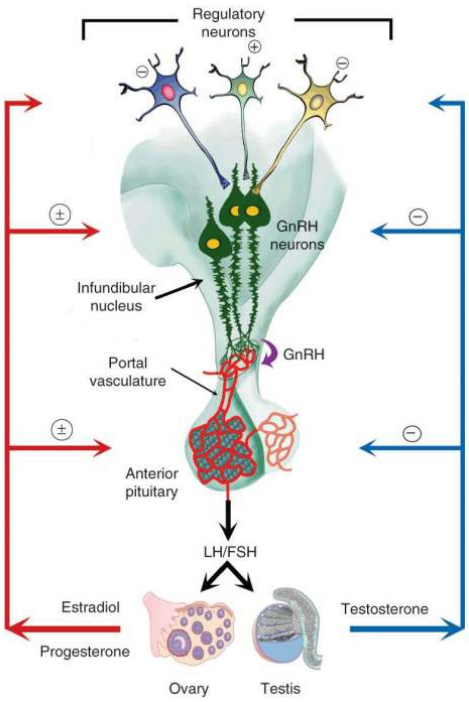
1.1 Action mode of hormones



1.2 Catalogue of hormone

Parts of the endocrine system	Cells, Structures and hormones
Dedicated endocrine organs	
Hypothalamus	Located at base of brain Produce AVP, oxytocin and regulatory hormones and somnatostatin that control secretion of anterior pituitary hormones **It is the "control centre" for endocrine systems**
Posterior pituitary gland	Store and release AVP and oxytocin
Intermediate pituitary gland	Secretes melanocyte stimulating hormone (MSH)
Anterior pituitary gland	 	6 in total: 5 tropic hormone + 1 hormone by 5 cell types • Somatotrophs: GH • Lactotrophs: PRL • Corticotrophs: ACTH • Thyrotrophs: TSH • Gonadotrophs: FSH & LH
Pituitary glands are called "master gland".	
Pineal gland	Located in the brain between the cerebral cortex & cerebellum

	Secretes melatonin that regulates sleep-wake cycle and modulates gonadal activity
Thyroid	 Located in neck, wrapped around the trachea Secretes T4, T3 and calcitonin. Receptos: TRH-R1 & TSHR • Thyroid follicles: follicles where thyroid hormones are stored • Capillaries: a rich network surrounds each follicle. • Colloid: lumen of each follicle, filled with colloid. It is mostly the protein thyroglobulin and bound T3 & T4 • Follicular cells: lining of follicles (epithelium). Secrete thyroid hormones when active.
Parathyroid (2 pairs, 4 in total)	4 small glands located on posterior surface of the thyroid gland\ Chief cells: the majority of cells. Secrete PTH to enhance calcium release from bone reservoirs, opposing calcitonin secreted by C cells of thyroid. Oxyphil cells: larger non-secretory cells.
Adrenal gland	 Located on top of the kidneys Consists of 2 distinct parts: cortex& medulla • Zona glomerulosa: Mineralocorticoids (aldosterone) • Zona fasciculata: Glucocorticoids (cortisol) • Zona reticularis: Androgens (DHEA) • Medulla (Chromaffin cells): adrenaline/noradrenaline
Pancreas	Located behind stomach The 'Islets of Langerhans' contain 4 major types of endocrine cells: • Alpha cells: Glucagon

	• Beta cells: Insulin • Delta cells: Somatostatin (Inhibits pancreatic alpha and beta cells) • PP-cells (or F cells): pancreatic polypeptide (Regulates endocrine & exocrine pancreatic secretions)
Ovary	 Secrete estrogens, progestins, inhibin and relaxion (only during pregnancy)
Testis	Leydig cells produces testosterone/androgens Seminiferous tubules produces sperm Sertoli cells: support cells and secrete inhibins Spermatogonia → Spermatocytes → Spermatids → Spermatozoa
Other endocrine organs	
Thymus	Secretes thymosin & thymostatin
Heart	Secretes atrial natriuretic peptide (ANP)
GI	Secretes gastrin, secretin, cholecystokinin (CCK)…
Kidney	Secretes erythropoietin, calcitriol, renin
Adipose	Secretes leptin
Placenta	hCG, placental lactogen, P4, E2

1.3 Hormone types

Property	**Steroid hormones**	**Amino acid derivatives**	**Peptide/polypeptide hormones**
Solubility	Typically lipophilic	Typically hydrophilic	Typically hydrophilic
Synthesis	Synthesised from cholesterol in the smooth endoplasmic reticulum of	These are modified amino acids: hormones synthesised from single amino acids	Synthesised in the rough ER → Golgi → secretory vesicles → cell exterior pathway

Property	Steroid hormones	Amino acid derivatives	Peptide/polypeptide hormones
	cells in the gonads and adrenal cortex		

Examples	• Corticosteroids:
	  ◦ Glucocorticoids: CORT
	  ◦ Mineralocorticoids: Aldosterone
	• P4 and E2
	• Testosterone
		Catecholamines such as adrenaline, noradrenaline, and T4	• Small peptides: TSH, Oxytocin, AVP
			• Large peptide: Glucagon, ACTH
			• Polypeptides: PTH, GH

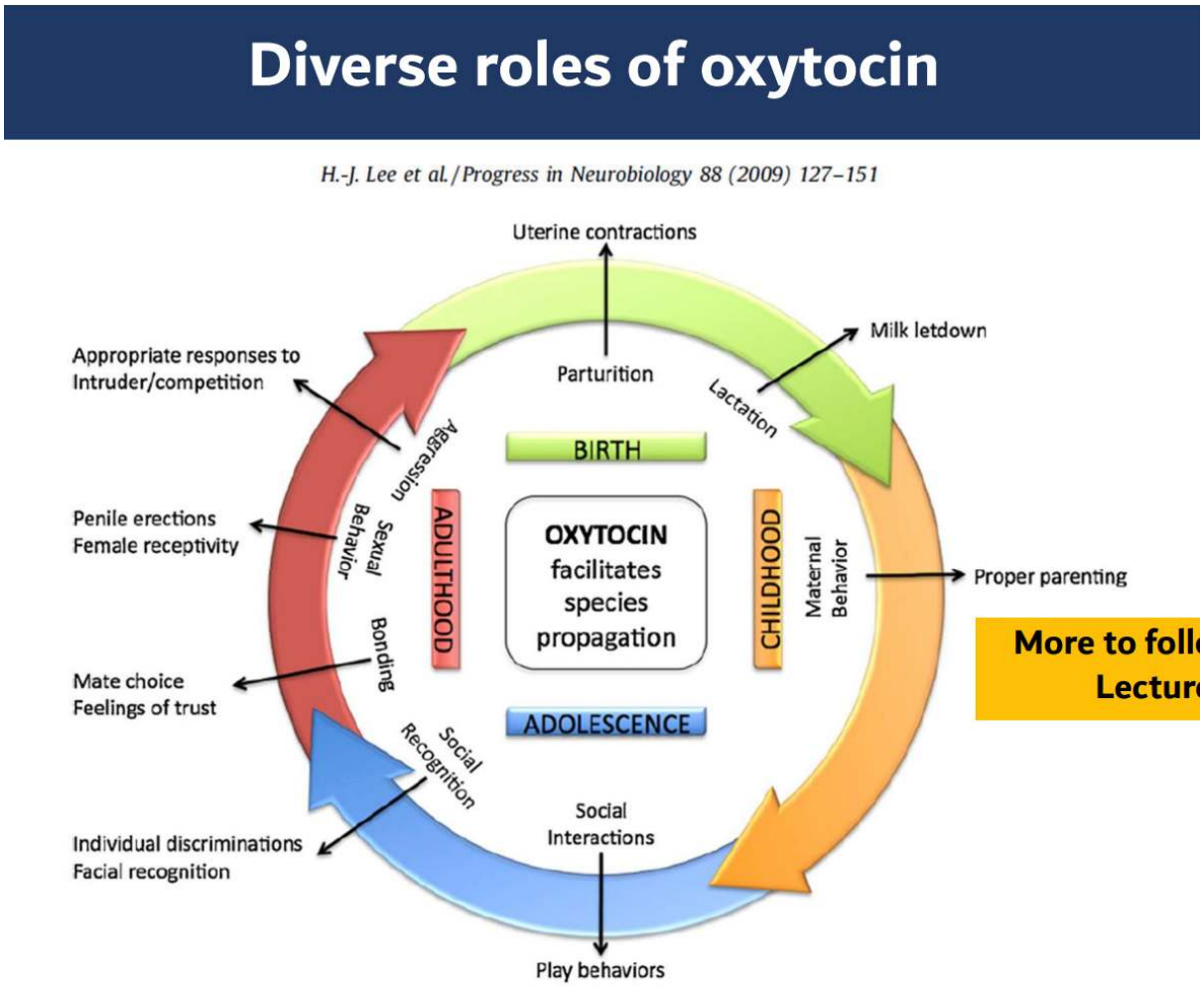
Secretion	Simply diffuse out of secreting cells & into the circulation. Therefore, steroid hormone release is controlled by the rate of synthesis.	—	Depolarisation-induced entry of sodium & calcium ions into the cell → Stimulation of peptide-hormone-secreting cells → cell membrane permeability increase → exocytosis
Transportation	Present in blood bound to carrier proteins (or binding proteins). Have longer half-lives as binding protein protects them from deactivation.	Dissolved in blood. Typically short half-lives as they are deactivated. However, T4 is bound to TBP.	Dissolved in blood. Typically short half-lives as they are deactivated.
Pulse	—	—	Usually in pulses. As each cohort of secretory granules bind to the cell membrane, a pulse of hormone is released into the blood.
Receptor	Cross the plasma membrane & bind to receptors in the cytoplasm. • Sex hormone-binding globulin (SHBG): binds testosterone, dihydrotestosterone, and E2 • Corticosteroid-binding globulin (CBG): binds glucocorticoids and P4	On cell membranes. But T3 is received by nuclear receptors (THR). • T3 cannot cross cell membranes like other lipophilic molecules, so it has to enter via thyroid hormone transporters. • In the nucleus, T3 binds to THR	On cell membranes: • G protein-coupled receptor (GPCR): CRH, vasopressin, GHRH, somatostatin, Ghrelin • Enzyme-linked receptor (most common: receptor tyrosine kinases): Insulin, leptin, PRL and GH

Property	Steroid hormones	Amino acid derivatives	Peptide/polypeptide hormones
	However, they can also bind to cell membrane receptors, causing rapid, non-genomic signalling via modulation of intracellular second messenger systems (e.g. MAPK).	retinoic acid receptors (RXR) heterodimers, which bind to the genomic thyroid-hormone responsive element (TRE) to regulate gene transcription.	• Ion channel-linked receptor
Signal transduction	• Genomic: The steroid hormone binds to a specific receptor in the cytoplasm or nucleus. The steroid-receptor complex enters the nucleus, binds to response elements on the DNA, causing up- or down-regulation of gene transcription. • Non-genomic: Membrane sex steroid receptors & Membrane corticosteroid receptors.	cAMP pathway: 1. Hormone binds to receptors on the plasma membrane 2. Receptor binding causes a conformational change initiating activation of adenylyl cyclase. 3. Adenylate cyclase catalyses the conversion of ATP to cAMP. 4. cAMP activates protein kinases that phosphorylate other cellular proteins to activate/deactivate them. 5. Phosphorylated proteins cause reactions that produce physiological responses. 6. After a brief period, an enzyme called phosphodiesterase inactivates cAMP, the cell's response is switched off.	cAMP pathway: 7. Hormone binds to receptors on the plasma membrane 8. Receptor binding causes a conformational change initiating activation of adenylyl cyclase. 9. Adenylate cyclase catalyses conversion of ATP to cAMP. 10. cAMP activates protein kinases that phosphorylate other cellular proteins to activate/deactivate them. 11. Phosphorylated proteins cause reactions that produce physiological responses. 12. After a brief period, an enzyme called phosphodiesterase inactivates cAMP, thus the cell's response is switched off. Or maybe PKC pathway.

1.4 Anatomy

Parvocellular neurosecretory cells	Magnocellular neurosecretory cells
Small cell bodies	Large cell bodies
In PVN only	In both PVN and SON
Release tropic hormones	Release oxytocin or vasopressin.

Release of magnocellular cells is mediated by somatodendritic release of vesicles.

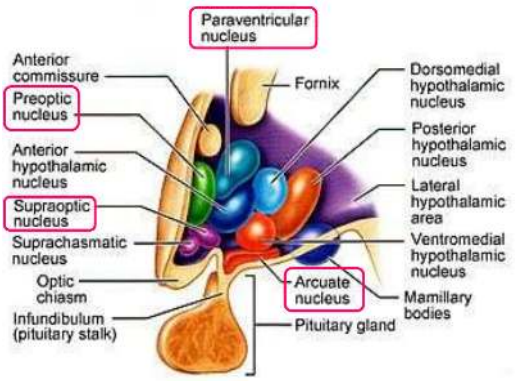


1.5 Hypothalamus

The hypothalamus is made of neurosecretory cells, a class of neuronal cells capable of endocrine functions into the blood.

- Magnocellular system: projects to the posterior pituitary
- Parvocellular system: projects to the median eminence
    - Nerve endings release their hormones into the hypophysial portal blood system

HYPO contains various nuclei. These nuclei contain neurosecretory cells that release hormones into the portal blood system that supplies the anterior pituitary gland.



1.6 Pituitary gland

Pituitary gland is also called hypophysis.

Hypothalamic hormone links pituitary hormone by hypophysiotropic hormones

Hypothalamic hormone	Action	Pituitary hormone
Corticotropin-releasing hormone (CRH)	Stimulatory	ACTH
Gonadotropin-releasing hormone (GnRH)	Stimulatory	LH and FSH
Thyrotropin-releasing hormone (TRH)	Stimulatory	TSH
Growth hormone-releasing hormone (GHRH) Somatostatin	Stimulatory Inhibitory	GH GH
Dopamine Prolactin-releasing factor (PRF)?	Inhibitory Stimulatory	Prolactin Prolactin?

* **Prolactin-releasing factor (PRF)**, is a hypothetical releasing factor – to date a specific PRF has not been identified

Prolactin is currently lacking a hypophysiotropic hormone, but under the control of (inhibited by) dopamine released from arcuate nucleus neurons. Also PRL does not have a target tissue that exerts negative hormonal feedback.

During pregnancy: prolactin levels rise to facilitate the development of the breast alveolar structure. This effect of prolactin is estradiol- and progesterone-dependent.

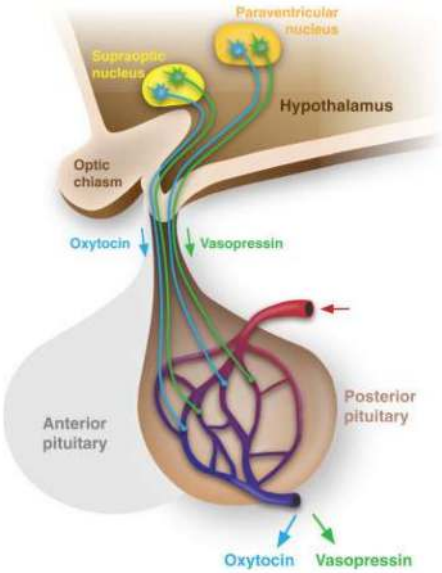
After pregnancy: prolactin is also critical for milk production, but is effective only when levels of estradiol and progesterone decline after parturition. The suckling stimulus* which produces surges of prolactin, is crucial for continued lactation via inhibiting dopamine.

2 POSTERIOR PITUITARY

The hypothalamus-posterior pituitary system is also called the hypothalamo-neurohypophyseal system.

The pituitary gland = adenohypophysis + neurohypophysis.

2.1.1 Content



It consists mainly of axons of magnocellular neurosecretory cells extending from the SON & PVN of the hypothalamus. In this way, it can be stated that the posterior lobe is an extension of the hypothalamus.

It also contains neurofilaments to provide structural support for the axons.

2.1.2 Hormone overview

OT and AVP are

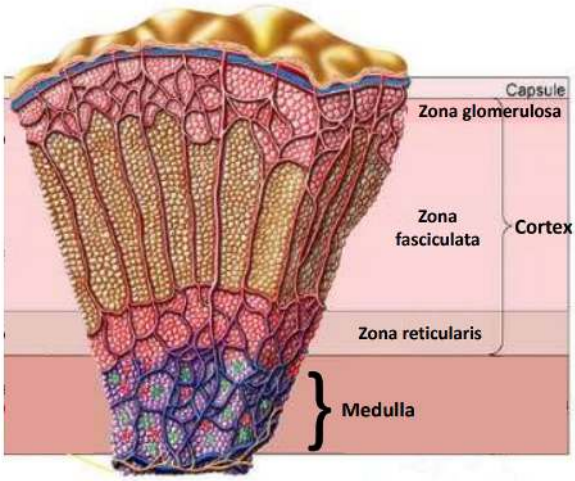
- PRODUCED in the cell bodies of the hypothalamus
- PACKAGED in neurosecretory granules
- TRANSPORTED to the nerve terminals
- STORED and RELEASED in the posterior pituitary
    - Secretion evoked by action potential firing.

3 STRESS

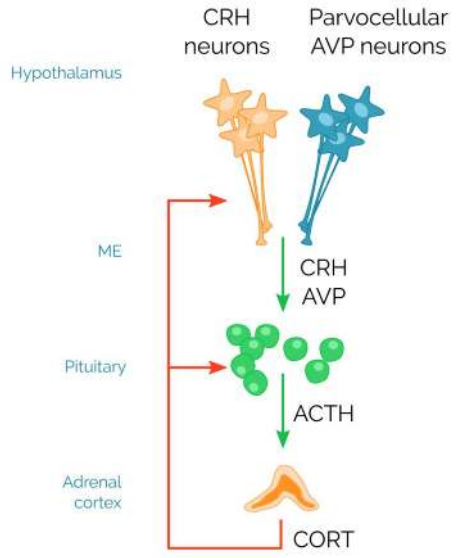
Stressors can be divided into psychological & physical stressors.

3.1 Adrenal cortex

3.1.1 Anatomy



3.2 HPA axis



Cortisol secretion is controlled by the hypothalamus-pituitary-adrenal (HPA) axis.

CRH neurons are in the PVN of the HYPO.

3.2.1 Hormones

3.2.1.1 Synthesis and storage

Cortisol is not managed in a storage way. It is always synthesized on the go. Hence, the acute need for increased cortisol secretion requires rapid activation of the initial synthesis step, where the rate-limiting enzyme needs to be activated.

3.2.1.2 Secretion

Both ACTH and cortisol secretion are pulsatile

On a large scale: A positive, delayed relationship exists between ACTH (before) and cortisol (after).

On a time scale: There is a **cortisol awakening response** effect, where cortisol levels peak within 20-30 min of awakening.

In short, CORT is secreted in an ultradian AND circadian pattern.

3.2.1.3 Response

The CRH neurones in the PVN integrate excitatory & inhibitory signals arising primarily from the brainstem & limbic brain regions into an appropriate neuroendocrine signal, where:

- Physical stressors signal through direct projections to CRH neurones from brainstem catecholaminergic neurons.
- Signals from psychological stressors utilize multisynaptic pathways via limbic structures

3.2.1.4 Action

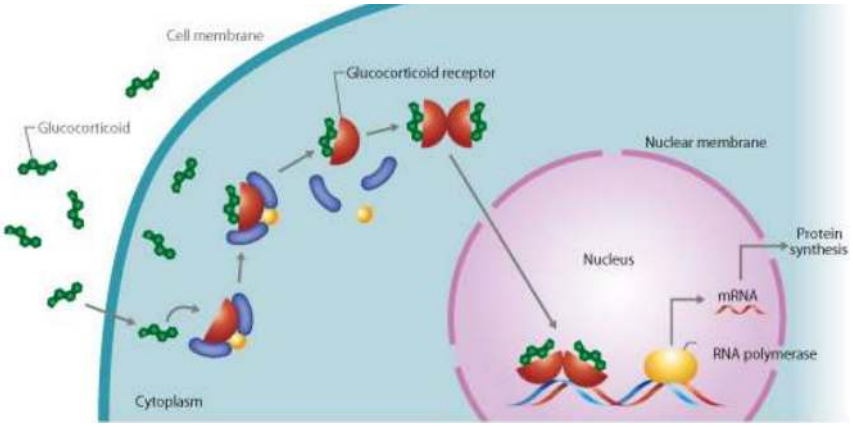
CORT can bind to both MR and GR. However, MR has a 10x higher affinity than GR for CORT. Corticosteroids bind to both GR and MR, while mineralocorticoids only bind to MR.

- GR is expressed in: hippocampus, PVN (of HYPO), AP and nearly all tissues in the body and almost ubiquitously expressed throughout the brain
- MR is expressed in: hippocampus.

Mode of action

- Genomic
- Non-genomic (less well understood)

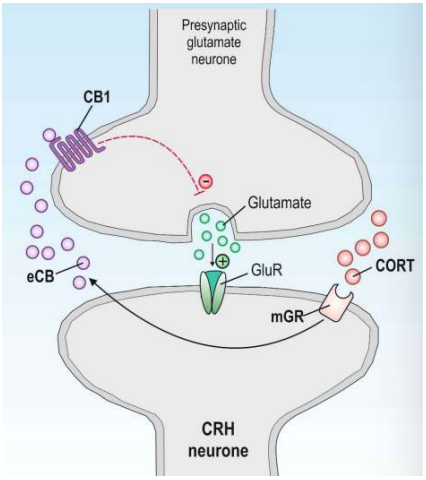
The classical effects of glucocorticoid signalling are via genomic actions. Ligand-bound GR homodimerizes in the nucleus and exerts transcriptional effects by binding to promoters of glucocorticoid target genes.



3.2.1.5 Negative feedback

Negative feedback is mediated by both MR and GR directly at the level of PVN-HYPO, AP and hippocampus.

GLUT negative feedback has two categories



- Rapid (non-genomic): act on secretion but not synthesis (***glucocorticoid-induced suppression of excitation***)
    - Mediated by increased glucocorticoid-induced endocannabinoid (eCB) synthesis in the PVN.
    - Followed by increased eCB1 release and increased eCB1-CB1 receptor binding on the presynaptic excitatory synapses.
- Slow (genomic): act on secretion AND synthesis

3.2.1.6 Effects

Increase blood glucose	1. Increase protein break down 2. Increase mobilization of amino acids 3. Stimulation of lipolysis in adipose tissue so fatty acids are used as an energy substrate for gluconeogenesis 4. Increase hepatic gluconeogenesis

	5. Inhibition of insulin-dependent glucose uptake & storage in muscle & adipose tissue
Immunosuppressive effects	1. Repression of pro-inflammatory genes 2. Activation of anti-inflammatory genes 3. Inhibit the two main products of inflammation - prostaglandins & leukotrienes 4. The induction of apoptosis in lymphocytes
Cognitive effects	1. Long-term memories are optimal when glucocorticoid levels are mildly elevated, whereas memory is impaired in a low-glucocorticoid state or a high-glucocorticoid state. 2. Elevated levels of glucocorticoids enhance memory for emotionally arousing events, but also lead to poor memory for material unrelated to the source of stress/emotional arousal.

3.2.2 Consequence

Adaptive Stress-Response	Stress-Related Disorder
Mobilization of energy	Type 2 diabetes, obesity
Increased cardiovascular tone	Hypertension, heart disease, stroke
Increased arousal	Insomnia, anxiety
Elevated circulating glucocorticoids	Cognitive impairments, depression
Suppression of immune system	Susceptibility to infection
Suppression of reproduction	Amenorrhea, impotency, loss of libido
Suppression of digestion	Gastric ulcers, colitis

3.2.3 Regulation

Major inputs of the HPA axis

- Stress
- Circadian

3.3 Clinical GCs

GCs have anti-inflammatory and immunosuppressive properties just like CORT. GCs are far more potent than non-steroidal anti-inflammatory drugs such as ibuprofen.

Hydrocortisone and cortisone are both used clinically.

- Cortisol (active) -11bHSD2-> Cortisone (inactive)
- Cortisone (inactive) -11bHSD1-> Cortisol (active), in the liver

Synthetic GCs are also used in the clinic:

Steroid	Relative potency		Half-life in plasma (h)	Biological half-life (h)
	GR	MR		
Hydrocortisone	1	1	1.5	8-10
Cortisone	0.8	0.8	0.5	8-10
Betamethasone	25-30	0.5	6.6	36-54
Dexamethasone	30	0.5	4	36-72
Prednisolone	4	0.8	3	12-36
Methylprednisolone	5	0.5	3	12-36

However, long term GC treatment could lead to adverse outcomes:

Atrophy of the adrenal glands (Exogenous glucocorticoids rapidly suppress HPA axis)

- Iatrogenic Cushing's syndrome
- Reproductive defects
- Metabolic defects

High GCs suppress reproductive function

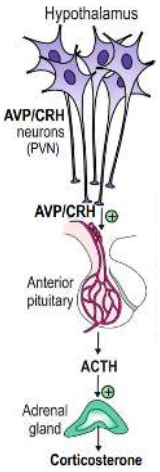
- **Hypothalamus -** decreased synthesis and release of GnRH, increased RFamide-related peptides (RFRPs – inhibitors of HPG axis).
- **Pituitary -** decreased synthesis and release of gonadotropins.
- **Gonads -** reduced response to gonadotropins and steroid biosynthesis in ovary & testes; increase in testicular oxidative stress, and apoptosis of Leydig and germ cells; antagonise oocyte maturation.

3.4 Disease

Addison's disease & Cushing's syndrome see PBL materials.

3.5 Other hormones' contribution

3.5.1 AVP as a stress hormone



- AVP is co-expressed with CRH in the PVN: CRH neurons in the PVN also synthesize AVP, which is co-stored & co-released WITH CRH at the median eminence
- Hypophysial-portal plasma concentrations of CRF & AVP are increased in a graded fashion in response to stress.
- CRH binds to CRHR1 receptors, while AVP binds to AVPR1B receptors on corticotrophs to stimulate ACTH secretion in response to acute restraint stressor chronic stress.
- While having minimal effects on its own, AVP synergizes with CRH to greatly amplify ACTH secretion.

The HPA axis habituates to repeated homotypic stressors

- There is adaptation or habituation of CRH to a repeated homotypic stressor, while its stress response to a heterotypic stressor is maintained.
- However, AVP responses persist after repeated restraint.
- When repeatedly restrained rats are exposed to a novel heterotypic stressor, CRH neurons still respond, but weakly, and AVP transcriptional responses are especially robust.
- Therefore, chronic repeated stress appears to shift PVN stress regulation away from a CRH-dominant response toward an AVP-dominant response.

3.5.2 Oxytocin as a stress hormone

- Stress increases oxytocin secretion into the blood, positively correlated with the cortisol response
- Oxytocin can also potentiate the ACTH response to CRH just like AVP.
- Oxytocin neurones are activated in response to stress, and their release is also activated.
- Oxytocin increases the pain threshold in rats by reducing nociception.
- Central oxytocin inhibits HPA axis responses to stress and administration of an oxytocin receptor antagonist into the brain increases HPA axis responses to stress (negative feedback)
- Oxytocin boosts recovery, rather than buffering stress reactivity
- Recovering from stress with social support promotes oxytocin release in the PVN. Oxytocin mediates the social buffering effect on stress responses.
- Oxytocin (OT) reduces anxiety and stress responses in 'solo' voles, while an oxytocin receptor antagonist (OTRa) prevents the social buffering effect in partnered voles

4 GROWTH

4.1 Overview

4.1.1 The discovery of GH

- Tom Thumb: Normal birth size does not guarantee normal postnatal growth, which depends on internal endocrine signals, especially GH.
- Explanation: In utero, GH has little effect on statural growth

4.1.2 Research models

- **Snell dwarf mice:** Pit-1 (pituitary-specific positive transcription factor 1) mutation. Pit-1 is a TF needed for development of certain pituitary hormone-producing cells.
- **Ames dwarf mice:** Prop-1 (paired-like homeobox 1). Another TF important for pituitary development.

4.2 Roles of GH

- **Development** (Canonical)
- **Bone:** Increase bone length and radial.
- **Protein synthesis:** Helps direct amino acids toward protein synthesis rather than other metabolic pathways.
- **Fat metabolism:** Promotes fat breakdown, helping provide energy.
- **Carbohydrate metabolism:** Increases gluconeogenesis, mainly by the liver.

So its metabolic effects are pretty much opposing CORT effects.

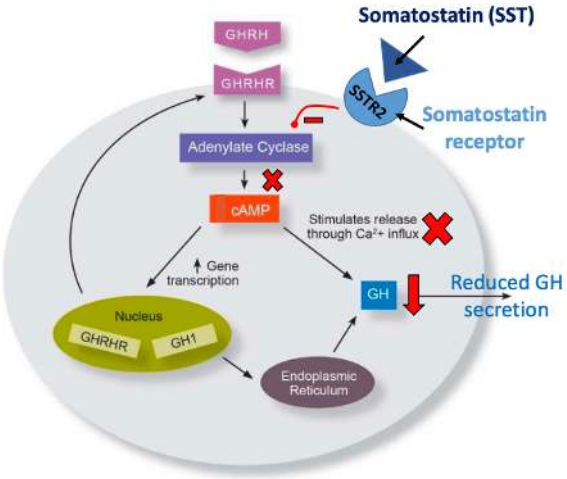
4.3 Regulation

4.3.1 HP axis

1. Hypothalamus releases GHRH by GHRH neurons in ARC nucleus
2. GHRH reaches the AP by portal blood
3. Somatotrophs respond
4. GH secretion increases

Somatotrophs are the most abundant cell type in the anterior pituitary gland. They form an organized network with plasticity. Around puberty, a time of high GH demand, the GH cell network shows a marked rise in the volume/surface ratio to satisfy that need.

Somatostatin (STT) secreted by STT neurons at PeV of HYPO, binds to somatostatin receptors on GH cells in AP to reduce GH secretion, not production. Delta cells of islets of Langerhans can also secrete somatostatin, but do not exert this effect



4.4 GH with other hormones

With thyroid hormone

- TH supports GHR expression
- GH inactivates T3

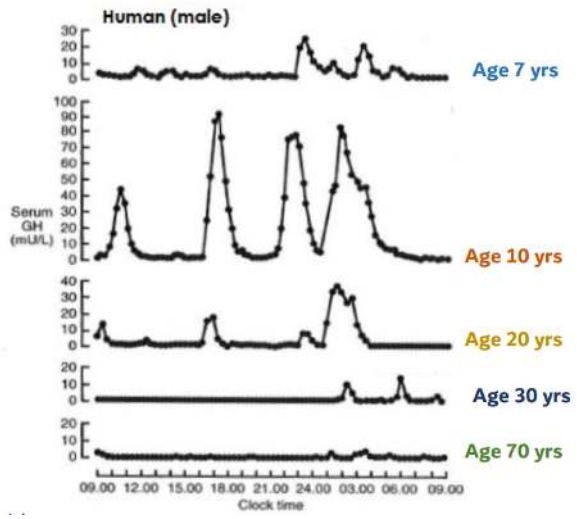
With CORT

- GH pulses increase 11β-HSD2, thus inactivating cortisol.
- Glucocorticoids reduce GHR expression

Estrogen and ghrelin can also stimulate GH secretion

4.5 GH secretion

4.5.1 Shared features



- GH has basal secretory activity.
- GH is secreted in pulses. GH pulses more effective in stimulating growth than continuous secretion.
- GH pulses are greater during sleep.
    - Sleep deprivation abolishes GH secretion, but daytime sleep can restore the sleep-related surge of GH secretion
- GH pulses is age dependent
- GHRH and SST together generate pulsatility. The pulsatility is actively generated by the hypothalamus.

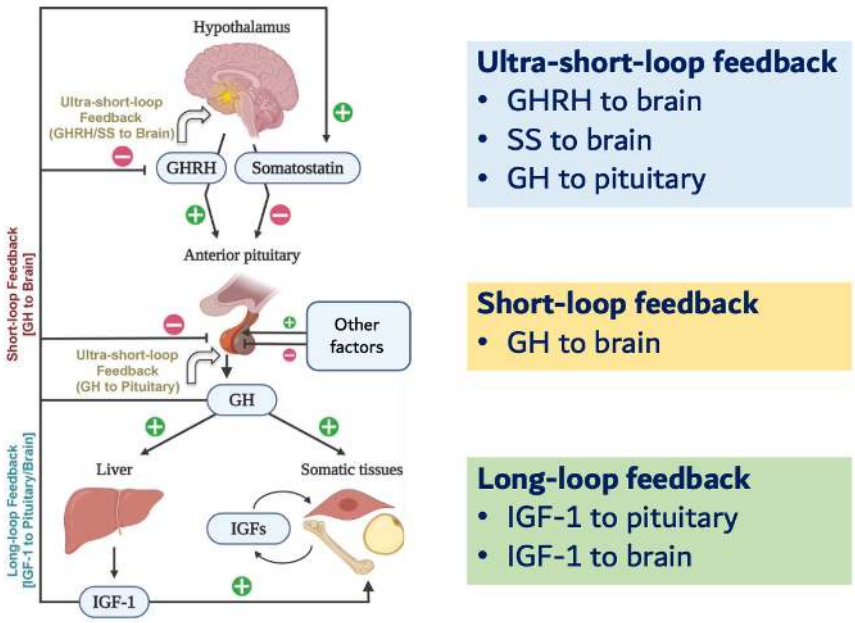
- GHRH stimulates somatotrophs
- Somatostatin inhibits somatotrophs

4.5.2 Sex-specific features

- Male: Large, separated pulses with low levels between pulses.
- Female: More frequent and less sharply separated.

4.5.3 Negative feedback

The negative feedback is more complicated in HP-GH axis.



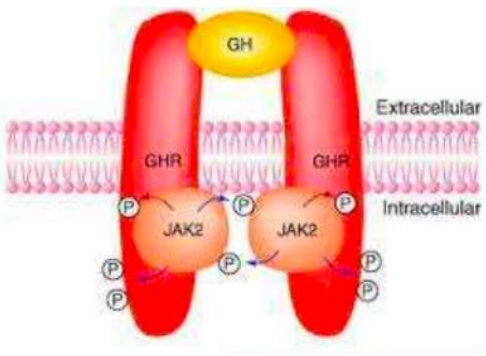
4.6 GH action

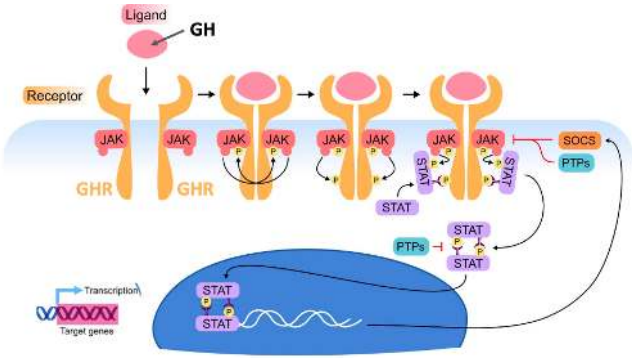
GH acts on Growth hormone receptor (GHR), not GR!

4.6.1 Features

- GHR has a wide tissue distribution, just like GR.
- GHR is a dimer.
- GHR expression is low in the fetus and rises after birth.
    - This explains why GH has little effect on fetal growth but becomes important after birth.
- TH supports GHR expression
- Glucocorticoids reduce GHR expression
    - So CORT can decrease GH's outcome by both directly acting on substrate, and also by acting on GHR's signalling transduction.

4.6.2 Signalling





GH → GHR → JAK2 phosphorylation → STAT5 phosphorylation → gene transcription

1. GH binds to the dimeric GHR at the cell surface.
2. This activates JAK2, a kinase that phosphorylates STAT5.
3. pSTAT5 moves toward the nucleus and stimulates transcription of GH-regulated genes.

SOCS protein family (SOC-1/2/3, uppressors of cytokine signaling) inhibites GH signaling by acting on JAK2.

4.6.3 Downstream of GH

- IGF-1 is the main mediator protein of GH.
- IGF-1 is produced by the liver (mainly, but also other organs) in response to GH-GHR binding.
- IGF-1 travels in the blood bound to IGFBP.
- IGF-1 then acts on target tissues such as bone, muscle, and adipose tissue through the IGF receptor.

4.7 GH-related diseases

Consequences

- Childhood - short stature
- Adult - increased fat mass, decreased lean body mass, increased mortality!

Treatment

- Childhood - inject GH
- GH treatment of adults?
- GH declines with age – GH treatment leads to increased muscle mass, decreased fat mass, increased muscle strength.
- BUT GH deficiency = reduced cancer risk.

- GH-secreting pituitary adenomas in children can cause accelerated height growth (gigantism)
- GH-secreting pituitary adenomas in adults can cause headache, visual impairment, seizures with neoplastic complications

5 ENERGY

Energy is directly influenced by **food intake**, determined by **appetite**, manifesting as **BW/BMI/Obesity outcome**, which has a close relationship with **glucose homeostasis regulation**.

5.1 Energy set point

There is no set point in energy homeostasis. Energy homeostasis is a dynamic concept. But there is an intervention point.

- Lower intervention point: a threshold below which the body defends against weight loss.
- Upper intervention point: a threshold above which the body might defend against weight gain.

5.2 Vagus nerve

The vagus nerve is the mediator of gut-brain communication, running from the brainstem to gut organs (stomach and intestines) and other organs (liver, pancreas)

Vagal afferents (gut → brain)

- They detect things like gut distension and gut contents.
- These sensory signals travel to the nucleus of the solitary tract (NTS) in the brainstem.
- From there, information is passed to other brain regions, including the AgRP of the hypothalamus.
- They can also sense anorexigenic gut hormones: GLP-1, CCK, oxytocin, …

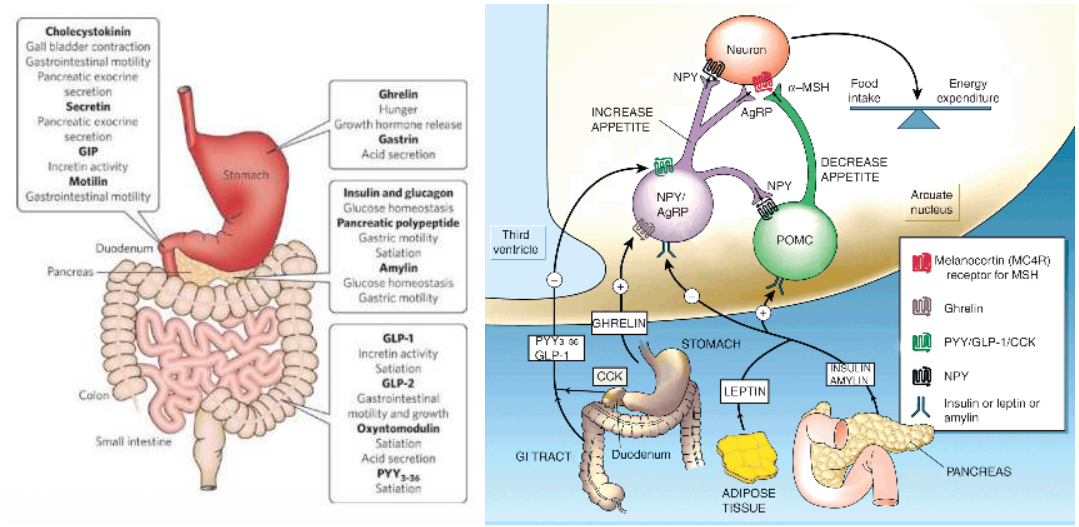
Vagal efferents (brain → gut)

- Affects GI peristalsis, stomach secretion, liver-related secretion, and pancreatic secretion.

5.3 Hormones

5.3.1 Overview

Below are all the GI hormones.



MC4R on MC4R neurons inhibits hunger

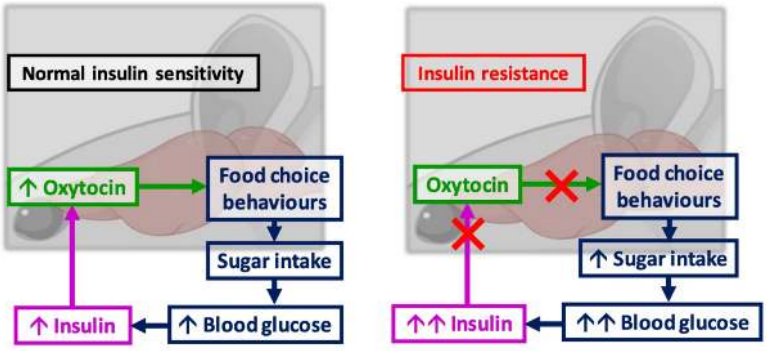
- MC4R neurons are inhibited by AgRP/NPY neurons' secretion of AgRP, NPY ligand, and GABA.
  - This is a hunger signal (Orexigenic effects)
- MC4R neurons are activated by POMC neurons' secretion of a-MSH ligand
  - This is a satiety signal (Anorexigenic effects)
- Note: NPY/AgRP neurons also have suppressive effects on POMC neurons.

5.3.2 Oxytocin

a-MSH also targets oxytocin neurones by evoking oxytocin release from dendrites of magnocellular cells.

Oxytocin and oxytocin receptor agonists act on the brain (VMH region, a complex and heterogeneous satiety centre) to reduce overall food intake and restrain palatable intake (such as sucrose, but not fat) in a dose-dependent manner.

High-sugar food activates oxytocin neuron activities, and insulin activates oxytocin neuron activities; hence, high sugar effects on oxytocin neurons are possibly mediated by insulin.



5.3.3 Ghrelin

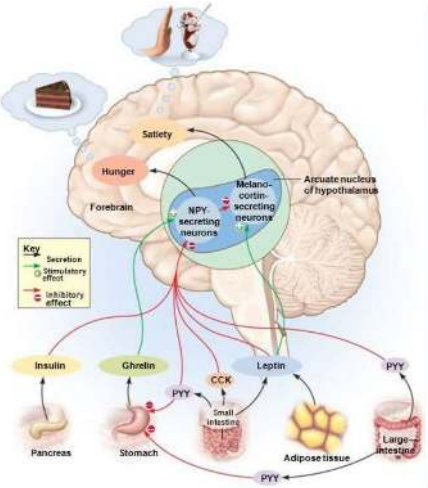
- Secreted by the stomach (X/A like enteroendocrine cells)
- Ghrelin can increase eating and body weight
- A GH-releasing acylated peptide, which means it can also stimulate GH secretion
- Rises especially before meals and signals hunger, decreases after meals.

5.3.4 GLP-1

- By stomach (L enteroendocrine cells)
- Via blood circulation as a hormone OR via vagal afferent nerves to signal.
- Decrease meal size, decrease meal duration, decrease refeeding after a fast.
- Released after nutrients enter the gut and contributes to signals of satiety and insulin-independent glucose homeostasis

5.3.5 Leptin

5.3.5.1 Properties



- **Leptin** (from adipose) acts on the leptin receptor (**Ob-R**) in the brain.
- In non-pregnant animals, leptin ***inhibits*** orexigenic **neuropeptide-Y** (NPY)/**agouti-related peptide-** (AgRP) neurones and ***stimulates*** anorectic **α-melanocyte-stimulating-hormone** (α-MSH)/**cocaine-and amphetamine-regulated-transcript** (CART) neurones in the arcuate nucleus.
- Leptin also activates **satiety** neurones in the ventromedial hypothalamus (VMH).
- In the non-pregnant state, leptin reduces appetite, reduces energy storage & increases expenditure.

Remember: The gene that encodes the leptin receptor is called Ob.

- Produced and secreted by fat tissue
- The body responds very strongly to falling leptin, which signals starvation risk. It responds much less reliably to high leptin, which would signal excess fat.

5.3.5.2 Leptin resistance

Obesity is not usually caused by a lack of leptin, but by leptin resistance

Leptin levels ARE higher in obese humans, BUT exogenous leptin treatment for obese people does not provide a strong effect on weight loss; rather, it works in non-obese people. Thus, it is concluded that obese individuals develop leptin resistance.

Luckily, Weight loss can reverse central leptin resistance.

- How?
  - Yes: Leptin still enters the brain. Leptin receptors are nit down regulated.
  - No: Signal transduction changed.

Leptin resistance is not only central (in the brain). It can also be peripheral, meaning in body tissues such as skeletal muscle, increasing the esterification/oxidation ratio of the fat in the brain.

5.4 Microbiota

Mammals have always co-existed with microbes, often in symbiosis. They are also a part of the gut-brain axis.

Evidence

- Obesity is associated with higher levels of bacteria that ferment carbohydrates to short-chain fatty acids. These provide an extra source of energy for the host's fat.
- Exposure of mice to a sustained high-fat diet can alter the microbiota ("dysbiosis").
- Transplanting gut microbiota from depressed humans to microbiota-depleted rats can induce characteristics of depression in these rats.
- The microbiome can release substances that stimulate enteroendocrine cells to secrete gut hormones and other signals.

5.5 Gravitostat

Gravitostat is a novel theory that proposes a system that senses body weight or mechanical loading by osteocytes. The body may detect physical weight-bearing and respond by reducing food intake. Gravitostat can also improve glucose homeostasis.

6 GLUCOSE HOMEOSTASIS

6.1 Overview

Glucose homeostasis is mainly controlled by the islets of Langerhans of the pancreas.

- **Beta cells**: insulin.
- **Alpha cells**: glucagon.

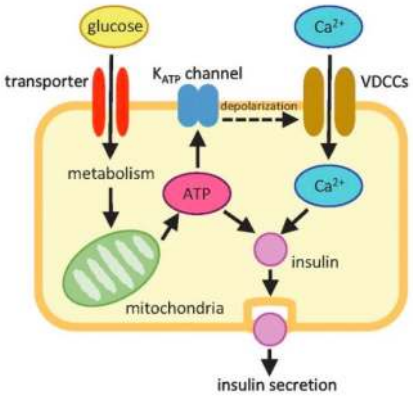
- **Delta cells**: somatostatin, which helps regulate insulin and glucagon secretion by paracrine signalling
- **Pancreatic polypeptide:** pancreatic exocrine secretion and gut motility. Gut motility means the movement of food through the digestive tract.

6.2 Insulin

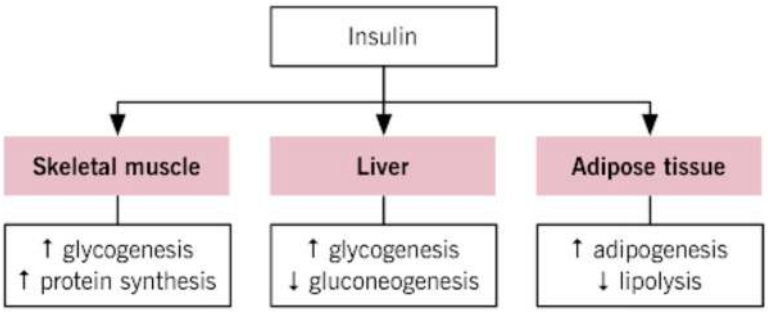
6.2.1 Secretion

Insulin is secreted in call of

- blood glucose and fatty acid
- GIP
- GLP



6.2.2 Action



Insulin can also act in the brain. Brain insulin can reduce food intake and BW by acting as a satiety signal.

6.3 Diabetes Mellitus (DM)

6.3.1 Test (Biomarkers)

- DM is defined by the oral glucose tolerance test (OGTT). In this test, a person drinks a fixed amount of glucose, and blood glucose is measured over time.
- However, HbA1c level testing can also work. HbA1c levels indicate the amount of glycation of haemoglobin. The higher the levels, the greater the degree of hyperglycaemia.

6.3.2 Category

Prediabetes: Where diabetes biomarkers are higher than normal but below the diagnostic threshold for diabetes. Prediabetes is not DM, but is a risk factor for type 2 DM.

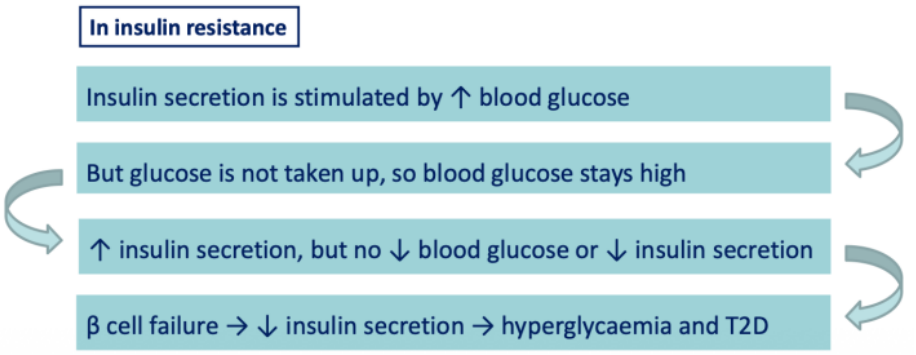
- Type 1 diabetes: a loss of beta cells
- Type 2 diabetes: insulin resistance

Also see PBL and practical materials.

6.3.3 Insulin resistance

Definition

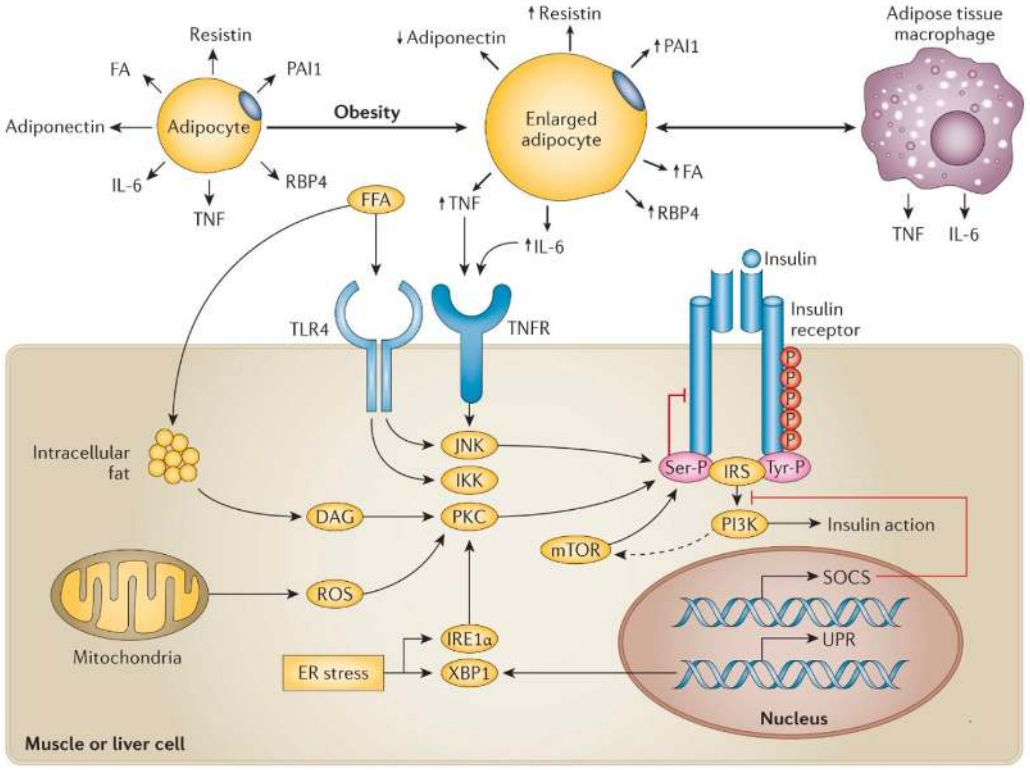
Insulin resistance = a reduction in the ability to reduce blood glucose.



6.3.4 Cause

1. Pathological visceral fat contains enlarged, dying, and abnormally-secreting **adipocytes** and more **M1 macrophages** with reduced **M2 macrophages**.
2. The abnormal adipocyte stage is thought to be caused by the abnormal macrophage populations' cytokines and adipocytes themselves by IL-6 and TNF secretion.
3. Hence, the anti-inflammatory adipokines are decreased, and pro-inflammatory adipokines and cytokines are increased, leading to altered insulin receptors' signalling by IRS-PI3K signalling.

- This insulin resistance can also happen in the brain. Also associated with changes in PI3K signalling.



6.4 Relationship with obesity

- Brain insulin can reduce food intake and BW by acting as a satiety signal, preventing obesity
- Obesity can induce insulin resistance.

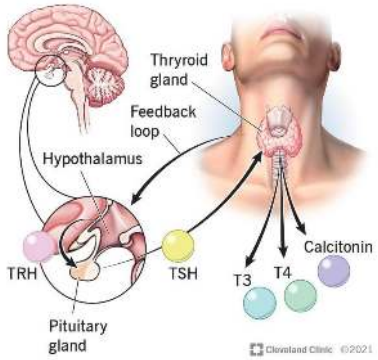
7 METABOLISM

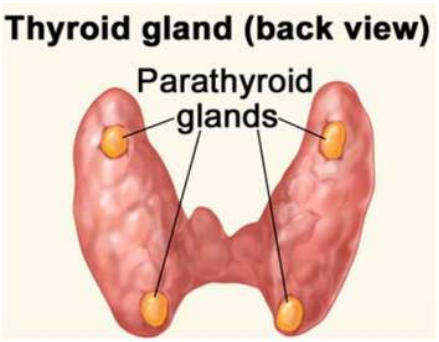
7.1 Gland

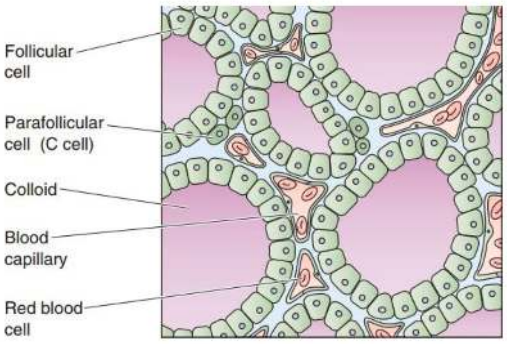
7.1.1 Properties

Location: anterior part of the neck, on both sides of the trachea. Just below the larynx.

Structure: Two lobes connected by an isthmus with a rich blood supply.







7.1.2 Parathyroid

During total thyroidectomy, the parathyroid glands may be damaged or accidentally removed. This can cause hypoparathyroidism.

7.1.3 Cellular composition

Thyroid follicular cell: makes **T4 and T3**

Parafollicular cell (C cell): produces **calcitonin**

7.2 Hormone

7.2.1 Overview

- T4: thyroxine/ tetraiodothyronine/thyroid hormone
  - A week of half-life
  - Larger amounts
  - Acts mainly as a storage or precursor hormone (prohormone)
  - T4 is converted into T3 in peripheral tissues, especially in the liver.
- T3: triiodothyronine
  - 18 hours of half-life
  - Lower amount
  - Biologically active
  - An intracellular hormone. It binds to nuclear receptors.

7.2.2 Effects

- **B**MR increase
- **B**eta-adrenergic effects
- **B**rain maturation
  - Supporting the formation of nerve terminals, synapses, dendrites, and myelin
- **B**one growth

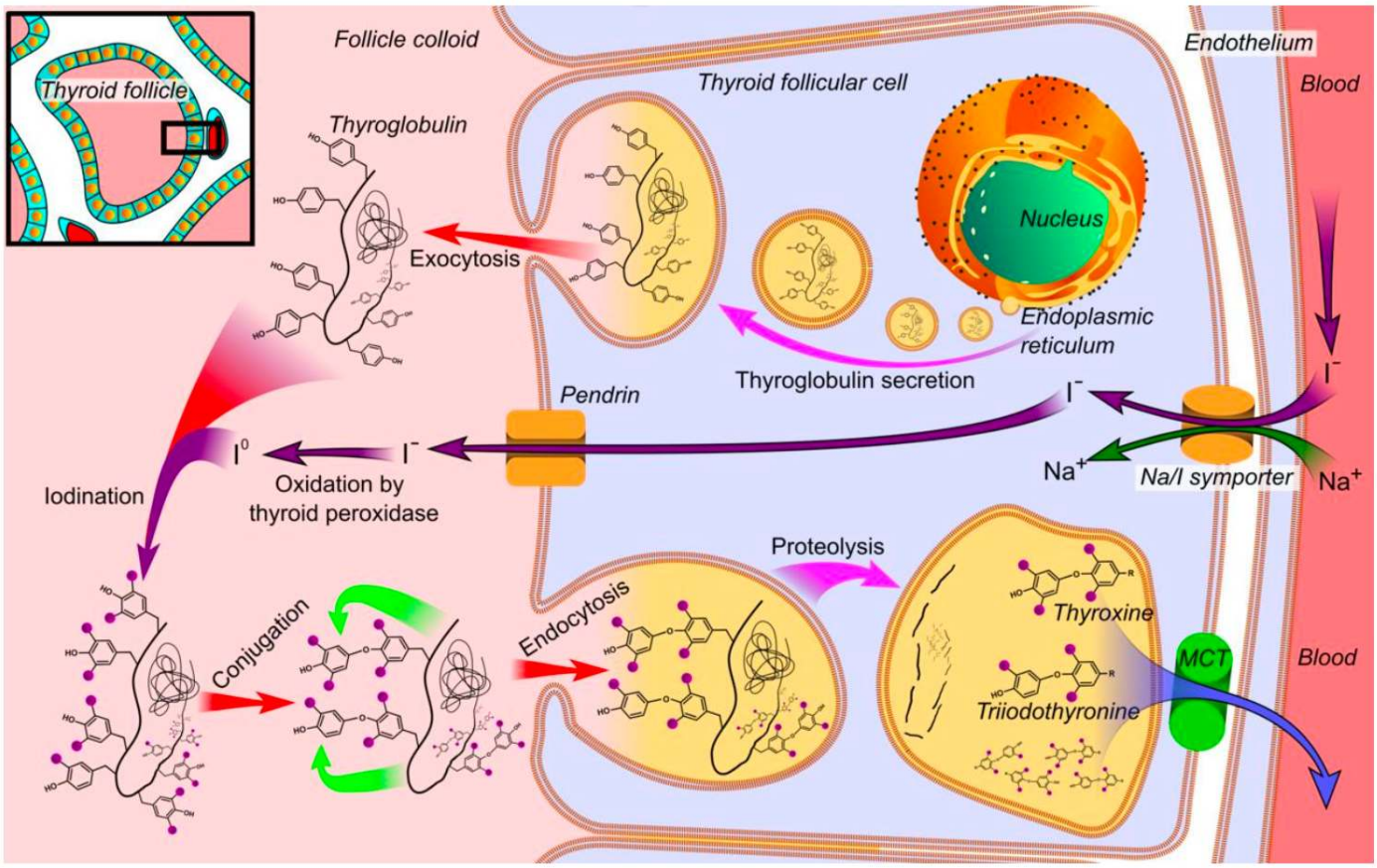
Growth and development effects are mediated by GH/IGF-1 production increase.

7.2.3 Biosynthesis

- T3 and T4 are made by iodination of tyrosine residues on thyroglobulin, synthesized and stored on the thyroglobulin.
- The source material is iodine in the form of ion and must be obtained from diet.

Steps include

1. Thyroglobulin is produced by the follicular cells and secreted into the follicle lumen
2. 'Iodide trapping' by Na^+/I^- cotransporter
3. Iodide is oxidised to iodine by thyroid peroxidase (TPO)
4. Iodination of tyrosine residues on the thyroglobulin (also by TPO)
5. TPO couples two iodotyroisine residues together
   a. MIT + DIT→T3
   b. DIT + DIT→T4



7.2.4 Features

- Storage: T4 is stored in follicles (specifically, the colloid part) in the form of thyroglobulin.
- Release
  - Stimulated by TSH
  - Even slower rate for T4 due to its higher affinity
  - Slow onset and long duration of action
- Transport: 99% are in the binding form, while only 1% is free. The TBP:
  - Thyroxine-binding globulin
  - Thyroxine-binding prealbumin
  - Albulmin
- Amount:
  - The thyroid produces essentially all circulating T4, but about 20% T3.
  - About 80% of T3 comes from the peripheral conversion of T4 into T3.
  - T4 is converted to T3 in peripheral cells (thyroid, BAT, kidney) and liver.
  - The conversion is catalyzed by deiodinases
- Application: Since T4 has a longer half-life, it is the best hormone for replacement therapy in most patients with hypothyroidism.

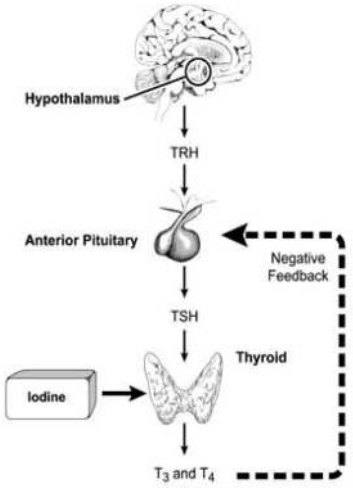
7.2.5 Mode of action

- T3 enters directly or T4 is converted to T3 in the cytoplasm
- T3 binds to coactivator or cosuppressor to act as TF to induce transcription

7.2.6 Activation and inactivation

- Activation occurs in the liver, kidney, and target organs, where T4 is converted into T3 by removing an iodine atom.
- Inactivation occurs mainly in the liver and kidneys, where thyroid hormones can be degraded or converted into inactive forms (rT3).

7.3 Regulation



Environmental factors such as cold and stress can activate the HPT axis as well.

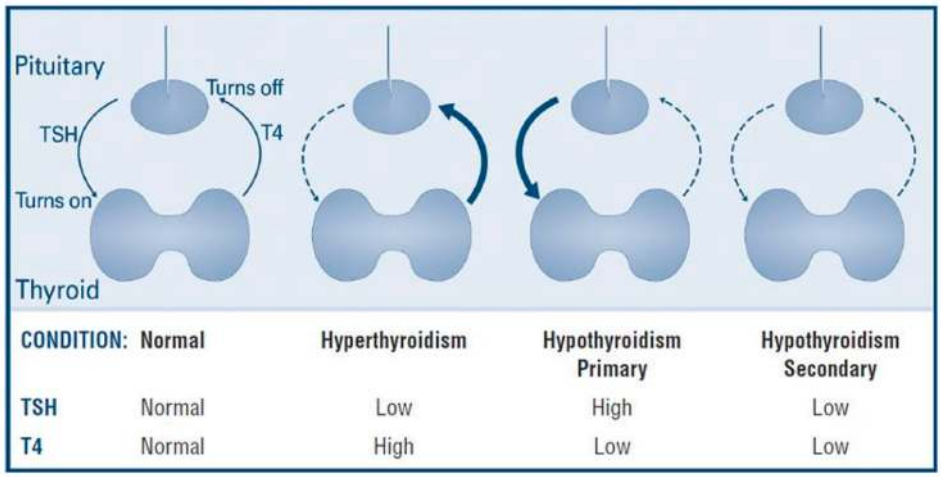
TSH stimulates the thyroid to

- Increase T3 and T4 synthesis + release
- Increase the size of thyroid cells

Negative feedback mechanism. Only T3 is the feedback hormone because only T3 is bioactive. In the hypothalamus and pituitary, deiodinases convert T4 to T3. That locally produced T3 is what signals the brain and pituitary to reduce TRH and TSH.

7.4 Disorders

7.4.1 Overview



CONDITION:	Normal	Hyperthyroidism	Hypothyroidism Primary	Hypothyroidism Secondary
TSH	Normal	Low	High	Low
T4	Normal	High	Low	Low

Key concepts:

- Thyrotoxicosis means the body is experiencing the effects of excessive thyroid hormone. It describes the clinical state, not necessarily the cause.
    - So, all hyperthyroidism causes thyrotoxicosis, but not all thyrotoxicosis is hyperthyroidism. For example, if thyroid hormone leaks out during thyroiditis, or someone takes too much thyroid hormone medication, they can have thyrotoxicosis without true overproduction by the gland.

7.4.2 Diagnosis

7.4.2.1 Method

TSH measurement is the BEST single screening test for thyroid function, because changes in TSH occur BEFORE measurable changes in T4 or T3 are present.

- LOW TSH = hyperthyroidism
- HIGH TSH = hyperthyroidism

7.4.2.2 Technique

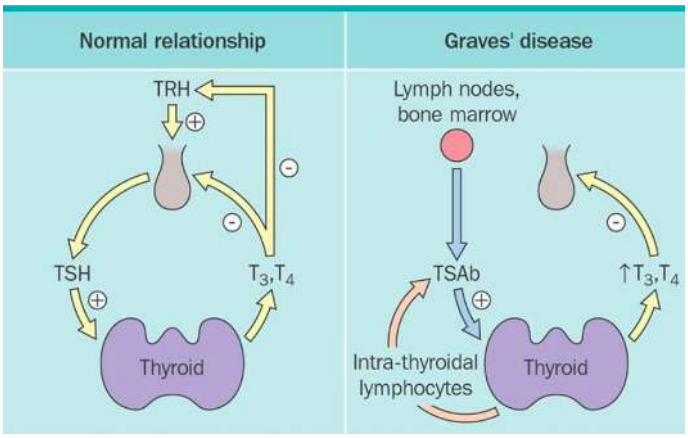
- Ultrasonography allows doctors to hear the bruiting voice of the thyroid, enabling diagnosis
    - Example: Ultrasound can help to diagnose Graves disease.
- Scintigraphy uses radioactive iodine to see how the thyroid takes up iodine.
    - Scintigraphy helps separate different causes of thyrotoxicosis.

7.4.3 Hyperthyroidism

Jod-Basedow effect: Excess iodide instead gets used as raw material to *make more* thyroid hormone, causing iodide-induced hyperthyroidism.

Grave's disease is the most common cause of hyperthyroidism**.**

Caused by autoimmune antibodies to the TSH receptor, which in turn leads to the excess of TSAb, too much thyroid hormone



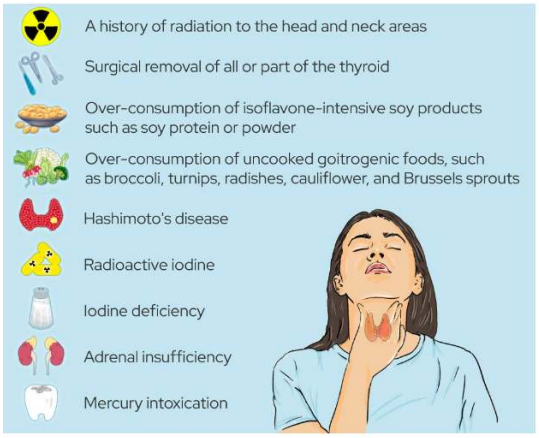
Symptoms: Feel hot, lose weight, have a fast heartbeat, tremor, sweating, anxiety, or diarrhea

7.4.4 Hypothyroidism

Wolff-Chaikoff effect: In response to iodine intake, the thyroid *shuts down* synthesis and trapping. This high-iodine inhibition is a protective mechanism. If a person suddenly receives a large iodine load, the thyroid temporarily reduces hormone production so it does not make excessive hormone.

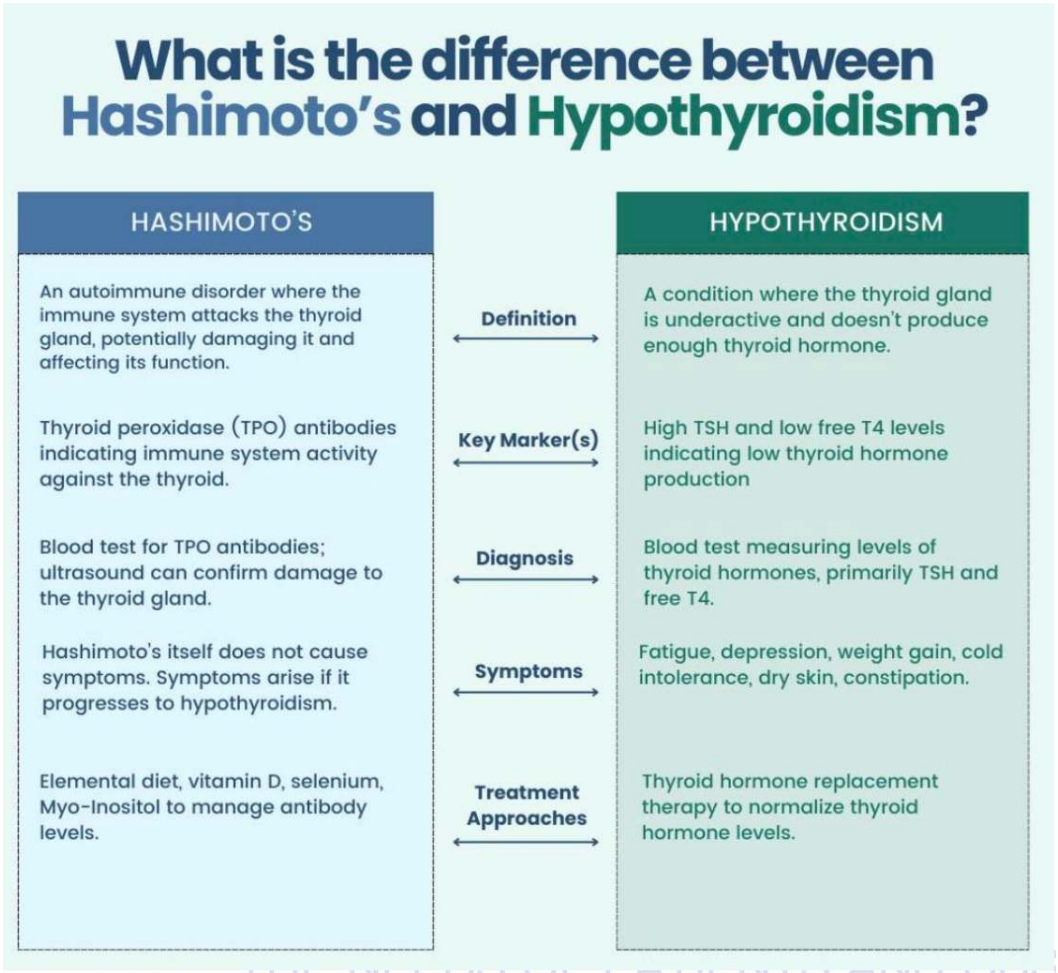
- Symptoms: Feel cold, gain weight, feel tired, have constipation, dry skin, slowed thinking, or a slow heart rate, or goitre
    - Goitre: A goitre is an enlarged thyroid gland. When iodine is low, the thyroid struggles to make enough T4 and T3. The pituitary may respond by increasing TSH, which stimulates the thyroid to grow. This can produce visible neck swelling.

Hashimoto's thyroiditis is the commonest cause of hypothyroidism. It is due to an autoimmune destructive process leading to hypothyroidism, or that leads to breakdown of thyroid follicles and spilling of stored thyroid hormone



- Endogenous
  - Autoimmune - Hashimoto's thyroiditis
  - Autoimmune - painless or post-partum (hypothyroid phase)
  - Subacute thyroiditis (hypothyroid phas
  - Congenital (1/4000 – detected by scre programs)
- Exogenous
  - Radioactive iodine, external radiation
  - Anti-thyroid drugs
  - Surgery
  - Iodine: radiocontrast dyes, amiodaror foods (kelp, iodine)
  - Ipilimumab, PD-1/PD-L1

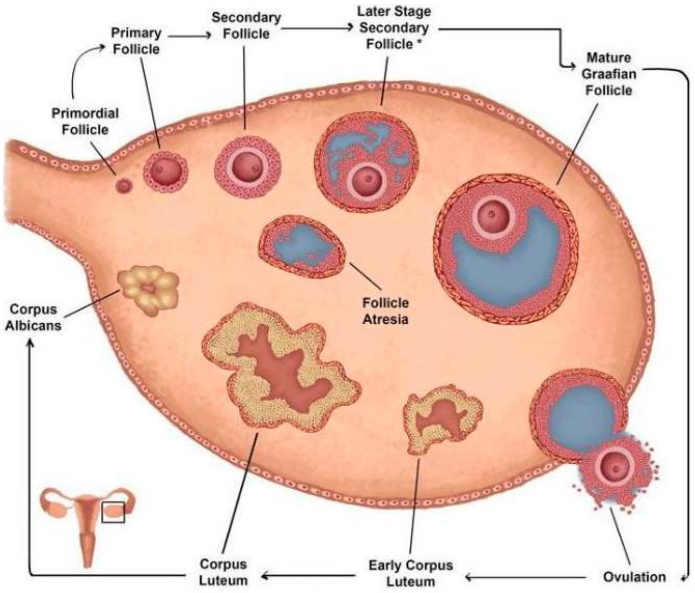
THE NEEDS OF PATIENTS AND CUSTOMERS COME FIRST



- There is subclinical hypothyroidism, marked by elevated TSH and normal FT4. The risk of progression from subclinical to overt hypothyroidism is related to TSH and the presence of anti-TPO Ab.

8 FEMALE REPRODUCTION

8.1 Overview



Oocyte resides in the follicle, and follicle resides in the ovary. The follicle = oocyte + surrounding somatic cells. When oocyte is mature enough, it will pop out of the ovary (e.a, out of the follicle). But before that, oocytes and follicles need to undergo the process of maturation, called oogenesis and follicular genesis, respectively.

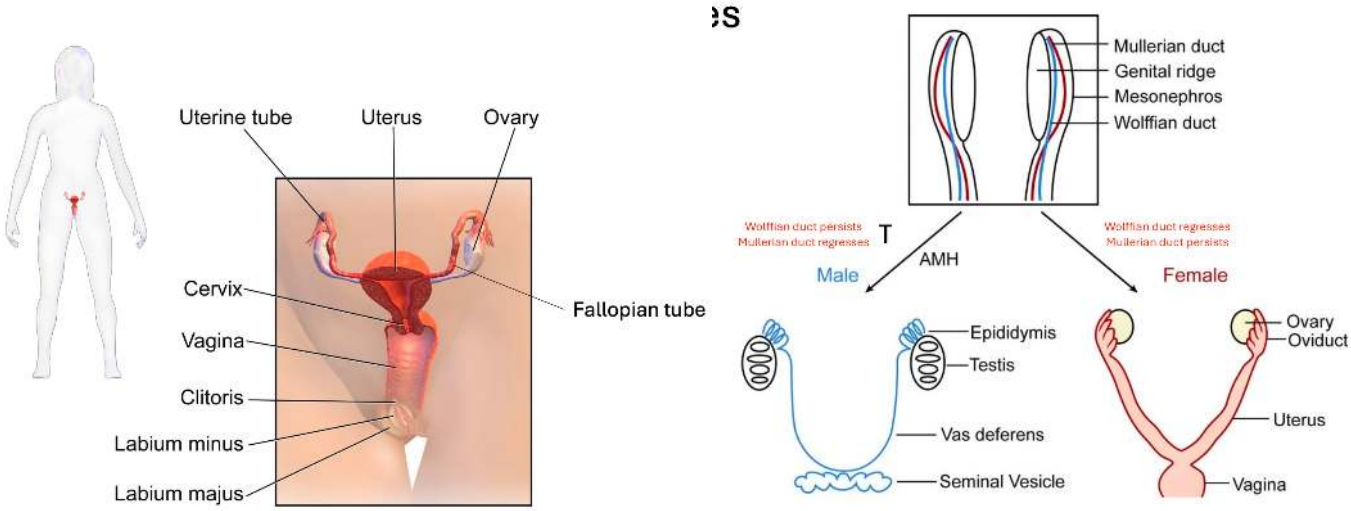
Postpubertal ovaries contain oocytes from all stages at the same time.

An oocyte is ovulated from the ovary, enters the Fallopian tube, may be fertilised there, and if fertilisation occurs, the early embryo travels to the uterus for implantation.

The follicle pool is finite. The ovary does not simply keep producing new follicles in life like sperm.

8.2 Anatomy

8.2.1 Overview



At the start of embryonic development, males and females have the same structure. The sex chromosomes determine hormone reproduction, which in turn instructs the development of gonads.

8.2.2 The Fallopian tubes

- An ovulated oocyte spends about 24 hours in the Fallopian tube
- Fertilisation may occur in the ampulla (median section of the Fallopian tube)
- Two types of cells reside in the Fallopian tube
    - Ciliated cells: beat towards uterus
    - Non-ciliated cells: are secretory

8.2.3 Uterus

- Endometrium = mucosal layer: implantation of fertilised embryo

- Myometrium = muscle layers: allow expansion during pregnancy and contractions during parturition
- Perimetrium = serosal layer

8.2.4 Cervix

- Allows flow of menstrual blood from the uterus into the vagina
- Directs sperm into the uterus after sexual intercourse
- Regulates initiation of parturition

8.2.5 Vagina

- An elastic muscular canal
- Joins the cervix to the vulva
- Can expand during parturition and sexual intercourse

8.2.6 Vulva

Also called external genitalia.

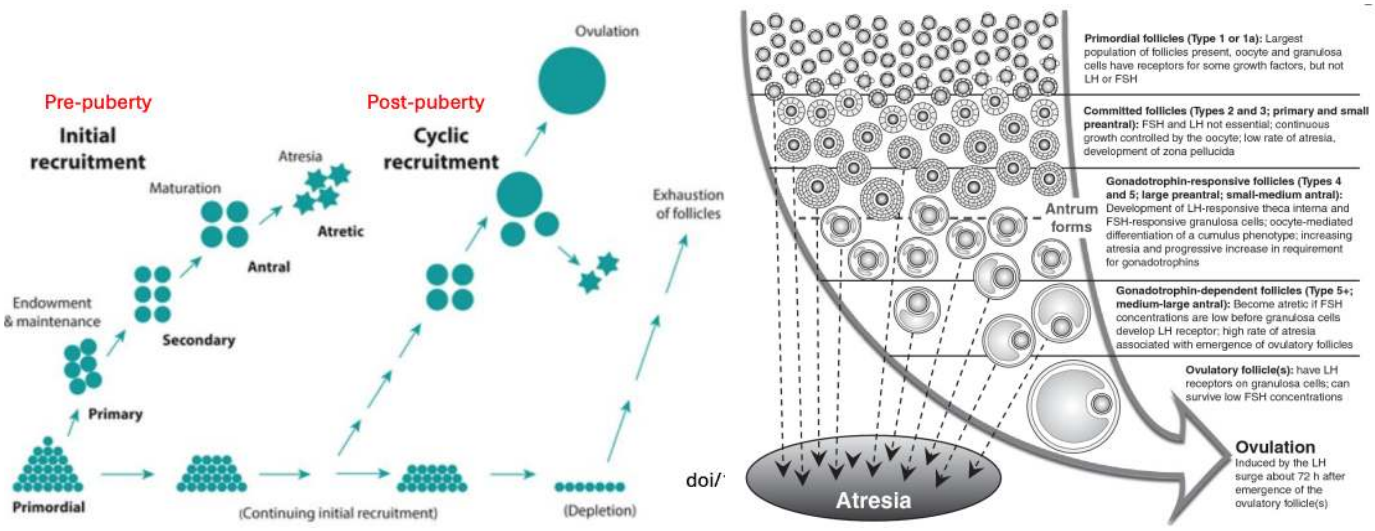
- The clitoris develops from the same embryonic structure (anlagen) as the penis, and contains the same number of nerve endings

8.3 Functional overview

The ovary in the female reproductive system has two primary functions: (1) oogenesis (which includes folliculogenesis) and (2) steroidogenesis.

8.4 Oogenesis

8.4.1 Overview



Before (include) the secondary follicle, the development of the oocyte is gonadotropin-independent. Hence, early follicle growth does not depend on FSH and LH but on local signals.

However, tertiary follicle growth and ovulation are controlled by gonadotrophins

Before puberty, follicles undergo atresia (degeneration and death) at the early antral stage.

After puberty, recruitment becomes cyclic, linked to the menstrual or ovarian cycle. Some follicles grow, most still undergo atresia, and usually one dominant follicle reaches ovulation.

8.4.2 The nonfollicular stage

- Oogonia are the diploid stem-cell-like starting population for oogenesis. They have not yet formed follicles. They divide themselves by mitosis.
- The mesonephros produces retinoic acid (RA), which initiates the meiosis of oogonia. From now on, this population is called oocytes.

8.4.3 The primordial follicle stage

- After being activated by RA, oocytes immediately arrest in prophase of meiosis I (MI) until ovulation, when they proceed to the follicular phase. After ovulation, oocyte goes into the primordial follicle phase, where it enters MII and arrests again.
    - Meiosis in oocytes is asymmetric
    - After MI the oocyte exudes the other set of chromosomes as a 'polar body' during ovulation
- An oocyte begins to be surrounded by a very thin layer of squamous granulosa cells (somatic cells). And the primordial follicles begin to form and mature.
- Primordial follicles do not activate simultaneously. Activation is controlled by a balance between local activatory signals and inhibitory signals. This stage is gonadotropin-independent.
    - AMH is produced by granulosa cells.
    - AMH acts as a brake. It inhibits primordial follicle activation, helping prevent too many follicles from leaving the reserve pool at once.
    - AMH inhibits FSH-stimulated granulosa cell mitosis and aromatase production to keep FSH's action restricted to later phases of oocyte development.

8.4.4 The primary follicle stage

- A primary follicle forms when the granulosa cells change shape from squamous to cuboidal.
- Zona pellucida begin to form. It is a specialized ECM between the oocyte and granulosa cells. It is made of ZP proteins (glycoproteins), repeating units of ZP2 and ZP3, crosslinked by ZP1.
- The zona pellucida is required for sperm binding during fertilisation.

8.4.5 The secondary follicle stage (pre-antral follicle stage)

- A secondary follicle has more granulosa cells and begins to recruit theca cells around, just outside of, the granulosa layer. Theca is divided into theca interna and theca externa.

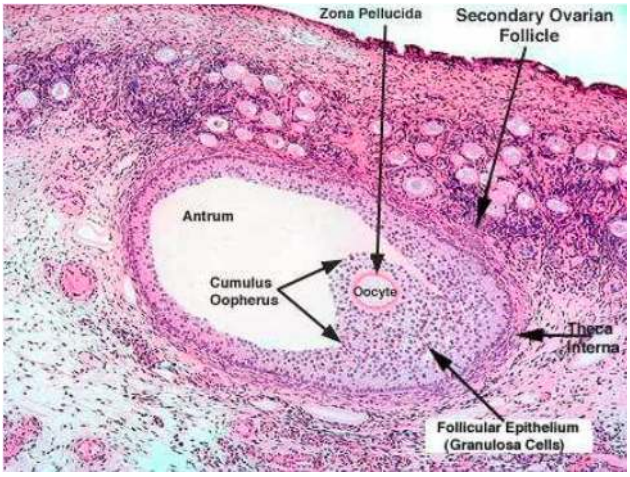
8.4.6 The tertiary follicle stage (antral follicle stage/Graafian stage)

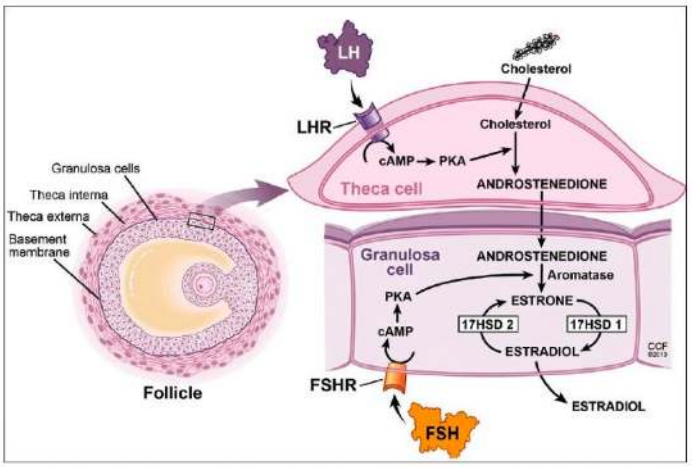
- Granulosa and theca cells undergo rapid mitosis, and follicle increases in volume.
- Cumulus granulosa forms, attaching the oocyte to the wall
- Mural granulosa cells form, producing E2

8.4.7 Ovulation

- Oocyte and cumulus granulosa cells are ejected from ovary into fallopian tube
- Remaining follicle becomes a corpus luteum

8.5 Structure

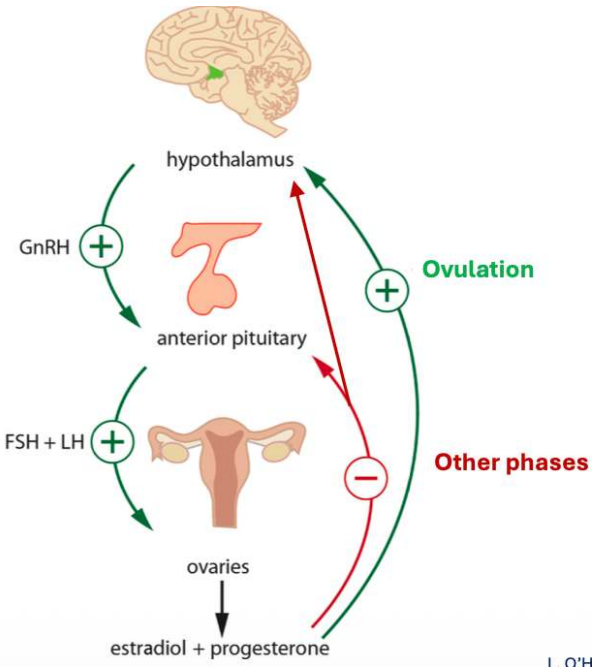




- Granulosa cells: Surround the oocyte. Support oocyte development and later support estrogen production.
- Theca interna cells: Sit outside the granulosa layer. They are vascularised and can make steroid hormone precursors.
- The theca externa**:** Supportive connective tissue, containing fibroblasts and structural cells.
- Somatic component of the ovary (theca and granulosa cells) are steroidogenic.

8.6 Hormone regulation

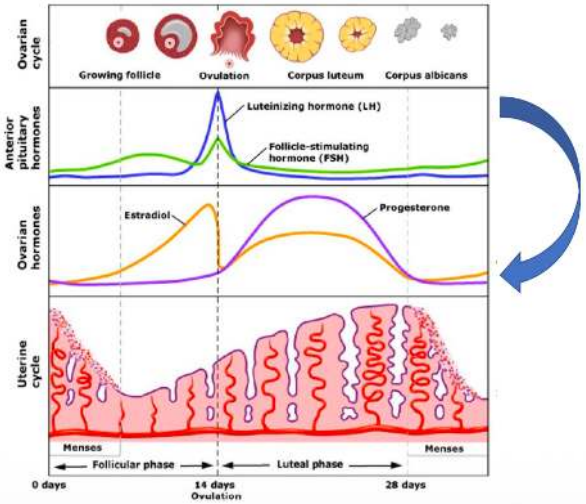
This axis is called HPG axis or HPO axis.

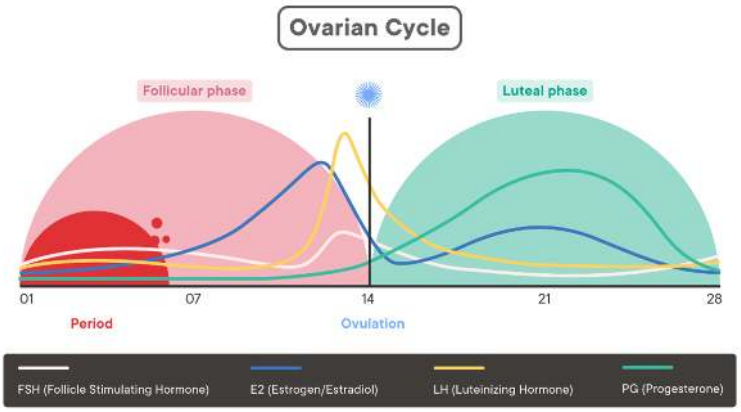


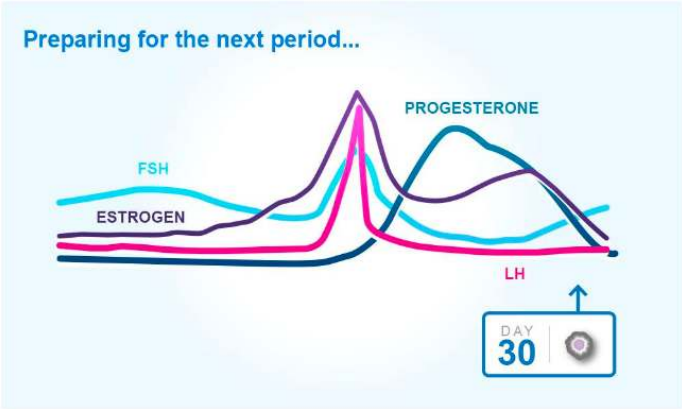
- GnRH is synthesized and released by GnRH neurons in a pulsatile way controlled by kisspeptin secreted by arc nucleus kisspeptin neurons. The receptor is KISS1R.
- GnRH receptor GnRHR is expressed by gonadotrophs of the anterior pituitary. GnHR-GnRHR binding stimulates LH and FSH release from gonadotrophs.
- FSH and LH are transcribed, translated, and stored in vesicles

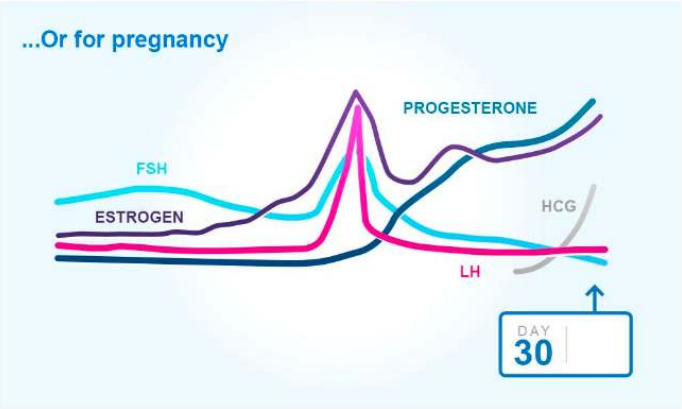
FSH	LH
Stimulates tertiary follicle growth, especially growth of the follicle's somatic supporting cells.	Stimulates final follicle development, ovulation, completion of meiosis I in the oocyte, and maintenance of the corpus luteum.
Pulsatile, but not obvious (constitutive)	Greatly pulsatile, matching GnRH pulses
Exocytosis via secretory vesicles	Exocytosis via secretory vesicles
Low E2 negative feedback	Strong E2 negative feedback

8.7 Cycle









8.7.1 The Follicular Phase

- FSH begins to rise; LH remains low
- FSH stimulates late secondary follicles to become tertiary follicles
- FSH stimulates granulosa cells to produce E2
    - Granulosa cells express E2 receptors and can also produce low levels of P4
- Rising E2 exerts negative feedback on the HPG axis, causing FSH levels to fall, since LH is not secreted at high levels, so its level appears stable
    - Low levels of FSH and LH mean that a lot of follicles cannot make it to survival.
    - However, the largest tertiary follicles survive this fall and become the dominant follicle
    - E2 production by the dominant follicle keeps rising. At a particular threshold, E2 stimulates positive feedback, which overcomes negative feedback
        - RP3V (AVPV) kisspeptin neurons are activated → GnRH surge release → dramatic increase in LH, smaller increase in FSH

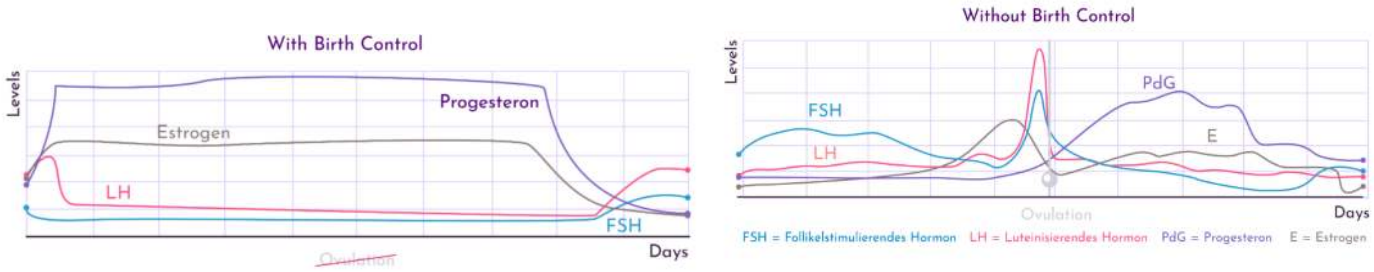
8.7.2 LH Surge at the Ovulation Point

- LH surge
    - Acts on the dominant oocyte to induce its ovulation and thus the oocyte's completion of the MI phase by reducing cGMP via mural granulosa cells
    - Just before ovulation, granulosa cells begin to express LHCGR too, probably triggered by slightly increased FSH
    - Connective tissue in the follicle wall breaks down after sensing LH, mediated by matrix metalloproteases (MMPs) and plasmin

8.7.3 The Luteal Phase

- The remaining follicle develops into a transient structure called the corpus luteum
    - Corpus luteum produces a lot of P4 and some E2
        - P4 prepares the endometrium for implantation by
            - Making the endometrium enter the secretory phase
            - Prompting the production of implementation-related proteins
            - Suppressing uterine contractions
            - Suppressing the immune response
    - This exerts negative feedback on LH and FSH release
- Different fates
    - The corpus luteum requires LH stimulation to survive
        - If no implantation occurs → LH level going down → luteolysis → fall in P4 → shedding of uterine lining (menstruation) → the negative feedback is inhibited, preparing for the next cycle
        - If fertilisation occurs → embryo produces hCG → hCG can also bind and stimulate LHCGR → the corpus luteum is maintained → FSH is kept low due to high estradiol, preventing new ovulation

8.8 Contraception



8.9 Polycystic ovarian syndrome (PCOS)

It is a disease that is characterized by anovulation, excessive ovarian cysts, and high androgen levels.

8.9.1 Causes

- Cysts are arrested follicles
- Initial recruitment of primordial follicles is increased
- Arrest at the early tertiary stage
- Do not ovulate

8.9.2 Features

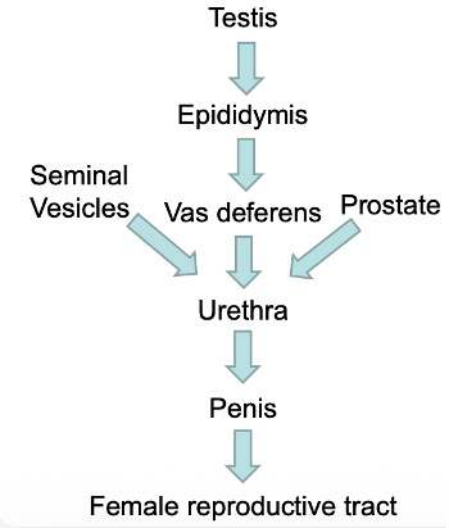
- The LH/FSH ratio is increased during the early follicular phase
    - FSH is similar between PCOS and controls, and thus E2 is normal in PCOS
    - LH is higher in PCOS → Every follicle survives → No dominating follicle → No large increase in E2 → No trigger for an LH surge
- Testosterone is increased.
    - Cause
        - Increased thickness of theca with intrinsic theca cell dysfunction → Theca cells make more testosterone in response to forskolin (Forskolin ↑ cAMP)
        - LH dominance over FSH → More androstenedione produced → Converted into testosterone
    - Consequence
        - Increased testosterone explains why LH is higher.
            - High androgens impair GnRH pulse generator sensitivity to negative feedback suppression → GnRH pulses are increased → Higher LH → Make every follicle survive
        - Premature expression of LHCGR in granulosa cells (↑ androgens may cause LHCGR expression to surge, occurring earlier in tertiary follicle development, thus arresting follicles)
- Adrenal hyperandrogenism can contribute to PCOS, possibly due to an increase in adrenal sensitivity to ACTH
- Insulin resistance (type II DM) can also cause PCOS since insulin can also stimulate theca cell, which stimulates intrinsic theca cell dysfunction and thus theca cells produce more androgens

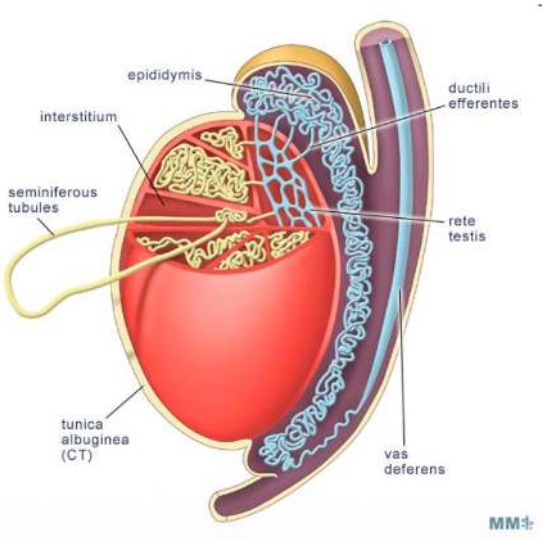
8.9.3 Treatment

- The COC pill can be used to suppress PCOS since exogenous progestogen and estrogen suppress pituitary LH and FSH, which in turn suppresses ovarian steroidogenesis.
- Ovarian steroid levels are low, but systemic estrogen and progestogens are high

9 MALE REPRODUCTION

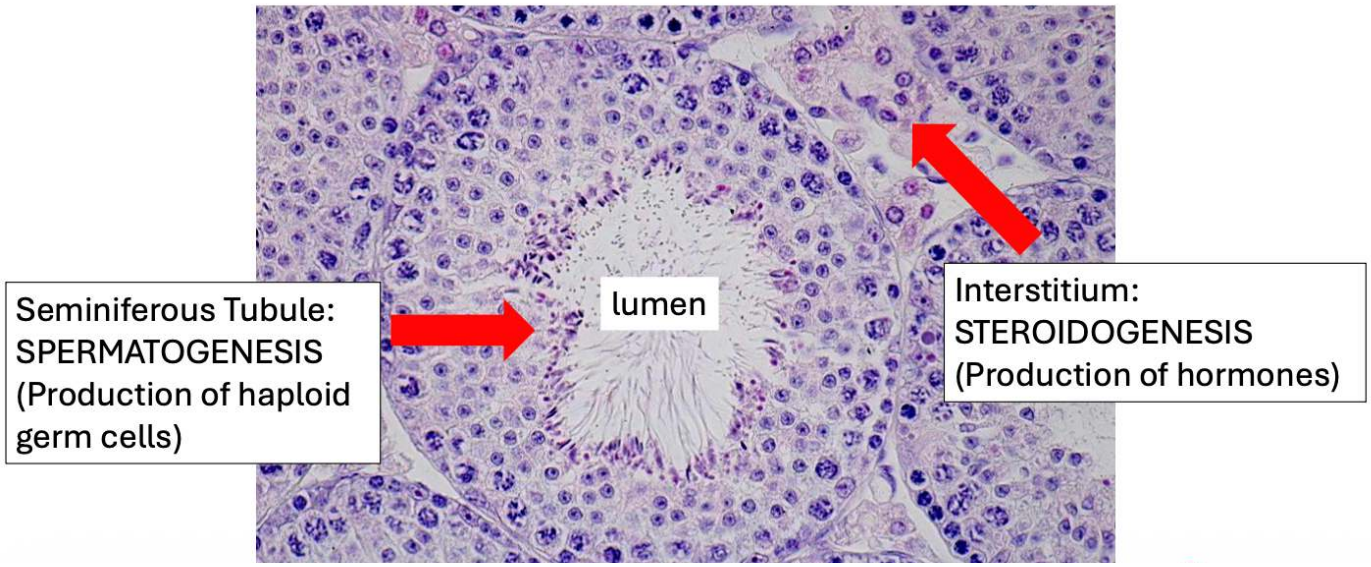
9.1 Structure





9.2 Testis

9.2.1 Function overview



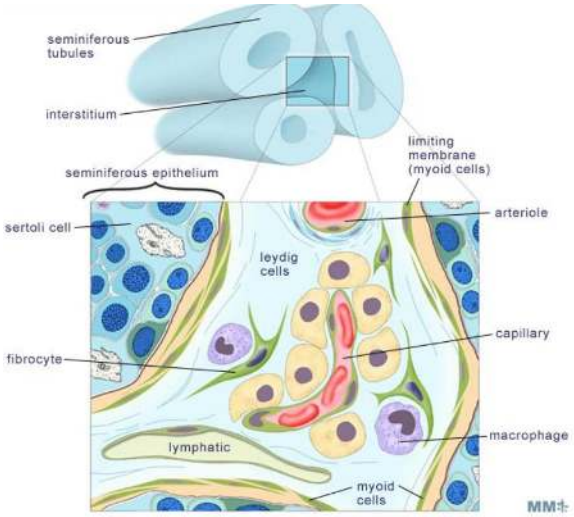
9.2.2 Spermatogenesis

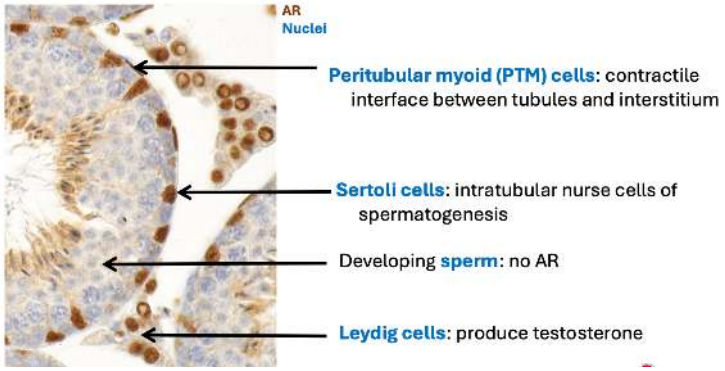
SSCs -differentiate-> **Spermatogonia** -miosis-> **Spematocyte** -meiosis-> **Spermatotid** -differentiate-> **Spermatozoa**

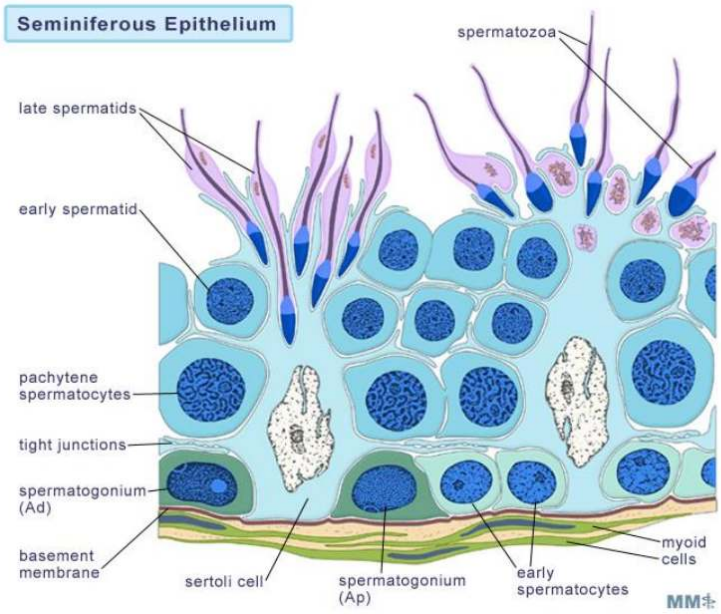
- Before spermatogonia: Diploid, round
- After spermatogonia: Haploid, Genetically diverse

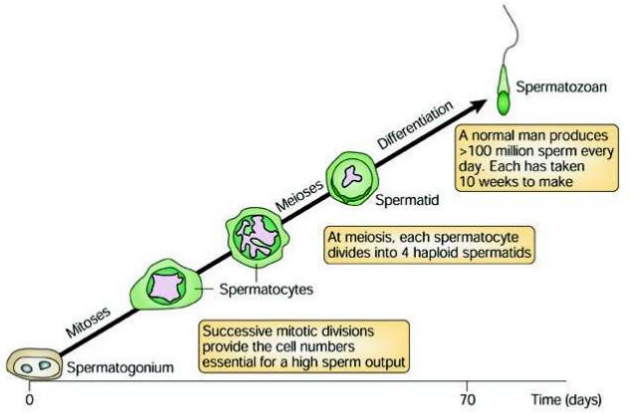
9.2.2.1 Overview

Germ cell maturation occurs from the basal to luminal surface of the seminiferous epithelium.





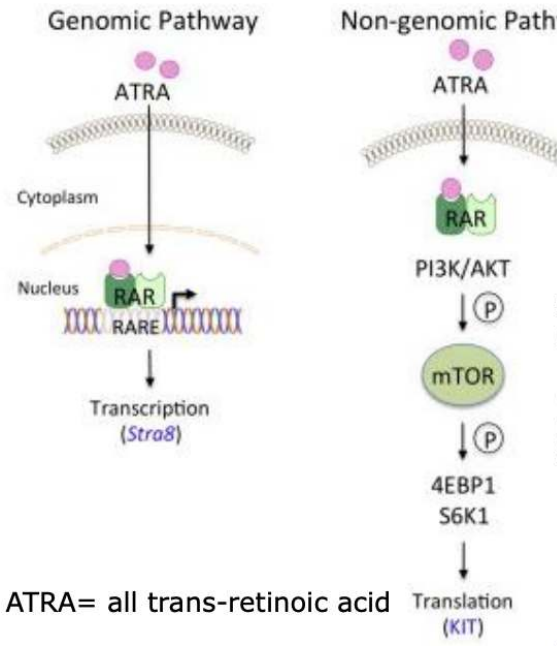






Below are the detailed steps of spermatogenesis

9.2.2.2 Step 1: Proliferation of spermatogonia



Spermatogonial stem cells (SSCs) can either self-renew or commit to differentiation.

The SSC niche is a specialised physical and chemical environment, and SSC self-renewal requires paracrine signalling from the niche.

The position of a spermatogonium relative to the niche affects the availability of mitogens and, therefore, whether it differentiates or self-renews.

- Self-renew: GDNF ligand produced by PMCs is essential for SSC self-renewal.
- Differentiation: Retinoic acid is required for spermatogonia differentiation by genomic and non-genomic pathways

9.2.2.3 Generation of genetic diversity by meiosis

SSCs undergoing meiosis are called spermatocytes.

Meiosis I results in the separation of homologous chromosomes (2n → n & exchange of genetic information), and meiosis II results in the separation of sister chromatids (n→ n)

Androgens are essential for progression through meiosis

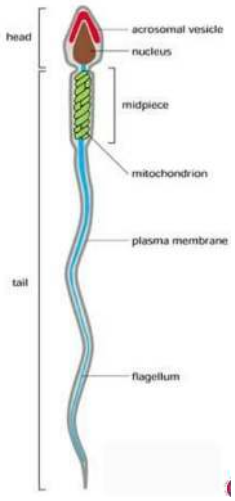
Germ cell attachment and movement: Germ-Sertoli cell adherens junctions are broken and reformed as the cells move across the epithelium

9.2.2.4 Spermiogenesis

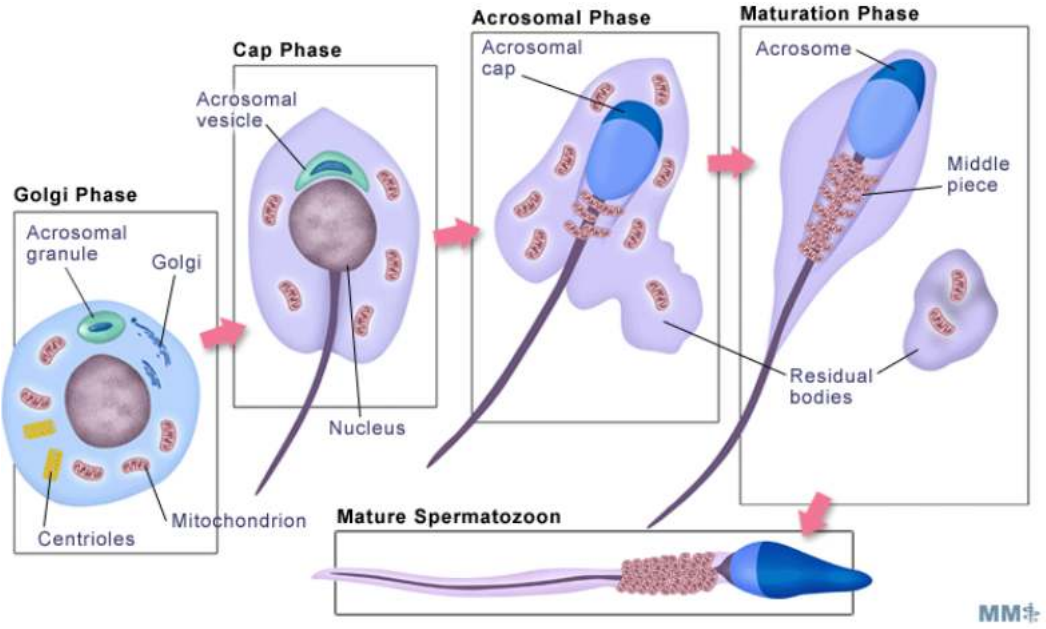
Also called cytodifferentiation.

It involves the Morphological changes in post-meiotic germ cells:

- Condensation of cytoplasm
- Formation of the acrosome and tail
- DNA compaction
- Cytoplasmic storage of mRNAs required for future translation

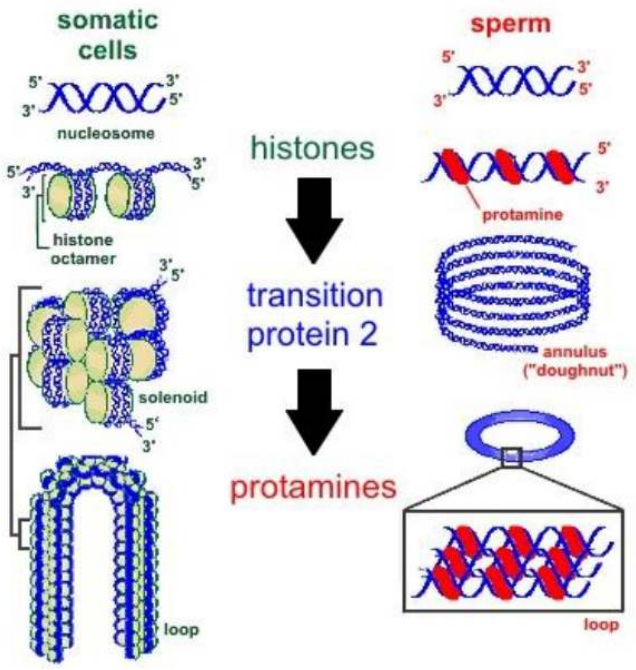


Phases of spermiogenesis



- Spermatogenic 'wave' is controlled by pulses of retinoic acid.
- Spermiogenesis is controlled by RA as well.
- Golgi phase: the round cell becomes polar. Golgi = head end, centrioles = tail end
- Cap phase: acrosomal vesicle forms cap over the nucleus, primitive flagellum forms
- Acrosomal phase: the acrosome begins to spread around to encapsule the nucleus

- Maturation phase: mitochondria gather in midpiece, excess cytoplasm lost as residual body which is then phagocytosed by Sertoli cell.



- Replacement of somatic histones with specialised histones
- Transcription stops
- Replacement of histones with transition proteins
- Replacement of transition proteins with protamines
- Histone → protamine approx. 85%

Then it experiences spermiation: Spermatozoa release from the Sertoli cell. Sertoli-germ cell junctions undergo extensive remodelling. When sperm are released, they leave behind a 'residual body' of cytoplasm, which is phagocytosed by the Sertoli cell.

9.2.2.5 Other cells

Cell type	Definition	Functions
Sertoli cells	the somatic supporting cells of spermatogenesis in the seminiferous epithelium	**Structural:** scaffold for germ cells connected with adherens junctions and hence able to move germ cells from basal to apical. Germ-Sertoli cell adherens junctions are broken and reformed as the cells move across the epithelium **Protective:** Form blood testis barrier, enabled by tight junctions. This forms after the appearance of spermatocytes to avoid the immune rejection of haploid cells **Secretory:** secrete fluid to form seminiferous tubule lumen, secrete androgen-binding protein to transport luminal androgens **Phagocytic:** absorb waste organelles of developing germ cells **Receive and transmit endocrine and paracrine signals:** they have receptors for FSH and testosterone, whereas germ cells do not
Leydig cells	the site of testicular steroidogenesis	After being stimulated by LH, it can produce testosterone.
Peritubular myoid (PTM) cells	surround seminiferous tubules	Endothelin-1-mediated contraction of PTM cells causes the contraction of seminiferous tubules, which brings sperm to the testis through the seminiferous tubule lumen.

9.2.3 Steroidogenesis

The interstitium consists of blood vessels, Leydig cells, and supporting cells. Leydig cells are the site of testicular steroidogenesis.

9.3 Other parts of the anatomy

9.3.1 Epididymis

- The epididymis is a single, highly coiled tubule. Sperm taken straight from the testis are unable to fertilise an oocyte in vivo.
- The epididymis requires androgens for normal development and function.
- When sperm pass through the epididymis, there are changes in sperm membrane proteins and lipids that are acquired from the secretion from epididymal epithelium
- The epididymis is like a 'school for sperm': for concentration, maturation, and storage.

The epididymis possesses epididymal stereocilia, which help to concentrate sperm by tall columnar epithelial cells to increase the surface area and increase the absorption of testicular fluid.

9.3.2 Vas deferens

- The vas deferens is a straight, muscular tube.
- In humans, spermatozoa are stored in the epididymis before ejaculation.
- The thick muscular layers contract to prompt the peristalsis of sperm along the vas deferens during ejaculation.
- The vas is a popular target for male contraceptives. For example, a vasectomy ligates the vas. Sperm are prevented from transiting the vas and are phagocytosed.

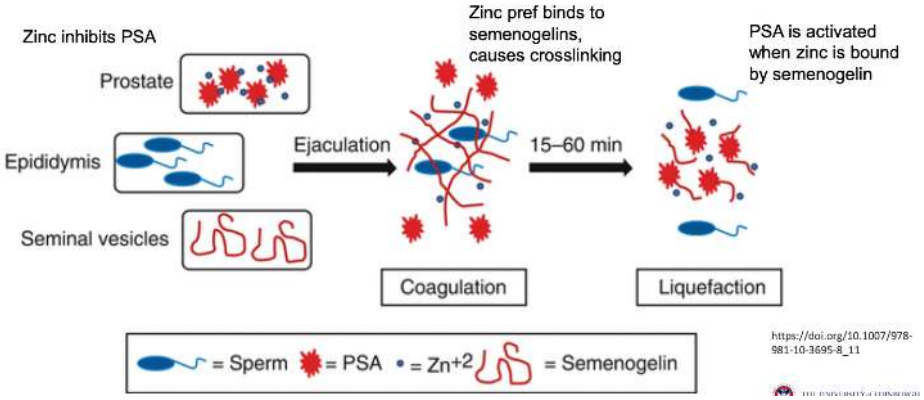
9.3.3 Seminal Vesicles

It is essentially the accessory gland of the male reproductive system.

- Seminal vesicle fluid makes up 60-75% of sperm.
- Contains fructose: provides sperm with energy.
- At ejaculation, semen coagulates. This is promoted by semenogelin I and II, expressed exclusively in the seminal vesicles.
- Seminal vesicles require androgens for their normal function, too.

9.3.4 Prostate

- Alkaline: helps sperm survive in the acidic female reproductive tract
- Prostate-specific antigen (PSA) is produced by prostate epithelial cells. PSA cleaves semenogelins to liquify coagulated semen, allowing sperm to swim freely (Semen coagulates immediately after ejaculation and then liquifies again later).
- Also contains Zn^{2+}, which inhibits PSA.
- The prostate requires androgens for normal function, too.
- It is not until ejaculation that 3 sources of fluids mix and coagulate immediately after ejaculation and then liquefy again later



9.3.5 *Penis*

- Smooth muscle cell relaxation → more blood enters → erection
- Erection is inhibited by nitric oxide (NO).

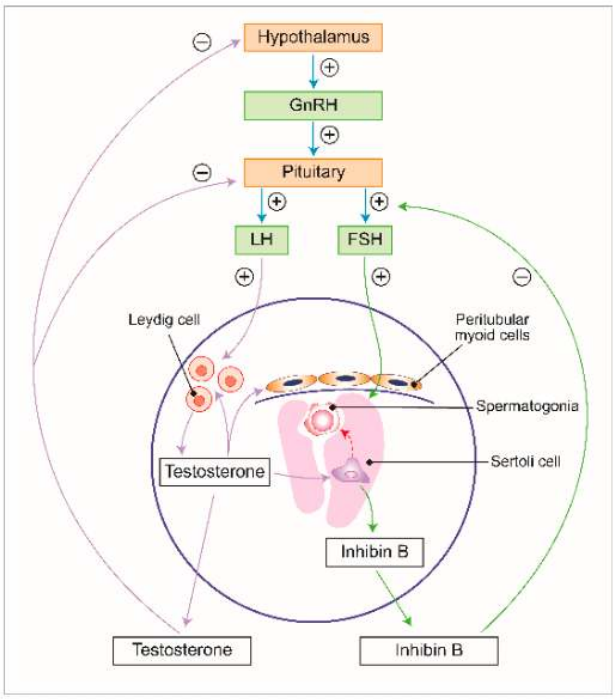
9.4 Hormone regulation

T + DHT = Androgens. Androgens act via the androgen receptor (AR)

HPG or HPT axis.

Optimal spermatogenesis requires two hormones:

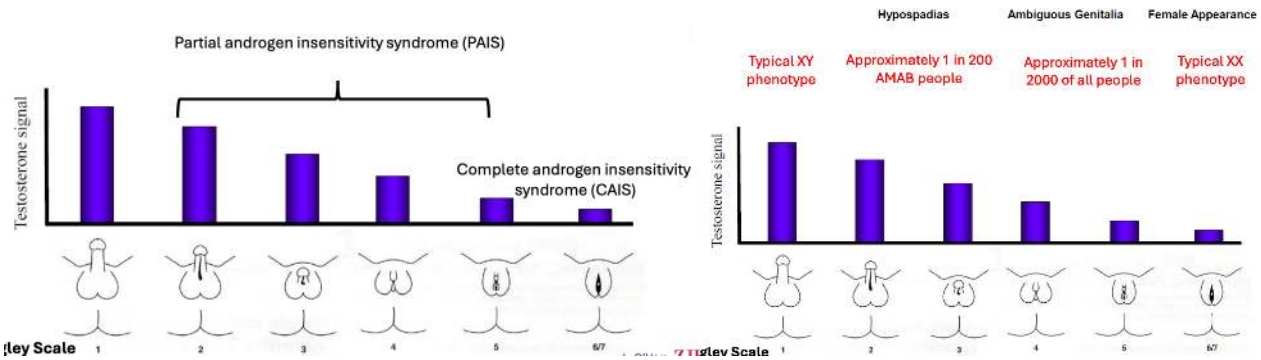
- Testosterone
  - Released from testicular Leydig cells, stimulated by LH
    - LH receptor (LHCGR) is expressed in Leydig cells
    - LHCGR signaling upregulates transcription of steroidogenic enzymes
    - Both testicular development and spermatogenesis are dependent on LH signalling through LHCGR (but not male sexual differentiation)



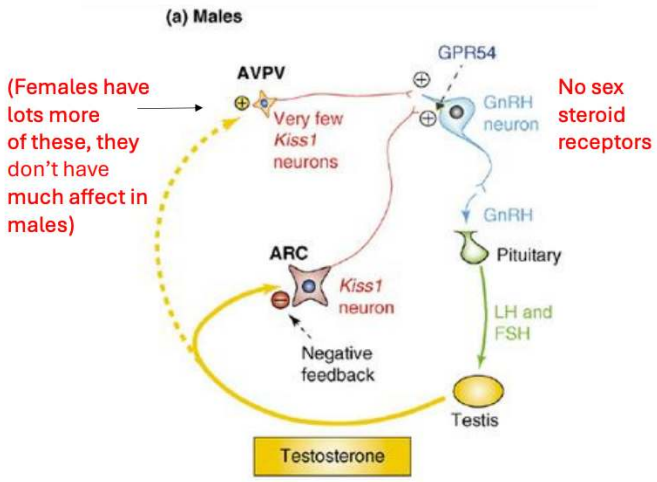
  - Testosterone can be converted to DHT in peripheral tissues by the action of the enzyme 5α-reductase type II
    - DHT is a more potent androgen than testosterone since it has 3x greater affinity for AR
    - DHT is needed for the masculinisation of organs distal to the testes, where testosterone concentrations are lower, since testicular testosterone levels are much higher than circulating
    - 5α-reductase type II deficiency would cause ‘eggs at 12’ syndrome: Penis does not usually develop enough for intercourse to be possible, but sperm is often mature enough for artificial insemination to be possible

- If AR undergoes inactivating mutations, one will develop Complete Androgen Insensitivity Syndrome (CAIS).
  - ‘Female’ external phenotype but no ovaries
  - Small, abdominal testes with no spermatogenesis
  - But also a short vagina, and no uterus, due to continued AMH production by testicular Sertoli cells
  - Infertile
  - Total androgen receptor knockout (ARKO) mice are a model for CAIS
- Sertoli cell-specific ARKO: Infertile with a block in spermatogenesis at meiosis because sperm are not able to move upward.
- PTM cell-specific ARKO: Infertile with a reduction of sperm at all stages of spermatogenesis, but not a block, because androgens act on PTM cells to simulate GDNF production.
- Leydig cell-specific ARKO: Fertile, but Leydig cells do not develop properly: apoptosis as the mouse reaches adulthood
- Developing sperm: has no AR.

- follicle stimulating hormone (FSH)
  - Stimulates fetal/neonatal Sertoli cell mitosis
  - Stimulates Sertoli cells to signal to and support developing germ cells
  - Stimulates the production of androgen-binding protein (ABP), which concentrates T in the seminiferous tubule fluid
  - Stimulates inhibin (alpha unit) production to exert negative feedback on FSH to preserve the balance



9.4.1 Negative feedback



- Steroid negative feedback on FSH and LH in the hypothalamus via Kisspeptin neurons
- Inhibin also represses FSH release. It is a heterodimer, acting on pituitary gonadotrophs and repressing pituitary gonadotrophin gene expression

Thus, injection of T could lead to spermatogenesis stop and testicular shrinkage by suppressing endogenous LH production (negative feedback of the HPG axis). Note: T injected into periphery does not keep testicular concentrations high enough for spermatogenesis

9.5 Testicular dysgenesis syndrome

9.5.1 Endocrine-disrupting chemicals (EDCs)

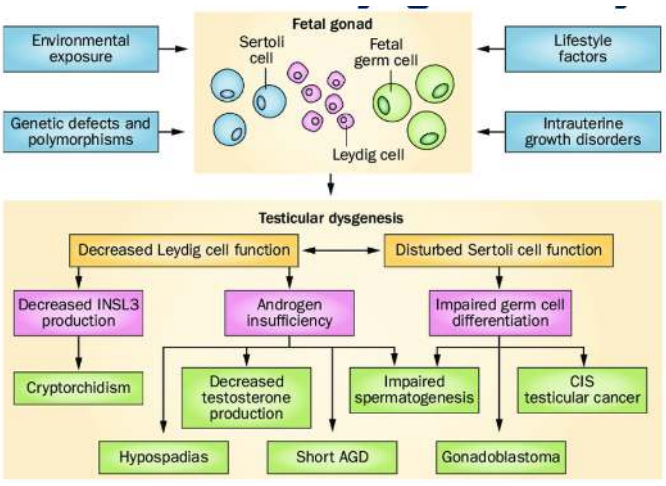
- Change (up or down) receptor/ligand binding
- Change (up or down) production of the hormone by its endocrine gland
- Change (up or down) the metabolism/inactivation of the hormone
- Change production of hormone binding proteins for circulatory transport

9.5.2 Terminologies

- Amount
  - Azoospermia = The testes are not producing ANY sperm (usually genetic cause)
  - Oligospermia = The testes are not producing enough sperm
- Function
  - Asthenozoospermia = <40% sperm are motile
  - Teratozoospermia = < 4% sperm have normal morphology
  - Necrozoospermia = sperm in the ejaculate are dead

9.5.3 Diseases

- Hypospadias
  - Can be a result of a hypomorphic AR polymorphism
  - But some babies are born with hypospadias and a fully functional AR gene
- Ambiguous genitalia
- Cryorchidism
  - This can be part of PAIS but can also have an idiopathic cause, similar to hypospadias
- Female appearance



Treating rats with an AR antagonist during fetal development causes:

- Hypospadias
- Cryptorchidism
- Decrease in AGD

9.5.4 Example of EDC

Summary of phthalate effects on testosterone production and male reproductive system physiology

- Rat studies show a strong and consistent association with phthalate treatment, reduced testosterone production in both fetal and adult rats
- Adult human observational studies on sperm quality and quantity are mixed and inconsistent
- Human studies in neonates show that certain phthalates are linked to shorter anogenital distance (AGD) in boys: a proxy for lower fetal androgen exposure.
- However, there is no direct evidence that phthalates reduce human fetal testis testosterone production

10 BODY FLUID HOMEOSTASIS

Homeostasis of body fluids is preserved primarily by the kidneys.

The kidney adjusts the amount of sodium it filters, reabsorbs, and excretes depending on whether the body is in deficit or excess.

- ADH: Water balance (retain water)
- Aldosterone: Sodium balance (retain sodium)
- Together: Body fluid balance

10.1 Aldosterone

10.1.1 Source

- Aldosterone is a class and main class of mineralocorticoid, secreted by the zona glomerulosa of the adrenal cortex.
- The zona glomerulosa cells express the aldosterone synthase (CYP11B2) to convert the previous molecule into aldosterone.
    - The source material is cholesterol

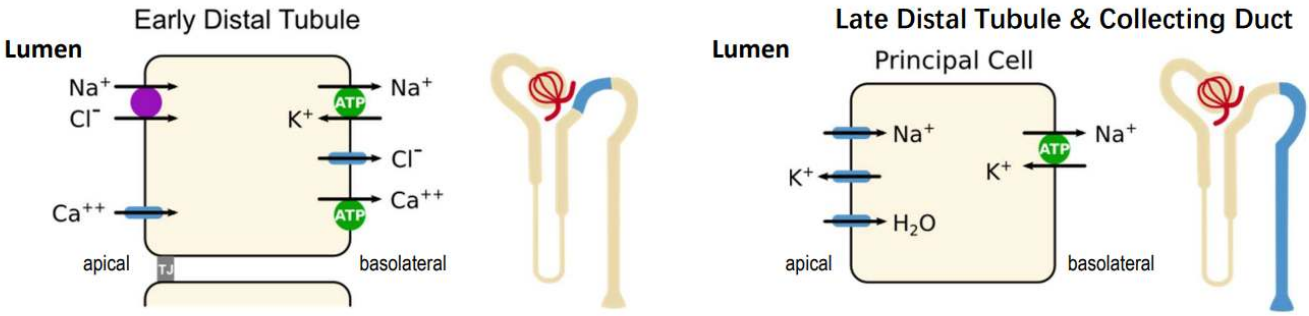
10.1.2 Function

- Besides reabsorbing sodium from the urine, it can also excrete potassium into the urine
- Thus, it increases water retention, blood volume, and BP.

10.1.3 Action

10.1.3.1Location

Aldosterone acts via mineralocorticoid receptors (MR). Aldosterone acts primarily on the nephron's epithelial cells, where MR is expressed. However, there are other sites of MR expression, including colon, CNS (hippocampus), heart, sweat glands, salivary glands & adipose tissue.



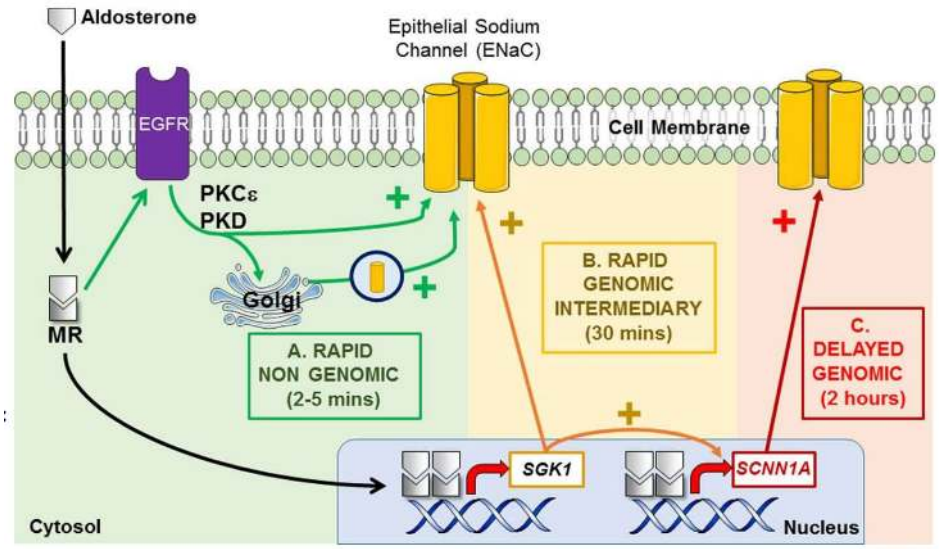
- Early distal convoluted tubules
  - Apical membrane: Sodium chloride cotransporters (NCC)
  - Basolateral membrane: Na+ /K+ ATPase pump
- Late distal convoluted tubules & Collecting ducts
  - Apical membrane: Epithelial sodium channels (ENaC)
  - Basolateral membrane: Na+ /K+ ATPase pump

10.1.3.2Receptor selection

MR has equal affinity for mineralocorticoids and glucocorticoids. Interestingly, there is a selective response in some tissues (e.g., kidney) for mineralocorticoids OVER glucocorticoids.

MR in the kidney is "protected" from glucocorticoids by co-localization of the GLUT-inactivation enzyme, 11β-HSD2.

10.1.4 MR transduction



- Genomic actions
  - Principle: Activation of MR results in its translocation to the cell nucleus, homodimerization, and binding of the homodimer to hormone response elements (HRE) in the promoter of target genes to initiate gene transcription (steroid hormone route).
  - Rapid: MR activation increases transcription of intermediary genes, which enhance the activity of ENaC and the sodium chloride cotransporter (NCC)
  - Sustained: MR activation also induces direct transcription of the gene that encodes the α- subunit of ENaC.

- Non-genomic actions
    - Rapid: MR transactivation of the EGFR leads to downstream activation of protein kinases, PKCε & PKD1. MR uses PKCε and PKD signalling to mediate aldosterone effects on ENaC subunit trafficking and membrane integration in renal epithelial cells. The results are enhanced intracellular trafficking of ENaC subunits and thus an increase in ENaC surface expression & activity.

10.1.5 RAAS

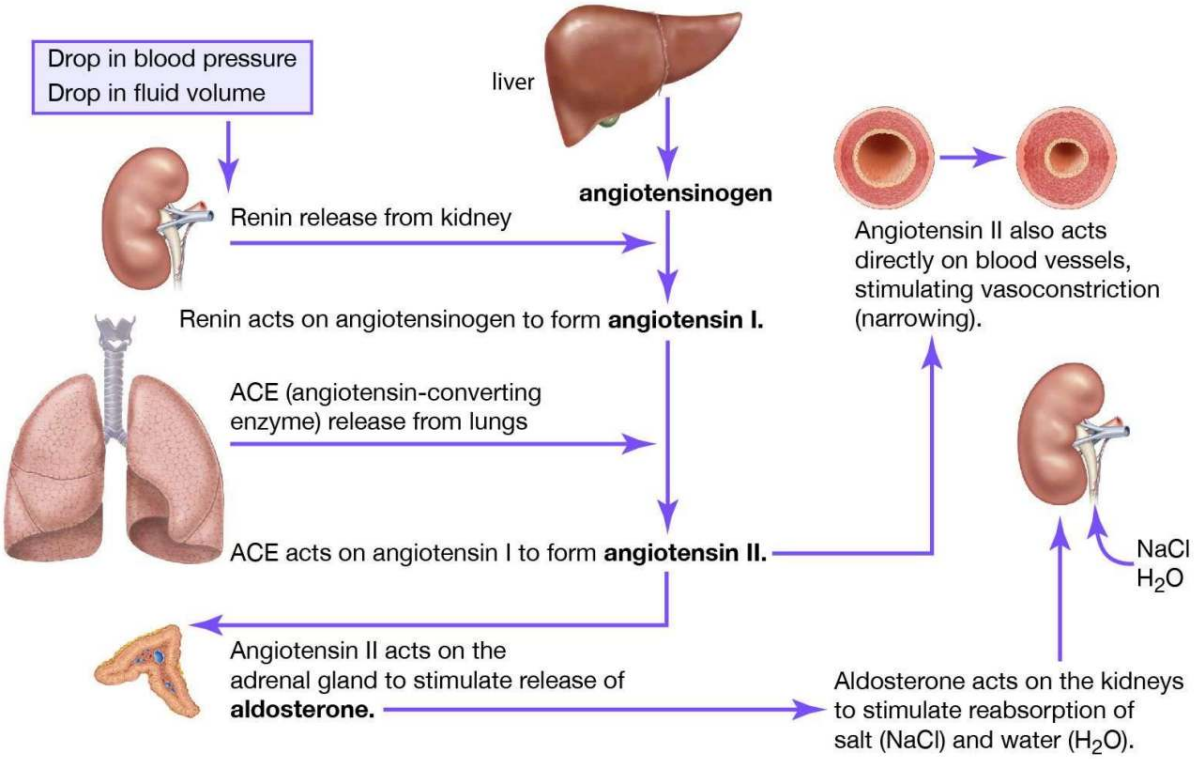
RAAS stands for **R**enin–**A**ngiotensin–**A**ldosterone **S**ystem

Activation of this system occurs when there is a decrease in blood flow to the kidneys following loss of blood volume OR a drop in BP.

RAAS regulates BP by increasing sodium and water reabsorption and vascular constriction.

The RAAS consists of three major substances, including

- Renin (an enzyme)
    - Secreted from the juxtaglomerular apparatus (JGA; juxtaglomerular cells) of the kidney
    - In response to
        - A decrease in arterial BP via stretch receptors in the vascular walls
        - A decrease in sodium load via the macula densa of the JGA
- Angiotensin II (a hormone)
- Aldosterone (a hormone)



10.1.6 Diseases

10.1.6.1 Primary Hyperaldosteronism (Conn's syndrome)

- Cause: The most common cause is an aldosterone-producing adrenal tumour or adrenal hyperplasia, causing them to produce too much aldosterone.

- Symptoms: Hypertension, stroke, Atrial fibrillation, coronary artery disease, and chronic kidney disease, low blood levels of potassium
- Treatment: Tumour removal; MR antagonist

10.1.6.2Secondary Hyperaldosteronism

- Cause: Health issues that cause hypofusion to the kidney, such as obstructive renal artery disease, renal vasoconstriction, heart failure, liver disease, and some kidney tumours.
- Symptoms: similar to primary hyperaldosteronism, but plasma renin levels are elevated in secondary hyperaldosteronism due to the reduced renal blood flow's stimulative effects.
- Treatment: treat the underlying cause of reduced renal blood flow; MR antagonist

10.1.6.3Addison's disease (primary adrenal insufficiency)

- Cause: Autoimmune destruction of the adrenal cortex
- Patients are deficient in both mineralocorticoids and glucocorticoids.
- Symptoms: hyponatremia, hyperkalemia, hypotension
- Treatment:
    - Hydrocortisone or prednisolone to replace glucocorticoids
    - Fludrocortisone (a synthetic MR agonist) to replace aldosterone to normalise blood pressure and serum potassium levels

10.2 Antidiuretic hormone (ADH)

- ADH is also called vasopressin and arginine vasopressin (AVP).
- It acts on the nephrons of the kidneys to facilitate greater reabsorption of water.
- AVP secretion is responsive to plasma osmolality, BV, BP, ANP, and Angiotensin.

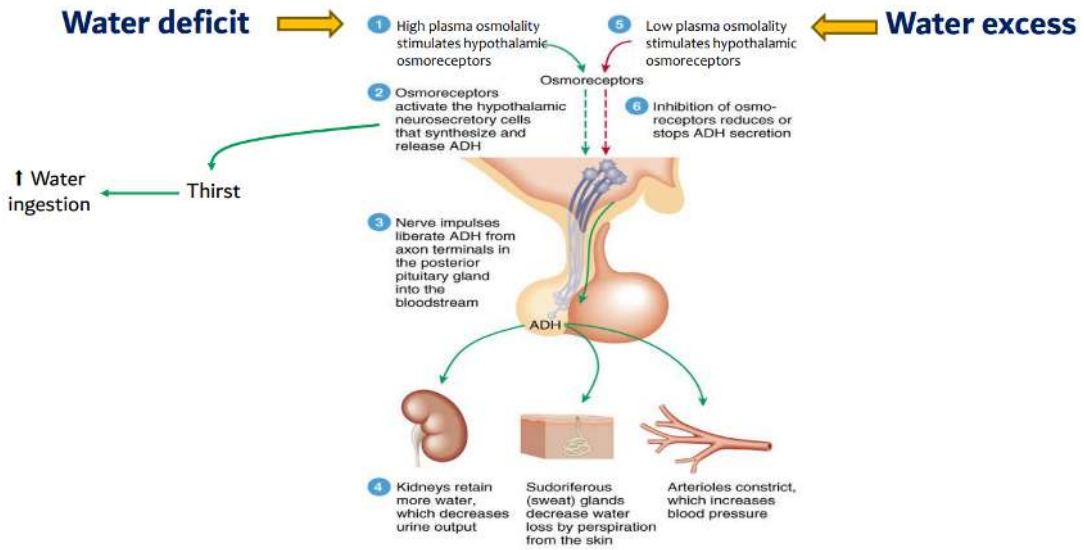
10.2.1 Receptors

AVP has different functions dependent on its acting receptors:

Receptor	Main Location	Primary Effect
V1aR	Vascular smooth muscle cells	Vasoconstriction → increased vascular resistance
V1bR	Anterior pituitary gland (corticotrophs)	Stimulates ACTH secretion
V2R	Collecting duct of kidney	Promotes water retention and concentrates urine

10.2.2 Osmoregulation

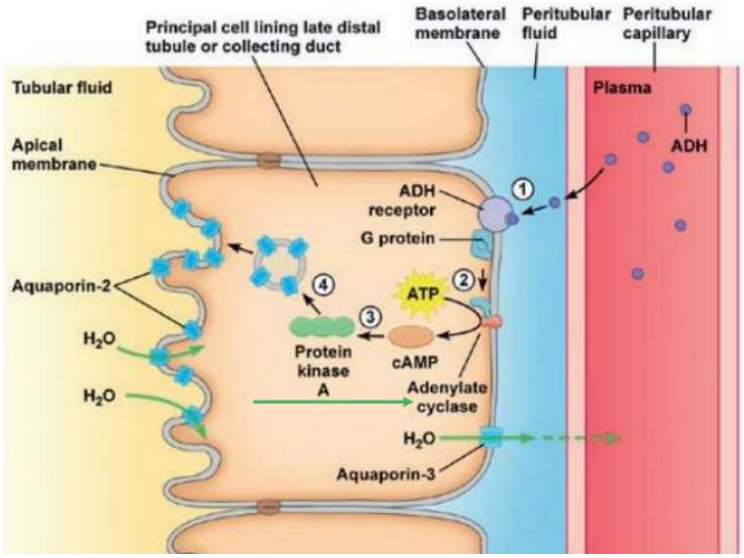
- Osmoreceptors are located in the hypothalamus (OVLT).
    - Destruction of the OVLT abolishes AVP secretion & thirst responses to hyperosmolality, but not their responses to hypovolemia.
    - In contrast, destruction of the magnocellular nuclei blocks dehydration-induced secretion of AVP, but does not alter thirst.



10.2.3 BP regulation

- High-pressure arterial baroreceptors sense heightened BP, which decreases the release of AVP
    - Located in the carotid sinus and aortic arch
- Low-pressure arterial baroreceptors sense heightened BP, which increases the release of AVP
    - Located in the atria and pulmonary venous system
- Signals from these receptors are relayed to the brainstem through cranial nerves IX and X.

10.2.4 Downstream action



1. AVP stimulates V2R on the basolateral membrane of collecting duct cells
2. V2R stimulation leads to cAMP pathway activation
3. This leads to aquaporin-2 water channels migrating to the apical cell membrane & facilitates passive water absorption across the collecting duct.
4. Urine osmolality increases and plasma osmolality decreases, leading to restoration of the water balance.

10.2.5 Diseases

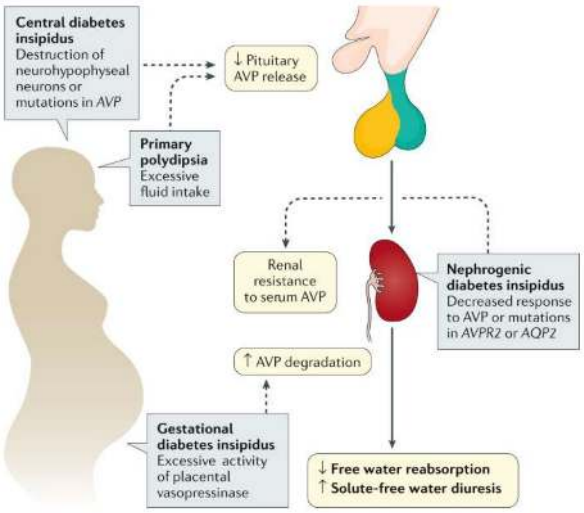
10.2.5.1Lack of vasopressin

Lack of vasopressin leads to diabetes insipidus (DI). This is a disease that produce large volume of urine in excess.

Cause:

- Mutation of vasopressin gene/V2 receptor gene/AQP2 gene
- Head trauma/pituitary surgery or tumour

Therapy: Desmopressin: a synthetic AVP analogue & selective V2 receptor agonist.



10.2.5.2Excess of vasopressin

Excess vasopressin secretion leads to Syndrome of Inappropriate Antidiuretic Hormone Secretion (SIADH).

Drinking water/reduced blood osmolality does not adequately suppress AVP secretion (lack of effective negative feedback) → water retention + concentrated urine → increased blood volume + hyponatremia.

Cause:

- Certain tumours produce AVP
- Infection/inflammation
- Drug stimulation

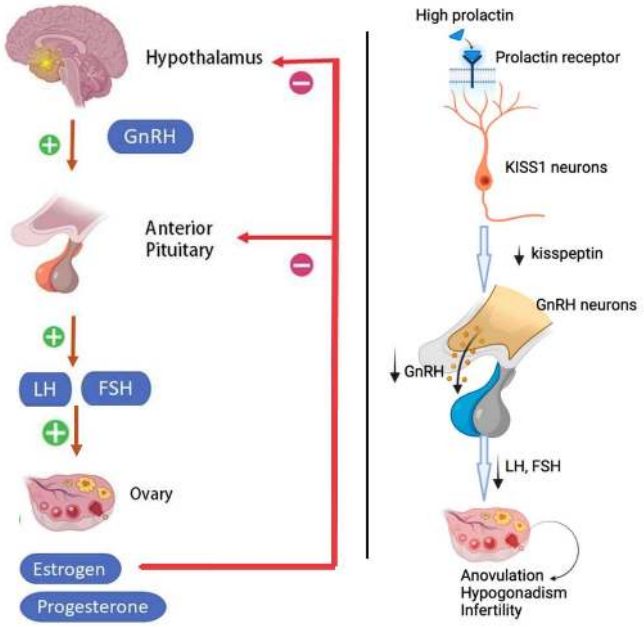
Therapy: Desmopressin: V2R antagonists

11 PREGNANCY

Physiological homeostatic set-points are reset in pregnancy.

11.1 HPG axis adaptation

- Pregnancy is a hypogonadotropic state, resulting in suppression of ovarian cyclical activity.
- Both basal and stimulated levels of LH & FSH decrease
- However, E2 and P4 level increase
    - Increased **E2 and P4** levels likely contribute to the inhibition of the LH and FSH
    - Estradiol (E2) generally has inhibitory effects on LH secretion and GnRH secretion
    - Progesterone inhibits LH pulse frequency & GnRH secretion and pulse frequency.



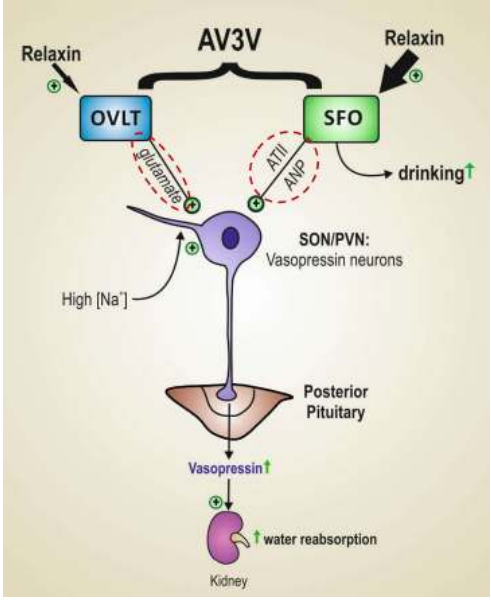
- **Inhibin** also increases
    - It indirectly inhibits FSH production by inhibiting activin
- **Prolactin** suppresses both the frequency and amplitude of LH pulses and suppresses GnRH secretion, possibly mediated by arc nucleus kisspeptin neurons.

11.2 Osmoregulation adaptation

Mechanisms regulating blood volume and osmolality are reset in pregnancy

- Phenomenon:
    - Blood volume increased by up to 50% by the end of pregnancy, which permits large increases in blood flow to the placenta & maternal organs supporting pregnancy
    - Pregnancy = Hyponatremia & Hypervolemia
- This is directly caused by
    - Increased drinking and water retention
    - Reduced sodium excretion
- The cause is the relaxin action

- Relaxin is produced by the corpus luteum to reset the osmoregulatory networks, drive water intake behaviour and increase water retention
    - This action does not need to cross the BBB
    - This action is dose-dependent
- Drive water intake's consciousness
    - Act on the brain's subfornical organ (SFO) neurones
- Increase water retention
    - Act on the brain's SFO and OVLT neurones + Reduction in the osmotic threshold at which vasopressin secretion is stimulated in pregnancy.
    - Increases the activity of the magnocellular vasopressin neurones
    - Acts on the kidney V2 receptors → water retention.



11.3 Food intake and metabolism adaptation

In pregnancy, there is a shift in the regulatory networks in the BRAIN to FAVOUR increased appetite and increased energy storage.

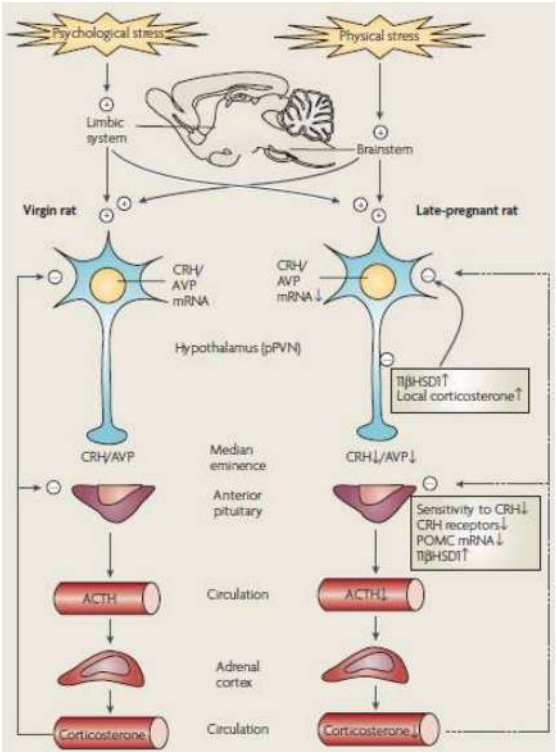
- Why?
    - Supplies the placenta, fetus, and uterus
    - Supplies increased maternal metabolism
    - Storage as adipose tissue
    - Prepares the mammary gland for lactation
- Cause
    - Resistance to anorexigens
    - Increased actions of orexigens
    - Normally, leptin secreted from adipose acts on Ob-R in the brain to
        - Inhibit NPY/AgRP neurons
        - Stimulate a-MSH and CART neurons in the ARC
        - Activate satiety neurons in VMH
    - However, leptin secretion increases during pregnancy
        - Leptin increases due to increased adipose tissue & secretion by the placenta.
    - However again, central leptin resistance occurs typically at mid-pregnancy, likely mediated by
        - Decreased Ob-Rb count in VMH during pregnancy
        - impaired LepR signalling downstream of Ob-Rb in the HYPO
        - increased leptin binding to circulating Ob-Re
    - Prolactin (& placental lactogen) induces similar changes in the VMH, indicating a role for prolactin in leptin resistance.

- P4 may have a role since it increases food intake, fat storage and decreases thermogenesis.
- Glucocorticoids are catabolic: in pregnancy energy is conserved by inhibition of HPA axis responses

11.4 HPA axis adaptation

The circadian variation of both ACTH and CORT secretion changes across pregnancy

- Corticosterone levels decrease in the first half of pregnancy but increase again in late pregnancy
- Loss of circadian increase in ACTH secretion as pregnancy advances
- Experimental evidence:
  - HPA axis responses to stress are suppressed in late pregnant rats, regardless of stressor type.
  - Stress responses are also suppressed in pregnant women.
- Cause: Reduced activation of the CRH neurones in the PVN during LATE PREGNANCY
  - Content
    - CRH mRNA in the PVN is decreased
    - CRH content in the median eminence is decreased
    - CRH receptors in the anterior pituitary are decreased



  - Sensitivity
    - The excitatory drive to the PVN neurones from limbic forebrain and brainstem nuclei is reduced
    - CRH and AVP neurones in the PVN are stimulated less by stressors
    - Pituitary corticotrophs less sensitive to CRH or AVP administration
  - Inactivation
    - Activity of 11β-HSD1is increased in the PVN and anterior pituitary
- Significance:
  - Minimise the risk of fetal programming by maternal glucocorticoids.
  - Conserve energy, as glucocorticoids have catabolic actions.

11.5 The role of oxytocin

- What: During pregnancy, oxytocin neurones in the SON and PVN are maintained in a quiescent state that limits oxytocin secretion.
- Significance:

    - Permits the accumulation of oxytocin stores in the posterior pituitary to meet the high demand at parturition and lactation.
    - Minimises the likelihood of premature oxytocin secretion that could lead to uterine contractions and preterm birth.
- How:
    - Oxytocin neurones show increases in auto-inhibitory mechanisms
    - The effectiveness of inhibitory inputs to oxytocin neurones is enhanced.
    - The inhibitory mechanisms become less effective at the end of pregnancy, permitting strong excitation of the oxytocin neurones at parturition.
        - Allopregnanolone in the blood & brain is increased → Upregulated brain-stem endogenous opioids → Inhibits IL-1β → NA release in the SON/PVN is decreased → quiescence of the OXT neurones AND the HPA axis in pregnancy is maintained (the release of CRH and OXT is decreased)
        - Allopregnanolone also potentiates inhibitory actions of GABA via actions on GABA receptors, which inhibit OXT activation
        - OXT neurones also produce NO, and NO inhibits OXT neurones directly
        - OXT released from the dendrites auto-stimulates endocannabinoid (eCB) production. In turn, eCBs inhibit glutamatergic inputs to the oxytocin neurones

11.6 The role of prolactin

- During pregnancy, prolactin increases to prepare the mammary alveoli for milk production.
    - Endogenous opioids increase → inhibit TIDA neurones → stimulate prolactin secretion
    - Enkephalin (enk) is up-regulated in TIDA neurones in late pregnancy → Acts on μ-/κ-opioid receptors → inhibit DA neurones and DA release
    - Dynorphin (dyn)-containing nerve terminals make contact with TIDA neurones & dynorphin → acts via κ-opioid receptors → inhibits TIDA neurones
- From mid-pregnancy onwards, placental lactogen suppresses maternal prolactin secretion.
- The night before parturition, prolactin secretion surges to act in the brain to promote the successful transition from pregnancy to motherhood
    - TIDA neurones become insensitive to negative feedback by prolactin & PL

12 PARTURITION

12.1 Disorders

- Preterm birth: fetal organ systems are not mature enough to survive outside the uterus, increased risk of a non-viable outcome
- Post-date fetuses: too large to pass through the birth canal, puts the survival of both the fetus and the mother at risk..

12.2 Overview

These are conserved events in parturition:

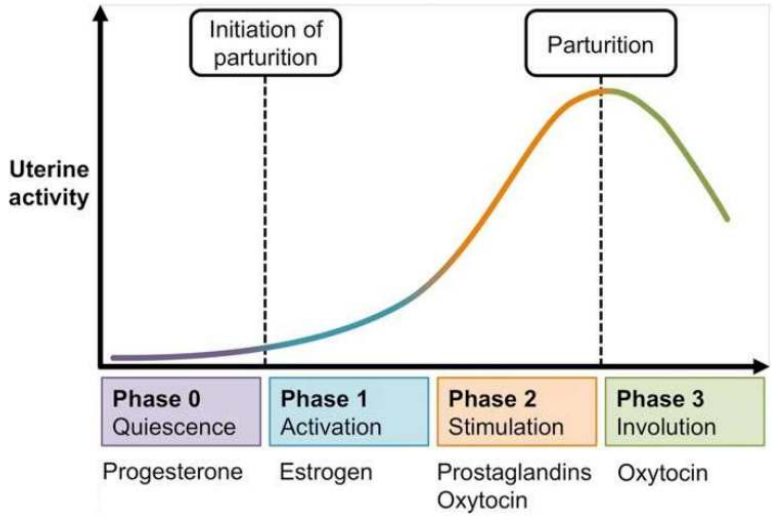
- Myometrium goes from a quiescent to a highly excitable state, where it contracts forcefully and rhythmically.

- Remodelling of the cervical connective tissue and smooth muscle, so it is capable of dilation and coordinated contractions to promote the passage of the fetus from the uterus.
- Rupture of the fetal membranes (amniotic and chorionic membranes) in response to paracrine interactions initiated by inflammation in the adjacent decidua.

Common features of hormonal regulation of parturition

- A paracrine loop within the gestational tissues that produce tissue-level inflammation
- A neuroendocrine loop that augments oxytocin secretion by the maternal pituitary gland

12.3 Phases



Note: E2 actually means estrogen.

Phase 0	Quiescence maintenance	• P4 dominance • P4 inhibits contraction-associated proteins (CAPs) expression • P4 inhibits ERa expression • Inhibition of P4 during this phase would cause preterm birth or even abotion

Phase 1	Myometrial activation	• From P4 to E2
		  ○ P4 withdrawal: Direct trigger for parturition
		  ▪ In rodents: P4 level decrease followed by luteolysis
		  ▪ In sheep: P4 level decrease followed by surge in cortisol from fetal HPA axis
		  ▪ In humans: Increase progesterone receptor-A and decreased progesterone receptor-B (functional progesterone withdrawal)
		  ○ E2 activation: Direct trigger for uterine activation at term
		  ▪ Upregulate OXTR
		  ▪ Promote prostaglandin
		  ▪ Increase the responsiveness of myometrial cells
		  ▪ Contraction-associated proteins (CAPs) increase: OXTR, prostaglandins (PGs)
		  ○ P4 decrease leads to E2 increase
		• Increased gap junction formation between myometrial cells
		• Increased influx of calcium ions into myocytes
		• OXT neurons became activated

		○ Central inhibitory mechanisms that restrain oxytocin neurones are down-regulated at parturition: P4 & AP fall, downregulated NO, loss of opioid
Phase 2	Myometrial stimulation	• Increased responsiveness to OXT & PGs • generate frequent, high intensity, coordinated, phasic contractions
Phase 3	Involution	Maternal oxytocin and uterine remodeling to expulse placenta to prevent postpartum hemorrhage

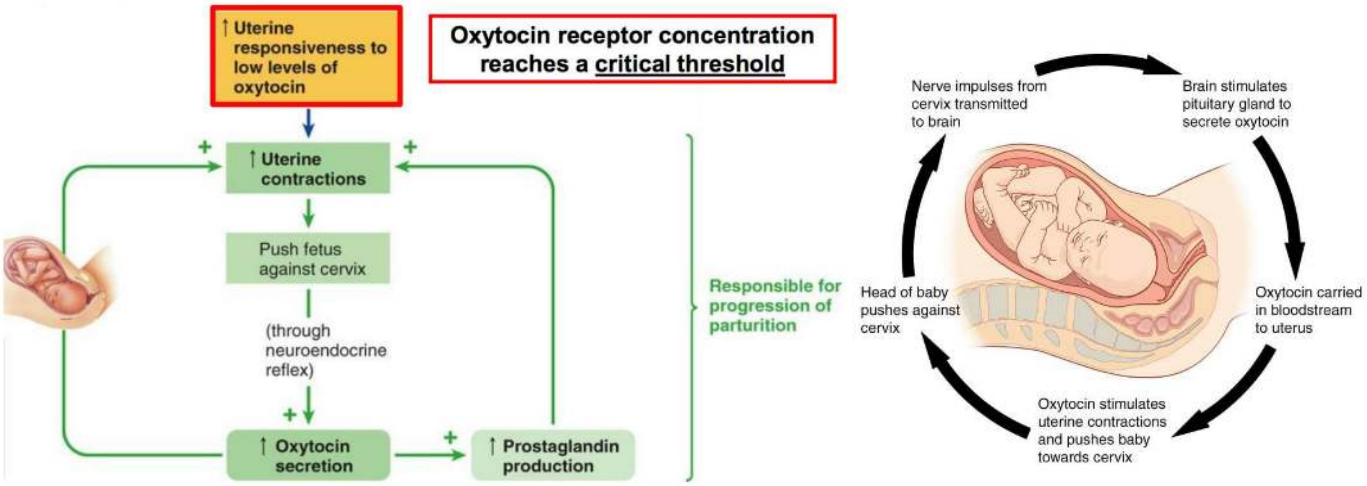
12.4 Orchestrated hormone action

12.4.1 Oxytocin

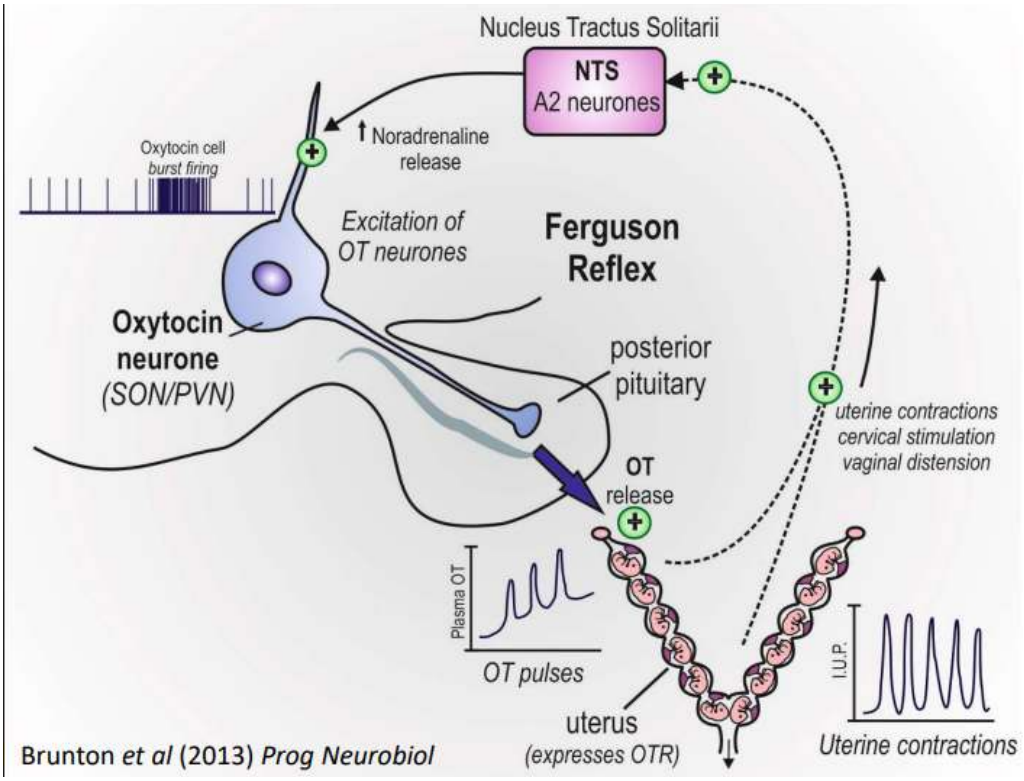
Overall, oxytocin increases the frequency and force of myometrial contractions to promote birth by both

- Directly: Induce myometrial contractions via actions on myometrial OTXR
- Indirectly: Stimulates the release of prostaglandins in the fetal membranes, which stimulate uterine contractility
    - PG levels are decreased by P4 and increased by E2, oxytocin, cortisol & CRH

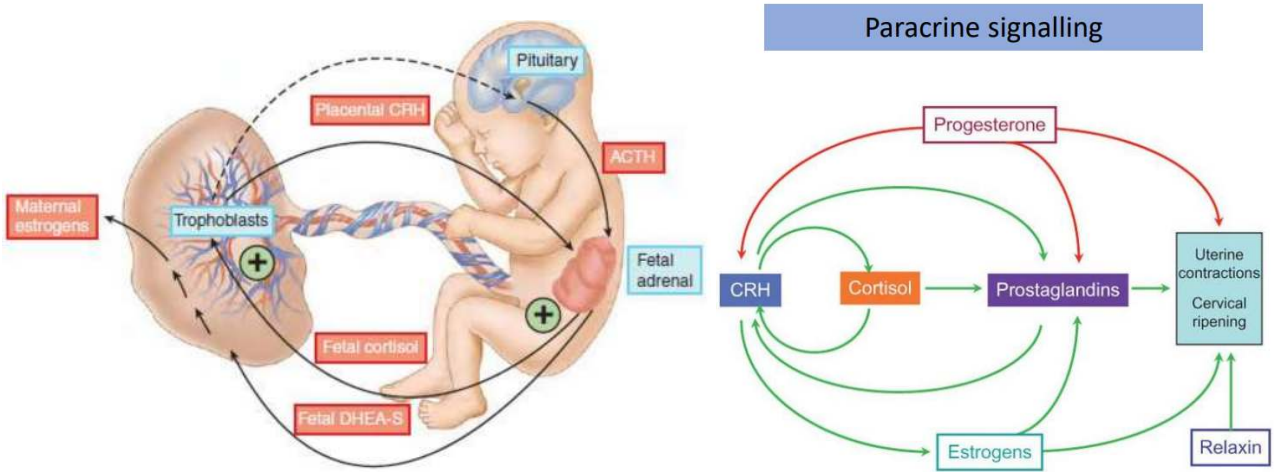
12.4.1.1 Positive feedback loop 1



12.4.1.2Positive feedback loop 2 (also called Ferguson Reflex)



12.4.2 Fetus HPA axis



12.4.2.1CRH

- In late gestation, placental CRH stimulates cortisol.
- Interestingly, placental cortisol has a POSITIVE FEEDBACK on CRH: A feed-forward cascade
- The placenta secretes CRH in increasing amounts from the 2nd trimester into the maternal body.
- The CRH peaks at term because CRH-binding protein (CRH-BP) in the blood renders circulating CRH inactive for most of pregnancy until the last 4 weeks, when CRH-BP levels decrease, so CRH activity increases.
- The rate of CRH increase is inversely related to gestation length.

12.4.2.2Estrogen

In late gestation, placental CRH also stimulates fetal adrenal production of DHEA-S, which is converted into E3 by the placenta.

13 Maternal behavior

13.1 Lactation

13.1.1 Functions

- Primary source of nutrition & energy for newborn
- Provides immune protection (e.g., antibodies)

13.1.2 Disorders

Excessive prolactin could cause disorders.

Increased prolactin acts via prolactin receptors on Kiss1 neurons in the ARC & nuclei to decrease kisspeptin gene expression. This, in turn, leads to

- Loss of the GnRH release
- Reduced LH and FSH
- loss of ovarian stimulation

Results in hypogonadism, infertility, and amenorrhea.

13.1.3 Stages

1. Mammogenesis: Growth & development of the mammary glands to a functional state

2. Lactogenesis: Synthesis & secretion of milk by the alveoli

3. Galactokinesis: Ejection of milk from the mammary glands

4. Galactopoiesis: Maintenance of lactation

5. Involution: regression and atrophy after lactation

13.1.4 Hyperphagia regulation

Food intake is significantly increased in lactation, driven by:

- Increased prolactin secretion (associated with suckling)
- A decline in leptin levels and reduced sensitivity to leptin (leptin resistance)

Thus, the energy requirement of lactation can be met.

Interestingly:

- As the pups get bigger, food intake increases correspondingly
- The extent of the increase in food intake depends on litter size.

This is promoted by prolactin in a dose-dependent manner.

- Suckling can increase prolactin secretion and NPY neuron expression in the hypothalamus.
- Plasma concentrations of leptin in decreased during lactation and so does leptin signalling.

- Central leptin resistance that develops from mid-pregnancy is maintained throughout lactation, caused by prolactin and is essential for the maintenance of hyperphagia.

13.1.5 Mammaogenesis (mammary gland development)

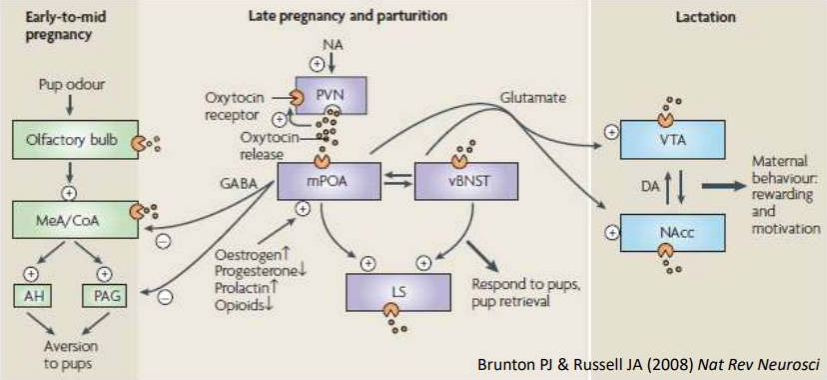
- Prepuberty to puberty: E2 and GH
- Puberty to post-puberty: Prolactin, E2, P4, GH
- Late pregnancy to lactation
  - Functional differentiation: proliferative phase DURING early pregnancy
    - P4: alveolar differentiation
    - Prolactin: alveologenesis
  - Secretory differentiation: Starts in mid-pregnancy to make the gland capable of secreting milk
    - Placental lactogen and progesterone prevent premature lactation
  - Secretory activation: Occurs at parturition
    - Triggered by high prolactin and a fall in P4

13.1.6 Galactopoiesis

Nursing of the young depends on two interrelated processes: milk production and milk ejection

- Milk production: Prolactin
  - Suckling activates somatosensory afferents that signal via the spinal cord and brainstem to the hypothalamus
  - Suckling inhibits TIDA neurones in the ARC nucleus, allowing elevated prolactin secretion into the blood.
- Milk ejection: Oxytocin
  - Initiation: Stimulation of nerve cells in the nipple produces impulses in spinal afferent nerves that signal to the magnocellular oxytocin neurones in the SON & PVN → OXT neurones fire in bursts → OXT acts on OTS on myoepithelial cells that surround alveoli → Coordinated contraction and rise in intramammary pressure → milk injection
- Maintenance: Prolaction + Oxytocin

13.2 Maternal behavior



- **Maternal behaviour** has several elements: nest-building, gathering, nursing, cleaning, protecting & non-aggression towards the young.
- Interconnected neural networks are responsible for each element.
- The **medial preoptic area** (mPOA) has a central role in the regulation of these maternal behaviours.

- Hormone priming of the **mPOA** by **E2, P4, PRL & OT** - receptors for these hormones are upregulated.
- Oxytocin released in the brain at parturition acts on **OTR** in the mPOA, nucleus accumbens (NAcc), ventral tegmental area (VTA), ventral bed nucleus of stria terminalis (vBNST), PVN, olfactory bulbs, lateral septum (LS) & amygdala, leading to the rapid initiation of maternal behaviour.
- Activation of the hormonally primed mPOA & vBNST overrides aversive neophobic responses to newborn odour & activates the mesolimbic dopaminergic **reward circuitry** (VTA & NAcc).

Oxytocin is necessary for proper maternal behaviour. Maternal behaviour is deficient in mice with impaired central oxytocin release, but is rescued by central oxytocin injections.

14 Developmental programming

14.1 Stress reprogramming

- Phenomenon: HPA axis responses to stress, in detail, CRH and AVP mRNA expression, are greater in adult prenatally stressed (PNS) rats.
- Direct explanations
  - GR and MR binding are lower in the hippocampus of PNS rats.
  - This suggests that impaired glucocorticoid negative feedback could explain the greater HPA axis responses to stress observed in PNS rats.
- During chronic stress,
  - More maternal GLUT reaches the fetus due to a reduction of 11b-HSD2 in the placenta
- Consequences
  - Increased stress could lead to lowered birth weight

14.2 Maternal care reprogramming

- Experiments: Cross-fostering pups
- Maternal behaviour is associated with long-term programming of anxiety behaviour in the adult offspring
- High-quality maternal care is associated with
  - Reduced CRH mRNA expression in the offspring
  - Reduced anxiety-like behaviour in the offspring
  - Increased hippocampal GR & enhanced glucocorticoid feedback sensitivity in the offspring
- Mechanism: Epigenetic changes

    - Maternal care increases DNA methylation of the GR promoter, reduce H3k3 acetylation, where stands the binding site for the TF, NGFI-A, stands
        - The HDAC inhibitor and TSA eliminate the maternal behaviour effect on histone acetylation, DNA methylation, and transcription factor binding
    - Less binding of the NGFI-A protein
- Childhood abuse is also associated with vulnerability to psychopathology.
    - This is also caused by reduced hippocampal GR expression & increased methylation of the GR promoter