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Embryology Explained

A Comprehensive Textbook for Medical Students & Junior Doctors

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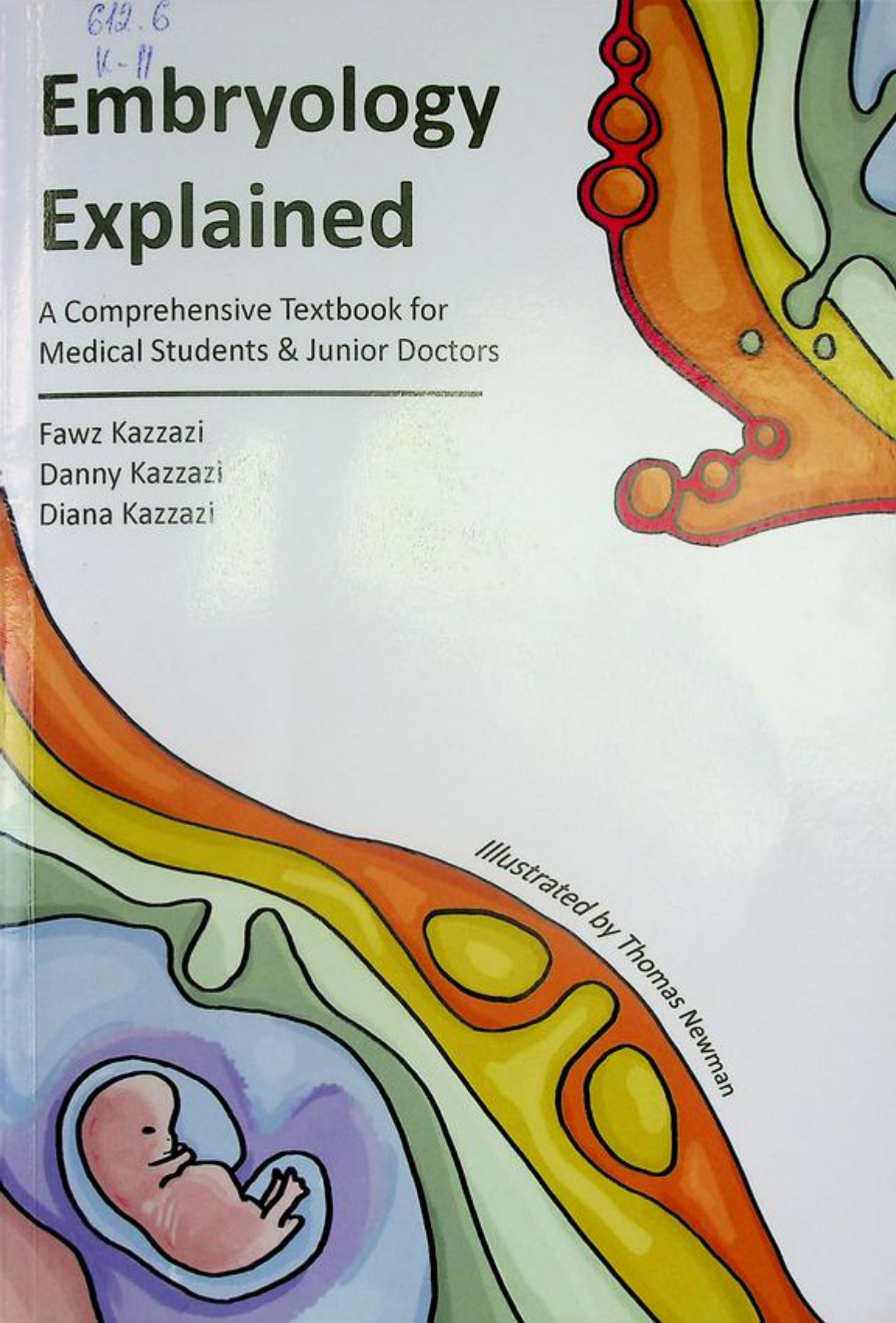

Illustrated by Thomas Newman

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Foreword 1

Embryology is a challenging subject. A comprehensive knowledge of the processes that govern embryo formation can help medical students and doctors appreciate the mechanisms behind the pathologies they come across in clinical practice. While some conditions have obvious embryological origins, the scope of pathologies extends into most medical and surgical specialities.

The difficulty with embryology teaching is that a good understanding is required to fully grasp the mechanisms underlying the formation of the human foetus; however, the depth of knowledge has not been matched in teaching schedules. This, coupled with low representation in exams, appears to have diminished the priority of the subject to the modern doctor.

This textbook aims to breakdown the complex theories into simple, manageable, and understandable blocks. Through this book, I hope you come to appreciate the beauty of the processes that create a human embryo.

Good luck and enjoy.

Basic Concepts 2

Embryology encompasses the processes and stages involved in changing an undifferentiated cell into a multi-organ structure. It involves key cell interactions that control the differentiation and architecture of organ systems. This section will explore the core concepts that are essential to understanding embryology.

Planes & Axes

Location within the embryo is described using a series of terms that specify a structure's position: (1) relative to other structures, (2) relative to key landmarks, and (3) along three-dimensional axes. These terms can be confusing as some are relative while others are fixed, and they can vary by the species (*figure 1*). They are listed below:

- *Rostral* – proximity to the head
- *Caudal* – proximity to the "tail" or feet
- *Dorsal* – lies to the back or spine
- *Ventral* – lies to the front
- *Medial* – lies closer to the midline of the trunk or limb
- *Lateral* – further from the midline of the trunk or limb
- *Proximal* – lies closer to the trunk
- *Distal* – further from the trunk
- *Inferior* – below; for the bipedal human this will be similar to *caudal*

- *Superior* – above; for the bipedal human this will be similar to *cranial*
- *Anterior* – to the front; for the bipedal human then this will be the same as *ventral*; for a four-legged/marine animal this will be equal to *rostral*
- *Posterior* – to the back; for the bipedal human, this will be the same as *dorsal*; for a four-legged/marine animal this will be equal to *caudal*

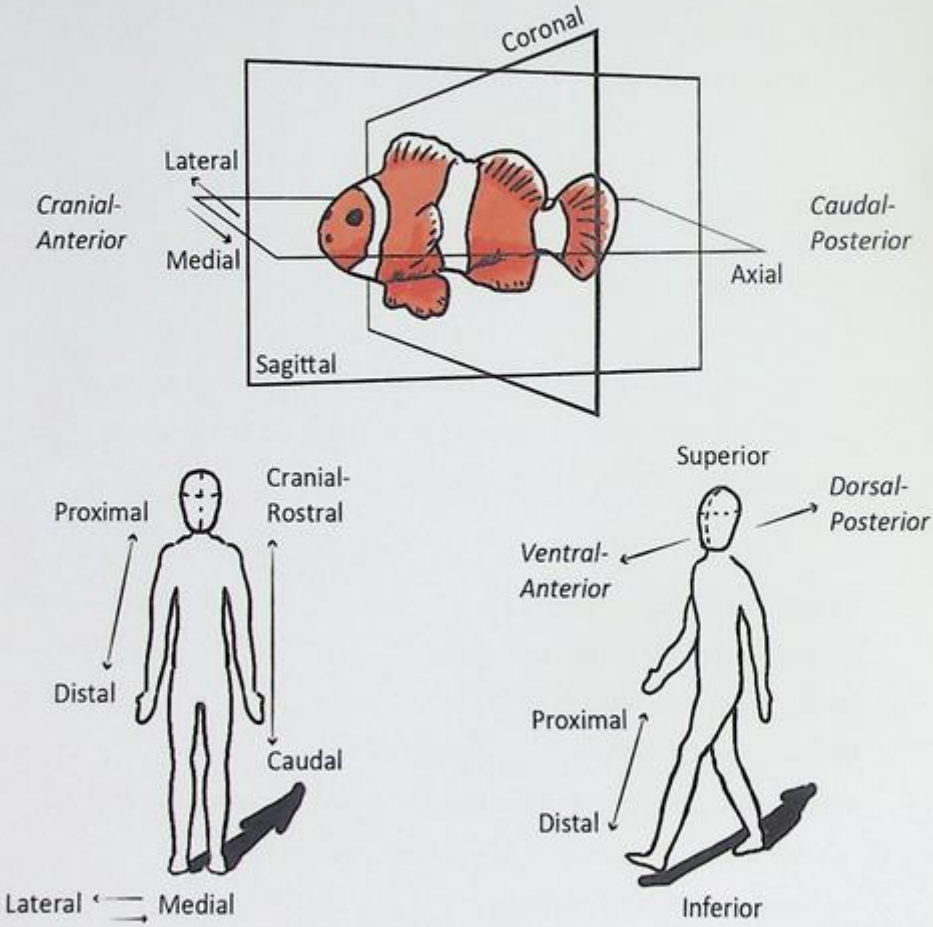

Figure 1: positional terms in a marine animal and human

Cellular Processes

In order for undifferentiated cells to organise and become complex structures, they undergo four key cellular processes:

- *Differentiation* – the process from which embryonic cells specialise and diverse tissue structures arise; the form and function of the cell will typically change.
- *Division* – the separation of a cell, often into equal identical parts.
- *Multiplication* – the division of a cell into further cells.
- *Chemotaxis* – chemical attraction of cells, leading to movement or differentiation of cells.
- *Convergent-Extension* – shortening in one axis and lengthening in another (*figure 2*).

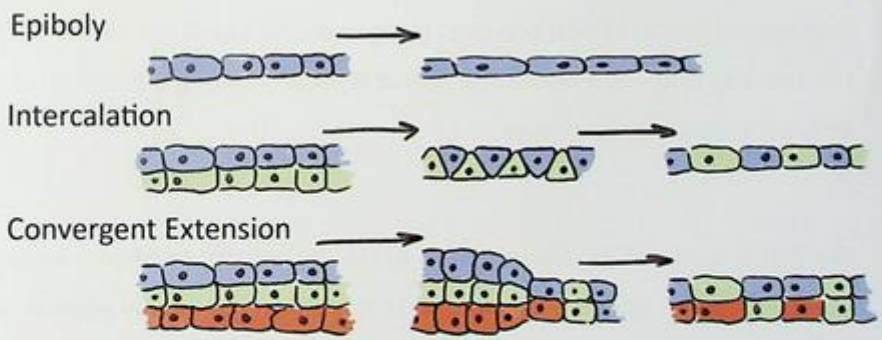

Figure 2: depiction of the cellular movement and directional growth of convergent-extension

In order to achieve these cellular processes in the embryo, cells utilise *morphogens* and *actin-myosin contraction*. A morphogen is a molecule whose non-uniform distribution (typically along a gradient) is detected by cells. The concentration is interpreted by cells to establish their position relative to other cells and give instructions for differentiation. Actin-myosin contraction occurs in individual cells to allow them to move towards or in response to a morphogen.

Understanding Timelines

The development of the embryo occurs in a near uniform order; and this is sometimes confused to mean it always occurs at a specific time (e.g. day 10). For some, it is useful to consider the sequential events of foetal development through the 23 *Carnegie Stages* that describe embryological features and events – rather than dates. When describing the weeks of embryonic development, they differ slightly from the *gestational age (GA)* that is described in clinical practice. The gestational age is calculated from the first day of the last menstrual period (typically adding 2 weeks to the embryonic development time).

The first 8 weeks of development (up to GA 10) are the *embryonic* period of development. Weeks 9 to 37 (GA 11 to 39) are the *foetal* periods of development. The first 4 weeks after birth are the *neonatal* period. Broadly, it can be said that the majority of organogenesis occurs in the first trimester; the second trimester involves developing and fine-tuning these organs, while the third trimester is the phase of rapid growth and

preparation for life outside the uterus. As such, the embryogenesis can be considered most vulnerable during the first trimester, where the processes of organogenesis can be affected by medication, alcohol, recreational drugs, infectious agents, tobacco, radiation, and other chemical agents.

Clinical Significance

The interruption or alterations of the steps of organogenesis lead to pathology in the newborn – some of which are compatible with life. For each chapter, this book will outline the pathologies relevant to the processes described.

What you may notice is that the pathologies often exist along a spectrum from mild defect (that may not influence function) to complete atrophy/absence of an organ. Some defects are symptomless while others require immediate or delayed intervention. "Clinical Significance" involves understanding your future patients as it is important to be able to identify both the symptoms (that the patient will report) and the clinical signs (that you will elicit on examination).

Foetal Warfarin Syndrome

The foetus of mothers who take warfarin during pregnancy may develop mutations in a dose-dependent manner. The risk of this is greatest if it is taken during the first trimester. The drug is teratogenic and can result in a wide spectrum of abnormalities. Most commonly, it leads to facial and skeletal variances including: scoliosis, nasal deformities/hypoplasia, brachydactyly (shortened fingers), short necks, and chest deformities. It can also result in congenital heart defects and central nervous system conditions including hydrocephaly, seizures, and microcephaly.

Warfarin crosses the placental barrier and inhibits vitamin K, which is necessary for clotting and bone growth in the embryo. Vitamin K is essential in the formation of clotting factors II, VII, IX, and X. It is also necessary for the correct form and function of a molecule known as *osteocalcin* – that is secreted by the osteoblasts and necessary for bone mineralization & maturation.

Foetal Alcohol Syndrome

This is the most severe of a spectrum of conditions that result from maternal consumption of alcohol during pregnancy. For a diagnosis of Foetal Alcohol Syndrome, all of the following criteria must be met:

- Growth deficiency: below the 10th percentile for weight or height
- Central nervous system damage: structural or functional impairment
- History of alcohol exposure in utero
- All of the following facial features (dose-dependent):
  - Smooth philtrum
  - Thin vermillion (particularly upper lip)
  - Small palpebral fissures

Neonatal Abstinence Syndrome

This occurs when babies begin to withdraw from drugs that they are exposed to *in utero*. This is commonly seen in the offspring of mothers that are dependent on opiates during pregnancy.

Relevant Molecules

For each chapter, there will be a summary of all the described cell signals, proteins, genes, and morphogens.

KEY POINTS

This section will list a summary of the high-yield facts from the section that regularly appear in written exams

- Embryology is the processes and stages involved in changing an undifferentiated cell into a multi-organ structure
- There is a subtle difference between cellular division and multiplication. Division involves the process of equal separation, whereas multiplication is the process of identical duplication
- Organogenesis predominantly occurs in the first trimester
- The embryonic period is the first 8 weeks of development. This is the same as the 10^{th} week of the gestational age, as the GA incorporates the 2 weeks since the last menstrual period
- A symptom is an experience that a patient describes
- A sign is a clinical finding that can be elicited on examination

Fertilisation & Implantation 3

This area of medicine is repeatedly assessed in both pre-clinical and clinical medicine as it contains important examinable knowledge for obstetrics, gynaecology, surgery, and emergency medicine.

Structures

Following ovulation, the female gamete (egg) is fertilised by the male gamete (sperm). The structures you need to be familiar with are:

- *Oocyte* – female gamete (egg, ova, ovum) prior to fertilisation.
- *Zona pellucida* – protective extracellular glycoprotein matrix surrounding the oocyte.
- *Zygote* – the fertilised (diploid) ovum.
- *Morula* – following fertilization and several rounds of mitotic division (to form identical cells), this structure is a ball of 16 cells (*morula* being Latin for mulberry).
- *Blastula* – the sphere of cells that forms after the morula, characterised by the existence of the inner fluid-filled cavity.
- *Blastocoel* – the cavity that the cells of the blastula surround.
- *Blastomeres* – the individual cells of the blastula.
- *Blastopore* – an opening in the blastula through which gastrulation occurs (process to be discussed in *Chapter 4*).

- *Trilaminar disk* – marks that gastrulation has occurred, this is the structure that forms containing the three germ layers.

Timeline

- Day 1 – Fertilization
- Days 1 to 3 – First Cleavage (2-16 cells)
- Days 4 to 6 – Blastocyst Enters Uterus & Implants
- Days 7 to 12 – Implantation Complete
- Day 13 – Formation of Primary Stem Villi & Primitive Streak
- Day 16 – Gastrulation

Fertilisation

This occurs in the *ampulla* of the oviduct (*figure 3*). Spermatic entry occurs by breaking through the oocyte's protective glycoprotein *zona pellucida*. First, the sperm's membrane fuses with the outer layer of the oocyte, releasing *acrosomal enzyme* (contained within the acrosome in the head of the sperm). This contains degradative substances, such as *hyaluronidase* and *acrosin*, to digest the outer membranes and allow entry – a process known as the *acrosomal reaction*. Following this there is cleavage of the ZP3 receptors on the zona pellucida to prevent the entry of further sperm (and additional chromosomes).

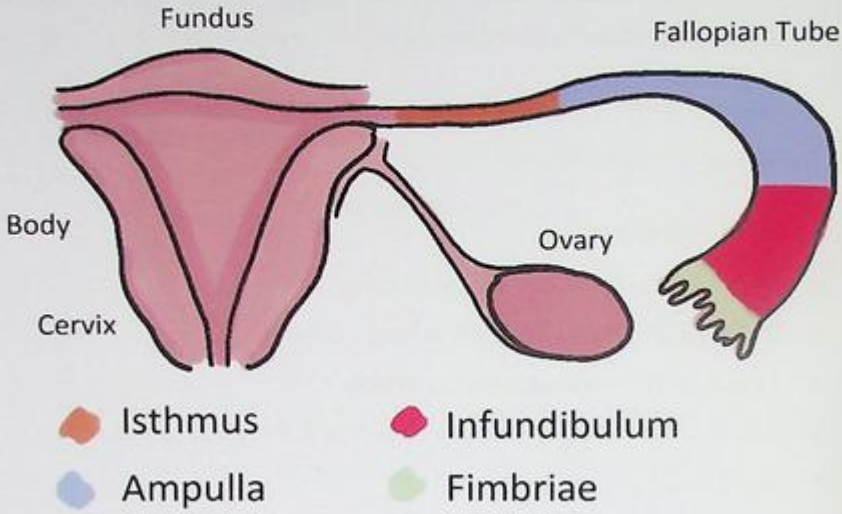

Figure 3: Uterus and the segments of the oviduct

Cleavage & Formation of Blastocyst

In mammals, the point of sperm entry determines the plane of the first division of the zygote. Central to the process of cleavage is that it occurs *without a change in size* – it is a pure division of cells into equal parts. The zygote will subdivide into blastomeres. At the 8-cell stage, the loose arrangement of blastomeres is compacted. When these blastomeres divide again into a 16-cell structure, it is called the *morula* (*figure 4*). The morula will reach the uterus around day 3 to 4.

Due to the size and structure of the morula, an inside-out axis (polarity) is formed). This is because there is now a difference between cells in the morula; they are not all the same. The inner-most cells are surrounded by cells on all sides, whereas the outer cells only contact cells on their inner surface. Therefore, the cells do not receive equivalent signals, as the inner-most cells will receive signals from all sides, whereas the outer cells only receive cell signals on their inner surface. The *inside-out polarity* is formed. This polarity allows cells to recognise their position in space and differentiate according to the transformation signals.

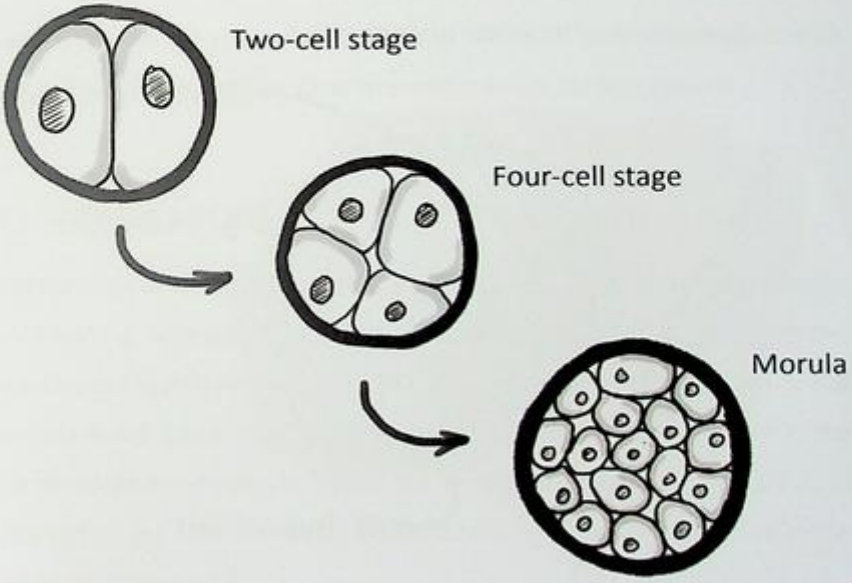

Figure 4: zygote sub-dividing (without an increase in size) into the morula

Formation of the Inner Cell Mass & Trophoblast

The inner cells of the morula will form the *inner cell mass* and the outer cells will form the *trophoblast*. The inner cell mass becomes the *embryo proper* (the cells which form the foetus). Due to the inside-out polarity, there is a discrepancy in the expression of sodium/potassium pumps. This leads to an influx of ions to the centre of the morula through which fluid follows (*water follows salt*). This creates a cavity in the middle of the morula, and the structure is now the *blastocyst* (*blastomeres* surrounding a central *blastocoel*). This pushes the inner cell mass to one end of the blastocyst (*figure 5*), such that there are two poles: the embryonic pole (where the *embryo proper* is) and the anesbryonic pole.

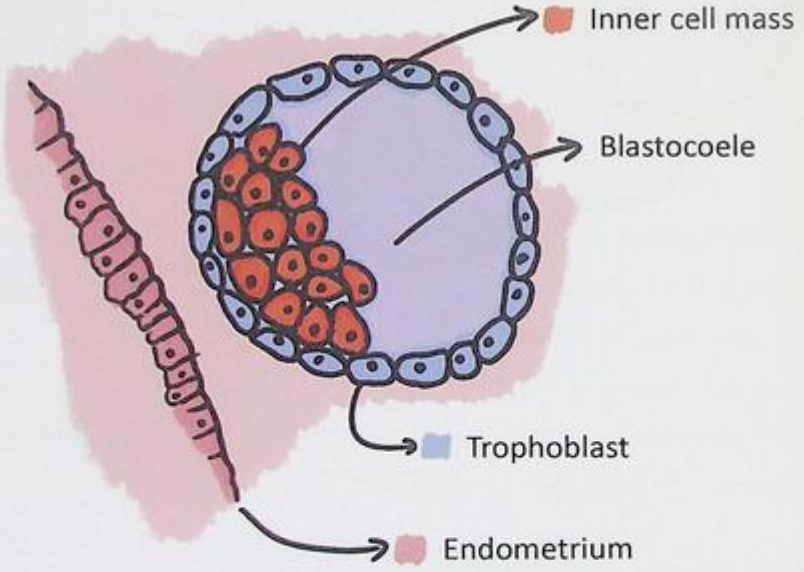

Figure 5: blastocyst with an inner cell mass at the embryonic pole heading towards the endometrium of the uterus

The inner cell mass (ICM) is *pluripotent* and can be cultured into embryonic stem cells. It is pluripotent rather that totipotent as it has partially differentiated into ICM and lost the ability to become trophoblast. The ICM will differentiate into a bilaminar (two-layered) structure containing *epiblast* and *hypoblast*. When these two layers form, the first asymmetry of the embryo forms – resulting in the *dorso-ventral axis*.

The trophoblast will become the *syncytotrophoblast* (STB) and the *cytotrophoblast* (CTB). As the name suggests, the STB is a syncytium (compare to the myocardial syncytium) because many cell nuclei are contained within a single cell membrane. The CTB (*cyto- meaning 'cell'*) is more like a typical cellular structure with a single nucleus per cell.

Implantation and the Formation of the Amnion

Implantation of the embryo is needed as oviductal nourishment (without a placenta; e.g. an egg) is too poor to support human embryo development, so a haemotrophic method of nutrient exchange is needed. On day 5, the blastocyst will hatch from the zona pellucida; between days 6 and 7, the STB develops and begins to invade the endometrium. The STB does this, as opposed to the CTB, due to its looser structure that allows invasion in the maternal uterine wall.

Trophoblastic lacunae open up within the STB and nearby maternal capillaries expand to form *maternal sinusoids* that rapidly anastamose.

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Proliferation of local CTB provides more structure to the invading STB and vascalature to form the *primary chorionic stem villi*. This implantation process will lead to the expression of β-human chorionic gonadotrophin by the trophoblast. These anastamoses will develop further to ultimately form the placenta; as the trophoblast proliferates, more β-HCG is produced.

At a similar time, an additional cavity will form between the ICM and the trophoblast at the embryonic pole – this is the *amnion* (*figure 6*). Specifically, the *amnion* will form between the epiblast (which sits closer to the endometrium) and the cytotrophoblast. Cells from the hypoblast will migrate around the blastocoel to form an inner layer, hence the hypoblast is sometimes called the 'primitive endoderm'. This will then form the *primary yolk sac* (extracoelemic cavity).

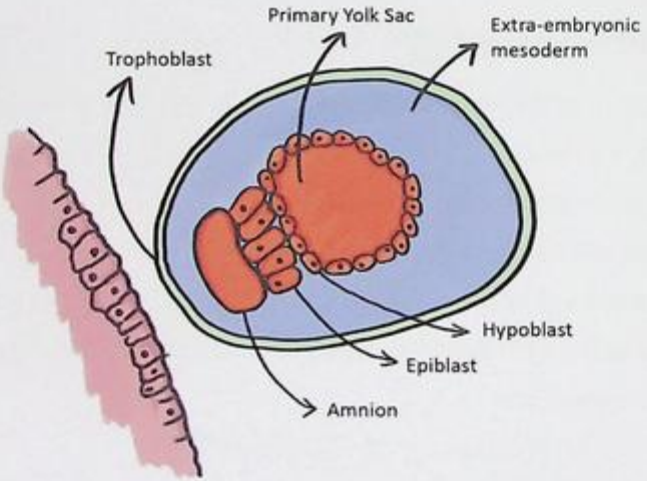


Figure 6: the embryo containing the epiblast & hypoblast (products of the ICM) and the amnion (between the epiblast and trophoblast)

Formation of the Yolk Sac and Chorionic Cavity

Cells from this yolk sac will divide the CTB into extra-embryonic mesoderm (*EEM*) (extra-embryonic as will not form part of the embryo proper). The cells of EEM will then separate into two forms: the somatic (parietal) EEM and visceral EEM (*figure 7*). The visceral EEM is the inner layer (closer to the embryo proper). The cavity that forms between the visceral and somatic EEM will be the *chorionic* cavity. After a further migration and lining by hypoblast cells, the primary yolk sac becomes the *secondary yolk sac*.

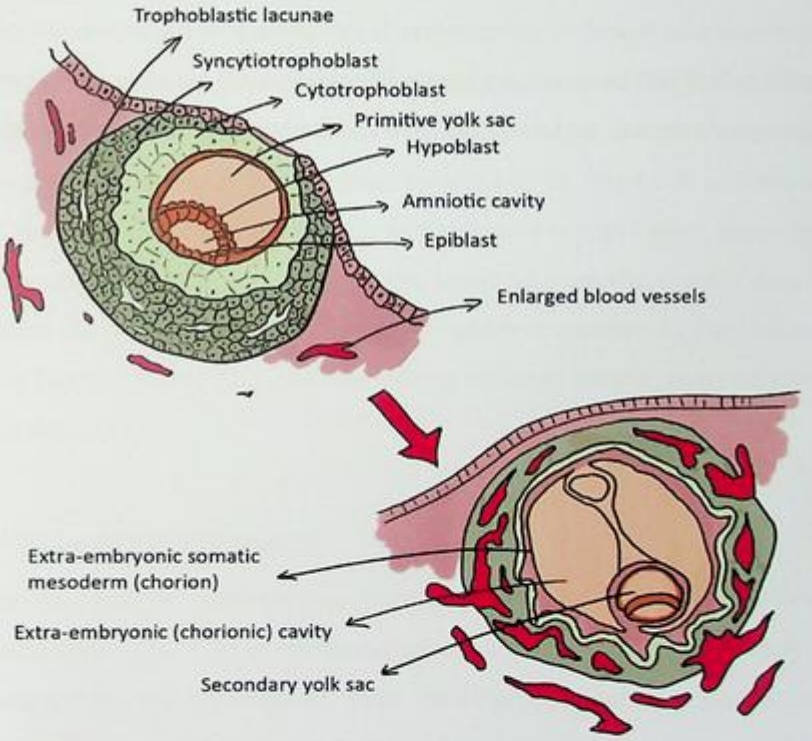


Figure 7: the formation of the yolk sac, amniotic cavity, and chorionic cavities

The EEM in humans forms the extra-embryonic membranes: amnion, yolk sac, chorion, and allantois. The collective role of the yolk sac, chorion, and amnion is for nutrient uptake and excretion – of more importance in non-mammals that do not have placentas. The extra-embryonic membrane system involves a small diverticulum, known as the *allantois*, that functions to excrete waste materials from embryos. The amnion cells are secretory epithelial cells that contribute to the formation of *amniotic fluid* which acts as a mechanical barrier to the embryo. The yolk sac is the first site of blood cell production and so contributes to early erythropoiesis, haematopoesis, and vasculogenesis. It also generates the *primordial germ cells* (which will be discussed later). The chorion will aid formation of the *chorionic stem villi* and contributes to the foetal portion of the placenta.

Clinical Significance

Ectopic Pregnancy

This occurs when the fertilised egg implants at a site other than the uterus. Most commonly, this happens in the fallopian tube. The likelihood of it occurring is increased in patients who have had previous operations, scarring, or chronic inflammation of their fallopian tubes. As the embryo cannot develop in this confined space, then a rupture of the tubes can occur – this is an emergency.

The symptoms of ectopic pregnancy include abdominal pain (often localised to the site), missed periods, bloody vaginal discharge, and discomfort on urination/defecation. If the fallopian tube ruptures, then the abdominal pain becomes diffuse or peritonitic, and the patient may report shoulder-tip discomfort. This occurs because the bleeding from the site of rupture irritates the underside of the diaphragm, which is supplied by the phrenic nerve (roots C3, C4, C5), and the pain is referred to the corresponding dermatome.

β-HCG Levels in Pregnancy

Pregnancy can be confirmed by detecting levels of β-human chorionic gonadotrophin (β-HCG) in the blood serum and the urine. This marker is produced by the trophoblast cells (STB initially) after implantation. Furthermore, by monitoring the level in the serum, the viability of the pregnancy can be determined.

The β-HCG levels are proportional to the volume of trophoblast / placental tissue. During the first four weeks of a viable pregnancy, the serum β-HCG levels would be expected to double every 2-3 days – as the trophoblast cells proliferate and the placenta grows. After six weeks, the level doubles every 4 days. In ectopic pregnancies, one can imagine that the trophoblasts and placenta cannot increase in size (and release β-HCG) due to a lack of viable tissue and space in the ectopic site. As such, if there is no doubling of the β-HCG serum levels, then the pregnancy may be ectopic or non-viable.

Every patient of child-bearing potential and age presenting to the Emergency Department with abdominal pain should have a urine β-HCG to exclude the existence of an ectopic pregnancy – as a delay in operating can be fatal.

Radiation-Risk in Pregnant Patients

Healthcare professionals should consider the risk of ionising radiation to the foetus in pregnant patients. This can increase the risk of mutation during organogenesis and subsequent disease in the offspring. As such, the type of scan should be considered in all patients who could be pregnant to minimise these risks. This may take the form of selecting an ultrasound scan as opposed to a CT scan (e.g. ultrasound abdomen rather than CT abdomen-pelvis to investigate acute abdominal pain). As such, all patients of child-bearing potential should have a urine pregnancy test prior to a scan.

Relevant Molecules

- **ZP3 receptors** – present on the zona pellucida; the sperm binds to these receptors to enter the oocyte.
- **β-HCG** – released by the trophoblast on implantation.

KEY POINTS

- Fertilisation occurs in the ampulla of the oviduct
- Division from zygote to blastomeres is mitotic
- Division to the morula stage occurs *without* an increase in cell mass – there is subdivision of the zygote without a change in size
- The inner cell mass (ICM) is pluripotent
- The ICM will form the epiblast and hypoblast
- The hypoblast is also known as the primitive endoderm
- β-HCG is released by the trophoblast (mainly STB) and used as a serum and urine marker for pregnancy
- Any patient of child-bearing age and ability who presents to the Emergency Department should have a β-HCG urine test to rule out ectopic pregnancy
- The yolk sac is the source of the primordial germ cells
- The chorion contributes to the foetal aspect of the placenta

Gastrulation & Axes 4

Gastrulation is considered by many to be the most important step in embryogenesis. By the end of this process, the definite body axes have been established and the basic architecture of human tissues are created. It results in the formation of three germ layers (endoderm, mesoderm, ectoderm) in a trilaminar disk, which will then undergo folding to generate the positioning of organs as we know them in the adult body.

Gastrulation

Around the 3rd week, the epiblast cells that lie *caudally* will condense to form the *primitive streak*. This marks the start of gastrulation. At the cranial end of this streak lies the *primitive pit* and the *primitive node*. The cells of the epiblast are *epithelial* and connected by a homodimer called *E-cadherin* (a strong adhesive molecule between epithelial cells). During gastrulation, these cells ingress at the primitive streak to create the three germ layers – endoderm, mesoderm, and ectoderm. In order to do this, the cells at the primitive streak release fibroblast growth factor 8 (FGF 8) to suppress the E-cadherin between epiblast cells so that they can mobilise (in a process known as *epithelial to mesenchymal transition*).

Now, with more fluidity in structure due to suppression of the connective bonds, these epiblast cells move towards the primitive streak then ingress through it to displace the underlying hypoblast (*figure 8*). The first migrating

epiblast cells will become the *definitive endoderm* (replacing the 'primitive endoderm' [the hypoblast]). The next cells to ingress will become the mesoderm. The remaining cells of the epiblast form the ectoderm. This creates a structure composed of three layers – the trilaminar disk. A useful list of the derivatives of each germ layer can be found in the *Clinical Significance* section.

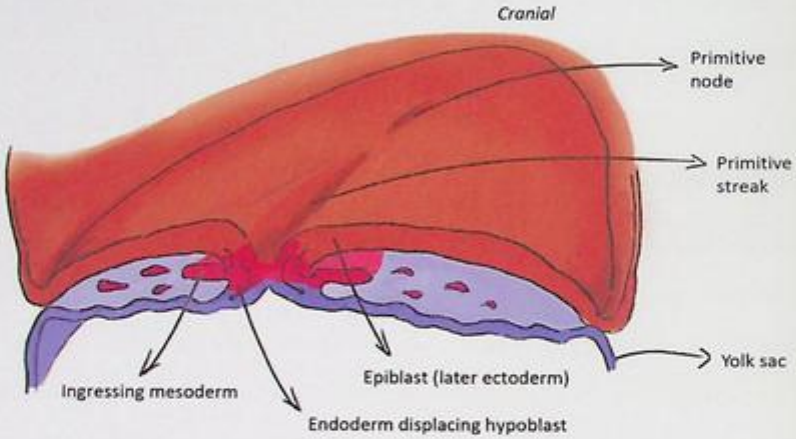


Figure 8: Epiblast cells ingressing at the primitive streak to displace the epiblast and form the three germ layers

Antero-Posterior Axis

At the cranial end of the embryonic disc, a portion of endoderm (the *'anterior visceral endoderm'*) modifies gene expression and morphogen levels to define this axis. It upregulates various transcription factors (*LIM1, HESX1, OTX1*) to secrete morphogens *lefty* and *cerebrus* ('head'/'cranial').

These inhibit the *nodal* molecules secreted by the primitive streak. This creates a gradient from the cranial (anterior) end of the disc to the caudal (posterior) end. This ensures that the appropriate genes for structures of the head are expressed anteriorly.

Mesoderm Subdivision

The mesoderm will divide into multiple sub-groups of cells defined by their position and laterality to the notochord: *paraxial*, *intermediate*, and *lateral mesoderm*. These are determined by the expression of signals from the notochord and the primitive streak.

BMP-4 is secreted in the throughout the embryonic disc. It is antagonised in the midline by the primitive node's expression of *chordin* and *noggin*. This signals to the nearby mesoderm to form the dorsal structures of the *notochord* and *paraxial mesoderm*. The non-antagonised expression of *BMP-4* will work with *FGF8* to signal to the remaining mesoderm to become *lateral plate* and *intermediate* mesoderm.

Dorsal-Ventral Axis

The formation of the three layers signifies the creation of the dorsal-ventral axis of the embryo. On a molecular level, this is due to *Nodal* in the primitive streak (positioned dorsally) upregulating a series of morphogens that stimulate cell differentiation and create different layers.

Left-Right Axis

The formation of the left-right axis is dependent on the *primitive node,* ciliated cells, and serotonin (5-HT expression). Ciliated cells waft morphogens onto the left side. *FGF8* on the left will upregulate expression of *Nodal* and *Lefty2* on this side and promote expression of 5-HT. This leads to left-sided features. On the right, *Snail* (a transcription factor) is upregulated and promotes right-sided genes; furthermore, uninhibited monoamine oxidase (MAO) enzymes break down the 5-HT to inhibit left-sided features.

Clinical Significance

Layers & Derivatives

Assessed in multiple-choice questions and spotter stations is the germ layer origin of organs or tissues. The following table acts as a quick-reference guide. A simplification to help generate a rough estimate is to consider that: the endoderm creates the inner-most tube (e.g. gut) and the organs that connect directly to it (e.g. gallbladder), the ectoderm forms the outer most tissues (e.g. skin), and that the mesoderm forms the tissues that lie between these two layers (e.g. kidneys).

Endoderm

Predominantly, this forms the gastrointestinal tube and structures that bud from or attach to it, including:

- Gastrointestinal tract (epithelial lining)
- Respiratory tract (epithelial lining)
- Auditory tube and middle ear
- Bladder and urethra
- Tonsils and thymus
- Liver glandular tissue
- Gallbladder
- Pancreatic glandular tissue
- Thyroid and parathyroid gland parenchyma (except C-cells)

Mesoderm

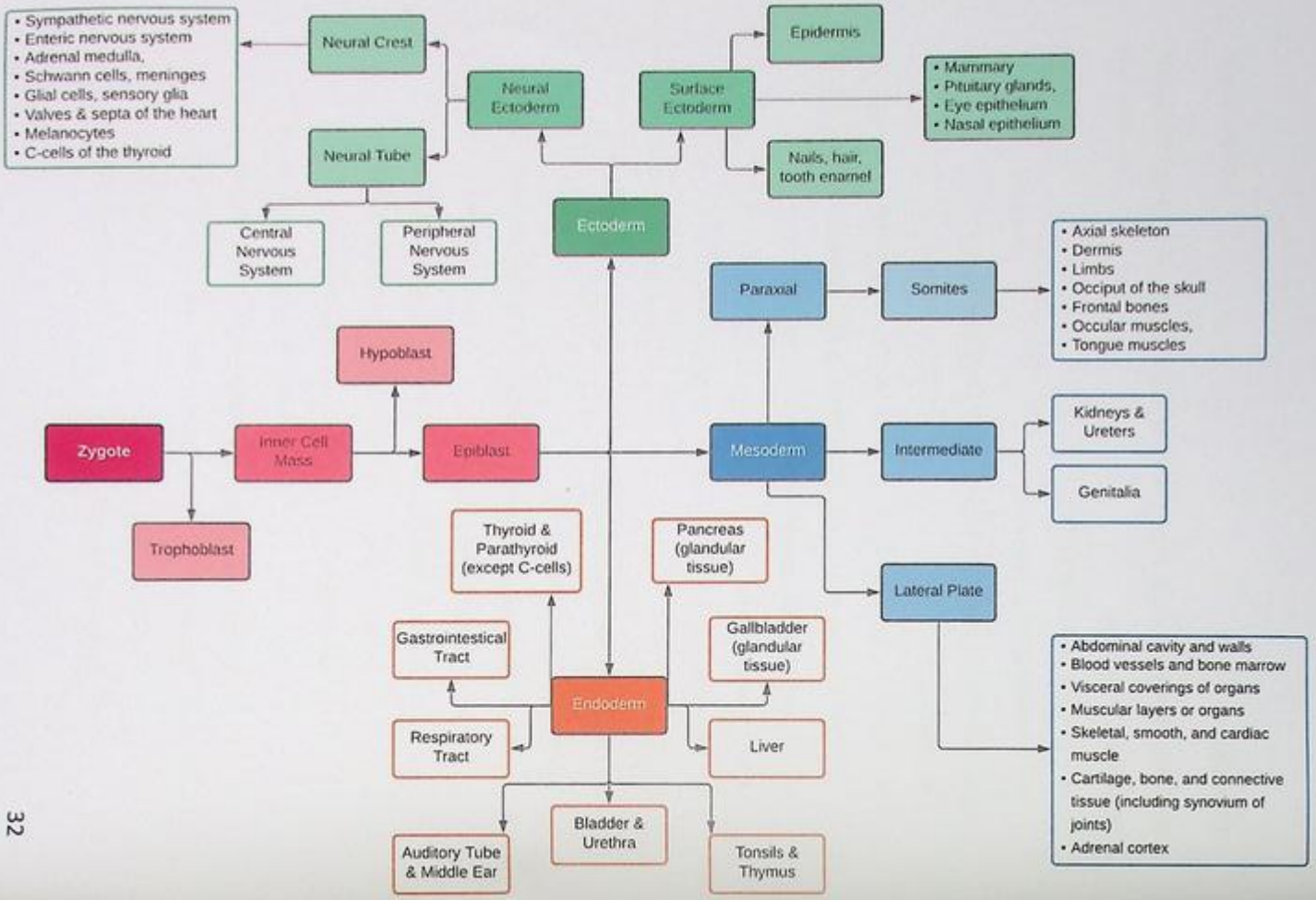
This generally forms organs that lie between the gastrointestinal tract and the epidermis:

- Paraxial
    - Somites
        - Axial skeleton (vertebrate and ribs) with overlying dermis and associated muscles (*NOT the epidermis*)
        - Limbs
        - Occipital part of the skill
        - Frontal bones (eyes, nose, inner ear)
        - Occular muscles
        - Tongue muscles
- Intermediate mesoderm
    - Kidneys
    - Ureters
    - External (gonads) genitalia and internal (reproductive) genitalia
- Lateral plate mesoderm
    - Abdominal cavity and walls
    - Blood vessels and bone marrow
    - Visceral coverings of organs
    - Muscular layers or organs
    - Skeletal, smooth, and cardiac muscle
    - Cartilage, bone, and connective tissue (including synovium of joints)
    - Adrenal cortex

Ectoderm

Broadly, the ectoderm forms sensory and epidermal (most external) structures, including:

- Peripheral neural system
- Cranial nervous system
- Epidermal skin including receptors and hair follicles
- Nails
- Teeth (note: odontoblasts have neural crest origin, whereas the enamel does not; teeth developed in vertebrates to allow fish to detect acidity/temperature of water)
- Mammary gland
- Pituitary gland
- Eye
- Sensory epithelium of nose
- Neural crest cells
    - Sympathetic nervous system
    - Enteric nervous system
    - Adrenal medulla
    - Schwann cells
    - Meninges
    - Glial cells
    - Valves/septum of the heart
    - Melanocytes
    - Sensory ganglia
    - C-cells of thyroid


• Sympathetic nervous system
• Enteric nervous system
• Adrenal medulla,
• Schwann cells, meninges
• Glial cells, sensory glia
• Valves & septa of the heart
• Melanocytes
• C-cells of the thyroid
Neural Crest
Neural Tube
Neural Ectoderm
Epidermis
Surface Ectoderm
• Mammary
• Pituitary glands,
• Eye epithelium
• Nasal epithelium
Nails, hair, tooth enamel
Central Nervous System
Peripheral Nervous System
Ectoderm
Paraxial
Somites
• Axial skeleton
• Dermis
• Limbs
• Occiput of the skull
• Frontal bones
• Occular muscles,
• Tongue muscles
Hypoblast
Zygote
Inner Cell Mass
Epiblast
Mesoderm
Intermediate
Kidneys & Ureters
Genitalia
Trophoblast
Thyroid & Parathyroid (except C-cells)
Pancreas (glandular tissue)
Lateral Plate
Gastrointestical Tract
Gallbladder (glandular tissue)
Endoderm
Respiratory Tract
Liver
Bladder & Urethra
Auditory Tube & Middle Ear
Tonsils & Thymus
• Abdominal cavity and walls
• Blood vessels and bone marrow
• Visceral coverings of organs
• Muscular layers or organs
• Skeletal, smooth, and cardiac muscle
• Cartilage, bone, and connective tissue (including synovium of joints)
• Adrenal cortex

Situs Inversus Totalis

This condition occurs in ~1 in 10,000 people and is characterised by the complete mirroring of the organs such that left-sided structures are on the right (and vice versa); for example, the liver is positioned on the left instead of the right. It occurs most commonly in an autosomal recessive pattern and patients are typically asymptomatic. The specific mutated genes are heterogenous and vary between families. In 10%, there are concurrent congenital heart conditions which can be severe.

Clinically, this conditioned may be encountered when patients present with atypical unilateral symptoms – for example migratory left-sided abdominal pain consistent with appendicitis.

Dextrocardia & Levocardia

In patients with dextrocardia, the heart is found of the right side of the thorax; it can occur as part of Situs Inversus or in isolation. For practical management of these patients, ECG leads should be mirrored. When occurring in isolation, it can be associated with defects of the heart and/or lungs – such as valve or septal malformations.

Primary Ciliary Dyskinesia & Kartagener's Syndrome

As discussed previously, ciliated cells waft molecules for early determination of right-left axis. Where there is a dysfunction in the motility of motor proteins in ciliated cells in patients, they are said to have *primary ciliary dyskinesia (PCD)*. As such, patients with PCD have a 50% chance of developing Situs Inversus – as the determination molecules have an equal

chance of lying either side of the midline when not being wafted by ciliated cells. PCD is an autosomal recessive disease and affects organs where flow or function is dependent on the efficient motility of cilia. As such, patients have defects in their respiratory tract, middle/inner ears, sinuses, and reproductive tracts/cells (e.g. sperm ejection). This manifests in conditions such as bronchiectasis, infertility, chest &sear infections, hearing loss, and sinusitis.

In Kartagener's Syndrome, patients have a *triad* of situs inversus, chronic sinusitis, and bronchiectasis.

Relevant Molecules

- *E-cadherin*: strong adhesive molecule between epithelial cells that is suppressed by FGF-8 in the epiblast cells to allow gastrulation
- *LIM1, HESX1, OTX1, cerebrus, lefty*: upregulated by the *anterior visceral endoderm* to define the anterior-posterior axis. These inhibit *Nodal*.
- *BMP-4*: secreted throughout the embryonic disc to generate morphogen gradients and determine cell differentiation
- *chordin, noggin*: inhibit BMP-4 in the midline to dorsalize the mesoderm and form the notochord and paraxial mesoderm
- *FGF-8*: works alongside BMP-4 to ventralize the mesoderm into intermediate and lateral aspects
- *Nodal*: secreted by the primitive streak to create axis that define the dorso-ventral axis
- *Lefty2, Nodal, FGF-8, 5-HT*: promote "left-sidedness" in the embryo

KEY POINTS

- Definition of the body axes occurs during gastrulation
- Hypoblast (primitive endoderm) is displaced by the definitive endoderm
- Epiblast will ingress to form the three germ layers
- The three-layered structure is the trilaminar disk and marks the formation of the axes
- The notochord is mesodermal in origin
- Ectoderm is the most dorsal layer
- A portion of endoderm defines the anterior-posterior axis
- The mesoderm is subdivided into regions according to proximity to the notochord (paraxial, intermediate, lateral)
- Endoderm generally forms the gut and structures which bud from it
- Organs not connected to the gut tend to be mesodermal, as well as the musculoskeletal and vascular structures
- Ectoderm generally forms the epidermis and sensory organs/tissues

Segmentation & Folding 5

Segmentation is a the formation of a structure in a linear series of repeating parts or segments. In the embryo, it establishes the *primary body map* to define where different structures will across the disk. This combines with folding in cranio-caudal, lateral, and ventral directions to determine the final positions of organs and structures in the embryo.

Segmentation & Somites

From around day 20 to day 28, the paraxial mesoderm condenses to form *somites*. These develop progressively in pairs (either side of midline), first cranially and then budding caudally – such that 3 to 4 paired somites form each day. In total, 42 to 44 pairs are created from the occiput base to the embryonic tail. In humans, those most caudal will disappear (we don't have tails!) leaving only 37 pairs.

The first 4 pairs will form the occipital part of the skull, the next 8 are cervical, then 12 thoracic, 5 lumbar, and 5 sacral. The ventral aspect of the somite becomes the sclerotome – which forms the vertebrae; the dorsal part form the dermomyotome that become the muscular, vascular, and dermal structures. The somite differentiates in response to ventral signals from *Sonic Hedgehog (SHH)* and *Noggin* genes; while the dorsal aspects respond to *WNT* and *BMP4* signals. A balance of these signals in the dermomyotome will activate the *MYF5* gene to form back muscle

when folding occurs it does so *ventrally*. The folding begins in the cranial portions and is completed caudally by day 28.

When the embryo is a disk, the most cranial structure is the *septum transversum* followed by the *cardiogenic area* and *oropharyngeal membrane*. These structures correspond to the presumptive diaphragm, heart, and mouth respectively. Folding repositions the *septum transversum* and *cardiogenic area* into the chest area, leaving the most cranial point as the *oropharyngeal membrane*; it also places the *septum transversum caudal* to the *cardiogenic area*.

After folding has completed, the embryo is covered by ectoderm everywhere except at the umbilicus – where the cord connects to the placenta. Internally, the body has divided into thoracic and abdominal cavities.

HOX Genes & Segment Identity

Somite boundaries are determined by the combination and patterns of Hox gene expression. These are numerical and sequential genes that are expressed together in a code-like manner to determine the location and differentiation of each somite and organ.

Clinical Significance

Dermatomes & Myotomes

Each spinal nerve that passes through the somite will supply an area of skin (dermatome); the exception is the C1 nerve for which there is no corresponding dermatome. The nerve will also supply the myotome that is generated from the corresponding somite, so that each spinal nerve supplies a muscle or group of muscles. The dermatomes can be mapped out (*figure 10*). While their distribution may be confusing at first, it is helpful to consider the bipedal human as a four-legged vertebrate, so that each dermatome acts as a slice.

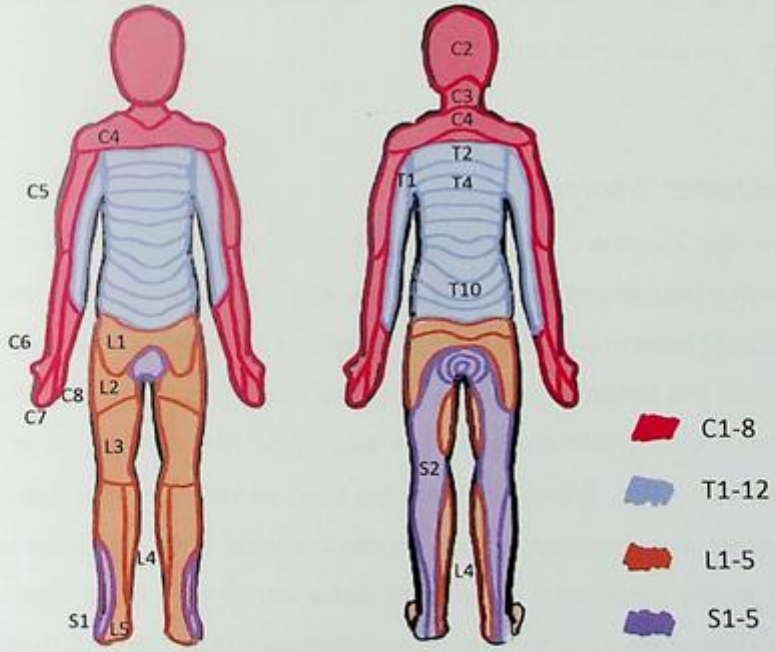


Figure 10: dermatomal distribution of spinal nerves

A good knowledge of the dermatomes allows clinicians to identify the likely lesion in neurological, vascular, trauma, and musculosketal disease/injuries. The commonly assessed dermatomes are:

- C5 – lateral upper arm in "regimental badge" area
- C6 – palmar/volar aspect of thumb
- C7 – palmer/volar aspect of middle finger
- C8 – palmar/volar aspect of little finger
- T4 – at the level of the nipples
- T10 – at the level of the umbilicus
- L3 – level of the knee
- L5 – great toe
- S1 – little toe
- S4/5 – perianal area & anus

Herpes Zoster Syndrome

This is a skin condition, also known as *shingles*, where a vesicular rash appears in a dermatomal distribution. Clinically, it is recognised by a rash that does not cross the midline and is isolated to a dermatome. The patient may report the sensation of being stung by nettles or ivy prior to the appearance of any skin changes. It is caused by the reactivation of a *Varicella Zoster Virus*. Previously, there has been an initial infection (which may present as chickenpox in their youth) following which the virus lay inactive in the dorsal root ganglia. Re-activation of may occur due to another inflammatory condition or physical/mental stress. The virus travels down the nerve body (of the corresponding nerve root) to create a

contained immune response in the dermatome. It can be managed with acyclovir and prednisolone.

Body Cavities

These are an essential part of the design of the human body. By having separate cavities (e.g. cranial, thoracic, abdominal), there can be different pressures and gradients within each component – allowing for specific function (e.g. negative pressure for inspiration). Clinically, this also reduces the spread of infection between body parts, while isolating injuries to specific areas without compromising function of all organs. For example, a penetrating injury to the abdomen will not immediately stop lung function and only risk infection/damage to that area initially.

Relevant Molecules

- *Sonic Hedgehog (SHH)* and *Noggin*: genes responsible for ventralizing the somite to form the sclerotome
- *WNT* and *BMP4*: morphogens that dorsalize the somite into the dermatomyotome
- *MYF5 gene*: generates back muscles from somite
- *MYOD gene*: codes for axial and limb formation from somites
- *NT-3 gene*: responsible for dermis formation
- *Hox genes:* these are sequential genes that, when expressed in certain patterns and combinations, instruct the location and outcome of somites

KEY POINTS

- Humans have 37 pairs of somites
- There are 8 cervical somite pairs but only 7 cervical vertebrae
- Somites will form the dermis (the ectoderm forms the epidermis)
- Each spinal nerve root innervates the dermis and muscle group of the somite that it penetrates; clinically this results in the dermatomal map and muscle groups
- Clinically, the dermatomes are very important in localising pathology
- Folding is essential for bring the presumptive diaphragm (septum transversum) and presumptive heart (cardiogenic area) into the thorax

Neurulation 6

The neural tube is formed during the third and fourth weeks from ectoderm and will produce the brain, pituitary gland, spinal cord, motor neurones, and retina. Its genesis occurs following four processes: formation, shaping, folding, and closure (*figure 11*).

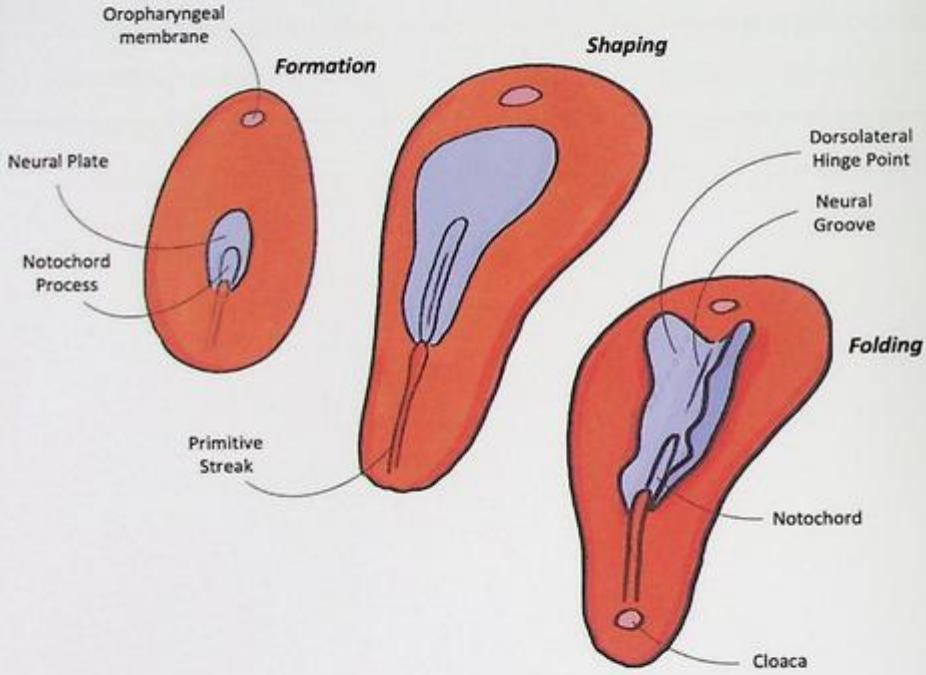


Figure 11: The neuroectoderm is established then shaped before folding form the closed neural tube

Formation

Ectodermal cells elongate and thicken in response to FGF signals from the notochord to form the neurectoderm; the cells in this single layer structure adhere to one another with *N-cadherin* homodimers ('N' for neural'). The FGF inhibits BMP-4 activity that would otherwise generate epidermis from the ectodermal cells – held together with E-cadherin homodimers ('E' for epithelial).

Shaping

The neural plate needs to form a lollipop-like shape such that it is broader in its cranial segment (the presumptive brain) and thinner caudally (presumptive spinal cord). This occurs due to differential rates of convergent-extension, such that the cells in the caudal region are lengthening at a greater rate. This occurs due different levels of morphogens in each region with *retinoic acid* being in higher concentrations cranially, and *WNT3a* in greater concentrations caudally.

Folding

The lateral edges of the neural plate elevate in order to commence the folding process. The plate folds over a central pivot overlying the notochord in a crease known as the *neural groove* – also known as the *median hinge point*. One may consider how a structure with varying widths along its length is able to close uniformly; this is achieved through additional hinge points in the broader cranial segment called the *dorsolateral hinge points.*

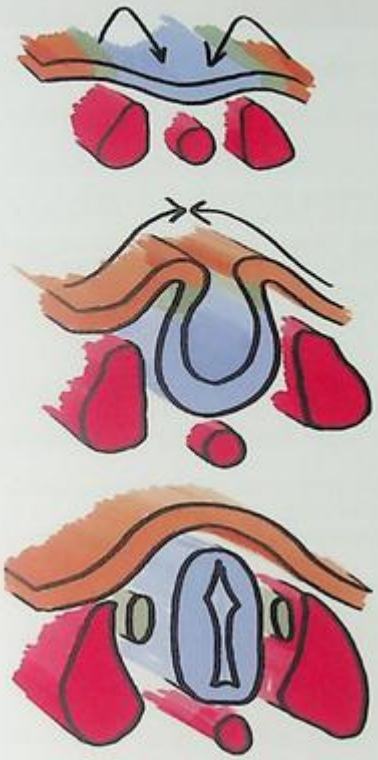
These ensure that the wider segments meet in the midline at the same time as the narrower portions.

Closure

There are different theories as to how this occurs. The most popular is that closure first starts in the midline and then progresses in cranial and caudal directions. Research in other animals, such as chickens, suggests that it may actually occur simultaneously at various points along the neural fold. Where the neural tube meets in the middle (*figure 12*), the expression of homodimers N-cadherin and E-cadherin ensures that equivalent cells join together and separate the neurectoderm from the surface ectoderm.

Neural Crest Cells

Cells lying between the neural tube and the epidermal ectoderm will form *neural crest cells* due to an intermediate gradient of *FGF* and *BMP-4*. These cells migrate to form many important structures around the body (*listed in the Clinical Significance section of Chapter 4*).



Orange
Epithelial (surface) ectoderm

Green
Cells that will become neural crest cells lying between surface and neural ectoderm

Blue
Neural plate (neural ectoderm) that forms the neural tube

Red
Mesoderm-derived notochord and somites

Figure 12: Closure of the neural tube over the central notochord

Clinical Significance

Neural Crest Cell Derivatives

Commonly assessed in exams are the derivatives of neural crest cells in the foetus. It is important to remember that these are ectoderm-derived structures. A useful mnemonic is MOTEL PASS:

- Melanocytes & Myenteric Plexus
- Odontoblasts
- Tracheal cartilage
- Endocardial cushions & Enterochromaffin cells
- Laryngeal cartilage
- Parafollicular 'C' cells & PNS Post-Ganglionic Neurones
- Adrenal medulla
- Schwann Cells
- Spinal Meninges (pia and arachnoid)

Neural Tube Defects (NTD)

NTDs are types of *spinal dysraphism*: a defect that occurs during formation or closure of the spine, spinal cord, or nerve roots. In its extreme, a total dysraphism of the brain known as *anencephaly* results in a normal spinal cord and absent brain. More typically, however, pathologies occur due to varying degrees of spinal closure – *localised dysraphism*. They can be subcategorised into *open* and *closed* neural tube defects, denoting whether the spinal cord is exposed.

The most common type of NTD is *spina bifida occulta*. This is a closed NTD that occurs in 5-10% of the population where there is a small defect in the spine (e.g. missing vertebral process) that is covered by overlying tissue (*figure 13*). It usually affects the lower spine and people will have no symptoms. It is recognised by a dimple, spot, hairy patch, or swelling in the midline of the back – at the point of the gap.

The other two forms of closed NTD are lipomyelomeningoceles and diastematomyelia. These occur due to a fatty lump or piece of bone in the spinal cord, respectively. The symptoms are associated with the meninges and spinal cord being trapped within these structures. This leads to: changes in sensation, bladder/bowel issues, back pain, or pain on movement. It is diagnosed using an MRI as there may not be a visible skin lesion and clinical assessment is needed. In the infant, it may be recognised by delayed toilet training, turning of the feet (talipes), or an unusual gait.

There are two forms of open NTD: *meningoceles* and *myelomeningoceles*, reflecting the contents of the cavity (*figure 13*). In meningoceles, the opening is slightly larger than in spina bifida occulta and the meninges overlying the spinal cord pass through a space between the vertebrae in a cerebrospinal fluid (CSF)-filled sac. Recall that *-cele* means *cavity* and the *meninges* are the covering layers of the spinal cord. The spinal cord is not in the protruding sac, as such cord development and function are often not affected. In a few patients, the spinal cord may tether to the sac leading to

symptoms associated with the tethered nerve root or below the lesion. Externally, meningoceles are visible as a red or purple sac.

Myelomeningoceles are the most severe form of NTD. *'Myelo'* is a combination of the terms meaning *"marrow"* and *"of the spinal cord"* – reflecting the myelinated tissues. In these, the spinal cord protrudes through the opening and may be accompanied by the meninges. In the uterus, the exposed nerves can be damaged by the amniotic fluid and this, combined with the structural disconfiguration, lead to symptoms in the newborn. Again, the resultant symptoms occurs at the level of or below.the sac. The higher up the lesion, the more significant the neurological deficit.

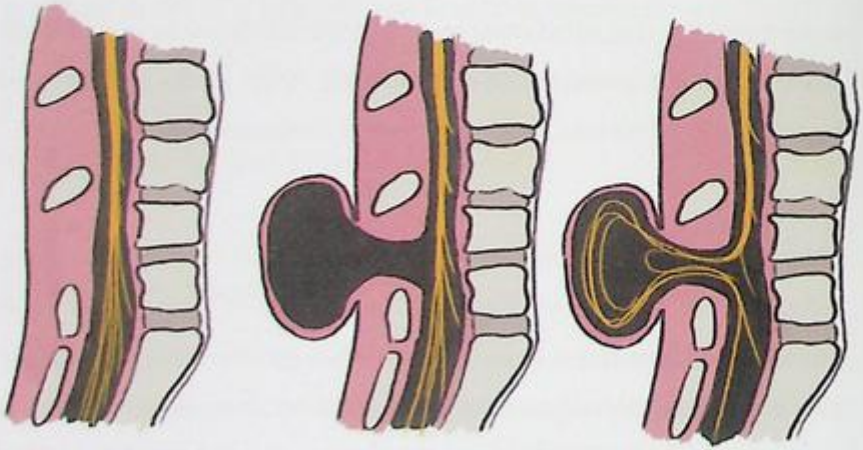


Figure 13: depictions of a neural tube defects. On the left, spina bifida occulta with a missing portion of the vertebrae leaving only soft tissue between the cord and skin. In the middle, a meningocele without the spinal cord. On the right, a myelomeningocele containing the spinal cord

Deficiencies in maternal folate can lead to NTDs. As such, pregnant patients are advised to take a daily (400 microgram) supplement of folic acid. This dose is increased to 5 milligrams if there any siblings are born with NTDs or there is a wider a family history of the condition.

Relevant Molecules

- *N-cadherin*: the homodimeric molecule between neuroectoderm cells
- *E-cadherin*: the homodimeric molecule between the epidermal ectoderm cells
- *FGF*: this inhibits BMP4 to promote the formation of the neural plate
- *BMP4*: this induces the formation of epidermal ectoderm
- *Retinoic acid*: expressed cranially to induce shaping and structures of the neural plate
- *Wnt3a*: expressed caudally to induce shaping and structures of the neural plate
- *Folate*: a maternal deficit in this substance leads to a higher incidence of neural tube defects

KEY POINTS

- The neural tube is derived from the ectoderm
- Neurulation occurs in four stages: formation, shaping, folding, closure
- Neural crest cells form from the ectoderm and form many key structures
- Spina bifida occulta occurs in up to 10% of the population
- Maternal folic acid deficiency leads to an increased incidence of neural tube defects

Craniofacial Development 7

The human face forms between weeks 4 and 12, initially with external structures and then the development of the intricate internal anatomy. In order to grasp the complex embryology occurring, it is useful to separate craniofacial development into distinct parts: external face, skull, palate, tongue, and pharynx. The neural crest cells and pharyngeal arches are central to the creation of many of these structures. The *pharyngeal apparatus* consists of the arches, clefts, and pouches. The pharyngeal arches are paired structures consisting of mesoderm and neural crest cells (*figure 14*). There are 5 arches numbered 1 to 4 and 6 (the 5th exists transiently and disappears). Between each two arches lies a *pouch* inside and a *cleft* outside; this means there are only 4 pharyngeal pouches and clefts. The inner pouch is lined by endoderm (like other internal structures), and the cleft lined by ectoderm (c.f. external surfaces).

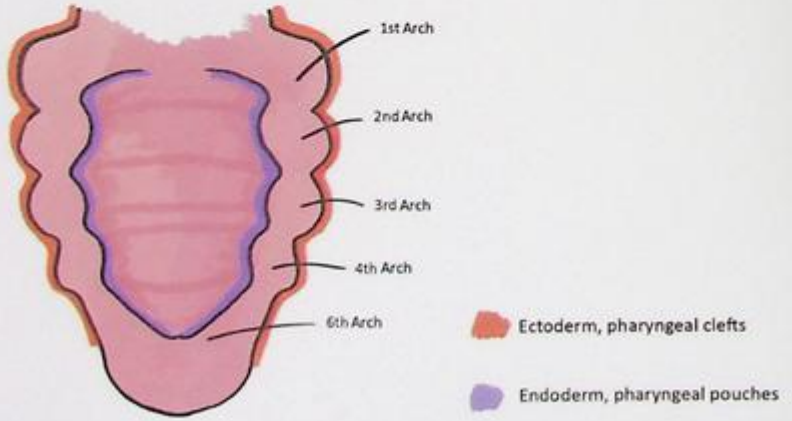


Figure 14: Pharyngeal apparatus consisting of arches, pouches, and clefts

External Face Development

The face is formed from 5 prominences: 1 frontonasal, 2 maxillary, and 2 *mandibular* (*figure 15*). These are mesenchymal proliferations of neural crest cells with the maxillary and mandibular prominences forming from part of the first pharyngeal arch. The space between the maxillary prominences is the *oral opening* and covered by *oropharyngeal membrane* – this is known as the *stomatodeum*. Within the ventrolateral aspect of the frontonasal prominence, two *nasal placodes* invaginate to form *nasal pits* with medial and lateral aspects. The expansion of maxillary prominences centres the nasal pits and creates a *nasolacrimal groove* between the presumptive maxilla and nose. This will obliterate leaving behind the nasolacrimal duct and lacrimal sac.

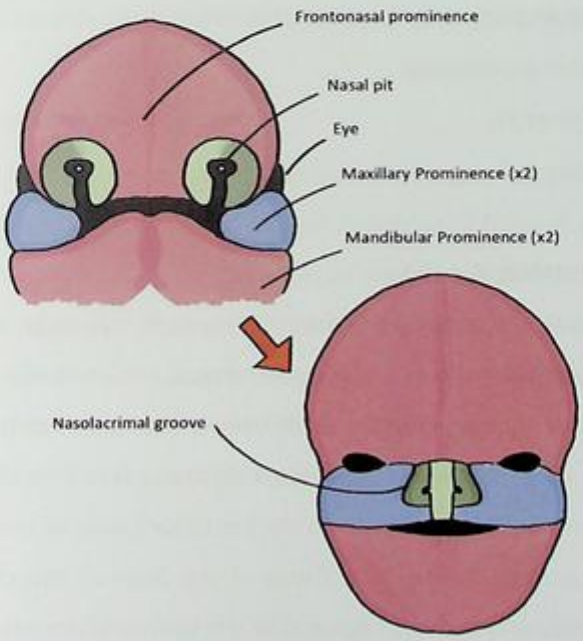


Figure 15: facial prominences and their re-organisation

The maxillary prominences continue grow and bring the medial nasal prominences together to meet in the midline to form the *philtrum* (*figure 15*). This then fuses with the maxillary prominences to complete the upper lip. The final structures of each prominence are listed below:

- Frontonasal
    - Forehead
    - Nasal bridge
    - Midline of nose (medial)
    - Philtrum and part of upper lip
    - Alae of nose (lateral)
- Maxillary prominence
    - Cheeks
    - Lateral upper lip (not the philtrum)
- Mandibular prominence
    - Lower lip
    - Jaw

Skull Development

The germ layer origin of the bones of the skull has been a historically challenging subject. There has been dispute over which bones are ectodermal in origin, and which result from mesodermal cells. It has been stated previously that the somites (paraxial mesoderm) contribute to the occiput. More specifically, they form the posterior bones of the cranial vault (parietal & occipital bones) and those in the *floor* of the cranial fossae (cribriform plate and *petrous* segment of the temporal bones).

The neural crest cells form the anterior bones of the facial skeleton (the *viscerocranium*), this includes: frontal, nasal, lacrimal, maxilla, volar, mandible, sphenoid, and zygomatic bones. They also form internal complex structures, such as: the inferior nasal conchae, ossicles (incus, malleus, stapes) and palantine bone (hard palate); as well as the *squamous* segment of the temporal bone.

The bones of the skull develop by one of two processes. The flatter bones that create the skull vault (*neurocranium*) form through *intraembranous ossification* – where bones develop from sheets of mesechymal connective tissue. Whereas those on the floor with complex shapes (*chondrocranium*) form through *endochondrol ossification* – a process by which hyaline cartilage is gradually replaced by bone (*chondro* meaning cartilage).

Palate Development

The developing palate consists of a small anterior primary palate and bony secondary palate posteriorly (*figure 16*). It begins to form shortly after the face – from weeks 6 to 12. For the anterior segment, a primary palate forms as the result of fusion of the medial nasal prominences and the anterior segment of the maxillary prominence. Then, a secondary palate develops from bilateral extensions of the maxillary prominences, called the *palantine shelves*, that meet in the midline and fuse

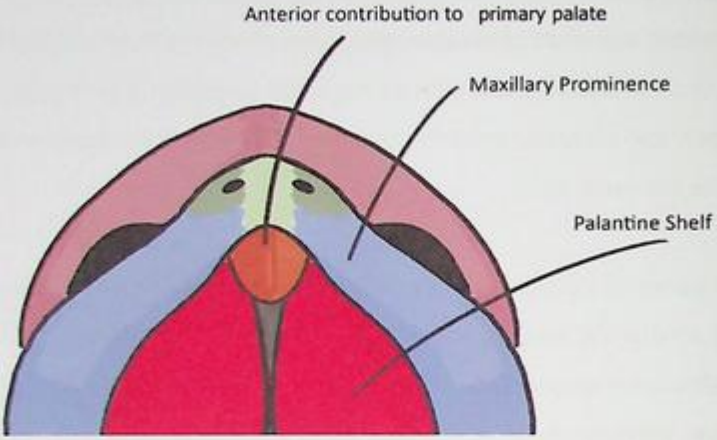


Figure 16: In yellow, the primary palate results from contributions of the maxillary prominences (blue) and nasal prominences (green). In red, the palantine shelves extending from the maxillary prominences to meet in the midline and form the secondary palate

Tongue Development

The anterior two-thirds and posterior one-third of the tongue form from different sources. The anterior segment is derived from the 1st pharyngeal arch, while the posterior third develops from swellings in the 3rd and 4th arches (note: there is *no* contribution from the 2nd arch). The point of fusion between anterior and posterior segments is marked by the *sulcus terminalis*; in the adult, this is bordered anteriorly by the *circumvallate papillae* (large & round taste buds). As a result of their separate development, the anterior two-thirds and posterior third of the tongue have different somatic sensory and special sensory (taste) innervations.

The hypoglossal nerve provides motor function to the entire tongue. However, the anterior tongue receives sensory innervation from the *lingual branch of the trigeminal* nerve and taste from the *chorda tympani branch of the facial nerve*. Meanwhile, the posterior tongue has sensory innervation from the *glossopharyngeal* and *vagal* nerves and taste from the *glossopharyngeal* nerve (with a small contribution from the *vagus*).

The intrinsic and extrinsic muscles of the tongue originate from the paraxial mesoderm that is accompanied by CN XII (hypoglossal nerve).

Pharynx & the Pharyngeal Apparatus

The pharynx is an intricate structure made of multiple parts derived from the parts of the *pharyngeal apparatus* (arch, cleft, and pouch). In embryology, the term '*pharyngeal*' is often interchanged with '*branchial*'. As stated previously, the arches are numbered, and this corresponds roughly the anatomical level of contribution (cranial to caudal). Each arch is associated with a cranial nerve, artery, and cartilage. Clefts are on the outside of the arches, while pouches lie on the inside.

Pharyngeal Clefts

The 4 clefts lie between the 5 pharyngeal arches. The 1st cleft is the only one to form a definitive structure in embryo – the *external auditory meatus*. Recall that the clefts are ectodermal, so it is logical for them to form the *external auditory* meatus. When the 2nd pharyngeal arch expands, it overgrows the 2nd, 3rd and 4th *clefts* – smothering and then oblitering them. If this is not complete, *cervical (branchial) sinuses* remain (note: remember

the cleft lies on the outside surface, so incomplete obliteration would leave a gap) – this is discussed in the *Clinical Significance* section later.

Pharyngeal Pouches

The pouches are endodermal in origin, and all four form internal structures. Similar to the 1st cleft, the 1st pouch will form the *eustachian tube* and *middle ear cavity*. The 2nd pouch forms the *palantine tonsils* (anatomically inferior to the eustachian tube). The 3rd pouch forms the *inferior parathyroid glands* and the *thymus*. The 4th pouch will form the *superior parathyroid glands* and the *parafollicular (C) cells of the thyroid*. The 3rd and 4th pouches are commonly assessed in exams as candidates often mistake their inferior/superior titles.

Pharyngeal Arches

There is no simple way to recall the long list of structures derived from each pharyngeal arch. It is helpful to recognise patterns and the progressive anatomy in cranio-caudal sequences to try and form some order. Each pharyngeal arch will generate arteries, muscles, bone and/or cartilage.

The nerves in the 5 arches (in ascending order) are CN V (1st), VII (2nd), IX (3rd), superior laryngeal branch of X (4th) and recurrent laryngeal of X (6th). The arteries are the maxillary (1st), stapedial (2nd), common & internal carotids (3rd), subclavian & arch of the aorta (4th), and the ductus arteriosus & pulmonary arteries (6th). If you are able to recall the function of the cranial nerves then it may make the structures easier to remember – particularly

when remembering that the facial nerve supplies the muscles of facial expression.

The 1st pharyngeal arch is the most cranial and is known as the *mandibular* arch. It consists of the maxillary and mandibular prominences and forms:

- Artery: maxillary
- Nerve: CN V (trigeminal)
- Muscular: muscles of mastication, mylohyoid, anterior belly of digastric, tensor veli palatini, tensor tympani
- Bone: maxilla, zygomatic, squamous temporal, palatine, vomer, mandible, incus, malleus
- Cartilage: Meckel's

Recall that the 1st cleft and pouch form other auditory structures – which aligns with the ossicles and muscles listed here. Also note how these are muscles which attach to the bones that they accompany (e.g. mylohyoid to the mandible). The *tensor veli* is the only muscle of the soft palate to be made by this arch (the rest from the 4th), so this is a regularly assessed fact. The *Meckel's cartilage* forms as a product of this arch, and is the precursor for endochondrol ossification for the incus and malleus of the middle ear.

The 2nd pharyngeal arch is known as the hyoid arch. Its derivatives are:

- Artery: stapedial (to supply the muscles of the sole ossicle in this arch)
- Nerve: CN VII (facial)
- Muscular: muscles of facial expression, posterior belly of digastric, stylohyoid muscle, stapedius

- Bone: upper half of hyoid body, lesser horn of hyoid, styloid process, stapes
- Cartilage: nil

Although it does not give rise to any specific cartilaginous structures, you may see the 2nd arch being referred to as *Reichart's cartilage*, this is the precursor to the bony structures that form by endochondral ossification.

The 3rd arch is unnamed and forms fewer musculoskeletal structures:

- Artery: common carotid & proximal internal carotid
- Nerve: CN IX (glossopharyngeal)
- Muscle: stylopharyngeus
- Bone: greater horn and lower half of the body of hyoid
- Cartilage: nil

Since it only forms a single muscle, and the stylopharyngeus is the only muscle of the pharynx not formed by the 4th arch. This is commonly assessed in multiple-choice question papers.

The 4th pharyngeal arch contributes significantly to the muscles and cartilage of the pharynx . It is also the first arch where there is an asymmetry between the products of the two sides. It forms:

- Artery: proximal subclavian (right) and arch of aorta (left)
- Nerve: CN X (superior laryngeal branch of the vagus)
- Muscle: muscles of the soft palate [except tensor veli] (levator veli palatini, palatopharyngeus, palatoglossus, musculus uvulae) and

muscles of the pharynx [except stylopharyngeus] (superior, middle, & inferior constrictors, and the salpingopharyngeus), cricothyroid, cricopharyngeus

- Bone: nil
- Cartilage: thyroid, cricoid, arytenoid, corniculate, cuneiform

Examiners often ask about the cricothyroid as it is the only intrinsic laryngeal muscle not generated from the 6th arch and, as a result, is innervated by the superior laryngeal nerve (rather than the recurrent laryngeal). It is also commonly assessed because it is the only tensor of the vocal cords – and this has significant clinical consequences (discussed later).

The 6th arch extends most inferiorly and the anatomy of its products make them easier to recollect:

- Artery: ductus arteriosus and pulmonary
- Nerve: CN X (recurrent laryngeal branch of the vagus)
- Muscle: intrinsic laryngeal muscles [except cricothyroid] and skeletal muscle of the (upper) oesophagus
- Bone: nil
- Cartilage: thyroid, cricoid, arytenoid, corniculate, cuniform

It is important to note that, while the 4th and 6th arches form different vascular and muscular structures, they both contribute towards the laryngeal cartilages. Recall that the recurrent laryngeal nerve loops under the ductus arteriosus on the left to rise cranially again to supply the intrinic laryngeal muscles.

The only muscles to not form from the pharyngeal arches or somites are the trapezius and sternocleidomastoid muscles, which are products of the lateral plate mesoderm that lies beside the first four somites. This mesoderm is accompanied by CN XI (accessory nerve).

Eye Development

The eye forms from ectodermal and mesodermal sources. The outer surface of the eye is formed by ectoderm – specifically the lens, corneal epithelium, and eyelid. Neural crest cells form the sclera, corneal endothelium, corneal basement membrane, and chondrocartilaginous components of the orbit. Somitic paraxial mesoderm contributes to the sclera, stroma, choroid, corneal endothelium, intra- & extra-ocular muscles, vessels and the vitreous. The retina and optic nerves (and epithelial lining of the ciliary body & iris) are formed from neuroectoderm in a process that follows closure of the neural tube.

As the neural tube closes, outpockets of neuroectoderm – the *optic vesicles* – grow cranially to induce *lens placodes* in the surface ectoderm. These placodes develop first into pits and then vesicles (note how similar this process is to formation of the nose). Meanwhile, the *optic vesicle* develops into the *optic cup* – the precursor for the definitive globe.

The development of the eye is dependent on the *PAX6* gene which modifies the tissue's responsiveness to nearby morphogens *BMP4* and Sonic Hedgehog (*Shh*). *Shh* suppresses *PAX6* to upregulate *PAX2* to divide the eye field in the neural plate into two; whereas *BMP4* works with locally

upregulated *FGF* to form the optic vesicle & cup (to form the retina), and the lens placode and vesicle.

Clinical Significance

Cleft Lip & Palate

Where the facial prominences do not completely align, a gap is the left between them – known as a *cleft*. This can occur in the lip, palate, or a combination of both. Recalling that there are multiple prominences that meet to form facial structures, the cleft lip pathologies are as below:

- Median cleft lip: incomplete fusion of nasal prominences in midline, leaving a cleft in the midline of the philtrum
- Oblique facial cleft: failure of the maxillary and nasal prominences to fuse – typically unilaterally, creating a cleft from the philtrum to the medial canthus of the eye (site of nasolacrimal system)
- Hare lip: failure of maxillary and medial nasal prominences to fuse bilaterally, leaving clefts at both lateral edges of the philtrum that meet the nostrils superiorly
- Frontonasal dysplasia: hyperplasia of the frontonasal prominence that leads to the widening of the gap between nasal prominences. This leads to incomplete fusion, a flattening of the nasal bridge and displacement of the tip of the nose

Incomplete fusion of the maxillary extensions in the secondary palate generates a cleft palate. Since the formation of the primary palate involves the maxillary and nasal prominences, then incomplete fusion of the palate often extends into the lip also – hence the pathologies often being co-

existent. An untreated cleft palate can lead to various issues. The baby uses its palate for sucking milk, by placing the bottle or nipple against the roof of its mouth. If it cannot latch on to drink milk, it may develop severe nutritional deficit as a result of being unable to feed. Sometimes the malnourishment is the sign that leads to a diagnosis of a cleft palate.

Clefts of the lip and palate can also hinder children development. Palatal clefts can lead to speech impediments that may hinder speech development. Meanwhile, the atypical anatomy can lead to an increased risk of recurrent sinus and ear infections that may affect hearing – resulting in speech and interaction milestone delays.

Branchial Cysts & Sinuses (Pharyngeal Cleft Pathology)

Failure of complete overgrowth and obliteration of the 2nd to 4th clefts by the 2nd arch leaves a space that these arches were occupying. This becomes a branchial *cyst* if it is sealed from the external surface and a *sinus* if there is a tract to the skin. These cysts/sinuses can grow large (compressing surrounding structures) or become infected – both indications for potential surgical excision. They can occur anywhere along the anterior border of the sternocleidomastoid (in the anterior triangle of the neck; SCM). The location of the cyst/sinus along the SCM depends on which cleft has not been obliterated, such that 2nd cleft cysts will be located more superiorly (typically inferoposterior to the mandible), 3rd cleft cysts are posterolateral to the laryngeal cartilage, and 4th in the thyroid region. The 3rd and 4th cleft

cysts can have an internal openings, which make them prone to recurrent infections.

Thyroids, Parathyroids & Thymus (Pharnygeal Pouch Pathology)

Most commonly, absence or anomalies in the derivatives of the 3rd and 4th pharyngeal pouches are noticed clinically: during head & neck operations, endocrine disorders (of calcium), or endocrine surgery. These disorders manifest as absences or dysplasia in the parathyroid, thymic, and parafollicular tissues.

Even under normal circumstances, the parathyroid glands have the most variable anatomy of all structures in the body, which is a challenge for endocrine surgeons. When the parathyroids form with the thymus (3rd pouch) and thyroid gland (4th pouch), they join them as they migrate from the neck into their final positions inferiorly. This leaves the 4th pouch parathyroids superiorly in position – typically posterior to the middle of each thyroid lobe. The 3rd pouch inferior parathyroids often deposit just below these; however, they can continue migrating anywhere between the thyroid in the neck and the thymus behind the sternum – so their position is very variable between individuals. The location of the parathyroids near the thyroid puts them at risk during thyroidectomies, as such it is routine post-operatively to check serum calcium and parathyroid levels to ensure the patient does not develop severe hypocalcaemia.

Di George Syndrome (3rd and 4th Pharyngeal Arches)

Also known as *velocardiofacial syndrome*, this condition is commonly assessed in examinations as its pathology involves multiple systems. It occurs due to a deletion in the long arm of chromosome 22 (*22q11*). This results in a hypoplasia of the 3rd and 4th pharyngeal arches and pouches leading to:

- Hypoplasia of the thyroid
- Thymic hypoplasia
- Hypoparathyroidism
- Heart outflow tract disorders (neural crest cells from this region contribute to conotruncal cushions of the heart)

This hypoplasia also impacts the 1st and 2nd arches leading to micrognanthia, sensoneural hearing loss (dysplastic ossicles), conductive hearing loss (recurrent infections), and cleft palate. For examinations, the mnemonic *CATCH-22* is a useful aide-mémoire: cardiac anomalies, abnormal facies, thymic hypoplasia, cleft palate, and hypocalcaemia.

Laryngeal Nerve Palsy

The 4th arch generates both the superior laryngeal nerve and the thyroid gland. In thyroidectomies, this nerve is at risk of damage. The external branch of the superior laryngeal provides motor function to the cricothyroid, while the internal branch provides sensation to the mucous membrane of the larynx. A unilateral external superior laryngeal nerve palsy

is associated with changes in pitch of the voice, while a bilateral palsy leads to hoarseness. This is in contrast to the recurrent laryngeal nerve whose unilateral palsy causes hoarseness, and bilateral palsy can cause fatal dyspnea if the cords lie in the abducted position and block the airway.

Relevant Molecules

- *PAX6 gene*: Responsible for initiating and regulating the complex development of the eye
- *BMP4*: promoted by PAX6 to upregulate FGF and develop the optic cup and lens of the eye
- *FGF*: works with BMP4 to locally induce cells to differentiate into optic structures
- *Shh*: promoted by PAX6, this then inhibits PAX6 to upregulate expression of PAX2 to divide the optic field into two (and avoid cyclopia)

KEY POINTS

- Pharyngeal arches are mesoderm
- Branchial and pharyngeal are interchangeable terms
- Clefts are on the outside surface of the pharyngeal apparatus and ectodermal in origin (cleft contains the letters 'ect')
- Pouches are on the inside of the apparatus and formed from endoderm
- The somites form the flat bones of the skull vault (neurocranium) through membranous ossification
- Endochondral ossification is used to form bones with more complex shapes
- The tongue develops in two parts with the anterior two-thirds and posterior third having different vasculature and innervation
- Only the 1st pharyngeal cleft forms a structure – the external auditory meatus
- The 3rd pharyngeal pouch forms the inferior parathyroid glands, while the 4th pouch forms the superior parathyroids
- There is no 5th pharyngeal arch in humans
- The stylopharyngeus is formed from the 3rd pharyngeal arch and is the only muscle of the pharynx not to be formed by the 4th arch
- The cricothyroid is the only intrinsic laryngeal muscle to be formed by the 4th arch (all others are formed from the 6th arch) and, therefore, has a different innervation to the other muscles
- The retina is formed from neuroectoderm

Heart & Vessel Development 8

The heart is the earliest functional organ to develop in the embryo, and the heartbeat can be detected in early booking or viability scans for pregnancy from as soon as six weeks. This milestone is occasionally used in arguments surrounding personhood of the foetus and has formed part of many controversial policies surrounding abortion. The heart begins pumping as a tube from day 22, and its development involves a significant remodelling to transform to a multi-chamber highly specialised organ. The process of heart development is highly reliant on 3 genes working synergistically: *NKX2.5*, *GATA4*, & *TBX5*.

Heart Tube

The early heart is tubular in shape. Its endocardium and myocardium develop from a *cardiogenic field* in the cranial third of mesoderm. Initially, a *primary heart field* forms and coalesces by the third week to create a *cardiac crescent* of progenitor cells. The epicardium (that also forms the coronary vessels) originates from a separate source of mesenchymal cells at a later stage in heart development. Lateral folding of the embryonic disk brings the sides of the cardiac crescent together to form a tube (*figure 17*), while cranio-caudal and ventral folding bring the heart precursor tissue into the centre of the chest.

At this point, the heart is suspended from the body wall by the *dorsal mesocardium*, which later obliterates to form the *transverse pericardial sinus*. In the developed heart, this lies posterior to the ascending aorta and pulmonary trunk, and anterior to the superior vena cava – separating the arterial and venous outflows of the heart. As such, it is used in cardiothoracic surgery to identify the vessels for temporary clamping.

The linear heart tube that results from folding has a caudal inflow end and a cranial outflow end. It consists of an endothelium surrounded by a contractile myocardium (*figure 17*). This tube will elongate in order to prepare for folding.

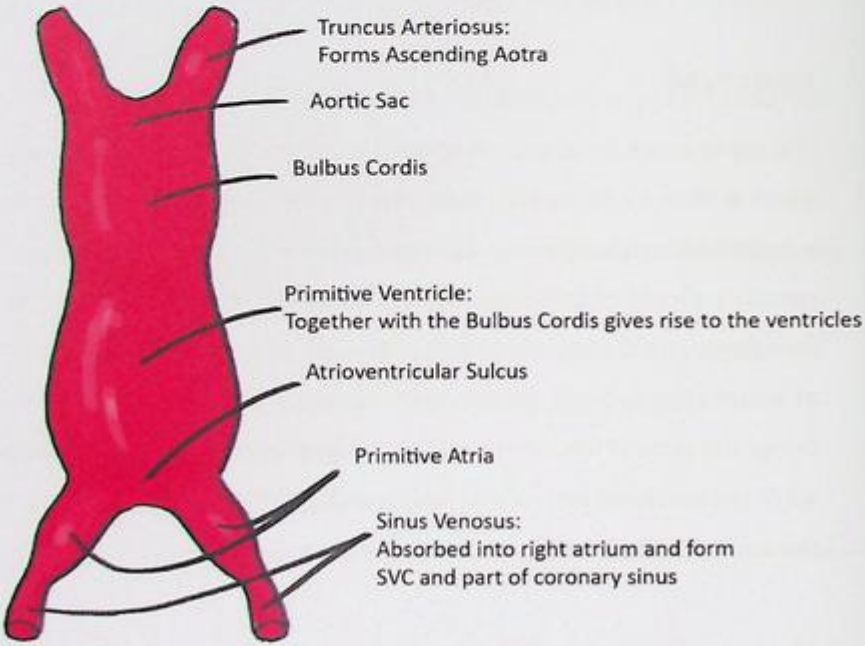


Figure 17: the linear heart tube and final products

Heart Folding

The looping of the heart determines the definitive positions of the chambers. It first lengthens and folds into a C-shape (with the right side as the outside curvature), before looping further into an S-shape (*figure 18*). In order to elongate, cardiac progenitor cells are recruited from a *secondary heart field* and added to the arterial and venous poles, this process displaces the structures as follows:

- Bulbus cordis inferiorly, ventrally and to the right – this moves the presumptive right ventricle anteriorly in the chest
- Primitive ventricle to the left – positioning the presumptive left ventricle
- Primitive atrium posteriorly and superiorly – placing the atria superior to the presumptive ventricles

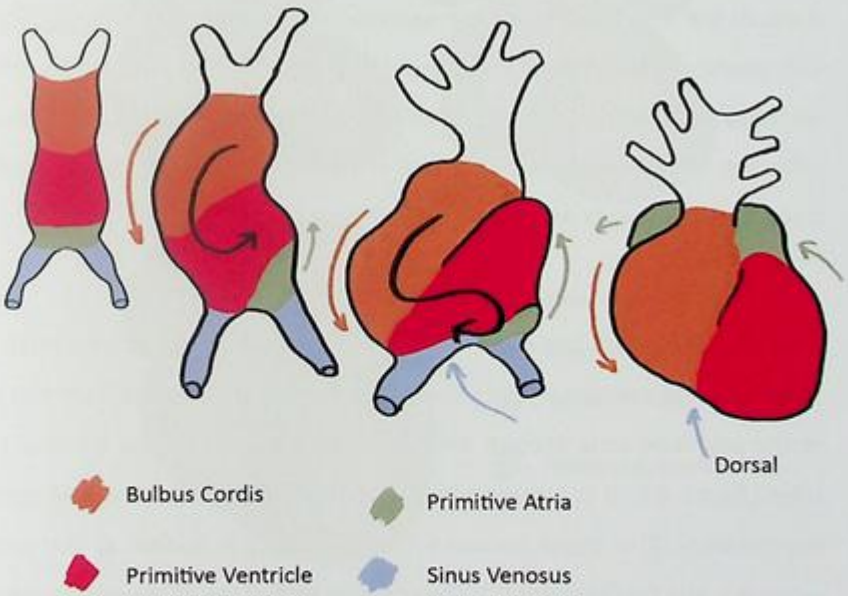


Figure 18: the linear heart tube folding into a 'C' and then 'S'

The primitive atria contribute little to the definitive atria and are incorporated into nearby structures. They remain as the *auricles* of the atria. The right atrium forms as a product of *right sinus horn*, while the left is produced by the *pulmonary outflow vessels*. The right ventricle is predominantly formed from the *bulbis cordis*, this structure also contributes to the outflow tract of the left ventricle. The bulk of the left ventricle is formed from the *primitive ventricle*.

Atrial Septation

Septations divide the folded heart tube into four chambers. Firstly, the atria and ventricles are separated by *endocardial cushions* growing inwards from the endocardial walls. These cushions are located dorsally & ventrally, and meet in the midline to form the *septum intermedium*. Their development and position is dependent on *retinoic acid* signally. This causes the cells to undergo *epithelial to mesenchymal transformation* to form tissue that is different to the endocardium and myocardium. This process is often disrupted in patients with *Down's Syndrome*.

Now that the heart has been divided into two to separate into atria and ventricles, the *septum primum* (Latin for "first fence") grows from the roof of the primitive atria around day 28 to separate them into left and right sides (*figure 19*). It is a membranous septum that grows towards *septum intermedium*. The space between the two septa is known as the *ostium primum* (Latin for "first door"). Towards the end of the 6th week, the *septum primum* fuses with the *septum intermedium* to obliterate the *ostium*

primum. Simultaneously, *apoptosis* occurs at the superior margin of the *septum primum* to create a new gap – the *ostium secundum* ("second door"). At its completion, the *septum secundum* ("second fence") develops from the roof of the atrium on the right side of the *septum primum* and *ostium secundum*. In contrast to the *septum primum*, the secundum is thick and muscular, but it does not reach the *septum intermedium*. As a result, the *foramen ovale* is produced in the communication between the right and left atria through the *ostium secundum*.

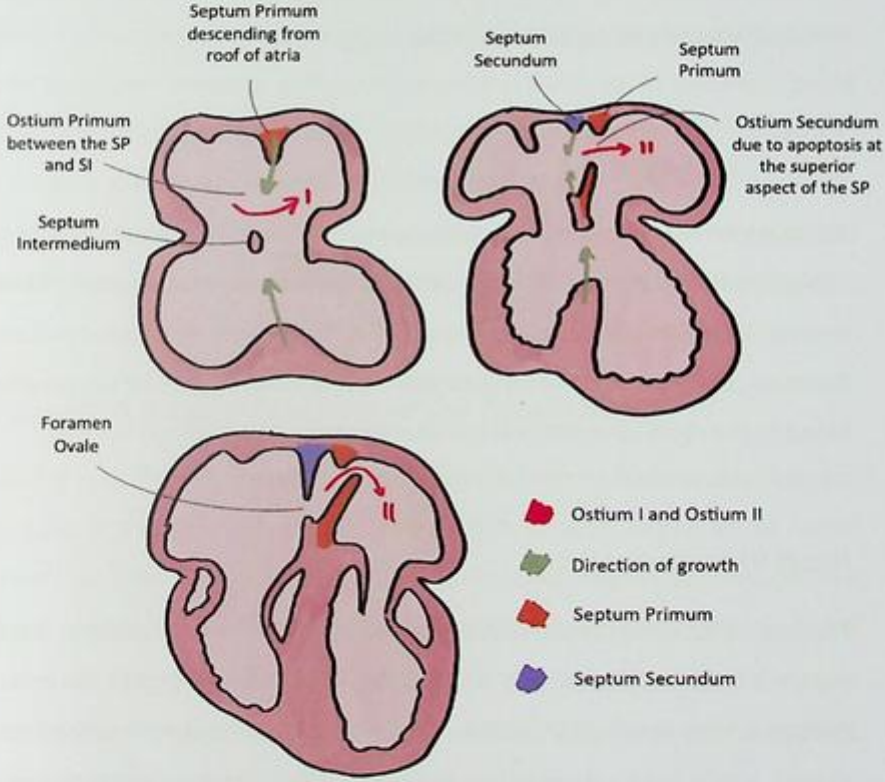


Figure 19: septation of the heart

It is important to recognise that the foetal blood pressure in the right atrium is higher than in the left atrium. This is a because it receives both oxygenated blood from the placenta and the deoxygenated blood from the body, meanwhile the left atrium receives little blood from the compressed (uninflated) foetal lungs. The blood flows down the pressure gradient from the right atrium to the left through the *foramen ovale*; this also prevents the *septum primum* and *secundum* from fusing as the force of flow pushes away the membranous *septum primum*. This right-to-left shunt is important in the embryo as it transfers oxygenated blood to the systemic side of the circulation for pumping to perfuse the body.

Ventricular Septation

An interventricular muscular septum develops from the caudal aspect of the ventricle around the 4th week of development. This grows towards the *septum intermedium* but does not meet it. This leaves an *interventricular foramen* such that both ventricles share an outflow tract and oxygenated blood in the right ventricle reaches the systemic (left-side) circulation.

Heart Valve Formation

There are four valves in the heart. The mitral (left) & tricuspid (right) divide the atria from ventricles, and aortic (left) & pulmonary (right) valves are located at the ventricular outflow tracts. Their development differs very slightly. While both sets originate from *endocardial cushions* (cardiac cells that have undergone epithelial-to-mesenchymal transformation), the

outflow valves require the additional ingression of *neural crest cells* into the cushions to form. The overall process is regulated by the receptor *Notch1* (and its ligand *Jagged1*).

Reversal of Heart Flow & Closure of the Foramen Ovale

The right-to-left shunting of the foetal circulatory system persists until birth in order to ensure that the systemic circulation receives maternal oxygenated blood. As the baby takes its first breath, expansion of the alveoli leads to dilatation of the pulmonary capillaries that lie on their surface. This decreases the pressure within the alveolar capillaries to generate blood flow through the lungs. This returns to the heart via the left atrium and increases the blood pressure of the left-side of the heart. As a result, the raised pressure in the left atrium pushes the septum primum away and closes the foramen ovale – although it can persist in <25% of individuals.

Formation & Spiral Septation of the Great Vessels

During the 5th week of development, septation of the outflow tract leads to closure of the interventricular foramen. This process begins when *neural crest cells* from the 4th and 6th pharyngeal arches migrate into the outflow tract (*truncus arteriosus*). They undergo *epithelial-to-mesenchymal* transformation to form two *truncus cushions*. These proliferate to meet in the midline and then grow to meet the interventricular septum – forming the membranous portion of this septum.

As these cushions meet in the midline, they spiral to separate the outflow tract into the aortic and pulmonary aspects. The pharyngeal arches will then contribute to the arterial vasculature (as discussed in *Chapter 7*) through aortic arches (*figure 20*). These arches will combine with a primitive *dorsal aorta* that has formed in a pair from the lateral plate mesoderm. The combined structures join in the midline to create the descending aorta. The *arch of the aorta* (not aortic arches) is generated from the heart tube, pharyngeal aortic arches, and dorsal aorta in the following segments:

- Initial: from the truncus arteriosus of the heart tube
- Ascending aorta: the aortic sac (the distal part of the truncus arteriosus)
- Transverse component: from the left 4th pharyngeal aortic arch
- Descending aorta: dorsal aorta (mainly left component)

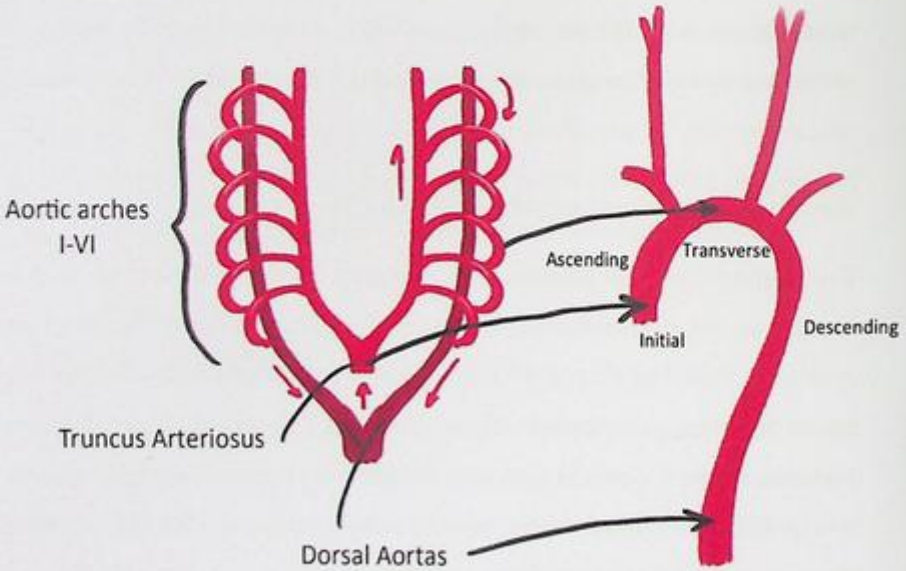


Figure 20: the primitive aortic structures and their corresponding part

When considering the *pharyngeal aortic arches* and their derivatives, they are as following:

- 1st aortic arch: maxillary artery
- 2nd: stapedial artery
- 3rd: common carotid and proximal internal carotid
- 4th (left): transverse component of anatomic arch of the aorta
- 4th (right): proximal right subclavian artery
- 6th (left): let pulmonary artery and the *ductus arteriosus*
- 6th (right): right pulmonary artery

The *ductus arteriosus* connects the trunk of pulmonary artery to the proximal descending aorta. This is to enhance the right-to-left shunt of the blood flow in the embryo and increase the oxygenated blood in the systemic circulation. When the foetus is born, the levels of placental *prostaglandin E2* drop and *bradykinin* is released from the ventilated lungs. This change closes the *ductus arteriosus* and forms the *ligamentum arteriosum* (that the left recurrent laryngeal nerve loops around).

Development of the Venous System

The Inferior Vena Cava (IVC) develops through extensive remodelling of the venous system. Initially, the vitelline veins from the yolk sac and the umbilical veins from the placenta are paired structures connected through extensive plexuses connecting their right and left parts. They work in a system with the cardinal veins – the early venous system of the 'body' of

the embryo. The *sinus venosus* (the inflow aspect of the heart tube) receives blood from the vitelline vein, umbilical vein, and the common cardinal vein.

As the liver develops and encroaches the vitelline vessels, the left vitelline system becomes the portal vein and the right vitelline becomes the suprahepatic portion of the IVC. An anastomosis forms between the left umbilical and vitelline veins called the *ductus venosus*. This allows oxygenated and nutrient-rich umbilical blood to bypass the liver (and first-pass metabolism) and directly enter the IVC to be delivered to the heart. This remains as the *ligamentum venosum* of the liver (that separates the caudate lobe from the left lobe).

In the embryo, the early venous system is named according to location relative to the heart with the *anterior cardinal (pre-cardinal) veins* draining the head, neck, upper torso, and upper limbs, while the *posterior cardinal (post-cardinal) veins* draining the body and lower limbs. As the embryo grows in size and the organs develop, the *posterior cardinal* veins are joined by the *subcardinal* and *supracardinal* veins. The anterior and posterior systems undergo significant remodelling to form veins draining into the superior vena cava and inferior vena cava, respectively.

Clinical Significance

Congenitial cardiovascular malformations occur in ~1% of all live births and account for ~20% of all congenital deformities.

Cyanotic Babies & Duct-Dependent Circulations

A central concept to understanding the clinical embryology of the heart is why cyanosis occurs following specific heart malformations. Central cyanosis occurs when the level of deoxygenated haemoglobin is above 5g/dL (with oxygen saturations below 85%). In the newborn, it is important to determine the timing and symptoms of the "blue baby", as the diagnosis varies on time since (*figure 21*). In blue babies, if the cyanosis worsens when it cries then the likely cause is a heart defect due to the raised blood pressure. If the cyanosis improves, the cause is likely respiratory and responding to the increased lung capacity & oxygenation.

If the baby is born pathologically cyanotic (i.e. persistently blue after the initial 5 to 10 minutes), then this means that the there is difficulty in oxygenating blood. The neonate may have a "right-to-left" shunt where deoxygenated blood bypasses the lungs and enters the systemic circulation through a shunt in the heart. The two most commonly assessed causes of this are *Tetralogy of Fallot* and *Transposition of the Great Arteries* (*figure 21*). However, it also occurs with *Total Anomalous Pulmonary Venous Return (TAPVR), tricuspid atresia, truncus arteriosus* and *hypoplastic left heart syndrome*; sometimes referred to as the "Five T's (& One H)".

If the baby is born normal colour in appearance and then becomes cyanotic in the first few days of life, this reflects a progressively cyanotic and *duct-dependent circulation*. This is a circulation that depends on the shunt of the *ductus arteriosus* between the pulmonary artery and the aorta to pump oxygenated blood systemically. The closure of the *ductus arteriosus* in these will reveal the life-threatening pathology, either: blood is unable to be oxygenated via the lungs (e.g. pulmonary atresia), or oxygenated blood is not reaching the systemic circulation (e.g. critical aortic stenosis). These newborns require medicating with Prostaglandin E1/2 to keep the duct open (recall that its levels decline with the removal of the placenta) while definitive surgical management is prepared.

Where the newborn is able to oxygenate the blood but there is mixing of arterial and venous blood (common mixing), then it will present with symptoms of severe anaemia – peripheral cyanosis and breathlessness. This most commonly occurs in atrio-ventricular septal defects. If the baby has signs of haemodynamic shock (e.g. low blood pressure), then one has to consider the function of the left side of the heart and its outflow. It may be that the pumping mechanism of the heart is ineffective and unable to generate sufficient pressure to perfuse the organs. For these patients, the differential includes conditions such as coarctation of the aorta or an interrupted aortic arch.

Type of Lesion	*Left-to-Right Shunt*	*Right-to-Left Shunt*	*Common Mixing*	*Well Children with Obstruction*	*Sick Neonates with Obstruction*
Symptoms	Breathless or asymptomatic	Blue	Breathless and Blue	Asymptomatic	Collapsed with shock
Causes	ASD VSD, PDA	ToF TGA	AVSD	AS PS, CoA	CoA HLHS

ASD - atrial septal defect; VSD - ventricular septal defect; PDA - patent ducts arteriosus; ToF – tetralogy of Fallot; TGA – transposition of the great arteries; AVSD - atrioventricular septal defect; AS - aortic stenosis; PS - pulmonary stenosis; CoA - Coarctation of the Aorta; HLHS - hypoplastic left heart syndrome.

Figure 21: presentation of heart defects and possible underlying causes (add extra column for duct-dependent circulations)

Septal Defects

These occur on a very broad clinical spectrum from asymptomatic to life-threatening. They can be auscultated as murmurs of the heart as they lead to turbulent flow; however, it is important to remember that a larger defect may have a quieter or absent murmur as blood easily passes through through the defect. Due to the higher pressures in the left-side of the heart, these typically cause a left-to-right shunt where oxygenated blood re-enters the pulmonary circulation. If the defect is sufficiently large, then this

can lead to signs of breathlessness – either due to insufficient oxygen delivery or an overloaded pulmonary system (pulmonary hypertension). Some patients may present in adulthood with signs of heart failure due to pathological cardiomegaly from the persistently elevated pressures.

Septal defects occur due to inadequate growth or closure of the septal processes in heart development. They often form part of a greater heart abnormality, clinical syndrome, or genetic mutation. Around 5-10% of all babies with congenital heart defects have some form of atrial septal defect (ASD), and ~33% of all congenital heart disease is due to a ventricular septal defect (VSD) – approximately 3 in every 1000 live births. For the ventricles, it is typically the membranous component of the septum that is affected.

Rarely, these may progress to *Eisenmenger's Syndrome* where the patient presents with cyanosis and dyspnoea several years later (typically aged 10 to 15). This happens because the higher pressures in left ventricle (or atrium) shunt blood across into the right side of the heart and cause pulmonary hypertension. The increased pressure causes hypertrophy of the pulmonary vessels and reduces their compliance, which in turn causes pathological hypertrophy of the muscles of the right-side of the heart. When the pressure in the right chambers rises sufficiently, the reverses and blood passes from the right to the left. This leads to deoxygenated blood entering the systemic circulation and the patient becomes cyanotic as a result.

Transposition of the Great Arteries & Outflow Tract Pathologies

These occur when there is an issue with the septation, positioning, or development of the great vessels of the heart. Most concerning is *transposition of the great arteries* where the aorta is connected to the right ventricle and the pulmonary artery to the left ventricle. If this occurs in isolation, then the baby is cyanotic at birth. However, if it exists in conjunction with another defect (e.g. septal defect) then admixture of the blood can occur – allowing some oxygenated blood to reach the systemic circulation (although the baby may remain cyanotic). Eventually all patients require surgery to correct the vessels. In ~20% of infants, an *atrial septostomy* is performed as a bridging measure pending further surgery. This involves passing a baloon catheter through the venous system and through the foramen ovale; the balloon is inflated in the left atrium and pulled through the atrial septum – to dilate the foramen ovale and tear the septum to ensure it does not close.

The other group of conditions are *outflow obstructions*. In the well child, these could be: mild coarctation of the aorta, aortic stenosis, or pulmonary stenosis. In the unwell infant, one should consider: severe coarctation of the aorta, hypoplastic left heart syndrome, and aortic arch interruptions.

Coarctation of the aorta occurs accounts for ~5% of congenital heart defects (~3 in 10,000 live births) and involves narrowing of the lumen of the aorta. It is classified according to the location of this narrowing relative to the *ductus arteriosus*: pre-ductal, ductal, and post-ductal. In severe pre-

ductal constrictions, the circulation is duct-dependent as no blood can pass through the aorta prior to the duct and the circulation is dependent on blood bypassing the obstruction through the *ductus arteriosus*. This condition is usually managed with surgery.

Interruption of the aortic arch occurs when the proximal and distal segments of the aortic arch are not connected (happens in ~1 in 10,000 live births). This is most likely due to malformations with the *pharyngeal aortic arches* – particularly the 4^{th} – that contribute to the transverse segment of the arch of the aorta. The condition is classified according to the location of interruption relative to the three arteries that branch off the arch: distal to left subclavian (type A), distal to left common carotid (type B), and distal to the braciocephalic (type C).

Mild to moderate stenosis of the aortic or pulmonary vessels will typically be asymptomatic and recognisable by a murmur on examination (ejection systolic murmurs). When severe, they may lead to cardiomegaly and heart failure; their treatment is with valve replacement.

Tetralogy of Fallot

This condition presents with cyanosis either at birth or shortly thereafter. It is a right-to-left shunt that is very frequently assessed in exams. It is the most common cyanotic congenital heart malformation (occuring in 1/1000 live births). It has four key features: over-riding aorta (that receives blood

from both ventricles), large ventricular septal defect, pulmonary stenosis (causing right outflow obstruction), and right ventricular hypertrophy (as result of the stenosis). Babies that do not present with cyanosis in the first few weeks of life, may be diagnosed in infancy with hypercyanotic spells and squatting on exercise. These spells are sudden episodes of profound cyanosis (occasionally with shock) due to hypoxia, and they typically occur during crying, defecating, or playing. The infant may squat as a compensatory mechanism to increase the peripheral vascular resistance in order to decrease the right-to-left shunt (by increasing the pressure in the left-side of the system and reducing the pressure gradient).

The management depends on the urgency of the presentation. The definitive intervention is surgery at ~6 months to correct the defects. If the baby is cyanotic in the neonatal period then an interim procedure is needed, either: the right ventricular outflow tract can be dilated, or a shunt can be placed between the subclavian artery and the pulmonary artery – known as a *Blalock-Taussing* shunt. The hypercyanotic spells are normally self-limiting but require medical management to correct the shock and metabolic consequences.

Tetralogy of Fallot can occur as part of *Alagille Syndrome* where a mutation in the *Jagged1* ligand leads to cardiovascular and hepatic malformations.

Patent Ductus Arteriosus

Approximately 10% of congenital heart disease is due to a patent ductus arteriosus (PDA). Except in the duct-dependent circulations, it is important to close the defect. The reason for this is two-fold. First, persistent shunting of blood from the aorta to the pulmonary artery can lead to pulmonary hypertension. Second, there is a continuous increased work for the left-side of the heart to pump sufficient blood through the aorta to compensate for losses through the PDA. As such, left untreated, one-third of patients die of heart complications by 40, and two-thirds by 60.

If recognised early by signs of tachycardia, shortness of breath, or a continuous "*machine-like murmur*", then prostaglandin levels can be reduced through use of non-steroidal anti-inflammatory drugs (COX inhibitors) to speed up the duct-closing process. Otherwise, patients require an operation to close the duct.

Blood Mixing

This is the result of arterial and venous blood mixing prior to expulsion via the outflow tract. It occurs in tricuspid atresia and severe atrioventricular septal defects (AVSD). The newborn is cyanotic with shortness of breath.

AVSD occurs in up to 10% of patients with Down's Syndrome. Its management, like septal defects, is dependent on its severity and is focused on interim measures prior to definitive surgical intervention at 3-6 months.

Tricuspid atresia (TA) is an absence of the tricuspid valve leading to a severely hypoplastic or absent right ventricle. The exact pathophysiology is unknown; however, blood flow through the linear tube is known to regulate the development and folding of the heart – and this defecit would severely affect this process. It order for viability of the embryo, TA needs to exist with an atrial septal defect (to transfer blood to the left atrium) and a ventricular septal defect (to allow the left ventricle to pump blood back into the right side of the heart). Procedures are performed to increase flow to the pulmonary circulation while reducing mixture, these include: the *Blalock Taussig shunt* (discussed earlier); connecting the superior vena cava to the pulmonary artery (*hemi-Fontan procedure* done at 6 months); and, later, connecting the inferior venca cava to the pulmonary artery (*Fontan procedure* at 3-5 years).

Ebstein's Anomaly

In this condition, the tricuspid valves are positioned too inferiorly (towards the apex) and located within the upper aspects of the right ventricle – despite the annulus of the valve being in a normal position. It is very rare, with an incidence of ~1 per 200,000 live births. It presents over a very broad spectrum from infanthood to well into adulthood; this is dependent on the severity/location and the presence of other defects – commonly an atrial

septal defect (ASD). In the presence of a severe malformation with ASD, patients present earlier with symptoms of right-to-left shunting (e.g. cyanosis). Where the defect is milder, patients present much later in life (sometimes in their 50s) with symptoms of right-sided heart failure.

Patent Foramen Ovale

Up to 25% of the population have a patent foramen ovale and the vast majority are asymptomatic. However, due to the communication between the pulmonary circulation and systemic circulation, then it may present with a *paradoxical embolus*. This occurs when a venous thrombus embolises and passes through the foramen to deposit within the arterial circulation.

Relevant Molecules

- *NKX2.5, GATA4, & TBX5*: genes required for development of the heart
- *Notch1 receptor*: this regulates the epithelial-to-mesenchymal transition of cells in the endocardial cushions
- *Jagged1*: the ligand for the *Notch1 receptor*
- *Retinoic Acid*: promotes growth of endocardial cushions from the dorsal and ventral walls of the heart to divide the chambers into atrial and ventricular segments
- *Prostaglandin E1 (PGE1)*: when the baby is born, serum levels of placental PGE1 drop and this leads to closure of the ductus arteriosus

KEY POINTS

- The heart is the earliest functional organ to develop and contracts at day 22
- Endocardium and myocardium are formed from mesodermal tissue
- Neural crest cells contribute to the formation of the aortic and pulmonary valves
- Folding involves reorganising the linear heart tube to correctly position each segment
- The foramen ovale forms due to apoptosis of the superior wall of the septum primum
- Right-to-left shunting is essential in the foetus for ensuring oxygenation blood reaches the systemic circulation; it is reversed at birth leading to closure of the foramen ovale
- The arch of the aorta receives contributes from the heart tube, pharyngeal aortic arches, and embryonic dorsal aortae
- The ductus arteriosus shunts oxygenated blood from the pulmonary artery to the aorta
- The ductus venosus shunts oxygenated and nutrient-rich blood from the umbilical vein directly into the inferior vena cava to bypass liver
- It is important to recognise congenital cardiac malformations that are dependent on shunting through the ductus arteriosus in order to prevent its closure
- Severe right-to-left shunt defects present with cyanosis immediately following birth
- Septal defects are the most common congenital malformation

Limb Development 9

Mesoderm & Ectoderm

The production of the limbs is deceivingly complex as it requires a high degree of patterning to establish position, axes and function. From the 4th to the 8th week, growth and patterning of the limb occurs. It is initiated in response to *HOX* genes that signal the correct position of upper and lower limbs. These, in turn, leads to expression of *TBX5* genes to initiate growth in the upper limb and *TBX4* for the lower limb. This signals mesodermal and ectodermal tissue to organise and grow in response.

The mesoderm provides tissue through its *paraxial* and *lateral plate* parts. Recall that the somites are formed from paraxial mesoderm and divide into a sclerotome and dermomyotome. The dermomyotome divides into a *dermatome* that forms the dermis, and a *myotome* that generates the muscles of the limb. The lateral plate mesoderm provides the bones, blood vessels, and connective tissue. Meanwhile, the ectoderm will provide the epidermis, nails, hair, and associated nervous system.

Limb Proximal-Distal Outgrowth & Patterning

Initially, the limbs are small buds of mesenchymal tissue surrounded by ectoderm on their outer surface. They grow outwards in response to *FGF* signals from the *apical ectodermal ridge (AER)* – a small area of ectodermal cells at the tip of the bud (*figure 22*). The AER uses FGF signals to encourage

proximal to distal growth of the limb bud, with *HOX* genes specifying the distinct elements of the limb (e.g. HOX11 for the forearm and HOX12 for the carpals). The more proximal sections of the limb will differentiate first, as the cells in closest proximity to the AER and FGF remain undifferentiated – creating a *progress zone*.

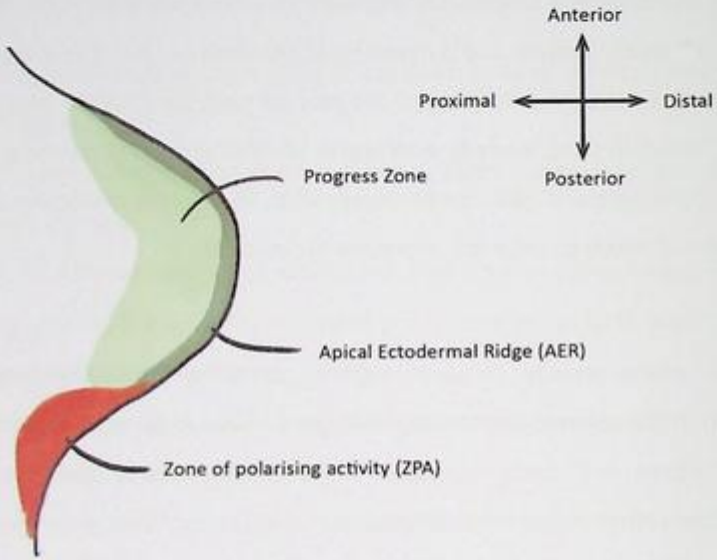


Figure 22: the limb bud growing in three axes in response to signals from the apical ectodermal ridge (distal) and the zone of polarising activity (posterior). The progress zone reflects the area where cells are becoming differentiated

Dorsal-Ventral Patterning

The AER demarcates the boundary between dorsal and ventral aspects of the limb. Its position in the midline occurs as a result of a difference patterning signals. Dorsally, the presence of *Wnt7* ensure that extensor structures form there, while *BMP* promotes the growth of flexor side muscles and structures. These two morphogens form a gradient by antagonising one another.

Cranio-Caudal Patterning

Patterning in this axis ensures that that the bones of the limbs are in the appropriate order – with caudal structures (e.g. little finger) developing before cranial structures (e.g. thumb). The region responsible for this is known as the *zone of polarising activity (ZPA)* and is located posteriorly/caudally. The morphogens being secreted from this area are *Sonic Hedgehog (Shh)* and *retinoic acid;* they provide positional information for the cells in the limb by creating a diffusion gradient. The molecules are dependent on the dorsoventral morphogens. The gradient is supported by *FGF8* secreted by the AER, which upregulates *Shh*. This in turn stimulates *FGF4* in the caudal aspects of the limb.

Digitalisation

Regulated and direct apoptosis of cells in the distal limb bud results in the formation of digits. This process is dependent on *BMP* signals interrupting the *Shh* signals from the ZPA.

Clinical Significance

Anomalies in limb formation can occur due to either disruption of growth or errors in patterning. These processes are heavily dependent on the correct expression of genes and appropriate signalling of morphogens.

Limb Bud Outgrowth Deformities

The limb bud grows out in response to *FGF* signalling from the AER, and disrupting this gradient leads to deformities dependent on the timing of the signalling loss. If it occurs early, it results in *amelia* and loss of the entire limb. Lower FGF concentrations or later loss of signal can result in either *meromelia* or *phocomelia*; the former is absence of a segment of the limb (e.g. forearm), and the latter is the shortening of the limb.

Digit Deformities

If *FGF* signalling is lost following the majority of bud outgrowth, then it results in *adactyly* (loss of digits) or *ectrodactyly* (absent middle finger).

If there is a disruption in the signalling of *Shh* from the ZPA or surrounding *BMP* levels then it results in *syndactyly* – fusion of digits; this most commonly affects the 3rd, 4th, and 5th digits. When there is upregulation of the *Shh* levels then it results in *polydactyly* (increased number of fingers).

Thalidomide

During the late 1950s and early 1960s, this medication was used as an anti-emetic for pregnancy-associated "morning sickness", as well as an anxiolytic in other patients (who could have been pregnant). It was found to be a *teratogen* – a molecule or substance that causes malformation of an embryo. Its use disrupted the *progress zone* and *FGF* signalling from the AER, which resulted in *phocomelia*. The issues associated with this drug led to international reforms in drug regulation and marketing.

Achondroplasia

This condition is also known as *dwarfism*. It results from an autosomal dominant mutation in the *FGF3* receptor gene that leads to a reduced response to signals from the AER and shortened limbs.

Relevant Molecules

- *Hox genes*: responsible for patterning the embryo
- *TBX5*: gene required for initiating upper limb development
- *TBX4*: gene required for initiating lower limb development
- *FGF*: signals released from the apical ectodermal ridge to promote outward growth of the limb bud
- *Wnt7*: signal promoted dorsally in the limb bud to promote development of extensor structures
- *BMP*: ventral signal to promote flexor structure development; also involved in digitalisation of the limb bud by disrupting Shh and promoting cell apoptosis
- *Shh & retinoic acid*: morphogens secreted by the zone of polarising activity (in the posterior/caudal limb bud) that is responsible for cranio-caudal patterning

KEY POINTS

- The limb is a highly patterned structure of the body whose development is dependent on positional signals
- The expression of morphogens leads to the formation of gradients in the axis to signal to limb bud cells for differentiation that is correct in orientation and size
- Disruption of morphogen signalling can either lead to shortening or absence of structures in the newborn

Foregut: Lungs & Diaphragm 10

After folding and formation of the "tube within a tube" structure, the endoderm becomes the innermost gut tube and runs from the *oropharyngeal membrane* (presumptive mouth) to the *cloacal membrane* (presumptive anus & urethral opening). This tube is divided into three parts with distinct blood supplies and derivatives. It was initially believed that that the arteries defined the segments of the gut tube; however, it is now known that the *HOX* genes code for the location of the arterial branches and organs.

The derivatives and blood supplies of the gut tube are very commonly assessed in written examinations and anatomy spotters, so they have been listed in the table below. For the foregut, it is important to remember that any organ or system which communicates with the gut tube is formed by the endoderm (e.g. liver and pancreas), whereas organs that do not connect to the gut are not (e.g. heart and spleen). The arteries are unpaired central branches of the descending aorta. These arteries will divide to supply all of the derivatives of the relevant segment of the gut through a named artery.

Subdivision	Derivates	Segment	Artery
Foregut	• Pharynx • Respiratory tract • Lungs • Larynx • Stomach • Duodenum (proximal 2 parts) • Liver • Pancreas	From the mouth to the second part of the duodenum (entry of the common bile duct)	Coeliac
Midgut	• Duodenum (distal 2 parts) • Ileum • Jejunum • Appendix • Caecum • Ascending colon • Transverse colon (proximal two-thirds)	From the entry of the common bile duct in the duodenum to two-thirds of the way along the transverse colon	Superior Mesenteric
Hindgut	• Tranverse colon (distal third) • Descending colon • Sigmoid colon • Rectum • Anal canal (proximal part)	From two-thirds across the transverse colon to the pectinate line of the anal canal	Inferior Mesenteric

Initially, the endodermal gut tube is surrounded by a layer of mesoderm, but this disperses to the edges to remain as the *dorsal mesentry*. This

structure suspends the gut tube from the abdominal cavity walls within a protective casing known as the peritoneum. The peritoneum has two parts: the visceral peritoneum from the *splanchnic mesoderm* that covers the organs, and a parietal peritoneum from the *somatic mesoderm* that lines the abdominal cavity (*figure 23*). What should also be evident here is that the dorsal mesentry is a double layer/fold of visceral peritoneum.

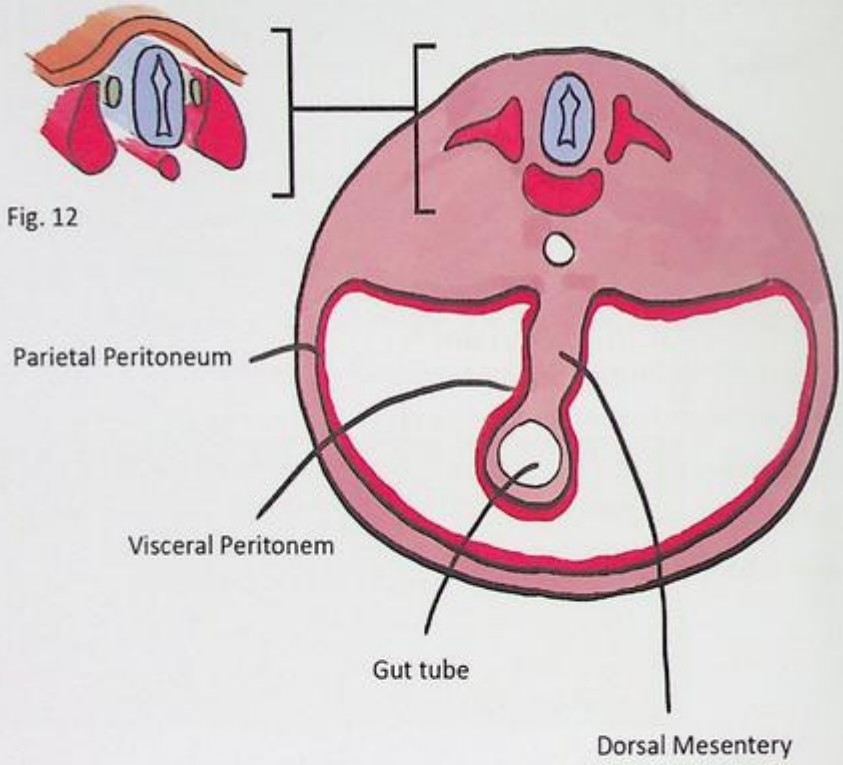


Figure 23: the dorsal mesentry of mesoderm with a suspended endoderm gut tube

Accordingly, the position of organs relative to the peritoneum in development and as final structures is important embryologically and clinically. Some organs develop and exist inside of the peritoneum, and are known as *intraperitoneal*. Others develop and exist outside the peritoneum, these are known as *primary retroperitnoeal* organs. The final group develop within the peritoneum to then lie outside of it, these are *secondary retroperitoneal* organs. This is another commonly assessed set of facts in exams. The organs there are retroperitoneal are listed below:

- Primary: kidney, adrenals, bladder, aorta, IVC, ureter, bladder, abdominal part of oesophagus, lower rectum, anal canal
- Secondary: ascending colon, descending colon, second part of duodenum, pancreas (head, body, neck), upper rectum

Trachea and Bronchial Tree

The trachea and lung begins to develop from the endodermal gut tube and surrounding mesoderm at the 4th week of gestation. An endodermal respiratory diverticulum (bud) grows ventrally from the foregut. This is surrounded by tracheo-oesophageal ridges of mesoderm that separate the budding lung from the oesophagus. This process is dependent on *retinoic acid* signalling.

The endoderm begins to proliferate to form a rod of tracheal epithelium that recanalizes (around 10th week). The U-shaped cartilage, posterior smooth muscle, and connective tissue of the trachea are formed from

mesoderm. During the 4th week, the rod of tracheal epithelium will divide in two to form the *primary bronchi*, and then continue to bifurcate (*figure 24*). The bifurcation of the endodermal buds occurs into the surrounding mesodermal tissue and is also regulated by *retinoic acid* levels.

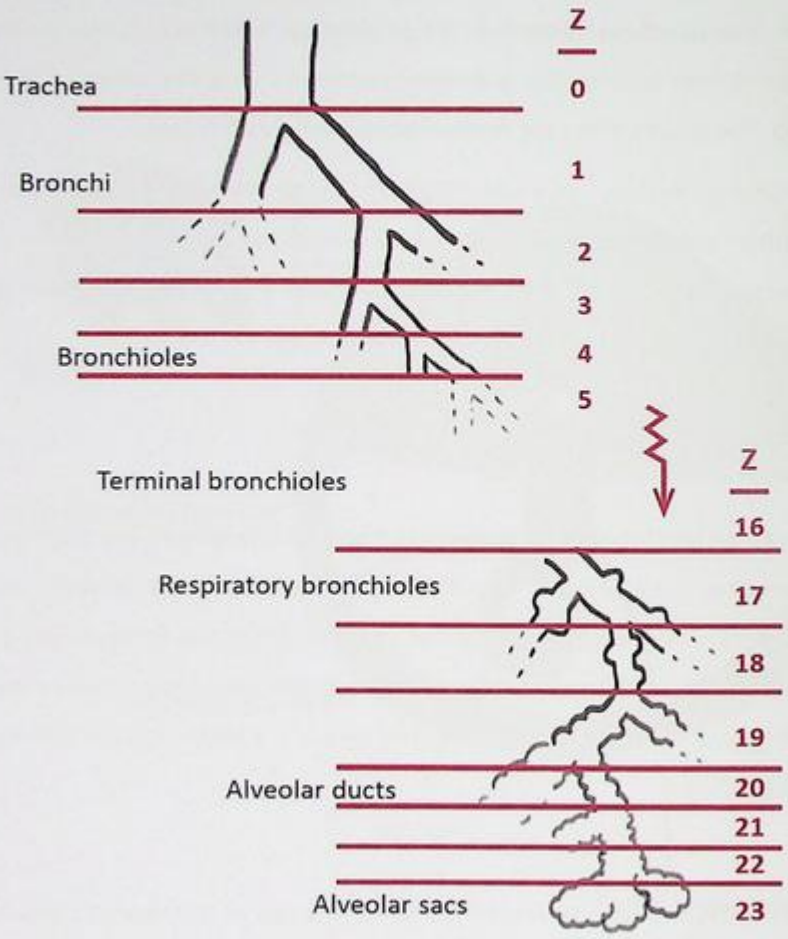


Figure 24: the different segments of the bronchial tree and bifurcation phases. 'Z' marks the number of branches in the logarithmic 2^Z

Lung Development

Once the bronchial tree has been established by the 5th week, the lung begins to form in four distinct stages. The first period is known as the *pseudoglandular* phase (weeks 5-16), in which the lung is a series of branched terminal bronchioles with no gas-exchange surfaces or alveoli. Therefore, the birth of a foetus at this phase is non-viable as respiration is not possible.

The *canalicular period* (weeks 16-26) follows and involves the formation of respiratory bronchioles and alveolar ducts with the emergence of a few terminal sacs; these sacs resemble early alveoli with vascularisation for the exchange of gases. With the occurrence of sacs, type II pneumocytes develop around week 24 to release surfactant. The role of the phospholipid surfactant is to prevent the collapse of airways and alveoli. It does so by maintaining the surface tension of the airways and alveoli; otherwise gradients of pressure would form between small and large alveoli leading to the collapse of the smaller alveoli. At this stage of development, respiration is poor but possible, and premature infants may survive – dependent on the presence of surfactant.

At this point, the basic outline of a respiratory tree is formed, and the remainder of lung development is for the maturation of the alveolar sacs into buds of alveoli – this is dubbed the *terminal sac* period (weeks 26-birth). There is a rapid increase in the number of terminal sacs/alveoli accompanied by thinning and increased vascularity of the alveolar walls to

maximise potential gas exchange. This generation of alveoli and remodelling continues until the age of 8, through a period of lung maturation known as the *alveolar period*; the child will ultimately have more than 6 times as many alveoli as it is born with.

As the lungs develop, they are separated from the heart during the 5th week. *Pleuropericardial folds* develop from the lateral body wall anterior to the developing lung. These folds grow to become *pleuropericardial membranes* that fuse in the midline to separate the pleural cavity (dorsal) from the pericardial cavity (ventral). These membranes become the fibrous pericardium.

Development of Diaphragm

The diaphragm isolates the thoracic cavity from the abdominal cavity. It forms from many parts: septum transversum, pleuroperitoneal membranes, mesentery of oesophagus, and myoblasts from 3rd to 5th cervical somites. The *septum transversum* is initially the most cranial structure in the embryonic disk; however, cranio-caudal folding brings it to lie between the presumptive heart and liver. This positions it at the level of C1, and it then it grows caudally with the development of the lungs – taking myoblasts from the 3rd to 5th somites with it. In doing so, it also takes the motor innervation of these myoblasts from C3, C4, & C5 to form the skeletal muscle component of the diaphragm. This is why the diaphragm is innervated by the phrenic nerve despite being a low thoracic structure (*"C3/4/5 keeps the diaphragm alive"*).

When this completes, there remains a channel between the pericardial area and the peritoneum – known as the pericardioperitoneal channel. Two *pleuroperitoneal folds* begin to grow from the lateral walls of the body to close this channel. They meet with the oesophageal mesentery and septum transversum to form *pleuroperitoneal membranes*.

Clinical Significance

Watershed Areas of the Gut

Watershed areas refers to zones that receive a dual blood supply from the distal end-branches of two large arteries. In the gut, this typically refers to the *splenic flexure* (*Griffiths's Point*) that marks the boundary between the midgut and the hindgut. It receives blood supply from the end-arteries of the superior and inferior mesenteric branches of the aorta. It can also refer to the *rectosigmoid junction* (*Sudek's Point*) between the zones of the inferior mesenteric and superior rectal arteries. During systemic hypoperfusion (e.g. during haemorrhage or shock), these areas are susceptible to ischaemia.

Retroperitoneal Organs

The peritoneum is known as the "policeman of the abdomen" because it migrates towards areas of infection/inflammation and puncture sites in stab wound injuries. This aims to mitigate the injury by reducing the zone of inflammation and infection. In stab injuries, it covers the puncture site to reduce additional risk of infection from the outside. An understanding of the location of organs relative to the peritoneum is important for surgeons to identify structures intra-operatively. Furthermore, it has led to the development procedures for retroperitoneal organs are possible without

affecting other organs or risking intra-peritoneal infection; these include: insertion of a suprapubic catheter, a renal biopsy, and an ascitic tap.

Tracheo-Oesophageal Fistula

If the tracheo-oestopheagul septum fails to separate the oesophagus from the trachea, then a fistula can occur. Typically, the oesophagus is interrupted into a proximal and distal end, with the proximal remaining as a short blind-ended sac and the distal adjoining the trachea via a fistula; however, many variations of this occur. The newborn will present with choking, coughing, vomiting, and cyanosis whenever they try to feed.

This condition can occur as part of a wider syndrome of associated conditions known as the VACTERL association. These patients will have at least three of the following features:

- Vertebral anomalies (spinal cord deformity)
- Anal atresia (imperforate anus)
- Cardiovascular abnormalities
- Tracheo-oesophageal fistula
- (o)Esophageal atresia
- Renal abnormalities
- Limb anomalies

Respiratory Distress Syndrome

This condition, also known as *Hyaline Membrane Disease*, is due to an absence or inadequate production of surfactant. It is usually caused by a lack of type II pneumocytes leading to insufficient surfactant production and collapse of the airways with a glossy hyaline membrane. Mothers of babies at risk of being born prematurely are given steroids to promote lung development and the formation of type II pneumocytes.

Congenital Diaphragmatic Hernia

Failure of the pleuroperitoneal membranes to close the pericaridoperitoneal canals can lead to a *congenital diaphragmatic hernia* (1 in 2000 live births) – where abdominal viscera protrude into the thoracic cavity. In the majority of cases, the herniation is on the left aspect of the aortic or oesophageal hiatus (85%), since the liver obstructs the route on the right. This is also known as a *Bochdalek hernia*. The presence of the abdominal contents within the thoracic cavity can lead to pulmonary hypoplasia and difficulty in breathing. When the defect is ventral, then it is known as a *Morgagni hernia*.

Relevant Molecules

- *Hox genes*: expression of these genes demarcates the gut into the foregut, midgut, and hindgut
- *Retinoic Acid*: signals of this molecule are needed to guide the separation of the trachea from the oesophagus, and the development of the bronchial tree

KEY POINTS

- The gut is divided into three regions: foregut, midgut, and hindgut
- Abdominal viscera are either intraperitoneal, primary retroperitoneal, or secondary retroperitoneal
- The lung is predominantly an endodermal foregut structure
- Phases of lung development reflect the presence of gas exchange surfaces
- Surfactant is essential to maintain surface tension and prevent the collapse of airways
- Viability of the foetus is linked to the development of the lungs

Foregut: Oesophagus & Stomach 11

The gastrointestinal tract is suspended from the abdominal wall via a series of mesentries. The dorsal mesentery connects to the length of the gut with with exception of the oesophagus and anus that are directly in contact with the body wall. The liver and stomach have additional mesenteries which regress to form structures adult. The *ventral mesentery* of the liver will become the *falciform ligament*, the *dorsal mesentery* of the stomach will form the *greater omentum*, and the *ventral mesentery* between the stomach and liver will become the *lesser omentum*.

Development of the Oesophagus

The oesophagus develops as a solid rod of thoracic foregut at the end of the 4th week. This occurs after the respiratory diverticulum buds ventrally and the tracheo-oesophageal ridges separate the presumptive oesophagus from the presumptive trachea. It elongates rapidly relative to the rest of the gut tube – being extended by the rapidly growing pharynx – in order to create the distance needed to reach through the thoracic cavity. The structure canalises around week 9 to form a tube.

Development of the Stomach

The stomach forms as a result of rotation, differential growth, and positional change of the gut tube. The process starts with a spindle-shaped dilatation of the foregut distal to the septum transversum. The dorsal

aspect begins to grow faster than the ventral side to create the greater curvature. Further expansion superior to the greater curvature forms the fundus and cardiac incisure, leading to the distinctive shape of the stomach (*figure 25*). As this happens, the thinning of the ventral mesentery leads to a 90-degree rotation in the cranio-caudal axis which:

- Brings the *dorsal border* to the *left* to form the *greater curvature*
- Places the *ventral border* to the *right* to form the *lesser curvature*
- Left-sided vagus nerve bundles become the *ventral vagus plexus*
- Right-sided vagus nerve bundles become the *dorsal vagus plexus*
- Dorsal and ventral mesenteries (mesogastrium) of the stomach become the *greater and lesser omentum*
- This rotation of the mesentery creates a space behind the stomach called the *lesser sac*
- Fuses the second part of the duodenum to the posterior wall, making it a *secondary retroperitoneal* organ

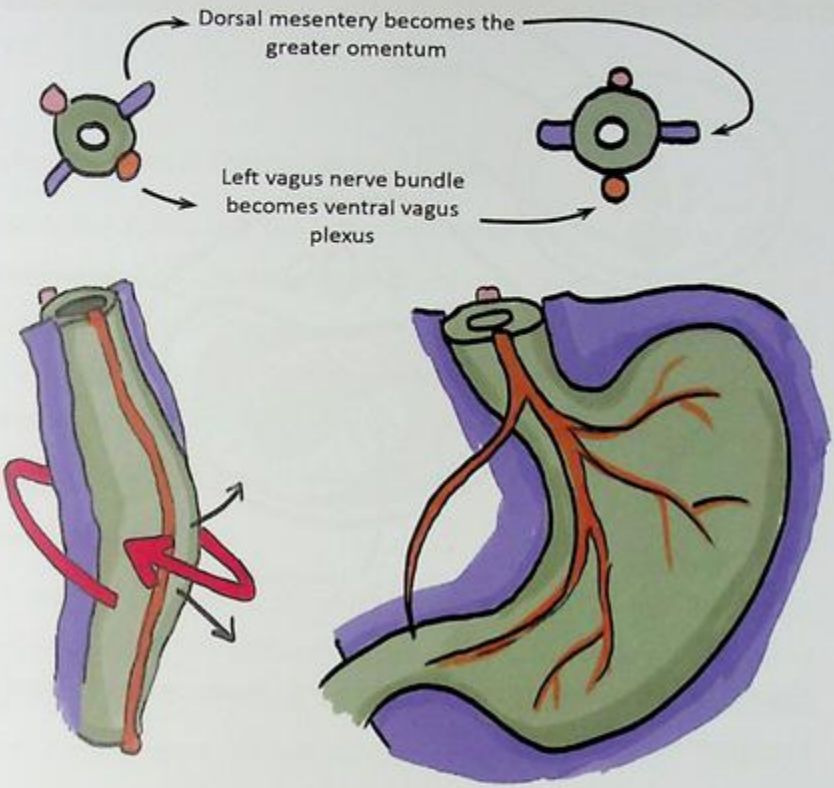


Figure 25: expansion and rotation of the stomach

During this process, two mesodermal processes are occuring. The first is that smooth muscle is recruited and proliferated in the distal aspect of the stomach to form the *pyloric sphincter*. Secondly, the spleen begins to form within the *dorsal mesogastrium* (*figure 26*).

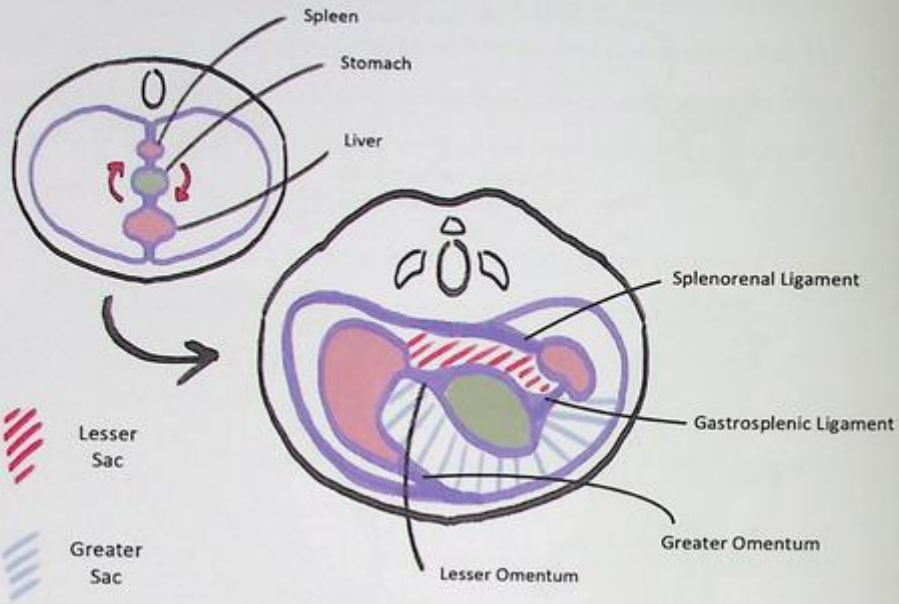


Figure 26: formation of the greater and lesser sac with rotational movements of the stomach

Clinical Significance

Polyhydramnios & Oesophageal Atresia

In utero, the foetus will use the surrounding amniotic fluid to aid the development of its internal organs. In order to prepare for breathing, it practises diaphragmatic movement to gain adequate strength & function to take a full breath at birth. This leads to a inflow and outflow of amniotic fluid through the lung tree, which stimulates the epithelium to promote maturation and development of the lung.

Polyhydramnios occurs in 1% of all births and occurs when there is an excess of amniotic fluid within the amniotic sac. It can be diagnosed during ultrasound scans of the foetus. It is a concern because it is associated with severe complications including: cord prolapse, placental abruption, premature birth, and perinatal death. It typically occurs with either a systemic disease in the mother or a congenital condition/infection in the foetus, so the newborn should be carefully examined for any issues.

Following canalisation of the oesophagus, the foetus will swallow the amniotic fluid to aid development of the gastrointestinal tract. If canalisation of the oesophagus at week 9 fails, then the foetus is unable to swallow intra-uterine amniotic fluid, leading to polyhydramnios. This is known as *oesophageal atresia* and is often associated with a tracheo-

oesophageal fistula. When the blockage is not complete, then it is known as *oesophageal stenosis*. A similar condition can occur when the duodenum is not fully canalised – *duodenal atresia*. The newborn will regurgitate, cough, or choke on attempted feeding, due to an inability to swallow. As with any patient with an impaired swallow, this leads to a risk of aspiration pneumonia.

Congenital Hiatal Hernia

In the last chapter, congenital diaphragmatic hernias were discussed due to inadequate formation of the diaphragm. If the oesophagus does not adequately elongate through the thoracic cavity, then the cardia of the stomach is pulled up into the thoracic cavity – leading to a hiatus hernia. The effects of this condition are similar to that of a congenital diaphragmatic hernia.

Hypertrophic Pyloric Stenosis

In this condition, the smooth muscle at the outflow of the stomach (pylorus) is hypertrophic (i.e. overproliferated). This leads to an outflow obstruction on ingestion of food. The standard description is that the newborn will projectile vomit ~1 hour after feeding, and the vomitus is non-bilous (as it has not mixed with bile salts in the duodenum). On clinical examination, a small hard lump (often described as an olive) may be palpable inferior to the sternum. It occurs in ~0.5% of births, and requires a pyloromyotomy procedure. The danger to the newborn is inadequate hydration/nutrition.

Relevant Molecules

- *Nil*

KEY POINTS

- As the epithelium of the gut tube proliferates, it develops as a solid rod that requires canalization – failure of this procedure leads to stenosis and atresia
- The rotation of the stomach is very important in positioning many of the adult organs and is responsible for the creation of the intra-abdominal cavities

The liver, gallbladder, and pancreas develop mainly from the endodermal foregut. The foetal liver acts as the first haematopoietic organ in the embryo until the third trimester where the function is performed by the bone marrow.

Development of the Liver & Gallbladder

The liver develops around day 22 as a small endodermal thickening overlying the ventral duodenum known as the *hepatic plate*. These endodermal cells will form the *hepatocytes, hepatic ducts, biliary cells* and *bili canaliculi*; mesoderm from the *septum tranversum* will generate the supporting stromal connective tissue, vascular sinusoids and the *Kupffer cells*. This mesoderm is very important in inducing the formation of the hepatic plate as it releases *BMP* to act on the nearby endoderm, which in turn makes this region highly sensitive to the *FGF2* signals from the cardiac mesoderm that induce hepatic development.

The hepatic plate will proliferate toward the septum tranversum to form a hepatic diverticulum around day 26. Caudal to the hepatic duct, a cystic diverticulum develops that will go on to form the gallbladder and share a common bile duct with the hepatic diverticulum (*figure 27*). It is important to note that, although it buds from the hepatic duct, the cystic diverticulum forms from a distinct group of cells on the ventral duodenum.

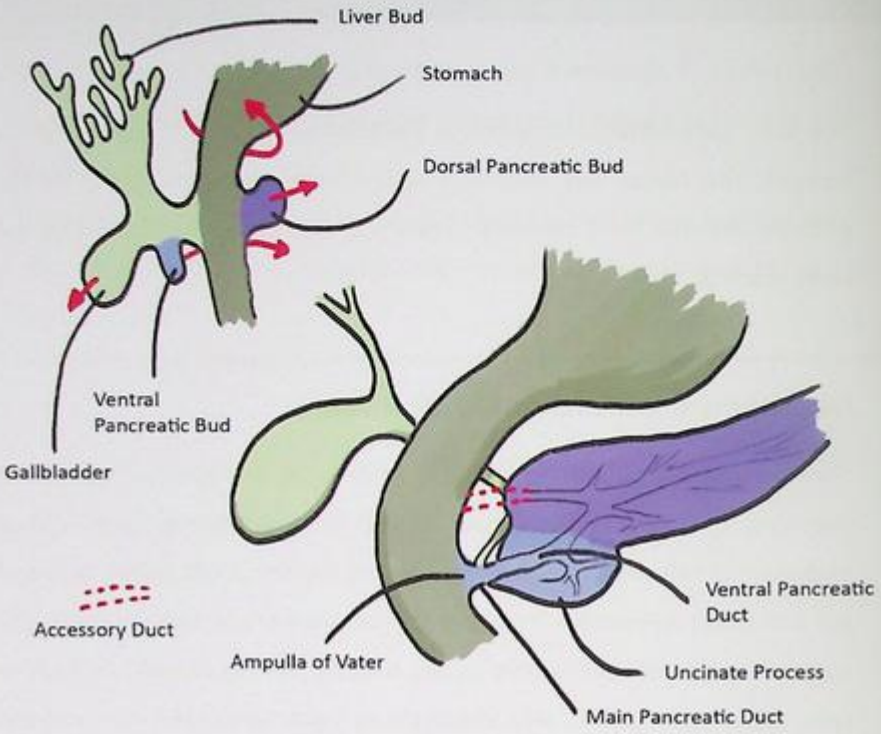


Figure 27: development of the endodermal organs from the foregut

Development of the Pancreas

The pancreas from a dorsal and ventral pancreatic bud. The ventral bud grows as an outpouching from the hepatic duct – caudal to the cystic diverticulum (*figure 27*). The dorsal bud proliferates from the dorsal duodenum, directly opposite the site of growth of the hepatic diverticulum, and grows into the dorsal mesentery. The ventral bud will form the hook-

like uncinate process of the pancreas, meanwhile the dorsal bud will form the head, body, and tail.

As the stomach and midgut rotate, it brings together the dorsal bud with the ventral bud and common bile duct, such that they open through a common opening into the second part of the duodenum – *the ampulla of Vater*. The dorsal and ventral buds will then fuse around the 6th week. The pancreatic duct within the dorsal bud usually degenerates, leaving the ventral duct as the main pancreatic duct; however, with inadequate fusion then accessory ducts may persist.

The initiation of pancreatic development is dependent on the expression of PDX1 genes, with expression of PAX1 and PAX6 specifically needed for differentiation of the endocrine (*Islets of Langerhan*) cell lineages.

Clinical Significance

Biliary Atresia

Similar to other disorders leading to atresia, this occurs when there is proliferation of epithelial tissue lining the intra- and extra-hepatic biliary tree with failure of canalisation of the lumen. It occurs in ~1/14000 live births and presents as one of the rarer causes of prolonged neonatal jaundice. Without intervention, it leads to progressive chronic liver failure that results in death. Once recognised, a *Kasai (hepatoportoenterostomy)* procedure can be performed within 60 days, in which a loop of jejunum is anastamosed to the porta hepatitis in order to allow biliary drainage. In ~80% of infants, this is successful if performed early enough – with the likelihood of success diminishing with age. Without this procedure, the infant requires a liver transplant; and biliary atresia remains the most common indication for a hepatic transplant in the paediatric population.

Annular Pancreas

This occurs when a ring of pancreatic tissue surrounds the second part of the duodenum and constricts the lumen. It results from a bifid ventral pancreatic bud or errors in the fusion process. In the case of the bifid bud, the tail of each ventral segment rotates in a different direction and adjoins the dorsal bud – leading to incomplete rotation and fusion (*figure 28*). Its symptoms would be similar to that of hypertrophic pyloric stenosis with

projectile vomiting occurring a short time (~1 hour) after feeding. However, it is distinguished by the vomitus being bile-stained as the food will have mixed with the bile.

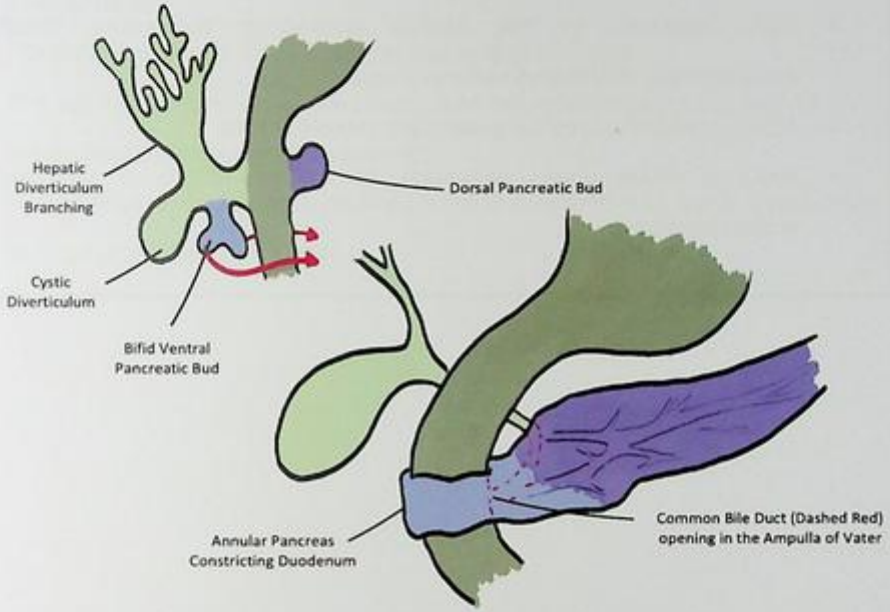


Figure 28: bifid ventral pancreatic bud (lilac) each rotating in a different direction and fusing with the dorsal pancreatic bud (purple) leading to annual pancreas

Relevant Molecules

- *BMP*: signals released from the septum transversum mesoderm makes the ventral duodenum responsive to FGF2 signals
- *FGF2*: released by the cardiac mesoderm to induce hepatic development of the endodermal tissue
- *PDX1*: genes required for pancreatic development
- *PAX1 & PAX6*: genes necessary for differentiation of pancreatic endocrine tissue

KEY POINTS

- The liver is endoderm-derived with the exception of the stromal connective tissue, vascular sinusoids, and Kupffer cells
- The gallbladder forms from endoderm that is caudal and distinct to the hepatic tissue
- The pancreas forms from a ventral bud and dorsal bud
- The dorsal bud forms the head, neck, and tail, while the ventral bud forms the uncinate process of the pancreas
- The main pancreatic duct is derived from the ventral bud, but it is common for accessory ducts to persist

Midgut Development 13

The midgut runs from the distal half of the duodenum to two-thirds across the tranverse colon. It starts precisely after the point of entry of the common bile duct at the Ampulla of Vater. Its development is dependent on differential rates of growth and directional rotation.

Growth & Herniation

Rapid growth of the midgut portion of the gastrointestinal tube leads to the formation of a *primary intestinal loop* that is shaped like a hairpin. This has cranial and caudal halves (*limbs*) along a central axis defined by the vitelline duct and superior mesenteric artery (*figure 29*). The cranial half will form the distal duodenum, jejunum and proximal ileum, while the caudal half forms the distal ileum, appendix, caecum, ascending colon and first two-thirds of the transverse colon.

The *vitelline duct* in the embryo is a communicating tract between the yolk sac and midgut. It receives a blood supply from left and right vitelline arteries, and acts as a transit of substances from the yolk sac into the embryo; however, the function in humans is not well understood. The left vitelline artery will involute, while the right will become part of the *superior mesenteric artery*. The duct usually obliterates by the 8th week, but may persist as a *Meckel's diverticulum*.

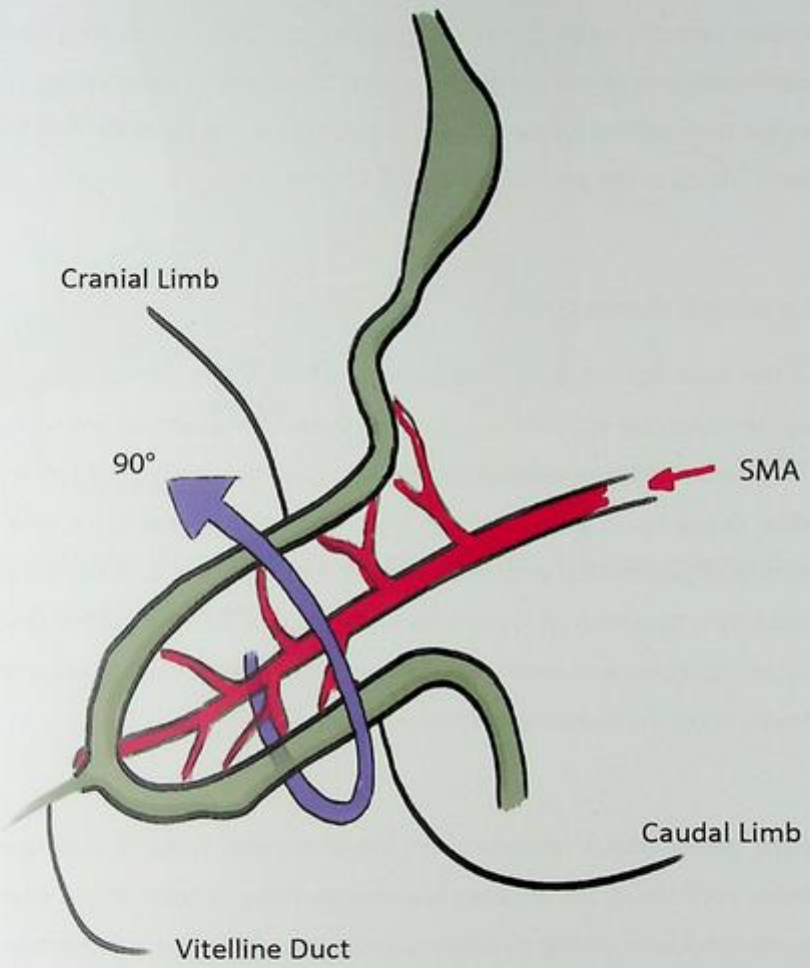


Figure 29: The primary intestinal loop and its axis of rotation/symmetry

Around the 6th week, the primary intestinal loop and liver are growing at a greater rate than the surrounding structures and expand to a volume beyond the size of the abdominal cavity. This leads to a herniation of the midgut through the presumptive umbilicus (where the vitelline duct runs); this is known as the *physiological umbilical herniation*.

Rotation & Retraction

As the loop herniates, it undergoes a *primary rotation* of 90 degrees *counter-clockwise* around the axis of the superior mesenteric artery (*figure 30*). This moves the cranial limb to the right and the caudal limb to the left. While doing so, it grows further and forms the jejunal loops and the vermiform (worm-like) appendix. Around week 12, the primary intestinal loop begins to retract. It is not fully understood if this is an active process due to regression and pulling or a passive process as a result of the relative growth of the surrounding abdominal cavity.

As the bowel loop is retracted into the abdominal cavity, it undergoes a further rotation of *180 degrees counter-clockwise* (a total of 270 degrees counterclockwise during herniation and retraction). This is important for the final positioning of the organs as it places the cranial limb to the left and the caudal limb to the right – resulting with the appendix in the right lower quadrant. The process is completed by the 11th week.

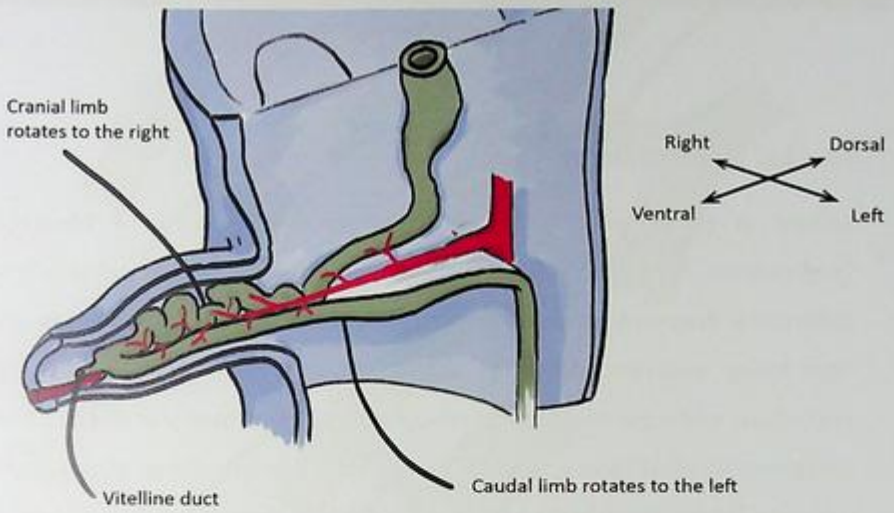


Figure 30: the herniated primary intestinal loop following growth of the presumptive small bowel

Clinical Significance

Meckel's Diverticulum

Failure of the vitelline duct to obliterate will result in a Meckel's Diverticulum. This is of relative importance in clinical medicine as it is a differential diagnosis for abdominal pain – particularly that migrating to the right lower quadrant (similar to appendicitis). It can be found in 2% of individuals and is the most common congenital abnormality of the gut. The persistent vitelline duct can exist as either: an outpouching of the small intestine (Meckel's diverticulum), cyst (with two closed ends), or fistula (a connection between two epithelial-lined organs) (*figure 31*).

A Meckel's can be considered a true diverticulum as it contains all three layers of the gastrointestinal tract. It typically contains gastric tissue, and this is identifiable using a 99-technetium (radioisotope nuclear) scan. The aetiology and pathophysiology of a Meckel's diverticulum can be recalled by the "Rule of 2's":

- 2% of the population
- 2 inches in length
- 2 feet proximal to the ileocaecal valve
- 2 years old is the most common age of presentation
- 2:1 male to female ratio of incidence
- 2 types of common ectopic tissue (gastric and pancreatic)

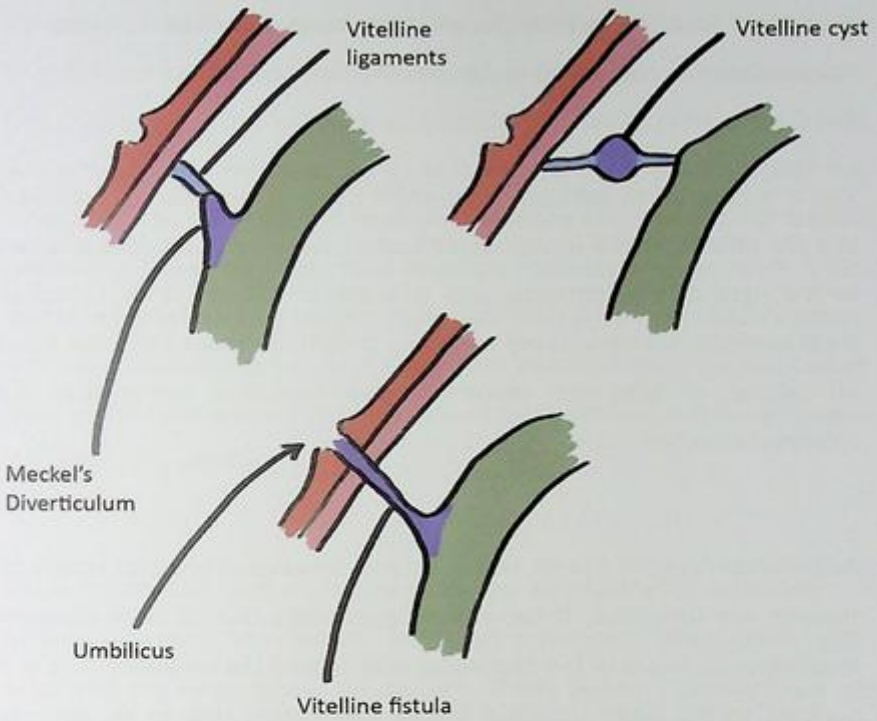


Figure 31: the differential forms of persistent vitelline duct tissue as a diverticulum, cyst, and fistula

Omphalocele, Gastroschisis, & Umbilical Hernia

An omphalocele, also known as *exomphalos*, is a herniation of the bowel (or other viscera) through the umbilicus and covered by a thin avascular membrane. It is a rare abdominal wall defect occurring in ~1/4000 births and it is associated with a high mortality rate. It is thought that a potential mechanism is malrotation during retraction of the midgut into the abdominal cavity, which leads to insufficient closure of the ventral

abdominal wall. Alternative causes have been described including the failure of somitic myotomes to form abdominal muscles.

This is in contrast to *gastrochisis* in which there is herniation of the bowel at a site other than the umbilicus *without a covering sac*. It typically occurs to the right of the umbilicus and at a similar rate (~1/2500 births) to omphaloceles. Similarly, there are many theorised causes but these focus on failures of adequate abdominal wall formation rather than gut rotation/retraction.

An *umbilical hernia* is a small, skin-covered protrusion of bowel or omentum through the umbilicus. It can occur congenitally due to an inadequate meeting and closure of the abdominal wall around the umbilicus. This is in contrast to the adult umbilical hernia that occurs due to an acquired weakness of the abdominal wall (e.g. obesity, post-operative or post-partum). In the neonate, it is most obvious when the baby cries as this increases the intra-abdominal pressure and protrudes the viscera. It is typically repaired at the age of 5. All forms of hernias are repaired electively (or as an emergency) due to the potential of the bowel to become stuck (non-reducible) or rotate, leading to strangulation, ischaemia, and necrosis.

Intestinal Malrotations

The congenital anomalies occur in ~1/3000 live births and the presentation is related to the final position of organs (not the degree of malrotation). It

can lead to midgut volvulus, in which the infant will present with symptoms of abdominal pain, sudden episodes of crying (due to cramps), constipation, or vomiting. The infant will undergo a *Ladd procedure* to remove the bands across the bowel. The bowel is then re-inserted into the abdomen with the small bowel on the right and large bowel on the left. The patient leads a normal life; however, if the infant develops appendicitis as an adult, it can lead to left-sided abdominal pain. Note that this is different to *Ladd's bands,* which are constricting bands of tissue that are a common cause of adult small bowel obstruction – typically caused by abdominal surgery or severe inflammatory pathology.

Similar to the post-Ladd procedure abdomen, a midgut which undergoes no rotation (*intestinal non-rotation*) will result in the small bowel being right-sided and the large bowel on the left. These patients usually have no symptoms. A small proportion develop signs as a result of kinking of the duodenum by the misplaced overlying bowel, which can lead to a blockage.

Relevant Molecules

- *Nil*

KEY POINTS

- The midgut runs from the distal half of the duodenum to two-thirds across the tranverse colon
- Increased growth of the midgut leads to the formation of a primary intestinal loop
- This loop grows faster than the surrounding abdominal cavity and so herniates through the umbilicus
- The herniated loop undergoes 90 degrees of counter-clockwise rotation
- As it retracts, it undergoes a further 180 degrees of counter-clockwise rotation
- In total, the midgut will rotates 270 degrees in a counter-clockwise direction

Hindgut & Bladder Development 14

The hindgut will form the: final third of the transverse colon, descending colon, sigmoid colon, rectum, and upper anal canal. It is supplied by the inferior mesenteric artery and its branches. The rotation and retraction of the midgut will position the hindgut appropriately. At its distal end, it terminates as an expanded pouch known as the *cloaca* – that will divide in two to contribute to the lower urogenital tract and the anorectal canal.

Enteric Nervous System

The bowel is innervated by an *enteric nervous system* that forms as a result of the migration of neural crest cells. It is a very complex neural plexus that is divided into the *submucosal (Meissner's) plexus* and *myenteric (Auerbach's) plexus*. It can operate independently of any central nervous system stimulation so is often dubbed the "second brain".

The myenteric plexus lies between the circular & longitudinal muscle layers of the bowel wall and is responsible for the peristalsis by controlling the tone and frequency of contractions. It provides both sympathetic and parasympathetic innervation to the gut. The submucosal plexus is found in the submucosa (between the mucosa and muscularis propria), and innervates the gut mucosa. It is responsible for the regulation of bowel secretions, local absorption, and local contraction.

Formation of the Anorectal Canal & Bladder

The hindgut ends in an endodermal-lined pouch known as the *cloaca* whose opening is covered by a *cloacal membrane* (presumptive anus). Within this structure, the endoderm of the hindgut meets the external ectoderm – marked by the *anal pit*.

Lying ventrally is the *allantois*, which is connected to the yolk sac. Its function in the human is not well understood, but it is used by reptiles and birds (who don't have placentas) for respiration and the removal of waste products. It is hypothesized that in mammals, it contains mesodermal tissue which is required for the formation of the umbilical arteries (to carry deoxygenated blood to the placenta) and to remove nitrogenous waste from the bladder. In the human, its proximal portion (the *urachus*) will form the superior part of the bladder, and the remainder regresses into a dense fibrous cord (*median umbilical ligament*) that connects the urinary bladder anteriorly to the umbilical region.

The expanded cloaca is divided by the growing urorectal septum into a *ventral urogenital sinus* and a *dorsal anorectal canal* (*figure 32*). The septum starts as two lateral segments that fuse in the midline and grow caudally. They meet with the cloacal membrane around the 7th week, to separate it into a *urogenital membrane* ventrally and an *anal membrane* dorsally. The anal membrane will perforate in the 9th week to form the anus. The urorectal septum will remain in the newborn as the *perineal body* – separating the genitalia from the anus as the perineum.

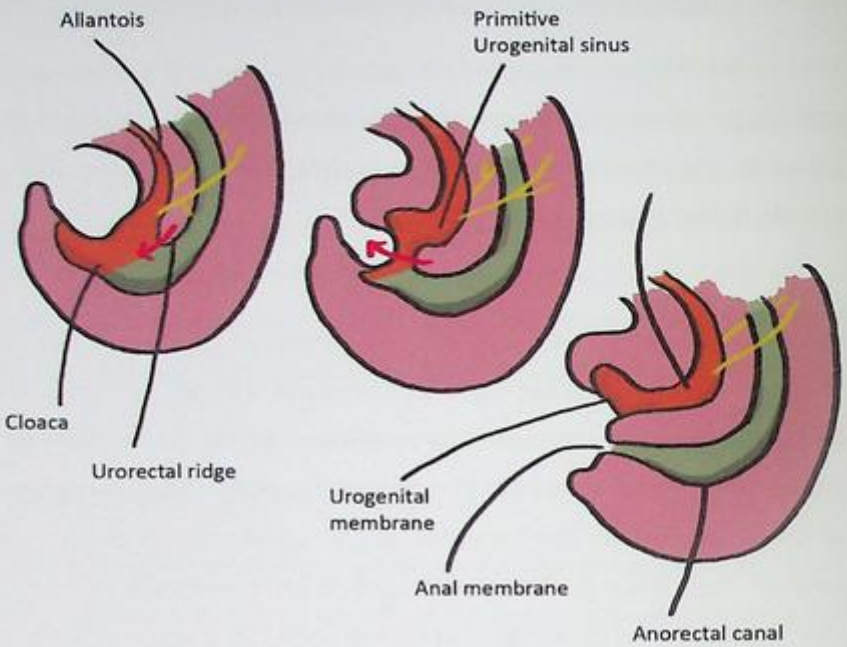


Figure 32: division of the cloaca into the urogenital sinus and anorectal canal

The endodermal anorectal canal will form the rectum and the upper two-thirds of the anal canal. This meets with the lower ectodermal anal canal at the *pectinate line* (also known as the *dentate line*). This is important as it marks a distinction between innervation, lymphatics and vascular supply of the two regions, as outlined below:

- Upper anal canal
    - Artery: *superior rectal artery* of the inferior mesenteric branch of the aorta

- Vein: *superior rectal vein* of the inferior mesenteric (draining into the *portal venous system*)
- Lymphatics: *internal iliac nodes*
- Nerve: visceral innervation via the *inferior hypogastric plexus*
- Sensation: only to *stretch*

- Lower anal canal
  - Artery: *inferior rectal artery* of the internal pudendal branch of the internal iliac artery
  - Vein: *inferior rectal vein* of draining into the systemic circulation via the internal pudendal vein
  - Lymphatics: *superficial inguinal nodes*
  - Nerve: somatic innervation via the *inferior rectal* branches of the *pudendal nerve*
  - Sensation: touch, pain, temperature, and pressure

A highly variable structure is the *middle rectal artery.* It is found in the majority of people and has been shown to supply both the upper and lower parts of the canal in different studies. It is typically found above the pectinate line, yet most commonly is a branch of the internal pudendal artery. It anastamoses with the superior rectal artery, inferior rectal artery, & the inferior vesical artery, and gives off an important branch to the supply the prostate in some individuals.

The ventral urogenital sinus will form the bladder (except the trigone) and sex-specific structures. In the female, it also forms the urethra and vagina.

In the male, it will form the prostate gland, prostatic urethra, and membranous urethra.

Clinical Significance

Haemorrhoids

These are vascular cushions that lie in the anorectal canal that have an important role for continence by protecting the internal and external anal sphincters. They are anastamoses of the vascular sinusoids between the superior & inferior rectal systems and can become engorged with blood, swollen, and inflammed in *haemorrhoid disease*.

Haemorrhoids are found at the left lateral, right anterior, and right posterior (3, 7, & 11 o'clock) positions. They can become pathological when other conditions cause increased intra-abdominal pressure and straining, these include: constipation or diarrhoea, low-fibre diets, ascites, chronic coughs, obesity, prolonged sitting, and intra-abdominal malignancies. Haemorrhoids can be internal or external dependent on their position relative to the pectinate line.

External haemorrhoids are not graded, whereas internal haemorrhoids are classified by their location. This is because they can exist above the pectinate line but, when severe, prolapse to lie at or below the line (*figure 33*). If minor, the patient presents with painless bright red bleeding per rectum, that might be seen on the toilet bowl, surface of the stool, or when wiping. Note that the blood would be on the surface and not mixed in –

which would be indicative of a malignancy or a gut inflammatory process. Haemorrhoids are associated with other symptoms such as pruritus, discharge or fullness. The symptom of pain is associated with thrombosis of the haemorrhoid and requires analgesia or surgical excision.

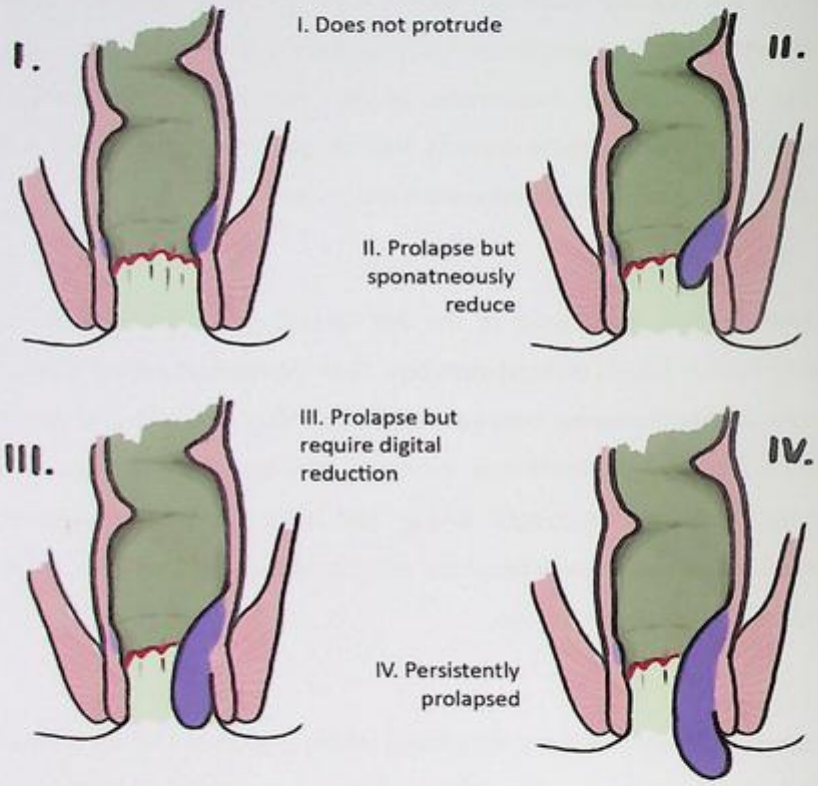


Figure 33: Grades of internal haemorrhoids

The haemorrhoidal disease is managed according to its severity and recurrence. Acutely, if presenting within 72 hours, external haemorrhoids

can be treated with topical analgesia or excised if they are particularly painful. For more chronic problems, haemorrhoid disease can be managed with non-operative changes such as weight loss, fibre diets or lifestyle modification (e.g. increased exercise). Where internal haemorrhoid disease is 1st or 2nd degree with recurrent inflammation, then rubber-band ligation can be performed under direct vision with a proctoscope. More severe disease may require haermorrhoidal artery ligation or haemorrhoidectomy.

Suprapubic Catheter

The bladder is a retroperitoneal structure and so can be accessed anteriorly for insertion of a suprapubic catheter. This may be used when access in not possible via the urethra, or when the bladder is reconstructed (e.g. with an ileal pouch).

Hirschsprung's Disease

If there is a failure of neural crest cells to migrate or differentiate into the neurones of the enteric nervous system then this is known as *Hirchsprung's Disease*. The lack of innervation leads to aperistalsis of the bowel followed by hypertrophy as the bowel is persistently contracted with no signal to relax. Typically, it is not the whole bowel which is affected with *aganglionic segments* most commonly occurring in the sigmoid colon. The infant presents with either: a distended abdomen due to the presence of a megacolon, or with failure to pass the first faecal movement (meconium). Unforgettably, on insertion of a finger into the newborn's anus for a digital

rectal exam (to rule out an imperforate anus), there is the expulsion of an explosive bolus of stool.

Imperforate Anus

This occurs due to inadequate perforation of the anal membrane. It can occur anywhere in the anorectal canal (high or low) and presents with a failure to pass stool. It is associated with other genetic conditions including VACTERL syndrome. It is treated with surgery to create a colostomy to allow the stool to bypass the anus.

Relevant Molecules

- *Nil*

KEY POINTS

- The hindgut will form the the final third of the transverse colon, descending colon, sigmoid colon, rectum, and upper anal canal
- Neural crest cells migrate to the bowel to form the enteric nervous system
- The cloaca is a pouch at the end of the hindgut that will be separated by the urorectal septum into a ventral urogenital sinus and dorsal anorectal canal
- The anal canal is separated into an upper and lower segment by the dentate line
- Structures superior to this line are hindgut-derived
- Inferior to the dentate line, structures are ectodermal in origin
- This distinction leads to difference in neurovascular supplies

Mesoderm: Spleen & Urinary 15

Mesodermal Tissues & Organs

The mesoderm forms key organs that lie between the endoderm-derived gut tube and surrounding cavity. This concept can be appreciated by looking at the map of the trilaminar disk (*figure 34*). The *intermediate mesoderm* forms the kidney, lower urinary tract, and reproductive system.

The *lateral plate mesoderm* forms the spleen, vessels, lymphatics, smooth muscle, and heart. It is divided by the *coelem* into the *somatic* and *splanchnic mesoderm*. The *coelem* creates the space between major organs and cavities (e.g. space within greater/lesser sacs). The *splanchnic mesoderm* forms the organs (*splanchnic* means 'relating to the viscera/organs), while the *somatic mesoderm* will form the peritoneal/pleural lining of the body's cavities (*somatic* means 'relating to the body').

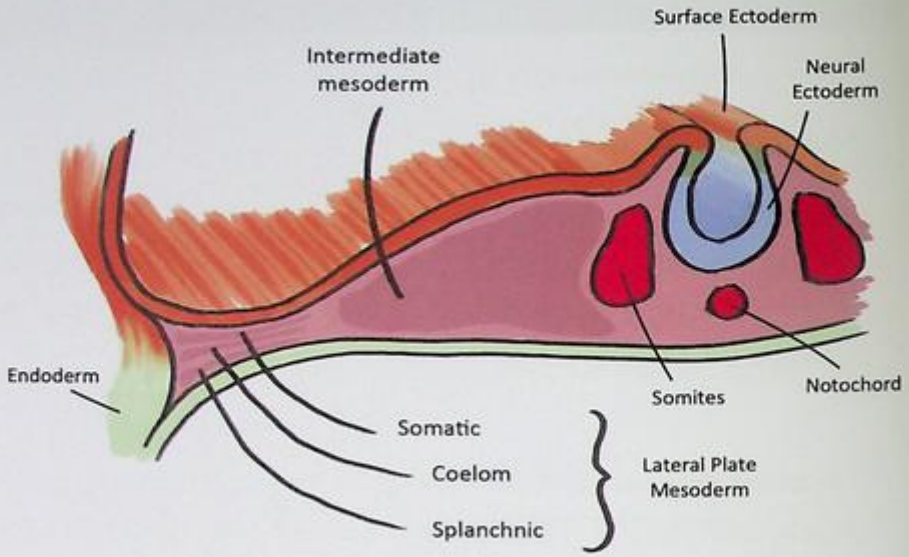


Figure 34: germ layer map of the trilaminar disk

Development of the Spleen

Around the 5th week of development, the spleen begins to form from the *splanchnic lateral plate mesoderm*. These cells proliferate within the mesogastrium of the stomach (dorsal mesentery) to form the spleen. Due to its location within the mesogastrium, rotation of the stomach positions the spleen into upper left quadrant of the abdomen.

Due to this movement, two key structures form (figure 35): first, the mesogastrium ventral to the spleen will become the *gastrosplenic ligament*; second, the mesogastrium dorsal to the spleen is brought into contact with

the surrounding peritoneum and left kidney to form the *lienorenal ligament*. The spleen functions as a haematopoietic organ within the foetus until the third trimester, but forms only lymphocytes and monocytes later in life.

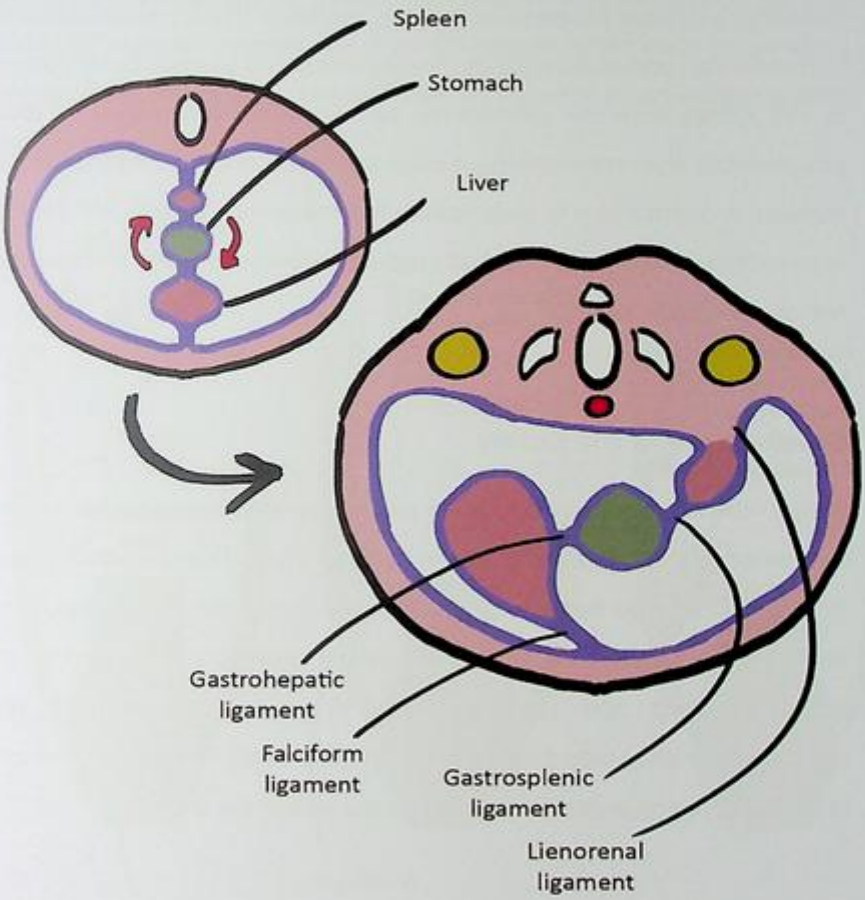


Figure 35: the rotation of the stomach leading to the final position of organs and their connecting ligaments

Development of the Urinary System

The *intermediate mesoderm* begins to organise and proliferate in the 4th week. It will form the kidneys, ureters, adrenal glands, gonads, and genital ducts. The development of the urinary system is a systematic process involving the staged progression through a series of structures: pronephros, mesonephros, and metanephros. These correspond to evolutionary stages of the kidney with the pronephros forming the adult kidney in some primitive fish, the mesonephros in small mammals, and the metanephros in humans. It is important to understand that the urinary system will develop in two distinct segments: one that produces urine (*excretory*) and one that removes it (*collecting*).

Development of the Kidney

Early in the 4th week, condensation of the *intermediate mesoderm* forms two *urogenital ridges* parallel to the midline. These ridges epithelialise at the cervical level to begin forming primitive rods – the *pronephric ducts*. Ventromedial to the rods, epithelial buds of *nephrotome* connect to the pronephric duct and communicate with vessels to create a very rudimentary kidney called the *pronephros* (*figure 36*). This exists superiorly in the embryo and entirely regresses by the end of the 4th week.

The rods continue to grow caudally to form the *mesonephric (Wolffian) ducts* which first appear in the thoracic and lumbar regions of the embryo. From the ducts, mesonephric tubules grow medially to meet small groups

of capillaries; here, the tubule expands to surround the vessels with a *glomerulus* to form the earliest *Bowman's Capsules*. This primitive system, called the *mesonephros*, is capable of producing a diluted urine that is functional between weeks 6 and 10. The mesonephric ducts continue to grow caudally towards the cloaca, such that 40 tubules develop in total. However, most will regress and ~20 will remain in the male for genitalia development. Importantly, the mesonephric (Wolffian) duct will completely regress in females.

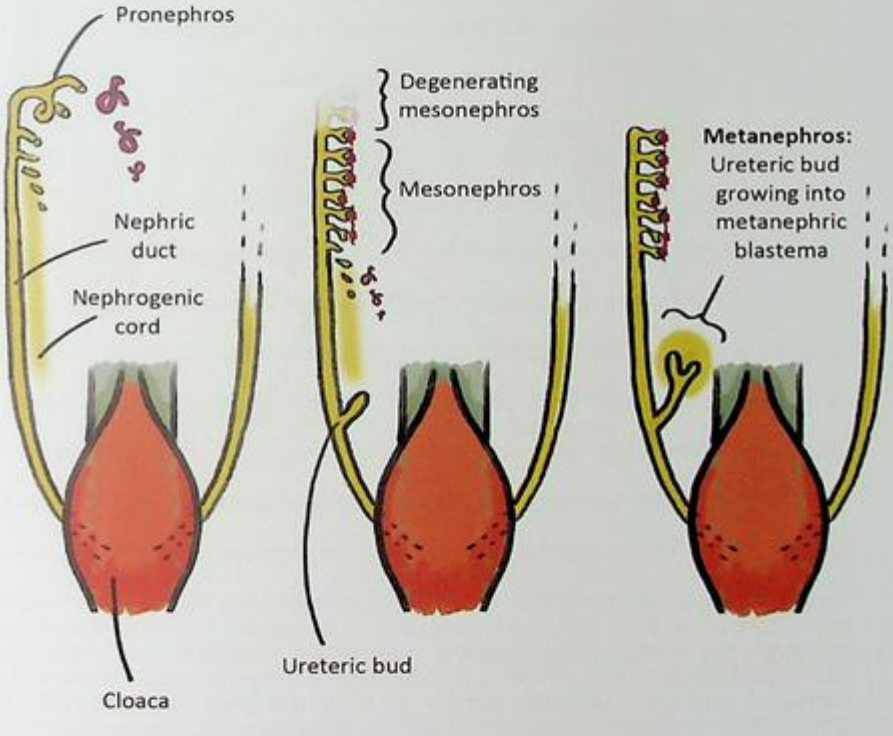


Figure 36: the developing kidney with formation of the pronephros, mesonephros, and metanephros

Around the 5th week, caudal to the mesonephros, the *metanephros* begins to develop in two separate components. A *ureteric bud* will branch from the metanephric duct. This will form the collecting segments of the urinary system including: the ureters, renal pelvis, major & minor calyces, and collecting ducts/tubules. The bud grows into the *metanephric blastema*, which forms the excretory components of the urinary system, and develops into: the bowman's capsule, proximal convuluted tubule, loop of Henle, and distal convuluted tubule. The *metanephric blastema* forms a cap around the growing *ureteric bud*. This bud elongate and bifurcate ~20 times in order to form the collecting ducts of 1-3 million nephrons (*figure 37*).

Meanwhile, the metanephric cap will respond to signals from the distal convuluted tubules to form *renal vesicles* – round collections of cap tissue. These vesicles will elongate to form a s-shaped tubule that communicates proximally with the glomerular capillaries as a *Bowman's capsule*, and distally with the collecting duct as the *distal convuluted tubule*. This is now a complete nephron that drains into the minor calyces. This kidney is functional from the 10th week of pregnancy and generates urine to contribute to the amniotic fluid surrounding the foetus. Recall that the metanephros is the caudal end of the duct (towards the cloaca), so the kidneys later ascend into the lumbar region to join the adrenals. As they rise through the foetus, they acquire new blood supplies. The ureters will descend and gain vascular supply as they do; they will attach to the posterior wall of the bladder (developed from the *ventral urogenital sinus*) and form the trigone.

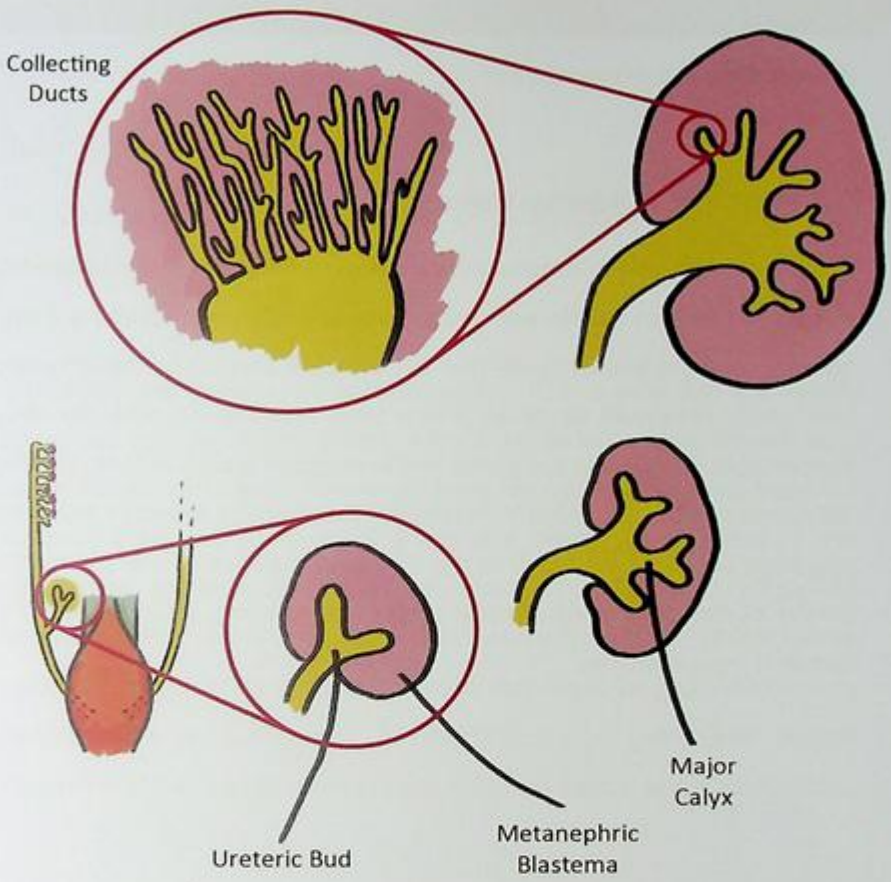


Figure 37: the bifurcating ureteric bud

The development of the kidney is dependent on *WT1* protein upregulation in the metanephric blastema, and *FGF2* & *BMP7* in the ureteric bud. *PAX2* and *WNT* proteins are expressed in the metanephric blastema to form the vesicles and tubules. There is constant communication between the bud

and cap through these molecules to ensure that the correct structures both form and adhere to one another.

Development of Adrenal Glands

Around the 4th week, *intermediate mesoderm* cells from the urogenital ridge will begin to proliferate. These cells will differentiate into a foetal adrenal cortex in the 6th week that will produce cortisol by the 8th week. This cortex continues to develop into birth and beyond, with the zona glomerulosa & fasiculata appearing late in pregnancy/early in birth, and the reticularis developing in early childhood. The medulla develops following migration of neural crest cells in the 7th week. These cells lie at the medial border of the developing adrenal cortex and are only enveloped by the cortex in late pregnancy.

Clinical Significance

Disorders of the Spleen

The pathologies of the spleen exist on a spectrum from absence to accessory tissue.

In *asplenia*, the spleen is completely absent. This is very rare but usually occurs as part of wider issues with mesoderm-derived tissues (e.g. cardiovascular disorders). Where *asplenia* happens in isolation, the child develops a *primary immunodeficiency*, and there is a high risk of life-threatening bacterial meningitis or sepsis. Patients with *congenital hyposplenia* also face these risks due to underdevelopment of the splenic tissue. This population must be treated the same as post-splenectomy patients with a full course of vaccinations – particularly against encapsulated bacteria (*pneumococcus*, *meningococcus*, and *haemophilus*).

The most common variance occurs in 10-20% of the population and involves additional nodules of splenic tissue – known as *accessory spleens*. They can be found anywhere within the abdomen but most commonly occur along the path of the splenic vessels, surrounding omentum/mesentery/ligaments, or towards the gonads. It is important to understand this phenomena as they can be identified during operations or on CT imaging.

Horseshoe

Fusion of the inferior poles of the kidneys leads to the formation of a horseshoe kidney that lies anterior to the aorta. As it ascends, it becomes trapped by the inferior mesenteric artery and cannot move further (*figure 38*).

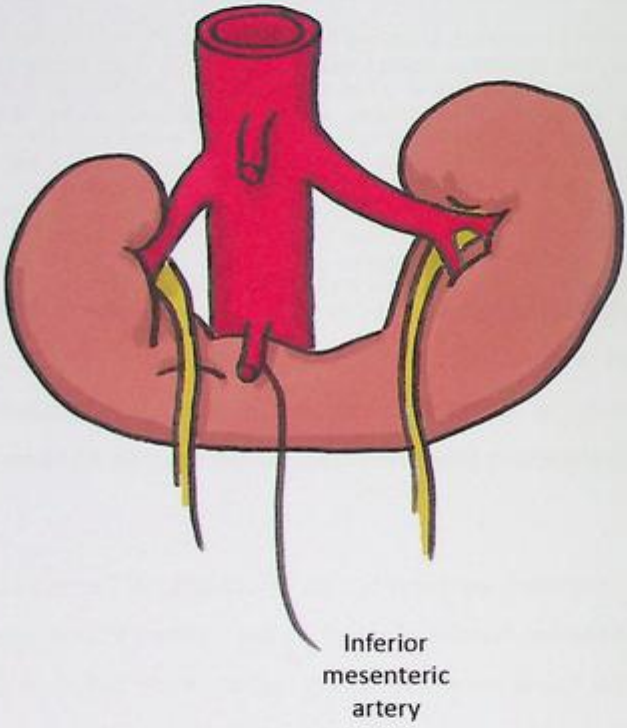


Figure 38: a horseshoe kidney unable to ascend beyond the inferior mesenteric artery

Supernumerary Arteries

Each kidney can have up to 3 arteries supplying it. They will have the renal artery branch of the aorta as their main supply, but may bring an accessory vessel from migration. This occurs in ~25% of patients and is important for identification in transplant operations. If the accessory artery perforates the substance of the kidney, rather than entering at the hilum, then it is known as an *aberrant* renal artery. This is commoner in horseshoe kidneys. Typically, aberrant arteries branch off the aorta to supply inferior poles.

Duplicated Kidney and Urinary Tract

This condition occurs when the ureteric bud prematurely bifurcates prior to penetrating the metanephric blastema to form a cap. It can lead to the formation of an accessory kidney, or additional urinary tracts. Neither of these confer any renal advantage but, rather, increase the susceptibility of the individual to urinary tract infections.

Pelvic Kidney

Occurs when there is a failure of the kidney to ascend and it remains in the pelvis. This is largely asymptomatic but can create greater difficulty in diagnosing renal pathology as the symptoms present atypically.

Renal Agenesis & Potter's Sequence

In this condition, there is complete failure to form renal tissue. It can be unilateral or bilateral, and occurs due to a lack of interaction between the ureteric buds and the metanephric blastema. This leads to oligohydramnios due to a reduction in the production of amniotic fluid. As a result of the reduced fluid, there is less space between the foetus and the mother's abdominal cavity. This leads to increased pressure on the embryo's body, which causes characteristic features of compression (also known as *Potter's Sequence*), such as: sloped forehead, flattened ("parrot beak") nose, shortened fingers, and compression of internal organs leading to hypoplasia.

Wilm's Tumour

Also known as a *nephroblastoma*, this condition occurs due to a mutation in the *WT1* (or *WT2*) gene. It is characterised by containing three types of cellular tissue: metanephric blastema, mesenchyme/stroma, and epithelium. It is diagnosed by the presence of a large, often painless, abdominal mass in the infant.

Renal-Coloboma Syndrome

Mutations in the PAX2 gene leads to defects in the kidneys and the eye. The mesenchymal blastema requires this gene to form the correct structures, and its modification leads to renal hypoplasia and vesicouretral reflux. The latter occurs when there is an inappropriate connection between the ureter

and the posterior bladder wall. Finally, a coloboma is a defect in the eye (iris, retina, and/or optic nerve) – most commonly a slit in the iris leading to disfigurement of the shape of the pupil. This occurs because PAX2 is important in the fusion of ventral parts of the eye.

Polycystic Kidney Disease

In this condition, there is dilatation of the excretory component of the urinary system – particularly in the tubules – leading to multiple cysts on the kidney. The most common cause of this is an autosomal dominant mutation in the *PKD1* gene. Less frequently, it can be due to an autosomal recessive mutation in the *PKD2* gene. These two genes are produce cilia on renal cells, but the mechanism for how this leads to cysts is less clear. It is thought to involve calcium regulation.

Mesoderm: Genitalia 16

It is important to know the individual components of sex determination within the embryo in order to understand how the processes occur. The key concepts to comprehend are:

- *Genetic sex*: the chromosome configuration (karyotype) of the embryo (e.g. XX or XY)
- *Gonadal sex*: the characterisation of the gonads that form within the embryo (e.g. testes)
- *Primordial germ cells*: these are the undifferentiated stem cells that will become either spermatozoa or oocytes
- *External genitalia*: sex organ of the embryo (e.g. penis)
- *Internal gonads*: an organ that produces gametes (e.g. ovary)
- *Gender*: not the same as genetic or gonadal sex; this refers to the invidual's identification of self (e.g. man, woman, non-binary) and is not determined in the embryonic stage of development

The gonads form from the *intermediate mesoderm* of the paired urogenital ridges. The genitalia develop from the *mesonephric (Wolffian) ducts)* in males, and from the *paramesonephric (Müllerian) ducts* in female. The gonads and genitalia are indistinguishable in males/females until the 7th week of development when the genetic sex influences their development; as such, the presumptive gonad tissue is considered *bipotential* – in that it can form either male or female gametes.

In XY individuals, the *sex-determining region (SRY)* leads to the formation of male gonads/genitalia; with its absence leading to female gonads/genitalia development. The *primordial germ cells (PGC)* appear in the 4th week and are derived from the *epiblast*. They begin to migrate during hindgut development and travel through the dorsal mesentery towards the urogenital ridges to lie medial to the developing ducts (*figure 39*).

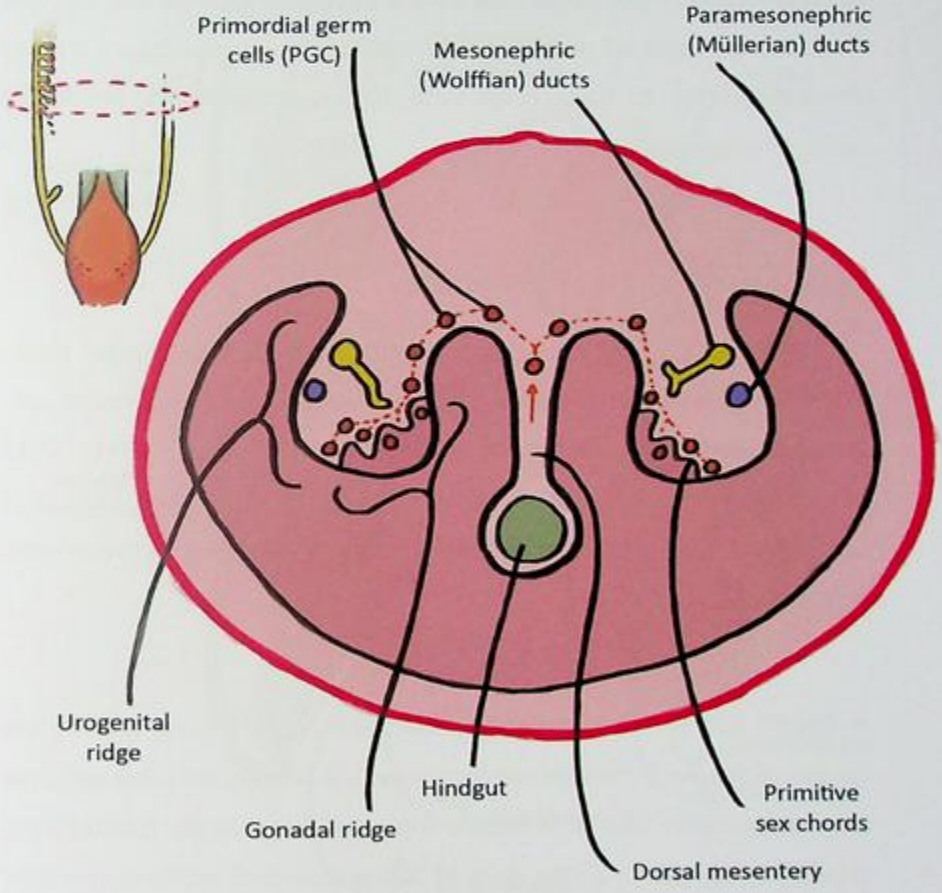


Figure 39: the primordial gonadal structures within the urogenital ridge

The gonads will develop from: intermediate mesoderm (mesenchyme), overlying coelemic epithelium (mesoderm/mesothelium on the posterior abdominal wall), and the primordial germ cells (PGC). The mesoderm and mesothelium profilerate to form the urogenital ridges, with a segment of these forming distinct *gonadal ridges* in the 5th week. Finger-like extensions of the coelemic cells, known *primitive sex cords,* grow inwards from the gonadal ridges. These ridges now have a surrounding *cortex* and an inner *medulla*. The testis will develop from *medulla*, whereas ovaries are created from the *cortex*. As these cords form, the *paramesonephric (müllerian) ducts* emerge laterally.

Development of the Male Gonads

The SRY region upregulates the autosomal gene for transcription factor *SOX9*, which in turn promotes the differentiation of the male gonadal cells by upregulating the production of *steroidogenic factor 1 (SF1)*. *SOX9* generates a positive feedback cycle for itself by stimulating *FGF9* that upregulates *SOX9* and causes the mesonephric tubules to grow towards the *rete testis*.

In the 6th and 7th weeks, the XY-containing PGCs invade the *primary sex cords* and release *testis-determining factor*. The *primary sex cords* will grow into the medulla and differentiate into the *testis cordis (seminiferous tubules)* and *Sertoli Cells*. The parts of the cord deepest within the medulla (deficient of PGCs) will develop the *rete testis cords*, while those in the most

peripheral medulla form the *tunica albuginea* (*figure 40*). The cortex will then degenerate. The *rete testis* will open into the most caudal parts of the *mesonephric (Wolffian) duct*. The *testis cordis* actually remains a rod until puberty, where the increase in testosterone levels lead to its canalisation and the formation of seminiferous tubules – capable to transporting sperm from the testis to the ejaculatory ducts.

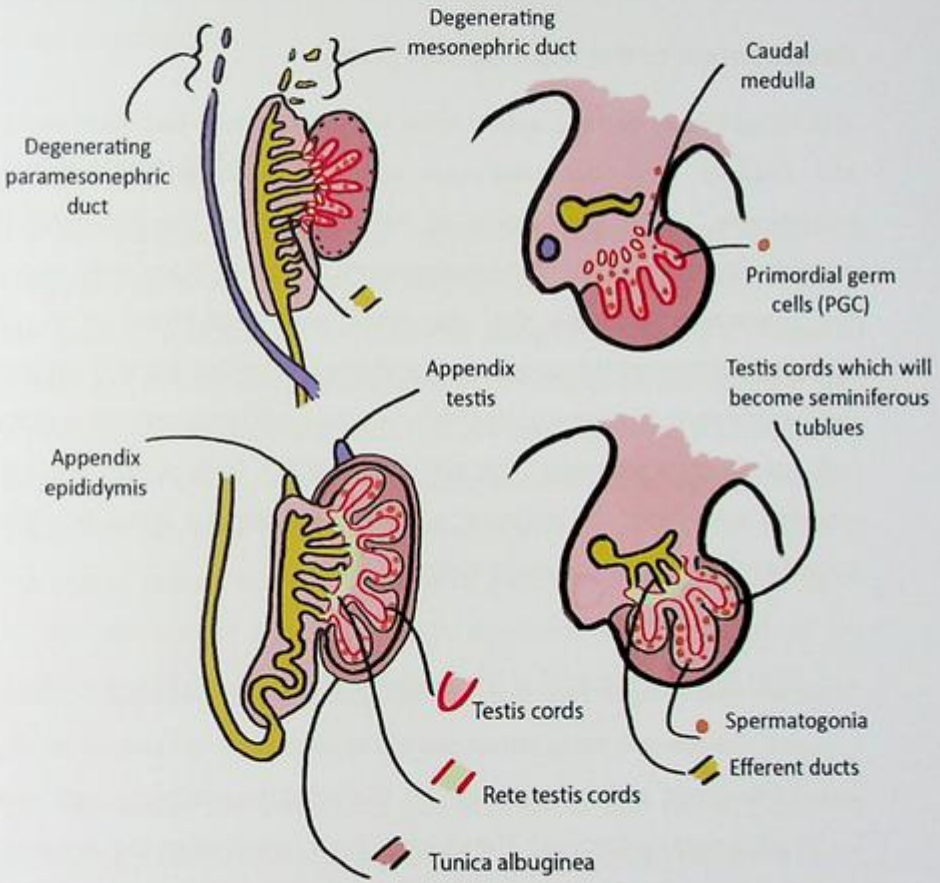


Figure 40: the development of the testes

The Sertoli Cells respond to *SF1* signals to secrete *Anti-Müllerian Hormone* (*AMH; Müllerian Inhibiting Substance*), which has a triple function. The first is to suspend the germ cells as spermatogonia in meiotic arrest (until puberty); the second is to stimulate *intermediate mesodermal* cells to differentiate into *Leydig Cells;* the final and most important function is to suppress the *paramesonephric (müllerian) ducts* so that they regress.

Development of the Male Genital Ducts

This forms in two parts: cranially from the mesonephric (wolffian) ducts, and caudally from the urogenital sinus. The *Leydig Cells* secrete *testosterone*, which acts locally to influence genital development. It promotes the persistence of the mesonephric (Wolffian) ducts and induces the connection between the *rete testis* and caudal-most 5 to 12 mesonephric tubules (bringing together the two systems). These drain into the mesonephric duct, which later becomes the *epididymis, seminal vesicles* and *vas (ductus) deferens*. The ejaculatory ducts form where the *vas deferens* opens into the posterior wall of the urogenital sinus, at a site known as the *verumontanum (seminal colliculus)*.

There are two vestigial remnants of the regressed paramesonephric ducts in males: the *apendix testis* (small cap of tissue on superior pole of testis), and the *prostatic utricle* (expansion on the prostatic urethra – used as a landmark in rigid cystoscopy). The utricle is likely homologous to the vagina, while the seminal collilus is supposedly homologous to the hymen.

The prostate forms in the 10th week as an *endodermal* outgrowth of the pelvic urethra (from the posterior urogenital sinus). Its development is stimulated by *dihydrotestosterone* (DHT), which is generated from testosterone after conversion by *5-alpha reductase*. The bulbourethral glands will form in a similar manner, by emerging as paired endodermal outgrowths from the membranous urethra (that is formed from the urogenital sinus).

Descent of the Testes

The testes develop in the lumbar region of the abdomen and descend to the groin area by utilising the contracture of tissues. Around the 2nd month, the testis is attached to the posterior abdominal wall by a urogenital mesentery; this becomes ligamentous as the *caudal genital ligament* with a mesenchymal component known as the *gubernaculum*. It connects the caudal pole of the testes to the labioscrotal swelling developing outside of the abdominal cavity. Ahead of the testes is a fold of peritoneum known as the *vaginal process* that follows a similar path through the inguinal region to the labioscrotal swellings. These structures are important as the abdominal wall muscles grow around them to form the inguinal canal.

The descent of the testes is a combination of two factors: first, the contractile proteins within the gubernaculum that actively move the testes; and secondly, the greater rate of growth of the abdominal cavity than the

gubernaculum, such that that testes (anchored by the ligament to the labioscrotal swelling) moves caudally. The active gubernaculum component of this descent is responsive to testosterone and dihydrotestosterone signalling.

Development of the Female Gonads

If the SRY gene is not present, then female gonads will form as default for the embryo. In the absence of SRY, the primary sex cords lose their structure and form loose clusters of mesenchymal cells within the medulla; these will later disappear to be replaced by highly vascular stroma (*figure 41*). Meanwhile, the PGC continue to proliferate within an expanding cortex into *oogonia*. Many of these oogonia apoptose and become reabsorbed into the cortical epithelium. This expands the size of the cortex to give rise to *cortical cords* that split into small clusters surrounding individual *oogonium* as *follicular cells*. By the 20th week of development, the oogonia have entered a suspended *prophase I* of *meiosis* to become *primary oocytes*.

This developmental process is dependent on activation of the *DAX1* gene. Due to the lack of *SOX9* production (due to no SRY), *WNT4* is not inhibited and its upregulation promotes the expression of *DAX1*. This in turn inhibits any expression of *SOX9*, in order to safeguard formation of the ovaries.

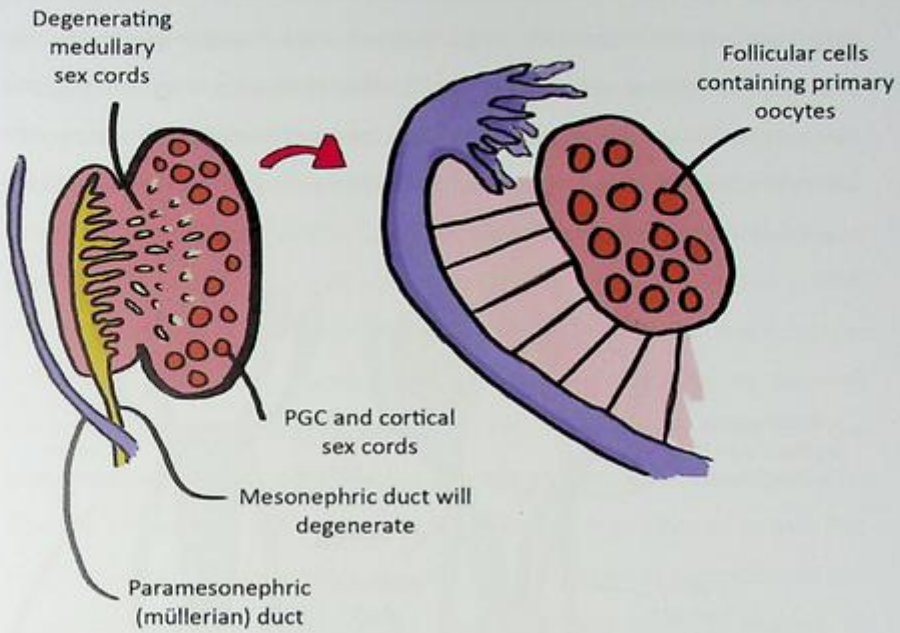


Figure 41: development of the ovaries

Development of the Female Genital Ducts

Once the ovaries have formed, the *mesonephric (Wolffian) ducts* will regress to remain only as vestigial *Gartner's ducts*. This leaves the *paramesonephric (Müllerian) ducts* and urogenital sinus to form the uterus and vagina.

In the 8th week, the two paramesonephric ducts will fuse in the midline (*figure 42*), bringing with them the peritoneal folds that form the *broad*

ligaments. Surrounding mesenchymal cells invade the primordial uterus to form the myometrium and endometrium. This process generates the presumptive uterus suspended within the ligaments that then grows towards the urogenital sinus. As the paramesonephric ducts make contact with the posterior urogenital sinus, two sinovaginal bulbs emerge from the sinus to form a solid *vaginal plate*.

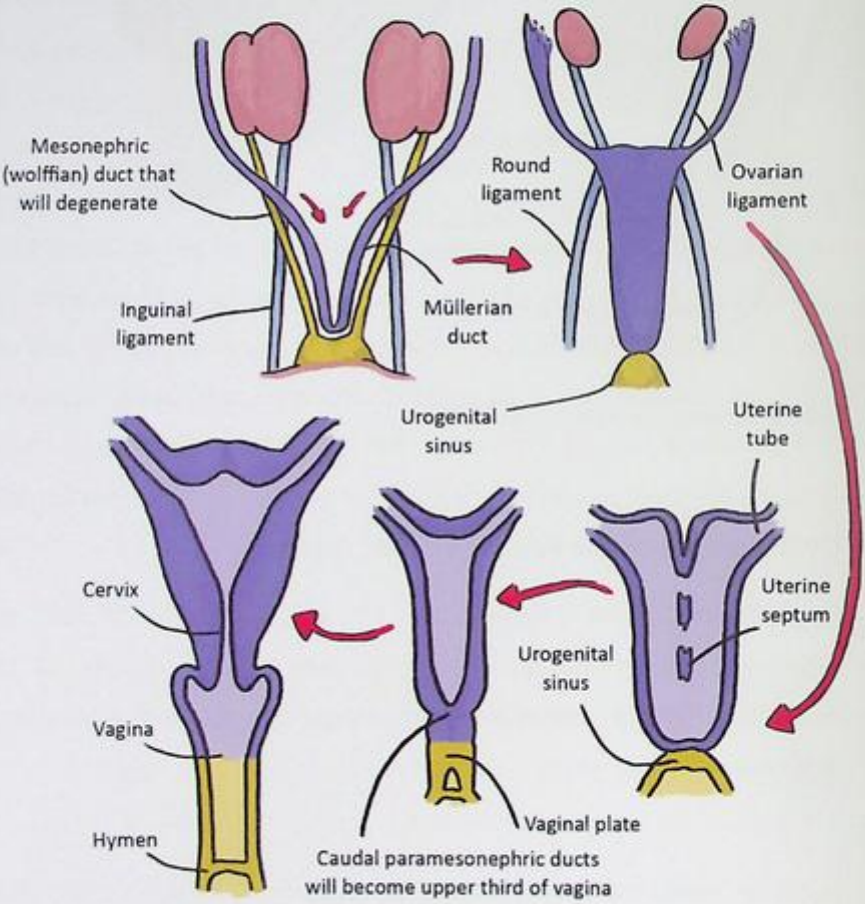


Figure 42: formation of the uterus and vagina

Starting in the 11th week and completing in the 5th month, this plate will canalise to complete the uterovaginal canal. The upper third of the vagina (the vaginal fornices) will be formed from the paramesonephric ducts, while the lower two-thirds develops from the urogenital sinus (*figure 42*). Furthermore, *vestibular (Bartholin) glands* develop as outgrowths from the urogenital sinus. Meanwhile, the fallopian tubes develop as unmerged cranial aspects of the paramesonephric (*Müllerian*) ducts with the cranial opening forming the *fimbrae*. Following regression of the mesonephric ducts and growth of nearby structures, the fallopian tubes become horizontal and the ligament connecting the mesonephric ducts and the paramesonephric duct becomes the *suspensory ligament* of the ovary. The opening of the vagina is marked by a *hymen* – a thin piece of tissue. The much shorter female urethra is generated by ventral components the urogenital sinus; its shorter length makes females more susceptible to urinary tract infections from the flora in the surrounding perineal areas.

Development of the Indifferent External Genitalia

The external genitalia initially develop in a uniform fashion from all three germ layers in both sexes: ectoderm forming overlying skin; lateral plate mesoderm the genital swellings; and, endoderm for urethral components. The swellings that surround the cloacal and urogenital membrane are indistinguishable until ~10th week, thus it is known as the *indifferent stage*.

Cloacal folds will surround the *cloacal membrane* to meet cranially at a *genital tubercle* (*figure 43*), which will lengthen early to form a phallus-

shaped structure that pulls caudal structures upwards. When the urorectal septum meets the cloaca and divides the *cloacal membrane*, the *cloacal folds* become *urethral folds*. Lateral to these, *genital swellings* appear to form the *labioscrotal swellings*. The urogenital membrane will narrow to form the urethral meatus. At this stage, the male and female external genitalia are indistinguishable, but these three core structures will form different structures in each sex, as described below:

Sex	*Genital Tubercle*	*Genital Folds*	*Genital Swellings*
Male	• Body & glans of penis • Corpora cavernosum • Corpora spongiosum	• Ventral penis • Penile raphe	• Scrotum • Scrotal raphe
Female	• Clitoral body & glans	• Labia minora	• Labia minora • Mons pubis

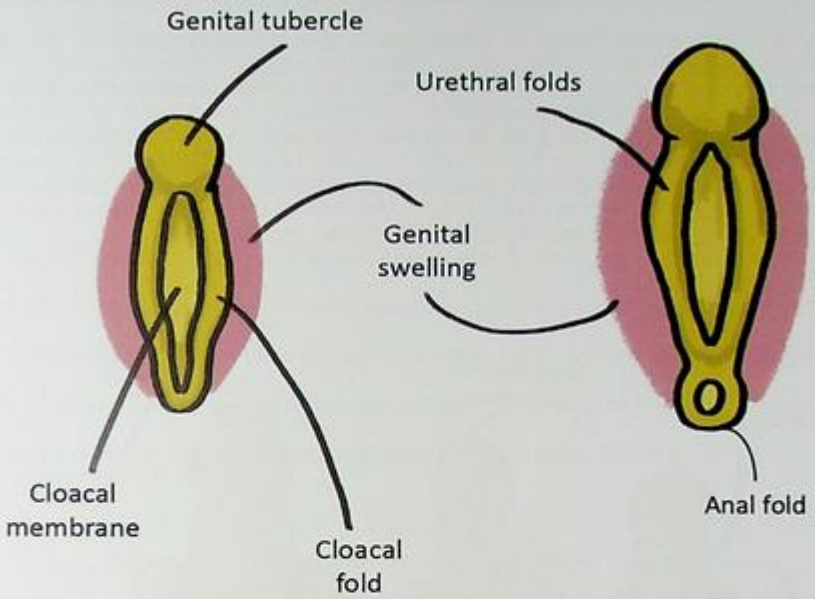


Figure 43: development of external genitalia in the indifferent stage

Development of the Male External Genitalia

All three germ layers are involved in the formation of the external genitalia. Under the influence of dihydrotestosterone, the external genitalia begin to undergo masculinisation (*figure 44*). The genital tubercle (*phallus*) elongates further, pulling the urogenital membrane (presumptive urethral meatus) cranially to form the *urethral groove*. The phallus expands cranially to form the *glans*. As such, there is now a *urethral groove* caudal to the phallus. This groove becomes lined with endoderm to form the *urethral plate*.

This *endodermal* plate brings new cells in to differentiate the urethra from surrounding genital tissue. Its introduction allows the *urethral folds* to close over the *urethral groove* in order to form the *penile urethra*. At the tip of the glans, an invaginating pit of ectoderm generates the *external urethral meatus* and the distal-most segment of urethra. Finally, the labioscrotal swellings will meet in the midline to form the scrotum.

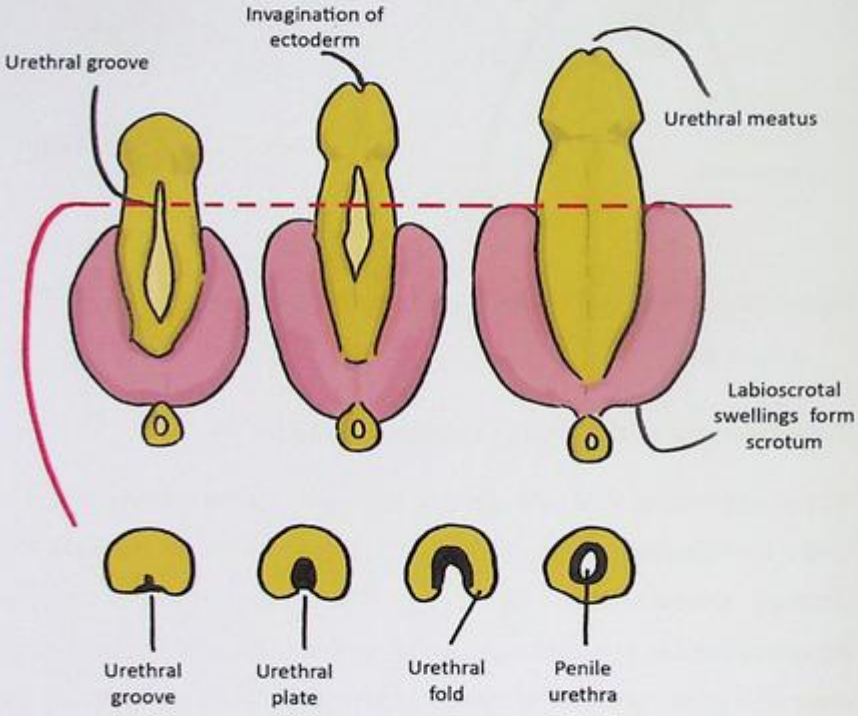


Figure 44: formation of the male external genitalia

Development of the Female External Genitalia

In females, the external genitalia begin to develop in response to secreted oestrogens (*figure 45*). The genital tubercle elongates only slightly to become a phallus that then forms the *clitoris* with erectile tissue (analogous to the cavernosum). The urogenital groove that lies between the urethral folds will remain open to contain the urethral opening ventrally/cranially and the vaginal opening dorsally/caudally; with the remainder forming the *vestibule* between the two. Meanwhile, the urethral folds do not overgrow the groove, but rather form the *labia minora* lateral to the vaginal opening. The labioscrotal (genital) swellings will form the *labia majora*.

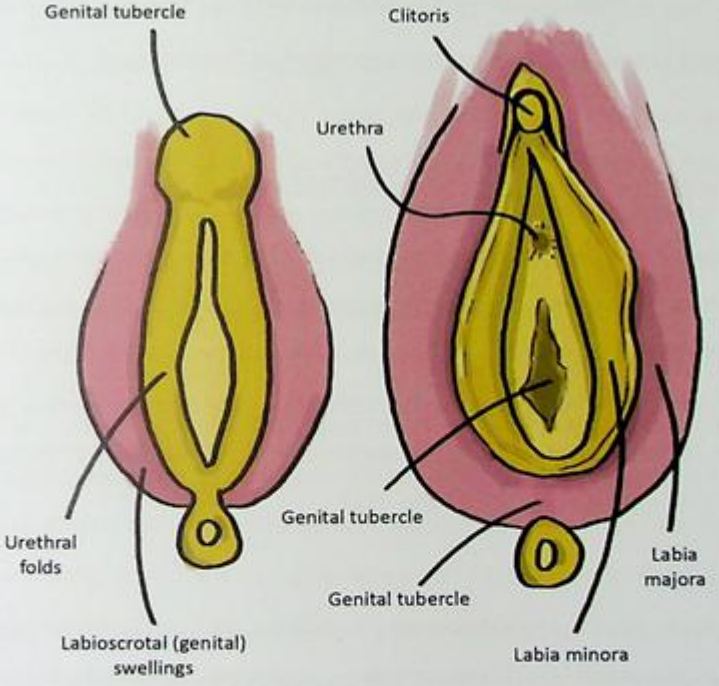


Figure 45: development of the female external genitalia

Clinical Significance

When considering the clinical syndromes associated with the formation of gonads and genitalia, it is useful to recall the initial descriptors of the different forms of sex: genetic, gonad, internal genitalia (genital ducts), and external genitalia. This will help learners understand the genetic and molecular pathways involved in each of the configurations. The terminology surrounding these conditions has changed to better reflect the underlying pathophysiology. Nomenclature such as "intersex" and "haemaphroditism" have been replaced with "differences of sex differentiation" (DSD), which assess the development from an underlying genetic perspective as "46XY", "46XX" and "sex chromosome" DSDs. Genitalia that lies between that of typical female or male genitalia is often described as "ambiguous".

Turner's Syndrome (Sex Chromosome DSD)

Most individuals carry 46 chromosomes of which 22 pairs are the autosomal chromosomes and 1 pair are the sex chromosomes. Genetic males have the configuration 46XY, and genetic females the configuration 46XX. In Turner's Syndrome, individuals have a genetic condition where the second X chromosome is either partly or completely missing – dubbed "45XO" (note that 45YO would not be compatible with life).

The classic presentation is females with a webbed neck, low-set ears, and short stature. The effect on fertility is dependent on the specific genetic mutation and proportion of the second X-chromosome deletion. Almost all

individuals are infertile due to ovarian dysgenesis, with only a small number of PGCs developing into oocytes. This also means that most of these individuals have *primary amenorrhoea* (failure to establish menstruation) due a lack of hormones produces by ovarian tissue. However, through hormone therapy (with oestrogen) and in-vitro fertilisation (IVF), individuals are able to undergo puberty and carry a pregnancy. This is because the primary reproductive defect is with the sex chromosome and primordial germ cells that influence the development of the gonads, and not with the internal female reproductive system (e.g. uterus).

Klinefelter's Syndrome (Sex Chromosome DSD)

Individuals with this condition have an additional X-chromosome (from the father) so carry the 47XXY karyotype. Individuals are male and generally asymptomatic, but will have infertility and hypoplasia of the testicles. At puberty, they may notice gynaecomastia, sexual anhedonia, or less hair growth. Generally, however, it is associated with a higher incidence of many other inflammatory and autoimmune conditions.

The hypogonadism occurs due to the lower levers of testosterone due to higher levels of follicule stimulating (FSH) and luteinising hormone (LSH) associated with the extra X-chromosome. The exact mechanism is not well understood and it is not as simple as a hormone imbalance, as it has been identified that the additional chromosome affects autosomal gene expression also. However, modern artificial insemination and sperm

extraction techniques have meant that these patients can have conceive children.

Androgen Insensitivity Syndrome (46XY DSD) [AIS]

Also known as "testicular feminisation syndrome", this occurs in individuals whose Leydig Cells produce testosterone but there is an inability of androgen receptors to bind the testosterone/DHT. This leads to the formation of undescended testes and female external genitalia. This is because the testes form in responsive to the SRY gene expression, while the external genitalia are unresponsive to the DHT and default to female development pathways. The lack of masculinsation of the external genitalia is sometimes described as *virilisation*. The insensitivity continues to puberty as there is a lack of response to testosterone.

It is important to note, however, that this condition exists on a spectrum relative to the extent of unresponsiveness to testosterone: *complete*, *partial*, and *mild*. In *Complete AIS*, there is total feminisation of the external genitalia; there is incomplete masculinisation in *Partial AIS*; and, in *Mild AIS* the genitalia appear male. In all cases, the Sertoli Cells continue to produce anti-müllerian hormones (AMH; müllerian inhibiting substance [MIS]) that will lead to the regression of the paramesonephric (müllerian) ducts; therefore, there is no formation of oviducts, uterus, or upper third of the vagina. Without the testosterone response, the male genital duct derivatives (e.g epididymis, vas deferens, and seminal vesicles) are typically absent.

5-Alpha Reductase Deficiency (46XY DSD)

This condition leads to a very similar clinical presentation to AIS, with internal male gonads and external ambiguous or female genitalia. This enzyme is responsible for the conversion of testosterone to dihydrotestosterone (DHT), which is required for signalling.

Interestingly, however, the mesonephric ducts and their derivatives persist as they require testosterone (not DHT) to continue development. The external genitalia may have the appearance of a micropenis with hypospadias (due to an enlarged clitoris with inferior-lying urethral opening). These individuals may have feminine features in childhood but then undergo a male puberty because the testes release testosterone – with the descent of the testes, deepening of the voice, and male-pattern hair development. As such, in some cultures, where the incidence of this deficiency is more common, then the entire process is celebrated with a marked occasion.

Furthermore, the release of testosterone can lead to lengthening of the clitoris (/micropenis) to form a phallus that may appear superficially like a penis. In these individuals, a hypospadias repair can be completed in order to place the external urethral opening on the tip of the phallus.

Cryptorchidism (Undescended Testes)

Up to 4% of full-term infant males will be born with unilateral or bilateral undescended testes. It is not treated immediately as the descent of the testes can continue into infancy. On examination, the healthcare

practitioner needs to determine if the testes are: impalpable (not found), palpable (perhaps in the inguinal or intra-abdominal regions), or retractile (appearing in the scrotum temporarility). It is important to monitor and appropriately manage these infants as untreated cryptorchidism leads to decreased infertility and increased risk of testicular malignancy (due to the inappropriate physiological temperature and environment).

If the testes have not descended by the 6th month, then a referral for surgical management (*orchidopexy*) should be completed. Post-surgically, individuals with unilateral cryptorchism have near-normal rates of fertility, whereas those with bilateral are reduced to 50%.

Persistent Müllerian Duct Syndrome (46XY DSD; PMDS)

In PMDS, there is either a mutation in the gene coding for production of Anti-Müllerian Hormone or in the gene encoding its receptor. The individual will develop male gonads and male external genitalia; however, there will be no regression of the paramesonephric (müllerian) ducts, leading to the formation of a small uterus, fallopian tubes, and upper third of the vagina. Due to the development of the uterus, a broad ligament forms which impedes the descent of the testes – leading to *cryptorchidism*. An operation can be performed to remove the Müllerian structures and correctly position the testicles.

Processus Vaginalis, Inguinal Hernias, & Hydroceles

As discussed earlier, the processus vaginalis is the fold of peritoneum that guides the inguinoscrotal descent of the testes. It will normally become

oblitered after birth, but persistence can lead to the formation of an inguinal hernia or hydrocele.

With inguinal hernias, a sufficiently wide patent processus vaginalis provides as route through the inguinal canal for an *indirect inguinal hernia*, such that bowel, mesentery, or intra-abdominal fat can pass through the superficial and deep rings to the scrotum. It presents with a swelling inferior and lateral to the pubic tubercle of the pubic bone (since the inguinal ligament attaches to the pubic tubercle medially with an opening lateral to this attachment site); this distinguishes it from a femoral hernia which lies superior and medial to the pubic tubercle. Inguinal hernias are at risk of strangulation following incarceration, so are usually operated on to avoid acute presentation of ischaemia/necrosis.

Hydroceles are benign swellings of the scrotum caused by the accumulation of serous fluid within the patent processus vaginalis. They can be visualised on ultrasound, and on examination will characteristically *transilluminate* when assessed with a torch. They can occur in up to 3% of births but are asymptomatic, and only require treatment due to a secondary pathology such as infection.

Hypospadias

This occurs due to the incomplete fusion of the urethral folds to enclose the urethral plate. It leads to a urethral orifice anywhere on the ventral/inferior aspect of the shaft of the penis. It can be corrected with surgery.

Congenital Adrenal Hyperplasia (46XX DSD) [CAH]

This occurs as a result of defects in the enzymes for the formation of glucocorticoids and mineralocorticoids, most commonly 21-hydroxylase deficiency. This means that all of the 17-hydroxyprogesterone precursor molecule is shunted to production of sex hormones (e.g. testosterone), rather than other steroids such as cortisol. The absence of other forms of steroid hormones means that there is limited negative feedback, leading to high levels of ACTH that stimulate the adrenals and cause hyperplasia.

Since there is no SRY gene, the individual will form female gonads (ovaries). The effect on the external genitalia is dependent on whether *androstenedione* (formed directly after the 17-hydroxyprogesterone on the sex hormone pathway) will be converted into testosterone or oestrogen compounds.

However, androstenedione itself is a potent virilising agent and if there is overwhelming production of androstenedione, then this will lead to virilisation (feminization) of the external genitalia. These individuals will have no male genital ducts as the mesonephric ducts regress due to a relative lack of testosterone; the lack of SRY also means that no Sertoli Cells (or AMH) is produced and so the female genital ducts (e.g. uterus) remain in situ.

On birth, this will appear as a scrotum with no palpable testes, so must be distinguished from cryptochordism. Individuals with this condition will

undergo female puberty, with the production of oestrogen. As such, re-assignment surgery can be undergone to feminise the genitalia – if desired.

Ovotesticular Disorder (46XX/XY DSD)

Also known as "true haemaphroditism", this condition is very rare and involves the presence of both male and female gonads with ambiguous external genitalia. Using traditional terminology, all previously described conditions in this chapter with incongruency between internal and external genitalia would be considered "pseudohaemaphroditism".

Most commonly, ovotesticular disorder is due to a division of the ovum into two separate haploid ova, that are then fertilised by different XX/XY sperm cells; these two fertilised ova (zygotes) then fuse together early in development. Alternatively, it may be due to a genetic mutation where there is mosaicism of the Y-chromosome onto the X-chromosome (translocation of the SRY).

Uterine Disorders

Malformations of the uterus can occur due to issues with the fusion of the paired paramesonephric (Müllerian) ducts, and leads to many anatomical variants.

In *uterus didelphys*, there is duplication of the uterus due to a lack of fusion between the two ducts (*figure 46*). These individuals can have two cervices and two vaginas, but will only have one horn from each uterus that links to the ipsilateral fallopian tube and ovary. Some studies have found this

condition to affect fertility. However, it is possible for each uterus to carry an individual pregnancy, with historical cases of triplets in the UK (twins in one uterus and single pregnancy in the other). Pregnant individuals with this condition require greater monitoring and most often require a Caesarean section. Like most disorders of the uterus, it is associated with higher complications of pregnancy including: preterm birth, malpresentation, placenta praevia (placenta attached near or oven the cervical opening), retained placental products, and premature rupture of membranes.

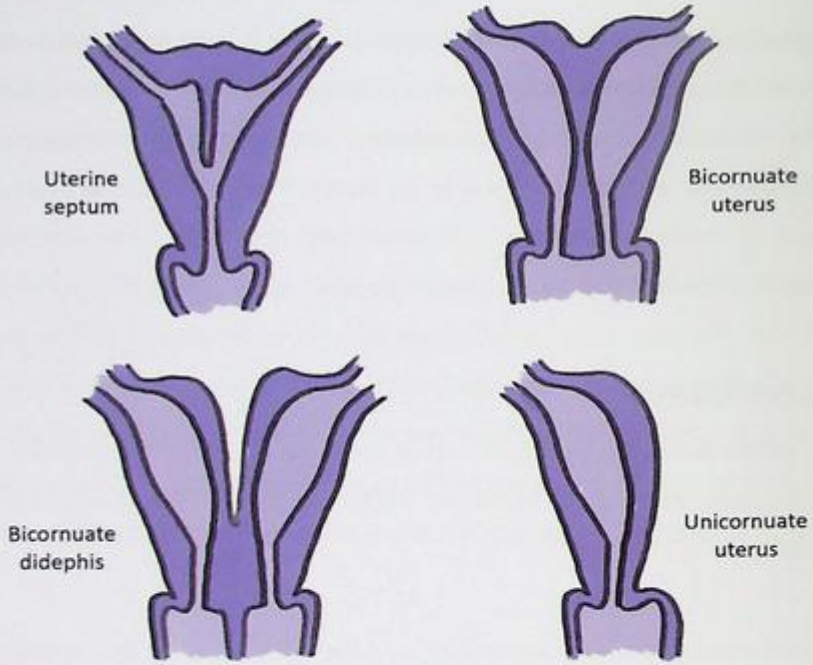


Figure 46: uterine disorders

A bicornuate uterus forms when the upper portion of the paramesonephric ducts do not fuse, while the lower portion does. These leads to a uterus with a septum extending from the uterine fundus to the cervical os – the extent of which is used for its classification. As before, it requires an increase in surveillance of pregnancy with higher risk of complications.

A unicornuate uterus occurs due to one of the paramesonephric ducts regressing or not forming appropriately. This uterus will communicate with the unilateral fallopian tube and ovary. It is managed with the same risks as the other outlined uterine disorders.

Relevant Molecules

- *SRY:* sex-determining region of the Y-chromosome that leads to the masculinisation of gonads, genital ducts, and genitalia in XY individuals
- *SF1:* steroidogenic factor 1 is a transcription factor that is required in the development of male gonads; it stimulates Sertoli Cells to produce Anti-Müllerian Hormone (AMH)
- *SOX9:* gene and transcription factor upregulated by SRY that leads to the formation of the testes
- *FGF9:* promoted by SOX9, this encourages a positive feedback cycle to self-propagate the production and expression of SOX9
- *Anti-Müllerian Hormone:* produced by the Sertoli Cells of the male genital ducts, this agent inhibits the Paramesonephric (Müllerian) Ducts in males leading to their regression
- *Testosterone:* secreted by the Leydig Cells, this hormone has local effects on the maintenance of the Mesonephric (Wolffian) Ducts, and distant effects on the virilisation of external genitalia
- *Dihydrotestosterone (DHT):* formed from testosterone, this stimulates tissues locally to form the male genital ducts and the external genitalia
- *5-Alpha Reductase*: the enzyme required to convert testosterone into DHT

KEY POINTS

- Genetic sex refers to the embryo's karyotype, which can be different to gonadal sex
- Gonads develop from the intermediate mesoderm of the urogenital ridges
- Gonads are indistinguishable until ~7th week of development
- Genitalia are indistinguishable until about ~10th week of development
- The SRY region on the Y-chromosome in genetic males will differentiate the bipotential genital primordia into male genitalia
- Spermatozoa and oocytes form from the primordial germ cells
- The Mesonephric (Wolffian) Ducts persist in males
- The Paramesonephric (Müller) Ducts persist in females
- Sertoli Cells secrete Anti-Müllerian Hormone (Müllerian Inhibitory Substance)
- Leydig Cells secrete testosterone
- The testes initially form in the lumbar region and undergo descend into the scrotum

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Illustrated by Thomas Newman

Embryology Explained is a comprehensive textbook for medical students and junior doctors. Original illustrations by Dr Thomas Newman help to guide the reader through the development of the embryo. The systematic approach provides clear explanations for each part of embryogenesis.

Each chapter is divided into four sections: embryology, clinical significance, molecules, and key points. This helps the reader to quickly identify and revise parts of their knowledge. The book has a particular focus towards explaining the parts of embryology that are commonly assessed in undergraduate and postgraduate medical exams, and would be a very useful aid for these examinations!

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